SYNOPSIS
This paper reviews some of the significant research done by staff of the Department of Anatomy, University of Singapore, during the past decade. It includes studies on the primate nasopharynx, innervation of the vertebrate gut, cuneate nucleus, intermediolateral nucleus of the spinal cord, mesencephalic nucleus of the trigeminal nerve and neuroglia.

THE PRIMATE NASOPHARYNX
Comparative anatomical, morphological, histological and developmental studies reveal that the anterior portion of the nasopharynx, in front of the tubal orifices, displays features similar to that of the nasal cavities while the posterior portion of the nasopharynx behind these orifices resembles the oropharynx. Thus the primate nasopharynx appears to be composed of an anterior 'nasal' and a posterior true 'pharyngeal' portion, the junction between the two occurring at about the tubal orifices where the first and third pharyngeal arches meet in embryonic life (Kanagasuntheram and Leela, 1974). This view is substantiated by (1) the presence of a transitional zone between the columnar ciliated and stratified squamous epithelia of the nasopharynx at about the level of the tubal orifices (Leela and Kanagasuntheram, 1972, 1973 a, b); (2) the innervation of the anterior and posterior portions of the nasopharynx by the trigeminal and glossopharyngeal nerves respectively (Leela et al., 1971); and (3) the absence of contractility in the bony walled anterior portion of the nasopharynx while the posterior portion, containing muscular elements is contractile as shown by contrast radiography and cine-fluorography (Khoo et al., 1967, 1977; Khoo and Kanagasuntheram, 1979). Consequently, the view that the entire nasopharynx belongs to the pharynx is erroneous.

The transitional zone lying between the two tubal orifices presents interesting features in that, in the embryo, this zone is the first part of the nasopharyngeal epithelium to become ciliated, a process which subsequently spreads into the entire nasopharyngeal region (Kanagasuntheram and Ramsbotham, 1968). In the older embryos, there is a replacement (metaplasia) of the ciliated epithelium into the stratified squamous type along the caudal part of the nasopharynx. Moreover, this zone shows the presence of varying types of epithelium ranging from stratified squamous, transitional to pseudostratified columnar ciliated epithelia. Such variations in the epithelium may...
represent arrested phases of development which give an insight into the process of ciliogenesis. It appears that the stem cells which give rise to ciliated cells pass through three different stages. The first stage is characterized by nonciliated cells containing vesicular inclinations of ‘mucus’ secretory granules. In the second stage, the cells begin to develop centrioles with a concomitant reduction of mucous droplets. The final stage is characterized by the movement of the basal bodies towards the surface of the cell and the formation of cilia. At the same time the mucous granules completely disappear from the ciliated cells. This view is also supported by the stages of wound healing in the anterior part of the nasopharyngeal region (Leela et al., 1975). During the regenerative process, the epithelial cells from the margin of the wound as well as cells lining the crypts of the glands move towards the centre of the wound where they exhibit characteristics of stem cells from which the ciliated epithelial cells are eventually differentiated. The healing process is usually rapid and takes approximately 48-72 hours. Moreover, the changing morphology of cells particularly in the transitional zone may perhaps account for the high incidence of cancer in the nasopharynx.

Contrast radiological and time lapse cinematographic studies reveal a wide ranging variation in the shape and size of the pharyngeal recess. Eustachian fossa and retroconchal space (Khoo et al., 1977; Khoo and Kana- gasuntheram, 1979). The presence of very large pharyngeal recesses and their contractility indicate that these outpouchings are not confined to the space above the superior constrictor but in actual fact are largely enclosed by this constrictor muscle. Moreover, the interior of some of the large recesses may not be revealed by posterior rhinoscopy and consequently radiography offers the best chance of an early diagnosis of tumours in this region.

INNERRATION OF THE VERTEBRATE GUT

The early studies were conducted on the toad (Bufo melanostictus). The submucous plexus in the small intestine was made up of a main plexus, just deep to the inner circular muscle coat (Wong et al., 1971 a). It interconnects with the myenteric plexus on the one hand and extends into the submucosa on the other. Although it has been claimed that ganglion cells are absent in the submucous plexus of lower vertebrates, with intravital methylene blue staining small to medium-sized ganglion cells could be demonstrated. The nerve fibres were thin or medium-sized and some stained positively for acetylcholinesterase (AChE). A subsequent study (Wong et al., 1971 b) using the AChE localization technique showed that the enteric plexuses in the toad from the oesophagus to the rectum could be differentiated into a submucous, a myenteric and a submucosal plexus. From the latter nerve fibres ran freely in the lamina propria to reach the epithelium. The appearance of the enzyme AChE in the enteric plexuses was studied in the developing foregut of the toad using post-hatched tadpoles between 1-57 days and juvenile toads (Wong and Sit, 1972). This study showed, relative to the localization of AChE in the enteric nerve plexuses, that the premetamorphic innervation pattern of the tadpole's foregut appeared well established about one week post-hatching and little change occurred until metamorphosis had advanced considerably. There was also a rostrocaudal developmental sequence in the appearance of AChE-positive neuroblasts, i.e., the latter appeared earlier in the pharynx and oesophagus than in the stomach. The myenteric plexus in the oesophagus of the toad was investigated at the ultrastructural level (Wong, 1973) and this showed that the nerve vancosities contained predominantly either large granular or agranular vesicles. These vesiculated nerve endings formed close neuromuscular contacts especially in the circular muscle coat.

Studies in the rat were focussed particularly upon the sympathetic innervation of the duodenum following acute chemical sympathectomy using 6-hydroxydopamine. Although most noradrenergic axon profiles were separated from the perikarya and dendrites of the submucous ganglion cells by glial processes, on rare occasions obvious membrane specialization consisting of thickened apposed membranes with subjacent dark deposit and interpreted as axosomatic synapses was observed (Wong et al., 1974). This study showed that the submucous ganglia may be directly innervated by sympathetic neurons. Further studies showed that, contrary to current axons, a direct sympathetic innervation to the muscularis externa could also be demonstrated in the duodenum of the rat (Wong, 1975, 1977).

The fine structure of the teleostean enteric plexuses was studied in the stomach of the coral fish, Chermon rostratus Cuvier (Wong and Tan, 1978). This study showed that the myenteric plexus was a prominent loose mesh containing nerve cells, myelinated and unmyelinated axon profiles, vesiculated axon profiles, Schwann cells, collagen and capillaries. Synapses were rarely observed. The fish myenteric plexus thus differed in a number of ways from the myenteric plexus in mammals which is usually taken as the standard description for all vertebrates. Thus the division of the myenteric plexus of the coral fish incorporated into compartments was reminiscent of other autonomic ganglia and suggest that the neurons were virtually isolated from each other, and so lack the possibility of interaction. Further, the rarity of synapses on the nerve cells in the coral fish myenteric plexus was in marked contrast to the light microscopic findings of previous workers. Until more evidence is available from more species at the ultrastructural level the schema of the innervation of the stomach in fish in which many excitatory preganglionic fibres are supposed to terminate on nerve cells must remain sub judice. Electron microscopy of the muscularis externa of the stomach in the coral fish showed that it consisted of four discrete layers — an outermost longitudinal layer followed by a circular, an oblique and an innermost layer (Tan and Wong, 1980). The number of nerve bundles per 1000 muscle fibres was 62 in the oblique layer, 30 in the circular layer and 28 in the longitudinal layer. Vesiculated axon profiles made up 55% in the oblique layer, 41% in the circular layer and 28% in the longitudinal layer. Axon profiles contained predominantly either round agranular, large granulated or clear flattened vesicles. The neuromuscular junctional gaps between vesiculated axon profiles and the surfaces of muscle cells showed great variability in width. Gaps of 11-30 mm, i.e., close gaps, formed 48% and 22% in the oblique and circular layers, respectively. Most commonly a vesiculated axon profile contacted a single muscle cell but vesiculated axon profiles contacting two, three and four muscle cells have been observed. Conversely, an innervated muscle cell was usually contacted by a single
axon profile but there were cases where two or even three axon profiles made contact with a single muscle cell. In sum, the study showed that the oblique layer was the most densely innervated and that the circular and longitudinal layers were about half as densely innervated.

THE CUNEATE NUCLEUS

The study has been devoted mainly to the neuronal and synaptic organization within this nucleus of the rat and monkey. In the rat, there are at least five morphologically different classes of neurons which can be identified under the electron microscope. In the monkey, however, three classes of neurons have so far been observed (Wen et al., 1978, 1980). In the rat at least two of the cell types have been shown to be cuneothalamic projection cells (Tan and Lieberman, 1978) and in the monkey, although no experiments have yet been carried out it is suggested that the major cell type may be thalamic projection cells.

In both the rat and monkey (Wen, 1979) the neurons have been found to be arranged in clusters. Most of the neurons are concentrated in the middle third of the nucleus along its rostrocaudal axis. This corresponds to the region of the obex. It is interesting that degeneration experiments have shown that primary afferent fibres terminate predominantly in this region of the nucleus (Wen, 1979). Hence it may be concluded that the majority of the neuronal types receive their afferents predominantly from dorsal root fibres.

The primary afferent terminals are large and of irregular shape; they contain round synaptic vesicles and establish Gray’s Type I synapses with dendrites of all diameters and rarely with neuronal perikarya (Wen et al., 1979). The primary afferent terminals are frequently presynaptically contacted by smaller boutons containing flattened vesicles through Gray’s Type II contacts. Such a configuration may well be the anatomical substrate for presynaptic inhibition in the cuneate nucleus. However, some of these presynaptic boutons are dendritic in origin (Wen et al., 1977). Many of the primary afferent terminals, the presynaptic boutons containing flattened vesicles and the postsynaptic dendrites are enclosed in astrocytic capsules to form synaptic glomeruli; in some, however, an astrocytic capsule appears to be lacking and these are called synaptic complexes or clusters (Tan and Lieberman, 1974; Wen et al., 1978).

The cortical terminals are all of small diameter (Wen et al., 1980). Following separate lesions of the motor and sensory cortices, most of the degenerating terminals of fibres arising from the motor cortex contained round vesicles and only a few contained flattened vesicles while most of the degenerating terminals arising from the sensory cortex contained flattened vesicles. All the cortical terminals did not enter into the formation of synaptic glomeruli nor were they ever presynaptic to primary afferent terminals.

THE INTERMEDIOLATERAL NUCLEUS OF THE SPINAL CORD

In the monkey at least three morphologically different types of neurons have been identified under the electron microscope (Wong and Tan, 1980). These neurons differ from each other in the morphology of their nucleus, arrangements of the rough endoplasmic reticulum and Golgi complexes, synaptic contacts and glia-neuronal relationships.

The axon terminals in the nucleus can be classified into those containing flattened vesicles and those containing dense-cored vesicles (Wong and Tan, 1974; Tan and Wong, 1975; Wong and Tan, 1980). Using the conventional method of fixation of tissues with paraformaldehyde/glutaraldehyde mixture only large dense-cored vesicles could be observed (Tan and Wong, 1975; Wong and Tan, 1980).

Many of the neural elements which establish synaptic relationships with each other are enclosed in astrocytic capsules to form synaptic glomeruli (Wong and Tan, 1974; Tan and Wong, 1975; Wong and Tan, 1980). Although axon terminals containing flattened vesicles are present in the nucleus of the rat (Tan and Wong, 1975) and monkey (Wong and Tan, 1980), it would appear that only in the rat that they formed axo-axonal synapses with axon terminals containing round vesicles.

Following chemical sympathectomy by administration of 6-hydroxydopamine there was degeneration of the noradrenergic terminals in the nucleus. These terminals contained large dense-cored vesicles. In the rat the drug was given systemically and degeneration was confirmed at the light (Wong, 1976) and electron microscopical (Wong and Tan, 1974) levels, thus showing that it crossed the blood-brain barrier. In the monkey both systemic and cisternal routes were employed. In each case there was degeneration of axon terminals containing large dense-cored vesicles.

THE MESENCEPHALIC NUCLEUS OF THE TRIGEMINAL NERVE

The mesencephalic nucleus of the trigeminal nerve is one of the longest nuclei in the brain stem and is an obvious feature in sections of the brain stem extending all the way from the lower pons to the level of the superior colliculus in the midbrain. Except for its function as the nucleus of termination for proprioceptors from the muscles of mastication no other definite function has been ascribed to it. As a first step in research into the possible functions of this nucleus, an exhaustive statistical study of the number of neurons in the nucleus was first undertaken, after defining its parameters (Sivanandasingham and Warwick, 1976) in over 45 animals covering four animal species. Following this the somata of proprioceptive fibres in extra-ocular muscles were localized to the mesencephalic nucleus in 12 cats by selective orbital resection of extra-ocular muscles and mapping of the areas showing unequivocal changes of retrograde degeneration (Sivanandasingham, 1977). These findings were subsequently confirmed in 20 more cats following intracranial surgical operations in which the oculomotor and ophthalmic nerves were divided both alone and in combination in several parts of their course. By using available histological and histochemical techniques, the peripheral pathway of proprioceptive fibres have been shown to be exclusively along the oculomotor, trochlear, and abducent nerves which are thus sensori-motor (Sivanandasingham, 1978).

The physiological role of the proprioceptive fibres from extraocular muscles which have been shown to terminate bilaterally in the mesencephalic nuclei have been discussed (Sivanandasingham, 1973).
NEUROGLIA

The initial studies were on the morphology and classification of the various glial cell types, i.e. astrocytes, oligodendrocytes and microglia in the corpus callosum of rat brain (Ling et al., 1973 a). The development of these cells was later studied in different age groups of animals by systematic cell enumeration and radioautography (Ling and Leblond, 1973; Ling et al., 1973 b). It was thus concluded that both astrocytes and oligodendrocytes were derived from common stem cells, i.e. the subependymal cells adjacent to the lateral ventricles of brain. However, the formation of microglia remained debatable. Subsequent studies were therefore performed in the hope to clarify the problem on the origin of microglia. For this purpose, the corpus callosum of early postnatal rats was examined and this resulted in the description of an additional glial type referred to as amoeboid microglial cells which were shown to be the precursors of microglia (Ling and Tan, 1974; Ling, 1976 a). It was found that amoeboid microglial cells were also distributed in other parts of the central nervous system (Ling, 1976 b). The nature of these cells were later ascertained and was shown to be active macrophages by using cytochemical methods (Ling, 1977). The next question then arose was the source of amoeboid microglial cells.

Since it is known that the majority of tissue macrophages are derived from circulating blood monocytes, it was therefore decided to investigate whether they too give rise to amoeboid microglia. The first approach was to label circulating monocytes by intravenous injection of carbon particles and to trace the fate of the carbon-labelled monocytes. Since it is unlikely that carbon particles introduced into the blood circulation can cross the blood-brain barrier, any carbon-labelled cells which appear in brain tissue must be derived from monocytes which have phagocytosed carbon particles in circulation. Indeed, by this method, the presence of carbon-labelled amoeboid microglial cells was demonstrated in the brain of postnatal rat (Ling, 1979 a). It was therefore deduced that amoeboid microglia arose from blood monocytes. Using the same method, a similar conclusion was reached for the epiplexus cells in the lateral ventricle of brain and subarachnoid macrophages (Ling, 1979 b).

Recently, a more direct method was designed in order to prove in a decisive manner the monocytic origin of amoeboid microglia. Briefly, in this study, carbon- or thymide-labelled monocytes were separated from donor rats and then injected into the blood circulation of syngeneic recipient postnatal rats. Again, the results showed the presence of labelled amoeboid cells in the brain tissues (Ling et al., 1980). We therefore concluded that amoeboid microglia are derived from blood monocytes which have infiltrated the brain tissue. A further evidence supporting this is the presence of peroxidase positive granules in some of the amoeboid microglial cells (Ling, 1980).

The origin of neural macrophages were also examined in rats under different experimental conditions using optic nerve and spinal cord as model for study (Ling, 1978, 1979 c). The results were in agreement with those of normal animals.

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REFERENCES

The primate nasopharynx


Innervation of the vertebrate gut


The cuneate nucleus


The intermediolateral nucleus of the spinal cord


The mesencephalic nucleus of the trigeminal nerve


Neurogia


