

The Pineal-Paraphyseal Complex of Sea Turtles

I. LIGHT MICROSCOPIC DESCRIPTION¹

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ABSTRACT The pineal-paraphyseal complex of sea turtles is an impressively large structure which projects dorsally and anteriorly above the prosencephalon. The complex was examined by light microscopy in several age classes of green sea turtles (*Chelonia mydas*) and from juvenile loggerhead turtles (*Caretta caretta*). The paraphysis is extensively fused to the distal portion of the pineal body, suggesting an interrelated function for these two tissues. No duct or canal was observed connecting the pineal lumen to the third ventricle. Two pineal cell types are described which appear to correspond to the neuroglial supportive cells and the secretory rudimentary photoreceptor cells of other amniotic vertebrates. A possible luminal secretion in the form of apical protrusions is produced by the latter cell type. No typical photoreceptive outer segments were observed.

Warren ('10-'11) described the development of the paraphysis in the aquatic turtle *Chrysemys picta marginata* as "a relatively complicated structure with many lateral tubules and a sinusoidal circulation approaching that of Amphibia." Since the Amphibia have the most elaborate vertebrate paraphyses, this suggests a well developed organ for chelonians. He also noted that the paraphysis grows backward from the dorsal-caudal telencephalon until it comes into contact with the rostrally projecting pineal gland. These observations are in agreement with our preliminary data for sea turtles (Owens and Ralph, '77). Shuangshoti and Netsky ('66) concluded after examining a few species (including a single lizard from the Reptilia), that the paraphysis is a modified extraventricular choroid plexus.

The pineal gland of the green sea turtle (*Chelonia mydas*) has been described as relatively massive (Humphrey, 1894; Tilney and Warren, '19). The drawings of Parker (1880), in his monograph on the development of the green turtle, show an embryonic pineal body of considerable size, though the author does not point this out. In fact, all of the few chelonian species examined appear to have well developed pineal glands (Tilney and Warren, '19; Combescot and Demaret, '63; and Vivien and Roels, '67), despite at least one suggestion to the contrary (Hoffman, '70).

The chelonian pineal body is of additional interest because of its intermediate form between the photoreceptive condition of lower vertebrates and the totally secretory appearance of snakes, birds and mammals. Thus, one might predict that the chelonian pineal gland is an evolutionary transitional form with a primary (possibly rather simple) secretory function. Since sea turtles are migratory, seasonally reproductive (Hirth, '71), possibly sensitive to photoperiodic cues (Ulrich and Owens, '74) and have a massive pineal body, we have taken the opportunity to look closely at the structure of this enigmatic organ.

In all turtle species in which the pineal body has been described, many of the pinealocytes which line the lumen produce apical blebs. These blebs appear to be shed into the lumen, suggesting to some workers the appearance of holocrine or exocrine gland activity (Combescot and Demaret, '63; Grignon and Grignon, '63). Thus, the question of whether the pineal lumen connects directly to the third ventricle, thereby providing an effective communication system to the circumventricular organs, is of particular interest in chelonians. The water turtle (*Emys leprosa*) (Combescot and Demar-

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et, '63) and the tortoise (*Testudo mauritanica*) (Grignon and Grignon, '63) were reported to have cavities which communicate directly to the third ventricle. However, Vivien ('64) stated that there is no direct connection in the slider (*Pseudemys scripta*) (now assigned to *Chrysemys*, Ernst and Barbour, '72). Similarly, Mehring ('72) found a closed lumen in *Testudo hermanni*. Thus, the examination of additional chelonian species is interesting from the standpoint of the possibility of a direct communication between the lumen of the pineal body and the third ventricle.

METHODS

A total of 25 immature green sea turtles (*Chelonia mydas*) were donated by Cayman Turtle Farm, Ltd. of Grand Cayman Island, British West Indies. These animals were purchased (as eggs) by the company from the nesting beach in Surinam and raised at the Grand Cayman facility. The brains from five turtles each at one month, four months and four years of age were prepared for light microscopy. In addition, two heads from wild-caught adult males were obtained at slaughter from Cayman turtle fishermen. These animals were reportedly netted off Nicaragua. We also obtained 18 loggerhead sea turtles (*Caretta caretta*) as eggs from the Florida Department of Natural Resources. Specimens one day, ten days and two months of age were examined.

The specimens were preserved in 10% formalin or Bouin's fluid, embedded in paraffin and sectioned at 5-7 μ m. Five of the smaller specimens were decalcified in a 22.5% formic acid - 10% sodium citrate solution prior to embedding. Ten additional specimens were preserved with 3% glutaraldehyde followed by 1% osmium post-fixation. These were embedded in epon 812 for semithin sectioning.

The paraffin sections were stained with either hematoxylin and eosin or luxol fast blue, Schiff's reagent and hematoxylin, according to the technique of Margolis and Pickett ('56). The epon sections were stained with toluidine blue.

Eight loggerheads which weighed 19.78 ± 0.39 g (mean \pm SEM) were utilized for measurements of the nuclear sizes of the pinealocytes and the parapyseal cell. The width and length of 30 nuclei for each cell type from near mid-sagittal paraffin sections were measured using an eyepiece micrometer. The turtles had been maintained in normal salt water

on a 14:10 (light:dark) photoperiod at 27°C day and 25°C night temperatures.

RESULTS

Gross morphology

The pineal body, in the two sea turtle species examined, arises via a stalk from the rostral aspect of the habenular region (fig. 1). Although the pineal lumen continues to the base of the stalk, we have found no opening from the lumen to the third ventricle (figs. 3-6). As the turtle ages, the pineal parenchyma enlarges via evaginations into the lumen. The lumen eventually fills loosely with pinealocytes, but is apparently never as densely packed as in snakes, birds and mammals.

The paraphysis, which is a continuation of third ventricular choroid plexus, attaches more or less firmly to the enlarged distal end of the pineal gland. In some specimens there is an extensive fusion of the pineal parenchyma and paraphysis (figs. 3-6), while in others, the two tissues border either side of vascular sinuses (figs. 6, 11).

A fluid-filled cavity is formed inferior to the large pineal-paraphyseal complex. The cavity is bounded laterally by pia mater, rostrally by the paraphysis and caudally by the pineal stalk. The cavity communicates with the third ventricle via a median aqueduct which courses anterior to the habenula and posterior to the massa intermedia (fig. 1).

The pineal bodies from a group of 12 approximately 2-month-old *C. mydas* that had been used in an unrelated experiment, were weighed upon dissection. The pineal gland wet weight was 2.6 ± 0.3 mg (mean \pm SEM) for turtles with body weights of 107.62 ± 1.56 g and curved carapace lengths of 92.16 ± 0.78 mm. It should be pointed out that in our dissection, we may have inadvertently included some parapyseal tissue which often adheres to the pineal body.

Cytology

The pineal parenchyma has the overall appearance of a pseudostratified columnar epithelium. It is composed of two primary cell types which appear for the most part in two distinct layers (fig. 7). Cell type A is found in the basal area of the tissue abutting the extensive capillary network. These cells are characterized by irregularly shaped nuclei which stain darkly with toluidine blue due to an abundance of dense heterochromatin. Cell type A is probably the supportive or neuroglial

cell of Collin ('71) and others. Cell type B contrasts markedly to type A due to its location on the lumen, larger size, ovoid nucleus with diffuse heterochromatin and large nucleolus and aggregations of dark staining material at the apical margin (figs. 7, 8, 11). In eight hatching loggerheads, cell type A nuclei had a length of $6.7 \pm 0.2 \mu\text{m}$ (mean \pm SEM) and a width of $2.7 \pm 0.1 \mu\text{m}$, whereas cell type B was $5.6 \pm 0.2 \mu\text{m}$ long and $3.6 \pm 0.1 \mu\text{m}$ wide. Cell type B is the "secretory rudimentary photoreceptor" cell (SRP) of Collin ('71). The type B cell is also the source of the abundant apical protrusions (blebs) which pass into the pineal lumen (figs. 7, 8). However, the typical outer segment structure of photoreceptors was not observed. The material in the lumen (fig. 8) is composed primarily of cell debris, including degenerating nuclei. Cells which appear to be macrophages are also occasionally seen in the lumen.

The parapineal cells have an apical brush border with tufts of cilia (fig. 9). In the hatching loggerheads, the nuclei were $5.7 \pm 0.1 \mu\text{m}$ in length and $4.1 \pm 0.1 \mu\text{m}$ in width. Aside from the close association with the pineal, these cells appear to be indistinguishable from choroid plexus at the light microscopic level.

Despite the use of the luxol fast blue stain, which is appropriate for myelin, we were unable to demonstrate nerve fibers in the pineal body at the light microscopic level. Recent electron microscopic observations (Owens and Morita, unpublished), however, have demonstrated the presence of sparse unmyelinated nerve bundles in marine turtle pineal tissue.

Although we have only examined young loggerhead turtles, the pineal morphology of this species appears similar to that of the green sea turtle at comparable ages (fig. 10). There are, however, two distinctive characteristics. The pineal lumen in the area of the stalk is more spacious in the loggerhead, while the dorsal sac appears better developed than in the green sea turtle.

DISCUSSION

The most striking observation regarding the pineal body of sea turtles is its large size. The Weddell seal is reported to have one of the largest mammalian pineal glands (Cuellar and Tramezzani, '69). Although interclass comparisons are hazardous, we estimate the sea turtle's pineal body to be two to five times larger than the Weddell seal's, as a function of body weight. Similarly, the sea turtle's pineal

gland would appear to be larger than those of the many mammalian species described by Legait and his coworkers ('76, '77).

To a limited degree, the close juxtaposition of the sea turtle's well developed parapineal adds to the overall impression of a massive pineal gland. While the pineal body continues to enlarge with age, the parapineal does not appear to keep pace in older animals. The close morphological relationship between the pineal gland and parapineal, particularly in younger turtles, suggests that the physiological roles played by these two tissues may be related.

Tilney and Warren ('19) described the sea turtle pineal body as bilobed. Although none of our specimens gave this appearance, it is possible that these authors mistook the parapineal as a second pineal lobe. Alternatively, they could have been referring to a drawing by Parker (1880) of a 2-cm embryo of *C. mydas* which appears to have a two-lobed pineal body. Unfortunately, Tilney and Warren ('19) did not cite Parker's work in relation to sea turtles. Rather they cited a paper by Rabl-Rückhard (1886) which did not mention sea turtles. It seems that the embryonic sea turtle may have a bilobed pineal body which, by the time of hatching, has taken the typical stalked form. The proximal lobe of the embryonic pineal may even become the pineal stalk.

Humphrey (1894) described the pineal complex of the green sea turtle as "... relatively enormous in size. It grows almost perpendicular to the longitudinal brain axis, not curving forward as it does in *Chelydra*. The epiphysis is very long and slender with an exceedingly tortuous narrow stalk and is much folded upon itself but has little widening at the distal end." Nearly all of the specimens which we have examined do in fact curve forward and are greatly widened at the distal end. We did, however, prepare one adult specimen in which the pineal was actually flexed posteriorly. Our initial conclusion was that this was an artifactual condition caused by inadvertent displacement during dissection (the pineal complex in turtles often adheres to the top of the skull when the skull is removed). An alternative explanation for these apparently unusual specimens (including Humphrey's specimen) is individual morphological variability.

The apical blebs, consistently seen in all turtle pineals, tempted previous workers to postulate that a hormone was being transported to the third ventricle via a duct or

opening in the pineal body. However, our observations, as well as those of Vivien ('64) on *Chrysemys* and Mehring ('72) on *Testudo hermanni*, do not support this possibility for turtles.

Our observations suggest that the presence of typical photoreceptor outer segments in the sea turtle pineal body is unlikely. Vivien-Roels ('70) reported finding typical-appearing outer segments in a very restricted portion of the posterior wall of the pineal gland in *Chrysemys scripta*. This observation has not been repeated in any other chelonian species (Collin, '71; Mehring, '72). We are not aware of any electrophysiological work in turtles that would support the conclusion that the chelonian pineal is photoreceptive.

Although we have been primarily interested in describing the morphology of the sea turtle's pineal body, the large size of the structure has tempted us to speculate briefly on its possible functions. Ralph ('75), after reviewing the pineal literature, hypothesized a positive correlation between the size and development of the pineal complex and the highness of the latitude that the animal inhabits. From this, he postulates a fundamental role for the pineal complex in thermoregulation. This suggestion seems particularly true for the lizard parietal eye (Gundy et al., '75) and may apply as well in mammals (Ralph, '75). As Ralph points out, however, an obvious exception is the arctic-dwelling whales (Cetacea) in which the pineal is rudimentary or absent (Oksche, '65). If one considers the relatively well buffered oceanic thermal environment of the deep diving whales, compared to the highly variable surface existence of seals, one can possibly salvage the basic hypothesis of a thermoregulatory role for the pineal complex. The involvement may not be merely thermogenic, but may include a fine tuning axis for variable environments. In other words, the pineal gland may provide some selective advantage to animals that normally experience extreme diurnal and seasonal fluctuations in temperature.

The large size of the sea turtle's pineal complex provides a further exception to the latitudinal hypothesis, though in the opposite extreme from whales, since the green sea turtle is primarily circumtropical. The idea of a thermoregulatory role for the pineal body, however, may still be germane for sea turtles. In this regard it is clear that sea turtles are far from being thermally passive. The leather-

back sea turtle (*Dermochelys coriacea*) is known to range into very high latitudes and has been shown to have body temperatures as high as 18°C above ambient when taken from cold water (Frair et al., '72). Green sea turtles are known to bask on the surface and on certain isolated beaches (Hirth, '71). Balaz and Ross ('74) have demonstrated that hatchlings will also bask under laboratory conditions. We have found (unpublished data) that hatchlings actually choose temperatures of around 30°C which is 2°-5°C above normal pelagic oceanic temperatures (hatchlings are commonly assumed to be pelagic). In addition, we have found that loggerhead hatchlings maintained at 31°C grew significantly larger than groups at 26° or 28°C (Owens and Ralph, unpublished data). Considering these obvious behavioral and physiological adaptations for thermoregulation in sea turtles, it seems plausible that these reptiles may also require some unknown integrative input from their large pineal glands.

A further possible function for the closely associated pineal-paraphyseal complex in sea turtles is in ionic and osmotic homeostasis. The choroid plexus has been clearly shown to function in this regard, while the pineal stalk of some species has been found to produce vasoactive peptides (Benson, '77). If the paraphysis functions similarly to choroid plexus, its uniquely close association to the glandular pineal may somehow facilitate handling the periodic salt loads that marine turtles must experience.

An additional possible function for the sea turtle pineal gland is as an annual time-keeper. It is possible that sea turtles are cueing on subtle day-length changes to synchronize their predictable migration and nesting seasons. Ulrich and Owens ('74) found that adult female *C. mydas* from a population nesting in Surinam, shifted their nesting season 2.5 months when they were relocated to the north by approximately 15° in latitude. Their new nesting season corresponded exactly to other nesting populations near the new latitude.

It is important to remember that with regard to morphology, size, glandular nature and photosensitivity, the pineal gland is possibly the most plastic of all vertebrate brain tissues. As Ralph has pointed out ('75), the pineal complex may serve several different functions in different species, or it may serve multiple functions in any single species.

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Abbreviations

AP, Apical protrusions (blebs)	O, Optic tectum
C, Cerebrum	OB, Olfactory bulb
Cap, Capillary	P, Pineal body
Ce, Cerebellum	Pa, Paraphysis
Cl, Cilia	PPC, Pineal-Paraphyseal cavity
DS, Dorsal sac	PS, Pineal stalk
HC, Habenular commissure	SC, Supportive neuroglial cell
L, Pineal lumen	SRP, Secretory rudimentary photoreceptor
LV, Lateral ventricle	VS, Vascular sinus
M, Microvilli	
MI, Massa intermedia	

PLATE 1

EXPLANATION OF FIGURES

- 1 Drawing of the sagittal section of the pineal complex and adjacent areas of the brain in a 2-month-old loggerhead sea turtle (*Caretta caretta*). Bar indicates 0.1 mm.
- 2 Dorsal-lateral view of the brain of a subadult green sea turtle (*Chelonia mydas*) which weighed approximately 30 kg. Bar indicates 1 mm. $\times 3$.

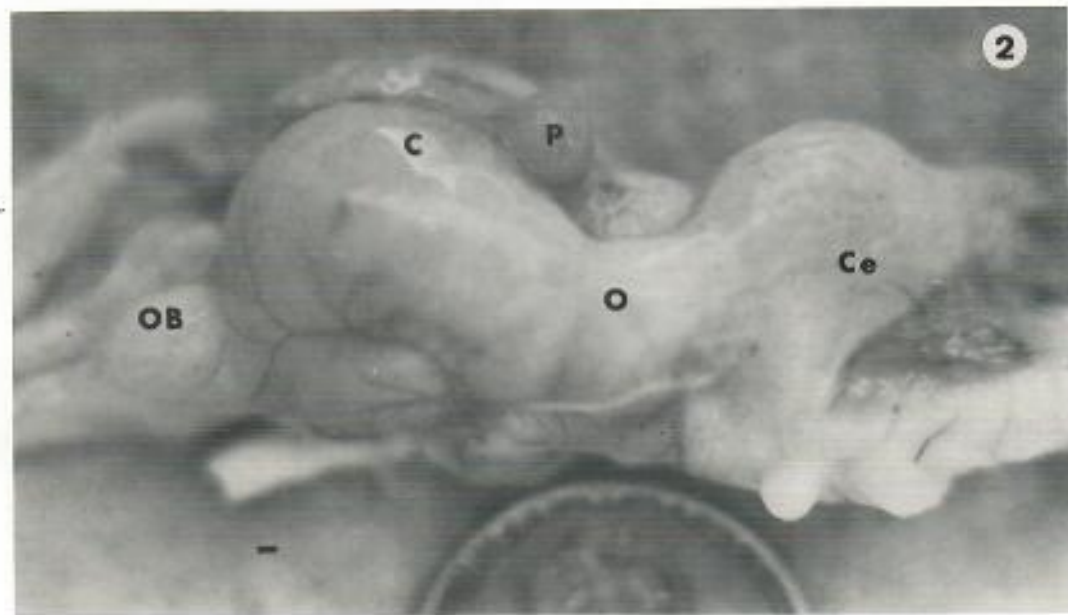
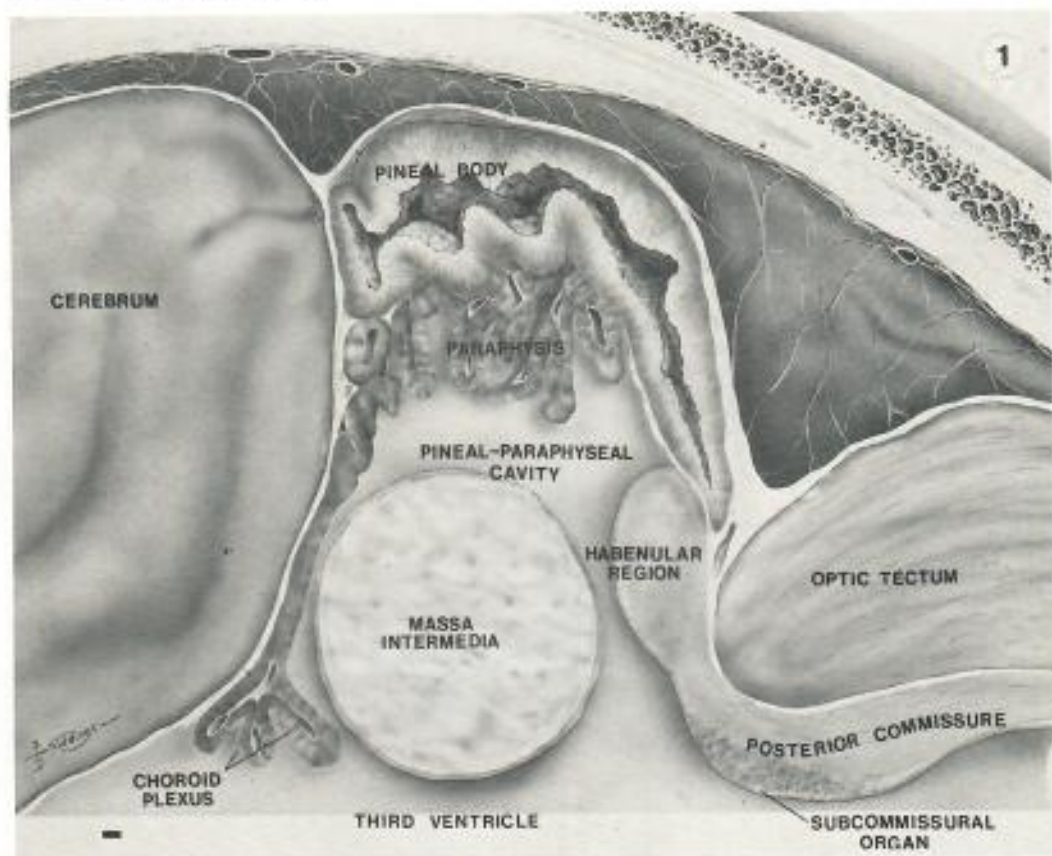


PLATE 2

EXPLANATION OF FIGURES

- 3 A 7- μ m parasagittal section of the pineal complex from a 1-month-old green sea turtle. Bar indicates 0.1 mm. Hematoxylin and eosin stain. \times 41.
- 4 A 7- μ m sagittal section of the pineal complex from a 4-month-old green sea turtle. The masses intermedia was washed off during staining. Bar indicates 0.1 mm. Laval fast blue, Schiff's reagent and hematoxylin stain. \times 33.
- 5 A 7- μ m parasagittal section from the pineal region of a 4-year-old green sea turtle. Bar indicates 0.32 mm. Hematoxylin and eosin stain. \times 12.5.
- 6 A 7- μ m parasagittal section from the pineal region of an adult male green sea turtle. Bar indicates 1 mm. Hematoxylin and eosin stain. \times 6.

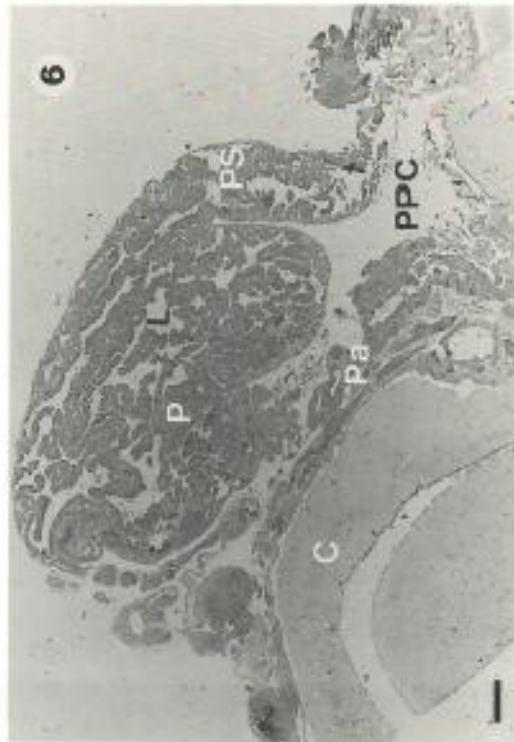
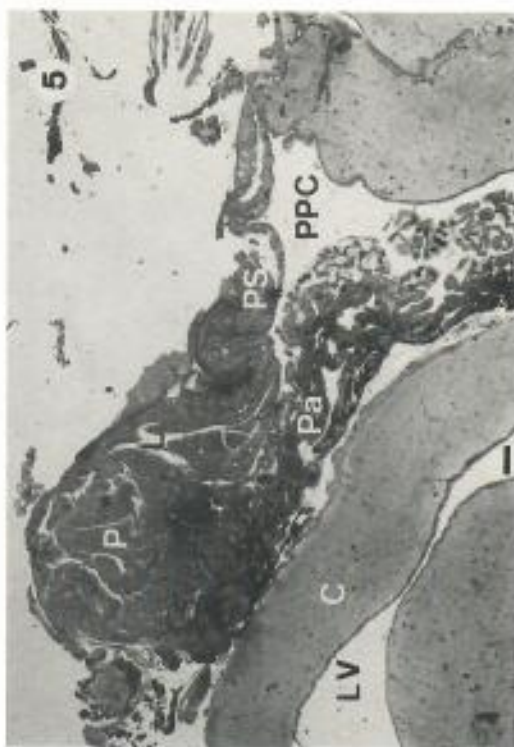
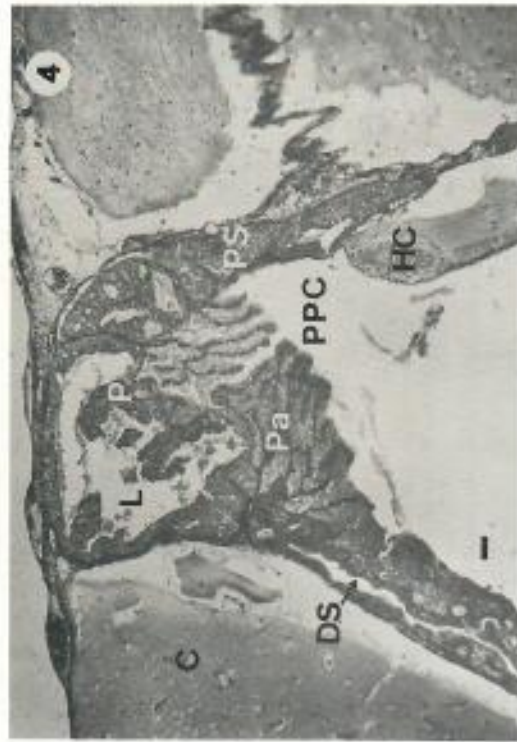


PLATE 3

EXPLANATION OF FIGURES

Figs. 7-11 One-micrometer epon sections stained with toluidine blue.

- 7 Two distinct pinealocytes are seen. The first cell is primarily associated with capillaries and has a more densely staining nucleus. The second cell type is located on the luminal margin, produces blebs and has a larger nucleus which does not stain darkly. Bar indicates $2.4 \mu\text{m}$. $\times 1,640$.
- 8 Apical margin of the secretory rudimentary photoreceptor pinealocyte showing the numerous blebs and occasional cilia. Bar indicates $1.6 \mu\text{m}$. $\times 2,430$.
- 9 Paraphyseal cells with tufts of cilia and dense border of microvilli. Bar indicates $2.2 \mu\text{m}$. $\times 1,690$.
- 10 Median sagittal section from a 1-month-old loggerhead sea turtle. The dorsal sac appears more prominent in this species and the lumen is larger in the stalk than in a gross sea turtle of the same age (fig. 3). Bar indicates 0.1 mm . $\times 30$.
- 11 Section from a loggerhead sea turtle pineal complex showing the close association of the pineal and paraphyseal tissue. A vascular sinus partially separates the two tissues in this specimen. Bar indicates 0.02 mm . $\times 200$.

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PLATE 8

