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HYPOTHALAMO-HYPOPHYSIAL CONNEXIONS IN THE CETACEA

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The anatomical connexions between the hypothalamus and adenohypophysis are of basic importance in the problem concerning neuro-endocrine relationships. The hypophysial stalk contains nerve fibres and blood vessels and either, or both, of these structures may form part of a pathway by which the hypothalamus influences the secretion of the anterior pituitary gland. It is well known from the work of Wislocki (1929) and Wislocki & Geiling (1936) that in the Cetacea the infundibular process is separated from the pars distalis of the hypophysis by a thick dural septum. This septum limits the area of contact between the adenohypophysis and neurohypophysis to the region where the pars tuberalis lies in contact with the tuber cinereum, and therefore, any structures of functional importance to hypothalamic-adenohypophysial relationship might be expected to be concentrated in this region. Drager (1944) has described the innervation of the adenohypophysis of the porpoise. He found that a few nerve fibres pass from the hypothalamo-hypophysial tract into the pars tuberalis, but that the pars distalis was free of nerve fibres. Wislocki & Geiling (1936) comment on the vessels of the infundibular stem of the whale. They state that 'pial arteries penetrating the stalk break up locally in the neural tissue into plexiform, sinusoidal channels. From these plexuses portal veins appear to arise which conduct blood via the pars tuberalis to the anterior lobe.' It was felt that further investigation of this region in the Cetacea might give evidence of general significance.

MATERIAL AND METHODS

Both adult and foetal material was examined.

Adult material. The pituitary glands of three adult blue whales (*Balaenoptera musculus*), fixed in formol within 5 hr. of death, were dissected and photographed. Blocks of tissue from representative areas of the pars distalis of these glands were embedded in wax, sagittally sectioned at 10 μ. thickness, and stained for nerve fibres by Davenport's method (Davenport, 1930), and by Bodian's method (Bodian, 1936), using both Bayer and Winthrop brands of protargol. To control the staining procedures, pieces of brain (rabbit's brain, also formol fixed, wax embedded and sectioned 10 μ.) were stained on the same slides as the whale pituitary sections. Other sections from these glands were stained for reticular connective tissue fibres by Gomori's method (Gomori, 1937) and again similar control tissue stained on the same slides. The fourth adult specimen was a male porpoise (*Phocoena phocoena*), weighing 62 kg. and 1·52 m. in length. This animal was injected with indian ink solution through the vertebral arteries. After fixation in formol, the hypothalamus, pituitary gland and surrounding structures were taken *en bloc*, serially sectioned in a sagittal plane at 150 μ. and stained with Biebrich scarlet.

Foetal material. The hypothalamus, pituitary gland and base of skull were removed, after formol fixation, from two foetal specimens, one, *Megaptera* (? *nodosa*), 360 mm. crown to tail tip, and the other *Balaenoptera* (? *physalus*), 290 mm. crown to tail tip. These specimens were decalcified in Jenkin's solution, embedded in celloidin, serially sectioned in a sagittal plane at 150 and 200 μ. respectively, and the sections stained with haematoxylin alone or haematoxylin and van Gieson. The brain and pituitary gland were removed in an intact dural envelope from a foetal Finback whale (*Balaenoptera physalus*), 2·44 m. long. After formol fixation a block of tissue containing hypothalamus and pituitary gland was embedded in celloidin, serially sectioned in a sagittal plane (200 μ.), and the sections stained with haematoxylin and van Gieson. The fourth foetal specimen was a female Lesser rorqual (*Balaenoptera acutorostrata*), 2·13 m. long and 101·8 kg. in weight. After attempted injection of the intracranial vessels with indian ink, the brain and pituitary gland were removed in the dural envelope and fixed in formol. A block of tissue containing the hypothalamus and pituitary gland was embedded in celloidin, serially sectioned in a sagittal plane (200 μ.), and the sections stained with haematoxylin and van Gieson, or mounted unstained.

RESULTS

Pars distalis of adult blue whales

The average dimensions of three specimens of the pars distalis of the blue whale were 3·2 cm. antero-posteriorly, 4·5 cm. transversely and 1·9 cm. supero-inferiorly, and their weights (after formol fixation) were 15·4, 20·8 and 16·6 g. From above, the glands appear kidney-shaped, the anterior pole of the gland corresponding to the hilum of the kidney. At this site is a structure closely resembling the zona tuberalis of the rabbit (Dawson, 1937; Harris, 1947). Through a foramen in the fibrous capsule which surrounds the pars distalis appears an extension of the glandular tissue which is grey in colour, semi-translucent and highly vascular as compared with the opaque white appearance of the rest of the gland. It is obvious that before removal of the brain and section of the hypophysial stalk, this region of the pars distalis was continuous with the pars tuberalis. On making a mid-line sagittal section through the gland, this tissue is seen to extend inferiorly around the anterior pole of the gland and to merge with the rest of the pars distalis. The vascular nature of this structure is again apparent on sectional view, and is well shown in Pl. 1 (figs. 3, 4). From its similarity to the zona tuberalis of the rabbit in appearance, continuity with the pars tuberalis, and relationship with the hypophysial portal vessels (see below), it is proposed to refer to this part of the adenohypophysis of the whale as the zona tuberalis.

The hypophysial portal vessels

Serial sections through the hypothalamus and pituitary gland of the four foetal whales show, in each case, that a connective tissue septum separates the pars distalis from the infundibular process, and that the pars tuberalis passes upwards anterior to the septum to form a collar around the tuber cinereum (Pl. 1, figs. 1, 2). In only one of these specimens, the Lesser rorqual, are blood vessels apparent. The indian ink injection had failed to perfuse the hypophysial vessels, but these vessels are distended with blood and therefore clearly visible. Large vessels running longitudinally in the pars tuberalis (Pl. 1, fig. 2) are seen to be in continuity, at one end with large capillaries penetrating the nervous tissue of the median eminence, and at the other end with the sinusoids of the pars distalis. These vessels are clearly seen in sagittal sections through the hypothalamus and pituitary gland of the adult porpoise (Pl. 1, figs. 3, 4). In this specimen the hypophysial vessels had been successfully injected with indian ink and the highly vascular pars tuberalis is seen to pass upwards anterior to the septum and to encircle the tuber cinereum. Although the infundibular stem of the porpoise is short and not clearly demarcated from the tuber cinereum or infundibular process, it was apparent from the vascular pattern that the hypothalamus, tuber cinereum (± infundibular stem) and infundibular process form three separate vascular territories. The tuber cinereum (± infundibular stem) contains a multitude of wide, tortuous, sinusoidal vessels (Pl. 1, fig. 3) which seem to drain into the mass of vascular trunks in the pars tuberalis (the trunks of the hypophysial portal vessels). These vessels in turn pass down into the zona tuberalis where they branch and are distributed posteriorly into the sinusoids of the pars distalis. The boundaries of the zona tuberalis are well shown by the vascular pattern, as illustrated in Pl. 1, fig. 4. The fact that the vessels in the more posterior region of the pars distalis are poorly filled with ink indicates that the portal trunks were injected in a direction from above downwards.

The nerve supply and reticular fibre framework of the pars distalis of the whale

Careful examination of many sections through representative parts of the pars distalis of the blue whale (*Balaenoptera musculus*) failed to reveal any structures that could be definitely identified as nerve fibres. Particular attention was paid to sections through the upper part of the zona tuberalis, for it might be expected that any innervation the pars distalis received from the hypothalamo-hypophysial tract would be concentrated in this region. Again, no nerve fibres were seen (Pl. 2, fig. 6). The control tissue of rabbit's brain (Pl. 2, fig. 5), stained on the same slides, showed good impregnation of nerve fibres both by Davenport's and Bodian's methods. The best impregnation was obtained with the Bayer brand protargol. (This particular sample of protargol had been used in previous work to stain the hypothalamus and pituitary glands of rabbits. Very good impregnation of the fine fibres forming the hypothalamo-hypophysial tract in the hypothalamus and in the neurohypophysis had been obtained.)

The reticular fibre framework of the pars distalis of the whale impregnates well by Gomori's method. Rich plexuses of reticular fibres envelop the hypophysial portal vessels and the sinusoids of the pars distalis generally. The reticular tissue extends throughout the pars distalis, and is related to the cells of the gland in that it forms basket-like frameworks around clusters of these cells (Pl. 2, fig. 8). The control tissue of rabbit's brain, stained on the same slides, showed an absence of stained fibres except in the walls of the larger vessels penetrating the brain substance and in the pia-arachnoid on the surface of the brain (Pl. 2, fig. 7).

DISCUSSION

The hypothalamus and pituitary gland are present in all living vertebrates. Clark (1938) states: 'That this neuro-glandular mechanism comprised by the hypothalamic nuclei and the hypophysis is of fundamental importance is sufficiently attested by its remarkable uniformity throughout the vertebrate series.' Since neural control of the adenohypophysis is of basic importance, it seems likely that the mechanism by which such control is exerted remains constant throughout the vertebrates. If such an assumption is made then the evidence derived from the examination of cetacean material supports the theory that hypothalamic control over the adenohypophysis is mediated via the hypophysial portal vessels, and not by a direct nerve supply.

Most workers have found, on microscopic examination, that the anterior pituitary gland possesses a scanty nerve supply (Berkley, 1894; Gentes, 1903; Herring, 1908; Tello, 1912; Croll, 1928; Bucy, 1932; Rasmussen, 1938; Brooks & Gersh, 1941, Drager, 1944; Green & Harris, 1947; Green, 1947, 1948). These findings, and the fact that the anterior pituitary gland possesses a rich reticular fibre framework which may stain by silver methods to give the appearance of nerve fibres, puts the onus of proof on those investigators who claim to have found many nerve fibres in the gland. Such claims now need the substantiation of two control procedures: first, to stain alternate sections for nerve fibres and reticular fibres and to compare the fibre patterns disclosed; and secondly, to stain on the same slide sections from a normal gland and a 'denervated' gland (after cervical sympathectomy and pituitary stalk section) in order to demonstrate the presence of nerve fibres in the former, and their absence in the latter organ. Without these control observations, statements that many nerve fibres have been found in the pars distalis are not convincing. The recent finding of Vasquez-Lopez (1949) that many nerve fibres are present in all parts of the adenohypophysis of the rabbit should be mentioned. The pituitary glands of normal rabbits, rabbits with encephalitis and newborn animals were investigated. Some animals (it is not clear which) had been used in other experiments and inoculated with Shope papillomata or injected with carbon tetrachloride, copper salts, thyroxine or oestrogen. This worker mentions the difficulty of distinguishing connective tissue fibres from nerve fibres. It is of interest that the best impregnation of 'nerve fibres' in the deeper parts of the pars distalis occurred in the pituitary glands of encephalitic rabbits. Vasquez-Lopez believes the explanation of this fact to be that nerve fibres take the silver best in abnormal conditions, but an alternative explanation is that the infection had caused an increase in the connective tissue element of the gland.

The literature regarding the innervation of the pituitary gland has recently been summarized (Harris, 1948). In contradiction to the scanty nerve supply, the hypophysial portal vessels or analogous vessels, form a constant and prominent feature of the pituitary glands of all animals (so far examined) from amphibians to man (Popa & Fielding, 1930; Wislocki & King, 1936; Green & Harris, 1947; Green, 1947, 1949, 1950).

The general statements made above hold true also for the pituitary gland of the Cetacea.

SUMMARY

1. The pituitary glands of four adult and four foetal whales have been examined with particular regard to the anatomical structures connecting the tuber cinereum and pars distalis.

2. The pars tuberalis extends upwards anterior to the thick septum which separates the infundibular process and pars distalis and enfolds the tuber cinereum in the way usual in other mammals. The region where the pars tuberalis merges with the pars distalis bears many points of similarity to the zona tuberalis of the rabbit, and the term zona tuberalis is applied to this region of the cetacean gland.

3. Nerve fibres were not seen in the pars distalis, though the presence of a rich reticular fibre framework was verified.

4. A prominent system of hypophysial portal vessels is associated with the pars tuberalis, linking the median eminence of the tuber cinereum and the pars distalis.

5. The functional significance of these findings is briefly discussed.

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EXPLANATION OF PLATES

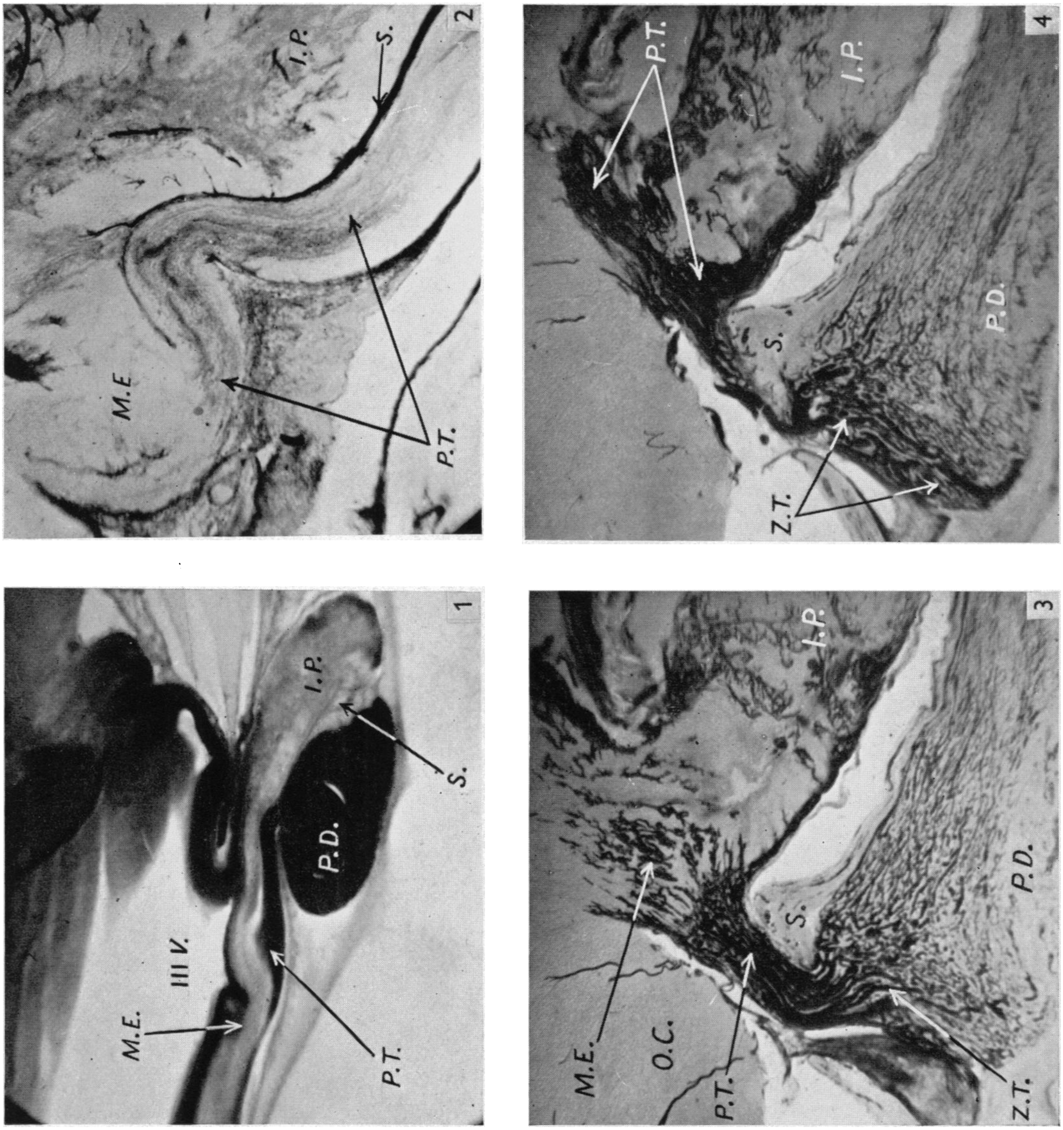
Plate 1

Fig. 1. Microphotograph of a mid-line sagittal section through pituitary gland and hypothalamus of foetal whale (*Balaenoptera ? physalus*). Note the pars tuberalis, *P.T.*, passing upwards anterior to the septum, *S.*, to come into intimate contact with the median eminence, *M.E.*, of the tuber cinereum. *I.P.*, infundibular process; *P.D.*, pars distalis; III *V.*, third ventricle. Section 200 μ. thick. Haematoxylin. $\times 17$.

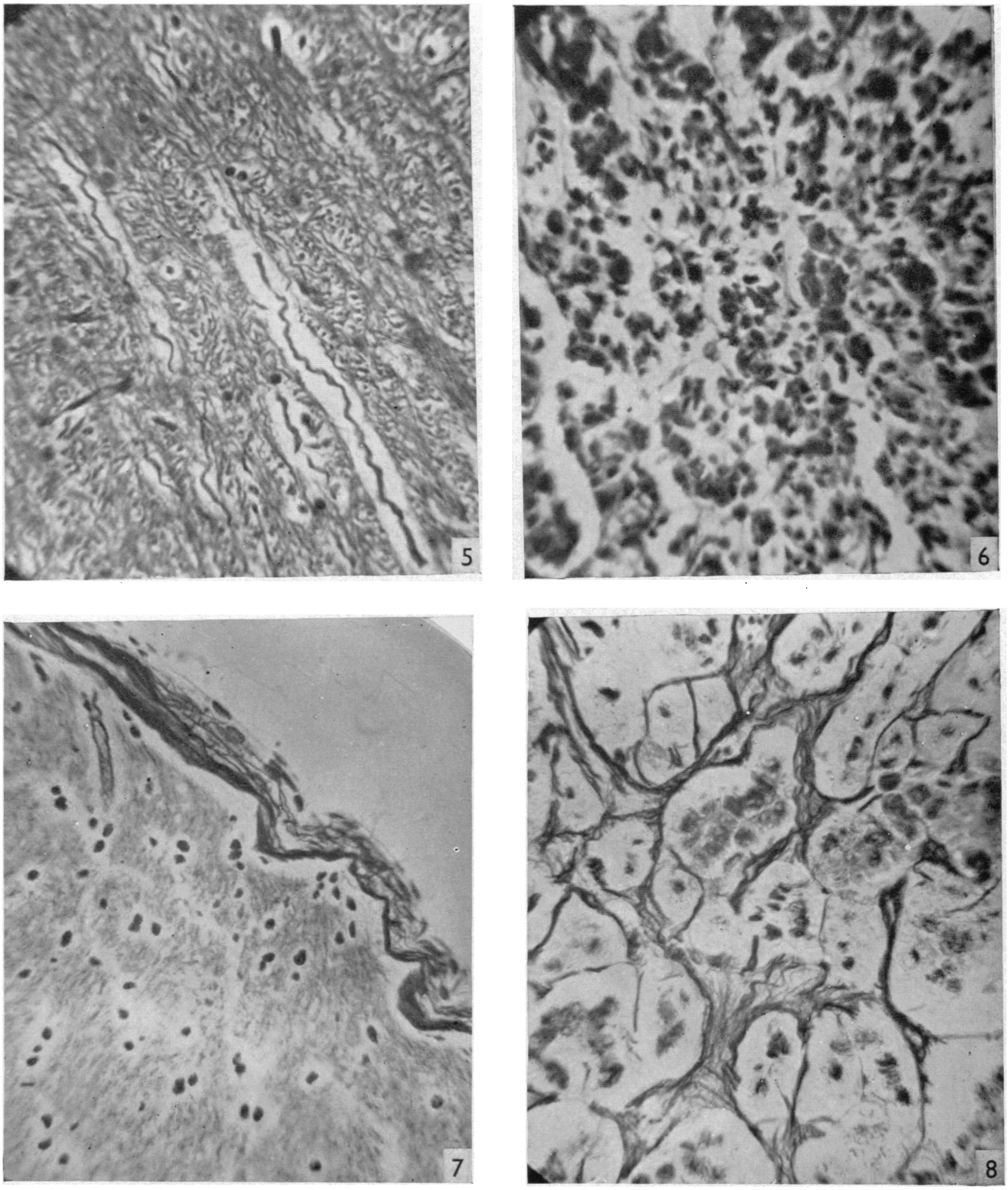
Fig. 2. Microphotograph of a mid-line sagittal section through the pars tuberalis and neurohypophysis of foetal whale (Lesser rorqual). *I.P.*, infundibular process; *M.E.*, median eminence of tuber cinereum; *P.T.*, pars tuberalis containing trunks of portal vessels; *S.*, septum. Section 200 μ. thick. Unstained. Blood vessels partially injected with indian ink. $\times 13$.

Fig. 3. Microphotograph of a mid-line sagittal section through the hypothalamus and pituitary gland of the porpoise (*Phocoena phocoena*). Note the pars tuberalis, *P.T.*, rises anterior to the septum, *S.*, to encircle the tuber cinereum, and that the mass of sinusoidal capillaries in the median eminence, *M.E.*, of the tuber cinereum connect with the trunks of the portal vessels in the pars and zona tuberalis, *Z.T.*, and so with the sinusoids in the pars distalis, *P.D. I.P.*, infundibular process; *O.C.*, optic chiasma. Section 150 μ. thick. Biebrich scarlet. Blood vessels injected with indian ink. $\times 16$.

Fig. 4. Microphotograph of a sagittal section through the lateral margin of the infundibular stem of the porpoise (*P. phocoena*). Legends as for fig. 3. Note the pars tuberalis with its vascular plexus extending around the lateral margin of the infundibular stem. The highly vascular nature of the zona tuberalis is well shown. Section 150 μ. thick. Biebrich scarlet. Blood vessels injected with indian ink. $\times 16$.



Figs. 1–4.



Figs. 5–8.

PLATE 2

Fig. 5. Microphotograph of a section through control tissue, rabbit's brain stained on same slide as pituitary gland shown in fig. 6. Note good impregnation of nerve fibres. Formol fixation. Section 10 μ. thick. Bodian's stain. $\times 300$.

Fig. 6. Microphotograph of a sagittal section through upper part of zona tuberalis of adenohypophysis of adult blue whale (*Balaenoptera sibbaldi*) stained on same slide as control tissue shown in fig. 5. The faint staining of vascular endothelium may be seen, but no structures that can be identified definitely as nerve fibres. Formol fixation. Section 10 μ. thick. Bodian's stain. $\times 300$.

Fig. 7. Microphotograph of a section through control tissue, rabbit's brain stained on same slide as pituitary gland shown in fig. 8. Note lack of impregnation of fibres in brain but well-stained reticular fibres in pia-arachnoid meninx on surface of brain. Formol fixation. Section 10 μ. thick. Gomori's stain. $\times 300$.

Fig. 8. Microphotograph of a sagittal section through upper part of zona tuberalis of adenohypophysis of adult blue whale (*B. sibbaldi*) stained on same slide as control tissue shown in fig. 7. The section shows the reticular fibre framework which exists around the clusters of glandular cells. Formol fixation. Section 10 μ. thick. Gomori's stain. $\times 300$.