Lactogenic Hormones

Ciba Foundation Symposium

LACTOGENIC HORMONES

A Ciba Foundation Symposium
in memory of Professor S. J. Folley

Edited by
G. E. W. WOLSTENHOLME
and
JULIE KNIGHT

CHURCHILL LIVINGSTONE
Edinburgh and London
1972

LACTOGENIC HORMONES

Photograph by Peter Grugeon

SYDNEY JOHN FOLLEY, F.R.S.

1906–1970

(*Frontispiece*)

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Symposium on Lactogenic Hormones,
held 11th–13th May 1971

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INTRODUCTION

J. C. Beck

It is my very real pleasure, as Chairman of this meeting, to pay specia tribute to Professor S. J. Folley, the man to whom this symposium is dedicated. His untimely death was a great loss and it is our profound regret that he did not live to join us in these deliberations.

This opportunity to pay tribute to one of the giants of endocrinology is an honour and privilege. The work of Sydney John Folley and his associates has laid much of the groundwork that brings this family of acquaintances and admirers together at this symposium. Inevitably, advances in technology and a new generation of creative younger scientists 'standing upon the shoulders of past giants' move the frontiers of our knowledge forward by a major stride, and I believe that in the course of the symposium we see this phenomenon demonstrated to an unusual degree. Observations made on the lactogenic hormones during the last quarter of a century are suddenly being brought into fresh focus. These observations are simultaneously generating new knowledge on the role of lactogenic hormones in health and disease and contributing unique information on the relationships between the structure and function of a group of polypeptide hormones at the molecular level.

At this point I would like to comment on 'relevant' research. It is almost irreverent today not to use this progressively more hackneyed term, but if there is one thing that the history of science has taught us, it is that no generation in its time is necessarily competent to judge which science of its day will ultimately be highly relevant or what datum may eventually prove 'the missing link' in the solution of some vital problem. It is upon the premises that were established 10, 20, 50 and 100 years or more ago that a so-called breakthrough or giant 'stride' suddenly takes place. It is difficult at a given time to know what is relevant to what.

At best we can only judge whether a worker is competent to do the job for which he seeks support, whether he is an honest individual, whether he works with care and meticulous detail, and whether he has built all the appropriate controls into his experimental design so that his work can be interpreted. If all these qualities are present, it is my belief that sooner or later the data obtained not only will be relevant, but will be highly useful. They will represent one more contribution to our culture's intellectual

'bank' and may one day provide an answer to what otherwise might remain a puzzle. I believe that in John Folley such a scientific personality is exemplified, and that in the course of this symposium we see an example of how some things suddenly fit together. After long years of toil in many places with a variety of tools and the accumulation of many data, the stage has finally been set.

Sydney John Folley obtained the degree of B.Sc. with first class honours in chemistry at the University of Manchester in 1927, and was awarded the Mercer Scholarship in chemistry. He then took an M.Sc. degree for research in colloid chemistry and joined the Department of Physiology as Research Assistant to H. S. Raper, who had recently succeeded Professor A. V. Hill as the Head of the Department. In 1931, he moved to Liverpool as an Assistant Lecturer in Biochemistry.

In 1932 he had to relinquish his teaching appointment at the University of Liverpool because of ill health, and through the influence of H. S. Raper went to the National Institute for Research in Dairying at Shinfield. At that time the Physiology Department occupied a converted back bedroom in the Manor House. The staff consisted of a biochemist from Oxford, G. L. Peskett, and a technical assistant, S. C. Watson. This is a far cry from the department which existed at John Folley's death—it is a modern facility with excellent equipment, and a staff which, together with visiting workers, sometimes exceeded fifty.

Initially in his new task, John Folley assisted Peskett in studies concerned with the relationship between blood electrolytes and the secretion of the lipid constituents of milk. When Peskett left the Institute in 1934, Folley wanted to carry on the physiological work, and with the new Director, Dr H. D. Kay, who was appointed in 1933, Folley collaborated in studies on the properties of the alkaline phosphatase of the mammary gland—his first venture into a new field which was later to become his life's work. Kay had recently returned from Canada and it was under his influence that Folley became interested in the relationship between the thyroid gland and lactation. This was his initiation into endocrinology; he rapidly became aware of the changes that were occurring in this field, particularly in the relationship between the knowledge of the anterior pituitary and the gonadal hormones and their influence on reproduction. His interest in the role and function of the mammary gland and in reproductive physiology was first aroused by A. S. Parkes' book entitled *The Internal Secretions of the Ovary*, and shortly thereafter Folley came into contact with the extremely active group headed by Parkes at the National Institute for Medical Research, then at Hampstead. This led to Folley's first experiments on the effects of the oestrogenic hormones on the composition of the milk of

cattle, and brought about the first of many contributions Folley made over his lifetime on the hormonal control of lactation.

Through his acquaintance with F. G. Young, one of A. S. Parkes' colleagues at Hampstead, Folley became interested in the anterior pituitary hormone, prolactin. At the time, Frank Young was primarily concerned with the effect of anterior pituitary hormones on carbohydrate metabolism, but their combined interest in the effects of anterior pituitary hormones on the mammary glands resulted in productive collaboration for a number of years. They were the first to suggest the concept of co-lactogens—that is, a complex of anterior pituitary hormones responsible for lactogenesis as well as for the maintenance of lactation. The role of anterior pituitary growth hormone in lactation led to the discovery of the galactopoietic action of highly purified bovine growth hormone. It is of interest that some years later a group of workers, headed by Dr A. T. Cowie in Folley's laboratory, first made the critical observation that after pituitary stalk section the pituitary continues to secrete prolactin but not the other anterior pituitary tropic hormones. This latter work also emphasized that prolactin and growth hormone were the most critical anterior pituitary hormones for the maintenance of lactation in the goat.

In the middle 1930's, F. H. A. Marshall invited Folley to contribute a chapter on lactation for a treatise on the physiology of reproduction. At a meeting of the contributors, Folley came into contact with other distinguished reproductive physiologists, among whom was Solly Zuckerman. Folley and Zuckerman joined forces in a study of the control of mammary growth in the rhesus monkey, and correlated this with the careful observations which Zuckerman had made on the menstrual cycle of primates. This began a long series of studies by members of Folley's department on various aspects of mammary growth and development in a wide variety of animal species, with a long-term objective of describing the conditions under which mammary glands might be grown by artificial means. During this time Folley repeatedly emphasized the importance of the traditional internal glandular morphology of the mammary gland.

During the Second World War, the major interests of the department concerned projects in the field of physiology of lactation and reproduction which might have practical applications to the efficiency of dairy farming. One of these projects was concerned with the hormonal stimulation of udder growth and milk secretion, whereby it was hoped that sterile cows and heifers might be brought into milk production. With a gift of stilboestrol from Charles E. Dodds, the Folley group were the first to show that its effects in the cow with respect to milk composition were comparable to those of natural oestrogens.

In the last decade the interests of Folley and his associates were in the neuroendocrine control of those hormones known to play an important role in lactation. Although they concentrated on two main themes—the factors concerned with the secretion of prolactin, and those involved in the mechanisms responsible for the removal of milk from the mammary gland during suckling—the interests of the group were mainly concerned with the latter. In the late 1950's the group developed a sensitive and specific method for estimating oxytocin, the milk ejection hormone, in biological fluids. The method depended upon measuring the milk ejection pressure in the cannulated mammary gland of the anaesthetized lactating guinea pig after retrograde arterial injection of the test solution; this enabled the group to study the release of oxytocin in the jugular blood of cows during machine milking, of sows during suckling and of goats during suckling and hand milking, and showed that the release of the hormone in the cow could be conditioned by auditory and visual stimuli.

The concept of species specificity of anterior pituitary protein hormones led Folley and his associates, together with Carl Gemzell, to study the prolactin-like activity of various preparations of human growth hormone. They found, as did others, that human growth hormone possessed weak crop gland-stimulating properties, but they went on to show that, by the mammary intraductal test for lactogenic activity in pseudopregnant rabbits, human growth hormone was as active as purified sheep prolactin. This series of observations together with some made by others caused uncertainty as to whether prolactin existed as a separate lactogenic hormone in primates as it does in animals such as the sheep and ox. The whole question of the possible existence of a separate human prolactin and the role of human placental lactogen in lactogenesis was raised as a fruitful area for future research by Forsyth and Folley in a recent paper (1970).

Folley was awarded the degree of D.Sc. by his university in 1940 and he was elected a Fellow of the Royal Society in 1951. In 1964 he was given the title of Research Professor in the University of Reading and in the same year he was awarded an honorary doctorate by the University of Ghent. In 1969 he was awarded the Dale Medal by the Society for Endocrinology. The medal was presented by Professor H. Heller at the Meeting House of the Zoological Society of London after Folley had delivered the annual Dale Lecture. His international contacts were many and varied and he maintained a keen interest in organizations fostering the study of endocrinology. He was a distinguished member of the Society for Endocrinology and one of its founder members; he was the Society's first secretary from 1946 to 1951 and was Chairman from 1951 to 1956. He attended to the

affairs of the *Journal of Endocrinology* with great energy and devotion and was Chairman of the Editorial Board from 1959 until his death.

John Folley's contacts with the Ciba Foundation have been many. He served as a member of the Ciba Foundation Scientific Advisory Panel from 1953 until his death. He participated in the first eight colloquia on endocrinology and in three symposia (*Isotopes in Biochemistry*, 1951; *Toxaemias of Pregnancy*, 1950; and *Mammalian Germ Cells*, 1952). It was through the aid of the Foundation that his classic monograph *Recherches récentes sur la physiologie et la biochimie de la sécrétion lactée* was translated into English (*The Physiology and Biochemistry of Lactation*, 1956).

In the time that I knew Folley, I was always reminded of the parallelism between his extra-scientific interests and those of J. S. L. Browne. Both had been beset through much of their careers with serious health problems and in later years with near blindness. Both had a deep and abiding appreciation of music and painting, and both had the same favourite composers. They both prided themselves on the capabilities of their high-fidelity equipment and would set the volume control at a level appropriate for a major concert hall. Folley's interests in art included the modern and he was a devotee of Picasso.

John Folley died on the 29th June 1970 after a brief illness and his untimely demise has saddened all who knew him. His career was a distinguished one and the dedication of this symposium to his honour is a fitting tribute to his major contributions to our understanding of the biochemistry and physiology of the lactogenic hormones.

Before closing, I want to comment briefly on the nomenclature of one of the lactogenic hormones discussed in the symposium. The new protein hormone isolated from human placenta was named human placental lactogen (HPL) by Josimovich and MacLaren (1962) and chorionic growth hormone-prolactin (CGP) by Kaplan and Grumbach (1964*a*, *b*). Subsequently it was designated as purified placental protein (human) (PPP(H)) by Bell and his associates (Florini *et al.* 1966) and placental protein, the most non-commital description of all, by Friesen (1965).

As a result of a discussion during a Round Table Conference on Human Placental Lactogen held at the University of Siena in September 1967, Li, Grumbach, Kaplan, Josimovich, Friesen and Catt proposed the name 'human chorionic somatomammotropin' (HCS) (Li *et al.* 1968). This was an attempt to prevent further confusion by the use of different terms for the same hormonal agent. Since the hormone was located in the syncytiotrophoblastic layer of the human placenta, according to immunofluorescence studies, and since it has both growth hormone (somatotropin) and lactogenic hormone (mammotropin) activities, the term was in line with

the established name for the other gonadotropin produced by the human placenta, human chorionic gonadotropin. 'HCS' thus indicated the origin of the hormone as well as the known biological properties. However, this name has not been entirely satisfactory or acceptable in practice, and readers will find on pp. 400–402 a discussion of the advantages of retaining, at least until we know more about its functions, the original name of human placental lactogen (HPL). In accordance with this view, HPL has been adopted as consistently as is feasible in this volume.

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RECENT KNOWLEDGE OF THE CHEMISTRY OF LACTOGENIC HORMONES

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THERE are three lactogenic hormones which have been isolated and chemically characterized from three different sources, namely the human pituitary, ovine pituitary and human placenta. These are: human growth hormone (HGH), ovine prolactin and human chorionic somatomammotropin (HCS; human placental lactogen, HPL). This paper summarizes briefly some aspects of the chemistry of these three lactogenic hormones.

HUMAN PITUITARY GROWTH HORMONE

HGH was isolated and characterized (Li and Papkoff 1956; Li 1957*a*) fifteen years ago and is a protein of molecular weight 21 500 (Li and Starman 1964) and isoelectric point pH 4·9 (Li 1957*a*). Some physicochemical properties of HGH are presented in Table I. It consists of a single polypeptide

TABLE I

SOME PHYSICOCHEMICAL PROPERTIES OF HGH, OVINE PROLACTIN AND HCS

Properties	*HGH*	*Ovine prolactin*	*HCS*
Molecular weight	21 500	23 300	21 600
Isoelectric point, pH	4·9	5·7	
Sedimentation coefficient, $s_{20,w}$	2·18	2·19	
Diffusion coefficient, $D_{20} \times 10^7$	8·88	8·44	
$[\alpha]_D^{25}$ (0·1M-acetic acid)	−39°	−41°	
Ellipticity $[\theta]$, at 221 nm	−19 700	−21 400	−16 700
α-Helix content, percentage	55	55	45
pK_a of tyrosine residues	10·8	11·2	10·9
$E_{1cm}^{0.1}$ at 277 nm	0·931	0·894	0·822

chain with one tryptophan residue and two disulphide bridges (Li and Papkoff 1956; Li 1957*a*). The complete amino acid sequence (Li, Liu and Dixon 1966; Li, Dixon and Liu 1969) of HGH was first proposed in 1966 to be a single chain protein of 188 amino acids with the tryptophan residue at position 25 and the two disulphide bridges formed by residues 68–162 and 179–186, as shown in Fig. 1. This proposed structure has