UPTAKE OF OESTRADIOL BY RAT AND HAMSTER UTERI *IN VITRO*: EFFECTS OF ETHYNYL OESTRADIOL AND AN ANTI-OESTROGEN

K. W. PERRY AND E. R. FERGUSON*

*Department of Endocrinology, Warner-Lambert Research Institute,
Morris Plains, New Jersey 07950, U.S.A.*

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Oestrogenic and anti-oestrogenic compounds that are effective antifertility agents for rats do not necessarily behave in a similar way for hamsters. The target tissues in the hamster are less sensitive to oestrogens and respond less to anti-oestrogens than those in the rat (Giannina, Butler, Popick & Steinetz, 1971). These investigators suggest that differences in metabolism or oestrogen affinity may explain their observations. Experiments *in vivo* show that hamster uteri retain significantly less tritiated oestradiol than is retained by rat uteri under identical experimental conditions (Ciaccio & Lisk, 1972). Prasad, Orsini & Meyer (1960) have shown that, unlike the rat, oestrogen is not required for implantation in the hamster.

In the present investigation, the uptake *in vitro* of tritiated oestradiol and the effects of ethynyl oestradiol or the anti-oestrogen, CN-55945-27 (1-[2-(p-{ α -(p-methoxyphenyl)- β -nitrostyryl}-phenoxy)ethyl] pyrrolidine monocitrate), on this uptake were compared in rat and hamster uterine tissue.

A tritiated oestrogen ([6,7- ^3H]oestradiol-17 β ; 40 Ci/mmol; New England Nuclear Corp., Boston, Mass.) with radiochemical purity $\geq 96.8\%$ and radioisotopic purity $> 99\%$ was used in these *in-vitro* studies. Benzene : ethanol (9 : 1) was used to dilute the tritiated oestradiol so that 0.16 ml of the diluted isotope in 400 ml incubation medium gave a final oestradiol concentration of 10^{-10} M.

The *in-vitro* system used for the uptake of tritiated oestradiol by uterine tissue was described by Jensen, De Sombre, Hurst, Kawashima & Jungblut (1967). Uteri were obtained from immature (40 to 50 g) Sprague-Dawley derived rats and outbred Lak : LVG golden hamsters. The slit uterine horns were stirred in beakers containing Krebs-Ringer-Henseleit glucose buffer (pH 7.3) at 37°C. Each beaker contained twenty uterine horns in 400 ml buffer with 10^{-10} M-tritiated oestradiol. The effects of the oestrogen and anti-oestrogen on the uptake *in vitro* of the tritiated oestradiol were determined by adding unlabelled ethynyl oestradiol (10^{-9} M) or CN-55945-27 (20 $\mu\text{g/ml}$ or 3.14×10^{-5} M) to the incubation medium. Five uterine horns were removed from each beaker at the end of each specific time period, frozen in a dry ice-acetone bath.

* Present address: Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois, U.S.A.

and freeze dried overnight. The dried uterine horns were weighed and then combusted for determination of d/min/mg dry weight.

The uptake *in vitro* of tritiated oestradiol by the hamster uterine tissue was significantly lower than that for the rat uterine tissue (Table 1). Tritiated oestradiol uptake by hamster uterine tissue (overall average) was 66% of that for rat uterine tissue. Ethynyl oestradiol and the anti-oestrogen (CN-55945-27) significantly inhibited the uterine uptake *in vitro* of oestradiol in a similar manner for both the rat and hamster. There appear to be no real differences in the trends of the effects produced by ethynyl oestradiol or the anti-oestrogen on the uptake of oestradiol by uterine tissue from the two species. The amounts of the uptake of tritiated oestradiol in the presence of ethynyl oestradiol were 41 and 44% of the controls for rat and hamster, respectively. In the presence of the anti-oestrogen, the amounts of the oestradiol uptake were 21 and 32% of the controls for the rat and hamster, respectively.

Table 1. The uptake of tritiated oestradiol by rat and hamster uterine tissue

Species	Incubation time (min)	Geometric average d/min/mg tissue (dry weight)*		
		[³ H]Oestradiol (10 ⁻¹⁰ M) (control)	[³ H]Oestradiol (10 ⁻¹⁰ M) + ethynyl oestradiol (10 ⁻⁹ M)	[³ H]Oestradiol (10 ⁻¹⁰ M) + CN-55945-27 (20 µg/ml)
Rat	30	3500	2121	1153
	60	5592	2708	1246
	120	8400	2955	1334
	180	9374	2505	1575
	**	6265	2554	1318
Hamster	30	2518	1519	1038
	60	4014	1823	1306
	120	5154	2026	1442
	180	5626	1909	1592
	**	4137	1809	1328

* Critical mean difference = 8.9%, $P = 0.05$, $n_1 = n_2 = 10$.

** Overall average, critical mean difference = 4.3%, $P = 0.05$, $n_1 = n_2 = 40$.

Analysis of variance of the radioactive data (logarithmic transformation) was carried out and is shown in Table 2. This analysis shows that highly significant differences existed between species, treatments, times and all of their interactions except for species versus time.

This *in-vitro* system provides a method for studying oestrogen-receptor interactions that excludes oestrogen biotransformation and metabolism that occurs *in vivo* in non-target tissues (Jensen, Numata, Smith, Suzuki, Brecher & De Sombre, 1969). Thus, any differences observed in the present study can only result from uterine tissue metabolism, uterine membrane transport, oestrogen-receptor affinity and/or number of receptor sites or molecules available.

In this study, the uptake *in vitro* of oestradiol by the hamster uteri (overall average) was 66% of that observed for the rat uteri. The uptake *in vivo* of oestra-

diol by hamster uteri was only 15 to 25% of that for the rat uteri (Ciaccio & Lisk, 1972). A comparison of these data indicates that some *in-vivo* factor(s) and/or mechanism(s) reduce the uptake of oestradiol by the hamster uteri. Giannina *et al.* (1971) have shown that seventeen and twenty times more oestradiol is required by the hamster to give uterotrophic and contraceptive activities equivalent to those observed for the rat. Thus, it appears that the differences between the hamster's and the rat's requirements *in vivo* for oestradiol are not solely confined to the uterus and its ability to take up oestradiol as shown by the *in-vitro* data.

The data in the present study indicate that there are no real differences in the effects of ethynyl oestradiol and the anti-oestrogen, CN-55945-27, on the uptake *in vitro* of oestradiol by the uteri from the two species. Giannina *et al.* (1971) reported that the effects of oestradiol can be completely antagonized by

Table 2. Analysis of variance of the radioactive data

	<i>d.f.</i>	<i>log V</i>	<i>F</i>
Species	1	0.71157	396.00*
Treatment	2	7.02964	3916.00*
Species × Treatment	2	0.19326	107.00*
Time	3	0.55555	309.00*
Species × Time	3	0.00066	0.37
Treatment × Time	6	0.10607	59.00*
Species × Treatment × Time	6	0.00861	4.80*
Residual	215	0.00179	

* $P < 0.001$.

1.0 mg of CN-55945-27/kg in the rat whereas more than 5.0 mg/kg is required for the hamster. Also, eighteen and 100 times more ethynyl oestradiol is required by the hamster to give uterotrophic and contraceptive activities equivalent to those observed for the rat. From the *in-vitro* data it appears that the differences in the *in-vivo* uterotrophic, anti-uterotrophic and contraceptive activities between the rat and hamster are not due solely to uterine metabolism or interactions with the uterine oestrogen receptors.

These data strongly indicate that the differences in the effects of oestrogens and anti-oestrogens *in vivo* on the uterotrophic, anti-uterotrophic and contraceptive activities between rats and hamsters may be due either to one or to a combination of the following: (a) non-target tissue metabolism, (b) action on target tissues other than the uterus, (c) uterine oestrogen receptor affinities and/or concentration, and possibly (d) assay sensitivities.

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REFERENCES

- CIACCIO, L. A. & LISK, R. D. (1972) Effect of hormone priming on retention of ^3H -oestradiol by males and females. *Nature, New Biology*, **236**, 82.

- GIANNINA, T., BUTLER, M., POPICK, F. & STEINETZ, B. (1971) Comparative effects of some steroidal and nonsteroidal antifertility agents in rats and hamsters. *Contraception*, **3**, 347.
- JENSEN, E. V., DE SOMBRE, E. R., HURST, D. J., KAWASHIMA, T. & JUNGBLUT, P. W. (1967) Estrogen-receptor interactions in target tissues. *Archs Anat. microsc. Morph. exp.* **56**, 547.
- JENSEN, E. V., NUMATA, M., SMITH, S., SUZUKI, T., BRECHER, P. I. & DE SOMBRE, E. R. (1969) Estrogen-receptor interaction in target tissues. *Devl Biol. Suppl.* **3**, 151.
- PRASAD, M. R. N., ORSINI, M. W. & MEYER, R. K. (1960) Nidation in progesterone-treated, estrogen-deficient hamsters, *Mesocricetus auratus* (Waterhouse). *Proc. Soc. exp. Biol. Med.* **104**, 48.