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THE ANATOMICAL RELATIONSHIPS, ULTRASTRUCTURE, AND FUNCTION OF THE PINEAL GLAND IN SOME COMMON LABORATORY RODENTS

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by

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DEDICATION

"The mission confided to you, therefore, ranks among the most noble, for you should be, in a sense, the discoverers of the inventions of God... Authorized interpreters of Nature, be you also the teachers who explain to their brothers the wonders which are unfolded in the universe, and which better than others, you see assembled as in a single book... Become, you who interpret creation, teachers eager to reveal its beauty, its power, its perfections, so that they may be enjoyed by others...teach others to behold, to understand, and to love the created world so that the admiration of splendors so sublime may cause the knee to bend and invite the minds of men to adoration."

Pope Pius XII

This thesis by Father Joseph Chester Gregorek is accepted in its present form as satisfying the thesis requirement for the degree of Doctor of Philosophy

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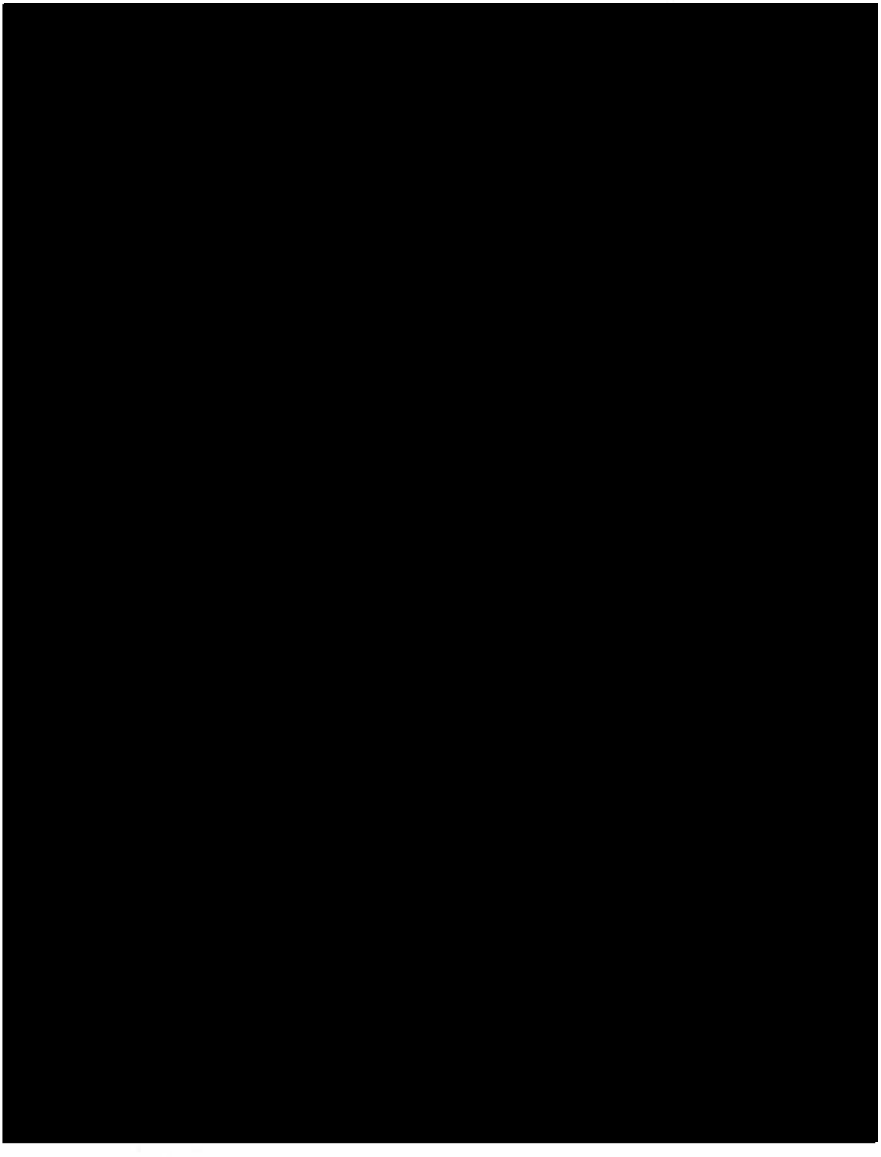

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LIST OF PUBLICATIONS

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CONTENTS

INTRODUCTION

Throughout the ages, the pineal gland and its functions have remained a great mystery. Galen, A. D. 129 to 199, wrote of the early Greek anatomists, "those who concern themselves with dissections", as the men who were aware that the pineal was the only single unpaired structure arising from the dorsal surface of the brain. They believed that this structure acted as a sphincter regulating the flow of thought. This basic concept also was proposed by Majendie in 1795. His theory was that the pineal acted as a tampon which would expand and shrink thereby regulating the flow of cerebrospinal fluid through the aqueduct of Sylvius. He stated that this function is obvious because of the location, structure, and form of the pineal body. Galen, however, thought the pineal to be a gland, similar perhaps to lymph glands. In 1871, Henle concurred with this thought.

Rene Descartes, in the 17th century, elevated the function of the pineal to a metaphysical level. He maintained that the pineal organ was the seat of the rational soul. The following three articles explain Descartes' thought as it appears in his work The Passions

of the Soul (Descartes, 1649).

Article XXXI

The pineal gland

Nevertheless, although the soul is joined with the entire body, there is one part of the body in which it exercises its functions more particularly than elsewhere. Some take this special part to be the brain, others the heart: The brain because of its connections with the organs of sense, and the heart because it appears to be the place where our deepest feelings are centered. But it seems to me quite clear, after carefully examining the matter, that the part of the body where the soul operates most directly is not the heart, nor is it the entire brain, but only the innermost portion of the brain -- a tiny gland situated in the very middle of it and suspended above the duct through which the animal spirits in the forward passages can communicate with those in the rear ones, so that the slightest movements which occur in it can greatly alter the course of those spirits, and conversely the smallest changes occuring in the animal spirits may greatly affect the movements of this gland.

Article XXXII

Evidence of its function

The reason why I feel sure, on reflexion, that this gland must be the one place where the soul operates directly upon the body is as follows. All the other parts of the brain are found in pairs, just as all the organs of our outer senses are double -- e.g., two eyes, two hands, two ears. Now our awareness of a particular thing is single and undivided. Hence, there must necessarily be some place where the double visual image and the other double impressions, which proceed from the several pairs of

sense-organs acted on by a single object, can unite before reaching the soul and thus represent to it not two objects, but one. It is easy to understand how these images and other impressions can unite in this gland by the agency of the animal spirits which fill the passages of the brain; but there is no other place in the body where they can be so united if not here.

Article XXXIV

Interaction of soul and body

The theory here set forth, then, is that the soul operates most directly upon the little gland which is poised in the middle of the brain. From here it radiates through all the rest of the body by means of the animal spirits, the nerves, and even the blood, which receiving impressions from the animal spirits, carries them via the arteries into all the body's members. I have already explained, in discussing the machinery of the body, that the tiny nerve-filaments are so distributed that according as one or another movement is excited in them by sense-objects they open in one or another way the pores of the brain, causing the animal spirits contained in these passages to enter in different ways into the muscles, and thereby they move the limbs in one or another of the ways in which they are capable of being moved. Any other causes, likewise, which affect the movement of the animal spirits will have similar results. I must now add that the small gland with which the soul is most directly connected functions in the following double way. It is so suspended between the passages containing the animal spirits that it can be moved by them in as many different ways as there are sense-differences in the object; and it carries this motion onto the soul, whose nature is such that she receives as many different perceptions as there are diverse movements in the gland. Conversely, the bodily machine is so constituted that whenever the gland is moved in one way or another by the soul -- it pushes the animals spirits which surround it

> toward the pores of the brain, through which they are conducted along the nerves to the muscles, thereby producing movement in the limbs.

Wurtman and Axelrod (1965) acknowledge Descartes' hypothesis by stating, "With the hindsight of 300 years of scientific development, we can admire this prophetic formulation of the pineal as a neuroendocrine transducer!" This statement is based on the present theory that the pineal transduces neural information and input into a hormonal output (Wartenburg, 1968; Wurtman and Anton-Tay, 1969).

Some researchers believed in a pineal-mind relationship. Voltaire thought the pineal should be viewed as a driver which guides the action of the cerebral hemispheres by means of two nerve bands. As Tilney and Warren (1919) state, "These nerve bands were long referred to by the anatomists as the 'reins of the soul'." Gunz in 1753 studied five cases of pineal calcification with patient psychosis. He believed the dementia was caused by the pineal body impeding the flow of spirits. In 1779, however, Morgagni doubted the existence of a consistant relationship between pineal calcification and psychosis. He stated, "And although you have heard above this disorder has been found with madness and without it also,

yet I would not have you forget there is not any one disorder wherewith it is so frequently found to be joined, as with madness."

Many authors, aware of the phylogenetic development of the pineal gland, attributed a vestigial function to this structure. Its relationship to the primitive eye of lower vertebrates indicated to them that this organ was a functionless remnant of a photoreceptive structure. Even a modern textbook states that "the pineal body of mammals is the vestige of the median eye which was probably a functioning organ in certain amphibia and reptiles that are now extinct" (Ham, 1965). This concept, and the absence of an apparent function of the pineal gland delayed interest and research into this organ.

In 1898, however, a German physician named Otto Heubner added to the knowledge of the pineal by publishing a study in which he noted precocious sexual development in a young boy suffering from a pineal tumor. A few years later Pellizzi reported two cases of precocious puberty which he attributed to pineal tumors. Other clinical observations of pinealomas in patients with pubertas praecos led to experimentation on pineal-gonadal relationships. These studies augmented the belief that the pineal gland

may regulate sexual development (Del Rio-Hortega, 1932; Cohen, et al., 1964; Kitay and Altshule, 1954). The function of the pineal thus progressed from a structure regulating the flow of thought, to an organ which may play a role in sexual development.

The Structure of the Pineal Gland

The pineal organ (also known as the pineal body, pineal gland, epiphysis, epiphysis cerebri, corpus pineale, glandula pinealis, konarion, or corpus turbinatum) has phylogenetically undergone great changes in form (Tilney and Warren, 1919; Bargmann, 1943; Oksche, 1965). The pineal organ of mammals, snakes, and many birds is a single solid parenchymal organ which varies in morphology in the different classes but always arises from the roof of the diencephalon between the habenular commissure and the posterior commissure.

The rodent pineal, which is the basis of this study, is generally an enlarged oval structure. Flesch (1887) describes it as being "more or less cylindrical" and Cutore (1910) identifies it as "cylindrococonical". It is located caudal to the occipital lobes of the telencephalon, rostral to the cerebellum and dorsal to the

corpora quadrigemina of the midbrain. It is always connected with the diencephalon by means of a stalk (Kappers, 1965; Sheridan and Reiter, 1970a). The pineal is also directly inferior to the lamboid suture of the skull and is usually attached to the ventral surfaces of the confluence of the superior sagital and transverse sinuses. These structures serve as anatomical landmarks in the excision of this organ.

The specific morphology of the pineal gland, its stalk, and other related structures varies in different rodents (Gregorek and Seibel, 1970). The albino rat has a pineal gland that is dorsocaudally flexed from its attachment to the diencephalon and a connective tissue stalk which may contain pinealocytes in certain areas. The kangaroo rat, however, has a pineal with a complete pinealocyte stalk.

The pineal complex of the golden hamster (Sheridan and Reiter, 1970a) and also of the Chinese hamster is divided into a deep pineal and a superficial pineal. The deep pineal body is a large mass of pinealocytes which is part of the diencephalic roof. The superficial pineal gland, which is comparable to the pineal in the rat, is considered the pineal gland proper. It is connected to

the deep pineal tissue via a connective tissue stalk containing pinealocytes in the golden hamster and a complete pinealocyte stalk in the Chinese hamster. Quay (1956) noted that the pineals of some North American rodents such as Peromyscus also have two subdivisions which he names the basal (anterior) part around the diencephalon and a distal (posterior) portion. Thus the deep pineal corresponds to Quay's basal portion and the superficial corresponds to the distal portion. Quay also observed great variation in pineal tissue of the stalk connecting both portions: 31% of the animals (Peromyscus) had a continuous strand of pineal tissue, 44% had an interrupted strand, 22% had scattered islets, and 3% had no pineal tissue in the stalk.

The dorsal sac is another structure which has aroused great interest because of its relationship to the pineal gland and stalk. It is a dilated vesicular pouch of eosinophilic cuboidal epithelial cells which is continuous and histologically identical to the choroid plexus of the third ventricle. This structure is so situated that its caudalmost portion is over the dorsal surface of the pineal body (Tilney and Warren, 1919) and is homologous to the human suprapineal recess. The observations of Sheridan et al. (1969) and Sheridan and Reiter (1970a)

concerning this pineal sac are that it is contiguous with the superficial pineal, is a part of the pineal stalk, and is continuous with the ventricular system of the brain. This has led them to speculate that the ventricular cerebrospinal fluid may serve as a means of transport of an anti-gonadic pineal substance.

Anton-Tay and Wurtman (1969) have investigated the question of whether melatonin, the unique pineal substance and suspected pineal antigonadotropic factor, is secreted into the blood or cerebrospinal fluid. Extremely small doses of melatonin injected into the ventricular system of the brain are retained in areas of the brain in higher concentrations than are massive doses injected systemically. This, however, is attributed to the rapid metabolism of melatonin by the liver in the systemic circulation. Brain implants of melatonin have been shown to antagonize the gonadotropic functions of the anterior pituitary (Mess, 1968). The synthesis of melatonin by the pineal gland of the brain is related to the pineal enzyme hydroxyindole - O - methyl transferase (HIOMT). The activity of this enzyme is increased when rats are kept in continuous darkness (Wurtman et al., 1963a). There is also a diurnal rhythm in this enzyme, its activity being greatest during

the daily dark period (Axelrod et al., 1965).

If the pineal secreted its hormones into the bloodstream as do the endocrine glands, then the vascular pathways would be especially significant. The arterial supply to the mouse pineal gland is by means of small arteries from the posterior cerebral arteries. The venous drainage of the pineal and stalk in mice is accomplished by two veins: the vena cerebri magna, which is homologous to the great cerebral vein of Galen and a more dorsal vein, the vena mediana prosencephalica. Both veins enter into the superior sagittal sinus rostral to the confluence of sinuses (von Bartheld and Moll, 1954). The venous drainage in the rat is similar to that of the mouse with two or very often a single large vena magna cerebri but they always enter into the confluence of sinuses (Kappers, 1960). A vascular link has not been demonstrated between the pineal and other parts of the brain or choroid plexus or dorsal sac. These findings and opinions concerning the secretory nature of the pineal have prompted further investigations (Sheridan et al., 1969) into the gross morphology and anatomical relationships in the pineal complex.

The terminology concerning nerve connections with the pineal gland is confusing since the pineal organ is part of the central nervous system. Some authors use the terms afferent and efferent in a pineal oriented sense and others use it in a CNS-centered sense. Kappers (1967) introduced the terms "pinealo-fugal" for nerves originating in the pineal and "pinealo-petal" for nerve fibers coursing to the pineal from other areas. Pinealo-fugal nerve fibers have never been demonstrated in the mammalian pineal.

Nerve connections to the rodent pineal gland are generally believed to be related to two sources: the habenulo-caudo-commissural epiphyseal fibers and the post-ganglionic autonomic fibers from the superior cervical ganglia (Kappers, 1960, 1965). Favaro (1904) was probably the first to describe nerve fibers entering the pineal from the habenular commissure, posterior commissure, stria medullaris, and possibly other areas of the brain. Le Gros Clark (1940) believes these commissural fibers are aberrant fibers which form loops and do not innervate pineal parenchyma. Kappers (1960) has shown that, in the rat, these commissural fibers either enter the surrounding leptomeningeal tissue or make a hairpin

loop through the pineal to return to the commissural area. Thus, these habenulo-caudo-commissural epiphyseal fibers are believed to be non-functional.

The functional nervous innervation of the rodent pineal is accepted to be the autonomic nervous system. Henle (see Kappers, 1960) is thoughtto be the first to observe sympathetic fibers in the epiphysis. Cajal (1904) indicated that they were extensions of the superior cervical ganglia. Kappers (1960), in a widely quoted paper, has shown that the autonomic innervation to the pineal of the rat is by means of two nervi conarii which enter the pineal under the confluence of sinuses after coursing in the tentorium cerebelli. These nerves were previously described in man by Kolmer and Lowy (1922) and by Le Gros Clark (1940). Kappers (1960) demonstrated that the nervi conarii, even to their terminal boutons within the pineal, would degenerate after bilateral superior cervical ganglionectomy.

The mammalian pineal parenchymal cell, the pinealocyte or pineocyte, differs from the extensively studied submammalian pineal cell. Ultrastructural studies of pineal cells from the saccular pineal organ of cold blooded vertebrates have shown these cells to be morphologically similar to retinal photoreceptor cells (Kelly, 1965). They have the

typical stacked lamellar structure of the outer segment, similarly appearing inner segments, and basal processes which enter into synaptic complexes as do other photoreceptor cells. There is also neurophysiological data that these cells produce neural responses with photic stimulation (Dodt, 1963). Thus, the pinealof submammalian species appears to be primarily a sensory structure.

The mammalian pinealocyte has been characterized on the light microscopic level as a large cell, with a large highly convoluted nucleus and prominantly staining large nucleolus, possessing many complex cytoplasmic processes (Del Rio Hortega, 1932). In mice, the cytoplasmic processes were also observed to have terminal boutons (Cajal, 1904). In addition to the parenchymal cell, there are other cell types found in the mammalian pineal. These include neuroglia cells, cells resembling those of the subcommissural organ, and ependyma-like cells. The stroma also contains lymphocytes, plasma cells, macrophages, mast cells, melanophores, fibroblasts, and fibrocytes (Quay, 1965).

The mammalian pinealocyte has been described ultrastructurally in many different rodents including the rat (Milofsky, 1956; Gusek and Santoro, 1960, 1961;

De Robertis and Pellegrino de Iraldi, 1961), the hamster (Sheridan, 1967, 1969; Sheridan and Reiter, 1968; Clabough, 1969, 1970) and the mouse (Ito and Matsushima, 1968; Pellegrino de Iraldi, 1969). In the above studies, the mammalian pinealocyte has been shown to lack the morphological similarity to retinal photoreceptor cells of submammalian pineal cells. They contain an abundance of membrane bound vesicles, many of which contain electron dense granules, in the terminations of their cell processes. These structures, correlated with the known physiological effects of the pineal suggest that the pineal organ of mammals may be primarily a secretory organ. Collin (1971) believed the pineal organ "to be a sensory and endocrine organ which during vertebrate phylogeny gradually loses its sensory activity and retains only its endocrine functions in snakes, mammals and probably in some, if not all, birds." Thus, as Scharrer (1964) has indicated, the pineal organ may be considered as a "photo-neuro-endocrine" organ functioning directly as such in lower vertebrates, and in an indirect manner in the higher vertebrates.

The Pineal Gland and the Endocrine System

The first recognized pineal-endocrine relationship was observed in the previously mentioned clinical cases of pineal tumors and precocious sexual development. These observations stimulated experimentation into endocrine effects of pinealectomy in animals.

Izawa (1926) reported hypertrophy of the testes, ovaries, seminal vesicles and uteri following pinealectomy in rats. Further studies in prepubertal female rats showed that pinealectomy of these animals resulted in increased ovarian weight (Simmonnet et al., 1951; Wurtman et al., 1959) but this increase was not observed in animals over 30 days old (Kitay, 1954). In the prepubertal male rat pinealectomy resulted in an increase of seminal vesicle and ventral prostate weights but not testicular weight (Roth, 1965). In the adult rat there was an increase in the proportion of daily vaginal smears showing estrous phases but no increase in the size of the ovaries (Gittes and Chu, 1965) and increased weights of testes, seminal vesicles, and ventral prostate (Motta et al., 1967) following pinealectomy. Pinealectomy was shown also to block the winter decrease in spermatogenesis in the adult hamster (Czyba et al., 1964), increase the

weight of testes and thyroid in the adult mouse (Houssay, et al., 1966), and result in earlier vaginal opening in rats (Kincl and Benagiano, 1967).

As conclusive as the previous papers may appear to be, many authors have shown conflicting results. The review of pineal literature by Kitay and Altschule (1954) emphasizes the contradictory nature of many studies. Wragg (1967) has questioned some of the above results and has reported that pinealectomy in neonatal rats has no demonstrable effect on the weight of the ovary, uterus, adrenal, or pituitary and has no effect on the onset of puberty or the estrous cycle. Reiter (1967) found that pinealectomy does not significantly affect the weights of the testes, accessory sexual organs, adrenals, and pituitaries of adult male hamsters or adult male albino rats. This work was confirmed by similar results in adult female rats (Bick, et al., 1969) and golden hamsters (Girod, et al., 1967).

Reiter and Fraschini (1969) believed that many of the problems in evaluating the early work of the relationship between the pineal and the gonads was the "failure of investigators to mention the lighting conditions under which the animals were maintained". Rowan (1925) demon-

strated that environmental lighting had an effect on gonadal function. Hammond (1954) and Ortavant et al. (1964) stressed the importance of environmental photoperiod in regulating mammalian reproduction. In humans, young girls who are blind because of retrolental fibroplasia or other eye damage reach menarche earlier than girls with light perception (Wurtman, 1969).

Short daily photoperiods or removal of the eyes of male and female golden hamsters resulted in the atrophy of the reproductive organs (Hoffmann et al., 1965). This effect, however, was prevented if the animals were pinealectomized (Hoffmann and Reiter, 1965b; Reiter et al., 1966; Reiter, 1967; Clabough and Seibel, 1968; Seibel and Bush, 1970). Atrophy was not observed in blinded animals sympathetically denervated by bilateral superior cervical ganglionectomy (Reiter and Hester, 1966). Constant illumination results in vaginal cytological changes of "persistant vaginal estrus" in the rat; this effect can be modified by pineal extracts (Jochle, 1956). According to Kinson and Robinson (1970), many of these responses may be animal and species specific. They have shown a difference in response in different strains of rats. In one strain of rat, light restriction affected pituitary weight but not seminal

vesicle, prostate or testis weight; in another strain, however, seminal vesicle and prostate weights were affected but not testes nor pituitary glands.

Reiter (1967) found that blinding, short light cycles, and pinealectomy did have an effect in NIH adult black rats. There was a significant difference in the weight of the accessory sexual organs, adrenals, and pituitaries between sham-pinealectomized and pinealectomized adult males kept in a short photoperiod. Blinded-sham-pinealectomized and blinded-pinealectomized females also responded positively by a significant difference in uterine and pituitary weights. In contrast, blinding or short photoperiods, with or without pinealectomy, did not affect the testes, accessory organs, adrenals, thyroids, pituitaries, ovaries, or uteri of adult albino rats. There was also no significant effect on the same structures in prepubertal or postpubertal male and female albino mice. In the male gerbil, however, optic enucleation did cause a decrease in the weight of the accessory sexual organs, but this effect was normalized by pinealectomy.

Androgen and estrogen sterilized animals have been used in studying the reproductive effects of the pineal gland and its relationship to light. The injection of gonadal steroids in the first weeks of life interferes

with the development of the preoptic-anterior hypothalamic area (Gorski, 1966; Barraclough, 1967). Animals thus treated have gonads and secondary sexual glands in adulthood which are significantly smaller than these same organs in untreated animals (Jacobsohn and Norgren, 1965). Kordon and Hoffman (1967) and Hoffman et al., (1968) have shown that in testosterone propionate treated animals the reproductive organs were very sensitive to the lack of light. Reiter et al. (1968, a,b,c) have shown that the additional reduction of the reproductive structures caused by a lack of light in androgen sterilized male and female rats could be overcome by pinealectomy.

Many authors investigating pineal-gonadal functions have employed anosmia in their studies since olfaction is known also to play an important role in mammalian reproduction (Parkes and Bruce, 1961; Bronson, 1968). Whitten (1956) has shown the importance of intact olfactory bulbs on the reproductive organs of mice since olfactoriectomy resulted in involution of the ovaries and uteri after six weeks. Olfactoriectomized immature rats demonstrated a lack of sexual maturation which was obviated by pinealectomy (Reiter, 1969). Adult rats, however, appeared to be less sensitive to removal of the olfactory bulbs.

Anosmic female rats showed no change in the frequency of ovulation, although the ovaries appeared to have slight morphological variation (Aron et al., 1970). No changes in the reproductive organs were observed to result from anosmia in males (Reiter et al., 1969).

Reiter (1967) has also shown that anosmia caused a uterine weight decrease which was reversable by pinealectomy in several experiments with adult female golden hamsters. One series of female and male golden hamsters did not exhibit a decrease in the reproductive organs, adrenals, or pituitaries with anosmia.

The Purpose of the Project

The purpose of this project was first to describe the epithalamus of different species of rodents, many previously undescribed. The gross morphology of the pineal gland, stalk, and pineal sac was investigated in the albino rat, kangaroo rat, golden brown hamster, Chinese hamster, PET mouse, and the monogolian gerbil. This was believed to present a general cross section of pineal morphology and related structures in the order, rodentia.

Secondly, the ultrastructure of the pineal glands of normal and blinded PET mice and gerbils was studied. These animals were chosen because at the commencement of this study the normal ultrastructure of the mouse pineal had not been described. To the present time, there has not been an ultrastructural study of the pineal of the blinded mouse, normal gerbil, nor blinded gerbil. The ultrastructure of the pineal sac, also previously unrecorded, was investigated.

Thirdly, a series of physiological experiments were performed to determine the effects of different parameters on pineal-endocrine functions. Adult male albino rats and golden hamsters were pinealectomized, blinded, hemicastrated, blinded-pinealectomized, hemicastrated-pinealectomized, hemicastrated-blinded, or hemicastrated-blinded-pinealectomized. The effects of these procedures were determined by the gross morphology, weight and histological appearance of the testes, accessory reproductive glands, adrenals and pituitaries. The same procedures, with the exception of anosmia replacing hemicastration, were performed in prepubertal male PET mice, adult male and female PET mice, prepubertal and adult testosterone proprionate treated (TP) male PET *mice, and* both male and female adult gerbils. This wide spectrum

of age, sex, and treatment was used in order to complete and correlate the different facets of pineal-endocrine function.

MATERIALS AND METHODS

The animals used in these experiments were procured from various sources. Albino rats (Wistar strain) and golden hamsters (Mesocricetus auratus) were obtained from the Lakeview Farms, Lakeview, New Jersey. Mongolian gerbils (Meriones unguiculatus) were purchased from Mr. Lester Embrey of Kansas City, Missouri. PET mice were generously supplied by Dr. Willie M. Reams, Jr., Department of Biology, University of Richmond. These mice are specific strain (PET/Wmr) which originated from a mixed stock of C3H and black petshop mice and are characterized by pigmented extraepidermal tissues and occasional mammary tumors in older females. The strain has been inbred to an extent of F80 by Dr. Reams and is a registered strain of mouse (Reams, 1967).

Animals for a particular experimental procedure were housed 5 to 8 animals per cage. Food and water were supplied ad libitum. The temperature in the animal room was maintained at approximately 22°C. During the entire experiment fluorescent lighting was regulated in the animal room in light-dark cycles of 14 hours of light

and 10 hours of darkness (lights on at 6 a.m. and off at 8 p.m.).

For pinealectomy the basic technique of Hoffmann and Reiter (1965a) was used. Sodium pentobarbital anesthetized animals (6 grains/100 gm body weight) were placed in a head holder apparatus; and after a midline skin incision was made to reveal the underlying skull, a circular area of bone (5 mm in diameter for the albino rat and golden hamster, 3 mm for the PET mouse and Mongolian gerbil) was removed from the cranial vault utilizing the lambda as a central point (Figure 1a, b, c). After piercing the superior sagittal sinus with jewelers forceps in the region of the confluence of sinuses, the pineal complex was grasped and removed. After removal of the pineal organ with fine watchmakers forceps, the circular area of bone was replaced, antibiotic powder was applied and the skin incision was closed with 9 mm animal surgical clips. For olfactoriectomy, the above modified drill saws were utilized for exposure of the olfactory bulbs; they were removed by suction with a Pasteur disposable pipette attached to a plastic hose which was connected to a water flow aspirator. In animals on which both pinealectomy and olfactoriectomy were performed, the two procedures

were separated at 3 day intervals to allow blood volume to return to normal levels after the initial surgery.

Orbital enucleation was accomplished by exerting pressure with open fine scissors around the eyes and severing the optic nerve after the pressure of the scissors elevated the eyes from the orbit. In this manner complete orbital enucleation was performed without the possibility of light sensitive retinal tissue remaining. Blinding and olfactoriectomy or pinealectomy were performed simultaneously without increased incidence of death due to blood loss.

The basic experimental design utilized the following groups: normal, bilateral enucleation, pinealectomy, hemicastration, bilateral enucleation-pinealectomy, hemicastration-pinealectomy, bilateral enucleation-hemicastration, or bilateral enucleation-pinealectomy-hemicastration. In experiments on the gerbil and a series of PET mice, olfactoriectomy, replaced hemicastration in basic design.

A series of testosterone treated animals was also included in the experiments. These animals were given 1 mg of testosterone propionate (Oreton® - Schering Corporation) in 0.5 ml of peanut oil on the third day

after birth. The injection was given subcutaneously in the suprascapular region. Upon reaching adulthood, these animals were sham pinealectomized, blinded, pinealectomized, or blinded-pinealectomized.

All animals were initially weighed and the experimental procedures were carried out under anesthesia. The animals were then maintained for 8 weeks in the animal rooms previously described. After 8 weeks, the animals were anesthetized, weighed, and decapitated. The cranial vault was opened by two lateral incisions with scissors through the foramen magnum. As the cranium was elevated, the pineal and a great portion of the stalk would adhere to the confluence of sinuses on the undersurface of the skull and could be easily removed and fixed for electron microscopy studies. At autopsy this procedure also served as a means of confirming the removal of the pineal in pinealectomized animals and the removal of the olfactory bulbs in the olfactoriectomized animals. The remaining brain tissue could then easily be scooped out of the cranial vault and fixed for histological confirmation of pinealectomy, thus causing a separation of the hypophysis from the rest of the brain in the area of the infundibulum. The meninges surrounding the hypophysis in the sella turcica could then be dissected free and the hypophysis

easily removed. The hypophysis was fixed in Bouin's fixative for 24 hours and weighed on a Roller-Smith balance (50 mg sensitivity) before the tissue was processed for light microscopic evaluation.

After decapitation of the animal, the testes, accessory sexual glands (seminal vesicles and coagulating glands), and uteri were also dissected free of all fat and connective tissues and immediately weighed on a Mettler type H5 balance. The ovaries and adrenal glands were weighed with a Roller-Smith balance.

The organ weights, expressed as percentages of the body weights, were then analyzed using an Olivetti-Underwood programma 101 desk computer. The Student's "t" test was used for statistical analysis.

In order better to preserve the anatomical relationships of the epithalamus area of the brains for gross morphological observations, a different procedure was used. After anesthetizing the animal several circular areas of bone were removed from the skull in the area of the pineal organ. The animals were perfused through the left ventricle of the heart first with 50 cc of physiological saline to exsanguinate the animals and then 50 cc of Bouins fluid by means of a syringe fitted with a 26 gauge needle.

After perfusion the animal was decapitated; the mandible, the anterior limits of the skull, the skin, muscles, and connective tissues of the skull were removed and the remaining brain and skull were placed in Bouin's fixative for 24 hours to assure adequate preservation. Following this procedure, the remaining cranium carefully was separated from the underlying meninges and a 0.6 mm square section of brain tissue including the occipital lobe of the brain, anterior parts of the cerebellum and underlying diencephalon and midbrain was removed and placed for another 24 hours in Bouin's fixative. This block of tissue then was processed histologically for light microscopy; and the anatomical relationships between the telencephalon, diencephalon, midbrain, cerebellum, pineal, pineal stalk, pineal sac, and meninges were well preserved. The author is endebted to Dr. Russell Reiter in whose laboratory the slides of this area of the brain of the Chinese Hamster, golden hamster, albino rat, and kangaroo rat were prepared and examined by the author.

The organs used for light microscopic studies were all histologically processed in the following manner. After fixation in Bouin's fluid, the schedule for dehydration, clearing, infiltration, and embedding was as follows:

Fixation	24 hours
70% ETOH	overnight
80% ETOH	30 minutes
95% ETOH	30 minutes
95% ETOH	30 minutes
100% ETOH	30 minutes
100% ETOH	30 minutes
Toluene	15 minutes
Toluene	15 minutes
Paraplast	45 minutes
Paraplast	3 hours
Embedding	

The embedded tissues were then sectioned at 5μ on a Spencer Lens Co. # 820 microtome. The serial sections were then stained with hematoxylin and eosin or Masson's Trichrome Stain (Luna, 1968).

The H and E staining procedure followed was:

1) Deparaffinization and hydration through an alcohol series to 100% H_2O
2) Stain in Delafield's hematoxylin - 3 minutes
3) Wash in tap water - 5 minutes
4) 35% ETOH - 1 minute

50% ETOH - 1 minute

70% ETOH - 1 minute

80% ETCH - 1 minute

alcoholic eosin - 1½ minutes

95% ETOH - 2 minutes

95% ETOH - 3 minutes

100% ETOH - 2 minutes

100% ETOH - 2 minutes

xylene I - 2 minutes

xylene II - 3 minutes

The Masson Triple Staining procedure followed was:

Xylene I - 5 minutes

Xylene II - 5 minutes

Dowanol I - 5 minutes

Dowanol II - 5 minutes

Hydration in distilled water - 5 minutes

Weigert's iron hematoxylin - 10 minutes

Wash in running water - 10 minutes

Rinse in distilled water - 1 minute

Biebrich scarlet-acid fuchsin stain - 2 minutes

Rinse in distilled water - 1 minute

Phosphomolybdic-phosphotungstic acid solution - 10 minutes

Analine blue - 15 minutes

Rinse in distilled water - 1 minute

Dowanol I - 1 minute

Dowanol II - 1 minute

Xylene I - 2 minutes

Xylene II - 3 minutes

All stained slides were mounted with Permount.

The pineal gland, pineal stalk, and pineal sac which were studied electronmicroscopically were processed in the following manner. Once removed from the overlying meninges, they were immediately fixed in a 3% glutaraldehyde solution of pH 7.3 at 0°-4°C for 1½ to 2 hours. The tissues then were placed in 3 changes of 0.1 M phosphate buffer (pH 7.3) at 0°-4°C. While still in cold buffer wash, the structures to be studied were separated from extranously adhering connective tissues and meninges and post-fixed for 1½ hours at 0°-4°C in a 2% solution of osmium tetroxide. Following post-fixation, the tissues were washed in buffer, dehydrated and cleared in accordance with the following schedule:

50% acetone - 10 minutes - 0°-4°C

50% acetone - 10 minutes - 0°-4°C

70% acetone - 10 minutes - 0°-4°C

85% acetone - 10 minutes - 0°-4°C

95% acetone - 10 minutes - 0°-4°C

95% acetone - 10 minutes - 0°-4°C

100% acetone - 10 minutes - room temperature

100% acetone - 10 minutes - room temperature

100% acetone - 10 minutes - room temperature

100% Propylene Oxide - 10 minutes - room temperature

1:1 Propylene Oxide - Durcupan mixture - overnight

Durcupan was used as the embedding material and was mixed in the following proportions:

Solution A (Epoxy resin) - 10 ml

Solution B (Hardener 964) - 10 ml

Solution C (Accelerator 964) - 0.25 ml

Solution D (Plasticizer) - 0.3 ml

After the 1:1 Propylene Oxide-Durcupan mixture treatment, the tissues were placed in a 100% Durcupan mixture for 8 hours, transferred to another Durcupan mixture overnight, and embedded for 72 hours in a pure fresh Durcupan mixture in Beem capsules at 60°C. The first few hours of this final embedding procedure were carried

out in a 60°C vacuum oven to help eliminate any air bubbles which may have been present.

The embedded tissues were then roughly trimmed with a razor blade, and 1μ thick sections were cut on a Porter-Blum MT 2 ultratome with glass knives broken on a LKB Knifemaker. The thick sections were stained with a 0.1% aniline blue solution and studied with an ordinary light microscope for orientation purposes.

Thin sections were then cut from the block of tissue utilizing the above microtome and a DuPont diamond knife. The thin sections were collected on 300 mesh copper grids. They were stained for 5 minutes with the lead citrate stain (Reynolds, 1963). The tissue then were studied and photographed with a RCA-EMU 3G electron microscope.

RESULTS

Gross Morphology of the Pineal Complex

PET Mouse

The pineal gland of the PET mouse is a solid parenchymous mass which originates from the diencephalon between the habenular commissure and the posterior commissure (Figure 2). Its length varies from 850μ to 1200μ and it is usually narrower near its proximal end which connects this structure with the commissures. The distal portion is enlarged and measures 350μ in diameter. There is always found a prominent pineal recess which is an extension of the third ventricle into the pineal body. It is lined with ependyma which is continuous with the habenular commissure and posterior commissure. This ependyma is specialized in the diencephalic roof posterior to the pineal recess and inferior to the posterior commissure to form the subcommissural organ. This structure, a secretory epithelium, is composed of simple columnar cells which are believed to release substances which affect water balance and sodium depletion.

The pineal gland parenchyma is composed of pinealocytes whose cell boundaries are difficult to distinguish histologically; the parenchyma has an epitheloid appearance. The cytoplasm is light staining, and the nuclei are oval or round and often appear indented. Most nuclei contain a prominently staining nucleolus.

The pineal sac, which is an extension of the choroid plexus of the third ventricle, forms the roof of the diencephalon rostral to the habenular commissure and pineal body. Only a small area of the sac is contiguous with pineal tissue. It is always found caudal to the corpus callosum of the cerebrum.

The venous drainage of the pineal is via the vena cerebri magna which adhers to the dorsal surface of the pineal. This large vein empties into the superior sagittal sinus before the latter vessel enlarges to form part of the confluence of sinuses (Figure 3).

Gerbil

The gerbil pineal is an elongated solid parenchymous structure which varies in length from 2400 μ to 2800 μ. The enlarged mass of pinealocytes superior to the habenular commissure measures 200 μ to 230 μ in thickness with a 50 μ to 100 μ thick stalk which follows the superior

surface of the midbrain. The dorsal sac is contiguous to the enlarged superhabenular pinealocyte mass but rarely abuts the pinealocyte stalk. As in the PET mouse, this sac is continuous with the choroid plexus of the roof of the anterior diencephalon. The cells have the same appearance as the cells of the dorsal sac of the PET mouse (Figure 4). The stalk terminates with a distal 280 μ to 350 μ pineal enlargement which is attached to the confluence of sinuses. The most enlarged area of the pineal gland and the most superficial parts of the pineal stalk are characterized by numerous circular intercellular spaces (Figure 5).

The vascular drainage of the epiphysis in the gerbil is by means of the vena cerebri magna which is dorsal to the dorsal enlargement of the pineal and the stalk. This vessel enters into the confluence of sinuses.

Golden Brown Hamster

The pineal complex in the golden brown hamster is composed of a deep pineal and superficial pineal organ with a connecting stalk composed of blood vessels, nerves and pineal sac. Cells with the appearance of those of the pineal parenchyma are occasionally found in the stalk

(Figure 6). The deep pineal component spans the area between the habenular and posterior commissures, averages 80 μ to 280 μ in thickness, and has a prominent pineal recess. The superficial pineal component, a large oval structure varying in length from 600 μ to 820 μ is connected to the deep portion of the gland by means of a 2700 μ to 3200 μ interconnecting stalk.

The pineal sac which arises as an extension of the tela choroidea of the roof of the third ventricle abuts the deep pineal portion, the pineal stalk, and the dorsal anteriormost portion of the superficial pineal component (Figure 7). This pineal sac is highly convoluted, possesses many villi which are lined with a simple cuboidal epithelium and contain a highly vascularized connective tissue core. The cells of the simple cuboidal epithelium average 11 μ in length and possess large, round, homogeneous, basally oriented nuclei which measure 6 μ in diameter. Histologically, the structure of the sac is identical to the choroid plexus (Figures 8 and 9).

In pinealectomy, the superficial pineal, pineal stalk, and pineal sac are removed. The pineal sac is separated from the co-extensive tela choroidea of the roof of the third ventricle in the area of the anterior habenular nuclei. In the process of pinealectomy in the golden

brown hamster, the deep pineal is rarely affected; post-surgically the roof of the third ventricle of the brain, in the area of the former dorsal sac, reforms with simple squamous epithelium.

Chinese Hamster

The pineal complex in the Chinese hamster is also composed of a deep pineal component joined by means of a pinealocyte stalk to a superficial portion (Figure 10). The superficial portion is oval in shape and ranges between 800 μ to 1300 μ in length and 350 μ to 735 μ in diameter. The deep component is a mass of pinealocytes which extend into the stalk and form a continuous, although narrow, parenchymal cell mass stalk connecting the deep and superficial parts of the pineal gland complex.

Histologically the pineal sac appears to be the same as the choroid plexus, except that the sac contains some cells which appear to be more active, with larger nuclei and less eosinophilic staining material in the cellular apices. The sac extends over the deep pineal portion and pinealocyte stalk but is always separated from the stalk by a prominent dorsal vessel, the vena cerebri

magna (Figure 11). The pineal sac in a few animals extends into the area of the superficial pineal component.

The Albino Rat

The albino rat possesses a pineal organ which varies in length between 1600 μ to 1950 μ with an average diameter of 820 μ. This pineal organ is comparable to the superficial portion of the pineal complex of the golden brown hamster. The rat pineal organ is connected by means of a connective tissue stalk which is a few cells thick and which occasionally possesses pinealocytes reaching the area of the dorsal surface of the diencephalon between the habenular commissure and posterior commissure. This area of the brain, which would be homologous to the area of the deep pineal gland of the golden brown hamster, appeared to lack a deep portion of the pineal complex or quantitative mass of pineal parenchyma cells, although some pinealoblast-like cells were present. Most conspicuously absent in the albino rat is a pineal recess. The habenular commissure is directly fused to the posterior commissure in the diencephalic roof (Figure 12). The pineal sac, moreover, consistently remained in the area of the connective tissue stalk and never abutted the

pineal organ itself.

The Kangaroo Rat

The kangaroo rat has a pineal organ which varies in length from 1200 μ to 2200 μ. The pineal organ is greatly enlarged at its most caudal end and thins out progressively more rostrally until it forms a pineal cell stalk. This pinealocyte stalk then connects to a sheet of pineal cells lying between the habenular commissure and the posterior commissure.

There is a pineal sac which abuts only the pineal stalk at its most anterior part (Figure 13). This sac was histologically identical to the choroid plexus. The nuclei of these cells differed from the nuclei of the sac cells of the other animals investigated by having more intensely staining, prominent nucleoli.

RESULTS

Ultrastructure of the Pineal Gland

Normal PET Mouse

The parenchyma of the pineal gland of the PET mouse is composed primarily of pinealocytes. Ultrastructurally, two pinealocytes are distinguishable: light cells and dark cells (Figure 14). This distinction is based upon the general electron density of the background cytoplasms of the two cell types. They do not differ in regard to nuclear or cytoplasmic structure or contents and probably represent different functional states of the same cell type. These pinealocytes are arranged rather haphazardly, and there does not appear to be any organization or orientation of the cells. The cells are not linked by desmosomes or other types of cellular junctions.

The pineal gland also has a great capillary network. The capillaries are composed of endothelial cells which are fenestrated. These fenestrations are closed by thin diaphragms (Figure 15). The capillary endothelial cells have a basal lamella and are surrounded by a perivascular space which frequently contains autonomic nerve fibers.

Pinealocytes are large polymorphic cells generally possessing highly convoluted large sized nuclei with a prominent nucleolus (Figure 16). There are pores appearing in many areas of the nuclear membrane. Generally the chromatin of the pinealocyte nucleus is homogeneous in appearance and resembles that of interphase nuclei. No mitotic figures were seen in any of the pinealocytes which were studied.

The cytoplasm generally contains smooth endoplasmic reticulum, although some rough endoplasmic reticulum is present. Free ribosomes (frequently found in rosette patterns), glycogen, and occasional lipid droplets are present in the cytoplasm. As in other cell types, pinealocytes have a Golgi body and a moderate number of mitochondria. The mitochondria vary in size from 0.25μ to 1.2μ and have irregularly arranged cristae. "Bulls-eye" mitochondria can often be seen.

The cytoplasm also contains vesicles, many of which exhibit an electron dense core. These membrane bound granulated vesicles vary in diameter from 800 $\mathring{A}$ to 1600 $\mathring{A}$ with a darkly staining core of 300 $\mathring{A}$ to 700 $\mathring{A}$ (Figure 17). Some authors believe that the dense core vesicles represent the pineal secretory products.

The pinealocytes have many complex cytoplasmic protrusions, called polar processes. These processes extend great distances from the perikaryon of the cells and intermingle with polar processes of other cells (Figure 18). The polar processes end as polar terminals which may abut the perikaryon of other pinealocytes (Figure 19), or more frequently, end in the pericapillary space of the pineal gland. The polar terminals which end on the membranes of other cells do so without a cellular junction or an accumulation of vesicles.

The polar terminals of the pinealocytes which end in the perivascular spaces of the gland frequently have an accumulation of the electron dense core vesicles (Figure 20). Many authors believe that these membrane bound substances are secreted into the perivascular space where they may then have access to the capillaries through the fenestrated endothelial cells.

Extensions of the autonomic nerve fibers from the superior cervical ganglia are also found in the perivascular space. These unmyelinated sympathetic nerve fibers are identified by small (250 Å to 500 Å) clear and granulated vesicles. Generally such adrenergic nerve fibers are encased in a Schwann cell (Figure 21) and are usually distinguishable from pinealocyte polar terminals by the

small size and arrangement of the vesicles and by their microfilamentous array in longitudinal sections. Myelinated nerve fibers frequently are found in the pineal capsule (Figure 22) but were not seen in the pineal gland itself.

The second cell type found in the PET mouse pineal gland is the interstitial cell. These small cells with attenuated cytoplasm most frequently are found in the capsule, intercellular spaces, and perivascular spaces. The cytoplasm of these cells exhibits abundant rough endoplasmic reticulum, a prominent Golgi body, and mitochondria with complexly structures cristae. The cells are characterized by nuclei with perinuclear chromatin distribution and many large chromatin aggregations throughout the nucleus. Often they are found in close proximity to collagen fibers (Figure 23).

No noticeable difference between pineal complexes of male and female animals was discernable. This was true without regard to the season of the year.

Enucleated PET Mouse

The pineal gland of the blinded PET mouse is very similar to the gland of the normal PET mouse. Light and dark cell pinealocytes are readily distinguishable by

cytoplasmic electron densities. Their arrangement and relationships to the capillaries and autonomic fibers in the pericapillary spaces appear the same. The interstitial cells, the second cell type found in the PET mouse pineal gland, and the autonomic nerve fibers appear identical in the pineal gland in normal and blinded mice. Astrocyte appearing cells were never seen in the pineal organs of normal or experimental animals. Two differences, however, do appear in the cytoplasm of the blinded mouse pinealocytes. The pinealocytes of the blinded mice have a greater number of large lipid droplets present and also have a great amount of small sized vacuoles in the cytoplasm (Figure 24).

The small sized vacuoles appear to have a prominently staining membrane. The vacuoles very frequently are found in close proximity to the outer mitochondrial membrane (Figure 25).

The other cytoplasmic structures of the blinded PET mouse pineal gland are similar to the normal gland. There may be, however, a greater number of small lipid droplets and a greater percentage of "bulls-eye" appearing mitochondria in the blinded PET mouse pinealocytes than in the normal animals. The dense core vesicles found throughout the cytoplasm of the normal pinealocyte appear at normal frequency in the perykaryon, polar processes, and polar

terminals of the blinded animals. The autonomic nerve fibers in the pericapillary space do not deviate from the normal in appearance (Figure 26).

Two of the blinded PET mouse pineal glands showed a remarkable modification of the nuclear membrane. The nuclear membrane, at different areas of the same nucleus, was lamellated and gave a myelin figure appearance (Figure 27).

Normal Gerbil

The normal gerbil pineal gland is similar in general organization to that of the PET mouse pineal gland. It is composed primarily of pinealocytes with polar processes; some interstitial cells are located primarily near the perivascular and intercellular spaces, and a capillary network surrounded by a pericapillary space containing autonomic nerve fibers is prominent.

The gerbil pineal gland, however, does differ in some respects from the PET mouse gland. The capillaries of the gerbil pineal gland are less numerous, and the endothelium lacks fenestrations. The gland of the gerbil also appears to have greater areas of intercellular spaces and a greater supply of autonomic nerve fibers than the gland of the PET mouse.

The gerbil pineal gland is composed of light and dark pinealocytes. The nuclei are pleomorphic and generally have an homogenous appearance with a prominent nucleolus (Figure 28).

The cytoplasm of the light cells primarily is made up of smooth endoplasmic reticulum which appears highly vesiculated. The dark cells appear to have more rough endoplasmic reticulum. Mitochondria have irregularly arranged cristae and frequently appear in close relationship to very darkly staining electron dense granules. Multiple Golgi bodies are found often, and a centriole may be observed occasionally (Figure 29). All normal gerbil pinealocytes have great accumulations of glycogen.

The cytoplasm of both the light and dark pinealocytes have two types of electron dense granules. The diameters of the larger granules varied in size from 1400 Å to 2800 Å and the smaller from 530 Å to 790 Å (Figure 30). It was very difficult to ascertain if either was membrane bound. The larger dense granules appeared similar to those found in close proximity to the mitochondria. The smaller granules appeared similar to granules of the same size found in the intercellular and perivascular spaces (Figure 31). They were more commonly found in the light

pinealocytes. Unstained tissues were also studied to confirm that these granules were not lead stain precipitate.

The dense core vesicles frequently found in the PET mouse pinealocytes were very rarely seen. In the PET mouse, the polar terminals of the pinealocytes had great accumulations of these vesicles while the gerbil seemed to have larger accumulations of small clear vesicles with the granular vesicles. The cytoplasm of the polar terminals appeared similar to the cytoplasm of the perykaryon.

The light pinealocytes were found in different stages which varied in appearance from the light cells closely resembling the dark cells to those which almost gave the appearance of degeneration or poor fixation. These latter cells had a cytoplasm which appeared "washed out"; that is, they lacked a well-developed endoplasmic reticulum, had little glycogen, light appearing nuclei, bizarre mitochondria, and an increase in the number of small granules (Figures 32 and 33).

Thick sections of the gerbil pineal organ which were used for orientation during ultramicrotomy revealed a similar picture of the different stages of the light cells. In some cells only a pyknotic nucleus was seen in a space

surrounded by other normal appearing cells. These intercellular spaces in the glutaraldehyde fixed gerbil pineal appeared similar to the intercellular spaces found in the gerbil pineal glands which had been fixed in Bouin's solution for light microscopy. These intercellular spaces were mentioned previously in this thesis as being characteristically found in gerbil pineal glands, q.v. Figure 5.

The pineal glands of both male and female gerbils were studied, and no sexual dimorphism was observed.

Blinded Gerbils

The pineal glands of enucleated gerbils differed from those of normal and blinded PET mice and of normal gerbils by the presence of large circular electron dense structures. These granules were found primarily in cellular processes in the perivascular spaces (Figure 34). Since serial sections of pineal tissues were not cut, the cells of origin of these processes could not be determined. However, both interstitial cells (Figure 35) and pinealocytes (Figure 36) were occasionally seen to have these large granules. It could not be determined if these large electron dense granules were limited by a unit membrane.

The granules were not homogeneous in appearance, and some appeared to be made up of smaller circular units (Figure 37).

In addition to large granules, the pinealocytes of the blinded gerbils differed from those of the normal gerbil by lacking the vast accumulation of glycogen granules which characterize the normal gerbil pineal cell.

The remainder of the blinded and normal gerbil pineal glands appeared the same. Pinealocytes contained the large and small granules previously described. The variation of stages of the light pinealocytes were identifiable, and autonomic nerves appeared identical in the normal and experimental group.

The Pineal Sac

The pineal sac is a large villous structure lined with a simple cuboidal epithelium and contains a highly vascularized connective tissue-capillary core. The epithelial cells are joined by junctional complexes (Figure 38).

The epithelial cells contain large convoluted nuclei. The cytoplasm contains endoplasmic reticulum primarily of the rough variety and the other usual cytoplasmic organelles. Mitochondria with lamellar cristae are numerous.

The free surface of the epithelial cells is characterized by numerous microvilli which have a very irregular orientation. Occasionally these cells also have cilia (Figures 38 and 39). The basal portions of the epithelial cells contain a series of highly convoluted membranes and are near perivascular cell processes which contain free electron dense granules (Figures 38 and 40). These structures suggest a possible secretory function for the pineal sac epithelial cells.

The capillaries in the connective tissue core of the pineal sac are thin walled and have numerous fenestrations (Figure 41). The fenestrations are bridged by thin diaphragms.

Thus, the ultrastructure of the pineal sac is similar to the ultrastructure of the choroid plexus of the diencephalon. Since these two structures are continuous, it appears that the pineal sac constitutes an extension and continuation of the choroid plexus rather than an unique, physiologically significant structure.

RESULTS

The Pineal Gland and the Endocrine System.

The Hamster

Fifty-two male golden brown hamsters were treated surgically with the following procedures: pinealectomy, hemicastration, blinding, pinealectomy-blinding, pinealectomy-hemicastration, hemicastration-blinding, and hemicastration-pinealectomy-blinding. There was a significant decrease in the weights of the testes and seminal vesicles (including coagulating gland and ventral prostate), expressed as proportions of the body weight in the blinded and hemicastrated-blinded animals (Figure 42). There was also a significant decrease in pituitary gland weight in the blinded animals (Table I).

These results indicate that after blinding there is a decrease in the weight of the testes and seminal vesicles. This decrease in weight is not prevented by hemicastration. Pinealectomy does prevent the decrease in testis and seminal vesicle weights caused by blinding, the blinded-pinealectomized animals showing normal weights of the testes and seminal vesicles.

TABLE I

EFFECTS OF VARIOUS TREATMENTS ON SEVERAL ORGANS IN THE MALE GOLDEN HAMSTER

Treatments	Number of Animals	Body Weight Grams	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Pituitary	Adrenal
Control	10	106±8[1]	1452±66	956±136	3.2±0.2	9.1±0.9
Pinealectomy	6	115±5	1285±36	805± 78	3.0±0.2	7.9±0.8
Hemicastration	6	124±5	1523±32	766±102	2.7±0.5	8.3±0.5
Blinded	6	121±5	184± 8[2]	238±5[3]	1.6±0.1[4]	9.0±0.3
Pinealectomy Blinded	6	123±6	1239±28	853±92	2.2±0.7	8.4±1.0
Pinealectomy Hemicastration	6	122±6	1529±154	825±139	2.2±0.1	8.5±1.0
Hemicastration Blinded	6	119±6	209±16[2]	363± 59[3]	2.7±1.8	9.1±0.5
Hemicastration Pinealectomy Blinded	6	119±7	1561±147	1008± 83	2.1±0.1	9.4±0.5

[1]Values are Means ± Standard Error
[2]Significant difference: $p = <0.001$
[3]Significant difference: $p = <0.01$
[4]Significant difference: $p = <0.05$

Histologically, the testes of the control animals are characterized by large seminiferous tubules with prominent lumina that are filled with spermatozoa. The seminiferous tubules are widely separated by connective tissue. Each tubule shows the typical arrangement of spermatogonia nearest the basement membrane, and a more luminal arrangement of primary and secondary spermatocytes, spermatids, and spermatozoa. So many spermatogenic cells are present in each seminiferous tubule that it is extremely difficult to distinguish the supporting Sertoli cells. The mitotic rate is high, indicating very active spermatogenesis (Figure 43).

The testes of the blinded and hemicastrated-blinded male golden hamsters are histologically different from the control animals. The seminiferous tubules are much smaller and are arranged more closely together. The Sertoli cells appear attached to the basement membrane. They are distinguishable as large cells which possess normal big, round to oval nuclei; they have cytoplasmic projections which extend towards the center of the tubules and frequently obliterate the lumen. The only spermatogenic cells present are spermatogonia and primary spermatocytes. The numbers of these cells are minimal, and they are

frequently found towards the center of the tubule. Many cells appear to have an unstained halo surrounding them, which may indicate a degeneration of the cytoplasm. Spermatids and spermatozoa are not found in the testes of the blinded or hemicastrated-blinded animals (Figure 44). This histological appearance is consistent with inactive, atrophic testes.

The histology of the testes of the hemicastrated, pinealectomized, pinealectomized-hemicastrated, pinealectomized-hemicastrated-blinded, and blinded-pinealectomized animals appeared to be identical to the untreated controls.

When compared with the normal control and other experimental animals, the involuted seminal vesicles of the blinded and hemicastrated-blinded animals appeared to be infantile. The lining epithelium was of the low cuboidal type and there was very little stainable material in the lumina of the glands. The seminal vesicles of the control animals were lined by a columnar epithelium with great amounts of basophilic staining material, suggesting that these seminal vesicles were highly secretory.

Although the size of the pituitary gland was significantly decreased in blinded hamsters, it was difficult to varify adenohypophyseal activity by histological techniques. A variability of uptake and intensity of staining made positive identification of pituitary cell types extremely uncertain. Even when cell types were easily distinguishable, there was uncertainty as to the specific sub-type of cell. We were not able clearly to distinguish the β-basophil (the presumed thyrotroph) from the δ-basophil, the putative gonadotrophin secreting cell.

The adrenal glands of all male hamsters studied showed no histological changes. The cortico-medullary ratio was similar, and the staining affinities were identical.

The Albino Rat

Adult male albino rats received operations similar to those performed in the golden brown hamster, i.e., animals were hemicastrated, blinded, pinealectomized, blinded-pinealectomized, blinded-hemicastrated, and pinealectomized-blinded-hemicastrated. There were no significant gross weight changes in the endocrine glands studied (Table II).

TABLE II

EFFECTS OF VARIOUS TREATMENTS ON ADULT MALE RATS

Treatments	Number of Animals	Body Weight	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Pituitary	Adrenal
Control	10	290±12[1]	510±40	130±12	3.0±0.3	7.0±0.9
Hemicastration	6	282±14	615±38	208±21	2.7±0.2	5.6±0.8
Blinded	6	296±19	528±65	142± 2	2.7±0.1	5.5±0.2
Pinealectomy	6	356±65	355±46	200±20	3.2±0.8	4.9±0.5
Blinded Hemicastration	6	194± 5	548±54	176±20	3.1±0.4	9.4±0.8
Blinded Pinealectomy	6	370± 2	445±18	155±27	2.7±0.3	5.6±0.3
Blinded Hemicastration Pinealectomy	6	259±56	629±75	190±11	3.1±0.2	8.0±0.6

[1]Values are Means ± Standard Error

Although there were no significant differences under our specific experimental conditions, the results suggested several interesting trends. Hemicastrated animals showed an enlargement of testes and seminal vesicle size. The same effect appears in the pinealectomized-blinded-hemicastrated and blinded-hemicastrated animals. The trend towards compensatory hypertrophy appears to be the factor most influencing gonadal and accessory reproductive structure weights. Even though these figures did not reach significant levels, they suggested the capability and the sensitivity of the albino rat gonads to compensatory hypertrophy.

There were no qualitative or quantitative microanatomical changes in the testes, seminal vesicles, adrenals, or pituitaries in the control and experimental adult male albino rats studied.

Adult PET Mice

The basic experimental procedures as previously described were also performed on groups of adult male PET mice. These experiments were done at three different seasons of the year. The first experimental series of mice operations was processed in March, and the animals

were autopsied in April to encompass the season of spring. The second series of experiments were undertaken in July and August to reflect the summer months; the third series of experiments occurred in the winter months of December and January. The results are listed on Tables III, IV and V.

These results do not demonstrate any significant difference in the gross weight of the testes, seminal vesicles and pituitary glands with blinding, hemicastration, pinealectomy, pinealectomy-blinding, pinealectomy-hemicastration, blinding-hemicastration, or blinding-pinealectomy-hemicastration. Further analyses suggest further that there is no seasonal variation in the response of these animals. The control and experimental groups do not differ significantly in organ weight with each other during the different seasons of the year. Regardless of the operative procedures or the season of the year, the general gross and microanatomic appearance of these organs is one of relative consistancy.

The only indications of variation are the data concerning elevated adrenal weights of the pinealectomized-blinded-hemicastrated and pinealectomized-hemicastrated animals during the winter months of December and January,

TABLE III

EFFECTS OF VARIOUS TREATMENTS ON ADULT PET MICE DURING MARCH-APRIL (SPRING)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Pituitary	Adrenal
Control	5	29.9±2.1[1]	31.8±1.6	186±13	249±33	6.9±0.2	6.7±0.8
Blinded	5	32.3±0.7	33.2±0.7	202± 4	252±23	7.3±0.3	5.6±0.3
Hemicastration	5	30.5±1.0	32.2±1.0	207± 7	244±18	7.1±0.4	6.5±0.5
Pinealectomy	5	32.3±0.7	33.3±0.7	199± 7	201±15	6.5±0.3	6.1±0.4
Pinealectomy Blinded	5	30.0±0.9	32.0±0.4	211± 8	223±21	7.0±0.3	5.8±0.5
Pinealectomy Hemicastration	5	32.2±0.7	32.4±0.5	207±14	245±22	6.9±0.3	5.4±0.5
Blinded Hemicastration	5	32.7±0.5	33.7±0.6	189± 4	227±29	5.8±0.6	6.5±0.3
Pinealectomy Blinded Hemicastration	5	30.7±1.0	33.1±0.8	219±13	223±35	7.1±0.1	6.0±0.2

[1]Values are Means ± Standard Error

TABLE IV

EFFECTS OF TREATMENTS ON ADULT PET MICE DURING JULY-AUGUST (SUMMER)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Testis	Seminal Vesicles	Pituitary	Adrenal
Control	4	25.5±2.2[1]	29.3±2.1	226±16	264±54	6.8±0.7	7.2±0.4
Blinded	4	31.1±1.1	33.0±1.0	190± 5	240±40	7.8±0.5	8.1±1.3
Hemicastration	4	30.5±0.4	32.9±0.3	234±23	282±33	8.5±0.6	7.9±0.7
Pinealectomy	4	29.2±1.4	30.9±2.0	199± 8	228±10	8.5±1.6	8.8±0.1
Pinealectomy Blinded	4	27.2±2.3	25.4±1.3	236±23	265±35	8.8±2.2	9.9±0.2
Pinealectomy Hemicastration	4	28.4±1.4	29.4±1.8	202± 6	231± 5	8.6±1.4	9.4±0.2
Hemicastration Blinded	4	32.5±0.8	34.4±0.5	197±18	263±41	8.0±0.5	6.9±0.4
Pinealectomy Blinded Hemicastration	4	31.0±1.4	31.0±2.7	237±24	227±24	8.4±0.2	7.9±0.7

[1]Values are Means ± Standard Error

TABLE V

EFFECTS OF VARIOUS TREATMENTS ON ADULT PET MICE DURING DECEMBER-JANUARY (WINTER)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Pituitary	Adrenal
Control	5	25.7±0.4[1]	30.4±0.3	215± 8	252±29	6.7±0.2	7.4±0.7
Blinded	7	24.1±0.9	31.7±0.7	221± 9	258±27	6.5±0.1	7.7±0.2
Hemicastration	4	26.2±1.3	28.9±0.9	225±10	297±37	6.6±0.5	8.5±0.8
Pinealectomy	5	26.2±0.5	29.1±0.4	243± 6	295±14	6.9±0.2	9.1±0.8
Pinealectomy Blinded	5	24.6±0.2	29.3±0.3	231±18	213±10	6.4±0.4	9.1±0.8
Pinealectomy Hemicastration	4	25.5±0.7	29.2±0.5	241±14	232±12	6.6±0.1	9.8±0.4[2]
Blinded Hemicastration	6	25.3±0.5	30.0±0.5	249± 7	294±22	6.0±1.5	8.1±0.3
Pinealectomy Blinded Hemicastration	4	25.3±2.1	29.1±0.1	267± 8	241±33	6.3±0.4	10.0±1.0[2]

[1]Value is Means ± Standard Error

[2]Significant difference: $p = <0.05$

and the summer series of pinealectomized-blinded and pinealectomized-hemicastrated animals.

To study the possible effects of anosmia on mice, a group of adult male PET mice were blinded, olfactoriectomized, olfactoriectomized-blinded, and pinealectomized-olfactoriectomized-blinded. Table VI illustrates the results. As shown, there was no statistical difference in the testes, seminal vesicle, or adrenal gland weights.

Reiter (1967) observed that the lack of light and pinealectomy had an affect on black rats but did not affect albino rats. This difference in results led us to test the effects of blindness, anosmia, blindness-anosmia, pinealectomy, and pinealectomy-blindness on a group of wild mice. These mice were offspring of PET mice females and wild mice males. All the F_1 generation of the black PET mice and brown wild mice were brown.

The results, listed on Table VII, show no significant effects on the weight of the testes, seminal vesicles, nor adrenals. An interesting point to be observed with these animals is that the testes weights are significantly larger and the seminal vesicle weights smaller in milligrams per 100 grams of body weight in this hybrid than in the pure species of the PET mouse.

TABLE VI

EFFECTS OF VARIOUS TREATMENTS ON ADULT PET MICE

Treatment	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Adrenal
Control	6	32.4±1.4[1]	32.0±1.5	216±28	190±12	5.9±1.6
Blinded	6	31.8±1.3	34.1±1.9	206±29	212±26	6.9±0.7
Olfactoriectomy	4	28.0±2.3	27.4±3.4	226±21	182±23	6.9±1.2
Olfactoriectomy Blinded	4	29.3±0.8	30.3±2.6	215±15	209±33	7.7±0.4
Olfactoriectomy Blinded Pinealectomy	4	33.0±0.4	32.0±2.1	242±32	218±17	4.8±0.8

[1]Values are Means ± Standard Error

TABLE VII

EFFECTS OF VARIOUS TREATMENTS ON ADULT MALE WILD MICE

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Adrenal
Control	4	30.7±2.5[1]	33.2±0.6	300±29	193± 3	6.6±1.1
Blinded	4	30.3±2.9	27.9±6.0	294±45	196± 5	7.5±1.2
Olfactoriectomy	4	28.8±0.6	31.4±1.1	298± 5	201±25	7.5±0.4
Olfactoriectomy Blinded	3	29.0±1.4	31.6±1.8	277±18	206±18	7.3±0.8
Pinealectomy	3	26.8±3.1	27.1±2.1	312±22	203±11	6.4±0.6
Pinealectomy Blinded	3	29.3±1.9	31.0±0.9	291±33	197±14	6.9±1.0

[1]Values are Means ± Standard Error

Experiments were also performed on adult female PET mice to discern if the general pattern of a non-gonadal response to the absence of light was to be found in the female as well as in adult male PET mice. Four groups of animals were used; there were control, blinded, pinealectomized, and blinded-pinealectomized animals.

There was no significant differences in the weight of the ovaries, uteri, nor pituitary, and adrenal glands of these animals (Table VIII). Histologically, all organs of the control and experimental groups appeared normal. All of the ovaries had corpora lutea and follicles in different stages of development. The uteri were in different stages of the estrus cycles and all appeared to be functional. The pituitary and adrenal glands appeared remarkably similar.

Prepubertal PET Mice

When the adult PET mice demonstrated a complete refractiveness to those procedures which had an effect on the golden hamster (viz., atrophy of testes and seminal vesicles after blinding, and the albino rat, viz., hypertrophy of the gonads and accessory sexual structures after hemicastration), we decided to duplicate some of these

TABLE VIII

EFFECTS OF TREATMENT ON ADULT FEMALE PET MICE

Treatment	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Ovaries	Uteri	Pituitary	Adrenal
Control	5	25.8±1.7[1]	27.7±1.0	44± 6	426±90	8.8±0.3	13.9±1.1
Blinded	7	24.7±1.7	28.0±1.1	47± 3	375±27	7.6±0.4	12.9±0.8
Pinealectomy	6	23.2±1.7	25.6±0.8	55± 4	459±58	9.4±0.5	13.2±1.7
Blinded Pinealectomy	6	21.9±1.5	25.7±0.8	51± 2	360±25	8.6±0.9	14.7±1.9

[1]Values are Means ± Standard Error

procedures on a prepubertal series of animals. The experiments were made to determine if these various treatments would affect the prepubertal animal before the gonadal-pituitary axis of endocrine function had been completely established as it is in the adult animal. Table IX summarizes the results.

As in the adult PET mice, the testes, seminal vesicles, pituitaries and adrenals were not affected by the operations. The seminal vesicles of the blinded animals, however, did show a trend towards decreased weight, but the weight loss was not significant. At autopsy, after the animals reached maturity, there was no histological difference in these organs studied; they all appeared to be normal and functional adult structures.

Testosterone Propionate (T P) Treated PET Mice

Animals treated with gonadal steroids shortly after birth have testes and seminal vesicles which are very sensitive to the absence of light. Orbital enucleation in these animals results in a significant decrease in testicular and seminal vesicular weight. This weight decrease can be modified by removal of the pineal gland, a procedure which results in gonads which are similar in weight to TP-treated controls.

TABLE IX

EFFECTS OF VARIOUS TREATMENTS IN PREPUBERTAL PET MICE

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testis	Seminal Vesicles	Pituitary	Adrenal
Control	11	13.9±0.5[1]	25.5±0.4	249± 7	395±18	6.4±0.2	8.8±0.3
Blinded	11	14.7±0.9	26.9±0.4	239± 8	346±10	6.0±0.1	8.9±0.5
Hemicastration	10	15.1±0.6	24.9±0.6	271±14	365±14	6.5±0.2	9.3±0.5
Pinealectomy	10	16.0±0.8	26.6±0.4	268±14	399±21	6.8±0.4	8.7±0.5
Pinealectomy Blinded	10	16.5±1.3	25.6±0.4	263±13	384±28	5.7±0.3	8.0±0.3

[1]Values are Means ± Standard Error

Adult PET mice which are treated with 1 mg. of testosterone propionate on the third day after birth were surgically treated to see if the increased sensitivity to the absence of light would have an effect on the reproductive organs. Table X summarizes the results.

The TP-treated adult PET mice reflected the same non-responsive pattern to the lack of light as did the untreated animals and prepubertal animals in the previous experiments. The testes, seminal vesicles, pituitary glands, and adrenalglands exhibited no significant difference in weight. The weight of the seminal vesicles in the blinded animals were somewhat reduced, but not significantly. These results parallel the results of the experiments with the prepubertal PET mice.

The Gerbil

Two series of male and female gerbils were treated surgically. In the first series of gerbil experiments, the operations occurred in September and the autopsies were performed in November to reflect the Fall season. The second series of animals received treatment during the winter months. The operations were performed in January and the post mortem examinations occurred in March.

TABLE X

EFFECTS OF TREATMENTS ON TESTOSTERONE PROPIONATE TREATED PET MICE

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight)			
				Testis	Seminal Vesicles	Pituitary	Adrenal
Control	5	29.8±0.9[1]	32.1±0.6	187±12	344±15	6.5±0.3	6.9±0.6
Blinded	5	29.6±0.7	28.0±1.5	201±12	298±10	5.8±0.2	7.8±0.6
Pinealectomy	5	27.0±2.0	30.6±1.1	182±43	349±22	5.9±0.3	6.7±0.8
Blinded Pinealectomy	5	26.6±2.9	33.0±1.1	202± 7	330±30	5.9±0.6	7.6±1.3

[1]Values are Means ± Standard Error

In the male animals receiving treatment during the fall season, there were significant effects (Table XI). The testes and seminal vesicles of the blinded and olfactoriectomized-blinded animals were significantly of less weight than control animals. The weights of the seminal vesicles were significantly less, and the adrenals were significantly greater in olfactoriectomized animals. The pituitary organs of the gerbils were not weighed because of the difficulty of removing the total structure. Generally the pituitary glands fractured, and there was no assurance of accurately weighing the total organ.

The testes of the operated animals, showing a decrease in weight, also exhibited microscopic changes. The testes of the blinded and blinded-olfactoriectomized animals had less interstitial space and smaller tubules. All appeared to possess normal function as was evidenced by the presence of all types of spermatogenic forms. To quantitate these findings more accurately, the following procedure was used.

Ten sections of testes from each of the control animals and blinded-olfactoriectomized animals were compared. There were five counts taken per section to record the number of tubules present in a 10x field. The five counts were taken

TABLE XI

EFFECTS OF VARIOUS TREATMENTS ON MALE GERBILS IN SEPTEMBER (FALL)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testes	Seminal Vesicles	Adrenal
Control	8	47.4±3.1[1]	70.6±3.5	1509±34	223±20	22.8±0.8
Blinded	5	69.1±3.8	65.9±3.1	1044±130[2]	87±14[2]	23.7±1.2
Pinealectomy	5	54.2±5.6	73.1±3.3	1532±51	224±24	26.5±1.5
Pinealectomy Blinded	7	70.7±3.3	71.3±3.2	1586±68	208±28	25.1±2.1
Olfactoriectomy	5	65.4±2.1	72.7±4.1	1383±80	146±19[3]	29.1±1.3[3]
Olfactoriectomy Blinded	5	68.4±6.3	64.6±4.1	954±88[2]	61± 3[2]	26.0±2.9

[1]Values are Means ± Standard Error
[2]Significant difference: $p = < 0.01$
[3]Significant difference: $p = < 0.05$

at the upper, lower, both sides, and middle areas of each testis. The number of lumina in the seminiferous tubules with adult sperm cells were also recorded. Measurements of individual tubules and lumen were also made. The results indicate that the control testes had a mean of 46 ± 2 tubules per 10x field. The blinded-olfactoriectomized animals had a mean of 92 ± 3 tubules per 10x field. The control animals had 42 ± 3 % tubules with adult sperm present, while only 16 ± 1 % of tubules contained adult sperm in the blinded-olfactoriectomized animals. The seminiferous tubules of the control animals ranged from 16 μ to 26 μ in diameter and the experimental group from 10 μ to 17 μ in diameter. Thus the control group had larger tubules, fewer tubules per field because of greater amounts of interstitial space, and a greater percentage of tubules with adult sperm. None of our experimental procedures caused a complete cessation of spermatogenesis.

The seminal vesicles of the blinded, olfactoriectomized, and blinded-olfactoriectomized animals showed significant histological variation from the control animals. The seminal vesicles of the control animals had a highly folded mucosa of columnar epithelial cells forming many crypts. These cavities generally were filled with a densely staining material (Figure 45). The mucosa had a

thin lamina propria. Peripherally, there was also a thin muscular coat with circularly arranged fibers. The seminal vesicles of the blinded, olfactoriectomized, blinded-olfactoriectomized animals had a poorly developed mucosa lined with low cuboidal cells. The lamina propria was almost indistinguishable; and there was a dense connective tissue infiltration into the muscular layer of the gland (Figure 46). The general appearance was that of a non-functioning gland.

The adrenal glands of the olfactoriectomized animals demonstrated histological changes indicative of increased organ weight. The zona glomerulosa of the control animals was composed of large cells with darkly staining cytoplasm. The cells of the zona fasciculata had a high lipid content, evidenced by their lack of staining affinity (Figure 47). In the enlarged adrenals of the experimental animals, however, changes were visible. The zona glomerulosa of these animals was undistinguishable from the zona fasciculata because of the high lipid content of these cells. There was also considerable hyperplasia of the cells of the zona glomerulosa as was evidenced by the greater number of very small sized cells (Figure 48). It thus appeared that the cells of the zona glomerulosa were

undergoing a higher mitotic rate and incorporating more lipid than the cells of the zona glomerulosa of the control animals. The cortical-medullary index of all the animals appeared to be the same.

The female gerbils during this same fall season experiment showed a decrease in the weight of the uteri in the blinded animals. (Table XII). The uteri were reduced in size, but did not give histological evidence of significant change of the mucosa or glands.

The same experiment on male and female gerbils was repeated during January to see if the previously recorded changes were consistent. Table XIII and Table XIV contain the results.

The male gerbils did not respond to the surgical procedures during the winter as dramatically as they did in the fall. Lack of light in the blinded and blinded olfactoriectomized animals resulted in a decrease in seminal vesicle weight. The histological appearance of these reduced seminal vesicles was similar to those previously described for the gerbil. The adrenal glands of the pinealectomized-olfactoriectomized and pinealectomized-olfactoriectomized-blinded animals showed an increase in weight. These adrenals were *histologically* identical to other enlarged adrenal glands previously

TABLE XII

EFFECTS OF VARIOUS TREATMENTS ON FEMALE GERBILS IN SEPTEMBER (FALL)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Ovaries	Uteri	Adrenals
Control	5	52.4±1.2[1]	57.6±1.2	29.4±3.2	229±28	25.5±1.7
Blinded	8	53.7±2.6	54.7±2.6	24.1±3.5	100± 7[2]	23.5±1.2
Pinealectomy Blinded	5	61.0±2.3	58.2±1.8	36.6±0.8	183±13	25.6±0.5
Olfactoriectomy	5	51.2±2.6	56.3±2.6	19.7±1.7	154±59	32.1±0.8[3]
Olfactoriectomy Pinealectomy	5	51.4±1.5	63.6±3.3	32.1±3.7	214± 8	31.9±5.5[3]
Pinealectomy	6	61.8±2.1	61.0±2.0	31.2±2.3	191±23	27.5±1.0

[1]Values are Means ± Standard Error
[2]Significant difference: $p = <0.01$
[3]Significant difference: $p = <0.05$

TABLE XIII

EFFECTS OF VARIOUS TREATMENTS ON MALE GERBILS DURING JANUARY (WINTER)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weight (mg/100 g body weight) Testes	Seminal Vesicles	Adrenal
Control	6	59±1	66±2	1644±78	435±37	22.8±0.7
Blinded	7	58±2	64±3	1554±52	317±31[3]	23.2±0.8
Olfactoriectomy	7	64±3	66±2	1521±64	381±18	24.3±0.6
Blinded Olfactoriectomy	5	70±2	69±1	1573±102	326±20	25.9±1.1
Pinealectomy	5	68±3	73±4	1544±67	347±40	24.0±1.8
Pinealectomy Blinded	5	63±3	66±4	1634±56	419±49	25.0±0.9
Pinealectomy Olfactoriectomy	6	54±2	61±2	1641±44	416±50	28.7±1.5[2]
Pinealectomy Olfactoriectomy Blinded	5	54±1	56±4	1674±33	455±15	30.4±2.9[2]

[1]Values are Means ± Standard Error
[2]Significant difference: $p = <0.01$
[3]Significant difference: $p = <0.05$

TABLE XIV

EFFECTS OF VARIOUS TREATMENTS ON FEMALE GERBILS DURING JANUARY (WINTER)

Treatments	Number of Animals	Initial Body Weight Grams	Final Body Weight Grams	Organ Weights (mg/100 g body weight) Ovaries	Uteri	Adrenal
Control	6	48±2[1]	60±3	46.1±7.1	243±34	24.5±1.3
Blinded	8	47±0.1	54±1	29.2±1.4[2]	82±6[2]	23.3±1.1
Olfactoriectomy	8	57±3	61±4	44.2±4.3	199±33	26.2±1.3
Blinded Olfactoriectomy	6	48±1	54±2	30.3±3.9[3]	113±16[2]	28.0±3.0
Pinealectomy	9	51±3	57±3	40.1±4.2	182±29	26.3±1.2
Pinealectomy Blinded	7	56±3	62±3	38.0±5.4	181±28	24.0±1.4
Pinealectomy Olfactoriectomy	5	55±1	61±1	55.3±13.6	174±5	30.9±1.4
Pinealectomy Olfactoriectomy Blinded	5	46±1	51±2	50.7±2.2	278±74	29.7±2.0

[1]Values are Means ± Standard Error
[2]Significant difference: $p = < 0.01$
[3]Significant difference: $p = < 0.05$

described.

The ovaries and uteri of blinded and blinded-olfactoriectomized gerbils treated during the winter season showed a significant decrease in weight. The results of the surgical procedures were verified by histological studies. The ovaries of the blinded and blinded-olfactoriectomized animals (Figure 49) showed an increase in medullary interstitial connective tissue and an increase in atretic follicles over the ovaries of the control unoperated animals (Figure 50). The ovaries did not exhibit complete atrophy since it was possible to identify ovarian follicles in different stages of development. However, there was a decrease in the size and number of follciles. The uteri of the blinded and blinded-olfactoriectomized animals were reduced in size and were diminished both in number and in size of uterine glands (Figure 51). The uteri of control animals may be compared in Figure 52. The vaginae of all the blinded and blinded-olfactoriectomized animals (Figure 53) failed to show an estrus vaginal cornification. Many of the slides of the control animals, however, did show that these control animals were in estrus as judged by vaginal corinification (Figure 54). The blinded-pinealectomized and blinded-olfactoriectomized-

pinealectomized animals were similar to the controls and did not demonstrate any histologically varied change from the controls. It thus appeared that pinealectomy compensated for the decrease in weight and histological changes in the ovaries and uteri of the blinded and olfactoriectomized-blinded animals.

Likewise in all animals which were studied, the decrease in reproductive organ size which resulted from the surgical procedures could be prevented by removal of the pineal organ.

DISCUSSION

Gross Morphology of the Pineal Complex

The pineal complex is extremely variable in rodents (Gregorek and Seibel, 1970). Although consistently present, the pineal gland and pineal stalk vary greatly in their morphology and anatomical relationships to the pineal sac, subcommissural organ, the habenular and posterior commissures, and the vena cerebri magna. These great variations are explained by Quay (1965) insofar as "the gross shapes seem to reflect the available space between adjacent areas of the brain, particularly cerebellum, cerebral hemispheres, and midbrain." Quay also speculates that the more dorsal location of the rodent pineal gland may be due to the fact that "early meningeal attachments of the pineal (sic) lead to its dorsal position, or a more superficial position may have had adaptive significance in reception of light, or dorsoposteriad migration of the pineal (sic) may have occurred along its path of primary innervation, the nervi conarii, which enter the rat pineal (sic) via the tentorium cerebelli."

The pineal sac in the PET mouse and the gerbil is contiguous with only a small area of pinealocytes near the habenular commissure. This sac is in contact with pineal-

ocytes of the anterior stalk in the kangaroo rat. It is never in contact with true pinealocytes in the albino rat, in which the stalk in this area is composed of connective tissues and pinealoblasts. These connective tissues and pinealoblasts appear to be non-functional pineal tissue. The pineal sac of the golden brown hamster abuts both superficial and deep components of the pineal complex which are similar except for one morphological feature of a higher concentration of glial cells, mainly fibrous astrocytes, in the deep pineal gland (Sheridan and Reiter, 1970b). The pineal stalk in the hamster includes the pineal sac, neural and vascular structures, and perhaps some pineal parenchymal components (Sheridan and Reiter, 1970a). The Chinese hamster, even though it has deep and superficial pineal glandular components, differs from the golden brown hamster by possessing a complete pinealocyte stalk with the dorsal sac generally abutting only the deep pineal portion and the pinealocyte stalk.

These variances have great implications on the possible mode of function of the pineal gland. Sheridan et al. (1969), demonstrated that the pineal sac of the hamster was contiguous to the superficial and deep pineal components, and continuous with the ventricular system of the brain; they speculated that the dorsal sac may be the route by

which pineal gland substances reach the ventricles and hence are transported to those parts of the brain which are believed to be the possible sites of action of these substances. Anton-Tay and Wurtman (1969) have also shown that melatonin, the suspected pineal antigonadotrophic substance, is incorporated more readily into areas of the brain nuclei if injected into the ventricles of the brain than if given systemically. Mess (1968) has also shown that brain implants of melatonin have antigonadic activity. Feldberg (1963) demonstrated that bromphenol blue, when perfused through the ventricular system, stained the pineal completely blue. This evidence, with the observation of Owman (1961) that embryonic ependymal pineal cells secrete into the cerebrospinal fluid of the third ventricle, supports this hypothesis.

Our observations concerning the anatomical relationships of the sac and pineal gland in different groups of rodents would indicate that pineal secretion through the dorsal sac is a possibility but may not be the prime physiological mechanism of transport. For the sac to be physiologically significant, there would have to be physical or vascular continuity of the pinealocytes with the sac or ventricles of the brain.

The pineal sac, however, demonstrates a variability which does not appear consistent with major function; it is consistently found only on the dorsal surface of the pineal organ and is contiguous with only small areas of pinealocytes. In the rat, moreover, the sac does not even abut true pineal parenchymal cells.

Also, the physical continuity of pinealocytes with the ventricles of the brain through the pineal recess does not appear to be physiologically significant since the number of pinealocytes lining the third ventricle is minimal, and in the rat brain, a pineal recess with pinealocytes is completely lacking. Sheridan and Reiter (1970a) observed, in the hamster that removal of only the superficial portion of the pineal gland elicited the effects of pinealectomy. One of the explanations offered was the possibility that the deep portion of the gland was non-functional.

The venous drainage of the pineal organs in the rodents under study was similar to that described both in the mouse by von Bartheld and Moll (1954) and in the rat by Kappers (1960). Drainage was by means of the vena magna cerebri which carried the venous blood into the systemic circulation via the superior sagittal sinus or the confluence of sinuses. There did not appear to be a

vascular link between the pineal organ and the pineal sac or other parts of the brain comparable to the hypothalamo-hypophyseal portal system. Thus it appears that the pineal organ may function more conventionally as an endocrine gland by secreting its products into the systemic circulation. This theory is even more appealing since it is known that pineal gland blood flow is as rapid as blood flow through the neurohypophysis and is probably surpassed only by renal blood flow (Goldman and Wurtman, 1964). Kappers (1971) also favors the concept of pineal secretory release into the vascular system rather than into the cerebrospinal fluid. He suggests that the presence of leptomeningeal coverings of the pineal sac, the leptomeningeal capsule of the pineal complex, and the dense glial membrane surrounding the pineal organs of ungulates and primates are convincing facts indicating that pineal secretory products cannot reach the cerebral spinal fluid either directly or indirectly.

Since it is continuous with the choroid plexus of the roof of the diencephalon and apparently identical to it, the dorsal sac may be simply a phylogenetically modified choroid plexus. Tilney and Warren (1919) observe "in mammals, however, in those forms in which the corpus callosum has made its appearance, the sac becomes much

flattened and is difficult to recognize because of the altered condition consequent upon the development of the corpus callosum." Thus the pineal sac, as primitive choroid plexus, may have filled in the available brain space. The connective tissue attachments of the pineal sac to areas of the pineal gland and stalk may be secondary connections of mesenchymal involvement, rather than a primary physiological pathway.

DISCUSSION

Ultrastructure of the Pineal Gland

Ultrastructural studies have been carried out on pineal glands of many groups of rodents. These include the rat (Milofsky, 1957; Gusek and Santoro, 1960, 1961; De Robertis and Pellegrino de Iraldi, 1961), the hamster (Sheridan, 1969; Sheridan and Reiter, 1968; Clabough, 1969) and the mouse (Ito and Matsushima, 1968; Pellegrino de Iraldi, 1969). To date, however, there has not been an ultrastructural study of the pineal organ of the gerbil.

The general anatomical features of the pineal gland in the PET mouse and gerbil are similar in most respects to those of other rodent species.

Most authors agree that there are two basic cell types in the rodent pineal gland: the pinealocyte, which is the major cell type or parenchyma, and a minority of stromal cells which have been identified as glial and/or interstitial cells (Wolfe, 1965; Sheridan and Reiter, 1968; Pellegrino de Iraldi, 1969). Glial cells, identified as astrocytes, are abundant in cat and monkey pineal organs (Wartenberg, 1968) but are absent or rarely seen in pineal organs of rodents. The pineal interstitial cells of the

rat differ from the pineal astrocytes which previously have been described (Wolfe, 1965) and appear to be an unique cell type. Our findings agree with those of Pellegrino de Iraldi (1969) who could identify distinct interstitial cell types but who found astrocytes lacking in the mouse pineal gland.

Othe cell types found on occasion in the mouse and gerbil have been mentioned previously by other authors. These include Schwann cells and fibroblasts.

The distinction between light and dark pinealocytes was made by Wartenberg and Gusek (1964, 1965) in the rabbit, by Arstila and Hopsu (1964), Gusek et al., (1965) and Arstila (1967) in the rat. Arstila (1967) observed that the dark cells differed from the light cells by having more numerous and smaller mitochondria. He also observed that the cytoplasm of dark cells was made up primarily of rough endoplasmic reticulum with great numbers of free ribosome-rosettes while the light cells were primarily made up of smooth endoplasmic reticulum with fewer free ribosomes. These features could be seen easily in the gerbil pineal gland but were difficult to confirm in the PET mouse pineal gland.

Although Wolfe (1965) did not identify light and dark cell types in the rat pineal organ, Arstila (1967) showed that these cell types were not artifactual because they were always present using a variety of methods of fixation and fixatives. Clabough (1971) identified two types of pinealocytes in the hamster, the P_1 and P_2 pinealocytes. These cell types are distinguished primarily by differences in mitochondrial structure.

The capillaries of the PET mouse and gerbil differed. The PET mouse had fenestrated capillaries while the gerbil did not. Fenestrated pineal capillaries have been reported in the rat (Milofsky, 1957; Wolfe, 1965), hamster (Clabough, 1971), and mouse (Ito and Matsushima, 1968). They were not observed in the cow or sheep pineal gland by Anderson (1965), the cat or monkey by Wartenberg (1968), nor in the hamster by Sheridan and Reiter (1968).

The identification of polar terminals and autonomic nerve fibers in the pericapillary space is very difficult. Milofsky (1957) published the first ultrastructural study of the pineal organ of the rat and observed processes with small vesicles in the pericapillary space which he interpreted as nerve endings. De Robertis and Pellegrino de Iraldi (1961) identified these structures as perivascular pinealocyte terminations. Wolfe et al. (1962)

showed that administered tritiated norepinephine became concentrated in these dense core vesicles, as in other known sympathetic nerve endings, and concluded that these vesicles were in fact nerve endings. Pellegrino de Iraldi, et al. (1965) abandoned their interpretation after noting a degeneration of these vesicles following bilateral superior cervical ganglionectomy. Rodin and Turner (1965) have obtained similar results.

Our identification of autonomic nerve endings was based on the commonly accepted principles of identification of other authors. The polar terminal processes were usually larger and more irregular in both the PET mouse and gerbil. Dense cored vesicles and mitochondria in the terminal processes were similar to those found in the pinealocyte perykaryon and polar processes. This was especially true in the PET mouse. The gerbil pinealocyte terminal process was more difficult to identify since it had greater concentrations of smaller clear vesicles which in many instances resembled the autonomic nerve fiber endings. However, even the gerbil terminal processes had larger vesicles and granules than the nerve terminals. The nerve fibers were identifiable by the great amount of small granular (cored) and agranular (clear) vesicles. Both

types of vesicles were similar in size, although on occasion larger vesicles were also found. These small vesicles are of the same relative size as those identified by other authors as nerve vesicles. They are also similar in size to those vesicles known to be synaptic vesicles of nerve fibers. Very frequently these nerve endings were more immediately adjacent to the capillary endothelium than the polar terminals of the pinealocytes. Many times, Schwann cells and their processes were near the nerve fibers.

Most authors believe that the pineal gland has secretory activity, but it is difficult to prove this from morphological data. Ito and Matsushima (1968) and Pellegrino de Iraldi (1969) have indicated that the large granular vesicles found in the cytoplasm and terminal processes may be the secretory material of the pinealocytes. Leonhardt (1967) has shown photomicrographs which suggest extrusion of a granule from a pinealocyte into the perivascular space.

The nature of the granules in pinealocytes is uncertain. Arstila et al. (1971) have demonstrated secretonin in pinealocytes by fluorescence microscopy, but have been unable to do so by electron microscopic-histochemical radioautographic techniques. Ultrastructural

localization of melatonin has not been accomplished. The histochemical technique of Wood (1967) to differentiate catecholamines and indolamines was negative in the perikarya and polar processes of the mouse pineal organ (Pellegrino de Iraldi, 1969).

Further histochemical studies should be done to identify the granules found in the PET mouse and gerbil pineal gland. Those of the gerbil pineal gland appear the most interesting. The small granules which permeate the intercellular spaces, pericapillary spaces, and the blood vessels appear noteworthy since granules of the same size and appearance are also found in the pinealocytes. Histochemical analysis is essential.

The larger granules found in the gerbil pinealocytes are also significant. Both appear to resemble structures identified as lysosomes by other authors and to be related to mitochondria by their frequent close association. Gusek and Santoro (1961) observed circular mitochondria in the rat which were similar to structures they identified as pigment granules. They considered the pigment granules as arising from the mitochondria. The relationships of these series of granules in the gerbil pinealocytes and surrounding structures may lead to further evidence of pineal functions and possible secretory activity.

Clabough (1971) described the pineal gland ultrastructure in experimentally blinded hamsters. She observed that blinded animals had a greater number of vesicles and had structures described as lamellar whorls. Our blinded animals did not exhibit lamellar whorls, although similar looking structures were found on rare occasion in normal and blinded PET mouse pineals. The blinded PET mouse, however, did show a great increase of clear vesicles. These observations are consistent with Clabough's results (1969).

The most significant finding in blinded and normal animals was the presence of the large electron dense structures in unidentifiable cell processes, interstitial cells, and pinealocytes. These structures were seen only in blinded gerbil pinealocytes and never in normal gerbil pinealocytes. The blinded gerbil pineal glands used were those of the fall experimental group which showed maximal pineal stimulation as evidenced by a drastic decrease in testis and seminal vesicle weight, q.v. Table XI. Olfactoriectomized-blinded gerbil pineals also should be studied ultrastructurally, since they exhibited maximal pineal stimulation during this time also. Furthermore, these tissues should be compared with the gerbil pineal

glands of the winter season which were less active as judged by their antigondotrophic effect, q.v. Table XIII. These studies would offer more convincing arguments for the presence and function of the large electron dense structures in blinded gerbil pineal organs. Above all, these structures should be identified histochemically. They may be lipofuchsin pigment, melanosomes, large stores of lipid, lysosomes, or even perhaps secretory products.

DISCUSSION

The Pineal Gland and the Endocrine System.

Bilateral orbital enucleation in male and female golden hamsters results in atrophy of the reproductive organs. This effect, however, is prevented if the animals are pinealectomized (Hoffman and Reiter, 1965b; Reiter et al., 1966; Reiter, 1967; Clabough and Seibel, 1968; Seibel and Bush, 1970). Our results in the hamster were in agreement with the published reports in the literature. Blinding and blinding-hemicastration resulted in a significant decrease in testicular and seminal vesicular weight. Both of these effects, however, can be prevented by pinealectomy.

The neuroendocrine-gonadal axis of the albino rat is less labile than this system in the hamster (Reiter and Sorrentino, 1970). Reiter (1967) reported that blinding or short photoperiods did not affect the testes, accessory sexual organs, adrenals, or pituitaries of adult male albino rats; the same results occurred in prepubertal and postpubertal male and female albino mice. In male gerbils, however, blinding and blinding-olfactoriectomy

did result in a decreased accessory sexual organ weight.

Our results in all these groups of blinded, hemicastrated, pinealectomized, pinealectomized-blinded, blinded-hemicastrated, blinded-pinealectomized and blinded-pinealectomized-hemicastrated hamsters are comparable with Reiter's results. The adult male albino rats, the prepubertal and post pubertal male PET mice, and the adult female PET mice did not show any decrease in the size of reproductive organs in response to a lack of light. The winter series of male gerbil experiments also gave results in accord with Reiter's findings, i.e., a decrease in accessory sexual organ weight with blinding. The blinded-olfactoriectomized animals of this winter season experiment also showed a similar effect although not quite as pronounced as in the blinded animals.

Reiter (1967), unfortunately, did not experiment with female gerbils nor did he indicate the season of the year in which he performed his experiments. Our experiments using females during this same winter season indicated a great responsiveness to the absence of light. The ovaries and uteri of the blinded and blinded-olfactoriectomized animals were decreased significantly in weight. Our gerbil experiments indicated a greater sensitivity in the

male to a lack of light in the fall than in the winter: the testes and seminal vesicles of the blinded and blinded-olfactoriectomized animals responded by a decrease in size as did the seminal vesicles of the olfactoriectomized animals. The females during this same fall season, however, were less responsive than the females of the winter group. In the fall experimental group, only the uteri of the blinded animals were sensitive to the surgical procedures. But, nevertheless, blinding, olfactoriectomy, and blinding-olfactoriectomy during the seasons investigated resulted in diminishing the size of the reproductive organs; the simultaneous removal of the pineal organ with these surgical procedures resulted in animals with normal weights for the reproductive organs.

Seasonal variations in regard to reproductive function are well-known in mammals. Richter (1954) has shown that domestication of the rat has been able to extend mating activities throughout the year. Furthermore, the reproduction of a laboratory rat is less seasonally oriented and influenced than its wild prototype (Richter and Uhlenhuth, 1954). Reiter et al. (1966) believe the selective inbreeding of albino rats for laboratory use may reduce the responsiveness to pineal antigonadal activity.

This selective inbreeding may be responsible for the lack of response to the pineal in our experiments on the albino rat. This may also be the reason for the unresponsiveness of the PET mouse. These animals have been bred selectively for their reproductive ability and may not be affected by pineal modifications.

The seasons of the year and the amount of light are important parameters of reproduction. Rowan (1925) showed that the gonads of the junco finch are regulated by the length of environmental lighting. Cusick and Cole (1959) indicated that increasing the daily light period in hamsters counteracted decreased fertility at different seasons. Our experiments on the PET mouse during different seasons showed a lack of response to darkness or pinealectomy during all seasons. Even though there are indications that hamsters show seasonal variations in reproductive capacities (Bruce and Hindle, 1934; Cusick and Cole, 1959), Reiter (1967) has shown that hamster reproductive organs are sensitive to a lack of light regardless of the season of the year in which they are studied. This observation may be significant since hamsters are non-obligatory hibernators (Mogler, 1958; Hoffman, 1964). The mongolian gerbil, an animal introduced into the laboratory relatively recently, (Marston and Chang, 1965) is not well-known as to its reproductive habits.

To our knowledge, no work has been done on determining the hibernation capacities of the gerbil. The responsiveness of hamster and gerbil gonads to a lack of light, which can be ameliorated by pinealectomy, may be related to their less domesticated natural history.

Reiter and Hester (1966) and Reiter, (personal communication), have indicated that coat coloration may play a role in pineal activity. Their experiments showed an effect of a lack of light on the reproductive system in black rats, but not in albinoes. On the basis of this, we chose to use the PET mouse, which is a black mouse, to see if the previous negative results in the albino mouse would be the same for the PET mouse. Our results indicate that skin pigmentation is not a determinant of pineal function in the mouse. It seems more probable that a species or strain difference in animals would be responsible for the difference in responsiveness between the black rats and the albino rats. A species and strain difference in rodents in relation to reproduction has been reported (Kinson and Robinson, 1970).

The response of prepubertal animals to blinding and blinding-pinealectomy differs in the hamster and rat (Reiter, 1968). Blinding of prepubertal (25 day old) male albino rats results in retarded development of the gonads

and accessory sexual organs until the animal is 150 days old. Blinded-pinealectomized albino rats and untreated control rats have a similar normal gonadal growth pattern. Blinded hamsters, however, do not show a delay of puberty, but once the reproductive structures become mature, there is an involution of the testes, seminal vesicles, and coagulating glands. Reproductive organs develop normally in blinded-pinealectomized and pinealectomized hamsters.

The exact age of the animals at the time of the surgical procedures also seems to be important. Kinson and Singer (1969) found no effect of pinealectomy on the weight of testes, seminal vesicles, or prostate when this procedure was performed on animals between 5 and 6 weeks of age and autopsied 30 or more days after the operation. If, however, pinealectomy was performed on day 28 after birth, i.e., one day after testicular descent, and sacrificed two weeks later, these animals had a significantly higher weight of seminal vesicles and prostate. Mature sperm, moreover, were present only in the testes of pinealectomized animals. These authors describe this effect as precocious puberty. The lack of response in our prepubertal series of PET mice may have been due to a lack of proper timing in performing the pinealectomy procedure or

sacrifice (8 weeks). However, since neither blinding nor pinealectomy modify gonadal size, the PET mouse may be less reproductively affected by external stimuli or pineal substances.

Studying the effect of short photoperiods or a lack of light on the reproductive abilities of hamsters has led one editor (Schwartz 1965/1966) to comment, "it would be a mightly cold day that had 1 hour of light and 23 of darkness and would occur at a helluva time for a self-respecting hamster to raise a family". Even though for the obvious fact that hamsters and rats are nocturnal animals and would have in fact diminished activity in light, these studies on the amount of light and gonadal response have stimulated much research on the pineal and reproductive physiology.

The pineal body is accepted presently as a gland which secretes an antigonadal substance. This theory is based on the well-founded facts that the effects of short daily photoperiods (1L:23D) or blinding which results in atrophy of the gonads of certain mammals can be counteracted by pinealectomy. The removal of the pineal thus removes the source of the antigonadotrophic substance thereby resulting in normal gonadal weight and function.

The pathways by which light affects the pineal are still in question. The response of the pineal to light is initiated by the retina (Wurtman et al., 1968). Feldman (1964) reported electrophysiological evidence for direct optic-hypothalamic projections in the cat. Lin (1972), however, denies connections between the retina and hypothalamus in the cat. Printz and Hall (1972) have described direct retina-hypothalamic projections in the golden hamster.

Many authors have suggested other pathways which relay photic information to the pineal. Axelrod et al., (1966) and Moore et al., (1967) have shown that bilateral electrolytic lesions in the medial forebrain bundle block the pineal response to light. The inferior accessory optic tract travels within the medial forebrain bundle (Hayhow et al., 1960). Even though this nerve fiber tract only extends to the midbrain tegmentum, there are other unknown tracts which connect this area of the brain to the superior cervical ganglia.

It is known that superior cervical ganglia are intimately involved in pineal function since bilateral superior cervical ganglionectomy in the hamster results in effects equivalent to pinealectomy (Hoffman and Reiter, 1966;

Reiter and Hester, 1966). This procedure most probably results in pineal denervation since Kappers (1965) has shown that the pinealo-petal fibers in the rat are post-ganglionic sympathetic nerve fibers from the superior cervical ganglia which innervate the pineal after traversing the tentorium cerebelli.

There have been many attempts to identify the pineal antigonadotropic substance. Many believe that it is melatonin, an indole, which is synthesized only by the pineal gland. Many of the studies on the pineal production of melatonin have been based on its rate limiting enzyme hydroxyindole-O-methyltransferase (HIOMT). This enzyme is responsible for the production of melatonin from N-acetylserotonin. HIOMT activity is increased in rat pineals with continuous darkness (Wurtman et al., 1963b It also undergoes a daily cyclic variation, and its activity is greatest during the daily dark period. Pineal HIOMT activity is not altered by continual darkness after superior cervical ganglionectomy (Wurtman, et al., 1964); and bilateral transection of the medial forebrain bundle shows no significant difference in HIOMT activity between animals in continuous light or continuous darkness (Axelrod et al., 1966).

Injection of melatonin has been reported to lower ovarian weight (Wurtman et al., 1963b; Sorrentino, 1968), inhibits testis growth in the rat (Kappers, 1962; Gaston and Menaker, 1967; Konig et al., 1971), inhibit the estrous phase of the estrous cycle in the rat and mouse (Chu et al., 1964), and affect seminal vesicle weights (Kappers, 1962; Moszkowska, 1965b). Other authors (Ebels and Prop, 1965; Kinson and Peat, 1971; Reiter, 1968) have been unable to confirm antigonadotrophic effects of melatonin on rat or hamster gonads.

Since bilateral ganglionectomy in the rat increases HIOMT activity and thus melatonin synthesis, one would expect the same with the hamster. This, however, is not the case; bilateral cervical ganglionectomy has no effect on the size of the reproductive organs. Its effects, moreover, are equivalent to pinealectomy. As Reiter (1967) states, "these findings suggest that either melatonin is not the pineal antigonadotrophic factor in the hamster or this compound is not synthesized (or released) in the denervated hamster pineal gland."

Melatonin, then, may not be the primary antigonadotrophic substance of the pineal. Other compounds such as serotonin, 5-methoxyindole acetic acid, and 5-methoxytryptophol have also been found in significant concentration in the pineal.

Methoxytryptophol has been shown to have inhibitory effects on growth of rat ovaries (McIsaac et al., 1964). Gromova, et al., (1967) have suggested that serotonin may be the biologically active pineal hormone. Recent studies have also revealed an active pineal antigonadotropin, believed to be a polypeptide, which has potent antigonadotropic activity (Matthews et al., 1971; Benson et al., 1972).

Whatever the pineal antigonadotropic principle is, it is certain that the pineal organ acts as a neuroendocrine transducer which is capable of activation and release of secretory products.

The actual site of responsiveness of the pineal substance is also unknown. This substance may affect the hypothalamo-pituitary axis, the target organs by means of the tropic hormones, or the target organs directly.

Hemicastration generally increases the weight of the remaining gonad (compensatory hypertrophy). There are many hypotheses concerning the mechanisms of action: compensatory hypertrophy(Jacobsohn and Norgren, 1965) due to an increase of gonadotropin secretion of the pituitary through the feedback of decreased circulatory androgen levels, accelerated growth (Addis and Lew, 1940) by a greater availability of a limited supply of pituitary gonadotropin to the remaining gonad, or neuroendocrine

reflex mechanisms (Aron, et. al., 1950) of proprioceptive nervous pathways and anterior pituitary gland secretion.

Edgren, et al. (1965) reported compensatory enlargement in the rat gonad following removal of the contralateral gland. Reiter et al. (1966) indicated that hypertrophy does not occur in the remaining gonad in hamsters following hemicastration. In our experiments all three species showed an increase in the size of the remaining gonad after hemicastration; however, other increases were not statistically significant. The rat testes appeared most sensitive and their enlargement neared significant values.

The pineal gland and the amount of light may alter the hemicastration enlargement result in rats. Reiter (1967) showed that the highest percentage increase in remaining ovarian weight after hemicastration in the rat was in the pinealectomized animals kept in a short photoperiod (one hour of light per day). The animals exhibiting the smallest percentage increase were the sham pinealectomized short photoperiod animals. The hemicastrated-blinded-pinealectomized animals of our experiments would parallel Reiter's experimental group. Our results indicate that these hemicastrated-pinealectomized animals also had the highest weight increase of all surgical procedures in the hamster, albino

rat, and PET mouse.

When the relationship between testicular weight loss following blinding and testicular weight increase of hemicastration are compared in the hamster, our results show that the hypertrophy of hemicastration is not sufficient to overcome the increased pineal antigonadal activity of blinding. The blinded-hemicastrated hamsters had atrophic testes. These results are in agreement with Clabough (1969) but are in contrast with Reiter et al., (1966) and Seibel and Schweisthal (1972). Reiter states that "growth stimulation to the remaining testes brought about by the hemicastration was sufficient to offset the normal inhibitory action of the pineal gland when animals were subject to short daily photoperiods." The animals in this experimental series of Reiter were kept in short daily photoperiods for only 4 weeks. Reiter (1967) later reported that "removal of the eyes of adult male hamsters followed within 5 to 7 weeks by involution of the gonads". The 4 weeks of short daily photoperiods may not then have been sufficient maximally to stimulate the pineal antigonadotropic principle thereby causing a false interpretation of the results. A possible explanation of Seibel's results may be that his animals exhibited remarkable compensatory hypertrophy and only a 65% decrease in testicular weight after blinding. Our

animals, however, demonstrated a 90% decrease in testicular weight after blinding, with only a slight increase in testicular weight after hemicastration. Thus, the net results of blinding far exceeded the results of hemicastration. Therefore, our results suggest that in our experimental animals, the atrophy of the gonads after blinding was not counteracted by compensatory enlargement after hemicastration.

A fruitful means of studying the reproductive effects of the pineal gland and the relationship of the gland to light has been the use of androgen and estrogen sterilized animals. Animals injected with gonadal steroids in the first week of life have gonads which are smaller than the reproductive organs of normal animals (Jacobsohn and Norgren, 1965). This occurs because the steroids interfere with the normal development of the preoptic-anterior hypothalamic area of the brain (Gorski, 1966; Barraclough, 1967). Kordan and Hoffman (1967) have shown that the absence of light further diminishes the size of the reproductive structures. Reiter (1968 a, b, c) has shown the reversability of this added decrease in reproductive organ size by pinealectomy.

Our experiments with testosterone propionate treated PET mice did not produce the expected results. The injected

control animals did not respond with smaller gonads than the untreated control animals from the three different seasons. The results of blinding, pinealectomy, and blinding-pinealectomy, also showed no significant difference. The seminal vesicles of the TP treated blinded animals were somewhat decreased in size, but not significantly. Our results are somewhat perplexing since TP treated neonatal animals usually respond and exhibit decreased gonadal size.

Olfaction also plays an important role in the reproductive patterns of many animals. Whitten (1956) reported that anosmia causes involution of the ovaries and uteri of female mice. In contrast, anosmia did not appear to affect the gonads of male mice. Aron, Roas, and Asch (1970) observed that anosmia in rats does not disburb the frequency of spontaneous ovulation, although there may be some morphological changes in these ovaries. Reiter, et al., (1969) show that there are no reproductive changes in anosmic male rats. Reiter (1967) found that olfactoriectomy did cause regression of the uteri in female hamsters. This regression could be counteracted by pinealectomy. He also noted that anosmia had no apparent effect on male hamsters or one series of female hamsters. The effect on

his other two series of females was transitory since it occurred only 8-12 weeks after surgery.

Our results show that olfactoriectomy did not affect the size of the gonads or accessory reproductive glands of PET mice. Olfactoriectomy likewise did not affect the male or female gerbils during the winter season, but did affect gerbils during the fall season. The gerbil experiments done during the fall show a significant decrease in the weight of the seminal vesicles of olfactoriectomized animals and a marked but not significant decrease in the weight of the testes, ovaries, and uteri.

In the male and female rats it has been shown that blinding and olfactory bulb removal resulted in a significant lack of development of the reproductive organs and delay in the onset of puberty, but that removal of the pineal organ at day 21 reversed these changes (Reiter et al., 1969; Reiter and Ellison, 1970). These effects occurred only when the animals were blinded and olfactoriectomized; if either procedure was done singly, there were no effects. The response of blinded-olfactoriectomized gerbils as compared with the blinded gerbils in our experiments does not indicate an additive effect. It appears that blinding is primarily responsible for the decrease in reproductive organ size.

The relationship between the pineal and pituitary glands is disputed frequently. Holmes (1956) and Wragg (1967) report that pinealectomy does not alter pituitary weight or morphology. Other authors, however, maintain that removal of the pineal results in pituitary hypertrophy or an increase in the number of pituitary basophils (Girod et al., 1964; Moszkowska, 1965a). Quay (1967) states that he has found pituitary adenomas in aged pinealectomized rats, although he points out that these tumors may not be related to pinealectomy. Malm et al., (1959) showed that removal of the pineal did not affect metabolic activity of the pituitary gland, as judged by utilization of P^{32}.

Reiter (1967) performed a series of experiments in which he contrasted pituitary weight in blinded and blinded-pinealectomized animals. There were no unoperated controls in these experiments. He reported that bilateral optic enucleation caused a significant decrease of pituitary weight in prepubertal and post pubertal albino rats. This decrease occurred 50, 75, and 100 days after the operations in males and 50 and 75 days after the operations in females. The 25 and 125 day post operative males and 100 day post operative females demonstrated no difference in pituitary weight. The same experiment in prepubertal and post pubertal

hamsters showed no pituitary weight change after bilateral optic enucleation after 25, 50 or 75 days in males or 25 and 50 days in females. There was a significant decrease, however, 75 days after blinding. Bilateral optic enucleation had no effect on male and female prepubertal and post pubertal albino mice 25, 50, 75, 100 or 125 days after surgery. There were also no effects on the pituitary glands of male gerbils sacrificed 10 weeks after blinding. Seibel and Schweisthal (1972)have shown no significant change in pituitary glands of normal or parabiosed golden hamsters after surgical procedures similar to our basic experimental design. Clabough (1969) reports the same results in albino rats and hamsters. These studies of Clabough and Seibel on male hamsters are in accord with those of Reiter.

Our results with golden brown hamsters and PET mice have shown that the only significant change in pituitary weight occurred in the blinded hamsters. Such a decrease in pituitary weight would be consistent with the concept that the decreased testicular activity is a result of a decrease in pituitary cellular numbers and activity and decreased gonadotropin secretion. This observed change is in accord with results of Reiter (1967), who observed pituitary weight decrease in male hamsters

sacrificed 6 and 8 weeks after bilateral orbital enucleation. This finding would be consistent with our results since the animals in our experiments were also sacrificed 8 weeks after surgery.

With such conflicting results on the same species of animals by the same researchers, much more research into the pituitary-pineal-gonadal interactions will have to be done. A possible explanation may be that weight measurement of the pituitary gland is not sensitive enough to detect changes. The extirpation of the anterior hypophysis *in toto* without elements of the pars intermedia or posterior hypophysis and the small size of the entire gland may be further complications to accurately detecting changes in the gland.

Wurtman *et al.* (1959) reported an increase in adrenal weight in female rats after removal of the pineal organ. Reiter *et al.* (1966) demonstrated that blinding or short daily photoperiods caused a significant decrease in adrenal weight in female hamsters. If the pineal complex of these animals also was removed, adrenal weights were similar to those in normal animals. In another series of experiments on prepubertal and postpubertal animals, Reiter (1967) found no significant difference between the adrenal glands of blinded and blinded-pinealectomized rats

on the 25th, 75th, 100th, and 125th day after surgery. Both male and female albino rats did show a significant decrease in adrenal weight on the 50th postoperative day. Prepubertal and postpubertal male and female hamsters showed no significant effect of absence of light on the weight of the adrenal glands on the 25th, 50th, and 75th day after blinding or blinding-pinealectomy. The same results were obtained for albino mice which were also autopsied on the 100th and 125th day as well as on the 25th, 50th, and 75th days. Male gerbils, sacrificed 10 weeks after bilateral optic enucleation, showed no significant difference in adrenal size. Clabough (1969) found no difference in adrenal weight in rats and hamsters after pinealectomy or blinding. Seibel and Schweisthal (1972) also reported no significant difference in adrenal weight of normal or parabiosed male and female hamsters after pinealectomy, blinding, blinding-pinealectomy, hemicastration, hemicastration-blinding, hemicastration-pinealectomy, and hemicastration-blinding-pinealectomy.

There was a significant increase in adrenal weight in pinealectomized-blinded-hemicastrated adult albino rats, pinealectomized-hemicastrated and pinealectomized-blinded-hemicastrated PET mice during the winter season, and

pinealectomized-blinded and pinealectomized-hemicastrated PET mice during the summer season. An increase during the fall season occurred in olfactoriectomized and olfactoriectomized-pinealectomized female and olfactoriectomized male gerbils. In the winter season experiments on gerbils, there was an increase in adrenal weight in pinealectomized-olfactoriectomized and pinealectomized-olfactoriectomized-blinded males but no such effect in the females. These results suggest that further research and a more thorough study of the data are needed before the results can be interpreted in relationship to the many variables affecting adrenal function.

Research on the pineal gland has now shown that this organ is not a primitive, non-functional vestige of phylogeny. Our experiments and the experiments of others have shown that the pineal organ plays a role in the reproductive function of mammals. Even though many results of pineal research have been inconsistent, the data which have accumulated show that pineal activity is related to reproductive activity. Many other factors may also enter into the overall effect of the pineal on reproduction: the age of the animals at pinealectomy, and the difference between strains of a species, which have already been

explained. Such other factors as early lighting conditions (Relkin, 1968), olfactory stimulation (Reiter et al., 1969), and methods of caging animals (Miline et al., 1968) also play undoubtedly a role in the interplay between the pineal organ and reproduction.

As Reiter and Sorrentino (1970) speculate, "it is possible that the pineal gland is more important for the slower, long term adjustments (e.g., seasonal) of the reproductive system to environmental conditions...Even under seasonal conditions the pineal gland may act as a relatively fine adjustor which attunes genital functions to external conditions."

Thus, the pineal organ which was once thought to act as a sphincter regulating the flow of thought, a gland, the seat of the soul, or an organ related to madness is in reality an organ which very subtlely regulates one of the main characteristics of all living things, the ability to reproduce. The overall effect of the knowledge of the pineal organ is expressed by Schwartz (1966/1967) "...all these extraordinary findings in a difficult field sustain the kind of admiration that the peasant holds for the dancing bear - not that the bear does it gracefully, but that he can do it at all."

SUMMARY

The pineal complex of rodents is made up of a pineal organ which developmentally always originates from the area between the habenular and posterior commissure and a pineal sac which is continuous with the choroid plexus of the third ventricle. This sac appears to be identical to the choroid plexus at both light and electron microscopic levels. The pineal sac abuts the deep and superficial pineal organs of the golden hamster. In the PET mouse, gerbil, kangaroo rat and Chinese hamster, the sac is contiguous with only small areas of pinealocytes. This sac never abuts true pineal parenchyma in the albino rat. The variability of the relationship between this sac and pineal parenchyma indicates that this structure may not be the main physiological route of pineal gland secretion.

The ultrastructure of pinealocytes from normal and blinded PET mice and normal and blinded gerbils indicates the possibility of secretory activity in pinealocytes. Pinealocytes in the PET mouse have many dense core vesicles which can be found in the perykaryon, processes, and polar terminals of the cells which end in a pericapillary space. This space is near a fenestrated capillary. The possibility exists that these dense cored vesicles may

be extruded from the cell terminals and pass into the fenestrated capillaries. Such fenestrated capillaries commonly are found in endocrine organs.

The gerbil pinealocyte has two types of granules. Their nature is unknown, and the relationship between the two is uncertain. Small granules, resembling the small granules in gerbil pinealocytes in size and density, can be found in intercellular and pericapillary spaces. The relationships between these two granules is equally in doubt.

The ultrastructure of pinealocytes of PET mice and gerbils reflects changes with blinding. Pinealocytes of blinded PET mice are more vesiculated and have greater numbers of lipid droplets. The pineal glands of those blinded gerbils, which were known to be highly functional by their physiological effects on the gonads, contained large electron dense structures. They were found in unidentified cell processes, interstitial cells, and pinealocytes. Pineal glands from a greater number of animals will have to be studied and compared with those of the same species reflecting low pineal activity in order to verify the relationship between pineal activity and these large electron dense structures. Histochemical analyses would be essential to identify and to clarify the

relationships and activities among these vesicles, granules, and electron dense structures in pinealocytes of PET mice and gerbils.

Blinding of the golden brown hamster and the gerbil resulted in a significant decrease of testis and seminal vesicle weight. This effect in the gerbil occurred during the fall season but when repeated during the winter season resulted only in the decrease of seminal vesicle weight. Female gerbils, however, responded to blinding by a decrease in ovarian and uterine weights during the winter season but only in uterine weight during the fall season. All of these effects of a lack of light on the gonads were prevented if the animals were also pinealectomized. The effects of olfactoriectomy which resulted in a decrease in seminal vesicle weight in one series of gerbils could also be overcome by pinealectomy. The pineal gland then may be responsible for production of an antigonadic substance. With the pineal gland removed the antigonadic source is removed, and the testis and/or seminal vesicle weights remain normal. A lack of light appears to stimulate the pineal gland to function since pinealectomy alone did not effect an increase in reproductive organ size in any of the animals which we studied.

The albino rat and PET mouse did not respond to a lack of light as did the hamster and gerbil. Moreover, in the PET mouse, blindness did not affect prepubertal or postpubertal animals, adults during different seasons of the year, or adults treated neonatally with testosterone propionate. Female PET mice and male wild mice were equally unaffected.

An effect of the pineal gland on the pituitary gland and adrenal gland is doubtful. Animals with or without a pineal gland exhibited no difference in pituitary or adrenal weights. More sensitive techniques will have to be employed to determine pineal-pituitary and pineal-adrenal interactions.

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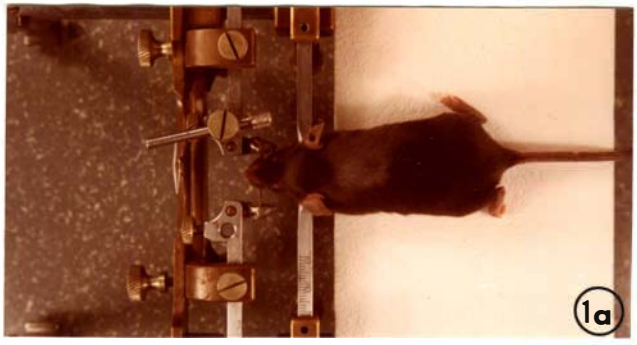
Figure 1

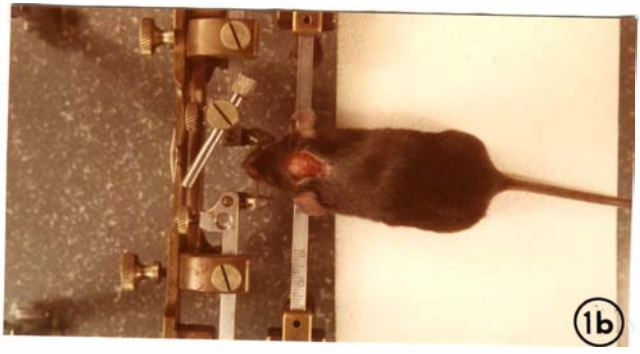
Surgical procedure in the PET mouse.

a. Animal is placed in a stereotaxic apparatus.

b. An incision is made to reveal the lambda of the skull.

c. Small circular area of the skull (→) is removed to allow access to the pineal gland.


1a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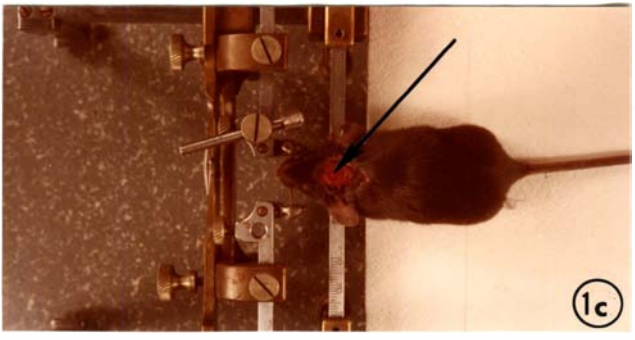
1c

Figure 2

The pineal complex of the PET mouse.

The pineal gland (P) arises between the habenular commissure (h) and posterior commissure (Pc) and has a prominant pineal recess (r) which is continuous with the third ventricle (III) of the diencephalon. The pineal sac (PS) abuts only the most proximal area of the pineal (→) and is separated from the most distal area of the pineal by the vena magna cerebri (V). The pineal organ is dorsocaudally flexed and is posterior to the cerebrum (C) and corpus callosum (cc) and is anterior and dorsal to the corpora quadrigemina (Q) of the midbrain (M). Subcommissural organ (s). X 245.

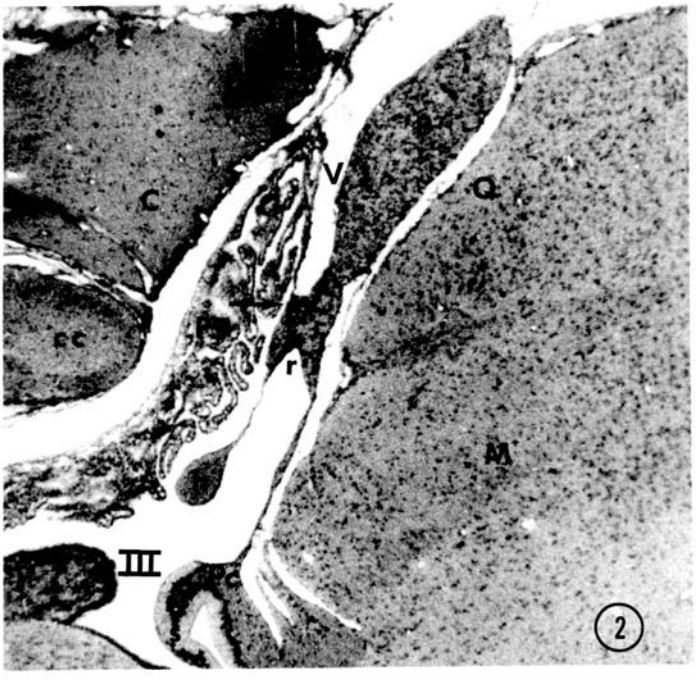
C
V
Q
cc
r
M
III
2

Figure 3

The venous drainage of the pineal gland in the PET mouse.

The vena magna cerebri (V) which contains the venous drainage of the pineal empties into the superior sagittal sinus (SS). The pineal sac (PS) may again be seen abutting only the lower area (→) of the pineal (P). Cerebellum (C). X 210.

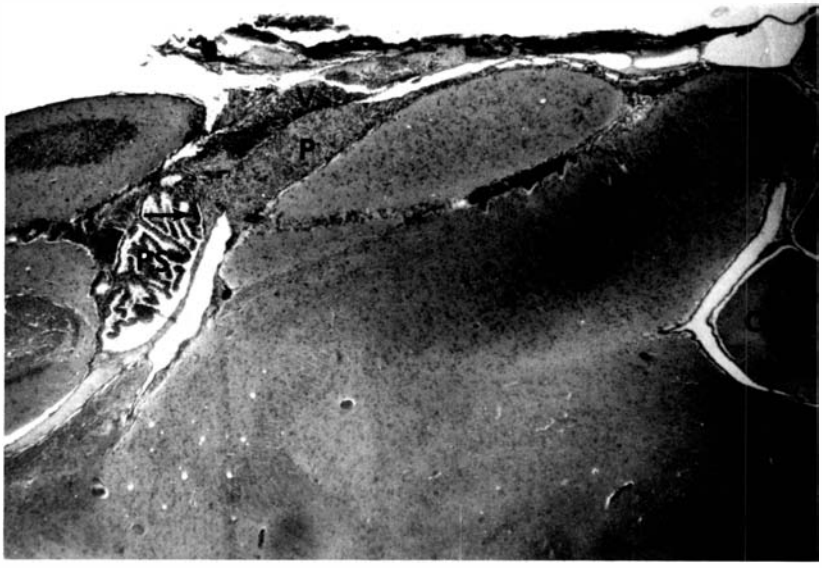
P

Figure 4

The pineal complex of the gerbil.

The pinealocyte stalk between the suprahabenular pinealocytes (P_1) and the distal pinealocyte enlargement (P_2) is out of the plane of section. The pineal sac (PS) abuts only the pinealocytes (P_1) above the habenular commissure (h). Vena magna cerebri (V). X 180.

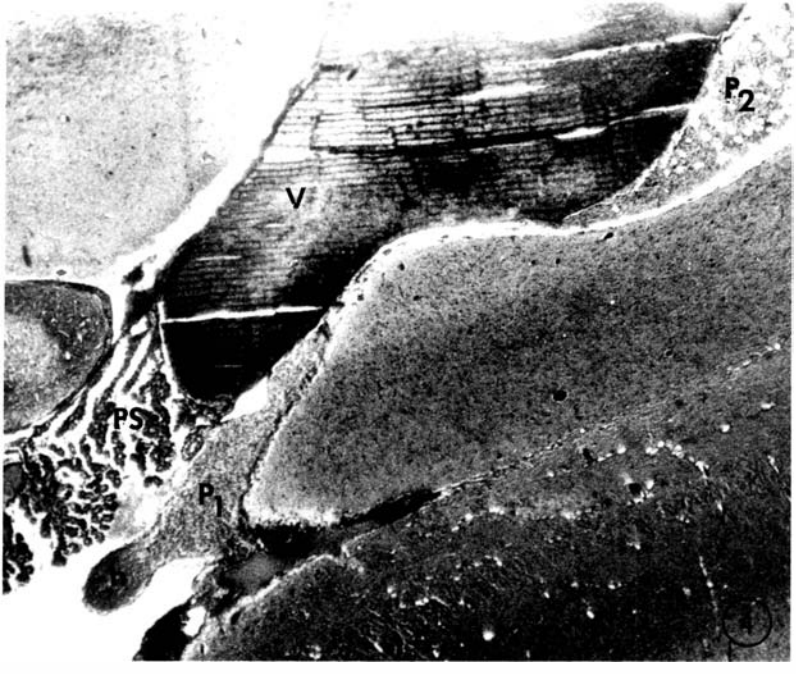
P2
V
PS
P1

Figure 5

The pineal gland of the gerbil.

Circular intercellular spaces (X) are characteristically found in the most superficial parts of the pineal stalk and most enlarged areas of the pineal gland in the gerbil. X 2011.

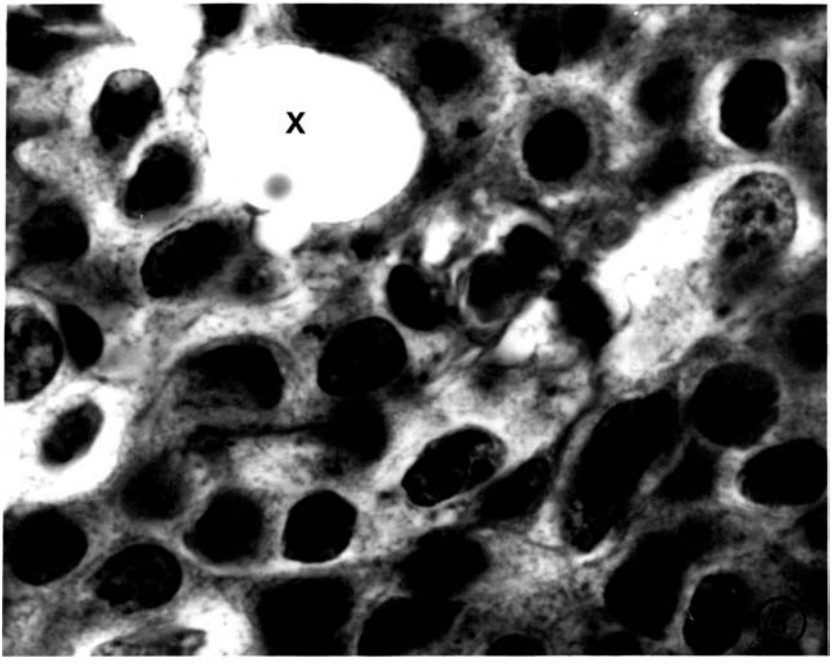
X

Figure 6

The Pineal Complex of the Golden Brown Hamster.

There is always found in the hamster a deep pineal (P_1) and a superficial pineal (P_2) structure. The pineal stalk (S) is composed of blood vessels, nerves, pineal sac (PS), and occasional pineal parenchyma appearing cells. The separation between the pineal sac and midbrain is artifactual. Habenular commissure (h). X 50.

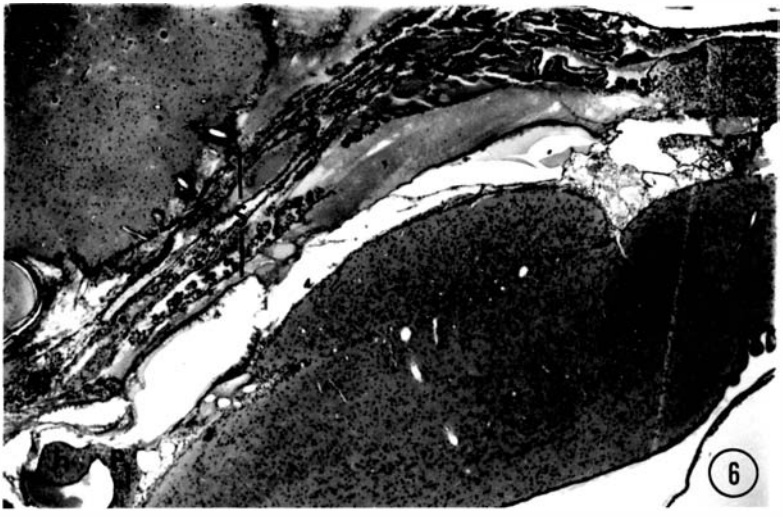
6

Figure 7

The superficial pineal (P) of the golden brown hamster abuts the pineal sac (PS). The vena magna cerebri (V) and the pineal sac are elements of the pineal stalk in the hamster. X 106.

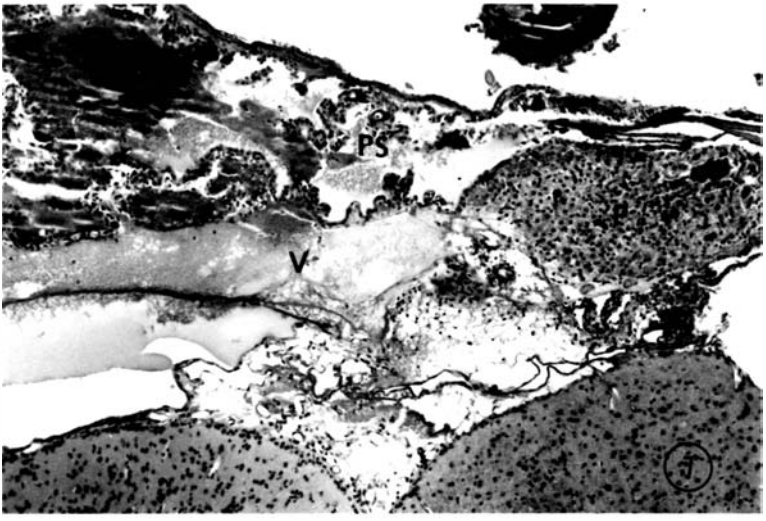
PS
V

Figure 8

The pineal sac of the golden brown hamster.

Arrows indicate the capillaries of the vascularized connective tissue core of the villous folds. X 780.

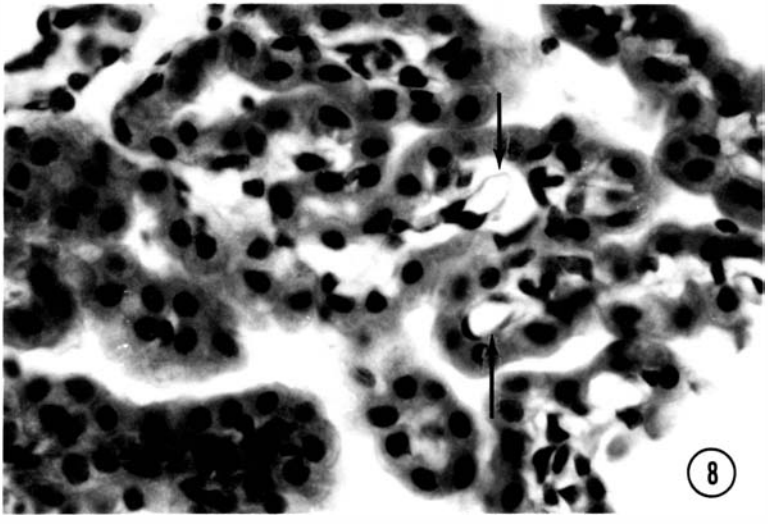
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Figure 9

The pineal sac of the hamster.

The cuboidal cells and the vascularized connective tissue core (X) of the pineal sac are illustrated.

X 1455.

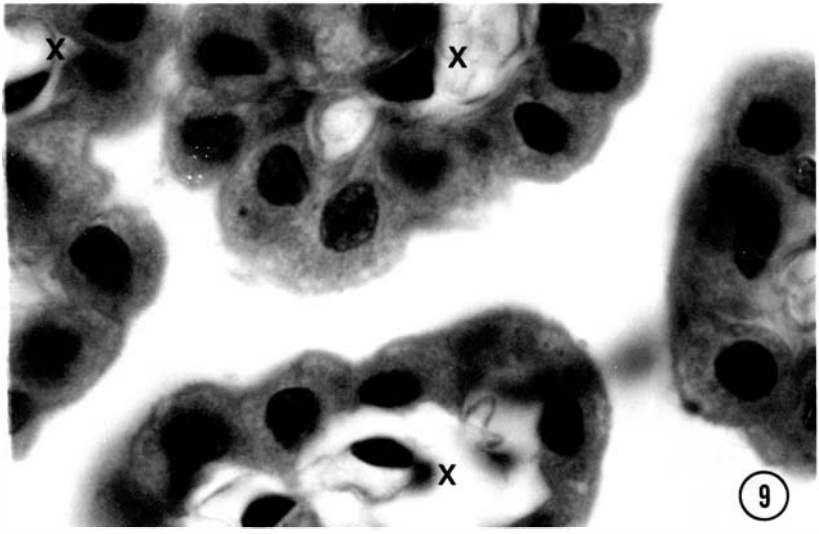

X
X
X
9

Figure 10

The Pineal Complex in the Chinese Hamster.

The superficial pineal (P_2) is joined to the deep pineal (P_1) by a thin pinealocyte stalk (out of plane of the section). The pineal sac (PS) is separated from the pineal stalk by the vena magna cerebri (V). Cerebrum (C), corpus callosum (cc), cerebellum (CB), midbrain (M). X 50.

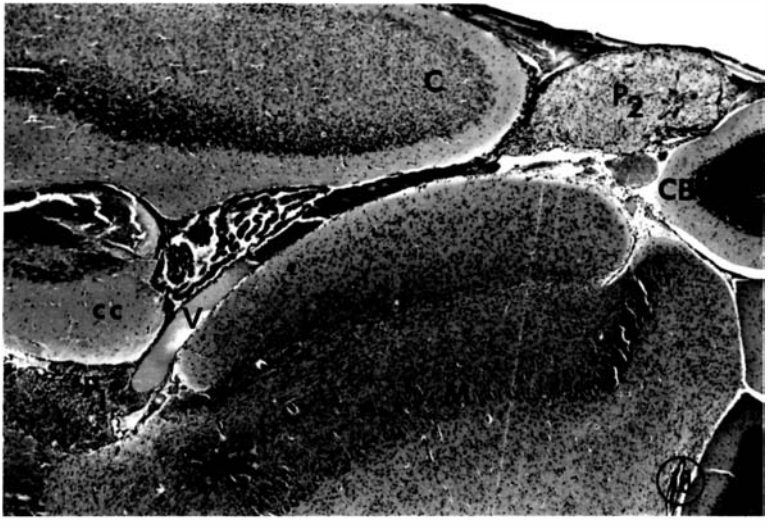

C
P2
CB
cc
V

Figure 11

The Pineal Complex of the Chinese Hamster.

The deep pineal (P_1) arises from the habenular commissure (h). The pineal sac (PS) only abuts the deep pineal (→). It is separated from the thin pinealocyte stalk by the vena magna cerebri (V). X 114.

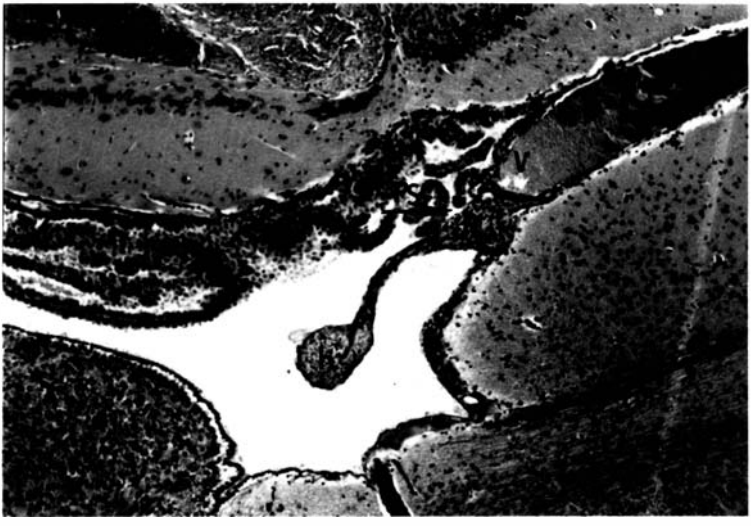


Figure 12

The pineal complex of the albino rat.

The habenular commissure (h) and posterior commissure (Pc) are fused in the diencephalic roof. There is a sheet of pinealoblast appearing cells (X) in close proximity to the pineal sac (PS). But the pineal sac never abuts true pineal parenchyma. The pineal stalk (St) is a connective tissue stalk with occasional pinealocyte appearing cells. Subcommissural organ (S), third ventricle (III). X 269.

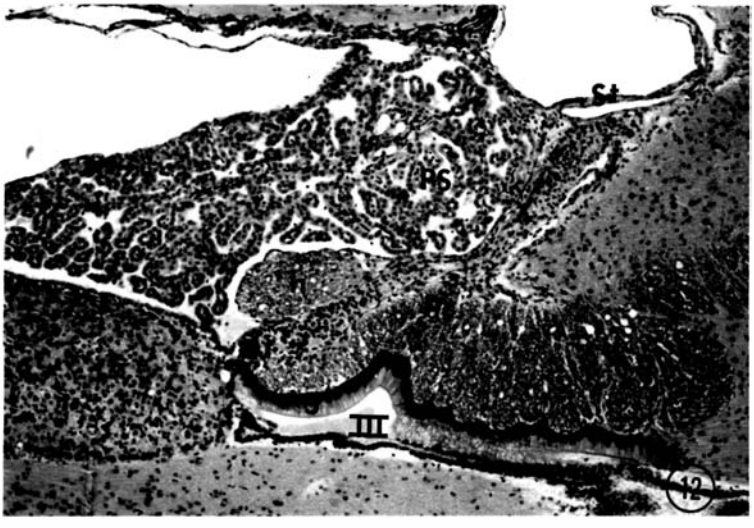

III
42

Figure 13

The pineal complex in the kangaroo rat.

The pineal sac (PS) is only contiguous with the most anterior aspects of the pineal stalk (→). Vena magna cerebri (V), pineal (P). X 45.


V
PS
OP

Figure 14

Normal PET mouse pineal gland.

The pineal organ is composed of light cells (L) and dark cells (D) and their processes. The light cell processes (lp) and dark cell processes (dp) are found among the pinealocytes. Nuclei (N) are very evident. X 8,925.

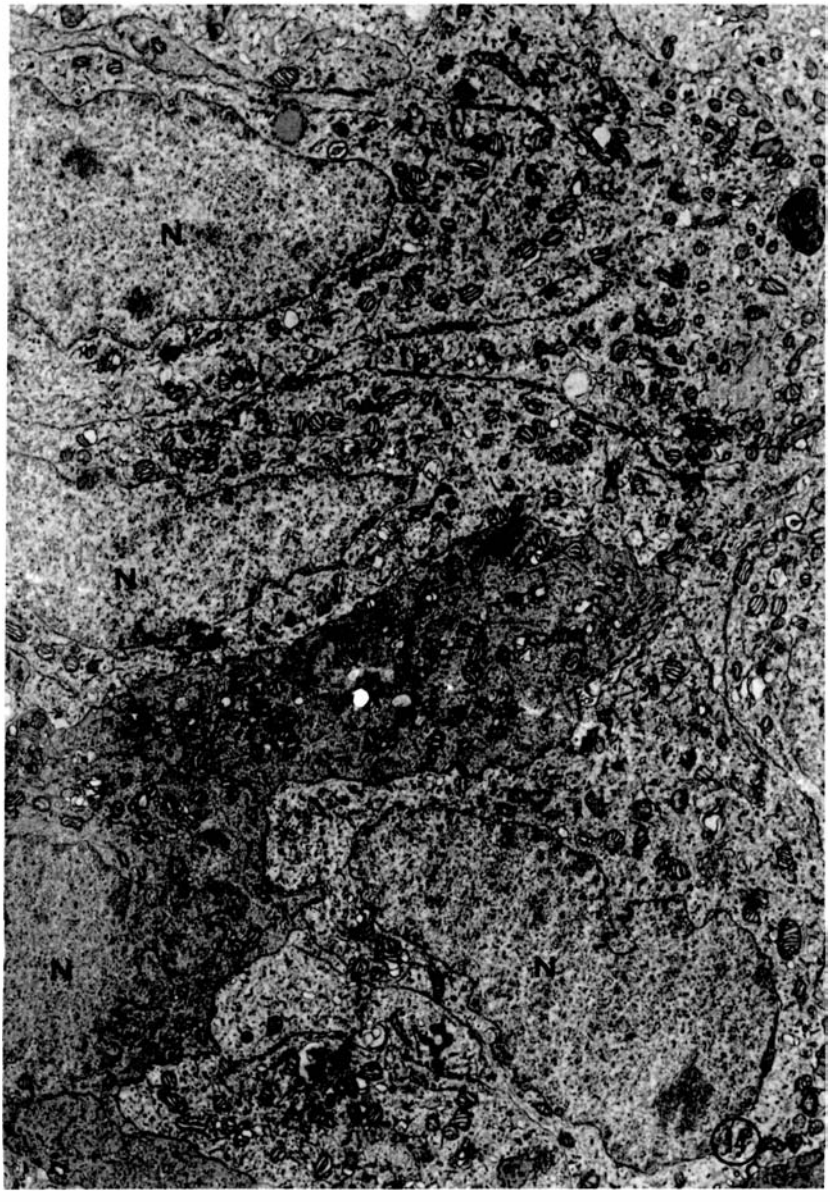

N
N
N
N

Figure 15

Normal PET mouse pineal gland.

The PET mouse pineal organ has numerous capillaries (C). The capillary endothelium is fenestrated (←f) and surrounded by a pericapillary space (PS). The pericapillary space contains autonomic nerve fibers (a) which often are in proximity to Schwann cell cytoplasmic extentious (s→). X18,120.

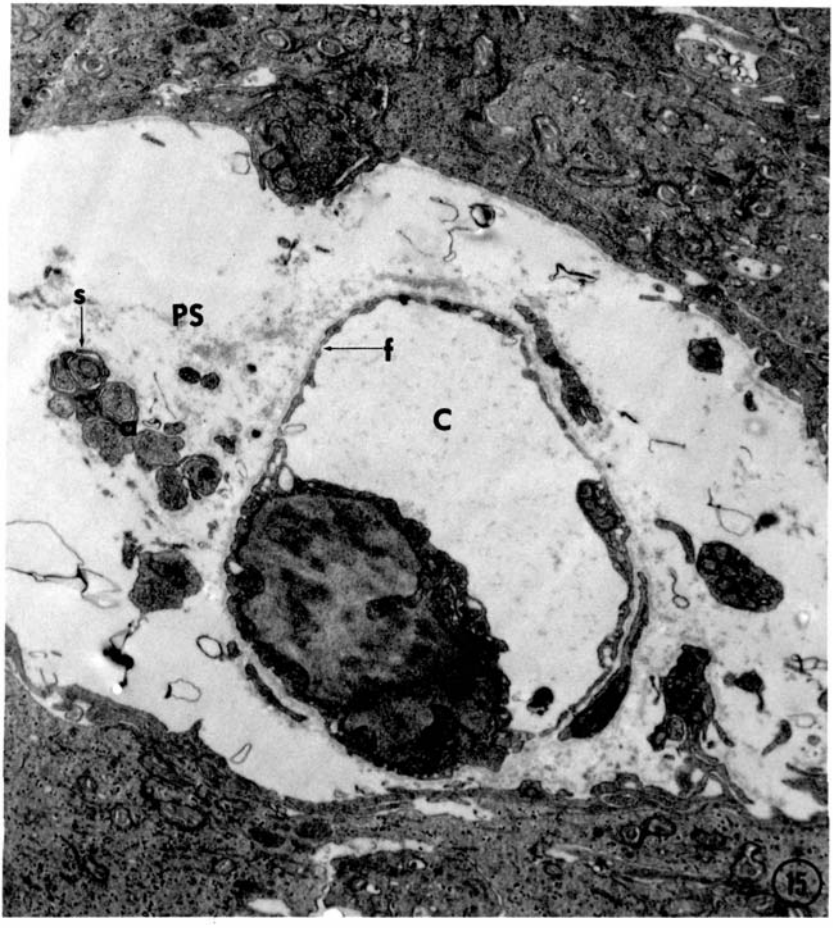

s
PS
f
C
15

Figure 16

Normal PET mouse pineal gland.

The nucleus (N) of the pinealocyte is highly lobulated and has a prominant nucleolus (n). A Golgi complex (G) and mitochondria (m) with irregular cristae are present.

The distinction between the light cell (L) and dark cell (D) is evident. A dense core vesicle is visible in the perikaryon of the dark cell (→) X 12,960.

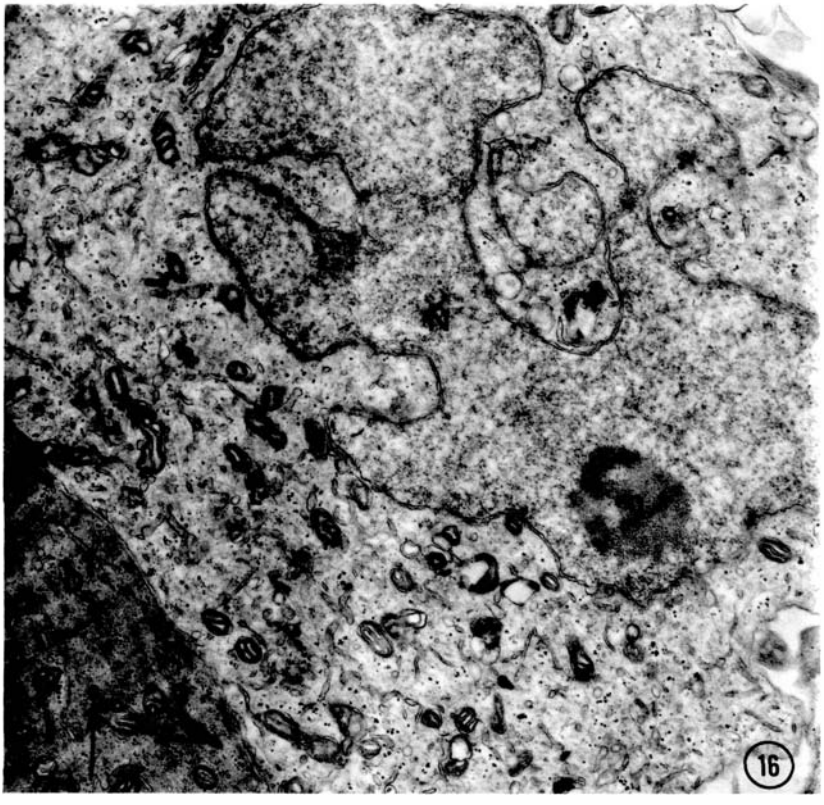

16

Figure 17

Normal PET mouse pineal gland.

Mouse pinealocytes may contain large stores of glycogen (G). Dense-core vesicles (→) are evident in both the dark and light cells. Nuclear pores, rough endoplasmic reticulum, smooth endoplasmic reticulum, and free ribosomes are seen in pinealocytes. Nucleus (N). X 22,260.

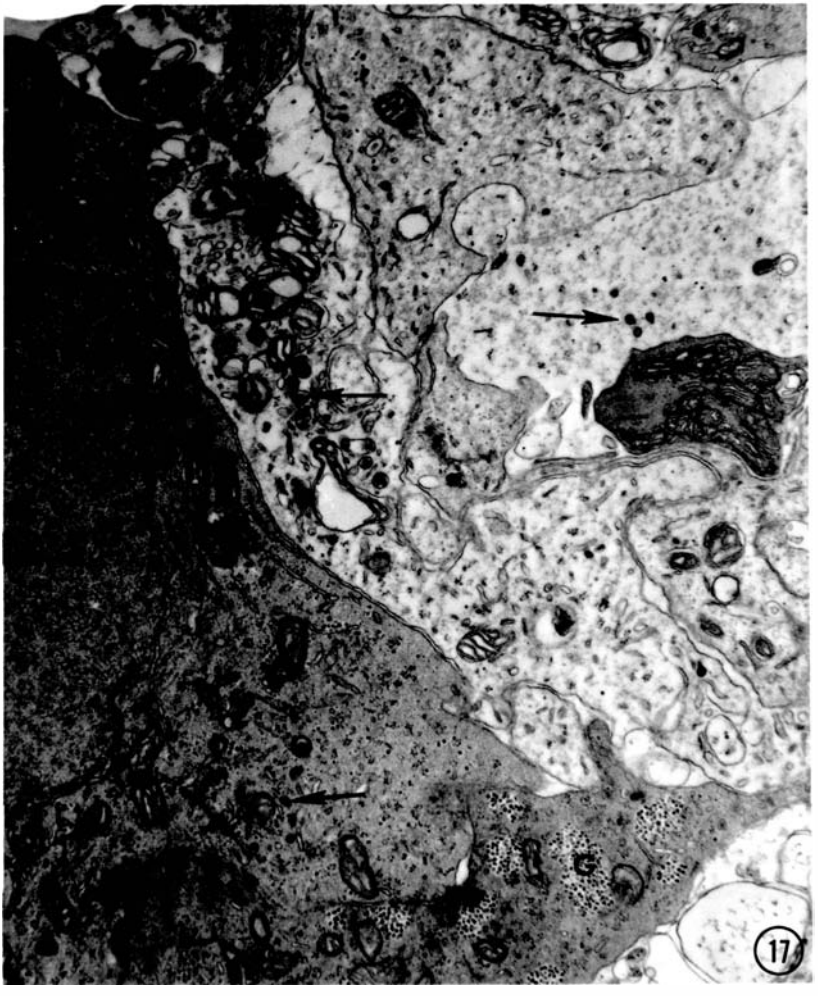

17

Figure 18

Normal PET mouse pineal gland.

The polar processes of pinealocytes intermingle with each other as they extend from their respective perikarya. These processes also contain dense cored vesicles (→). X 27,290.

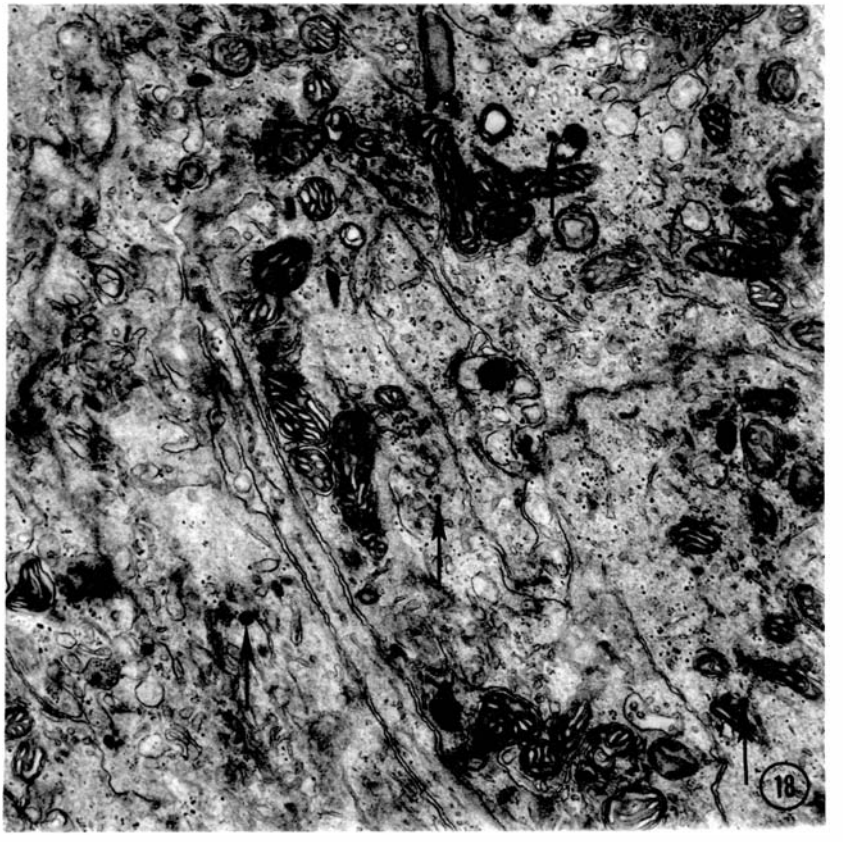

18

Figure 19

Normal PET mouse pineal gland.

The polar terminations (PT) of the pinealocyte polar processes may end on the perykaryon of the other pinealocytes (→). There is a cleft between the membranes and no modifications of the cytoplasm. Junctional complexes were not seen in the PET mouse pineal gland. X 18,840.

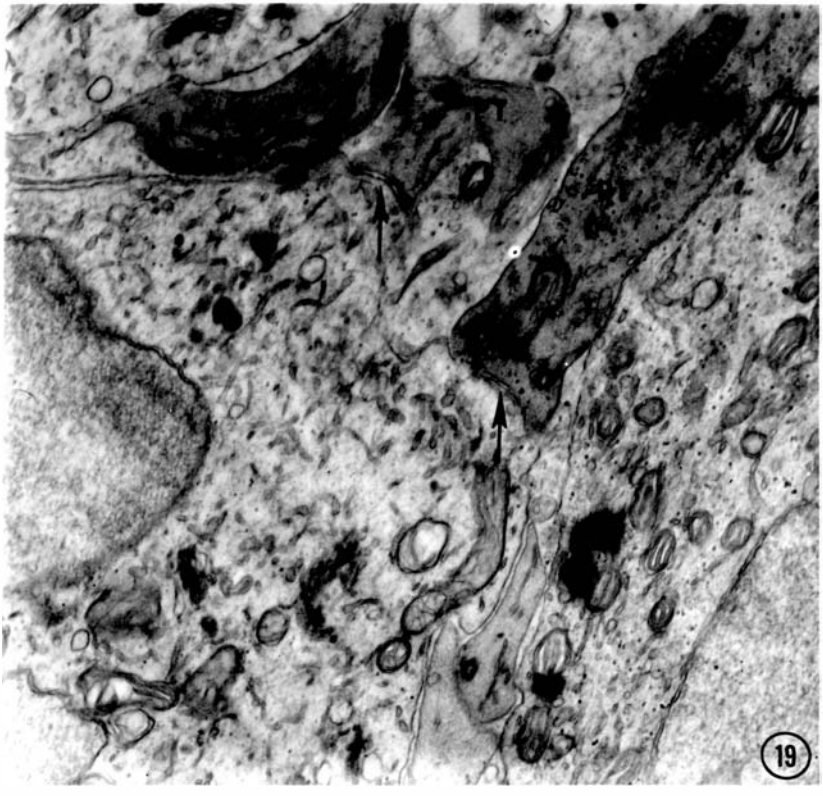

19

Figure 20

Normal PET mouse pineal gland.

The pericapillary space (PS) contains pinealocyte polar terminals (PT). These terminals have the same type of dense cored vesicles which were found in the pinealocyte perikaryon and processes. A capillary (C) endothelial fenestration can be seen to be closed by a thin diaphragm (→). Red blood cell (R). X 30,050.

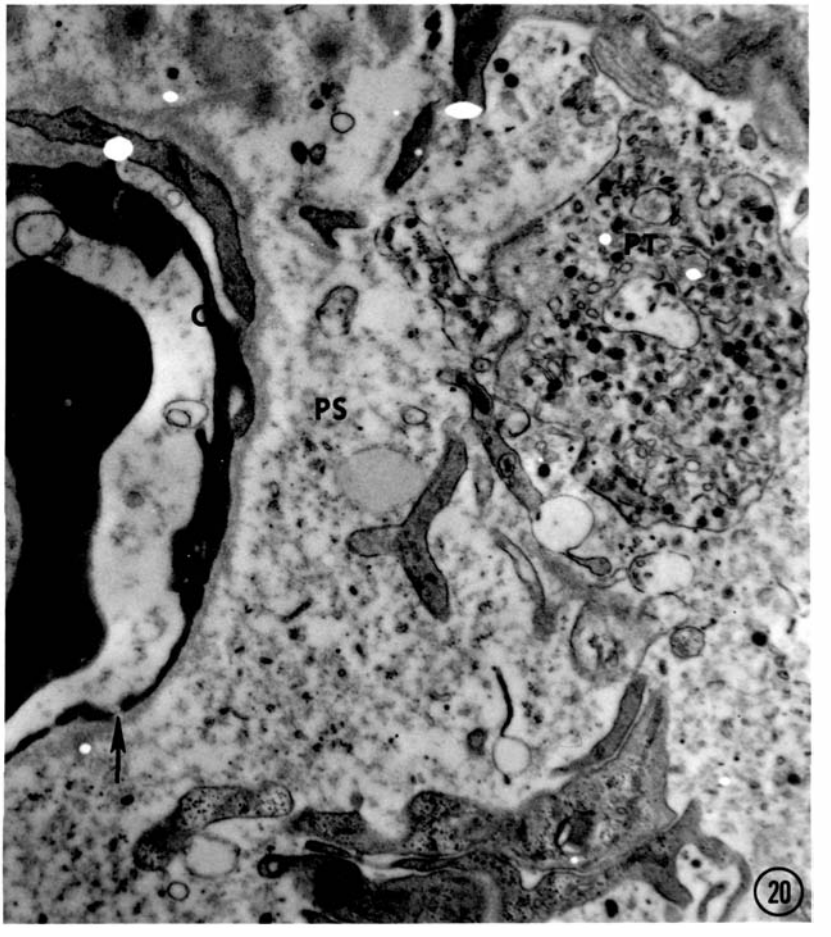

PT
PS
20

Figure 21

Normal PET mouse pineal gland.

The autonomic nerve fibers (a) in the pericapillary spaces are frequently embedded in Schwann cells (S). The autonomic fibers are made up of small granular (→g) and agranular (c →) vesicles. X 38,850.

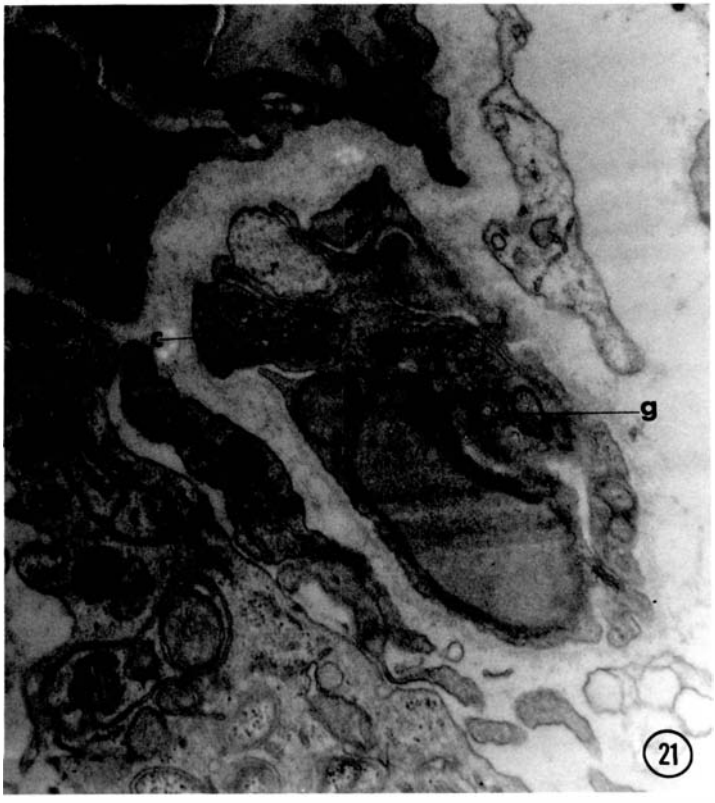

g
21

Figure 22

Capsule of the PET mouse pineal gland.

Many myelinated (M) and unmyelinated nerves (U) are found in the capsule of the PET mouse pineal gland. Myelinated nerves, however, were not seen in the pineal gland itself. X 13,590.

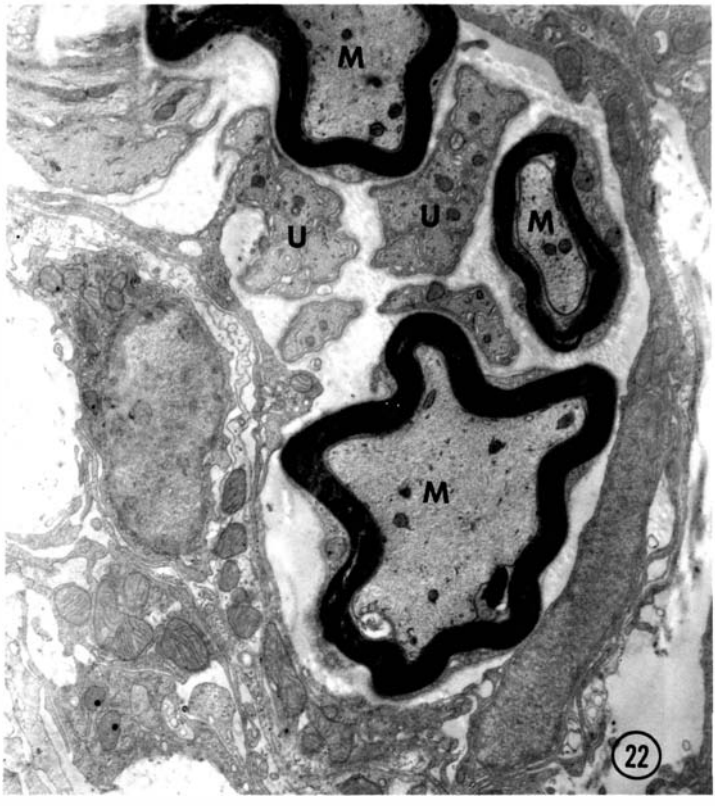

M
U
U
M
M
22

Figure 23

Normal PET mouse pineal gland.

Interstitial cells (IC) are frequently found near the pericapillary and intercellular spaces. They are characterized by their elongated nuclei with great amounts of chromatin found in thick aggregations in the nucleus. Collagen (c). X 10,520.

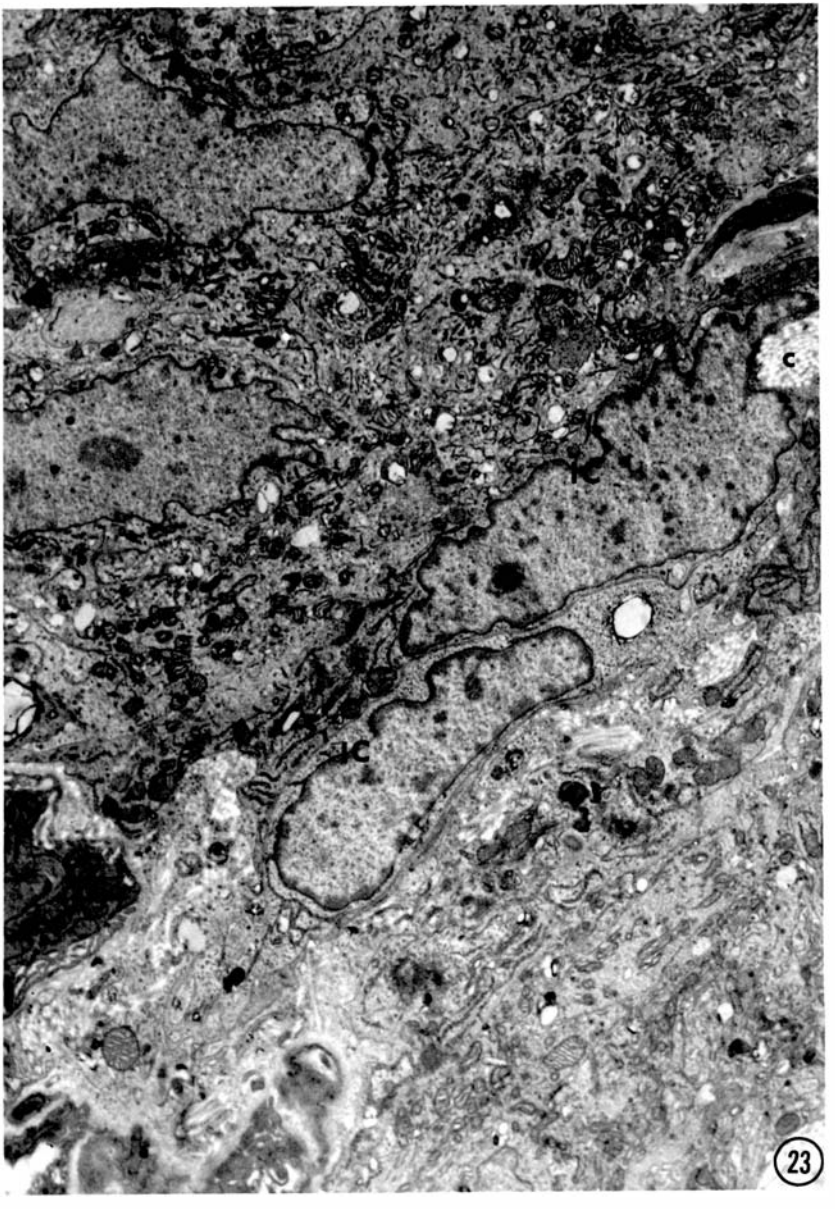

C
C
23

Figure 24

Blinded PET mouse pineal gland.

Light (LP) and dark (DP) cell processes are distinguishable in the pineal gland of blinded PET mice. The cytoplasm appears more highly vesiculated (V) than in the normal gland. X 8,925.

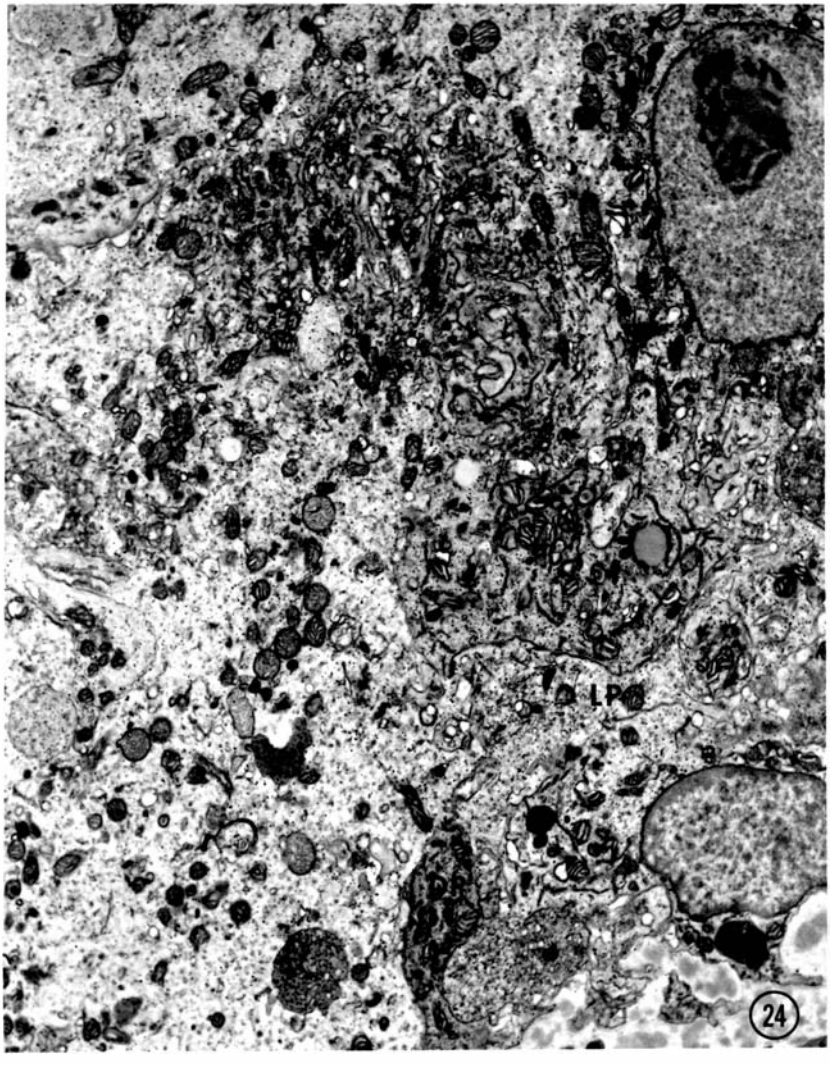

LP
24

Figure 25

Blinded PET mouse pineal gland.

Many vesicles (→) appear in close association with mitochondria in the blinded animals. Some mitochondria appear "bulls-eye" in shape (B). X 10,050.

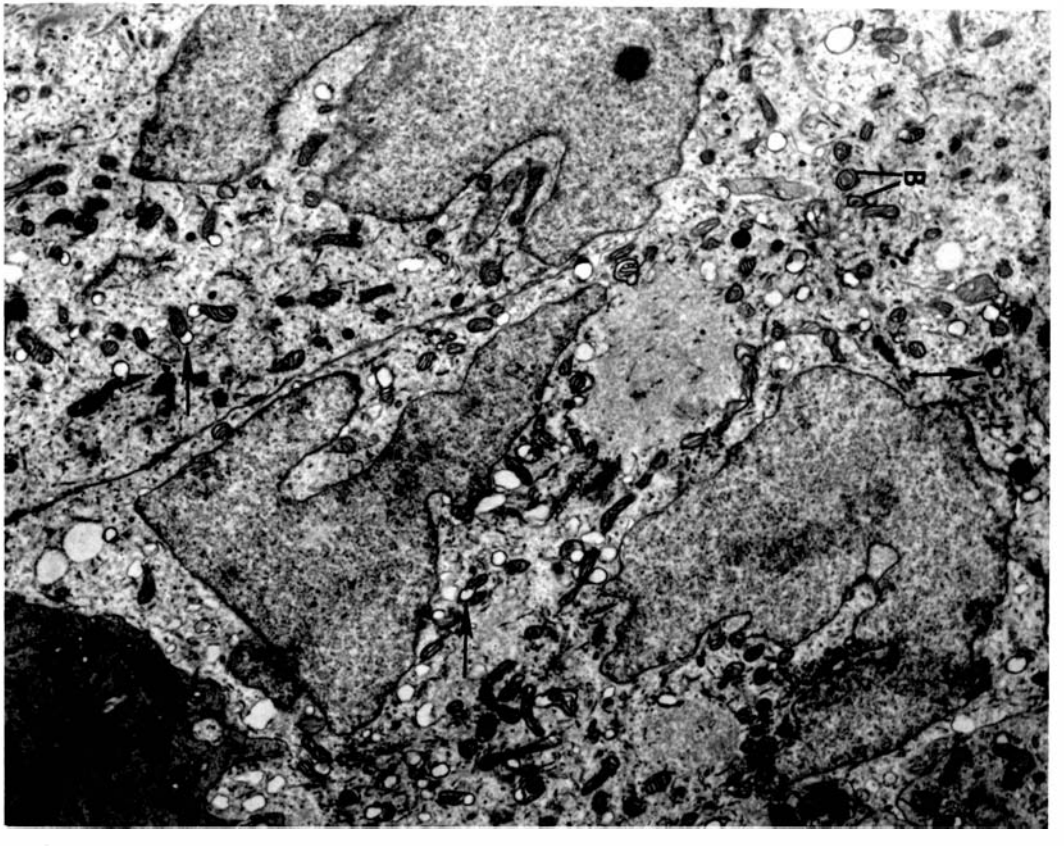

B

Figure 26

Blinded PET mouse pineal gland.

Autonomic nerves (a) are found in the pericapillary space (PS). X 23,190.

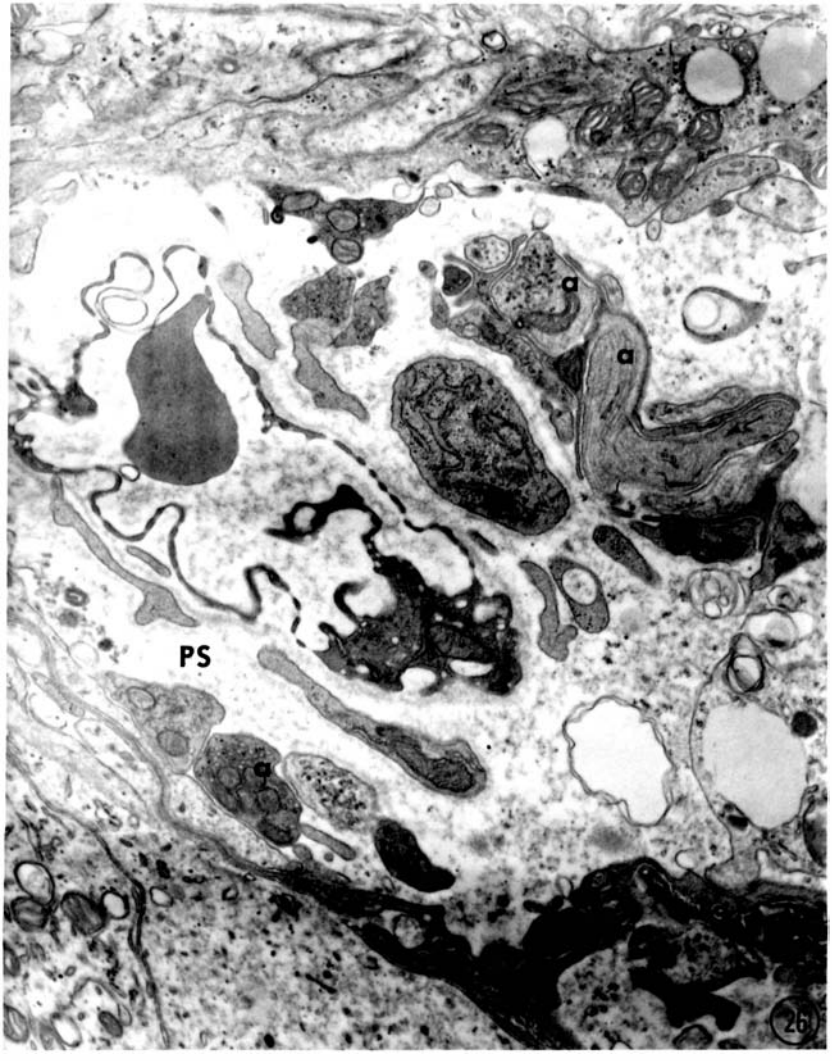

a
a
PS
26

Figure 27

Blinded PET mouse pineal gland.

In some blinded animals, the nuclear membranes (→) of some pinealocytes are lamellated in areas and give a myelin-figure appearance. The cristae of many mitochondria are "bulls-eye" in configuration (B). X 8,925.

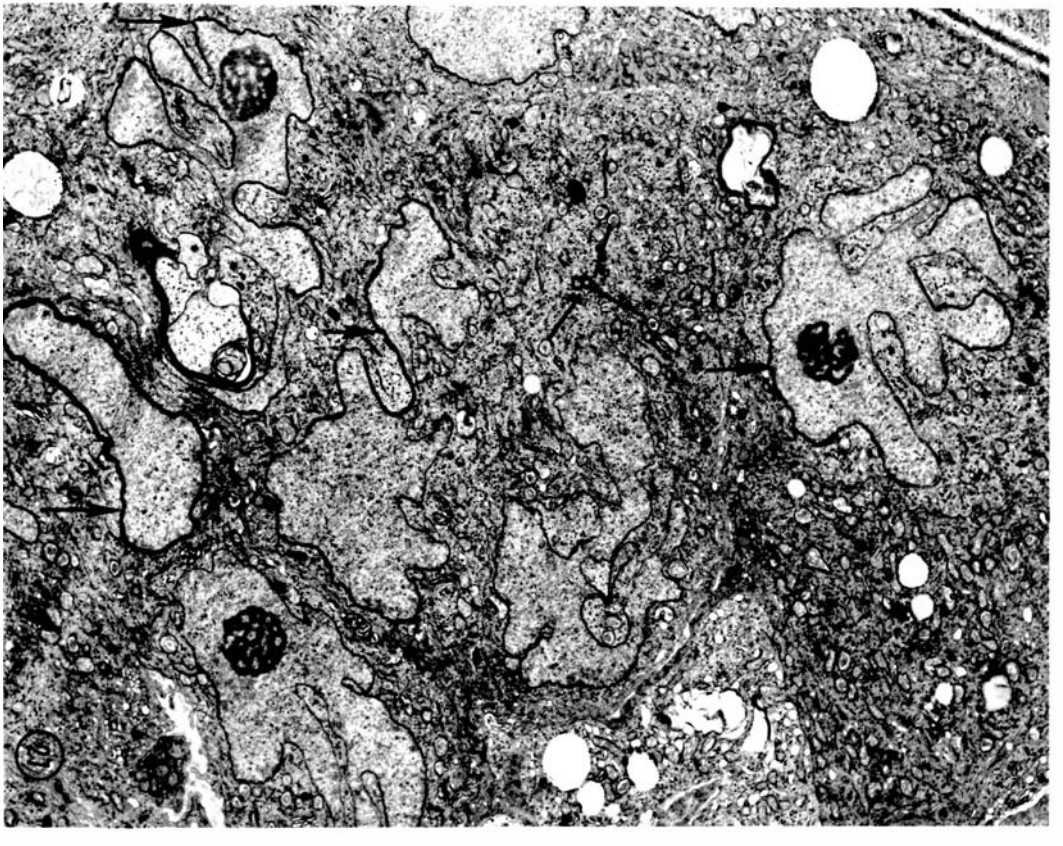


Figure 28

Normal gerbil pineal gland.

Light cells (L) and dark cells (D) are found in the pineal gland. Many intercellular spaces (is) are visible. Junctional complexes were not seen in the gerbil pineal gland. X 14,730.

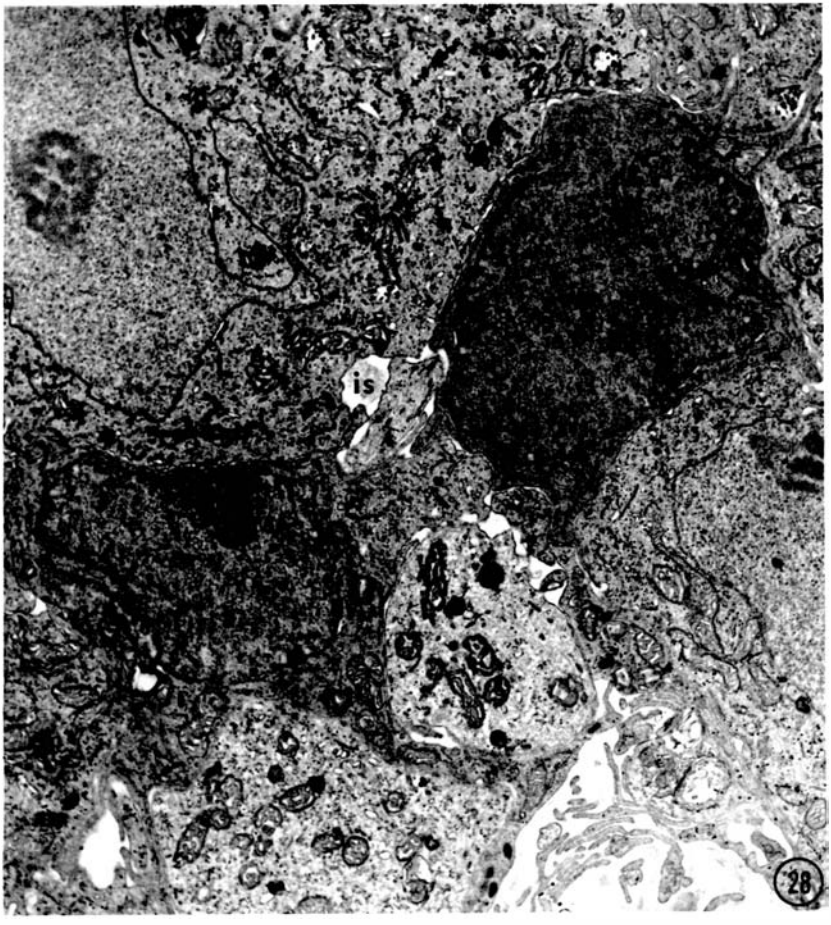

is
28

Figure 29

Normal gerbil pineal gland.

Normal gerbil pinealocytes have large accumulations of glycogen (X). Well developed Golgi zones (G) and on occasion a centriole (→) can be seen. X 19,640.

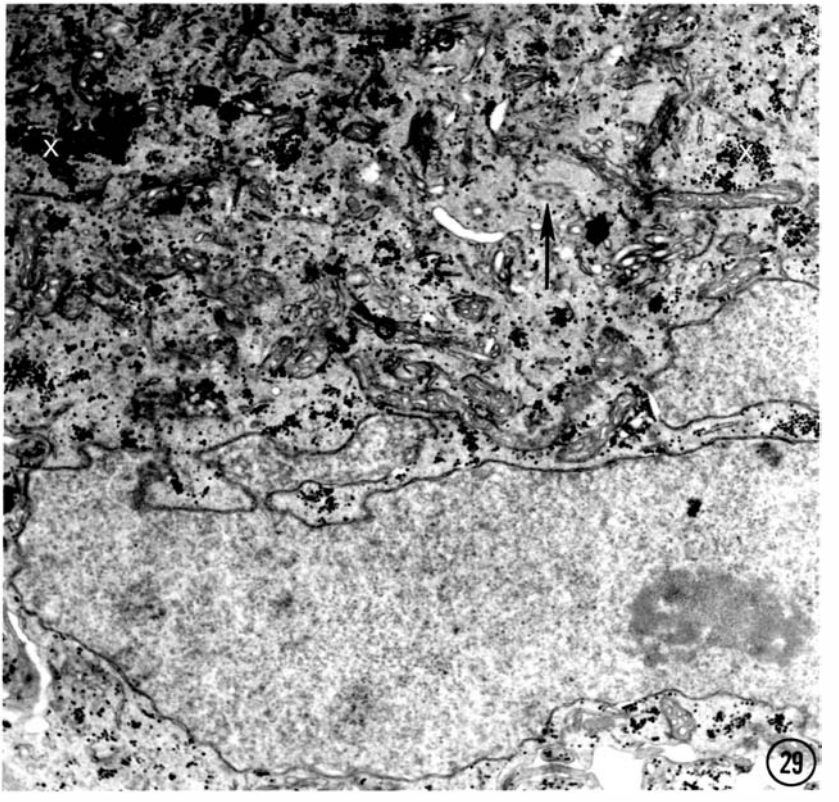

X
X
29

Figure 30

Normal gerbil pineal organ.

Large granules (LG→) and small granules (SG→) are always found in gerbil pinealocytes. The larger ones are frequently found in association with mitochondria. X 21,495.

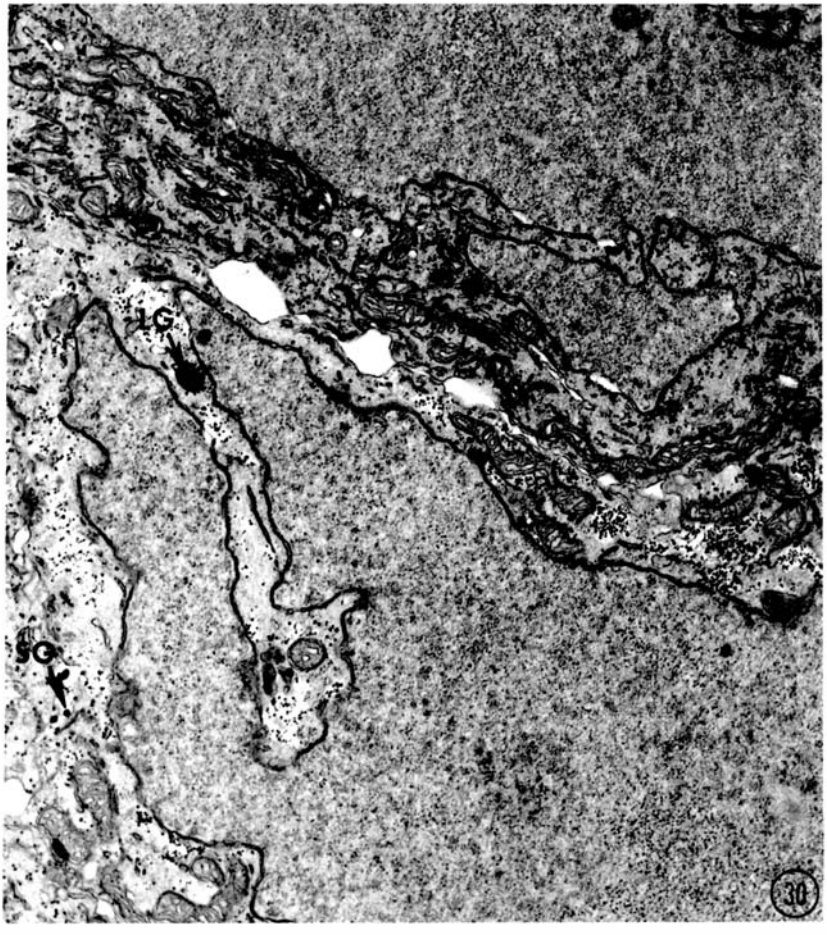

LG
SG
30

Figure 31

Normal gerbil pineal gland.

The granules in the intercellular spaces (IS) have a similar appearance to the small granules (→) found in the pinealocytes. These granules are sometimes found within an enlargement of the pinealocyte cell membrane and appear to be connected by fine filaments (X →). X 32,875.

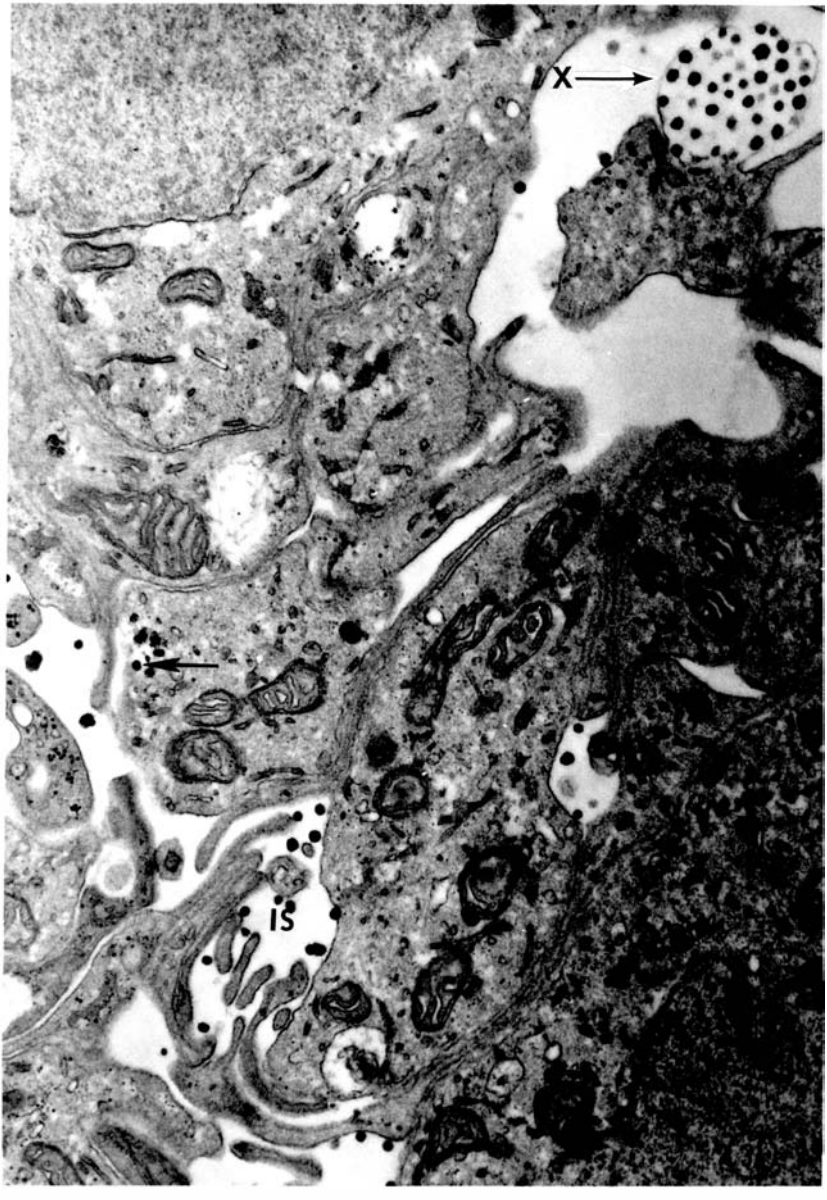

X
IS

Figure 32

Normal gerbil pineal gland.

Many light pinealocytes appear to have a "washed out" cytoplasm with an increase in large (→) and small (sg) granules. The larger granules appear related to mitochondria. X 13,590.

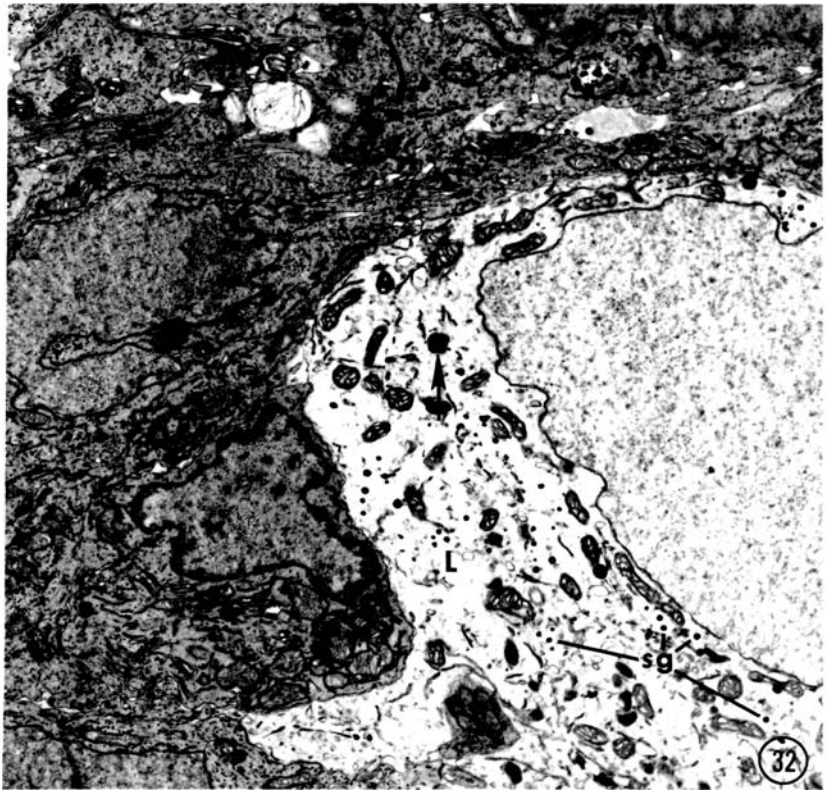

L
sg
32

Figure 33

Normal gerbil pineal gland.

Two variations in appearance of adjoining light pinealocytes. The light cell with a "washed out" appearing cytoplasm has many more granules than the other light cell. X 31,760.

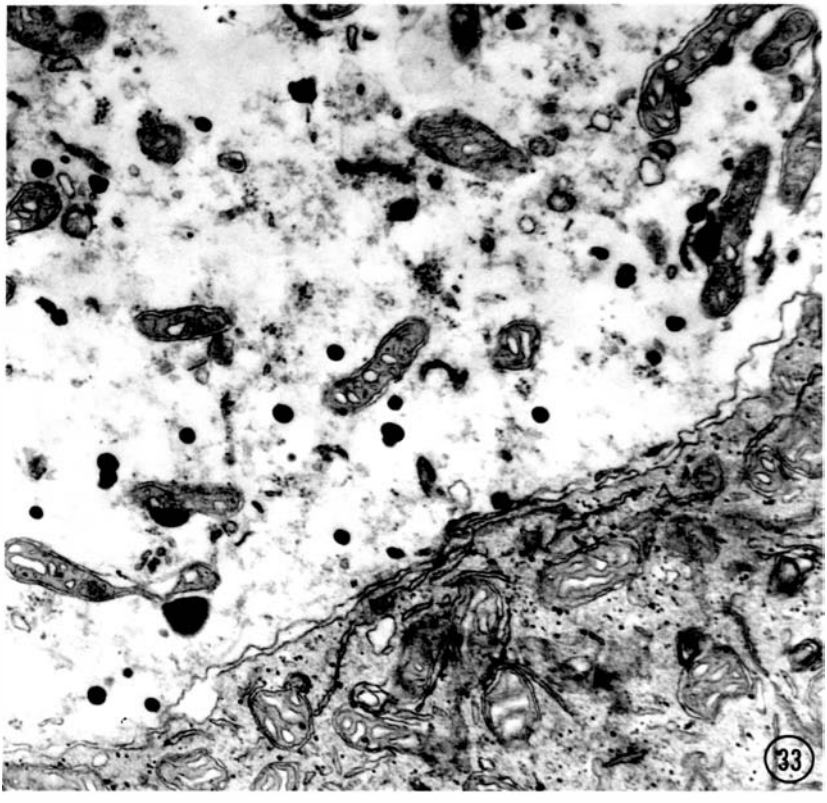

33

Figure 34

Blinded gerbil pineal organ.

Large electron dense structures are found in cell processes in the pericapillary space (PS).

X 12,410.

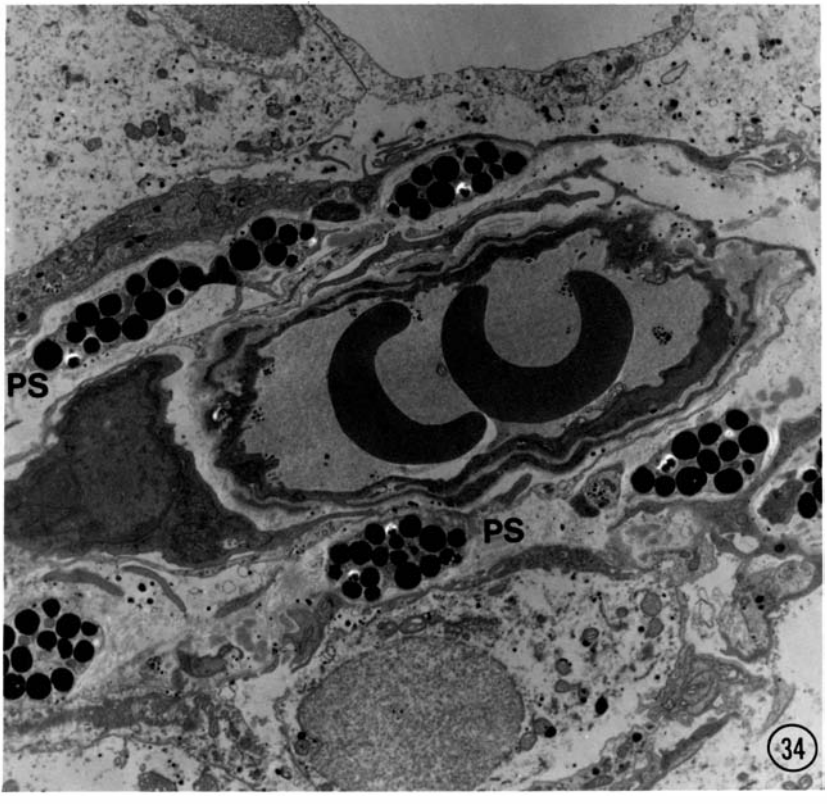

PS
PS
34

Figure 35

Blinded gerbil pineal gland.

An interstitial cell with large electron dense structures in its cytoplasm is illustrated. Collagen (C). X 19,250.

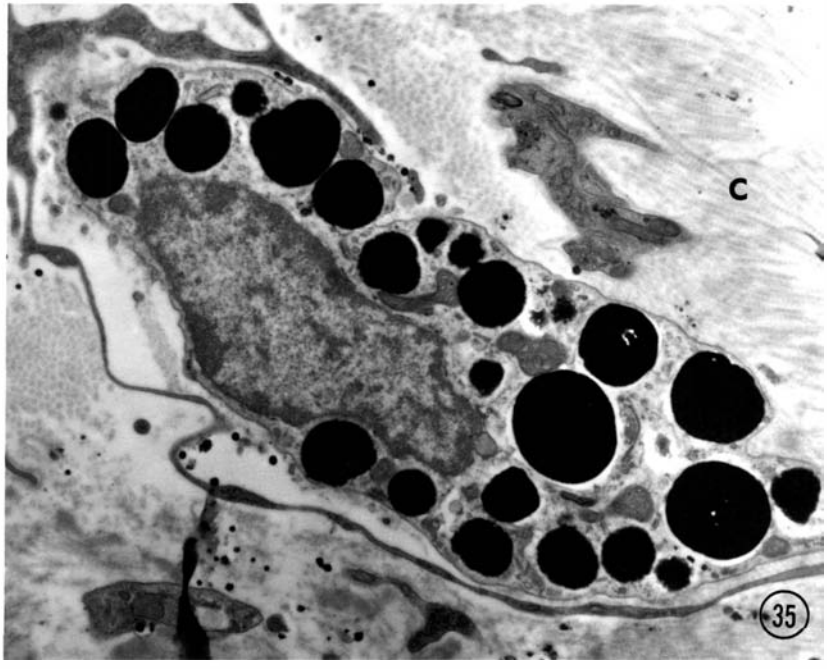

C
35

Figure 36

Blinded gerbil pineal gland.

Large electron dense granules similar to those found in cell processes and interstitial cells can also be found in pinealocytes. X 18,480.

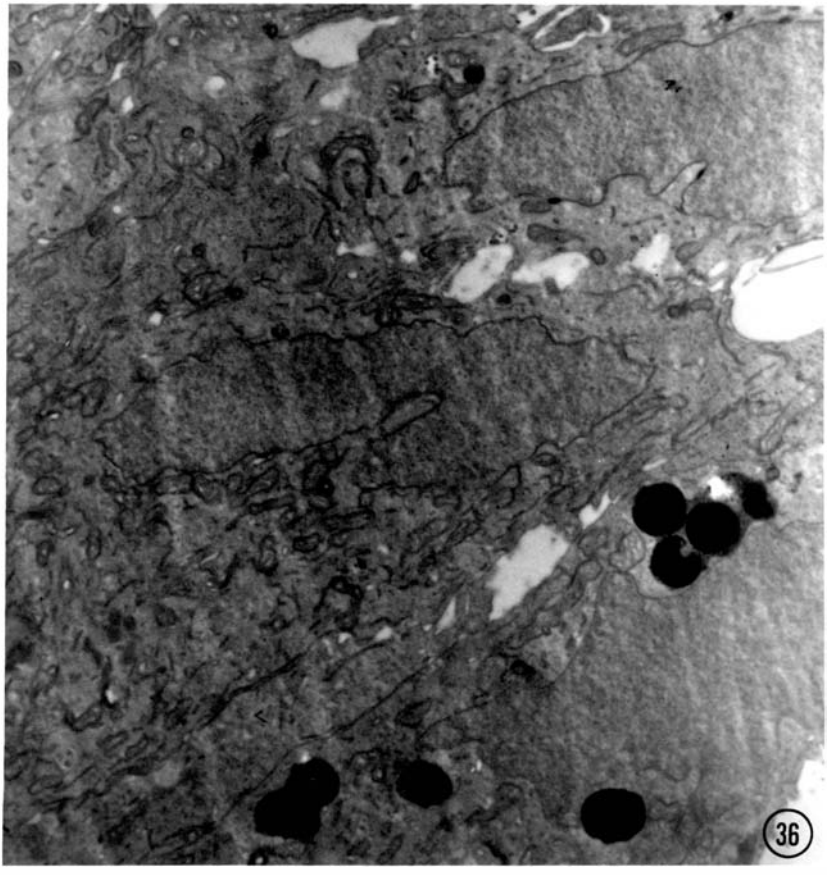

36

Figure 37

Blinded gerbil pineal organ.

Many times, large electron dense structures appear to be forming from smaller circular structures (→). A dark cell (D), dark cell process (dp), and a "washed out" appearing white cell (L) can still be recognized in blinded gerbil pineal organs. X 7,325.

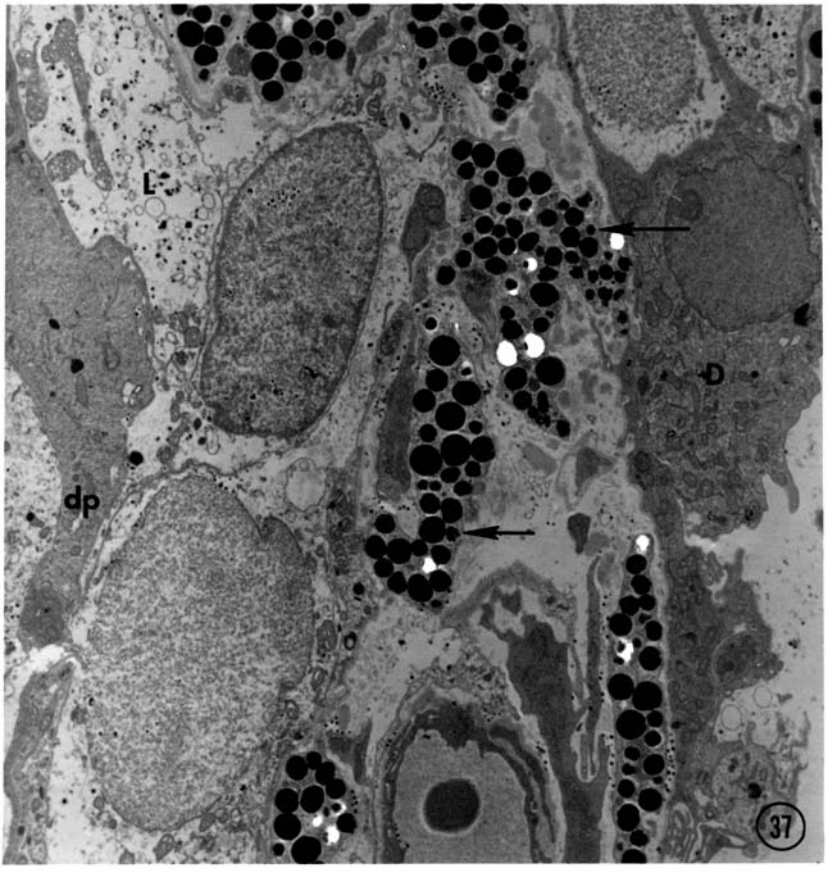

L
D
dp
37

Figure 38

Pineal Sac.

The cuboidal epithelial cells of the pineal sac are joined by junctional complexes (→). Their apical end has numerous microvilli (MV) and infrequent cilia (C). The basal portion of the cell which is nearest the vascularized core (VC) exhibits infolding of the cell membrane (X). X 10,870.

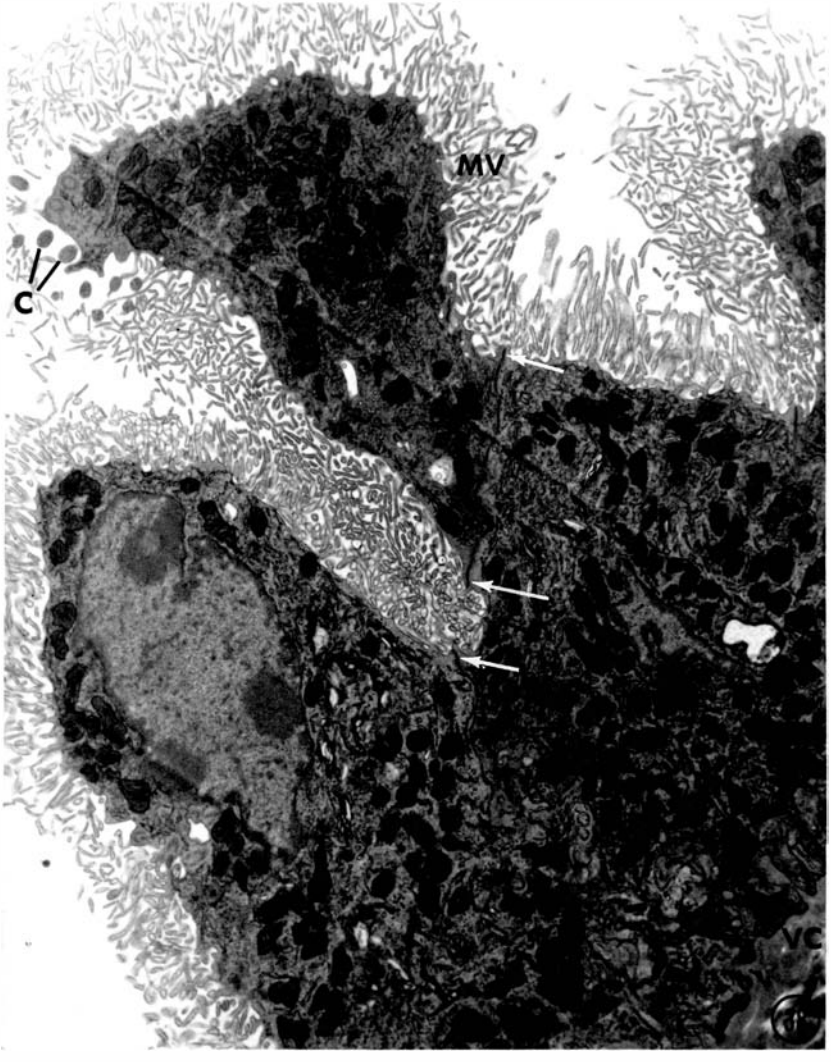

MV
C
VC

Figure 39

Pineal Sac.

A high power view of the apical end of a pineal sac epithelial cell with its nucleus (N), microvilli (MV), a mitochondrion (M), and a cilium (C). X 30,050.


C
MV
M
N
39

Figure 40.

Pineal Sac.

Perivascular cell processes (P) can be seen with granules and a canalicular system within the cell. The great infolding of the epithelial cell membrane at the basal end of the cell can also be seen (X). Microvilli (M) are apparent. X21,495.

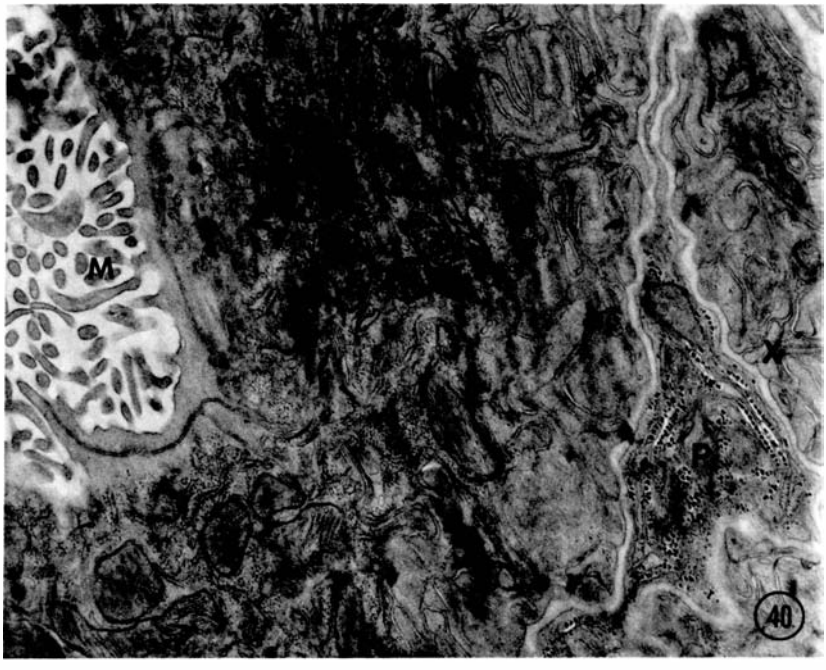

M
X
P
40

Figure 41

Pineal Sac.

A pineal sac epithelial cell (E) can be seen near a capillary (C). The capillary has a fenestrated endothelial cell membrane (→). X 14,950.

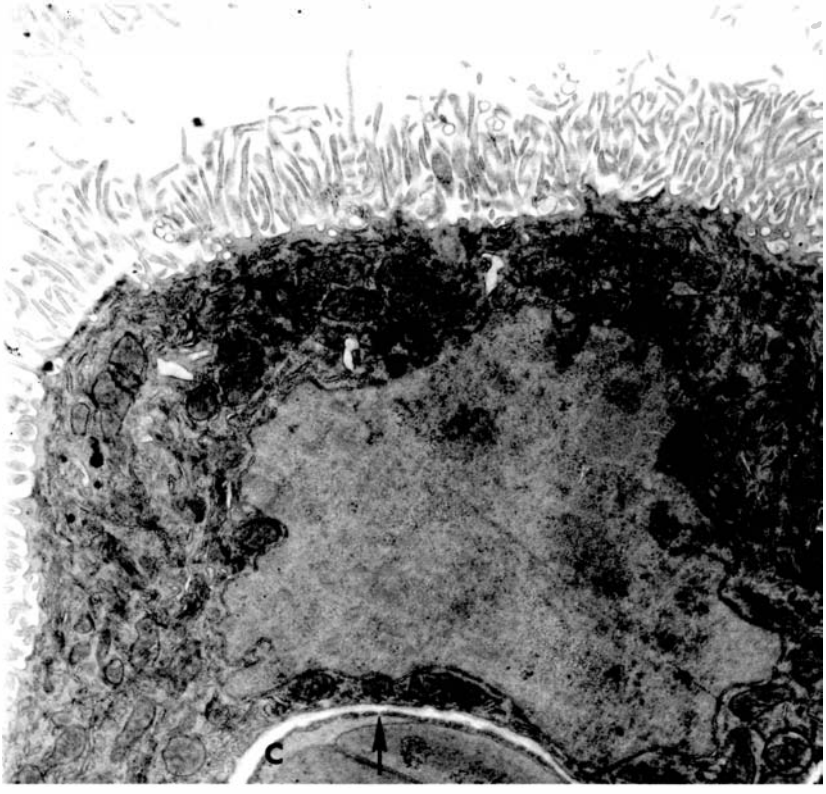

C

Figure 42

A testis reveals the decreased testicular weight which occurs in hamsters after blinding. The testis of a blinded-pinealectomized hamster however appears normal in size.


CONTROL
BLINDED
PINEALECTOMIZED
HEMICASTRATED
BLINDED & PINEALECTOMIZED
42

Figure 43

Normal hamster testis.

The normal hamster testis is made up of large seminiferous tubules with much intertubular space. The cells of the tubules have all spermatocytogenic and spermiogenic cell types possible. There are mature sperm in all lumina. The spermatogonia have a high mitotic rate which is indicative of active spermatogenesis. X 224.

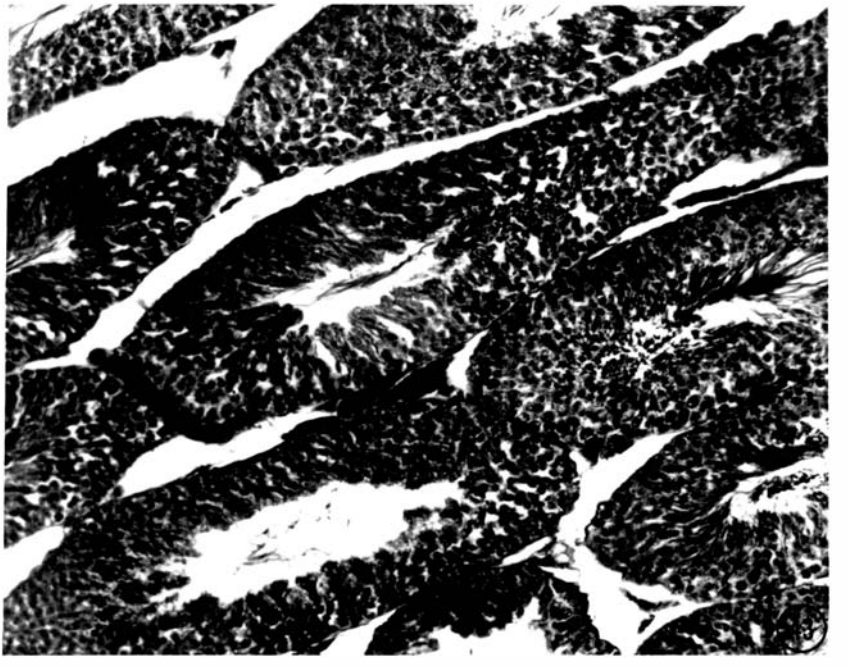


Figure 44

Testis of a blinded hamster.

The tubules of these testes are smaller in size with less intertubular space than the tubules of normal hamster testes. The lumina lack mature sperm and spermatids, but have extensions of Sertoli cell (SC) cytoplasm. The spermatogonia (→) frequently have light halos surrounding them. X 375.

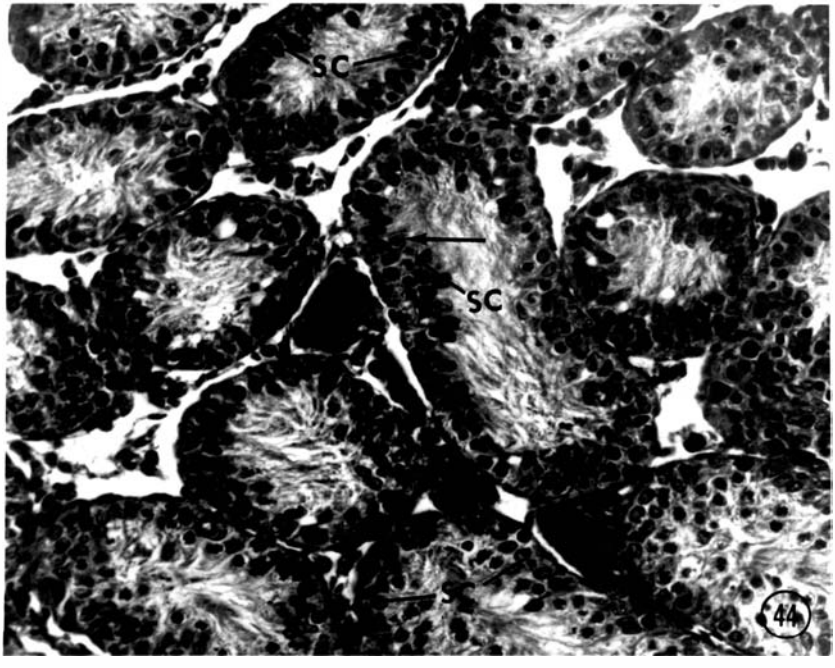

SC
SC
44

Figure 45

Seminal vesicles of control gerbils.

The highly folded mucosa is made up of columnar epithelial cells. The cavities of these glands are generally filled with a densely staining material (X). X 375.


45

Figure 46

Seminal vesicles of a blinded gerbil.

The mucosa of the gland is composed of low cuboidal cells which appear to have a scattered, non-luminal array (X). There is also a great infiltration of dense connective tissue (DCT) separating the lobules of the gland. X 375.

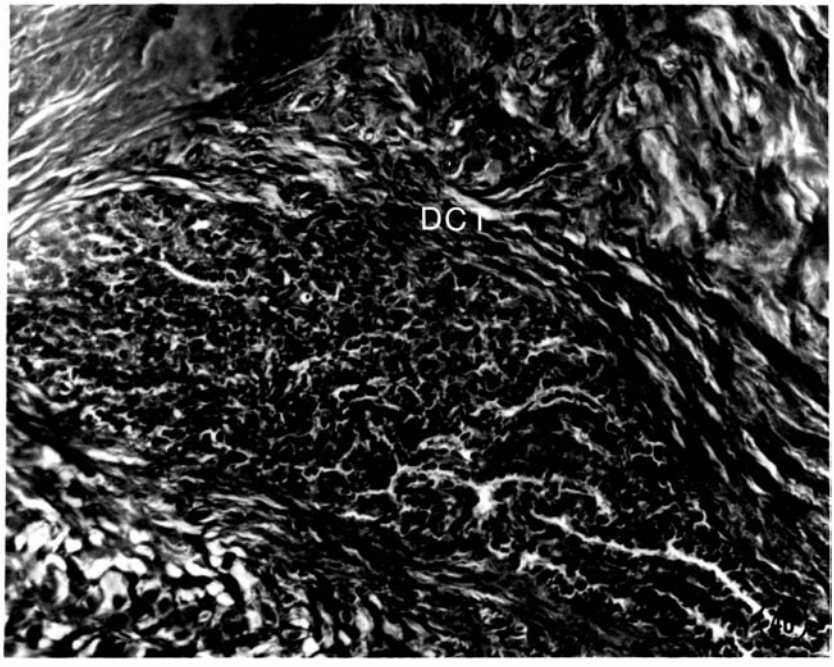

DCT

Figure 47

Normal gerbil adrenal gland.

The zona glomerulosa (ZG) is composed of large cells with darkly staining cytoplasm. The zona fasciculata (ZF) has large cells with low staining affinity because of the ligh lipid content. Zona reticularis (ZR). X 240.

ZR
47

Figure 48

Adrenal gland of some experimental gerbils.

The zona glomerulosa (ZG) is not clearly distinguishable from the zona fasciculata (ZF) because of the low staining affinity of some of the zona glomerulosa cells. The cells of the zona fasciculata in these animals are smaller in size than those of the control animals. There is also a higher rate of cellular proliferation as is evidenced by the rows of small cells (→). Zona reticularis (ZR). X 240.

ZG

Figure 49

Ovary of a blinded gerbil.

The ovaries of blinded and blinded-olfactoriectomized gerbils generally have an increase of connective tissue (CT) and atretic follicles (AF). X 310.

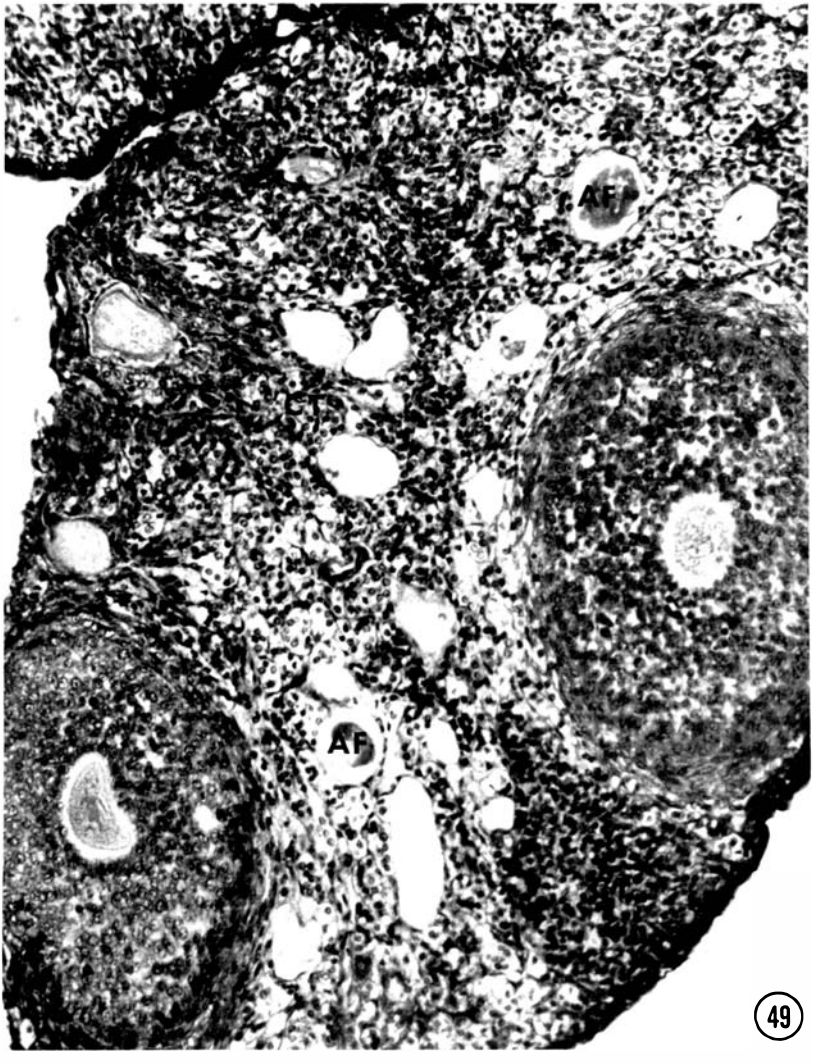

AF
AF

Figure 50

Ovary of a control gerbil.

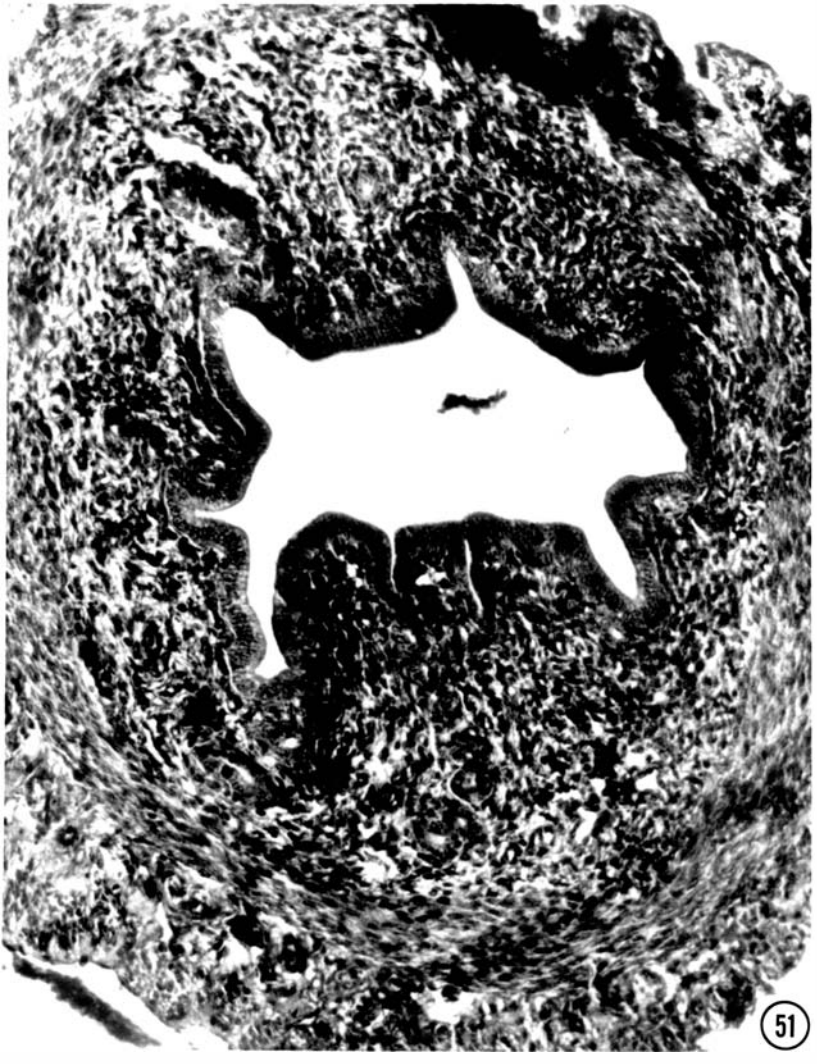
The ovaries of control gerbils have numerous primordial follicles (PF) and vast accumulations of lutein cells (LC). Primary follicle (F). X 310.

T.LG

Figure 51

Uterus of a blinded gerbil.

The uteri of blinded and blinded-olfactoriectomized gerbils are smaller in diameter than the uteri of control animals. The number and size of the endometrial glands are decreased. X 320.



51

Figure 52

Uterus of a Control gerbil.

The uteri of control gerbils have a large, extensive lumen (L) with many endometrial glands (g). X 124.

52

Figure 53

Vagina of a blinded gerbil.

The vaginae of blinded and blinded-olfactoriectomized gerbils have a thin squamous epithelial lining. None of these animals showed an estrus vaginal cornification. X 384.

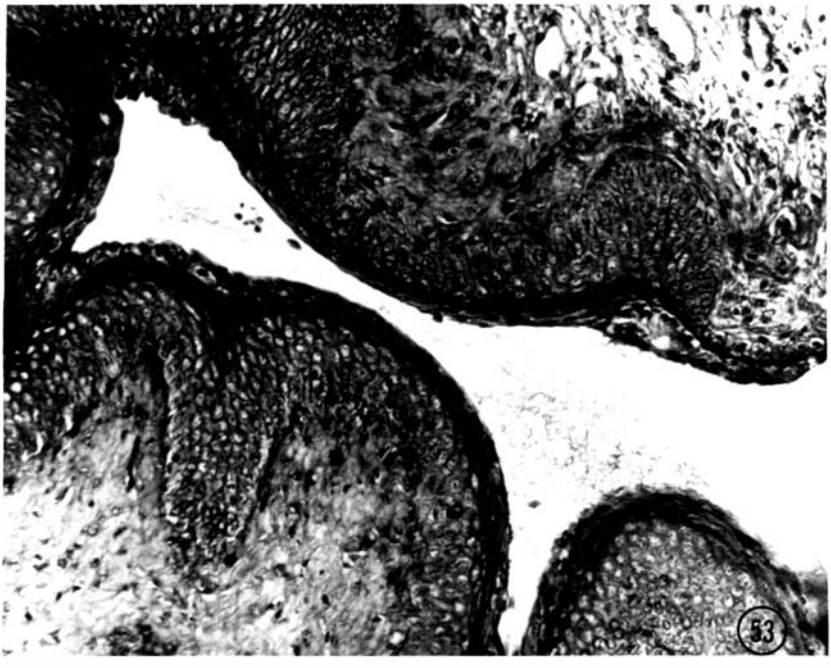
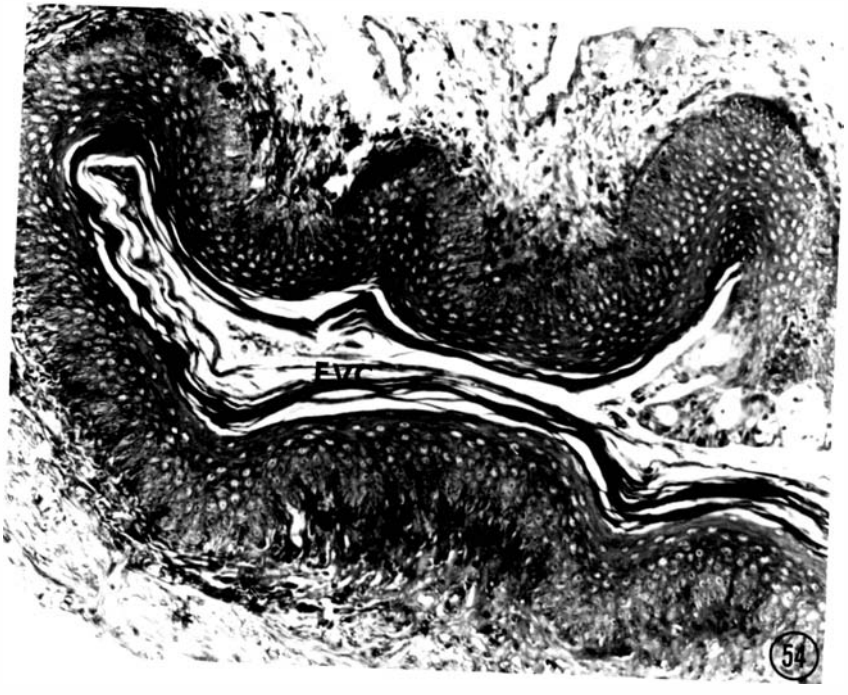

53

Figure 54

Vagina of a control gerbil in estrus.

Vaginal cornification (EVC) and a thick vaginal epithelium were found in many control gerbils indicating properly cycling estrus animals. X 268.


Evc
54