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The Histology of the Pituitary and the Adrenal Glands of the Dugong. *(Dugong Dugung)*

BY

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There is little information available on the glandular structures of aquatic mammals, more particularly of the dugong of the Indian waters. The opportunity of being present at the slaughter of one of these animals was afforded me recently. This particular specimen was a male which weighed over 200 pounds and measured over five feet in length. (Although these animals are sold by weight the fish merchants were rather reluctant to give me the correct weight). Phillips (1927) states that adults have been known to measure as much as nine feet. Fuller accounts of the dimensions of these animals are lacking.

The dentition—4 molar teeth in each side of the upper and lower jaws—would suggest that this specimen was that of a young adult. However, no tusks (incisor teeth), a feature of the dentition of adult males, were found even after dissection. Further a well-developed thymus gland was found.

The pituitary gland

The brain case was opened as soon as the animal was decapitated. The brain was removed after the severing of the hypophyseal stalk at its union with the base of the brain. The gland was dissected out, weighed and immersed in 10 per cent. neutral formalin. No attempt was made to preserve the integrity of the meninges about the gland. When the gland was of sufficient hardness, it was sectioned sagittally into two halves and fixation was continued for 24 hours. One half was cleared in terpineol, embedded in paraffin and sectioned at 6μ. The other half was double embedded in celloidin-paraffin. The sections were stained with haematoxylin and eosin, Mallory's triple stain and Heidenhain's Azan, and some were stained by the P.A.S. technique (Hotchkiss, 1948) and for acid muco-polysaccharides (Ritter and Oleson, 1950). The gland weighed 0·54 g. It consisted of two well defined lobes. The anterior lobe was applied to the ventral and lateral surfaces of the diminutive posterior lobe, leaving the dorsal and posterior surfaces of this latter lobe uncovered (Fig. 1). The appearance was not unlike that of the Florida manatee (Trichechus latirostris) described by Wislocki (1940) except that in this case the hypophyseal stalk was considerably longer and thinner. The infundibular recess of the third ventricle tapered into the stalk and the surface of the recess was smooth, unlike that of the Florida manatee.

Microscopically, the anterior lobe was demarcated into lobules by well marked strands of connective tissue. The sinusoidal blood vessels were well marked but not so prominent a feature as in some other pituitary glands, for instance, that of the

horse. The cells were arranged in cords though in some areas the arrangement was more irregular. Follicles lined by cuboidal cells were common in the posterior part of the pars anterior. These follicles in some cases contained small quantities of colloid which were P.A.S. positive but not positive for acid polysaccharides. There was no evidence of a pars intermedia. A cleft lined by cuboidal epithelium, one cell of which was strongly eosinophilic was situated in the posterior part of the anterior lobe. This cleft did not appear to show any branching even on examination of the more lateral sections and did not contain any colloid or cell debris. However, posterior to the cleft, normal pars distalis cells were found in all sections. The anterior lobe was separated by a very definite layer of collagenous fibres from the pars nervosa.

Mallory's triple stain differentiated the cells of the pars distalis into four types :

(*a*) The acidophil cells were situated more peripherally but distinct clumps of eosinophil cells were present in the more central areas of the gland.

(*b*) The large slate grey basophils described by Oldham *et al* (1938) in the pituitary of the South American manatee (Trichechus inunguis) are seen in the pituitary of the dugong too. These cells, the largest cells in the pars anterior, are situated centrally and posteriorly in the lobe.

(*c*) The small basophil cells appear to be most numerous and these are distributed somewhat like the acidophils and again as in the South American manatee appear to show varying degrees of basophilia.

(*d*) The chromophobes are probably the least numerous. Their cytoplasm is scant and their nuclei appear to be more vacuolated than those of the other cell types.

Staining with the P.A.S. technique revealed the presence of cells similar in size and appearance to the eosinophil cells although a very few appeared to be more of the size of the large basophils. The number of P.A.S. positive cells appears to be much less than the number of eosinophil cells shown up by the triple stain.

The pars tuberalis

The pars tuberalis is greatly reduced. It is present on the front and back of the hypophyseal stalk (Fig. 3) and in this gland appear to stop short of the base of the brain. Inferiorly, however, typical tuberal cells are present on and in close contact with that part of the hypophyseal stalk that is embraced by the pars distalis (Fig. 5). These cells are completely separated from the pars distalis by dense connective tissue fibres. No collections of tuberal tissue were found in close association with the actual bulbous distal extremity of the pars nervosa, namely, the posterior lobe proper. The pars tuberalis showed the typical hyperchromic nuclei and clefts filled with colloid which was P.A.S. positive but did not give a positive reaction for the ' acid polysaccharide ' test. The tuberal cells gave a diffuse positive reaction to both the P.A.S. and acid polysaccharide methods. The cells lining the colloid containing clefts were, however, not stained by either of these methods.

Pars nervosa

No specific methods were adopted to demonstrate the cells of the pars nervosa. The nuclei of the pituicytes are numerous. The vascular tufts described by Wislocki (1940) in the Florida manatee were not seen.

PLATE I

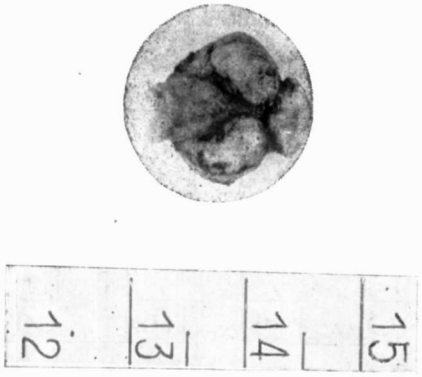

Fig. 1
Dorsal view of the pituitary gland

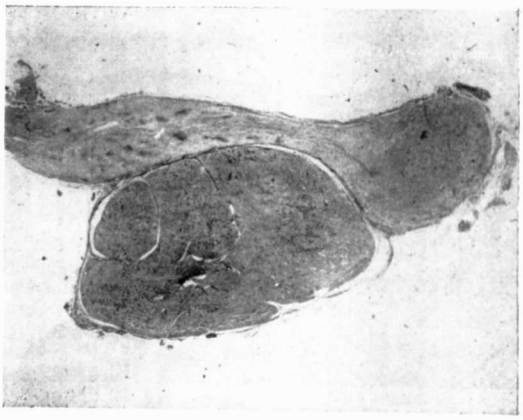

Fig. 2
Sagittal section of the pituitary gland showing the discrete pars distalis. Paraffin H & E × 5

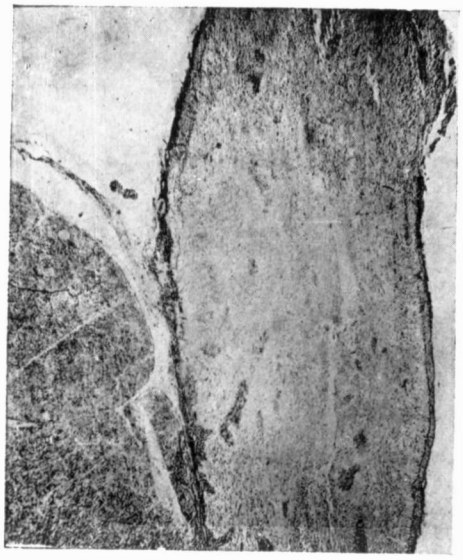

Fig. 3
Shows the hyperchromic cells on the front and back of the hypophyseal stalk
Paraffin H & E × 19

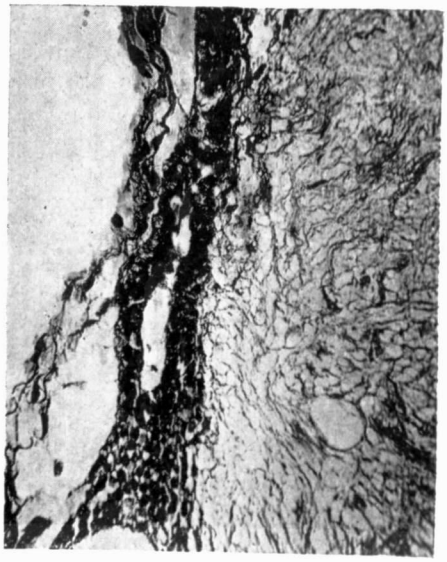

Fig. 4
Pars tuberalis showing colloid vesicles and typical hyperchromic nuclei
Paraffin H & E × 250

Discussion

The pituitary of the dugong appears to resemble the Florida manatee macroscopically and the South American manatee microscopically. The large basophil cells are clearly seen in this ' fresh ' specimen of the dugong pituitary and it is unlikely that post-mortem changes are responsible for the appearance of these large cells (Oldham *et al*, 1938).

Opinion is divided as to whether the P.A.S. positive cells of the mammalian pituitary gland are basophils or acidophils (Leblond, 1950). In the dugong it would appear that cells similar in size and distribution to the acidophil cells showed a positive reaction. A very few cells resembling the large basophils were also P.A.S. positive. The total number of cells that were P.A.S. positive, however, was less than the number of acidophils in the section. The findings in the dugong, then, do not shed any light on the problem of the reaction of the pars anterior cells to the P.A.S. stain.

The positive reaction of the colloid to P.A.S. but not to the acid polysaccharide technique is of some interest. McManus *et al* (1951) express their doubts as to the specificity of the latter stain for acid polysaccharide in that nuclei of the cells of the kidney tubules gave a positive reaction. Whatever the outcome of such findings it is of interest to note the differences in the staining reactions of colloid to the two polysaccharide techniques. Preliminary investigations of colloid material from pituitary glands of other mammals show that the colloid is positive in some cases to P.A.S. and in other cases to both tests. A comprehensive study of the material is being made with particular reference to the calcium content of these colloids in pituitary glands and will form the subject of a separate paper.

The lower limit of distribution of the tuberal cells—descending on the hypophyseal stalk behind the collagenous barrier of the pars distalis—appears to be similar to that observed by Wislocki (1940) in his re-examination of the elephant pituitary and also to that of Oldham (1938) in the armadillo. The distribution of this tuberal material of the dugong differs then from that of the South American manatee although in other histological aspects the two glands are closely similar.

The adrenal glands

Bourne (1949) in his monograph on the mammalian adrenal gland states that ' There are very few records of the adrenals of these creatures '. He goes on to state that Perrault (1676) made reference to the glands in the dugong or the manatee and that Meckel probably described the macroscopic appearances of the glands of the now extinct Stellar's sea cow. There is in all probability no accurate, comprehensive study of the adrenals of the dugong.

These glands in the dugong were surprisingly small (Fig. 7). Both glands together weighed 5·29 g. The glands were triangular in shape and were 3·5 cm. high, 2 cm. wide, and 0·5 cm. thick. These glands were situated about 5 to 7 cm. from the upper end of the fusiform kidneys which were 21 cm. long and 7 cm. wide. It was not possible under the circumstances in which the material was obtained to define the precise anatomical relations or the arterial and venous connexions of the glands. Within half-an-hour of the slaughter of the animal, the glands were cut into sections about 0·5 cm. thick, most of which were fixed in Zenker-formol and embedded in

paraffin or celloidin-paraffin. A few pieces were fixed in 10 per cent. neutral formalin. Inadvertently, this formalin material was immersed in 70 per cent. alcohol for nearly 48 hours after the washing. However, these blocks were embedded in gelatine and frozen sections were made. Microscopically the adrenals are covered by a well-developed collagenous capsule. Mallory's stain demonstrated trabeculae running centrally and forming a dense network around clumps of cells in the reticular zone. There was no evidence of a definite fibrous lamina between the cortex and medulla of the gland. The cortex showed distinct, glomerular, fascicular and reticular layers. The glomerular layer was composed of cells arranged as ' acini ' or as tall narrow arches surmounting the fascicular layer. Each fasciculus consisted of three to four parallel rows of cells separated from other fascicles by the distinct fascial septa. Distinct straight blood vessels were seen to run with the trabeculae. The fascicles were on the average about one and a half times as long as the glomerular zone. Marked vacuolation was noted in the more central cells of the zona fasciculata. The reticular layer was four to six cells wide. A large majority of the cells were vacuolated. Perhaps the most striking feature of the cross-sections was the vascularity of the reticular layer so well demonstrated by the Mallory and azan stains. The connective tissue of the medulla formed a close network around clumps of medullary cells which appeared to be closely packed. Medullary cells appeared as light or dark ones with all the stains used. Eosin-azure II staining revealed the presence of a few nerve cells. No green staining cells were, however, noticed.

Islets of cortical tissue were observed (Fig. 8).

Histochemical tests

Frozen sections were stained after the manner of Yoffey and Baxter (1949). It will be remembered that the tissue was left in 70 per cent. alcohol for nearly 48 hours.

(*a*) *Sudan B.* The sections stained an intense black except in the glomerular zone which appeared to be more lightly impregnated.

(*b*) *Schultz reaction.* The green coloration was similar in distribution to that of Sudan B.

(*c*) *Plasmal reaction.* All three zones stained with equal intensity.

(*d*) 2, 4 *Dinitrophenylhydrazine reaction.* The method of Albert and Leblond (1946) was used. The staining was no different to that of the plasmal reaction.

Attempts have been made in the past to correlate the weight of the adrenal glands with that of other organs. For the sake of the record, weights of some organs of the dugong are appended.

Adrenal glands	2·68 g and 2·61 g
Thyroid gland	13·0 g
Kidney	395·0 g
Pituitary	0·54 g
Brain (formalin fixed for two weeks) ..	237·0 g

The glands are typically mammalian.

There appear to be no descriptions of the adrenals of other Sireniae except for that of Stellar's sea cow where the glands have been said to be 3 inches long (Bourne, 1949). The gland of the dugong, however, is very much smaller, and when considering the size of the animal, surprisingly small. The thyro-adrenal ratio in the dugong is

PLATE II

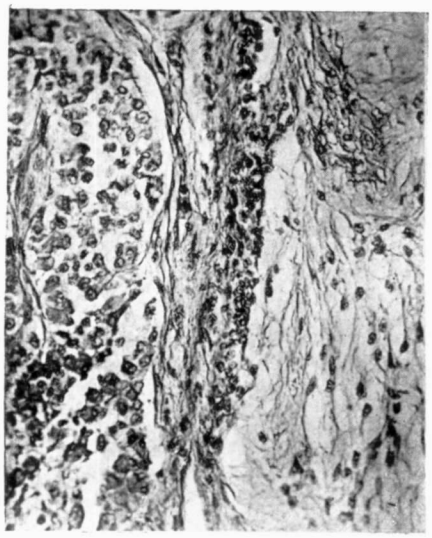

Fig. 5
Section showing the lower limit of tuberal tissue in close contact with the pars nervosa (on right) and separated by a collagenous barrier from the pars distalis (on left) Paraffin Mallory × 200

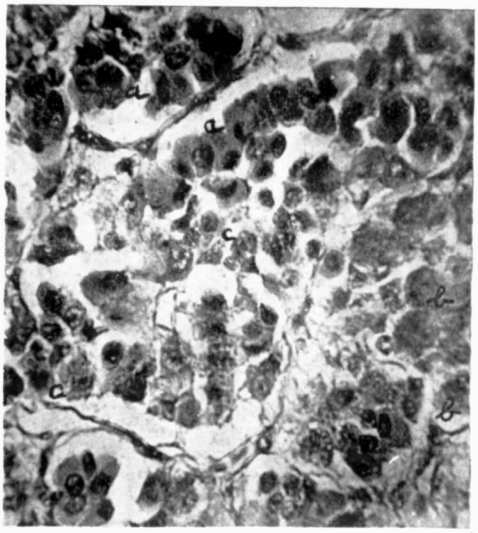

Fig. 6
The cells of the pars distalis (*a*) eosinophils (*b*) large basophils (*c*) small basophils Paraffin Mallory × 400

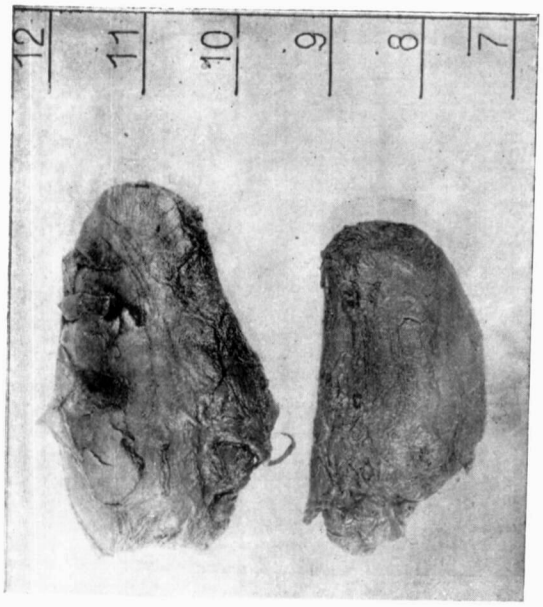

Fig. 7
The adrenal glands of the dugong

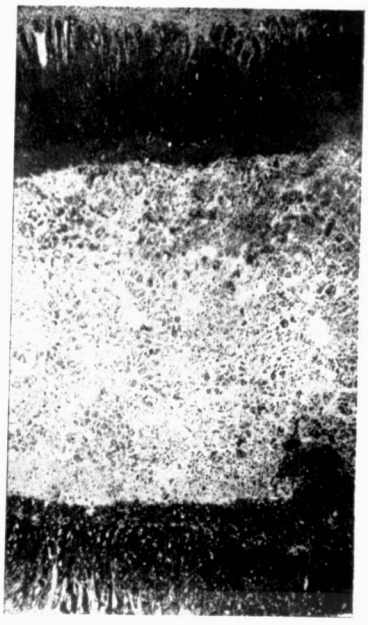

Fig. 8
Frozen section of adrenal stained with Sudan B showing Medulla at its widest part. × 13

1:0·41. It would be profitless on this one examination to speculate as to the reasons for this small size. It may not be out of place, however, to state that the breeding season of these animals is round about December and January of each year. The animal described here was netted in the early part of May.

The histochemical tests do not reveal anything distinctive. Differences in the intensity of staining of the different zones with Sudan B and 'plasmal reactions' are known to occur in other adrenals and at present these differences have not been adequately explained.

Summary

1. The pituitary and adrenal glands of the dugong are described probably for the first time.

2. The fresh pituitary gland of the dugong is similar to that of the South American manatee in that there is no pars intermedia and the cells of the pars distalis are of four types. The pars tuberalis however would appear to be better developed.

3. The reactions of the cells and colloids to P.A.S. and to acid polysaccharide test are discussed.

4. The adrenal glands are surprisingly small for an animal of such proportions. The arrangement of the cells of the cortex and medulla closely follow the mammalian pattern. There was no evidence of a fibrous lamella separating the cortex from the medulla.

5. The histochemical tests, Sudan B and the Schultz reaction stain the zona glomerulosa less intensely than the rest of the cortex and thus differs from the staining by plasmal and phenyl-hydrazine reactions, which stain the cortex more evenly. These differences have been previously noted in other glands and have not been adequately explained.

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Plate I

Figure I—Dorsal view of the pituitary gland.

Figure II—Sagittal section of the pituitary gland showing the discrete pars distalis.
Paraffin H & E × 5.

Figure III—Shows the hyperchromic cells on the front and back of the hypophyseal stalk.
Paraffin H & E × 19.

Figure IV—Pars tuberalis showing colloid vesicles and typical hyperchromic nuclei.
Paraffin H & E × 250.

Plate II

Figure V—Section showing the lower limit of tuberal tissue in close contact with the pars nervosa (on right) and separated by a collagenous barrier from the pars distalis (on left).
Paraffin Mallory × 200.

Figure VI—The cells of the pars distalis (*a*) eosinophils (*b*) large basophils (*c*) small basophils.
Paraffin Mallory × 400.

Figure VII—The adrenal glands of the dugong.

Figure VIII—Frozen section of adrenal stained with Sudan B showing Medulla at its widest part. × 13.