METABOLISM OF DEHYDROEPIANDROSTERONE SULPHATE 
AND OESTRONE SULPHATE FOLLOWING IN SITU 
PLACENTAL PERFUSION AT MIDPREGNANCY

By

J. Schwers, T. Vancrombreucq, M. Govaerts, 
G. Eriksson and E. Diczfalusy

ABSTRACT

Two midgestation placentas were perfused in situ with a combination of 
[7α-3H] dehydroepiandosterone sulphate and [4-14C] oestrone sulphate and metabolites were isolated from the placentas, perfusates and maternal urine specimens. Approximately 70 per cent of the perfused radioactive 
material was recovered from these three sources. 
The bulk of the administered radioactive material was recovered in an 
unchanged form from the perfusates; some 2–4 per cent was excreted 
in the urine and less than 0.5% was found in the placentas. 
The tritium to carbon-14 ratio of the unconjugated material isolated from 
the perfusates and placentas was higher, and that of the conjugated material 
recovered from the same sources was lower than the ratio of the 
administered material. In addition, more tritium than carbon-14 labelled 
material was present in the urine. 
Approximately 2 per cent of the perfused dehydroepiandosterone sulphate 
was recovered in the form of phenolic steroids, mostly from the urine. 
From this source double labelled oestrone, oestriol, 16α-hydroxy-oestrone 
and 16-epioestriol were isolated. The tritium to carbon-14 ratio of all 
oestrogens isolated from the urine was higher than that of the perfused 
material. From the urine specimens 10 to 15 times more double labelled 
oestriol than oestrone was isolated.

A preliminary report has been presented at the third International Congress of Endocrinology, Mexico City (Govaerts et al. 1968).
It is concluded, that:
the placental hydrolysis, metabolism and transfer of dehydroepiandrosterone sulphate exceed those of oestrone sulphate,
the midgestation placenta secretes considerably more oestrogens to the maternal than to the foetal circulation,
the extent of maternal 16α-hydroxylation of oestrone and 17β-oestradiol at midgestation seems to exceed that found in non-pregnant subjects.

Previous studies demonstrated that midgestation placentas perfused with dehydroepiandrosterone sulphate (DHAS) hydrolyze this compound and convert the liberated dehydroepiandrosterone (DHA) to oestrone (OE1) and 17β-oestradiol (OE2). This conversion takes place via the formation of androstenedione (A), and the oestrogens formed are transferred mainly to the maternal organism and to a smaller degree to the umbilical circulation (Bolté et al. 1964a,b,c; Kirschner et al. 1966; Lamb et al. 1967). The same sequence of reactions takes place also at term (Bolté et al. 1969). Oestrone sulphate (OE1-S) which is formed in the foetal organism from placental OE1 and OE2 (Schwers et al. 1965b) is also hydrolyzed by the human placenta and the unconjugated oestrogens are transferred mainly to the mother and in part to the foetus (Kirschner et al. 1966).

The purpose of the present study was to compare the placental hydrolysis and metabolism of DHAS with that of simultaneously administered OE1-S and to investigate the contributions of these two precursors to the oestrogen present in pregnancy urine.

EXPERIMENTAL

Clinical material
This study was carried out on two physically healthy subjects, who were admitted to the hospital for the termination of pregnancy. Permission for interruption of gestation was granted by the Swedish National Board of Health and Welfare upon the request of the patient under the statute of 1938 as amended in 1946 and 1955. The period of gestation was around the 19th week.

Placental perfusion. The technique of in situ placental perfusion was the same as previously reported (Bolté et al. 1964a). Perfusion times was 15 minutes.

Abbreviations and trivial names
(10)-oestratriene-3,16α,17β-triol), 16-epi-\(OE_3\): 16-epioestriol (1,3,5(10)-oestratriene-3,16β,17β-triol, \(OE_4\): oestreltol (1,3,5(10)-oestratriene-3,15α,16α,17β-tetrol), \(T\): testosterone (17β-hydroxy-4-androsten-3-one).

Other abbreviations: \(CPC\): column partition chromatography, \(dpm\): disintegrations per minute, \(HBV\): hold back volume, \(K\): partition coefficient, \(PPC\): paper partition chromatography, \(S.A\): specific activity.

Material injected. This was the same as that used in the study of Kirschner et al. (1966). In each of the two perfusions 11.8 \(\mu\)Ci of \([7\alpha-^{3}H]\)DHAS and 2.4 \(\mu\)Ci of \([4\alpha^{14}C]\)OE_{1}-S were administered.

Countercurrent distribution. Twenty-four transfer distributions were carried out, using the following solvent systems:

- \(CPC-1\): n-Hexane, benzene, ethanol, water (5:5:0.2:9.8)
- \(CPC-2\): Benzene, water (1:1)
- \(CPC-3\): Petroleum ether, methanol, water (2:1:1)
- \(CPC-4\): Ethyl ether, water (1:1)

Paper partition chromatography. This was carried out at 22°C, using the following systems:

- \(P-1\): ligroin, methanol, water (5:4:1) for 10 hours
- \(P-2\): toluene, n-butanol, 1.5 \(\mu\) \(NH_4\)OH (3:1:2) for 12 hours
- \(P-3\): Benzene, heptane, methanol, water (7:3:8:2) for 8 hours

Column partition chromatography. Celite columns were prepared according to the method of Sitteri (1963). The following systems were used at room temperature:

- \(C-1\): Ethylene glycol, isoctane, ethyl acetate (gradient)
- \(C-2\): n-Hexane, ethyl acetate, methanol, water (17:3:7:3)
- \(C-3\): n-Hexane, ethyl acetate, methanol, water (17:3:9:1)
- \(C-4\): n-Hexane, ethyl acetate, methanol, water (15:5:7:3)
- \(C-5\): n-Hexane, chloroform, methanol, water (8:12:7:3)
- \(C-6\): Isooctane, t. butanol, methanol, water (5:2:1:2)
- \(C-7\): Isooctane, t. butanol, methanol, water (20:8:3:9)
- \(C-8\): Isooctane, t. butanol, methanol, water (10:4:1:5)
- \(C-9\): Water, \(NH_4\)OH (39:1) and
  - a) isoctane, t. butanol (3:5)
  - b) isoctane, t. butanol (2:5)
- \(C-10\): Isooctane, ethyl acetate, n-butanol, methanol, 0.1 \(\mu\) \((HN_4\)\)\(_2\)SO\(_4\), 13.36 \(\mu\) \(NH_4\)OH (20:40:7:20:30:0.1)

Formation of derivatives and colour reaction for oestrogens were carried out as described in previous papers (Schuers et al. 1965a,b).

Measurement of radioactivity. Tritium and carbon-14 were measured simultaneously in a liquid scintillation spectrometer (Packard Model 3380) with the absolute activity analyzer.

Collection of material for analysis. Upon completion of the perfusions the placenta was removed, the membranes and the cord were separated and the placental tissue weighed. The volume of the perfusate was measured. Twenty-four hour urine specimens were collected for 4 days.

Extraction procedure and separation of ether soluble (unconjugated) and water soluble (conjugated) radioactive material was carried out as described by Mikhail et al. (1963).

Hydrolysis of conjugated material. Urinary steroid conjugates were hydrolyzed by a Helix pomatia enzyme preparation, using the conditions described previously.
(Reynolds et al. 1968). This was followed by solvolysis according to Burstein & Lieberman (1958).

Isolation of individual steroids. The method used for the isolation of individual steroids present in the extracts of placentas and perfusates is indicated in Fig. 1, whereas the method employed for the isolation of urinary oestrogens is shown in Fig. 2.

RESULTS

Distribution of radioactive material

The distribution of radioactive material recovered from the placentas, perfusates and urine specimens is indicated in Table 1.

Some 70 per cent of the administered radioactive material was recovered. In both experiments the recovery of carbon-14 was higher than that of tritium. Relatively more tritium than carbon-14 was present in the urine, whereas the opposite was true in the perfusates. The unconjugated material recovered from the perfusates and placentas showed a tritium to carbon-14 ratio which was higher than that of the administered material. On the other hand, the isotopic ratio of the conjugated material present in the placentas and perfusates was lower than that of the perfused material. The data of Table 1 also indicate that – in contradistinction to the placentas – the bulk of the radioactive material recovered from the perfusates was in a conjugated form.

The amount of phenolic tritium labelled material recovered from the various sources is indicated in Table 2.

Certain neutral steroids behave as pseudophenols when partitioned between toluene and sodium hydroxide (Dell’Acqua et al. 1967a). Therefore, the amount of tritium labelled phenolic material formed was calculated on the basis of the carbon-14 labelled material recovered from each phenolic fraction. These figures were adjusted on the basis of the lowest tritium to carbon-14 ratio of radiochemically homogeneous oestrogens recovered from that particular source. Hence the figures presented in Table 2 were not corrected for methodological losses. It is likely therefore that they represent underestimates.

The data of Table 2 indicate that somewhat less than 2 per cent of the perfused DHAS was converted to phenolic material. The bulk of this was recovered from the urine, which contained 7 to 15 times more tritium labelled phenolic material than the perfusates.

Isolation of individual oestrogens

The tritium to carbon-14 ratios of the oestrogens isolated from the various sources are indicated in Table 3.

The data of Table 3 indicate that all of the urinary oestrogens and 3 of the
Fig. 1.
Scheme of the method used for the isolation of various metabolites from the extracts of placentas and perfusates. X indicates crystallization to constant specific activity.

Extracts of placentas or perfusates

ether

N NaOH

C-2

HBV 1

+ carrier OE₁

X

OE₁

HBV 3

+ carrier OE₂

X

OE₂

toluene

[¹⁴C] T

+ carrier

[¹⁴C] DHA

[¹⁴C] A

P-1

acetylation

acetylation

+ carrier A

acetylation

X

X

X

T

DHA

A

unknown

OE₂ 3-S

water

C-9

HBV 5-6

C-10

HBV 3

HBV 4-8

no carbon-14

P-2

18-23 cm (OE₂ 3-S)

26-29 cm (OE₁ 3-S)

32-37 cm (DHAS)

+ carrier OE₂ 3-S

acetylation

X
Fig. 2.
Scheme of the method used for the isolation of urinary oestrogens. X stands for crystallization to constant specific activity.

Urine
Hydrolysis and ether extraction
N NaOH
C-1
HBV 4
C-3
HBV 2-3 + carrier OE₁

H
K = 1.04
KBH₄

C-4
CCD-1

K = 0.18
acetylation

HBV 10
K = 2.23
+ carrier OE₃

HBV 11
K = 0.37
+ carrier OE₃

CCD-1
acetylation

HBV 17
X

CCD-2
acetonide

HBV 19
C-6
HBV 4
HBV 4
HBV 7-10
C-7
HBV 6-8
C-8
CCD 4

OE₁
OE₂
16-OH-OE₁
16-oxo-OE₂
16-epi-OE₃
OE₃
unknown

K = 0.89

a) The specific activities and isotopic ratios of the material contained by the 9 peak tubes of this distribution were constant. The mass was estimated by a Kober reaction.
Table 1.
Distribution of unconjugated (U) and conjugated (C) radioactive material recovered from the placentas, perfusates and urine specimens following placental perfusion with 11.8 µCi of [7α-3H] dehydroepiandrosterone sulphate and 2.4 µCi of [4-14C] oestrone sulphate. Figures indicate percentage of the administered material and isotopic ratios.

<table>
<thead>
<tr>
<th></th>
<th>Placenta</th>
<th>Perfusat</th>
<th>Urine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>C</td>
<td>U</td>
<td>C</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>³H</td>
<td>0.35</td>
<td>0.08</td>
<td>0.70</td>
<td>64.08</td>
</tr>
<tr>
<td>¹⁴C</td>
<td>0.29</td>
<td>0.15</td>
<td>0.16</td>
<td>69.89</td>
</tr>
<tr>
<td>³H/¹⁴C</td>
<td>5.9</td>
<td>2.7</td>
<td>22.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>³H</td>
<td>0.36</td>
<td>0.09</td>
<td>0.98</td>
<td>64.72</td>
</tr>
<tr>
<td>¹⁴C</td>
<td>0.29</td>
<td>0.12</td>
<td>0.17</td>
<td>74.70</td>
</tr>
<tr>
<td>³H/¹⁴C</td>
<td>6.0</td>
<td>3.8</td>
<td>23.1</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Table 2.
Amount (dpm) of tritium labelled phenolic material recovered from the placentas, perfusates and urine specimens. Figures in parentheses indicate percentage of administered material. For further particulars consult Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Placenta</th>
<th>Perfusat</th>
<th>Urine</th>
<th>Total</th>
<th>Perfusat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76.400 (0.3)</td>
<td>26.000 (0.1)</td>
<td>392.000 (1.3)</td>
<td>494.400 (1.9)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75.500 (0.3)</td>
<td>43.900 (0.2)</td>
<td>299.000 (1.2)</td>
<td>418.400 (1.7)</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.
Tritium to carbon-14 ratios of radiochemically homogeneous oestrogens isolated from placentas, perfusates and urine specimens. The isotopic ratio of the perfused material was 5.0. For further particulars consult Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Placenta</th>
<th>Perfusat</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OE₁OE₂</td>
<td>OE₁OE₂</td>
<td>OE₁OE₂</td>
</tr>
<tr>
<td>OE₁OE₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16α-HO-</td>
<td>16-epi-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-oxo-</td>
<td>OE₁OE₂</td>
<td>OE₁OE₂</td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>5.8</td>
<td>6.1</td>
<td>*</td>
</tr>
<tr>
<td>5.6</td>
<td>*</td>
<td>5.5</td>
<td>6.6</td>
</tr>
<tr>
<td>5.4</td>
<td></td>
<td>6.4</td>
<td>7.0</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>4.9</td>
<td>5.3</td>
<td>5.0</td>
</tr>
<tr>
<td>5.5</td>
<td>*</td>
<td>5.2</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* Insufficient material for isolation.
4 placental oestrogens isolated exhibited a tritium to carbon-14 ratio higher than that of the perfused material.

As indicated in Fig. 2, from the urine extracts a metabolite was isolated, which was more polar than OEs, or even OE4. Lack of material precluded identification of this compound.

Attempts to isolate \([4-^{14}C]\) 17\(\beta\)-oestradiol-3-sulphate

In both experiments attempts have been made to isolate carbon-14 labelled OE2-3S from the placentas and perfusates, using the procedure outlined in Fig. 1. All carbon-14 labelled material was dissociated from the authentic carried after three crystallizations.

Oestrogen ratios in various sources

As expected from our earlier studies, no OE3-like radioactive material was found in the extracts of placentas and perfusates. However, from the urine specimens 10 to 15 times more OE3 than OE1 was isolated in a radiochemically homogeneous form. There was no significant difference in this respect between the tritium and carbon-14 labelled materials, as indicated by the data of Table 3.

The amount of radiochemically homogeneous OE2 isolated from the placentas exceeded 3 to 5 times that of OE1. This was true for both labels. On the other hand, more OE1 than OE2 was present in the perfusate in Exp. No. 2.

Neutral steroids

Attempts were also made to isolate DHA, A and T from the placentas and perfusates. Insufficient material precluded the isolation of these steroids from the placental extracts. However, small amounts of radiochemically homogeneous \([^{3}H]\) DHA were isolated from both perfusates, and \([^{3}H]\) A from one of the two perfusates. In the case of \([^{3}H]\) T-like material constant S.A. was not achieved.

Finally, it should be pointed out that more than 90 per cent of the unconjugated tritium labelled material present in the neutral fraction of the perfusates exhibited an \(R_f\)-value higher than that of A when chromatographed in system P-1. This material was completely dissociated from the carbon-14 labelled A, T and DHA added as internal standards. The identity of this material could not be established.

DISCUSSION

The data reported in this paper indicate that the placental uptake, hydrolysis and transfer of DHAS and its metabolites is greater than that of OE1-S and
its metabolites. However, the mass of the OE1-S administered exceeded that of DHAS by a factor of 8.7. Furthermore, although the concentrations of DHAS and OE1-S in cord blood at midgestation are unknown, it could be anticipated that the former greatly exceeds the latter. Thus, the possibility cannot be excluded, that the amount of OE1-S administered was unphysiologically high.

The present data confirm and extend the findings of previous studies indicating that more OE2 than OE1 is present in the placentas, whereas the opposite is the case as far as the perfusates are concerned (Bolté et al. 1964a; Schwers et al. 1965a). Also our failure to find any labelled OE3 in the placentas and perfusates is in agreement with the results of previous studies indicating that the midgestation human placenta is not able to carry out a significant 16α-hydroxylation (Bolté et al. 1964a; Schwers et al. 1965a; Dell’Acqua et al. 1967b; Reynolds et al. 1968; Jackanicz & Diczfalusy 1968). The present isolation of DHA and A from the perfusates confirms previous data reported by Lamb et al. (1967) and indicates that small quantities of unconjugated neutral steroids are secreted by the placenta to the foetus.

On the other hand, we were unable to find any conversion of OE1S to OE2-3S in the placentas or in the perfusates. The possibility that such a conversion might take place in the placenta was suggested by Alonso & Troen (1966).

The data reported in this study indicate that the midgestation human placenta secretes much more OE1 and OE2 to the maternal circulation than to the foetal compartment. This is in agreement with the conclusions reached by Kirschner et al. (1966) and Gurpide et al. (1966, 1967).

In a previous study, Kirschner et al. (1966) injected tritium labelled DHAS and carbon-14 labelled OE1-S into the intact umbilical circulation at laparotomy, prior to the removal of the foetus. They found a much higher tritium to carbon-14 ratio in the urinary OE3 than in OE1 isolated from the same source and concluded that a major 16α-hydroxylation of DHAS took place in the foetal compartment. Their conclusion is supported by the finding that in our present study (which was carried out in the absence of a living foetus) the tritium to carbon-14 ratio of urinary OE1 and OE3 agreed within the limits of experimental error. That midgestation human foetuses are capable of 16α-hydroxylating major quantities of both DHA and DHAS was demonstrated by Bolté et al. (1966).

Finally, the amount of both tritium and carbon-14 labelled OE3 in the urine exceeded that of OE1 10 to 15 times. This ratio is markedly different from that found following the administration of OE1 or OE2 to non-pregnant subjects (Bauld et al. 1956; Brown 1957), but is in good agreement with the results obtained in pregnant subjects by Davis et al. (1963) and Kirschner et al. (1966). Hence our data seem to support the concept advanced by Davis et al. (1963)
that in pregnancy adaptive changes take place in oestrogen metabolism by maternal tissues, which results in an enhanced conversion of $OE_1$ and $OE_2$ to $OE_3$. Such an enhanced conversion can also be induced in males by the administration of large doses of long-acting $OE_2$ preparations (Engel 1960).

ACKNOWLEDGMENTS

The expenses of this investigation were defrayed by research grants from the Ford Foundation, Swedish Medical Research Council, by Grant No. 411 from the North Atlantic Treaty Organization and by Grant No. 1032 from the Fond de la Recherche Scientifique Medicale, Brussels.

REFERENCES

Bolté E., Mancuso S., Eriksson G., Wiqvist N. & Diczfalusy E.: Acta endocr. (Kbh.) 45 (1964c) 574.

646

Received on August 1st, 1970.