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Transfer of [^{3}H]estrone-[^{35}S]sulfate Across Guinea Pig Fetal Membranes

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The possible role of fetal membrane deconjugating activity in the movement of a charged steroid conjugate between fetal and maternal compartments was investigated. The ability of amnion and chorion laeve to transfer [^{3}H]estrone-[^{35}S]sulfate was assessed in both orientations of guinea pig tissue at 45 days and near parturition. While early amnion was impermeable, late tissue transferred approximately 50% (w/w) of the substrate in a bidirectional process that was non-saturable and independent of either deconjugation or ATP. Transfer across early chorion was similar to late amnion. Saturation curves from each tissue were superimposable, as were those of the time course. Transfer across both early and late chorion proceeded in the absence of deconjugation, with no effect of tissue orientation or ATP depletion. However, late chorion exhibited a decrease in estrone-sulfate transfer, as verified by concentration dependency and time course analyses, though transport across the tissue remained non-saturable. The results in amnion were congruous with the presence and absence of tight junctions in the epithelium of early and late tissue, respectively. However, sulfoconjugate transfer across early chorion proceeded in the presence of a paracellular barrier, suggesting specialized regulation of the transport process which extended late into gestation. **© 1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

There are several possible routes which may mediate molecular exchange between fetus and mother [1]. The path across the fetal membranes is common to all eutherians [2] and has the advantage of a large surface area and direct contact with the uterus and amniotic fluid. In most mammals, including the human [3], rhesus monkey [4] and guinea pig [5], attachment of chorion to endometrium late in gestation would appear to facilitate communication between maternal and fetal compartments, although such a role has not been clearly defined. The presence of estrogen conjugating and deconjugating activities which are temporally expressed in the amnion and chorion laeve of several species [6], raises the possibility of hormonal regulation within the intrauterine cavity and perhaps between fetal and maternal compartments.

Previous results demonstrated that conjugated steroids were transferred differentially across guinea pig fetal membranes, depending on the specific tissue and stage of gestation [7]. While it is often assumed that the polar nature of sulfated and glucuronidated compounds would exclude these charged groups from the epithelial plasma membrane, transfer of estrogen and pregnenolone so conjugated was not always accompanied by much evidence of deconjugation. Microscopic analyses demonstrating tight junctions in fetal membranes [8] coupled with the dynamic nature of paracellular barriers [9] suggested that specialized pathways might exist to transport steroid conjugates from one side of the tissue to the other in the absence of deconjugation. For example, transfer of estrone-glucuronide (E1G) across guinea pig chorion laeve met the criteria for unassisted movement across the trophoblast monolayer [10]. Until now, the factors regulating the passage of estrone-sulfate (E1S) had not been studied in any depth.

Whether or not estrone sulfotransferase (ST) [11, 12] and estrone sulfatase [13, 14] activities in fetal membranes had a specific role in the transmembranous transfer of E1S was plausible but unknown [15]. The present report examines the movement of E1S across amnion and chorion laeve of the developing guinea pig. Tissue from 45 days and beyond 60 days of gestation was incubated with

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[^{3}H]estrone-[^{35}S]sulfate in an effort to understand the relationship between steroid deconjugation and transfer across the fetal membranes. The ratio of ^{3}H/^{35}S indicated the balance between conjugated and deconjugated estrogen. In addition, the transfer of E1S was assessed with regard to tissue orientation, saturability and the effect of ATP depletion.

EXPERIMENTAL

Chemicals and reagents

[6,7-^{3}H]-(*N*)-Estrone (E1), 60 Ci/mmol, and sodium [^{35}S]sulfate, 1218 Ci/mmol, were purchased from New England Nuclear, Boston, MA, and Du Pont, Mississauga, Ontario, respectively. [^{3}H/^{35}S]Estrone-sulfate (E1S) was prepared by combining [^{3}H]E1S with E1-[^{35}S]S, each derived enzymatically (see below). Unless otherwise noted, reagents were of analytical grade and purchased from either Sigma, St. Louis, MO, BDH, Toronto, Ontario, or Fisher Scientific, Fair Lawn, NJ.

Synthesis of [^{3}H/^{35}S]E1S

This was prepared using a biological protocol [16] adapted for guinea pig liver. Tissue (20 g) was perfused with saline, washed and homogenized using three 10 s bursts of the Polytron at power setting 6, in 20 ml of 10 mM Tris–HCl pH 7.5/250 mM sucrose. Incubations consisted of 400 μl of cytosol (105,000 $\times g$ for 60 min) containing ST activity, 20 μl of steroid in methanol (15 pmol [^{3}H]E1 or 30 μg unlabelled E1) and 500 μl of buffered ATP (0.09 M KH_2PO_4 pH 7.5, 1.5 mM $MgCl_2$, 33 mM ATP·Na_2, and 9 mM Na_2SO_2; for the synthesis of E1-[^{35}S]S, 100–300 pmol [^{35}S]sulfate was added and 9 mM NaCl was substituted for Na_2SO_4). After 60 min at 37°C, products were separated by DEAE LC [17] on Sephadex A25, Pharmacia, Baie d'Urfe, Quebec, and further purified by Sep Pak filtration (C_{18}, Millipore, Bedford, MA) and ether extraction. Solvolysis (see below) of [^{3}H]E1S and TLC [18] of the free [^{3}H]estrogen confirmed the synthesis of conjugated E1. E1-[^{35}S]S coeluted with [^{3}H]E1S on DEAE LC.

Tissues

Amnion and chorion laeve (chorion) were obtained at two stages of gestation from English shorthair guinea pigs, bred in our animal facility [8]. Early or 45 day fetal membranes (42–47 days) were compared to late tissues, obtained after relaxation of the pubic symphysis (beyond 60 days). Animals were sacrificed by CO_2 inhalation, complying with guidelines issued by the Canadian Council on Animal Care.

Steroid transfer incubation

Incubations were conducted as previously described [10]. Fetal membranes were removed from fetuses sequentially, allowing for incubations with tissue oriented either fetal side up (FSU) or maternal side up (MSU). After washing with saline, tissue was positioned over a 5 ml beaker containing 10 mM Tris buffered saline (TBS) pH 7.4, such that a pocket of tissue was formed in complete contact with lower buffer. Previous studies have confirmed the viability of tissue incubated under these conditions [7, 8]. Unless otherwise noted, incubations were conducted with 15 nM [^{3}H/^{35}S]E1S: 1.13 pmol [^{3}H]E1S (1.5×10^5 dpm) and 0.02–0.10 pmol E1-[^{35}S]S (3.0×10^4 dpm), with the remaining weight from unlabelled E1S, in a total volume of 1.0 ml. This gave a starting (reference) ^{3}H/^{35}S ratio of about 5. Substrate was incubated above the tissue for 90 min at 37°C in 10 mM TBS pH 7.4, containing 0.001% phenol red to detect any leaky tissue. Incubations were stopped by the sequential removal of upper buffer, tissue and lower buffer. Volumes recovered from above and below the tissue were 0.72 ± 0.01 ml and 2.84 ± 0.07 ml, respectively ($n = 77$). Recovery of radiolabel was $88 \pm 10\%$.

Saturation of E1S transfer was examined over a 10,000-fold change in substrate concentration: 1.5 nM to 15 μM. Each sample contained 0.75 pmol [^{3}H]E1S, with the remainder as unlabelled E1S. The effect of energy inhibition on transfer was assessed by depleting tissues of ATP stores in a 30 min preincubation with one of the following inhibitors: 40 μg/ml valinomycin (Calbiochem, La Jolla, CA), 20 μg/ml oligomycin or 20 μM carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP). Evidence of energy depletion without disrupting cellular integrity has been published [8]. Following preincubation, 15 nM [^{3}H/^{35}S]E1S in buffer with inhibitor was incubated above the tissue for an additional 90 min at 37°C.

Analysis of products

Radiolabel was quantitated on a Beckman model 3801 liquid scintillation counter using single or double label programs, as appropriate. The efficiency of counting for ^{3}H was 35–45%, while that for ^{35}S was 70–75%. Samples were prepared for counting in Formula 989 scintillation fluid, Amersham, Oakville, Ontario.

Estrogen-3-sulfates were identified by solvolysis [19]. The desired aliquot of steroid-sulfate was dried, placed in 250 μl methanol and mixed with 1.0 ml glacial acetic acid:ethyl acetate, 1:9 (v/v). Samples were incubated 16–20 h in a 50°C shaking water bath. Desulfation was routinely monitored by ether extraction and TLC of the free estrogen [18].

Statistics

Replicate experiments are reported as the mean $\pm$ standard error (SE). Statistical significance ($P < 0.05$) was assigned using Student's *t*-test [20].

RESULTS

Transfer of [$^{3}H/^{35}S$]E1S

Figure 1 shows the concentration of E1S above and below the tissue after 90 min of incubation above 45 day and late fetal membranes. The distribution of ^{3}H and ^{35}S in each buffer compartment was the same prior to ether extraction. An average of 1–5% of the radiolabel was sequestered in the tissue compartment (data not shown) and was not analyzed further. At 45 days (Fig. 1(a)), amnion posed an almost complete barrier to E1S movement across the tissue, transferring less than 0.5 pmol/ml. After symphysis dilation (Fig. 1(b)), amnion transferred approximately 50% (w/w) of the incubated steroid sulfate for an average of 2.9 pmol/ml in the lower buffer (45 days vs. late: $P < 0.001$, for FSU and MSU incubations). The constant ratios of $^{3}H/^{35}S$ (Table 1) indicate very little change in the conjugation of E1S after incubation and extraction, these FSU results also representative of MSU incubations.

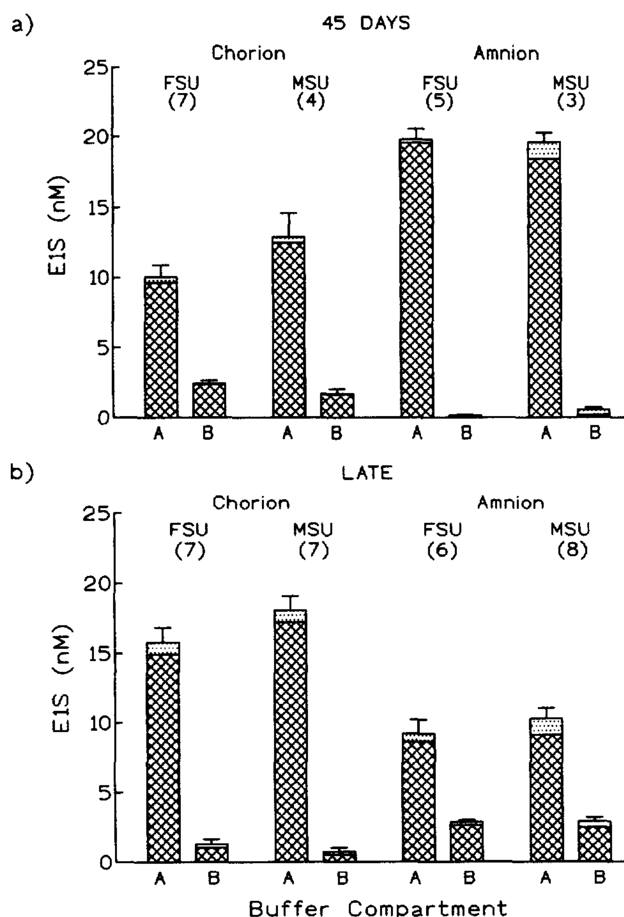

Fig. 1. Concentration of radiolabel above (A) and below (B) the tissue after incubation of 15 nM [$^{3}H/^{35}S$]E1S with fetal membranes at (a) early and (b) late stages of gestation (hatched bar, aqueous phase; stippled bar, ether phase; replicate experiments in parentheses).

Table 1. Isotope ratios after incubation of [$^{3}H/^{35}S$]E1S with fetal membranes

	FSU chorion	FSU amnion
	45 Days	
	(3)[a]	(3)
Reference[b]	5.8 ± 0.3[c]	6.0 ± 0.3
Above[d]	6.2 ± 0.8	7.2 ± 0.9
Aqueous[e]	6.2 ± 0.7	7.1 ± 0.9
Below[f]	9.1 ± 1.8	6.9 ± 0.5
Aqueous	8.8 ± 1.2	6.4 ± 0.3
	Late	
	(5)	(5)
Reference	6.7 ± 0.7	6.7 ± 0.7
Above	7.0 ± 0.7	7.0 ± 0.7
Aqueous	6.7 ± 0.7	6.7 ± 0.8
Below	7.1 ± 0.6	7.0 ± 0.7
Aqueous	5.9 ± 0.5	6.7 ± 0.8

[a]Replicate experiments.
[b]Ratio before incubation.
[c]Mean ratio ± SE.
[d]Ratio in the upper buffer after incubation.
[e]Ratio in the aqueous phase after ether extraction.
[f]Ratio in the lower buffer after incubation.

In contrast to amnion, early chorion was capable of transferring large amounts of E1S in the absence of deconjugation (45 day amnion vs. chorion: $P < 0.001$, FSU; $P < 0.05$, MSU). The 40–50% (w/w) of radiolabel transferred at 45 days decreased near term, reducing the concentration of E1S in the lower buffer from 1.7–2.4 pmol/ml to 0.7–1.3 pmol/ml (45 days vs. late: $P < 0.05$, for FSU chorion; late amnion vs. chorion: $P < 0.001$, for FSU and MSU incubations). In late chorion, a minor decrease in the $^{3}H/^{35}S$ ratio after extraction of lower buffer (Table 1) was negatively correlated with steroid transfer across the tissue, suggesting little effect of small amounts of deconjugation on transmembranous movement of E1S. Incubations in the presence of 100 mM phosphate to inhibit sulfatase activity [13] had no effect on transfer or $^{3}H/^{35}S$ ratios (data not shown). Tissue orientation did not significantly influence E1S transfer under any of the conditions tested.

Concentration dependence of [^{3}H]E1S transfer

Figure 2 demonstrates a linear log relationship between the amount of E1S placed above the tissue and the amount transferred to the lower buffer. A 10,000-fold increase in the substrate concentration failed to saturate the transport process. The behavior of 45 day chorion was identical to that for late amnion. By comparison, the ability of late chorion to transfer E1S was reduced. The latter two results were also observed in time course experiments (data not shown).

Energy inhibition and [$^{3}H/^{35}S$]E1S transfer

Figure 3 indicates the pattern of E1S transfer across energy depleted fetal membranes at 45 days

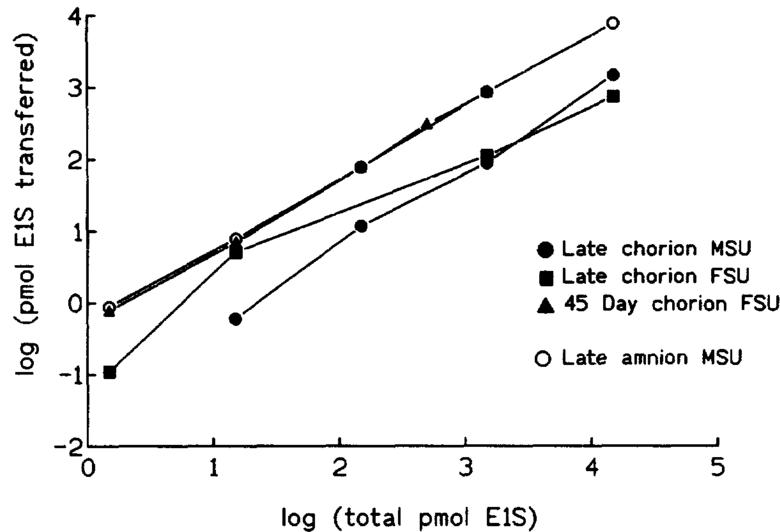

Fig. 2. Concentration dependence of [^{3}H]E1S incubation with early and late fetal membranes (n = 2–4 experiments; SE ranges between 1 and 19%).

and late gestation. Prior to extraction, the distribution of ^{3}H and ^{35}S in each buffer compartment was the same. The combined results of energy inhibitors is presented since neither the concentration of radiolabel above and below FSU tissue, nor the amount of deconjugation was affected by the removal of tissue ATP stores (compare Figs 1 and 3). Early amnion continued to exhibit barrier function, while the same tissue near term transferred approximately 50% (w/w) of the label or 3.2 pmol/ml (45 days vs. late: $P < 0.001$). Also in agreement with Fig. 1 data, early chorion transferred 30–40% (w/w) of the steroid conjugate (2.0 pmol/ml; 45 day chorion vs. amnion: $P < 0.001$), while late in gestation 20–30% (w/w) of the radiolabel was recovered below the tissue (1.8 pmol/ml). The transfer of E1S across chorion in either direction or stage of gestation was not statistically different from the energy replete to the ATP depleted state. A similar low level of desulfation was recorded by ^{3}H/^{35}S ratios whether or not tissue was preincubated with inhibitors of electron transport (compare Tables 1 and 2). These results also suggest that the possibility of transfer associated with substantial deconjugation and reconjugation from a mixture of labelled and unlabelled phosphoadenosine 5′-phosphosulfate is unlikely.

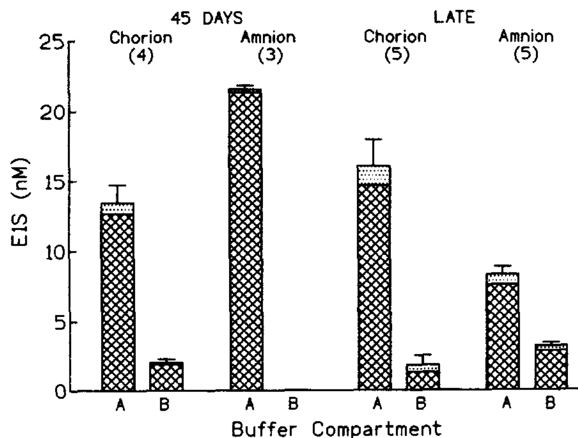

Fig. 3. Concentration of radiolabel above (A) and below (B) the tissue after incubation of 15 nM [^{3}H/^{35}S]E1S with fetal membranes depleted on ATP (hatched bar, aqueous phase; stippled bar, ether phase; replicate experiments in parentheses).

Analysis of products after [^{3}H/^{35}S]E1S transfer

Although a minor component of the total radioactivity, TLC analysis of free steroid collected above

Table 2. Isotope ratios after incubation of [^{3}H/^{35}S]E1S with fetal membranes depleted of ATP

	FSU chorion	FSU amnion
	45 Days	
	(4)[a]	(3)
Reference[b]	11.4 ± 3.2[c]	9.2 ± 2.9
Above[d]	12.6 ± 3.5	13.5 ± 4.9
Aqueous[e]	12.2 ± 3.2	13.2 ± 4.5
Below[f]	20.0 ± 6.9	nd[g]
Aqueous	18.1 ± 5.8	nd
	Late	
	(5)	(5)
Reference	7.4 ± 0.8	7.3 ± 0.7
Above	7.2 ± 0.7	7.5 ± 0.9
Aqueous	6.8 ± 0.9	7.1 ± 1.0
Below	7.8 ± 0.9	7.9 ± 0.9
Aqueous	6.1 ± 0.7	6.8 ± 1.2

[a]Replicate experiments.
[b]Ratio before incubation.
[c]Mean ratio ± SE.
[d]Ratio in the upper buffer after incubation.
[e]Ratio in the aqueous phase after ether extraction.
[f]Ratio in the lower buffer after incubation.
[g]nd, not detectable.

Table 3. Isotope ratios of conjugated steroid before and after solvolysis[a]

	Late chorion FSU		Late amnion FSU	
	above	below	above	below
	(3)[b]	(2)	(3)	(3)
Aqueous sample[c]	1.00	1.00	1.00	1.00
Incubation[d]	1.25 ± 0.03[e]	1.38 ± 0.18	1.14 ± 0.09	1.13 ± 0.11
Solvolysis[f]	0.20 ± 0.08	0.26 ± 0.21	0.22 ± 0.09	0.31 ± 0.13

[a]Reference ratios before incubation are 6.07 ± 0.03, chorion; 6.15 ± 0.23, amnion.
[b]Replicate experiments.
[c]Ratio after incubation and ether extraction, normalized to assimilate decay of ^{35}S between experiments and arbitrarily assigned a value of 1.00.
[d]Ratio after Sep Pak filtration to remove [^{35}S]sulfate hydrolyzed during incubation.
[e]Mean ratio ± SE.
[f]Ratio after solvolysis and extraction of the conjugated steroid.

and below late fetal membranes indicated some conversion of E1 to 17β-estradiol (E2, data not shown). Enzymic reduction was greater in chorion than amnion, converting 45–60% of E1 to E2 in the former tissue versus less than 25% of E1 to E2 in the latter. These results were representative of both tissue orientations.

Radiolabelled material which remained in the aqueous phase after incubation and ether extraction was solvolyzed to determine the content of estrogen-sulfate (Table 3). Prior to solvolysis, ^{3}H/^{35}S ratios revealed consistently small amounts of deconjugation in both late amnion and chorion, as previously noted. After solvolysis and extraction ^{3}H/^{35}S ratios decreased 4 to 6-fold, verifying the presence of estrogen-3-sulfate. In general, 45 day ratios (data not shown) indicated even less desulfation during incubation and more thorough cleavage by solvolysis. Analysis of the deconjugated steroid by TLC showed virtually no conversion of E1S to 17β-estradiol-3-sulfate in either tissue, tissue orientation or stage of gestation (data not shown).

In samples where greater than 10% of the water-soluble steroid after incubation was not susceptible to solvolysis, a β-glucuronidase digestion was performed on the solvolysis-resistant material. Digestion in these samples produced less than 20% steroid cleavage (data not shown), dispelling the notion that much glucuronide had been formed. DEAE LC of aqueous phases from the lower buffer of late FSU incubations confirmed the presence of E1S and the absence of E1G (Fig. 4). Minor peaks eluting ahead of E1S were not further analyzed.

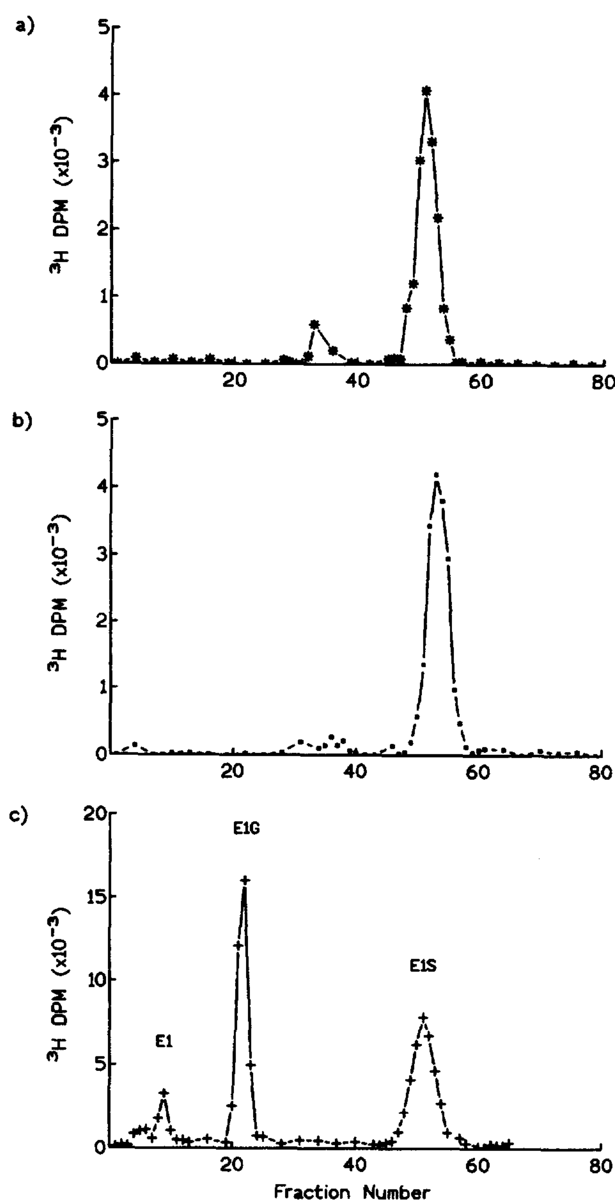

Fig. 4. DEAE LC of water-soluble products in the lower buffer after incubation of 8 nM [^{3}H]E1S with late FSU (a) chorion and (b) amnion ((c) [^{3}H]standards).

DISCUSSION

Transfer of E1S across guinea pig amnion and chorion depended on the specific tissue and stage of gestation but not on the direction of transfer. The present studies show that the movement of E1S proceeded without requirements for deconjugation of the sulfate group or a reserve of ATP. In addition, E1S

transfer was not a saturable process. The similarity between transfer characteristics of E1S and E1G [10] (Fig. 5) suggests that modification of the steroid conjugate was not, in itself, the main factor regulating transmembranous passage. The impermeable nature of early amnion is consistent with the presence of tight junctions in the cell monolayer. Near parturition, this epithelium undergoes extensive remodelling, including loss of tight junctions and luminal surface structure [8]. Consistent with this, charged estrogen conjugates are freely permeable across late amnion. In contrast, guinea pig chorion maintains the integrity of its trophoblastic epithelium throughout gestation, though a decrease in tight junctions may account for the permeability to E1S and E1G near term. Deconjugation of E1G on the maternal side of late chorion proceeded independently of conjugate transfer [10] and demonstrated tissue polarity. It is possible that E1S transferred to the maternal surface may be modified by uterine estrogen sulfatase (see below). That E1S and E1G were transferred intact across the tight epithelium of 45 day chorion suggested specialized transport features of this tissue, which could not be reconciled to the properties of transcellular pathways such as receptor-mediated transport.

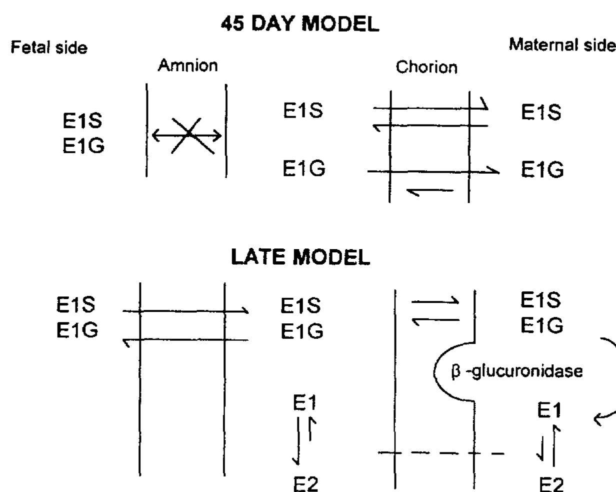

Fig. 5. Model of estrogen conjugate transfer across 45 day and late fetal membranes (the line drawing reflects *in vivo* positioning of amnion and chorion next to fetal and maternal compartments, respectively). In contrast to 45 day amnion, which was impermeable, both E1S and E1G were transferred across the leaky epithelium of late tissue. Both conjugates were likewise transferred intact across late chorion, in a process that was possibly attenuated by the fetal side cell layer or the patchy distribution of tight junctions observed in the trophoblast at this stage of gestation [8]. In addition, E1G but not E1S was subject to deconjugation when incubated with late chorion MSU [10]. Transfer of E1S and E1G across the tight epithelium of 45 day chorion, in a process that did not involve deconjugation, receptor-mediation or ATP stores, should be considered in view of the dynamic nature of tight junctions.

Transfer of E1S across 45 day chorion was very similar to the pattern of E1S movement across late amnion in several respects. Both tissues transferred 2–3 pmol/ml of steroid conjugate to the lower buffer in the virtual absence of desulfation. This pattern of transfer was neither energy-dependent nor saturable, suggesting a passive form of transport that is probably not receptor-mediated. In addition, the respective curves constructed from saturation and time course data from each tissue were essentially superimposable, indicating similar concentration dependency and rate of transfer. That these two tissues and stages of gestation reflected different mechanisms of E1S transport is supported by microscopic evidence of their permeability and viability [8]. Although transfer across the leaky epithelium of late amnion was expected, it is still not clear how a charged steroid conjugate was transported across the barrier presented by trophoblast of 45 day chorion. Barring an endocytic pathway [21], the results presented here suggest that alternative modes of regulation affect the permeability of tight junctional barriers in chorion early in fetal development.

After relaxation of the pubic symphysis, the transfer of E1S across chorion was more restricted than earlier in gestation, exhibiting decreases in the rate and saturability of transfer compared to 45 day tissue. These results were again in opposition to the microscopic evidence for increased permeability in late chorion [8], suggesting that other factors contributed to the regulation of steroid conjugate movement across the epithelium. The lack of either deconjugation or saturation of E1S transfer indicates that the properties of the tissue are probably more important determinants of conjugate transport than steroid modifying activities. At this late stage of gestation, chorion had undergone a number of physical changes which may account in part for changes in the transfer of E1S. This near to term, chorion was firmly associated with the uterine wall and a layer of metabolically active squamous cells appeared on the fetal side of the tissue. The latter constituted somewhat more of a cell barrier than did the maternal side trophoblast; although the paracellular junction was permeable with time to molecules of less than 1000 M.W. [8]. While uterine effects on E1S transfer were not directly measured in this study, late chorion has been exposed to the influences of the endometrium, which may be sufficient to confer the permeability characteristics observed *in vitro*.

A prehormone role for E1S has been postulated [22] and evidence in guinea pig [14, 23], rat [24], pig [25] and human [26] suggests that estrogen sulfatase activity in the uterus facilitates the local production of active steroid. In guinea pig, both endometrium and myometrium sustain high levels of hydrolytic activity toward E1S between 50 days of gestation and term [27]. A lower level of estrogen sul-

fatase activity found in chorion nonetheless increases toward term, correlating with a steady increase in E2 formation in this tissue. In the present study, the permeability of chorion to E1S is consistent with a role for fetal E1S in supplying the uterus with substrate for conversion to active estrogen.

The barrier function of 45 day amnion toward E1S transfer persisted regardless of ATP depletion, defying an increasing number of reports which implicate ATP in the maintenance of tight junctions [21, 28, 29]. In studies with Madin–Darby canine kidney (MDCK) monolayers, transcellular electrical resistance (TER) was the usual assay of epithelial permeability. Whether or not steroid transfer corresponds with TER measurements has not been established, and potentially explains the discrepancy in results. Alternatively, TER and paracellular permeability may represent two different properties of an epithelium which are regulated independently [30, 31]. Staddon *et al.* [32] demonstrated that tyrosine phosphorylation of proteins at intercellular junctions coincided with increased permeability in epithelial and endothelial cells, despite the absence of microscopic evidence for changes in tight junction structure. In view of this latter report, ATP depletion may actually serve to counter the activity of kinases by limiting substrate, and hence maintaining the barrier or gate function.

As for early and late chorion, transfer of E1S across amnion near term proceeded without the assistance of deconjugation or receptor-mediation, and was unaffected by the removal of ATP stores. The similarity of these data to transfer results obtained with other free and conjugated steroids [7], together with the microscopic evidence of epithelial cell lesions [8], supports the view that transfer across late amnion occurs in the presence of little or no regulation. In this case, the adherent chorion-uterine layer may be the more important regulator of hormonal signalling [33] between fetal and maternal compartments near term. Since late amnion contains additional layers of connective tissue fibers and some cells [3, 34, 35], it could be useful as a control for steroid transfer behavior not associated with a viable epithelium. Late amnion was certainly a dramatic contrast to 45 day tissue, emphasizing the general need for studies at multiple stages of gestation.

In conclusion, the transfer of E1S across guinea pig fetal membranes depended on tissue and stage of gestation. Neither energy nor sulfatase inhibition significantly altered the pattern of transfer: E1S was translocated intact across two different epithelia in a non-saturable and bidirectional process. Knowledge of changes in fetal membrane permeability during gestation should be exploited in future investigations as a means of approaching an understanding of the basis of steroid transfer across these tissues. Several investigators have proposed that tight junction permeability (gate function) can be dynamically regulated, depending on the physiological state [9, 32, 36]. Given the multiplicity of changes ongoing in the developing fetus and the absolute necessity for interchange between the maternal and fetal compartments during this process, the transmembranous movement of steroids may be at least partly regulated by the intercellular junction.

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