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Some *in vivo* and *in vitro* Interactions of Oestrogen Sulphates with the Mouse Uterus and Vagina

G. M. Stone

Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W. 2006.

Abstract

The *in vivo* and *in vitro* uptake and metabolism of [^{3}H]oestradiol 3-sulphate and [^{3}H]oestrone 3-sulphate by the mouse uterus and vagina were examined. After intravaginal injection of either sulphate, radioactivity disappeared from the vagina at a rate which was greater than that seen with [^{3}H]-17β-oestradiol, similar to that seen with [^{3}H]oestrone, but somewhat less than that previously observed with some non-oestrogenic steroids. Sixteen minutes after intravaginal administration of either sulphate there was significant hydrolysis in the vagina, but the greater part of the radioactivity was still associated with the sulphates. No interconversion of the conjugates was observed, but the formation of oestradiol was favoured following hydrolysis of the conjugates to the free steroids. Plasma levels of radioactivity were very low and would have directly accounted for little of the tissue radioactivity.

With *in vitro* incubation of uterine and vaginal minces from ovariectomized animals, rapid hydrolysis of both sulphates was observed and this was greater than the non-specific effect seen in muscle tissue. Uterus was more active than vagina and no significant difference in the hydrolysis of tritiated oestradiol sulphate and oestrone sulphate was observed. Again, no interconversion of the sulphates was detected, and in the free steroid fraction the reduction of oestrone was favoured. Incubation of uteri from mice at days 5 or 19 of pregnancy showed an increased sulphatase activity towards the end of pregnancy and increased reduction of oestrone. In these incubations there were suggestions of the formation of [^{3}H]oestradiol sulphate from [^{3}H]oestrone sulphate.

The results suggest that, in the mouse, oestrogen target tissues such as the uterus and vagina have a greater ability than some non-target tissues to hydrolyse circulating oestradiol sulphate or oestrone sulphate. The ability, at least in the uterus, is increased towards the end of pregnancy.

Introduction

The sulphate conjugates comprise a considerable portion of the circulating oestrogens in man and other species (Fishman *et al.* 1969; Loriaux *et al.* 1971; Ruder *et al.* 1972; Thorburn *et al.* 1972) and may be particularly important towards the end of pregnancy (Thorburn *et al.* 1972; Wong *et al.* 1972). Whether these conjugates function merely as metabolites of the free steroids or are able to interact directly with the oestrogen target tissues is not clear. Certainly, administered oestrone sulphate elicits normal oestrogenic responses (Davies *et al.* 1950), and some oestrogen target tissues have the ability *in vitro* to hydrolyse oestrone 3-sulphate and oestradiol 3-sulphate (Pack and Brooks 1970; Gurpide *et al.* 1971; Payne *et al.* 1973). However, under such *in vitro* conditions this sulphatase activity is also seen in non-target tissues (Pack and Brooks 1970; Payne *et al.* 1973). The aim of the present experiments was to examine some *in vivo* and *in vitro* interactions of oestrone sulphate and oestradiol sulphate with the mouse uterus and vagina in both ovariectomized animals and during pregnancy.

Materials and Methods

Solutions

[2,4,6,7-^{3}H]-17β-Oestradiol (specific activity 370 μCi/μg) ([^{3}H]E2), [2,4,6,7-^{3}H]oestrone (370 μCi/μg) ([^{3}H]E1) and the potassium salt of [6,7-^{3}H]oestrone 3-sulphate (1·2 μCi/μg) were obtained from the Radiochemical Centre, Amersham, England. Stock solutions of radioactive and carrier steroids (Sigma Chemical Co.) were maintained at −20°C in ethanol (free steroids) or alkaline ethanol (sulphates) (Rosenthal *et al.* 1972). Radioactive and non-radioactive oestradiol 3-sulphate was prepared from the respective oestrone sulphate by borohydride reduction (Griffiths *et al.* 1963) and all sulphates were purified before use by thin-layer chromatography using solvent system D of Flickinger and Touchstone (1968). Purification of [^{3}H]E2 and [^{3}H]E1 before use and the preparation of all compounds for intravaginal injection in 5 μl were as previously described (Stone and Baggett 1965*a*).

For *in vitro* experiments, aliquots of the [^{3}H]E2 sulphate and [^{3}H]E1 sulphate solutions were dried under nitrogen and the residue dissolved in 0·01M tris–0·01M KCl–0·001M EDTA buffer (pH 7·4) (buffer A) such that the incubation media contained 5×10^{-2} μg of the appropriate sulphate per gram of tissue incubated (approximately 30 nM).

Animals

Randomly bred mice of the QS strain were ovariectomized at 20–25 g body weight, primed 1–2 weeks later with 1·0 μg of subcutaneous oestradiol, and used 1 week later. For the *in vivo* studies the vaginal lumen was rinsed through before dissection (Stone 1971) and the rinses discarded. Mature females were mated and the morning that the copulation plug was observed was designated as day 1 of pregnancy. Animals were killed by cervical dislocation, or if plasma was required, by decapitation. In the latter case the blood was collected into heparinized tubes and the plasma obtained by centrifugation.

Tissue Preparation and Measurement of Radioactivity

Radioactivity estimates on whole vaginae were made as previously described (Stone 1971). For *in vitro* experiments the vaginae were dissected onto ice, minced with scissors and tissues pooled to yield portions weighing 0·7–1·7 g. These were incubated in buffer A at 37°C in air for 30 or 120 min. Tissue was separated from the medium by centrifugation, washed once by resuspending in medium at 4°C and the tissue and medium extracted separately. As a check on tissue specificity and solvolysis induced by the extraction procedure, incubations of skeletal muscle or boiled tissue were included. Tissues were homogenized in alkaline 80% ethanol and the residue extracted three times with ethanol. The extract was partitioned in chloroform–water (1 : 1 v/v) and the aqueous phase extracted twice more with chloroform. The residual aqueous phase was extracted three times with an equal volume of n-butanol. Media or plasma were extracted three times with an equal volume of chloroform and then with n-butanol as above. Radioactivity in the media, the total tissue extracts, the chloroform fractions (free steroids), the butanol fractions (sulphates) and the residual aqueous fractions was measured.

Thin-layer chromatograms of the extracts were developed in chloroform–acetone (92 : 8 v/v) (free steroids) or system D of Flickinger and Touchstone (1968) (sulphates). The respective zones were located using a radiochromatogram scanner (Packard Instrument Co.), iodine vapour (free steroids) or sulphuric acid (sulphates). The radioactivity was measured following elution with methanol or suspension in Insta-gel (Packard Instrument Co.).

For binding studies 105 000 *g* supernatant preparations of vaginal or uterine homogenates were prepared in 0·01M tris–0·4M KCl–0·001M EDTA buffer (pH 8·5) as described by Stone and Pollard (1973). Aliquots of the supernatant were incubated with [^{3}H]E2 sulphate or [^{3}H]E1 sulphate (1 ng per organ equivalent) for 2 h at 4°C and the binding studied by charcoal absorption or density gradient centrifugation (Stone and Pollard 1973). Gradient fractions (0·2 ml) were collected using an Isco gradient fractionator (Instrumentation Specialties Co.). Total radioactivity in the fractions was measured or the nature of the compounds in the peak areas of radioactivity was examined as described for the media from the incubations.

Results

In the experiment of Table 1, groups of ovariectomized mice received intravaginal injections of [^{3}H]E2, [^{3}H]E1 or their respective sulphates and were killed 1, 4, 16 or

64 min later. The retention of radioactivity by the vagina was measured and expressed as a percentage of the injected dose for statistical analysis. After [^{3}H]E2 injection the expected prolonged retention was observed, and this at all times was significantly greater than seen with any other compound. The time–response slopes did not differ after injection with either [^{3}H]E2 sulphate or [^{3}H]E1 sulphate, but the overall retention, which was somewhat greater than previously observed with some non-oestrogenic steroids (Stone and Baggett 1965*b*), was greater after [^{3}H]E2 sulphate injection. The results suggested that there could be some oestrogen sulphatase activity in the vagina, or that tissue binding of the sulphates themselves might occur, or both.

Table 1. Uptake of free tritiated oestrogens and their sulphates by the vagina *in vivo*

Mice were killed at varying times after the intravaginal injection of 50 pg of [^{3}H]-17β-oestradiol (E2) or [^{3}H]oestrone (E1) or 1 ng of [^{3}H]E2 3-sulphate or [^{3}H]E1 3-sulphate. Results are expressed as the percentage of the injected radioactivity in the vagina at the time of killing (means from five mice per group). In the analysis of variance, non-significant single degree of freedom effects are pooled as remainder

Steroid injected	Time after injection (min): 1	4	16	64
Oestradiol	76·3	70·4	56·6	53·6
Oestrone	71·7	60·1	23·9	14·4
Oestradiol sulphate	69·6	56·8	22·9	8·6
Oestrone sulphate	61·1	49·6	16·1	5·6

Summary of analysis of variance

Source of variation	D.F.	Mean square	*F* ratio
I. Time			
(a) Linear	1	32010·58	684·7***
(b) Cubic	1	1458·86	31·2***
II. E2 *v.* E1	1	4708·90	100·7***
III. E2 *v.* E1 sulphate	1	514·80	11·0**
IV. E2 + E1 *v.* E2 sulphate + E1 sulphate	1	6644·84	142·1***
Ia × II	1	1985·76	42·5***
Ia × IV	1	1146·16	28·5***
Remainder	8	38·13	0·8
Error	64	*46·75*	

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

To examine the former suggestion, mice were given intravaginal injections of [^{3}H]E2 sulphate or [^{3}H]E1 sulphate and the distribution of radioactivity in the vaginae and plasma was examined. The experimental design, results for the whole vaginae and plasma radioactivity and the distribution between the free steroid and sulphate fractions are given in Table 2. Again a greater percentage of the injected dose was retained after [^{3}H]E2 sulphate (21·4) than after [^{3}H]E1 sulphate (17·0) ($P < 0{\cdot}05$). Based on an average plasma volume of 1·2 ml/mouse (Spector 1956), the total radioactivity in the plasma was low, did not differ between sulphates and in no case exceeded 2% of the injected dose. As expected, in each case essentially

all of the radioactivity recorded was in either the chloroform (free) or butanol (sulphate) fraction, with no significant difference between tissue and plasma or between the two sulphates in this respect.

Table 2. Distribution of radioactivity after injection of tritiated oestrogen sulphates

Replicate groups of 30 mice received 1 ng of [^{3}H]oestradiol 3-sulphate (E2 sulphate) or [^{3}H]oestrone 3-sulphate (E1 sulphate) intravaginally and were killed 16 min later. Within each replicate the vaginae and plasma collected at killing were separately pooled and extracted. Values are the percentage of the recovered radioactivity in chloroform (free steroid) and n-butanol (sulphate) fractions of extracts of the tissues (see Methods)

	Vagina:		Plasma:	
Steroid injected	Free steroid	Sulphate	Free steroid	Sulphate
E2 sulphate	21·1	78·8	29·5	68·9
E1 sulphate	13·4	86·6	55·1	41·6

Summary of analysis of variance

Source of variation	D.F.	Mean square	*F* ratio
I. Free steroids *v.* sulphates	1	6138·72	78·22***
II. E2 *v.* E1 sulphates	1	0·64	0·01
III. Vagina *v.* plasma	1	5·52	0·07
I × II	1	347·82	4·44
I × III	1	2656·25	35·12***
II × III	1	0·72	0·01
I × II × III	1	1169·64	14·91**
Error	8	*78·48*	

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

Although some sulphatase activity was evident in the vaginal tissue, the greater part of the radioactivity was still associated with the sulphate fractions (Table 2). In the plasma this proportion was decreased. The sulphate was more readily removed from [^{3}H]E2 sulphate than from [^{3}H]E1 sulphate in the tissue, while in the plasma the reverse was observed. Chromatography of the free tissue fractions indicated that all of the radioactivity was associated with either E2 or E1. After [^{3}H]E2 sulphate injection almost all of the free radioactivity (95·5%) was associated with E2, while after injection of [^{3}H]E1 sulphate only 65% was associated with E1. In the tissue sulphate fractions, essentially all (>95%) of the activity was associated with the injected compound. Due to the low radioactivity levels the radiometabolites in the plasma were not examined.

The possibility of direct oestrogen sulphate binding by the vagina was examined by charcoal absorption and density gradient centrifugation as described in the Methods section. No binding was seen with either technique. With the gradients essentially all of the radioactivity remained in the 'free' area at the top and was still associated with the incubated compounds.

In the experiment of Table 3, minced uterus or vaginal tissue from ovariectomized mice was incubated with [^{3}H]E2 sulphate or [^{3}H]E1 sulphate; incubations with

muscle and boiled tissue were included as controls. Uptake of radioactivity was greater in uterus than in vagina and increased with time in both tissues. Considerable non-specific uptake was evident, however, as in each type of control more than 50% of the incubated radioactivity was recovered in the tissue at 120 min. In both uterus

Table 3. Distribution of radioactivity after incubation of tritiated oestrogen sulphates with mouse uterus and vagina *in vitro*

Uteri, vaginae or skeletal muscle tissue from ovariectomized mice were incubated with [^{3}H]oestradiol sulphate (E2 sulphate) or [^{3}H]oestrone sulphate (E1 sulphate) (50 ng/g of tissue). The percentage of the recovered radioactivity in chloroform (free steroids), n-butanol (sulphates) and aqueous fractions of extracts of tissues and media were separately measured. Values are means from two replicates per group

Incubation time (min)	Tissue	Steroid incubated	Tissue uptake (%)[A]	Percentage of recovered radioactivity in free steroid fraction: Tissue	Medium	T.I.[B]
30	Uterus	E2 sulphate	65·3	95·8	48·3	77·5
		E1 sulphate	57·7	94·4	52·8	76·6
30	Vagina	E2 sulphate	36·6	85·0	25·5	49·1
		E1 sulphate	25·2	82·3	24·9	44·2
120	Uterus	E2 sulphate	77·9	95·5	63·9	88·1
		E1 sulphate	70·8	94·9	62·4	83·7
120	Vagina	E2 sulphate	54·7	92·2	66·7	79·1
		E1 sulphate	50·8	87·9	65·1	76·5
120	Muscle	E2 sulphate	58·4	55·1	55·8	55·6
		E1 sulphate	63·4	64·1	56·2	61·1
120	Boiled tissue	E2 sulphate	69·8	1·7	1·5	1·6
		E1 sulphate	59·6	1·6	1·7	1·7

Summary of analysis of variance

Source of variation	Degree of freedom	Uptake	*F* ratio: Tissue	Medium	T.I.
I. Steroid	1	4·54	1·42	0·01	1·88
II. Tissue	1	55·15***	19·30**	14·48**	68·57***
III. Incubation time	1	24·50**	2·96	80·81***	74·39***
I × II	1	0·00	0·46	0·19	0·05
I × III	1	0·32	0·01	0·34	0·02
II × III	1	0·16	2·82	22·40**	22·98**
I × II × III	1	0·24	0·10	0·17	0·38
Error	8	*49·36*	*14·28*	*35·19*	*21·59*

[A] Measured as percentage of radioactivity incubated with the tissue.
[B] Total incubation.
** $0{\cdot}01 > P > 0{\cdot}001$. *** $P < 0{\cdot}001$.

and vagina after 30 or 120 min incubation more than 80% of the tissue activity was associated with the free steroid fraction, with significantly greater hydrolysis being shown by the uterus. Less of the medium radioactivity was associated with the free steroid fraction, but the difference decreased with incubation time, and by 120 min the greater part of the added radioactivity in the total incubations had been hydrolysed in both target tissues. In boiled tissue incubations little artifactual solvolysis was evident,

essentially all of the added radioactivity being recovered in the sulphate fractions. In the muscle tissue incubations considerable hydrolysis was observed by 120 min, but this was less than seen in the target tissues, and with both [^{3}H]E2 sulphate and [^{3}H]E1 sulphate the proportion of free radioactivity in tissue and medium was similar. This contrasted with the pattern seen in uterus and vagina.

After chromatography of the sulphate fractions from the above experiment essentially all of the radioactivity remained with the respective incubated sulphate. In the free steroid fractions, however, considerable interconversion of E2 and E1 was observed (Table 4). Although equilibrium was not reached, even at the longer time of incubation, the formation of E2 was favoured and this was more evident in the vagina. A similar pattern was seen in both tissue and medium.

Table 4. Radiometabolites in free steroid fractions of uterus and vagina after incubation with tritiated oestrogen sulphates

Chloroform extracts (free steroid fraction) of uterine and vaginal tissues and media from the experiment described in Table 3 were chromatographed, and the radioactivity associated with the respective carrier areas was measured. Results are expressed as the radioactivity associated with the hydrolysed incubated steroid as a percentage of that associated with both oestradiol and oestrone (means from two replicates per group)

Incubation time (min)	Tissue	Steroid incubated	Radioactivity (%): Tissue	Medium
30	Uterus	E2 sulphate	87·4	92·3
		E1 sulphate	90·8	70·6
30	Vagina	E2 sulphate	92·8	94·1
		E1 sulphate	65·8	65·9
120	Uterus	E2 sulphate	73·1	77·7
		E1 sulphate	53·0	51·6
120	Vagina	E2 sulphate	86·4	91·2
		E1 sulphate	51·1	45·9
120	Muscle	E2 sulphate	92·5	94·5
		E1 sulphate	88·9	92·0

Summary of analyses of variance

Source of variation	D.F.	*F* ratio: Tissue	Medium
I. Steroid	1	46·05***	103·52***
II. Organ	1	0·49	2·72
III. Incubation time	1	39·64***	22·37**
I × II	1	15·37**	4·62
I × III	1	7·47*	3·26
II × III	1	7·10*	0·81
I × II × III	1	1·70	1·14
Error	8	*33·75*	*35·62*

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

Uterine minces prepared from mice at days 5 or 19 of pregnancy (designated day-5 and day-19 tissues respectively) were also incubated with [^{3}H]E2 sulphate or [^{3}H]E1 sulphate for 30 or 120 min. Boiled day-19 tissue was included as control. The results (Table 5) show that tissue uptakes were generally less than seen in the previous experi-

ment (Table 3), in which tissues from ovariectomized animals were used. Although the uptake increased with time of incubation, this effect and the total uptake were significantly less with the day-19 tissues. However, the high non-specific uptake shown by the control tissues was again observed and in fact exceeded that shown by normal (unboiled) day-19 tissues.

Table 5. Distribution of radioactivity after incubation of tritiated oestrogen sulphates with uteri from pregnant mice

Uteri from mice at days 5 and 19 of pregnancy were incubated with [^{3}H]oestradiol 3-sulphate (E2 sulphate) or [^{3}H]oestrone 3-sulphate (E1 sulphate) (50 ng/g of tissue). The percentage of recovered radioactivity in chloroform (free steroids), n-butanol (sulphates) and aqueous fractions of extracts of tissues and media were separately measured. Values are means from two replicates per group. Some non-significant single degree of freedom effects are pooled as remainder in the analysis of variance

Day of pregnancy	Incubation time (min)	Steroid incubated	Tissue uptake (%)[A]	Percentage of recovered radioactivity in free steroid fraction: Tissue	Medium	T.I.[B]
5	30	E2 sulphate	34·4	75·9	9·9	28·2
		E1 sulphate	26·5	70·6	10·7	23·3
5	120	E2 sulphate	59·9	87·8	33·8	68·7
		E1 sulphate	62·1	87·3	27·3	62·3
19	30	E2 sulphate	25·5	80·4	41·8	49·9
		E1 sulphate	24·2	81·0	40·2	48·9
19	120	E2 sulphate	33·1	87·0	81·6	83·4
		E1 sulphate	34·0	85·2	75·7	78·8
Boiled control	120	E2 sulphate	56·8	2·6	1·3	2·0
		E1 sulphate	57·7	2·5	2·7	2·6

Summary of analysis of variance

Source of variation	Degree of freedom	*F* ratio: Uptake	Tissue	Medium	T.I.
I. Day of pregnancy	1	63·68***	2·77	824·01***	57·04***
II. Incubation time	1	90·01***	11·01*	443·64***	155·15***
III. Steroid	1	0·44	1·35	5·73*	2·68
I × II	1	27·64***	1·49	39·99***	2·41
Remainder	3	1·09	0·39	1·61	0·20
Error	8	*16·92*	*27·02*	*7·55*	*26·94*

[A] Measured as percentage of radioactivity incubated with the tissue.
[B] Total incubation.
* $0{\cdot}05 > P > 0{\cdot}01$. *** $P < 0{\cdot}001$.

With each incubation the greater part of the radioactivity in the tissue was associated with the free fraction (Table 5), and this increased with incubation time. Overall, the hydrolysis of the incubated sulphates was greater with day-19 tissues, where the initial rate was approximately double that observed for day-5 tissues. The free radioactivity in the medium was less in the [^{3}H]E1 sulphate incubations, but overall this effect was not significant. Again, essentially all of the radioactivity in the boiled control incubations remained associated with the sulphates.

Chromatography of the tissue extracts from the above experiment indicated again that, in the free fraction, the formation of E2 was favoured (Table 6). The conversion

of E1 to E2 was greater in the day-19 tissue and a similar pattern was seen in tissues and media. In the sulphate fractions, after incubation with [^{3}H]E2 sulphate essentially all of the radioactivity was associated with the incubated compound, while in [^{3}H]E1 sulphate incubations there was evidence for the formation of [^{3}H]E2 sulphate. This effect was greater in the day-19 incubations.

Table 6. Radiometabolites in fractions of uteri from pregnant mice after incubation with tritiated oestrogen sulphates

Chloroform and n-butanol extracts (free steroid and sulphate fractions respectively) of both uterine tissues and media from the experiment described in Table 5 were chromatographed, and the radioactivity associated with the respective carrier areas was measured. Results are expressed as the radioactivity associated with the hydrolysed incubated steroid as a percentage of that associated with both oestradiol and oestrone (free steroid fraction) or as the radioactivity associated with the incubated steroid as a percentage of that associated with both of the oestrogen sulphates (sulphate fraction). All results are means from two replicates per group

Day of pregnancy	Incubation time (min)	Steroid incubated	Radioactivity (%) in tissue: Free	Sulphate	Radioactivity (%) in medium: Free	Sulphate
5	30	E2 sulphate	93·2	97·1	91·3	99·9
		E1 sulphate	84·6	89·0	80·7	88·9
5	120	E2 sulphate	83·7	97·4	81·0	99·5
		E1 sulphate	53·2	86·6	56·7	77·8
19	30	E2 sulphate	86·7	98·2	84·3	95·1
		E1 sulphate	73·2	81·1	70·6	75·0
19	120	E2 sulphate	75·3	97·9	72·1	99·3
		E1 sulphate	43·6	78·7	41·3	65·2
Boiled control	120	E2 sulphate	—	99·9	—	100·0
		E1 sulphate	—	99·4	—	99·7

Summary of analysis of variance

Source of variation	Degree of freedom	*F* ratio: Free[A]	Sulphate[A]	Free[B]	Sulphate[B]
I. Steroid	1	94·57***	114·83***	38·11***	48·72***
II. Incubation time	1	87·14***	0·87	35·64***	1·88
III. Day of pregnancy	1	17·42**	7·63*	10·71*	6·36*
I × II	1	20·62**	0·87	5·63*	3·93
I × III	1	0·57	11·19*	0·50	2·98
II × III	1	0·00	0·02	0·36	0·23
I × II × III	1	0·23	0·01	0·05	0·07
Error	8	*19·01*	*6·61*	*40·89*	*38·84*

[A] Tissue. [B] Medium.
* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $P < 0{\cdot}001$.

Discussion

These experiments indicate that mouse oestrogen-responsive tissues, such as uterus and vagina, have a significant ability to hydrolyse E1 and E2 sulphates *in vivo* and *in vitro*. Similar observations *in vitro* with some oestrogen target tissues of the rat and sheep have been reported (Pack and Brooks 1970; Payne *et al.* 1973). A non-target tissue, skeletal muscle, also showed high sulphatase activity *in vitro*, but differences

compared with the target tissues were noted with respect to the lower overall hydrolysis of both sulphates by muscle and the proportion of total tissue radioactivity associated with the free steroid fraction. In the target organ incubations, most of the tissue radioactivity was associated with free steroids and the proportion was generally greater than that associated with free steroids in the media. By contrast, in muscle the proportion was similar in tissue and medium. Payne *et al.* (1973) noted no difference between a number of sheep brain tissues in their ability to hydrolyse E1 sulphate and Pack and Brooks (1970) found that mature, but not immature, rat oesophagus had a similar hydrolytic ability to uterus.

Less cleavage of the sulphates was observed *in vivo* than might have been expected from the extensive hydrolysis found *in vitro*. The difference is rather puzzling, as it might be anticipated that any unconjugated E2 formed from hydrolysis of E2 sulphate or reduction of unconjugated E1 would be bound to the target tissue receptors (Martin and Stone 1965; Stone and Martin 1965). Other differences between the *in vivo* and *in vitro* results were noted with respect to the interconversion of E2 and E1 and to the formation of E2 sulphate from E1 sulphate. Under *in vivo* conditions there was evidence of little oxidation of E2 and extensive reduction of E1 as expected (Stone and Martin 1965). The former might be partly explained by a loss of any weakly bound E1 from the tissue as it was formed. Extensive interconversion of the two steroids was noted *in vitro*, although the formation of E2 was favoured. Pack and Brooks (1970), however, demonstrated interconversion of E2 and E1 in uteri from intact mature animals but not from immature or castrate animals. The formation of E1 was favoured in that case. In the present experiments the higher rate of reduction of E1 and the lower uptakes seen in the day-19 tissues may reflect higher circulating free and conjugated levels of oestrogen at this time. Evidence of some formation of E2 sulphate from E1 sulphate has also been noted by Mathur (1969) in the hen and Pack and Brooks (1970) in the rat uterus.

In view of the ability of whole tissue, in particular the boiled controls, to concentrate radioactivity during incubation, and of the ready binding of at least oestrone sulphate to protein (Rosenthal *et al.* 1972), the lack of any sulphate binding by cytosol fractions was rather surprising. This may be partly explained by the lower temperatures used in the cytosol incubations to minimize hydrolysis, and by a high non-specific binding that has been observed in similar whole-tissue incubations (Stone and Baggett 1967).

The increase in uterine sulphatase activity towards the end of pregnancy when circulating sulphate levels might be expected to be rising (Wong *et al.* 1972) is of interest. In the mice used in the present experiments plasma levels of E2, E1, E2 sulphate or E1 sulphate were too low to be satisfactorily estimated by the sensitive protein-binding method of Shutt and Cox (1973) in castrate or day-5 animals, but there were indications of rising E1 and E1 sulphate levels by day 19 (Shutt, personal communication).

The demonstrated increased ability of the mouse uterus in late pregnancy to hydrolyse oestrogen sulphates and to reduce oestrone might be of physiological significance.

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