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The Mammalian Pineal Gland, a Survey

By

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With 5 Figures

Introduction

During the past 10–15 years many fascinating investigations have been performed on the structure, innervation, biochemistry and function of the mammalian pineal gland. Although, especially as far as pineal function is concerned, our present knowledge is not absolutely satisfying in detail, it can be stated that the pineal gland, far from being an insignificant phylogenetic relic, is a most active organ synthesising a number of compounds. Its secretory products, released into the systemic circulation, exert an effect on several endocrine organs, most probably via the hypothalamo-pituitary system. Because its secretory products are synthesised in organ-typical cells, the pinealocytes, and conveyed to their target cells by the blood, and because the organ, as far as its specific cells are concerned, is ontogenetically derived from neural epithelium, the pineal gland is an endocrine organ of neural origin. It should not be termed a neuro-endocrine organ because the synthesis of its secretory products does not happen in neurones but in pinealocytes. These cell elements are quite different in structure and function from neurones although they also originate from the neuro-epithelium.

The present paper gives a survey of the phylogenetic and ontogenetic development, the structure, innervation, biochemistry and function of the mammalian pineal gland, and the factors regulating its function will also be considered. As the literature on the subjects mentioned has been growing tremendously during the last years, it has been necessary not only to make a somewhat personal choice from the

many data obtained, but also to summarize them shortly, generalizing in such a way that they are of relevance in view of the special aim for which this paper is written.

Historical Data

The organ has been termed corpus pineale, glandula pinealis or pineapple gland because, especially in man, it shows the shape of a pineapple or pine cone. In the ancient Greek literature the term "konareion" (cone-shaped) occurs. This was later latinized to "conarium", a term which still lives in the name for the nerves of the gland, the conary nerves. In the West, the epiphysis cerebri, as the organ is also called, was first described by Herophilos of Alexandria (325–280 B. C.) who held that the organ would function as a tap regulating the stream of "spiritus" from the third to the fourth brain ventricles. This opinion was connected to the idea, developed at those times, that the brain ventricles and their contents are basic in regulating the somatic and psychic functions of man. Twenty centuries later this same view was extensively elaborated in a more refined physico-mechanistic way by Descartes (1596–1650), still a firm believer in several kinds of spiritus containing particles of different size and function. As is generally known, this philosopher, physicist and mathematician regarded the epiphysis as the seat of the soul, and more especially as the seat of imagination and consciousness, while he also thought that the organ would play an important role in memory (see Kappers 1954, 1965a, also for literature). After the 17th century, especially when Stensen (1638–1686) had refuted Descartes' hypothesis on the all-important role of the organ and Kant had argued that, in principle, no organ could be indicated as the seat of the soul, further study was completely neglected. Interest revived in the 19th century along with the development of comparative anatomy. The structure of the pineal organ in lower vertebrates was then investigated but interest in the mammalian epiphysis remained slight.

In 1910 a future professor of physiology in Amsterdam wrote a doctorate thesis on the function of the hypophysis. He concluded that the mammalian hypophysis is probably the rudiment of an organ which might have been of functional significance in earlier phylogenetic stages but had lost all importance in man and other mammals. Until recently, and even still now, the opinion in the medical profession on the importance of the pineal gland was and is not very different from that just cited about the hypophysis in 1910. Truly, pineal concrements form a welcome point of orientation for the radioneurologist and the neurologist, neurosurgeons and neuropathologists know of the

existence, symptomatology and structure of various types of pineal tumours, but some earlier opinions about the function of the organ and its medical implications seem to have been entirely forgotten or thought unworthy of serious consideration.

As early as 1898 Heubner described a boy suffering from a pinealoma and showing symptoms of premature puberty, while Marburg in 1913 and 1930 named this special syndrome—characterized by pinealoma, premature development of the primary and secondary sex organs and occurrence mainly in boys—pubertas praecox or genitosomia praecox. According to Marburg, pubertas praecox could be caused by inadequate function of the pineal gland, or hypopinealism, due to pineal degeneration or to destruction of the specific pineal parenchyma. This author was already of the opinion that the epiphysis is an endocrine gland which, in youth, would exercise an inhibiting influence on the hypothalamus and, via this part of the diencephalon, on the development and function of the gonads. Any failure of this inhibiting pineal effect would cause pubertas praecox. This at that time still unproven theory explains why the epiphysis was called "Keuschheitsdrüse" or "chastity gland" by German authors of those days. It was also maintained that hyperpinealism could occur and, by hyperfunction of the gland, cause an excessively slow and eventually incomplete development of the sex glands, giving general adiposity as a secondary symptom. Berblinger (1926, 1930) also attributed an inhibiting action on the gonads to the pineal while Engel (1936) was of the opinion that pineal extracts contained an antigonadotropic hormone. Jöchle (1956), experimenting with such an extract, termed glanepin, stated that the epiphysis probably contained an antigonadotropic as well as a gonadotropic factor.

Once it became known that hypothalamic centres are involved in the regulation of sexual development and function the above-mentioned theories met with much criticism. Disturbances of the reproductive system were not thought to be due to the nature of pineal tumours, but to the pressure exerted by them on these hypothalamic centres or to hypothalamic hyperplastic malformations such as hamartomas which are often found in the region of the tuber cinereum and are sometimes associated with precocious puberty (Bustamente 1942, Bustamente *et al.* 1942, Lange-Cosack 1951, Spatz 1951, 1953, 1955, Schmidt *et al.* 1958).

A most important new impulse for carrying out endocrinological and other research on the pineal gland was given by Kitay and Altschule in 1954. These authors critically surveyed the previous work on the organ. They concluded that of all effects attributed to the gland that on the reproductive organs was statistically the best founded.

Pineal Morphology

Phylogenetic Development

In submammalian Vertebrates the pineal and accessory pineal organs, such as the parapineal organ in fishes, the frontal organ in anuran amphibians, and the parietal eye of lizards, are, for the most part, vesicular, saccular or tubular epithalamic structures. Their epithelium contains neurosensory photoreceptor cells, sensory nerve cells and supporting elements. Photic stimuli directly reach the epithelium of these organs which are in a superficial position on or just beneath the roof of the skull in the midline between the lateral eyes. Light is perceived by the photoreceptor cells which transduce these stimuli. The axons of the photoreceptor elements synapse with the intraepithelial sensory nerve cells which convey the impulses, by way of their axons which form a nerve bundle, to the brain.

In frogs and fishes axons originating in the pineal complex have been shown to run to the dorsal thalamus, the habenular ganglion, the pretectal region, the optic tectum, the subcommissural organ, and the mesencephalic periventricular grey matter (Paul *et al.* 1971, Zimmermann and Paul 1972, Hafeez and Zerihun 1974) which suggests that this complex has an important function. The conduction of transduced photic stimuli by the pinealo-fugal nerve bundle has also been proved by a number of experimental neurophysiological investigations in amphibians and reptiles which have been extensively surveyed by Dodt (1973). It appears that, *i. a.*, photic stimuli inhibit the spontaneous firing of the pineal sensory nerve cells and that there are achromatic and chromatic responses. About the exact function of the photoreceptor activities of the pineal and accessory pineal organs in the physiology and behaviour of lower Vertebrates more theories than facts, so far, exist.

Next to a direct photosensory function, the pineal of submammals has a secretory activity. The amphibian pineal, for instance, contains melatonin and the enzymes that synthetize this indoleamine (Axelrod *et al.* 1965, Van de Veerdonk 1967). Melatonin causes a contraction of the pigment granules of the melanophores in frogs and fish, administration of this compound rapidly blanching their skins. The same effect is obtained by adding rat pineal to the water in which a frog larva is swimming, an experiment which is easily performed. As we will see later, melatonin is also produced by the mammalian pineal gland. In mammals, however, this compound does not affect skin melanophores.

During phylogenetic development the structure of the pineal photoreceptor cells which, ultrastructurally, resembles that of the rods in the retinas of the lateral eyes, changes. The cell organelles, involved

in direct photoperception, are gradually lost and the photoreceptor cells change to what have been called by Collin (1971) rudimentary secretory photoreceptor cells which synthesise indoleamines. Along with ultrastructural changes in the cells their function becomes exclusively secretory. As the photosensory function of these cells is lost they can no longer convey photic stimuli to the brain via pineal sensory neurones, which means that these become redundant and disappear. This phylogenetic developmental process is best studied in lizards and in some families of birds in which intact photoreceptor cells are found next to rudimentary photoreceptor elements while the numbers of sensory nerve cells are decreased. This process is fully completed in snakes and in some families of birds the pineals of which no longer contain true photoreceptor cells or sensory neurones. When sensory nerve cells are entirely lacking in the pineal, pinealo-fugal nerve fibres, which are the axons of these elements, are also absent. Instead of a pinealo-fugal innervation, a pinealo-petal one consisting of peripheral autonomic nerve fibres now comes to the fore.

In the mammalian pineal gland neither true photoreceptor cells nor sensory nerve cells are found. In most mammals the adult pineal is a parenchymatous organ which contains pinealocytes with an exclusively secretory function, next to some other cell types to be mentioned later, and which is exclusively innervated by the peripheral autonomic nervous system. It will be shown that the function of the mammalian pineal gland is influenced via its autonomic innervation by light so that the organ is indirectly photosensitive in contrast to the pineal in many submammalian Vertebrates which is directly photosensitive by way of its neurosensory photoreceptor cells.

Ontogenetic Development

As in submammalian Vertebrates, the primary pineal anlage in mammals is formed by an evagination of the neuro-epithelium constituting that part of the roof of the diencephalon which is situated between the habenular commissure rostrally and the posterior commissure caudally. The organ, therefore, is of epithalamic origin. In the rat (Kappers 1960), its further ontogenetic development is simple. The neuro-epithelial matrix layer forming the wall of the saccular anlage proliferates giving rise to lobules and pseudofollicles which may, for some time, contain a lumen derived from the originally relatively large pineal recess of the third ventricle. Soon the embryonic mesenchyme surrounding the pineal anlage produces connective tissue strands and blood vessels growing in between the cell cords and pseudofollicles of neuro-epithelial derivation. Along with these, vessels, fibro-

blasts and other mesenchymal elements such as lemmoblasts, and sometimes mast cells and plasma cells, move into the anlage of the organ which becomes ensheathed by a leptomeningeal covering. At birth the rat pineal gland is a massive parenchymatous organ which is well vascularized, while the pineal recess has become very small. During the first postnatal weeks the rat epiphysis grows in a caudal and a dorsal direction, its connection with the roof of the third ventricle between the posterior and habenular commissures only consisting of a very thin pineal stalk. The tip of the organ is in close topographical connection with the floor of the confluens sinuum. In the hamster the adult pineal consists of two parts. A proximal part is situated deeply, keeping its direct connection with the roof of the third ventricle, while the other, distal, part is comparable with the rat pineal gland lying more dorso-caudally in a superficial position. The two parts are connected by a thin strand of pineal tissue like the rat pineal stalk.

In other mammals, such as rabbit and man, the adult organ is formed by two more or less separate anlagen. A first, saccular, one which develops in the way described for the rat pineal, is, in rabbit, covered dorsally and in part laterally by and fuses with a solid second anlage which arises in the habenular region from the neuro-epithelium of the diencephalic roof just in front of the first anlage (Turkewitsch 1937). In man the solid anterior anlage invades the posterior anlage which is also solid (Hülsemann 1971). Further differentiation of the organ is similar to that in mammals showing a single pineal anlage.

In the rat, pineal autonomic innervation by peripheral fibres develops during the first postnatal weeks while that in man occurs in the 5th embryonic month (Hülsemann 1971).

Topography of the Adult Organ

In man the cone-shaped pineal gland is a small solid organ which is still closely connected with, and forms, part of the epithalamic roof (Fig. 1). Its rostral and dorsal end merges with the habenular commissure while its caudal and deeper tapering part continues into the subcommissural organ and the posterior commissure. Most of the organ is situated in the septum interpositum, a space filled with connective tissue of pia-arachnoidal origin just dorsal to the roof of the third ventricle, in which many vessels, especially veins, run. The gland contains a small pineal recess, a third ventricle evagination which is the most proximal non-obliterated part of the originally much larger lumen of the pineal anlage. The tip of the organ is situated just dorsal to the pretectal region in front of the superior colliculi of the quadrigeminal lamina.

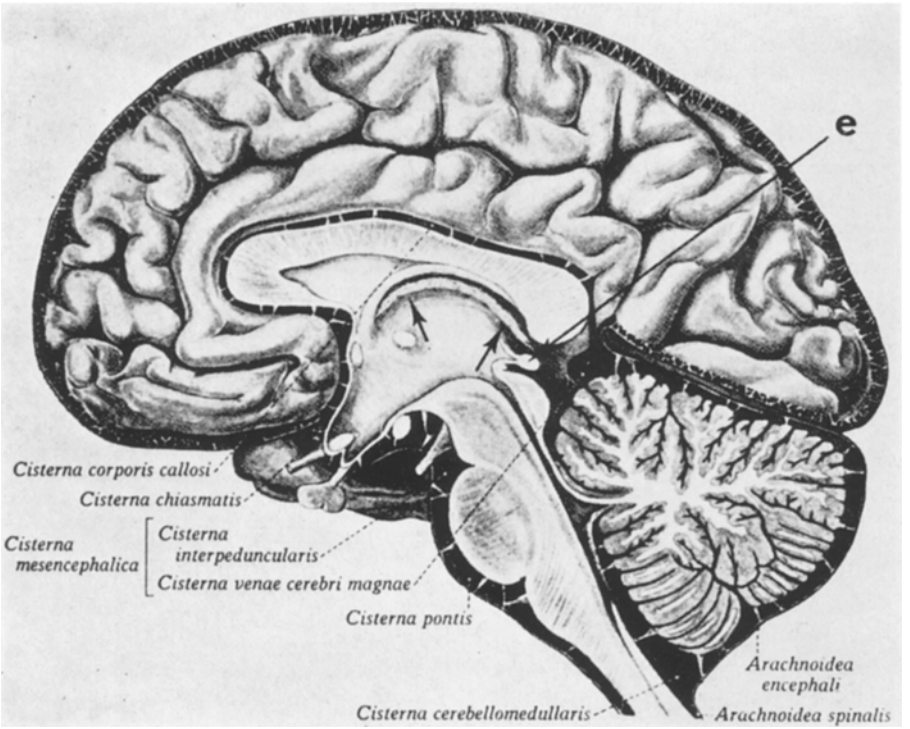

Fig. 1. Topography of the pineal gland (*e*) in man. The arrows indicate the position of the velum interpositum which is rich in cerebral veins. [From M. W. Woerdeman (1950), Atlas of Human Anatomy, II. Wetensch. Uitg., Amsterdam; Plate 459, slightly altered]

In some mammals, such as the rat and the rabbit, part of the dorsal surface of the gland is covered by the so-called suprapineal recess of the third ventricle. In the rat the tip of the organ lies in close contact with the floor of the confluence of the sinuses while in the rabbit the pineal invaginates into the confluence and even into either the left or the right transverse sinus. In these animals the gland is closely surrounded by large cerebral veins such as the internal cerebral veins, the great cerebral vein, and the deep dorsal cerebral vein.

Microscopic Anatomy and Cytology

In mammals the pineal gland shows a great variability in size, shape, and differentiation. In most species in which the structure and function of the organ has been investigated it is a well vascularized

parenchymatous structure, constituted of cell strands or cords, still showing a pseudofollicular arrangement, which are incompletely separated by stromal septa containing arterioles and venules. In the present review only those light and electron microscopical data which seem to be of special relevance will be mentioned. Due to the fact that the human pineal gland cannot be fixed as fresh as is necessary for electron microscopical examination, the ultrastructural data are mostly derived from subhuman mammals.

The characteristic cell element of the pineal parenchyma is the *pinealocyte*. Its perikaryon contains a nucleus which is either spherical or oval and may also be lobulated or indented. In the latter case the nuclear membrane shows deep infoldings, the cytoplasm seemingly penetrating the nucleus. In earlier days this situation gave rise to a wrong concept of nuclear secretion. The perikaryon contains rough, granular and smooth endoplasmic reticulum. Because, at least in some mammals such as the rat, the association of ribosomes with the membranes constituting the plasmatic reticulum is not so close and frequent in the cytoplasm of pinealocytes as it is in other cell types, this endoplasmic reticulum has been termed a mixed or intergrade reticulum (Wolfe 1965). In the pinealocytes of the rabbit, however, a well-developed rough next to a smooth endoplasmic reticulum can be distinguished. The perikaryon contains some extensive Golgi complexes and numerous mitochondria. From the perikaryon extend one or two processes which may be long. Their endings form terminal buds which, for the most part, show close topographical relationships with the outer membranes of the pericapillary spaces or the membranes of the intraparenchymal lacunae which, in fact, are extensions of the pericapillary spaces. According to the species of mammal investigated the terminal buds of the processes may be either separated by thin glial processes from these membranes, they may be in direct contact with them, or they may even penetrate into the pericapillary spaces, either being covered by the outer membrane bounding the spaces or hanging freely into them. These terminal buds contain much smooth endoplasmic reticulum, which may be either tubular or vesicular, and dense-core granules which are produced by the Golgi complexes in the perikaryon and which have migrated into the terminal buds. These buds also contain mitochondria.

Besides the cell organelles mentioned, synaptic ribbons or vesicle-crowned rodlets which are situated close to the cell membranes, acanthosomes or spiny bodies, and so-called multivesicular bodies also occur in pinealocytes. Vesicle-crowned rodlets were first demonstrated in the photoreceptor cells of the retina. They have also been observed in the photoreceptor elements present in the frontal and pineal organs

of amphibians as well as in the pineal rudimentary photoreceptor cells of other submammalian vertebrates. In general, they are found in several types of receptor elements (see Wartenberg 1968, also for references). This corroborates the view that the mammalian pinealocyte is phylogenetically derived from a neurosensory element. The exact function of this organelle is not known, but it has been supposed that it is involved in impulse transmission from one pinealocyte to another.

In some species a small number of pinealocytes contain ciliar structures. Although these have been observed in several cell types of ectodermal origin, and even in nerve cells, their presence in pinealocytes may point to the derivation of these cells from photoreceptor elements.

It is generally held that the secretory products of the pinealocyte are contained in the granular vesicles produced by the Golgi complexes in the perikaryon and in the clear vesicles originating from the smooth endoplasmic reticulum, abundantly present in the terminal buds of the cell processes to which the dense core vesicles migrate. Extrusion of the content of these vesicles has been best described and illustrated by Leonhardt (1967) in the rat pineal gland. These products are released into the pericapillary spaces and into the parenchymal lacunae. It has also been observed that experimental conditions which are known to influence pineal function, such as sympathectomy and continuous illumination of the animal, are accompanied by alterations in the production of dense-core vesicles by the Golgi complexes and in the structure of the endoplasmic reticulum in the terminal buds of the processes of rabbit pinealocytes (Romijn 1975).

The secretory products, once released from the terminal buds mentioned into the pericapillary spaces by penetrating the outer limiting membranes of these spaces, reach the capillary blood via the pericapillary basement membrane which is the inner lining membrane of the pericapillary space, and the capillary endothelium which, in many mammals, is fenestrated. The pericapillary spaces in the mammalian pineal gland vary in width, but are often rather wide. They contain collagenous fibrils, fibrocytes, single autonomic fibres or bundles of such nerve fibres, and autonomic nerve terminals.

Besides the organ specific pinealocytes, the mammalian pineal gland contains varying amounts of *glial cells* of the fibrous astrocytic type which are characterized by large bundles of neurofilaments of indeterminate length. Evidently these fibrous astrocytes originate, as do pinealocytes, from the embryonic neuro-epithelium constituting the pineal anlage. Some cytoplasmic glial cells have also been described in mammalian pineals. The amount of astroglia may vary considerably according to the species investigated. The same holds for the topo-

graphical relationship between the glial processes and the terminal buds at the outer border of the pericapillary spaces. Pineal glial elements have sometimes rather confusingly been termed "interstitial cells". *Lemmocytes* or Schwann cells showing long nuclei accompany unmyelinated nerve fibres and are also present in bundles of nerve fibres which are seldom myelinated.

Furthermore, the mammalian pineal gland may contain cells of mesenchymal origin such as varying numbers of *fibrocytes*, *plasma cells*, and *mast cells*, which are topographically related to the connective tissue strands. As has already been mentioned, fibrocytes also occur in the pericapillary spaces. In the mammalian pineal gland the presence of some few single *striated muscle fibres* has occasionally been observed (Quay 1959, Kappers 1960). Their function, if any, is unknown.

The presence of intrapineal *autonomic nerve cells* will be dealt with in connection with pineal innervation.

As is well-known, cysts, plaques, and acervuli can be often observed in the pineal gland. We will deal shortly with these formations here.

Pineal cysts may be either primary or secondary in origin. The first category can be explained by the persistence of remnants of the original lumen of the pineal anlage, more specifically remnants of the lumina of the pseudofollicles. Secondary cysts are probably formed in plaques.

Pineal plaques are not formed by pinealocytes, the characteristic secretory pineal cells, but by glial elements. They show all the characteristics of glial plaques. The number of pineal glial cells is known to increase somewhat with advancing age.

Acervuli, also called psammomal bodies, corpora arenacea, or brain sand, are calcareous deposits varying in number in the pineal gland, but also present in other parts of the brain, such as the habenular commissure, the choroid plexus, and other tissues. For the röntgenologist pineal corpora arenacea, if not too small, are of importance for localizing the organ which can then be used as a point of reference in relation to other structures. They have been regarded as a sign of pineal atrophy and involution in man, while they have also been related to brain disease. As our review of the epiphysis cerebri is not concerned with neuropathology, only the following points which we quote from Quay's survey (1974) will be mentioned here.

Corpora arenacea are not found in the pineal organ of all mammalian species. They have, for instance, never been observed in the laboratory rat, carnivores, insectivores, or primates excluding man. They are, however, present in the pineals of ungulates. It is generally known that their occurrence in man increases with age, but that there is a great individual variation in their number at all ages. It is of im-

portance that the site of origin of these corpora is primarily glial and stromal, the brain sand not being formed within pinealocytes. They may contain calcium, magnesium, phosphorus and iron while their primary microcrystalline structure resembles that of hydroxyapatite.

Much has been speculated on the causes of their origin and their relations, if any, with neuropathological symptoms and with endocrine as well as other diseases. In general it can be said that no unequivocal proof has been observed for such a direct relation. It is also noteworthy that no correlation was observed between pineal enzyme activity levels and pineal calcification. On the contrary it has, for instance, been found that the activity of the enzyme hydroxyindole-0-methyltransferase, which is involved in the synthesis of melatonin, an important pineal biogenic amine, from serotonin, is scarcely lower in the aged human than it is in the young (Wurtman *et al.* 1964). This agrees with the localization of these deposits in stromal cells and glial elements, but not in pinealocytes.

Sometimes corpora arenacea resemble amyloid bodies and amyloid-like plaques which have also been described in aged human pineal organs but which have been rarely reported in laboratory animals. The structure and histochemistry of corpora amylacea in the pineal and in other parts of the brain have been specially studied by Quay (1971) in the orangutan.

Vascularization

The vascular supply of the pineal gland is from branches of the posterior cerebral arteries which penetrate the organ all over its surface. In the gland the arterioles run in connective tissue septa between the cell cords, but the capillaries form an extensive network within the pineal parenchyma. Arterioles and capillaries are surrounded by perivascular spaces which have been dealt with earlier in this paper. Pericapillary spaces are also characteristic of other endocrine systems. The cappillary blood is collected in venules and veins which drain directly into the large internal cranial veins with which the gland is topographically closely related, such as the internal cerebral veins and the great cerebral vein. In rabbits the distal part of the pineal may even protrude into either the right or the left transverse sinus, its pia-arachnoidal envelope then being immediately surrounded by venous blood.

It should, therefore, be noted that the pineal venous blood reaches the systemic blood circulation directly. There is no kind of portal vascular system by which venous pineal blood, containing the pineal secretory products, could reach special brain centres.

For a proper understanding of pineal function the following facts should be noted: (1) the characteristic pineal cell, the pinealocyte, shows all the characteristics of a secretory cell; (2) most probably the secretory products are released from the terminal buds of the pinealocytic processes into the blood of the extensive parenchymal capillary network via the pericapillary spaces and the intraparenchymal lacunae which are extensions of these spaces; (3) the pineal venous blood, containing the secretory products, is drained directly into the systemic circulation; (4) the organ is enveloped by a pia-arachnoidal sheath and, sometimes, by a glial layer; (5) the suprapineal recess of the third ventricle which, in some mammalian species, covers part of the dorsal surface of the pineal gland, is lined by ependymal cells which are nowhere in immediate contact with pineal parenchyma, pia-arachnoidal tissue being situated between the ependymal wall of this recess and the surface of the gland; (6) the wall of the pineal recess of the third ventricle, if present, consists of non-pineal specific ependymal cells which, at least in the adult stage, are not in immediate contact with the processes of pinealocytes. From the above it can be deduced that pineal secretory products cannot reach the cerebrospinal fluid directly but are released into the systemic blood circulation. This, of course, does not absolutely exclude the possibility that injection of pineal secretory products into the ventricular system could be of functional significance.

Innervation

Although a part of the central nervous system by origin, the adult pineal gland receives an afferent innervation from peripheral autonomic nerve fibres, while no nerve fibres originate from it and run to the brain. Most of the autonomic pineal afferent innervation is from postganglionic nordrenergic nerve fibres originating in the superior cervical ganglia and reaching the organ in two somewhat different ways. Many fibres, which are mostly unmyelinated, enter the gland along the vessels penetrating over the entire surface of the organ. These, and also fibre bundles consisting of sympathetic fibres, run in the perivascular and pericapillary spaces where many of them terminate, while some may enter the pineal parenchyma bordering the pericapillary spaces. Postganglionic sympathetic fibres reach the gland also by way of two bilateral symmetrical nervi conarii which, in some mammalian species such as man, may fuse before entering the pineal gland at its caudal pole. The nervi conarii run through the tentorium cerebelli and may contain both myelinated and unmyelinated fibres. These nerves branch extensively within the pineal parenchyma to terminate between the processes of the pinealocytes (Figs. 2, 3). Some fibres may also

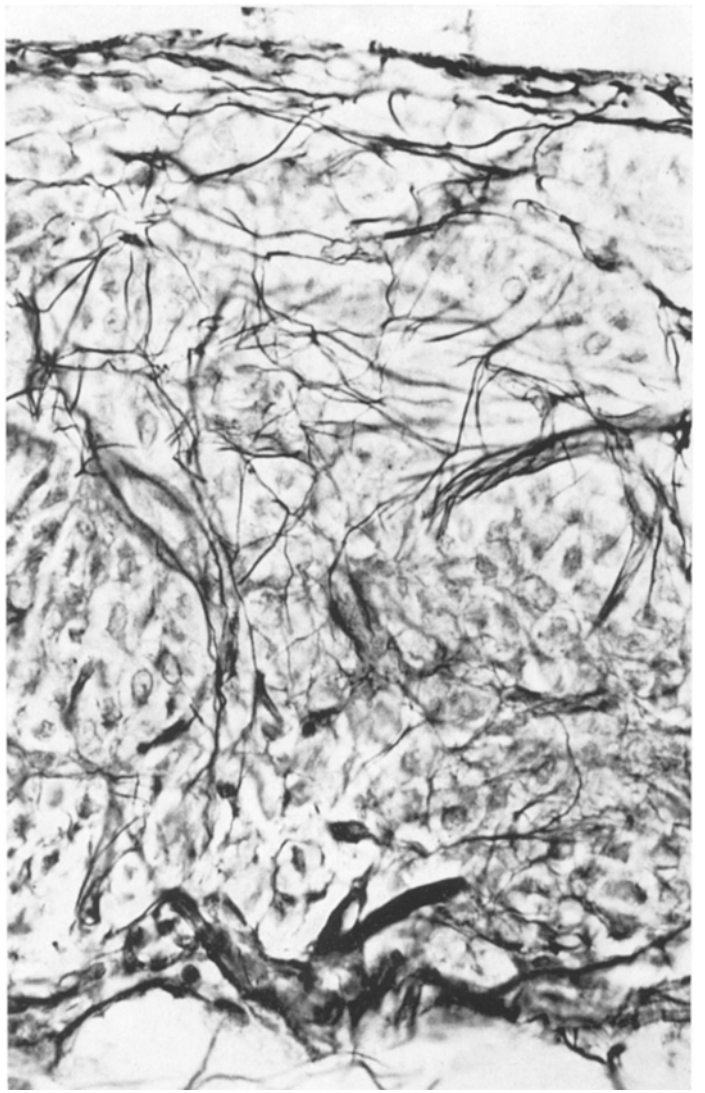

Fig. 2. Sympathetic innervation of the rabbit pineal gland. Bodian staining. ×500. (By courtesy of Dr. H. J. Romijn)

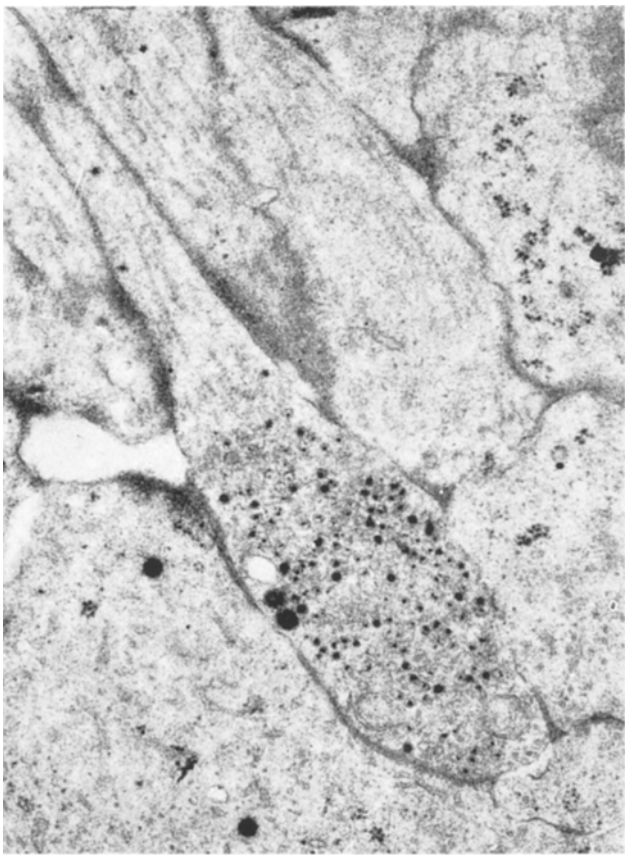

Fig. 3. Rabbit pineal gland. Ending of noradrenergic nerve fibre, characterized by many small and a few larger dense-core vesicles containing noradrenaline and dopamine, respectively. The ending does not form synaptic junctions with adjacent pinealocytes, lying freely in the pineal parenchyma. ×27,620. (By courtesy of Dr. H. J. Romijn)

enter the pericapillary spaces from the side of the parenchyma. Conclusive experimental evidence for the presence of a pineal sympathetic innervation, which had been observed by Cajal (1911) in the mouse, was obtained by removal of both superior cervical ganglia in the rat. This was followed by the disappearance of this type of innervation in the gland (Kappers 1960, 1965b, also for references to earlier literature).

By light and electron microscopy, by enzyme histochemistry, and experimentally, Romijn (1972, 1973a, b, 1975) demonstrated in rab-

bits that the pineal gland is not only innervated by sympathetic but also by parasympathetic fibres. Earlier, a parasympathetic innervation of the macaque epiphysis was briefly described by Kenny (1961, 1965, see for other earlier literature Romijn 1975). The following description of pineal parasympathetic innervation is based on data obtained by Kenny and, more fully, by Romijn.

Preganglionic acetylcholinergic fibres, probably originating in the superior salivary nuclei, leave the brain stem with the facial nerves to run with the greater superior petrosal nerves. Some of these fibres synapse with postganglionic parasympathetic nerve cells situated along these nerves, often forming small ganglia The postganglionic cholinergic parasympathetic fibres originating in these cells reach the pineal gland by way of the conary nerves, and probably also with the blood vessels to the surface of the gland. Other preganglionic parasympathetic fibres, however, reach the organ, some at least with the conary nerves, to synapse either with the perikarya or with the dendrites of intramural pineal postganglionic parasympathetic nerve cells. As in other organs, the intramural position of these cells can be explained by ontogenetic migration in a peripheral direction toward and even into the organ which is to be innervated by them. In this connection it is interesting that in some species a juxtamural pineal ganglion has been observed at the posterior pole of the organ (ganglion of Pastori). Its cells have also been supposed to be parasympathetic. The postganglionic acetylcholinergic parasympathetic nerve fibres originating from the intrapineal nerve cells, the presence of which had earlier been demonstrated by light microscopy also in primates including man (Bargmann 1943), terminate in the pineal parenchyma, while some fibres penetrate into the pericapillary spaces from the pineal parenchyma. The terminal endings of these postganglionic cholinergic parasympathetic nerve fibres contain the characteristic clear "synaptic" vesicles while those of the sympathetic postganglionic noradrenergic fibres as well as their preterminal varicosities show the typical dense-core or granular vesicles. Often sympathetic and parasympathetic nerve fibres run in the same nerve fibre bundle while some terminal endings containing dense-core noradrenergic vesicles exclusively have been observed to synapting the dendrites of intramural pineal postganglionic nerve cells next to contact terminal endings containing only clear acetylcholinergic vesicles. This agrees with modern opinions, on which we cannot dwell here, about the functional cooperation between the sympathetic and the parasympathetic system.

It is of interest that both free cholinergic and noradrenergic nerve terminals have been observed in the pineal parenchyma between the processes of the pinealocytes. In contrast to our earlier opinion (Kap-

pers 1969, 1971) it would now seem that endings of postganglionic fibres do not form true synaptic junctions with the perikarya of pinealocytes or their processes. The rare synapses earlier observed and interpreted as such were probably synapses between postganglionic nerve endings and dendrites of intramural postganglionic parasympathetic cells. It is now considered most likely that the sympathetic and parasympathetic nerve terminals form so-called "synapses à distance" which means that the neurotransmitter substance released at these terminals reaches the effector cells, *i.e.* the pinealocytes, by way of diffusion along a shorter or longer distance, not by specialized synaptic junctions.

Autoradiographically, Wolfe *et al.* (1962) demonstrated that dense-core vesicles in the terminal endings of the sympathetic nerve fibres do, indeed, contain noradrenaline as a neurotransmitter. Besides this compound these vesicles not infrequently contain serotonin, which has probably been taken up from the pinealocytes which, in many mammalian species, contain an abundant amount of this indoleamine (see later). However, these dense core vesicles may also contain endogenously synthetized serotonin (see Kappers 1969, for references). The parasympathetic nerve fibres and their terminals are acetylcholinesterase-positive as has been shown by Romijn (l. c.).

Before it was finally demonstrated that pineal innervation is by the peripheral autonomic nervous system, the opinion was held that nerve fibres originating in the habenular nuclei reached and innervated the gland. It is true that some habenular commissural fibres as well as posterior commissural fibres may penetrate into the proximal part of the pineal organ. Most of these fibres, however, form hairpin loops, heterolaterally returning to the commissural system to which they belong. Obviously these fibres are aberrant commissural nerve fibres (Kappers 1960, 1965b, Kenny 1965, Romijn 1972, Smith 1972). David and Herbert (1973) observed degenerative changes in axon terminals ending on intramural pineal nerve cells in the ferret, after making lesions in the habenular nuclei. So far this pinealo-petal innervation by fibres originating in the habenular nuclei has not been observed in other species.

As will be shown below, the sympathetic innervation of the pineal gland is of the utmost importance for its function. The role of the parasympathetic innervation of the organ has still to be elucidated.

Pineal Chemistry

For an excellent and comprehensive survey on pineal biochemistry we may refer to Quay (1974). In the present paper only some of the many investigations on pineal chemistry which seem of special im-

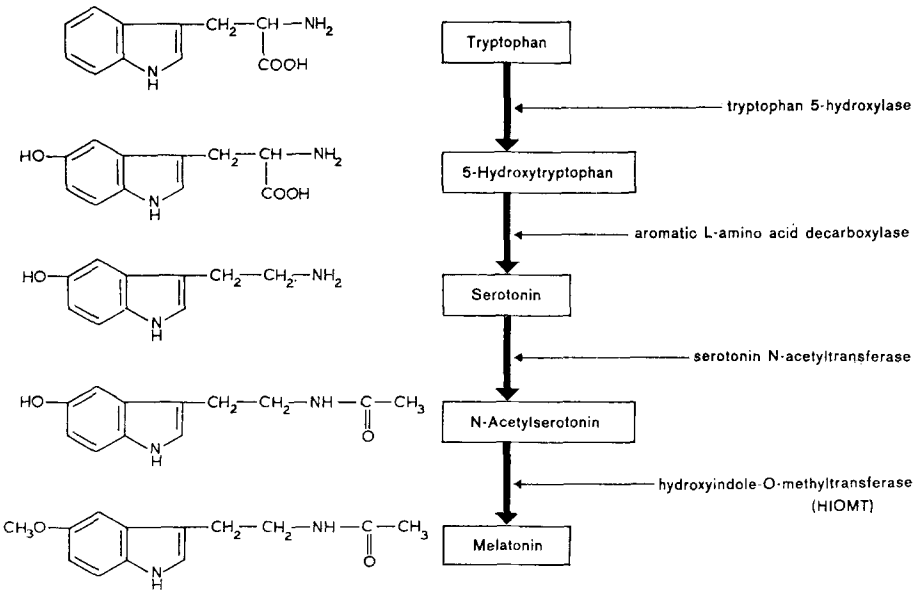

Fig. 4. Biosynthesis of melatonin from tryptophan in the pineal gland. [From H. J. Romijn (1972); Fig. 1]

portance in relation to the function of the organ can be mentioned shortly.

In general it has been demonstrated that the pineal gland shows a high substance and energy metabolism. The organ contains a number of biogenic amines, catecholamines as well as indoleamines. The most important catecholamine is noradrenaline which is found particularly in the sympathetic nerve fibres innervating the organ. Using the Falck-Hillarp histochemical fluorescence technique and its modifications, these fibres show a green fluorescence, characteristic of primary catecholamines, which disappears after denervation of the gland by bilateral superior ganglionectomy. Indole compounds are numerous, including 5-hydroxy and 5-methoxy indoles. In the pineal gland of many mammalian species, the pinealocytes contain 5-hydroxy-tryptamine (5-HT) or serotonin which shows a yellow fluorescence demonstrably by the Falck-Hillarp technique. It has been found (see Wurtman and Axelrod 1965, Axelrod 1971) that 5-HT is a link in a chain of indoleamines which are synthesised in the pineal. The basic compound of this synthesis is tryptophan which reaches the epiphysis by way of its vascular supply. In figure 4 the synthesis of some pineal indoles and the enzymes involved in their synthesis are illustrated.

A pineal compound to which considerable functional importance has been attributed is N-acetyl-5-methoxytryptamine or melatonin. This substance was isolated by Lerner *et al.* (1960) from bovine epiphyses and was also chemically identified (Lerner *et al.* 1959). Lerner termed it melatonin because it aggregates the pigment granules in the melanophores in amphibian skin causing this to turn pale. As is shown in figure 4, tryptophan is converted by the enzyme tryptophan-5-hydroxylase into 5-hydroxytryptophan from which 5-HT is synthetized by 5-hydroxytryptophan decarboxylase or aromatic L-aminoacid decarboxylase, an enzyme which occurs in high concentration in the pineal. It has been shown by McIsaac and Page (1959) that, in the epiphysis, 5-HT is converted to N-acetylserotonin by means of the N-acetylizing enzyme N-acetyltransferase. In its turn, N-acetylserotonin is the precursor substance of melatonin into which it is converted by the enzyme hydroxyindole-0-methyltransferase (HIOMT) as was shown by Axelrod and Weissbach (1960). Later this enzyme was obtained by these authors (1961) in its pure form. The synthesis of melatonin from tryptophan as described takes place in the pinealocytes. Serotonin or 5-HT, an important precursor substance of melatonin, is present in many mammalian epiphyses in higher concentration than in any other neural structure (Giarman and Day 1959). It is found also in man and in apes (Giarman *et al.* 1960). Serotonin and the enzyme HIOMT have also been demonstrated in the pineal gland of the elderly human (for literature see Feldstein *et al.* 1970).

Of other indole derivatives isolated and identified in the mammalian pineal gland 5-methoxyindole-3-acetic acid and 5-hydroxyindole-3-acetic acid can be mentioned. Probably both compounds are products of 5-HT metabolized by monoamine oxydase (see Quay 1965, 1974, for indole derivatives known and postulated to occur in the pineal and their metabolic pathways). Moreover, 5-hydroxytryptophol and 5-metoxytryptophol have been shown to occur in pineal tissue (Delvigs *et al.* 1965). The latter compound has a similar and even stronger inhibitory effect on the rat pituitary-gonadal axis than has melatonin (McIsaac *et al.* 1964) (see later).

Of the many other chemical compounds now known to occur in the mammalian pineal gland we will only here mention the proteins. As has been shown by gel micro-electrophoresis, the concentration of proteins in rat pineal is particularly high in comparison with other parts of the brain (Pun and Lombrozo 1964). The pinealocytes contain, among other peptides, a protein which is rich in tryptophan (Smith 1972) and which does not occur in any other part of the brain, the nuclei arcuatus and ventromedialis of the hypothalamus excepted (Smith and Kappers 1975). These nuclei belong to the hypophyseotropic

area and are involved in the synthesis of the luteotropic releasing hormone (LH-RH). Histochemically, a striking activity of aminopeptidase in rat pineal parenchyma (Niemi and Ikonen 1960) and in the parenchyma of the human pineal gland (Bayerova and Bayer 1967) has been shown. Biochemically Jouan and Rocaboy (1966) demonstrated that pineal aminopeptidase activity is very similar to that in the cortex cerebri and in the hypophysis. On these grounds it has been postulated that the organ synthesises proteins very actively. As will be mentioned below, a number of authors are of the opinion that the pineal effector substances are indeed polypeptides. Ford (1965) observed an intense uptake of labelled amino acids which could be considered additional proof of the glandular activity of the organ.

Pineal Physiology

As has been mentioned, Kitay and Altschule concluded in 1954 that, of the many investigations on pineal function so far performed, those showing an influence on sexual development and function were statistically most sound. Since then much work has been done in this field. In general it would appear that the mammalian pineal gland suppresses the function of other endocrine organs. The regulation by the pineal of the hypothalamo-anterior pituitary functional system and, in this way, pineal regulation of the function of those endocrine organs depending on anterior pituitary function has received special attention. Pineal antigonadotropic function has been studied most extensively. Therefore, this principally will be dealt with here, because its implication for the medical profession is obvious.

Removal of the organ, pinealectomy, under normal conditions, the most common experiment to establish the function of an endocrine organ, has not consistently furnished unequivocal results. In prepuberal rats exposed to normal conditions of illumination, pinealectomy resulted in increase of the weight of the ovaries and the uterus while precocious opening of the vagina was observed (Simonnet *et al.* 1951, Kitay 1954, Wurtman *et al.* 1959). According to Roth (1965), pinealectomy in puberal rats caused a significant increase in size and weight of the seminal vesicles and the ventral prostate while testicular weight did not change much, but Thiéblot and Blaise (1963) and Thiéblot (1965) found that the weight of the testes increased in this situation. Interestingly, pineal grafts in the anterior chamber of the eye compensated for the structural differences of the ovaries observed by some authors after pinealectomy. At the author's Institute, no changes in either testicular weight or weight of the seminal vesicles and prostate was observed after pinealectomizing male adult rats (Van Bronswijk

et al., to be published). Wragg (1967), criticizing the results obtained after pinealectomy, concludes that only within a restricted age range, *i.e.* operation at 26 to 30 days and sacrifice at 50 to 54 days, can reasonable evidence be obtained that pinealectomy indeed induces ovarian hypertrophy.

Changes in weight of primary sex organs should, in the opinion of the present author, always be followed by histological examination of the testes and the ovaries. In the former spermatogenesis and the Leydig cells, in the latter the formation of follicles and of corpora lutea, should be microscopically examined before conclusions can be drawn. It should also be stressed that histological control of the pinealectomies performed, evaluation of the data obtained by sound statistical methods, and the use of sufficient experimental and control material, are absolutely necessary. Variations in response are, moreover, now known to be attributable to a number of exogenous and endogenous factors. Of the exogenous ones, environmental illumination of the experimental animal is one of the most important. Among other factors the function of the pineal at any given moment greatly depends on the quantity of light to which the animal has been subjected at the time of the investigation, as we will see shortly.

Administration of pineal extracts to normal maturing rats resulted in a significant decrease in ovarian weight while opening of the vagina was delayed. In middle-aged rats anoestrus followed injection of such extracts (Wurtman *et al.* 1959, 1961) which also prevented permanent oestrus and pineal weight decrease in rats exposed to permanent illumination (Wurtman *et al.* 1963). After administration of hydrosoluble pineal abstracts to female guinea pigs Moszkowska (1967) observed retardation and even suppression of vaginal opening and an arrest of ovarian follicular growth. Moreover, she found a delay of the first oestrus in young rats and mice, and a decrease in weight of the seminal vesicles and the prostate in male rats and mice. Furthermore, an inhibitory effect of such pineal extracts could be demonstrated by this author on the permanent oestrus and the decrease in pineal weight occurring after exposure to permanent illumination. That the pineal gland probably exerts its antigonadotropic influence via the anterior pituitary was already shown by Wurtman *et al.* in 1961 by administering pineal extracts to hypophysectomized rats in which normal ovarian size was maintained by injections of follicle stimulating hormone (FSH). In these animals the administration of pineal extract did not show any effect while it did in non-hypophysectomized controls.

As has been mentioned, it is now know that the quantity of light to which an animal is subjected is related to pineal function, as it is to the development and function of the sex organs. The effect of long

periods of either light or darkness on the primary and secondary sex organs of mammals was observed earlier. More recently, they could be related to pineal function. Prolongation of daily photoperiods, and continuous light, cause, in the rat, an increase in weight of the ovaries and precocious oestrus (Fiske 1941, Wurtman *et al.* 1961, Fiske *et al.* 1962). Under these conditions pineal serotonin content, lipid content and pineal weight were found to be decreased (Fiske *et al.* 1960, Wurtman *et al.* 1961, Quay 1961, 1962, 1963a). The pinealocytes are smaller, while they also show a decrease in nucleolar size, RNA-content and HIOMT activity (Quay 1961, 1962, 1963a, Roth 1965). The activity of 5-hydroxytryptophan decarboxylase, on the other hand, is increased. In rats exposed to excessive quantities of light, pineal weight also decreases after removal of the thyroid, hypophysis, adrenal glands and gonads, which proves that pineal weight decrease is not due to any indirect effect caused by these other endocrine organs (Fiske *et al.* 1962). It was also demonstrated that these effects of constant light on the pineal only occur if the optic nerves are intact (Quay 1961, 1963a, Quay and Halevy 1962). From these facts it can be concluded that pineal function is generally inhibited in rats exposed to long periods of light, which evidently reaches the organ by way of the eyes, while the sex organs are stimulated. Permanent illumination may even lead to permanent oestrus and vaginal cornification, but ovulation does not occur.

Prolongation of daily dark periods or exposure of the animals to constant darkness, on the other hand, shows opposite effects. In young female rats the oestrous cycle starts later (Fiske 1941) while in male hamsters testicular weight significantly decreases (Hoffman and Reiter 1965, Hoffman *et al.* 1965). In female hamsters ovarian weight is about normal. Histologically, however, the ovaries show minimal follicular development, and the weight of the uterus is decreased (Reiter *et al.* 1966). Female rats in permanent darkness show a more or less permanent anoestrus. Their pineals increase in weight, the pinealocytes are larger and contain more lipids and RNA, while the activity of the melatonin producing enzyme HIOMT is increased. Blinding of the experimental animals produces the same effect as do long periods of darkness (Reiter and Hester 1966). From the above facts it can be concluded that long periods of darkness generally stimulate the function of the pineal gland, while the function of the sex organs is inhibited. The blinding experiments, moreover, demonstrate that darkness, or the loss of photic stimuli, influences pineal function by way of the visual system.

As has been demonstrated by Reiter and coworkers in many experiments, in scarcely any mammalian species studied is the pineal organ more convincingly antigonadotropic than in the hamster. By

pinealectomy as well as by superior cervical ganglionectomy, by which pineal sympathetic innervation is removed, atrophy of the sex organs following exposure to reduced light quantities or blinding can be prevented (Hoffman and Reiter 1965, 1966, Reiter and Hester 1966), while pinealectomy performed on hamsters during winter time, when the testes are atrophic, is followed by resumption of spermatogenetic activity (Czyba *et al.* 1964, 1965).

From the above, the general conclusion can be drawn that the mammalian pineal gland is involved in the regulation of the function of the reproductive system. It appears that pineal activity tends to inhibit this system while inactivity of the organ stimulates reproductory capacity. Furthermore, it is apparent that the state of pineal function, the organ being either in an active or inactive state, depends, among other factors, on environmental illumination. If the visual system and pineal innervation are intact, photic stimuli depress pineal activity while this activity is enhanced by lack of such stimuli caused either by darkness in the environment, by blinding, or by denervation of the pineal gland.

Before dealing with the neural pathways of the visual system which are now known to be involved in the functioning of the pineal organ, and the biochemical mechanism in the pinealocytes caused by light and darkness by means of the peripheral sympathetic innervation of the gland, we will first mention which pineal hormones have been held responsible for the antigonadotropic function of the organ and deal with the problem in which way they might influence the function of the gonads.

The discovery by Lerner and colleagues of the pineal indoleamine *melatonin* and of its synthesis, in the pineal, by the enzyme hydroxyindole-0-methyltransferase (HIOMT), led to the supposition that melatonin would probably be the pineal antigonadotropic hormone par excellence (Wurtman and Axelrod 1965), the more so because it was thought for a considerable time that the enzyme HIOMT occurs in the pineal organ only. It is, however, now known that this enzyme is also present in the mammalian retina (Cardinali and Rosner 1971) and in the Harderian gland in the rat orbit (Vlahakes and Wurtman 1972), which means that melatonin can also be produced in these organs. The antigonadotropic function of melatonin has meanwhile been substantiated, at least in rat, by a number of experimental investigations, some of which will be mentioned here. Daily administration of this compound to maturing female rats was followed by retardation of increase in ovarian weight, delay of vaginal opening time (Wurtman *et al.* 1963, Wurtman and Axelrod 1965), and decrease in incidence

of oestrus (Wurtman *et al.* 1963, Chu *et al.* 1964). High incidence of oestrus after pinealectomy could also be blocked by melatonin administration. Chu *et al.*, however, mention that this effect was merely statistical and could not be obtained in all animals investigated. Wurtman *et al.* (1963) not only observed that the incidence of oestrus was halved the day after the administration of melatonin to female rats which were first exposed to permanent illumination, but also that constant illumination reduced the uptake of circulating labelled melatonin by one half in the ovaries and the pineal organ. Injections of melatonin into male adult rats resulted in a decrease in size of the seminal vesicles (Kappers 1962, Moszkowska 1965).

It should, however, be mentioned that not all experiments in which melatonin was administered gave results (Ebels and Prop 1965). These authors suggested that inconsistency of results may depend on different sensitivity to melatonin among different rat strains.

Moreover, pineal indoles other than melatonin have been reported to have an at least equal, if not stronger, antigonadotropic effect. This holds, for instance, for 5-methoxytryptophol, another product of HIOMT, while N-acetylserotonin, 5-hydroxytryptophol and serotonin or 5-HT have also been shown to exert antigonadotropic activity. The role which might be played by hypothalamic serotonergic neurones in the inhibition of the production of gonadotropins in the anterior pituitary will be mentioned later.

The work by Motta *et al.* (1967) has contributed to an understanding of the way in which melatonin may influence the reproductive organs in the rat. According to these authors pinealectomy performed in sexually mature rats causes an increase in the weight of the testes as well as of the prostate and the seminal vesicles. These data suggested that pinealectomy stimulates both the production and release of LH and FSH in the anterior pituitary. Motta *et al.* also showed that treatment of 30-day-old rats with high doses of melatonin did not cause any significant changes in testicular weight, but that a significant atrophy of the prostate and of the seminal vesicles occurred. This would, following these authors, imply that melatonin primarily reduces LH secretion from the anterior pituitary, but would not modify FSH secretion. Fraschini (1969) observed after pinealectomy a significant increase of both LH and FSH in the anterior pituitary. Also, from earlier work, it became clear that pineal function influences the gonadotropic function of this part of the hypophysis. According to Reiter and Fraschini (1969) the pineal gland would normally inhibit the synthesis and the secretion of both gonadotropic hormones, LH and FSH, while melatonin is involved only in the ultimate release of LH from the anterior pituitary. It should, however, be mentioned that, at our

Institute, Van Bronswijk *et al.* (to be published), using the radio-immunoassay technique, were not able to show a rise of serum gonadotropin levels after pinealectomy performed in adult male rats, but even demonstrated a temporary decrease of the serum LH level after 7 days. This could mean that the increase of gonadotropins in the anterior pituitary occurring after pinealectomy, observed by the authors cited earlier, is due to excessive storage caused by a decrease in release of these hormones, or at least of LH. It might, therefore, be possible that the gonadotropic factor, also known to be present in the pineal gland, would normally enhance the release of gonadotropins from the anterior pituitary. Pinealectomy, then, would cause inhibition of this release and excessive storage of gonadotropins in the pituitary.

As far as the probable regulation of the function of the anterior pituitary by the pineal gland and therefore possibly pineal regulation of hypophyseotropic hypothalamic centres is concerned, we will mention shortly the investigations of Clementi *et al.* (1966). These authors studied changes in ultrastructure and gonadotropin content in the anterior hypophysis after pinealectomy as well as after implantation of pineal tissue and of melatonin into the median eminence of the hypothalamus and into the midbrain reticular formation (see also Fraschini *et al.* 1968). Their results suggested that the pineal gland would exert an inhibiting influence on the hypophyseal gonadotropic cells via hypothalamic and even mesencephalic centres. Changes observed in the fine structure of gonadotropic cells induced by implants of pineal tissue or of melatonin were identical with those obtained by inhibition of the synthesis and release of hypothalamic gonadotropic releasing hormones (LH-RH and FSH-RH).

It is known that peripherally administered melatonin elevates the level of serotonin in the brain (Anton-Tay *et al.* 1968) and that serotonergic nuclei are present in the mesencephalon, the neurones of which project, *i.a.*, on the nerve cells in the basal medial hypothalamus. It can be speculated that in such a way midbrain serotonergic neural pathways might influence the hypothalamo-pituitary system. Another possibility would be that specific melatonin receptors are present in the median eminence (Fraschini *et al.* 1971).

Recently serotonin-containing nerve cells have been observed by Smith and Kappers (1975), using fluorescence histochemistry, in the nucleus arcuatus and in part of the nucleus ventromedialis of the hypothalamus. These nuclei belong to the hypophyseotropic hypothalamic area and are known to be specially involved in the production of LH-RH. It was found that pinealectomy reduced the number of serotonin-containing neurones in these nuclei, while their normal number was restored by administration of pineal extract. From

earlier work by other authors it is known that serotonin inhibits, via the hypothalamus, gonadotropin—and specially LH production in the anterior pituitary—and also that serotonin inhibits synthesis and release of the gonadotropin releasing hormones in the hypothalamus (Moszkowska *et al.* 1973). Smith and Kappers, therefore, suggested as a working hypothesis, that pineal compounds exert their antigonadotropic influence via this hypophyseotropic hypothalamic area by stimulating the production of serotonin in neurones in this region which then inhibit, in the same nuclei, the production of gonadotropic releasing hormone, especially LH-RH. It is well known that the arcuate and the ventromedial nuclei contain neurones with short axons synapting with other neurones in the same nuclei so that an anatomical basis for this hypothesis seems to be present. This work, however, could not give, so far, conclusive evidence concerning the pineal compound or compounds which influence the hypothalamic hypophyseotropic area.

Although the antigonadotropic activity of melatonin seems to have been fairly well-established in the rat, experimental results lately obtained by Reiter *et al.* (1974) suggest that this compound is not the pineal antigonadotropic factor in the golden hamster, melatonin even inhibiting pineal antigonadotropic activity. It therefore appears that there are at least interspecies differences as far as the activity of this pineal substance is concerned.

It is of interest that exogenous melatonin has also been found to modify the rhythm of sleep and of locomotor activity, and it also modifies the electroencephalogram (for references see Lynch *et al.* 1975). This may be due to modification of serotonin content of the brain by melatonin. Lynch *et al.* (*l. c.*), using a bio-assay technique, were also able to demonstrate a circadian rhythm in the melatonin content of human urine. The rate of melatonin excretion is greatest during the night, that is at the time when melatonin synthesis is also greatest in the rat pineal gland. During daytime the content of melatonin of human urine proved to be considerably lower.

By many authors, among whom Thiéblot and Blaise (1963) and Thiéblot (1965) were probably the first, pineal polypeptides, not pineal indoles, have been claimed to be the antigonadotropic substances produced by the gland. Thiéblot and Menigot (1971) found both an antigonadotropic and a gonadotropic principle in pineal extracts. These authors isolated a melatonin-free fraction which contained five peptides and showed antigonadotropic activity. Earlier, several authors had already observed that pineal extracts could have a gonadotropic effect (Jöchle 1956, Reiss *et al.* 1963a, b, see also Moszkowska *et al.* 1965, Thiéblot 1965 for literature). Antigonadotropic as well as gonadotropic

fractions have also been isolated from acetone-dried pineal powder by Moszkowska *et al.* (1965), Ebels *et al.* (1965) and Moszkowska and Ebels (1971). *In vitro*, a partially purified antigonadotropic fraction which was most probably a polypeptide inhibited the release of gonadotropins from the anterior pituitary. It furthermore appeared that this fraction increased the hypothalamic FSH- and LH-RH content *in vitro* and *in vivo*, which is suggestive of a decrease in release of these hypothalamic releasing hormones under the influence of this factor (Moszkowska *et al.* 1973).

From bovine pineal extracts Benson *et al.* (1971, 1972a, b) isolated a pineal polypeptide showing a 60–70 times stronger antigonadotropic activity than melatonin. Two melatonin-free bovine pineal extracts, when administered to rats, inhibited compensatory ovarian hypertrophy, delayed vaginal opening time and reduced the incidence of light-induced constant oestrus while they significantly reduced the serum levels of LH in long-term castrated male rats (Orts *et al.* 1974).

Earlier, Milcu *et al.* (1963) isolated from bovine pineals a polypeptide which showed not only a pressor and oxytocic but also, in immature mice, an antigonadotropic activity. This compound was identified as arginine-vasotocin, while Pavel and Petrescu (1966) demonstrated that the activating effect of gonadotropin on mice uteri was indeed inhibited by both purified pineal arginine-vasotocin and the same substance in its synthetic form. Cheesman (1970), and Cheesman and Fariss (1970), also isolated from bovine pineal glands a cyclic nonapeptide, 8-arginine-vasopressin showing, antigonadotropic activity. Moszkowska and Ebels (1968) confirmed the inhibition of gonadotropic stimulation by arginine-vasotocin in immature mice. These authors, however, hold that this effect is the result of a direct action on the gonads or on the gonadotropic hormones and not on the depletion of gonadotropic hormones, an action which the antigonadotropic pineal peptide, previously demonstrated by these authors, possesses. It is also known that arginine-vasotocin occurs in parts of the brain other than the pineal gland so that the question can be raised whether this compound is specifically produced by this organ or not.

Pun and Lombrozo (1964) demonstrated in the rat pineal four proteins which do not occur in cortex, hypothalamus or putamen and have been held to be pineal-specific by these authors. In 1972 Smith found in rabbit pinealocytes a yellow autofluorescing peptide, next to formaldehyde-induced yellow fluorescing 5-HT, which was identical to one of the four peptides mentioned by Pun and Lombrozo. Contrary to the earlier opinion of Smith (1972) this peptide, which is very rich in tryptophan, is not pineal-specific as it also occurs in the nucleus arcuatus and nucleus ventromedialis of rat hypothalamus but not

in any other part of the brain (Smith and Kappers 1975, also for literature). If, under the influence of pinealectomy, the number of serotonin-containing nerve cells in these nuclei decreases (see earlier in this paper), the number of nerve cells containing this autofluorescent peptide increases. At present, it is not yet known whether this special peptide is related to the function of the reproductive system because it has not yet been isolated and experimentally tested.

From the above it appears that the pineal gland indeed exerts an antigonadotropic function while both, pineal indoleamines and polypeptides, may show antigonadotropic activity. It has, however, been mentioned that the pineal indoleamine, melatonin, does not exert this activity in all species while, according to Thiéblot and Menigot (1971), melatonin is even gonadotropic when administered in high doses. Moreover, it is now known that some pineal polypeptides are antigonadotropic whereas others are gonadotropic, which complicates matters further. It should also be realized that the function of the reproductive system depends on many factors, the function of the pineal gland being only one of them. It is generally known that the type of activity exerted by a compound on its effector cells is at any moment conditioned by the state of these cells which in turn depends on various exogenous and endogenous factors. This may, at least in part, explain the difference in results obtained by different authors.

Where the site of action of pineal antigonadotropic factors is concerned, it can be stated that most investigations point to the anterior pituitary and, even more probably, primarily to the hypothalamus. It should, however, be mentioned that *in vitro* experiments performed by some authors gave evidence of a gonadal site of melatonin action. After adding melatonin to testicular incubates Peat and Kinson (1971) observed a reduction of androgen production from pregnenolone, while Ellis (1969) found that melatonin interfered with the formation of testosterone in incubated testicular tissue. In inhibiting testosterone production melatonin was approximately 500 times more active than serotonin.

The problem whether pineal gland function is regulated by feedback mechanisms either by way of the anterior pituitary, or of the primary or secondary sex organs alone or in combination, has so far scarcely been touched by pineologists.

Data relating to pineal regulation of the production of hormones other than gonadotropins also synthesised in the anterior pituitary, such as prolactin, growth hormone, adrenocorticotropin and thyrotropin, are excellently summarized in a paper by Reiter (1974) to which the reader is refered. This author comes to the conclusion that, probably, the pineal gland produces a "heterogeneous group of sub-

stances and that these active materials may modulate the secretion of each of the adenohypophyseal hormones".

It should also be mentioned that pinealectomy inhibits the production of adiuretin or vasopressin in the magnocellular hypothalamic nuclei, that is in the nucleus supraopticus and the nucleus paraventricularis (De Vries, Kappers 1971, De Vries 1972a, b), which might explain the symptom of diabetes insipidus which not uncommenly occurs in cases of pineal tumours. Evidently the pineal gland does not exert its influence only on the parvocellular hypothalamic neurosecretory system and, thus, on the hypothalamo-anterior hypophyseal axis, but also on the magnocellular hypothalamic neurosecretory system and the hypothalamo-posterior hypophyseal axis.

Before concluding this paper, we will summarize some recent data on the control of the synthesis of pineal indoleamines by environmental photic stimuli which are mediated by the peripheral sympathetic innervation of the organ. In addition we shall deal with the visual pathways by which this innervation is now supposed to be influenced.

Regarding the first subject, reference is made to figure 4 illustrating the synthesis of melatonin from tryptophan. Quay (1963b) first discovered that the pineal serotonin content in rat varies during the day showing a peak about midday and a rapid fall soon after night fall. Evidently, pineal serotonin content displays a circadian rhythm. This rhythm persists or "free-runs" when rats are subjected to permanent darkness and after enucleation of the eyes (Snyder *et al.* 1965), which is the reason why it has been termed "endogenous". Constant illumination, however, abolishes the circadian rhythm of 5-HT which is also suppressed by bilateral superior ganglionectomy denervating the pineal gland (Snyder *et al.*, *l. c.*), deafferentiation of the superior cervical ganglia, and depletion of brain serotonin and noradrenaline by reserpine (Snyder and Axelrod 1965). Reversing the lighting schedule by exposing the animals to darkness during normal daytime and to light during the night changed the pineal serotonin rhythm by 180° within 6 days (Snyder *et al.* 1967). Denervation, however, does not affect this rhythm in rats aged 0–8 days (Machado *et al.* 1969a, b) while, just as in adults, in very young animals whose eyelids are still closed or which wear a hood over their skulls the normal fall of pineal 5-HT during the night does not occur under the influence of continuous illumination (Zweig *et al.* 1966). These experiments suggest that in very young rats, but not in adults, environmental stimuli can reach the pineal gland, even when denervated, by extra-retinal pathways. It does, however, appear that even in very young animals this rhythm is abolished by permanent environmental lighting. The difference between very young and adult rats is that in the former light evidently

influences the pineal gland via extra-retinal pathways, perhaps directly as is the case in submammalian vertebrates, whereas in the latter light stimuli are mediated to the gland via visual pathways and its autonomic innervation. The fact that denervation of the gland in very young rats living under normal conditions of illumination does not suppress the circadian serotonin rhythm, whereas constant illumination does, also suggests that in these young animals pineal sympathetic innervation is not yet fully established. It has indeed been found by Machado *et al.* (1968) that only after 21 days is pineal innervation, as investigated by fluorescence histochemistry, similar to that in the adult rat.

Because in both young and adult rats external illumination influences pineal 5-HT content, the "endogenous" circadian rhythm of pineal serotonin appears to be synchronized, but not caused, by this exogenous stimulus at all ages, although in a different way.

N-acetyltransferase, the enzyme which converts serotonin to N-acetyl-serotonin, also shows a marked circadian rhythm which is 180° out of phase with that of serotonin. This rhythm, which can be inverted by reversing environmental lighting (Nir *et al.* 1974), is abolished by ganglionectomy and denervation of the superior cervical ganglia as well as by continuous illumination, but is not suppressed by continuous darkness or enucleation of the eyes, therefore also being "endogenous" (Klein and Weller 1970, Klein *et al.* 1971). Brownstein and Axelrod (1974) likewise found a circadian rhythm in the turnover of noradrenaline present in the pineal sympathetic nerves. More of this neurotransmitter is utilized at night than during the day, which is probably due to its release from the terminals of these nerves and their preterminal varicosities. This circadian rhythm in noradrenaline turnover persisted in blinded rats, but was abolished in continuous light suggesting, according to the authors cited, that the circadian rhythms of serotonin, N-acetylserotonin, melatonin and N-acetyltransferase are generated by diurnal differences in release of noradrenaline. The enzyme hydroxyindole-0-methyltransferase (HIOMT) which converts N-acetylserotonin to melatonin likewise shows such a rhythm although not consistently. The rhythm of all indole compounds and enzymes mentioned is 180° out of phase with that of serotonin, their pineal contents or activity being low during daytime and high at night (for a table summarizing the circadian rhythms of the compounds and enzymes involved in the synthesis of melatonin and the influence exerted on their rhythm by ganglionectomy, decentralization of the ganglia, continuous light, continuous darkness and enucleation of the eyes see Kappers *et al.* 1974). *In vitro* studies indicated that cyclic AMP plays an important role in mediating, within the pineal gland, the effect of noradrenaline

on the synthetic activities of the pinealocytes. Increased discharge of noradrenaline during the night stimulates the β-adrenergic receptors in the membranes of these cells causing incerased synthesis of N-acetyltransferase by way of an adenylate cyclase system. Because N-acetyltransferase activity rises during the night, pineal serotonin content decreases while that of N-acetylserotonin increases. An increased production of melatonin then follows as a result of activity by the enzyme HIOMT which mostly also shows high activity during the night. In this way, the fall of pineal serotonin content during the night is explained by conversion of most of this substance to N-acetylserotonin by the activity of N-acetyltransferase which is at a high level at night. For more details and literature concerning these biochemical processes the reader is referred to Axelrod (1974) and Quay (1974).

In contrast to earlier opinions it has thus been established that it is not the activity of the enzyme HIOMT, but the conversion of serotonin to N-acetylserotonin by the enzyme serotonin N-acetyltransferase which is the rate-controlling step in the final production of melatonin (Klein 1973). As has been mentioned, pineal serotonin N-acetyltransferase activity shows an "endogenous" circadian rhythm which is controlled by photic stimuli finally reaching the pineal gland by its sympathetic innervation. Moore and Klein (1974) studied experimentally the visual pathways which mediate this regulation in the rat. It appeared most probable that the suprachiasmatic nuclei in the hypothalamus are involved. They are now definitely known to receive direct fibres from the large nerve cells in the retinae. This retinohypothalamic bundle is both crossed and uncrossed (Moore, Lenn 1972, Moore 1973). Selective ablation of the suprachiasmatic nuclei abolished the circadian rhythm of pineal serotonin N-acetyltransferase activity while destruction of the optic tracts, the accessory optic tracts, or both of these visual pathways together did not show any effect on this activity. The authors, speculating on the probably multisynaptic descending pathway from the suprachiasmatic nuclei to the intermediolateral nucleus in the upper part of the spinal cord which is involved in pineal sympathetic innervation, remark that the medial forebrain bundle could possibly be part of this pathway since its bilateral destruction likewise abolished the circadian rhythm of N-acetyltransferase activity. Recently the efferent connections of the suprachiasmatic nuclei have been studied by autoradiography and the horseradish peroxidase technique by Swanson and Cowan (1975). These authors found indeed an extensive projection of these nuclei through the medial forebrain bundle to nuclei in the posterior hypothalamus, to the thalamus, and also to the midbrain tegmentum. It is known that a tegmentospinal tract originating from a nucleus in the deep tegmentum

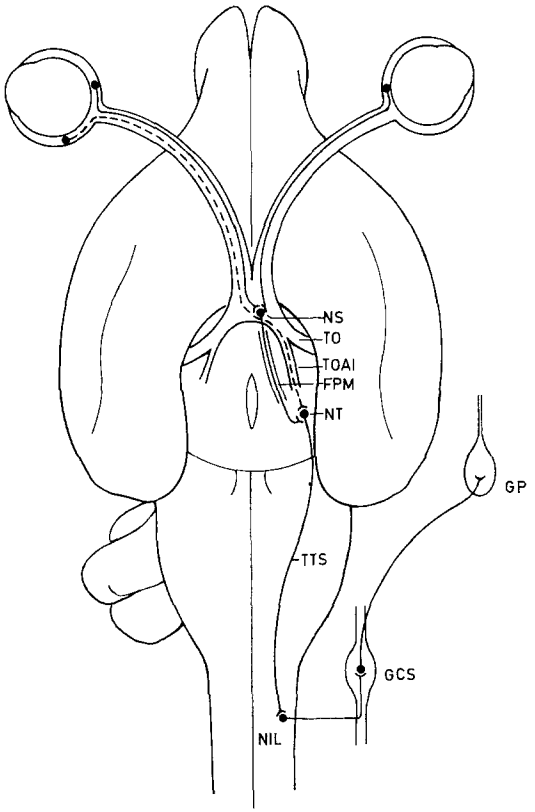

Fig. 5. Diagram of the rat central neural visual pathways mediating photic stimuli to the pineal gland by way of its sympathetic innervation. Broken line: retinotegmental, exclusively crossed, visual pathway. Drawn lines: retinohypothalamic and hypothalamotegmental visual pathways and all other connections. *FPM* fasciculus prosencephali medialis (medial forebrain bundle); *GCS* ganglion cervicale superius; *GP* glandula pinealis; *NIL* nucleus intermediolateralis; *NS* nucleus suprachiasmaticus in the hypothalamus; *NT* nucleus tegmenti receiving afferents from visual pathways; *TO* tractus opticus; *TOAI* tractus opticus accessorius inferior; *TTS* tractus tegmentospinalis. [From R. Y. Moore and D. C. Klein (1974), Elsevier Scientific Publ. Co., Amsterdam; Fig. 9 with many alterations]

of the midbrain which also receives fibres of the optic tectum, projects *i.a.* on the intermediolateral nucleus, the tecto-tegmento-spinal system being involved in the sympathetic pupillary reflex. In view of these facts the present author suggests that possibly the neural pathway mediating retinal stimuli to the intermediolateral nucleus via the

suprachiasmatic nuclei does not consist of so many links as was postulated by Moore and Klein (1974).

Unlike serotonin N-acetyltransferase activity, pineal HIOMT activity does not consistently show a significant circadian rhythm, although this activity is mostly high at night under normal conditions of illumination while after blinding, pineal activity of this enzyme is also high (Klein 1973). In an earlier study, Moore *et al.* (1967) demonstrated, also in the rat, that the response to light of HIOMT activity is mediated by the inferior accessory optic tract. Transsection of this tract gave the same results as blinding. Destruction of the suprachiasmatic nuclei did not, however, influence the pineal HIOMT response to light. From this it would appear that pineal HIOMT activity regulation by light is independently mediated by a neural pathway coursing from the retinae via the crossing inferior accessory optic tracts and the midbrain tegmentum to the spinal intermediolateral nuclei and, next, via the superior cervical ganglia to the pineal gland (Fig. 5). It has been speculated (see Moore, Klein 1974, also for literature) that the accessory optic system mediates continuous or tonic effects of light, whereas the retinohypothalamic pathway is involved in rhythmic or phasic effects.

Conclusions

1. The mammalian pineal gland, far from being a phylogenetic relic or a vestigial organ, is an endocrine gland. Its characteristic cells, the pinealocytes, are of neuro-epithelial origin. Their secretory products are released into the systemic circulation. In man, enzymatic activity in the pineal gland has been demonstrated even in the aged.

2. Although the gland develops as a part of the brain, the organ is innervated by peripheral autonomic nerve fibres, unlike any other part of the brain. This, and the fact that in some mammalian species in which the pineal is well-developed the organ shows few, if any, morphological connections with the brain at the adult stage, speaks for its relative independence of the brain.

In some mammalian species the presence of a peripheral parasympathetic innervation next to a sympathetic innervation of the gland has now been established. Demonstration in other species of this type of pineal innervation, the function of which is so far unknown, will certainly follow.

3. In general, the pineal gland appears to inhibit the function of other organs or systems. The organ has an antigonadotropic function also, however, secreting a gonadotropic compound.

4. Pineal indoleamines, especially melatonin, but also pineal polypeptides have been demonstrated to be antigonadotropic. Melatonin

would specially inhibit the function of one of the gonadotropins, luteinotropic hormone (LH) or interstitial cell stimulating hormone (ICSH). However, it appears that melatonin does not show an antigonadotropic activity in all species, while it has also been maintained that this compound may even be gonadotropic. As far as pineal polypeptides are concerned one or more are now known to be antigonadotropic whereas another is gonadotropic.

5. There are many indications that pineal secretory products exert their inhibitory activity on the synthesis and, perhaps, the release of anterior pituitary hormones, gonadotropins as well as other hormones, by way of hypothalamic hormone secreting centres. Therefore the gland has been termed a "regulator of regulators". Its direct inhibiting influence on the anterior pituitary and even on peripheral effector organs, *e.g.* the gonads, has also been demonstrated, at least *in vitro*. So far, little is known about feed-back mechanisms influencing pineal function from the anterior pituitary or the gonads.

6. A number of indoleamines and enzymes involved in their biosynthesis show a circadian rhythm in so far as their quantity or activity in the organ is concerned. Most of these rhythms depend on the daily rhythm of serotonin N-acetyltransferase, the enzyme which converts serotonin to N-acetylserotonin, and of hydroxyindole-0-methyltransferase, the enzyme which synthetizes melatonin from N-acetylserotonin. The pineal activity of these enzymes is controlled by photic stimuli, which are mediated by different visual pathways, and by the pineal gland's peripheral sympathetic innervation which controls the amount of noradrenaline released at the sympathetic nerve fibre terminals and consequently regulates biosynthesis in the pinealocytes.

In many submammalian vertebrates the pineal organ is directly photosensory. It is evidently indirectly photosensory in the adult mammal by way of its innervation.

Rhythms showing a longer periodicity than just one day have also been demonstrated, but they could not be dealt with in the present paper.

From the above conclusions it can be followed that many problems are still to be solved in the field of pineology and that they are rather more complicated than has often been thought before. Investigations on this fasciating organ ask for a modern multidisciplinary approach, which means that specialists in various disciplines should cooperate. Clinicians, by carefully recording endocrinological symptoms in patients suffering from pineal tumours, and neuropathologists can certainly help the purely scientific researchers.

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References

For some special books on the pineal gland we refer the reader to:

The Pineal, R. J. Wurtman, J. Axelrod, and D. E. Kelly. New York and London: Academic Press. 1968.

The Pineal Gland, a CIBA Foundation Symposium. G. E. W. Wolstenholme and J. Knight, eds. Edinburgh and London: Churchill Livingstone. 1971.

Pineal Chemistry in Cellular and Physiological Mechanisms, W. B. Quay. Springfield, Ill., U.S.A.: Ch. C Thomas. 1974.

Anton-Tay, F., Chou, C., Anton, S., Wurtman, R. J., Brain serotonin concentration: elevation following intraperitoneal administration of melatonin. Science *162* (1968), 277—278.

Axelrod, J., Neural control of indoleamine metabolism in the pineal. In: The Pineal Gland, CIBA Foundation Symposium. G. E. W. Wolstenholme and J. Knight, eds., pp. 35—47. Edinburgh and London: Churchill Livingstone. 1971.

— The pineal gland: a neurochemical transducer. Science *184* (1974), 1341—1348.

— Weissbach, H., Enzymatic 0-methylation of N-acetylserotonin to melatonin. Science *131* (1960), 1312.

— — Purification and properties of hydroxyindole-0-methyltransferase. J. Biol. Chem. *236* (1961), 211—213.

— Quay, W. B., Baker, P. C., Enzymatic synthesis of the skin-lightening agent, melatonin, in amphibians. Nature *208* (1965), 386.

Bargmann, W., Die Epiphysis cerebri. Handb. mikrosk. Anatom. d. Menschen. W. von Möllendorff, ed., VI/4., pp. 309—502. Berlin: Springer. 1943.

Bayerova, G., Bayer, A., Beitrag zur Fermenthistochemie der menschlichen Epiphyse. Acta Histochem. *28* (1967), 169—173.

Benson, B., Matthews, M. J., Orts, R. J., Presence of an antigonadotropic substance in rat pineal incubation media. Life Sci. *11* (1972a), 669—677.

— — Rodin, A. E., A melatonin-free extract of bovine pineal with antigonadotropic activity. Life Sci. *10* (1971), 607—612.

— — — Studies on a non-melatonin pineal antigonadotrophin. Acta Endocrinol. *69* (1972b), 257—266.

Berblinger, W., Die Glandula pinealis. Handb. spez. pathol. Anatom. Histol. *8*. Berlin: Springer. 1926.

— Physiologie und Pathologie der Zirbel (Epiphysis cerebri). Ergebn. ges. inn. Med. *14* (1930), 245.

Brownstein, M., Axelrod, J., Pineal gland: 24-hour rhythm in norepinephrine turnover. Science *184* (1974), 163—165.

Bustamente, M., Experimentelle Untersuchungen über die Leistungen des Hypothalamus, besonders bezüglich der Geschlechtsreifung. Arch. Psychiatr. Nervenkr. *115* (1942), 419—468.

— Spatz, H., Weisschedel, E., Die Bedeutung des Tuber cinereum des Zwischenhirns für das Zustandekommen der Geschlechtsreifung. Dtsch. med. Wschr. *68* (1942), 289—292.

Cajal, S. R., Histologie du système nerveux de l'homme et des vertébrés. Tome II, pp. 484—487. Paris: A. Maloine. 1911.

Cardinali, D. P., Rosner, J. M., Retinal localization of the hydroxyindole-0-methyltransferase (HIOMT) in the rat. Endocrinol. *89* (1971), 301—303.

Cheesman, D. W., Structural elucidation of a gonadotropin-inhibiting substance from the bovine pineal gland. Biochem. Biophys. Acta *207* (1970), 247—253.

— Fariss, B. L., Isolation and characterization of a gonadotropin-inhibiting substance from the bovine pineal gland. Proc. Soc. Exp. Biol. *133* (1970), 1254—1256.

Chu, E. W., Wurtman, R. J., Axelrod, J., An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinol. *75* (1964), 238—242.

Clementi, F., De Virgiliis, G., Fraschini, F., Mess, B., Modifications of pituitary morphology following pinealectomy and the implantation of the pineal body in different areas of the brain. Proc. VIth Intern. Congr. Electr. Microsc., Kyoto, pp. 539—540. Tokyo: Maruzen. 1966.

Collin, J.-P., Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In: The Pineal Gland, CIBA Foundation Symposium. G. E. W. Wolstenholme and J. Knight, eds., pp. 79—120. Edinburgh-London: Churchill Livingstone. 1971.

Czyba, J. C., Girod, C., Durand, N., Sur l'antagonisme épiphyso-hypophysaire et les variations saisonnières de la spermatogenèse chez le Hamster doré (*Mesocricetus auratus*). C. R. Soc. Biol., Paris, *158* (1964), 742.

— — Curé, M., Durand, N., Sur les corrélations épiphyso-testiculaires chez le Hamster doré (*Mesocricetus auratus* Waterh.). Bull. Assoc. Anatom., 50e Réun., Lausanne, 324—333. 1965.

David, G. F. X., Herbert, J., Experimental evidence for a synaptic connection between habenula and pineal ganglion in the ferret. Brain Res. *64* (1973), 327—343.

Delvigs, P., McIsaac, W. M., Taborsky, R. G., The metabolism of 5-methoxytryptophol. J. Biol. Chem. *240* (1965), 348—350.

De Vries, R. A. C., Influence of pinealectomy on hypothalamic magnocellular neurosecretory activity in the female rat during normal light conditions, light-induced persistent oestrus, and after gonadectomy. Neuroendocrinol. *9* (1972a), 244—249.

— Some aspects of the functional relationship of the rat pineal gland to the hypothalamic magnocellular neurosecretory nuclei under different experimental conditions. Med. Thesis, Univ. Amsterdam. 1972b.

— Kappers, J. Ariëns, Influence of the pineal gland on the neurosecretory activity of the supraoptic hypothalamic nucleus in the male rat. Neuroendocrinol. *8* (1971), 359—366.

Dodt, E., The parietal eye (pineal and parietal organs) of lower vertebrates. Handb. Sens. Physiol. VII/3, pp. 113—140. Berlin-Heidelberg-New York: Springer. 1973.

Ebels, I., Prop, N., A study of the effect of melatonin on the gonads, the oestrus cycle and the pineal organ of the rat. Acta Endocrinol. *49* (1965), 567—577.

— Moszkowska, A., Scemama, A., Etude *in vitro* des extraits épiphysaires fractionnés. Résultats préliminaires. C. R. Acad. Sci., Paris, *260* (1965), 5126—5129.

Ellis, L., The direct action of melatonin and serotonin on testicular androgen production *in vitro*. J. Reprod. Fert. *18* (1969), 159.

Engel, P., Die physiologische und pathologische Bedeutung der Zirbeldrüse. Ergebn. inn. Med. Kinderheilk. *50* (1936), 116—171.

Feldstein, A., Williamson, O., Tedeschi, L. G., The pineal body: neurochemical correlates. In: Neuropathology, Methods and Diagnosis. G. C. Tedeschi, ed. Boston, Mass., U.S.A.: Little, Brown & Comp. 1970.

Fiske, V. M., Effect of light on sexual maturation, estrous cycles and anterior pituitary of the rat. Endocrinol. *29* (1941), 187—196.

— Bryant, G. K., Putnam, J., Effect of light on the weight of the pineal in the rat. Endocrinol. *66* (1960), 489—491.

— Pound, J., Putnam, J., Effect of light on the pineal organ in hypophysectomized, gonadectomized, adrenalectomized or thiouracil-fed rat. Endocrinol. *71* (1962), 130—133.

Ford, D. H., Uptake of ^{131}I-labelled triiodothyronine in the pineal body as compared with the cerebral grey and other tissues of the rat. Progr. Brain Res. *10* (1965), 530—539.

Fraschini, F., The pineal gland and the control of LH and FSH secretion. In: Progr. in Endocrinol. Proc. IIIrd Intern. Congr. Endocrinol., Mexico. A. Gual, ed., pp. 637—645. Amsterdam: Excerpta Med. Found. 1969.

— Collu, R., Martini, L., Mechanisms of inhibitory action of pineal principles on gonadotropin secretion. In: The Pineal Gland, CIBA Foundation Symposium. G. E. W. Wolstenholme and J. Knight, eds., pp. 259—273. Edinburgh-London: Churchill Livingstone. 1971.

— Mess, B., Piva, F., Martini, L., Brain receptors sensitive to indole compounds: function in control of luteinizing hormone secretion. Science *159* (1968), 1104—1105.

Giarman, N. J., Day, M., Presence of biogenic amines in the bovine pineal body. Biochem. Pharmacol. *1* (1959), 235.

— Freedman, D. X., Picard-Ami, L., Serotonin content of the pineal glands of man and monkey. Nature *186* (1960), 480—481.

Hafeez, M. A., Zerihun, L., Studies on central projections of the pineal nerve tract in rainbow trout (*Salmo gairdneri*, Rich.), using cobalt chloride iontophoresis. Cell Tissue Res. *154* (1974), 485—510.

Hoffman, R. A., Reiter, R. J., Pineal gland: influence on gonads of male hamsters. Science *148* (1965), 1609—1611.

— — Responses of some endocrine organs of female hamsters to pinealectomy and light. Life Sci. *5* (1966), 1147—1151.

— Hester, R. J., Towns, C., Effect of light and temperature on the endocrine system of the golden hamster (*Mesocricetus auratus*, Waterh.). Comp. Biochem. Physiol. *15* (1965), 525—533.

Hülsemann, M., Development of the innervation in the human pineal organ. Z. Zellforsch. *115* (1971), 396—415.

Jöchle, W., Über die Wirkung eines Epiphysenextraktes (Glanepin) auf Sexualentwicklung und Sexualcyclus junger weiblicher Ratten unter normalen Haltungsbedingungen und bei Dauerbeleuchtung. Endokrinol. *33* (1956), 287—295.

Jouan, P., Rocaboy, J.-C., Etude de l'activité peptidasique de la glande pinéale du porc. C. R. Soc. Biol., Paris *160* (1966), 859.

Kappers, J. Ariëns, Opvattingen over de bouw en de functie van het ventrikelsysteem in de hersenen, van de Griekse tijd tot aan het begin van de 19de eeuw. Ned. Tijdschr. Geneesk. *98* (1954), 3569—3587.

— The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. Z. Zellforsch. *52* (1960), 163—215.

— Melatonin, a pineal compound. Preliminary investigations on its function in the rat. Gen. Comp. Endocrinol. *2* (1962), 610—611.

— Preface. Progr. Brain Res. *10* (1965), IX—XII.

— Survey of the innervation of the epiphysis cerebri and the accessory pineal organs of vertebrates. Progr. Brain Res. *10* (1965b), 87—151.

— The mammalian pineal organ. J. Neuro-Visc. Rel., suppl. IX, (1969). 140—184.

— The pineal organ: an introduction. In: The Pineal Gland, CIBA, Foundation Symposium. G. E. W. Wolstenholme and J. Knight, eds., pp. 3—34. Edinburgh-London: Churchill Livingstone. 1971.

— Smith, A. R., De Vries, R. A. C., The mammalian pineal gland and its control of hypothalamic activity. Progr. Brain Res. *41* (1974), 149—173.

Kenny, G. C. T., The nervus conarii of the monkey. An experimental study. J. Neuropathol. Exp. Neurol. *20* (1961), 563—570.

— The innervation of the mammalian pineal body. A comparative study. Proc. Austr. Assoc. Neurol. *3* (1965), 133—140.

Kitay, J. I., Effects of pinealectomy on ovary weight in immature rats. Endocrinol. *54* (1954), 114—116.

— Altschule, M. D., The Pineal Gland. Common Wealth Fund Book. Cambridge, Mass., U.S.A.: Harvard Univ. Press. 1954.

Klein, D. C., Circadian rhythms in indole metabolism in the rat pineal gland. In: The Neurosciences-Third Study Program. F. O. Schmitt and F. G. Worden, eds., pp. 509—516. Cambridge, Mass., U.S.A.: MIT Press. 1973.

— Weller, J. L., Indole metabolism in the pineal gland: a circadian rhythm in N-acetyltransferase. Science *169* (1970), 1093—1095.

— — Moore, R. Y., Melatonin metabolism: neural regulation of pineal serotonin: acetyl coenzyme A N-acetyltransferase activity. Proc. Nat. Acad. Sc., U.S.A., *68* (1971), 3107—3110.

Lange-Cosack, H., Verschiedene Gruppen der hypothalamischen Pubertas praecox. Dtsch. Z. Nervenheilk. *166* (1951), 499.

Leonhardt, H., Über axonähnliche Fortsätze, Sekretbildung und Extrusion der hellen Pinealozyten des Kaninchens. Z. Zellforsch. *82* (1967), 307—320.

Lerner, A. B., Case, J. D., Heinzelman, R. V., Structure of melatonin. J. Amer. Chem. Soc. *81* (1959), 6084.

— — Takahashi, Y., Isolation of melatonin and 5-methoxyindole-3-acetic acid. J. Biol. Chem. *235* (1960), 1992—1997.

Lynch, H. J., Wurtman, R. J., Moskowitz, M. A., Archer, M. C., Ho, M. H., Daily rhythm in human urinary melatonin. Science *187* (1975), 169—171.

Machado, C. R. S., Machado, A. B. M., Wragg, L. E., Circadian serotonin rhythm control: sympathetic and nonsympathetic pathways in rat pineals of different ages. Endocrinol. *85* (1969a), 846—848.
— Wragg, L. E., Machado, A. B. M., A histochemical study of sympathetic innervation and 5-hydroxytryptamine in the developing pineal body of the rat. Brain Res. *8* (1968), 310—318.
— — — Circadian rhythm of serotonin in the pineal body of immunosympathectomized immature rats. Science *164* (1969b), 442—443.
Marburg, O., Die Klinik der Zirbeldrüsenerkrankung. Ergebn. inn. Med. Kinderheilk. *10* (1913), 146—166.
— Die Physiologie der Zirbeldrüse (Glandula pinealis, Epiphyse). Handb. norm. pathol. Physiol. *13* (1930), 493—590.
McIsaac, W., Page, I. H., The metabolism of serotonin (5-hydroxytryptamine). J. Biol. Chem. *234* (1959), 858—864.
McIsaac, W. M., Taborsky, R. G., Farrell, G., 5-Methoxytryptophol: effect on estrus and ovarian weight. Science *145* (1964), 63—64.
Milcu, S. M., Pavel, S., Neascu, C., Biological and chromatographic characterization of a polypeptide with pressor and oxytocic activities isolated from bovine pineal gland. Endocrinol. *72* (1963), 563—566.
Moore, R. Y., Retinohypothalamic projection in mammals: a comparative study. Brain Res. *49* (1973), 403—409.
— Klein, D. C., Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Res. *71* (1974), 17—33.
— Lenn, N. J., A retinohypothalamic projection in the rat. J. Comp. Neurol. *146* (1972), 1—14.
— Heller, A., Wurtman, R. J., Axelrod, J., Visual pathways mediating pineal response to environmental light. Science *155* (1967), 220—223.
Moszkowska, A., Contribution à l'étude du mécanisme de l'antagonisme épiphyso-hypophysaire. Progr. Brain Res. *10* (1965), 564—575.
— Etude des extraits épiphysaires fractionnés—Physiologie. Biol. Méd. *56* (1967), 403—412.
— Ebels, I., A study of the antigonadotrophic action of synthetic arginine vasotocin. Experientia *24* (1968), 610—611.
— — The influence of the pineal body on the gonadotropic function of the hypophysis. J. Neuro-Visc. Rel., suppl. X. (1971), 160—176.
— — Scemama, A., Etude *in vitro* des extraits fractionnés d'épiphyses d'agneau. C. R. Soc. Biol., Paris, *159* (1965), 2298.
— Scemama, A., Lombard, M. N., Héry, M., Experimental modulation of hypothalamic content of the gonadotropic releasing factors by pineal factors in the rat. J. Neur. Transm. *34* (1973), 11—22.
Motta, M., Fraschini, F., Martini, L., Endocrine effects of pineal gland and of melatonin. Proc. Soc. Exp. Biol., New York *126* (1967), 431—435.
Niemi, M., Ikonen, M., Histochemical evidence of amino peptidase activity in rat pineal gland. Nature *185* (1960), 928.
Nir, I., Hirschmann, N., Kremer, N., Sulman, F. G., Inversion of pineal N-acetyltransferase rhythm by reversed environmental lighting. Neuroendocrinol. *15* (1974), 231—235.
Orts, R. J., Benson, B., Cook, B. F., Some antigonadotrophic effects of melatonin-free bovine pineal extracts in rats. Acta Endocrinol. *76* (1974), 438—448.

Paul, E., Hartwig, H.-G., Oksche, A., Neurone und zentralnervöse Verbindungen des Pinealorgans der Anuren. Z. Zellforsch. *112* (1971), 466—493.

Pavel, S., Petrescu, S., Inhibition of gonadotrophin by a highly purified pineal peptide and by synthetic arginine vasotocin. Nature *212* (1966), 1054.

Peat, F., Kinson, G. A., Testicular steroidogenesis *in vitro* in the rat in response to blinding, pinealectomy and to the addition of melatonin. Steroids *17* (1971), 251—253.

Pun, J. Y., Lombrozo, L., Microelectrophoresis of brain and pineal proteins in polyacrylamide gel. Analyt. Biochem. *9* (1964), 9—20.

Quay, W. B., Striated muscle in the mammalian pineal organ. Anatom. Rec. *133* (1959), 57—61.

— Reduction of mammalian pineal weight and lipid during continuous light. Gen. Comp. Endocrinol. *1* (1961), 211—217.

— Metabolic and cytologic evidence of pineal inhibition by continuous light. Amer. Zool. *2* (1962), 550.

— Cytologic and metabolic parameters of pineal inhibition by continuous light in the rat, *Rattus norvegicus*. Z. Zellforsch. *60* (1963a), 479—490.

— Circadian rhythm in rat pineal serotonin and its modification by estrous cycle and photoperiod. Gen. Comp. Endocrinol. *3* (1963b), 473—479.

— Indole derivatives of pineal and related neural and retinal tissues. Pharmacol. Rev. *17*, 321—345.

— Structure and histochemistry of corpora amylacea in the brain of an orangutan (*Pongo pygmaeus*). J. Comp. Path. *81* (1971), 89—95.

— Pineal Chemistry in Cellular and Physiological Mechanisms. Springfield, Ill., U.S.A.: Ch. C Thomas. 1974.

— Halevy, A., Experimental modification of the rat's pineal content of serotonin and related indole amines. Physiol. Zool. *1* (1962), 1—7.

Reiss, M., Davis, R. H., Sideman, M. B., Mauer, I., Plichta, E. S., Action of pineal extracts on the gonads and their function. J. Endocrinol. *27* (1963a), 107—118.

— Mauer, I., Sideman, M. B., Davis, R. H., Plichta, E. S., Pituitary-pineal-brain interrelationships. J. Neurochem. *10* (1963b), 851—857.

Reiter, R. J., Pineal-anterior pituitary gland relationships. In: MTP International Review of Science. Physiology, ser. I, *5*: Endocrine Physiology. S. M. McCann, ed., pp. 277—308. Baltimore: Butterworths, London-University Park Press. 1974.

— Fraschini, F., Endocrine aspects of the mammalian pineal gland. Neuroendocrinol. *5* (1969), 219—255.

— Hester, R. J., Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod in the regulation of the endocrine systems of hamsters. Endocrinol. *79* (1966), 1168—1169.

— Hoffman, R. A., Hester, R. J., The role of the pineal gland and of environmental lighting in the regulation of the endocrine and reproductive system of rodents. Edgewood Arsenal Technical Report, 4032. Res. Laborat. US Army, Edgewood Arsenal, Maryland, pp. 1—43. (1966).

— Vaughan, M. K., Blask, D. E., Johnson, L. Y., Melatonin: its inhibition of pineal antigonadotropic activity in male hamsters. Science *185* (1974), 1169—1171.

Romijn, H. J., Structure and innervation of the pineal gland of the rabbit, *Oryctolagus cuniculus* (L.), with some functional considerations. A light and electron microscopic investigation. Thesis Fac. Sci., Free Univ. Amsterdam. 1972.
— Parasympathetic innervation of the rabbit pineal gland. Brain Res. *55* (1973a), 431—436.
— Structure and innervation of the pineal gland of the rabbit, *Oryctolagus cuniculus* (L.). I. A light microscopic investigation. Z. Zellforsch. *139* (1973b), 473—485.
— Structure and innervation of the pineal gland of the rabbit, *Oryctolagus cuniculus* (L.). III. An electron microscopic investigation of the innervation. Cell Tissue Res. *157* (1975), 25—51.
Roth, W. D., Metabolic and morphologic studies on the rat pineal organ during puberty. Progr. Brain Res. *10* (1965), 552—562.
Schmidt, E., Hallervorden, J., Spatz, H., Die Entstehung der Harmartome am Hypothalamus mit und ohne Pubertas praecox. Dtsch. Z. Nervenheilk. *177* (1958), 235—262.
Simonnet, H., Thiéblot, L., Melik, T., Influence de l'épiphyse sur l'ovaire de la jeune rate. Ann. Endocrinol., Paris *12* (1951), 202—205.
Smith, A. R., Conditions influencing the serotonin and tryptophan metabolism in the epiphysis cerebri of the rabbit; a fluorescence histochemical, microchemical and electrophoretic study. Thesis Med. Fac., Univ. Amsterdam. 1972.
— Kappers, J. Ariëns, Effect of pinealectomy, gonadectomy, pCPA and pineal extracts on the rat parvocellular neurosecretory hypothalamic system. A fluorescence histochemical investigation. Brain Res. *86* (1975), 353—371.
Snyder, S. H., Axelrod, J., Circadian rhythm of pineal serotonin: effect of monoamine oxidase inhibition and reserpine. Science *149* (1965), 542—544.
— — Zweig, M., Circadian rhythm in the serotonin content of the rat pineal gland: regulating factors. J. Pharmacol. Exp. Ther. *158* (1967), 206—213.
— Zweig, M., Axelrod, J., Fischer, J. E., Control of the circadian rhythm in serotonin content of the rat pineal gland. Proc. Nat. Acad. Sci. U.S.A. *53* (1965), 301—305.
Spatz, H., Neues über die Verknüpfung von Hypophyse und Hypothalamus. Acta Neuroveg. *3* (1951), Heft 1—2.
— Das Hypophysen-Hypothalamus-System in seiner Bedeutung für die Fortpflanzung. Verh. anatom. Ges. *51*, (1953), 46—86.
— Das Hypophysen-Hypothalamus-System in Hinsicht auf die zentrale Steuerung der Sexualfunktionen. 1. Symp. Dtsch. Gesellsch. Endokrinol., Hamburg, pp. 1—44. Berlin-Göttingen-Heidelberg: Springer. 1955.
Swanson, L. W., Cowan, W. M., The efferent connections of the suprachiasmatic nucleus of the hypothalamus. J. Comp. Neurol. *160* (1975), 1—12.
Thiéblot, L., Physiology of the pineal body. Progr. Brain Res. *10* (1965), 479—488.
— Blaise, S., Influence de la glande pinéale sur les gonades. Ann. Endocrinol., Paris *24* (1963), 270—286.
— Menigot, M., Acquisitions récentes sur le facteur antigonadotrope de la glande pinéale. J. Neuro-Visc. Rel., suppl. X. (1971), 153—159.

Turkewitsch, N., Eigentümlichkeiten in der Entwicklung des Epiphysengebiets des Kaninchens (*Lepus cuniculus* L.). Gegenbauers Morphol. Jb. *79* (1937), 634—649.

Van de Veerdonk, F. C. G., Demonstration of melatonin in amphibia. Curr. Mod. Biol. *1* (1967), 175—177.

Vlahakes, G. J., Wurtman, R. J., A Mg^{2+} dependent hydroxyindole-0-methyltransferase in rat Harderian gland. Biochim. Biophys. Acta *261* (1972), 194.

Wartenberg, H., The mammalian pineal organ: electron microscopic studies on the fine structure of pinealocytes, glial cells and on the perivascular compartment. Z. Zellforsch. *86* (1968), 74—97.

Wolfe, D. E., The epiphyseal cell: an electron-microscopic study of its intercellular relationships and intracellular morphology in the pineal body of the albino rat. Progr. Brain Res. *10* (1965), 332—376.

— Potter, L. T., Richardson, K. C., Axelrod, J., Localizing tritiated norepinephrine in sympathetic axons by electron microscopic autoradiography. Science *138* (1962), 440—442.

Wragg, L. E., Effects of pinealectomy in the newborn female rat. Amer. J. Anat. *120* (1967), 391—402.

Wurtman, R. J., Axelrod, J., The formation, metabolism and physiologic effects of melatonin in mammals. Progr. Brain Res. *10* (1965), 520—528.

— Altschule, M. D., Holmgren, U., Effects of pinealectomy and of a bovine pineal extract in rats. Amer. J. Physiol. *197* (1959), 108—110.

— Axelrod, J., Barchas, J. D., Age and enzyme activity in the human pineal. Endocrinol. *24* (1964), 299—301.

— — Chu, E. W., Melatonin, a pineal substance: effect on the rat ovary. Science *141* (1963), 277—278.

— Roth, W., Altschule, M. D., Wurtman, J. J., Interactions of the pineal organ and exposure to continuous light on organ weights of female rats. Acta Endocrinol. *36* (1961), 617—624.

Zimmermann, P., Paul, E., Reaktionsmuster verschiedener Mittel- und Zwischenhirnzentren von *Rana temporaria* L. nach Unterbrechung der Nervenbahnen des Pinealcomplexes. Z. Zellforsch. *128* (1972), 512—537.

Zweig, M., Snyder, S. H., Axelrod, J., Evidence for a nonretinal pathway of light to the pineal gland of newborn rats. Proc. Nat. Acad. Sci., U.S.A. *56* (1966), 515—520.

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