Hormones in Blood

Edited by

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and

A. L. BACHARACH

Formerly of Glaxo Laboratories Ltd

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Preface

In the first chapter of this book we set out why and for whom it was written. Briefly, we had thought it time that a mass of existing publications purporting to record the amounts of some 15 hormones (or groups of hormones) in human blood or plasma should be critically surveyed. This meant not only that suitable experts should try to re-evaluate the bearing of plasma hormone levels on health and disease, but also, equally important, that they should have a good and critical look at the methods by which the published results had been obtained—and possibly even any unpublished results to which they may have access. In the first chapter we have, moreover, also tried to define and to clarify some of the questions that in our view need to be answered before the basic problems can be solved.

Everything included in the volume bears directly or indirectly on some aspect of the matters under review, for we have considered it essential not only to provide accounts and critiques of the analytical methods available or proposed, but also to survey the hormones' chemistry, biochemistry, anabolism, catabolism and physiology as a background to any valid discussion of the relation between "normal" hormone levels and the subject's state of health. For this purpose consideration of analogous matter in relation to laboratory, domestic and farm animals seemed to us to be far from irrelevant, indeed vital, as has also been some consideration of hormone levels in other tissues than blood.

We have had the co-operation of experts from both medical and agricultural Institutions, and their qualifications range over a wide field of biological and chemical science. So anxious were we that our contributors should be the most suitable experts that in approaching them we largely ignored international boundaries, our only restriction being that they could and would write in the English language.

When a book is written by over twenty authors from two or more countries and from both sides of the Atlantic, Editors have to face certain special problems. We have, therefore, thought it our duty to get rid, as far as we could, of some small and perhaps almost unnoticed inconsistencies that we believe to affect the reader of a book, albeit often subconsciously, with an irritating sense of uncertainty about author's (and Editors') logic.

When we had trans-atlantic inconsistencies to resolve, it was inevitable that we should come down on the British side of the fence, and this not only in matters of ordinary spelling—we have, for example, used sulphur, tumour, technique, analogue and labelled, instead of our colleagues' sulfur, tumor, technic, analog and labeled—and words, but also in matters of scientific nomenclature and terminology generally. As far as it seemed to us practicable in a book covering fairly wide fields of both biology and chemistry, we have followed the instructions of the Royal Society for symbols and abbreviations and have adhered to the rules promulgated by the Chemical Society for the

nomenclature of chemical, and in particular organic, compounds. These rules are moreover, close to, when not identical with (which they usually are), the agreed practices recommended by the relevant International Scientific Unions and the International Council of Scientific Unions.

Thus it comes about that the designations of the many steroids mentioned in this book, though we hope them to be self-consistent and logical, may not be those equally consistent and logical ones to which some of our readers, and indeed a few of our contributors, are best accustomed. We have, incidentally, used the suffix aetio-, rather than etio-; we have throughout used the prefix oxo- rather than keto- whenever this was chemically permissible. Similarly, we have used, again following Chemical Society and similar practice, oestro-, not estro, -aemic, not emic and litre, not liter. Our decision to use the suffix -trophin rather than -tropin was not made because we necessarily accepted the claimed etymological legitimacy of either, but simply because in our estimate most British endocrinologists favour the former. We have preferred ovariectomy to oöphorectomy on the grounds of greater familiarity, in spite of its hybrid derivation.

We have banned the, to our mind, illogical expression mg (or g) per cent, replacing it when necessary by mg (or g) per 100 g or ml, and we have used ml in preference to cc (or ccm) throughout. Also we have accepted the spelling tryptophan since, in accordance with international agreement, English-writing chemists dropped the final e several years ago.

When referring to the state of several hormones in the blood, more particularly the steroids and thyroxine, we have used the descriptions "free" and "unconjugated" each with its own meaning, which we believe will be clear from the context. Suffice it to say here that a "free" steroid is one not bound to protein, whether it be conjugated or not. In matters of typography we have tried to avoid everything that would distract the reader, including any excess of punctuation or italics; thus we have printed throughout such words and phrases as in vivo, in vitro, vice versa and et al. in ordinary roman type.

We have adopted the "Harvard" rather than the serial system of giving references because in our view the former has for this kind of publication fewer disadvantages than the latter. Nobody is really satisfied with either. For the titles of journals we have largely adhered to abbreviations given in the "World List".

On several diverse points we have exercised editorial dictatorship. Thus we have followed the suggestion of Costello, P. D. (*Science*, 1947, **105**, 474) in disallowing references to revolutions per minute of a centrifuge and have converted the figure to multiples of *G* when available information made this possible. When it did not, we have had perforce to leave a meaningless mention of r.p.m. We have distinguished between the (observed) standard deviation—of a single observation—and the (observed) standard error—of the mean—whenever our contributors were in a position to help us do so, using the abbreviations s.d. and s.e.m., respectively. Unfortunately the information available in some of the papers to which they refer makes the

necessary discrimination impossible; in consequence the original author's figures for the "standard error" may also be meaningless.

We have throughout referred to determinations, not estimations, when we considered that the author believed, rightly or wrongly, a described method to be capable of giving results with some quantitative meaning.

In order that no one shall be left guessing by what he may regard as editorial whims with little, if any, rational foundation, we have tried to collect in one Table (p. xi) all the abbreviations and symbols used in this book, even those that may seem to conform to universally accepted scientific or general practice. Such a Table may indeed, possibly have an incidental value in indicating where there is need for further international agreement on such matters.

C. H. GRAY

A. L. BACHARACH

Symbols and Abbreviations

I. Mass, length and time and their derivatives

g	gram(s)	mμ	millimicron(s) (10^{-9} m)
k	kilo	cm^2	square centimetre(s) and similarly
kg	kilogram(s) (10^3 g)		
m	milli	l	litre(s)
mg	milligram(s) (10^{-3} g)	ml	millilitre(s) (10^{-3} l)
μ	micro	μl	microlitre(s) (10^{-6} l)
μg	microgram(s) (10^{-6} g)	mμl	millimicrolitre(s) (10^{-9} l)
ng	nanagram, millimicrogram(s) (10^{-9} g)	in.	inch(es)
		ft.	foot(feet)
m	metre(s)	sec.	second(s)
c	centi	min.	minute(s)
cm	centimetre(s) (10^{-2} m)	hr.	hour(s)
mm	millimetre(s) (10^{-3} m)	v/v	volume in volume
μ	micron(s) (10^{-6} m)	w/w	weight in weight per

II. Miscellaneous

b.p.	boiling point	μu	microunit(s) (10^{-6} u)
%	percent(age)	s.d.	standard deviation
V	volt	s.e.m.	standard error of the mean
mV	millivolt (10^{-3} V)	P	probability of random occurrence
c	curie(s)		
mc	millicurie(s) (10^{-3} c)	λ	index of precision
μc	microcurie(s) (10^{-6} c)	v.	see
mμc	millimicrocurie(s) (10^{-9} c)	cf.	compare
G	gravitational constant	c.	about
u	international unit(s)	p.	page
mu	milliunit(s) (10^{-3} u)	pp.	pages
		et seq.	and following

All temperatures are in °C(entigrade)

III. Amino acid residues

Amino Acid	*Abbreviation*	*Amino Acid*	*Abbreviation*
Alanine	ala	*Iso*leucine	ileu
Arginine	arg	Leucine	leu
Asparagine	asp-NH_2	Lysine	lys
Aspartic acid	asp	Methionine	met
Cysteine	cys	Phenylaline	phe
Glutamic acid	glu	Proline	pro
Glutamine	glu-NH_2	Serine	ser
Histidine	his	Threonine	thr
Hydroxylysine	hylys	Tryptophan	try
Hydroxyproline	hypro	Tyrosine	tyr
		Valine	val

Acknowledgments

EVERY contributor to this book has assured us that he has obtained all the permissions necessary to allow him to include in his chapter any required quotation, table or diagram from his own or another worker's published writings. Nevertheless we, as editors, wish to express our sincere thanks to all those, authors, editors and publishers, whose "permission to reprint" has been so generously and courteously given to us, directly or indirectly. It would be impossible to mention all by name and invidious to mention only some.

Nevertheless we feel bound to make a single breach in that anonymity. Mrs. Tonia Mason's services to one of us (CHG) during all the preliminary stages of preparing this book for the press were so exceptionally helpful that both of us would advise all future editors to try and get equally willing and skilful secretarial assistance. But we don't think that they will succeed.

C. H. GRAY

A. L. BACHARACH

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Chapter I

Introductory

C. H. Gray and A. L. Bacharach

Objectives

In experimental as well as in clinical endocrinology it is often necessary to assess the ability of an endocrine organ to perform its function. This may sometimes be done at post mortem, but it is not infrequently undesirable for the experimental animal and never really satisfactory for the human subject. Methods for determining hormones and their metabolites in body fluids have therefore been extensively developed. The higher concentrations frequently found in urine, and its ready availability in large quantities, inevitably led to the development first of methods for determining hormones in urine. However, the conversion of some hormones to a large number of metabolites, which are then excreted in the urine, frequently made these analyses either complicated and time-consuming or difficult to interpret in terms of endocrine function. The latter handicap is also exaggerated because the quantities of metabolites excreted can only represent the average excretion and the average endocrine activity during any given time; especially when the organ is subject to short bursts of abnormal activity, analyses only of urine collected during a period may be unhelpful.

For this reason, many workers have preferred to determine the hormone content of the blood, in the belief that this may give information as to the functional state of the endocrine organ at the precise time when the sample is collected. This somewhat naïve view has not been entirely confirmed in practice, for the concentration of a substance in the blood is only proportional to the rate at which it is secreted into the blood if the rate of removal is itself constant. For this reason, therefore, it seems that, except when we can compare the hormonal content of a systemic blood sample with the content of the endocrine organ involved, possible in certain animal experiments and sometimes on human subjects during surgical operations such as adrenalectomy, a complete picture of endocrine organ function is likely to be obtained only by investigating both blood and urine. Sometimes such observations need to be supported by determining blood or urine levels, or both, before and after an endocrine organ has been stimulated by a trophic hormone or some other mechanism for increasing activity.

Except for a number of specialised centres, most laboratories have concentrated attention on methods of urine analysis, and many of the generally accepted techniques appear in numerous current books on methods of clinical chemistry. The minute amounts of hormones present in blood and the smaller quantities available have demanded more sensitive techniques: some of them are biological and often only to be satisfactorily performed in departments having special experience in this field; others make tremendous demands on ultra-micro techniques. Nevertheless, many of them are now well established, and it seemed that the time had come when they might be surveyed together.

We would only add our awareness of having lifted for the title of this book the phrase "Hormones in Blood", which already belongs to an admirable publication of the Ciba Foundation (1957). This, however, was the report of a Symposium and was never meant, unlike our own more ambitious project, to cover the whole field and some of the adjacent territory. We can only apologise to the authors and editor of the Symposium report for any plagiarism of which they may think us guilty.

This book is thus aimed primarily at those interested or working in the medical and cognate sciences, from biochemistry and physiology to clinical practice and applied nutrition; it has been assumed that the readers will all be qualified in some relevant branches of pure and applied science, though different ones will be qualified in different ways and few, if any, in the speciality of a particular contributor. Consequently, the extent to which terms and concepts seemed to need explanation varies directly as the extent to which they are exclusive to the writer's subject, and this applies alike to techniques and equipment.

It may be thought at first blush that the contributors to this book have been induced publicly to attempt the instruction of their endocrinological grandmothers. Certainly this was not our intention, nor do we believe that further consideration would substantiate the charge. There are working in the endocrinological field many experts whose activities have so far been wholly or largely confined to studies of one hormone or group of hormones derived from one particular gland or having a particular biological function. From time to time such an expert may have to turn his attention to a different hormone, to which the specialist skills with which he has already become familiar may not apply, and for this he will probably need entirely new techniques. Moreover, we suspect that this is likely to occur with increasing frequency as our knowledge of glandular interdependence increases. It is in such circumstances that we hope this book will prove both informative and time-saving.

A fortiori we believe it should help an increasing number of biochemical analysts who suddenly find themselves faced for the first time with the need to conduct hormone determinations in blood, that is, at the microgram or millimicrogram level. If what has been written here in any way helps him to a solution of his immediate practical problem, while at the same time supplying him with some essential theoretical background, both chemical and physiological, this book will have achieved one at least of its deliberate objectives.

Although the Editors realised that special considerations might need to dictate minor modifications, it was hoped that all the chapters would conform fairly closely to the same pattern.

A. Chemical constitution and main physical properties (as far as these are known with reasonable certainty).

B. Organs or tissues of origin, with brief outline of path or paths of biosynthesis (again, as far as this is known).

C. Methods of determination in blood and plasma: (1) chemical and physico-chemical; (2) biological; (3) meaning of differences between 1 and 2 above.

D. Plasma levels.
   1. Amounts in normal plasma and other sites (e.g., corpuscles, organ of origin) given as ranges and means (with their standard errors, if known). Physiological variations (e.g., with sex, age, variations in temperature, etc.).
   2. Amounts in plasma during disease of (a) originating endocrine organ; (b) other endocrine organs; (c) non-endocrine organs.

In most chapters this order of presentation has been substantially observed by the authors. When it has not, there was a justification for the departure—either a gap in our knowledge or because for some special reason an alternative presentation appeared more logical.

The order of presentation of the chapters has been a matter of some concern to the editors; a straightforward scheme, beginning with simple peptides, complex peptides and protein hormones, succeeded by a separate series of chapters describing the different steroids, seemed an obvious choice, but the relation between the thyroid hormones and the thyroid-stimulating hormones clearly demanded that they should be treated in close apposition, especially as the same authors were responsible for the two subjects. For several reasons, we decided to take insulin out of the chapters on complex peptides. It is the most completely characterised peptide hormone, and we thought that logically, or at least chronologically, it could precede the others. It has seemed best to deal next with another well-characterised group, the thyroid