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BIOLOGICAL ACTIVITIES *of* ALPHA$_1$-FETOPROTEIN

Volume I

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Biological Activities of Alpha$_1$-Fetoprotein

Volume I

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PREFACE

The idea for this volume stemmed from a satellite symposium of the VIIth International Congress of Endocrinology, entitled "Alpha-fetoprotein as a Modulator of Estrogen Response", held under the auspices of the Faculty of Medicine of Laval University in Quebec City on June 30, 1984. It had become evident from the recent scientific literature that alpha-fetoprotein (AFP) is not merely a passive blood protein, but is rather a molecule that can in vivo and in vitro elicit diverse biological responses, some of which are remarkably intense. The Symposium brought together participants drawn from diverse biological disciplines, who had encountered such AFP phenomena in their own fields of investigation, and who subsequently contributed to this volume.

AFP is a glycoprotein of molecular weight 70,000 which is elaborated by the yolk sac and fetal liver. Its concentration in the fetal plasma is high (2 to 5 mg/mℓ), and this condition may persist in the neonate for several weeks postpartum. The AFP molecules of some animal species bear high-affinity binding sites for the classical steroidal estrogens. In those species, the impaired response to estrogens seen in fetal and neonatal tissues is not unexpected, since estrogen binding to AFP molecules in the plasma would limit entry of the hormone into responsive cells. This long-held explanation is probably not correct. Recent investigations in several laboratories have disclosed that there is yet another (and possibly the major) manner in which AFP affects tissue responses to steroids and other substances, and that is by altering the sensitivity of the tissues to these agents. Those findings suggest that the AFP molecule has intrinsic cellular regulatory activity; depending on circumstances the cell exposed to AFP may display either greater or lesser response to steroid hormones and to other compounds than it would exhibit in the absence of AFP. While the present clinical utility of AFP measurement is restricted to its use as a serum marker for the detection of neural tube defects and of AFP-secreting tumors, the research now being reported suggests that this "regulatory" property of AFP may also apply to human tissues and be clinically important. This observation deserves and is receiving intensive study.

The contributors to this volume represent the investigators most actively studying the regulation of various biological activities by AFP. They describe the results of their recent investigations performed in cell-free systems, in cell and/or organ cultures, as well as in vivo. Studies in cell-free systems concern the functional topography of the AFP molecule, and especially the biochemistry of its various binding sites. The cell/organ culture participants report AFP effects on estrogen-induced cell proliferation and the detection of AFP synthesis within those cells. The reports of in vivo studies include effects of AFP on estrogen responses in the central nervous system, testes, uterus, and ovary. These reports, and the included abstracts of the presentations at the Quebec Symposium will provide the reader with a clear understanding of the state of the art concerning the biological activities of AFP.

G. J. Mizejewski, Ph.D.
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Dr. Mizejewski graduated in 1961 from Duquesne University, Pittsburgh, Pennsylvania, with a B.S. degree in Biology/Chemistry and obtained his M.S. degree in 1965 from the Zoology Department at the University of Maryland, College Park, Maryland. He earned the Ph.D. in Zoology/Biochemistry in 1967 at the University of Maryland majoring in embryology and physiology. Dr. Mizejewski served a two-year post-doctoral tenure at the University of Michigan Medical School from 1968 to 1970, in immunobiology.

Dr. Mizejewski is a member of the New Academy of Sciences, the American Federation of Clinical Research, Society of the Sigma Xi, American Society of Zoologists, the American Association for the Advancement of Science, the American Institute of Biological Sciences, the Society for the Study of Reproduction, the Reticuloendothelial Society, the American Society of Microbiology, the International Society for Oncodevelopmental Biology and Medicine, and the Society for Experimental Biology and Medicine.

Among other awards, he has received the University of South Carolina Research and Productive Scholarship Award, a Ford Foundation Teaching Award, the Chesapeake Bay Research Fund Award, and has received clinical investigative support from commercial companies such as Amersham Corporation, Cal-Biochem, Behring Corporation, Hoffman-LaRoche, Abbott, and the Upjohn Pharmaceutical Company. Dr. Mizejewski was a National Science Foundation Research Fellow and has served on numerous grant review boards including the American Cancer Society, the University of South Carolina Cancer Review Board, and the March of Dimes. Dr. Mizejewski was invited to organize and conduct an alpha-fetoprotein Satellite Symposium for the 7th International Congress of Endocrinology in Quebec, Canada. He also served as Chairman for the Comparative Physiology and Biochemistry Sections for the American Institute of Biological Sciences.

Dr. Mizejewski has presented over 30 oral presentations at national meetings, over 20 invited lectures at regional meetings, and approximately 50 guest presentations at Universities and Institutes. He has served as senior editor on three books and has contributed five chapter presentations in various textbooks. He has published more than 130 works including manuscripts and abstracts. Dr. Mizejewski has also reviewed manuscripts for *Clinical Chemistry,* the *International Journal of Cancer,* and other journals. His current major research interests include the biological role (function) of alpha-fetoprotein in animals and man, the biochemistry and physiology of oncofetal antigens, immune scintography, the immunobiology of cancer, and the development of animal models for cancer and reproductive research.

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Dr. Jacobson's research has been supported by grants and contracts from the National Institutes of Health, the Ford Foundation, the American Cancer Society, the Damon Runyon Cancer Research Foundation, and by funds from industry. His research career encompasses 35 years of active investigation in the areas of molecular endocrinology and the biology of endocrine cancer. He is currently investigating the role of alpha-fetoprotein in the control of hormone-dependent neoplasia. He has published more than 100 research reports in national and international journals, has participated in and has chaired international symposia, has contributed chapters to edited volumes, and has served as co-editor of published symposium proceedings.

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SECTION I

Alpha-Fetoprotein — Binding Substrates and their Properties

Chapter 1

ESTROGEN AND FATTY ACID BINDING PROPERTIES OF MURINE ALPHA-FETOPROTEIN: A GUIDE TO EXPLAIN SOME BIOLOGICAL ACTIVITIES OF THIS PROTEIN

E. A. Nunez, C. Benassayag, N. Christeff, M. Essien, J. Hassid, M. E. Martin, L. Savu, G. Vallette, and R. Vranckx

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I. INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein which is present in the plasma of numerous species at a high concentration (up to milligrams per milliliter) during developmental and pathological circumstances characterized by an important cellular multiplication and differentiation.[1] It is poorly produced in the normal adult and is present at a very low concentration in the plasma (nanograms per milliliter). This concentration can rise during some pathological proliferating processes which may be neoplastic or not (e.g., hepatoma, cirrhosis). This protein is first synthetized in the yolk sac, then later synthesized in the liver. Synthesis sites in the gut,[2] the nervous system,[3] and the uterus[4] have been described in the young rat or mouse, but some of these results remain controversial.[5] However, many authors have observed that AFP (as well as some other serum proteins) is found in the cytoplasm during the development and the differentiation of some fetal and neoplastic cells.[6-9] More work is needed to definitively determine whether the presence of the fetoprotein in the cells is due to a selective penetration and/or synthesis that only occurs in some circumstances depending on the age, the cellular cycle, the cell type, and the pathological status. Moreover, some studies suggest that there could exist a preferential synthesis of one of the numerous molecular forms of the AFP depending on the organ involved.[10] These isoforms vary from one to another by the nature of their glucidic structure.[11] The concentration of these different molecular forms can vary during the ontogenesis and some physiopathological circumstances like carcinogenesis.[12]

Murine AFP (mAFP) has the specific and remarkable ability to bind natural estrogens with high affinity (Ka = $10^7 M^{-1}$).[13-15] This property is not shared by the AFPs of other species studied until now.[16] Human AFP does not bind estrogen.[17] Furthermore, we have observed that the estrogen binding properties of the various molecular forms of rat AFP vary very significantly[18] as do the number of binding sites for estradiol when the protein is diluted (from 1 to 0.3 sites).[19] These binding variations can modify the in vivo functions exerted by the protein from one situation to another.

By contrast, the fatty acids are bound by all AFPs studied until now.[20,21] For the mAFP, the higher affinity constant is observed for the polyunsaturated fatty acids (Ka = $10^7 M^{-1}$).[22,23] This binding, which can reach 15 mol of fatty acid per mole of rat AFP, turns this protein into a highly hydrophobic glycoprotein. The binding differences, particularly for estrogens, observed from one species to another have prompted us to consider that AFPs of different species do not necessarily exert the same functions — or if these functions are the same, the mechanisms by which they are exerted may differ from one species to another.

A mutual inhibition is observed between estrogens and unsaturated fatty acids as far as murine AFP is concerned.[24-26] Both ligands are bound with albumin, but the binding constant of the estradiol 17β (E2) is 10^2 to 10^3 lower than that of the AFP.[26] Furthermore, albumin binds them independently.[26] These latter observations show that AFP and albumin in spite of important similitudes in the structure may be dissimilar for some important functions. It is clear from all the data presented above that it can be considered that AFP exerts its various functions in relation to its structure (isoforms), the nature and concentration of its ligands, and to the receptivity of the cells on which the protein acts. It can also easily be assumed that all these parameters change according to age, nutritional, and pathological circumstances.

Thus, we have observed that the concentration of the non-esterified fatty acids (NEFAs) decreases according to the age in the postnatal period of the rat. The percentage of the polyunsaturated fatty acids (PUFAs) decreases concomitantly.[27] Important variations of the estrogen and glucocorticoids concentration also occur during this period.[28] These hormonal variations could play a major role in the concentration of the

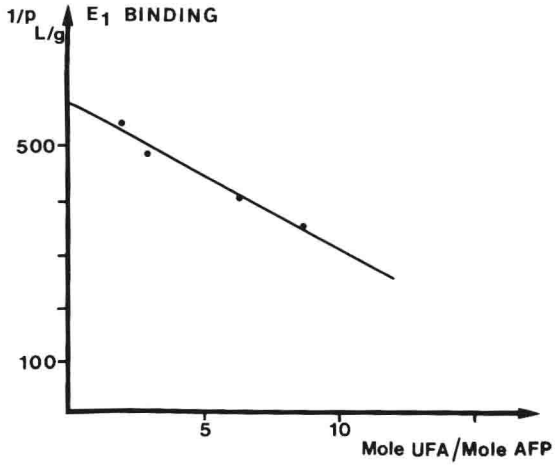

FIGURE 1. Correlation between the concentration of endogenous bound unsaturated fatty acids (UFA) (for method see Reference 21) and estrone (E1) binding to AFP. Binding is determined by an equilibrium dialysis technique described in Reference 14.

free fatty acids during this period. Thus, we have shown that adrenalectomy considerably raised the NEFA concentration while ovariectomy made them decrease.[27] These variations are not observed if the operations are carried out on animals older than 28 days. Any modification occurring in the environment of the AFP can no doubt exert an important effect on its biological function(s). Thus, the estrogen binding capacity is lowered when the concentration of the bound NEFAs is high (Figure 1). In these conditions a "purified AFP", which is to be used on a biological model to investigate the role of this protein, must be necessarily submitted to a previous precise quantification and analysis of its various identified ligands and eventually be submitted to the analysis of its isoforms.

As regards the ligands, Table 1 shows that the fatty acid and the phenolsteroid content of a purified AFP vary from one batch to another according to the biological origin of the protein and the purification method used. This fact can partly explain the contradictory results obtained by various authors as regards the role of AFP on the immune system.[29] In these circumstances, it appeared to us to be worth opting for a new experimental approach which relies on the hypothesis that AFP acts by modulating the action of the NEFAs and/or that NEFAs can modify the conformation of the AFP and consequently its function. The role of estrogens or other unknown ligands on these activities remains to be precisely defined.

Under these conditions, using endocrine and immune experimental protocols, we have planned to study the action of the free fatty acids and estrogens, introduced alone or together. Once results are obtained in these conditions, we will introduce an AFP whose content in estrogen and fatty acids is properly determined and we will then compare the biological effects of these various ligands in the presence and absence of AFP. The same studies can be performed to understand the biological role of other steroid binding proteins like the sex binding protein (SBP).[30] This is the only specific estrogen and androgen binding protein in the human. We hypothesize, by analogy with the murine AFP, that some properties of this protein can be modified by the NEFAs. The results of these experiments are also presented in this work.

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