THE EFFECTS OF SOME ANTI-OESTROGENS AND ANTI-METABOLITES ON THE BINDING OF TRITIATED OESTRADIOL BY THE VAGINA OF THE OVARIECTOMIZED MOUSE

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Abstract

The antimetabolite actinomycin D inhibited the retention of [^{3}H]oestradiol-17β and tetrazolium reduction in the mouse vagina when administered 1 and 25 hr before [^{3}H]oestradiol. Inhibition of retention in both 105,000 *g* pellet and supernatant fractions of vaginal homogenates was observed with the pellet fraction more sensitive to the inhibitor. Actinomycin D inhibited tetrazolium reduction, but not the retention of [^{3}H]oestradiol, when administered 1 hr before [^{3}H]oestradiol.

p-Hydroxymercuribenzoate (PHMB) inhibited the retention of [^{3}H]oestradiol with a maximum effect when administered 10 min before the oestradiol. Again the 105,000 *g* pellet fraction was more sensitive to inhibition, indicating, with PHMB and actinomycin D, a possible interference with oestrogen receptor transformation from the 9S to the 5S form. The inhibitory effect of PHMB on [^{3}H]oestradiol retention was more transient than the effect on the tetrazolium response. When administered up to 16 hr after the oestradiol, PHMB did not displace more than 50% of the radioactivity from the tissue, suggesting that *in vivo*, in contrast to *in vitro*, the formation of the receptor oestrogen complex affords some protection to the receptor –SH groups.

The anti-oestrogens dimethylstilboestrol and 7α,17-dimethyl-19-nortestosterone inhibited the retention of [^{3}H]oestradiol and again a greater effect was found in the pellet fraction.

I. INTRODUCTION

The ability of the oestrogen-responsive tissues, such as the uterus and vagina, to incorporate physiological levels of tritiated oestradiol-17β from low blood levels *in vivo* following parenteral injection (Jensen and Jacobson 1962; Stone, Baggett, and Donnelly 1963), from the vaginal and uterine lumen following direct application (Martin 1964; Stone and Martin 1964), or during *in vitro* incubations in buffered salt solutions (Jacobson *et al.* 1964; Stone and Baggett 1965*a*; Rochefort and Baulieu 1969), is now well documented. The retention of oestradiol is obligatory for its subsequent oestrogenic action in these tissues (Stone 1964). Homogenization of the tissue, labelled *in vivo* or *in vitro* with [^{3}H]oestradiol, has shown that almost all of the radioactivity is associated with either the 800 *g* (nuclear) or 105,000 *g* (supernatant) fraction, the relative proportion associated with each fraction depending on the time after administration or incubation and the conditions of fractionation (Noteboom and Gorski 1965; Jensen *et al.* 1968; Stone 1968). In the nuclear fraction the oestradiol is associated with a 5S component (Puca and Bresciani 1968; Jensen *et al.* 1968) and in the supernatant with a 9S component (Toft and Gorski 1966), both protein in nature. There is evidence that the transfer of oestradiol from the cytoplasm to the nucleus is effected while bound to the 9S component (Baggett and Stone

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1966; Jensen *et al.* 1968) and that the form extracted from the nucleus is a 5S complex (Jensen *et al.* 1968; Korenman and Rao 1968; Jensen *et al.* 1969).

Various anti-oestrogens and anti-metabolites have been shown to interfere with oestrogen binding by these tissues or with the responses of the tissues to oestradiol or both. In the present experiments a number of such compounds has been examined for their effects on the *in vivo* retention of [^{3}H]oestradiol by the mouse vagina, on the distribution of oestradiol between 105,000 *g* pellet and supernatant fractions of vaginal homogenates and on the normal oestrogenic response. It was thought that this approach might provide some information on the relative roles of these two tissue fractions in oestrogen action and on the anti-oestrogenic action of the inhibitors.

II. Materials and Methods

(*a*) *Solutions*

[6,7-^{3}H]Oestradiol-17β (specific activity 160–170 μCi/μg) was obtained from New England Nuclear Corp. and purified before use as previously described (Stone and Baggett 1965*a*). The purified compound was stored at −20°C in 10% benzene in methanol and for each experiment aliquots were dried under nitrogen and the residues dissolved in distilled water to give the required weight of steroid for injection in 5 or 10 μl. Actinomycin D was kindly provided by the Merck Institute for Therapeutic Research. The dry powder was dissolved in propylene glycol and Krebs–Ringer phosphate buffer, pH 7·4 (Umbreit, Burris, and Stauffer 1959), was added to give the required level of the compound in 10 μl of 10% propylene glycol. *p*-Hydroxymercuribenzoate, sodium salt (Sigma Chemical Co.), was dissolved in a 0·01M tris(hydroxymethyl)aminomethane (Mann Research Laboratories) buffer, pH 7·4. Dimethylstilboestrol (DMS) was the compound described by Emmens and Cox (1958) and 7α,17-dimethyl-19-nortestosterone was provided by the Upjohn Co. Aliquots of ethanolic stock solutions of the latter compounds were dried under nitrogen and dissolved in 10% propylene glycol in distilled water to give the required level in 5 μl.

(*b*) *Animals*

Randomly bred mice of the QS strain, bred in this laboratory and of weight 20–25 g, were ovariectomized, primed 2 weeks later with 1·0 μg of subcutaneously injected oestradiol-17β, and used 8–11 days after priming. Within each experiment mice were randomized to experimental groups. Intravaginal applications were made with an Agla micrometer syringe. Mice were killed by cervical dislocation and where indicated the vaginal lumen was rinsed through *in situ* from the cervix with 1 ml of 0·9% NaCl and the rinses collected. The tetrazolium assay (Martin 1960) was used to follow the effects of the inhibitors on the oestrogenic response. This sensitive assay measures the increased reduction of 2,3,5-triphenyltetrazolium chloride in the vaginal epithelium 24 hr after oestrogen administration. The reduced formazan was extracted with 2 ml of a mixture of ethanol–tetrachlorethylene (3:1 v/v) and estimated at 495 nm. For statistical analysis each absorbance was transformed to 100 $\log_{10}$ (absorbance).

(*c*) *Tissue Preparation and Measurement of Radioactivity*

For radioactivity estimations on whole vaginae the tissues were digested in 0·5 ml of hydroxide of hyamine-10X (Packard Instrument Co.) at 60°C until complete when 10 ml of toluene containing 0·4% (w/v) 2,5-diphenyloxazole (PPO) and 0·01% (w/v) 1,4-bis(4-methyl-5-phenyl-oxazol-2-yl)benzene (dimethyl POPOP) were added. For tissue fractionation studies the vaginae were dissected onto ice, minced with scissors, and homogenized at 0°C in 0·01M Tris–0·1M KCl–0·001M EDTA buffer (pH 7·4) with an all-glass Dual tissue grinder (Kontes Glass Co.), using a motor-driven pestle. The homogenate was centrifuged at 4°C for 60 min at 105,000 *g* using a type 40 rotor in a Spinco model L265B ultracentrifuge to separate the 105,000 *g* pellet and supernatant fractions. The pellet fraction, which essentially represents oestradiol association with the nucleus, was extracted three times with 10 ml of ethanol and three times with 10 ml of chloroform–methanol (2:1 v/v), the extract dried under vacuum, and the radioactivity measured.

Aliquots of the 105,000 *g* supernatant were taken for radioactivity estimation. Where indicated the supernatant fraction binding of [^{3}H]oestradiol was studied either by dialysis or density-gradient centrifugation. For dialysis aliquots of the supernatant were dialysed against 4 litres of distilled water for 4 hr with agitation and the change in [^{3}H]oestradiol concentration measured. No correction for change in volume of the non-dialysed fraction was made. For the density-gradient study, 0·25-ml aliquots of supernatant were layered over 4·7 ml of a 5–20% linear sucrose gradient and centrifuged at 39,000 r.p.m. for 15 hr at 4°C in a type SW50L rotor. Successive 0·2-ml fractions were collected from the gradient and assayed for radioactivity or absorbance at 220 nm. Beef liver catalase (Sigma Chemical Co.) was used as a reference standard for calculation of approximate sedimentation coefficients (Martin and Ames 1961).

The pellet extracts and small aqueous volumes (< 0·5 ml) were taken up in diotol scintillation fluid (Herberg 1960) while larger aqueous volumes were taken up in a scintillator consisting of toluene–Triton X-100 (2:1 v/v) (Patterson and Greene 1965) containing 0·4% PPO and 0·01% dimethyl POPOP. Radioactivity was measured in either a Nuclear Chicago or Packard liquid scintillation spectrometer and the counts were corrected to disintegrations per minute using a [^{3}H]palmitic acid internal standard.

TABLE 1

EFFECT OF ACTINOMYCIN D ON [^{3}H]OESTRADIOL RETENTION

Actinomycin D (0·3 μg) was administered intravaginally either 1 hr (A) or 1 and 25 hr (B) before 25 pg of [^{3}H]oestradiol-17β. At 1 hr the vaginal retention, the distribution of radioactivity between 105,000 *g* supernatant and pellet fractions of vaginal homogenates, and oestradiol binding by the supernatant fraction were measured. At 24 hr the tetrazolium response was measured

Treatment	Tissue Radioactivity				Tetrazolium Response ($A_{495\,nm}$)
	Total (% of injected)	Supernatant Fraction (% of recovered)	Total in Fractions (% of injected)	Dialysis (% of initial concn.)	
Control (C)	57·6	25·4	56·3	61·1	0·348
A	61·5	21·8	57·1	63·7	0·001
B	22·8	35·7	19·5	62·7	0·001
No oestradiol					0·068

Summary of Analyses of Variance

Source of Variation	D.F.	Mean Square†	D.F.	Mean Square‡			D.F.	Mean Square§
A *v.* C	1	37·2	1	26·8	1·1	13·6	1	649·64***
C + A *v.* B	1	4501·9***	1	388·01***	3700·2***	0·3	1	182·25**
Within group	12	*75·4*	9	*8·96*	*7·13*	*30·7*	12	*14·93*

** 0·01 > *P* > 0·001. *** 0·001 > *P*. † Total radioactivity. ‡ Radioactivity in fractions. § Tetrazolium response.

III. RESULTS

In the first experiment the effect of an intravaginal injection of 0·3 μg of actinomycin D at either 1 hr or 1 and 25 hr before treatment with 25 pg of [6,7-^{3}H]-oestradiol-17β was studied. Within each treatment five groups, each of five animals, were killed 1 hr after the administration of the oestradiol. In one group total radioactivity in each organ was measured while tissues in the remaining four groups were

separately pooled, 105,000 *g* pellet and supernatant fractions prepared, and aliquots of each fraction taken for radioactivity estimation. A further aliquot of each supernatant was dialysed for 4 hr. The remaining supernatant fractions within each treatment were pooled and an aliquot subjected to density gradient centrifugation at 39,000 r.p.m. for 15 hr. The effect of the treatments on the 24-hr tetrazolium response was also measured. Control animals received 10 μl of 10% propylene glycol 1 hr before the [^{3}H]oestradiol. Results are shown in Table 1. Although the tetrazolium response was completely inhibited with each actinomycin D pretreatment, only administration at both 1 and 25 hr inhibited the retention of [^{3}H]oestradiol. Total recoveries of radioactivity from the tissue fractionations were in good agreement with those from whole tissue and showed a similar inhibition. However, the inhibition

TABLE 2

EFFECT OF ACTINOMYCIN D PRETREATMENT ON *IN VITRO* [^{3}H]OESTRADIOL BINDING

Actinomycin D (0·3 μg) or the vehicle were administered 25 hr before killing. Aliquots of 105,000 *g* supernatant fractions of the vaginal homogenates were incubated with varying levels of [^{3}H]oestradiol-17β and dialysed for 4 hr at 4°C. The change in [^{3}H]oestradiol concentration was measured

Level of Oestradiol (pg/organ)	Percentage of Initial [^{3}H]Oestradiol Concentration		Summary of Analysis of Variance		
	Control	Treated	Source of Variation	D.F.	Mean Square
10	44·4	37·1	Oestradiol		
			Linear (D_L)	1	680·81***
50	37·2	29·7	Quadratic (D_Q)	1	9·63
250	23·1	21·5	Actinomycin D (A)	1	90·20**
			Oestradiol × actinomycin D		
			$D_L \times A$	1	16·25*
			$D_Q \times A$	1	6·41
			Within group	6	*2·61*

* 0·05 > P > 0·01. ** 0·01 > P > 0·001. *** 0·001 > P.

was significantly greater in the pellet than in the supernatant fraction. No significant treatment effect was observed in dialysis while during density gradient centrifugation essentially all of the radioactivity was associated with a single 9S peak. Radioactivity in the luminal rinses was uniformly low in all treatments representing 0·2–0·4% of the injected radioactivity and indicated that actinomycin D pretreatment did not prevent the uptake of [^{3}H]oestradiol into the vaginal tissue. In a further experiment, in which the effect of a single injection of actinomycin D 25 hr before the oestradiol was studied, results similar to those obtained with both 1 and 25 hr pretreatment were observed.

The effect of 25 hr pretreatment with actinomycin D on the supernatant fraction binding of [^{3}H]oestradiol was further studied in the experiment of Table 2. Ten mice were treated with either 0·3 μg of actinomycin D, or the vehicle, 25 hr before killing. Vaginae within each treatment were pooled and 105,000 *g* supernatant fractions prepared. Portions of each were incubated for 1 hr at 4°C with three levels of [^{3}H]oestradiol (10, 50, or 250 pg per organ), and the change in [^{3}H]oestradiol con-

centration, after dialysis of replicate aliquots for 4 hr against 4 litres of distilled water, was measured. At the low and intermediate levels of [^{3}H]oestradiol, actinomycin D pretreatment caused a 15–20% decrease in binding while little inhibition was noted at the high level probably due to a saturation of receptors in both treated and control groups. At the low levels of oestradiol the inhibition was considerably less than when this tissue fraction was labelled *in vivo*.

TABLE 3

EFFECT OF *p*-HYDROXYMERCURIBENZOATE ON [^{3}H]OESTRADIOL RETENTION

p-Hydroxymercuribenzoate (10 μl of an 0·07M solution) was intravaginally administered 30 (A) or 10 min (B) before or simultaneously (C) with 50 pg of [^{3}H]oestradiol-17β. At 1 hr the vaginal retention and distribution of radioactivity between 105,000 *g* supernatant and pellet fractions were measured. At 24 hr the tetrazolium response was measured

Treatment	Tissue Radioactivity: Total (% of injected)	Tissue Radioactivity: Fractionation (% of recovered in pellet fraction)	Tetrazolium Response ($A_{495\ nm}$)
A	21·1	54·2	0·006
B	3·9	39·2	0·002
C	9·0	54·7	0·004
[^{3}H]Oestradiol alone	50·8	69·1	0·309
No oestradiol			0·063

Summary of Analyses of Variance: Source of Variation	D.F.	Mean Square†	D.F.	Mean Square‡
A *v.* C	1	367·2***	1	0·6
A+C *v.* B	1	415·2***	1	305·0*
Within group	12	*14·8*	3	*28·1*

* $0{\cdot}05 > P > 0{\cdot}01$. *** $0{\cdot}001 > P$. † Total radioactivity. ‡ Radioactivity in fraction.

In the experiment of Table 3, mice were injected with 10 μl of 0·07M *p*-hydroxymercuribenzoate (PHMB) in 0·01M Tris buffer, pH 7·4, 30 or 10 min before, or simultaneously with, 50 pg of [^{3}H]oestradiol. Pilot tests had established that higher levels of PHMB were no more effective. The mice were killed 60 min after the [^{3}H]oestradiol administration, each vaginal lumen rinsed *in situ* with 1 ml of 0·9% sodium chloride and the radioactivity in the rinses measured. Within each treatment the vaginae from five mice were digested and counted individually and the tissues from two groups, each of 10 mice, were separately pooled and 105,000 *g* pellet and supernatant fractions were prepared. The radioactivity in each fraction was measured. The effect of the treatments on the tetrazolium response was studied in further groups, each of five mice. Control animals received the vehicle with or without the [^{3}H]oestradiol. PHMB at all times inhibited the retention of radioactivity by the vagina, with maximum inhibition when administered 10 min before the [^{3}H]oestradiol. The proportion of the recovered radioactivity associated with the pellet fraction was also decreased by all treatments with PHMB and again the maximum sensitivity was seen at 10 min. However, with this parameter there was no difference between administration 30 min before and simultaneously with the [^{3}H]oestradiol. The uniformly low levels of radioactivity in the luminal rinses with all treatments indicated that PHMB did not prevent the uptake of [^{3}H]oestradiol by the vagina. With each antimetabolite treatment the tetrazolium response was significantly less than vehicle-injected controls, indicating that the interference of PHMB with the oestradiol binding might be more transient than the interference with the tetrazolium reduction.

The effect of PHMB, administered varying times after [^{3}H]oestradiol, was studied in the experiment of Table 4. Mice received 50 pg of [^{3}H]oestradiol followed

TABLE 4

EFFECT OF *p*-HYDROXYMERCURIBENZOATE ON [^{3}H]OESTRADIOL RETENTION

p-Hydroxymercuribenzoate (PHMB, 10 μl of an 0·07M solution was administered at varying times after 50 pg of [^{3}H]oestradiol-17β. Mice were killed 1 hr after the PHMB to measure the vaginal retention of radioactivity and 24 hr after the [^{3}H]oestradiol for the tetrazolium response. Five mice per group

Treatment (PHMB or buffer)	Tissue Radioactivity (% of injected)		Tetrazolium Response ($A_{495\ nm}$)	
	Buffer	PHMB	Buffer	PHMB
Simultaneously with oestradiol	58·4	32·6	0·399	0·034
After 1 hr	36·6	24·8		0·021
After 2 hr	23·1	16·8		0·023
After 4 hr	17·4	10·8		0·023
After 8 hr	6·6	4·5		0·049
After 16 hr	4·2	2·0		0·058
No oestradiol				0·078

Summary of Analyses of Variance

Source of Variation	Tissue Radioactivity		Tetrazolium Response	
	D.F.	Mean Square	D.F.	Mean Square
Time				
Linear (T_L)	1	4817·75***	1	4608**
Quadratic (T_Q)	1	137·41**	1	2128*
Substance (S)	1	416·45***		
Substance × time				
$S \times T_L$	1	138·5**		
Remainder	5	12·64	2	686
Within group	36†	*11·12*	20	*451*

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

† Reduced for four missing observations. Non-significant single degrees of freedom pooled as remainder.

by 10 μl of 0·07M PHMB or the vehicle at the time indicated. One hour after the PHMB the mice were killed, the vaginae removed, digested, and counted. The effect of PHMB at these times on the 24-hr tetrazolium response was also measured. At all times PHMB decreased the retention of [^{3}H]oestradiol although the rate of loss of radioactivity from the tissue was slightly greater in the control animals as seen in the significant substance × linear time interaction in the analysis. At no time, however, was more than 50% of the radioactivity in the tissue removed by treatment with PHMB. The tetrazolium response was decreased below that of vehicle-injected controls with all PHMB treatments, and, although the inhibition was significantly

less at the later times, the responses were probably too low for any meaningful interpretation.

The effect of 25 ng of dimethylstilboestradiol (DMS) and 50 ng of 7α,17-dimethyl-19-nortestosterone (U-10997) on the binding of 50 pg of [^{3}H]oestradiol by the mouse vagina was studied in the experiment of Table 5. The oestrogen and anti-oestrogen were administered together as a single intravaginal injection in 10 μl of 10% propylene glycol in distilled water. The animals were killed 1 hr after injection.

TABLE 5

EFFECT OF DIMETHYLSTILBOESTROL AND 7α,17-DIMETHYL-19-NORTESTOSTERONE ON OESTRADIOL RETENTION

Dimethylstilboestrol (DMS) (25 ng) and 7α,17-dimethyl-19-nortestosterone (U-10997) (50 ng) were administered intravaginally simultaneously with 50 pg of [^{3}H]oestradiol-17β. At 1 hr the vaginal retention, the distribution of radioactivity between 105,000 *g* supernatant and pellet fractions, and the [^{3}H]oestradiol binding by the supernatant fractions were measured. At 24 hr the tetrazolium response was measured. Five mice per group

Treatment	Tissue Radioactivity			Tetrazolium Response ($A_{495\ nm}$)
	Total (% injected)	Supernatant (% of recovered)	Dialysis (% of concn.)	
[^{3}H]Oestradiol	36·9	36·2	63·9	0·349
[^{3}H]Oestradiol+DMS	6·4***	48·6***	68·1	0·116***
[^{3}H]Oestradiol+U-10997	23·3**	40·0**	67·5	0·253*

* $0·05 > P > 0·01$, ** $0·01 > P > 0·001$, *** $0·001 > P$ (significance of differences from [^{3}H]oestradiol alone).

Within each treatment the vaginae from one group of mice were individually digested and counted and the vaginae from four groups each of five mice were separately pooled and 105,000 *g* supernatant and pellet fractions were prepared. The binding in the supernatant fraction was studied after 4 hr dialysis against 4 litres of distilled water. The effect of DMS and U-10997 on the 24-hr tetrazolium response was also measured. Both DMS and U-10997 significantly decreased the retention of radio-activity by the vagina. the proportion of tissue radioactivity associated with the pellet fraction and the tetrazolium response to [^{3}H]oestradiol. At the doses tested DMS was more effective than U-10997. Neither compound affected the binding of [^{3}H]oestradiol in the supernatant fraction during dialysis.

IV. DISCUSSION

The effect of actinomycin D pretreatment at 25 and 1 hr, but not at 1 hr alone, in decreasing by more than half the whole tissue retention of oestradiol may be explained in at least two ways. There may be a rapid turnover of receptor even in this unstimulated ovariectomized state and thus the effect would represent a decrease in total amount of receptor. Alternatively actinomycin D may inhibit the synthesis of enzymatic mechanisms acting in the initiation or the maintenance of the receptor–oestradiol complex, if such actinomycin-sensitive mechanisms are involved. The

ability of at least the 9S component to show *in vitro* binding after considerable purification (Soloff and Szego 1969; Ellis, personal communication to Emmens) and previous findings (Stone and Baggett 1965*a*) that at least 17 hr after both oestradiol and actinomycin D no inhibition of retention is observed suggests that a decrease in receptor level is involved. Actinomycin D decreased the amount of [^{3}H]oestradiol associated with both the 105,000 *g* supernatant and pellet fractions with a relatively greater inhibition in the latter. This possibly represents an interference with the mechanisms involved in transfer of the 9S receptor complex from the cytoplasm to the nucleus or with the suggested transformation of the 9S to the 5S form. It is still not clear whether two distinct binding components exist, in which case the observed results would suggest a more rapid turnover of the nuclear component, or whether the 9S to 5S transformation represents a true conformational change or merely an artifact induced by the extraction procedure (discussed by Korenman and Rao 1968; Jensen *et al.* 1969). If the 5S complex is consumed during oestrogen action in the nucleus an effect opposite to that observed might have been expected during actinomycin inhibition. With all treatments only the 9S component was observed in the supernatant in contrast to *in vitro* experiments in the uterus where 8S to 4S subunit formation has occurred at this time (Rochefort and Baulieu 1969).

PHMB interferred with the binding of [^{3}H]oestradiol by components of both the cytoplasmic and nuclear fractions, indicating participation of sulphydryl (–SH) groups in the binding of oestradiol. A similar observation with PHMB and other –SH blocking agents has been reported by Jensen *et al.* (1967). However, these authors found that tissue, labelled *in vivo* or *in vitro* with [^{3}H]oestradiol, rapidly lost essentially all of the radioactivity when subsequently incubated with PHMB. In the present completely *in vivo* system a similar effect was observed only when the PHMB was administered just prior to, or simultaneously with, the [^{3}H]oestradiol. With administration between 1 and 16 hr after the [^{3}H]oestradiol no more than 50% of the radioactivity was displaced from the tissue, indicating that *in vivo* the formation of the receptor–oestradiol complex might afford some protection to the receptor –SH groups. The rate of association of PHMB with the receptor –SH groups appears slower than the rate of formation of the receptor–oestradiol complex *in vivo* as administration 10 min before the oestradiol was required to show the most effective inhibition. The association of the inhibitor with the –SH groups of the receptor appears quite transient considering the significantly lower inhibition with administration 30 min, as opposed to 10 min, before the oestradiol and the inhibition of the mechanisms involved in the tetrazolium response, which at all times of PHMB treatment, was reduced to a level below that obtained for vehicle injected controls. Differences in the strength of association of PHMB with –SH groups in different proteins have been reported (Madsen 1963). As observed with actinomycin D the PHMB decreased the movement of oestradiol from the supernatant to the nucleus indicating either that the nuclear component is more sensitive to –SH group interference or an inhibition of the transfer or transformation mechanisms from the 9S to the 5S form.

Although DMS is apparently oestrogenic *per se* with a low receptor affinity (Martin 1969), in low doses it acts as an anti-oestrogen (Emmens and Cox 1958) by competing with oestradiol for binding (Pollard and Martin 1967). The present results indicate that competition with both nuclear and cytoplasmic receptors is involved with the nuclear component again being the more sensitive. U-10997, although less

potent, had a similar action to DMS in both tissue fractions. This may partly explain the antifertility action of this compound in early pregnancy in the mouse (Emmens *et al.* 1967).

A feature common to all of the inhibitors examined was a greater decrease in nuclear retention than in cytoplasmic retention of [^{3}H]oestradiol. While an explanation for this effect, representing an interference either with receptor synthesis or receptor transformation from the 9S to the 5S form, can be envisaged for actinomycin D and PHMB, the reasons for a similar effect for the anti-oestrogens DMS and U-10997 are not clear. The phenomenon of supernatant to nucleus transfer, together with mechanisms by which oestradiol is lost from rather than incorporated by the tissues is being studied.

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