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Steroid Desmolase Synthesis by *Eubacterium desmolans* and *Clostridium cadavaris*

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The synthesis of a steroid desmolase was demonstrated in two obligate anaerobes: a new bacterial species, *Eubacterium desmolans*, isolated from cat fecal flora, and *Clostridium cadavaris*, recovered from sewage of New York City. The enzyme cleaves the C-17–C-20 bond of corticoids possessing hydroxyl functions at C-17 and C-21. The conversion is quantitative, provided the substrate concentration is less than 100 μg/ml and the organisms are in the log phase. The velocity of transformation parallels the bacterial growth curve and in the log phase is higher for *E. desmolans* than for *C. cadavaris*. In addition, both organisms synthesize a 20β-hydroxysteroid dehydrogenase.

Side-chain cleavage of 17α-hydroxysteroids is performed by a group of enzymes referred to as desmolases, which are synthesized by gonadal tissue (9), some free-living fungi (8), and the obligate anaerobe *Clostridium scindens* (12). The latter organism, present in the normal colonic flora of humans, converts cortisol to 11β-hydroxyandrostenedione. Cortisol, however, does not undergo enterohepatic circulation in humans (3, 4, 6) and is therefore not the natural substrate for the desmolase synthesized by *C. scindens* (1, 5). In cats, on the other hand, tetrahydrocortisol is a normal biliary steroid (10); therefore, cats could be one of the natural hosts for bacteria with steroid desmolase activity. In this paper we describe the isolation of two desmolase-synthesizing organisms, one from the fecal flora of a cat and one from New York City sewage. Except for the production of a rare enzyme, these two organisms have little in common and share few characteristics with *C. scindens*.

MATERIALS AND METHODS

Media. Supplemented peptone broth was purchased from Becton Dickinson and Co., Paramus, N.J.; chopped meat broth was from Carr-Scarborough Microbiological, Inc., Decatur, Ga.; and prereduced brain heart infusion was obtained from Scott Laboratories, Fiskeville, R.I. Brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) was supplemented with cysteine as previously described (12). Ready-made Columbia agar plates supplemented with 5% sheep blood were purchased from BBL.

Source of microorganisms. Fecal samples from a healthy female cat fed Purina cat food and table scraps were collected from the litter box and processed within 1 h of defecation. Samples of supernatant fluid of precipitated sewage from New York City were obtained from the Randall's Island plant.

Isolation of microorganisms. Desmolase-active organisms were recovered by the modified dilution technique (2). Samples of feces decimally diluted in supplemented peptone broth (0.1 ml) were seeded in brain heart infusion broth supplemented with cysteine and cortisol (20 μg/ml). *Escherichia coli* and other members of the family *Enterobacteriaceae* present in fecal flora created the anaerobic conditions (2). Media inoculated with dilutions higher than 10^7 were further supplemented with 0.1 ml of a 24-h-old culture of *E. coli* (2). The strain of *E. coli* used was known not to metabolize cortisol. However, desmolase activity was present only in cultures diluted up to 10^7-fold (7). After 7 days of incubation at 37°C, 5-ml samples were extracted and tested for desmolase activity by thin-layer chromatography (1, 12). The culture, representing the highest dilution with desmolase activity, was then decimally diluted in supplemented peptone broth; samples (0.1 ml) were seeded in brain heart infusion broth supplemented with cysteine and cortisol and spread on three sets of blood agar plates. After incubation of the liquid media the endpoint of desmolase activity was determined. The plates were incubated at 37°C in GasPak jars (BBL) for 2 to 3 days; the colonies were counted, and the growth was harvested in 2 ml of supplemented peptone broth and tested for desmolase activity. When plates with less than 100 colonies were positive, their sister plates from the second set of plates were selected for isolation of the organism synthesizing desmolase. When a positive plate had more than 100 colonies the above-described enrichment procedure was repeated one or more times until positive plates with less than 100 colonies were obtained. All isolates were tested for desmolase activity, and positive cultures were identified by conventional bacteriological methods (11).

Growth curves. Prereduced brain heart infusion (50 ml) was inoculated with 0.25 ml of an 18-h-old culture and incubated at 37°C. The increase in optical density was followed spectrophotometrically at 430 nm in a Gilford 250 instrument (Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

Steroids. Steroids used as substrates or reference compounds were purchased from Sigma Chemical Co., St. Louis, Mo.

Conversion experiments. Prereduced brain heart infusion (50 ml) supplemented with 20 to 300 μg of steroids per ml, depending on the type of experiments, was seeded with 0.25 ml of an 18-h-old culture of the organism to be investigated. The cultures were incubated at 37°C; steroid conversion and growth were checked at appropriate intervals (12).

Separation and identification of steroid metabolites. The compounds were separated and identified by thin-layer chromatography, gas-liquid chromatography, and gas-liquid chromatography–mass spectrometry as previously described (1, 12).

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TABLE 1. Characteristics of metabolites obtained from incubation of cortisol with cat fecal flora (A through D) and *E. desmolans* (E and F)

Metabolite	Structure[a]	R_f[b]	RRT[c]
5β-Androstan-3α,11β-diol-17-one	A	0.50	0.94
5α-Androstan-3β,11β-diol-17-one	B	0.53	1.04
5α-Androstan-11β-ol-3,17-dione	C	0.63	ND
5β-Androstan-3α,11β,17β-triol	D	0.23	0.89
20β-Dihydrocortisol	E	0.69	2.11
11β-Hydroxyandrostenedione	F	0.60	1.14

[a] See letter designations in Fig. 1 and 2.
[b] Determined by thin-layer chromatography on Bakerflex silica gel (I B2F; J. T. Baker Chemical Co., Phillipsburg, N.J.). The solvent system was isooctane-ethylacetate-acetic acid (4:25:0.2, vol/vol/vol).
[c] RRT, Retention time relative to 5α-cholestane. Gas-liquid chromatography was performed on a Hewlett-Packard 5880A instrument with a flame ionization detector under the following conditions: fused silica capillary column, OV 101; gas carrier, helium; oven temperature, 180 to 230°C with an increase of 3°C/min. Steroids were derivatized as methoxime-trimethylsylil and purified on a Lipidex column (3). ND, Not determined.

RESULTS

Isolation and identification of a desmolase-synthesizing organism from cat feces. Two liquid enrichment procedures were required to obtain desmolase activity in suspensions harvested from plates with less than 100 colonies. Chromatographic characteristics of the metabolites of these suspensions are shown in Table 1 and Fig. 1. Isolation of the colonies from one of the duplicate plates yielded two identical, obligately anaerobic, desmolytic cultures. Morphological examination and fermentation products indicated that the organism belonged to the genus *Eubacterium*. It has few biochemical reactions; inositol is the only carbohydrate fermented, and indole is produced. Thus it has characteristics unlike those of other described species in the genus. The name *Eubacterium desmolans* was proposed for this new species (7).

Isolation and identification of a desmolase-synthesizing organism from sewage. The metabolic products obtained after incubation of sewage in conversion medium showed that organisms with desmolytic activity were present. After three consecutive liquid enrichment procedures, blood agar plates with suitable numbers of colonies for isolation were obtained. Eight strains identified as *Clostridium cadaveris* all synthesized steroid desmolase.

Steroid metabolism by *E. desmolans*. *E. desmolans* synthesized two enzymes with activity on the steroid molecule: a C-17–C-20 desmolase and a 20β-hydroxysteroid dehydrogenase (20β-HSDH) (Fig. 2).

(i) The desmolase cleaved the side chain of cortisol, 11β-deoxycortisol, cortisone, tetrahydrocortisol, 20β-dihydrocortisol, and 20β-cortol but not the side chain of 21-deoxycortisone, 20α-cortol, 17α-hydroxyprogesterone, 17α-hydroxypregnenolone, and 17-deoxycorticoids and did not alter bile acids. The cleavage of cortisol to 11β-hydroxyandrostenedione was quantitative in media containing up to 100 μg of substrate per ml. In cultures with higher substrate concentrations, desmolase activity reached a plateau, and 20β-dihydrocortisol appeared in increasing amounts up to a maximum of 100 to 125 μg/ml regardless of the duration of the incubation beyond 24 h. Kinetic experiments were performed with cortisol at a concentration of 100 μg/ml to avoid interference from 20β-HSDH. Side-chain cleavage occurs during the log phase of bacterial growth and is essentially completed after an incubation period of about 16 h (Fig. 3). Moreover, small amounts of 20β-dihydrocortisol formed in the early phases of the incubation were quickly converted by the desmolase to 11β-hydroxyandrostenedione.

(ii) 20β-HSDH, as noted above, may be demonstrated in kinetic experiments but is far more obvious in conversion experiments with high concentrations of cortisol. The enzyme has the same substrate specificity for 17α-hydroxysteroids as desmolase, but in contrast to the latter enzyme it can also use 17α-hydroxyprogesterone and certain 17-deoxycorticoids as substrates, e.g., deoxycorticosterone and 11-dehydrocorticosterone. It does not metabolize closely related structures such as corticosterone, progesterone, and aldosterone.

(iii) Both steroidal enzymes of *E. desmolans* are constitutive, as evidenced by their undiminished synthesis after

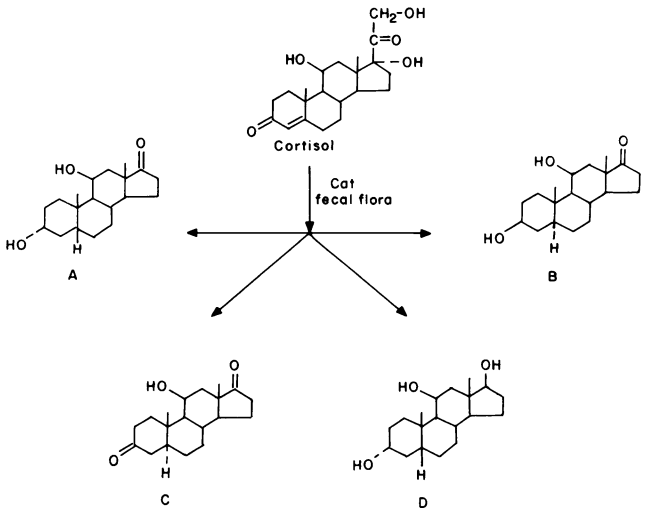

FIG. 1. Bacterial metabolites of cortisol in cat fecal flora. A, 5β-Androstan-3α,11β-diol-17-one (11β-hydroxyetiocholanolone); B, 5α-androstan-3β,11β-diol-17-one; C, 5α-androstan-11β-ol-3,17-dione; D, 5β-androstan-3α,11β, 17β-triol.

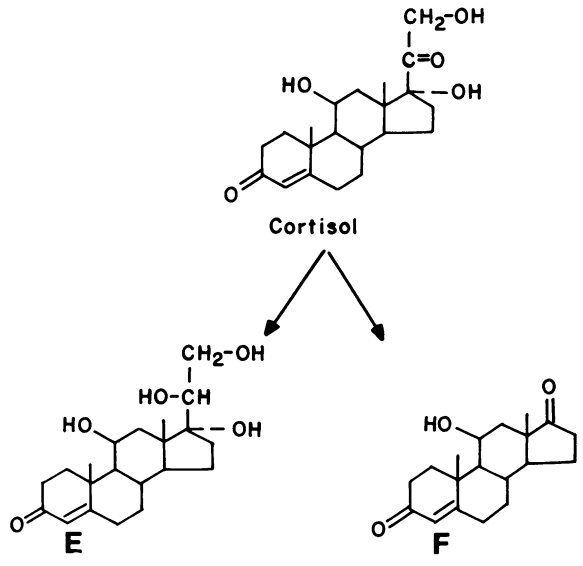

FIG. 2. Metabolism of cortisol by *E. desmolans* and *C. cadaveris*. E, 20β-Dihydrocortisol; F, 11β-hydroxyandrostene-dione.

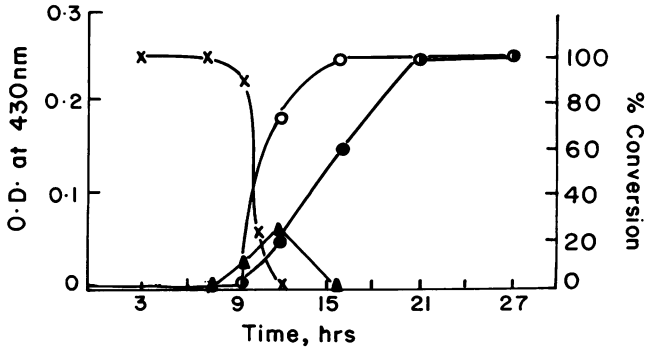

FIG. 3. Relationship between growth and enzymatic activity of *E. desmolans*. Symbols: ×, unconverted cortisol; ○, 11β-hydroxyandrostenedione; ▲, 20-β-dihydrocortisol; ●, growth curve. The cortisol concentration was 100 μg/ml. O.D., Optical density.

weekly transfer of the organism for 6 months in corticoid-free media.

Steroid metabolism by *C. cadavaris*. The desmolases of *C. cadavaris* and of *E. desmolans* differ only on a few minor points. First, the conversion begins earlier in the *C. cadavaris* culture (Fig. 4) but parallels the growth of the organism, which proceeds along a steeper slope. Second, 20β-dihydrocortisol continues to accumulate until the ratio of this component to 11β-hydroxyandrostenedione is 2:1. The ratio declines with time; i.e., more and more of the 20β-dihydrocortisol undergoes side-chain cleavage, until at the end of 1 week about 93% of the substrate has been converted to androgen (Table 2). The steroidal enzymes synthesized by *C. cadavaris* are also constitutive.

DISCUSSION

Taxonomy. The organism recovered from cat feces is evidently a new organism for which the name *Eubacterium desmolans* has been proposed to the International Committee on Systematic Bacteriology (7).

Desmolase-synthesizing organisms of intestinal origin. Together with the previously described *C. scindens* (1, 12) from human feces, we now know of two fecal organisms and one sewage organism capable of synthesizing steroid desmolase. The working hypothesis that the cat would turn out to be the natural host for *C. scindens* because cortisol in this animal undergoes enterohepatic circulation was not supported by our findings. Furthermore, it was a surprise that the most common desmolase-synthesizing organism in sewage from New York City was unrelated to its most common counterpart in the human gut. On the other hand, since *C. cadavaris* is a normal inhabitant of the intestinal flora of humans and rodents, it may indicate that humans harbor more than one desmolase-synthesizing organism, or it may reflect the contribution of rodents to the metropolitan sewage system.

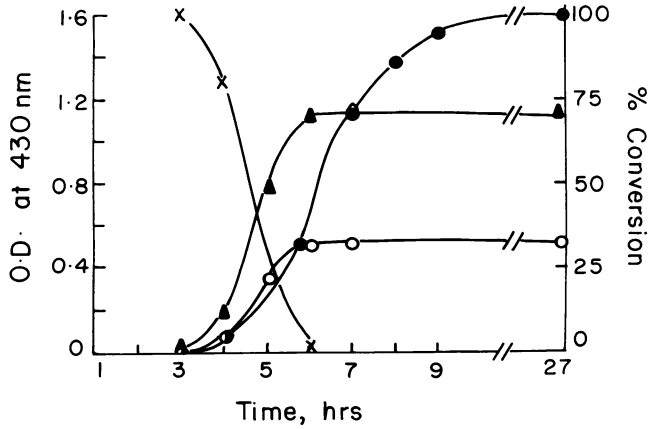

FIG. 4. Relationship between growth and enzymatic activity of *C. cadavaris*. Symbols and substrate concentration are as in Fig. 3. For conversion days 2 through 7, see Table 2.

TABLE 2. Metabolism of cortisol by *E. desmolans*[a]

Cortisol concn (μg/ml)	11β-Hydroxyandrostenedione (μg/ml) on day:			
	1	3	7	14
20	15	17	18	18 to 19
50	20	35	45	45
100	40	45	80	80
200	80	100	180	180
300	100	140	280	280

[a] The balance of cortisol which did not undergo side-chain cleavage was converted to 20β-dihydrocortisol.

Substrate requirements for desmolase. Desmolases synthesized by *E. desmolans*, *C. cadavaris*, and *C. scindens* (1, 12) require hydroxy groups at C-17 and C-21 and a keto or hydroxy group at C-20. Interestingly, *E. desmolans* and *C. cadavaris*, synthesizing a 20β-HSDH, can use a 20β-hydroxylated steroid but not the 20α epimer as a substrate. The reverse is true for *C. scindens*, which synthesizes a 20α-HSDH.

Although the side chains of 20α- and 20β-hydroxysteroids serve as substrates for their respective bacteria, we do not know whether these products are intermediary metabolites between substrates and 11β-hydroxyandrostenedione.

Substrate requirements for 20β-HSDH. 20β-HSDH has a less restricted substrate specificity than desmolase. In general, hydroxy groups at C-21 and C-17 are beneficial, but they are not mandatory, since the enzyme reduces 17α-hydroxyprogesterone (a 21-deoxy compound), deoxycorticosterone, and 11-dehydrocorticosterone (both 17-deoxy products).

Purpose of steroidal enzymes. The most likely explanation for the bacterial synthesis of these constitutive enzymes seems to be that they are designed for a purpose other than the metabolism of substrates used in our experiments. The organisms do not depend on these substrates for growth and in certain cases will even have to expend energy to accomplish the conversion. Moreover, there is no evidence that the metabolites are used by the bacteria.

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