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Review

Steroid hydroxylations: A paradigm for cytochrome P450 catalyzed mammalian monooxygenation reactions ☆

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Abstract

The present article reviews the history of research on the hydroxylation of steroid hormones as catalyzed by enzymes present in mammalian tissues. The report describes how studies of steroid hormone synthesis have played a central role in the discovery of the monooxygenase functions of the cytochrome P450s. Studies of steroid hydroxylation reactions can be credited with showing that: (a) the adrenal mitochondrial enzyme catalyzing the 11β-hydroxylation of deoxycorticosterone was the first mammalian enzyme shown by O^{18} studies to be an oxygenase; (b) the adrenal microsomal enzyme catalyzing the 21-hydroxylation of steroids was the first mammalian enzyme to show experimentally the proposed 1:1:1 stoichiometry (substrate:oxygen:reduced pyridine nucleotide) of a monooxygenase reaction; (c) application of the photochemical action spectrum technique for reversal of carbon monoxide inhibition of the 21-hydroxylation of 17α-OH progesterone was the first demonstration that cytochrome P450 was an oxygenase; (d) spectrophotometric studies of the binding of 17α-OH progesterone to bovine adrenal microsomal P450 revealed the first step in the cyclic reaction scheme of P450, as it catalyzes the "activation" of oxygen in a monooxygenase reaction; (e) purified adrenodoxin was shown to function as an electron transport component of the adrenal mitochondrial monooxygenase system required for the activity of the 11β-hydroxylase reaction. Adrenodoxin was the first iron–sulfur protein isolated and purified from mammalian tissues and the first soluble protein identified as a reductase of a P450; (f) fractionation of adrenal mitochondrial P450 and incubation with adrenodoxin and a cytosolic (flavoprotein) fraction were the first demonstration of the reconstitution of a mammalian P450 monooxygenase reaction.

Keywords: Photochemical action spectra; Oxygenase stoichiometry; Steroid 11β hydroxylase; Steroid C21-hydroxylase; Adrenodoxin; Cholesterol; Progesterone

The history of discovery of steroids in humans reads like a thrilling adventure novel. The structural characterization of steroids, determination of their physiological function, and definition of the biochemical reactions that catalyze the sequential transformations of steroid hormones serve as the foundation of our present knowledge of this important class of lipids. The experimental challenges that had to be overcome in defining the enzymology of these reactions have been formidable. Today we profit from the elegant research involved in unraveling the role that these naturally synthesized endogenous chemicals play in the maintenance of human cellular homeostasis.

Background

During early chemical studies of steroid hormones it was recognized that functional hydroxyl-groups on the steroid nucleus play a major role in determining the physiological actions of different steroids. An excellent summary of the early history of the discovery and identification of human

☆ This manuscript is dedicated to Osamu Hayaishi and the late Howard Mason. These two giants led the world of science in the exploration of reactions of oxygen. Their pioneering discoveries nearly 50 years ago continue to set the stage for elegant research studies on enzymes that react with atmospheric oxygen.

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steroid hormones is described in the Presentation Speech given by Professor G. Liljestrand as the 1950 Nobel Prize in Physiology and Medicine was awarded to Edward Kendall, Tadeus Reichstein, and Philip Hench [1].

In the early part of the 20th century, the role of the adrenal gland was an undefined mystery. It was recognized that adrenal glands were essential for life and that injections of extracts of the adrenal into humans could produce remarkable effects on the treatment of a number of medical diseases. Originally, it was believed that there was a single adrenocortical hormone designated "cortin." Increased sophistication in the extraction, isolation, purification, and characterization of such extracts identified the empirical formulae of over 30 different forms of steroids in humans which had differences in their biological activities. At that time these steroids were identified as "chemicals built up of 21 carbon atoms, but the number of oxygen atoms in the molecule is three, four or five." Interestingly, there is an absence of references to studies which questioned the source of the oxygen in the hydroxyl- or keto-functional groups in the different steroid molecules.

Cholesterol as the source of steroids

The next 20 years, following the isolation and characterization of human steroids, were occupied by experiments attempting to understand the physiological differences and biochemical inter-relationships of the various steroid hormones. Central to these advances was the introduction in the 1940s of isotopes that could be used to label and identify metabolic products formed during metabolism. These studies are the basis of our current definition of many of the pathways of metabolic transformations of steroids in mammalian tissues.

A seminal discovery was reported in 1945 by Bloch [2] who showed that cholesterol was metabolized to pregnanediol when a woman in her 8th month of pregnancy was given deuterio cholesterol. This in vivo experiment clearly demonstrated that cholesterol was the precursor of biologically active steroid hormones in humans.

In vitro studies of steroid transformations

The decade from 1945 to 1955 provided a wealth of new results describing the in vitro metabolism of a large number of different steroids. A fountainhead for much of this research was the pioneering work of investigators such as Gregory Pincus, Ralph Dorfman, Oscar Hecter, Mika Hayano, and many others working at the Worcester Foundation in Shrewsbury, MA. (e.g., Levy et al. [3] described the "Chemical Transformations of Steroids by Adrenal Perfusion" and the review [4] by Oscar Hecter and Gregory Pincus summarizing the "Genesis of the Adrenalcortical Secretion"). The workers at the Worcester Foundation became experts in the use of a single or multiple pass perfusion techniques applied to the beef adrenal gland combined with development of various analytic methods for evaluating the steroid metabolites formed. These studies defined [4] the pathway of metabolism of pregnenolone (the first metabolite formed from cholesterol) via progesterone to corticosterone and 17-hydroxycorticosterone. A large literature soon accumulated during this period identifying new steroids, proposing new pathways of steroid transformation, and possible schemes of inter-conversion of steroids (a few of the giants who contributed to our knowledge of steroid metabolism include Max Sweat, Jim Tait, Alex Zaffaroni, Don Nelson, Carl Djerassi, Leo Samuels to name just a few). However, little was known about the nature of the enzymes involved in these reactions and the mechanism of action(s) of such enzymes remained a mystery for many years. Questions remained unanswered as to the source and mechanism of incorporation of the different oxygen atoms (as hydroxyl or keto functions) in the different steroid molecules. One textbook described the mechanism of hydroxylation of steroids as "via simple stereospecific replacement of hydrogen by hydroxyl" (quoted in an article by Holloway [5]).

The discovery of oxygenases

The mid-1950s were an exciting time of discovery related to the biotransformation of various chemicals (including steroids) by oxygen. In 1955, Mason et al. [6] and Hayaishi et al. [7] reported essentially simultaneously the properties of a new family of enzymes called "Oxygenases." The paper by Mason et al. [6] appeared first, having been submitted on April 11, 1955 and published in the May 20, 1955 issue of the Journal of the American Chemical Society. In their paper, [6] they provided evidence that the *plant copper enzyme* called "phenolase complex," catalyzes the conversion of 3,4-dimethylphenol to 4,5-dimethylcatechol in a manner that "all oxygen enzymatically introduced as hydroxyl into the benzene ring of the substrate comes from molecular oxygen." Shortly thereafter Hayaishi et al. [7] published in the same journal (received on August 31, 1955 and published October 20) a paper titled "Mechanism of the Pyrocatechase Reaction." Using a *bacterial enzyme* that catalyzes the oxidative cleavage of the aromatic ring of catechol to *cis–cis*-muconic acid, Hayaishi et al. [7] observed "a new class of metallo-protein enzymes which introduce two oxygen atoms directly across the aromatic bond adjacent to the phenolic group with simultaneous rupture of the aromatic structure."

Less well recognized is a third contributor to these exciting discoveries. Hayano et al. [8] from the Worcester Foundation for Experimental Biology submitted a Letter to the Editor of Archives of Biochemistry and Biophysics on September 9, 1955. This paper was titled "On the Mechanism of the C-11β-hydroxylation of Steroids: A Study with H_2O^{18} and $O_2{}^{18}$" and was published in December 1955. This study, using a *mammalian enzyme* system, showed that incubation of the steroid, 11-deoxycortisol, with a mince prepared from bovine adrenal cortex, "conclusively demonstrates that molecular oxygen, but not water, is utilized

by the adrenal 11β-hydroxylase in the C-11β-hydroxylation of steroids.''

Thus, in the short period of six months ''Oxygenases'' were born and shown to participate in the enzymatic oxidative functions of plants, bacteria, and mammalian tissues.

At about this same time two seemingly unrelated areas of biomedical research were also in an active phase of development: (a) the study of the reactions of drug metabolism and detoxification led by a group at the National Institutes of Health under the direction of Brodie et al. [9] and (b) the study of the metabolism of xenobiotics and the associated reactions of chemical carcinogenesis carried out at the McCardle Research Foundation at the University of Wisconsin by a group led by Elizabeth and Jim Miller [10]. In subsequent years, these reactions (steroids, drugs, and xenobiotics) were found to share a common denominator—the P450 Oxygenases—as the essential catalytic reaction center for metabolism.

Each of these areas of study revealed a number of common features: (a) the enzymatic reactions were catalyzed by membrane-bound enzymes that appeared refractory to purification; (b) the obligatory role of atmospheric oxygen was a requirement for each type of reaction; and (c) the requirement for reduced pyridine nucleotides (NADPH) as a source of reducing equivalents for the hydroxylation reactions was essential. These requirements fulfilled the definition of a ''mixed-function oxidase'' (monooxygenase) as proposed by Mason [11]. However, the enzymology and mechanism of such reactions remained elusive and many hypotheses were proposed.

The C-21 hydroxylation of 17α-hydroxyprogesterone

A seminal paper appeared in the Journal of Biological Chemistry in 1957 in which Ryan and Engel [12] described their studies on the C-21-hydroxylation of 17α-hydroxyprogesterone by a particulate preparation (microsomes) isolated from the cortex of the bovine adrenal gland. This study has served as the groundwork for many subsequent studies of steroid hydroxylation reactions and systematically defined the conditions for obtaining optimal rates of enzymatic conversion of 17α-hydroxyprogesterone to Reichstein's Substance S (17α,21-dihydroxyprogesterone or 11-deoxycorticosterone). Like the studies of drug and xenobiotic metabolism catalyzed by the microsomal fraction of liver [9] [10], Ryan and Engel [12] showed that the C-21-hydroxylation of steroids required reduced triphosphopyridine nucleotide and atmospheric oxygen. Of particular interest was the demonstration that the reaction was inhibited in the dark when incubated under an atmosphere of 90 percent carbon monoxide and 10 percent oxygen, and that this inhibition was reversed by shining white light on the reaction vessel. In the Discussion section of this paper [12] Ryan and Engel proposed the general concept that the reaction shares the ''common features of the incorporation of atmospheric oxygen into the substrate'' in the manner proposed by Mason [6]. This hypothesis served as the critical link to the earlier work on Oxygenases. However, the work of Ryan and Engel [12] left many questions unanswered—in particular the nature of the steroid hydroxylating enzymes and the stoichiometry and mechanism of the reactions. Ryan and Engel [12] were limited by the unavailability of the required spectrophotometric and polarographic instruments needed to observe the presence of additional hemeproteins in the microsomal fraction of the adrenal cortex. Instead, they focused their attention on the microsomal pigment cytochrome b_5 (then called cytochrome m) and proposed a role for the function of this hemeprotein in the hydroxylation reaction.

David Cooper, Otto Rosenthal, and the Johnson Foundation of the University of Pennsylvania

In 1959, I returned to the Johnson Foundation for Medical Physics at the University of Pennsylvania after a year of study at the Molteno Institute, University of Cambridge with David Keilen (the discoverer of cytochromes). On my return to Philadelphia I renewed my friendship with a young surgeon named David Cooper. David Cooper and Otto Rosenthal were carrying out experiments similar to those described by Ryan and Engel [12] for measuring the C-21 hydroxylation of 17α-OH progesterone catalyzed by microsomes isolated from bovine adrenal cortex [13]. We had many discussions about the results described by Ryan and Engel [12] and since my academic appointment was in Physical Biochemistry at the Johnson Foundation with Britton Chance I had access to a number of sophisticated instruments that permitted us to do experiments to answer questions raised by the Ryan and Engel study. One of the first experiments we did was to examine the spectrophotometric properties of the microsomal fraction isolated from the adrenal cortex. The rapid and sensitive spectrophotometers developed by Chance [14] allowed us to work with turbid suspensions of microsomes (i.e., in the absence of any detergent). Further, I was aware of the work of my laboratory colleagues Klingenberg [15] and Garfinkel [16] who had observed a unique carbon-monoxide binding pigment in liver microsomes with a spectrophotometric maximum of the complex at 450 nm.

Our first experiment showed the presence in adrenal microsomes of the CO-binding pigment with a maximum at 450 nm (we soon recognized that Ryan and Engel [12] had not seen this pigment because they had used deoxycholate as a detergent to clarify their samples for spectrophotometric analysis using a conventional spectrophotometer).

Could we establish a link between Oxygenases and this CO-binding pigment with a maximum at 450 nm?

The first challenge was to carry out experiments to show that the C-21-hydroxylation reaction of 17α-OH progesterone fulfilled the stoichiometry of a monooxygenase reaction as proposed by Mason [11].

Establishment of the stoichiometry of the steroid 21-hydroxylase reaction

While at the Johnson Foundation I became quite expert in the construction and use of a polarographic instrument designed to measure oxygen uptake during studies of oxidative phosphorylation by isolated rat liver mitochondria [17]. David Cooper and I tried this technique using microsomes isolated from bovine adrenal cortex and the experiment failed. The oxygen electrode was not sufficiently sensitive to measure the small rates of oxygen uptake catalyzed by adrenal microsomes under our conditions of experimentation.

I redesigned the electronics of the oxygen electrode circuitry to increase sensitivity greater than tenfold and I included a compensating voltage circuit to permit the electronic offset of background oxygen uptake by the microsomal preparation oxidizing NADPH in the absence of steroid substrate. The redesigned oxygen electrode worked [18] and we were able to experimentally determine that 1 nmol of steroid substrate (17α-OH progesterone) was hydroxylated for every nanomole of oxygen utilized. But, we also wanted to know the balance of reducing equivalents derived from NADPH for this reaction. However, as indicated above, the use of turbid microsomal preparations excluded the use of conventional spectrophotometric or fluorometric methods for measuring the time-dependent changes in the oxidation of NADPH during the oxygenase reaction, because of large light scattering problems. Earlier I had applied [19] a front-surface reflectance fluorometric method (developed by Eppendorf) to studies of turbid mitochondria for monitoring changes in the steady state reduction of mitochondrial reduced pydridine nucleotides. When we applied this approach for the measurement of NADPH oxidation by bovine adrenal microsomes, it worked. And we soon calculated the balance of nanomoles of NADPH oxidized to the nmoles of deoxycorticosterone formed. Our results [20] established a stoichiometry of approximately 1 nmol of NADPH oxidized per 1 nmol of 17α-OH progesterone hydroxylated. In this way we confirmed the earlier proposal by Mason [11] that a stoichiometry of equimolar amounts of reactants (i.e., 1 nmol of steroid substrate; 1 nmol of oxygen utilized; and 1 nmol of NADPH oxidized) participated in the mixed-function oxidase (monooxygenase) reaction. Interestingly, very few investigators have subsequently used this procedure to establish a similar stoichiometry for their oxygenase reactions.

Measurement of the photochemical action spectrum identifying P450 as an oxygenase for the steroid C-21 hydroxylation reaction

As described above, we had already observed the presence of the carbon-monoxide binding pigment, described by Klingenberg [15], in our preparations of adrenal cortex microsomes. We asked ourselves: was there any relationship between this carbon-monoxide binding pigment seen spectrophotometrically and the carbon-monoxide inhibition of the hydroxylation of 17α-OH progesterone as catalyzed by adrenal microsomes? Britton Chance was an expert [20] in the application of Warburg's photochemical action spectrum technique [21] for characterizing carbon-monoxide-binding oxidases of bacterial and cellular electron transport systems. Ryan and Engel [12] had earlier reported studies showing that carbon monoxide inhibited the C-21-hydroxylation of 17α-OH progesterone, as catalyzed by adrenal cortex microsomes, and that this inhibition could be reversed by shining white light on the incubating sample. The answer was simple: do the experiment [22]. Cooper [23] and Estabrook [24] have each independently written our reflections of this exciting phase of collaborative research. The reader interested in a more detailed description of the experimental conditions used for these critical experiments is referred to these two publications.

A few of the salient highlights of the experiments we carried out to measure the photochemical action spectrum identifying P450 as an Oxygenase are the following:

(a) our first experiment that we conducted to show the reversal of carbon-monoxide inhibition of the C-21-hydroxylase reaction was carried out using adrenal microsomes suspended in a cuvette and irradiated with monochromatic light generated by a spectrophotometer. This experiment failed. We know today that the intensity of the light in the spectrophotometer was insufficient to cause dissociation of CO from the heme-iron of the reduced P450;
(b) we soon learned that preparation of explosive mixtures of differing ratios of carbon monoxide and oxygen under high pressure is not a task for the faint-hearted;
(c) we were delayed because of the difficulty in finding a laboratory in the hospital that was sufficiently dark and with windows that opened to allow venting of the continuous stream of carbon monoxide needed to maintain the CO: oxygen atmosphere that was flowing over our incubating experimental samples;
(d) working with an old set of manometers (brought to the United States by Otto Rosenthal when he left Berlin in 1936) suspended in a leaky fish tank and irradiating the samples by a high intensity heat-generating zenon arc football stadium light left little room for mistakes;
(e) obtaining high quality heat-stable light filters with a sufficiently narrow half-band width and of the required wavelength and learning how to use a bolometer to standardize light intensities impinging on the samples required mastering new skills;
(f) and many more "war stories."

Using the photochemical action spectrum technique developed by Warburg [21], Estabrook et al. [22] were able to demonstrate the role of the hemeprotein, named cytochrome P450 [25], as the oxygen-activating terminal

oxygenase for the steroid C-21-hydroxylation reaction of adrenal microsomes. This seminal contribution served as the initial step in defining the major class of Oxygenases that we now recognize as P450s. The results showing that the steroid C-21-hydroxylation reaction was catalyzed by a P450 were soon followed by photochemical action spectra experiments studying the demethylation of codeine and monomethyl-4-aminopyrine and the para-hydroxylation of acetanilide using liver microsomes. These studies [26] provided the link between P450 and the earlier work on drug metabolism [9] and xenobiotics [10] and serve today as the foundation of a large body of research showing the role of P450 in the oxidative metabolism of a large inventory of different chemicals. This memorial volume for the 50th Anniversary of the Discovery of Oxygenases is testimony to the major impact that the discovery of P450 as an Oxygenase has had on science.

The mechanism of function of P450 as an oxygenase: the solubilization of the 11β-hydroxylase system and the discovery of the spectral change associated with substrate-binding to P450

The laboratory of Cooper et al. [27] were determined to solubilize and fractionate the proteins of the adrenal steroid hydroxylation reactions in order to reconstitute the reactions and thereby better understand the enzymatic control of steroid hormone biosynthesis. They focused their attention on solubilizing the electron transfer components of adrenal cortex mitochondria [27]. Earlier, Tompkins et al. [28] and Sharma et al. [29] had reported limited success in separating, by centrifugation, the disrupted adrenal mitochondria into three fractions which, when combined, could reconstitute 11β-hydroxylase activity. At about this time Harding et al. [30] reported that the mitochondria of the adrenal cortex contained a CO-binding pigment that resembled the CO-binding pigment we had reported to be present in the microsomal fraction of the adrenal cortex.

Cooper et al. [27] separated a sonicated preparation of adrenal mitochondria by ultracentrfugation into a particulate fraction and a soluble fraction. They noted [27] that the soluble fraction had the required 11β-hydroxylase activity but that the activity decreased precipitously on dilution. Subsequent studies showed that the soluble fraction could be further separated into three fractions by prolonged high speed centrifugation.

Similar attempts to resolve the C-21-hydroxylase activity of adrenal microsomes were not successful [31]. Science had to wait for another five years before the classic report by Lu et al. [32] described the conditions required for the resolution of the liver microsomal cytochrome P450 system into fractions needed for reconstitution of monooxygenase activities. However, Narasimhulu et al. [31] did successfully clarify the adrenal microsomes with the non-ionic detergent Triton N-101 and this solubilized preparation retained enzymatic activity. While studying the spectrophotometric properties of the Triton-clarified adrenal microsome preparations Narasimhulu [31] made a startling observation. The addition of the steroid substrate, 17α-OH progesterone, to a sample of clarified adrenal microsomes placed in a cuvette in a spectrophotometer caused a concentration-dependent change in the optical density of a pigment absorbing light in the Soret region of the spectrum at about 418 nm (later identified as oxidized P450). This was the birth of the "substrate-binding spectrum" of P450 and the opening key for understanding the multi-step sequence of reactions that we know today represents the cyclic function of P450. We rapidly exploited this observation by Narasimhulu et al. [31] and confirmed the general properties of the "substrate-binding spectra" by using turbid liver microsomes (in the absence of any detergent) that were rich in P450 [33]. We observed two types of spectral changes (called Type I and Type II). At about the same time Imai and Sato [34] reported the observation of a similar substrate-binding spectra on addition of aniline to microsomal P450. We soon applied the use of EPR spectroscopy for characterizing the states of oxidized P450, i.e., the "low spin" (substrate-free) and "high spin" (substrate-bound) forms of P450 [35]. Concomitantly, we initiated spectral and kinetic studies of microsomal pigments [36] and established the reversibility of substrate-binding to P450 by the loss of the substrate-induced spectral absorbance during metabolism following the addition of NADPH and the time-dependent steady-state transient reduction of cytochrome b_5 using varying concentrations of reducing equivalents (NADPH) in the presence and absence of different substrates.

P450 reduction by the electron-transport proteins of adrenal mitochondria: the discovery of the iron-sulfur protein, adrenodoxin

In 1964, Tsuneo Omura joined our laboratories at the University of Pennsylvania as a Research Fellow and soon he was working on resolving the proteins functional for P450 reduction in the 11β-hydroxylase P450 of adrenal mitochondria. Harding and Nelson [37] as well as Bryson and Sweat [38] were also studying these reactions—but we had an advantage. Cooper et al. [27] had already resolved the mitochondrial 11β-hydroxylase system of adrenal mitochondria and had mastered the methodology for measuring the steroid hydroxylating activity of the reconstituted system. Soon Tsuneo Omura was running columns to purify the proteins that had been solubilized from adrenal mitochondria by David Cooper. One day Tsuneo Omura proudly showed us his latest DEAE column which had a bright red band. The timing of this discovery was critical since Anthony San Pietro was visiting Britton Chance at that time and they were carrying out experiments studying the role of the plant iron-sulfur protein (ferredoxin) on the photosynthetic cycle of plant chloroplasts. I showed Tony San Pietro the absorbance spectra of the red protein purified by Omura and San Pietro predicted it was a 2Fe–2S iron–sulfur protein similar

to ferredoxin—and he encouraged us to measure the electron-spin resonance properties of the reduced protein. We did and we were excited to see the signal at $g = 1.94$, a signature signal for a ferredoxin-type iron-sulfur protein. We first published this result at the Symposium on Non-Heme Iron Proteins held in Yellow Springs, Ohio,

Fig. 1. (1) Picture of Osamu Hayaishi taken at the United States–Japan Symposium on Oxygenases, Kyoto, Japan, May 16–19, 1966; (2) Picture of Howard Mason taken during an NIH Study Section meeting in 1964 at Bethesda, MD; (3) Mika Hayano of the Worcester Foundation. [picture copied from the J. Steroids (1965); 58, (Suppl. 1): 1–10. dedicated to her memory]; (4) Tsuneo Omura as a post-doctoral Fellow in Philadelphia, PA 1964; (5) The David Cooper laboratory in 1964 with the photochemical action spectra apparatus (Individuals shown from left to right): Shak Narasimhulu, Sydney Levine, Acie Slade, Tsuneo Omura, Olga Foroff, Otto Rosenthal, and David Cooper; (6) Britton Chance in 1965 at a lunch seminar at the Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA.

Fig. 2. (7) Martin Klingenberg. Picture taken about 1974; (8) David Cooper and Otto Rosenthal (about 1965) in the laboratory at the Harrison Department of Surgical Research, Hospital of the University of Pennsylvania, Philadelphia, PA; (9) A group picture of the participants of the United States-Japan Symposium on Oxygenases, Kyoto, Japan, May 16–19, 1966; (10) Tokuji Kimura taken in his laboratory in Tokyo in 1966; (11) Column chromatography of adrenodoxin (about 1964); (12) Koji Suzuki while a post-doctoral Fellow at the Johnson Foundation, University of Pennsylvania about 1966.

in the Spring of 1965 [39] and subsequently in Archives of Biochemistry [40]. But science operates in an unusual way. At essentially the same time Suzuki and Kimura [41] published similar results identifying the adrenal iron–sulfur protein they had isolated in Tokyo and they showed its function as an electron transfer agent for the reduction of the adrenal mitochondrial P450 that catalyzes the 11β-hydroxylation of deoxycorticosterone. Thus, in a matter of a few months adrenodoxin had been isolated, purified, and characterized in two laboratories as an electron carrier for P450 reduction. Adrenodoxin was the first iron–sulfur protein isolated and purified from mammalian tissues and the first soluble electron-carrier characterized as a P450 reductase [42].

Concluding remarks

Today we are overwhelmed by the number, distribution, and diversity of action of the superfamily of proteins known as P450 [43]. Clearly, the fifty years since the first characterization of Oxygenases has seen an explosion in science, in particular in the arena of monooxygenases. This publication attests to the elegance of the studies carried out to better characterize the oxygenase chemistry and function with a staggering number of different chemical entities.

The present report illustrates the central role that understanding the reactions participating in steroid hormone synthesis has played in the development of this field of oxygenase research. It is rewarding to reflect on how the series of biomedically important reactions of steroid hormone synthesis dominated the origin of our knowledge of monooxygenase reactions and how the emphasis has shifted to focus on the reactions of drug and xeobiotic metabolism.

The pictures of many of the individuals who played key roles in the development of this history of Oxygenases are shown in Figs. 1 and 2. Pictures from the personal collection of Ronald Wstabrook.

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The author is indebted to Britton Chance for his mentoring during those formative years of development of my scientific career. The encouragement and friendship of Brit has remained for over 50 years and his dedication to science and insatiable curiosity have served as the template for my participation in the studies described in this article. In addition, I thank David Cooper and the late Otto Rosenthal for sharing with me their interest in steroid hormone metabolism and the discovery of the role of P450 in these reactions.

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