

CIBA FOUNDATION COLLOQUIA. ON ENDOCRINOLOGY

VOLUME 13

Human Pituitary Hormones

In honour of

PROFESSOR B. A. HOUSSAY, FOR. MEM. R.S.

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME,

O.B.E., M.A., M.B., M.R.C.P.

and

CECILIA M. O'CONNOR, B.Sc.

With 36 Illustrations

ALL RIGHTS RESERVED

*This book is protected under the Berne Convention.
It may not be reproduced by any means, in whole
or in part, without permission. Application with
regard to reproduction should be addressed to the
Publishers.*

© J. & A. CHURCHILL LTD. 1960.



THE Ciba Foundation, a unique international institution, owes its inception to the generosity of CIBA Limited, Basle. However, being established under British trust law, it enjoys complete independence in practice and policy.

Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, the proceedings of which are published in book form in the manner of the present volume. The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a black sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

THE CIBA FOUNDATION

for the Promotion of International Co-operation in Medical and Chemical Research

41 PORTLAND PLACE, LONDON, W.1.

Trustees

THE RIGHT HON. LORD ADRIAN, O.M., F.R.S.

THE RT. HON. LORD BEVERIDGE, K.C.B., F.B.A.

SIR RUSSELL BRAIN, BT.

THE HON. SIR GEORGE LLOYD-JACOB

SIR RAYMOND NEEDHAM, Q.C., F.S.A.

Executive Council

SIR RUSSELL BRAIN, BT., *Chairman*

PROFESSOR A. HADDCW, F.R.S.

SIR ARTHUR VERE HARVEY,
C.B.E., M.P.

THE HON. SIR GEORGE LLOYD-JACOB

PROFESSOR DR. DR. h.c. R. MEIER

PROFESSOR F. G. YOUNG, F.R.S.

Director, and Secretary to the Executive Council

DR. G. E. W. WOLSTENHOLME, O.B.E.

Assistant Secretary

MISS N. BLAND

Scientific Assistant

MISS CECILIA M. O'CONNOR, B.Sc.

Editorial Assistant

MISS MAEVE O'CONNOR, B.A.

Conference Assistant

MRS. NANCY G. SPUFFORD

PREFACE

It was a suggestion by the late Professor Braun-Menendez which led to the organization by the Ciba Foundation of a colloquium in Buenos Aires immediately before the 21st International Congress of Physiological Sciences. During its preparation, an air crash caused the tragic death of Professor Braun-Menendez, a scientist, a colleague and a friend whose memory all will cherish with respect and affection.

Professor B. A. Houssay, whom the Ciba Foundation was privileged to honour by holding in his name this colloquium on Human Pituitary Hormones, a subject owing so much to his own original and classical work, generously spared time from the multitude of his responsibilities for the major Congress to obtain important governmental and local decisions which enabled the Foundation, despite many difficulties, to arrange its meeting.

Dedicated and untiring assistance was given in Buenos Aires by Dr. M. R. Malinow, and much help also by Dr. E. Montuori in finding the path—there seemed to be no correct way—through the intricate maze of airline prices and reservations. The colloquium was held in the City Hotel, whose manager, Mr. R. Papernik, co-operated most readily with Dr. Malinow in meeting the detailed requirements of the Foundation.

The Foundation's own team for this distant occasion consisted of the Director, with his Scientific Assistant, Cecilia O'Connor, and his Conference Assistant, Nancy Spufford. They received essential advice and encouragement in all the planning, the journeys, and the technical and social aspects of the colloquium from a Member of the Executive Council, Professor F. G. Young. In addition, Professor Young's Chairmanship was a major factor in establishing the maturity and excitement of the scientific proceedings.

The papers, and the discussions they aroused, are here reproduced. The editors hope that these contributions from workers in eleven countries, assembled in the Argentine by a British Trust of Swiss origin, will provoke interest and stimulate further research in many parts of the world.

**List of those participating in or attending the Colloquium
on "Human Pituitary Hormones",
6th-8th August, 1959**

EVELYN ANDERSON	.	.	National Insts. of Health, Bethesda, Md.
R. A. ATRIA	.	.	Hospital del Salvador, Santiago de Chile
F. A. DE LA BALZE	.	.	Endocrine Inst., National Inst. of Health, Buenos Aires
J. C. BECK	.	.	McGill University Clinic, Royal Victoria Hospital, Montreal
P. M. BOTTARI	.	.	Dept. of Medicine and Pharmacology, Univer- sity of Brussels
E. B. DEL CASTILLO	.	.	Endocrinological Dept., Hospital Rivadavia, Buenos Aires
J. M. CERVIÑO	.	.	Inst. of Endocrinology, Hospital Pasteur, Montevideo
A. B. DE ULHÔA CINTRA	.	.	Faculty of Medicine, University of Sao Paulo
*E. J. CONWAY	.	.	Dept. of Biochemistry and Pharmacology, University College, Dublin
G. W. CORNER	.	.	Rockefeller Inst. for Med. Res., New York
A. TACHELLO COSTA	.	.	Soc. Argentina de Endocrinología y Meta- bolismo, Buenos Aires
*V. G. FOGLIA	.	.	Inst. of Physiology, University of Buenos Aires
RUSSELL FRASER	.	.	Postgraduate Medical School, London
C. A. GEMZELL	.	.	King Gustaf V Research Inst., Stockholm
F. GÓMEZ-MONT	.	.	Hospital de Enfermedades de la Nutricion, Mexico, D.F.
J. I. HARRIS	.	.	Dept. of Biochemistry, University of Cam- bridge
A. B. HOUSSAY	.	.	Centro de Endocrinología, Buenos Aires
B. A. HOUSSAY	.	.	Inst. de Biología y Medicina Experimental, Buenos Aires
C. H. LI	.	.	Hormone Research Laboratory, University of California, Berkeley
J. A. LORAINÉ	.	.	Clinical Endocrinology Research Unit (Medi- cal Research Council), Edinburgh
R. LUFT	.	.	Dept. of Endocrinology and Metabolism, Karolinska Hospital, Stockholm
M. R. MALINOW	.	.	Inst. of Physiology, University of Buenos Aires
J. MORATÓ-MANARO	.	.	The Endocrinology Laboratory of the Inst. of Endocrinology, Ministry of Public Health, Montevideo

* Unable to participate in the colloquium because of illness.

O. H. PEARSON . . .	Western Reserve University School of Medicine, Inst. of Pathology, Cleveland, Ohio
M. S. RABEN . . .	New England Center Hospital, Boston, Mass.
C. H. READ . . .	Dept. of Pediatrics, State University of Iowa, Iowa City
R. R. RODRIGUEZ . . .	Inst. de Fisiología, Universidad Nacional de la Plata, La Plata
JANE RUSSELL . . .	Dept. of Biochemistry, Emory University, Georgia
M. SONENBERG . . .	Memorial Center for Cancer and Allied Diseases, New York
ELEANOR VENNING . . .	McGill University Clinic, Royal Victoria Hospital, Montreal
A. E. WILHELMI . . .	Dept. of Biochemistry, Emory University, Atlanta, Georgia
F. G. YOUNG . . .	Dept. of Biochemistry, University of Cambridge

CONTENTS

	PAGE
Chairman's opening remarks	
F. G. YOUNG	1
The radiological anatomy of the human pituitary	
by G. F. JOPLIN and RUSSELL FRASER	9
<i>Discussion:</i> DE LA BALZE, BECK, CORNER, RUSSELL FRASER, GÓMEZ-MONT, LORAINÉ, LUFT, MALINOW, PEARSON, RODRIGUEZ, SONENBERG, WILHELMI, YOUNG	17
Systematic fractionation of human pituitaries	
by A. E. WILHELMI	25
<i>Discussion:</i> LI, LORAINÉ, RABEN, WILHELMI	42
Studies on human pituitary growth and gonadotropic hormones	
by C. H. LI	46
<i>Discussion:</i> BECK, GEMZELL, LI, LORAINÉ, RABEN, SONENBERG, WILHELMI	64
Immunological studies of human growth hormone	
by C. H. READ and G. T. BRYAN	68
<i>Discussion:</i> BECK, RUSSELL FRASER, GEMZELL, LORAINÉ, READ, WILHELMI	84
Growth hormone and the mobilization of fatty acids	
by M. S. RABEN and C. H. HOLLENBERG	89
<i>Discussion:</i> DE LA BALZE, BECK, RUSSELL FRASER, LI, LUFT, MALINOW, PEARSON, RABEN, READ, WILHELMI, YOUNG	102
Aspects of the metabolic action of human growth hormone	
by D. IKKOS and R. LUFT	106
<i>Discussion:</i> BECK, DE ULHÔA CINTRA, RUSSELL FRASER, GEMZELL, GÓMEZ-MONT, LI, LUFT, PEARSON, RABEN, SONENBERG, VENNING	129
The effect of growth hormone on urinary calcium excretion	
by RUSSELL FRASER and M. HARRISON	135
<i>Discussion:</i> DE LA BALZE, BECK, DE ULHÔA CINTRA, RUSSELL FRASER, GÓMEZ-MONT, LI, LORAINÉ, LUFT, PEARSON, RABEN, SONENBERG, WILHELMI	148
The variability in physiological response to growth hormone	
by J. C. BECK, E. E. MCGARRY, I. DYRENFURTH, R. O. MORGEN, E. BIRD and ELEANOR VENNING	156

*Short communication:***Growth hormone and aldosterone secretion**

by ELEANOR VENNING and O. J. LUCIS 174

Discussion: ANDERSON, BECK, RUSSELL FRASER, LI, LORAINÉ, LUFT, PEARSON, RABEN, RODRIGUEZ, RUSSELL, SONENBERG, WILHELMI, YOUNG 182

Human pituitary follicle-stimulating hormone**I. Clinical effect of a partially purified preparation**

by C. A. GEMZELL, E. DICZFALUSY and K.-G. TILLINGER . . 191

II. Preparation of a highly active fraction

by P. ROOS and C. A. GEMZELL 209

Discussion: BOTTARI, CORNER, RUSSELL FRASER, GEMZELL, A. B. HOUSSAY, LORAINÉ, MALINOW, MORATÓ-MANARO, RABEN, VENNING 212

Some observations on the clinical value of pituitary gonadotropin assays in human urine

by J. A. LORAINÉ 217

Discussion: DE LA BALZE, DE ULHÔA CINTRA, GEMZELL, LI, LORAINÉ, LUFT, PEARSON, READ, SONENBERG, VENNING, WILHELMI 233

A method of determining interstitial-cell stimulating hormone in urine: some results in normal and pathological cases

by J. MORATÓ-MANARO, J. M. CERVÍÑO and J. MAGGIOLO . . 238

Discussion: LORAINÉ, MORATÓ-MANARO, WILHELMI 249

Adrenocorticotropic hormone and melanocyte-stimulating hormone from human pituitary glands

by T. H. LEE, A. B. LERNER and VINA BUETTNER-JANUSCH . 251

A melanocyte-stimulating hormone from the human pituitary gland

by IEUAN HARRIS 266

Discussion: HARRIS, LI, SONENBERG, YOUNG 273

Blood concentration of thyrotropic hormone in normal subjects and in patients with thyroid disease

by P. M. BOTTARI 275

Discussion: ANDERSON, DE LA BALZE, BECK, BOTTARI, RUSSELL FRASER, A. B. HOUSSAY, LORAINÉ, PEARSON, RABEN, READ, SONENBERG 294

General Discussion: ANDERSON, ATRIA, DE LA BALZE, BECK, DE ULHÔA CINTRA, CORNER, RUSSELL FRASER, GEMZELL, GÓMEZ-MONT, HARRIS, A. B. HOUSSAY, LI, LORAINÉ, LUFT, MALINOW, PEARSON, RABEN, RODRIGUEZ, RUSSELL, SONENBERG, WILHELMI, YOUNG 302

CHAIRMAN'S OPENING REMARKS

F. G. YOUNG

It is with great pleasure and, indeed, with pride that I take the chair at a Ciba Foundation Colloquium held in honour of Prof. Houssay, in this great capital city of Argentina. Perhaps the only claim that I have for appointment to this position is that I am a member of the Executive Council of the Foundation. Furthermore, I believe that I have known Prof. Houssay and admired his work as long as, or possibly longer than, anybody in this room, apart from those who live in Argentina. Nobody will disagree with me when I say that Prof. Houssay is one of the most distinguished citizens that Argentina has ever had, and that he is the most outstanding scientist in South America. Not only has he been responsible for much important original research, about which I shall speak a little later, but also he has been a rock standing firm in a sea of political trouble. The tide comes, the tide recedes, but the rock stands as it always did. It is with special pleasure that we all meet here today to honour one who has withstood so much and who has survived with dignity and calm.

In 1950 I had the most interesting experience of lecturing in a number of countries in this vast subcontinent of South America. Although I knew before how internationally distinguished Prof. Houssay was, I had not realized until then how, in all of South America, he had also become a symbol of independent science. In every country which I visited he was, I found, a scientific and a personal inspiration. His activities have meant much more to the world than has been provided by the facts that his researches have elucidated, vitally important though these facts have been.

The poet Shakespeare, who is known in this country as well as he is known in other parts of the world, wrote: "The toad,

ugly and venomous, wears yet a precious jewel in his head." That Shakespeare had the pituitary gland in mind when referring to the jewel in the head of the toad is unlikely, but Prof. Houssay's researches clearly established what a valuable jewel the hypophysis of the toad really is, and how important it is to have an experimental animal in which the different parts of the pituitary body can be separately removed with relatively little interference to the rest of the gland. The jewel in the head of the toad has turned out to be a very valuable prize indeed.

It is perhaps rather surprising now to recall that in the early nineteen-twenties the metabolic importance of the pituitary gland as a whole was generally ascribed to the posterior portion, the anterior lobe being considered by many as of much less significance. I believe I am right in saying that it was the investigations of Prof. Houssay and Dr. Dora Potich with the toad in the late nineteen-twenties that first revealed the important metabolic effects of removal of the pars glandularis of the anterior pituitary gland, especially on carbohydrate metabolism and experimental diabetes. One disadvantage of the toad is that it wears its "anterior" pituitary lobe in a posterior position, so that it is not correct to call the pars glandularis of its pituitary gland "the anterior lobe". In the hands of Prof. Houssay and his colleagues the toad proved to be an immensely useful experimental animal, and paved the way for the demonstration in mammals—the dog was important in the later investigations—of what came to be known as the "Houssay phenomenon", the alleviation of experimental diabetes by removal of the pituitary gland or of its anterior portion alone. Subsequently Houssay and Biasotti, as a corollary of the earlier findings, showed that an extract of the anterior pituitary lobes of oxen exerted a diabetes-inducing effect in partially depancreatized dogs. This was in the early nineteen-thirties. I personally recall the scepticism with which the description of the Houssay phenomenon and of the diabetes-inducing activity of anterior pituitary extracts was greeted at that time in some apparently authoritative

quarters. A distinguished physiologist, long since dead, told me at that time that he thought these results must be wrong, because the evidence from other laboratories showed that the posterior lobe of the pituitary was of predominant importance in the control of metabolic processes. Nevertheless, I was able myself to show that if one exactly repeated Prof. Hous-say's preparation of an extract of fresh ox anterior tissue, administering this extract to a normal intact dog, however, one could demonstrate a clear diabetes-inducing effect of this material.

It would be dangerous to say of any scientist that nothing that he has published has ever been contradicted; but I can say of Prof. Houssay that nothing that I know about which he has published has ever turned out to be wrong. People have failed sometimes to obtain the same results, but in my experience their failure has usually been attributable to the fact that those attempting to repeat the experiments did not use precisely similar conditions.

There is no doubt that Prof. Houssay is one of the few responsible for the recognition of the importance that we now ascribe to the anterior pituitary gland. Others whose names come to mind in this connexion are Drs. Philip E. Smith, Herbert M. Evans, J. B. Collip and Hans Selye. More recently the investigations of others, including in particular those of Dr. C. H. Li—coming as he does from the same laboratory as Philip Smith and Herbert Evans—have placed the identity and chemistry of certain anterior pituitary hormones on a firm basis.

In reviewing the nature and mechanism of action of hormones, I recently pointed out that somewhat confusingly we are now less certain of the precise identity of some hormones than we were, say, ten years ago. I raised the question of whether the substance that we ought to call a hormone is that which is present in the endocrine gland itself or in the blood flowing from the endocrine gland; or is it the substance which may be formed, by metabolic changes in the blood stream or in non-target organs, from the material originally liberated by

the secretory tissues? Or is it whatever may ultimately appear as the result of metabolic changes in the tissues on which the hormone acts, and which may itself be capable of evoking a biological response in the target tissues which the chemical substance in the endocrine gland or in the blood would not be capable of producing? Discussion of these questions appears to me not to be very useful at the present time, although the relevant experimental facts are, of course, of great importance. When we come to discuss anterior pituitary growth hormone during this colloquium, we shall surely find that this hormone provides an example of some of these complications. Moreover, it seems possible that many hormones, and the pituitary hormones in particular, are not the specific substances that we once thought they were and that a substantial overlap of properties may exist between different hormones and their relations. Perhaps also no single target organ exists for many or all of them. Indeed, some of the rigid ideas of the past appear to be in need of modification. We shall no doubt have further information on these points at the end of the present colloquium.

There are five main stages in the development of experimental research concerning the endocrine activity of any particular organ or tissue. Clinical observations and investigations often play a part in the early stages and sometimes in the later ones; but the five experimental stages which I distinguish are as follows: (i) the results of removing the endocrine organ, or of damaging the secretory tissue, in suitable experimental animals are first described; (ii) crude extracts of the organ or of the tissue are then prepared and attempts are made by administration of these crude extracts to alleviate the symptoms which develop in the animal in which the organ or tissue is missing or damaged; (iii) chemical isolation of active material is then effected and the chemical structure of the active principle or principles is elucidated; (iv) the active substance or substances are produced in the laboratory by artificial means and the influence on biological activity of variations in chemical structure is assessed; (v) the

mechanism of action of the substance or substances is then investigated and in these researches the activity of metabolites, and of chemical relations of the naturally occurring substances which may or may not naturally exist, may be of particular importance. Investigation of the mechanism of action of a hormone may run concurrently with all the four other phases, but the results are most likely to be meaningful when pure, preferably chemically synthetic, preparations of the active hormones are available. This is also true with respect to evidence that multiple activities may be ascribable to one hormone or that hormones may share activities in common. It is reasonable to say that with respect to anterior pituitary hormones we are in stages (iii), (iv) and (v), although we can still argue quite strongly about stage (iii)—the chemical isolation of the pure substance and its chemical structure—with respect to some of these hormones at the present time.

The production of peptide hormones in the laboratory by artificial means is in its infancy. But the infant is a lusty one and is crowing loudly at the present time. As a biochemist I am delighted that some, at least, of the discussions during this colloquium can be in terms of known chemical structures and, moreover, in some instances, about chemical structures that can either be reached by synthesis already, or will almost certainly be synthesized in the not far distant future. On the other hand, the mechanism of action of anterior pituitary hormones is still a matter of considerable doubt and discussion. I do not think that this is a subject on which there will be wide agreement during the present meeting.

One striking point that has emerged from studies on peptide hormones in recent years is the existence, in some instances, of clearcut chemical differences between hormones prepared from different species of animal. In his brilliant work on the structure of insulin, Sanger clearly demonstrated that differences exist in the structure of insulin from different species of animal, although these differences were of a relatively minor character, and associated with the same small portion of the A chain of the insulin molecule. Recent evidence

suggests that some hormones of the anterior pituitary gland exhibit much bigger species differences than have so far been found for insulin. The growth hormone of the human pituitary gland differs very strikingly in chemical properties and structure from that of the ox pituitary gland. This is a matter that will no doubt come up for consideration during this meeting. Advantages may result from these species differences since one is sometimes able to produce antisera to certain hormones, which is of great value for investigation and assay of the antigenic hormone. This is an important development which we shall hear discussed during the present meeting.

Another striking fact which has arisen in the course of recent investigations of the chemistry of pituitary hormones, is the existence of a close chemical relationship between the melanocyte-stimulating hormones of the pars intermedia, and corticotropin. This is another subject that will be considered in the course of our discussions, and I shall therefore say no more about the work of C. H. Li, J. I. Harris and A. Lerner in this connexion, except to enquire perhaps, whether we might reasonably hope that some similar identities of sequences may exist between other pituitary hormones. Certainly the possibility that some common building blocks may exist from which can be fashioned anterior pituitary hormones of different sorts according to different needs is a fascinating one.

Species differences in anterior pituitary hormones may well account for the apparent lack of activity of the hormone from one species in another, in certain instances. The lack of growth-stimulating action of ox growth hormone in the human being has been a mystifying and, indeed, a depressing fact, until relatively recently. But the chemical differences now known to exist between the ox and the human hormones may well account for the facts as we know them at present. The effect of human growth hormone in human beings will be a matter for fruitful discussion during our colloquium.

When Prof. Houssay and his colleagues were carrying out their careful investigations on the hormonal effects of removal