ESSAYS ON APS CLASSIC PAPERS

Follicular origins of modern reproductive endocrinology

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IN THE 1920S, the field of reproductive endocrinology was just beginning to take form. Much of what was known about the reproductive cycle was learned from anatomic dissection and histological examination, but the driving forces behind the cyclical changes observed remained a mystery. When Edgar Allen and Edward Doisy published their landmark paper, “The induction of a sexually mature condition in immature females by injection of the ovarian follicular hormone,” in 1924, they stirred strong feelings in at least one already established researcher in the field and changed the way research on the ovarian hormone would then be conducted (3).

The “modern theory of menstruation,” as Emil Novak described it in 1921, placed emphasis on the ovary as a secretory organ that governs menstruation by the release of a hormone (13). Supporting this theory were experiments showing cessation of menstruation after castration and the resumption of menstruation after transplantation of ovarian tissue and after the administration of ovarian extracts. The popular theory of the time, propagated by Fraenkel and Cohn in Germany, held the corpus luteum as the source of this ovarian hormone (9). Several camps of researchers were racing to isolate and purify the ovarian hormone, and most were focusing on the corpus luteum.

Edgar Allen (Fig. 1) was fairly new to the academic scene when he published his landmark article in 1924. He had completed his graduate degree at Brown University in biology and moved to St. Louis to become Instructor and Associate in Anatomy at Washington University in St. Louis in 1919. His first publications, in 1922 and 1923, described the estrous cycle in the mouse and oogenesis during sexual maturity (1, 4). While studying oogenesis, he recognized the relationship between the development of ovarian follicles and the maturation of other reproductive organs. He became convinced that the follicle was the source of the ovarian hormone and not the corpus luteum, which never preceded the first Graafian follicle (1). He teamed with Edward Doisy, a biochemist at St. Louis University, to challenge the corpus luteum theory of menstruation.

Allen and Doisy’s experiment involved taking follicular fluid from hog ovaries, creating an extract, injecting it into immature and castrated rats over several days, and examining the rodents for artificially induced sexual maturity (3, 5). They used the opening of the rat vaginal orifice as the marker for sexual maturity instead of ovulation, since they had removed the ovaries of some of their rats. The first part of their study looked at the efficacy of follicular fluid extract to induce vaginal opening. The second part evaluated the changes seen in the uterus and vagina as a result of the extract and whether it correlated to normal rats in estrus. They found that the follicular fluid caused sexual maturity, that the uterus became hyperemic and swollen with secretions, and that the vaginal epithelium became well developed and cornified. Importantly, the changes in the vaginal epithelium caused by sexual maturity could be seen by examining a vaginal smear of the animal (3, 5).

Fig. 1. Edgar Allen.
Allen and Doisy published a preliminary report on their discovery in 1923 describing their test method, their purification process, and their early results. In that paper, they mentioned that Robert Frank, an advocate of the corpus luteum theory, had earlier published positive results from using the follicular fluid but had perhaps overlooked their importance. They also reported entirely negative results from using corpus luteum extracts. Frank criticized their preliminary report in a letter to the editor stating that he had indeed shown follicular fluid to contain the ovarian hormone in 1922, well before Allen and Doisy’s paper that he devoted five pages of his book, The Female Sex Hormone, to establishing himself as the first to obtain positive results from follicular fluid and again criticized Allen and Doisy as “newcomers” who tried to “cast doubt on the observations of all their predecessors because they (Allen and Doisy) could not obtain positive results with corpus luteum by means of their test”. Despite Frank’s criticisms of Allen and Doisy, he did recognize their study as being important in the description of a new method of assaying and titrating the ovarian hormone by examining the vaginal smear of living rats.

Prior to Allen and Doisy’s article, the methods for testing ovarian extract efficacy were often cumbersome. Frank noted that uterine contractions in rats slowed during estrus, but that was a complicated and impractical test of hormonal influence. Most researchers were using rabbits or guinea pigs, which were more expensive and difficult to keep than rats, and their longer estrous cycles extended the time needed to complete an experiment. Additionally, test animals were typically euthanized to examine their uteri for effects of an extract. In 1917, Charles Stockard and George Papanicolaou discovered that the ovulation period in the guinea pig, corresponding to estrus, was accompanied by hypertrophic changes in the vaginal epithelium, which could be seen in a vaginal smear. Allen and Doisy had found similar results in the rat, and with their publication in 1924, they described an easy, quick, and economical method to test extract efficacy without having to sacrifice the animal.

Allen and Doisy not only confirmed a new way to test extract efficacy, they also standardized it by defining the Rat Unit (R.U.). The rat unit was defined as the “minimal quantity which will induce full estrus growth in the genital tract of a spayed adult rat 48 hours after the first of three 1-cc injections given at intervals of 4 to 6 hours.”

The development of the vaginal smear test for extract efficacy, coupled with the definition of the R.U., vastly accelerated the search for the ovarian hormone. Allen and Doisy’s method was used to evaluate many other tissues for the presence of the ovarian hormone, including blood, urine, feces, bile, liver, male tissues, and even plants. The search for the ovarian hormone, now known as estrogen, cumulated in the isolation of the hormone in crystalline form by Allen and Doisy in 1930, and a few months later by Butenandt and von Ziegner in Germany.

By use of the Allen and Doisy method, it was demonstrated that the corpus luteum was almost completely devoid of estrogen. This prompted the search for another ovarian hormone and ultimately resulted in the discovery of progesterone in 1929. The next several years saw the identification of the hypothalamic-pituitary-ovarian axis that we now know today.

This classic paper being one of his first publications, Edgar Allen made an early mark in the burgeoning field of reproductive endocrinology and accelerated the discovery of the two female hormones estrogen and progesterone. He went on to publish 147 articles and edit two editions of the book Sex and Internal Secretions and received several awards both abroad and in the United States. He died in 1943 while serving in the Coast Guard Auxiliary in Long Island Sound.

REFERENCES