A Comparative Anatomical Study of the Pineal Gland in Mammals

Sood
A COMPARATIVE ANATOMICAL STUDY OF THE PINEAL GLAND IN MAMMALS

by

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CHAPTER 1

INTRODUCTION

The pineal organ is one of the most recent organs to have been rediscovered and subjected to modern investigational techniques. Wurtman (1965a), Krabbe (1961), and Adam (1957), reviewed the presence of the pineal gland in all present day vertebrates. With this nearly universal distribution of the pineal organ in vertebrates there evolved a vast range of morphological diversity. Most of the recent anatomical investigations have been devoted to elucidating the diversity in hopes of correlating it with emerging physiological evidence. Comparative studies indicated the diversity at the gross and light microscopic levels.

The form of the mammalian pineal organ varies between species. There is an elongated type similar to that of birds, situated between the telecepha] and cerebellum, and attached to the dura mater, (e.g. guinea pig) and a conical type (carnivora), or pear-shaped (cattle, man), (Oksche, 1965). A capillary network courses deeply through the parenchyma of the mammalian pineal. In the monkey, a rich central plexus is evident, while vessels are sparse in other areas. (Le Gros Clark, 1939-1940). This pattern is not necessarily the rule in other mammalian species.

In the sub-mammalian species, the pineal system is complex, consisting of two different organs. An intracranial component,
epiphysis cerebri, is supplemented by a second extracranial component known as parapineal or frontal organ. (Fakin, 1964, Oksche, 1965). The pineal organs of cyclostomes, most fish, amphibians and lizards show the characteristics of a sense organ. (Adam, 1957). Among these, several cyclostomes, fish and lizards have a well developed parapineal organ.

Some investigators thought of the pineal organ in the sub-mammalian species as a third eye. Franz Leydig (1872) investigated many questions regarding the evolution of the pineal. When did nature invent the third eye? Were these prevertebrate antecedents? How large was the eye in ancient reptiles and amphibians? Was it paired? What changes did it undergo during the evolution of birds and mammals, etc.? His studies reported that third eyes were not preserved in any known fossil vertebrate, but their presence was indicated by a hole, the parietal or pineal foramen, on the top of the skull of extinct fishes, amphibians and reptiles.

Pineal differentiation in most lower vertebrates is also associated with the acquisition of morphological structures resembling the known photoreceptors in the vertebrate retina. The concept that lower vertebrate pineal organs are photoreceptors is strongly reinforced by neurophysiological data (Dodt, 1963, Dodt and Jacobson, 1963, and Morita, 1965). Work showed that potentials were evoked along pineal tracts, when the organ was exposed to light of varying wave-lengths or darkness.

The mammalian pineal has been described as lacking sensory cells.
The specific mammalian pineal parenchymal cell or pineocyte is generally held to be secretory. The mammalian epiphysis is an endocrine organ, the parenchymal cells producing and storing specific components, which are secreted into the rich pineal vascular system (Oksche, 1965).

Recent physiological studies have shown that the mammalian pineal responds to environmental light indirectly. Wurtman and Axelrod (1965a) have shown that the mammalian pineal functions as a biological clock in response to photoperiods. The photoperiods produce secretions, probably hormones, in the pineal which regulate timed events such as mating or migrations.

Recent work in this laboratory on rodents' (laboratory rat and wild rat) pineal in situ has shown that this structure is not buried deeply within the brain as stated in the literature. Part of the pineal lies immediately under a relatively thin bony fossa and secondarily extends down into the thalamic area.

A comparative study of mammalian pineal was undertaken to determine the placement of this organ relative to the skull structures. The animals studied included an equal number of herbivores to carnivores.
CHAPTER II
LITERATURE SURVEY

The existence of the pineal body has been known for at least 2,000 years. Galen, writing in the second century A.D. quoted studies of earlier Greek anatomists, who were impressed with the fact that the pineal was perched atop the aqueducts of the cerebrum in the human and was a single structure rather than a paired one. He concluded that it served as a valve to regulate the flow of thought out of its "storage bin" in the lateral ventricles of the brain (Wurtman, R.J., 1965a). In the 17th century Rene Descartes (1662) embellished this notion; he believed that the pineal housed the seat of the rational soul. In his formulation, the eyes perceived the events of the real world and transmitted what they saw to the pineal by way of strings in the brain, (Fig. A).

In the late 19th and 20th centuries the pineal was connected to a physiological response. In 1972, Tapp, E., a physician, published a case report of a young boy who had shown precocious puberty and was also found to have a pineal tumor.

The association of pineal tumors and sexual malfunction gave rise to hundreds of research projects designed to test the hypothesis that the pineal was a gland whose function was to inhibit the gonads. Little appeared to result from the early efforts. In 1954 Kitay and Altschule, directors of internal medicine at McLean Hospital in Waverly, Massachusetts, reviewed the entire world
Figure A. Seat of the rational soul was the function assigned to the human pineal by Rene Descartes in his mechanistic theory of perception. According to Descartes, the eyes perceived the events of the real world and transmitted by way of "strings" in the brain. The pineal responded by allowing animal humors to pass down hollow tubes to the muscles, where they produced an appropriate response. The size of the pineal is exaggerated in this wood carving, which appeared first in 1662.
literature on the pineal. There were some 1,800 references, about half of which dealt with the pineal-gonad question.

Description of the Pineal in the Lower Vertebrates

Fran Leydig (1872), stated that among the lower vertebrates the pineal organ is a sense organ containing receptor and nerve cells, in some forms resembling an eye. In the roof of the vertebrate brain, there are other organ-like differentiations, the parapineal organ, which is closely associated with the pineal organ (Oksche, 1965).

Both these organs are old, having first appeared in certain Devonian tetrapods, the ancestors of recent amphibians and lizards. A well marked pineal foramen is found in the skulls of both branchosaurs and lepospondyles (Noble, 1931). The pineal and parapineal organs can be divided into the parts as follows, according to Tilney and Warren, 1919. (Table A).

Epiphysseal Complex in Reptiles

In this class, there is a spectrum of epiphysseal complex types ranging from the completely non-existent crocodilian, to the most morphologically intricate known lizards.

Crocodilians have a prominent finger-like projection on the mid-line of the brain, extending between the cerebral hemispheres and the optic lobes. This is precisely where one would expect to find the epiphysis. Thus, earlier investigators mistook it for "glandula pinealis" (Reese, 1910). The situation was cleared up by
Reese (1910), who pointed out that the embryological origin of this evagination is telencephalic, and therefore it is paraphysis, the epiphysis develops from the diencephalon.

Sphenodon (Rhyncocephala) and the lacertilians were most interesting in this class. Some authors (Beraneck, 1892, 1893, cited by Bargman, 1943; Dendy, 1899, 1910, 1911, and Nowikoff, 1910), held that the pineal would arise independently from two separate anlagen situated behind each other in the diencephalic roof. Other authors, on the contrary, such as Warren (1911), and Boveri (1925), Preisler (1942), and Steyn (1957), were of the opinion that the epiphysis and the parietal eye parapineal are derived from one single diencephalic evagination containing the anlagen of both these organs.

The Epiphyseal Complex in Amphibians

The amphibians' epiphyseal complex includes a second diencephalic evagination known as the frontal organ or Stirnorgan, which is visible externally as a small, pellucid spot on the midline of the head between the lateral eyes. Light microscopy revealed that the frontal organ consists primarily of sensory cells having inner and outer segments as in lateral eye rods and cones. They are capable of photoreception and wave-length discrimination (Dodt and Heerd, 1962).

The frog's epiphysis develops as a hollow sac between the habenular commissure rostrally and subcommissural organ caudally.
TABLE A
DIVISIONS OF THE PINEAL AND PARAPINEAL ORGANS
(Tilney and Warren, 1919)

1. The pineal organ, consisting of:
   1. an end-vesicle
   2. a stalk
   3. a proximal portion
   4. a peduncle

II. The parapineal organ, consisting of:
   1. an end-vesicle
   2. a stalk
   3. a proximal portion

Parietal organs (Studnicka, 1905):
   1. Caudal parietal organ = epiphysis cerebri
   2. rostral parietal organ = parapineal organ (Petromyzon, Ganoides, Teleostei) = parietal eye (Sauria)

Epiphyseal complex (Tilney and Warren, 1919) in lizards
   (Steyn, 1957) = epiphysis cerebri + parietal organ (eye).

Pineal complex in Anura = frontal organ (Stirmorgan, organ frontale, pineal end vesicle) + epiphysis cerebri.
In the adult it becomes flattened, and its lumen appears to be in contact with the third ventricle, (Van De Kamer, 1965). Some evidence has been presented that an epiphyseal secretion, melatonin, may play a role in amphibian color change responses (Charlton, 1966; Bagnara and Hadley, 1970).

The Epiphyseal Complex in Fishes

Teleosts

The epiphyseal complex in teleosts consists of an elongated sac-like epiphysis that lies in close contact with the skull. The highly folded epiphyseal walls enclose a large continuous lumen that may communicate with the third ventricle. (Hafeez and Ford, 1967).

Murphy (1971) suggested that the teleost epiphysis acts as a light sensor, also a characteristic indole of epiphysis, melatonin, has been identified in the Pacific salmon, Oncorhynchus tshawytscha (Fenwick, 1970).

Elasmobranchs

An epiphyseal complex occurs in all elasmobranchs studied, except members of the genus Torpedo, where both epiphysis and parapineal are absent. In adult elasmobranchs, the epiphyseal complex consists of an extremely elongated tubular epiphysis which arises from a single evagination of the diencephalon. The distal end of this evagination, referred to as the epiphyseal end
vesicle, lies in close contact with the brain case. This end vesicle is connected to the habenular-posterior commissure region by a long hollow epiphyseal stalk, which is continuous with the third ventricle, and shows very little epithelial folding (Rudeberg, 1969a).

**Cyclostomes**

The epiphyseal complex in cyclostomes is present in members of the order *Petromyzontia* (lampreys), but is absent in the order *Myxinoidia* (slime eels and hagfishes), (Wurtman et. al., 1968). In adult lampreys the epiphyseal complex appears as two stalked vesicles extending rostrally from the habenular-posterior commissural region, to a position just under the cartilaginous skull. The more superficial of these two vesicles is the epiphysis (Tilney and Warren, 1919).

**The Epiphysis in Birds**

Birds have a diencephalon that forms a single epiphyseal evagination. The epiphysis is located just under the skull on the mid-line between the cerebral hemispheres and the cerebellum. Three basic avian types were described by Studnicka (1905). A tubular epiphysis may appear simply as a tubular sac with a large lumen. It may be semi-compartmentalized by projections from the epiphyseal wall. A follicular epiphysis contains many distinct groups of columnar ependymocytes, each surrounding a separate lumen. A solid epiphysis consists of many distinct groups of columnar
ependymocytes compacted to the extent that very few lumina are present.

The Epiphysis in Mammals

The diencephalon roof in mammals gives rise to a single epiphyseal evagination. In most mammals studies, the organ originates along the midline between the habenular commissure anteriorly and the posterior commissure and subcommissure organ posteriorly. Altogether these structures produce the roof of the third ventricle. The structure evaginates toward the brain roof and the final form is a "head" or bulbous structure connected by a stalk to the diencephalon area. The "head" of the pineal in mammals extends toward the roof of the brain, but has been described as fully covered by the cerebral hemispheres. In the adult mammal the epiphyseal gland is transformed from a saccular gland into a solid mass of tiny follicular cell aggregations, each of which no longer has a common connection to a lumen (Quay, 1965).

In the external form of the mammalian pineal organ, extensive interspecific differences exist (Oksche, 1965). There is an elongated type, similar to that of birds, situated between telencephalon and cerebellum and attached to the dura mater (e.g. guinea pig, rat) and conical (carnivora) or pear-shaped (cattle, man) types covered by the telencephalon. The lobular structure is extremely pronounced in some species as in man. These forms can be seen in Figure 18 in the results section.
Cytology of the mammalian epiphysis

In mammals, the pineal gland is thought to be exclusively secretory. Morphologically, the pineal cells have lost all trace of the external segment, except for a ring of nine ciliary microtubules at the distal end of the cells. Such cells are termed pinealocytes. They are innervated by autonomic nerve fibers from the superior cervical ganglia. The pinealocytes have short processes which end on the basement membrane of the pineal organ, or at intracellular spaces which communicate with perivascular spaces.

The pineal body of mammals is homologous with the parapineal or third eye of some more primitive vertebrates. Although the pineal body of mammals has lost all known structural resemblances to eyes or photoreceptors, and appears to be primarily glandular, its cytology and its metabolic activities, including melatonin formation and levels, are markedly affected by light and darkness (Quay, 1965).

Vascularization of the mammalian pineal

In the mammalian pineal, the capillary network courses deeply through the parenchyma. In the monkey, a rich central plexus is evident while vessels are sparse in other areas, (Le Gros Clark 1939-1940), but this pattern is not necessarily the rule in other species.

Innervation of mammalian epiphysis

Le Gros Clark (1939-1940) suspected that the nerve fibers
coursing into the epiphysis from the habenular and posterior commissural regions might actually turn and course back out without terminating. In fact, Ariens-Kappers (1960-1965) subsequently did show in the albino rat that probably all these pineal fibers do reverse without terminating and that the primary and perhaps exclusive innervation of the organ is from sympathetic fibers which enter the pineal via paired nervi conarii from the tentorium cerebelli and intimately with the parenchyma (Kappers, 1965).

**Histological study of the mammalian pineal**

The characteristic and the most prevalent cell type in the mammalian pineal organ, is the pinealocyte, also termed as pineocyte, pineal parenchymal cell, epiphysial cell, and a host of other, less specific names (Wolfe, 1965). Pinealocytes are complex cells having processes which may trail off from the cell body for some distance before ending in some other cells, or near interstitial trabeculae, perivascular areas, or residual lumina (Quay, 1965).

Milofsky (1957) was the first to study the pineal parenchyma utilizing the electron microscope. The fine structures of pinealocytes seemed to be remarkable. They appeared to contain the same amount of organelles but abundantly supplied with mitochondria, and prominent golgi complex. Indications of a regressive development or degeneration are manifested in man in form of cysts or calcium concrements.
Mammalian pineal uptake of radioactive phosphorus

In the cat, guinea pig, rabbit, pig and rat, pineal uptake of radioactive phosphorus was reported to be three times that of the pituitary gland and twenty times that of the cerebellum (Borreli, V. and Orstrom, A., 1945, 1957). Thus, the organ can be assumed to be a highly metabolizing structure.

Studies on differences between mammalian skulls

Rodents

In the skull, at the confluence of the sagittal and the coronal sutures, a depression or fossa was visible immediately over the pineal gland (Hoffman, Roger A. and Reiter, Russel J., 1965).

Larger Herbivores

The deer and cow skull were heavier with numerous sinuses. In butting animals, more protection to the brain is required. According to Letellier, et. al., (1965), the external protuberance over the pineal area was very prominent and represented the superior aspect of the occipital bone as it met the parietal bone. It could easily be palpated through the skin.

Carnivores

The skull was much thinner than in the case of butting animals. Few sinuses were present. The fossa at the confluence of the sagittal and the coronal sutures above the pineal was present,
According to the variations in skull structures and brain evolution, the mammalian pineal was no longer exposed directly to the light. However, there are good evidences that its function continues to be related somehow to environmental lighting. A study done on rats also determined that continuous environmental lighting brought about a decrease in the weight of their pineals (Legait, H., Legait, E., 1977).

This study continues an investigation of the exact placement of the mammalian pineal organ in relationship to the skull. Animals with a wide variation in skull structures were studied, carnivores, rodents and grazing mammals being included.
CHAPTER III

MATERIALS AND METHODS

The white laboratory rat pineal had been used by investigators in our laboratory for physiological investigations. The investigators were surprised to find that the pineal was not buried deeply within the brain, but was relatively accessible. It was located above the brain masses in a sinusoidal area between the cerebral hemispheres and the cerebellum. Furthermore, it was noted that the skull area immediately above the pineal was modified, the bone being thinned to form a depression or fossa. This led to the question that is being investigated here: Are other mammalian pineals located immediately below the skull? If this were true, then one may propose that the pineal might act directly as a photoreceptor, as in the case of most of the lower vertebrates.

Other categories of mammals were used for this study because of their availability; rodents, larger herbivores and carnivores. At least two species from each category were dissected. In the rodent category, the laboratory rat, wild rat, microtus mouse and the white footed deer mouse were studied. In the carnivorous group, the domestic cat and dog were used. The larger herbivorous mammals included the cow and deer.

Dissection of the Rat Pineal

A modified procedure of Hoffman, Roger A., and Reiter,
Russel J., 1965) was used as follows: Killing of animals was
effected in a CO₂ atmosphere. The animals were then prepared in the
following way; the bases of their ears were slit to expose the ear
canals. These openings served to immobilize the head in the stereo­
taxic apparatus (see Figure 1). An incision was made at the base
of the neck so that the excess blood was allowed to flow out.
Draining the blood was helpful so that the vascular sinus immediate­
ly around the pineal did not flood over the operating area. The
skin was then peeled off, and the attachments of neck and mouth
parts muscles were scraped off to one end, to reveal the skull
fossa (Figures 2 and 3). The head of the animal was then placed in
a wooden stereotaxic base. By means of set screws placed in the
ear the head was immobilized.

With a sharp scalpel, the attachment of the jaw muscles on the
occipital ridge of the skull was scraped off. At this point, the
confluence of the sagittal and occipital sutures was clearly
visible (Figures 4 and 5). The rounded depression, or fossa, could
be clearly seen. Previous work in the laboratory had demonstrated
that the pineal lay immediately underneath a vascular membrane
(tela choroidea), which covered the sinus under the fossa.
Keeping this in mind, a dental drill was used to penetrate the
skull. The drill was centered on the confluence of the superior
sagittal and the transverse sinuses. Rounded craniotomy was
performed. The hole was drilled, taking precaution not to damage
the soft membranes underneath. The disc of bone was removed
Figure 1. Stereotaxic base used for the dissection of the Rodent brain. Diagrammatic representation. 1/4 X.
Figure 2. Superficial muscles of the rat head and face, dorsal view. Magnification 2X. Similar in the mouse.
Figure 3. Superficial muscles of head and face—Lateral view. Magnification, 2X. Similar in the mouse.
Figure 5. Top view of the skull in rat. 2X. Similar in the mouse.
before reaching the underlying vascular membrane, tela choroidea (Figure 6). A dissecting microscope magnification 14X to 60X was then placed over the hole to study the underlying tissue in more detail. Excessive blood was removed by means of blotting paper. The membrane was removed by means of forceps and the pineal gland was studied.

Study of the Sagittal Section

A few drops of formalin were dropped on the brain tissue to harden it. The bony case over the brain was now entirely removed. After a few hours, the entire brain was lifted from the bony case. It was then placed in Bouin's solution for fixation. A sagittal section was then made to study the placement of the pineal gland in relation to the third ventricle.

Dissection of the Cow Pineal

The procedure used above for the dissection of the rat pineal was modified to account for the heavier skull size.

The cow head was obtained from a local butcher shop, shortly after slaughter. The skin had already been removed. The lateral view of the muscles can be seen in Figure 7. Due to the larger size of the head, a stereotaxic base was not needed for immobilization. The head was placed in a large dissecting tray.

Upon removal of the muscles, the skull of the cow was seen to be pyramidal in shape. The cranium was quadrangular and large externally, the larger size being due mainly to the great extent of
Figure 6. Top view of part of the brain; as seen underneath the fossa using a dissecting scope. (Mag. 10X-60X). The skull has been outlined. 2X. Similar in the mouse.
Figure 7. Muscles of the head of the cow. Lateral view. 1/8 X.
the frontal sinuses. The frontal surface was formed by the frontals, nasals and the premaxillae. The frontal part was quadrilateral and very extensive, the greatest width being at the orbits (Figure 8). It presented a central depression on its anterior part, and on either side were the supraorbital grooves and foramina. Behind was the median frontal eminence, and at the lateral angles the "horn cores" projected in the cow skull (Figures 8, 9 and 10).

The lateral surface was triangular in appearance. It was limited dorsally by a crest which extended from the posterolateral angle of the frontal bone to the supraorbital process (Figure 9). The top part of the skull on one half was removed to study the extensive distribution of the sinuses above the cranial cavity (Figure 10).

A rectangular craniotomy was performed using a large drill. The structure under the hard bone was found to be porous. Sinuses filled a depth of approximately two inches. A large amount of bone was removed, using hammer and bone chips, and a hack saw. After removal of bone, the brain was studied. A sagittal section was made using the same method as in the case of the rat brain.

Dissection of the Deer Pineal

The deer head was dissected by a similar procedure as with the cow. The deer head was obtained from a fellow student. The deer had been slain at an earlier time and the head had been frozen.

In the deer, the external occipital protuberance was very prominent, and represented the superior aspect of the occipital bone.
Figure 8. Top view of the cow skull. 1/8X.
Figure 9. Side view of the cow skull. 1/8x.
Figure 10. Skull of the cow; Dorsal view. Sinuses opened on one side. 1/8 X.
as it met the parietal bone. It could be easily palpated through the skin. The caudal limit of the skin flap extended to within 2.5 cm. rostrad at the occipital protuberance. The flap was hinged laterally over the ipsilateral ear (Figure 11). The periosteum and muscle were very adherent in the deer and were removed by sharp dissection.

Upon removal of the muscles, the skull was visible. The deer skull was similar to the cow skull in many ways. It had numerous sinuses also. The superior sagittal sinus was clearly seen as the midline bone was removed. The sinus had a distinct purple color. The occipital and coronal sutures formed a raised ridge as in the case of the cow. The skull formation over the pineal region can be seen in Figures 12 and 13. Craniotomy was performed by a method similar to the one used in the case of the cow head. The pineal was then studied.

Dissection of the Dog Pineal

The dog was killed immediately before dissection by injecting it with a 20 mg. dose of phenobarbitol. The superficial muscles of the dog head can be seen in Figure 14.

Upon removal of the muscles, the skull was observed. The skull of the dog is a complex of bones formed in both membrane and cartilage around the brain, sense organs and entrances to the digestive and respiratory systems. The dog's skull varies more in shape among different breeds than does the skull of other species of domestic animals.

The paired frontal and parietal bones formed the dorsum of the
Figure 11. Muscles of the deer head. Lateral view. 1/8X.
Figure 12. Side view of the deer skull. 1/8x.
Figure 13. Top view of the deer skull. 1/8X.
Figure 14. Superficial muscles of the dog head. Lateral view. 1/4X.
neurocranium. Caudally, the parietal bones met the occipital bone to form the caudal surface of the skull. A depression or fossa at the confluence of the sagittal and the occipital sutures was observed (Figure 15). Rounded craniotomy was performed at the point of the fossa and the pineal studied.

Dissection of the Cat Pineal

Studies were performed on a freshly killed cat. The dorsal view of the superficial muscles of the cat head can be seen in Figure 16. Upon removal of the muscle, the skull structure was observed.

The skull of the cat was very much similar to that of the dog. It was, however, more rounded than in the case of the dog. The skull was the widest at the point of the orbits. A depression or fossa at the confluence of the coronal and the occipital sutures was observed (Figure 17). Rounded craniotomy was performed at the point of the fossa. Upon removal of the fossa, the pineal was studied.
Figure 15. Dorsal view of the dog skull. 1/4X.
Figure 16. Superficial muscles of the cat head. Dorsal view. 1/4X.
Figure 17. Dorsal view of the cat skull. 1/4X.
Histological Study of the Pineal

To study the cellular structure of the pineal gland, the pineal was removed in its entirety and slides were prepared, using a modified procedure outlined by Humanson (1972).

Fixing

The gland was placed in Bouin's solution and was left in solution for three days.

Dehydration

Fixed glands were placed in 70% alcohol for washing. A series of alcohol baths was set up in succession of increasing alcohol concentration of 70%, 80%, 95%, 100% and 100%. The tissue was kept in each bath for two hours, and then transferred to the next higher alcohol concentration, taking care not to stop at any point along the line.

Xylene infiltration

A series of baths was set up as follows, with the following ratios of alcohol: xylene: paraffin. 1) Alcohol:xylene 1:3. 2) xylene:alcohol, 3:1. 3) xylene. 4) xylene:paraffin (melted) 3:1. 5) paraffin:xylene, 3:1. 6) paraffin.

The tissues were placed in each of the above series for two hours. They were then embedded into the paraffin mold.
Preparation of the paraffin mold

This technique must be carried out carefully but quickly, since room temperature hardens the paraffin. The paraffin was allowed to harden at once around the embedded tissue in order to get good sections later. The paraffin was melted in a paraffin pitcher. The metal molds were now sprayed with mold releaser. Melted paraffin was poured into the mold until it was one-half full, with the plastic holder in place. The tissue to be embedded was dropped into the melted wax, orienting it for the best position for sectioning. A dissecting needle heated in a Bunsen burder flame kept the paraffin melted for manipulation of the tissue. The preparation was now set aside for cooling, the metal mold was removed from the preparation. The excess paraffin was removed from the tissue. Sections of 10 micron thickness were now obtained using a microtome.

Hydration of the sections

The sections were cut off from the paraffin mold and were affixed to a clean glass slide with an adhesive and allowed to set on a slide warmer overnight. The tissue was now ready for replacement of the water so as to get it ready for staining.

A series of baths was set up as follows: xylene; xylene; alcohol, 100%; alcohol, 100%; alcohol, 95%; alcohol, 70%; tap water. The slides were placed in each of the above baths successively for five minutes. The sections were now ready for staining.
**Staining**

The Mallory staining method was used (Humanson, 1972)

1. Deparaffinize and hydrate slides to water.
2. Wash with Lugol's solution ................. 2-3 minutes.
3. Wash with sodium thiosulfate solution .... 3-5 minutes.
4. Stain in Mallory I ............................ 15 seconds.
5. Rinse in distilled water to differentiate reds. 10 seconds.
6. Treat with phosphomolybdic acid .......... 1-5 seconds.
7. Stain in Mallory II ............................ 2 minutes.
8. Rinse in distilled water ..................... 5 minutes.
9. Differentiate aniline blue in 90% EtOH.
10. Dehydrate in absolute alcohol, clear and mount.

**Photographs**

Photographs of the slides were taken by means of a Nikkon camera, through a microscope (Magnification 10X-100X). The microscope had a camera fitted on the top. The slide was placed on a stage and focused through the microscope objective and the camera lens, and the picture was taken at 30 and 60 second time exposures, for maximum clarity.

A black and white film 110X pan with 12 exposures was used.
CHAPTER IV

RESULTS AND DISCUSSION

The pineal body in the different species of mammals was compared at different levels. Comparisons were made as follows:

1). Study of the skull structures. 2). Position of the pineal gland in relation to the skull. 3). Relationship of the pineal gland to the third ventricle. 4). Structure, size, shape and weight of the gland. 5). Histological study of the pineal gland in three categories- rodents, larger herbivores and carnivores.

The pineal gland in mammals studied was observed to be a small rounded structure ranging from 1 mm. to 3.5 mm. in diameter at the proximal end. It was positioned at the posterior-most region and between the cerebral hemispheres, lying slightly above the hemispheres, at the junction with the cerebellum. The rounded, proximal end was immediately visible after removal of the tela choroidea, or the vascular membrane, that covered the triangular space housing the pineal gland.

Upon parting the cerebral hemispheres, one could trace downward the length of the pineal organ. The gland was observed to be positioned on the midline, in front of the two rostral colliculi. The corpus callosum transversely crossed the midline rostral and dorsal to the pineal gland. The colliculi were arranged in pairs (superior and inferior), and were separated from one another by a cruciform sulcus. The longitudinal part of the sulcus expanded
superiorly to form a slight depression, above which the pineal lay. It was seen that the stalk of the pineal gland originated from the roof of the third ventricle region, below the corpus callosum, and then merged with the thalamic region. The base of the stalk was slightly expanded to form a pineal recess.

Upon removal of the entire brain and its fixation, a sagittal section was obtained to study the placement of the gland in relation to the third ventricle. The triangular area of descent to the pineal gland was bordered by the left cerebral hemisphere laterally, and the tentorium cerebelli caudally. Underneath the base of the gland, the subcommissural organ was observed. Some individual variations in pineal glands in the different animals can be seen (Figure 18). In the rodents, the pineal was elongated with a thin, long stalk. In the herbivorous mammals studied, the head of the gland appeared to be pear-shaped with a thicker stalk than in the rodents. The carnivorous mammals had a conical shape to the head, with the stalk being thickest in this case. The individual differences in the different mammalian pineals is presented as follows.

**Rat Pineal**

The skull of the rat was comparatively thin. At the confluence of the sagittal and the occipital sutures, a rounded depression or fossa was observed. As anticipated, due to former experimentation, upon removal of the fossa the tela choroidea was visible. Upon removal of the vascular membrane, the pineal was immediately visible. It was seen to be a small rounded structure about 1.5 mm. in size at
Figure 18. Summary of various shapes of mammalian pineal glands.

- Elongated type pineal e.g. rat. 2X.
- Pear shaped pineal e.g. cattle. 2X.
- Conical pineal e.g. cat. 2X.
the proximal end (Table 1, Figure 19) in the case of the laboratory rat and 2 mm. in size in the wild rat. The pineal in the laboratory rat was white in color whereas the pineal in the wild rat was seen to be pink in color immediately after dissection. Its relationship to the third ventricle was similar to the general description. This is demonstrated in Figure 20. An enlarged side-view of the rat hindbrain was drawn in Figure 21 to clearly demonstrate the origin of the pineal gland in relationship to the third ventricle. The gland was 1 cm. in length in the laboratory rat and 1.2 cm. in the wild rat. It weighed .01 gms. in both the wild and the laboratory rat. (Table I).

Mouse Pineal

The skull structure was similar to the rat. Thus, only one set of diagrams was used to show the placement of the pineal in the rat and mouse. (Figures 19, 20, 21 and 22).

The pineal was observed to be a small, rounded pea-shaped structure about 1 mm. in diameter at the proximal end. It was about 7 mm. long in the microtus mouse and 8 mm. long in the deer mouse. It weighed .001 gms. (Table I).

The position of the gland in relation to the skull and the third ventricle was similar to the rat as shown in Figures 20 and 21. An enlarged view of the gland is shown in Figure 22 to better illustrate the shape of the pineal in the rat and mouse.
Figure 19. Magnified view of the rat brain; as seen underneath the fossa with a dissecting scope. (Mag. 10X-60X). 10X. Similar in the mouse.
Figure 20. Side view of the brain of the rat; as it is positioned in the cranial cavity. The skull has been outlined. 2X. Similar in the mouse.
Figure 21. Enlarged side view of the rat hind-brain showing the relationship of the pineal to the skull and the third ventricle. 8X. Similar in the mouse.

Figure 22. Enlarged view of the rat pineal gland. 10X. Similar in the mouse.
Cow Pineal

The skull of the cow was large in appearance, the large size being due mainly to the great extent of the frontal sinuses. The frontal part of the skull was quadrilateral, whereas the lateral part was more triangular in appearance.

The cranial cavity was small compared to the large skull size. Most of the skull was filled with sinuses (Figure 23). The frontal sinus was very large, involving almost all of the frontal bone and a large part of the posterior wall of the cranium. It also extended for a variable distance into the horn processes. The major compartment comprised the portion of the sinus lying posterior to the orbits, whereas the minor compartments laid in front of the major compartments and between the orbits. The other major sinuses were maxillary, palatine, sphenoidal and lacrimal. This is clearly demonstrated in Figure 23.

Upon removal of the bony casing, the pineal gland was observed to lay underneath the frontal eminence (Figure 24). It was observed to be a pear-shaped organ about 3 mm. in diameter at the proximal end (Table 1, Figure 25). An enlarged dorsal view of the opening was drawn to clearly illustrate the position of the gland in relationship to the cerebral hemispheres and the cerebellum.

The relationship of the pineal gland to the third ventricle was very much similar to the general description. It is illustrated by Figure 26, which is an enlarged side-view of the hind-brain in the cranial cavity. An enlarged view of the pineal gland was drawn to
Figure 23. Side view of the cow brain in the cranial cavity. 1/8X.
Figure 24. Top view of the cow brain as seen after the hole has been drilled. 1/8X.
Figure 25. Enlarged view of the cow brain underneath the drilled hole. 1/2X.
Figure 26. Enlarged side view of the cow hind brain; showing the relationship of the pineal to the third ventricle. 1/2X.

Figure 27. Enlarged view of the cow pineal. 2X.
show clearly the shape (Figure 27). The gland was pink in color, about 1.5 cm. long, and weighed .199 gms. (Table 1).

Deer Pineal

The deer skull was similar to the cow skull in many ways. It also had numerous sinuses. The superior sagittal sinus in the deer was clearly seen as the midline bone was removed. The sinus had a distinct purple color. The occipital and coronal sutures formed a raised ridge as in the case of the cow. The cranial cavity was extremely small, as compared to the skull sinuses. Figure 28 demonstrates the relationship of the cranial cavity to the entire skull. Upon removal of the frontal eminence, the pineal was seen to lay underneath the vascular membrane. It was observed to be a pineapple-shaped structure, purple in color, and about 4 mm. in diameter at the proximal end (Table 1, Figure 29).

The falx cerebri in the deer brain was very shallow, with only a depth of 1 cm.; therefore, the medial aspect of both hemispheres could be readily observed from the ipsilateral side, without the falx cerebri obstructing the view. Figure 30 is an enlarged side-view of the hind-brain to show the exact placement of the pineal gland in relationship to the third ventricle and the skull. As can be noted, the head of the gland is immediately under the frontal eminence. An enlarged view of the pineal gland is illustrated in Figure 31 to show the shape of the gland. The gland is 2 cm. long and weighed .18 gms.
Figure 28. Side view of the deer brain as seen in the cranial cavity. 1/16.
Figure 29. Top view of the deer brain as seen underneath the dissected bone. 1/8x.
Figure 30. Enlarged side view of the deer hind brain as seen in the cranial cavity. 1/2X.

Figure 31. Enlarged view of the deer pineal. 2X.
Dog Pineal

The dog skull was much thinner than in the case of the herbivorous mammals. It had a fossa as in the case of the rat. Upon removal of the bony casing, the pineal lay immediately underneath the fossa and the vascular membrane. This is illustrated in Figure 32, which is an enlarged view of the portion underneath the fossa. It was a conical structure about 3 mm. in diameter at the proximal end. It was 2 cm. in length and weighed .12 gms. (Table 1, Figure 33). Figure 33 also illustrates the brain size as compared to the entire skull. It demonstrates the location of the pineal gland under the vascular membrane. An enlarged side-view of the hind part of the dog brain in the cranial cavity was drawn to illustrate the relationship of the pineal to the third ventricle (Figure 34). An enlarged view of the pineal gland is shown in Figure 35 to show the shape of the gland. The stalk was very thick as compared to the herbivirous and rodent glands, and also the size of the head of the gland was almost the same as the stalk of the gland.

Cat Pineal

As in the case of the dog, the skull of the cat was thin and the cat pineal was seen immediately underneath the fossa upon removal of the tella choroidea. An enlarged dorsal view of the drilled fossa is shown in Figure 36 to illustrate the position of the gland between the cerebral hemispheres and the cerebellum.

The pineal was conical in shape and was about 3.5 mm. in
Figure 32. Top view of the dog brain as seen underneath the fossa. 2X.
Figure 33. Side view of the dog brain as seen in the cranial cavity. 1/8X.
Figure 34. Enlarged side view of the dog brain. 2X.

Figure 35. Enlarged view of the dog pineal. 4X.
Figure 36. Enlarged view of the cat brain as seen after removal of the bone. 2X.
diameter at the proximal end (Table 1). Its placement with regards to the cranial cavity was similar to that in the dog, and is illustrated in Figure 37. An enlarged side-view of the hind part of the cranial cavity was drawn to clearly illustrate the position of the pineal gland with regards to the third ventricle (Figure 38). An enlarged view of the pineal was drawn to illustrate the size and shape of the head of the gland in comparison to the pineal stalk (Figure 39). The gland was 2.5 cm. in length and weighed .13 gms. (Table 1).

Histological Results

A histological study of the pineal gland of certain mammals was attempted to show the differences in the cellular structure and the amount of secretions present in the mammals of the different categories. Slides were prepared using the gland from one mammal in each category.

Rodents

Upon studying the histological structure of the rat pineal under high magnification, it was found to contain numerous cell bodies which stained purple with Mallory stain. Neurosecretions were limited to the inner part and stained red. Some connective tissue was found on the outer boundaries of the gland and stained blue (Figure 40).
Figure 37. Side view of the cat brain as seen in the cranial cavity. 1/2X.
Figure 38. Enlarged side view of the brain in the cat skull. 2X.

Figure 39. Enlarged view of the cat pineal. 4X.
Figure 40. Sagittal section of the head of the rat pineal. The cell bodies stained purple. Neurosecretions stained red. The outer boundary with connective tissue stained blue. Mallory stain was used. Photographs were taken at 100X magnification.
**Herbivores**

In this category of mammals, the cow pineal was used for the preparation of the slides. Upon studying it under the microscope it was seen that the cell bodies were more numerous toward the outer boundaries and stained deep purple. Toward the median part of the gland secretions were observed and stained blue. Connective tissue was not seen in this gland, however, some fibers were found interspersed and stained red (Figure 41).

**Carnivores**

The cat pineal was studied in this category of mammals. The cell bodies in this case tended to aggregate into bunches toward the center, and stained purple with shades of brown. A few secretion droplets stained red were observed interspersed with the cells. No connective tissue was seen. Some collagenous fibers were seen toward the outer boundaries and stained blue (Figure 42).
Figure 41. Sagittal section of the cow pineal. The cell bodies stained deep purple. The neurosecretions stained red, and the interspersed fibrous material stained blue. Mallory stain was used. Photographs were taken at 100X magnification.
Figure 42. Sagittal section of the median portion of the cat pineal. The nervous secretions stained purple and dark brown. Collagenous fibers stained blue and nervous secretions stained red. Mallory stain was used. Photographs were taken at 100X magnification. Note the aggregation of cells toward the outer boundary.
<table>
<thead>
<tr>
<th></th>
<th>Microtus</th>
<th>Deer Mouse</th>
<th>Lab Rat</th>
<th>Wild Rat</th>
<th>Cow</th>
<th>Deer</th>
<th>Dog</th>
<th>Cat</th>
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<tr>
<td><strong>Shape at proximal end</strong></td>
<td>Round</td>
<td>Round</td>
<td>Elongated</td>
<td>Elongated</td>
<td>Pear shaped</td>
<td>Pineapple shaped</td>
<td>Conical</td>
<td>Conical</td>
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<td>1 mm.</td>
<td>1 mm.</td>
<td>1.5 mm.</td>
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<td>4 mm.</td>
<td>3 mm.</td>
<td>3.5 mm.</td>
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<tr>
<td><strong>Diameter of pineal body at proximal end</strong></td>
<td>7 mm.</td>
<td>8 mm.</td>
<td>1 cm.</td>
<td>1.2 cm.</td>
<td>1.5 cm.</td>
<td>2 cm.</td>
<td>2 cm.</td>
<td>2.5 cm.</td>
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<tr>
<td><strong>Length of gland from point of origin</strong></td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Pink</td>
<td>Cream</td>
<td>Purple</td>
<td>White</td>
<td>White</td>
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<tr>
<td></td>
<td>.001 gm.</td>
<td>.001 gm.</td>
<td>.01 gm.</td>
<td>.01 gm.</td>
<td>.199 gm.</td>
<td>.18 gm.</td>
<td>.12 gm.</td>
<td>.13 gm.</td>
</tr>
</tbody>
</table>
CHAPTER V

SUMMARY

As a result of this investigation, it was seen that the thin fossa was present always immediately above the region of the pineal, except for the butting animals. But even in these animals, numerous fossae produced a comb-like structure immediately above the pineal, so that the final product was quite translucent to light.

The pineal was seen to lay directly underneath the fossa in the rodents and carnivores. In the larger herbivores, the pineal was covered slightly by the cerebral hemispheres.

The size of the gland changed markedly in the different mammals. In the rodents, the pineal "head" was small and the stalk was long and thin. In the larger herbivores, the head was larger and the stalk thicker than in the case of the rodents. In the carnivores, the size of the "head" of the pineal equaled the length of the stalk, and the stalk was the thickest.

As a result of this investigation, it could be suggested that the pineal may have retained its photo-sensory properties, along with being a secretory organelle.
BIBLIOGRAPHY


