Dehydroxylation of 16α-hydroxyprogesterone by fecal flora of man and rat

V. D. Bokkenheuser,[1] J. Winter, P. B. Hylemon, N. K. N. Ayengar, and E. H. Mosbach

Department of Pathology, St. Luke's-Roosevelt Hospital Center, New York, NY 10025;[2] Department of Microbiology, Medical College of Virginia V.C.U., Richmond, VA 23298;[3] and Lipid Research Laboratory, Department of Surgery, Beth Israel Medical Center, New York, NY 10003[4]

Abstract 16α-Hydroxyprogesterone, precursor of biliary 16α-hydroxypregnanolone, was incubated with mixed fecal flora of humans and rats. The major steroid metabolite formed in both systems was 3α-hydroxy-17α-pregnan-20-one. These results demonstrated that the fecal flora reduced the Δ^4-3 keto structure, removed the hydroxy group at C-16 and isomerized the side chain from the β to the α configuration. Ring-A reduction of the substrate resulted in a 5β-compound with human flora and a 5α-product with rat bacteria. The prevalence of 16α-dehydroxylating organisms varied considerably in human fecal flora and was approximately 10^5/g of feces in the three rats tested. Rat fecal flora dehydroxylated 16α-hydroxyprogesterone after 4–5 days incubation at 37°C, at pH 6.5–7.5, and with a substrate concentration of 20–80 μg/ml (optimal condition). Preliminary evidence suggests that 16α-dehydroxylase is exclusively of bacterial origin and is synthesized by an obligate anaerobe.—**Bokkenheuser, V. D., J. Winter, P. B. Hylemon, N. K. N. Ayengar, and E. H. Mosbach.** Dehydroxylation of 16α-hydroxyprogesterone by fecal flora of man and rat. *J. Lipid Res.* 1981. **22:** 95–102.

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In 1962, Calvin and Lieberman (1) demonstrated that 16α-hydroxyprogesterone administered intravenously to a human was excreted in the urine as 3α-hydroxy-5β,17α-pregnan-20-one (17α-pregnanolone). Thus, prior to excretion the molecule had undergone ring-A reduction, dehydroxylation at C-16, and a change in the configuration of the side chain from β to α.

In humans, 16α-hydroxyprogesterone is reduced in the liver to 16α-hydroxypregnanolone (2–5). In rats, the steroid is reduced to 3α,16α-dihydroxy-5α-pregnan-20-one (6–8). In both species the 16α-hydroxysteroids are excreted in the bile, undergo enterohepatic circulation (4–5), and, therefore, become exposed to the intestinal flora. In conventional rats the biliary 3α,16α-dihydroxy-5α-pregnan-20-one is dehydroxylated to 3α-hydroxy-5α,17α-pregnan-20-one (9) which is excreted in the feces. In contrast, germ-free rats do not alter 16α-hydroxysteroid (9). These data implicate the fecal flora in the 16α-dehydroxylation process. The contention is further supported by experiments in which 3β,16α-dihydroxy-5α-pregnan-20-one was incubated with cecal bacteria of rat (9) and intestinal flora of man (10). In both cases, the major metabolite was 3β-hydroxy-5α,17α-pregnan-20-one. Thus, it follows that 16α-hydroxyprogesterone is the precursor for urinary 17α-pregnanolone, and that intestinal bacteria are responsible for the molecular alterations.

With a view eventually to isolating and identifying the organisms synthesizing the specific enzymes, we investigated the metabolism of 16α-hydroxyprogesterone by fecal flora of human and rat; the results are reported here.

EXPERIMENTAL PROCEDURES

Media

Dehydrated brain-heart infusion (BHI) broth and Mueller Hinton (MH) broth (Difco Laboratories,

Abbreviations: 16α-hydroxyprogesterone, 16α-hydroxy-4-pregnene-3,20-dione; 16α-hydroxypregnanolone, 3α,16α-dihydroxy-5β-pregnan-20-one; 17α-pregnanolone, 3α-hydroxy-5β,17α-pregnan-20-one; pregnanolone, 3α-hydroxy-5β-pregnan-20-one; TMS, trimethylsilyl; MO-TMS, O-methyloxime trimethylsilyl; TLC, thin-layer chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry; PC, partition chromatography; HPLC, high pressure liquid chromatography. Pregnan or 17β-pregnan refers to a pregnane with a side chain in the 17β-position. 17α-pregnan refers to a pregnane with the side chain in the 17α-position.

[1] Reprint requests should be addressed to Dr. V. D. Bokkenheuser, Department of Pathology, St. Luke's-Roosevelt Hospital Center, Amsterdam Avenue at 114th Street, New York, NY 10025.

[2] V. D. Bokkenheuser and J. Winter.

[3] P. B. Hylemon.

[4] N. K. N. Ayengar and E. H. Mosbach.

Inc., Detroit, MI) were reconstituted, sterilized by autoclaving at 121°C for 20 min, and stored at 4°C for no longer than 48 hr before use.

BHIC broth. Dehydrated BHI broth (37 g), 0.5 g of cysteine hydrochloride, and 1 g of $NaHCO_3$ were dissolved in 1 liter of distilled water, distributed in 50-ml amounts in screwcapped vials, and sterilized as above.

MHC broth. Dehydrated MH broth (21 g), 0.5 g of cysteine hydrochloride, and 1 g of $NaHCO_3$ were dissolved, distributed, and sterilized as above.

PR broth. Prereduced BHI (45 ml/vial) was purchased from Scott Laboratories, Inc., Fiskeville, RI.

SPB broth. Supplemented peptone broth, 18 ml/tube, was purchased from Becton, Dickinson & Co., Rutherford, NJ.

Eh and pH

Eh and pH were measured on a Beckman Zeromatic II instrument (Beckman Instruments, Inc., Fullerton, CA). Eh measurements were made with a platinum electrode. Readings, checked regularly with quinhydrone-saturated buffers at pH 4 and 7, invariably were within 10 mV of the theoretical values.

Source and preparation of microorganisms

Human. Fecal samples from healthy adults on a "Western diet" were collected in stool cups under ordinary atmospheric conditions; processing began within 30 min after defecation (11). Serial 10-fold dilutions of the specimens were prepared in SPB broth.

Rat. Wistar rats fed Wayne Lab-Blox (Chicago, IL) were used in these experiments. Fecal samples were collected and processed as described above. Rats, excreting swarming proteus in the stools, were rejected, since these organisms might interfere with the isolation of 16α-dehydroxylating bacteria. To keep the animals free of proteus, they received autoclaved drinking water in sterile bottles.

Pure cultures. The neotype strain of *Eubacterium lentum* (VPI 0255) was kindly supplied by Dr. L. V. Holdeman, Virginia Polytechnic Institute, Blacksburg, VA. Strain 116, an organism phenotypically similar to *E. lentum* was isolated in our laboratories (12, 13). *Escherichia coli* (14, 18) and *Clostridium paraputrificum* (14) were isolates from human fecal flora, and *Bacteroides ruminicola*, an organism with an efficient Δ^4-3-keto reductase was recently isolated in our laboratories from the fecal flora of rats.

Solvents. Solvents for thin-layer chromatography (TLC) and partition chromatography (PC) were reagent grade except for methanol, which was technical grade. Solvents for high pressure liquid chromatography (HPLC) with a cut-off point below 195 nm were purchased from J. T. Baker, Chemical Co., Phillipsburg, NJ.

Separation of steroids

Thin-layer chromatography. A 5-ml aliquot of incubated culture was extracted with 5 ml of methylene chloride for 30–60 sec. The organic phase was separated, dried over sodium sulfate, and evaporated under nitrogen gas at 40–50°C. The residue was redissolved in 50 μl of acetone and spotted on Bakerflex silica gel (1B 2F, J. T. Baker). Three solvent systems were employed; A, benzene–acetone 50:50 (v/v); B, chloroform–acetone 95:5 (v/v); and C, isooctane–ethyl acetate–acetic acid 5:25:0.2 (v/v/v). The steroid spots were located either under ultraviolet light (254 nm) or by spraying with a mixture of sulfuric acid–ethanol 1:1 (vol/vol) as previously described (11).

Partition chromatography. Tritium-labeled steroids were chromatographed on a celite partition column, and quantitated and purified as previously described (11).

High pressure liquid chromatography. To prepare the samples for HPLC, culture extracts were subjected to preliminary purification by TLC. The silica gel corresponding to the steroid spot was transferred to a vial and extracted with 5 ml of acetonitrile. After filtration and evaporation under a stream of nitrogen gas, the residue was redissolved in 0.5 ml of acetonitrile and passed through prefilter AP and Millipore filter FH, 0.5 μ (Millipore, Bedford, MA). An aliquot of the ensuing filtrate (1–10 μl) was injected into a Varian model 5000 HPL chromatograph (Varian, Palo Alto, CA) using a Micropak MCH-10 column preceded by a guard column packed with Vydac Reverse Phase (Varian). The steroids were eluted at room temperature with acetonitrile–water 70:30 under a pressure of 110 atm, and a flow rate of 2 ml/min. A variable wave-length ultraviolet detector set at 195 nm was used for the detection of ring-A reduced structures and at 254 nm for the Δ^4-3-keto steroids. The instrument was operated at 0.1 AUFS. It should be noted that the prefilter used in the purification process gave off a component with a retention time of 4.2 min.

Gas–liquid chromatography–mass spectrometry was carried out on a Hewlett-Packard 5992 B instrument (Hewlett-Packard Co., Avondale, PA) equipped with a jet separator. The conditions for the analysis were as follows: injection temperature, 250°C; oven temperature, 234°C; source electron impact, 70 electron volts; column packed with 2% OV-210 on 100/120 gas chrom Q (Hewlett-Packard) or 1% SE-30; source pressure 1×10^{-6} torr.

Preparation of derivatives. Oximes and silyl derivatives of the steroids were performed as previously described (15).

Steroids

16α-hydroxyprogesterone, progesterone, and isomers of pregnanolone were purchased from Steraloids, Wilton, NH and Makor Chemicals Ltd., Jerusalem; 17α-pregnanolone was kindly donated by Dr. D. K. Fukushima, Montefiore Hospital, NY.

The following steroids, not commercially available, were prepared in our laboratories:

16α-Hydroxypregnanolone. Ninety ml of PR broth supplemented with 16α-hydroxyprogesterone (20 μg/ml) was seeded with 0.25 ml of a 24 hr culture of *C. paraputrificum* (14). The culture was incubated at 37°C for 7 days, precipitated with 5 vol of methanol while stirring, left 24 hr at room temperature, filtered, and the methanol was evaporated in vacuo leaving an aqueous residue. The residue was diluted to 100 ml with distilled water and extracted three times with 50 ml of methylene dichloride. The combined extract was dried over sodium sulfate, filtered, evaporated under vacuum, and purified by TLC using solvent system A. Retention time on HPLC and the mass spectrum were determined.

17α-Pregnanolone. Five mg of 17β-pregnanolone was dissolved in 10 ml of ethanol to which three drops of 20% NaOH were added (9). The mixture was refluxed for 4 hr and then cooled to room temperature. The steroids were extracted with 40 ml of ethyl acetate. The solution was washed with distilled water until the pH was about 6.0, and then evaporated. 17α-pregnanolone was separated from its isomer by TLC using solvent system B. The retention time on HPLC was identical to that of an authentic sample and the GLC–MS peaks and their relative intensities matched the spectrum published by Eriksson, Gustafsson, and Sjövall (9).

17α-Progesterone was prepared from 17β-progesterone as described above for 17α-pregnanolone. The GLC–MS pattern was typical for 17α-progesterone as published by Hammerum and Djerassi (16).

Labeled 16α-hydroxyprogesterone. The labeled compound was tritiated by Wilzbach's method (17) in the laboratories of New England Nuclear Corp., Boston, MA. The crude sample was diluted with 10 mg of unlabeled 16α-hydroxyprogesterone. Purification was achieved by repeated chromatography on 500 μm silica gel G plates using solvent system B. The bands were detected by brief exposure to iodine vapor. The ensuing steroid was at least 98% pure as measured by TLC and radioassay of the labeled compound.

TABLE 1. Properties of steroid metabolites formed by incubation of 16α-hydroxyprogesterone with human fecal flora

Characteristics	Steroid Metabolites		
	Human		Rat
	#1[a]	#2[b]	#3[c]
U.V. absorption at 254 nm, solvent system B	Neg	Neg	Neg
R_f on TLC, solvent system B	0.25		
R_f on TLC, solvent system C	0.43	0.15	0.51
R_f on TLC, solvent system A		0.39	
HPLC, Rt in min	3.1	1.7	3.1
GLC (column 1% SE-30) Rt			
Underivatized[d]	0.52		
TMS derivative[e]	0.54		0.52
MO-TMS derivative[f]		1.21	

[a] 17α-Pregnanolone (3α-hydroxy-5β,17α-pregnan-20-one).
[b] 16α-Hydroxypregnanolone.
[c] 3α-Hydroxy-5α,17α-pregnan-20-one.
[d] Retention time in min; conditions listed in Methods section.
[e] Trimethylsilyl derivative.
[f] O-Methyloxime trimethylsilyl derivative.

Conversion experiments

One mg of the appropriate steroid, dissolved in 0.25 ml of methanol, was added to 50 ml of culture medium to give a concentration of 20 μg steroid/ml. The medium was then seeded with 0.25 ml of fecal suspension decimally diluted in SPB broth or with 0.25 ml of a pure, young bacterial culture. Nonprereduced media were supplemented with 0.1 ml of a 24-hr culture of *E. coli* to ensure a low Eh (12, 18). After 7 days incubation at 37°C, if not otherwise stated, Eh and pH were determined and the steroids extracted, separated, and identified.

RESULTS

Metabolism of 16α-hydroxyprogesterone

Incubation with human fecal flora. 16α-Hydroxyprogesterone incubated with a 10^3 fecal dilution in BHIC broth yielded two metabolites. The properties of the products are listed in **Table 1.** Metabolite 1 was reduced in ring-A, and gave an R_f value on TLC (solvent system C) and a retention time on GLC identical with those of pregnanolone. However, by TLC with solvent system B and by HPLC, metabolite 1 could be separated from pregnanolone and behaved like 17α-pregnanolone (**Fig. 1**). Mass spectrum of the TMS derivative (**Fig. 2**) displayed a M^+ of 390 amu. The fragmentation pattern and relative intensities of the fragments were similar to those of 17α-pregnanolone. The base peak of metabolite 1 was m/e 230 which is characteristic of the 17α-isomer while a base peak of m/e 300 is typical for the 17β-isomer (9).

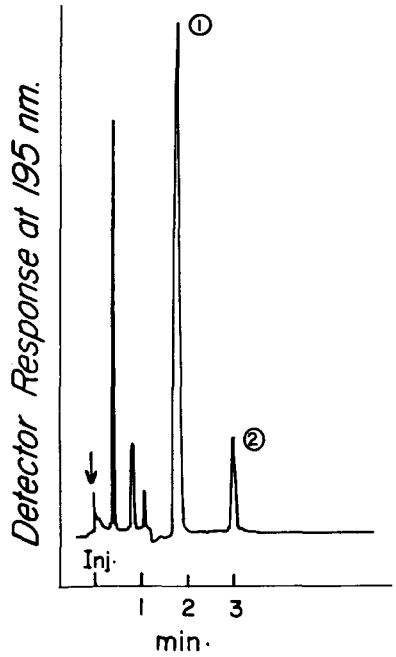

Fig. 1. Separation of 17α- and 17β-pregnanolones by HPLC. Column, Micro Pak MCH-10, 25 cm × 2 mm; solvent, acetonitrile–water 70:30; flow, 2 ml/min; detection, UV at 195 nm; AUFS, 1.0; peak #1, 17β-pregnanolone; peak #2, 17α-pregnanolone.

Metabolite 2 was clearly different from metabolite 1 (Table 1). In particular, metabolite 2 decomposed (dehydrated) during GLC. To obtain a satisfactory chromatogram, the OH-groups and the keto function were protected by derivatization. The properties of metabolite 2 matched those of 16α-hydroxypregnanolone, a steroid also obtained by the biological reduction of 16α-hydroxyprogesterone with *C. paraputrificum*. The mass spectra of the derivatives (MO-TMS) of metabolite 2 and 16α-hydroxypregnanolone revealed identical molecular ions (M^+, 507), base peak (m/e 476), and identical fragmentation patterns. The fragment at 188 is noteworthy since it represents the side chain and a part of ring D, demonstrating that the metabolite had retained the OH-group at C-16 (19).

Incubation with rat fecal flora. Metabolism of 16α-hydroxyprogesterone with rat fecal material yielded a major compound, metabolite 3, which did not absorb at 254 nm. It exhibited an R_f in solvent system C of 0.51 (different from that of metabolite 1) but with the same retention time on HPLC. The mass spectrum of the TMS derivative of metabolite 3 (**Fig. 3**) showed the same molecular ion and fragmentation pattern as metabolite 1 but the relative intensities of the fragments differed. They matched those obtained by Eriksson, et al. (9) for 3α-hydroxy-5α,17α-pregnan-20-one. Thus, rat fecal flora 16α-dehydroxylated the substrate, changed the configuration of the side chain from 17β- to the 17α-position, and reduced ring-A of the substrate to a 5α-compound (human fecal flora yielded the 5β-product).

Prevalence of bacteria synthesizing 16α-dehydroxylase in feces of human and rat

To determine the prevalence of converting organisms, fecal flora of humans and rats were decimally diluted in SPB. BHIC, supplemented with 20 μg of 16α-hydroxyprogesterone per ml, was seeded with

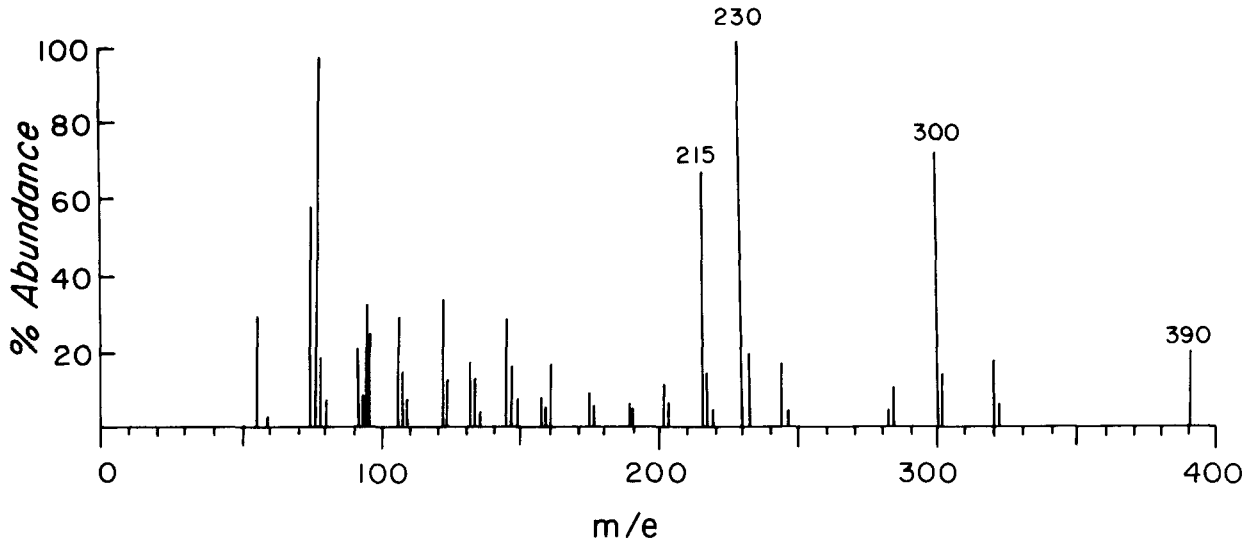

Fig. 2. Mass spectrum of metabolite #1; TMS derivative of 17α-pregnanolone. Note the base peak at m/e 230 characteristic of 17α-isomer (17α-pregnanolone).

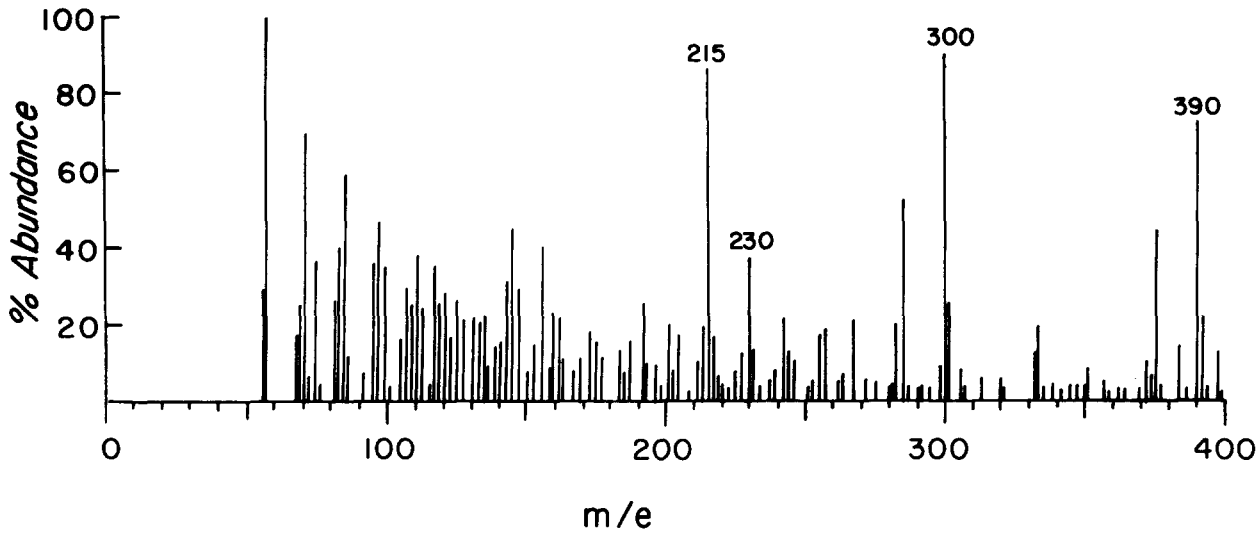

Fig. 3. Mass spectrum of metabolite #3; TMS derivative of 3α-hydroxy-5α,17α-pregnan-20-one. Note the base peak at m/e 300.

0.25 ml diluted fecal suspension; 0.1 ml of a 24-hr culture of *E. coli* was added to all cultures originating from rats and to human cultures diluted 10^6 or more. The data in **Table 2** show that the prevalence of 16α-dehydroxylating organisms varied considerably in the three normal humans examined while it was constant in the excreta of rats.

The levels of 16α-dehydroxylating organisms in lyophilized human excreta was 10^2 and in cecal contents of rat, 10^1 per gram.

Media supporting 16α-dehydroxylase activity

With fecal material from humans or rats, BHIC (brain heart infusion broth supplemented with cysteine) was the superior conversion medium (**Table 3**); PR was a close second. MHC did not support good growth of converting organisms.

Preliminary conclusions

The data indicated that rat feces would be the best source of material for the eventual isolation of organisms synthesizing 16α-dehydroxylase, and that BHIC was the preferred conversion medium. Hence, the experiments described below were performed exclusively with rat fecal flora incubated in BHIC.

Duration of incubation. The complete dehydroxylation of 16α-hydroxyprogesterone required an incubation period of at least 4 days (**Table 4**). Moreover, with highly diluted specimens, conversion continued for a long time after the cultures had reached the stationary phase.

Incubation temperature. Parallel cultures of rat fecal flora, diluted 10^3, dehydroxylated 16α-hydroxyprogesterone at 37°C, but not at 28°C or at 42°C.

Effect of pH. Buffered BHIC supported maximum 16α-dehydroxylation at pH 6.5–7.5. Conversion was suppressed at pH 5.5 and 8.4. In these experiments 0.2 M buffers were needed to neutralize the acids produced by *E. coli* and allied organisms during the growth period.

Substrate concentration. The inhibitory effect of high concentrations of steroids on the specific enzymatic activity has been noted several times (11, 20). Accordingly, we examined the conversion in the presence of increased concentrations of 16α-hydroxyprogesterone. 16α-Dehydroxylation in cultures seeded with rat feces was quantitative at substrate concentrations up to 80 μg/ml, and partial at concentrations of 160 μg/ml or more.

Yield of metabolites. To quantitate the yield of metabolites and detect minor products, if any, 10^3 to 10^7 dilutions of rat fecal flora were seeded in BHIC supplemented with 0.1 ml *E. coli* culture and tritiated 16α-hydroxy-progesterone, 20 μg/ml, 10^6

TABLE 2. Dehydroxylation of 16α-hydroxyprogesterone by progressively diluted fecal flora[a]

Log 10 fecal dilutions	Humans			Rats		
	#1	#2	#3	#1	#2	#3
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	–	–	+	+	+	+
5	–	–	+	+	+	+
6	–	–	+	–	–	–
7	–	–	–	ND	ND	ND[b]

[a] Media, BHIC; substrate, 16α-hydroxyprogesterone, 20μg/ml; inoculum, 0.25 ml fecal suspension; incubation, 7 days; humans 1–3, different individuals; rats 1–3, different rats; +, 16α-dehydroxylase activity; –, no 16α-dehydroxylase activity.
[b] Not determined.

TABLE 3. Media supporting 16α-dehydroxylase activity

Media	Human	Rat	Eh mV
	Log 10 Highest Converting Dilution[a]		
BHIC	3	5	−260 ± 30
PR	2	5	−200 ± 20
MHC	1	1	−220 ± 30

[a] Metabolite 17α-pregnanolone.
Experimental conditions see legend for Table 2.

counts per culture. Partition chromatography revealed that the substrate in the 10^3 dilution of fecal material had been metabolized to two compounds: an unidentified steroid product constituting about 20% of the radioactivity and a major product (60%) that was identified as 3α-hydroxy-5α,17α-pregnan-20-one (metabolite 3). The latter metabolite was the sole steroid structure present in cultures of 10^4 to 10^6 fecal dilutions.

Pure culture. None of the following stock cultures showed 16α-dehydroxylase activity: strain 116, synthesizing a 21-dehydroxylase (12); *E. lentum* neotype, synthesizing a 21-dehydroxylase and a 3α-hydroxy steroid dehydrogenase (6); *Cl. paraputrificum*, synthesizing a Δ^4-3-keto reductase (15, 18, 21); *B. ruminicola* which, during the course of these observations, was shown to reduce the Δ^4-3-keto structure; and *E. coli* which was used in many experiments to bioreduce the culture media.

DISCUSSION

Sequential formation of metabolites

Incubation of 16α-hydroxyprogesterone with human fecal flora resulted in the formation of two ring-A reduced structures (**Fig. 4**), one with an OH group at C16 (metabolite 2) and one without (metabolite 1). In the normal sequence of events, it is likely that the Δ^4-3CO reductase from *C. paraputrificum* first transforms the substrate to metabolite 2 (12). By analogy

TABLE 4. Effect of incubation period on 16α-dehydroxylation by rat fecal flora

Days of Incubation	Max. Converting Dilution (log 10)	Conversion[a] %
2	None	None
3	5	50
4–21	5	100
28	7	100

[a] 3α-Hydroxy-5α,17α-pregnan-20-one (metabolite 3).
Experimental conditions: see legend to Table 2.

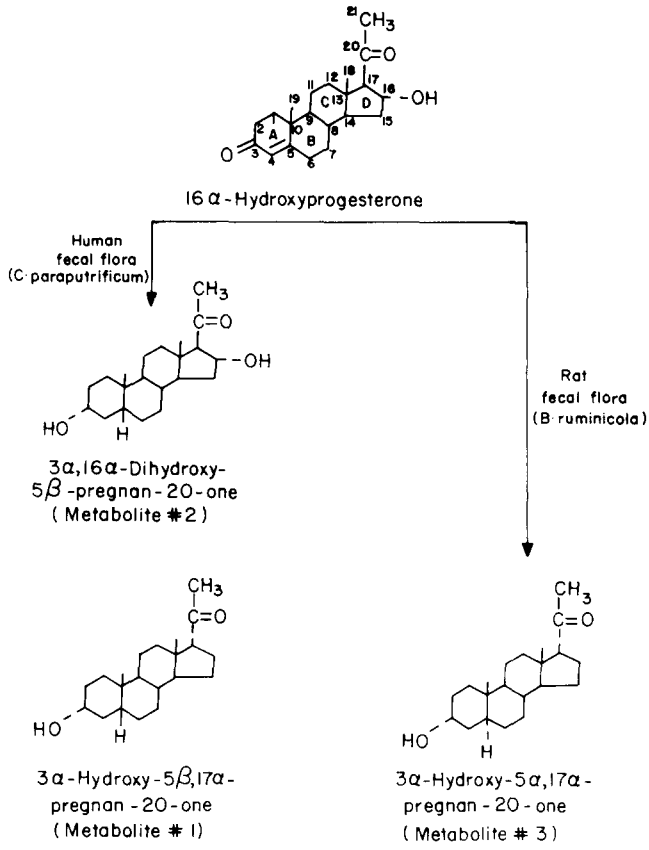

Fig. 4. Metabolism of 16α-hydroxyprogesterone by fecal flora from human and rat.

with the 21-dehydroxylation (14), this structure in turn is converted to metabolite 1 by the slower 16α-dehydroxylase. However, it is quite possible that the order of conversion can be reversed. Our failure to detect 17α-progesterone could be due to the high velocity of the ring-A reduction.

Only one product, metabolite 3, was observed in experiments with rat feces. It may be assumed that the 16α-dehydroxylating organisms from rats synthesize a more potent enzyme than do their human counterparts.

Origin of 16α-dehydroxylase

Our findings, together with those of Eriksson et al. (9) and Eriksson and Gustafsson (10), provide strong evidence that the urinary 17α-pregnanolones in man and in the rat are bacterially mediated metabolic products of 16α-hydroxyprogesterone. The observations that 3α,16α-dihydroxy-5α-pregnan-20-one in the germ-free rat is the predominant fecal $C_{21}O_3$ steroid, and that the corresponding 16α-dehydroxylated compound is lacking (9, 19) suggest that 16α-dehydroxylase is exclusively of bacterial origin. The inability of mammalian tissue to synthesize 16α-

dehydroxylase is paralleled by its failure to make 21-dehydroxylase (20) and may well reflect a necessity on the part of the host to protect the integrity of the specific OH functional groups.

Mechanism of 16α-dehydroxylation

Mammalian tissues synthesize C-21 steroids, including 16α-hydroxyprogesterone with the side chain in the 17β-position. During 16α-dehydroxylation the position of the side chain is changed to the α-configuration. In contrast, 21-dehydroxylation does not affect the configuration of the side chain (12, 20, 22). Thus, the stereochemistry of the 17-side chain of urinary pregnanolones can indicate their biological origin.

Calvin and Lieberman (1) suggested that the conversion of 16α-hydroxyprogesterone to 17α-pregnanolone takes place via the intermediate C21-Δ^{16}-steroid. The latter steroid is metabolized in vivo to 17α-pregnanolone (1). Moreover, it is present in the feces of conventional rats, but not in germ-free rats (9). We did not observe the C21-Δ^{16}-steroid in our cultures, presumably because it was rapidly reduced. Alternatively, the C21-Δ^{16}-steroid never leaves the enzyme surface or can only exist as an enzyme complex because of its inherent instability. Clarification of this point must await kinetic experiments with pure cultures of strains synthesizing 16α-dehydroxylase.

17α-Pregnanolone recovered from substrate incubated with human fecal flora has the 5-hydrogen in the β-position. In identical experiments with rat fecal flora, the 5-hydrogen was in the α-position. Thus the Δ^4-reductase found in the human intestinal flora appears to have a different specificity than that found in rat flora.

Nature of the converting organisms

The present experiments with the 16α-dehydroxylating bacteria in fecal material did not yield information as to the possible identity of the converting organisms. The narrow temperature range of conversion suggests that it is an obligate mammalian parasite. The low Eh required for 16α-dehydroxylation, together with the reductive nature of the reaction, is indicative of anaerobic organisms. If that is correct, it is also predictable that the organisms are quite resistant to molecular oxygen. For example, samples containing converting organisms could be manipulated under *aerobic* laboratory conditions, and *aerobic* media (BHIC) served well for conversion purposes provided they were supplemented with *E. coli*, which played the role of a bio-reducing agent.

It is difficult to predict whether 16α-dehydroxylase is an exoenzyme or an endoenzyme, but it is interesting that under certain conditions conversion continues for weeks after the culture has reached the stationary phase. In this respect, the enzyme behaves like the 21-dehydroxylase (11).

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