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A Search for Lymphatic Tissue in the Mouse Pineal Gland

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A SEARCH FOR LYMPHATIC TISSUE

IN THE MOUSE PINEAL GLAND

(TITLE)

BY

John B. Hutchinson

THESIS

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ABSTRACT

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A SEARCH FOR LYMPHATIC TISSUE

IN THE MOUSE PINEAL GLAND

by

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ABSTRACT

There is recent evidence that the pineal gland in some species may have lymphatic qualities during early post natal life. In this study the development and structure of the pineal complex in the CDF_1 mouse is examined in fetal, neonatal, juvenile, and adult stages. The gland appears as an out growth of the dorsal wall of the third ventricle of the brain and at ten days gestation attains a mean size of 90 microns in the transverse and 35 microns in the median saggital plane. General and specific staining shows fetal and early post partum tissue to be compact and mitotic with scant vascularity and poorly developed connective tissue structure. Mitotic activity ceases around three weeks post partum marking the beginning of the juvenile period. Neuroglia become evident as cell volume increases throughout the juvenile stage. Parenchyma cells reach a size of 15 microns in the late juvenile and adult glands. In the adult the gland has grown to a mean diameter of 630 microns in the transverse by 390 microns in the median saggital plane. At this stage the gland is supported by an extremely fine connective tissue framework which is continuous with the capsule. There is no evidence of lymphatic aggregations or germinal centers as described in other species, although occasional lymphocytes are seen in the capsule of the gland and adjacent meningeal tissues of juvenile specimens.

ACKNOWLEDGEMENTS

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LITERATURE REVIEW

According to Reiter (1), the scientific community has
recognized the existence of the pineal for some 2000 years.
Galen, in the second century AD, cited observations of
Herophilus and Erasistratus who were intrigued with the
gland's central location and with its singularity. Galen
interpreted the pineal to be a valve that controlled the
flow of thought from one brain region to another. His
theory, along with that of Rene DeCartes who decided the
pineal was "The Seat of the Soul", marked the beginning of
the period of superstition in pineal studies.

By the end of the nineteenth century investigators
began to suspect the pineal of having endocrine properties.
One of the studies that marked this period was published by
the German physician Otto Heubner (2,3), correlating preco-
cious sexual behavior with tumors of the pineal gland. In
an effort to support this idea, early investigators studied
the effects of pineal extracts, implants, and pinealectomy
on the major endocrine glands of the body. An extensive
review by Kitay and Altschule (4) of the early experiments
using pineals, revealed a general inconsistency in the
results. Only a small percentage of the studies they
examined showed adequate procedure and controls for statis-
tical validity. Though a relation between the pineal and

gonads was suggested, there was little to characterize it.

Correlations of pineal function with the effects of light on melatonin synthesis (5-9), gonadal activity (10-15), and pigment responses (16-19) are now well established. The pineal gland's activity is controlled primarily by environmental light. Whether directly or indirectly depends upon the vertebrate animal studied. The gland originates embryologically as part of the brain and retains a connection, providing a visual tract in lower vertebrates. In birds and mammals this neural connection disappears almost entirely by birth or shortly thereafter, leaving the pineal of higher vertebrates to receive its neural messages via the peripheral sympathetic nervous system (20).

The pineal gland's influence on various tissues is known to be hormonal, and the gland displays a characteristic histological picture supporting a secretory nature. Parenchymal, expendymal, and other glial cell types, mostly astrocytic in structure, comprise the ectodermal derivatives (21). The parenchymal cell, or pinealocyte, makes up 90% of the tissue (22) arranged in follicles, cords, and incompletely separated lobules depending upon species and age. Neuroglia cells are fewer in number compared to other nervous tissues (23).

Mesodermal components pass to the interior of the gland through the meningeal capsule with the blood supply. Connective tissue trabeculae, continuous with the outer capsule,

contain the vascular channels and separate the lobular formations. In the mouse, arterial branches penetrating the pineal completely lack accompanying venules and are absent in the center of the gland (24). Venules occur in the center, and in the gland's capsule, remaining just beneath the latter until they reach considerable size. Here they drain into large trunks that join in a common sinus adjacent to the gland and just below the junction of the lambdoidal and sagittal sutures.

Pinealocytes and many of the glial cells communicate with capillaries via slender cytoplasmic processes that end in close proximity with the vessels and their adjacent connective tissue spaces suggesting an environment of intense aerobic metabolism (21). Vascular studies in the mouse (24) and analysis of pineal blood content in the rat (25) produce results to the contrary.

Proof of the pineal's secretory character was first obtained in a study that demonstrated the melanophore con-tracting effect of bovine pineal tissue on tadpoles (16). Subsequent studies report similar results using human and pig pineal extracts on larval and adult Xenopus (26). Efforts to isolate the pineal "hormone" resulted in the extraction of an unsaturated nitrogenous base that possessed melanophore contracting ability (27). This compound was identified as melatonin in 1958 (18). The pineal gland produces melatonin from N-acetylserotonin (28-31). The

enzyme necessary for this reaction, hydroxyindole-o-methyl transferase (HIOMT), is found to be specific to pineal tissue despite efforts to isolate it from various other organs in mammals (31). It has been suggested that the activity of HIOMT may serve as the rate limiting factor for melatonin synthesis since the tissue contains large amounts of the substrate serotinin (7).

Lighting changes have been seen to evoke pigment responses in fish (32, 33), amphibians (34), lamprey (35), and mammals (36, 37). Prolonged exposure to light reduces production of melatonin by the pineal (8) and also has been shown to alter its mass (5), morphology (38), and chemical characteristics (39). Concomitant gonadal involvement in response to light variation has been another observation. The gonads have been associated with pineal function since Heubner first described pineal tumors in individuals experiencing precocious sexual development (2). Further evidence supporting a relationship between the pineal and gonads comes from a study that demonstrated concentration of radioactive melatonin in the gonads and pituitary (40). It has also been shown that melatonin injection will decrease the incidence of estrus and ovarian weight in rats, and that constant exposure to light will obviate the effects of exogenous melatonin (41).

Despite the wealth of literature on the histology, chemistry, and morphology of the pineal, information concern-

ing the occurrence of lymphatic germinal centers in pineal tissue is scarce to nonexistent. Little has been published on the subject since the reviews of Bargmann in 1943 (42) and Kitay and Altschule in 1954(4). Lymphatic nodules in pineal connective tissue occur more often in certain species of birds than in mammals (21). They are characteristic in the pineal of domestic chickens, appearing as early as four days post hatching and lasting in some cases into adulthood (43, 44). Romieu and Jullien (45) described aggregations of lymphocytes in the capsule appearing one month post hatching. They report the subsequent appearance and disappearance of lymphocytes within the gland from the end of the first through the sixth month. These authors maintain that these cells do not invade the gland but rather differentiate in situ to become the source of lymphocytes for the meninges and cerebro-spinal fluids. Spiroff (43) later confirmed these findings except to disagree as to the true origin of the cells. He noted masses of lymphocytes in the gland's capsule in addition to groups entering the tissue within the walls of blood vessels. At certain points the two groups of cells are continuous indicating an extra-glandular origin and contradicting the previous in situ theory. A more recent study (46) has reinforced this idea. In an effort to remove all existing lymphatic elements, newly hatched chicks were bursectomized, thymectomized and irradiated. No lymphatic tissue developed in the pineal glands of these

birds indicating that the gland may be an interim tissue for the maturation and/or sensitization of lymphocytes.

In mammals, pineal lymphatic elements are quite rare and have been mentioned only in passing in the rat (47) and white footed mouse (48). It is obvious that the frequency and definition of the occurrence of lymphatic tissue differs markedly between birds and mammals; however, no one offers an explanation of this difference in the literature.

The impetus for this study comes from research reported by Cogburn and Glick (46). They described numerous lymphatic nodules within the interlobular spaces and along the basement membrane of the choroid plexus in the pineal glands of chickens 32-64 days old. The purpose of this study is to observe the post natal development of the mouse pineal gland at the light microscope level, determine if lymphatic nodules or germinal centers are present, and if so at what point in development do they appear and later disappear.

METHODS

Observations were made on pineal glands from 130 CDF_1 mice during fetal, neonatal, juvenile, and adult stages. Pineal glands from fetuses were observed at ten and seventeen days gestation. Neonatal and juvenile pineal glands were studied on a weekly basis from parturition through ten weeks. Pineal glands at twenty-one weeks represent the adult animal. Two or more mice of each sex representing each age group were observed.

Animals were killed by decapitation and the brain peeled away from the skullcap to expose the pineal gland. Skullcaps with the gland intact were fixed for twenty-four hours in Bouin's or Kahle's solutions. The glands were carefully dissected from the meninges, taken up in a $2\frac{1}{2}$ ml. syringe, and imbedded in wax. Entire fetal heads were sectioned at ten microns in the transverse plane. Neonatal, juvenile, and adult glands were sectioned at seven microns in the transverse plane and mounted in complete series. Tissue fixed in Bouin's solution was stained with Harris's hematoxylin and eosin for general histological detail (49). Tissue fixed in Kahle's solution was stained with Lehman's polychrome to differentiate connective tissue from blood vessels and illustrate any change in cell chemistry with age (50).

Glandular dimensions in the transverse plane were determined with an ocular micrometer calibrated in .01 mm divisions. All measurements were recorded to the nearest 10.0 microns. Parenchyma cell diameters were also obtained in this manner. Since all glands were sectioned in the transverse plane, glandular dimensions in the median saggital plane were determined by counting the number of sections in a series and multiplying by the section thickness.

RESULTS

Fetal Stage

Three specimens were studied from each age group at
ten and seventeen days gestation. At ten days gestation
the pineal appears as an elliptical outgrowth from the
dorsal wall of the third ventricle with a mean diameter of
90 microns in the transverse plane, by 30 microns in the
median saggital plane. See Fig. 1 for an explanation of
the transverse and median saggital plane.

Fetal tissue in general is compact and mitotic with
cells averaging 7-10 microns in size. Cells are packed in
random fashion and are morphologically indistinguishable
from those in the wall of the brain from which they develop.
At this stage neuroglia and vascular elements appear to be
absent from a cell mass containing only the parenchymal
neuro-secretory cells (Plates 1 & 2).

By seventeen days the gland is nearly a separate
structure, remaining attached to the roof of the third
ventricle by a thin connective tissue stalk that is contin-
uous with the gland's capsule and the pia mater. At this
stage the gland has grown to a mean diameter of 170 microns
in the transverse and approximately 180 microns in the
median saggital plane. It has attained a relatively
symmetrical fusiform shape with its apex at the connection

with the roof of the brain (Fig. 1). Nuclei are surrounded
by scant cytoplasm and often arranged in rosettes, about 30
microns in diameter, giving some cells a columnar appear-
ance (Plate 3). Occasionally the cells in rosettes will
approach 10 microns in size. Capillaries are scarce but
they are larger and easier to characterize than those in
older tissue. Neuroglia are still not apparent. Connective
tissue is underdeveloped in the gland at this age. A
capsule is distinct, however cell packing is so tight that
a structural extension of the connective tissue into the
gland is not evident. Male and female fetal pineal tissues
are indistinguishable.

Neonatal Stage

Pineal tissue obtained within eight hours of parturition
and at one and two weeks post partum is considered neonatal.
Nineteen specimens were studied from this age group (Table 1).
At birth, glands have a mean width of 200 microns in the
transverse and 200 microns in the median saggital plane,
growing to 260 and 350 microns respectively by two weeks.
Parenchyma cells remain at 7-10 microns in size arranged in
a dense random mosaic supported by a developing connective
tissue framework of delicate trabeculae (Plates 4 & 5).
Rosettes are no longer present, and mitotic activity
gradually abates during this time, becoming all but absent
in juvenile specimens. There is no apparent difference
between male and female tissue. The gland appeared to

contain only parenchyma cells at birth. By two weeks there
is evidence of neuroglia appearing as darkly staining
nuclear bodies in the extra-cellular spaces (Plate 6).
Capillaries are more numerous, however their walls are
extremely thin and difficult to distinguish.

Juvenile Stage

Animals from three through ten weeks of age are consi-
dered juveniles. One hundred and thirteen specimens were
studied from this period (Table 2). The pineal is slightly
larger at three weeks than at two weeks; however, throughout
juvenile development it doubles its width in the transverse
plane. Glands from animals at ten weeks of age reach a
mean width of 560 microns in the transverse, and 370 microns
in the median saggital plane. By three weeks of age the
parenchyma cells begin to increase their cytoplasmic volume,
attaining an average diameter of 12-13 microns. Occasion-
ally three or four cells are enclosed into lobules by a fine
connective tissue framework. The connective tissue framework
is evident throught the gland and continuous with the
capsule by six weeks (Plate 7). In all juvenile tissue,
dark staining nuclei characteristic of microglia and
obligodendria are present and are more numerous than the
lighter staining nuclei of astrocytic neuroglia. The
chromatin of neuroglia nuclei stain dark blue with either
staining method. Numerous capillaries accompany the con-
nective tissue and larger vessels are seen in the gland's

capsule. Throughout the juvenile period, diffuse lymphocytes are seen in the capsule and surrounding meninges, but not associated with the structural framework of the gland itself.

Cell volume and connective elements increase gradually during the juvenile stage to bring about a histological picture at ten weeks quite similar to that seen in the adult (Plates 8 & 9).

Most of the parenchymal cells at ten weeks have attained adult size, approximately 15 microns (Plate 8). Many are smaller and will hypertrophy by maturity contributing to the size of the gland at this age. Parenchyma cell outlines are distinct. Cytoplasm is relatively clear with a large dark staining nucleus that takes on a shape similar to that of the entire cell. A nucleolus is evident.

Glands from male and female juvenile mice show no apparent morphological or histological differences. They are indistinguishable.

Adult Stage

Eight adult specimens at twenty-one weeks of age were studied. Tissue at this age differs from that at ten weeks in the amount and definition of connective tissue elements, in further hypertrophy of parenchyma cells, and hence in the size of the gland itself (Plate 9). The gland averages 630 microns across in the transverse and 340 microns in the median saggital plane. The slight growth

appears due to maturation of the connective tissue frame-
work and hypertrophy of smaller parenchymal cells. Cell
outlines and connective tissue septa are very distinct. No
significant change in vascularity was observed and male and
female tissues are indistinguishable. Mitotic activity was
entirely absent in glands of adults and there was no change
in staining character over that of younger tissue.

Table 1. Number of glands and sexes studied from fetal and neonatal stages with parameters in microns. Mean appears above standard deviation, range is in parenthesis.
M = male, F = female

Stage	Age	# glands Studied	M	F	Parameters in microns Transverse	median saggital
Fetal	10 days gest.	3	–	–	90 10 (80-100)	35 5 (30-40)
	17 days gest.	3	–	–	170 20 (150-200)	180 10 (170-190)
Neonatal	Birth	6	3	3	200 20 (140-230)	200 10 (200-230)
	1 week	6	3	3	200 10 (200-210)	350 10 (350-370)
	2 weeks	7	3	4	260 20 (250-300)	350 20 (300-390)

Table 2.   Number of glands and sexes studied from juvenile
           and adult stages with parameters in microns.
           Mean appears above standard deviation, range is
           in parenthesis. M = male, F = female.

Stage	Age	# glands Studied	M	F	Parameters in microns Transverse	median saggital
	3 weeks	8	4	4	290 10 (270-320)	390 10 (370-420)
	4 weeks	13	9	6	310 20 (290-340)	360 10 (350-380)
	5 weeks	15	5	10	360 10 (340-390)	390 10 (370-420)
	6 weeks	15	10	5	390 20 (340-410)	390 20 (350-410)
Juvenile	7 weeks	20	9	11	400 20 (350-450)	380 20 (320-410)
	8 weeks	10	5	5	450 20 (410-470)	390 20 (350-410)
	9 weeks	15	7	8	470 30 (400-510)	400 30 (340-410)
	10 weeks	17	10	7	560 30 (500-610)	370 20 (350-400)
Adult	21 weeks	8	4	4	630 20 (600-660)	390 20 (350-410)

DISCUSSION

The mouse pineal gland is seen to arise from the dorsal surface of the third ventricle at a point just posterior to the developing choroid plexus. During uterine and early post partum development only cells of a parenchymal nature, accompanied by occasional capillaries, are evident within the gland. The cells have very little cytoplasm, hence the gland appears as a densely packed mass of nuclei within a capsule that is continuous with the pia mater and the walls of adjacent veins.

It has been observed in the rat that post partum glandular growth is due primarily to hypertrophy of parenchyma cells (47). Mitotic activity in rat pineal tissue is reported to disappear during the second and third week post partum (51). This appears to be true of the mouse pineal as well. Mitotic activity ceases by the juvenile stage as parenchyma cells begin to acquire more cytoplasm, separating their nuclei within a fine reticular framework. Dark staining nuclei of neuroglia cells accompany an increasing number of capillaries and intercellular spaces.

Lehman's polychrome stains hemoglobin bright yellow and was used with the intent of contrasting vascular elements against their connective tissue surroundings. The procedure proved to be no better than the hematoxylin and

eosin method.

Although extensive, the histological development of the mouse pineal during its juvenile life is very gradual. An obvious change in cell volume, neuroglia and connective tissue content, vascularity, etc., is seen when comparing specimens from juveniles of significantly differing ages, for example, three and nine weeks. These histological criteria mature too slowly to make it possible to distinguish between tissue from animals of very similar ages, i.e., four and five, or six and seven weeks. In order to delineate pineal tissue according to the histological characteristics mentioned above, you must compare specimens from juvenile mice differing by several weeks in age.

By the end of the juvenile stage, approximately ten weeks, most parenchymal cells have attained adult size, 15 microns. Adult tissue is nearly indistinguishable from that seen at ten weeks, differing mostly in the extent of capsular and intraglandular connective tissue development. All of the parenchyma cells appear to have reached maximum diameter making a final contribution to the dimensions of the glands at this stage. The connective tissue lattice separates small groups of parenchyma cells into obscure lobules that are more accurately defined in adult than in juvenile tissues.

The mouse pineal presents some histological characters in contrast with those seen in pineal tissue of other

species. For example, none of the tissue observed in this study illustrates a histological delineation separating the pineal into anterior and posterior portions (21). Serial sections in the transverse plane do not indicate a difference in cell density or morphology in any part of the gland. Every cell picture is congruous within age groups and no portion of any gland appears to differ significantly from another. Also there is no evidence of striated muscle fibers in any of the mouse tissue as reported in the rat (52, 53).

In spite of considerable interest in the pineal, little has been done to investigate the presence of lymphatic tissue either in a single species or on a comparative basis. Studies showing lymphatic elements in the pineal tissue of birds reported the establishment of germinal centers early within the first few months of post hatching development, and their complete disappearance by six months (43, 45, 46). Similar work dealing with mammals cite only small fractions of the pineal tissue observed as having lymphatic elements, if at all (47, 48). The rare occurence of lymphatic aggregations in mammals, as compared to the apparent natural occurrence in birds, suggests a pathological explanation rather than a functional homology between the two classes. The significance of finding germinal centers in mammalian pineal tissue lies in their potential as a site for lymphatic sensitization, homologus to the bursa in birds.

Such a tissue has yet to be identified in mammals. Cogburn and Glick (46) demonstrated the extraglandular origin of pineal lymphatic elements in chickens, and in view of the fact that these germinal centers establish themselves and disappear within a six month period, offers grounds for suspecting the gland as a possible site for lymphocyte maturation. Hence the search for lymphatic aggregations in mammalian pineal tissue.

The picture presented by the mouse pineal gland in this study is characteristic of that seen in other rodents with respect to lymphatic content (21). Lymphocytes inhabit the capsule and meningeal tissues of juvenile and adult mice throughout development with no sign of grouping or convergence, and they are not observed in dispedesis as is seen during the establishment of germinal centers in the pineal of chickens (46). It appears, that in no way, do these few cells aggregate into nodules or germinal centers like those described in domestic fowl (43, 46). Rather they are present only in extraglandular tissues, in a random manner throughout most of the juvenile development and on to maturity. It is conceivable, considering the short life span and early maturity of the mouse, that germinal centers could establish themselves and disappear during the short time interval not studied from eleven through twenty weeks. These cells most likely represent the normal tissue population of lymphocytes for this

animal rather than a stem cell line for germinal activity, sensitization or a reaction to some mild pathological condition. The fact that they are not observed migrating into the gland from the blood supply, as is described in the chicken, is further evidence against consideration of the mouse pineal as a target organ for lymphatic involvment.

This study verifies and extends some previous observations on pineal tissue, but the primary objective has been to search the mouse pineal gland for lymphatic germinal centers as have been described elsewhere (43, 46). It seems clear that lymphatic nodules do not normally occur in mammalian pineal tissue as they do in the chicken, and that the role or activity of the lymphocyte in mouse, or mammalian pineal tissue in general, is entirely different from that in certain species of birds.

Figure 1.   This drawing of the rat pineal gland is intended
to illustrate the shape of the pineal and to de-
fine the planes of reference described in this
study. The morphology of the pineal in the rat
is similar enough to that in the mouse so that
it may be used to illustrate the following
points. The gland is pictured as seen in the
median saggital plane. The red lines define
the parameters of the gland as are referred to
in this study in the median saggital plane (a).
The blue line (b), represents the transverse
plane which runs perpendicular to the page. All
the glands in this study were sectioned in the
transverse plane. The greatest diameter in this
plane was recorded for each gland and used for
the calculation of data in tables 1 & 2. The
number of sections for each gland in the
transverse plane were multiplied by the section
thickness to obtain dimension (a) in the median
saggital plane. Taken from Wurtman, R.J., and
Axelrod, J. 1965. The Pineal Gland. Sci. Am.,
213:50.

Plate 1.   The mouse pineal gland (P) at ten days gestation.
Third ventricle (V). H & E stain. (100X).
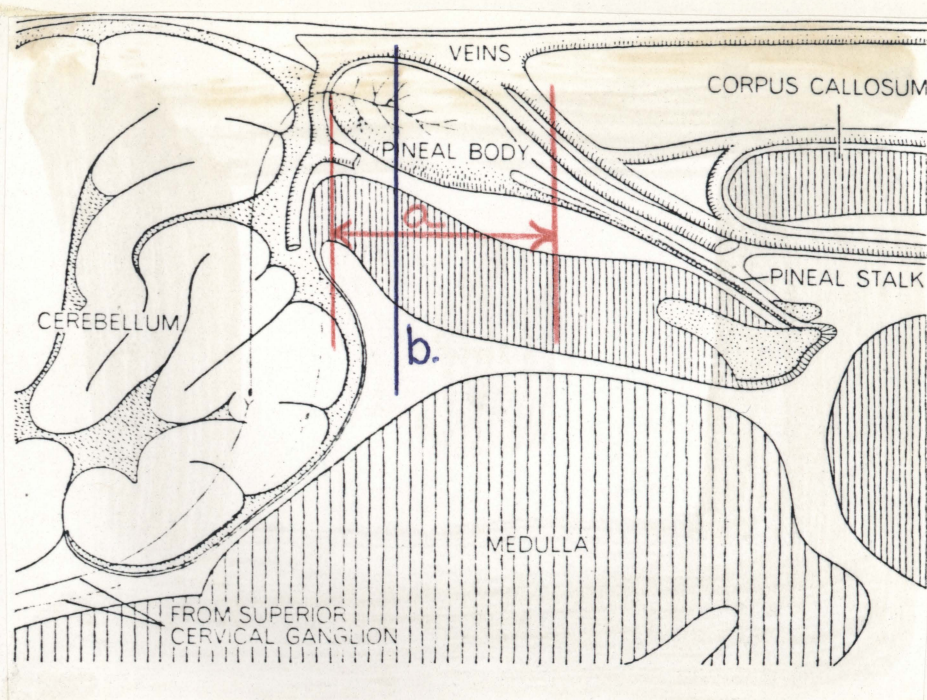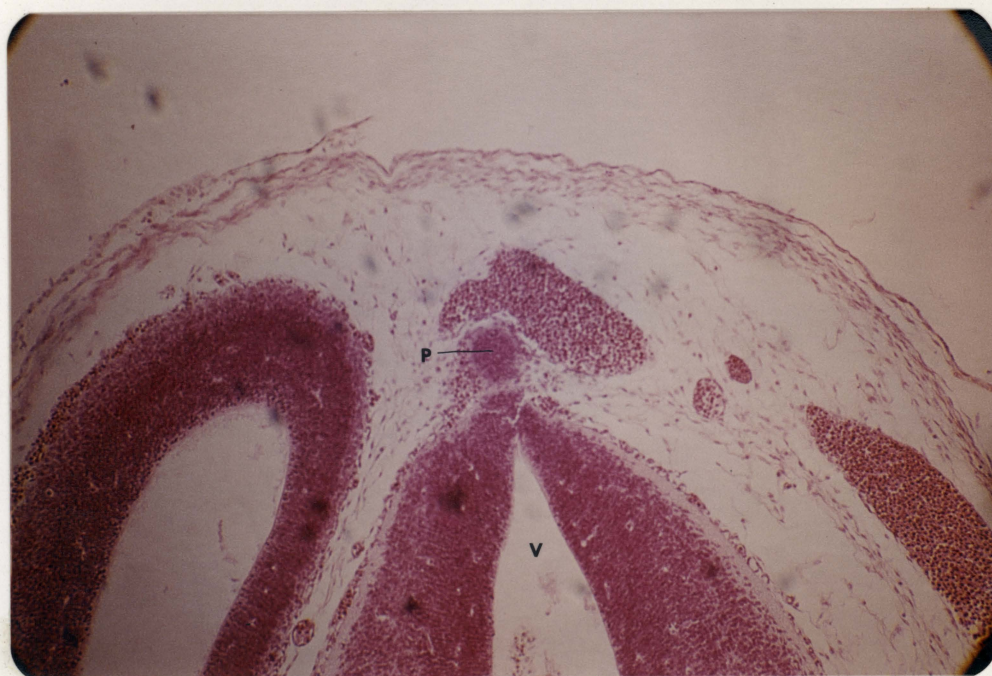
Figure 1.

Plate 1.

Plate 2.   The mouse pineal gland at ten days gestation.
           Nuclei are packed tightly together with very
           little cytoplasm. Note several cells are in
           mitosis (M). H & E stain. (450X).

Plate 3.   The mouse pineal gland at seventeen days
           gestation. Unique to this age is the
           arrangement of parenchyma cells into
           rosettes approximately 30 microns in
           diameter (R). Blood vessels (B) are
           larger and more evident during early
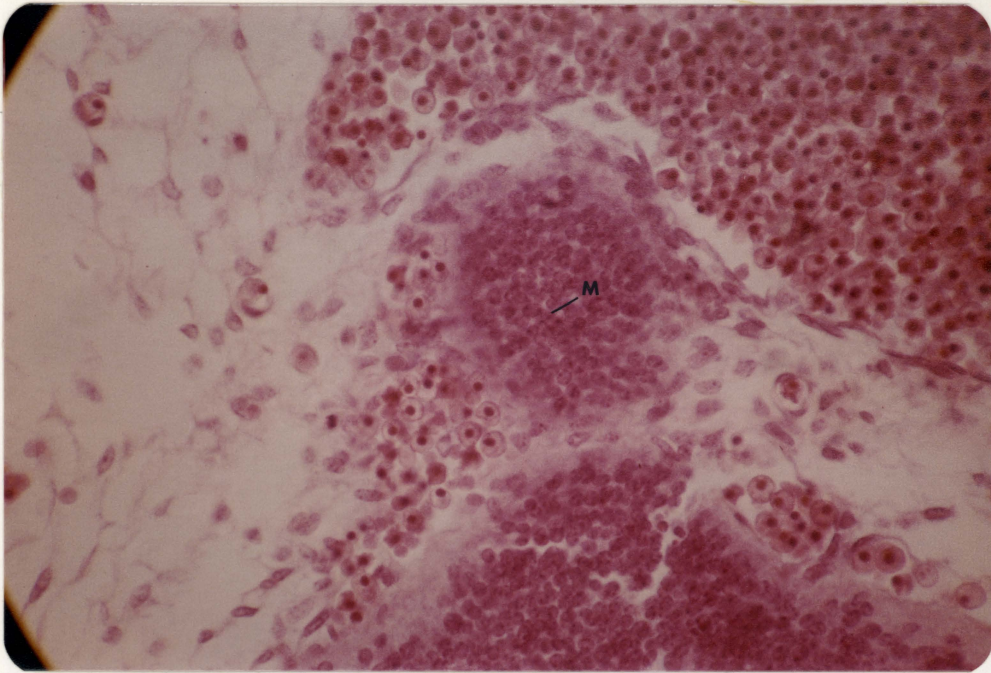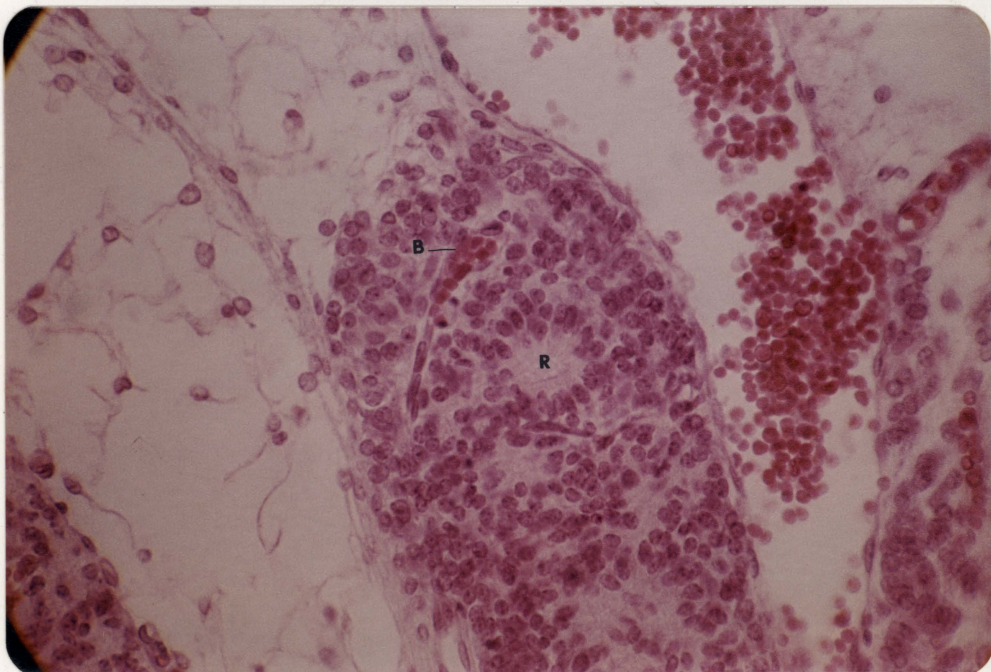   development. H & E stain. (450X).

Plate 2.

Plate 3.

Plate 4. The mouse pineal gland (P) at birth. H & E stain.
          (100X).

Plate 5. The mouse pineal at birth. Note the slight in-
          crease in extracellular spaces (EX), and that
          mitotic cells (M) are still present. Several
          blood vessels (V) are also evident. H & E stain.
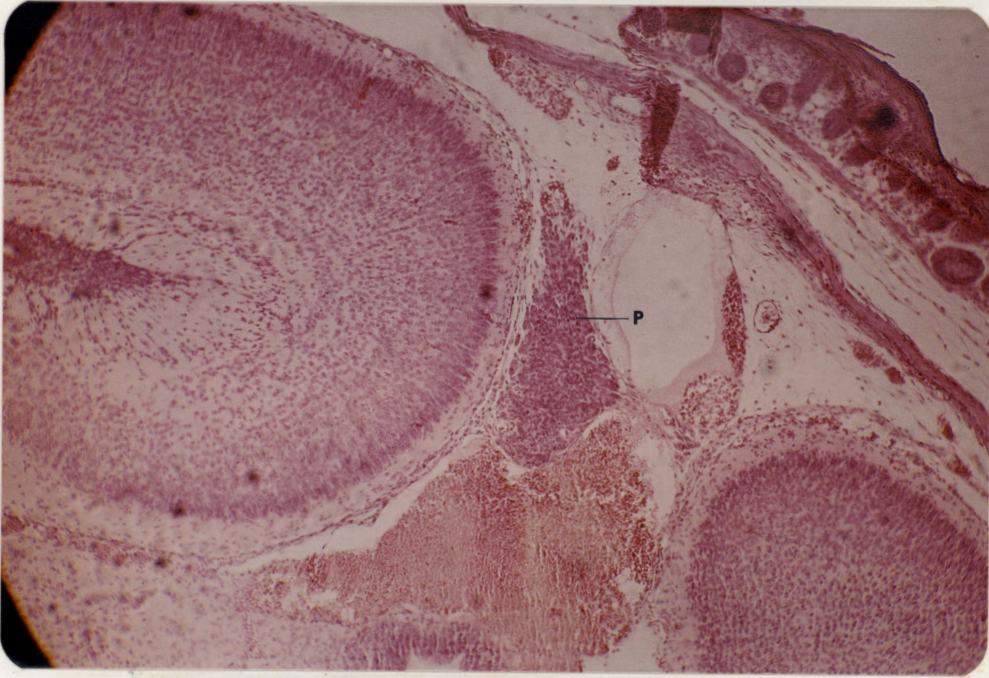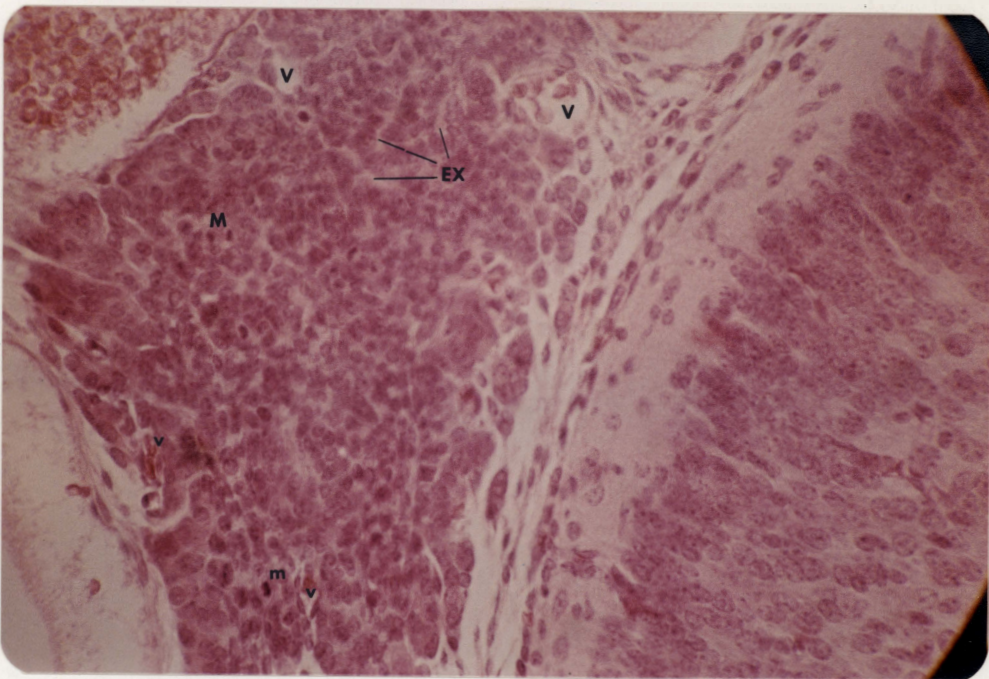   (450X).

Plate 4.

Plate 5.

Plate 6.   The mouse pineal gland at two weeks post partum.
           Note the increase in nuclear spacing over that
           in Plate 5. Neuroglia (N) are evident and very
           few cells are in mitosis (m). Mitotic activity
           ceases shortly after two weeks of age. Lehman's
           polychrome stain. (100X).

Plate 7.   The mouse pineal at six weeks post partum.
           Note the increase in cytoplasm and spacing of
           nuclei. Cell outlines are relatively obscure
           however a fine connective tissue framework is
           evident that separates the parenchyma cells into
           lobules (L). The dark staining nuclei of
           neuroglia are identified as microglia (m), and
           oligodendria (o). H & E stain. (450X).
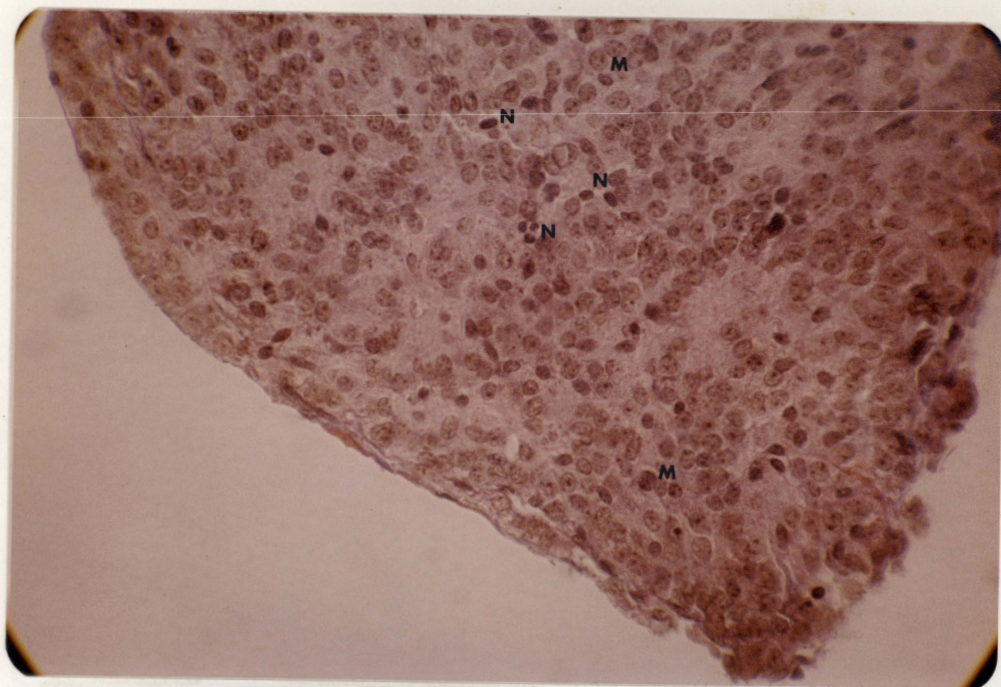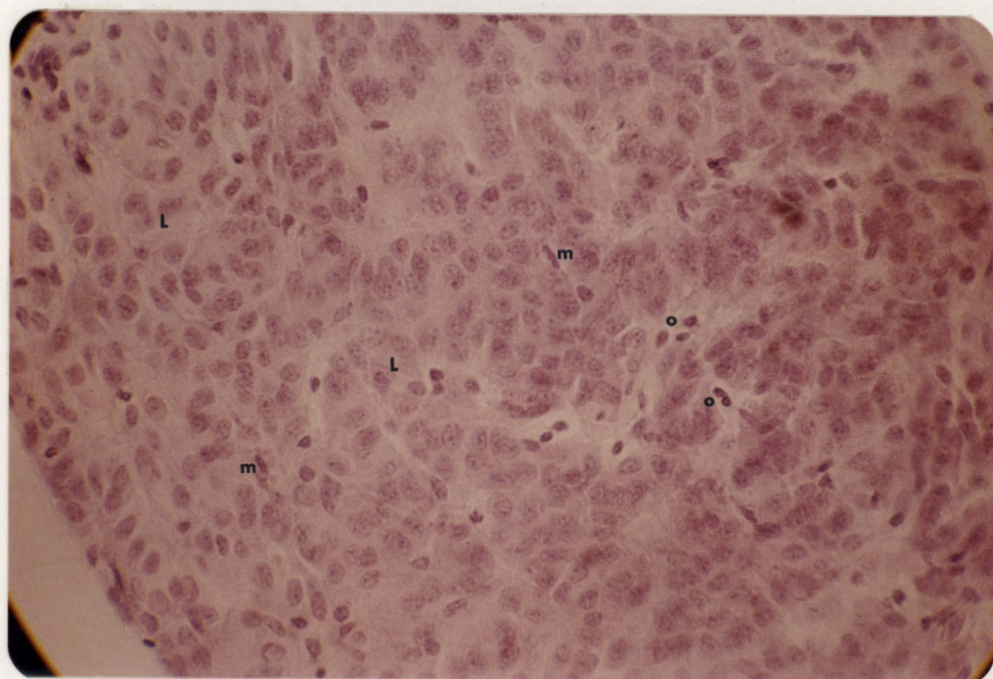
Plate 6.

Plate 7.

Plate 8.   The mouse pineal gland at ten weeks post partum.
           Note the definition in cell outline and
           connective tissue elements. Most of the cells (P)
           in this example are approximately 10-15 microns
           in size. Lehman's polychrome stain. (450X).

Plate 9.   Adult tissue at twenty-one weeks. There is a
           slight increase in glandular size and con-
           nective tissue content over that at ten weeks.
           Note the thickness of the capsule (c) as
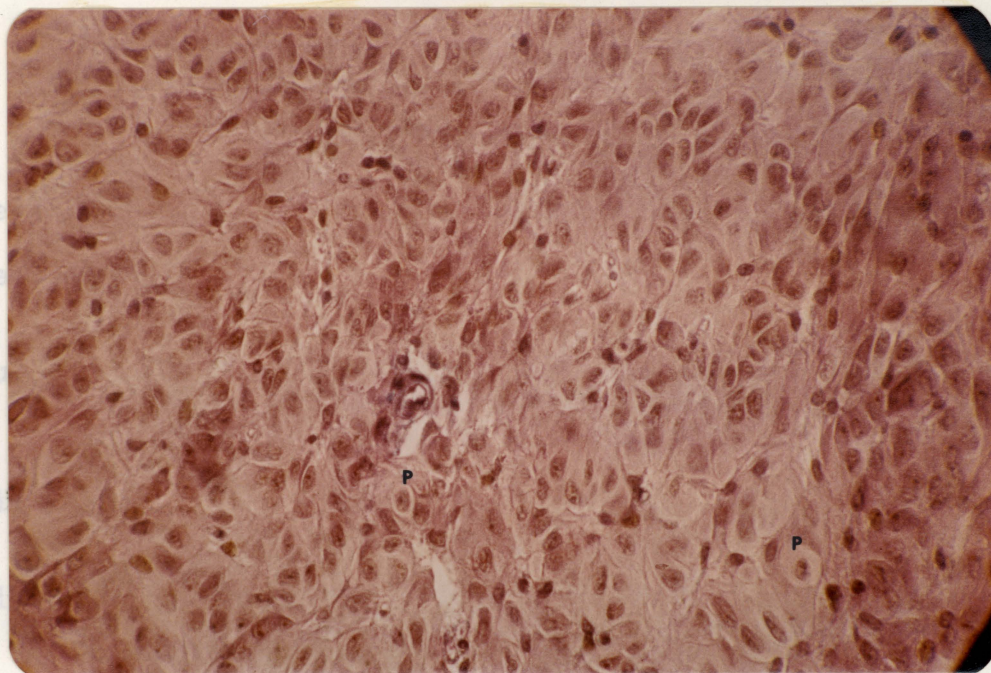   compared to earlier stages. H & E stain. (450X).

Plate 8.

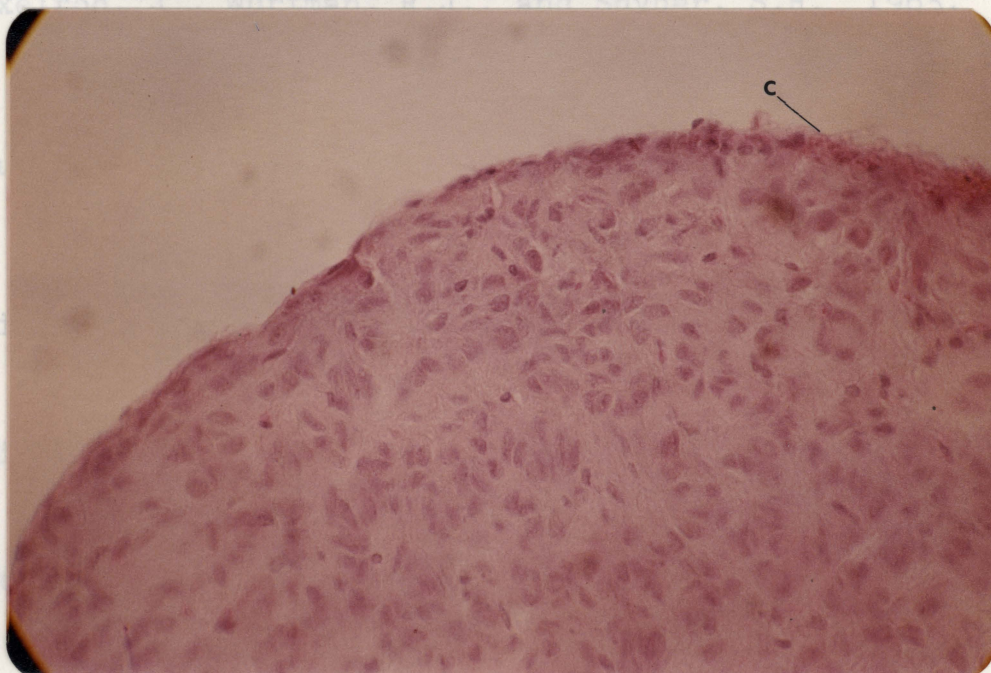Plate 9.

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