The subgingival microbial flora during pregnancy

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The subgingival bacterial flora from 2 gingival sites was cultured and characterized monthly in twenty periodontitis-free women during pregnancy and again post-partum. Monthly plaque samples were also cultured in eleven age and disease matched non-pregnant women. Plaque was processed anaerobically on selective and nonselective media and the predominant colony types were characterized. A portion of each plaque sample was tested for bacterial uptake of C^{14}-estradiol and C^{14}-progesterone. Plasma levels of estrogens and progesterone were measured four times in each subject. The number of gingival bleeding sites, the Gingival Index and the Plaque Index were determined at each sampling period.

In the second trimester there was a significant increase in gingivitis, the ratio of anaerobic to aerobic bacteria, and the proportional levels of *Bacteroides melaninogenicus* ss. *intermedius*. In the third trimester both gingivitis and the levels of *B. melaninogenicus* ss. *intermedius* decreased. Plaque uptake of C^{14}-steroids increased significantly during pregnancy and paralleled the plaque levels of *B. melaninogenicus* ss. *intermedius*. In the second trimester, recovery of *B. melaninogenicus* ss. *intermedius* was strongly correlated with plasma levels of estrogens and progesterone. No changes were observed in clinical parameters or the subgingival flora of non-pregnant subjects.

Pregnancy and specifically steroid hormones appear capable of influencing the normal bacterial flora and inducing alterations in the subgingival ecology.

Introduction

Recent studies in humans have suggested that the subgingival bacterial flora associated with periodontal disease is composed of elevated proportions of certain Gram-negative microorganisms (Slots 1976, Newman & Socransky 1977, Slots 1977b). These organisms are also found in lower numbers in gingival health and in gingivitis (van Palenstein Helderman 1975, Slots 1977a, Slots et al. 1978, Loesche & Syed 1978). The determination of factors which induce or allow a shift in the bacterial flora found in gingival health may provide insights into the etiology of the transition from health to gingivitis and to periodontitis.

An increase in gingival inflammaton has been reported for several systemic conditions, such as puberty, menstruation, pregnancy, and stress, which are associated with fluctuations in the levels of serum steroids. Cross-sectional and longitudinal studies have consistently shown a gradual increase in gingivitis during pregnancy with apparent resolution following parturition (Löe & Silness 1963, Silness & Löe 1964, Hugoson 1970, Cohen et al. 1971). In all of the studies, plaque scores were essentially low and stable throughout pregnancy. The increase

in gingivitis independent of a change in clinically detectable plaque mass led to the suggestion that altered steroid levels increased the inflammatory response to a given plaque challenge (Lindhe, Attstrom & Bjorn 1968, Hugoson 1970, Lindhe, Hellden & Lundgren 1972). No investigations of the bacteriology of plaque from pregnant subjects have been reported. This study monitored the gingival crevice microbial flora in pregnant and nonpregnant women to determine if gestation induces alterations in the gingival crevice flora and if these changes can be associated with pregnancy gingivitis.

Clinical Methods

Twenty women, age 20–33, whose pregnancies were thought to be less than 13 weeks at the initial dental examination, were seen monthly until delivery and again post-partum in the University of Michigan Women's Hospital Obstetrics Clinic. Eleven nonpregnant women, age 22–36, were seen monthly for four consecutive months. Each control subject was asked to record the dates of her menstrual period so that each visit occurred at approximately the same stage in the menstrual cycle as the first visit. Preliminary studies suggested that a four month sampling period would be adequate for determining the stability over time of clinical and bacteriological evaluations in nonpregnant subjects. At the initial examination none of the subjects had any evidence of loss of periodontal attachment on probing. All subjects had moderate to good oral hygiene and had not received a dental prophylaxis for at least 6 months prior to the study. At each visit the Gingival Index (G.I., Löe & Silness 1963) and the Plaque Index (Pl. I., Silness & Löe 1964) were determined for the mesial of the maxillary right second premolar (Site 1) and of the mandibular left cuspid (Site 2) in each subject. An interproximal eval-

uation of the bleeding score (B.S.) for each subject was determined by counting the interproximal sites which bled to any extent after gentle probing with a wooden interdental stimulator (Stim-u-dent®). A maximum of 22 interproximal sites were available in each patient, mesial to the first molars. No distinction was made between spontaneous and induced bleeding.

Supragingival plaque was removed from both sample sites by means of a scaler and discarded. Subgingival plaque samples were taken from each site by means of a sterile curette and placed in a vial of reduced transport fluid without ethylenediaminetetraacetate (RTF, Loesche, Hockett & Syed 1972).

Bacteriology

Plaque samples were taken into the anaerobic chamber (Aranki et al. 1969) and were dispersed by sonication ((Kontes Cell Disrupter, Syed & Loesche 1978) and diluted in RTF. Dilutions were plated on selective and nonselective media by means of an automatic diluting and plating device (Spiral Systems, Bethesda, Md.).

Enriched Trypticase Soy Agar (ETSA) which consists of a trypticase soy agar and yeast extract base supplemented with sodium lactate (600 μg/ml), sodium succinate (500 μg/ml), sodium formate (500 μg/ml), potassium nitrate (500 μg/ml), cysteine (400 μg/ml), dithiothreitol (100 μg/ml), hemin (1 μg/ml), menadione (1 μg/ml) and 3 % sheep blood was used for nonselective anaerobic and aerobic growth. *Bacteroides melaninogenicus* was quantitated by both ETSA and ETSA with 50 μg/ml kanamycin sulfate (Loesche, Hockett & Syed 1971, Syed, Svanberg & Svanberg 1979). The gelatin, metronidazole, cadmium sulfate (GMC) agar of Kornman and Loesche (1978) was used to quantitate *Actinomyces viscosus* and *Actinomyces naeslundii*. MM10 agar with 5 % sucrose (Loesche et

al. 1972) was used to quantitate *Streptococcus sanguis.*

Differential counts and biochemical characterization of isolates were keyed to those organisms currently thought to undergo proportional shifts associated with periodontal disease. These organisms are *Actinomyces naeslundii, Actinomyces odontolyticus, Actinomyces viscosus, Bacteroides melaninogenicus* ss. *intermedius, B. melaninogenicus* ss. *melaninogenicus, Bacteroides asaccharolyticus, Bacteroides (Capnocytophaga) ochraceus, Fusobacterium nucleatum, Streptococcus sanguis,* and surface translocating bacteria (STB).

From each medium, two precisely measured areas or sectors of the agar including more than 20 colonies per sector were quantitated by means of a binocular dissection microscope. The total number of colonies and the number of selected colony types were determined for each sector. Classification of isolates was based on colony morphology, Gram-stain morphology, motility by dark field microscopy, aerobic growth, indole production, esculin hydrolysis, nitrate reduction, nitrite reduction, gelatinase activity, catalase activity, pH in glucose broth, pH in glucose-1-phosphate broth, pH in mannitol broth, and acid end products by gas liquid chromatography.

Isolates were identified to the species level, where possible, using the classification scheme in Table 1. The scheme allowed many of the predominant organisms to be identified with a limited number of tests which were keyed to a presumptive identification based on characteristic colony morphology. The taxonomic scheme incorporated data from the Virginia Polytechnic Institute Anaerobe Laboratory Manual (4th Edition, 1977), Loesche & Gibbons 1965, Holmberg & Nord 1975, Holbrook, Duerden & Deacon 1977, Duerden, Holbrook & Collee 1976, Syed & Loesche 1978 and Facklam 1977.

Steroid uptake by plaque samples

Duplicate 100 μl. portions of each dispersed plaque sample were incubated anaerobically with estradiol and progesterone to evaluate uptake of these substrates by the plaque sample.

The assay was carried out in round bottom plastic microtiter plates.

Into each well was placed:

50 μl Estradiol (4-C^{14}, Amersham-Searle, 1.82×10^{-3} μmoles/50 μl)

50 μl Progesterone (4-C^{14}, 3.40×10^{-3} μmoles/50 μl)

50 μl RTF + NADH (1.64×10^{-4} μM) + $MgCl_2$ (1.00×10^{-4} μM)

100 μl dispersed plaque suspension. Controls contained RTF instead of plaque or plaque heat-killed at 80°C for thirty minutes.

After 60 minutes incubation at 37°C, 1 μl samples were removed by means of a Hamilton syringe with a Cheney Adaptor and the samples were spotted on silica gel thin-layer chromatography (TLC) sheets. TLC plates were removed from the anaerobic chamber, developed vertically in chloroform/acetaldehyde (80/20 v/v), visualized with vanillin (0.5 %) in 1 % H_2SO_4 and ethanol (1:4) (modified procedure of Matthews 1963). The estradiol and progesterone spots were identified by comparisons with standards and were cut out and quantitated by means of liquid scintillation counting. The quantities of C^{14}-estradiol and C^{14}-progesterone in the assay wells after 60 minutes were subtracted from RTF control wells to calculate the amount of substrate utilized by the plaque samples. Substrate uptake was expressed as moles per μg of bacterial protein (fluorescamine protein assay, Weigele et al. 1972) in the assay well.

Serum steroid determination

Thirty ml. of peripheral blood was removed from the median vein in the ante-

Table 1

	Colony Morphology Characteristic on	Gram Stain	Growth Aerobically +CO$_2$	Acid from Glucose	Motility	Esculin Hydrolysis	Indole	Reduction of Nitrate	End-products by GLC	Others
Gram-Positive Rods										
Actinomyces		G+ branching							ALS	Gelatinase– MOH+
israelii			–	+		±	–	+		
naeslundii	white convex on GMC		+	+		+	–	+		
odontolyticus	red convex on ETSA		–	+		–	–	+		
viscosus	white convex on GMC		+	+		+	–	+		Catalase+
Bacillus		spores	+	+						Gelatinase+ Catalase+
Propionibacterium acnes		branching	–	+		–	+	+	AP	Gelatinase+ Catalase+
Clostridium		spores	–							
Arachnia			–	+		–	–	+	AP	
Eubacterium			–			–	–	+	APB	
Gram-Positive Cocci										
Micrococci			+	+				–		Catalase+
Streptococci			+	+				–		Catalase–
miteor						–				MOH–
mutans	mm 10 heaped					+.				MOH+
sanguis	mm 10 adherent					+				MOH–
salivarius	mm 10 mucoid					+				MOH–
Peptostreptococci			–						A*	Catalase–
Peptococci			–						A*	Catalase+
Gram-Negative Rods										
Bacteroides asaccharolyticus	ETSA		–	–		–	+	–	AP*	
melaninogenicus ss. intermedius	ETSA		–	+		–	+	–	SA	
ss. melaninogenicus	ETSA		–	+		+	–	–	SA	
corrodens	ETSA		–	–		–	–	+	SA	urease+ oxidase+
oralis	ETSA		–	+		+	–	–	SA	
ochraceus (Capnocytophaga)	ETSA	fusiform	+	+		+	–	–	SA	
Eikenella corrodens	ETSA		+	–		–	–	+	SA	oxidase+ urease–
Fusobacterium nucleatum	ETSA	fusiform	–	–		–	+	–	ABL	
Selenomonas sputigena	curved rod		–	+	+	+	–	–	L$_p$	
Campylobacter sputorum	curved or straight rod		–	–	+	–	–	+	none	catalase– nitrite reduction

Table 1 (contd.)

Colony Morpho- logy Charac- teristic on	Gram Stain	Growth Aero- bi- cally +CO₂	Acid from Glu- cose	Moti- lity	Escu- lin Hy- dro- lysis	In- dole	Re- duc- tion of Ni- trate	End- pro- ducts by GLC	Others
Gram-Negative Cocci									
Neisseria		+	+						catalase+
Veillonella alcalescens		−	−	−	−		+	AP	catalase−
parvula		−	−	−	−		+	AP	catalase+

* others variable

cubital fossa of each subject 3 times during pregnancy and again post-partum. Blood samples were taken at each visit from five of the control subjects. The peripheral blood leukocytes were used to monitor the *in vitro* lymphocyte transformation response to the predominant microorganisms found in the subgingival plaque during pregnancy and will be reported separately. Plasma levels of progesterone and estrogens, including both estradiol and estrone, were determined by radioimmunoassay as described by Yalow and Berson (1971) using reagents obtained from New England Nuclear (NEA-044,042).

Statistical analysis

Differences between the mean data from different time periods were evaluated by means of a paired t-test. The strength of the linear relationship between parameters was evaluated by computing the product-moment correlation coefficient, including a t-test of the null hypothesis that the population correlation was zero.

Results

Clinical measurements revealed an increase in gingivitis in the pregnant subjects (PG) as indicated by gingival bleeding. The mean number of bleeding sites per patient in-

creased significantly between 13–28 weeks gestation, with the peak at 21–24 weeks (Fig. 1). The mean Gingival Index at Site 1 and Site 2 increased significantly ($p < 0.05$, pairwise t-test) from 1.16 initially to between 1.44 and 1.61 by 13–28 weeks. The mean Plaque Index at both sites (1.13) was essentially unchanged throughout the pregnancy and post-partum. Clinical parameters (B.S., GI and Pl I) in the nonpregnant (NP) group did not change during the 4-month sampling period, with a mean bleeding score of 3.7 ± 0.1 (standard error), mean GI of 1.17 ± 0.18, and mean Pl I of 1.00 ± 0.36.

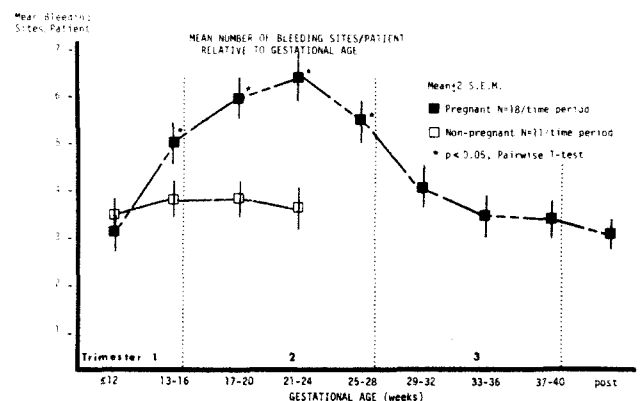In the second trimester the mean number of bleeding sites and the GI were signifi-

Fig. 1. Mean number of interproximal gingival bleeding sites in the entire mouth for pregnant and nonpregnant subjects. A maximum of twenty sites per subject was available for scoring.

Table 2

Bacteriological Findings in Pregnant Subjects

Organism	Sampling Time			
	First Trimester ($\leq$ 13 weeks)	Second Trimester (21–24 weeks)	Third Trimester (33–36 weeks)	Post-Partum
Total Count ($\times 10^6$)	11.00 ± 15.52[1]	7.30 ± 13.60	4.35 ± 11.00	10.21 ± 12.35
Anaerobe/Aerobe Ratio[2]	5.94 ± 0.36	19.86 ± 2.29	6.20 ± 0.52	6.14 ± 0.53
A. naeslundii (%)[3]	2.65 ± 6.01	4.87 ± 5.33	4.88 ± 5.67	1.78 ± 5.51
A. odontolyticus (%)	2.36 ± 3.18	4.35 ± 4.12	1.99 ± 3.14	1.97 ± 3.18
A. viscosus (%)	5.80 ± 3.40	7.62 ± 4.86	1.70 ± 3.24	4.32 ± 3.78
B. asaccharolyticus (%)	2.24 ± 4.48	7.65 ± 10.84	2.83 ± 5.60	2.52 ± 5.02
B. melaninogenicus ss. intermedius (%)	2.43 ± 2.19	10.10 ± 7.74	3.20 ± 4.00	2.18 ± 3.47
B. (Capnocytophaga) ochraceus (%)	2.42 ± 2.37	7.21 ± 7.45	2.94 ± 3.99	2.04 ± 2.29
F. nucleatum (%)	4.80 ± 4.42	7.30 ± 6.48	4.78 ± 6.56	4.39 ± 4.66
S. sanguis (%)	19.00 ± 10.42	26.32 ± 8.04	19.25 ± 7.23	22.16 ± 8.46

1. Mean ± 2 standard errors of the mean N = 36/time period.
2. Ratio of the total number of organisms growing anaerobically to the total number growing aerobically on primary isolation.
3. Percentage of total colony forming units.
□ Values within box are significantly different (p < 0.01, pairwise t-test) from all other values in row.

Table 3

Bacteriological Findings in Non-Pregnant Subjects During Four Successive Samplings

Organism	Sampling Time			
	0	4 Weeks	8 Weeks	Weeks
Total Count ($\times 10^6$)	3.50 ± 7.63[1]	4.25 ± 9.65	3.20 ± 7.15	3.10 ± 7.60
Anaerobe/Aerobe Ratio[2]	3.72 ± 0.76	4.25 ± 0.32	5.43 ± 0.48	4.51 ± 0.55
A. naeslundii (%)[3]	4.60 ± 8.30	6.04 ± 7.45	2.34 ± 3.98	2.32 ± 4.06
A. odontolyticus (%)	0.49 ± 1.67	0.53 ± 2.07	0.93 ± 4.50	1.25 ± 4.40
A. viscosus (%)	5.96 ± 2.80	4.45 ± 3.64	3.89 ± 2.64	4.44 ± 3.20
B. asaccharolyticus (%)	0.70 ± 0.60	0.52 ± 0.68	0.54 ± 0.55	0.72 ± 0.53
B. melaninogenicus ss. intermedius (%)	1.98 ± 1.81	2.65 ± 1.78	2.32 ± 1.90	2.83 ± 1.87
B. (Capnocytophaga) ochraceus (%)	3.70 ± 2.97	3.12 ± 3.90	3.21 ± 3.56	3.64 ± 3.99
F. nucleatum (%)	2.24 ± 2.98	3.98 ± 5.05	1.50 ± 2.50	2.19 ± 2.45
S. sanguis (%)	18.46 ± 7.46	18.73 ± 8.22	22.27 ± 8.04	21.74 ± 7.92

1. Mean ± 2 standard errors of the mean N = 22/time period
2. Ratio of the total number of organisms growing anaerobically to the total number growing aerobically on primary isolation.
3. Percentage of total colony forming units.

cantly higher (p < 0.05, Student's t-test) than those values in the non-pregnant subjects. The Pl I exhibited no differences between groups at any time period.

A total of 416 subgingival plaque samples were cultured during the course of the study. A minimum of 36 samples from the pregnant subjects were analyzed at each of the 9 time periods. A minimum of 22 samples from the nonpregnant subjects were evaluated monthly for four months. Bacterial changes over time were evaluated by means of pairwise t-tests comparing the bacteriology at each time period with the initial sampling period. Statistically significant changes were observed in the pregnant subjects for the anaerobe/aerobe ratio and the proportions of *B. melaninogenicus* ss. *intermedius*.

Table 2 gives the bacterial findings representative of the trimesters of gestation and the post partum samples. Data for the four samples in the nonpregnant subjects are in Table 3.

In the pregnant women the total count of colony forming units (CFU) per sample was $4-11 \times 10^6$ at all time periods. Total counts from NP sites were in the range $3-5 \times 10^6$ at all sampling periods (Tables 2,3).

The anaerobe/aerobe ratios for NP samples were stable over time in the range of 4–7 (Fig. 2), but the ratios for the PG samples increased significantly at the second sampling period. The initial mean anaerobe/aerobe ratio of 5.9 increased to approximately 35 at 13–20 weeks and remained at an elevated level through 24 weeks (Fig. 2). Samples returned to initial values by 25–28 weeks and remained at that level through the postpartum sample.

Recovery of *B. melaninogenicus* ss. *intermedius* from the PG group increased significantly (p < 0.05) as pregnancy progressed. The mean recovery of *B. melaninogenicus* ss. *intermedius* went from 2.2 % of the initial cultivable flora to 10.1 % at 21–24
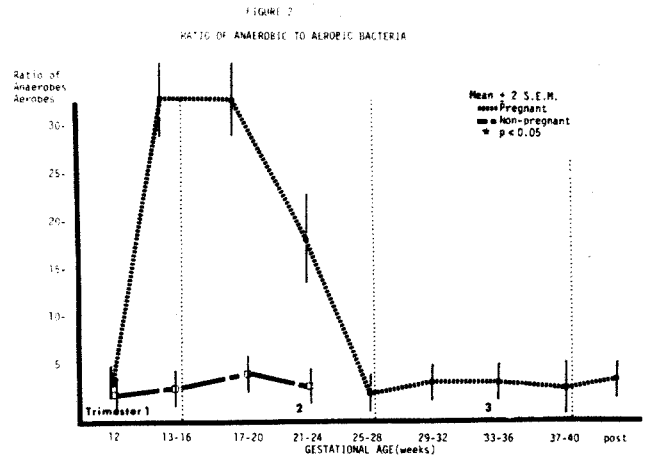

Fig. 2. Ratio of the total number of colony forming units on a nonselective medium (ETSA) incubated anaerobically to the total number on the same medium incubated aerobically.

weeks (Fig. 3a, Table 2). The proportions of this organism decreased by 29–32 weeks and returned to the initial levels at the postpartum sample. No similar change was observed in the NP samples.

The mean proportions of *B. asaccharolyticus* increased at 21–28 weeks, but the variation was great and statistical significance at p < 0.05 was not achieved (Fig. 3b, Table 2). The apparent increase in this organism was due to two subjects from whom very high levels (50–65 %) were recovered at 21–28 weeks. *Bacteroides (Capnocytophaga) ochraceus* also appeared to increase at 21–28 weeks (Fig. 3c, Table 2), but the changes were not statistically significant by pairwise t-tests.

Actinomyces viscosus exhibited a significant (p < 0.05) decrease in the mean recovery from samples at 33–36 weeks (Fig. 3d, Table 2). The initial mean level of 5.8 % declined to 1.7 % of the cultivable flora. In the post partum samples *A. viscosus* accounted for 4.3 % of the flora. Other bacteria which were found consistently in all subjects did not change significantly during pregnancy (Table 2). At all sampling periods from both pregnant and nonpregnant subjects *A. naeslundii* represented 4.9 % of the cultivable flora, *F. nu-*
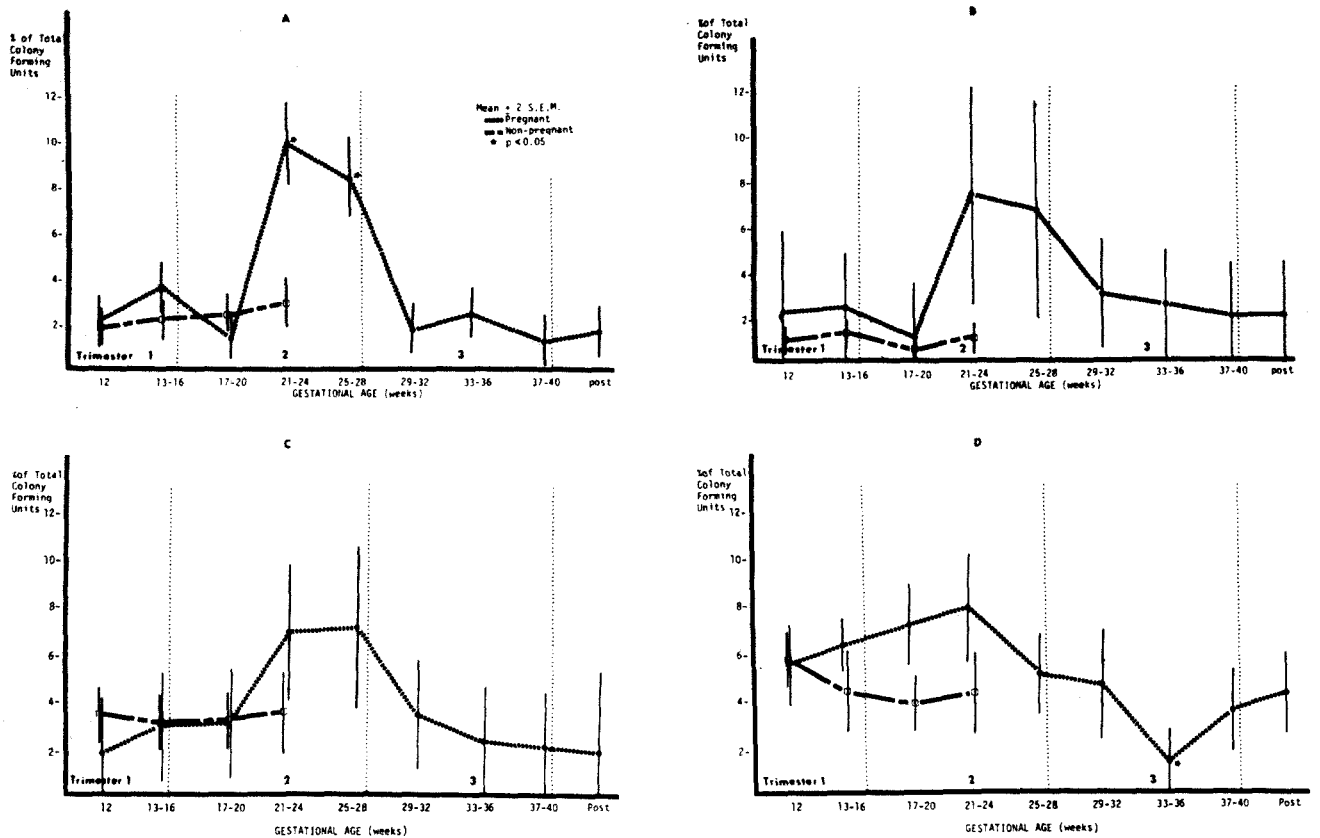
Fig. 3. Recovery of each indicated organism as a percentage of the total colony forming units on a nonselective medium (ETSA) incubated anaerobically.

A. Recovery of *Bacteroides melaninogenicus* ss. *interm edius* as evaluated on a nonselective medium (ETSA).

B. Recovery of *Bacteroides asaccharolyticus* as evaluated on ETSA.

C. Recovery of *Bacteroides (Capnocytophaga) ochraceus* as evaluated on ETSA.

D. Recovery of *Actinomyces viscosus* as evaluated on gelatin-metronidazole-cadmium sulfate medium.

cleatum 5.0 % of the flora, *A. odontolyticus* 3.3 % of the flora, *S. sanguis* 20.5 % of the flora, and *Veillonella* species 4.8 % of the flora. Gram positive anaerobic rods represented 10–15 % of the flora except for low levels (3.4 %) recovered in the second trimester. Great variability was observed in recovery of individual species but most samples included at least two of the following: *A. israelii*, *A. odontolyticus*, *Propionibacterium acnes*, and *Eubacterium* species.

No significant changes were observed in any of the clinical or bacteriological parameters from the NP group (Table 3).

In the second trimester samples from the PG group, the anaerobe/aerobe ratio and the proportions of *B. melaninogenicus* ss. *intermedius* and *B. asaccharolyticus* differed significantly ($p < 0.05$, Student's t-

test) from the NP group. No other group differences were observed.

At the initial sampling period (12 weeks) Gram-negative anaerobic rods accounted for approximately 10 % of the total flora. At the time of maximum detectable bleeding (21–24 weeks) the Gram-negative anaerobic rods had increased to 39 % of the flora. The proportions of the Gram-positive facultative organisms, which included primarily *Actinomyces* species and *Streptococcus sanguis* remained unchanged throughout pregnancy. Although no single species decreased significantly during pregnancy, Gram positive anaerobic rods and Gram negative anaerobic cocci together accounted for 19.7 % of the flora initially and decreased to 5.4 % of the flora in the second trimester.
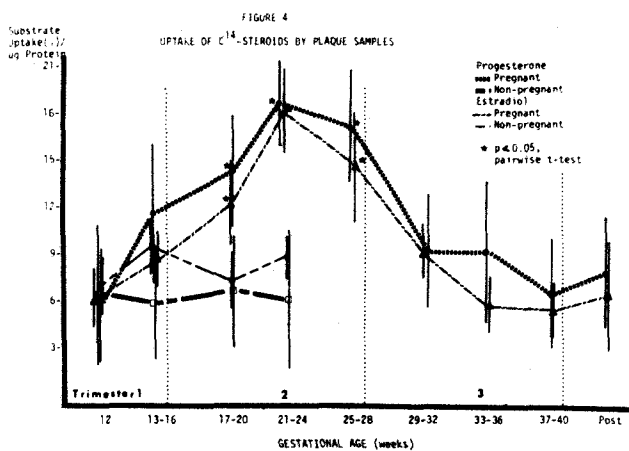
Fig. 4. Uptake of C¹⁴-esstradiol and C¹⁴-progesterone by plaque samples from pregnant and non-pregnant subjects. Data are expressed as substrate taken up relative to total substrate added.

Steroid uptake by plaque samples

The uptake of estradiol by PG plaque samples increased significantly ($p < 0.05$) from 4.4 % of the available steroid taken up initially to 18.1 % at 21–24 weeks (Fig. 4). After that time estradiol uptake decreased so that by 29–32 weeks the values had returned to those seen initially.

Progesterone uptake by PG plaque samples increased significantly ($p < 0.05$) from an initial mean uptake of 4.1 % to 18.4 % by 21–24 weeks (Fig. 4). Uptake of estradiol or progesterone by plaque samples from the NP group did not change during the experimental period (Fig. 4). Negligible steroid uptake occurred with heat killed plaque or when the reaction mixtures were incubated at 4°C.

In the pregnant subjects, mean plasma estrogens ranged from 5.8 to 6.4 ng/ml. in the first and second trimesters and post-partum. The mean estrogen level in the third trimester was 21.2 ng/ml. Progesterone ranged from 24.2 to 41.5 ng/ml. except for the third trimester samples which averaged 200.4 ng/ml. In the nonpregnant subjects mean estrogen levels ranged from 0.16 to 0.20 ng/ml. and mean progesterone levels ranged from 6.28 to 8.48 ng/ml.

These values are consistent with previous reports which used comparable techniques (Abraham et al. 1971, Wu & Lundy 1971).

In the PG group, recovery of *B. melaninogenicus* ss. *intermedius* had a positive correlation ($r = 0.93$, $p < 0.01$) with progesterone levels at 25–28 weeks and a positive correlation ($r = 0.55$, $p < 0.05$) with estradiol at 21–24 weeks. *F. nucleatum* had a positive correlation (0.67, $p < 0.05$) with serum progesterone at 33–34 weeks, and *B. (Capnocytophaga) ochraceus* had a positive correlation ($r = 0.68$) with serum estradiol at 21–24 weeks. *B. asaccharolyticus* exhibited a significant negative correlation with serum estradiol levels at 17–20 weeks ($r = -0.80$, $p < 0.05$) and at 21–24 weeks ($r = -0.68$). The plaque species in the NP group presented no significant correlations with serum steroid levels.

Discussion

In the present study gingivitis increased significantly between 13–28 weeks gestation and then decreased. Cross-sectional studies (Löe & Silness 1963, Silness & Löe 1964) described a gradual increase in gingivitis during pregnancy with apparent resolution following parturition. One longitudinal study (Hugoson 1970) confirmed that pattern, and another (Cohen et al. 1971) observed a statistically significant increase in gingivitis at six months gestation and a slight decrease in the third trimester. In previous studies (Löe & Silness 1963, Cohen et al. 1971), plaque remained essentially constant throughout the pregnancy. Hugoson (1970) reports, however, a decrease in the Plaque Index in the third trimester. In the present study the Plaque Index did not fluctuate but the viable count of organisms recovered from the samples showed a marked decline in the later stage of pregnancy.

The subgingival flora changed to a more anaerobic flora as pregnancy progressed.

The anaerobe/aerobe ratio increased significantly at an early stage of pregnancy (13–16 weeks) and remained high until the third trimester. The only organism whose proportions increased significantly during pregnancy was B. melaninogenicus ss. intermedius. At the peak of gingival bleeding the plaque proportions of this organism were five-fold greater than initial levels.

Minor increases were observed in other Gram-negative anaerobic rods, such that at 21–24 weeks all Gram-negative anaerobic rods together accounted for 39 % of the flora. This is greater than the proportions of Gram-negative anaerobes reported for chronic gingivitis i.e. 26–29 % (Slots et al. 1978, van Palenstein Helderman 1975), experimental gingivitis i.e. 20 % (Loesche & Syed 1978), and less than the levels in juvenile periodontitis i.e. 55–73 % (Slots 1976, Newman & Socransky 1977).

The increase during pregnancy in B. meloninogenicus ss. intermedius appears to be associated with elevations in systemic levels of estradiol and progesterone. This is based on the finding that there was increased steroid uptake by plaque samples which paralleled increased plaque proportions of B. melaninogenicus ss. intermedius. In addition, serum levels of estradiol and progesterone showed a positive correlation with plaque levels of B. melaninogenicus ss. intermedius until the late stages of pregnancy. Subsequent pure culture studies have shown that estradiol or progesterone can substitute for menadione as an essential growth factor in B. melaninogenicus ss. intermedius and B. melaninogenicus ss. melaninogenicus but not in B. asaccharolyticus or B. (Capnocytophaga) ochraceus (Kornman & Loesche 1979).

The effects of endogenous steroids on the normal microbial flora are essentially unknown. Ovarian function has been associated with changes in the vaginal tract bacterial flora, with a cyclic increase in aciduric lactobacilli being the most prominent finding (Weinstein et al. 1936). A recent study in rats (Larsen, Markovetz & Galask 1977) observed that anaerobic bacteria became nondetectable in genital samples after animals were ovariectomized. Exogenous estradiol administered to ovariectomized rats produced a prolonged increase in vaginal bacterial levels from 5×10^4 to 1×10^8 organisms/sample. Other investigations have demonstrated that steroid hormones inhibit the growth of pure cultures of a variety of Gram-positive bacteria (Yotis 1967), as well as Neisseria gonorrhoeae (Morse & Fitzgerald 1974).

Although it is difficult to assess the involvement of specific bacteria in pregnancy gingivitis, at the peak of gingival bleeding Gram-negative anaerobic rods had increased four-fold over initial levels.

Of perhaps greater importance is the observation that shifts in endogenous steroid levels may have a significant influence on the indigenous gingival crevice flora. Since the disease-associated microflora appears to represent an alteration in the normal subgingival ecology, the identification of factors which induce a shift in the flora may be of importance in determining what initiates periodontal disease. Subsequent reports will describe a potential biochemical mechanism for steroid interaction with Bacteroides species, and the cell-mediated immune response to oral antigens during pregnancy.

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