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Sex Steroid Hormones and Cell Dynamics in the Periodontium

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ABSTRACT: The biological changes that occur in tissues of the periodontium during puberty, the menstrual cycle, pregnancy, menopause, and oral contraceptive use have heightened interest in the relationship between sex steroid hormones and periodontal health. These clinical observations coupled with tissue specificity of hormone localization, identification of hormone receptors, as well as the metabolism of hormones have strongly suggested that periodontal tissues are targets for androgens, estrogens, and progestins. The etiologies of periodontal endocrinopathies are diverse; nonetheless, periodontal pathologies may be a consequence of the actions and interactions of sex steroid hormones on specific cells found in the periodontium.

KEY WORDS: androgens, estrogens, progestins, periodontium, gingiva, epithelial cell, fibroblast, periodontal diseases.

And take upon's the mystery of things, As if we were God's spies. Shakespeare, King Lear, v, 3.

I. INTRODUCTION

Homeostasis of multicellular organisms is contingent on communications between the endocrine, nervous, and immune systems. If any component of this triad falters, the survival of the organism is at stake. Therefore, life is dependent on a functioning endocrine system whose role is to maintain the internal milieu of a multicellular organism by using specific chemical messengers that recognize specialized macromolecules in sensitive cells to transduce a signal into a distinctive response.

The central focus of endocrinology revolves around specific regulatory molecules (i.e., hormones) that govern reproduction, growth and development, maintenance of the internal environment, as well as energy production, utilization, and storage. As a result of these global demands within the organism, it is not surprising that the actions of hormones are complex and diverse in nature. A single hormone may elicit a different outcome in a variety of tissues or a variety of hormones may be required to produce a single, particular effect in a group of tissues. For example, estrogens can function independently to stimulate growth of the breast (promotion of fat accumulation, connective tissue development, and ductal growth), yet must work in concert with other hormones (prolactin, progesterone, placental lactogen, glucocorticoids, thyroxine, and oxytocin) to regulate lactation. Because of the complex and diverse nature of hormones, it is difficult to arrange these chemical agents into discrete groups; nonetheless, they can be categorized into two classes according to their chemical structure. The peptide/amino acid derivative hormones represent a large and diverse group of molecules that range from complex polypeptides (luteinizing hormone) to single amino acid derivatives (catecholamines). The other large hormone group contains the steroid hormones. Steroid hormones are derivatives of cholesterol and consist of a combination of three rings of six carbon atoms each (phenanthrene) and one ring of five carbon atoms (cyclopentane) to form a complex hydrogenated cyclopentanoperhydrophenanthrene ring system (see Figure 1). This group can be further divided into three principal sets: corticosteroid hormones (glucocorticoids and mineralcorticoids),

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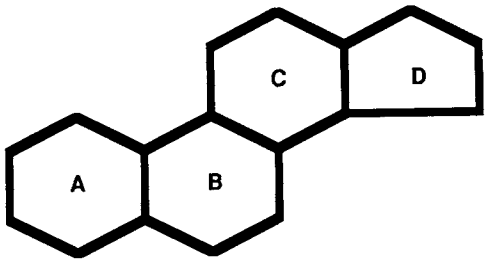


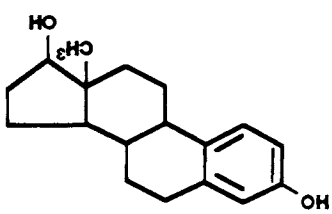
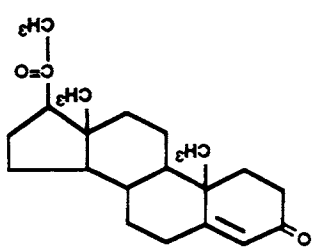
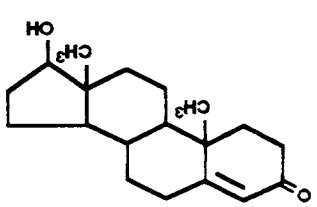
FIGURE 1. Schematic diagram of the cyclopentanoperhydrophenanthrene ring system. The three rings of six carbons each and one ring of five carbon atoms are identified as A, B, C, and D rings.

calcium-regulating steroid hormones (vitamin D and its metabolites), and gonadal or sex steroid hormones (estrogens, androgens, and progestins) (see Table 1).

The past 50 years have dramatically improved our perceptions concerning the actions of sex steroid hormones in health and disease. Although there is no doubt of the importance of sex steroid hormones in reproductive endocrinology, evidence has accrued that gonadal hormones have a much broader role in human tissues. Androgens, estrogens, and/or progestins are now believed to be directly or indirectly involved in the regulation of various, diverse tissues such as the brain, heart, kidney, skin, liver, and periodontium. Reports of the effects of sex steroid hormones in the periodontium, a unique structure composed of two fibrous (gingiva and periodontal ligament) and two mineralized (cementum and alveolar bone) tissues, have been noted for over a century. The effect of sex steroid hormones on each periodontal tissue has heightened interest in defining the specific relationship among androgens, estrogens, and progestins to normal function and disease in the periodontium.

The goal of this article is to provide the reader with current information about the relationship between sex steroid hormones and cells of the periodontium. To accomplish this goal, three prin-

TABLE 1
The Chemical Formula and Sources of Secretion for the Principal Sex Steroid Hormones

Hormone	Principal Source	Chemical Formula	
Estradiol	Ovary		$C_{18}H_{24}O_2$
Progesterone	Ovary, Placenta		$C_{21}H_{30}O_2$
Testosterone	Testis		$C_{19}H_{28}O_2$

cipal areas are explored. First, a broad overview of steroid hormone physiology is considered. A general understanding of hormone transport, metabolism, and mechanism of action provides the background necessary for understanding hormone action in the periodontium. Second, the significance of sex steroid hormone effects in the periodontium is reviewed. The reported clinical phenomena observed during times of fluctuations in hormone levels, the retention and metabolism of sex steroid hormones, as well as the identification of steroid receptors are important evidence for the periodontium being a target tissue for sex steroid hormones. Finally, various theories of the roles of steroid hormones in pathogenesis in the periodontium are critically evaluated. An understanding of the etiology of periodontal endocrinopathies is essential for the prevention and/or treatment of sex steroid hormone-sensitive periodontal diseases.

II. SEX STEROID HORMONE PHYSIOLOGY

A. Androgens

All natural androgens are derived from a 19-carbon tetracyclic hydrocarbon nucleus known as androstane. One of the most potent androgenic hormones, testosterone (17-hydroxy-androst-4-en-3-one), is synthesized by the Leydig's cells of the testes, the thecal cells of the ovary and the adrenal cortex. In men, testosterone is the principal plasma androgen and is reduced to dihydrotestosterone (17-hydroxy-5-androstan-3-one), the mediator of most actions of the hormone (Mooradian *et al.*, 1987) (see Figure 2). The irreversible metabolic conversion of testosterone to dihydrotestosterone (DHT) occurs only in tissues that contain the enzyme 5α-reductase (Wilson, 1975). Testosterone (but not DHT) can also be aromatized to estradiol by a number of extragonadal tissues (primarily adipose tissue and skeletal muscle), a common route of estrogen production in men. In women, the major plasma androgen is androstenedione (androst-4-ene-3,17-dione), which can be secreted into the bloodstream or converted into either testosterone or estradiol by the ovary. Once secreted into the bloodstream, the majority of androgens are transported to their sites of action by a hepatic-secreted carrier protein designated as

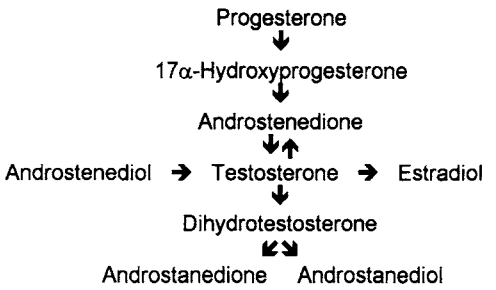


FIGURE 2. Biosynthesis and metabolism of testosterone.

sex hormone-binding globulin (44% bound), as well as serum albumins and other proteins (54% bound) (Dunn *et al.*, 1981). Secreted plasma androgens are also metabolized to physiologically weak or inactive molecules consisting of either 17-ketosteroids or polar compounds (diols, triols, and conjugates) for excretion by the kidney or liver (Kochakian and Arimasa, 1976).

The biological activities of androgens can be observed in virtually every tissue of the body. The more important functions of androgens include: (1) male sexual differentiation of wolffian ducts, external genitalia, and brain *in utero*; (2) development of adult male phenotype, including growth and maintenance of male sex accessory organs as well as anabolic actions on skeletal muscle, bone, and hair; (3) facilitation of human sexual behavior, and (4) regulation of specific metabolic processes in the liver, kidney, and salivary glands (Mooradian *et al.*, 1987).

B. Estrogens

The naturally occurring estrogens, estrone (3-hydroxyestra-1,3,5(10)-triene-17-one), estradiol (estra-1,3,5(10)-triene-3,17-diol), and estriol (estra-1,3,5(10)-triene-3,16,17-triol), are characterized by an aromatic A ring, a hydroxyl group at C-3, and either hydroxyl groups (C-16 and C-17) or a ketone group (C-17) on the D ring. Estradiol is the most potent estrogen and is secreted by the ovary, testis, placenta, as well as by peripheral tissues. Estrone is also secreted by the ovary; however, the principal source in both women and men is through extragonadal conversion of androstenedione in peripheral tissues (Siiteri and MacDonald, 1973). In premenopausal women the

most abundant physiological estrogen is estradiol, and in men and postmenopausal women the most abundant estrogen in the plasma is estrone (Weinstein *et al.*, 1974; Yen, 1977). Like other lipid-soluble hormones, estrogens are transported in the blood principally bound to carrier proteins; for example, estradiol in the plasma is bound by either albumin (60%) or sex hormone-binding globulin (38%), leaving only 2% of the hormone free (Wu *et al.*, 1976). Both estradiol and estrone are metabolized principally to estriol, which is the major estrogen detected in the urine (see Figure 3).

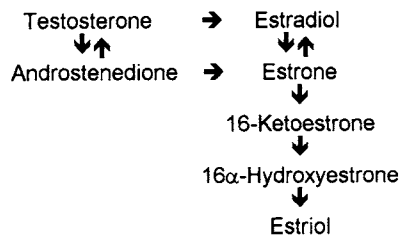


FIGURE 3. Biosynthesis and metabolism of estradiol.

The biological activities of estrogens in women include: (1) development, growth, and maintenance of secondary sex characteristics; (2) uterine growth; (3) pulsatile release of luteinizing hormone (LH) from the central nervous system; (4) thickening of the vaginal mucosa; and (5) ductal development in the breast. In the male, the physiological significance of estrogens is largely unknown but may be involved in the regulation of plasma androgen and estrogen levels as well as sexual behavior (Mawhinney and Neubauer, 1979).

C. Progestins

The natural progestins, or steroids that have progestational activity, are derived from a 21-carbon saturated steroid hydrocarbon known as pregnane. Corticosteroids are also derived from pregnane but differ from progestins because they contain an α-ketol group at C-17 and a ketone or hydroxy group at C-11. The principal progestational hormone secreted into the bloodstream is progesterone (pregn-4-ene-3,20-dione), which is synthesized and secreted by the corpus luteum, the placenta, and the adrenal cortex. Similar to androgens, the vast majority of progesterone is transported in the bloodstream by plasma proteins; however, progesterone in the human is primarily nonspecifically bound to globulin and albumin proteins (MacDonald *et al.*, 1991). The fate of plasma progesterone is dependent on hepatic, extrahepatic, and extraadrenal metabolism. Both 5α-dihydroprogesterone and deoxycorticosterone (21-hydroxy-4-ene-3,20-dione) are the most probable active progesterone metabolites; nonetheless, metabolic inactivation of progesterone to pregnanediol (5-pregnane-3,20-diol) is accomplished by the liver (MacDonald *et al.*, 1991) (see Figure 4).

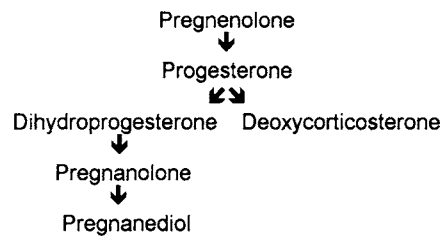


FIGURE 4. Biosynthesis and metabolism of progesterone.

The biologic activities of progestins are principally observed during the luteal phase of the menstrual cycle and pregnancy. Progesterone is necessary for glandular endometrial development prior to nidation, development of mammary lobules and alveoli as well as the maintenance of pregnancy (i.e., endometrial gland function, decreased excitability of myometrium and possible effects on the immune system to decrease rejection of the developing fetus). Progesterone also decreases hepatic secretion of VLDL and HDL, diminishes insulin action, stimulates the hypothalamic respiratory center, elevates basal core body temperature at ovulation, and enhances sodium excretion by the kidneys.

III. MECHANISM OF ACTION OF SEX STEROID HORMONES

In the bloodstream, sex steroid hormones exist in extremely low concentrations (in the femtomolar

to nanomolar range) yet are capable of regulating differentiation and growth in selected tissues distant from the site of secretion. The actions of sex steroid hormones become even more intriguing when one considers that the distinct biological effects of these hormones depend on nominal differences between relatively small (molecular weight approximately 300 g) molecules. For example, testosterone, which is capable of powerful virilizing effects, differs from estradiol only by one carbon atom and four hydrogen atoms (see Table 1). These apparently superficial differences in molecular structure of steroid hormones can alter the molecule's shape and qualitatively change biological activity. Specificity of hormone response also depends on the presence of intracellular proteins called receptors, which specifically recognize and selectively bind the hormone and act in concert with the hormone ligand to regulate gene expression.

Initial observations in the 1960s that sex steroid hormones bind to intracellular proteins with specificity and high affinity (Jensen *et al.*, 1968; Gorski *et al.*, 1968) have led to the predominant theory that steroid hormones act via receptors to initiate biological responses. In the past 3 decades, further insight into the actions of steroid hormones has been gained and there have been several comprehensive reviews on this subject (Hansen *et al.*, 1988; Sheridan *et al.*, 1988; Savouret, 1989; Martin *et al.*, 1990; Funder, 1991; O'Malley, 1991; Wilson, 1991). The current hypothesis of sex steroid hormone action (see Figure 5) begins with the secretion of the hormones into the bloodstream, where they circulate, principally bound (approximately 98%) to plasma proteins. In the circulation, the unbound or free hormone can enter the cell by diffusion and bind to macromolecules called receptors. These large intracellular protein receptors are located in both the cytoplasm and the nucleus of the cell. Depending on the type of steroid hormone, the intracellular localization of the receptor will vary. Gonadal hormones principally reside in the nuclear component of target cells, but whether the native receptor is confined exclusively to the nucleus is an object of current research. When the steroid hormone is bound to the receptor, it transforms the receptor to an active configuration and the activated receptor-steroid hormone complex binds with high affinity to specific nuclear sites (e.g., discrete DNA sequences, the nuclear matrix, non-

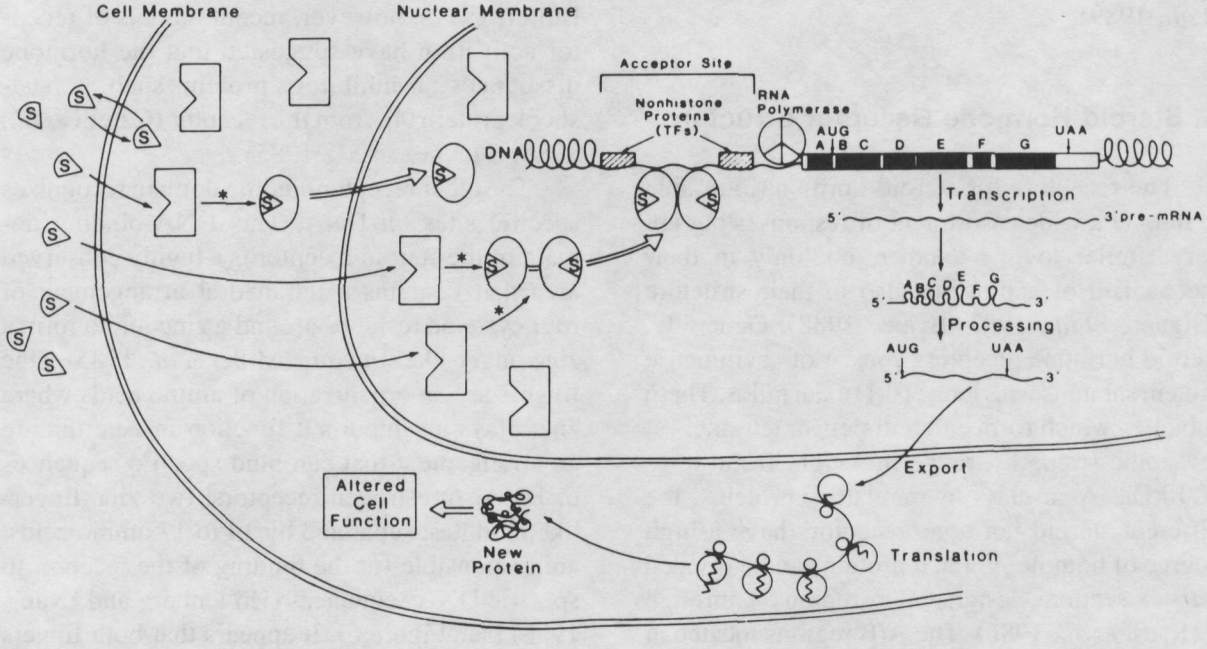


FIGURE 5. Mechanism of action of steroid hormones. The free steroid hormone (S) diffuses across the cell membrane and binds to a cytosolic or nuclear receptor. Once bound the receptor is activated and binds to a nuclear acceptor site where transcription of RNA occurs. (Reproduced with permission from Clark *et al.*, 1992.)

histone proteins, and the nuclear membrane). The activation step of this process may occur in the cytoplasm or the nucleus. Once the receptor-hormone complex is bound to nuclear regulatory elements, gene activation and transcription of messenger RNA occurs. Following the nuclear interaction, the receptor-hormone complex disassociates, leaving an unoccupied receptor and the steroid hormone. The dissociated receptor is thought to be in an inactive configuration that requires conversion to a form that can bind the steroid again and the steroid hormone is metabolized and eliminated from the cell. The steps of dissociation and receptor recycling are poorly understood at this time.

Although the regulation of gene transcription by hormone-receptor complexes in the nucleus appears to be the major biological action of sex steroid hormones, these molecules also have other behaviors that are independent of the genome. Recent studies have shown that androgens, estrogens, and progestins have membrane effects and can influence the production of second messenger systems. Sex steroid hormones can affect neural transmission (Ke and Ramirez, 1990; Lan *et al.*, 1990), modify the transport of calcium ions into cells (Blackmore *et al.*, 1990), and stimulate the intracellular concentration of polyamines (Koenig *et al.*, 1989).

A. Steroid Hormone Receptor Structure

The receptors for steroid hormones are able to initiate a wide assortment of responses but are very similar to one another, not only in their mechanism of action but also in their structure (Giguere *et al.*, 1987; Evans, 1988). Generally, steroid hormone receptors consist of asymmetric protein subunits with long (10:1) axial ratios. These subunits, which form either dimers or tetramers at low ionic strengths, range in weight from 80 to 100 kDa. As a class of regulatory proteins, the different steroid hormone receptors have a high degree of homology. Each protein can be divided into six sections designated as regions A through F (Krust *et al.*, 1986). The A/B regions located at the N-terminal are exceedingly variable in size (50 to over 500 amino acids) and have negligible amino acid similarities among the different receptors. The C region located between the N- and C-terminus is a remarkably conserved area that contains the DNA binding domain. The hydrophilic D region is not conserved in length or sequence but may serve as a hinge between the hormone- and DNA-binding domains. The E/F regions located at the C-terminal are similar in size (250 to 300 amino acids), have moderate amino acid homology among the different steroid receptor proteins, and contain the hormone-binding domain. Areas in both the N- and C-terminal are responsible for the transcriptional activation of the DNA (Gronemeyer *et al.*, 1987; Kumar *et al.*, 1987).

From these six regions, two important binding domains are present for sex steroid hormone receptors. In one binding domain, the functional activation of the receptor is dependent on a distinct, high-affinity binding site for a specific hormone. This steroid hormone binding domain is a large hydrophobic region located near the C-terminal. It has been suggested that the tertiary structure of receptor proteins forms a hydrophobic pocket that recognizes areas on both the A and D rings of the cyclopentanoperhydrophenanthrene ring system. Early models of steroid hormone action proposed that the hormone-induced allosteric changes in the receptor influenced activation (O'Malley and Buller, 1977); however, recent models of receptor activation have suggested that the hormone dissociates an inhibitory protein, such as heat-shock protein 90, from the receptor (Catelli *et al.*, 1985; Sanchez *et al.*, 1985).

The other receptor binding domain recognizes specific sites on DNA. This DNA-binding domain of the steroid receptor is a highly conserved area that contains a tetrahedral arrangement of four cysteine residues around a zinc ion to form a zinc finger-like structure (Miller *et al.*, 1985). Zinc fingers are an organization of amino acids where zinc plays an important function in determining an arrangement that can bind specific sequences of DNA. In estrogen receptors, two zinc finger-like modules, separated by 14 to 17 amino acids, are responsible for the binding of the receptor to specific DNA sequences (Hollenberg and Evans, 1988) (see Figure 6). It appears that both fingers are necessary for DNA binding; however, amino acids found in the proximal portion (P box) of the first finger and the distal portion (D box) of the second finger are crucial for determining the speci-

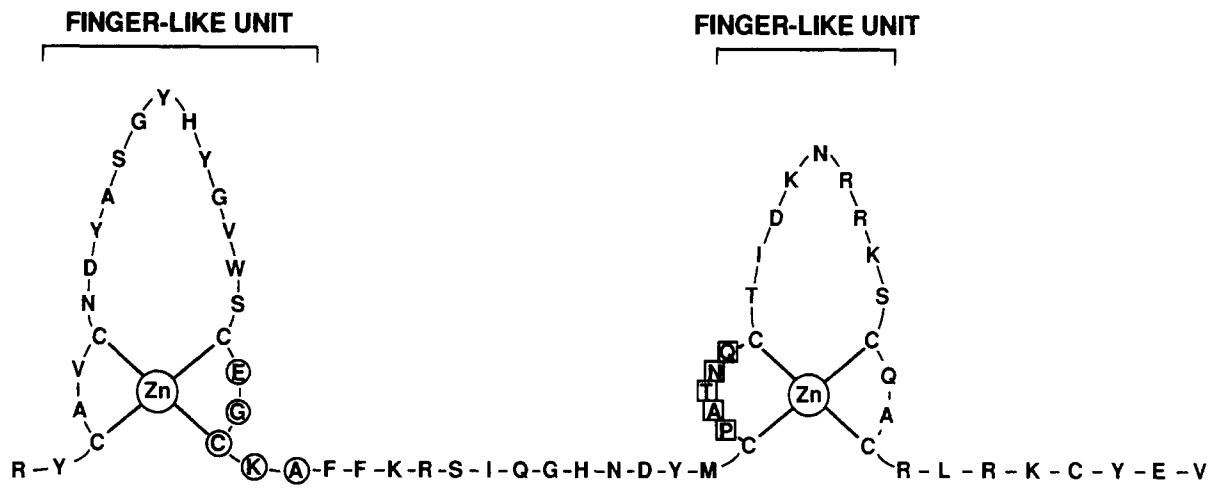


FIGURE 6. Estrogen receptor zinc finger-like units. The estrogen receptor contains two amino acid domains that independently fold around a zinc ion (Zn), forming a unique structure. The amino acids thought to interact with DNA bases are located in the proximal box (O) and the amino acids thought to stabilize the receptor are located in the distal box ([]).

ficity of binding (see Table 2). Green *et al.* (1988) have suggested that the N-terminal zinc finger-like domain determines target gene specificity, while the C-terminal zinc finger-like domain acts to stabilize the interaction between receptor and DNA.

TABLE 2
Sex Steroid Hormone DNA-Binding Domains

Receptors	P Box	D Box
Androgen	GSCKV	ASRND
Estrogen	EGCKA	PATNQ
Progesterone	GSCKV	AGRND

Note: The amino acid sequence of the DNA-binding domains found in the proximal box (P box) of the first finger-like region and the distal box (D Box) of the second finger-like region are listed.

After activation of the receptor, the receptor-steroid complex binds to a specific site on the DNA that is referred to as a steroid responsive element (SRE). SREs are unique for each receptor but have common nucleotide characteristics. In general, SREs for sex steroids contain two hexanucleotide sequences separated by a trinucleotide spacer (see Table 3). Once bound to a distinct DNA sequence, the hormone-receptor complex will regulate specific transcriptional events. The elements responsible for the regulation of transcription are not isolated to a single piece of DNA but are arranged in complex chromatin structures. For gene activation, steroid receptors probably must interact with transcriptional factors and/or with components in the chromatin structure. The exact nature of the interactions between steroid-hormone receptors and the constituent proteins in the nucleus responsible for gene activation is only beginning to be elucidated.

IV. THE PERIODONTIUM AS A TARGET TISSUE FOR SEX STEROID HORMONES

The homeostasis of the periodontium is a complex, multifactorial relationship that involves,

TABLE 3
Sex Steroid Hormone Binding Sites on DNA

Receptors	Steroid responsive elements
Androgen	GGTACA-N_3-TGTTCT
Estrogen	AGGTCA-N_3-TGACCT
Progesterone	GGTACA-N_3-TGTTCT

Note: Steroid responsive elements contain two hexanucleotide sequences separated by a trinucleotide spacer.

at least in part, the endocrine system. Evidence has accrued to suggest that tissues of the periodontium are modulated by androgens, estrogens, and progestins. Although all four tissues of the periodontium are regulated by sex steroid hormones at one time or another, most of our information about hormone actions and effects involve the gingiva. For this reason, this review focuses on the actions of androgens, estrogens, and progestins in the gingiva.

A. Clinical Phenomena

One piece of evidence implicating the gingiva as a target tissue for sex steroid hormones deals with clinical phenomena described during periods of hormone fluctuations. These clinical observations have confirmed an increased prevalence of gingival diseases with fluctuating sex steroid hormone levels, even when oral hygiene remained unchanged. In large part, the periodontal changes have been characterized in females, because they have distinctive cycles of sex steroid hormone secretion. Therefore, it is not surprising that the majority of clinical observations were derived from women at various times in their life.

1. Puberty

Epidemiological data have shown that gingivitis in children is a ubiquitous condition (Sutcliffe, 1972; Hefti, 1981; Cutress, 1986). It has been hypothesized that the incidence and severity of gingivitis in childhood is influenced by plaque levels, dental caries, mouth breathing, crowding of the teeth, tooth eruption, and puberty. Puberty, the complex process of sexual maturation resulting in an individual capable of reproduction, induces changes in physical appearance and behavior that is the direct result of increases in sex steroid hormones, primarily testosterone in males and estradiol in females. The dramatic rise in steroid hormone levels during puberty in both sexes was believed to have a transient effect on the inflammatory status of the gingiva (Marshall-Day, 1951); however, data to support this concept has been fragmentary. Several studies have demonstrated an increase in gingival inflammation in circumpubertal age individuals of both sexes, but data were not available on the pubertal status of the individuals (Parfitt, 1957; Sutcliffe, 1972; Hefti *et al.*, 1981). Sutcliffe (1972) examined 127 school age children in a 6-year longitudinal study and found an abrupt and transitory increase in the incidence of gingivitis without a change in plaque levels. The mean age at which girls and boys reached their maximum gingivitis experience was 12 years and 10 months and 13 years and 7 months, respectively. In a cross-sectional study examining 7380 children, gingival inflammation increased at age 11 in both sexes (Hefti *et al.*, 1971), while plaque levels remained constant in all age groups (personal communication from Dr. Hefti). In most longitudinal or cross-sectional studies, the data strongly indicate that there is a short period of time when children experience an exaggerated gingival inflammatory response to plaque. The crux of the reported relationship between the increased incidence of gingivitis and onset of puberty has depended on the chronological age of the cohort examined. However, it should be noted that chronological age is a poor predictor of the onset of puberty, and these data should only be considered circumstantial evidence that puberty-induced increases of plasma sex steroid hormones affect gingival tissues.

2. Menstrual Cycle

Following menarche, there is a periodicity of estrogen and progesterone secretion that is an important component for continued ovulation until the menopause. This rhythm of sex steroid hormone secretion over a 25- to 30-d period has been described as the menstrual cycle (see Figure 7). In humans, the menstrual cycle can be divided into a follicular or proliferative phase and a luteal or secretory phase. During the follicular phase, estrogen levels rise and prior to ovulation the preovulatory follicle significantly increases estrogen secretion, initiating a luteinizing hormone surge that stimulates progesterone secretion and ovulation. After ovulation, the luteal phase is marked by increased progesterone and estrogen secretion. During the final days of the luteal phase, if fertilization has not occurred, the plasma levels of progesterone and estradiol decline because of the demise of the corpus luteum.

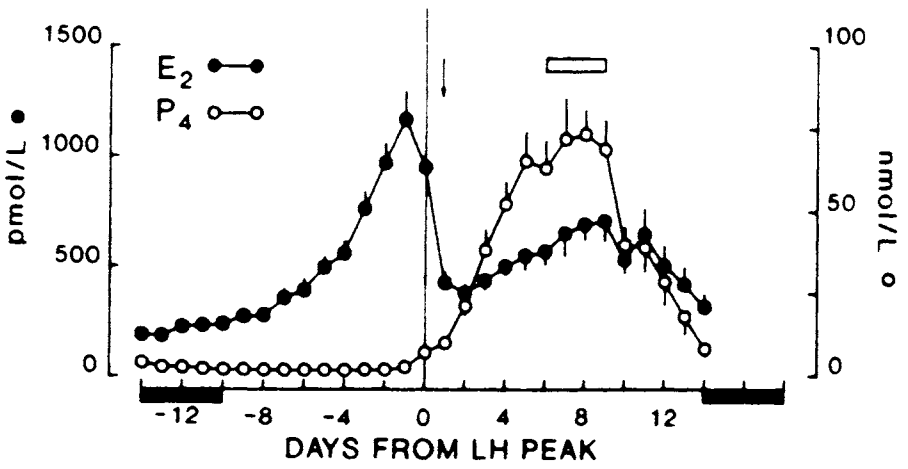


FIGURE 7. The pattern of circulating hormone levels in the human menstrual cycle. The plasma concentrations of progesterone (o) and estradiol (•) are depicted in the menstrual cycle. The follicular or proliferative phase occurs 12 d prior to the LH peak (days -12 to 0), and the luteal or secretory phase occurs 12 d after the LH peak (days 0 to 12). The solid bars represent menstrual periods and the open bar represents the time of implantation during the luteal phase. The arrow indicates the time of ovulation. (Reproduced with permission from Yen, 1991a).

In general, the periodontium does not exhibit clinically obvious changes during the menstrual cycle. Nonetheless, two different clinical findings have been noted in the oral cavity. One observation deals with the inflammatory changes that develop in gingival tissues of the cycling human female (Klein, 1934; Muhlemann, 1948; Lindhe and Attstrom 1967). In a very small percentage of women, ulcerations of the oral mucosa, vesicular lesions, and bleeding have been described several days before menstruation. Muhlemann (1948) clinically and histologically described a case of "gingivitis intermenstrualis" where bright red hemorrhagic lesions of the interdental papilla developed prior to the menses. The more common inflammatory changes that develop in the gingiva seem to involve less dramatic clinical alterations in the gingiva. For example, during the menstrual cycle, gingival exudate, an indicator of gingival inflammation, has been shown to increase during ovulation. Lindhe and Attstrom (1967) described an increase in the production of gingival exudate during ovulation, which returned to baseline during menses. In 88% of the subjects evaluated, there was at least a 20% increase in gingival crevicular fluid during ovulation. Holm-Pedersen and Loe (1967) also examined exudates from gingival crevices but found no change in gingival exudates during the menstrual cycle. Perhaps Holm-Pedersen and Loe (1967) were unable to verify changes in crevicular fluid flow during the menstrual cycle for several reasons. First of all, they failed to verify ovulation on the day samples were collected. It is well known that the time at which humans ovulate varies and these investigators may have missed the event since they relied only on a set date to collect samples from subjects. In addition, the subjects in the Holm-Pedersen and Loe study had very low levels of gingival inflammation (approximately 94% of sites scored had a GI = 0). In contrast, the cohort of patients examined by Lindhe and Attstrom (1967) had a preexisting mild gingival inflammation. Therefore, manifestation of hormonal influences in the gingiva of susceptible individuals may depend on the simultaneous presence of gingival inflammation. Finally, the technique used by Holm-Pedersen and Loe (1967) to collect gingival crevicular fluid has low sensitivity in detecting hormone-induced changes in crevicular fluid (Lindhe *et al.*, 1968a).

The other significant observation that has been described during the menstrual cycle is the appearance of aphthous ulcers. Whether aphthae

develop as a result of hormonal changes during the menstrual cycle remains controversial. Several investigators have described specific populations of women who develop aphthous ulcers during the luteal phase of the menstrual cycle (Strauss, 1947; Ferguson *et al.*, 1978). Dolby (1968) examined 20 women suffering recurrent oral aphthae and demonstrated maximal ulceration in the luteal period, but did not ascertain whether these changes were statistically significant. In another study, 415 women were examined by questionnaire and a significant incidence of recurrent oral ulcerations was reported during the menstrual period in regularly cycling women (Ferguson *et al.*, 1984). In contrast to these studies, other investigators have been unable to demonstrate any influence of the menstrual cycle on aphthous ulcer formation (Ship *et al.*, 1961; Segal *et al.*, 1974). For example, in one 36-month prospective study, 104 student nurses were examined for the incidence of aphthous lesions. Using daily diaries to monitor the time of menses and the onset of aphthae, no association was found between recurrent aphthous ulcers and the menstrual cycle (Segal *et al.*, 1974). If the prevalence of hormonally sensitive aphthous ulcers in the population is low, the population of patients examined by Segal and colleagues (1974) may not have been sufficiently large to distinguish if menstrual-related mucosal ulcers develop. Anecdotal reports of individuals with aphthous ulcer formation in the luteal phase of the menstrual cycle suggest that there is a population of women whose oral mucosa exhibits cyclic changes; however, what role, if any, sex steroid hormones play in aphthous ulcer formation is not understood.

3. Oral Contraceptives

Oral contraceptive agents are one of the most widely utilized class of drugs. In the U. S., it has been estimated that approximately 10,000,000 women are currently using these agents (Murad and Kuret, 1990). Current oral contraceptives consist of low doses of estrogens (50 µg/d) and/or progestins (1.5 mg/d); however, it should be noted that initial hormone contraceptive formulations contained higher concentrations of sex steroid hormones and that early clinical studies examined gingival conditions in women using these higher doses of estrogens and/or progestins (see Table 4).

Numerous clinical studies have recorded gingival changes that develop as a result of the use of oral contraceptive agents. Several case reports described gingival enlargement induced by oral contraceptives in otherwise healthy females with no history of gingival hypertrophy or hyperplasia (Lynn, 1967; Kaufman, 1969; Sperber, 1969). In all cases, the gingival overgrowth was reversed when oral contraceptive use was discontinued or the dosage reduced. In addition, various clinical

TABLE 4
Concentrations of Sex Steroid Hormones in Oral Contraceptives Reported in Case Reports and Clinical Studies

		Oral Contraceptive	
Reference	**Type of Report**	**Progestin**	**Estrogen**
El-Ashiry *et al.* (1970)	Clinical study	4 mg megestrol acetate	50 µg ethenyl estradiol
Kaufmann (1969)	Case report	1 mg ethynodiol diacetate	100 µg mestranol
Knight and Wade (1974)	Clinical study	Steroid not reported no dose reported	Steroid not reported; no dose reported
Lindhe and Bjorn (1967)	Clinical study	5 mg megestrol acetate	100 µg mestranol
		components and concentrations of Gestadydral (produced by Hoffman-La Roche & Co.) were not reported.	
Lynn, (1967)	Case report	30 mg daily of an oral contraceptive that contained both norethindrone and mestranol.	
Pankhurst *et al.* (1981)	Clinical study	Steroid not reported between 0.15–4.0 mg	Steroid not reported; between 20–50 µg
Sperber (1969)	Case report	2 mg norethindrone	100 µg mestranol

studies have demonstrated that women using hormonal contraceptive drugs have a higher incidence of gingival inflammation in comparison to women who do not use these agents (Lindhe and Bjorn, 1967; El-Ashiry *et al.*, 1970; Pankhurst *et al.*, 1981). The use of oral contraceptives has also been associated with changes in periodontal attachment level. Knight and Wade (1974) found a statistically significant loss of attachment in women taking exogenous sex steroid hormones for over 1 1/2 years despite no differences in gingival inflammation between oral contraceptive users and nonusers. In contrast, Pankhurst *et al.* (1981) detected no change in the periodontal attachment level despite an increase in the prevalence of gingival inflammation in women taking oral contraceptives when compared with controls. Although the Pankhurst *et al.* (1981) study found no difference in attachment levels among the groups, a trend of increased attachment loss was evident in women taking oral contraceptives. The failure to find statistical significance in this study may be due to the degree of error involved in the manual measurement of attachment levels, because Knight and Wade (1974) used a modified method to measure attachment loss to obtain "greater accuracy."

4. Pregnancy

Some of the most remarkable endocrine alterations accompany pregnancy. The increases in sex steroid levels that begin prior to fertilization during the luteal phase of the menstrual cycle continue once implantation of the embryo occurs and are maintained until parturition (see Figure 8). For example, pregnant women near or at term produce large quantities of sex steroid hormones (20 mg of estradiol, 80 mg of estriol, and 300 mg of progesterone) on a daily basis. This prominent increase in plasma hormone levels over several months has a dramatic effect on the periodontium throughout pregnancy.

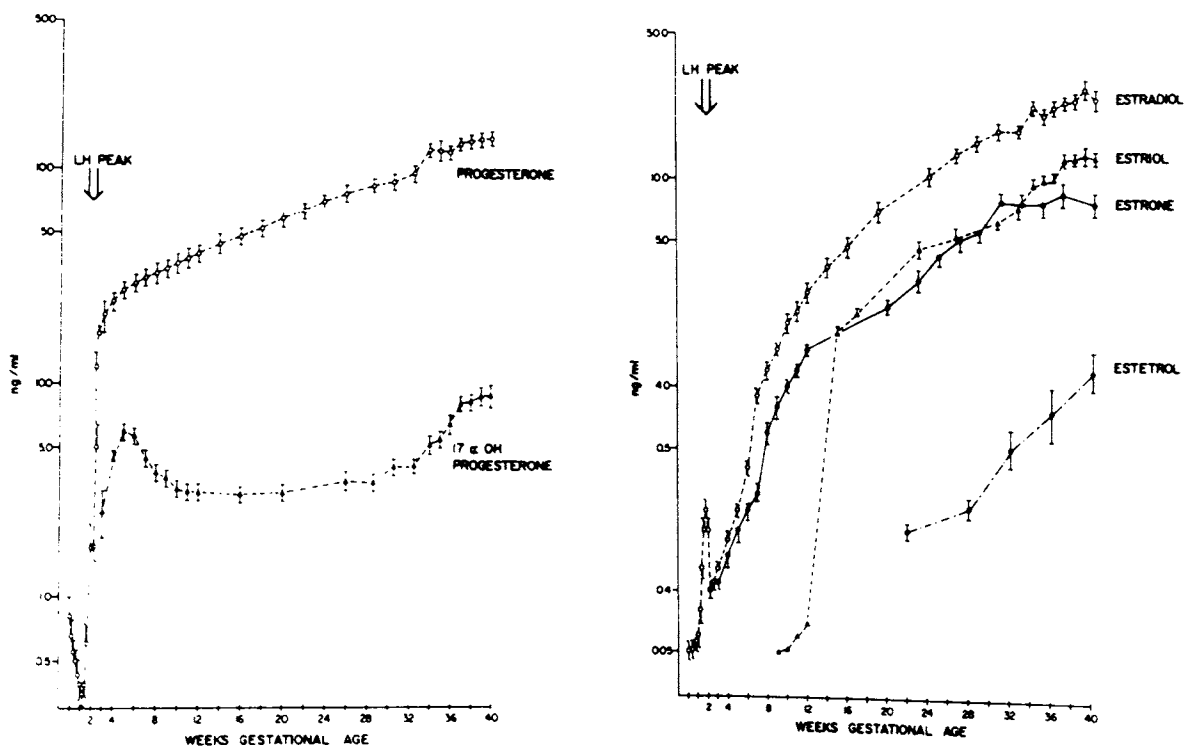


FIGURE 8. Circulating hormone levels during human pregnancy. The plasma concentrations of estrogens (right panel) and progestins (left panel) from the beginning of the menstrual cycle (week 0), to fertilization (week 2), to parturition (week 40) are illustrated for a typical woman. The human gestational period can be separated into trimesters. In these figures the first, second, and third trimesters range approximately 2 to 15 weeks, 15 to 27 weeks, and 27 to 40 weeks, respectively. (Reproduced with permission from Yen, 1991b.)

During pregnancy, the prevalence and severity of gingivitis have been reported to be elevated (Loe and Silness, 1963; Loe 1965; Hugoson, 1971; Arafat, 1974). Loe and Silness (1963) used a cross-sectional study to examine 121 pregnant and 61 postpartum women for changes in gingival inflammation. During pregnancy, 100% of women exhibited gingival inflammation when compared with postpartum controls. The prevalence and severity of the gingival inflammation were significantly higher in the pregnant vs. the postpartum patients, even though plaque scores remained the same between the two groups (Silness and Loe, 1963). Similar results were obtained in a longitudinal study that examined 26 women during and following pregnancy (Hugoson, 1971). In addition to confirming that the severity of gingival inflammation was exacerbated throughout pregnancy and reduced following parturition, this investigation also demonstrated that the severity of gingival inflammation was correlated with sex steroid hormone levels during pregnancy and not with the amount of plaque. Furthermore, gingival probing depths are larger (Loe and Silness, 1963; Hugoson, 1971; Miyazaki *et al.*, 1991), bleeding on probing or toothbrushing is increased (Arafat, 1974; Miyazaki *et al.*, 1991), and gingival crevicular fluid is elevated (Hugoson, 1971) in pregnant females. Finally, women who are pregnant also exhibit a low prevalence (0.5 to 0.8%) of localized gingival enlargements (Maier and Orban, 1949; Loe and Silness, 1963). These pregnancy-induced gingival overgrowths are reversed following parturition (Ziskin and Nesse, 1947).

5. Menopause

In contrast to pregnancy when hormone levels are significantly elevated, during menopause ovarian function is declining and there is a reduction in the production and secretion of sex steroid hormones. Since the average age of menopause is 51 years (McKinlay *et al.*, 1972), a high percentage of women will spend, on average, one third of their lives with no ovarian function. In the next 2 decades, approximately 40 million women in the U.S. will pass through menopause.

The changes observed in the gingiva during and after menopause are quite different from other times in a woman's life. There are no endogenous hormone-induced increases in gingival inflammation or size; instead, desquamations of gingival epithelium have been commonly reported. The question has arisen as to whether gingival vesiculobullous lesions, which develop in women after the climacteric, are a manifestation of one of several different vesiculobullous diseases, a variant of a single vesicular dermatologic disorder, or a distinct disease under hormone control.

Desquamative gingival diseases were described in the late nineteenth century by Tomes (Tomes and Tomes, 1894), who noticed "a singular modification of chronic inflammation of gums, in which, instead of becoming thickened and irregular on the surface, they seem rather to decrease in size, assuming a very smooth and polished surface and mottled aspect. The patients suffering from this complaint for the most part seem to be poor, middle-aged women in whom menstruation was becoming irregular or had altogether ceased." Early investigators believed gingival lesions that developed in postmenopausal women were primarily the result of a change in their hormonal status. However, in the mid-twentieth century, McCarthy *et al.* (1960) concluded, after a review of the literature and the study of 40 cases over 12 years, that chronic desquamative gingivitis was probably a manifestation of several diseases with multiple etiologies. If there are several different disease entities, the contribution(s) of sex steroid hormones in the initiation and progression of specific desquamative lesions is (are) largely undefined.

Circumstantial clinical data are available to suggest that sex steroid hormones may play a role in some types of desquamative gingival lesions. First of all, most patients with desquamative gingival lesions are middle-aged and approximately 80% are female (Nisengard and Rogers, 1987). Second, some lesions such as benign mucous membrane pemphigoid (Shklar and McCarthy, 1971; Silverman *et al.*, 1986) and lichen planus (Kovesi and Banoczy, 1973; Silverman and Griffith, 1974) are reported to have a female sex predilection. Finally, exogenous estrogens have been used to successfully treat desquamative lesions (Richman and Abarbanel, 1943a,b; Ziskin

and Zegarelli, 1945; van Minden, 1946). This final piece of evidence suggests that some lesions are estrogen-sensitive; however, the studies must be viewed cautiously because the investigators were not blinded, placebos were not used, and the types of lesions were not identified.

Similar to desquamative gingival lesions, the association between periodontitis and postmenopausal osteoporosis is not well defined. The evidence that osteoporosis, following surgical or natural menopause, may be involved with the loss of periodontal attachment is circumstantial. Although the theories for the pathogenesis of osteoporosis are diverse, it is known that estrogen deficiency is an important factor in bone loss (Richelson *et al.*, 1984). In addition, positive correlations between estrogens and bone density have been demonstrated (Johnston *et al.*, 1985; Steinberg *et al.*, 1989). Considering these findings, it is not surprising that bone mass from edentulous mandibles has been shown to differ by age and sex. Several cross-sectional studies have demonstrated decreased bone mass and density (Kribbs, 1990; Moshchil' *et al.*, 1991), as well as reduced bone mineral content (von Wopwern, 1988) in edentulous mandibles of postmenopausal females. A variety of studies have attempted to provide insight into the relationship of osteoporosis to periodontitis, but the results of these studies have been equivocal (Groen *et al.*, 1968; Phillips and Ashley, 1973; Ward and Manson, 1973; Manson, 1976; Baum, 1981; Daniell, 1983; Kribbs, 1990). To ascertain if osteoporosis is a risk factor for periodontal attachment loss in postmenopausal women, well-designed, long-term longitudinal investigations controlling for temporal period following the menopause, plaque levels, medications used (e.g., hormones, etc.), age, race, and social habits (e.g. smoking, drinking, etc.) are needed.

B. Tissue Specificity of Localization

Steroid hormone receptors are not ubiquitous but are located in high concentrations in hormone-sensitive tissues termed target tissues. Cytoplasmic and nuclear receptors that bind specific hormones lead to the preferential accumulation and retention of hormones in target tissues. In a variety of species, the accumulation and localization of androgens, estrogens, and progestins have been observed in the periodontium. Exposure of murine gingiva to dilute concentrations (136 ηg/100 g body weight) of tritiated estradiol *in vivo* results in the accumulation and retention of labeled estradiol similar to that seen in the uterus at 1 and 4 h after subcutaneous injection (Formicola *et al.*, 1970). Estrogen treatment for 7 d reduced the concentration of labeled estradiol in murine gingiva and uterus by 86.5 and 54.6%, respectively (Formicola *et al.*, 1970). In addition, autoradiographic studies have demonstrated nuclear localization of estradiol in human gingival epithelium (Vittek *et al.*, 1982) as well as human (Vittek *et al.*, 1982) and primate (Aufdemorte and Sheridan, 1981) gingival fibroblast cells. Similar to estrogens, nuclear localization of methyltrienolone (R1881), a synthetic androgen that binds primarily to androgen receptors, in human gingival epithelial cells and fibroblasts has also been confirmed using autoradiographic procedures (Vittek *et al.*, 1985). In contrast to estradiol and methyltrienolone, autoradiographic localization of progesterone in gingival epithelial cells has not been demonstrated. Mohamed (1974) found no uptake of progesterone into gingival epithelium and only a slight accumulation in the cytoplasm of gingival fibroblasts from sexually immature rabbits. Using a radiolabeled synthetic progestin (^{3}H-ORG 2058) in bilateral oophorectomized and adrenalectomized primates that were pretreated for 3 d with estradiol benzoate (50 μg/kg body weight), ^{3}H-ORG2058 was observed in the nuclei of fibroblasts found in the lamina propria of the gingiva, whereas gingival epithelial nuclei did not concentrate the radiolabeled progestin (Weaker *et al.*, 1988).

C. Sex Steroid Hormone Metabolism

The metabolism of sex steroid hormones in target tissues will either degrade and inactivate the hormone or alter the hormone and increase the potency. The gingiva of man and animals contains the necessary enzymatic machinery to metabolize all sex steroid hormones by common metabolic pathways. To begin, the conversion of estrone to estradiol occurs in both healthy and chronically inflamed human gingiva (ElAttar and Hugoson, 1974a) and may represent a bio-

activation process. Gingival slices, incubated with radiolabeled estrogens, were capable of metabolizing estrone to estradiol. The mean rate of conversion for estrone to estradiol was threefold higher in inflamed vs. healthy gingival tissues. In either inflamed or healthy gingival tissues, there was little or no detectable conversion of estradiol to estrone. The enhanced metabolic conversion of estrone to estradiol has also been documented in inflamed canine gingiva (ElAttar and Hugoson, 1974b).

The metabolism of androgens has been reported in human and murine gingiva. In the adult male rate, radiolabeled testosterone incubated with gingiva and vestibular oral tissue was converted primarily to 5α- and 5β-reduced steroids (Vittek *et al.*, 1974). The administration of medroxyprogesterone acetate for 2 weeks caused a twofold increase in 5α-reductase activity (Vittek *et al.*, 1974). In human males and females, the conversion of androstenedione to testosterone (ElAttar, 1974; Vittek *et al.*, 1979) as well as the conversion of testosterone to various 5α- and 5β-reduced steroids (ElAttar, 1974; Vittek *et al.*, 1979; Ojanotko *et al.*, 1980) is characteristic of gingiva. Similar to estrogen metabolism, inflamed human gingiva is much more efficient in converting androstenedione to testosterone (ElAttar, 1974; Vittek *et al.*, 1979) and testosterone to 5α- and 5β-DHT (Vittek *et al.*, 1979).

Unlike the conversion of androgens and estrogens to metabolically active forms in the gingiva, the metabolism of progesterone is principally to inactive metabolites; however, similar to androgens and estrogens, progesterone metabolism is elevated in inflamed gingival tissue (ElAttar *et al.*, 1973; Ojanotko-Harri, 1985; Ojanotko-Harri *et al.*, 1991). In human gingiva, progesterone is metabolized to a variety of metabolic products, and the principal metabolites include 20α-hydroxy-4-pregnen-3-one (ElAttar *et al.*, 1973; Ojanotko-Harri, 1985), 5α-pregnane-3,20-dione (ElAttar *et al.*, 1973; Ojanotko-Harri, 1985), 3β-hydroxy-5α-pregnane-20-one (Ojanotko-Harri, 1985), 20α-hydroxy-5α-pregnan-3-one (Ojanotko-Harri, 1985), and some other "more polar" metabolites (Ojanotko-Harri, 1985). Of the major metabolites, only 20α-hydroxy-4-pregnen-3-one retains any progestational activity (Ojanotko-Harri, 1985).

D. Sex Steroid Hormone Receptors

Target tissues for steroid hormones contain proteins that specifically recognize, retain and initiate the actions of hormones. In the periodontium, intracellular binding proteins have been partially characterized for estrogens (Vittek *et al.*, 1982b; Musajo *et al.*, 1984; Lewko and Anderson, 1986; Staffolani *et al.*, 1989), androgens (Southern *et al.*, 1978; Vittek *et al.*, 1985), and progesterone (Vittek *et al.*, 1982a). The human gingival cytosol receptor for estrogen is a high-affinity (Kd 340 *fM*), low-capacity (4.5 *f*mol/mg protein), heat- and proteolytic enzyme-sensitive protein that exhibits steroid specificity of binding to estradiol but not cortisol, progesterone, or testosterone (Vittek *et al.*, 1982b). During periods of gingival inflammation, the number of gingival estrophiles is elevated by almost tenfold (Staffolani *et al.*, 1989). Human gingiva also contains an androgen cytosol receptor that binds with high-affinity (Kd 2.2 η*M*) and low-capacity (190 *f*mol/mg protein) to a heat-sensitive protein that exhibits steroid specificity to DHT (the principal mediator of androgen action in adult tissues) but not to progesterone, dexamethasone, cortisol, androstenedione, or estradiol (Southern *et al.*, 1978). Additional techniques using immunohistochemical detection have identified androgen receptors in the nuclei of basal gingival epithelial cells and gingival fibroblasts (Ojanotko-Harri *et al.*, 1992). Little is known about the progesterone receptor in human gingiva except that progesterone recognizes a cytosolic protein (Vittek *et al.*, 1982c); the specificity and affinity of the protein remain to be described. In contrast to humans, progesterone receptors have been characterized in rabbit gingiva and exhibit high-affinity (Kd 2.7 η*M*), low-capacity (10 *f*mol/mg protein) binding to a heat- and proteolytic enzyme-sensitive protein that demonstrates a pattern of steroid specificity similar to progesterone receptors obtained from other target tissues (Vittek *et al.*, 1982a).

V. ETIOLOGIES OF PERIODONTAL ENDOCRINOPATHIES

A great deal of evidence has accumulated to implicate the periodontium as a target tissue for steroid hormones; nonetheless, the specific rela-

tionship of sex steroid hormones to periodontal endocrinopathies remains an enigma. The role of gonadal hormones in periodontal diseases is obscure; however, several explanations have been forwarded in an attempt to describe how androgens, estrogens, and progestins affect tissues of the periodontium. To date, the most prominent explanations used to describe hormone action in the periodontium have dealt with hormone effects on microbial organisms, the vasculature, the immune system, and specific cells in the periodontium (see Table 5). To be sure, the response of the periodontium in disease is probably not because of a single mechanism but rather multifactorial in nature; nevertheless, each theory of how sex steroid hormones induce disease in the periodontium is examined.

A. Hormones and Microbial Organisms

Gingivitis is considered to be primarily a microbial disease that can be modulated by different systemic and environmental factors (Stamm, 1986). Therefore, it was natural to assume that exacerbations in gingival inflammation observed

TABLE 5
Summary of Potential Etiologies for Gingival Endocrinopathies

References	Proposed mechanism	Comments
Delaney *et al.* (1986) Wojcicki *et al* (1987)	Increased prevalence of gingivitis at puberty is due to elevated levels of specific bacteria	Changes in microbiota were not correlated with changes in hormone status; Yanover and Ellen (1986), found no change in oral flora in a longitudinal study of human females at puberty
Kornman and Loesche (1980) Kornman and Loesche (1982)	Increased incidence of gingivitis during pregnancy is due to an increase in *Provetella intermedia*; estradiol and progesterone accumulated by *P. intermedia* and used as a substitute for vitamin K	Longitudinal study of 22 pregnant women found increases in *Provetella intermedia only* during the second trimester; may be a nonspecific accumulation of estrogen and progesterone by *P. intermedia*; only pharmacologic concentrations of sex steroid hormones are effective as a substitute for vitamin K; Jonsson *et al.* (1988) observed no changes in oral flora in pregnant women.
Lindhe and Branemark (1967a, b, c)	Increased incidence of gingivitis in women using oral contraceptives is due to reduction in corpuscular flow rate, increased vascular permeability, and vascular proliferation in gingiva	Only pharmacologic concentrations of sex steroid hormones were effective; examined in hamster cheek pouch or depilated ear of rabbit
Raber-Durlacher *et al.* (1992)	Increased incidence of gingivitis during pregnancy is due to elevated levels of CD1, CD3, and CD4 cells	Evidence to support theory that pregnancy gingivitis may be a consequence of reduced immunoresponsiveness, resulting from directed cytotoxicity against B cells and macrophages by a subset of helper T cells, is highly speculative.
Fukuda (1971) Kofoed(1971) Nicolau *et al.* (1979) Mariotti *et al.* (1990) Mariotti (1991)	Changes in gingival phenotype (i.e., gingival enlargement, epithelial desquamation etc.) are due to the stimulation of specific populations of fibroblasts and epithelial cells by sex steroid hormones; secretion of hormone-stimulated extracellular matrix components has a permissive or instructive effect on cells of gingiva	Evidence to support dynamic reciprocity of periodontium is limited, particularly in respect to the actions of sex steroid hormones

during increases in plasma sex steroid hormones were due to hormone-induced alterations in the microbial flora of the gingival sulcus. Unfortunately, data to support a transient increase in a specific microorganism during puberty or pregnancy have been equivocal, and in some cases the speculative interpretation of specific results may have been overzealous.

Several investigators have described a transient increase in black-pigmented, Gram-negative obligate anaerobic rods in children during puberty. Using three indicators of sexual maturation (menarche, breast development and pubic hair development), Delaney *et al.* (1986) grouped 22 girls into four stages of pre- and/or postmenarchal development and examined the changes in the percentage of *Actinomyces* sp. (*A. naeslundii*, *A. viscosus*, *A. odontolyticus*), *Actinobacillus actinomycetemcomitans*, total surface-translocating bacteria (*Capnocytophaga*, *Wolinella*, and *Eikenella* sp.), *Fusobacterium nucleatum*, spirochetes and black-pigmenting bacteria (*Prevotella intermedia*, *Bacteroides melaninogenicus*, *Bacteroides denticola*, *Porphyromonas gingivalis*) during these periods of time. Unfortunately, the data provided were a composite of the four groups examined; nonetheless, the authors did state that black-pigmented bacteria "in the predominant cultivable microbiota of the subgingival plaque in the 22 subjects were related to only one developmental assessment, that of menarchal stage, or composite sexual maturation (Kruskall-Wallis: $p < 0.05$)". Wojcicki *et al.* (1987), using bone age to confirm puberty, grouped 21 girls and 21 boys into three stages (prepubertal, pubertal, and postpubertal) and examined *Fubosbacterium* sp., total black-pigmented bacteria and *Prevotella intermedia*, *Bacteroides melanogenicus*, and *Bacteroides denticola/Bacteroides loeschii* in each stage. In contrast to Delaney *et al.* (1986), they found that the increase in black-pigmenting bacteria at puberty was not reversed in postpubertal subjects; however, a significant increase in *P. intermedia* did occur and only in subjects judged to be in puberty. Neither study examined the changes in hormone status of the children as changes in the microbiota developed. Contrary to the previous two studies, Yanover and Ellen (1986) were unable to find any changes in oral flora during puberty. Their study evaluated longitudinal changes in black-pigmenting bacteria in 18 subjects progressing normally through puberty and cross-sectional changes in 9 subjects with precocious puberty. They found that *P. intermedia* was not correlated with physical maturation in either group. Furthermore, plasma estradiol levels were not correlated with the levels of black-pigmenting bacteria.

Additional controversy exists as to whether subgingival bacterial flora is influenced in women during pregnancy. In a cross-sectional study, Jonsson *et al.* (1988) found no difference in levels of *P. intermedia* at any time during pregnancy or between pregnant and nonpregnant control subjects. In contrast, a longitudinal study of 22 women examined several species of bacteria during all three trimesters and found a significant increase in the percentage of total colony-forming units for *P. intermedia* but only during the second trimester (21 to 24 weeks) (Kornman and Loesche, 1980). In addition, gingival plaque samples from pregnant patients in the second trimester were reported to accumulate significantly more estradiol and progesterone than plaque samples from other time periods. To explain these results, a later study demonstrated that both estradiol and progesterone were selectively accumulated by *P. intermedia* and both ovarian hormones could be used by *P. intermedia* as a substitute for vitamin K; therefore, progesterone and/or estrogens could foster the growth of this microorganism (Kornman and Loesche, 1982). Although the data suggest that *P. intermedia* increased during the second trimester as a result of elevated ovarian hormone levels (Kornman and Loesche, 1980), the increase of *P. intermedia* during the second trimester of pregnancy may be independent of estrogens or progesterone and occur for other reasons. There are several interesting observations relating to why *P. intermedia* may not be dependent on ovarian hormones. The first observation deals with the transient increase of *P. intermedia* during the second trimester followed by the decline of this microorganism to postpartum control values during the third trimester, despite elevated hormone levels (see Figure 8). If bacterial growth was dependent on sex steroid hormones, then increases that develop in the second trimester would also be evident in the third trimester and would decrease following parturition as do plasma hormone levels. Further, the accumulation of estradiol and progesterone in second trimester plaque samples

or pure cultures of *P. intermedia* may be a nonspecific accumulation. Since competitive inhibition with other steroid-like molecules was not examined, it is unknown if the accumulation of estradiol or progesterone was steroid specific or purely dependent on the lipophilic nature of the plaque sample. Finally, if certain bacteria can use sex steroid hormones as a substitute for vitamin K, these bacteria should be able to convert to estradiol or progesterone at physiologic concentrations. This does not appear to be the case because the concentrations of estradiol and progesterone used as a substitute for menadione, a vitamin K analog, were pharmacologic (micromolar concentrations) in nature and may not represent what is seen in physiologic situations, such as in pregnancy.

Whether the increases in plasma estrogen and progesterone during puberty or pregnancy stimulate specific bacterial species remain inconclusive. The current data indicate that *P. intermedia* may be elevated during pregnancy and around the period of puberty. Nevertheless, whether this bacterial species is responsible for the observed gingival inflammation during puberty and pregnancy and is increased as a result of increasing steroid hormone concentrations remain to be determined.

B. Hormones and the Gingival Vasculature

In both intact and hormone-treated castrate animals, one of the early responses of gonadal hormones in accessory reproductive tissues involves increased blood volume, flow rate, hyperemia, and enlarged microvascular surface (Spanziani, 1975). In females, estrogen, in physiologic concentrations, is the principal sex steroid hormone responsible for alterations in blood vessels. For example, in the uterus estrogen will stimulate blood flow (Kalman, 1958; Greiss and Gobble, 1970) and increase the movement of fluid and plasma proteins across blood vessel walls within minutes of administration (Hechter *et al.*, 1942; Friederici, 1967). During the human menstrual cycle, endometrial blood flow increases concomitantly with the rise in plasma estrogen levels in the follicular phase; moreover, endometrial blood flow decreases during the luteal phase of the menstrual cycle when estrogen levels are decreasing and progesterone levels are elevated (Prill and Gotz, 1961).

Although evidence exists for estrogen-induced changes of vascular function, how the responses are mediated remain obscure. Several putative mechanisms by which estrogens may control blood vessel tone include inhibiting movement of calcium ions through the potential sensitive calcium channels of uterine arteries after metabolic conversion to catechol estrogens (Stice *et al.*, 1987), influencing release (Bengtsson, 1978), or disposition (Hamlet *et al.*, 1980) of sympathetic transmitter, or affecting alpha adrenoceptor number or affinity (Hoffman *et al.*, 1981; Culocci *et al.*, 1982). Estrogens may increase capillary permeability by stimulating the release of various mediators (e.g., adenosine, bradykinin, vasoactive intestinal polypeptide, neurotensin, Substance P, various prostaglandins, AMP, ADP, ATP, cAMP, guanosine, thymidine, histamine, cytidine, uridine, acetylcholine, isoproterenol, and glycosaminoglycans); however, none of these mediators have been able to mimic the qualitative and quantitative changes in blood flow induced by estrogen (Magness and Rosenfeld, 1992). In contrast to the principal effects induced by estrogen on blood vessels, progesterone may have little or no direct effect on the vasculature (Magness and Rosenfeld, 1992). Progesterone has been reported to antagonize the actions of estrogen, presumably by reducing estrogen receptor numbers (Magness and Rosenfeld, 1992). In males, testosterone, which can be metabolized to estradiol, will cause a sharp, transient dilation of arterioles and venules in sex accessory organs (Knisely *et al.*, 1957).

The gingival vasculature also appears sensitive to sex steroid hormones. Several clinical studies have correlated elevated gingival crevicular fluid with the presence of sex steroid hormones. Because the net flow of gingival crevicular fluid is related to an increase in the permeability of dentogingival blood vessels and the movement of interstitial fluid into the sulcus (Cimasoni, 1983), ovarian hormones may affect the integrity of the vasculature. In pregnant human females, gingival crevicular fluid is elevated by as much as 54% when compared with gingival crevicular fluid levels from postpartum controls (Hugoson, 1971). Furthermore, exogenous estrogen and/or progesterone administration will significantly increase the amount of crevicular fluid in either inflamed

or noninflamed canine dentitions (Lindhe *et al.*, 1968a,b; Hugoson and Lindhe, 1971).

Several studies have implied that ovarian hormones, particularly progesterone, were responsible for a reduction in corpuscular flow rate (Lindhe and Branemark, 1967a), increased vascular permeability (Lindhe and Branemark, 1967b), and vascular proliferation (Lindhe and Branemark, 1967c). However, the results of these studies must be placed in proper perspective since pharmacologic doses of estrogens (up to 400,000,000 times the plasma concentration found in nonpregnant human females) and progesterone (up to 1,000,000 times the plasma concentration found in nonpregnant human females) were used to examine the effects of ovarian hormones in nonperiodontal tissues such as the hamster cheek pouch and the ears of rabbits. In a nonblinded, nonparametric, histological study that used only progesterone, Mohamed *et al.* (1974) described transient morphologic changes leading to increased permeability in rabbit gingival blood vessels.

Estrogens are primarily responsible for vascular changes in target tissues, such as the uterus, yet several studies have suggested that increased vascular permeability in the gingiva is essentially the result of progesterone. As noted, these studies were principally descriptive in nature and/or used concentrations of hormones far higher than what is normally found in women. As in other target tissues, the actions of estrogen and progesterone on the gingival vasculature are complex and yet to be defined.

C. Hormones and the Immune System

Immunological reactions play an important role in the pathogenesis of periodontal diseases (Genco, 1992) and our understanding of the relationship between sex steroid hormones and the immune system is developing rapidly. Although it is not within the scope of this review to examine the actions and interactions of steroid hormones with the immune system, there are several salient observations that suggest that changes in the periodontium may develop as a result of the influence of sex steroid hormones on the immune system. First, there are a number of autoimmune diseases that exhibit a gender-related susceptibility. For example, there is a female sex predilection of 13:1 for systemic lupus erythematosus (Inman, 1978) and 9:1 for Sjogren's Syndrome (Whaley and Buchanan, 1980). Second, estrogens can also modulate many autoimmune diseases. For instance, rheumatoid arthritis (Hench, 1949), autoimmune thyroiditis (Amino *et al.*, 1977a), Graves disease (Amino *et al.*, 1977b), polymyositis/dermatomyositis (Gutierrez *et al.*, 1984), systemic lupus erythematosus (Mund *et al.*, 1963), and idiopathic thrombocytopenic purpura (Lorz and Frumin, 1961) are all affected during pregnancy. Third, sex steroid hormones have been shown to modulate the production of cytokines, such as interleukin-6 (Tabibzadeh *et al.*, 1989). Finally, sex steroid receptors have been identified on components of the immune system and may act to modulate the actions of these cells (Ahmed, 1988). For example, low concentrations of estradiol (1.5 ηM) have been shown to reduce polymorphonuclear leukocyte chemotaxis by as much as 26.8% (Miyagi *et al.*, 1992). In addition, a number of immune-sensitive cells, including CD1 cells (primarily Langerhans cells) and CD3 cells (majority of mature T lymphocytes) in the oral gingival epithelium as well as CD4 cells (helper T cells) in the oral and sulcular gingival epithelium were elevated during pregnancy (Raber-Durlacher *et al.*, 1993).

Observations concerning gender-related disease susceptibility, hormone regulation of immune cells, and hormone modulation of autoimmune diseases offer initial evidence to support the premise that some immunologic reactions in the gingiva are probably affected by sex steroid hormones. The type and degree of influence that sex steroid hormones exert on the immune system in the gingiva remain to be ascertained.

D. Hormones and Cells of the Periodontium

Sex steroid hormones exert considerable influence, both directly and indirectly, on cellular differentiation, proliferation, and growth in target tissues. In the oral cavity, androgens, estrogens and progestins are known to affect several cell types, and in the gingiva, reports dealing with the actions of sex steroid hormones have focused primarily on two cell groups, the keratinocyte and the fibroblast.

Many of the histologic studies that examined the effects of gonadal hormones on gingival epithelial cells were purely descriptive in manner and the investigators were usually not blinded to the treatment modalities. Keeping this in mind, several investigators perceived that estrogens increased epithelial keratinization and stimulated proliferation (Ziskin *et al.*, 1936; Richman and Abarbanel, 1943). Trott (1957) noticed a reduction in keratinization of marginal gingival epithelium in postmenopausal women when plasma estrogen levels were declining. In senile mice, estrogens were reported to increase the downgrowth of epithelial attachment (Nutlay *et al.*, 1954). Beagrie (1966) described an estrogen-induced increase in thymidine uptake in murine oral epithelium and epithelial attachment; however, no analysis was attempted to determine if these differences were significant. In one of the few studies to quantify changes induced by estrogens in epithelial cells, Litwack *et al.* (1970) found the length of rete pegs, number of basal epithelial cells per area of basement membrane, and thymidine labeling of epithelial cells in oral mucosa to be significantly increased after estrogen administration to castrated adult female squirrel monkeys. Moreover, estrogen also stimulated an increase in the number of basal epithelial cells per area of basement membrane in the gingiva of squirrel monkeys. Similar to estrogens, the actions of androgens and progestins on gingival epithelium are also ill-defined at the present time. In humans, simians, and rodents, androgens were perceived to stimulate an increase in epithelial cell number (Ziskin, 1941; Shklar *et al.*, 1967). In another study, the daily administration of norethisterone acetate, a progestin, to nine healthy women between day 3 to 27 of the menstrual cycle, resulted in a significant reduction in the keratinization index and karyopyknotic index from gingival smears (Klinger *et al.*, 1981). The authors suggest that the reduction in gingival proliferation was not due to the direct effects of the progestin but rather to a reduction of plasma estradiol induced by daily progesterone administration. Circumstantial evidence exists that sex steroid hormones have an effect on gingival epithelium, but the responses of keratinocytes to sex steroid hormones remain both obscure and complex.

The extracellular matrix of the periodontium is an intricate mosaic of cells (e.g., fibroblasts, mesenchymal cells, mast cells, endothelial cells, etc.) interspersed among a diverse number of macromolecules (Mariotti, 1993). The actions of sex steroid hormones on the extracellular matrix are a prime example of the dynamic response of cells in gingival connective tissue during times of hormone fluctuations. Early studies examining the effects of sex steroid hormones on the gingival extracellular matrix were primarily descriptive in nature and ascribed histologic changes in the composition of the entire tissue. For example, initial histologic studies in humans described the maintenance of gingival connective tissue in women receiving exogenous estrogen (Ziskin and Zegarelli, 1945), whereas androgen treatment was effective in stimulating proliferation of connective tissue elements in humans, castrate rhesus monkeys (Ziskin, 1941) and rats (Shklar *et al.*, 1967). Later studies began to biochemically analyze the changes that developed in the presence of androgen or estrogen. A sexual dimorphism was reported in the amount of gingival sialic acid (Nicolau *et al.*, 1979). Moreover, in young Wistar rats, normal female gingiva was found to contain as much as 42% more *N*-acetylneuraminic acid in comparison to age-matched males. Testosterone was also found to have an effect on extracellular matrix components. Kofoed (1971) demonstrated that gingival hyaluronic acid but not heparan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, or dermatan sulfate was androgen sensitive. More specifically, castration of adult rats induced a 54% reduction in hyaluronic acid that could be prevented with subcutaneous injections of testosterone propionate. Investigations examining the effect of estrogen on collagen synthesis have been limited in the gingiva. Dyer *et al.* (1980) found no significant effect of a single dose of estrogen on hydroxyproline-specific radioactivity in gingival or palatal tissues of ovariectomized, nulliparous rats. Although the amount of newly synthesized gingival collagen was not statistically different between estrogen-treated castrate and castrate control animals, differences between these two groups may be masked either by the amount of error around the means or because these experiments allowed for a castration-induced regression prior to estrogen treatment. Evidence from various epithelial cell lines and tissues show that hormone-sensitive cells fail to respond or have diminished sensitivity to steroid hormones after a

time period of reduced hormone secretion. Investigations using human epithelial tumor cell lines have demonstrated a loss or reduction of estrogen-sensitive cell growth after estrogen deprivation (Darbre and King, 1988; Daly and Darbre, 1990). In addition to epithelial cells, stromal components have been shown to lose sensitivity to estrogen after a period of hormone deprivation. Research into the maintenance and restoration of estrogen action has demonstrated that estradiol-induced increases in extracellular matrix components are lost after a castration-induced regression (Mariotti and Mawhinney, 1982).

The fibroblast is the principal cell type found in the extracellular matrix of the gingiva (Narayanan and Page, 1983) and contemporary research is demonstrating that gingival fibroblasts are affected by all three sex steroid hormones. Androgens have an inhibitory effect on fibroblast proliferation. Testosterone has been shown to significantly reduce the proliferative rate of fibroblasts derived from either phenytoin-enlarged human or newborn feline gingiva in media supplemented with 10% fetal bovine serum (FBS) (Fukuda, 1971). Human gingival fibroblasts also metabolize testosterone to 5α-DHT, 4-androstenedione, and 5α-androstanediols in cell culture (Sooriyamoorthy *et al.*, 1984). Additional studies using fibroblast monolayers or fibroblast cytosols have shown a significant increase above controls in the metabolism of testosterone to 5α-DHT and 4-androstenedione by fibroblasts derived from either phenytoin-, nifedipine-, or cyclosporine-induced gingival tissues (Sooriyamoorthy *et al.*, 1988; Sooriyamoorthy *et al.*, 1990). At this time, it is unclear whether the increase in androgen metabolism by fibroblasts derived from drug-induced gingival enlargements is because of the inflamed nature of the donor tissue or the drug involved in increasing tissue size. Similar to the actions of testosterone on gingival fibroblasts cultures, the effects of progesterone on human and feline gingival fibroblast cell cultures revealed an inhibition of gingival fibroblast proliferation (Fukuda, 1971; Willershausen *et al.*, 1986). Progesterone has been shown to significantly reduce the proliferative rate of fibroblasts derived from either phenytoin-enlarged human or newborn feline gingiva in media supplemented with 10% FBS (Fukuda, 1971). A later study confirmed these results in humans, demonstrating that 20 μg/ml of progesterone induced a significant reduction in DNA synthesis and 40 μg/ml of progesterone reduced protein synthesis by as much as 50% (Willershausen *et al.*, 1986). In contrast to testosterone and progesterone, estrogens appear to be stimulatory in nature in gingival fibroblast cell cultures. Estradiol was capable of stimulating proliferation of fibroblasts derived from either feline or human drug-enlarged gingiva (Fukuda, 1971). In a study examining fibroblasts derived from clinically healthy human gingiva of premenopausal women, physiological concentrations of estradiol were found to stimulate cell proliferation *in vitro* (Mariotti, 1991). More specifically, gingival fibroblasts derived from the papilla of young, medically healthy premenopausal women were seeded in culture plates at a low density (11 cells/mm^2) in media (MEM) supplemented with 10% FBS. After 24 h, media were removed, cells washed twice with serum-free MEM, and media replaced with MEM containing 1% charcoal-treated FBS. Charcoal treatment of serum was used to remove any endogenous steroids. Upon quiescence, cells were incubated with concentrations of estradiol ranging from 1 μ*M* to 1 *fM*. It was found that 1 η*M* estradiol stimulated cell proliferation significantly above control levels in premenopausal fibroblast strains. In fact, estradiol stimulated cell proliferation anywhere from 50% to 310% above control values. Further studies characterizing fibroblasts from premenopausal women demonstrated distinctive cell populations. A fluorescent-activated cell sorter revealed two distinct premenopausal gingival fibroblast populations: one containing a fluorescent-labeled estrogen probe and the other population consisting of cells that did not accumulate the probe (Mariotti *et al.*, 1990). An interpretation of these preliminary results suggests that a subpopulation of estrogen-sensitive gingival fibroblasts exist in premenopausal women. Hence, estrogen-stimulated proliferation of human premenopausal gingival fibroblasts result in the proliferation of a distinct population of estrogen-sensitive cells.

There is evidence to suggest that gonadal hormones mediate the actions of some gingival fibroblasts and epithelial cells and therefore contribute to the maintenance of this tissue. It is known that gingival tissues and/or cells metabolize sex steroid hormones, contain hormone re-

ceptors and proliferate in the presence of specific steroids. Some of the hormonal changes that develop in cellular elements associated with inflammatory periodontal diseases have been identified, including alterations in metabolism of sex steroid hormones (ElAttar *et al.*, 1973; ElAttar, 1974; ElAttar and Hugoson, 1974a; Vittek *et al.*, 1979; Ojantko-Harri, 1985; Ojantko-Harri *et al.*, 1991) and the number of hormone receptors (Southern *et al.*, 1978; Vittek *et al.*, 1982b; Staffolani *et al.*, 1989). Despite the observed influence of sex steroid hormones on the gingiva, the specific effects of gonadal hormones on cellular function in this tissue remain to be elucidated. For example, the molecular mechanisms that steroid hormones use to affect cell differentiation, proliferation, and growth remain ill-defined. Furthermore, it is unknown if gonadal hormones, such as estradiol, act directly on cells or whether other hormone-stimulated autocrine and paracrine growth factors, such as IGF-I, IGF-II, TGF-α, TGF-β, EGF, and FGF (Dickson and Lippman, 1988; McCarty and McCarty, 1991) are responsible for modulating cell function.

VI. SUMMARY

The assertion that hormone-sensitive periodontal tissues exist relies on several salient observations, including an increased incidence and severity of periodontal diseases during periods of hormone fluctuations, retention and metabolic conversion of sex steroid hormones and the presence of steroid hormone receptors in periodontal tissues. Much of the data examining hormone behavior in the periodontium has focused on the actions of estrogens in the gingiva; unfortunately, less is known of the actions of androgens or progestins in gingiva or the behavior of any sex steroid hormone on the periodontal ligament, cementum, or alveolar bone. Moreover, our current knowledge of the specific actions of sex steroid-hormones in the gingiva, periodontal ligament, cementum, or alveolar bone is quite limited.

During the 1960s and 1970s there was an explosion of clinical research examining the actions of sex steroid hormones in the gingiva. Concepts were formulated that hormones act in the gingiva by modulating blood vessel integrity or by serving as an alternative growth factor for bacteria; however, it would be naive to think that the actions of these diverse biological molecules are limited to these responses alone. Contemporary models for hormone action in the periodontium will depend on understanding the actions and interactions of different hormones with the resident population of cells in a specific tissue (see Table 5). For example, the secretion of soluble (i.e., growth factors, cytokines, etc.) and insoluble (i.e., extracellular matrix components) signals from estrogen-sensitive cells may dictate gingival phenotype and the response of the gingiva to environmental insults.

When one considers the primary functions of sex steroid hormones, the periodontium would appear to be an odd target; however, given the influence of sex steroid hormones on periodontium, health and lifestyles of women are significantly impacted. Further, the prominent use of exogenous hormones for contraception or hormone replacement expands the importance of understanding the actions of sex steroid hormones in the periodontium. Future research must investigate the relationship of hormones to periodontal diseases in pre- and postmenopausal women as well as the possibility of a sexual dimorphism of hormone action. Throughout life in both sexes, the association between estrogens, progestins, and androgens and desquamative gingival diseases, inflammatory periodontal diseases, drug-induced gingival overgrowths, maxillary and mandibular osteoporosis, immunologic oral diseases, and oral neoplasms require further investigation. The actions of androgens, estrogens, and progesterone in tissues of the periodontium remain an enigma in many ways; nonetheless, future investigations into the actions of sex steroid hormones will provide an extraordinary understanding of periodontal endocrinology.

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