Journal of Steroid Biochemistry, 1971, Vol. 2, pp. 11–18. Pergamon Press. Printed in Great Britain

UTILIZATION OF INGESTED DEHYDROISOANDROSTERONE SULFATE FOR THE PRODUCTION OF ESTROGENS BY NORMAL AND ADRENALECTOMIZED PREGNANT SUBJECTS

ERLIO GURPIDE* and RAYMOND L. VANDE WIELE†
Departments of Obstetrics and Gynecology and Biochemistry, Columbia University, New York, U.S.A.

(*Received* 29 *June* 1970)

SUMMARY

The transfer of ingested dehydroisoandrosterone sulfate (DS) to systemic circulation was measured in two normal pregnant women, in one adrenalectomized pregnant woman and in one non-pregnant woman, by oral administration of ^{14}C-labeled or unlabeled DS and by intravenous injection of tritiated DS. The isotope ratios in urinary DS, and the decrease in the specific activity of this metabolite with the ingestion of DS, indicated that more than 60 per cent of the ingested steroid appeared in the bloodstream. The placental conversion of DS to estrogens was unaffected by the increased amounts of circulating DS. Consequently, ingestion of DS resulted in significant increases in the rate of production and urinary excretion of estrogens. An adrenalectomized pregnant patient at term increased her sub-normal rate of production of estrone plus estradiol (5 mg/day) to 16 mg/day by ingesting 60 mg of DS daily.

INTRODUCTION

ESTROGENS are necessary during the early and possibly late stages of pregnancy [1] but their exact role in the maintenance of human pregnancy or upon the development of the fetus remains to be clarified. Whether a low level of estrogens is only a symptom rather than a cause of habitual abortion remains equally uncertain.

One approach to the study of estrogen effects in pregnancy would be to administer estrogens or their precursors to pregnant subjects who produce them at a reduced rate. The use of a precursor which is converted to estrogens in the placenta, the site where they are normally produced, is to be preferred since estrogen administration to the mother may be ineffective because of their limited passage from maternal to fetal circulations [2]. Dehydroisoandrosterone sulfate (DS)‡ was chosen for this study because it is an effective precursor of estrogens in pregnancy [3] and is apparently devoid of physiologic effects in non-pregnant subjects. Oral administration of DS, if effective, would provide a convenient method of increasing the concentrations of this steroid and the output of placentally produced estrogens during pregnancy.

The study reported here was undertaken to evaluate the effectiveness of orally

*Present address: Departments of Obstetrics and Gynecology and of Biochemistry, College of Medical Sciences, University of Minnesota, Minneapolis, Minn. 55455, U.S.A.

†Career Scientist, Health Research Council of the City of New York.

‡The following trivial names are used: dehydroisoandrosterone: 3β-hydroxy-5-androsten-17-one: 16α-hydroxy-dehydroisoandrosterone: 3β,16α-dihydroxy-5-androsten-17-one; epiestriol: 1,3,5(10)-estratriene-3,16β,17β-triol.

administered DS as a precursor of estrogens during pregnancy. The experimental designs involved the intravenous administration of tritiated DS and ^{14}C-labeled estradiol (E_2). In some experiments, labeled or unlabeled DS was administered orally.

The extent of transfer of ingested DS to systemic circulation was determined by comparing the $^{3}H:^{14}C$ ratio in the urinary DS with the isotope ratio administered as labeled DS, intravenously and orally. The isotope ratios in estrogens isolated from urine in the same experiment were compared with the $^{3}H:^{14}C$ ratio in urinary DS to test for the existence of paths on which ingested DS might convert to estrogens without involving circulating DS.

The efficiency of ingested DS as a precursor of estrogens was determined by measuring "production rates" of estradiol and rates of urinary excretion of estrogens.

The influence of the production rate of circulating DS on the extent of conversion of this compound to estrogens was studied in a pregnant adrenalectomized woman whose low rate of estrogen production was markedly increased by oral administration of DS.

EXPERIMENTAL

Tracers. 4-^{14}C-17β-estradiol was purchased from New England Nuclear Corp. and purified as described in a previous paper[2]. Radiochemical homogeneity of the purified tracer was verified by crystallization with carrier.

7-^{3}H- and 4-^{14}C-dehydroisoandrosterone sulfate were prepared and purified as described elsewhere[4]. Radiochemical purity was tested by crystallizing from methanol a portion of the tracer mixed with carrier DS, ammonium salt.

^{14}C and ^{3}H were measured simultaneously in a 3 channel liquid scintillation counter (TriCarb, Model 3003, Packard Instruments), using a solution of PPO (2,5-diphenyl-oxazole) and POPOP (1,4-bis-5-phenyloxazolyl-benzene) in toluene or in a 1:5 methanol–toluene mixture. All data are reported in counts per minute, at the efficiency corresponding to counting in toluene.

Subjects. Subjects N1 and N2 were young normal pregnant women and were studied at term. Subject Ax had been bilaterally adrenalectomized 1 year before the study. She required steroid replacement therapy. At the time of the study she was 23 years old and was in the 34th week of gestation. Subject NP was a 36-year-old, normal non-pregnant woman.

Administration of tracers and unlabeled DS. Table 1 summarizes the amounts of tracers and unlabeled DS used in each experiment and the corresponding routes of administration. Two consecutive experiments were conducted on subject Ax (experiments Ax-1 and Ax-2). Figure 1 shows the schedule of oral administration of gelatin capsules containing crystalline sodium DS during experiment Ax-2. Subject NP was given 500 mg of the same compound daily for 3 days immediately preceding administration of the tracer, as well as during the period of urine collection.

Isolation of urinary metabolites. In each experiment, collected urine was pooled, and 8/10 of it was treated with β-glucuronidase (Ketodase) for 5 days, at 37°C and pH 5, and then extracted with ethyl acetate. The residue obtained by evaporation of the solvent was partitioned between benzene and 1 *N*-NaOH. The alkaline layer was acidified to pH 1 and extracted with ether. The neutral ether extract was then chromatographed on Celite using the gradient system of Siiteri

Table 1. Amounts of tracers and DS administered in each experiment

Experiment	Intravenous route		Oral route		Days of urine collection
	^{3}H-DS (cpm)	^{14}C-E_2 (cpm)	^{14}C-DS (cpm)	DS sodium salt	
N-1	$8{\cdot}53 \times 10^6$	—	$2{\cdot}08 \times 10^6$	422 mg	3·5
N-2	$12{\cdot}2 \times 10^6$	$1{\cdot}17 \times 10^6$	—	—	3
Ax-1	$8{\cdot}84 \times 10^6$	$0{\cdot}57 \times 10^6$	—	—	5
Ax-2	$8{\cdot}84 \times 10^6$	$0{\cdot}57 \times 10^6$	—	60 mg/day	4
NP	$2{\cdot}25 \times 10^6$	—	—	500 mg/day	3

Abbreviations: DS = dehydroisoandrosterone sulfate; E_2 = estradiol.

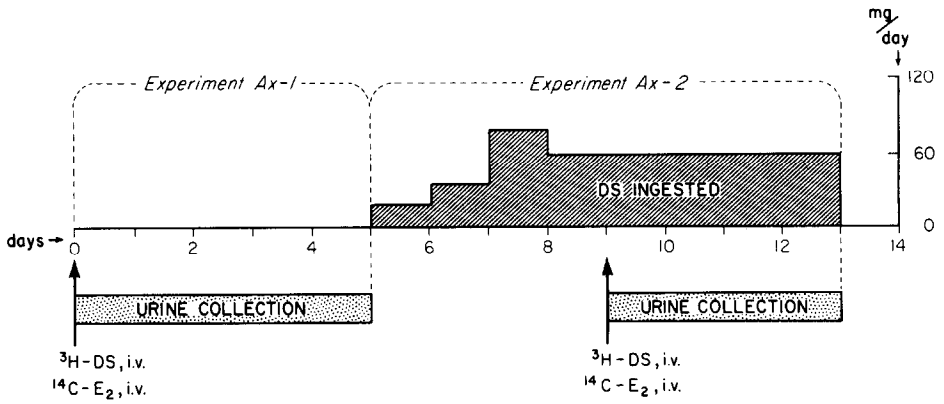

Fig. 1. Schedule of intravenous injection of tracers, of ingestion of DS and of urine collection in experiments Ax-1 and Ax-2.

[5]. The radioactive peaks corresponding to estrone (E_1), E_2, and 16-epiestriol (epi-E_3), were purified by chromatography on Celite, using systems described in a previous publication[2]. Each of these fractions was further purified by filtration through a small alumina bed, and by acetylation and chromatography of the acetates on a thin layer of Silica Gel G, using the system cyclohexane: ethyl acetate, 1 : 1 (v/v).

The fraction corresponding to estriol (E_3) in the gradient elution chromatography was rechromatographed on Celite using the system hexane: chloroform: methanol: water, 8:12:7:3 (by vol.). The radioactive material eluted in the 3rd–4th hold-back volume was crystallized from a methanol-benzene mixture. Some of the crystalline E_3 was acetylated; estriol triacetate was then crystallized from hexane.

In some experiments, DS and 16α-hydroxydehydroisoandrosterone-3-sulfate (16HO-DS) were extracted from the urine according to the procedure of McKenna and Norymberski[6] and chromatographed on Celite using the system isooctane: *t*-butanol: 1 *M*-ammonia, 2:5:5 (by vol.)[7]. The steroid sulfate fraction, eluted in the 2nd–3rd hold-back volume, was solvolyzed with $HClO_4$ in tetrahydrofuran[8] and chromatographed on paper using a Bush A system. The radioactive material eluted from the origin was rechromatographed on paper using the system benzene: heptane: methanol: water, 7:3:8:2 (by vol.). Scanning of the

paper (Vanguard Instrument Co. Model 880) and development of the chromatogram with Blue Tetrazolium indicated coincidence of the radioactive zone with the blue tetrazolium-positive compound and with a sample of 16α-hydroxydehydroisoandrosterone* run in a parallel strip.

Measurements of specific activities. Mass measurements to determine specific activities were performed by colorimetry, using the Kober reagent[9] for free and acetylated estrogens, and the Zimmermann reagent[10] for dehydroisoandrosterone. When possible, specific activities of E_3 and estriol triacetate were determined by direct weighing and counting.

Measurement of estrogen excretion (experiments Ax-1 and Ax-2). The daily excretion of E_1 and E_2 by subject Ax, before and during ingestion of DS, was estimated by isotope dilution methods. About 1/10 of the pooled urine was treated with β-glucuronidase and extracted with ethyl acetate. Known amounts of crystalline E_1 and E_2 were added to the extract. These compounds were again isolated and purified as described above, and their specific activities determined by weighing and counting the crystallized products. The amount of radioactivity present as E_1 and E_2 in the examined aliquot of urine was estimated by multiplying these specific activities by the amounts of carriers added. The total amount of E_1 and E_2 in the collected urine was calculated using these data and the specific activities of these metabolites in the pooled urine, to which no carriers were added.

RESULTS AND DISCUSSION

The data presented in Tables 2 and 3 have been "normalized," i.e. made independent of both the length of urine collection and the amount of radioactivity administered, so as to facilitate comparison between experiments. Normalized specific activities have been defined[2] as the observed specific activities, expressed in cpm per millimole, multiplied by the number of days of urine collection and divided by the administered amount of isotope (in cpm). It should be recalled that the product of the specific activity and the time of urine collection becomes constant when no additional labeled metabolite is excreted.

The $^3H:^{14}C$ ratios shown in Tables 2 and 3 are the ratios of the corresponding normalized specific activities and therefore imply a ratio of administered isotopes equal to 1.

As shown in Table 2, the isotope ratio of DS isolated from urine in experiment N-1 is 1·6. Assuming that urinary DS is entirely derived from circulating DS, this isotope ratio indicates that 63 per cent of the ingested ^{14}C-DS was transferred to systemic circulation[11]. The similarity of the ratios of DS, 16 HO–DS and the 3 estrogen metabolites indicates that the conversion of ingested DS to placental estrogens occurred through the intermediary of circulating DS and that there was no preferential 16α-hydroxylation of the ingested tracer.

It is possible that the extent of transfer of ingested DS to the bloodstream, estimated from the isotope ratios in urinary DS and urinary estrogens (all products of circulating DS), may be smaller than the actual absorption through the digestive tract. The orally administered tracer is more exposed to hepatic metabolism than is the tracer injected into a peripheral vein, and some loss might be expected. However, since the metabolic clearance rate of plasma DS is very small, only

* Kindly provided by Dr. S. Bernstein, Lederle Labs., New Jersey, U.S.A.

Table 2. Results from experiments N-1 and N-2

			E_1	E_2	E_3	epi-E_3	DS	16 HO-DS
Experiment N-1	Normalized specific activities	^3H	3·3	—	0·17	—	—	—
		^{14}C	2·3	—	0·13	—	—	—
(^3H-DS i.v. + ^{14}C-DS oral)	Normalized $^3H/^{14}C$		1·5	1·6	1·3	—	1·6	1·6
Experiment N-2	Normalized specific activities	^3H	10·4	9·8	1·5	—	29·5	—
		^{14}C	31	30	2·1	—	0	—
(^3H-DS i.v. + ^{14}C-E_2 i.v.)	Normalized $^3H/^{14}C$		0·34	0·33	0·72	0·53	∞	—

Normalized specific activities are defined as the observed specific activities (cpm/m mol) multiplied by the number of days of urine collection and divided by the administered amount of isotope (cpm). Normalized isotope ratios correspond to the ratio of normalized specific activities. Abbreviations: E_1, estrone; E_2, estradiol; E_3, estriol; epi-E_3, 16-epiestriol; DS, dehydroisoandrosterone sulfate; 16 HO-DS, 16α-hydroxydehydroisoandrosterone-3-sulfate.

Table 3. Results from experiments Ax-1 and Ax-2

			E_1	E_2	E_3	epi-E_3
Experiment Ax-1	Normalized specific activities	^3H	36·4	29·0	1·70	8·14
		^{14}C	68·8	52·6	1·84	14·2
(^3H–DS i.v. + ^{14}C–E_2 i.v. before ingestion of DS)	Normalized $^3H/^{14}C$		0·52	0·55	0·92	0·57
	Urinary excretion (μg/day)		330	96	—	—
Experiment Ax-2	Normalized specific activities	^3H	9·59	9·05	1·36	4·52
		^{14}C	20·3	16·8	1·75	7·01
(^3H–DS i.v. + ^{14}C–E_2 i.v. during ingestion of 60 mg DS per day)	Normalized $^3H/^{14}C$		0·47	0·54	0·77	0·64
	Urinary excretion (μg/day)		1200	200	—	—

See footnote to Table 2.

8 liters/day in a non-pregnant woman[4], the hepatic extraction could be estimated to be less than 1 per cent[12].

The normalized specific activities of the estrogen metabolites (Table 2) were much higher in subject N-2 than in subject N-1, also at term, who ingested approximately 400 mg of DS. The low specific activity of the metabolites in N-1 can be attributed to the dilution of circulating DS by the ingested compound.

The results from experiment N-2 shown in Table 2 serve to illustrate the extent of the conversion of DS to estrone and estradiol, at term, in a normal pregnancy (see references 3 and 13 for a description of the pertinent calculations). In this subject, approximately 10 mg of DS (expressed as unconjugated dehydroisoandrosterone) and 9 mg of $E_1 + E_2$ entered *de novo* into the maternal bloodstream each day ($PR_{DS} = 1/29{\cdot}5$ mmole per day; $PR_{E_1+E_2} = 1/30{\cdot}5$ mmole per day). Approximately 33 per cent of the circulating *DS* was converted to $E_1 + E_2$ ($\rho_{DS\,to\,E_1+E_2}$ = normalized $^3H/^{14}C$ in E_1 or E_2). About 35 per cent of these estrogens were derived from maternal DS ($\Delta_{E_1+E_2\,from\,DS} = 10{\cdot}1/29{\cdot}5$) but only 5 per cent of the estriol originated in that source ($\Delta_{E_3\,from\,DS} = 1{\cdot}50/29{\cdot}5$). The lower specific activity of E_3, compared to the specific activities of E_1 and E_2, indicates a major additional source for this steroid, most likely 16α hydroxylated neutral steroids of fetal origin[3].

The results from experiment N-2 are in complete agreement with those obtained by Siiteri and MacDonald[3] in their pioneering and extensive studies on this topic.

Table 3 presents the results obtained from the pregnant adrenalectomized patient. In both experiments conducted on this subject, a mixture of tritiated DS and ^{14}C-labeled estradiol was injected intravenously. The first experiment (Ax-1) was performed prior to the ingestion of DS, and the second (Ax-2) was carried out while the subject was daily being fed 60 mg of DS in gelatin capsules.

The normalized specific activities of the urinary estrogens in experiment Ax-1 are significantly larger than those obtained with a normal pregnant subject

also at term (N-2). The higher specific activities in Ax-1 indicate a lesser degree of dilution of the injected tritiated estradiol than that observed in subject N-2. The rate of production of $E_1 + E_2$ by Ax, measured from the specific activity of urinary E_2, was only half of that estimated for the normal subject N-2. This result is in agreement with the subnormal urinary excretion of estrogens recently reported in another adrenalectomized pregnant woman (14 and references therein). The ingestion of DS by Ax increased this rate from 5 mg/day to 16 mg/day. The increase in estrogen production was also indicated by the rates of urinary excretion of estrogen metabolites shown in Table 3.

It is noteworthy that the $E_2 : E_3$ ratio of specific activities, with respect to tritium, is 2·5 times higher in subject Ax than in the normal subject N-2. This observation could be explained by considering that in the adrenalectomized patient an even larger proportion of E_3 than in the normal was derived from precursors other than those of maternal E_2. It is therefore not surprising that in subject Ax, the effect of ingestion of DS on the value of the specific activities was more pronounced in E_1 and E_2 than in E_3. The specific activities of epi-E_3 lie in between those of $E_1 + E_2$ and E_3, in agreement with previous reports [2, 15].

It is interesting to note that the fraction of intravenously injected DS converted to $E_1 + E_2$ did not change appreciably, despite the increased amount of DS in circulation. Thus in subject Ax, the conversion of DS to E_2, calculated from the isotope ratio in urinary E_2, was 55 per cent under basal conditions and 53 per cent after ingestion of 60 mg of DS per day. It can be concluded that the placental enzymic systems involved in the conversion of DS to estrogens have a large capacity. This finding is in agreement with the observations of Siiteri and MacDonald, who detected no changes in the fractional conversion of DS to E_2 by either suppressing or stimulating maternal production of DS by administration of dexamethasone or ACTH [16].

In another experiment, a non-pregnant woman (NP) was daily fed 500 mg of DS, sodium salt, also in gelatin capsules. In this subject, the average rate of production of circulating DS, determined by intravenous injection of ^{3}H-DS and measurement of the specific activity of urinary DS, increased from a base level of 7·3 mg/day to 416 mg/day. These values represent an 80 per cent transfer of ingested DS to systemic circulation.

It can then be concluded from these results that an effective increase in estrogen production in pregnant women is achieved by oral administration of DS. The bulk of the ingested steroid is transferred to the maternal circulation and aromatized in the placenta, the normal site of production of estrogens in pregnancy. The estrogens formed in this manner can be expected to have the same metabolic rate as the endogenously produced hormones. Ingestion of DS thus appears to be a convenient means of generating estrone and estradiol which may be available to the fetus.

ACKNOWLEDGEMENTS

The authors are indebted to Drs. P. Siiteri and S. Lieberman for contributing valuable comments. Miss Margaret Welch provided excellent technical assistance. The work was supported by Grant AM-110 USPHS.

REFERENCES

1. R. Deanesly; in *Marshall's Physiology of Reproduction* (Edited by A. S. Parker). Little, Brown & Co., Boston, Vol. 3 (1966) p. 891.

2. E. Gurpide, J. Schwers, M. T. Welch, R. L. Vande Wiele and S. Lieberman: *J. clin. Endocr.* **26** (1966) 1355.
3. P. K. Siiteri and P. C. MacDonald: *J. clin. Endocr* **26** (1966) 751.
4. E. Sandberg, E. Gurpide and S. Lieberman: *Biochemistry* **3** (1964) 1256.
5. P. K. Siiteri: *Steroids* **2** (1963) 687.
6. J. McKenna and J. K. Norymberski: *Biochem. J.* **76** (1960) 60P.
7. P. K. Siiteri, R. L. Vande Wiele and S. Lieberman: *J. clin. Endocr.* **23** (1963) 588.
8. S. Burnstein and S. Lieberman: *J. biol. Chem.* **233** (1958) 331.
9. J. Ittrich: *Hoppe Seyler's Z. physiol. Chem.* **312** (1958) 1.
10. W. Zimmermann: *Z. physiol. Chem.* **245** (1936) 47.
11. E. Gurpide, P. C. MacDonald, R. L. Vande Wiele and S. Lieberman: *J. clin. Endocr.* **23** (1963) 346.
12. J. F. Tait and S. Burstein: in *The Hormones* (Edited by G. Pincus, K. V. Thimann and E. B. Astwood). Academic Press, N.Y., Vol. 5 (1964) p. 441, pp. 457–8.
13. J. Mann and E. Gurpide: *J. clin. Endocr.* **26** (1966) 1346.
14. D. Charles, R. A. Harkness, F. M. Kenny, E. Menini, A. A. A. Ismail, J. W. Durkin and J. A. Loraine: *Am. J. Obstet Gynec.* **106** (1970) 66.
15. R. Hobkirk and M. Nilsen: *J. clin. Endocr.* **26** (1966) 625.
16. P. C. MacDonald and P. K. Siiteri: *J. clin. Invest.* **44** (1965) 465.