THE PINEAL GLAND

A Ciba Foundation Symposium

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

CHURCHILL LIVINGSTONE
Edinburgh and London
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Contents

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L. Martini (Chairman)	Istituto di Farmacologica e di Terapia, Università degli Studi, Via Vanvitelli 32, 20129 Milano, Italy
F. Antón-Tay	Instituto de Investigaciones Biomédicas, Universidad Naçional Autonoma, Apartado Postal 70228, Mexico City 20, D.F., Mexico
A. Arstila	Laboratory of Electron Microscopy, University of Turku, Turku 3, Finland
J. Axelrod	Mental Health Intramural Research Programme, National Institute of Mental Health, 9000 Rockville Pike, Bethesda, Maryland 20014, U.S.A.
J. P. Collin	Laboratoire de Biologie Animale, Université de Clermont-Ferrand, Complexe Scientifique des Cézeaux, B.P. No. 45, 63 Aubière, France
E. Dodt	William G. Kerckhoff-Herzforschungsinstitut der Max-Planck-Gesellschaft, 6350 Bad Nauheim, Germany
Virginia M. Fiske	Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts 02181, U.S.A.
F. Fraschini	Istituto de Farmacologia e di Terapia, Università degli Studi, Via Vanvitelli 32, 20129 Milano, Italy
J. Herbert	Department of Anatomy, University of Cambridge
J. A. Kappers	Nederlands Centraal Instituut voor Hersenonderzoek, Ijdijk 28, Amsterdam-O, The Netherlands
D. E. Kelly	Department of Biological Structure, University of Miami, School of Medicine, P.O. Box 875, Biscayne Annex, Miami, Florida 33152, U.S.A.
C. Kordon	Laboratoire d'Histophysiologie, Collège de France, 4 Avenue Gordon-Bennett, Paris 16e, France
A. B. Lerner	Section of Dermatology, School of Medicine, Yale University, New Haven, Connecticut, U.S.A.
B. Mess	Department of Anatomy, University Medical School, Dischka utca 5, Pécs, Hungary
S. M. Milcu	Institutul de Endocrinologie, Academia de Stiinte Medicale, Bulevardui Aviatorilor 34, Bucharest, Rumania
R. Miline	Medicinski fakultet, Institut za histologiju i embriologiju, Novi Sad, Yugoslavia
A. Moszkowska	Laboratoire d'Histophysiologie, Collège de France, 4 Avenue Gordon-Bennett, Paris 16e, France

Marcella Motta	Istituto di Farmacologia e di Terapia, Università degli Studi, Via Vanvitelli 32, 20129 Milano, Italy
I. Nir	Department of Applied Pharmacology, Hebrew University Medical School, Jerusalem, Israel
A. Oksche	Anatomisches Institut, Justus Liebig-Universität, Friedrichstrasse 24, Giessen 63, Germany
C. Owman	Institute of Anatomy and Histology, Department of Anatomy, University of Lund, Biskopsgatan 7, 223 62 Lund, Sweden
Amanda Pellegrino de Iraldi	Instituto de Anatomia General y Embriologia, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Buenos Aires, Republica Argentina
R. J. Reiter	Department of Anatomy, School of Medicine and Dentistry, The University of Rochester, 260 Crittenden Boulevard, Rochester, New York 14620, U.S.A.
H. M. Shein	Department of Psychiatry, Harvard Medical School, Belmont, Massachusetts 02178, U.S.A.
Bertha Singer	Department of Physiology, The Medical School, University of Birmingham, Birmingham, 15
R. J. Wurtman	Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.

Preface

At the Third International Congress of Endocrinology in Mexico City in 1968, Professor R. J. Wurtman approached the Director of the Ciba Foundation, Dr Wolstenholme, to suggest that one of the Foundation's small international symposia should be held to discuss recent progress in research on the pineal gland. There was a certain nobility about this proposal, in that *The Pineal* (Academic Press, 1968), the excellent and comprehensive review by Professor Wurtman, Dr J. Axelrod and Professor D. E. Kelly, was in press at the time. It was already obvious, however, that research on the pineal was going so well that there was much promise of a useful symposium in two years' time. Further talks were held in Stresa, Italy, in 1969 between Professor Wurtman, Professor L. Martini, Professor J. Ariëns Kappers and Dr Wolstenholme, which helped to decide the scope and membership of the Foundation's symposium, and in the event, in June 1970, the wealth of new material with all its interesting potentialities surprised even those most closely involved in this area of research.

The pineal gland may well be about to attract the same limelight as the adrenal cortex some twenty years ago, with an equal impact on the better understanding of human functioning and behaviour, in health and disease.

The editors are indebted to all contributors, particularly those named above, for unstinted and enthusiastic cooperation in the preparation of this book.

The Ciba Foundation

The Ciba Foundation was opened in 1949 to promote international cooperation in medical and chemical research. It owes its existence to the generosity of CIBA Ltd, Basle (now CIBA-GEIGY Ltd), who, recognizing the obstacles to scientific communication created by war, man's natural secretiveness, disciplinary divisions, academic prejudices, distance, and differences of language, decided to set up a philanthropic institution whose aim would be to overcome such barriers. London was chosen as its site for reasons dictated by the special advantages of English charitable trust law (ensuring the independence of its actions), as well as those of language and geography.

The Foundation's house at 41 Portland Place, London, has become well known to workers in many fields of science. Every year the Foundation organizes six to ten three-day symposia and three or four shorter study groups, all of which are published in book form. Many other scientific meetings are held, organized either by the Foundation or by other groups in need of a meeting place. Accommodation is also provided for scientists visiting London, whether or not they are attending a meeting in the house.

The Foundation's many activities are controlled by a small group of distinguished trustees. Within the general framework of biological science, interpreted in its broadest sense, these activities are well summed up by the motto of the Ciba Foundation: *Consocient Gentes*—let the peoples come together.

CHAIRMAN'S INTRODUCTION

PROFESSOR L. MARTINI

The first thing I want to say is that all of us must be very grateful to the Ciba Foundation and to Dr Wolstenholme in particular for having arranged this meeting for us. Secondly, I wish to point out a few peculiar things about the pineal gland.

The first peculiarity is that scientists have not been really interested in this gland until very recently. I believe one of the reasons why this happened is that the pineal gland has interested the philosophers for a long time; it is possible that endocrinologists do not have much respect for philosophers. Another possible reason is that animals normally survive pinealectomy (provided the surgeon is a good one); this puts the pineal gland in a kind of second rank among endocrine structures. In addition, the effects of pinealectomy are apparent for some time, but later on pinealectomized animals recover and become perfectly normal again.

A second peculiarity of the pineal gland is that you cannot bring back a pinealectomized animal to its normal status by administering systemically crude pineal extracts or the more refined compounds which have recently been isolated from the gland. Transplants of pineal tissue are also ineffective (Reiter and Fraschini 1969). The reason why you cannot reverse the effects of pinealectomy by transplanting the pineal gland is now apparent. Thanks to studies which will be reviewed at this meeting we now know that the pineal gland has a peculiar sympathetic innervation which is essential to its function; a transplanted pineal gland cannot be re-innervated in the same way (Wurtman, Axelrod and Kelly 1968). This brings me to a third peculiarity of the pineal gland: this gland is located in strict contact with the brain; however, its innervation does not originate from the nervous structures which are close to the gland.

Another peculiarity of the pineal gland is the fact that the receptors sensitive to its hormones are localized almost exclusively in the brain. Published evidence for a nervous site of action of pineal hormones includes (*a*) the studies by Wurtman and his co-workers (Antón-Tay and Wurtman 1969) indicating that exogenous melatonin is concentrated by nervous structures; (*b*) the observation that melatonin may modify some biochemical processes taking place in the brain (as, for instance, the metabolism of serotonin) (Antón-Tay *et al.* 1968); and (*c*) the data obtained in my

laboratory showing that brain implants of melatonin, but not intrapituitary ones, may reduce gonadotropin secretion (Fraschini, Mess and Martini 1968). I am sure that additional data along these lines will be presented at this meeting.

The final peculiar thing about the pineal gland is indicated by the type of scientists who have become interested in it. A general pattern is usually seen in science. A scientist normally wants to make a reputation for himself and, in order to do so, he picks up a respected topic and devotes himself fully to it. Exactly the reverse happened in the case of the pineal gland. A group of distinguished and already recognized scientists decided to make this gland fully and, we hope, definitely respectable.

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THE PINEAL ORGAN: AN INTRODUCTION

J. ARIËNS KAPPERS

The Netherlands Central Institute for Brain Research, Amsterdam

RESEARCH on the pineal organ in various vertebrates has now so much intensified that it is impossible to offer a comprehensive survey of all its different aspects as an introduction to this symposium. In the present paper, therefore, I shall deal with a limited number of subjects, drawing your attention to some problems still to be solved.

First and foremost I shall deal with the phylogenetic development of the pineal. I am sure that Dr Collin and Professor Oksche will give more details (see pp. 79 and 127).

Far from being a new acquisition in mammals, the pineal has a long and interesting evolution during which it shows a striking transformation in structure, function and innervation pattern. It is increasingly clear, however, that the way in which the mammalian pineal functions has very old phylogenetic roots.

PINEAL ORGAN IN ANAMNIOTES

In fishes and amphibians the development and structure of the epiphysis is, in principle, very similar to that of the retina of the eye, although some differences exist (Kappers 1965). In the epiphysial epithelium three cell types can be distinguished: (1) neurosensory photoreceptor cells of a ciliary type, (2) supportive elements which may show certain specializations, and (3) sensory nerve cells. All the cells mentioned derive from the embryonic neuroepithelium which forms, in every vertebrate, the primary anlage of the organ.

The pineal photoreceptor elements are neurosensory or primary sensory cells, not neurons. They are sensitive to photic stimuli and convey the transduced photic impulse to the next element, the sensory neuron. The outer segment of the photoreceptor cell, which shows a complicated structure, is sensitive to photic stimuli and can be termed the photic pole of the cell. The basal process of the cell is directed toward the basement membrane of the pineal epithelium. Its terminal ending which, in fishes and amphibians, does not generally reach the basement membrane, is in

synaptic contact with either the dendrites or the soma of the intraepithelial sensory nerve cells. These neurons are the homologues of the large nerve cells of the inner layer of the retina of the eye, the axons of which constitute the optic nerve. In the pineal, the axons of these cells constitute the pineal tract which runs to the epithalamic region of the brain. The synaptic ending of the basal process of the photoreceptor cell contains clear vesicles (Oksche and Vaupel-von Harnack 1963 and Ueck 1968*a*, *b* in anuran amphibians; Rüdeberg 1966, 1968, 1969 in fish), but often also synaptic ribbons or "vesicle-crowned rodlets" (e.g. Collin 1969*b* in lamprey; Kelly 1967 in newt). The synaptic ribbons recall similar organelles present in the presynaptic endings of photoreceptor cells in the retina.

After reaching the epithalamus the sensory pineal fibres mostly join those of the posterior commissure, spreading in a lateral and ventral direction (Kappers 1965 in fish; Ueck 1968*b* in anuran amphibians). It is uncertain whether some join the fibres of the habenular commissure. It is a serious handicap to our understanding of the function of this direct photosensory pineal pathway in lower vertebrates that the site of termination of these fibres in the brain is not known with certainty (see Kappers 1965 for references).

In summary, it appears that the direct photosensory function of the organ depends on two types of cells which are synaptically connected, namely the neurosensory photoreceptor cell and the sensory neuron. Evidently, this function will be impaired if the photoreceptor cell loses its photosensitivity or if the nerve cells are lost. Loss of the nerve cells is a very crucial point because the basal processes of the photoreceptor cells are not connected directly with the brain.

The direct photosensitivity of the pineal of fishes and amphibians has also been demonstrated electrophysiologically. It was shown (Morita 1966*a*) that the constant train of impulses fired normally by the pineal nerve cells of trout decreases when the organ is illuminated. A sustained discharge of action potentials was also recorded from the pineal stalk of anuran amphibians in the dark. This activity was likewise inhibited by direct illumination (Morita 1965; Morita and Dodt 1965). For details of the light response of the anuran pineal, see the papers by Dodt and Jacobson (1963), Dodt and Morita (1964) and Morita (1969).

Formerly, it was generally held that the pineal of fishes and amphibians has a photosensory function only, but there is increasing evidence for a second, secretory function. Dense-cored vesicles, measuring about 100 nm (1000 Å) in diameter, have been observed to originate in the Golgi zone of the photoreceptor cells and to accumulate in the basal process (Rüdeberg 1969 in dogfish; Collin 1969*b*, *c* in lamprey; Oksche and Vaupel-von

Harnack 1963, Ueck 1968*a*, *b* and Charlton 1968 in anuran amphibians). Their chemical composition is so far unknown. ^{14}C-labelled 5-hydroxytryptamine and methyl methionine, precursors of melatonin, were shown to be selectively incorporated in the epiphysis of *Xenopus* (Charlton 1964, 1966*b*) and the enzyme hydroxyindole-*O*-methyltransferase was also demonstrated in the amphibian pineal region (Axelrod, Quay and Baker 1965; Quay 1965). This is indirect proof of the synthesis of melatonin, which was also directly demonstrated in the *Xenopus* pineal by a special fluorescence technique (Van de Veerdonk 1965, 1967; Balemans and Van de Veerdonk 1967; Balemans, Van de Veerdonk and Van de Kamer 1967) after an earlier failure to demonstrate 5-hydroxy- and 5-methoxyindoles in the pineal of *Hyla* (Eakin, Quay and Westfall 1963) by spectrofluorometry. On the ground of comparative structural and functional considerations it can be assumed that the compound is produced by the photoreceptor elements which therefore show both a photosensory and a secretory function.

The uptake of labelled precursors of melatonin by the pineal of *Xenopus* depends upon the colour of the background on which the animals are kept after injection (Charlton 1966*b*), while the organ may directly control the primary response to colour change in eyeless *Xenopus* (Charlton 1966*a*). This suggests that production of melatonin by the photoreceptor cells may depend on photic stimuli directly received by these same cells. Much work has been done on pigment regulation in lower vertebrates in relation to the function of the pineal (see Bagnara 1965 and Kappers 1969*b* for references).

No difference was found in thyroid and interrenal gland activity in pinealectomized goldfish from that of controls (Peter 1968), while the gonadosomatic index also remained unchanged. Recently, however, melatonin was identified by thin-layer chromatography in the pineal of the Pacific salmon (Fenwick 1970). The amount stored in the pineal was approximately six times as great in immature salmon as in mature fish, suggesting that the pineal melatonin store is related to gonadal function. Intraperitoneal injections of melatonin into goldfish inhibited the increase in gonadal size which accompanied increased daily light exposure in those animals which received placebo injections. The melatonin-treated fish also showed larger pituitary gonadotropic cells. This points to a light-dependent effect of pineal melatonin on the hypophysio-gonadal axis in a fish.

Because, in the mammalian pineal, the function of the pinealocytes is known to depend, at least partly, on the sympathetic innervation of the organ, it is of interest whether the pineal of anamniotes also shows such an

innervation. Oksche and Vaupel-von Harnack (1963, 1965*a*) postulated the autonomic origin of unmyelinated fibres containing dense-cored vesicles and running in the pineal tract as well as in the pineal perivascular spaces in *Rana*. Similar fibres were demonstrated by Ueck (1968*a*) in the perivascular spaces and in the epithelium of anuran pineals. No membrane thickenings pointing to true synaptic contacts between terminals of autonomic fibres and photoreceptor cells were observed. By fluorescence histochemistry, pinealo-petal catecholamine-containing nerve fibres have also been described in *Bufo* (Iturizza 1967). Neither the exact origin nor the function of these fibres is, as yet, known.

PINEAL ORGAN IN REPTILES

Among reptiles, lacertilians and turtles show an interesting evolutionary transformation of the pineal organ. The lacertilian pineal photoreceptor cell rarely shows a normally developed outer segment (Oksche and Kirschstein 1968; Wartenberg and Baumgarten 1968; Petit 1969*a*; Collin 1967*b*, 1969*b*). Many are rudimentarily developed or modified by disorganization or disintegration. In general, the basal processes are not in synaptic contact with sensory neurons, but end on the basement membrane of the epithelium. The number of sensory nerve cells is decreased (Kappers 1967). They can be demonstrated only with difficulty in electron micrographs (Petit 1969*a*; Collin 1969*b*). Accordingly, the number of sensory fibres running in the pineal tract is also reduced (see Kappers 1967 and Petit 1969*a* for their interepithalamic course).

Notwithstanding the tendency to regression of the photosensory apparatus of the lacertilian pineal, electrophysiological investigations have corroborated the morphological evidence that this function is not altogether lost. The lizard pineal, for example, shows spontaneous electrical activity which is inhibited by illumination of the organ. Increasing illumination causes increasing inhibition until there is a complete cessation of firing for the duration of the stimulus (Hamasaki and Dodt 1969).

The well-developed Golgi zone of the chief cells produces dense-cored vesicles of about 100 nm (1000 Å) in diameter (see e.g. Collin 1967*b*, 1969*b*; Collin and Kappers 1968; Petit 1969*a*). Small and larger clear vesicles have also been observed. The vesicles migrate to the basal processes of the cells and their content is released into the pericapillary spaces, while the capillary endothelium can be fenestrated (Petit 1969*a*; Wartenberg and Baumgarten 1969*a*). These facts suggest a secretory function of the chief pineal cells, which have been termed secretory rudimentary photoreceptor cells by Collin.

Indoleamine, probably 5-hydroxytryptamine (5-HT), was histochemically demonstrated in the pineal of *Lacerta* (Collin 1967*a*, 1969*a*), the reaction being strongest in those cell zones in which the dense-cored vesicles are observed. By fluorescence histochemistry a yellow compound, probably 5-HT, was also demonstrated (Kappers 1967; Quay, Jongkind and Kappers 1967; Collin 1968*a*, 1969*b*; Wartenberg and Baumgarten 1969*a*). Fluorescence is not always localized in granules but more often diffusely spread throughout the cell. Possibly there are two pools of 5-HT in the pinealocytes, an intravesicular, relatively stable one which is resistant to reserpine treatment, and an easily available extravesicular pool (Wartenberg and Baumgarten 1969*a*). 5-HT has also been demonstrated biochemically in the lacertilian pineal (Quay and Wilhoft 1964) as well as a considerable amount of hydroxyindole-O-methyltransferase (Quay 1965). All these facts suggest the synthesis of melatonin in the lizard pineal.

The pineal pericapillary spaces contain some, probably sensory myelinated nerve fibres (Collin and Kappers 1968) and bundles of unmyelinated fibres the endings of which contain the three types of vesicles characteristic of noradrenergic nerve terminals (Collin and Kappers 1968; Oksche and Kirschstein 1968; Wartenberg and Baumgarten 1968, 1969*b;* Petit 1969*a*). Autonomic nerve endings in the pineal epithelium are rare and they do not make true synaptic contacts with the secretory cells (Collin and Kappers 1968; Wartenberg and Baumgarten 1969*b*). As the exact function of the sympathetic innervation is not known, it can only be surmised that it is involved in the regulation of the production and/or the excretion of pineal cell compounds.

In principle, the pineal of Chelonia (turtles) shows the same structural features (Vivien 1964*b*; Lutz and Collin 1967; Mehring 1970; Vivien and Roels 1967, 1968; Vivien-Roels 1969; Collin 1969*b*). The outer segments of the chief cells are rudimentarily developed and the basal processes either reach the basement membrane of the pineal epithelium, showing a so-called vascular polarity, or end on the membranes limiting the intercellular spaces which are extensions of the pericapillary spaces (Mehring 1970). No secretory granules were observed in *Testudo hermanni* during the winter (Lutz and Collin 1967) but they are present in animals living under conditions of artificial lighting (Mehring 1970). Seasonal variations in secretory activity may, therefore, be present. In most chelonians pineal secretory activity is obvious. Endothelial pores can be present (Vivien-Roels 1969).

Features pointing to a direct photosensory function, such as sensory nerve cells, some rare synaptic contacts between basal processes and nerve cells (Vivien-Roels 1969), and a pineal tract are still present but vary according to species. In *Testudo hermanni* the sensory neurons show signs

of degeneration in the adult, as do their axons which are in part myelinated (Mehring 1970). In *Pseudemys*, the tract is better developed than in *Testudo*.

Adrenergic fibres and their endings are present in the perivascular spaces as well as within the pineal epithelium (Mehring 1970). The intraepithelial endings form simple appositional contacts with the secretory cells. In summary, it appears that the pineal of Chelonia, like that of Lacertilia, shows a varying tendency to regression of its direct photosensory function, a distinct secretory function and a sympathetic innervation.

In Crocodilia even the anlage of the pineal organ is missing. In Ophidia (snakes) the epiphysis is solid and parenchymatous. In *Tropidonotus*, the Golgi zone of the chief cells produces secretory granules which are emptied into the pericapillary spaces (Petit 1969*b*). Sympathetic fibres run in these spaces as well as in the pineal parenchyma. Here they show close appositional contacts with the basement membrane as well as with the basal processes of the secretory chief cells (Vivien 1964*a*, 1965). The observation of green-fluorescing nerve fibres (Quay, Kappers and Jongkind 1968) corroborates the findings of sympathetic fibres by other methods. So far, no clear proof of a pineal photosensory function has been found in snakes.

A distinct development of ergastoplasm and Golgi zone in *Tropidonotus* pinealocytes and a nearly complete disappearance of secretory granules, by comparison with normal animals, was observed in adults injected with chorionic gonadotropin (Vivien 1965). The secretory as well as the excretory function of the cells would be stimulated by the injections. The presence of a pinealo-petal nerve tract in *Tropidonotus* running along the stalk and originating from cells in the hypendyma of the subcommissural organ (Petit 1969*b*) needs corroboration.

PINEAL ORGAN IN BIRDS

Although the structure of the avian pineal varies widely, most authors agree that the outer segments of the chief cells are rudimentary, regressed or disintegrated (Oksche and Vaupel-von Harnack 1965*b*, 1966; Collin 1966*a*, *b*, 1967*c*, 1969*a*, *b*; Oksche and Kirschstein 1969; Bischoff 1969; Oksche, Morita and Vaupel-von Harnack 1969; Ueck 1970). The basal process reaches the basement membrane of the epithelium and may invaginate into the pericapillary space. Here it is sometimes devoid of its basement membrane covering (Collin 1969*a*, *b*). Again, secretory granules measuring 70–100 nm (700–1000 Å) in diameter and originating in the Golgi zone accumulate in the terminal buds of the basal processes. Synaptic ribbons have been observed in these buds and in the somata of the

cells but they are not associated with synaptic contacts (Collin 1968*b*, 1969*a*, *b*; Ueck 1970).

Pineal tract fibres have been found (unpublished observation by the present author; Quay and Renzoni 1963, 1967; Oksche and Kirschstein 1969; Oksche, Morita and Vaupel-von Harnack 1969; Ueck 1970), while rare nerve cells and synaptic junctions between receptor elements and nerve cells have been demonstrated by electron microscopy in *Passer* (Ueck 1970). In some birds at least, a rudimentary pineal photosensory function still exists, although the photic poles of the chief cells are practically absent. In the basal part of the pineal, which is continuous with the stalk, sustained, low-amplitude spikes can be recorded which are not influenced by light (Morita 1966*b*, and Oksche, Morita and Vaupel-von Harnack 1969 in the pigeon; Ralph and Dawson 1968 in *Coturnix* and *Passer*). Pineal electrical activity could not be elicited either by direct illumination of the organ or by photic stimulation of the eyes. Nevertheless the avian pineal plays a role in the effect exerted by light on the reproductive system, as will be shown later.

The avian organ is innervated by noradrenergic fibres (Bischoff and Richter 1966; Gonzalez and Hidalgo 1966; Collin 1969*a*, *b*, also for references; Oksche and Kirschstein 1969; Oksche, Morita and Vaupel-von Harnack 1969; Hedlund 1970; Ueck 1970). Nerve fibre trunks extend dorsally along the venous sinuses and pierce the pineal capsule to enter the organ (Quay and Renzoni 1963; Oksche and Kirschstein 1969). Some rare adrenergic fibres were observed in the nerve bundle accompanying the pineal stalk (Oksche and Kirschstein 1969). The fibres, which have also been demonstrated by fluorescence histochemistry, run in the interlobular connective tissue strands along the blood vessels in the perivascular spaces. They either penetrate the basement membrane of the epithelium to enter the parenchymal compartment (Bischoff and Richter 1966; Gonzalez and Hidalgo 1966; Collin 1969*a*, *b*; Hedlund 1970) or not (Ueck 1970; Hedlund 1970), probably according to species. Bilateral superior cervical ganglionectomy proved that the avian sympathetic fibres originate primarily if not exclusively in these ganglia (Hedlund 1970).

A yellow fluorescence (Fuxe and Ljunggren 1965; Hedlund 1970; Ueck 1970) pointing to the presence of 5-HT is equally distributed over the entire parenchyma and does not seem to be bound specifically to granular material (Ueck 1970). Considerable amounts of 5-HT as well as other hydroxy- and methoxyindoles have been found biochemically in the pigeon pineal (Quay 1966). A daily rhythm in pineal 5-HT content can be triggered partly by light and darkness although its regulation appears to be basically endogenous. Hydroxyindole-*O*-methyltransferase activity is at least 200 times higher in the chick than in the rat pineal, but, in contrast to

the condition in the rat pineal, this activity increases in light and decreases in darkness (Axelrod and Wurtman 1964). Neither bilateral enucleation of the eyes nor pineal sympathetic denervation prevented the elevation of enzyme activity induced by light (Lauber, Boyd and Axelrod 1968). Evidently, in contrast to the mammalian pineal gland, neither the retinas nor an intact pineal sympathetic innervation are essential for environmental control of melatonin production in the chick pineal. This casts some doubt on the regulation of biochemical processes in avian pinealocytes by the sympathetic system, or at least on its exclusive role in this regulation. It has been suggested that the thin avian skull allows light to penetrate and reach the organ directly. This idea is supported by recent experiments in the canary (Munns 1970). It was demonstrated that birds in constant light without an opaque covering of the pineal region by a black polyester resin layer had more than a two-fold elevation in pineal HIOMT activity when compared to birds either in constant darkness or with opaque coverings over the pineal region. Moreover, these birds showed reduced spermatogenesis. It may be that, in birds, the chief pineal cells are directly affected by light, the photic stimuli regulating the synthesis of compounds present in the same cells. In contrast, the functioning of the mammalian pinealocyte is regulated by light transmitted by way of the eyes and the sympathetic innervation.

That the sympathetic pineal innervation in birds is not without any function, however, follows from the observation that bilateral superior ganglionectomy in quail is followed by a decrease in egg production (McFarland, Homma and Wilson 1968; Sayler and Wolfson 1968). After a time the birds regain their preoperative level of egg production. Bilateral ganglionectomy in immature quail results in a delay of the onset of egg laying. Both ganglionectomy and pinealectomy delay the onset of laying and both effects are transitory (Sayler and Wolfson 1967). Evidently, pineal sympathetic innervation in quail is somehow involved in the functioning of the reproductive system, but whether photic stimuli are transmitted to the organ by this innervation is not clear from these experiments. Although a number of papers claim that the avian pineal is involved in the reproductive process, pinealectomy was found ineffective in preventing gonadal inhibition or atrophy in immature and in mature quail in non-stimulatory photoperiods (Arrington, Ringer and Wolford 1969). The rate of sexual maturation of 8-week-old females given stimulatory photoperiods was not affected by pinealectomy and there was only a trend in the results indicating that pinealectomy reduced or delayed gonadal atrophy in sexually mature males when put under non-stimulatory photoperiods—that is, in increasing periods of darkness. It may be that pinealectomy or

pineal denervation exerts an influence on the reproductive system only in normal illumination or in stimulatory photoperiods.

For a discussion of the circadian rhythm and photoperiodism in connexion with extraretinal light perception in birds, as postulated by Menaker, see a paper by Oksche and Kirschstein (1969) and the contribution by Professor Oksche to this symposium (p. 127).

PINEAL ORGAN IN MAMMALS

In the pineal organ of mammals the specific cell is the pinealocyte which, in its adult state, shows no vestiges of an outer segment. Remnants of a ciliary apparatus may, however, be present. The cells show one or more long processes which end in terminal buds. For an excellent survey of the microscopic anatomy and cytology of the mammalian pineal, see Bargmann's (1943) extensive paper (see also Kappers 1969*a*).

Although the pineal gland of mammals is part of the brain it does not show either afferent or efferent nervous connexions with the brain proper. Fibres of the habenula and posterior commissure may intermingle with the pineal parenchyma but, so far, it has not been demonstrated by anatomical or by physiological methods that they play a functional role in pineal innervation.

It is agreed that the mammalian pineal is exclusively innervated by the peripheral autonomic nervous system (Kappers 1960, 1965, also for references; Kenny 1965) which forms a rich network of fibres, associated with lemmocytes, except in a few species (Owman, 1965). In most mammals these fibres are sympathetic postganglionic fibres originating in the superior cervical ganglia. This means that the gland is an end organ of the peripheral sympathetic system, which is of great consequence for its function.

The fibres can enter the organ by two ways: (1) all along its surface, either in association with the pineal blood vessels or not, and (2) by way of the nervi conarii. It follows that total denervation of the gland can only be achieved by bilateral superior ganglionectomy and not by cutting the nervi conarii alone, if this were technically feasible.

In the macaque monkey, preganglionic parasympathetic fibres coursing in the greater superior petrosal nerves enter the pineal and synapse with intrapineal nerve cells (Kenny 1961). This may mean that parasympathetic cholinergic fibres contribute considerably to the pineal innervation in primates.

Intrapineal nerve cells (autonomic intramural ganglion cells) constantly occur in the pineals of primates including man. They have also been

observed in the pineals of many non-primates (see Kappers 1960, 1965 for references). Recently, such cells were demonstrated in the rabbit pineal by my co-worker H. Romijn (unpublished) and by Trueman and Herbert (1970) in the ferret pineal. The latter authors showed that these cells did not contain monoamines but that acetylcholinesterase was present. Arvy (1961) was probably the first to observe a few acetylcholinesterase-positive fibres in the pineal of cattle, sheep and pig. They have also been demonstrated in the rat pineal (Kappers, unpublished; Machado and Lemos 1970). Penetrating from the capsule into the gland in 36–48-hour-old rats, they form a network associated with pineal vessels. Fibres were also seen in close topographical relationship with pinealocytes. All these fibres disappear after bilateral superior ganglionectomy, which suggests their sympathetic origin (Machado and Lemos 1970).

So far, acetylcholinesterase-positive pineal nerve fibres, which are probably cholinergic, have been detected only by histochemical methods and light microscopy. In electron micrographs some nerve endings of fibres running in the pericapillary spaces of the rabbit pineal show exclusively accumulations of clear vesicles measuring about 50 nm (500 Å) in diameter and some few dense-cored vesicles of the large type while, on the other hand, most endings show small clear as well as dense-cored vesicles next to large dense-cored vesicles (H. Romijn, unpublished). Possibly the endings containing small clear vesicles exclusively belong to the acetylcholinesterase-positive fibres. Further electron microscopic studies using different fixation methods are needed to prove this hypothesis. In view of the experimental evidence of a parasympathetic innervation of the monkey pineal mentioned above, it would be especially worthwhile to look for cholinergic fibres in the pineals of primates.

Autonomic fibre bundles coursing in the pericapillary spaces and in the pineal parenchyma can be readily observed by electron microscopy. The latter bundles can reach the parenchyma in two ways: (1) by leaving the perivascular space and penetrating the basement membrane of the pineal parenchyma which is also the external limiting membrane of that space, or (2) directly, without having first accompanied the vessels. Penetration of fibres from the perivascular space into the parenchyma is supposed to occur rather often although it has been demonstrated only once in a mammalian pineal (Wartenberg 1968). A much larger number of sympathetic fibres can certainly reach the parenchyma directly by the second way—that is, either along the nervi conarii or along the entire surface of the gland.

Sympathetic fibres coursing in the pericapillary spaces often end in these spaces. The endings show the usual characteristics of noradrenergic fibres.

As the structure and function of varicosities occurring along the preterminal part of such fibres is quite similar to that of their terminals, some of the structures hitherto described as nerve endings may have been varicosities.

The neurotransmitter, most probably noradrenaline, released from these varicosities and endings will stimulate the pinealocytes by diffusing through the basement membrane lining the pineal parenchyma. The neurotransmitter can reach the terminal buds even more easily if they are invaginated into the pericapillary space and devoid of their basement membrane covering. This mode of transmission of autonomic impulses is rather similar to that generally occurring in the stimulation of, for instance, smooth muscle cells by autonomic fibres. In that case also the fibre terminals are often separated by a considerable distance and a basement membrane from their effector cells.

The intraparenchymal sympathetic fibres end in close appositional contact with processes of pinealocytes and their terminal buds but never on the somata of these cells. The nature of this contact was first dealt with by Wolfe (1965; for a discussion on this subject see also Kappers 1969*a*). In general, no morphological characteristics of true synaptic junctions are observed. True synaptic contacts between endings of noradrenergic intraparenchymal fibres and pinealocytes have been illustrated only by Wartenberg (see Kelly 1967) and by the present author (Kappers 1969*a*) in, respectively, the cat and the rat pineal. Here, pre- and postsynaptic membrane thickenings, a subsynaptic web and electron-dense material in the synaptic cleft were present.

Considering that these are terminals of sympathetic postganglionic fibres which, in general, never form true synaptic contacts with their effector cells, it is rather remarkable that rare synaptic junctions of this type have been observed at all in the pineal gland. The transmitter released at intraparenchymal nerve endings can certainly reach the pinealocytes quicker by diffusion than the transmitter released at the endings in the pericapillary spaces because, in the first case, no basement membrane is interposed between these endings and the effector cells. In the exceptional cases in which true synaptic contacts occur, a still quicker and more specifically directed impulse transmission seems warranted. It should be mentioned that, in some cases, rather intimate contacts between axons and smooth muscle cells have been demonstrated by some earlier authors (see Kappers 1964 for references, and Taxi 1965). Recently, membrane specializations have been observed in certain neuromuscular contacts in the vas deferens of the rat (Ivanov 1970). From this it would appear that also in other systems innervated by autonomic fibres, more or less specialized neuroeffector contacts do occur.

The mammalian pinealocyte is a secretory cell which produces, stores and excretes pineal-specific compounds. Storage and possibly also part of the production of secretory substances occurs in the terminal buds of the pinealocyte processes. They contain dense-cored vesicles, varying in number according to species (see Pellegrino de Iraldi 1969, also for references and the nature of their content), clear vesicles and much larger spaces which are membrane-bound and may be part of the smooth endoplasmic reticulum. We shall hear more about these organelles in Dr Arstila's paper (pp. 147–164). Most often the buds terminate either on the basement membrane of the pineal parenchyma or on intercellular spaces which communicate with the pericapillary spaces.

In some species processes of gliocytes intervene between the terminal buds and the basement membrane (Wartenberg 1968) while in others the buds may invaginate into the pericapillary space. In the latter case the bud may even lose its basement membrane covering, then hanging naked in the pericapillary space, as has also been described in birds. As in some Sauropsida the intercellular space sometimes shows an electron-dense content, indicating the presence of extruded secretory products. Extrusion of the content of granules present in the buds has been demonstrated in the rabbit pineal (Leonhardt 1967; H. Romijn, unpublished). In some mammals, such as the rabbit, the pericapillary spaces form long extensions in the parenchyma. These extensions are narrow clefts containing bundles of collagenous fibres. Terminal buds of pinealocytes end on these clefts and it can be assumed that they are receptacula for the pineal secretory substances extruded into them.

In rat (Milofsky 1957) and in mouse (Ito and Matsushima 1968) the pineal capillary endothelium is distinctly fenestrated. If no pores seem to be present, the endothelial wall is often extremely thin locally.

All the structural features mentioned suggest that the pinealocytes extrude their products into the blood circulating in the capillaries via the basement membrane of the pineal parenchyma, the pericapillary spaces, the basement membrane covering the capillary endothelium, and the endothelial wall. In the parenchyma extrusion occurs also into intercellular spaces which are in open communication with the pericapillary spaces.

FUNCTIONAL EVOLUTION OF THE PINEAL

Some general conclusions on the structure, function and innervation of the pineal can be drawn on the basis of its phylogenetic development already described.

(1) The mammalian pinealocyte is phylogenetically derived from the neurosensory photoreceptor element present in the pineal of anamniotes. It is not a modified nerve cell. During its evolution this cell loses its outer segment or photoreceptive pole while its synaptic pole also disappears. Likewise, the nervous apparatus conducting the transduced photic stimuli from the photoreceptor cells to the brain is gradually lost. This means that the pineal loses its capacity to convey photic stimuli to the brain. If some intraepithelial pineal sensory nerve cells are left in any given pineal it can be assumed that this type of direct pineal photosensory function is still intact, however restricted. Very early in pineal phylogeny signs are found of a secretory function of the chief pineal cells. It is quite probable that, in one and the same cell type, a photosensory and a secretory function are combined. Gradually the chief pineal cell loses its photoreceptive capacity and becomes preponderantly a secretory element which, however, possibly remains directly photosensitive or becomes indirectly photosensitive by a circuitous route, via the retinas and a complicated neural pathway to be mentioned later. The transformation described is most clearly shown in the pineal of Sauropsida. In one pineal epithelium the chief cells may show different gradations of the process of transformation, but, in principle, there is only one single cell type. A clear distinction between two basically different types of chief pineal cells cannot be made.

(2) Alongside the gradual loss of the pinealo-fugal sensory innervation pattern there is an increasing development of an autonomic pinealo-petal motor innervation pattern. Already distinct in some fishes and amphibians and very evident in Sauropsida, this type of innervation pattern is the only one present in the mammalian pineal. As is known, the sympathetic fibres innervating the mammalian pineal mediate (among other things) photic stimuli to the organ. It would be reasonable to accept that, because the autonomic pineal innervation regulates the photo-dependent synthesis of compounds in the mammalian pinealocyte, the same will be true for the pineal photoreceptor elements and the secretory rudimentary photoreceptor cells in non-mammalian vertebrates. On the ground of some of the observations cited, however, it cannot be excluded that in non-mammals the production and/or the excretion of pineal substances may be regulated by photic impulses directly received by the same cell which produces the pineal secretory substances. In these cases, the chief pineal cells are directly photosensitive although they may have lost their photoreceptor capacity. As we have seen in birds this does not mean that the sympathetic innervation is of no consequence for the regulation of reproductive processes via the pineal but probably only that in non-mammals

also, other, non-photic impulses may be conveyed to the pineal via its sympathetic innervation which influence its function.

(3) In the phylogenetic development of the autonomic innervation pattern of the pineal a distinct trend toward an ever more extensive and efficient stimulation of the chief cells by autonomic fibres can be observed. Three stages can be distinguished.

Stage (*a*): invasion of the organ by postganglionic fibres along the perivascular spaces. At this stage the fibres end exclusively within these spaces and do not contact chief pineal cells, which can only be reached by the transmitter released at the varicosities and endings of the fibres by diffusion through the basement membrane of the pineal epithelium.

Stage (*b*): the sympathetic fibres may leave the perivascular spaces to enter the pineal cell compartment by penetrating its basement membrane. Appositional contacts with chief cells are made by the endings. In this way a somewhat quicker impulse transmission by diffusion is realized than at stage (*a*).

Stage (*c*): the pineal is invaded by postganglionic fibres entering the organ not by way of the perivascular spaces but via the nervi conarii and all along the surface of the pineal. These fibres can distribute directly in the pineal parenchyma. Their endings make close appositional contacts with the secretory pinealocytes and, in rare cases, even well-developed true synaptic junctions are formed. Preganglionic fibres may also enter the organ, synapsing with intramural autonomic nerve cells which give rise to postganglionic fibres distributing in the pineal parenchyma.

The sympathetic innervation pattern of stage (*a*) is the simplest and theoretically the phylogenetically earliest one. It is possibly realized in lower fishes, as can be seen in an illustration of the structure of a lamprey pineal by Collin (1969*a*). In amphibians and Sauropsida both stages (*a*) and (*b*) are realized. Stage (*c*), side by side with stages (*a*) and (*b*), occurs in the mammalian pineal.

The transformation of photoreceptor cells into pinealocytes is traceable not only in the phylogenetic development of the pineal; it is also evident during its ontogenetic development in mammals. Cilia-bearing bulges of cytoplasm extending into the lumen of the pineal anlage in foetal hamsters and rats recall the rudimentary development of outer segments (Clabough 1970). In adult pinealocytes synaptic ribbons are commonly observed, as are remnants of a ciliary apparatus. The function of the synaptic ribbons is still open to discussion (see Kappers 1969*a*, also for references).

Considering the embryonic neuroepithelial origin of the mammalian pinealocyte, its secretory function, the excretion of its products into the general circulation, and its sensitivity to indirect photic stimulation, the