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Effects of Estradiol and Progesterone on *Bacteroides melaninogenicus* and *Bacteroides gingivalis*

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Bacteroides melaninogenicus subsp. *intermedius* increases in the subgingival microflora during pregnancy. These studies evaluated direct interactions between hormonal steroids and oral *Bacteroides* species. Resting cell suspensions of pure cultures of plaque organisms were incubated anaerobically with [^{14}C]estradiol and [^{14}C]progesterone. Uptake of labeled compound per microgram of bacterial protein was determined by thin-layer chromatography and liquid scintillation counting. *B. melaninogenicus* subsp. *intermedius* and *B. melaninogenicus* subsp. *melaninogenicus* took up 2.6×10^{-4} to 5.4×10^{-4} μmol of estradiol or progesterone per μg of cell protein. Minimal steroid uptake was observed with *B. gingivalis* and five other organisms. Uptake of steroids by *B. melaninogenicus* subsp. *intermedius* was temperature dependent and resulted in a labeled product as detected on thin-layer chromatography. Growth curves indicated that *intermedius* and *melaninogenicus* subspecies of *B. melaninogenicus* but not *B. gingivalis* could substitute progesterone or estradiol for vitamin K, an essential growth factor. Growth of *B. melaninogenicus* subsp. *intermedius* in steroids was concentration dependent. Addition of fumarate to resting cells of *B. melaninogenicus* subspecies as well as *B. gingivalis* increased steroid uptake by 70 to 500% and resulted in the gas-liquid chromatographic detection of succinate. Cultures given fumarate alone or steroids alone produced no succinate. Steroids appeared to directly interact with the fumarate reductase system and foster the growth of *B. melaninogenicus* subsp. *intermedius*. This interaction may be of ecological significance.

In studies of the plaque flora associated with periodontal disease, the proportions of certain bacterial species are greater than the proportions observed in gingival health (19, 26–28, 32, 35). If periodontal disease involves increases in specific components of the indigenous flora (17), the determination of the factors which allow or initiate a shift in this microflora is essential to an understanding of the etiology of the disease.

The subgingival bacterial flora of pregnant individuals changes as pregnancy progresses (13). In the second trimester, a significant increase was observed in the ratio of anaerobic to facultative bacteria and the proportional levels of *Bacteroides melaninogenicus* subsp. *intermedius*. In addition, uptake of [^{14}C]estradiol and [^{14}C]progesterone by dental plaque samples increased significantly during pregnancy and paralleled the plaque proportions of *B. melaninogenicus* subsp. *intermedius*. This suggests that endogenous steroids may influence the microbial ecology of the gingival sulcus.

In this communication, we report the identification of a role for steroid utilization in the metabolism of *B. melaninogenicus* subsp. *intermedius* and *B. asaccharolyticus* (*B. gingivalis* sp. nov. 6).

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MATERIALS AND METHODS

Microbiological methods. Pure cultures of *Actinomyces naeslundii*, *A. viscosus*, *B. gingivalis*, *B. melaninogenicus* subsp. *intermedius*, *Capnocytophaga ochraceus*, *Fusobacterium nucleatum*, and *Streptococcus sanguis* were isolated from subgingival plaque samples from a bacteriological study of pregnancy gingivitis. *B. melaninogenicus* subsp. *melaninogenicus* ATCC strain 25845 was obtained from the American Type Culture Collection. Each organism was grown anaerobically to exponential phase in basal anaerobic broth (BAB; 30) with menadione (0.5 μg/ml, 2.9 μM), centrifuged at 16,000 × *g* for 15 min, and washed two times in reduced transport fluid (RTF; 18) without EDTA. Washed cell concentrates were assayed for protein (fluorescamine; 34) and diluted with RTF to 0.5 to 1.5 μg of protein per 50 μl.

[^{14}C]steroid uptake. Fifty-microliter portions of the cell suspension were dispersed ultrasonically (Kontes model K88144, 5 s) and incubated anaerobically with radioactive estradiol and progesterone to evaluate uptake of these substrates by bacterial cell suspen-

sions. All steroid uptake assays were run three times, each in duplicate.

Radioactive estradiol and progesterone were obtained from Amersham/Searle as follows: [4-^{14}C]estradiol, specific activity of 56.7 mCi/mmol in benzene/ethanol; [4-^{14}C]progesterone, specific activity of 58.8 M mCi/mmol in benzene. Both steroids were taken to dryness in a stream of nitrogen and diluted in ethanol (absolute) to a concentration of 1.82×10^{-3} μmol/50 μl for estradiol and 3.40×10^{-3} μmol/50 μl for progesterone.

The assay was carried out in round-bottom plastic microtiter plates (Falcon Plastics). Preliminary studies (data not shown) evaluated the effect of divalent cations, AMP, NAD, and NADH on the uptake of estradiol and progesterone by *Bacteroides* species. The following components were found to give optimal steroid uptake: 50 μl of [^{14}C]estradiol (1.82×10^{-3} μmol); 50 μl of [^{14}C]progesterone (3.40×10^{-3} μmol); and 50 μl of RTF plus NADH (1.64×10^{-4} μM) plus $MgCl_2$ (1.00×10^{-4} μM).

The microtiter plate was covered with a self-adhesive sealing tape, placed on a microtiter plate shaker, and taken into an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, Mich.) with an atmosphere of 85% nitrogen, 5% carbon dioxide, and 10% hydrogen. The assay was initiated by lifting the sealing tape and placing 50 μl of each sonified cell suspension, RTF, or heat-killed controls into the triplicate wells. The plate was resealed and vibrated at low speed for 10 s initially and again at 30 min.

At 60 min, the sealing tape over each well was punctured with a 10-μl Hamilton syringe, and 1 μl of the cell suspension was removed by means of a Cheney adapter and spotted at the origin of a silica gel thin-layer chromatography (TLC) sheet (Eastman Kodak 13179 without fluorescent indicator). The TLC plates were removed from the anaerobic chamber, and to each sample spot was added 1 μl each of unlabeled estradiol (2% wt/vol) and progesterone (2% wt/vol). Plates were developed vertically in chloroform-acetaldehyde (80/20, vol/vol) to a solvent height of 100 mm. Steroids were visualized by means of a vanillin spray (0.5% vanillin in 1 part H_2SO_4 [1.0%] and 4 parts absolute ethanol; 29).

The TLC spots with R_f values corresponding to the estradiol and progesterone were cut out and placed in liquid scintillation vials with 10 ml of scintillation fluid (3% Biosolv [Beckman Instruments, Inc.] and 15 g of Omnifluor [New England Nuclear Corp.] in 1 gallon [ca. 3.8 liters] of technical-grade toluene) and quantitated by means of a liquid scintillation counter (Beckman LS 100). The quantities of [^{14}C]estradiol and [^{14}C]progesterone remaining in the bacterial suspensions were subtracted from the RTF controls to calculate the amount of substrate taken up by the bacterial suspensions. The quantity of substrate uptake was calculated per microgram of bacterial protein added to the reaction well. Before the steroid uptake studies were started the TLC procedure was evaluated for quantitative recovery of [^{14}C]estradiol and [^{14}C]progesterone. Since steroids are frequently destroyed by TLC procedures, the visualization reagents, scintillation fluids, and developing solvents were tested for effects on quantitation (data not included). The TLC procedures eventually used were found to give optimal quantitative recovery.

Heat-killed controls were prepared by heating a portion of the cell suspension in a water bath at 80°C for 30 min. A culture plate was streaked with the heated suspension and incubated to confirm the lack of viable cells. Attempts to determine steroid uptake during cell growth were not successful due to nonspecific oxidation-reduction of the estradiol and progesterone when incubated for prolonged periods in the reduced medium.

The effect of temperature on steroid uptake was also evaluated. Microtiter plates were prepared without bacteria, sealed, and placed at 0°C for 2 h. The plates were then taken into the anaerobic chamber, and the sealing tape was removed. Cell suspensions or RTF were added to appropriate wells to initiate the reaction. The plates were then resealed with two layers of adhesive plastic strips and immediately returned to 0°C for 60 min before taking 1-μl samples for the TLC assay of steroid uptake.

Autoradiographs using no-screen X-ray film (Kodak NS54T) were prepared from the TLC sheets to determine the location of the ^{14}C label after the steroid uptake assay.

Gas-liquid chromatography. Succinate and fumarate were sought for in the supernatants of the steroid uptake assays. Their methyl derivatives were prepared by the method of Holdeman et al. (10). A Varian Aerograph series 2700 gas-liquid chromatograph was used with a flame ionization detector. Temperatures were maintained at 150°C in the column, 200°C in the detector, and 175°C at the injector port. Standard succinate and fumarate solutions were freshly methylated and chromatographed on the day of sample analysis. Detection of fumarate and succinate standards in the range of 2.0 to 7.0 μmol/100 μl of fumarate and 0.2 to 0.7 μmol/100 μl of succinate was found to be linear.

Growth studies. Vitamin K, or an analog such as menadione, is essential to the growth of most strains of *B. melaninogenicus* and *B. asaccharolyticus* (8, 16). Structural similarities with regard to electron transfer between estradiol, progesterone, and vitamin K suggested the possibility that steroid hormones might be able to substitute for vitamin K compounds in the growth of pigmented *Bacteroides* species. Therefore, growth of these organisms was evaluated in the presence of estradiol or progesterone and in the absence of menadione in the medium.

The following organisms were maintained in pure culture on enriched Trypticase soy agar plates (31): *B. gingivalis* strains W, 165.5, and 208.1; *B. melaninogenicus* subsp. *intermedius* strains 155.6, 166.5, and 167.4; and *B. melaninogenicus* subsp. *melaninogenicus* strain ATCC 25845. A single colony was subcultured to 5 ml of BAB and incubated until the culture reached an optical density of 0.30 at 540 nm as measured in a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc.) present within the anaerobic chamber.

Cultures were Gram stained and streaked on enriched Trypticase soy agar plates to confirm purity, and 0.1 ml was inoculated into the following media: (i) BAB containing 0.5 μg of menadione per ml (2.9 μM); (ii) BAB without menadione; (iii) BAB without menadione plus estradiol, equimolar or twice (5.8 μM) or three times (8.7 μM) the concentration of menadione in BAB; and (iv) BAB without menadione plus proges-

terone, equimolar or twice or three times the concentration of menadione in BAB. All broth media were reduced and incubated within the anaerobic chamber for 48 h before use.

Optical density at 540 nm was monitored until a stationary phase was reached. At that time 0.1 ml was transferred to a second tube of the same medium, and growth was again monitored. If growth occurred in the second transfer, then 0.1 ml of that stationary-phase culture was used to inoculate a third tube of the same medium, and growth was again monitored. This procedure was used to prevent influences on growth from carryover of nutrients present in the original 0.1-ml inoculum from BAB. In separate experiments, optical density was related to dry weight by means of a standard curve derived for each organism. Growth curves represent the mean (± standard deviation) of three separate studies.

RESULTS

Uptake of ^{14}C-labeled steroids by pure cultures of bacteria. Steroid uptake was consistently observed in strains of oral *Bacteroides*, with *B. melaninogenicus* subsp. *intermedius* and subsp. *melaninogenicus* taking up substantially more estradiol and progesterone than *B. gingivalis* (Table 1). There was no detectable uptake of either [^{14}C]estradiol or [^{14}C]progesterone by *C. ochraceus*, *F. nucleatum*, or *S. sanguis*) Table 1). The uptake of both steroids by *A. viscosus* was similar to uptake observed with *B gingivalis*, whereas *A. naeslundii* took up progesterone but not estradiol. Six different batches of strain 155.6 of *B. melaninogenicus* subsp. *intermedius* consistently showed steroid uptake, with the mean uptake of the six experiments being 4.06 ± 1.22 × 10^{-4} μmol/μg of protein for estradiol and 2.21 ± 0.30 × 10^{-4} μmol/μg of protein for progesterone.

The reaction mixtures from steroid uptake studies with *B. melaninogenicus* subsp. *intermedius* strain 155.6 were spotted on TLC sheets and developed. The radioactivity in various spots was demonstrated by autoradiography. Reaction mixtures with RTF instead of bacteria (Fig. 1A) revealed labeled spots with R_f values of 0.69 and 0.97 which were compatible with the locations of progesterone and estradiol, respectively. Reaction mixtures B to G, to which were added increasing concentrations of bacteria, also revealed labeled spots for residual progesterone and estradiol and at an R_f of 0.53 as in the control. Additional labeled spots were evident at the origin and with R_f values of 0.25 and 0.84. The labeling at the origin represents ^{14}C-labeled compounds which were cell bound, and the spot at R_f 0.25 appears to represent diffusion of cell-bound material which did not increase with the increase in bacterial concentration from B to F. The labeled spot at R_f 0.84 was not present in the control and appears to be an unknown product of the labeled steroid utilization, since it increased with bacterial concentration. Of the total uptake, 26% was accountable in this unknown spot and 62% was located at the origin, apparently bound to the bacterial cells deposited with the sample. Uptake of ^{14}C-labeled steroid was found to be temperature dependent. Uptake of progesterone by viable *B. melaninogenicus* subsp. *intermedius* incubated at 0 and 37°C was 9.6 and 20.9 times the uptake by heat-killed cells at 37°C. Uptake of estradiol by viable cells at 0 and 37°C was 16.7 and 32.9 times the uptake by heat-killed cells.

Utilization of steroids for growth of *B. melaninogenicus*. *B. gingivalis* failed to grow in medium depleted of menadione (Table 2). The substitution of estradiol or progesterone for menadione did not support growth of *B. gingivalis*. *B. melaninogenicus* subsp. *intermedius* strains also

TABLE 1. Uptake of ^{14}C-labeled estradiol and progesterone by pure cultures of oral bacteria

Organism	Strain	Uptake[a] of:	
		Estradiol	Progesterone
A. naeslundii	401N	0	1.09 ± 0.22
A. viscosus	378.5	0.79 ± 0.09	1.76 ± 0.28
B. gingivalis	w	0	0.41 ± 0.06
	167.5	0.71 ± 0.08	1.76 ± 0.82
	208.1	0.42 ± 0.10	0.83 ± 0.44
B. melaninogenicus subsp. *intermedius*	155.6	2.15 ± 1.44	2.41 ± 0.42
	166.5	3.35 ± 0.48	3.12 ± 0.81
	167.4	5.48 ± 0.68	2.48 ± 0.39
B. melaninogenicus subsp. *melaninogenicus*	ATCC 25845	2.96 ± 0.42	4.12 ± 1.70
C. ochraceus	146.1	0	0
F. nucleatum	395.4	0	0
S. sanguis	399.5	0	0

[a] Mean micromoles uptake × 10^{-4}/microgram of cell protein ± standard deviation for triplicate samples.

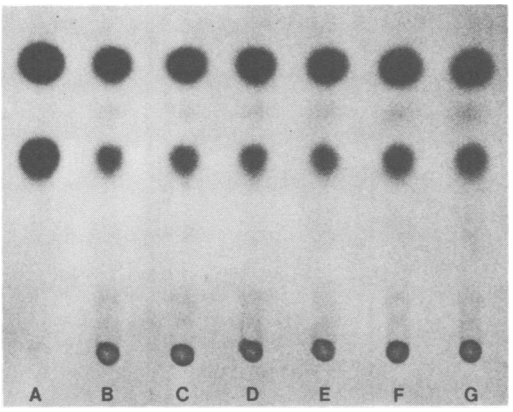

FIG. 1. Autoradiograph of TLC of ^{14}C-labeled steriod uptake. (A) Standard steroid uptake system to which was added estradiol, progesterone, and RTF, but no bacteria. (B–G) Standard steroid uptake system to which was added estradiol, progesterone, RTF, and increasing quantities of *B. melaninogenicus* subsp. *intermedius* strain 155.6.

did not grow in the absence of menadione. However, when estradiol (2.91 μM) was substituted for menadione, growth approached that observed with menadione (Table 2 and Fig. 2). A twofold increase in estradiol inhibited growth slightly, whereas a threefold increase markedly reduced growth. Progesterone at 5.8 and 8.7 μM substituted for menadione in the growth of *B. melaninogenicus* subsp. *intermedius*, and no reduction in growth was observed at the higher concentrations (Table 2 and Fig. 3).

The growth pattern of *B. melaninogenicus* subsp. *melaninogenicus* was different from that of the other subspecies, as this subspecies grew to some extent in the absence of menadione. The substitution of estradiol (2.9 and 5.8 μM) stimulated growth, but not to extent seen with menadione. A higher concentration of estradiol (8.7 μM) inhibited growth. Progesterone stimulated growth of this organism only at the highest concentration.

Interaction of steroids with the fumarate system. The addition of fumarate to the assay for uptake of ^{14}C-labeled steroids (Table 3) increased the uptake of estradiol and progesterone by *B. gingivalis* and the subspecies of *B. melaninogenicus*. Uptake of steroids by *B. gingivalis* strain W was negligible but, with the addition of fumarate, increased to a level intermediate between those of the two subspecies of *B. melaninogenicus*. When the supernatants of the uptake assay were methylated and analyzed by gas-liquid chromatography, the addition of fumarate gave rise to trace amounts of succinate. However, fumarate plus steroids greatly enhanced succinate production (Table 4).

DISCUSSION

Changes in endogenous steroid levels have been associated with alterations in the vaginal tract microflora (6, 9, 14), the urinary tract flora (21, 25, 29), the gastrointestinal tract flora (1, 7), and the oral subgingival flora (13). Most investigators have attributed hormonally associated mircobial shifts to steroid-induced tissue and immunological changes and not to direct effects of the steroids on bacteria. Studies of gram-positive organisms have shown that various steroids inhibit in vitro growth and enhance cell leakage (15, 36).

Progesterone has also been reported to inhibit growth of *Neisseria gonorrhoeae* and to have no effect on the growth of 14 other facultative gram-

TABLE 2. Effect of menadione and steroids on growth of *B. melaninogenicus* and *B. gingivalis*

BAB additives	*B. gingivalis*		*B. melaninogenicus* subsp. *intermedius*		*B. melaninogenicus* subsp. *melaninogenicus*	
	Doubling time[a]	Maximal cell mass[b] (%)	Doubling time	Maximal cell mass (%)	Doubling time	Maximal cell mass (%)
Menadione,						
2.9 μM	21	100	14	100	12	100
None	NG		NG		24	32.4
Estradiol						
2.9 μM	NG		16	82.0	12	62.1
5.8 μM	NG		20	28.3	12	62.8
8.7 μM	NG		NG		24	27.0
Progesterone						
2.9 μM	NG		14	98.6	NG	
5.8 μM	NG		12	102.6	NG	
8.7 μM	NG		9	99.3	12	148.0

[a] Hours required for cell mass, as measured by turbidity, to double during the exponential growth phase. NG, No growth.

[b] Maximal turbidity obtained over 96 h was converted to cell mass by means of standard curves relating turbidity to dry weight for each organism. Growth in complete medium (BAB) plus 2.9 μM menadione was taken as 100%.

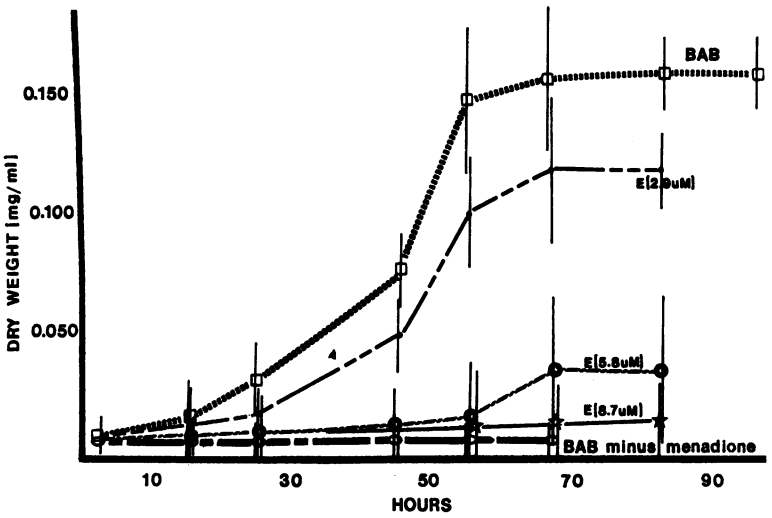

FIG. 2. Growth in complete medium (BAB which included 2.9 μM menadione), BAB without menadione, or BAB without menadione to which was added estradiol in equimolar, twice (5.8 μM), or three times (8.7 μM) the concentration of menadione in BAB. Data represent mean cell masses of strains 155.6, 166.5, and 167.4.

negative species (23). In the present study, *B. melaninogenicus* was found to have an affinity for estradiol and progesterone which was not demonstrated by other tested oral bacteria.

Some aspects of steroid uptake may reflect binding to the cell wall rather than actual utilization. Morse and Fitzgerald (23) observed that binding of [^{14}C]progesterone was three times greater to *Neisseria* species which were metabolically sensitive to the steroid than to insensitive cells. In our study, the uptake of steroids appeared to reflect an active metabolic process, since the uptake was temperature dependent and resulted in the detection of an unknown labeled by-product with an R_f value intermediate between those of progesterone and estradiol. Preliminary chromatographic studies (data not shown) suggest that this compound may be 5-β-pregnane 3-20 dione which would result from 5-β reductase activity on progesterone. Further analyses of this unknown product are in progress.

Mixed cultures of human fecal bacteria have been shown to dehydroxylate or dehydrogenate

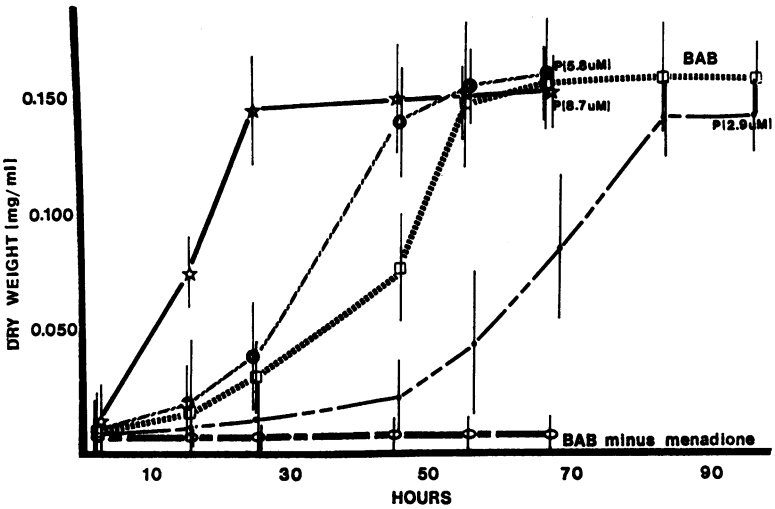

FIG. 3. Growth of *B. melaninogenicus* subsp. *intermedius* in complete medium (BAB which included 2.9 μM menadione), BAB without menadione, or BAB without menadione to which was added progesterone in equimolar, twice (5.8 μM), or three times (8.7 μM) the concentration of menadione in BAB. Data represent mean cell masses of strains 155.6, 166.5, and 167.4.

TABLE 3. Effect of fumarate on uptake of ^{14}C-labeled estradiol and progesterone

Organism	Fumarate added[a]	Total steroid uptake[b]	Steroid uptake fumarate+/fumarate−
B. melaninogenicus subsp. *intermedius* strain 155.6	−	4.56 ± 0.93	1.5
	+	6.70 ± 0.76	
B. gingivalis strain W	−	0.41 ± 0.03	21.4
	+	8.76 ± 1.07	
B. melaninogenicus subsp. *melaninogenicus* strain ATCC 25845	−	5.71 ± 1.40	2.2
	+	12.37 ± 1.50	

[a] Fumarate added to standard reaction mixture to final concentration of 0.5%.
[b] Mean micromoles uptake of labeled estradiol and progesterone × 10^{-4}/microgram of cell protein ± standard deviation for triplicate samples.

deoxycorticosterone (2, 12), bile salts and androsterone (20), estrone and estradiol (11), and progesterone (1). In addition, *B. fragilis* (V. C. Aries and M. J. Hill, Biochem. J. **119**:37p–38p, 1970) and *B. thetaiotaomicron* (24) have been shown to exhibit 7-α-hydroxysteroid dehydrogenase activity in vitro, and pure cultures of *B. fragilis* appear capable of reducing estrone to estradiol (11).

However, chemical changes resulting from bacterial action and binding of a compound may represent reactions which are not truly involved in physiological processes. The hypothesis that steroids directly affect subspecies of *B. melaninogenicus* is strengthened by the observation that estradiol and progesterone can substitute for vitamin K compounds, an essential growth factor, in the metabolism of these organisms. The ability of the steroids to substitute for vitamin K is compatible with earlier observations that the enzymes involved in vitamin K utilization by *B. melaninogenicus* appear to have broad substrate specificity (8, 16).

TABLE 4. Effect of steroids and fumarate on succinate detection

Organism	Steroids added[a]	Fumarate added[b]	Succinate detected[c]
B. gingivalis strain W	+	−	0
	+	+	0.48
	−	−	0
	−	+	<0.01
B. melaninogenicus subsp. *intermedius* strain 155.6	+	−	0
	+	+	0.52
	−	−	0
	−	+	0.03
B. melaninogenicus subsp. *melaninogenicus* strain ATCC 25845	+	−	0
	+	+	0.61
	−	−	0
	−	+	0.04

[a] Estradiol was added at 1.82 × 10^{-3} μmol; progesterone was added at 3.77 × 10^{-3} μmol.
[b] Fumarate was added at 8.60 μmol.
[c] Micromoles/100-μl sample as detected by gas-liquid chromatography.

Steroids have not been previously reported to enhance bacterial growth. In fact, gonadal steroids have been shown to inhibit growth of a variety of gram-positive organisms (3, 36) as well as *N. gonorrhoeae* (23). The various growth effects of different concentrations of estradiol and progesterone as substitutes for vitamin K in *B. melaninogenicus* are consistent with previous reports (16) of a narrow concentration range for vitamin K stimulation of *B. melaninogenicus*, with inhibition of growth observed at higher vitamin K concentrations. Substrate inhibition was also reported for a hydroxysteroid dehydrogenase isolated from *B. thetaiotaomicron*, in which enzyme K_m values were low and substantial inhibition was observed at steroid concentrations greater than 1 mM (24).

In the present study, fumarate, a terminal electron acceptor in anaerobic respiration, increased steroid uptake by *B. melaninogenicus* subspecies and *B. gingivalis* and was reduced to succinate in the presence of estradiol and progesterone. Naphthoquinones, such as vitamin K or menadione, have been implicated as electron carriers in the bacterial anaerobic reduction of fumarate to succinate (33). In *B. thetaiotaomicron*, fumarate has been shown to markedly increase 7-α-hydroxysteroid dehydrogenase activity (24). Therefore, the effect of fumarate on the uptake of estradiol and progesterone adds further support to the finding that steroids may substitute for vitamin K analogs in these microorganisms. The fact that *B. gingivalis* does not routinely produce succinate as an end product, yet is able to do so if given exogenous fumarate plus estradiol and progesterone, raises the possibility that this organism has a block in fumarate synthesis.

In general, a role for estradiol and progesterone in the metabolism of *B. melaninogenicus* and *B. gingivalis* is suggested by four observations: (i) there was a significant increase in the uptake of [^{14}C]estradiol and [^{14}C]progesterone by dental plaque samples taken from pregnant patients in the second trimester, and the increased uptake paralleled increased plaque proportions of *B. melaninogenicus* subsp. *intermedius* (13); (ii) pure cultures of *B. melaninogenicus* and *B. gingivalis* took up ^{14}C-labeled estradiol and progesterone; (iii) estradiol and progesterone substituted for menadione, an essential growth factor, in the growth of *B. melaninogenicus* subspecies; and (iv) the uptake of estradiol and progesterone was increased by the addition of fumarate, with fumarate apparently being reduced to succinate in the presence of steroids.

These findings strongly suggest that estradiol and progesterone are involved in the fumarate reductase system of subspecies of *B. melaninogenicus* and *B. gingivalis*. In addition, the interchangeability of menadione and gonadal steroid raises the possibility that estrogens or progesterone, which are readily available in the gingival sulcular fluid (4), may be a primary growth factor in vivo for *B. melaninogenicus*. Steroid hormones therefore appear to have the potential for altering the subgingival microbial ecology by directly influencing the metabolic pathways of *B. melaninogenicus*.

Several mucosal diseases, including periodontitis and urinary tract infections, are known to be caused by bacteria but have been frequently associated with altered hormone levels. The identification of the potential for endogenous steroids to directly induce a shift in the normal microflora may provide new insights to the association of periodontal disease with conditions characterized by altered steroid levels.

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LITERATURE CITED

1. **Adlercreutz, H., F. Martin, P. Jarvenpaa, and T. Fotsis.** 1979. Steroid absorption and enteroheoptic recycling contraception. J. Steroid Biochem. **20**:201–223.
2. **Bokkenheuser, V. D., J. Winter, and W. G. Kelly.** 1978. Metabolism of biliary steroids by human fecal flora. Am. J. Clin. Nutr. **31**:S221–S226.
3. **Casas-Campillo, C., D. Balandrano, and A. Galzrza.** 1961. Antimicrobial properties of 21,-dimethoxy progesterone and other progesterone analogues. J. Bacteriol. **81**:366–375.
4. **Cimasoni, G.** 1974. The crevicular fluid. Monogr. Oral Sci. **3**:1–74.
5. **Coykendall, A. L., F. S. Kaczmarek, and J. Slots.** 1980. Genetic heterogeneity in *Bacteroides asaccharolyticus* (Holdeman and Moore 1970). Finegold and Barnes 1977 (Approved Lists 1980) and proposal for two new species: *Bacteroides gingivalis* sp. nov. and *Bacteroides macacae* comb. nov. Int. J. Syst. Bacteriol. **30**:559–564.
6. **Cruickshank, R., and A. Sharman.** 1934. The biology of the vagina in the human subject. II. The bacterial flora and secretion of the vagina in relation to glycogen in the vaginal epithelium. J. Obstet. Gynaecol. Br. Emp. **41**:208–266.
7. **Drasar, B. S., and M. J. Hill.** 1974. Human intestinal flora. Academic Press, Inc., New York.
8. **Gibbons, R. J., and J. B. MacDonald.** 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. J. Bacteriol. **80**:164–169.
9. **Goplerud, C. P., J. J. Ohm, and R. P. Galask.** 1976. Aerobic and anaerobic flora of the cervix during pregnancy and the puerperium. Am. J. Obstet. Gynecol. **126**:858–868.
10. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore** (ed.). 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
11. **Jarvenpaa, P., T. Kosunen, T. Fotsis, and H. Adlercreutz.** 1980. *In vitro* metabolism of estrogens by isolated intestinal micro-organisms and by human fecal microflora. J. Steroid Biochem. **13**:345–349.
12. **Kelly, W. G., O. DeLeon, J. Winter, and V. D. Bokkenheuser.** 1977. Exchange of hydrogen at C-21 during dehydroxylation of deoxycorticosterone by mixed cultures of human fecal flora. J. Steroid Biochem. **8**:73–75.
13. **Kornman, K. S., and W. J. Loesche.** 1980. The subgingival microbial flora during pregnancy. J. Periodontal Res. **15**:111–112.
14. **Larsen, B., A. J. Markovetz, and R. P. Galask.** 1976. Quantitative alterations in the genital microflora of female rats in relation to the estrous cycle. J. Infect. Dis. **134**:486–489.
15. **Lester, G., and O. Hetcher.** 1966. Effects of deoxycorticosterone on growth of microorganisms. J. Bacteriol. **92**:627–635.
16. **Lev, M.** 1959. The growth-promoting activity of compounds of vitamin K group and analogues for a rumen strain of *Fusiformis nigescens*. J. Gen. Microbiol. **20**:697–703.
17. **Loesche, W. J.** 1976. Chemotherapy of dental plaque infections. Oral Sci. Rev. **9**:65–107.
18. **Loesche, W. J., R. Hockett, and S. A. Syed.** 1972. The predominant cultivable flora of tooth surface plaque removed from institutionalized subjects. Arch. Oral Biol. **17**:1311–1325.
19. **Loesche, W. J., and S. A. Syed.** 1978. Bacteriology of human experimental gingivitis II. Effect of plaque and gingivitis score. Infect. Immun. **21**:830–839.
20. **MacDonald, I. A., G. R. Webb, and D. E. Mahony.** 1978. Fecal hydroxysteroid dehydrogenase activities in vegetarian Seventh-Day Adventists, control subjects, and bowel cancer patients. Am. J. Clin. Nutr. **31**:S233–S238.
21. **Marrie, T. J., C. A. Swantee, and M. Hartlen.** 1980. Aerobic and anaerobic urethral flora of healthy females in various physiological age groups and of females with urinary tract infections. J. Clin. Microbiol. **11**:654–659.
22. **Marshall, S. and J. Linfoot.** 1977. Influence of hormones on urinary tract infection. Urology **9**:675–678.
23. **Morse, S. A., and T. J. Fitzgerald.** 1974. Effect of progesterone on *Neisseria gonorrhoeae*. Infect. Immun. **10**:1370–1377.
24. **Sherrod, A., and P. B. Hyleman.** 1977. Partial purification and characterization of NAD-dependent 7-alpha-hydroxysteroid dehydrogenase from *Bacteroides thetaiotaomicron*. Biochim. Biophys. Acta **486**:351–358.
25. **Silk, M., and D. B. Perez-Vorela.** 1970. Effects of oral contraceptives on urinary bacterial growth rate. Invest. Urol. **8**:239–245.

26. **Slots, J.** 1977. Microflora in the healthy gingival sulcus in man. Scand. J. Dent. Res. **85**:274–254.
27. **Slots, J.** 1977. The predominant cultivable microflora of advanced periodontitis. Scand. J. Dent. Res. **85**:114–121.
28. **Slots, J., D. Moenbo, J. Langebaek, and A. Frandsen.** 1978. Microbiota of gingivitis in man. Scand. J. Dent. Res. **86**:174–181.
29. **Stahl, E.** 1969. Thin layer chromatography, 2nd ed. Academic Press, Inc., New York.
30. **Syed, S. A.** 1980. Characteristics of *Bacteroides asaccharolyticus* from dental plaques of beagle dogs. J. Clin. Microbiol. **11**:522–526.
31. **Syed, S. A., M. Svanberg, and G. Svanberg.** 1980. The predominant cultivable dental plaque flor of beagle dogs with gingivitis. J. Periodontal Res. **15**:123–136.
32. **Tanner, A. C. R., C. Haffer, G. T. Bratthal, R. A. Visconti, and S. S. Socransky.** 1979. A study of the bacteria associated with advancing periodontitis in man. J. Clin. Periodontics **6**:278–307.
33. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
34. **Udenfriend, S., S. Stein, and P. Bohlen.** 1972. Fluorescamine: a reagent for assay of amino acids, peptides, protein, and primary amines in the picomole range. Science **178**:871–872.
35. **Van Palenstein-Helderman, W. H.** 1975. Total viable count and differential counts of vibrio (*Campylobacter*) *spurtorum*, *Fusobacterium nucleatum*, *Selenomonas sputigena*, *Bacterioides ochraceus*, and Veillonella in the inflamed and non-inflamed human gingival crevice. J. Periodontal Res. **10**:294–301.
36. **Yotis, W. W.** 1967. *In vivo* and *in vitro* action of norethindrone on staphylococci. J. Bacteriol. **94**:1353–1358.