THE ENDOCRINE FUNCTIONS OF THE MAMMALIAN OVARY*

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The complete expression of sexuality in the female involves the secretion of female sex hormones, ovulation, copulation and the birth of young. The internal secretions of the ovary regulate the sex accessories, the secondary sex characters and the mating reactions. Numerous factors in the external environment are known to influence the cyclicity of the female tract and it is probable that most of these effects are mediated by means of neuro-endocrine mechanisms. Not all of the genetic, endocrine, neural and nutritional phenomena involved in the control of ovarian functions have been elucidated, though substantial progress has been made in this direction during the last few decades. In natural environments there is a basic tendency for females to permit copulations only during recurrent periods when the best opportunities prevail in the female tract for the union of spermatozoa and eggs and for the differentiation of the zygotes.

In newborn mammals a large number of primary follicles are identifiable in the ovaries and, in certain species at least, there is evidence that this number is augmented by proliferations from the germinal epithelium during postnatal life. Only a small proportion of the original follicles ever complete their differentiation, the majority of them undergoing atresia without ovulating. As the individual approaches pubescence, certain of the ovarian follicles increase greatly in size and rupture in order to free their ova. The periodic release of eggs from the ovary is termed ovulation.

After ovulation the collapsed wall of the follicle undergoes rapid structural changes. The cells of the granulosa and, in most species, the cells of the theca interna enlarge, become fat-laden and highly vascularized, thus forming a compact body called the corpus luteum. If impregnation ensues, the corpus grows and persists functionally until near the end of gestation. In the absence of fertilization it persists for a shorter period which varies according to the species. The primary function of the graafian follicle is the nourishment and discharge of the ovum, but it is also associated with an incretory function. The corpus luteum seems to be entirely incretory and its secretion is distinct from that of the graafian follicle.

I. OVULATION

Ovulation is a complicated process involving both hormonic and nervous mechanisms. Only within recent years has experimental evidence made possible a satisfactory explanation of this phenomenon. Morphological studies indicate that the graafian follicle enlarges appreciably during proestrus and early estrus. Extensive mitoses occur in the follicular walls and the secretion of liquor folliculi distends the antrum. The cumulus oöphorus loosens and frees the ovum and its surrounding corona radiata. The tissue between the graafian follicle and the surface of the ovary thins out somewhat and an avascular area, the stigma, appears on the surface of the follicle. As the pressure within the antrum increases, the whole avascular area is caused to bulge. The weakest point on the avascular area bulges still further, producing a nipple or cone which finally ruptures. Slight hemorrhage into the antrum may precede rupture of the follicle. Direct observations indicate that rupture of the follicle is a non-explosive process somewhat similar to the breaking of an abscess. A small jet of thin liquor folliculi first spurts from the burst follicle but the remainder of the fluid is more viscous and leaves the follicle more slowly. Ovulation in the mammal requires less than a minute, whereas in the frog the release of an egg from the ovary occupies a period of from four to ten minutes.

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In the past there have been many unsuccessful attempts to explain ovulation on the basis of local changes in the graafian follicle and the adjacent wall of the ovary. Most of the earlier views held that ovulation resulted from increased intra-follicular pressure, supposedly brought about in various ways, or from the enzymatic dissolution of the follicular wall. While both of these may be important auxiliary factors, they are for the most part incidental. The modern view is that ovulation is a differentiational phenomenon which is initiated by the gonadotropic hormones of the anterior lobe of the hypophysis cerebri. During the past few years the following pertinent points have been established:

1. The administration of purified FSH produces huge cystic follicles which do not ovulate. Thus ovulation cannot be explained on the basis of increased secretion of liquor folliculi and a consequent elevation of pressure within the antrum.

2. Hypophysectomy prevents pre-ovulatory swelling and rupture of the graafian follicle.

3. Ovulation can be induced in a variety of vertebrates by the administration of a proper mixture of FSH and LH. A correct ratio between FSH and LH seems to be absolutely essential for the full expression of the ovarian follicle. While ovulation in the rabbit is conditioned normally by cervical stimulation, it may be induced in normal or hypophysectomized subjects in the absence of sexual excitement by the administration of both FSH and LH. This seems to justify the assumption that ovulation is a growth phenomenon which occurs only in the presence of the proper anterior lobe principles.

4. Ovulation is not dependent upon the nerve supply to the ovaries. Normal ovulation may occur in ovarian grafts and in intact ovaries deprived of all nervous connections when the proper hypophyseal hormones are present.

5. The release of the ovulatory hormones from the anterior pituitary is almost certainly regulated by means of nervous pathways. Rabbits continue to copulate after surgical section of the infundibular stalk but ovulation does not occur in these animals. Under these conditions ovulations can be induced by the administration of FSH and LH. In normal estrous rabbits ovulation may be induced by electrical stimulation of a certain area in the pre-optic region of the brain, but this becomes impossible after section of the infundibular stalk. An increasing amount of evidence justifies the assumption that the secretion and/or release of ovulatory principles from the anterior hypophysis is controlled by nerve fibers which extend to it from the hypothalamic region of the brain stem.

The continued removal of eggs from the nests of certain birds may prolong the period of egg-laying very appreciably. In the light of what is known about ovulation in other vertebrates, one might suppose that this response is affected by means of a neural mechanism which prolongs the release of gonadotropins by the hypophysis rather than by a direct nervous stimulation of the ovaries. Endocrinologists are just beginning to appreciate the possibility that stimuli originating in the central nervous system may profoundly modify the functional states of endocrine glands.

II. THE OVARIAN HORMONES

A. The estrogenic hormone of the graafian follicle.—The first experiments leading to the identification of the ovarian hormones were attempts to prevent castration atrophy in the female by means of transplanting ovarian tissue. Near the turn of the present century, several investigators demonstrated that castrate atrophy of the uterus could be prevented by incorporated ovarian grafts. These first experiments demonstrated clearly that the atrophic changes following castration resulted from the withdrawal of an ovarian secretion rather than from nervous disturbances.

During the next few years there were attempts to develop desiccated preparations and ovarian concentrates which would be as effective as subcutaneous ovarian grafts in correcting the syndrome resulting from castration. At this period, the ovary was regarded as performing an incretory function but the ovarian secretion was conceived of as a single substance. While Adler's work was unconvincing, he claimed in 1912 that he was able to prepare aqueous extracts of ovarian tissue which had the property of restoring some degree of sexual activity in spayed females.

The next observations of importance were detailed histological studies of the ovary and the female accessories during the course of the normal cycle. It became possible to correlate ovarian changes with differences in the sex accessories and secondary sex characters. The estrous cycle of the guinea pig was described in minute detail in 1917 by Stockard and Papanicolaou (1). Similar studies were made on the rat by Long and Evans (2) and on the mouse by E. Allen (3). These observations were extremely significant since they suggested that the graafian follicle is the source of the estrogenic hormone, and since they indicated sensitive physiological indicators for the assay of estrogens. In these laboratory rodents, it was determined that a rhythmic sequence of changes occurred in the vagina and that these changes, followed by the vaginal smear technique, corresponded to rhythmic modifications of the ovary. Following oöphorectomy the vaginal rhythm ceases and the diestrous condition prevails.

The final physiological identification of an ovarian hormone awaited the epochal experiments of Allen and Doisy (4). These workers aspirated liquor folliculi from the vesicular follicles of fresh sow's ovaries and injected it into oöphorectomized mice and rats. Within fifty hours subsequent to the injection of fresh liquor folliculi, or alcoholic extracts of the fluid, to spayed animals the vaginal smear contained cornified epithelial cells typical of normal estrus. Histological examination of the sex accessories indicated that the vaginal wall had attained maximal growth, the superficial layers being cornified as during estrus. The uteri were hyperaemic and distended with fluid. Following withdrawal of the injections, the castrate condition supervened. When administered to immature rats and mice these extracts produced premature canalization of the vagina. The property of estrogenic substances to produce cornification of the vagina of the spayed rat or mouse was adopted as a simple and accurate method of bioassay.

Frank (5), using castrate mice as test animals, demonstrated that an estrogenic substance having the same properties as follicular fluid was present in menstrual and circulating blood of the human female. During pregnancy the amount in the blood was increased. In 1928 Zondek (6) reported that large amounts of estrogen were present in the urine of pregnancy. This was a timely discovery inasmuch as the urine from pregnant women and mares provided a cheap source of tremendous amounts of estrogen for chemical studies. This substantial background of animal experimentation culminated quickly in the chemical isolation and purification of the follicular hormone and chemically related estrogens.

During the 1920s many investigators believed that the ovary secreted only one hormone, i. e., estrogen. Here was a substance which fulfilled many of the functions ordinarily attributed to the ovary. Since it appeared likely that estrogen, when properly purified and physiologically tested, would substitute completely for the ovaries in castrate rodents, there seemed to be little incentive to look further for additional ovarian hormones. Other workers, however, remained skeptical and insisted that it was necessary to account for certain observations which had been made during the first decade of the present century.

B. The Hormone of the Corpus Luteum.—Long before any experimental evidence became available, there were many speculations regarding the function of the corpora lutea. Beard in 1897 suggested that the corpora lutea constituted an "organ of pregnancy" and speculated that this organ exerted an inhibitory influence upon ovulation and that it prolonged the cycle. Born (1900) was aware that the corpora lutea attained maximal differentiation during the period when blastocysts were ready to implant in the uterine mucosa and when the placental connections were being established. He indicated to his student, Ludwig Fraenkel, that he believed that the corpora lutea produced a substance which prepared the uterus for the reception and inplantation of the developing embryos. Born died before having opportunity to test experimentally his hypothesis and Fraenkel (7) proceeded to do so. Fraenkel bilaterally oöphorectomized rabbits immediately after mating, or removed the corpora lutea from mated individuals, and found that under these conditions implantation and pla-

centation did not ensue. He found that the removal of the corpora lutea previous to the twentieth day of pregnancy resulted either in absorption or abortion of the young.

Additional evidence that the corpora lutea are important in conditioning uterine reactions was provided by the classical experiments of Leo Loeb (8). He allowed estrous guinea pigs to mate with vasectomized males and, several days later, laparotomized the females and traumatized the uteri. He found that a tumor of decidual cells differentiated at the sites where the uteri were injured. This indicated that the corpora lutea produce the hormone which makes it possible for the endometrium to undergo decidual changes in response to the irritating effect of the blastocysts. Loeb (9) demonstrated also that extirpation of the corpora lutea hastened the next estrus and that removal of other parts of the ovary did not give this effect. Later it was found that ablation of the corpora during pregnancy might be followed by ovulations. On the basis of these early experiments, many believed that the corpora lutea secreted the ovarian hormone and that this was the only hormone concerned in the reproductive cycle.

Ancel and Bouin (10) extended Loeb's work to include the pseudo-pregnant rabbit. Since ovulation in this species occurs ten hours subsequent to copulation or cervical stimulation, luteinization can be induced in the absence of pregnancy by artificially stimulating the cervices or by mating with a vasectomized male. These two French investigators found that during pseudopregnancy the uterus underwent a type of proliferation which simulated that normally occurring during pregnancy. They found that this type of uterine growth, now designated as progestational proliferation, did not occur after the ablation of the corpora.

Herrmann (11) prepared lipoid extracts of corpora lutea and placental tissue and showed that they produced uterine growth and congestion.

In 1921, it was reported that corpora lutea could be shelled out of the ovaries of the non-pregnant cow by means of rectal palpation. This procedure was followed within two days by ovulation and estrus.

The chain of evidence establishing the incretory function of the corpus luteum was completed by Corner (12) and his collaborators. These workers prepared lipoid extracts of corpora lutea obtained from the ovaries of pregnant swine and found that such extracts produced progestational proliferation in the uteri of castrate adult rabbits, a reaction which cannot be elicited by estrogen alone. The recognition of this physiological endpoint in the rabbit was as instrumental in the isolation of the luteal hormone as was the vaginal response of the mouse and rat in the physiological identification of estrogen. These extracts, administered to rabbits which had been castrated eighteen hours after fertile matings, maintained pregnancy to term. They sensitized the uterus so that decidual reactions resulted from uterine trauma and, in short, produced all of the effects which earlier experiments indicated as attributable to the corpus luteum. The active extract of the corpus luteum was named "progestin". By 1933 several groups of investigators had announced the isolation of the hormone in crystalline form. Shortly thereafter, it became possible to synthesize the hormone of the corpus luteum, progesterone, from stigmasteral and from pregnanediol.

C. Androgenicity of the Mammalian Ovary.—There is substantial evidence indicating that the ovary may become capable of liberating appreciable amounts of androgen during unusual or abnormal circumstances. Steinach and Kun (13) reported that the luteinized ovaries of the guinea pig may exert masculinizing effects. Lipschütz (14) found that an ovarian graft persisting for three years in a castrate male guinea pig had restored fully the seminal vesicles and prostatic glands of the host. The experiments of Hill and collaborators (15, 16) indicate that ovarian homotransplants into the ears of castrate mice frequently become capable of maintaining secretion in both the seminal vesicles and prostate. A similar androgenic action of ovarian grafts persisting in the ears of rats has been reported by Deanesly (17). She did not find that the androgenicity of the ovarian grafts was conditioned by temperature as Hill maintains for mice.

Several workers have shown that crystalline progesterone produces masculinizing effects. It has not been proved,

however, that the chemical configuration of the progesterone molecule is not altered by the organism before androgenic effects are elicited. Nelson (18) found that progesterone resembled androgens inasmuch as it was capable of maintaining spermatogenesis in the gonads of hypophysectomized rats. Turner (19) described a spontaneous lesion of the rat's ovary which rendered it hyper-estrogenic and definitely androgenic. The absence of corpora lutea provided presumptive evidence that the masculinizing action of these ovaries was not due to progesterone.

The identity of the androgenic substances which derive from ovaries has not been ascertained. While the administration of large amounts of progesterone elicits masculinizing effects in certain laboratory rodents, the evidence seems to indicate that the ovaries may librate another androgenic compound which is similar to but not identical with that secreted by the testis. Deanesly found that gonadotropins causing extensive luteinization of the granulosa did not alter appreciably the androgenicity of the ovarian ear grafts in the rat. From studies upon the growth curves of male accessory glands, Hill and Strong concluded that the physiological response induced by ovarian grafts in the ears is not nearly duplicated by the experimental administration of testosterone propionate plus estrogens.

On the basis of studies undertaken in this laboratory, we believe that the androgenicity of the ovary is correlated with hypertrophy and hyperplasia of the theca interna of follicles which are forced experimentally to become atretic. In the case of ovaries persisting in the ears of castrate males, the hypophysis stimulates the differentiation of many vesicular follicles. These cannot ovulate because of an improper endocrine balance in the male and because of the complete encapsulation of the graft by dense tissue. When the cords of epithelioid cells derived from the theca interna persist and become abundant the ovary is made capable of secreting enough androgen to maintain secretion in the male sex accessories.

A permanent impairment of the rat's hypophysis and ovary results from the daily administration of 100 IU of estrogen during the first ten days of postnatal life. The follicles become atretic before reaching full maturity. The thecal cells become epithelioid and persist after other elements of the follicles have deteriorated. Some of the ovaries from adult animals of this type induce secretion in the seminal vesicles and prostate when such gonads are transplanted to the kidneys of long-time castrate male hosts. Since both kidney and ear grafts sometimes possess androgenic potencies, it appears that the temperature of the transplantation site is not the principal factor determining the androgenicity of the ovary.

In conclusion, an attempt has been made to present evidence upon which a modern theory of ovulation is based, and to outline the major events which led to the physiological identification of the ovarian hormones. Evidence has been presented which indicates that the ovary, under certain conditions at least, may secrete a male-sex-hormone-like compound which seems not to be progesterone.

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