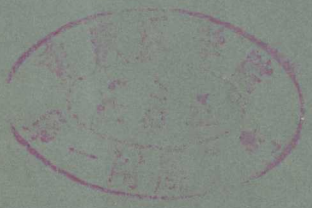
# Mammary Cancer and Neuroendocrine Therapy





# Mammary Cancer and Neuroendocrine Therapy

*Edited by*

**BASIL A. STOLL**

*Honorary Consultant to the Radiotherapy Departments, St. Thomas Hospital and Royal Free Hospital, London*


BUTTERWORTHS

ENGLAND: BUTTERWORTH & CO. (PUBLISHERS) LTD.
LONDON: 88 Kingsway, WC2B 6AB

AUSTRALIA: BUTTERWORTHS PTY. LTD.
SYDNEY: 586 Pacific Highway 2067
MELBOURNE: 343 Little Collins Street, 3000
BRISBANE: 240 Queen Street, 4000

CANADA: BUTTERWORTH & CO. (CANADA) LTD.
TORONTO: 14 Curity Avenue, 374

NEW ZEALAND: BUTTERWORTHS OF NEW ZEALAND LTD.
WELLINGTON: 26–28 Waring Taylor Street, 1

SOUTH AFRICA: BUTTERWORTH & CO. (SOUTH AFRICA) (PTY.) LTD.
DURBAN: 152–154 Gale Street



Suggested U.D.C. Number: 618·19–006: 615·252·43
Suggested Additional Number: 618·19–006:615·357

I.S.B.N. 0 407 26440 X

*Printed in Great Britain by*
*The Camelot Press Ltd, London and Southampton*

# List of Contributors

R. Chemama, M.D., Physician to the Department of Medicine, Fondation Curie, Paris*

James A. Clemens, Ph.D., Research Physiologist, Department of Physiological Research, The Eli Lilly Research Laboratories, Indianapolis, Indiana.

A. T. Cowie, B.Sc., Ph.D., D.Sc., M.R.C.V.S., Head of the Physiology Department, National Institute for Research in Dairying, Shinfield, Reading, Berkshire

V. M. Dilman, M.D., Professor of Endocrinology, Petrov Research Institute of Oncology, Leningrad

A. Ennuyer, M.D., Head of the Department of Medicine, Fondation Curie, Paris

K. Fotherby, Ph.D., F.R.I.C., Reader in Biochemistry in the University of London at the Royal Postgraduate Medical School

H. G. Friesen, M.D., Professor of Experimental Medicine, McGill University, Montreal

H. J. Guyda, M.D., Assistant Professor of Pediatrics, McGill University, Montreal

M. Hayem, Associate Oropharyngeal Surgeon, Institut Gustave Roussy, Villejuif, France

J. C. Heuson, M.D., Deputy Director, Department of Medicine, Institut Jules Bordet, Brussels

P. Hwang, M.B., B.S., Research Fellow, McGill University, Montreal

Frances James, B.Sc., Ph.D., Research Fellow, Royal Postgraduate Medical School, London

M. F. Jayle, Professor of Biochemistry, U.E.R. Biomédicale des Saints—Pères, Paris

P. Juret, Chief of the Department of Medicine, Centre Françoise Baclesse, Caen, Frane

Howard D. Kolodny, M.D., Director of the Department of Medicine, Queens Hospital Center Affiliation of the Long Island Jewish–Hillside Medical Center, Jamaica, New York; Associate Professor of Medicine, School of Medicine, State University of New York at Stony Brook

* Deceased.

LIST OF CONTRIBUTORS

Robert M. MacLeod, B.S., M.S., Ph.D., Professor of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia

Joseph Meites, Ph.D., Professor of Physiology, Michigan State University, East Lansing, Michigan

Charles S. Nicoll, Ph.D., Department of Physiology, University of California, Berkeley, California

Simone Saez, M.D., Department of Medical Biology, Centre Léon Bérard, Lyon, France

Lawrence Sherman, M.D., Associate Director, Department of Medicine, Queens Hospital Center Affiliation of the Long Island Jewish–Hillside Medical Center, Jamaica, New York; Associate Professor of Medicine, School of Medicine, State University of New York at Stony Brook

Basil A. Stoll, Honorary Consultant to the Radiotherapy Departments, St. Thomas' Hospital and Royal Free Hospital, London

Karen C. Swearingen, Ph.D., Department of Physiology, University of California, Berkeley, California

R. Tchao, B.Sc., Ph.D., Assistant Professor, Department of Pathology, Medical College of Pennsylvania, Philadelphia

L. Terenius, Ph.D., Associate Professor of Pharmacology, University of Uppsala

Clifford W. Welsch, Ph.D., Associate Professor of Anatomy, College of Human Medicine, Michigan State University, East Lansing, Michigan

# Tribute to Marcel Dargent (1908–1972)

The tragic news of the untimely passing of Marcel Dargent broke during the summer of 1972 while this Symposium was being written. It was thought appropriate that it should be dedicated to his memory.

Marcel Dargent was a man of enormously wide interests embracing science and literature, art and philosophy, but oncology was a field well suited to his talents, energy and enthusiasm. He was to reveal himself an extremely able administrator in the building up of the Centre Léon Bérard in Lyon. Under his leadership, and with the enthusiastic help of an outstanding team of co-workers whom he gathered around him, this centre rapidly grew from modest beginnings into one of the leading cancer institutes in Europe.

However, to ask such a man as Marcel Dargent to limit his activities to organization and administration would have been an impossible demand. He trained as a surgeon and its practice remained his vocation. He did not accept the popular concept of super-specialization in cancer surgery, but operated just as skilfully whether it was on the larynx, breast or abdomen. Nevertheless, he was not one to ignore the limitations of surgery, and this led him to familiarize himself with complementary and new approaches to cancer treatment, such as hormonal therapy, chemotherapy and immunotherapy.

He initiated a number of projects designed to combine these newly developing forms of therapy with surgery. He made his mark in the field of endocrine ablation therapy in breast cancer by being the first to carry out successfully, and to make a routine of, the portalization of the left adrenal gland. This procedure, which he performed on hundreds of patients with breast cancer, permitted many to enjoy the remission to be expected from standard bilateral adrenalectomy, without the burden of cortisone replacement therapy.

Those who had the privilege to be close to Marcel Dargent when he gave free rein to the flow of memory and fancy, quickly became aware of his cultural breadth. Served by a prodigious memory, a dazzling talent for story-telling and an incomparable sense of humour, he was an ideal companion at meetings and conferences. The pleasure of his companionship between and after the official sessions permitted a welcome escape from the rigours of oncology into the less austère world of witty anecdote and widely ranging speculation. He will be sadly missed both as a fighter and a teacher in the struggle against cancer, and also as a wonderful personality and companion.

Paul Juret

# Preface

The last few years have seen rapid advances in our knowledge of the hypothalamic neurohormones which control anterior pituitary secretion, and also of psychopharmacological agents able either to stimulate or depress the hypothalamic centres. New methods of radioimmunoassay of both polypeptide and steroid hormones have been developed, including the separation of prolactin from growth hormone in the human. This new knowledge has considerable bearing on the endocrine control of mammary cancer in the human.

Endocrine therapy of both experimental and human mammary cancer in the past 25 years has been characterized by a multiplicity of treatment methods, and no one hypothesis has been found satisfactory to explain the mechanism of all the methods used. A major reason for this is that the homeostatic mechanisms of the endocrine system create difficulty in distinguishing between the primary effects of additive or ablative hormonal manipulation and the secondary effects resulting from compensatory changes developing in the other endocrine glands. Furthermore, the evaluation of the cancer response in the human has been clouded by different methods of selecting cases for treatment and by different criteria of response.

Although still at an early stage, it is likely that pharmacological influences on the hypothalamus may simulate the effect of surgical ablation of the pituitary, *but in a selective fashion.* To clarify the possible mechanisms it appeared essential to bring together in one volume, relevant contributions from some research workers responsible for the advances in knowledge mentioned above. The Symposium might point the way to rational and effective therapy of hormone sensitive breast cancer in the human.

Basil A. Stoll

# Contents

PART I

# Endocrine Mechanisms in Normal and Malignant Mammary Growth

# PART I

# Endocrine Mechanisms in Normal and Malignant Mammary Growth

# 1

# Hormonal Factors in Mammary Development and Lactation

A. T. Cowie

This chapter aims to review the relative roles, in laboratory animals and primates, of the anterior pituitary, ovarian and adrenal hormones, and of other factors in the normal growth and development of the mammary glands and in the control of milk secretion.

## MAMMARY DEVELOPMENT

Purified ovarian hormones became available in the 1930s and studies on a variety of laboratory animals soon showed that oestrogens and progesterone had the ability to induce duct growth and lobulo-alveolar development of the mammary gland. In some species oestrogens induced only duct growth while lobulo-alveolar development required a combination of oestrogen and progesterone, whereas in other species oestrogen alone could induce both duct and lobulo-alveolar development. It was then noted that the mammary glands of animals from which the pituitary had been surgically removed showed little or no growth response to the ovarian hormones, and it became apparent that one or more hormones from the anterior pituitary played an essential role in the induction of mammogenic (i.e. mammary growth-stimulating) effects in response to ovarian hormones. In the 1940s purified anterior pituitary hormones became available and Lyons and his colleagues initiated their classical studies on the analysis of the hormonal requirements for mammary duct growth and lobulo-alveolar development in rats; similar detailed studies were later carried out in mice by Nandi.

Attention also became focused on the placenta as an organ affecting mammogenesis when its ability in some species to secrete steroids became

apparent in the 1940s. Over the last 20 years substances having mammotrophic (i.e. both mammogenic and lactogenic) activities have been detected in the placenta of rodents, ruminants and primates. A mammotrophic hormone has been isolated from human placenta and designated human placental lactogen or human chorionic somatomammotrophin.

In the early 1960s much research was centred on the preparation of human pituitary hormones. Attempts to isolate prolactin from primate pituitaries were initially unsuccessful. Human growth hormone, however, showed mammotrophic properties similar to those of ruminant prolactin. The belief grew that in primates the growth hormone with its intrinsic prolactin-like properties served the functions of both the growth hormone and the prolactin of non-primate mammals. However, in 1971 the existence of a separate prolactin was established in both human and monkey pituitaries.

From the above brief review of some of the more important landmarks in the study of the endocrine factors concerned in mammary growth it will be clear that the hormonal mechanisms concerned in mammary growth are complex (see reviews by Folley, 1952; Cowie and Tindal, 1971; Forsyth and Edwards, 1972).

It is now intended to examine selected aspects relative to the control of mammogenesis in the higher mammals and man.

## Mammogenesis in laboratory animals

Much of our information on the role of hormones in mammary growth in rodents derives from the studies of Lyons and of Nandi. Lyons recognized the futility of attempting to analyse the role of the various hormones in mammogenesis by injection of the hormones into intact animals (i.e. into animals whose endocrine glands were already secreting some or all of the hormones under study). He therefore used triply operated (i.e. hypophysectomized, ovariectomized, adrenalectomized) animals, since only when the animal was deprived of the endogenous hormones could interactions between injected and endogenous hormones be avoided, and responses in mammary growth be reliably related to the hormone(s) injected, or to their metabolites.

In triply-operated young rats, Lyons (1958) noted that normal mammary duct growth could be induced with bovine growth hormone + oestrone + adrenal steroids, but the addition of progesterone + sheep prolactin was necessary for lobulo-alveolar development. Such studies indicate that the mammary growth responses in the intact or ovariectomized rat in response to the administration of ovarian steroids are dependent on the presence of endogenous anterior pituitary and adrenal hormones. Subsequently Talwalker and Meites (1961) were able to induce moderate lobulo-alveolar development in triply-operated rats in the absence of ovarian or adrenal steroids by *thrice*

*daily* injections of bovine growth hormone and sheep prolactin. These observations do not negate the role of steroid hormones in normal mammogenesis in rats but they do suggest that the steroids may sensitize the mammary tissue to the action of the pituitary hormones (see p. 11).

Nandi's studies (1958, 1959) revealed slightly different hormonal requirements for mammogenesis in mice. Some duct growth occurred in triply-operated mice in response to a combination of oestrogen and adrenal steroids (i.e. in the absence of growth hormone or prolactin)—a response which does not occur in the triply-operated rat. Nandi further observed that in this strain (C3H/HeCrgl) prolactin was not essential for lobulo-alveolar development although in other strains of mice prolactin was essential (Nandi and Bern, 1960).

Hormones of the thyroid gland play no essential role in mammary growth in the rat since ablation of the thyroid by surgery, or by radio-iodine or by a combination of these techniques did not, in triply-operated rats, affect lobulo-alveolar growth induced with the hormonal complexes already discussed (Chen et al., 1955). However, thyroid hormones may well *modify* the mammary growth response since there is evidence from *in vitro* studies on mouse mammary tissue that when levels of prolactin in the culture medium are optimal for lobulo-alveolar development then the addition of thyroxine inhibits this development; however, if levels of prolactin are suboptimal then thyroxine at low concentrations increases lobulo-alveolar development whereas at higher concentrations it again inhibits development (Singh and Bern, 1969). These observations may offer some explanation of the conflicting reports of the effects of thyroid hormones on mammary growth *in vivo* (for references see Singh and Bern, 1969).

Although the study of hormone administration to hypophysectomized rats and mice has permitted a much better understanding of the hormonal factors concerned in mammary growth by overcoming the difficulties of interaction with endogenous hormones, the technique may introduce other complications. The loss of the pituitary gland depresses a variety of essential metabolic processes which may affect the ability of the mammary gland to respond to exogenous hormones (Jacobson, 1958, 1961). Thus, treatment of the hypophysectomized rat with insulin will allow a limited growth-response of the duct system to steroid hormones, a response which can be enhanced with thyroxine but is counteracted by cortisone. The overall response is, however, small compared with that observed in intact rats.

The above studies have provided much information on the hormonal requirements for experimental mammary growth in rats and mice and undoubtedly reflect normal mechanisms. However, in pregnancy a mammotrophic hormone is known to occur in the placenta of both rats and mice (Cowie and Tindal, 1971) and no doubt supplements or synergises with the hormones from the anterior pituitary.

In the rat there is evidence that full lobulo-alveolar growth of pregnancy is dependent also on an intensified licking of the nipple skin over the mammary glands which is a behavioural characteristic of pregnant rats. If the rat is prevented from licking itself in this way then the normal mammary development of pregnancy is partially inhibited (Roth and Rosenblatt, 1968; McMurtry and Anderson, 1971). There is little doubt that this response is mediated through the release of anterior pituitary hormones. An allied reflexly-induced response is noted later in the non-pregnant human female (see page 7).

Limited attempts to analyse the hormonal requirements for mammary growth by replacement studies have been made in two other species—the rabbit and goat. Norgren (1968) has studied mammogenesis in the hypophysectomized rabbit: while the mammary parenchyma remained partially responsive to ovarian, adrenal and thyroid hormones, with no combination of these was the response comparable to that obtained in the presence of an intact pituitary. A study on a few hypophysectomized–ovariectomized goats by Cowie, Tindal and Yokoyama (1966) suggests that in the goat the ovarian hormones have little or no mammogenic effect in the absence of adenohypophysial or placental hormones.

## Mammogenesis in primates

Speert (1948), in an extensive study on normal and experimental mammary growth in intact and ovariectomized rhesus monkeys, showed that prolonged treatment with oestrogens alone can induce extensive lobulo-alveolar growth. The analysis of the hormonal requirements for developmental mammary growth in hypophysectomized monkeys using replacement therapy with anterior pituitary hormones has not, so far as I am aware, been carried out. Pituitary hormones are most probably involved in mammogenesis since atrophy of the mammary glands occurs in immature monkeys after destruction of the pituitary by deuteron irradiation (Simpson et al., 1959).

The existence of prolactin in the monkey pituitary has recently been established (Friesen et al., 1972a). In the monkey, however, levels of prolactin in the blood are low during pregnancy (Friesen et al., 1972a) suggesting an important role for the placental mammotrophic hormone (which has recently been obtained in a highly purified state by Shome and Friesen, 1971) in stimulating normal mammary growth: indeed, hypophysectomy of the pregnant monkey does not substantially affect mammary lobulo-alveolar development (Agate, 1952).

Information relevant to the endocrine control of mammary growth in man has necessarily to be derived from clinical observations. Oestrogens and progestagens have been widely used in therapeutic regimens for inadequate development in the size of the breasts (e.g., Bishop, 1969). The results of such

therapy tend to be unsatisfactory, which is perhaps hardly surprising since in the non-pregnant woman breast size is likely to depend on the volume of stromal tissue present. In the young woman the mammary stroma is mainly fibrous tissue (Dabelow, 1957; Mayer and Klein, 1961; Sandison, 1962) and little information is available concerning the factors regulating the growth of this tissue. Only recently Paape and Sinha (1971) have made the somewhat unexpected observation that ovariectomy increases the rate of growth of the mammary fat pad in the rat! From reports in the literature on gynaecomastia it appears that the male breast readily increases in size in response to oestrogens (e.g. Bishop, 1969)

Prolactin is now known to occur in the human pituitary (Forsyth and Edwards, 1972; Friesen et al., 1972a) and since both it and human growth hormone are mammotrophic in laboratory animals, both hormones may well act in conjunction with the ovarian hormones in stimulating normal mammary growth. During pregnancy it is possible that placental lactogen plays an important role in lobulo-alveolar development although levels of prolactin in the blood are also high in women during the second half of pregnancy (Friesen et al., 1972a; Tyson et al., 1972b). In women it was noted many years ago that the presence of the ovaries is not essential for mammary growth during pregnancy (Halban, 1905). This is hardly surprising since it is now known that during gestation the placenta is the chief source of oestrogens and progesterone (Hytten and Leitch, 1971).

Numerous reports indicate that the suckling stimulus, if prolonged and repeated, may induce lobulo-alveolar proliferation in the breast and eventually milk secretion in non-pregnant, non-parturient women (Knott, 1907; Foss and Short, 1950; Deanesly and Parkes, 1951; Grishchenko and Grischenko, 1967; Cohen, 1971; Mobbs and Babbage, 1971). No doubt this response is mediated through a neuro-endocrine reflex leading to the release of anterior pituitary hormones. It may be relevant to note that lobulo-alveolar development and lactation can be induced in ovariectomized virgin goats by the repeated application of the milking stimulus and that this response is abolished when the pituitary stalk is surgically transected (Cowie et al., 1968; see also p. 18 for a discussion of the possible role of the cerebral cortex in prolactin release).

## Local mechanisms affecting mammary growth

So far we have discussed the hormones required for mammary growth and we have noted that lobulo-alveolar development, to the extent noted in pregnancy, can be induced experimentally in the virgin rodent by injecting the necessary hormones. There are, however, also local control mechanisms regulating mammary growth. In the cyclic female rodent the mammary duct system just before the onset of oestrus enters a phase of rapid growth when the

ducts are growing at a rate some three to eight times that of the body surface as a whole (Cowie and Tindal, 1971). By the time the animal has reached three months of age the ducts have attained their maximum extension and duct proliferation virtually ceases. This phase of rapid growth is initiated and maintained by oestrogens from the ovary acting in conjunction with pituitary growth hormone; if the ovaries are removed before the onset of oestrous cycles, the phase of rapid proliferation does not occur; that is, the mammary ducts grow but only at the same rate as the body as a whole.

In the intact animal the phase of rapid growth ends at about three months of age although ovarian hormones are still present and the animal is still undergoing regular oestrous cycles. Some mechanism now overrules the hormonal stimulus. Studies in mice by Faulkin and DeOme (1960) have shown that it is the gland stroma which exerts this control and which regulates both the spacing of the ducts and the extent of their growth. In the infantile mouse it is possible to excise the mammary parenchyma from the stromal pad and later to transplant other mammary duct tissue into the empty pad. If a portion of duct tissue is taken from a gland which has attained maximum size (i.e. has ceased to grow) and is transplanted into an empty pad it will again respond to the circulating hormones and will proliferate until it fills the fat pad.

Although the mammary ducts in the virgin rodent appear to fill the fat pad, only a small portion of the total volume of the pad is occupied and the ducts do not touch one another except at their point of origin. It appears that there is a cylinder of stroma around each duct into which adjacent ducts do not enter. Thus the spacing of the ducts seems to be determined by some growth inhibiting factor imparted to the stroma by the existing ducts. Peripheral duct growth ceases at the border of the fat pad beyond which ducts do not grow.

The circulating hormones are thus the 'go' stimulus for mammary growth, but this stimulus can be superseded by a local duct-inhibiting 'stop' signal. When the animal becomes pregant, the hormonal environment changes and this local regulating system also appears to change; ductules and alveoli develop and occupy previously unoccupied interductal spaces until the whole stroma is almost entirely replaced by a tightly packed parenchyma.

Interesting effects of ageing on the mammary parenchyma and its responses to hormones have been noted in serial transplantation studies (Daniel et al., 1968, 1971; Hoshino, 1970; Daniel and Young, 1971) and also in mammary explants in organ culture, but there is little known about the relationship of the hormonal environment to the structural changes which occur in the human mammary gland with advancing age. These structural changes include atrophy of the lobules of parenchyma, a deposition of fat and a hyalinization of the fibrous tissue (see reviews by Geschickter, 1945; Dabelow, 1957).