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EXCRETION OF PROGESTERONE METABOLITES AND ESTRIOL IN FAECES FROM PREGNANT WOMEN DURING AMPICILLIN ADMINISTRATION

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SUMMARY

Progesterone metabolites and estriol were determined in urine and faeces collected daily from three pregnant women (33–37 weeks) before and during ampicillin administration (2 g/day orally).

Two of the three subjects showed marked changes in their faecal steroid excretion during ampicillin administration: the faecal progesterone-metabolite pattern changed from containing 69–79% unconjugated metabolites and 19–26% glucuronides under control conditions, to high steroid sulphate content (28–44%); the faecal elimination of 3β-hydroxy-5α-pregnan-20-one and 5α-pregnane-3β,20α-diol glucuronide all but ceased; two 16α-hydroxylated progesterone metabolites were detected in significant amounts in faeces during ampicillin administration but not under normal conditions. Steroid sulphate hydrolysis, epimerization of 3α,5α- to 3β,5α-steroids and 16α-dehydroxylation are all well known actions of intestinal bacteria on biliary steroids. It thus seems clear that the changes found in the faecal progesterone metabolite pattern are due to the reduction of the intestinal flora by ampicillin.

Under control conditions the bulk of the faecal estriol was unconjugated. During ampicillin administration this excretion remained unchanged but in addition large quantities of conjugated estriol appeared in the faeces, apparently as a result of inhibition of bacterial deconjugation.

Ampicillin administration also caused decreased urinary excretion of estriol and pregnanediol glucuronide. It seems likely that these well documented effects of ampicillin on urinary steroid excretion are caused by an interruption of the enterohepatic circulation of steroids which results from the inhibition of intestinal steroid metabolism described above.

INTRODUCTION

The urinary excretion of estriol [1] and progesterone metabolites [2, 3] during pregnancy can be markedly reduced by oral administration of the antibiotic ampicillin. Both estrogens and progesterone metabolites take part in an extensive enterohepatic circulation [4–6]. During this they are subjected to the influence of the intestinal microflora which is known to effect deconjugation, epimerization and dehydroxylation of steroids [7–10]. The reduction of the intestinal microflora by an antibiotic might eliminate these reactions and could probably cause changes in the urinary excretion of steroids by altering their enterohepatic circulation.

The purpose of the present investigation was to test this hypothesis by measuring the faecal and urinary excretion of certain progesterone metabolites and unconjugated and conjugated estriol in pregnant subjects before and during ampicillin administration.

EXPERIMENTAL

Three apparently normal hospitalized, noninfected mothers, 33–37 weeks pregnant participated in the study. Urine and faeces was collected daily for 6 consecutive 24 h periods (days 1–6). On days 3, 4 and 5 each patient received 500 mg ampicillin (Doktacillin®, Astra, Sweden) 4 times daily. Excreta were stored at −20°C until analyzed.

Extraction of faeces

The steroids were extracted from the faeces as described by Laatikainen and Vihko[11]. The extracts were stored in chloroform–methanol (1:1 v/v) at +4°C until analyzed.

Analysis of faecal progesterone metabolites

Unconjugated steroids and steroid mono- and disulphates. One per cent of each faecal extract in chloroform–methanol (1:1) was applied to a 4 g Sephadex LH-20 column (220 × 10 mm) packed in the same solvent but containing 0·01 mol/l NaCl [12]. The column was eluted with the latter solvent and fractions between 8 and 28 ml (unconjugated steroids) and 28–50 ml (steroid monosulphates) collected. The column was then eluted with 150 ml of methanol (steroid disulphates). These elution volumes were determined by adding [^{3}H]-testosterone and [^{3}H]-testosterone monosulphate to some of the samples (subject A, days 1, 2, 4 and 5) and monitoring their elution from the column. The fractions were evaporated to dryness, the steroid mono- and disulphate

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fractions solvolyzed and the liberated steroids recovered as described by Jänne *et al.*[12]. The remaining steps in the analytical procedure are described below.

Steroid glucuronides. A suitable amount of [^{3}H]-testosterone glucuronide (50,000 c.p.m.) was added to 3·3% of each faecal extract which was then evaporated to dryness. The dry residue was suspended in 25 ml of warm (40°C) methanol:water (7:3 v/v) and the suspension left at −20°C overnight. The sample was then centrifuged at 1000 *g* for 30 min at −20°C and the resultant supernatant evaporated to dryness. The dry residue was partitioned between equal volumes (10 ml) of water and diethyl ether and the ether phase discarded. The aqueous phase was purified on a column of Amberlite XAD-2 (BDH Chemicals, Poole, U.K.) as described by Viinikka and Jänne[13]. The sample was then chromatographed on a 20 g column of Sephadex LH-20 and the unconjugated plus steroid glucuronide fraction collected [2, 14]. This fraction was partitioned between 30 ml of 8·4% $NaHCO_2$ and 30 ml of ethyl acetate [15] and the glucuronide fraction thus obtained was dissolved in 0·5 mol/l sodium acetate–acetic acid buffer pH 5·0, 666 units/ml of Ketodase® (Warner–Lambert Pharmaceutical Co., Morris Plains, N.J., U.S.A.) added and the solution, final volume 15 ml, was incubated at 39°C for 24 h. Thereafter, a further 666 units/ml of Ketodase were added and the incubation continued for a further 16 h. After hydrolysis the liberated steroids were extracted as described by Laatikainen and Vihko [14] and further analyzed as outlined below.

Silic acid chromatography, gas–liquid chromatography (g.l.c.) and gas chromatography–mass spectrometry (GC–MS)

Silicic acid chromatography, quantitative steroid determination by g.l.c. and GC–MS was carried out exactly as described recently [2].

In general, the peaks were quantitated after chromatography on 3% QF-1 but 5β-pregnane-3α,20α-diol and 5α-pregnane-3α,20α-diol in the monosulphate fractions were quantitated after chromatography on a 1% HI-EFF 8 BP (Applied Science Laboratories, Inc., Pa., U.S.A.) liquid phase as all impurities did not separate from these peaks on QF-1 or SE-30. The values were not corrected for methodological losses.

Analysis of faecal unconjugated and conjugated estriol

One per cent of each faecal extract in chloroform–methanol was evaporated to dryness, dissolved in 25 ml of warm methanol–water (7:3 v/v) and left at −20°C overnight. After centrifugation the supernatant fraction was analyzed essentially as described by Adlercreutz and Luukkainen[16] excluding all unnecessary steps for other estrogens. The values were corrected for losses incurred during purification on the basis of simultaneous recovery experiments using bile samples.

Analysis of urinary estriol

Urinary estriol was determined by gas–liquid chromatography by the method of Adlercreutz and Luukkainen[17]. The values were corrected for losses incurred during the procedure by simultaneous recovery experiments with estriol. In some instances radioactive estriol was used as internal standard.

Analysis of urinary 5β-pregnane-3α,20α-diol glucuronide

A suitable amount of [^{3}H]-testosterone glucuronide (100,000 c.p.m.) was added to a 10 ml sample of each 24 h urine collection. The samples were then processed exactly as described in [2], except that the steroids liberated by Ketodase were subjected to chromatography on columns of Lipidex™-5000 (Packard Instrument International S.A., Zürich, Switzerland) (100 × 3 mm) packed in petroleum ether (66–68°C boiling point): chloroform (95:5 v/v) (18). The columns were eluted with the same solvent and a fraction between 8 and 12 ml collected which contained the 5β-pregnane-3α,20α-diol. For quantitation a suitable amount (30 μg) of 5β-pregnane-3α,17α,20α-triol was added as internal standard and the samples silylated and subjected to g.l.c. on a 3% QF-1 column. The purity and identity of the analytical peak was assessed by GC–MS. The values were corrected for methodological losses.

RESULTS

Progesterone metabolites in faeces

The progesterone metabolites in the faeces of the three subjects on days 1 and 2 (control days) and days 4 and 5 (the second and third day of ampicillin administration) of the study were analyzed. The latter two days were chosen because in previous studies [1, 2, 19] the greatest ampicillin-induced impairment of urinary steroid excretion was seen at this time. The daily faecal excretion of the C_{21}-unconjugated steroids, glucuronides and mono- and disulphates are given in Table 1. These values have not been corrected for methodological losses but some indications of recoveries obtained in the steps after faecal extraction and prior to g.l.c. can be given: the recovery of added [^{3}H]-testosterone and [^{3}H]-testosterone monosulphate to the samples from subject A were 100 and 51–55%, respectively. The recovery of [^{3}H]-testosterone glucuronide added to all samples ranged from 59 to 84%. Under control conditions the total daily faecal progesterone metabolite excretion varied to quite a large extent between the subjects studied (Table 1). Ampicillin administration had no consistent effect on total faecal progesterone metabolite excretion by the three subjects (Table 1).

The distribution of the steroids determined in this study between the unconjugated, glucuronide and mono- and disulphate fractions is shown in Fig. 1. Subjects A and B showed the same excretion pattern

Table 1. The excretion of unconjugated, glucuronide and mono- and disulphated C_{21} neutral steroids (mg/24 h) in the faeces of three pregnant subjects (33–37 weeks) before and during ampicillin administration (2 g/day orally)

Steroid	Subject	Day 1	Day 2	Day 4[x]	Day 5[x]
Unconjugated					
	A	7.4	6.1	7.7	3.9
3α-hydroxy-5β-pregnan-20-one	B	7.3	10.6	6.4	5.9
	C	3.9	1.6	4.9	2.3
	A	4.3	3.6	6.1	4.6
5β-pregnane-3α,20α-diol	B	10.1	15.8	9.6	6.6
	C	5.1	2.1	5.4	2.8
	A	1.2	0.9	1.3	1.3
5α-pregnane-3β,20α-diol	B	6.2	7.9	2.3	1.0
	C	1.0	0.8	2.4	1.3
	A	1.1	0.6	1.8	1.9
5α-pregnane-3β,16α,20α-triol	B	0.9	1.2	0.3	> 0
	C	n.f.	n.f.	n.f.	n.f.
total	A	14.0	11.1	16.9	11.7
	B	24.5	35.5	18.6	13.5
	C	10.0	4.5	12.7	6.4
Glucuronides					
	A	0.33	0.39	0.12	0.12
3α-hydroxy-5α-pregnan-20-one	B	0.44	0.41	0.05	0.18
	C	0.10	0.04	0.03	0.03
	A	1.30	1.40	0.43	0.53
3α-hydroxy-5β-pregnan-20-one	B	2.27	2.06	0.49	1.48
	C	0.42	0.19	0.07	0.23
	A	0.65	0.73	0.23	0.03
3β-hydroxy-5α-pregnan-20-one	B	0.51	0.57	0.03	n.f.
	C	0.05	0.03	0.01	0.01
	A	0.90	1.00	0.44	0.83
5β-pregnane-3α,20α-diol	B	3.51	3.36	0.71	1.72
	C	0.70	0.31	0.13	0.49
	A	0.58	0.69	0.36	0.13
5α-pregnane-3β,20α-diol	B	1.82	2.10	0.10	0.04
	C	0.17	0.14	0.04	0.13
	A	0.18	0.24	0.16	0.38
5α-pregnane-3α,20α,21-triol	B	0.20	0.10	0.11	0.24
+ estriol[+]	C	-[xx]	-	-	-
	A	0.33	0.16	0.09	0.03
5α-pregnane-3β,20α,21-triol	B	0.10	0.08	0.04	0.03
	C	-[xx]	-	-	-

continued

Table 1 (continued)

Steroid	Subject	Day 1	Day 2	Day 4[x]	Day 5[x]
3α,16α-dihydroxy-5α-pregnan-20-one + 3β,16α-dihydroxy-5β-pregnan-20-one	A	<0.03	<0.03	<0.03	0.37
	B	<0.03	<0.03	0.09	0.03
	C	-[xx]	-	-	-
total	A	4.3	4.7	1.9	2.4
	B	8.9	8.7	1.6	3.7
	C	1.4	0.7	0.3	0.9
Monosulphates					
5β-pregnane-3α,20α-diol	A	0.2	1.3	1.9	5.8
	B	0.3	0.3	3.3	3.8
	C	4.1	2.0	4.9	4.2
5α-pregnane-3α,20α-diol	A	0.9	0.2	0.2	1.1
	B	0.2	-[xx]	2.5	2.4
	C	0.6	0.3	0.2	0.5
5-pregnene-3β,20α-diol	A	0.2	0.04	0.1	0.4
	B	0.02	0.02	0.2	0.3
	C	1.3	1.0	0.1	0.7
5α-pregnane-3β,20α-diol	A	0.04	0.1	0.1	0.3
	B	0.2	0.2	0.4	0.7
	C	1.2	0.9	0.3	0.9
5α-pregnane-3α,20α,21-triol + estriol[+]	A	0.01	0.05	0.3	4.2
	B	0.1	0.1	0.5	0.9
	C	0.5	0.5	1.6	1.0
5α-pregnane-3β,20α,21-triol	A	0.02	0.2	0.4	0.3
	B	n.f.	n.f.	0.1	0.1
	C	0.3	0.2	1.1	n.f.
total	A	1.4	1.9	3.0	12.1
	B	0.8	0.6	7.0	8.2
	C	8.0	4.9	8.2	7.3
Disulphate					
5α-pregnane-3α,20α-diol	A	0.2	0.2	0.1	1.1
	B	0.1	0.1	1.1	3.5
	C	0.5	0.1	0.9	1.5
Total excretion	A	19.9	17.8	21.9	27.3
	B	34.3	44.9	28.3	28 9
	C	19.9	10.2	22.1	16.1

\- n.f., not found

x Days 1 and 2 are control days and days 4 and 5 the second and third day of ampicillin administration.

\+ A certain amount of estriol is retained during this procedure and is quantitated with the 5α-pregnane-3α,20α,21-triol.

xx These peaks could not be quantitated because of interfering compounds.

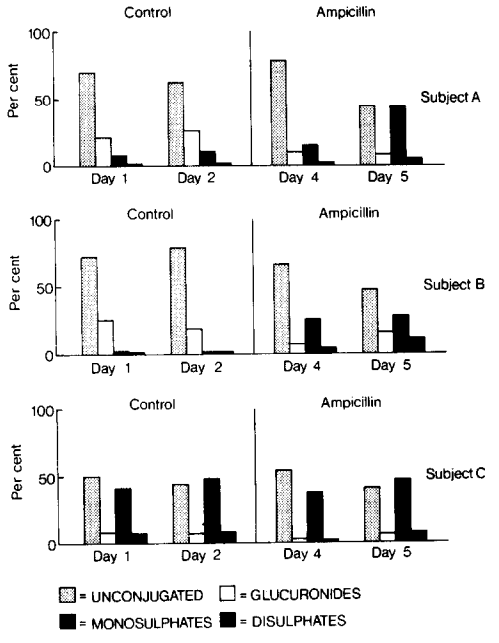

Fig. 1. The relative excretion of unconjugated steroids, steroid glucuronides and mono- and disulphates in the faeces of three pregnant women (33–37 weeks) before and during ampicillin administration (2 g/day, orally). The amount of steroids in each fraction is expressed as a percentage of the total excretion measured. Days 1 and 2 are control days and days 4 and 5 the second and third days of ampicillin administration.

on the control days: the majority (69–79%) of the steroids were unconjugated but considerable amounts (19–26%) of the steroids, were excreted as glucuronides. Subject C on the other hand excreted large amounts of monosulphates (Fig. 1). During ampicillin treatment the overall pattern of progesterone metabolite excretion was altered drastically in subjects A and B (Fig. 1). Smaller proportions of unconjugated and glucuronide conjugated metabolites and increased amounts of mono- and disulphates were now excreted. This effect only became evident in subject A on the last day of ampicillin treatment (day 5) but was already evident in the case of subject B on the previous day. These changes were not seen with subject C.

Four unconjugated steroids were quantitated (Table 1). Of these 5β-pregnane-3α,20α-diol and 3α-hydroxy-5β-pregnan-20-one were excreted in greater amounts than any other progesterone metabolite by all three subjects. Subject B also excreted large amounts of 5α-pregnane-3β,20α-diol. During ampicillin administration (days 4 and 5, Table 1) striking decreases were seen in the excretion of 5α-pregnane-3β,20α-diol and 5α-pregnane-3β,16α,20α-triol in subject B, and clear decreases occurred in the excretion of the other two compounds measured. In subject A a clearly decreased excretion was seen only with 3α-hydroxy-5β-pregnan-20-one. Whereas total unconjugated metabolite excretion was markedly decreased in one of the subjects (B). this was not the case with subject A. No clear pattern could be seen in the unconjugated steroid excretion by subject C (Table 1).

In the glucuronide fraction three pregnanolones, two pregnanediols, two pregnanetriols (one of the peaks containing some estriol) and a peak containing 3α,16α-dihydroxy-5α-pregnan-20-one and 3β,16α-dihydroxy-5β-pregnan-20-one were quantitated (Table 1). Ampicillin administration caused a dramatic depression in the concentration of the 3β-hydroxy-5α-pregnane steroids excreted as glucuronides (Table 1). In addition, the levels of 3α,16α-dihydroxy-5α-pregnan-20-one and 3β,16α-dihydroxy-5β-pregnan-20-one increased from less than 0·03 mg/day (subjects A and B) under control conditions to 0·37 (subject A) and 0·09 mg/day (subject B, Table 1). During ampicillin treatment the total faecal glucuronide concentrations were clearly depressed (subjects A and B, Table 1). Subject C excreted smaller amounts of glucuronides. Interfering compounds precluded quantitation of four of the g.l.c. peaks in the samples from subject C. However, among the steroids determined a clearly decreased excretion was seen on the second day of ampicillin administration (day 4).

Under control conditions only low faecal excretion of sulphate conjugated steroids was apparent in the case of subjects A and B (Table 1). Ampicillin administration caused a very marked increase in the excretion of all of the monosulphates and the disulphate determined in both subjects (Table 1). In the case of subject B the increase was already quite apparent on day 4, while with subject A the increase occurred mainly on day 5. Subject C showed high faecal monosulphate excretion throughout the study (Table 1).

Unconjugated and conjugated estriol in faeces

The faecal excretion of unconjugated and conjugated estriol by the three subjects on control days 1 and 2 and on the second and third days of ampicillin administration (days 4 and 5) are given in Table 2. The percentage distribution of estriol between the two fractions in these samples is shown in Fig. 2. To the best of our knowledge values for estriol excretion in faeces in late pregnancy have not been pub-

Table 2. The excretion of unconjugated and conjugated estriol in the faeces of three pregnant subjects before and during ampicillin administration

Subject	Steroid		Day 1	Day 2	Day 4[x]	Day 5[x]
A	Estriol	Unconjugated	748[xx]	513	873	1886
		Conjugated	23	25	16	1596
B	Estriol	Unconjugated	544	886	293	844
		Conjugated	16	22	324	820
C	Estriol	Unconjugated	823	212	115	197
		Conjugated	97	10	17	14

x The three subjects, 33-37 weeks pregnant, received ampicillin, 2 g/day orally, on days 3, 4 and 5.

xx Values are expressed as µg/day.

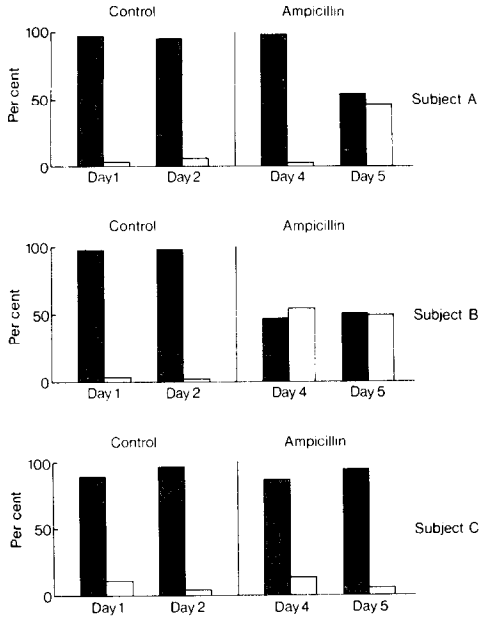

Fig. 2. The relative excretion of unconjugated (hatched bars) and conjugated (open bars) estriol in the faeces of three pregnant women (33–37 weeks) before and during ampicillin administration (2 g/day, orally). The amount of steroids in each fraction is expressed as a percentage of the total excretion measured. Days 1 and 2 are control days and days 4 and 5, the second and third days of ampicillin administration.

lished previously: from this series, normal daily unconjugated estriol excretion would seem to be in the range of 500–900 μg with conjugated estriol levels being less than 25 μg/day.

While estriol was excreted almost exclusively in unconjugated form in faeces under control conditions, during ampicillin administration equal amounts of unconjugated and conjugated estriol were present in the faeces of subjects A and B (Table 2, Fig. 2). As observed with the faecal progesterone metabolites the ampicillin-induced changes in estriol metabolism were seen already on day 4 with subject B while they did not become apparent in subject A until day 5. In absolute terms the total faecal estriol excretion increased from mean control levels of 654·3 and 733·9 μg/24 h to maxima of 3482 and 1664 μg/24 h in subjects A and B, respectively. No such changes were seen in subject C.

Table 3. The excretion of estriol in the urine of three pregnant subjects before, during and after ampicillin administration

Subject	Day 1	Day 2	Day 3[x]	Day 4[x]	Day 5[x]	Day 6
A	33.0[xx]	34.8	32.1	17.4	24.4	21.3
B	8.7	8.2	10.0	7.6	6.6	7.6
C	8.9	13.7	20.2	10.6	10.3	20.2

x The three subjects, 33-37 weeks pregnant, received ampicillin, 2 g/day orally, on days 3, 4 and 5.

xx Values are expressed as mg/24 h.

Table 4. The excretion of 5β-pregnane-3α.20α-diol glucuronide in the urine of three pregnant subjects before, during and after ampicillin administration

Subject	Day 1	Day 2	Day 3[x]	Day 4[x]	Day 5[x]	Day 6
A	58.5[xx]	39.5	26.4	19.6	55.4	44.5
B	15.2	19.9	19.3	6.2	13.3	9.6
C	9.1	8.1	13.5	2.6	10.9	13.8

x The three subjects, 33-37 weeks pregnant, received ampicillin, 2 g/day orally, on days 3, 4 and 5.

xx Values are expressed as mg/24 h.

Urinary estriol

The urinary estriol excretion values for the three subjects over the six day study period are given in Table 3. Subjects A and B exhibited a reasonably typical response to ampicillin administration with diminished excretion on days 4 and 5 (see 1–3, 19). Subject B showed a tendency to return to normal on day 6, but with subject A the post ampicillin level was still low. The third patient did not show a typical pattern, the variation in estriol output was great and ampicillin administration did not seem to have any effect.

Urinary 5β-pregnane-3α,20α-diol glucuronide

The urinary 5β-pregnane-3α,20α-diol glucuronide values for the three subjects are given in Table 4. The response to ampicillin administration by all three was as found in our previous study [2], with a marked reduction in excretion on the second day of ampicillin administration (day 4) and a tendency towards returning to normal levels on the next day.

DISCUSSION

Under control conditions the faecal excretion of progesterone metabolites by the three subjects was very variable, between 10·21 and 44·91 mg/day (Table 1). Eriksson *et al.*[7] have reported a daily faecal neutral steroid excretion of 33·3 mg for a woman in the 37th week of pregnancy. The bulk of the steroids excreted by their subject was unconjugated as were the majority of those found in this study (Table 1, Fig. 1). However, here, considerable amounts of glucuronide conjugated progesterone metabolites were also detected in the faeces (Table 1, Fig. 1). While no endogenous glucuronide conjugated neutral steroids were found in the faeces of normal subjects [10, 11], small amounts have been detected in the faeces of a subject with intrahepatic cholestasis of pregnancy [8].

All of the individual progesterone metabolites in the unconjugated and mono- and disulphate conjugate fractions have previously been identified as such in normal, human pregnancy faeces [7], or faeces from women with intrahepatic cholestasis of pregnancy [8]. Four of the glucuronides, 3α-hydroxy-5β-pregnan-20-one, 3β-hydroxy-5α-pregnan-20-one, 5β-

pregnane-3α,20α-diol and 5α-pregnane-3β,20α-diol were also detected in the latter study [8].

Of the three subjects studied, subject C, will not be considered in this discussion. While this patient showed the usual response to ampicillin in regard to urinary 5β-pregnane-3α,20α-diol glucuronide (Table 4 [2]), the urinary estriol pattern was unusual (Table 3 [1, 2, 19]). In addition, the changes seen in faecal steroids in the other two subjects were not apparent in this patient (Tables 1 and 2; Figs. 1 and 2). Subject C showed high faecal neutral steroid sulphate excretion under control conditions (Fig. 1). Eriksson and Gustafsson[10] have also described an apparently normal non-pregnant female subject who excreted most steroids as monosulphates in faeces. The reason for this different pattern is not clear.

During ampicillin administration marked changes were seen in the faecal excretion of progesterone metabolites in subjects A and B (Table 1, Fig. 1). The excretion of all sulphate-conjugated metabolites was markedly increased. The excretion of unconjugated metabolites tended to decrease, but this trend was much more noticeable in subject B than subject A. There was also a general decrease in the excretion of all glucuronide conjugated metabolites, except the 16α-hydroxylated compounds, by both subjects (Table 1, Fig. 1). In addition, in the glucuronide conjugated and to some extent in the unconjugated steroid fraction, the excretion of 3β-hydroxy-5α-pregnane derivatives was specifically decreased by ampicillin administration (Table 1). Another apparent change in the glucuronide fraction was the clear increases in the excretion of 3α,16α-dihydroxy-5α-pregnan-20-one and 3β,16α-dihydroxy-5β-pregnan-20-one (Table 1). It seems that all of these changes may be associated with altered intestinal steroid metabolism.

The role of the intestinal microflora in the metabolism of steroids in the rat has been studied in great detail by comparing the steroids excreted by germ-free and conventional rats and by incubating steroids with caecal contents [reviewed in 20–22]. Among the major reactions catalyzed by the intestinal bacteria in the conventional rat are: deconjugation of neutral steroid sulphates [23, 24], epimerization of 3α-hydroxy-5α-steroids to the equatorial 3β/5α form [25–27] and 16α-dehydroxylation of C_{21} steroids to yield pregnane isomers with a 17α side chain [27–29]. That the human intestinal microflora can carry out these reactions is also reasonably certain. The high level of unconjugated steroids in normal pregnancy and non-pregnancy faeces (Table 1 [7, 10, 11]) contrasts markedly with the high proportion of sulphate conjugated steroids in pregnancy and non-pregnancy bile [30, 31]. In addition, a much greater proportion of 3β,5α-pregnane isomers and 17α-pregnane derivatives occur in pregnancy faeces [7] than in pregnancy bile [30]. The administration of intestinal sulphonamides, which effectively reduce the intestinal bacteria, to pregnant and non-pregnant subjects increases the excretion of steroid sulphates and leads to the disappearance of 3β,5α isomers and pregnane isomers with a 17α side chain from faeces [9, 10]. In addition, human intestinal bacteria contain the enzymes necessary for 16α-dehydroxylation of C_{21} steroids [10]. Thus, it can be concluded that the principal effects of ampicillin administration on the pattern of progesterone metabolites in pregnancy faeces may all be associated with the inhibition of intestinal bacterial steroid matabolism.

Estriol is secreted in pregnancy bile in conjugated form only [4]. From this study (Table, Fig. 2) it is clear that under normal conditions almost all of the estriol excreted in faeces is in the unconjugated form. The administration of ampicillin caused a huge increase in the faecal excretion of estriol conjugates by subjects A and B (Table 2, Fig. 2). As the human intestinal contents hydrolyze estrogen conjugates rather efficiently [32, 33] it seems clear that the reduction of the intestinal microflora by ampicillin is also responsible for this dramatic change in the faecal estrogen pattern.

The administration of ampicillin to pregnant women also causes significant reductions in the urinary excretion of estriol and progesterone metabolites (Tables 3 and 4). In previous studies [2, 19] it has been shown that these urinary decreases are mainly confined to specific metabolites: in the case of estriol, to estriol-3-glucuronide a specific intestinal metabolite [34, 35] and in the case of the progesterone metabolites to glucuronide conjugated steroids. Both estrogen and progesterone metabolites undergo considerable biliary secretion [4, 29] and enterohepatic circulation [4–6]. Therefore, the decreased excretion of estriol-3-glucuronide and the pregnane glucuronides most probably arise from an interruption of the enterohepatic circulation of steroids during ampicillin administration. The efficient reabsorption of the biliary steroids from the intestine may depend to some extent on their deconjugation by the intestinal bacteria [32]. The impairment of this process, as is shown here to occur during ampicillin administration, results in faecal loss of steroid conjugates and is most likely directly responsible for the decreased urinary excretion of specific estrogen and progesterone metabolites.

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ADDENDUM

The steriodologically exceptional third case had symphyseolysis and mild toxemia. She gave birth to an icteric baby (girl, 4000 g) with a presacral fistula. The child required blood exchange during the 3rd day of life for an unknown reason.

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