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# Some Fundamentals Of Gonadal Development And Function

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# SOME FUNDAMENTALS OF GONADAL DEVELOPMENT AND FUNCTION*

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The traditional division of animal life into male and female forms is based on obvious biological differences, but these distinctions become less striking when we realize that life, in a sheer physico-chemical sense, is a spectrum of sexuality — that maleness and femaleness are relative terms. Our conceptual devotion to a two compartment universe is apparent in many areas of life, sociologic, moral, legal, spiritual or biologic. Although reproductive obligations remain clear, albeit increasingly restricted, man's greater social sophistication is molding an order in which underlying biological distinctions of the two sexes are sometimes obscured by the potent solvents of culture, leisure and intellect. The purpose of this essay is to review some fundamental aspects of gonadal development and function, presenting similarities as well as differences between the male and female forms. Since few areas of biology have been favored with such significant contributions as have come recently to the fields of genetics and embryology, it is logical to begin with a brief review of these before discussing the prevailing concepts of gonad endocrinology.

The classical concept that the germinal epithelium of the vertebrate gonad has its origin in the primitive coelomic epithelium has been challenged by the Weismann[1] viewpoint which has received support in the studies of Fuss[2], Witschi[3] and McKay et al.[4] It proposes that the primordial germ cells, arising *de novo* in the yolk sac endoderm, migrate through the mesenchyme to their final destination and become the fetal gonad. As shown in Figure 1 the hormone-producing structures of ovary, testis

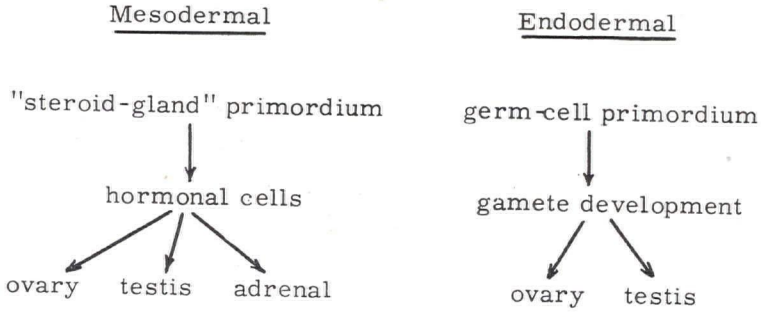


Figure 1
Embryologic derivation of adrenal and gonadal tissue.

and adrenal are derived from one select area of the mesoderm which might be labeled the steroid-gland primordium. The gonadal ridges are apparent in the human embryo at about the fourth week of fetal development, and until the seventh week the gonads of the genetic male and female embryos are indistinguishable (see Fig. 2). Fusing of the bipotential primordial germ cells with the hormonal cells of mesodermal origin results in the embryonic gonad, consisting of the primary sex cords surrounded

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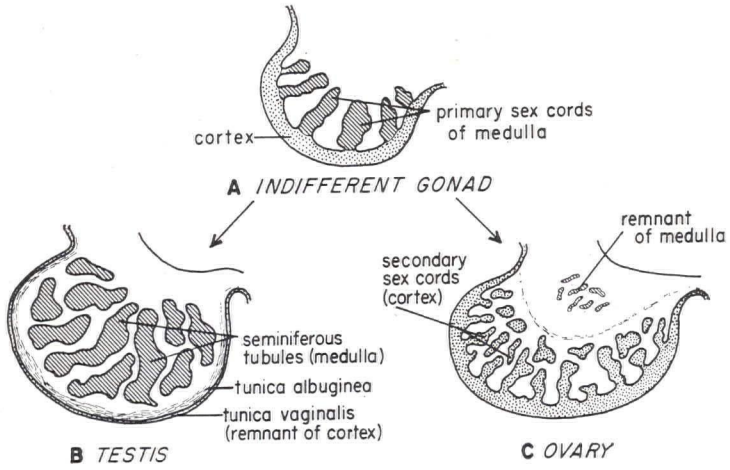


Figure 2

Embryologic development of the human gonad. Male and female structures are indistinguishable until after the 7th week (from Barr,[5] by permission of the author).

by the cortex. At this critical junction the fate of the indifferent gonad is decided by subtle forces which have been termed "inductors". As stated by Barr,[5] germ cells assume the male or female sex character not because of their respective genic make-up but in response to inductive stimuli. Simultaneously, gonia begin differentiation along spermatogenetic lines if originally located in the medulla, or along ovogenetic lines if the primordial germ cells reside in the gonadal cortex. At about the seventh week in embryos of XY chromosomal complex, the medulla begins to develop and the cortex to regress while the sex cords, differentiating to early seminiferous tubules, are interspersed with interstitial cells. The gonadal cortex in male embryos develops ultimately into the thin, visceral layer of the tunica albuginea. At the eighth or ninth week of embryos with XX chromosomal pattern, the gonadal cortex is invaded by sex cords, the medulla regresses and the gonad develops into an ovary. Disruption of the critical differentiation in the bipotential gonad at this point may result in sex anomalies. Such interference may be the result of a mutant gene, a defective chromosome carrying sex determiners, or a nongenetic factor such as a chemical or hormone, either of endogenous or exogenous origin.

Experimental grafting of embryonic rat ovary and testis into castrated adult hosts results in suppression of the differentiation of the embryonic ovary while the testis remains unaffected.[6,7] Holyoke and Beber[8] augmenting these experiments, made tissue cultures of the paired embryonic gonads from both rabbit and rat. The embryonic testes grew well in all combinations, whereas development of ovaries cultured with young testes was markedly retarded (Table I.). In some instances masculinizing effects occurred as shown by the presence in ovaries of testicular cords in the medullary portions. It was deduced that the embryonic testis produces a substance capable of altering the embryonic ovary. That this substance is a hormone distinct from adult male hormone is concluded from the observation that

Table I

Effects of Hormonal Environment on the Development of the Mammalian Ovary (rat and rabbit embryos)*

| Host | Hormonal Environment | Ovary Development |
|---|---|---|
| Castrate adult male | O | normal |
| Intact adult male | male hormone | normal |
| Intact adult male | testis | normal |
| Castrate adult male | embryonic ovary | normal |
| Castrate adult male | embryonic testis | retarded |
| Organ culture | embryonic ovary | normal |
| Organ culture | embryonic testis | retarded |

*based on report of Holyoke and Beber.[8]

the same embryonic ovary can develop normally in an adult male even when grafted to the host testis.

The secondary sex structures, already in existence when gonadal differentiation begins, also have a developmental bipotential (Fig. 3). The ovaries are apparently

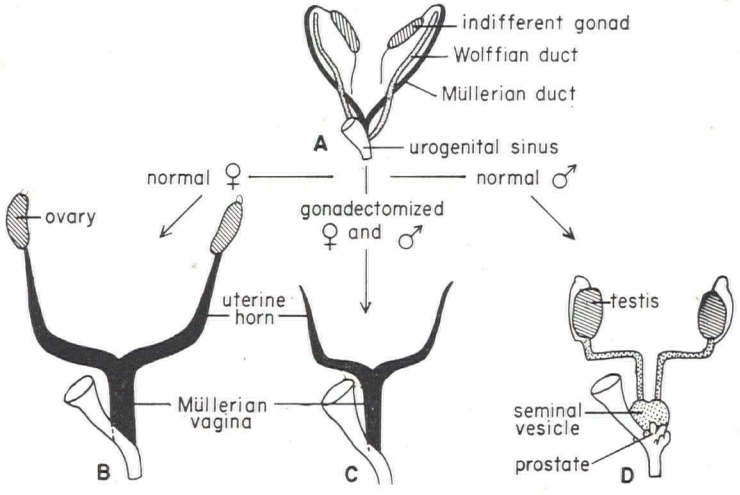


Figure 3

Development of the sex ducts in normal and gonadectomized rabbit embryos (from Jost,[9] by courtesy Cambridge University Press).

not essential for continuing differentiation and growth of the Müllerian system but the embryonic testis, through a presumed hormonal inductor, is critical for Wolffian duct maturation. Barr[5] has stated "the requirement of a masculine evocator of testicular origin to counteract a tendency of all embryos to feminize is a keystone in current concepts of the pathogenesis of congenital errors of sex development". The inherent feminizing propensity of embryos seems genetically regulated and not the result of maternal estrogen or some exogenous factor. The genes involved must

not be those for the male and female determiners of gonadal differentiation since female duct development can occur even in the absence of a normal XX sex chromosome complex and a maturing ovary. Finally, from recent studies of patients with sexual anomalies it appears that the Y chromosome is not genetically passive but carries potent male-determining genes.

The development of a histologic test for sex chromatin determination has contributed greatly to the understanding of the mechanisms involved in both normal and abnormal sex differentiation. This relatively simple technique, introduced by Moore and Barr[10] in 1953, makes possible the accurate recognition of chromosomal sex, thus providing the critical link between gonadal morphology and phenotype in man. The feature which identifies the nuclei of females is a large, dense chromatin particle, usually in proximity to the nuclear membrane and found often to be a bipartite body. In properly prepared sections of female tissue it can be identified in 60 to 70 per cent of the nuclei. Barr[5] states that the densely staining body probably represents the heterochromatic regions of the two X chromosomes. Its presence, however, does not necessarily mean a normal XX structure since unusual chromosomal complexes have been described for patients with Klinefelter's syndrome (XXY)[11] and for the "super-female" individual (XXX)[12]. Conversely, its absence

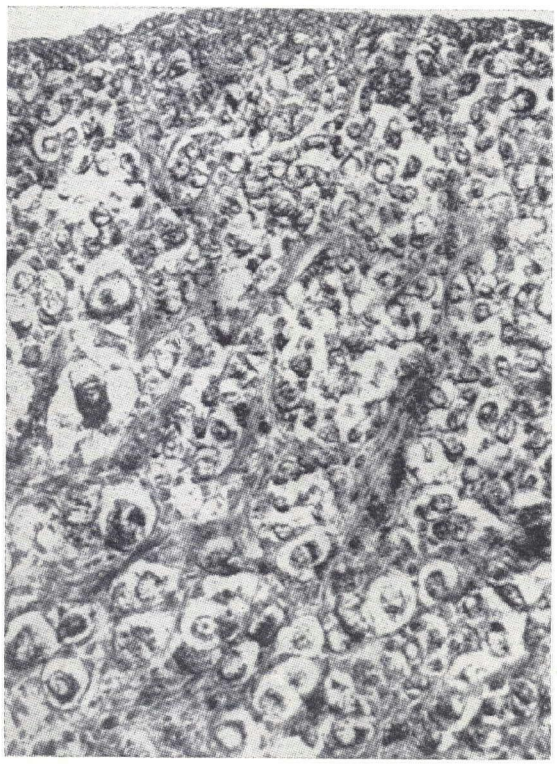

Figure 4

Microscopic (X400) structure of an ovary from a fetus 25 weeks old (ovulation age). Primordial germ cells and 'nurse' cells are almost indistinguishable in cortical area (right upper half of photograph). Germ cells are better differentiated from 'nurse' cells in lower half of the section where the latter are seen surrounding the primordial ova.

indicates that two X chromosomes are not present, which could mean either a normal XY complex or an abnormal XO structure (Turner's syndrome).

## DIFFERENTIATION AND MATURATION OF THE OVARY

In the normal subject with XX chromosome complex, the joining of the germ cells and mesenchymal hormonal cells at the locus of the fetal gonad results in the maturing ovary. In Figure 4, taken from a publication by Garcia and Rock,[13] are shown the primitive elements in an ovary of a 25 week old fetus. The mesenchymal interstitial cells, or "nurse" cells, are the precursors of the granulosa cells which eventually surround each primordial ova. By the time of birth nearly all germ cells have been so surrounded. The actual number of primordial follicles existing at birth appears to vary widely but approximates 200,000 per ovary. Only a small fraction show any signs of further maturity. The weight of evidence favors the idea that aging of the ovary begins at birth and no new primordial ova are formed, the attrition through ova-death or ovulation progressively depleting the gonad. By age 40 to 50 only a few thousand primordial follicles can be found. A progressive thinning of the cortex ensues, the surface wrinkles and the medulla appears relatively conspicuous, having become the repository for multiple corpora albicantia.

It is generally held that FSH accelerates the growth and maturation of the ovarian follicles through its effect on the granulosa cells which, surrounding each ovum throughout fetal and juvenile life, have promoted a slow process of follicular maturation (Fig. 5). As granulosa cell activity increases, a local stimulus results

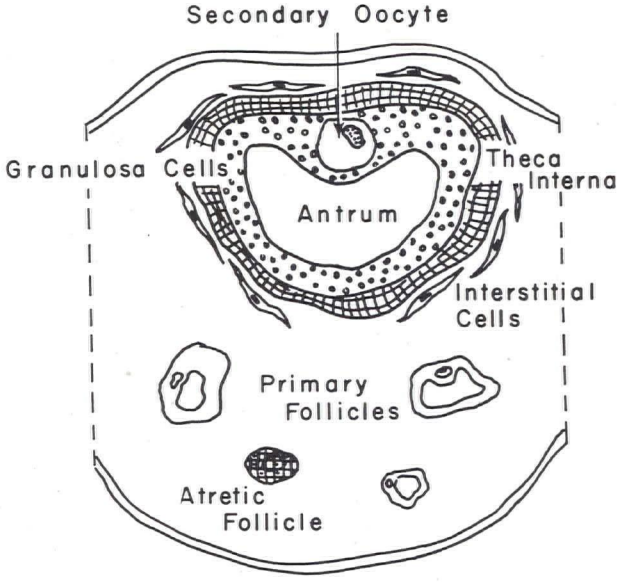


Figure 5

Schematic representation of the micro-anatomy of the ovary, showing follicular maturation and structure of the Graafian follicle.

in the formation of a surrounding theca interna which in turn autonomously elaborates estrogen as estradiol. This hormone now becomes the key factor in further gonadal

and follicular growth. It directly affects the amount and composition of the follicular fluid, while the follicle expands eccentrically, forming the antrum and the Graafian follicle. The FSH-induced increase in estrogen production by the follicle provokes the pituitary into releasing the luteinizing hormone which in combination with FSH furthers the follicular maturation. The theca interna begins elaborating small amounts of progesterone, the follicle becomes distended and the stage is set for ovulation. At this point the action of the third tropic substance, luteotropin or prolactin, leads to release the ovum. During this time the ovogonium has matured through mitotic and reductional division to a secondary oöcyte with 23 chromosomes. This cell is approximately five to seven times larger than the primordial germ cell, excluding the zona pellucida. The exact hormonal chain of events at dehiscence of the follicular wall is not known. Luteinization of the ruptured follicle follows, and with this proliferation of hormonally active cells, increased amounts of estrogen and progesterone are produced. FSH release by the pituitary is suppressed and luteotropin dominates ovarian control with emphasis on progesterone formation until the end of the luteal phase.

That this chain of events applies to man is still unproved since gonadotropin assays have not been perfected to the degree that luteotropin can be separated with confidence from FSH and LH in human urine. Furthermore, the experimental induction of ovulation in primates has proved difficult, Knobil et al[14] only recently having reported the achievement in one hypophysectomized Rhesus monkey receiving thyroid, primate FSH and human chorionic gonadotropin. The capacity of purified FSH alone to induce marked follicular hypertrophy, however, has been amply demonstrated both in primate and infra-primate species. In a 1958 report from Sweden, Gemzell and associates[15] claimed that the injection of human pituitary FSH followed by chorionic gonadotropin produced polycystic ovaries and ovulation in amenorrheic women.

Progress in the isolation, purification and characterization of pituitary gonadotropins, especially from human sources, has been disappointingly limited.[16-17] Small amounts of highly purified sheep FSH and LH are becoming available, and data are accumulating from the comparative assays of these substances with human urinary gonadotropins.[18,19] Yet the latter are excretory products and thus may be chemical disfigurements of the original pituitary secretion. As long as bioassay in animals remains the best assay procedure, varying as it does from laboratory to laboratory, and as long as the source and methods of isolation and purification differ widely, the identity and functional interrelationships of human gonadotropins will not be readily resolved. In Figure 6 are graphed the results obtained in our laboratory for the comparative properties in the immature mouse of human gonadotropins from urine of postmenopausal women and young men, and of purified follicle-stimulating hormone from sheep pituitaries. Although no differences in biologic response under conditions of this assay could be demonstrated between the human urinary gonadotropins, the sheep FSH produced a significantly different response. This difference, which has not been explained, may be due to species specificity or to the result of metabolic tranformation or alterations produced in preparation of the hormone.

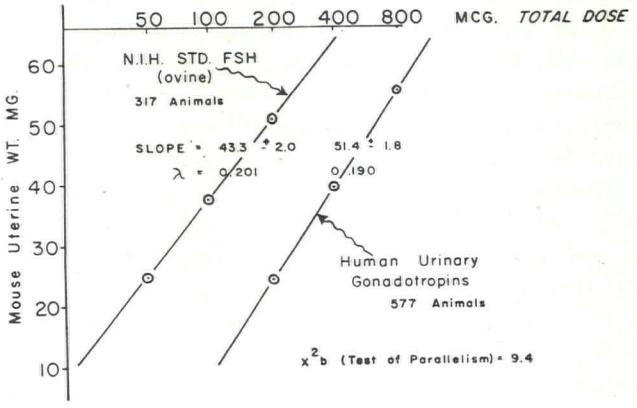


Figure 6

Comparative effects of human urinary gonadotropins and sheep pituitary FSH in the immature mouse uterine weight assay. The slopes of the dose-response curves are significantly different.

## DIFFERENTIATION AND MATURATION OF THE TESTIS

The three main cellular components of the developing testis are the germinal epithelium, the Sertoli cells and the interstitial cells of Leydig. In Figure 7 is

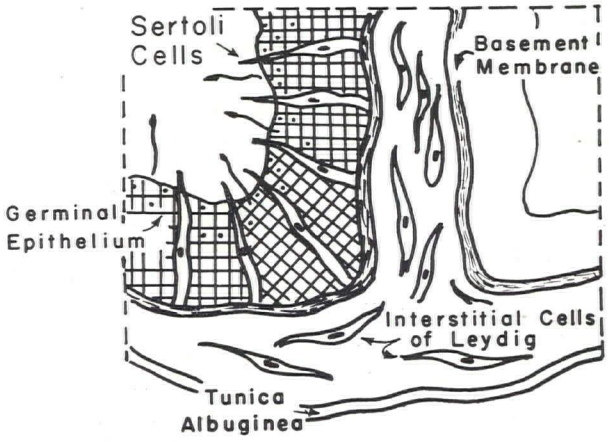


Figure 7

Schematic representation of the micro-anatomy of the mature human testis.

presented in schematic fashion the microscopic differentiation of the major components of the adult human testis. The germinal epithelium of the late fetal and infantile gonad is well developed, and except for the lack of spermatogenesis, the architecture is essentially that of the adult. The seminiferous tubules are solid i.e. lacking a central tubular lumen, and the germinal epithelium consists of resting spermatogonia.

Spermatogenesis begins at age 11 to 18 years and continues well into the sixth decade of life or beyond. The chain of events producing the spermatozoa

involves both a primary and secondary maturation division. The development of the spermatocytes containing 46 diploid chromosomes is believed to occur in response to local hormonal stimulation. In man this maturation is dependent upon gonadotropic hormones, but in the rat it is known to proceed without FSH. The miotic division to secondary spermatocytes with 23 chromosomes also apparently depends on FSH action, but whether this is a direct effect or is mediated through the Sertoli cells remains to be established.

The true function of the Sertoli cells in man is unknown, but they appear intimately involved in the process of sperm maturation, possibly serving as a nutrient source. One school, perhaps dominant, attributes testicular estrogen production to the Sertoli cells, while others have reported evidence which assigns this function to the interstitial cells. Whatever is produced, be it substance "X" of Howard[20] or estradiol, the Sertoli cell often shows histologic evidence of secretory activity, and in the dog, Sertoli cell tumors are often highly productive of estrogens.

The third functional element of the testis, the interstitial cell of Leydig, is the major site of hormonal production. Well developed in the embryo, the interstitial cells undergo a period of relative dormancy from birth to puberty at which time a resurgence of hormonal activity occurs. That this function is controlled by the pituitary through the luteinizing hormone (LH) is a universally accepted concept.

## BIOGENESIS OF GONADAL HORMONES

Among the recent significant developments in endocrinology has been the elucidation of the biosynthetic pathways of the gonadal hormones. It has long been held that estradiol is the chief estrogen produced in the ovary, that testosterone is the testicular androgen and that estrogens and androgens of lower potency arise in the adrenal cortex. There is evidence for estrogen production by the human testis but that for androgen synthesis in the human ovary is less conclusive.

The basic pattern of chemical events in the biosynthesis of both adrenal and gonadal steroids (Fig. 8) has been deciphered primarily through studies of patients with hyperfunctioning neoplasms of endocrine glands. As depicted by Dorfman,[21] the chief substrate, acetate, undergoes a series of molecular couplings yielding cholesterol. Probably initiated by the action of pituitary tropic hormones on specific enzymes, cholesterol undergoes a side-chain hydrolysis and cleavage, with oxidation of $C_{20}$, to become pregnenolone. The next steps, common to the biosynthesis of all adrenal and gonadal steroids, are the oxidation of pregnenolone through the action of 3$\beta$-ol-dehydrogenase to progesterone, and the 17$\alpha$-hydroxylation of the latter compound to 17$\alpha$-hydroxyprogesterone. The recent report by Landau[22] that administration of large amounts of chorionic gonadotropin to intact males results in an increased excretion of pregnanetriol (a urinary metabolite of 17-hydroxyprogesterone), supports the concept that 17-hydroxyprogesterone is an intermediate in testicular steroid biosynthesis. This latter steroid in turn undergoes oxidative removal of the side chain at $C_{17}$ yielding a 19-carbon steroid, $\Delta^4$-androstene-3:17-dione and acetic acid. Testosterone results from reduction of the $C_{17}$ ketone group to a 17$\beta$-hydroxyl group. The major urinary metabolites of testosterone are androsterone,

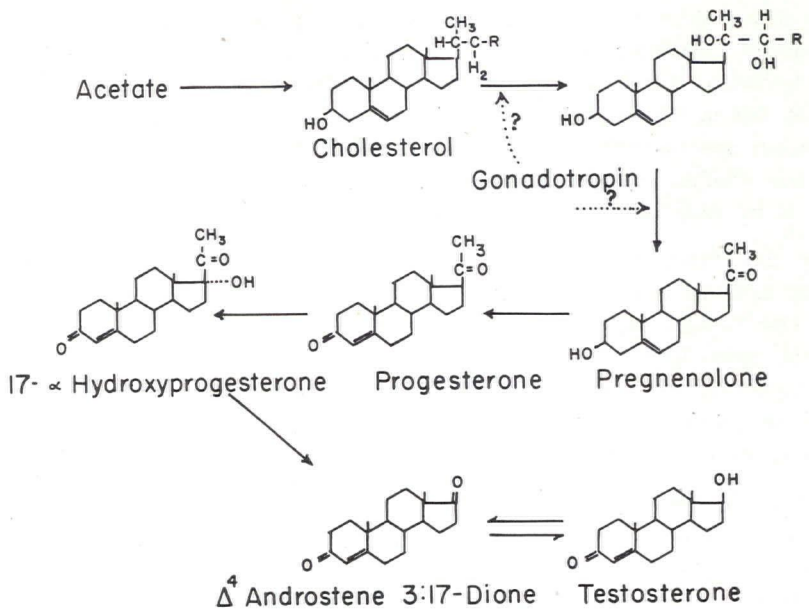


Figure 8

Chemical pathways in the biosynthesis of the adrenal and gonadal steroid hormones, as proposed by Dorfman.[21]

epiandrosterone, androstane 3, 17-dione and three etiocholanes: 3, 17-dione, 3$\alpha$, 17$\alpha$-diol and 3$\alpha$-o1, 17-one.

As previously mentioned, the interstitial cells of the testis are the locus of androgen biosynthesis in the male. That testosterone is actually produced in the human testis has been demonstrated in recent years by Lucas and associates[23] who isolated the hormone from the spermatic vein. Hollander and co-workers[24] more recently confirmed this observation and found a progressive decrease in concentration of the hormone with age. Although no pure androgen has been recovered from the ovary, androgenic activity in ovarian extract was demonstrated 20 years ago.[25] More recently, Johnson[26] has demonstrated that implanted ovaries in parabiotic male castrate rats when stimulated with gonadotropins can maintain the male secondary sex characteristics. It is probable that androgens are synthesized in the ovary in a manner similar to that described for the testis. Baggett and co-workers[27] have demonstrated in human ovarian tissue the conversion of $C^{14}$-labeled testosterone to 17$\beta$-estradiol.

That the adrenal cortex secretes androgenic steroids is evident in the sharp increase in urinary 17-ketosteroids which occurs in the puberal period. That a major portion of these ketosteroids are of adrenal origin is apparent in the marked reduction in their excretion which occurs during adrenal-suppressive therapy with the glucocorticoids. During early adult years, women excrete approximately 30 per cent less urinary androgens and 17-ketosteroids than do contemporary males. No quantitative change has been observed during the menstrual cycle. Shown in Figure 9 are data concerning the widely recognized decline in urinary neutral

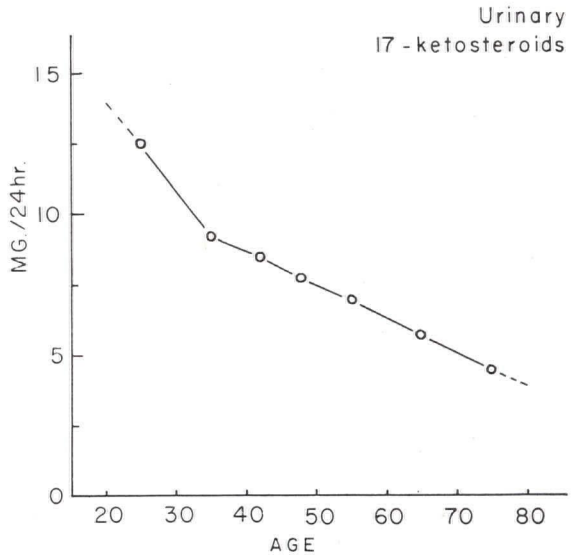


Figure 9

Composite of urinary 17-ketosteroid excretion of 147 women, normals and osteoporotics, studied by the authors.

17-ketosteroid excretion which occurs with age. A similar progressive lowering of 17-ketosteroid excretion ocurs in the aging male. The studies of Pincus and associates[28] have shown that this decline in ketosteroid excretion for both sexes is much greater for the 11-deoxy fraction than for the 11-oxygenated steroids. That this is a change in adrenocortical steroidogenesis and not a result of waning ovarian secretion is suggested by the fact that bilateral oophorectomy in the pre-menopausal female does not significantly reduce the urinary 17-ketosteroid level. It could be interpreted as suggesting that the primary function of cortisol biosynthesis requires progressively less pituitary ACTH as the individual ages, resulting in a proportional decrease in androgen secretion. In that sense, adrenocortical function in the aged is the reverse of that seen in patients with congenital virilizing adrenal hyperplasia a syndrome in which deficient cortisol production permits excessive ACTH release and accelerated adrenal androgen formation.

In the young woman, estradiol biosynthesis probably occurs for the most part in the theca interna, possibly in the granulosa cells. When the ovary atrophies at menopause, however, these structures are replaced by functionless corpora albicantia, and the interstitial stroma remains as the only potential source of gonadal estrogens. That the adrenal cortex contributes estrogen is beyond question, but the extent and biological significance of this secretion (probably estrone) are not entirely known.

The three major estrogens are 17$\beta$-estradiol, estrone and estriol (Fig. 10). 17$\beta$-Estradiol is considered the parent compound, while estrone and estriol are its metabolic derivatives. Experimental evidence indicates that estriol can not be converted to estrone or estradiol, although interconversion between estradiol and estrone

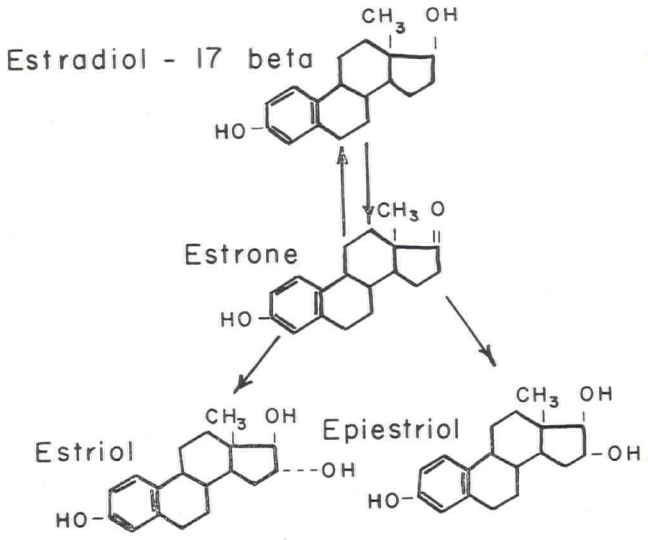


Figure 10
Chemical relationship of the three major estrogens.

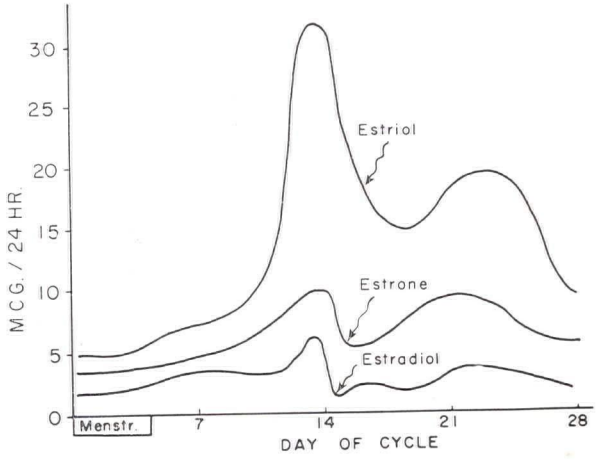


Figure 11
Urinary estrogen levels during the menstrual cycle (adapted from Brown[29]).

is possible. In urine estriol constitutes from 50 to 70 per cent, estrone 20 to 40 per cent and estradiol 12 to 15 per cent of the total estrogens, expressed in micrograms per day. The excretion values for these three substances during typical menstrual cycles are shown in Figure 11, data for which have been taken from the studies of Brown.[29]

With advancing years women excrete progressively smaller amounts of all estrogens, with a fairly rapid drop occurring in the fourth decade. The greatest reduction occurs in the estriol fraction. This change is shown in Figure 12, representing data from

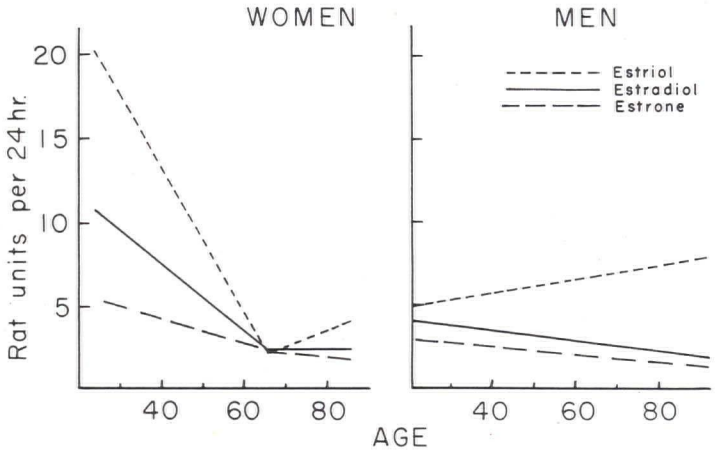


Figure 12

Urinary excretion of estrogens by men and women, from maturity to senescence (adapted from Pincus[28]).

the published studies of Pincus and associates.[28] These investigators demonstrated that by the time of menopause, total urinary estrogen levels were no greater than those found in the urine of men of comparable age. The great difference between the sexes in the fourth to sixth decades of life was the progressive rise in the estriol fraction of male urine. Based on this observation, the authors suggested that age effects the metabolic conversion of estrogens differently in the two sexes.

These data concerning estrogen excretion by women 20 to 40 years postmenopausal have been confirmed by others, but whether such estrogens are the products of the postmenopausal ovary is open to question. Wotiz and associates[30] have reported the *in vitro* conversion of $C^{14}$-labeled testosterone to estrone, estriol and, to a lesser extent, estradiol by tissue obtained from the ovary of a woman 20 years postmenopausal. The stroma of this ovary was described as being hyperplastic. Such evidence might be considered to support the theory of Sommers[31] that stromal hyperplasia in postmenopausal women represents a functioning tissue, the presence of which can be correlated with the occurrence of breast carcinoma. On the other hand, West and co-workers[32] have demonstrated in castrated, adrenalectomized women the conversion of testosterone to estrogens, a process presumably occurring in the liver. Paulsen and associates[33] administered hog FSH to six intact, postmenopausal women and found in two an increased excretion of urinary estrogens, measured by biological assay. These authors observed that the level of urinary estrogens of intact postmenopausal women were about twice that of contemporary castrates. McBride[34] of Scotland reported in 1957 that the quantity of the three major estrogens in urine of women averaging 10 years postmenopause was comparable to that of women at the low point in the menstrual cycle. No cyclic variation was noted in the former group, and the level of estrogen was unrelated to the histologic pattern of the endometrium. Furthermore, bilateral oophorectomy in postmenopausal women led to no reduction in levels of urinary estrogens.

Gynecologists and internists long have recognized clinical evidence that great differences exist in levels of ovarian function in postmenopausal women. Clinical data concerning this point (Table II) were recently collected by the authors. Levels

Table II

Estrogen Effects on Vaginal Epithelia

| Clinical Categories | No. Subjects | Cytologic Rating* | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Normals | 58 | 12.0 | 44.8 | 32.8 | 10.4 |
| Osteoporosis | 27 | 3.7 | 17.8 | 55.6 | 22.9 |
| Breast Cancer | 58 | 0.0 | 31.0 | 34.5 | 34.5 |

*Per cent distribution, adjusted for time after menopause
A=cornified and noncornified superficial cells
D=basal and parabasal cells
B and C=intermediate groups

of estrogen effect were judged by the degree of maturity of the vaginal epithelium, and two groups of patients (breast cancer and osteoporosis) were compared to contemporary normal subjects who had been found free of any neoplasm at a large cancer detection clinic. While a relatively high percentage of all subjects maintain mature epithelia years after the menopause, differences of probable significance were found between normals and the two patient groups. Whatever the source of estrogens in postmenopausal women it appears that they are of some biological importance to the host. An understanding of hormonal functions of the elderly is at least equal in importance to problems concerning reproduction and fertility, because these functions bear heavily on such complications of the aging process as osteoporosis, atherosclerosis and carcinoma.

Progesterone has two important roles, as an intermediate in corticoid, androgen and estrogen biosynthesis, and as a hormone secretion itself. As a major intermediate in the biosynthesis of adrenocorticoids, progesterone normally undergoes a rapid hydroxylation at $C_{17}$ with only small amounts excreted in urine as the pregnanediol derivative. However, selective enzymatic deficiencies in the adrenal cortex may result in failure of hydroxylation at $C_{21}$, as in the syndrome of congenital adrenal virilism. Under these conditions of impaired cortisol synthesis and compensatorily increased ACTH release, the accelerated adrenal steroidogenesis produces 17-hydroxyprogesterone in large amounts. This latter compound is reduced and excreted as pregnanetriol in urine, one of the biochemical hallmarks of the adrenogenital syndrome.

Progesterone is considered phylogenetically to have become significant in reproductive physiology when ovoparity was superseded by viviparity in the animal kingdom.[35] Its metabolism to pregnanediol, the major excretion product in urine, involves a series of reduction steps. The four pregnanediol isomers represent only one group of the several urinary metabolites that have been identified. Pregnanediol,

excreted as a glucuronide conjugate, can be recovered in the urine during the luteal phase of the menstrual cycle in amounts of approximately 2 to 4 mg. daily.

Many of the concepts concerning mechanisms of steroid synthesis in ovary and testis have been derived to a great extent from *in vitro* studies of human tumor tissue. The hazard of error is great in assuming that knowledge of hormone biosynthesis by tumors can be applied fully to function of the normal human gonad. Competent pathologists and endocrine histologists admit the difficulties of distinguishing, for instance, luteomas from Leydig cell tumors, and these from certain adrenocortical tumors arising from adrenal rests in the gonad. There have been reports[36] in recent years of gonadal tumors synthesizing steroids hydroxylated at the $C_{11}$ position, which might suggest that the gonads normally contain an 11-$\beta$ hydroxylating enzyme, as does the adrenal cortex for the biosynthesis of the glucocorticoids. There is yet no evidence for this in the normal gland, however. The critical issue in such studies of gonadal tumors is which method, the histological or biochemical, will be accepted as the final determinant of whether the tumor was derived from gonadal or displaced adrenal tissue.

As to the human *in vivo* studies most data have been derived chiefly from analyses of excretory products. Until more is known of metabolic pathways, blood concentrations, gonadal blood flow and the renal handling of steroids and their metabolites, it will be difficult to describe with any precision the steroid productivity of the gonads.

In addition to the gonadal steroids there is one non-steroid hormone of the ovary, relaxin, which can be given passing recognition. This substance has been demonstrated only during pregnancy, and apparently is of both ovarian and placental origin. Hisaw[37] in 1929 first isolated relaxin from corpus luteum extracts and defined its action in the softening and relaxation of pelvic ligaments of parturient rodents. The results of several clinical studies concerning the efficacy of relaxin in the treatment of premature labor[38,39] have been conflicting.

## HORMONAL INTERRELATIONSHIPS

The gonads have long been viewed as victims of thyroidal and adrenal dysfunction, yet much of the accusation has been based on highly circumstantial clinical evidence, particularly in respect to the thyroid. Despite the almost overwhelming clinical testimony, fortunately not all published, regarding the therapeutic efficacy of desiccated thyroid in disturbances of gonadal function, there have been few scientific demonstrations of an intimate interrelationship. It has been claimed that patients with untreated myxedema have prominent ovarian cysts, and Janes[40] reported a high incidence of cystic ovaries in rats with long-standing induced hypothyroidism. Meites[41] has shown that such animals are more responsive to chorionic gonadotropin, which seems to exhibit more FSH-like action in the hypothyroid animal than in the euthyroid. Beierwaltes[42] found normal amounts of gonadotropins in the urine of myxedematous women, an observation confirmed in our laboratory in the assay of urine from postmenopausal women with spontaneous myxedema. Goldsmith and associates[43] have studied the menstrual pattern of women with disturbed thyroid

function. In 70 per cent of patients with hyperthyroidism, ovulation occurred regularly, yet practically all had a marked degree of oligomenorrhea. Seven of ten premenopausal females with myxedema demonstrated ovulatory failure, while two had normal ovulation and menses. All were restored to normal menstrual cycles after proper treatment with desiccated thyroid. The investigators considered the menstrual defects to be the result of inadequate production or effectiveness of the luteinizing hormone.

The evidence that the level of basal metabolic rate determines to a great extent the rate of estrogen disposal is quite consistent with the frequently encountered clinical observation of altered menstrual patterns in women with thyroid dysfunction. The known acceleration of rates of estrogen conjugation and excretion in a thyroxin-induced hypermetabolic environment may acount for the hypo- and oligomenorrhea characteristic of the hyperthyroid female. Contrariwise, delayed metabolism of estrogen in experimentally produced hypothyroidism is consistent with the menorrhagia which often occurs in patients with myxedema. This important hormonal relationship is probably liver dependent, since this organ is the major site of estrogen metabolism. There is less evidence of significance relative to the effects of thyroxin on androgen metabolism. However, in 1956 Bradlow and associates[44] reported high urinary etiocholanolone to androsterone ratios in myxedema patients, and a partial correction toward normal when triiodothyronine was administered.

There is evidence that estrogens in turn can modify certain aspects of thyroid function, although the biological significance of this relationship has not been defined. The best example is the known action of estrogens to increase the concentration of circulating protein-bound iodine. The demonstration of increased thyroxin-binding protein, both in pregnancy and in estrogen-treated women, seems to explain the phenomenon since the high PBI concentrations are unassociated with evidence of an increased metabolic action of the circulating thyroxin. That the effect is dependent on several factors is suggested by the studies of Stoddard and associates[45] who reported no consistent change in concentrations of serum precipitable iodine after bilateral oophorectomy of women in their reproductive years, and of Engbring and co-workers[46] who have shown that the thyroid gland is not necessary for the estrogen-induced rise in PBI concentration. They concluded from their studies that the increased PBI is due to a prolongation of the thyroxine survival time. Thus, the sequence of action can be outlined as follows: 1) increased thyroxine-binding capacity of serum; 2) increased concentration of PBI; 3) decreased utilization of thyroid hormone; 4) decreased BMR; 5) increased pituitary secretion of TSH; 6) increased thyroid gland activity. Testosterone, on the other hand, appears to lower serum PBI levels, but the mechanism has not been defined.

Clinicians have postulated that the cycles of follicular and luteal activity in the normal female, by virtue of sequential stimulation and suppression of hypophyseal function, including TSH release, result in cyclic variations in thyroid function and ultimately in goiter, nodular or otherwise. Experimental support of this concept in man is lacking, however. Reineke and Soliman[47] have reviewed evidence for a functional interrelationship of thyroid and gonad in animals and demonstrated that at estrus the metabolic rate is often maximal and, by the criterion of thyroidal uptake

of $I^{131}$, that thyroid function is also maximal. In ovariectomized rats, the administration of small doses of estrogen results in an increased $I^{131}$ uptake by the thyroid.

Existence of a complex interrelationship between the gonad and adrenal may be anticipated by virtue of the common embryologic origin of these glands. Clinical endocrinologists in the past three or four years, recognizing the potential significance to the ovary of adrenal function, normal and abnormal, have successfully used the glucocorticoids in the treatment of ovarian dysfunction and infertility[48,49,50].

That an ovarian-adrenal relationship exists in the normal female is evident in the conspicuous change in adrenocortical function which occurs in the years just before and during menarche. The data shown in Figure 13 have been taken from

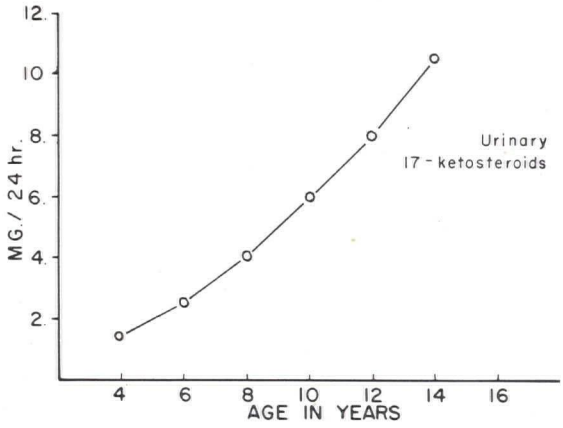


Figure 13

Urinary 17-ketosteroid excretion from childhood to adolescence.
Summation of data from Nathanson[51] and Oesting.[52]

the well-known studies of Nathanson and associates,[51] and Oesting and Webster.[52] The rise in urinary 17-ketosteroids from prepubertal levels of one or two milligrams to adult levels of 14 or 16 milligrams represents an impressive alteration in adrenal function brought on as a result of, or at least simultaneously with, the rise in ovarian function. Evidence that these ketosteroids are adrenal and not ovarian in origin has been previously cited. Reifenstein et al.[53] once postulated that pituitary LH, released in increasing amounts from the pituitary through the effects of estrogens from the maturing ovary, resulted in the so-called adrenarche. This concept has never been substantiated. Furthermore, recent evidence is available to indicate that chorionic gonadotropin, possessing LH action, does not stimulate the adrenal cortex, judged by levels of urinary 17-ketosteroids and 17-hydroxycorticoids.[54] Patients with primary hypogonadotropic hypogonadism and those with gonadal dysgenesis have been found to excrete low levels of urinary 17-ketosteroids despite normal rates of glucocorticoid biosynthesis.[55,56]

Recently, several groups of investigators have shown an effect of estrogens on one interesting aspect of adrenal function.[57,58] Administration of stilbesterol or ethinyl

estradiol in moderate amounts to a normal person results in materially increased levels in plasma of free 17-hydroxycorticoids. This phenomenon has been shown to result from a decreased rate of corticosteroid reduction in liver and to an increased plasma binding of cortisol. However, in contrast to the increasing levels of urinary 17-ketosteroids observed during gonadal activation at puberty, the opposite effect has been observed during estrogen administration. In Table III are data from our

Table III

Effect of Oral Estrogen in Nine Virilized Women.

| Treatment (No. of assays) | 17-KS mg./24 hr. mean ± S.E. | P value |
|---|---|---|
| None (22) | 18.2 ± 1.7 | — |
| Estrogen (26) | 12.7 ± 1.7 | 0.05 — 0.02 |

recent studies of a group of virilized women. In these subjects, 17-ketosteroid excretion was observed to fall from 18.2 to 12.7 mg. during the ingestion of Estinyl.® Hermann and associates[59] have also observed reduced urinary 17-ketosteroids during periods of estrogen administration. By fractionation analyses of the 17-ketosteroids, they found the decreased excretion to involve steroids of both adrenal and gonadal origin. Furthermore, this phenomenon occurred in their study at physiologic levels of administered estrogen.

In addition to suppressing gonadal steroidogenesis via inhibition of gonadotropin release, administered estrogens could also lower urinary 17-ketosteroids by: 1) inhibiting the conversion of corticoids to 17-ketosteroids; 2) suppressing adrenal androgen secretion through ACTH inhibition, via elevated titers of plasma cortisol; and 3) the increased plasma binding or altered metabolism of the androgen precursors themselves. Since estrogen administration can reduce the urinary 17-ketosteroid response to infused ACTH without altering the plasma cortisol response, Hermann and associates[60] concluded that estrogens increase the plasma binding of 17-ketosteroid precursors.

In several species, under both natural and experimental conditions, adrenal hyperplasia has been induced by the administration of estrogens.[61,62] This effect is ACTH dependent, and Witschi[63] has postulated from his experiments that in the amphibia at least, the hypothalamic-hypophyseal release mechanism, essential to ovulation, is dependent not only on the gonadotropic but also on the adrenotropic system. The postulated mechanism of ovulation is depicted in Figure 14. Whether or not this observation applies to human females is problematical, but the presently inexplicable successes reported to follow glucocorticoid administration to infertile, non-virilized women may involve some such system.

Concerning the effect of the glucocorticoids on gonadal function, it might be reasoned on ontogenetic ground that a condition of relative permissiveness exists, since complex gonadal functions evolved under the influence of a much older adrenocortical system. That is, within physiological limits, changing adrenal function

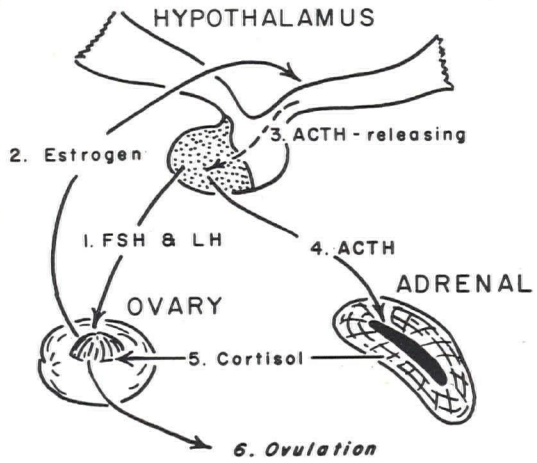


Figure 14

Endocrine interrelationships during ovulation in the amphibia. The postulated role of the adrenotropic system is based on the observations of Witschi.[63]

has only a minor effect on gonadal function. Clinical experience with patients having adrenocortical insufficiency provides support for this concept, since undisturbed gonadal function is often observed in patients with Addison's disease. In instances of complete adrenocortical insufficiency, amenorrhea in women and impotence in men may result, but the mechanism would seem to be an adaptation for survival rather than a reflection of endocrine dependency. Continued ovulation and, indeed, fertility with resulting pregnancy were not rare experiences for the Addisonian female treated in the pre-cortisone era with sodium chloride and deoxycorticosterone. Continuation of pregnancy under these conditions may have been dependent to a great extent on placental synthesis of glucocorticoids, although results in recent studies do not support this idea.[64]

Physicians have had a rich opportunity to observe the effects of increased glucocorticoid levels on gonadal function as a result of the widespread use of corticoids in clinical practice. Interference with function does occur, but is usually observed quite late in the course of treatment, at least if conventional dosages are used. Irregular menses, amenorrhea and reduced potency result, but they are not common in otherwise healthy persons. With high dose glucocorticoid therapy particularly for persons with systemic disease, the aforementioned effects are likely to occur earlier. The beneficial results of glucocorticoid therapy in women with ovarian dysfunction are considered due to improvement of pre-existing endocrine abnormalities.

One action by which adrenal steroids could alter hypophyseal-gonadal function was first reported by Sohval,[65] and independently by Smith[66] in 1950. Markedly increased levels of gonadotropins were observed in urine of patients receiving glucocorticoid or corticotropin therapy. The patients under study were in the initial phases of treatment with conventional but physiologically abnormal doses of the hormones. Gonadotropin titers were increased two to six fold in both studies. The

mechanism remains unknown but increased renal clearance of the gonadotropin as a result of the corticoid effect on kidney function is considered one explanation. Whatever the basis, disturbed gonadal function manifested as amenorrhea and oligomenorrhea with anovulatory cycles, might be the anticipated result if the corticoid effect on gonadotropin release were prolonged. Study of patients with Cushing's syndrome provides conflicting evidence, since low or absent gonadotropin excretion has been observed.[67] These low titers may reflect the general protein depletion of prolonged hypercorticism, with curtailment of gonadotropin formation such as occurs in starvation. Although increased renal loss of gonadotropin may have occurred in the early phases of the hypercorticism, there is no supporting data for this thesis. However, Iannaccone and associates[68] observed that ovaries of premenopausal Cushing's patients were small and white, with diminished follicular activity. Primordial follicles were sharply reduced and the overall picture was that of premature aging. These observers cited no gonadotropin data, stating that "disordered pituitary gonadotropic function cannot be excluded as a possible contributory cause". It seems likely that depleted gonadotropin secretion could have been a very important factor even though the ovaries histologically were not much different from those seen in postmenopausal women with high gonadotropin output.

This essay, keyed to the clinically oriented reader, is an attempt to integrate established knowledge with certain of the recent contributions in the study of gonadal development and function, particularly as it relates to man. Without question, some areas of the broad subject have been insufficiently discussed, and of the many available references, only a few have been cited. For more extensive information, the reader may turn to several monographs and texts recently published.[69,70-71] Just as in the case of insulin, therapeutic triumphs in clinical endocrinology are often achieved before the *modus operandi* are known. Yet the past ten years of accumulating insight regarding adrenal pathophysiology and biochemistry have prepared the way for gaining the more elusive knowledge concerning the gonads. A broad conceptual framework of gonadal form and function is helpful for understanding the contributions to come. The present review may serve this purpose for those not intimately concerned with endocrine research.

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