

On the trail of steroid aromatase: The work of Kenneth J. Ryan

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The sexes in humans and other animals typically display numerous differences. This belies the fact that the two major hormone classes responsible for these differences, androgens and estrogens, differ only subtly in their chemical backbones. Androgens have a six-carbon nonaromatic ring—the “A ring” (Fig. 1)—in their steroid skeleton, whereas estrogens have an aromatic A ring. Remarkably, a single protein, steroid aromatase (also called estrogen synthase), is the only known enzyme capable of “aromatizing” the A ring in androgens such as testosterone and androstenedione to produce estrogens such as estradiol and estrone.

Females and males require both sex hormones in a balance appropriate for each sex. A chief role of steroid aromatase, along with those of other enzymes that modify or metabolize steroid hormones, is to maintain this healthy balance, for example, during development and pregnancy and in reproductive tissues. Accordingly, researchers have long sought to better understand steroid aromatase activity in order to reduce pregnancy complications, develop hormone-based contraceptives, and manage estrogen-responsive cancers.

Three papers published in the *Journal of Biological Chemistry*, authored by Kenneth J. Ryan and recognized as Classics here (1–3), laid the groundwork for understanding the role of steroid aromatase and other steroid-modifying enzymes in estrogen biosynthesis.

Before embarking on his studies of steroid aromatase, Ryan, along with co-author Lewis Engel, discovered that microsomal fractions of adrenal glands from beef contain an enzyme activity that hydroxylates carbon 21 in the hormone progesterone and several of its derivatives (1).

This finding clarified the enzymatic nature of this pivotal step in steroid hormone production in adrenal tissues. It revealed that this step consists of an oxidative reaction that requires microsomes, NADPH, and molecular oxygen. It also helped establish an experimental system that Ryan could then use to probe how estrogens are made from androgens.

“[He was] trying to figure out the physiology of pregnancy, what the estrogens were doing, and how they how they arose,” says Richard Auchus, Professor of Internal Medicine and Pharmacology at the University of Michigan and a researcher with a long-standing research interest in steroid production and modification.

The *in vivo* conversion of an androgen to estrogenic activity was noted more than 80 years ago, when Steinach and Kun (4), using a bioassay that measured hormone activities only indirectly, reported that men given testosterone have higher estrogenic activity in urine. This finding suggested that estrogens can be formed from androgens, and further studies in the 1950s

with extracts from animal tissues hinted at the enzymatic nature of this conversion (5, 6).

However, the activities in these preparations were insufficient to study the nature of the androgen-to-estrogen conversion and its roles in human health and reproduction. This dearth in available methods to investigate this conversion prompted Ryan, then a clinician-researcher at Harvard Medical School, to try to boost estrogen production, extract these hormones more efficiently, and begin to characterize the enzymes involved.

“He was one of the first people who did that sort of stuff,” says Auchus. Moreover, Ryan was an obstetrician, meaning that he had a ready supply of tissue ideal for investigating estrogen production—human placentas obtained immediately after delivery.

Preparing and analyzing these biological materials was laborious, requiring many time-consuming steps now seldom performed in biochemistry labs. Ryan had to carefully extract the enzyme activities from kilograms of placental tissues, and even small glitches in the experimental protocols could result in loss of activity after hours of preparation. “These were some of the first very brave steps to try to figure out where [estrogens] were coming from,” says Auchus.

This effort bore fruit when, in 1959, Ryan confirmed that the formation of the aromatic A ring is the result of enzymatic activity leading to aromatization (2). “He figured out that [the enzyme activity] was in the microsomal fraction and that it required NADPH, and he developed the chromatography to measure it,” Auchus says.

Ryan’s findings suggested that the aromatization involved an enzyme system that used molecular oxygen to achieve A-ring aromatization—yet, quite paradoxically, no extra oxygen atoms were retained in the estrogen products. With the tools available at the time, Ryan could not delineate the exact biochemical sequence of events, nor could he isolate the enzyme(s) responsible or the reaction intermediates that bore the tell-tale oxygen atoms.

According to Auchus, initially it wasn’t even clear whether the aromatization was performed by one enzyme or by several because the reaction sequence required at least three oxidations, which yielded some, at the time, unusual products. “[It] was fascinating that you got an aromatic A ring, [but] this did not really make a lot of sense to people back then.” says Auchus. “The reaction was weird because it involved both loss of a methyl group and formation of the aromatic A ring.”

The reactions remained black boxes, but Ryan’s work had begun to pry the lids open. “Now people could start to look at the mechanism,” says Auchus.

Studies in the 1980s revealed that the aromatizing activity is indeed performed by just one enzyme, a cytochrome P450

Fred Guengerich at Vanderbilt University nominated this series as Classics.

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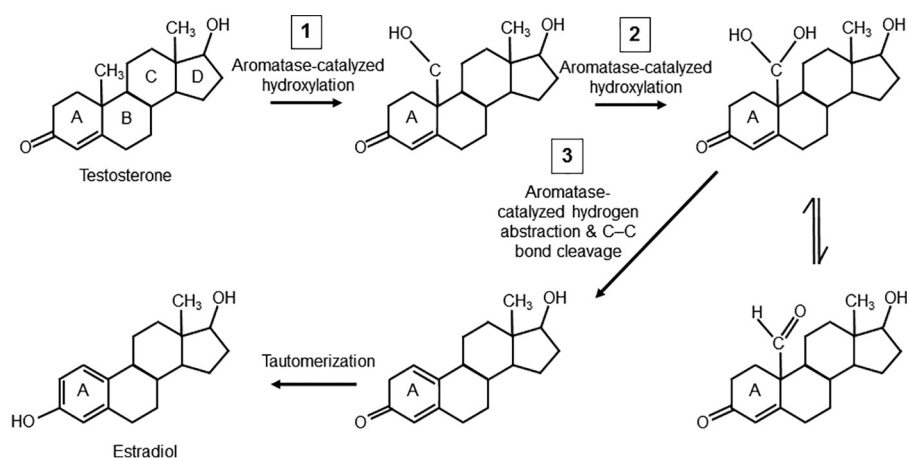


Figure 1. Steroid aromatase (estrogen synthase) is a cytochrome P450 enzyme that aromatizes androgens to estrogens. Kenneth Ryan developed a protocol for improved extraction of steroid aromatase activity from human placentas (2). This improvement opened up the way for later in-depth studies of this enzyme, revealing that steroid aromatase is a P450 monooxygenase that catalyzes three reactions (indicated by boxed numbers above/beside the 1st three arrows) in the conversion of androgens such as testosterone to estrogens such as estradiol. These reactions include two hydroxylations at carbon 19 and an uncommon third oxidation step leading to multiple molecular rearrangements (10), resulting in carbon 19 elimination, reduction of the A-ring keto group to a hydroxyl, and A-ring aromatization.

monooxygenase (7–9). This enzyme, aptly named steroid aromatase, oxidizes carbon 19 (a methyl group attached to the A ring in androgens) in a multistep reaction sequence, starting with two sequential hydroxylations and culminating in an unusual C–C bond cleavage, leading to C-19 elimination and formation of the phenolic A ring in estrogens (Fig. 1).

However, as Auchus notes, the details of the third aromatase-catalyzed step remained long unresolved and were somewhat controversial. “People fairly quickly figured out where the first two reactions occurred, but the third reaction [resulting in formic acid release from carbon 19 and the A-ring aromatization] was a vexing step that people could not work out for a long time.”

Recent work has now shown that this reaction involves abstraction of a hydrogen atom from carbon 1 of the 19-*gem*-diol, rearrangement with loss of the formic acid prior to oxygen rebound, and, finally, keto-enol tautomerization to form the phenolic A ring (10). “Suffice it to say, it is a unique chemical reaction,” Auchus concludes.

Ryan next set his sights on establishing the biochemical origins of another important estrogen, estriol, now a standard biomarker in routine pregnancy care. One classic pathway of estriol formation involved the hydroxylation of carbon 16 in the estrogens estradiol and estrone. However, in an earlier study, Ryan had found preliminary evidence for another pathway in which estriol also could be produced by aromatization of C-16–hydroxylated androgens (11).

Using his placental microsomal enzyme system, Ryan confirmed his earlier finding of estriol production from 16 α -testosterone. He also clarified that another estrogen, 16 α -hydroxy-estrone, is an intermediate in the classic estriol-producing pathway (3). These discoveries underscored the utility of Ryan’s assay and represented key early steps in untangling the biochemical complexities in estrogen production.

Born in 1926 in New York City, Ryan grew up during the Great Depression, working on farms in his teen years and, following graduation from high school, serving in the United States Navy during World War II (12). Upon returning from the

war, he enrolled in Northwestern University for his undergraduate studies and then joined Harvard Medical School, graduating magna cum laude in 1952.

During a couple of residencies at hospitals in the Boston area, Ryan landed a biochemistry fellowship shared with Nobel Laureate Fritz Lipmann, enabling him to pursue his interest in the roles of estrogens in the biology of pregnancy.

In the 1960s, thanks to his managerial skills and knack for anticipating future directions in clinical research, Ryan began taking on administrative duties, successively becoming chairman of several obstetrics and gynecology departments across the country. In the early 1970s, he returned to Harvard where he helped build an academic OB-GYN department. In addition to his work in the clinic and extensive research activities, Ryan trained and mentored many students and residents.

He also became active in medical ethics. “He actually got involved with a lot of ethical issues, like fetal tissue research,” Auchus says. “In his later years, he became a pretty prominent person in that field.”

Notably, Ryan chaired the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. This commission produced the Belmont Report in 1979, whose guidelines for protecting the rights and dignity of human subjects in research continue to provide an ethical framework for research and health providers in the United States to this day. Ryan also was an early and strong proponent of reproductive choice.

He died in 2002 at the age of 75.

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