

Edgar Allen and Edward A. Doisy's Extraction of Estrogen from Ovarian Follicles, (1923) ^[1]

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In the early 1920s, researchers Edgar Allen and Edward Adelbert Doisy conducted an experiment that demonstrated that ovarian follicles, which produce eggs in mammals, also contain and produce what they called the primary ovarian [hormone](#) ^[2], later renamed [estrogen](#) ^[3]. In their experiment, Doisy and Allen extracted [estrogen](#) ^[3] from the ovarian follicles of hogs and proved that they had isolated [estrogen](#) ^[3] by using a measurement later renamed the Allen-Doisy test. Allen and Doisy's 1923 experiment to isolate [estrogen](#) ^[3] showed that [estrogen](#) ^[3] is made within the ovaries and also established a method for isolating the sex [hormone](#) ^[2]. That method provided a basis for future research on [hormones](#) ^[4]. Later researchers showed that [estrogen](#) ^[3] functions in the menstrual cycles of primates by signaling for the tissue lining the [uterus](#) ^[5] ([endometrium](#) ^[6]) to thicken in preparation for possible [implantation](#) ^[7] of a [fertilized egg](#) ^[8].

In 1923, Allen and Doisy were instructors in anatomy and biochemistry, respectively, at the [Washington University](#) ^[9] School of Medicine in St. Louis, Missouri. Doisy worked with [sex hormones](#) ^[10] and vitamin K, and he later received the Nobel Prize for Physiology or Medicine in 1943 for his work discovering the chemical nature of vitamin K. Allen worked with mice and their estrous cycle, the reproductive cycle of non-primates similar to [menstruation](#) ^[11] in [humans](#) ^[12]. Allen helped map the changes that occur during the estrous cycle in mice.

By the 1920s, researchers had discovered that a particular [hormone](#) ^[2] caused the cyclical changes in the reproductive systems of mammals. Referring to that [hormone](#) ^[2] as the primary ovarian [hormone](#) ^[2], researchers attempted to identify where it was produced. In the early 1920s, Allen and Doisy began researching where primary ovarian [hormone](#) ^[2] was produced. At that time, some researchers said that primary ovarian [hormone](#) ^[2] was produced at a site called the [corpus luteum](#) ^[13], which developed on the [ovary](#) ^[14] and caused nutrients to build up on the uterine lining after the [egg](#) ^[15] was released. Allen, while conducting his research on the estrous cycle in mice, noticed that multiple corpus luteum sites (corpora lutea) at any given time were in many different stages of degeneration. Some old sites were broken down and healing, while new and intermediate sites were forming at the same time. The estrous cycle first changes the thickness of uterine tissue and then releases eggs from the [ovary](#) ^[14]. Because researchers could see the corpora lutea in different but simultaneous stages of formation, the corpora lutea alone could not control [hormone](#) ^[2] release by a progressive or stepwise series of tissue changes during a reproductive cycle. Allen, after arriving at that conclusion, ruled out the corpora lutea as producing the primary ovarian [hormone](#) ^[2] in 1921.

Still searching for where the primary ovarian [hormone](#) ^[2] was made, Allen observed that uterine tissue change occurred parallel to [egg](#) ^[15] production (follicle development) in the ovaries of mice. Allen hypothesized that the changes observed in the lining of the [uterus](#) ^[5] during the estrous cycle were caused by production of the primary ovarian [hormone](#) ^[2] during ovarian follicle development. Allen hypothesized that the changes in the uterine tissue resulted as a direct response to a [hormone](#) ^[2] produced by the ovarian follicles. He further hypothesized that the ovarian follicles produced the primary ovarian [hormone](#) ^[2].

In 1923, Allen tested his hypothesis with Doisy's help in their lab at the Washington University School of Medicine. The researchers tested their hypothesis in a series of steps. First, they isolated the primary ovarian [hormone](#) ^[2] from ovarian follicles, which contained unwanted products such as proteins and fats. Next, Allen and Doisy developed a method to remove the unwanted products, to leave behind pure [hormone](#) ^[2] compound. Lastly, the researchers injected the isolated the primary ovarian [hormone](#) ^[2] compound into mice to prove that they had properly isolated it. Allen and Doisy performed the animal tests to determine if their extraction restored natural endocrine functioning in mice with their ovaries removed. If Allen and Doisy had isolated the [hormone](#) ^[2], the animals would show normal reproductive cycle changes and development. If the researchers had not isolated the primary ovarian [hormone](#) ^[2], reproductive changes would not take place in the mice, as [hormone](#) ^[2] is needed for the reproductive responses to occur.

To complete the first step of procuring the primary ovarian [hormone](#) ^[2], Allen and Doisy used hypodermic needles to remove the liquid content of the ovarian follicles from hog ovaries. Doisy and Allen selected hog ovaries for their large follicles, each about half the size of a pencil eraser (5mm in diameter), and because of hogs' long reproductive cycles. The long reproductive cycle made it easier to retrieve the content of the follicles, as the follicles do not degenerate as fast compared to [humans](#) ^[12]. Hog ovaries could be used for tests on mice because the [hormones](#) ^[4] of both animals have identical molecular structures, though the researchers did not know that at the time. The researchers gathered approximately one hundred milliliters of follicular liquid for every pound of ovarian tissue. The amount of liquid gathered was close to one small bottle of cologne or perfume. Because the untreated follicular liquid contained eggs (ova), proteins, and fats (lipids) as well as the suspected primary ovarian [hormone](#) ^[2] ([estrogen](#) ^[3]), Allen and Doisy had to devise a means to separate the contents to isolate the primary ovarian [hormone](#) ^[2].

Allen and Doisy's next task was to develop and test a procedure to separate the primary ovarian [hormone](#)^[2] from the mixture of substances. Allen and Doisy extracted the primary ovarian [hormone](#)^[2] from the follicular liquid by adding it to a solution containing ninety-five percent alcohol that caused the proteins to clump together (coagulate), enabling them to remove the proteins from the mixture. Next, the researchers separated the alcohol from the [hormone](#)^[2] and the remaining fats using distillation, in which they boiled the substance to evaporate the alcohol from the sample. After distilling the alcohol out of the solution, the researchers needed to remove excess water, and they added ether (an organic solvent) to separate the water. The procedure is similar to how vegetable oil reacts with water and forms two distinct layers. Allen and Doisy then added acetone to the solution to cause the lipids in the remaining [hormone](#)^[2] solution to change states from liquid to solid (precipitate) and allow for the removal of the now solid lipids. Doisy and Allen were left with only the primary ovarian [hormone](#)^[2].

After purifying the primary ovarian [hormone](#)^[2] from hog ovarian follicles, Allen and Doisy performed four tests to show that they had properly extracted and located the primary ovarian [hormone](#)^[2]. In the first test, they injected the primary ovarian [hormone](#)^[2] into spayed mice, which had had their ovaries removed, to determine if normal endocrine activity resumed. Endocrine activity in female mice depends on the presence of sex [hormones](#)^[4] like [estrogen](#)^[3], which Allen and Doisy hypothesized was produced in the ovaries. Without ovaries, the mice had no endocrine activity. After the injection with Allen and Doisy's extraction, the mice experienced a normal estrous cycle, even though they had no means of producing the primary ovarian [hormone](#)^[2] by themselves without ovaries. The results showed that the [hormone](#)^[2] Doisy and Allen extracted produced normal cycle responses in mice, thus verifying that the substance was the primary ovarian [hormone](#)^[2]. That [hormone](#)^[2] was later named [estrogen](#)^[3]. Allen and Doisy's procedure with spayed mice and the primary ovarian [hormone](#)^[2] was later called the Allen-Doisy test. The Allen-Doisy test is a procedure that uses spayed mice, as they no longer have endocrine functioning to detect the presence of [estrogen](#)^[3] in a sample. Researchers inject a sample into spayed mice and if their reproductive cycle starts, [estrogen](#)^[3] is present in the sample.

To further verify that they had isolated the primary ovarian [hormone](#)^[2], Allen and Doisy used the extract to determine if it could induce normal sexual behavior in spayed mice. The researchers again used spayed mice because they are not inclined to mate without [estrogen](#)^[3] to provoke their sexual drives. After the injection of extracted [hormone](#)^[2] into spayed female mice, the mice allowed males to mate with them, signifying resumption of normal endocrine activity. As [estrogen](#)^[3] functions in a [mouse](#)^[16]'s sexual drive (heat) during its reproductive cycle, the injection of a different substance would not produce those results.

The third test performed by Allen and Doisy showed that injections of the primary ovarian [hormone](#)^[2] in three-week-old mice caused the mice to sexually mature in only two to four days, approximately thirty days in advance of normal development. Allen and Doisy concluded that the extracted [hormone](#)^[2], when injected in mice, led to the sexual maturation of sex organs and the development of secondary sexual characteristics, such as the enlargement of mammary glands.

Using lab tests to compare compounds, Allen and Doisy's final experiment compared what they hypothesized was the primary ovarian [hormone](#)^[2] with a solution taken from the corpora lutea, where other researchers claimed the primary ovarian [hormone](#)^[2] was produced. The tests showed that the corpora lutea, just as Allen and Doisy predicted, did not contain the primary ovarian [hormone](#)^[2]. That confirmation allowed them to reject the old theory that the corpora lutea produced the primary ovarian [hormone](#)^[2].

The results from the four tests supported Allen and Doisy's hypothesis that the production of the primary ovarian [hormone](#)^[2] occurred in the follicles as they develop in ovaries. Allen and Doisy concluded that the compound extracted from the ovarian follicles produced the primary ovarian [hormone](#)^[2] ([estrogen](#)^[3]), because it caused natural endocrine processes and functions that mimicked the naturally occurring estrous cycle. Allen and Doisy's work uncovered that [estrogen](#)^[3] is made within the ovarian follicles of the ovaries, replacing the theory that the [corpus luteum](#)^[13] primarily produced [estrogen](#)^[3].

Allen and Doisy's results and their process for [estrogen](#)^[3] extraction led to the development of therapeutic tests involving [estrogen](#)^[3] injections. Scientists soon made [estrogen](#)^[3] available via injection for women with lower levels of [estrogen](#)^[3], as a method for its extraction had been developed and proven to work.

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In the early 1920s, researchers Edgar Allen and Edward Adelbert Doisy conducted an experiment that demonstrated that ovarian follicles, which produce eggs in mammals, also contain and produce what they called the primary ovarian hormone, later renamed estrogen. In their experiment, Doisy and Allen extracted estrogen from the ovarian follicles of hogs and proved that they had isolated estrogen by using a measurement later renamed the Allen-Doisy test. Allen and Doisy's 1923 experiment to isolate estrogen showed it was made within the ovaries and also established a method for isolating the sex hormone. That method provided a basis for future research on hormones. Later researchers showed that estrogen functions in the menstrual cycles of primates by signaling for the tissue lining the uterus (endometrium) to thicken in preparation for possible implantation of a fertilized egg.

Subject

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