

Hormones In Human Plasma

NATURE AND TRANSPORT

Edited by

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Foreword by

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Foreword

Delineation of the role of hormones in the maintenance of man's physical and psychological integrity as well as his capacity to adapt and reproduce has stimulated the co-ordinated efforts of chemist, physiologist, immunologist and clinical investigator. The present volume, *Hormones in Human Plasma*, brings together a comprehensive and authoritative account of a seriously neglected area in human endocrinology — namely, the nature of the transported hormone and its involved carrier system.

Historically, astute clinicians, pathologists and physiologists such as Addison and Gull, Graves and Basedow, Berthold and Brown-Séguard, Von Mering and Minkowski, Able and Cushing set the stage for biochemical advance in our understanding of the nature and action of hormones. As might have been anticipated, the most rapid strides in human studies occurred in those areas in which progress in methodology such as steroid analyses paralleled the ready availability of biological material, i.e., the steroid content of urine. Inferences regarding quantitative secretory activity of the endocrine glands in man were necessarily tentative in view of the inability to assess accurately changes in intermediary metabolism of the hormone prior to its excretion and, of course, the difficulties inherent in attempting to analyze blood for its microgram quantities of secretory products. The major breakthrough which we are now experiencing in this area of medicine had as its cornerstone the development by Professor Edwin J. Cohn and his associates of physicochemical methods for separating plasma into its various components by a technique designed to minimize alteration in chemical structure or biological activity of the contained protein constituents. However, even this important advance could not be fully explored until the investigator had also at his command microchemical, chromatographic and immunological techniques capable of identifying extremely small quantities of hormonal substances, improved microbiological assays and isotopic techniques available for identifying hormones, their degradation products and the losses inherent in the analytical procedures.

It is scarcely necessary to emphasize the importance of understand-

ing the means by which hormones are transported in the blood and the cyclical variations in plasma level which may occur throughout the 24 hours if one is to understand the role of hormones in regulating metabolic reactions. The knowledge that elderly patients may exhibit a high normal blood level of thyroid hormone in the presence of subnormal secretory activity of the thyroid gland immediately suggests that the peripheral tissues of the septuagenarian may not be able to utilize thyroid hormone at a rate comparable to that in his youth. By what means do estrogens increase the capacity of the plasma to transport thyroid hormone? Is a lack of diurnal variation in the plasma level of adrenal steroids capable of inducing Cushing's disease despite a relatively normal total 24-hour secretion of 17-hydroxycorticosteroids? From considerations such as these, the importance of being able to determine the plasma level of hormones is evident, and knowledge regarding the form in which hormones are transported in the blood is essential. There is every reason to believe that the plasma concentration of hormone is critical in regulating the rate of glandular secretion of hormone as well as its peripheral or tissue effect.

The desirability of summarizing our knowledge of hormones in human plasma may be illustrated by the marked differences which are known to exist in the physiological activity of growth hormone derived from species other than man and primates. In addition, in man it is interesting to speculate on the potential difference between the form in which insulin exists in the peripheral blood of normal subjects after its secretion by the pancreas and passage through the liver as compared to insulin injected subcutaneously in a diabetic patient which reaches the peripheral tissues before transportation through the portal system. In the case of adrenal steroids, one observes the reverse; normally, the adrenal secretes its hormone directly into the systemic circulation, whereas hydrocortisone taken by mouth must first traverse the gastrointestinal tract and portal circulation prior to reaching peripheral tissues.

In organizing this volume on *Hormones in Human Plasma*, Dr. Harry N. Antoniades has profited from his long association with important members and associates of the late Professor Edwin J. Cohn at the Harvard Medical School. Strength in this undertaking has been attained by selecting outstanding investigators of international reputation who are at present contributing in an important way to progress in their respective fields. Breadth has been attained by including investigators from countries other than the United States — and

where methodology is so vitally important, different techniques have been presented by their own proponents. The background of physical chemistry has been provided by Professor J. L. Oncley, Professor of Biological Chemistry at Harvard, Dr. Robert B. Pennell, Director of Protein Foundation Laboratories, Jamaica Plain, and Dr. Harry N. Antoniades, at present a member of both Protein Foundation and the Department of Gynecology at the Harvard Medical School.

During recent years, although there have been a number of conferences sponsored for the purpose of exchange of information regarding advances in endocrinology, there has not been an opportunity, because of limited time, to discuss extensively the many aspects concerned with the nature, secretion and transport of hormones in human plasma. It is natural that a volume such as this should have its roots in the laboratories originally occupied by Professor Cohn. It is tribute to his vision, as well as his chemical skill, that so many of the distinguished contributors to this volume have been, in part, collaborators of Professor Cohn and his associates. Dr. Antoniades is to be congratulated on envisioning and activating the publication of this monograph. It is certain to fill a need felt by many for an authoritative statement by experts on the problems peculiar to human function. For those concerned with technical advance in one small area of the field, it will provide a panorama of progress and the possibility of stimulating interaction among scientists. A volume of this type may be expected to stimulate research and integration at all levels, including, on the one hand, the molecule, its configuration and physical chemistry, and on the other, the patient exhibiting a serious physiological disability. The success of the present volume will be measured by the inability, ever again, to contain within one single volume all information pertinent to this important subject!

GEORGE W. THORN, M.D., LL.D., S.D. (Hon.)

Preface

The basic significance of experimental inquiry into blood and blood components has been recognized for centuries. As early as 1771, in his treatise on *An Experimental Inquiry into the Properties of the Blood*, William Hewson wrote:

An inquiry into the Properties of the Blood, it is presumed, will be thought, in a particular manner, interesting, since there is no part of the human body upon which more physiological reasoning is founded, nor any from which more inferences are drawn for the cure of diseases. And, as the inquiry is made by experiments upon the blood as near as possible to the state in which it circulates in the vessels, it is hoped that the conclusions made from them will stand the test of a candid examination, and lead to further observations and improvements.

Such an inquiry upon the blood under conditions "as near as possible to the state in which it circulates" was made feasible only recently, following the development of adequate tools. The ingenious work of Edwin J. Cohn (1894 - 1953) and his associates on blood and blood components and their fractionation provided invaluable new techniques and information on the composition, the preparation, the nature, and the preservation of many blood plasma components. It also provided a philosophy for the approach to problems concerned with the study of the state of plasma components under conditions close to their natural state.

These new techniques, in turn, along with the remarkably sensitive methods of hormone assay developed by biologists, provided the necessary instruments for experimental search into the state of hormones in blood plasma. Although the concept of hormones as chemical messengers that transport stimuli from tissue to tissue via the blood stream was introduced by Bayliss and Starling almost half a century ago, hormones had rarely been investigated in the state in which they circulate in the blood. In recent years, however, the concentrated effort of investigators has produced remarkable progress. Many of the hormones in human plasma have been identified. Non-protein hormones such as steroid hormones, thyroxine, epinephrine and norepinephrine appear to be transported in the blood by plasma proteins. Hormones

of protein nature may also be in close association with other proteins in blood. The physiological significance of this function is the object of current investigations. Methods have been developed for the preparation of hormone concentrates from human plasma for potential clinical use.

More sensitive techniques suitable for the identification and estimation of hormones in blood plasma or serum have been proposed, and current efforts for the development of immunological techniques for hormone assay are most promising.

Questions as to the nature of hormones and their transport mechanism in plasma, both in normal and abnormal states, are being eagerly explored. The attempt to answer these questions has brought scientists from various fields together in a joint effort to provide an answer, working under most delicate conditions in a most complex system, the blood plasma.

The present volume reflects this collaborative effort. It hopes to provide, along with authoritative scientific information, the stimulus for an ever more vigorous effort in the numerous areas of this field.

I am most grateful for the invaluable advice and encouragement so generously given by Dr. George W. Thorn in the preparation of this volume.

I also wish to thank Dr. Don H. Nelson and Dr. Albert E. Renold for their kind co-operation and assistance at all times throughout this work.

The excellent collaboration and understanding of the staff of the Medical Book Department of Little, Brown and Company, the manager, Mr. Fred Belliveau, Mrs. Mary Rackliffe and Mr. Howland P. Hall is greatly appreciated. My thanks also to Miss Marie Gleeson for her typing assistance. To my wife, whose help was extended throughout this work, my deepest gratitude.

HARRY N. ANTONIADES

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PART ONE

*Methods of Blood Collection
and Plasma Fractionation*

CHAPTER I

Blood Collection

Robert B. Pennell

CHANGES IN SHED BLOOD

Serum

Plasma

TECHNICAL ASPECTS OF BLOOD COLLECTION

Collection of blood serum

Use of citrate solutions for collection of plasma

Use of ACD solutions for collection of blood

Collection of blood through cationic exchange resins

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Much of the work discussed in this volume is concerned with *in vitro* examination of blood and its components. The collection of blood and its separation into cellular elements and plasma may be accomplished in numerous ways. These methods of collection and separation often influence the *in vitro* findings. It is intended here to describe the various procedures in sufficient detail as to make reference to the pertinence of collection and separation techniques readily understandable.

It is probable that, however collected, progressive changes in the character of blood begin at the time it is withdrawn from the vascular system. These changes can be modified in various ways by the manner of collection. The type of modification deemed most compatible with the given purpose can be directed, to a certain extent, by the collection procedure chosen. The manner in which a given collection system may be expected to influence the blood obtained will be considered. Technical details of the various methods will then be presented separately.

CHANGES IN SHED BLOOD

Serum

When blood is drawn without precaution, change is obvious in the formation of a clot within a short period. This clot at first encom-