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THE PHYSIOLOGY OF MENSTRUATION

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CURRENT STATUS OF THE PHYSIOLOGY OF MENSTRUATION

Sexual maturation is apparently a gradual, not a cataclysmic, process which is dependent upon maturation of the central nervous system. Specifically, this seems to involve the concentration of neural hormones, adrenergic and cholinergic transmitters in the hypothalamus. The process is accomplished by transmission of the hormones from the site of origin, along nerve tracts which have their endings in the hypothalamus. An example of this mechanism, which has been nicely documented by neurophysiology, is illustrated. (Fig. 2. 2) Serotonin is synthesized in the retina, presumably under light stimulation and transmitted through nerve tracts to the posterior hypothalamus in the region of the arcuate nucleus, the area closely associated with the cells which product tonic FSH and LH releasing hormone (LH-RH). The concentration of these neurotransmitters in the hypothalamus, as in the cerebral cortex, can be shown to increase with the age of the individual, presumably related to the quality of quantity of sensory stimuli exposure. As the amount of stimulatory transmitters reach the level compatible with physiologic activity, the effects of the gonadotrophic stimulation of the pituitary by the hypothalamus are evidenced in the response of the end organs, and pubertal development begins.

In the experimental animal an inhibitory control seems to be operational in the immature individual. Thus, if lesions are made in the amygdaloid region or the stria terminalis, the tract leading from this complex to the hypothalamus, precocious development is initiated. The occurrence of precocious puberty in children with central nervous system scars would seem to be compatible with the same type of early inhibitory control in the human. The ectual presence of such inhibitory neural hormones in the hypothalamus of infancy and preadolescence has not been demonstrated.

The hypothalamus can be shown to have two anatomically distinct nervous systems (1) the tubero-infundibular tract, which originates within the basal hypothalamus, secretes dopaminergic transmitters, which stimulate, and indolamines, which inhibit, LRH synthesis, and (2) the noradrenergic (catecholaminergic) neurons, which have cell bodies outside the hypothalamus and axons only within the hypothalamus. These neurons also stimulate LRH synthesis. Estrogen has been shown to inhibit catecholamine stimulation of LRH release (Fig. 2. 1).

The noradrenergic neurons(catecholaminergic system) have the ability to grow postnatally and actively regenerate by sprouting after injury or when given access to areas previously innervated by other fibers. They can be stimulated in a dose-dependent relation by neural growth factor. These characteristics of ontogenicity and plasticity of the central monoaminergic neurons has led Ruf to hypothecate that when the adrenergic neurons reach the limit of their growth potential, puberty is initiated by the adult level

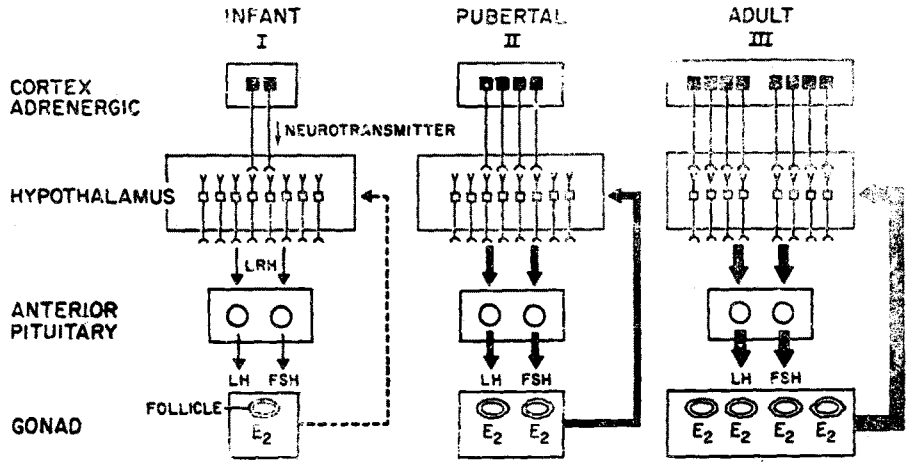


Fig. 2.1. Interaction between the developing adrenergic nervous system, hypothalamus, pituitary and ovary from infancy to adulthood. (Derived from the theory of K.B.Ruf, Z. Neurol., 204:95. 1973.).

of terminal aborization (and possibly definitive numbers of cells) of adrenergic neurons synapsing with neurons of hypothalamic cells synthesizing GnRH. This results in increased follicle growth and estrogen production(Fig. 2. 1).

The appearance of precocious puberty following brain injury or lesions in amygdala or stria terminalis therefore can be due not so much to interruption of inhibition as to the capacity of the aminergic fiber system to show increased sprouting and regeneration after injury and to its ability to avidly innervate areas previously innervated by injured neural fibers.

Gonadal control by the hypothalamus is dependent upon cells in the posterior hypothalamus in the region of the arcuate nucleus which synthesize tonic FSH and LH release hormone designated as LRH, LH-RH and FSH-RH. Sometimes these hypothalamic hormones are referred to as GnRH, gonadotrophic hormones which cause the synthesis and release of pituitary LH and FSH. LRH has been isolated and synthesized and can be shown to cause LH stimulation in a reproducible manner. It also causes stimulation of FSH but usually to a lesser degree and in a more unpredictable fashion. Johannson et al. have attempted to isolate an FSH-RH and, although initial investigations looked promising, no confirmation has been forthcoming. This work indicated that the FSH-RH might also contain the potential for stimulating both FSH and LH, but in reverse ratios to that seen by LRH. The hypothalamic hormones, two of which have been synthesized, are small polypeptides, LRH being a decapeptide and are, therefore, not species specific and are active by all routes of administration.

In the anterior hypothalamus in the region of the supraoptic nucleus, a group of cells, the mechanism of action of which is not well understood, control the cyclic release of LH responsible for ovulation. It is this center which is especially responsive to steroid modulation. Cells of the anterior hypothalamus, in addition to containing estrogen receptor protein, have the

capabillty of aromatizing C-19 steroids to estrogen. This center is blocked in the male during embryonic life apparently by androgen. In the rat, but not the primate, the block is permanent.

Modulation of hypothalamic function occurs by way of (1) steroid feedback, the long loop, (2) pituitary feedback, the short loop, and (3) the ultra short loop, the intra-hypothalamic feedback. All of these mechanisms can be demonstrated under special conditions, but the importance of the specific control mechanism varies with the specific hypothalamic and pituitary hormone complex in question.

The functional hypothalamic unit requires a cell or cells which are programmed to synthesize hypothalmic hormones, neurotransmitters to initiate the release of these hormones and perhaps to regulate the synthesis by both stimulation and inhibition. The cells must also contain steroid receptor proteins to allow for modulation by the long loop steroid feedback and a membrane receptor for the pituitary hormones to permit the short loop feedback.

Both tonic FSH and LH are suppressed by estrogen, FSH being most responsive and LH secondarily so. LH is suppressed by testosterone and certain of the progestational drugs. However progesterone per se is not effective in inhibiting pituitary gonadotrophins in humans. Kraftin et al. have postulated that the major site of steroid suppression of pituitary gonadotrophins occurs at the hypothalamus or higher, because pretreatment with oral contraceptives, estrogen and progestational drugs (Lyndiol) did not prevent the release of LH and FSH after the administration of LRH. However, as modulation of pituitary gonadotrophin production also occurs at the pituitary level, it has been extremely difficult to pinpoint the exact site of action. FSH is also suppressed by some nonsteroidal substance or substances secreted by the testicular tubules and the Graafian follicles in the ovary.

LH and, to a lesser extent, FSH are secreted by the pituitary in an episodic fashion. Reichert and ward have presented evidence using a bicassay for LRH that the LRH hypothalamic secretion is likewise episodic and that periodicty is in an inverse relation suggesting that the loop feedback is operational.

The so-called positive estrogen feedback is the initating of the LH surge by the anterior hypothalamic cells apparently under the influence of a specific amount of estrogen. In addition to the effect on the anterior hypothalamus, estrogen also apparently exerts an effect on the ability of pituitary cells to release LH. Arimura et al., using a sensitive immunoreactive LH-RH assay in plasma, have demonstrated an apparent increase in this hypothalamic hormone at mid-cycle, substantiating these authors belief that an incrased LRH is responsible for the LH surge. Other authors, Nett et al., Keye et. al., have been unable to demonstrate this relationship. Whatever the etiology, however, the important facts are that given a specific amount of estrogen at the mid-cycle, some mechanism is initiated in the anterior hypothalamus which triggers a flood of LH from the pituitary.

HYPOTHALAMUS

The estrogen effect on pituitary gonadotrophin secretion, although initially though to be a direct one, was later ascribed by neurophysiologists to the hypothalamus. The characteristic effect is a suppression of both FSH and LH presumably due to suppression of GnRH in the posterior tonic release center. FSH is most sensitive to estrogen suppression in the human, if not the rat. LH suppression requires a longer estrogen administration. This is the long-loop negative feedback. If estrogen is given acutely

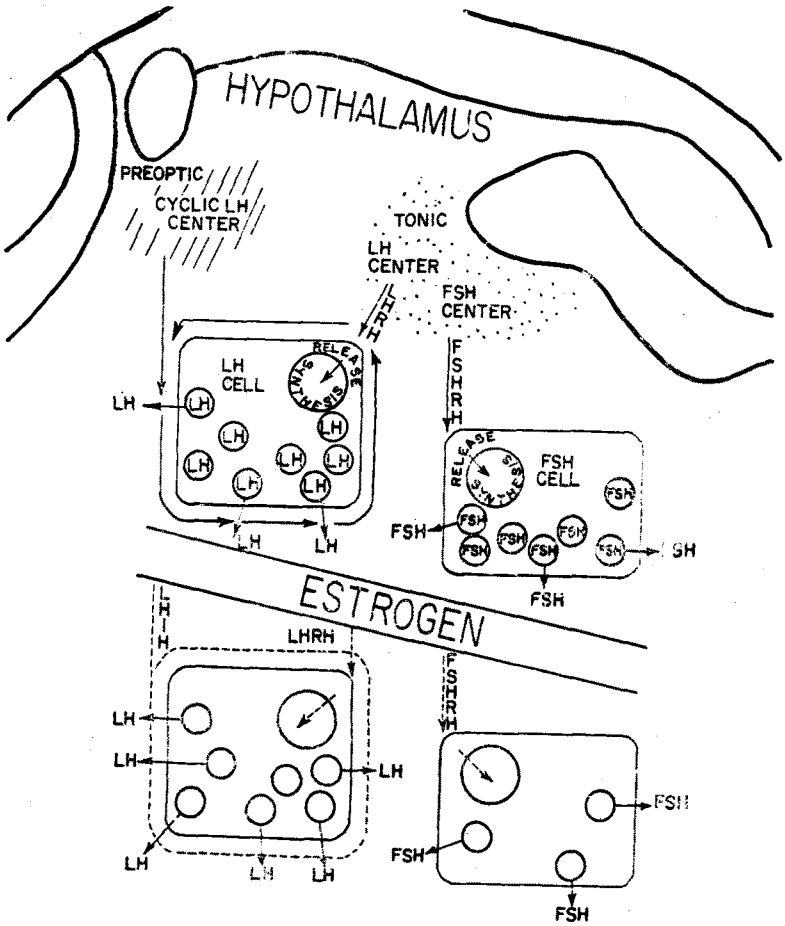


Fig. 2. 12. Effect of hypothalamic hormones on the pituitary LH cell. Explanation of the apparent positive estrogen feedback on the anterior cyclic-LH center by assuming that these cells are producing an inhibitory LH-releasing hormone (LH-IH). Estrogen can then be assumed to have an inhibitory effect in both anterior and posterior hypothalamic centers. (From S. Aksel and G.E.S. Jones, Qbstet. Gynec., 44:1, 1974).

in high dosage or chronically at intermediate levels, a "so-called" positive feedback or stimulation of LH occurs. This effect is though to be mediated by the anterior hypothalamic cyclic-LH center, as it is abolished by destruction of these cells or interruption of axons between the suprachiasmatic area and the median eminence. It is probably via the hypothalamus rather than pituitary, because no acute stimulation effect of estrogen on Lh release occurs after an LHR infusion. As discussed elsewhere the "positive" feedback could be the result of a negative feedback of an inhibition, e.g., an inhibition of an inhibition (Fig. 2. 12).

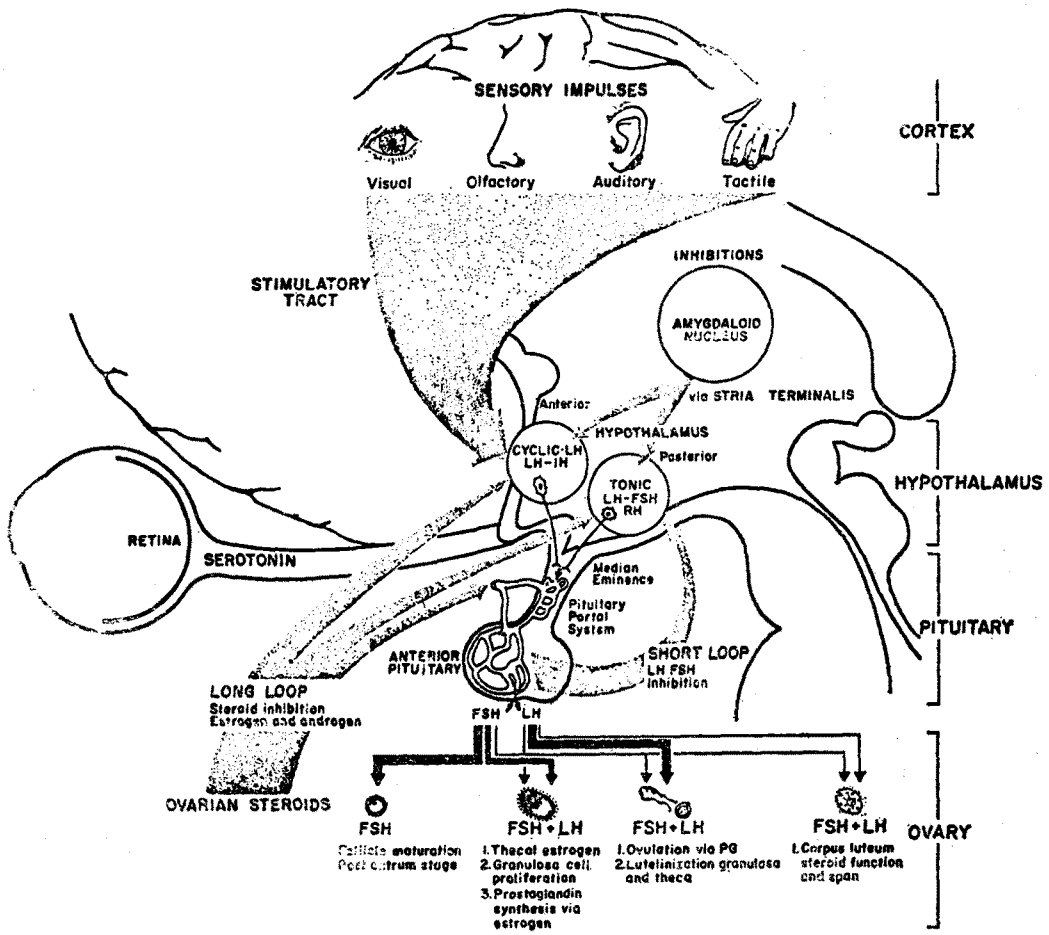
The ensuing corpus luteum function after ovulation results in production of sufficient estrogen and, perhaps, progesterone to inhibit the posterior hypothalamic centers decreasing

pituitary FSH and LH. As the corpus luteum regresses, approximately 2 to 4 days prior to menstruation, steroid levels reach a sufficiently low concentration to allow the pituitary to increase FSH secretion. This initiates the growth of follicles for the next cycle. FSH continues to rise during the first 7 to 10 days of the follicular phase associated with a slightly delayed LH rise. This rising LH in the first part of the follicular phase is responsible for the production of estrogen by the theca surrounding the developing follicles. However, during this phase of the cycle, the estrogen remains relatively low. In the preovulatory swelling phase, approximately 4 days prior to ovulation, there is a rapid increase of estrogen apparently from the ovulatory follicle in the "active ovary," and it is this estrogen surgen which then triggers the LH surge.

The LH surge which is associated with ovulation initiates luteinization of the granulosa cells and corups luteum formation. Ovulation per se is dependent upon a vascular phenomenon, the stigmata that can be shown in experimental animals to depend upon prostaglandin which, in turn, may be stimulated by estrogen.

The ability of the ovary to respond to the pituitary gonadotrophins depends primarily upon the normalcy of the chromosomal content. In the absence of two normal X chromosomes, it has been shown that meiosis does not occur and, as oocytes begin to go into meiotic division during the second or third month of embryonic life, the germ cells disappear from the genital ridge leaving only stroma and forming a "streak ovary". Given two normal X chromosomes and a normal complement of oocytes, these eggs organize around them granulosa cells, which in turn organize the theca. The normally programmed granulosa cell contains FSH gonadotrophic receptor sites, as the follicle will not proceed beyond the antrum stage in the absence of pituitary stimulation. Channing has presented some evidence which indicates that the FSH stimulation is also responsible for the induction of LH receptor sites in the granulosa and theca cells. The theca cells, under the stimulation of LH, are responsible for estrogen production and use as their preferential substrate in the follicular phase of cycle dehydroepiandrosterone, a $\triangle^5$ steroid. In the luteal phase, the preferential substrate has been shown to be progesterone, a $\triangle^4$ steroid. Immediately after the estrogen surge which triggers the LH surge and ovulation, there is a fall in the estrogen concentration followed by a secondary rise which parallels the serum progesterone elevation. The etiology of this interruption of estrogen secretion at midcycle is as yet undetermined, but because of temporal relationships with the assay of serum 17-hydroxyprogesterone, an intermediate metabolite between the synthesis of progesterone and estrogen, it can be postulated that this hiatus is due to the lag which occurs in the change over from the $\triangle^5$ to the $\triangle^4$ substrate.

The normalcy of corpus luteum function depends upon the initial programming of the granulosa cells to contain proper gonadotrophin receptor sites and the necessary enzymes for progesterone production, secondly upon a proper FSH stimulation beginning in the prior cycle to initiate normal numbers and composition of granulosa cells, and finally adequate residual tonic LH stimulation during the luteal phase to insure an adequate 14-day span. It is as yet unresolved whether the corpus luteum fixed life span depends upon its failing response to minimal LH, or upon some active luteolytic factor. Prostaglandin F_2a has been suggested as such an agent. Under the aegis of estrogen production, the corpus luteum cells also apparently produce prostaglandin F_2 and both of these substances can be shown to be luteolytic in the experimental animals.



CHEMISTRY OF STEROID HORMONES

NOMENCLATURE

The ovarian, testicular, and adrenal hormones are steroids derived from the same basic molecular structure as cholesterol. The steroid nuclei from which these hormones derive their names, estrane, androstane, and pregnane, with the designation of the rings and numbering of the carbon atoms, are shown in Figure 2. 4. The major structural differences are the absence of the side chain (C_{20} and C_{21}) in the androstane nucleus, from which the androgens are derived, and the absence of both the side chain and a methyl group at C_{19}, in the estrane nucleus, from which the estrogens are derived. Progesterone and the adrenal corticoids belong to the pregnane series.

Stereoisomerism can occur at any of the asymmetric carbon atoms, 5, 8, 9, 10, 13, and 14, and is important as it affects the biological activity of the compound. The Greek letters, α and β, are used to designate the sterometric position of the hydrogen atom or substituents in relation to the angle of the methyl groups at carbon atom 18 or 19 or both, β being in the same plane and α in the opposite. When drawn on a formula, solid lines are used for the β

position and dotted lines are used for the position. The β position, which is present in biologically active steroid, is also referred to as cis while the α position may be called trans. The terms allo and epi are some-times used to denote the α and β positions. However, allo is used only in relation to the hydrogen atom at C_5, while epi can be used in relation to any other carbon atom.

The presence of a double bond is noted in the nomenclature by changing the suffix "ane" to "ene". Two double bonds are denoted by the suffix "diene" and three by "triene". The number of the lowest sequential carbon designates the position of the bond and this number should be placed between the name of the parent nucleus and the prefix. The Greek letter, $\triangle$, placed before the parent nucleus name, with the superior carbon number, e.g., ($\triangle^5$), has also been used for this purpose.

The presence of a hydroxyl group is denoted by the prefix "hydroxy" or the suffix "ol" and a ketone group by the prefix "oxo" or the suffix "one." The absence of a substituent group or a carbon is designated by the prefixes des or nor.

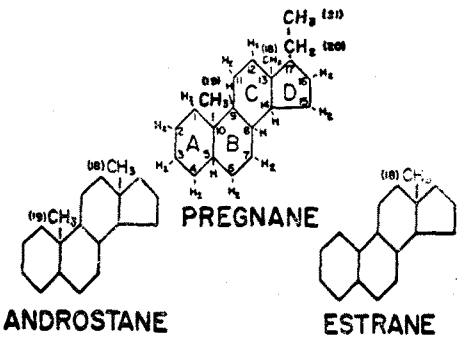


Fig. 2. 4. The steroid hormone parent nuclei from which the nomenclature is derived. The ring designations and the carbon atom numbers are shown in the pregnane nucleus. Hydrogens are not shown on the androstane or estrane nuclei.

BIOSYNTHESIS

As might be expected from the chemical similarity of their formulae, the biosynthetic pathways for the production of estrogens, progesterone, androgens, and adrenal corticoids seem to be equally interrelated. The ovary, adrenal, and testis apparently all posses, in some degree capabilities for biosynthesis of all steroids.

In general, two major pathways seem to exist. The first utilizes cholesterol as the substrate (Fig. 2. 5). After hydroxylation at C_{20} and C_{22}, the sidechain is split off with the formation of pregnenolone and isocaproic acid. Reduced nicotinamide adenine dinucleotide phosphate(TPN H) is an essential cofactor. By and large, TPNH is necessary wherever an hydroxylation occurs. Nicotinamide adenine dinucleotide (DPN) seems to be essential for certain of the dehydrogenase reactions, e.g., removal of hydrogen.

Progesterone is formed from pregnenolone by removal of the hydrogen at the three position and shifting of the double bond from the B ring to the A ring (Fig. 2. 6). The first reaction utilizes a 3β-hydroxydehydrogenase which is catalyzed by DPN. The isomerization reaction which shifts the double bond from the $\triangle^5$ to the $\triangle^4$ position can be accomplished either enzymatically or chemically.

Progesterone has been said to be the precursor of all sex steroids, as it is possible to proceed from progesterone to corticoids, testosterone, or estrogens through 17α-hydroxyprogesterone. This has been called the $\triangle^4$ pathway. Following the formation of the 17α-hydroxyprogesterone the side chain can be removed, forming $\triangle^4$-androstene-3, 17-dione which can be converted readily to either testosterone (Fig. 2. 6) or estrogens (Fig. 2. 7). Evidence for the occurrence of this pathway in the human corpus luteum and luteinized follicle was furnished by

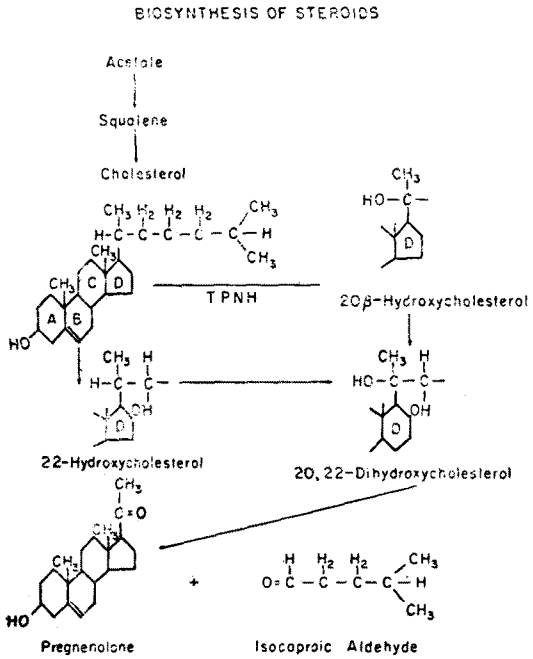


Fig. 2. 5. Steroid biosynthesis showing the pathway through cholesterol to pergnenolone.

Zander who identified both of these steroids, and Baggett et. al. who demonstrated the in vitro conversion of testosterone to estradiol by human ovarian slices. While studying this reaction, Longchampt et al. identified a steroid with an hydroxyl group at C_{19}, $\triangle^4$, 19-hydroxyandrostene-3, 17-dione, and it now appears proved that before the A ring can be aronatized, i.e., unsaturated, it is necessary to change the methyl group at C_{19} to an hydroxyl radical (Fig. 2. 7). The aromatization mechanism has not been elucidated although Morato et al., in 1962, suggested several theoretical pathways.

The second pathway, the $\triangle^5$ pathway, demonstrated by in vitro experiments with human Graafian follicles through dehydroiscandrosterone, DHA, rather than through pregnenolone and progesterone. This pathway has become more

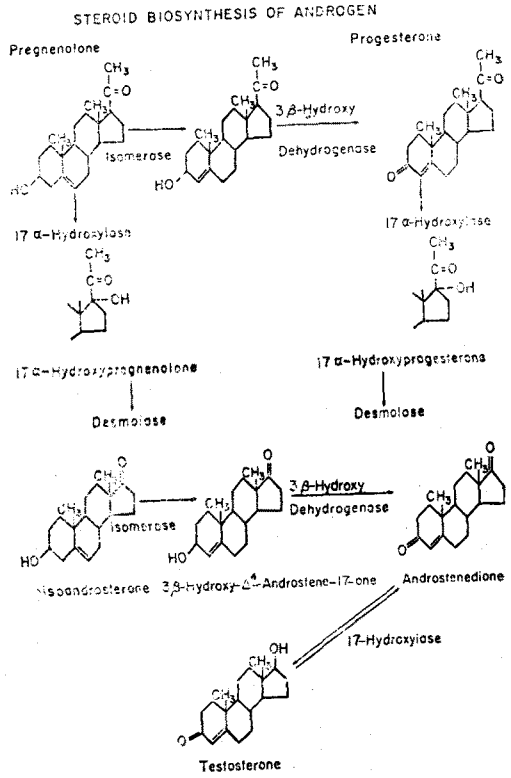


Fig. 2. 6. Steroid biosynthesis showing the pathway from pregnenolone through progesterone to androstenedione or testosterone.

interesting since it has become apparent that the dehydroisoandrosterone pool in the blood represents a constant source of steroid substrate.

Currently it seems that the $\triangle^5$ pathway, directly through DHA, is probably most active in estrogen production by the theca and interstitial cells of the ovary during the proliferative stage of the cycle, while the $\triangle^4$ pathway, through pregnenolone and progesterone, is probably the one of choice following luteinization and corpus luteum formation.

with a better understanding of the chemical reactions involved in the production of the various steroids, it is easy to understand the

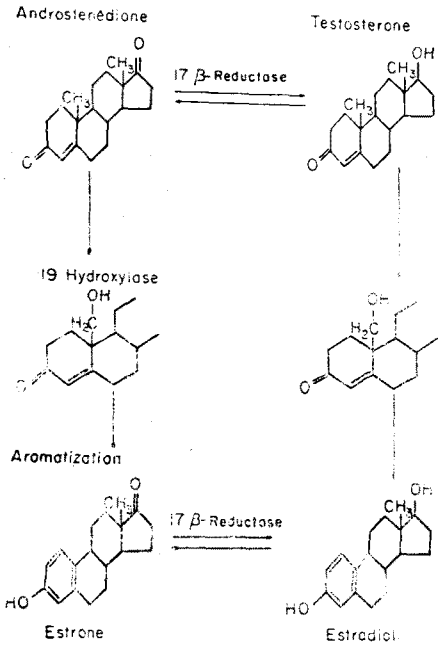


Fig. 2.7. Steroid biosynthesis showing the pathway from androstenedione to estrone and estradiol. Androstenedione may be derived from either progesterone through 17α-hydroxyprogesterone or from dehydroepiandrosterone.

amount of interconversion which can and does occur. The final synthetic potential of the adrenal, ovary, or testis depends upon the amount of enzymes and cofactors present which, in turn, is probably under the control of pituitary hormones. These factors determine to what extent estrogens, androgens, progesterone, or corticoids are to be produced by the gonads and adrenal.

In addition to these sources, however, it has been shown by Mac Donald and Siiteri and others that for estrogens and androgens peripheral conversion of steroid substrates occurs in the skin and appendages. The presence of subcutaneous fat has a positive influence on the efficiency of these mechanisms.

HORMONE CONTENT OF TISSUE AND BODY FLUIDS, METABOLISM FUNCTION AND MECHANISMS OF ACTION

ESTROGENS

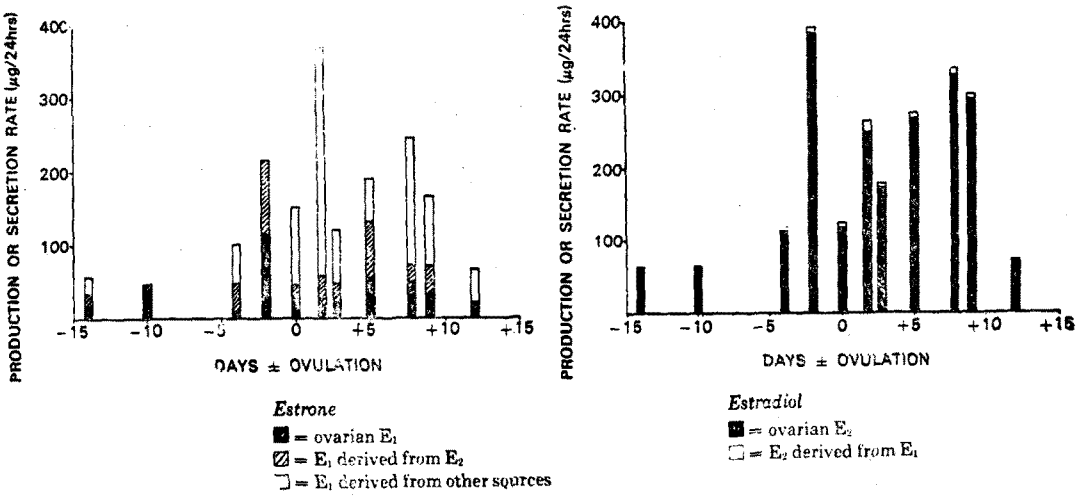
TISSUE CONTENT AND METABOLISM

Estrogens have been recovered from follicular fluid, human placenta as well as urine, blood, feces, and bile of pregnant and menstruating women. Free circulating estrogens are bound to a specific protein for transport and are conjuated in the liver for excretion into the urine or feces as glucuronides or sulfates. Estradiol and estrone are the major components of Graafian follicle fluid; estriol represents the largest urinary estrogen component. Although all three major estrogens have been identified in placental extracts, estriol seems to be the predominant placental estrogen.

DEFINITIONS: Secretory rates equal the estimated contribution of a gland to the total blood hormonal concentrations. Production rates equal the total blood hormone concentration from whatever source. The metabolic clearance rate equals the volume of blood which is cleared of hormone per unit of time.

OVARY: Follicular Fluid. Since 1935, when Mac Corquodal working in Doisy's laboratory first crystallized estradiol from follicular fiuid of sow ovaries, this estrogen has been considered to be the naturally occurring ovarian hormone. Zander and his associates isolated and chemically identified estradiol and estrone from human ovaries in 1959. Estrone is found in lesser amounts and since the conversion reaction between the two hormones is apparently completely reversible, an eqilibrium is probably established.

Nakayama et al. concluded, from experiments with perfused human ovaries, that estradiol



(From D. T. Baird and Fraser, J. Clin. Endocrinol. Metab., *38:* 1009, 1974.)

Fig. 2.8. (*Left*) Blood production rate for estrone (P_S^{E1}) or ovarian secretion rate (S_{ov}^{E1}) (*Right*) Estradiol (E_2) throughout the normal menstrual cycle. Values have been plotted by the estimated day of ovulation (O) for each subject. Total blood production rate for each subject is indicated by the height of the bar. The amount of estrone or estraciol from each steroid is indicated by the corresponding codes:

might be the sole estrogen synthesized. Baird and Fraser have published estrone and estradiol values for peripheral blood and ovarian vein blood with simtltaneous studies on follicular fluid (Table 2.1) throughout the normal menstrual cycle. From these they have calculated blood production and ovarian secretion rates (Fig. 2. 8).

BLOODD: Knowledge of estroged transportation in the blood has been handicapped, prior to immunoassay, by the extremeliy low amounts of physiologically actve circulating estrogen. As early as 1925, Lowe and Frank independently reported estrogen activity in human blood, and Frank and Goldberger in 1926, with bioassay techniques, were able to describe the double peak found during the normal menstrual cycle.

Using a sensitive radioimmunoassay, Thorneycorft et al. measured estrone and estradiol in

Table 2.1 Concentration (ng/100ml) of estrone (E^1) and estradiol (E_2) in follicular fluid

Patient No.	Right Ovary E_1	Right Ovary E_2	Left Ovary E_1	Left Ovary E_2
1	—	—	2.86*	11.44*
2	2.40*	44.1*	1.04	10.19
	6.39*	130.7*	—	—
	8.65*	66.0*	—	—
	5.90	88.63	—	—
	5.37	83.12	—	—
3	—	—	4.94*	127.5*
4	—	—	1.55	7.35
5	0.33	2.23	0	0.80
6	0	1.46	0	0
7	0.42*	12.78*	—	—
8	24.4*	380.5*	—	—
9	16.5*	375.0*		

* All follicles of diameter 1cm or greater.
(Baird and Fraser: J. Clin. Endo. Metab., 38: 1009, 1974.)

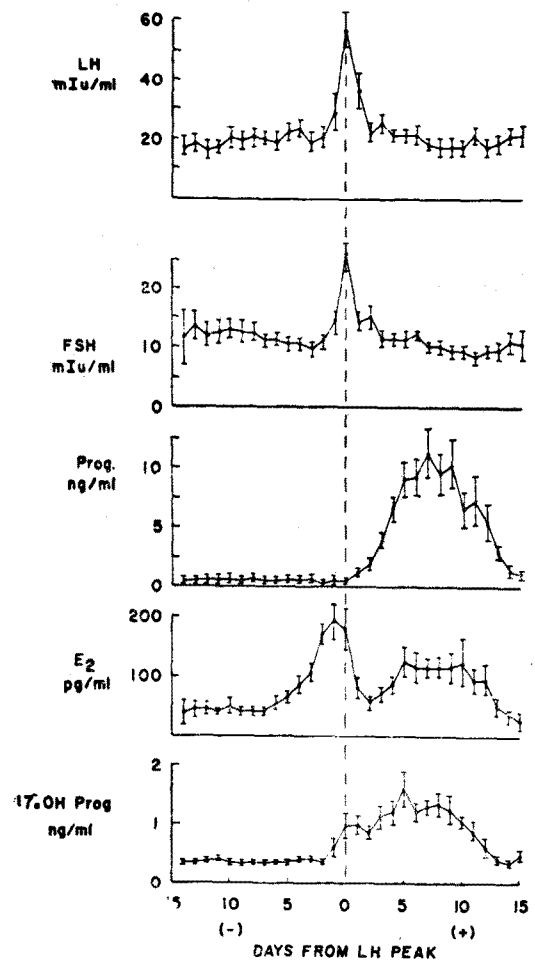


Fig. 2. 9. Serum estradiol (E_2) values throughout the normal menstrual cycle, related to gonadotrophin FSH and LH and progesterone values. (From Thorneycroft et al., Amer. J. Obstet. Gynec., 111:947, 1971.)

the normal menstrua cycle in relation to other steroids, as well sa to pituitary FSH and LH (Fig. 2. 9). The range for estradiol is between 20 and 500 pg/ml and for estrone between 50 and 400 pg/ml. Menopausal values for estradiol are below 10 pg/ml and below 30 for estrone. Male values are in the range of 15 through 25 pg/ml for estradiol and 40 through 75 for estrone.

The metabolic clearance rate of estradiol is reported by Longcope et al. to be 1,360 L/day for females and 1,600 L/day for males, while the clearance rate of estrone is the same for males and females, approximately 2,000 L/day.

URINE: A number of urinary metabolites of estradiol have been recovered. These are apparently conjugated as glucuronides or sulfates or double conjugates: glucronide sulfates. Only the three classic estrogens, estradiol, estrone, and estriol, have been measured throughout the menstrual cycle. However, some idea about the relative importance of each metabolite can be obtained by the work of Gallagher and his coworkers who have measured the various fractions after administering radioactivec arbon-labeled estradiol. The percentage recovery in the individula fractions is shown in Figure 2. 10. The urinary estrogen curve parallels the serum levels. There is a gradul rise after menstruation, reaching a peak prior to ovulation after which a drop in the hormone levels occurs. This is followed by a secondary rise, corresponding to maximum corpus luteum function with a fall just before menstruation. The amounts of estriol, estrone, and estradiol recoverable from the urine during the various phases of the menstrual cycle can be seen in Table 2. 2.

BILE AND FECES: The recovery of substantial amounts of estrogen from the feces has been reported following estrogen administration. Sieke and Schuschania reported equal amounts of estrogen in the feces and the urine of normally menstruating women. Dohrn and Faure report high estrogen titers in the feces of pregant women. Autopsy findings indicate that the liver of pregnant women is high in estrogen concent and Cantarow et al. report that the bile concent is 3 times that of the blood in human term pregnancy.

These combined experimental observations suggest an enterohepatic estrogen circulation.

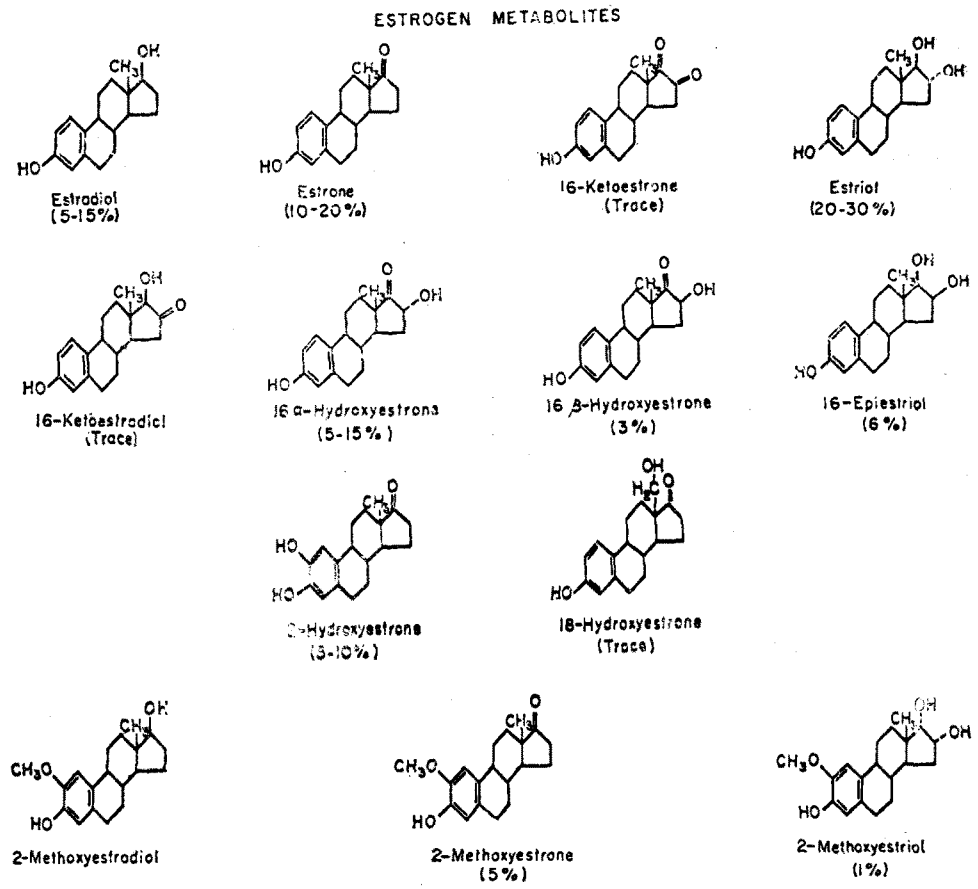


Fig. 2.10. Human urinary metabolites of estrogen with the estimated total urinary radioactivity found on recovery experiments. (Compiled with the assistance of F. Gallagher.)

Table 2.2 Estrogen Levels Found at Various Times during Menstrual Cycle*

Time in Cycle	Estrogens Excreted(μg. per 24 hr,)					
	Average			Range		
	Es-triol	Es-trone	Estra-diol	Es-triol	Es-trone	Estr-adiol
Onset of menstruation	6	5	2	0—15	4—7	0—3
Ovulation peak	27	20	9	13—54	11—31	4—14
Luteal maximum	22	14	7	8—72	10—23	4—10

* From Brown, J.B.: Lancet, 1:320, 1955.

A consideration of the enterohepatic circulation is of importance in determiningthe biologic effect of administered estrogenic drugs. Either the mode of administration or the chemical configuration of the drug can change its circulation time and the access of liver cells to the steroid.

CONTRIBUTION OF ANDROGENIC STEROIDS TO ESTROGEN MILIEU: Tesosterone is secreted presumably by the hilus cells of the ovary. Abraham has confirmed a midcycle peak and slightlyhigher values in the luteal phase as compared to the follicular phase (Fig.2.11). The range for normal menstruating females is between 20 and 50μ g/ml. The ovarian contribu-

tion to the peripheral testosterone value is estimated at 33% during the follicular and luteal phases and 60% at the mid-cycle. The remainder is due to adrenal function. Testosterone contributes relatively little to the E_2 blood production rate.

Androstenedione, ADD, is the steroid preferentially secreted by the ovarian stromal cells, according to Rice et al. Ovarian vein studies of Lloyd ed al. indicate it is the major androgen secreted by the ovary, and Abraham has confirmed this. The range for normal menstruating females is between 100 and 220μ g/ml. An appreciable amount of ADD is also secreted by the adrenal but, under normal conditions, the contribution of the ovary makes up as much as 70% of peripheral ADD at mid-cycle (Table 2. 3). ADD is an important steroid, as it is a precursor for estrone. In the menopause little or no estrogen is secreted by the ovary. All of the serum estrone is derived by peripheral conversion of ADD which at this time of life is

Table 2. 3 Ovarian and adrenal contribution to peripheral androgens (ng/ml)

Steroid	Ovarian contribution F	M	L	Adrenal contribution
T	0. 1 (33%)	0. 3 (60%)	0. 1 (33%)	0. 2 (40—66%)
DHT*		0. 1 (50%)		0. 1 (50%)
A	0. 5 (45%)	1. 5 (70%)	0. 8 (60%)	0. 6 (30—55%)
DHEA*		0. 8 (20%)		3. 2 (80%)
DHEA-S	80 (4%)	200 (10%)	80 (4%)	2000 (80—96%)

( )=percent contribution calculated by comparing conrtol cycles with a cycle in which the adrenal cortex was suppressed by dexamethasone.

* = ovarian contribution not influenced by phase of menstrual cycle.

F = early follicular; M=midcycle; and L=late luteal phase.

T = testosterone, A=androstenedione, DHT=dihydrostestosterone, DHEA=dehydroepiandrosterone, DHEAS=dehydroepiandrosterone sulfate.

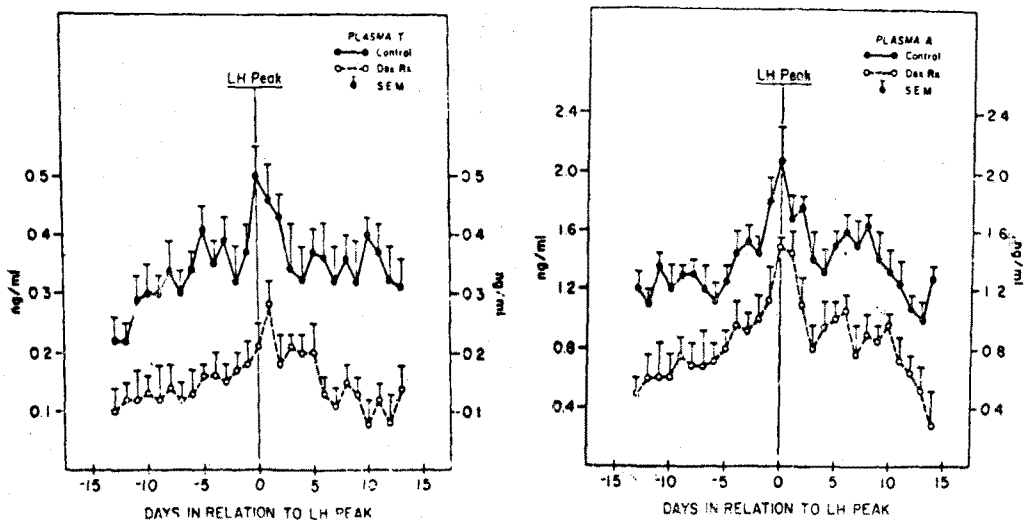


Fig. 2. 11. Patterns and levels (mean+SEM) of serum testosterone (*T*) and androstenedione (*A*) during 2 consecutive menstrual cycles in 6 premenopausal women. The first cycle served as control and the adrenal corten was suppressed with dexamethasone (*Dex Rx*) during the second cycle.

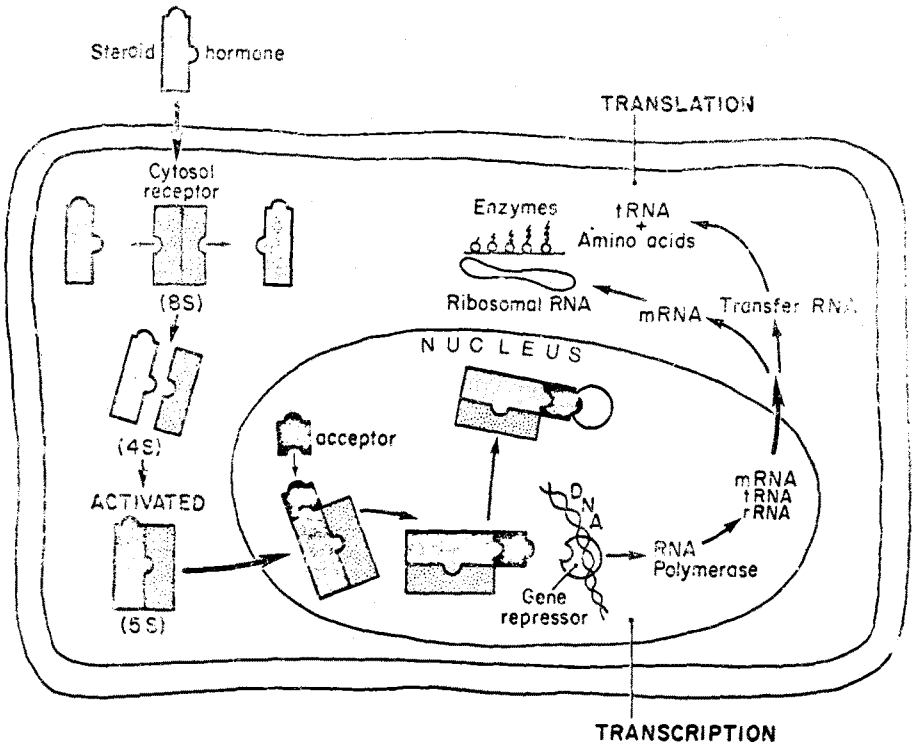


Fig. 2. 14. Intracellular mechanisms of action of estrogen by gene activation

thought to come mainly from the adrenal.

ADD can also be aromatized to estrogen by cells of the anterior. but not posterior, hypothalamus furnishing the ovary with a differential feedback control mechanism of the cyclic and tonic centers.

SUMMARY: In summary, the theca cells of the ovary apparently produce estradiol which is immediately in equilibrium with estrone. It is transported in the blood by a specific estrogen binding protein. The lliver is the site of conjugation and conversion of estradiol and estrone to estriol glucuronide through 16-ketoestrone. This conjugated steroid is the major metabolite excreted in the urine. Substantial amounts of estrone are excreted in the bile and feces of estrone sulfate. or estrone glucuronide sulfate.

Androstenedione, which is the major steroid secreted by ovarian interstial cells, is the steroid precursor for estrone by peripherial conversion. Estrone constitutes the major estrogen in menopausal women.

MECHANISMS OF ACTION

The current theory for the intracellular mechanism of estrogenic action is that of gene activation. Estrogen target cells contain specific loci, receptor proteins. which bind estrogens. These proteins have a sedimentation rate of approximately 8S. As thehormone is bound, the protein splits to form a hormone complex and a 4S (S =sedimenting) protein. The hormone receptor complex must then beactivated in order for it to move into the nucleus where it combines with an acceptor protein. This nuclear receptor protein is not hormone specific. It will accept other molecules such as insuline, glucagon and aminopeptides. Once in the nucleus, the hormone acceptor complex can act as a gene derpressor by combining with a repressor protein on the

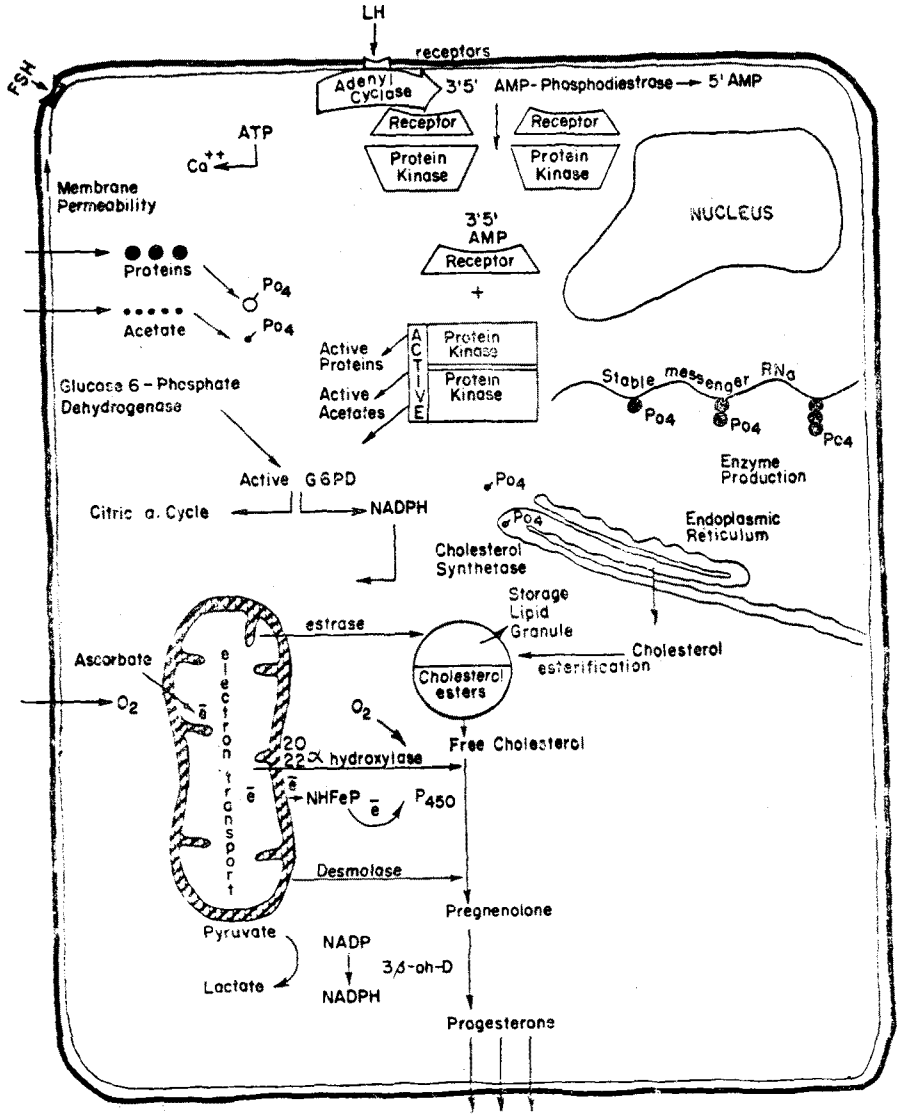


Fig. 2. 18. Intracellular mechanism of action of protein hormones by activation of "the second messenger" cyclic aPenosine monophosphate, CAMP. (From S. J. Behrman and R. W. Kistner (eds.), *Progress in Infertility*, 2nd Ed., Little Brown & (Co., Boston, 1975.)

surface of the gene; it removes the repression and allows gene activation. This results in replication of RNA polymerase, which in turn increases ribosomal RNA and transfer RNA, thus setting into motion all of the necessary reactions for the synthesis of proteins (enzymes) which are characteristic of the target cell response to estrogen (Fig. 2. 14) (Fig. 2. 18).

PROGESTERONE

TISSUE CONTENT AND METABOLISM

Progesterone has been isolated from ovarian tissue, placental tissue adrenal, and testis.

OVARY: CORPUS LUTEUM: In 1948, Hoffmann and Van Lam assayed human corpora lutea of diffe-ent ages and found a measurable amount of progesterone on the first ovulatory

day. This increased to a maximum by the 16th cycle day and remained elevated until the 24th cycle day. Appreciable amounts of progesterone were, however, still present at the onset of menstruation and traces were defectable in the corpora of the previous cycle. Zander, studying the corpus luteum of pregnancy, identified two additional pregestational steroids (Table 2. 4). However, there is some question as to the physiological activity of these compounds in the human. The 20 β-ol steroid is said to be inactive and the activity of the A compound may depend upon the ability of the body to convert it to progesterone. Thus, although there are many naturally occurring steroids which are estrogenic to some degree, three seems to be only one naturally occurring steroid which has appreciable progestational activity.

BLOOD: Progesterone is transported in the blood as are other steroids by a specific binding protein. Raadiolimmunoassay or a competitive protein binding method, adapted from the Murphy technique, indicates a low baseline serum progesterone level in the follicular phase of the menstrual cycle compatible with adrenal function. Just prior to ovulation, a slight increase can be detected, apparently due to luteinization of the granulosa cells of the preovulatory follicle. Following ovulation, there is a gradual rise to a plateau between days 19 and 21 and a rather sharper decline to baseline values again at the time of menstruation (Fig. 2. 15). The value of approximately 0. 5mg/ml in males and menopausal women is the same as that in the follicular phase of the cycle and represents the adrenal component.

The metabolic clearance rate of progesterone in males and ovariectomized females, according to Little et al., is 2, 100 L/day. The rapid removal of progesterone from the blood and its equally rapid conversion to a biologically inactive steroid must be obviated for effective administration of this hormone. This has been accomplished by frequent intramuscular administration, vaginal absorption or chemical changes in the molecule which protect it from metabolism.

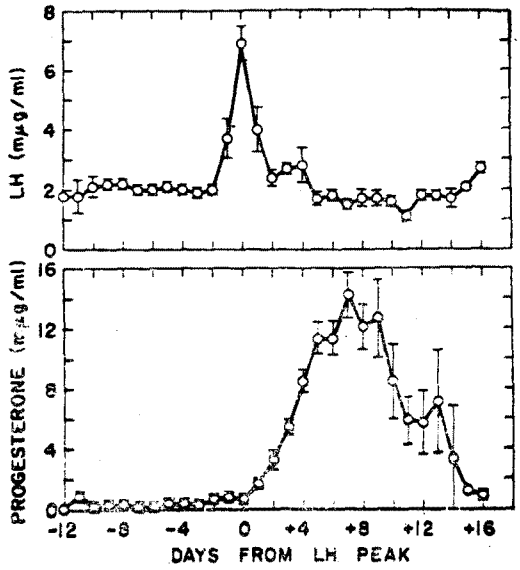


Fig. 2. 15. Serum progesterone during menstrual cycle. (From Neill, *et al.*: *J. Clin Endocr.*, 27:1167, 1967.)

Table 2. 4 Progestional Compouns Isolated from Human Corpora Lutea

Progesterone=△⁴-3-ketopregnene-20α-one[a]
1/2—1/3 activity of Progesterone =△⁴-3-ketopregnene-20α-ol[b]
1/5—1/10 activity of Progesterone =△⁴-3-ketopregnene-20β-ol[b]

[a] Isolated by Corner and Allen, 1929, and identified by Butenandt and Schmidt, 1934.

[b] Isolated by Zander, Forbes, Von Munstermann, and Neher, 1958.

URINE: In 1937, Venning and Browne presented evidence that sodium pregnanediol glucuronide was the metabolic product of progesterone and published their result on the urinary excretion throughout the menstrual cycle. Before ovulation, between 0. 2 and 1 mg of pregnanediol per 24 hours can be excreted. This amount

presumably represents the contribution from the adrenal gland. After ovulation, the excretion rapidly rises to between 3 and 6 mg per 24 hours at the peak of the luteal phase and falls again before menstruation. When measured as free pregnanediol, this metabolite represents approximately 20% of injected progesterone.

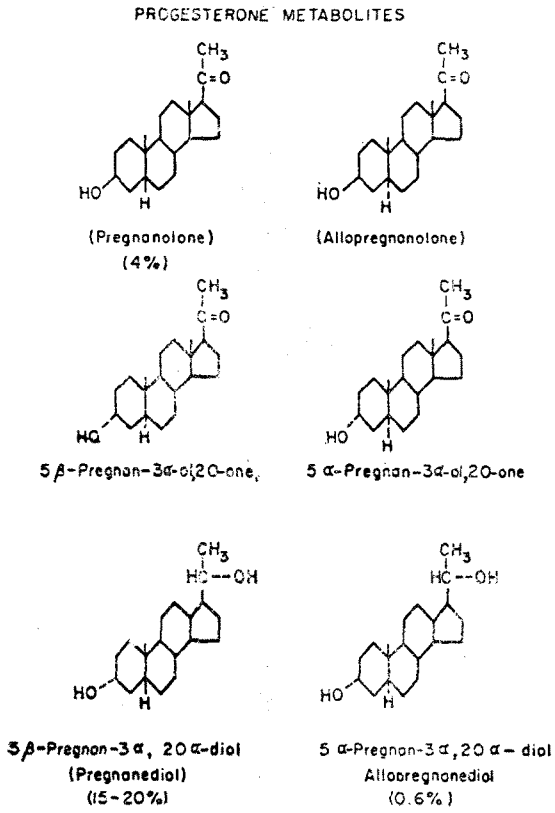


Fig. 2. 16. The metabolic products of pregesterone isolated from human urine.

Although other metabolic products of progesteeron have been isolated from human urine(Fig. 2. 16), only one, pregnanolone, is of any importance.

BILES AND FECES: Sodium pregnanediol glucuronide has been identified in the blood and has also been isolated from bile. However, in the feces pregnanediol is found in the free form, indicating that hydrolysis occurs in the gut.

SUMMARY: In summary, progesterone is probably the only naturallly occurring progestational agent of any significance. Small amounts may be synthesized by the cells of the follicle in the provulatory swelling phase; however, the major production is by the corpus luteum cells of the ovary during the luteal phase of the cycle. It is constantly produced in small amounts by the adrenal gland and by the testis in the male. In the adrenal and the testis, it probably serves as the precursor for corticoids and androgens. It is transported in the blood by a specific binding protein and metabolized and conjugated in the liver into sodium pregnanediol glucuronide which also circulates in the blood. Approximately 20% is excreted in the urine as sodium pregnanediol glucronide; pregnanolone represents a minor metabolic product. The pregnanediol which is excreted in the bile is enzymically hydrolyzed by the gut so that the pregnanediol recovered in the feces is in the free form.

(本演題는 1975. 5. 19. ~5. 24에 있었던 존스·홉프킨스大學校 國際產婦人科 韓國敎育硏究院(JHPIEGO/KOREA)의 第1次學術大會時에 講演하였든 것이며 著者의 承認을 얻어서 本誌에 게재함).