Fritz Fuchs
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Endocrinology of Pregnancy

THIRD EDITION

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Edited by

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Preface

No longer can a medical textbook merely be updated every five or six years; it must be rewritten to keep pace with the avalanche of new information. The third edition of *Endocrinology of Pregnancy* has retained only the format of the previous editions; the content is completely new. In fact, several chapters have new authors, who have changed both the style and the substance of these chapters.

One entirely new chapter on the endocrinology of lactation has been added for a number of reasons. The information contained in this chapter is important for our readers but has hitherto not been easily available. Lactation is an immediate and, for survival of the human race, indispensable sequel to pregnancy and parturition, and the hormonal preparation of the breasts for their vital function begins during gestation.

Two chapters have been omitted, but their essence has been preserved by incorporation into other chapters. Two indispensable chapters on estrogens and androgens, respectively, have been combined into one because of the inter-relationship in pregnancy of these two groups of steroids.

The success of the previous editions has convinced us that there is a real need for a review within a single volume of the diverse aspects of the endocrinology of pregnancy, parturition, and lactation. *Endocrinology of Pregnancy* covers both physiological and clinical aspects of endocrinology. It deals with all the hormones directly involved with pregnancy, including those of the fetoplacental unit, as well as with the effects of pregnancy on all the endocrine glands, which must adjust their secretion of various metabolic and regulatory hormones to the special demands of gestation. For the clinician to be able to evaluate the results of hormone assays, he must have a working knowledge of the technology of increasingly sophisticated assay methods, including their sensitivity and limitations. During the last few years, the importance of specific binding sites in or on target cells for the mechanism of action of hormones has become clarified, overshadowing the importance of the dynamics of hormone production and metabolism, which no longer is deemed to deserve a separate chapter.

We prepared this edition, as we had the previous ones, with the intention of making available to obstetricians the ideas and technical advances in scientific research in the field and of

providing them with a survey of the clinical aspects of the hormones in pregnancy and puerperium. We particularly had in mind the obstetrician in training, the resident preparing for the American Board Examinations, the registrar reading for the Membership Examination of the Royal College of Obstetricians and Gynaecologists, and their equivalents in other parts of the obstetric world. We believe that students of pediatric and medical endocrinology may also profit from this book.

Again, we wish to thank not only our contributors, but also those who assisted them. Our publishers, Harper & Row, Inc., have spared no effort to make this edition better than the previous ones, having again coped with the particular idiosyncracies of the editorial team.

Fritz Fuchs, M.D., Dr. med., F.A.C.O.G.
Arnold Klopper, M.D., Ph.D., F.R.C.O.G.

ENDOCRINOLOGY OF PREGNANCY

Contents

Mechanism of Steroid Hormone Action

1

Stanley R. Glasser

The ability to regulate the function of cells in distant and apparently unrelated tissues is basic to the process of homeostasis and central to the evolutionary success of mammalian species. Steroid and peptide hormones, secreted by the endocrine glands, play a pivotal role in ordering these specific cellular responses. It is the nature of this specificity that poses a paradox. All cells are exposed to hormones, but only a very few of them respond (Fig. 1-1). Even more perplexing are the detailed cellular responses to the steroid hormones, which are small molecules. It is not obvious how such molecules can contain enough information in their structure to guarantee adequate specificity and to account for their diverse influences.

Historically, animal scientists and physicians whose main interest has been the study of reproductive biology have been engrossed with the analysis of hormonal modulation of the patterns of growth and development. This interest derives from the observation that steroid hormones stimulate certain cells by controlling the synthesis of particular proteins. Protein synthesis is a central process in the metabolism of the cell and directly expresses the information in the genes. In their search for those mechanisms by which hormones regulate gene expression and thereby the information that controls the synthesis of particular proteins, the reproductive endocrinologists have turned to the methods of molecular biology.

BLOOD BINDING OF STEROIDS AND ENTRY INTO CELLS

The extent to which a cell responds to a steroid hormone depends on the quantity of free hormone available for entry into the cell (Fig. 1-2). Steroids bind to many of the macromolecules that are borne by the blood. The affinity of these steroid binders, which are often present in high concentration,[2,22] varies from $K_d \sim 10^{-3}$ M (weak) to $K_d \sim 10^{-10}$ M to 10^{-8} M (strong). Thus, these blood proteins can influence steroid hormone action by restricting the amount of free hormone. Assays of blood hormones that fail to distinguish free from bound fractions can be misleading.

A major blood binding protein is α-fetoprotein (α-FP, testosterone-estrogen-binding globulin), which binds estradiol (E_2) and testosterone (T) with equal affinity (i.e., $K_d \sim 10^{-10}$ M).[22,35] During gestation, α-FP binds and inactivates E_2 and thereby protects the fetus from hyperestrogenization. Following parturition, the concentration of α-FP declines from high to very low titers before puberty,[28] progressively increasing the amount of free steroid available for cell interactions.

Other important blood steroid-binding proteins include serum albumin and corticosteroid-binding globulin (CBG), which binds and inactivates glucocorticoids, thereby protecting the fetus from an excess of these

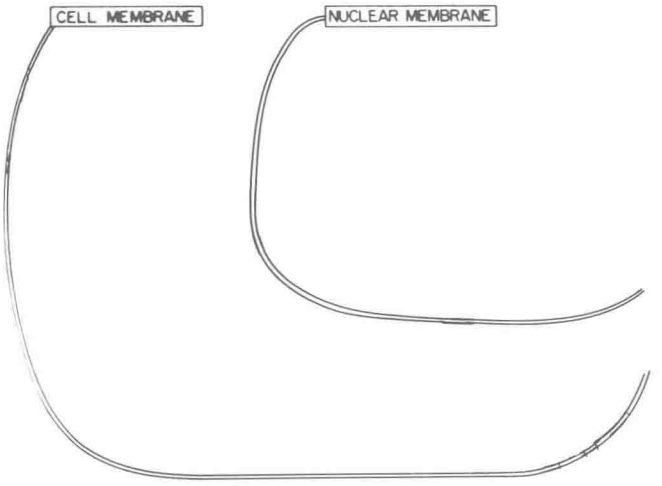

FIG. 1-1. A diagrammatic view of a cell showing the bilaminar lipoprotein cell (plasma) membrane and the membrane that limits the nucleus. These are features common to all cells, and their presence raises the question of why only certain cells respond to a hormone.

hormones. Serum albumin has only a weak affinity to E_2 ($K_d \sim 10^{-4}$ M to 10^{-5} M), but its concentration in the blood is approximately 4%;[41] thus, it remains a significant estrogen binder. The affinity of serum albumin for E_2 is 10- to 100-fold greater than for estriol (E_3).[1] Consequently, when these estrogens are administered, the amount of E_3 that remains free and available for tissue interaction is significant. Thus, although the K_d values (4°C) for receptor binding of E_2 and E_3 are 10^{-10} M and 10^{-9} M, respectively, the elevated values of free E_3[2] may result in approximately equal receptor binding and stimulation of early uterotrophic events (Table 1-1).[3]

The actual entry of steroid hormones into cells is probably by diffusion. There are recurrent studies that suggest that steroids enter by means of saturable transmembrane proteins.[27] While the possibility of a carrier-mediated process has not been eliminated, the consensus of studies of entry kinetics[11,26] supports the idea that E_2 partitions between medium and tissue (target and non-target) in a nonspecific, passive manner suggestive of simple diffusion. In non-target cells, steroid molecules leave as rapidly as they enter so that the concentration of steroid in these cells does not exceed that of the blood. In target cells, however, very few molecules leave, and their concentration within the cell increases as more hormone is sequestered.

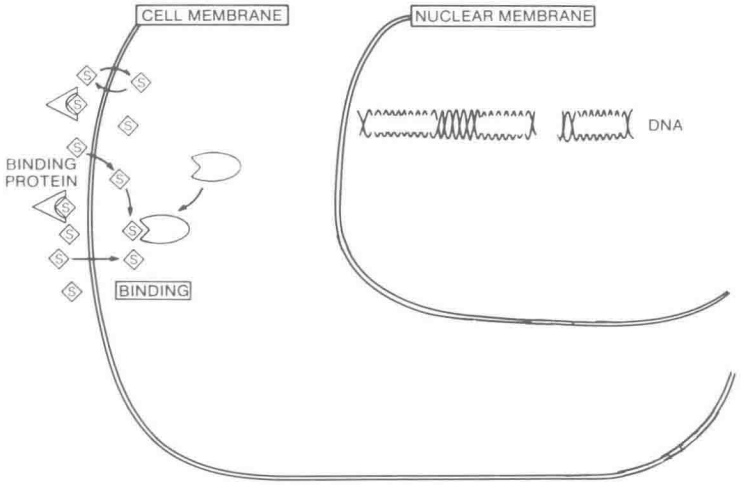

FIG. 1-2. The amount of free hormone in the blood determines the extent to which a cell can respond to the hormone. Amongst the binding proteins of the blood are albumin and α-fetoprotein, which are important determinants in plasma steroid binding.

Table 1-1.
The temporal sequence of biosynthetic and metabolic events elicited in the rat uterus (immature or ovariectomized) by a single *in vivo* injection of estradiol-17β at time zero.*

LOG Time	Biosynthetic Responses	Metabolic Responses
5 min	E_2 binding to cytoplasmic and nuclear receptors	Histamine mobilization; hyper-; emia; electrolyte increases; water imbibition
10 min	Increased incorporation of RNA and protein precursors	Increased uptake of RNA and protein precursors
15 min	Increased lipid synthesis	Increased albumin; lysosome labilization
30 min	1° increase in RNA polymerase II activity; synthesis of induced protein and its mRNA; increased phosopholipid synthesis	Increase in cyclic nucleotides; increase in prostaglandins and associated enzyme
60 min	Increased synthesis of GLU-6-P dehydrogenase; decreased activity of RNA polymerase II; elevation of type II E binding sites	Increased glucose metabolites and associated enzymes; increased Ca^{2+}
2 hr	2° increased activity of RNA polymerase II; 1° increased activity of RNA polymerase II	
4 hr	Increased acidic proteins; increased synthesis of RNA and protein; increased protein synthesis (GENL); continued retention at nuclear E receptor; increased DNA polymerase activity	Water imbibition (peak); beginning water loss
10 hr	Continued stimulation of RNA polymerase I, II; increased dry weight	Continued activation of many enzymes
15 hr	Increased synthesis of RNA and protein (GENL); increased synthesis of DNA	
20 hr	Increased synthesis of acidic proteins, histones	
24 hr	Hypertrophy; cell division	

*Note that the time of uterine response is expressed on a logarithmic scale. (Adapted from Katzenellenbogen & Gorski, 1975).

These data suggest that some agent, present in target cells only, binds the steroid and prevents its release.

STEROID BINDING WITHIN CELLS

Jensen and Jacobson suggested the presence of an intracellular receptor that could be involved in binding the steroid hormone.[15] The association of the relatively simple steroid molecule with a larger "helper" or receptor molecule would serve to sequester the hormone within the cell and also to increase its complexity, thereby allowing for a greater diversity of effects.

Retention of steroid hormones at a cellular level was demonstrated by the use of autoradiography. Exposure of a special photographic emulsion to tissue sections containing radioactively labeled steroid hormones indicated that the labeled hormone entered the

target minutes after injection of the experimental animal. Very rapidly, the hormone-associated label accumulated in the nucleus of the target cell. These changes preceded all other observable alterations in the target cell and persisted long after the radioactive hormone had left the non-target cell.

AN EARLY MODEL OF STEROID HORMONE ACTION

If we advance the hypothesis that certain cell functions are regulated by the hormonal synthesis of particular proteins, we must identify a cellular apparatus for protein synthesis. An additional mechanism by which a steroid hormone is sequestered by a target cell and engages the apparatus for protein synthesis must also be demonstrated.

The first important clue to this particular mechanism of steroid hormone action derived from studies on gene expression in bacteria. The sequence of protein-synthetic events proves to be essentially the same in bacteria and higher organisms; the basic process is one by which information is transferred by transcription of a segment of DNA, representing a gene, to a corresponding length of messenger RNA (mRNA). This process, by which the double-stranded helix of DNA is transcribed, is controlled by the enzyme RNA polymerase. The single-stranded mRNA is subsequently associated with organelles called ribosomes. At the ribosomes, the RNA messenger is translated by means of a genetic code in which groups of three nucleotide bases specify the amino acid to be added to the protein molecule.

While the genetic code can explain how a gene can specify the structure of a protein, it does not explain why a particular assortment of proteins is synthesized by any given cell. In a bacterial cell, there are thousands of genes but only a few are functioning at any one time. It follows that systems exist that regulate the selective expression of genes. Models proposed for bacteria do not readily apply to higher organisms, who share the same genetic code but differ fundamentally in organization. Bacteria are prokaryotes: their genetic material is distributed throughout their cytoplasm. Higher plants and animals are eukaryotes: they possess a distinct nucleus which confines the DNA within a nuclear membrane and separates it from the cytoplasm.

Within the nucleus, the genetic material is structurally assembled into chromosomes which include the nucleic acids of DNA as well as a variety of specialized proteins not found in prokaryotes. These materials are organized into a complex substance termed chromatin (Fig. 1-3). Two main classes of proteins, present in an approximately 1:1 ratio, are recognized constituents of chromatin. The first class is composed of histones, which are highly alkaline proteins and appear to be very similar from one tissue to another and, indeed, from

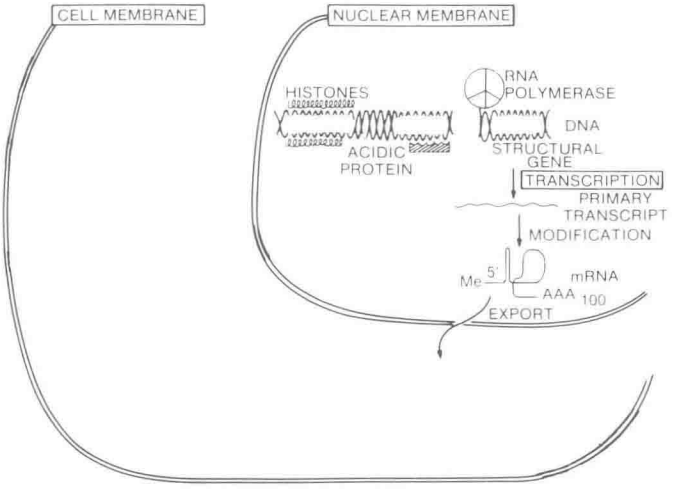

FIG. 1-3. Early models of steroid hormone action in eukaryotic cells assigned the unique response of those cells to specific regulatory proteins whose synthesis was induced by a hormone. The text describes the genetic apparatus for this transcription and translation that is confined within the nuclear membrane.

one organism to another. Histones are presumed to play a structural role. The second class consists of non-histone chromosomal or acidic proteins. The acidic proteins vary considerably from tissue to tissue and from organism to organism. For this reason, they are thought to be determinant factors in qualitative gene expression, although this point remains to be proven. Chemically, the acidic proteins have yet to be satisfactorily isolated and characterized.

An average 10μ-diameter mammalian nucleus contains approximately one meter of DNA. Although densely packaged by a process yet to be described, the genetic material of the individually packaged chromosomes must remain accessible if the information it contains is to be retrieved from it.

The synthesis of new mRNA yields a primary transcript containing information pertinent to a particular hormone response and is probably the primary event in hormonal action and "rate-limiting" with respect to the induction of protein synthesis (see Fig. 1-3). Transcription of new mRNA species is initiated by a class of enzymes, the DNA-dependent RNA polymerases I and II. RNA polymerase II, found in the nucleoplasm, directs the synthesis of DNA-like RNA which includes the messenger species. RNA polymerase I, found in the nucleolus, codes for ribosomal-RNA (rRNA) and transfer-RNA (tRNA) synthesis. Estrogen stimulates the activity of both polymerases in the uterus.

Translation of mRNA follows as the logical sequence of the editing, processing, and accumulation of mRNA species in the nucleus and their transfer to the cytoplasm. This protein synthesis, initially dependent on the type and amount of new mRNA produced, may be further amplified during a subsequent growth phase of the target cell. It is this latter growth phase-related amplification that was erroneously identified as the mechanism of hormone action in the literature of only 15 years ago. It is imperative to note that induction of cellular regulatory processes by steroid hormones, through activation of the genome, requires neither the obligatory nor facultative involvement of amplification. Differentiation and growth are both liable to the influence of steroid hormones. Whereas the inductive actions of the hormone at the level of the genome are not amplification-dependent, the secondary or deferred hormonal responses may be sensitive to both amplification and metabolic regulation. Thus, processes involved in the production of true growth can be influenced by steroid hormones at a variety of determinative loci.[2,8,25]

EXPERIMENTAL EVIDENCE

To this point, we have offered a scheme to advance the idea that a steroid hormone (i.e., estrogen) elicits a cellular response by stimulating the genome and inducing the transcription of a specific mRNA. This mRNA is subsequently translated into a newly synthesized protein. This scheme can be tested experimentally using recombinant DNA techniques to assay short term expectations and validate the data. It can be noted (Fig. 1-4) that in response to estrogenic stimulation (diethylstilbestrol [DES]) the concentration of mRNA is increased in the tubular gland cells of the chick oviduct. This increase in the putative message for ovalbumin occurs before any immunospecific product (ovalbumin) can be detected by assay of the cell-free translation system.

The mRNA isolated from such oviducts can be visualized by use of the electron microscope.[42] Isolated mRNA was suspended in hydrophobic media (formamide-urea) and spread. Each strand of mRNA (Fig. 1-5) comprises 1082 nucleotides and contains all the information necessary to translate ovalbumin. The data inset into Figure 1-4 demonstrate the sensitivity of the methods used. Within 60 min after DES stimulation, the number of molecules of mRNA (ovalbumin) increases from approximately 7 to 80 $\times 10^3$ per tubular gland cell.

Protein synthesis confers new cellular capacities and functions on the target cells (Fig. 1-6). Many are regulatory in nature and are the *conditio sine qua non* of the differentiating cell. The final events of this sequence take place in the cytosol with the translation of the transcript exported from the nucleus. Additionally, protein synthesis requires an increase in the population of ribosomes and in the amount of tRNA and initiation factors as well as increases in the activity of a number of enzymes, including RNA polymerase I (which

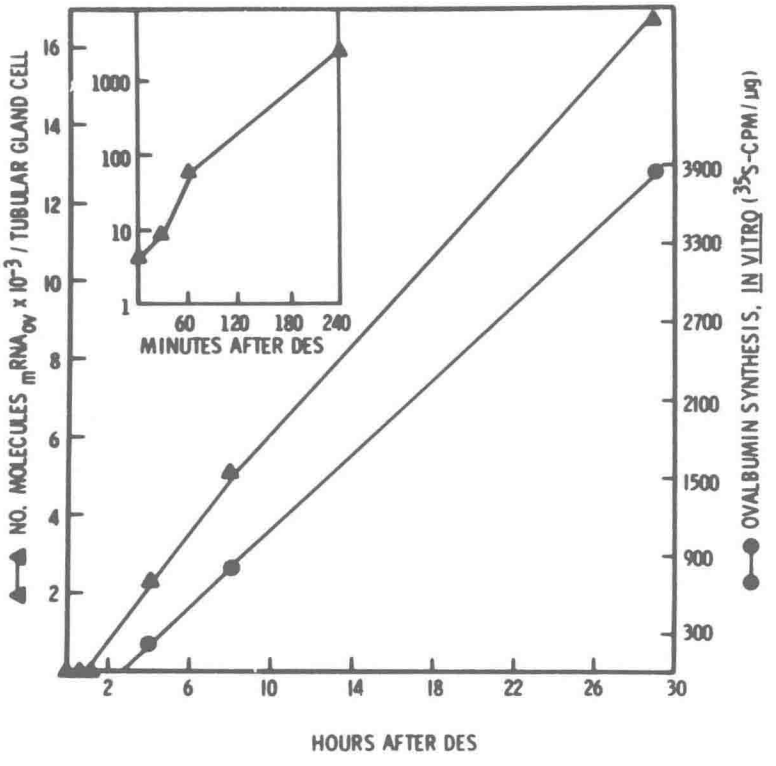

FIG. 1-4. Transcription is a steroid hormone inducible event. The oviduct of the immature chick is undifferentiated and produces no ovalbumin. Estrogen stimulation stimulates the differentiation of oviductal tubular gland cells which synthesize ovalbumin. Preceding protein synthesis is an increase in the concentration of mRNA for ovalbumin which occurs before detectable amounts of protein can be detected. The inset expands the scale of mRNA changes during the first four hours. (Woo SLC, Rosen JM, Liarakos CD, Choi YC, Busch H, Means AR, O'Malley BW: Physical and chemical characterization of purified ovalbumin messenger RNA. J Biol Chem 250:7027, 1975)

FIG. 1-5. Electron micrograph of mRNA-ovalbumin strands isolated from the estrogen stimulated chick oviduct. (Woo SLC, Rosen JM, Liarakos CD, Choi YC, Busch H, Means AR, O'Malley BW: Physical and chemical characterization of purified ovalbumin messenger RNA. J Biol Chem 250:7027, 1975)