Epiplexus Cells in the Embryos of the Turtle, 
*Trionyx sinensis*

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Received November 21, 1984

Summary. The epiplexus cells in the embryos of the turtle, *Trionyx sinensis* were examined in scanning and transmission electron microscopy. Although the cells showed diverse morphological forms, the majority of the cells however had a stellate cell body each with 3-5 radiating long processes with secondary processes terminating on the ventricular surface of the choroid epithelial cells. The cell body was relatively smooth. Occasional epiplexus cells displayed blebs on their cell bodies and processes. In the transmission electron microscope, the nucleus contained coarse chromatin clumps. The cytoplasm was endowed with large lysosomal bodies. Based on these observations, it is suggested the epiplexus cells in the turtle embryos are phagocytic even though most of them probably are at the resting stage.

Much of the information on epiplexus cells in the cerebral ventricles has been derived from the observations made on the materials of mammalian species using transmission and scanning electron microscopy (Carpenter, McCarthy and Borison, 1970; Hosoya and Fujita, 1973; Chamberlain, 1974; Peters, 1974; Allen, 1975; Sturrock, 1978, 1979; Ling, 1979, 1981, 1983), yet the first description of these cells was by Kolmer (1921) and subsequently by Ariens-Kappers (1953) in lower vertebrates mainly in amphibians especially in salamanders. From the literature surveyed, there is little, if any, description about the ultrastructure of epiplexus cells and in particular, their surface morphology in turtles and other reptiles. The present study was undertaken to find out whether the epiplexus cells in the embryonic turtles, *Trionyx sinensis* displayed similar morphology to those found in the higher vertebrates.

MATERIALS AND METHODS

Embryonic turtles, *Trionyx sinensis* of 27, 34, 41, 45 and 52 days of age were used in this study. Between 3-5 animals from each of the age groups were perfused intracardially under hypothermic anaesthesia with a mixed aldehyde fixative consisting of 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer. After perfusion which lasted for about 20 min, the brain was removed. The lateral ventricles and their containing choroid plexuses were exposed by making a coronal section at a midpoint

*This study was supported by a donation from the Singapore Turf Club to Drs. E. A. LING, P. GOPALAKRISHNAKONE and F. C. T. VOON.*
Fig. 1. A tuft of choroid plexus extending into the lumen of the lateral ventricle. Arrows indicate a few epiplexus cells resting on the furry surface of the plexus. 27 days embryo. ×210

Fig. 2. Scores of epiplexus cells amongst the cilia and microvilli from the choroid epithelial cells. Most of the cells are spherical. 34 days embryo. ×780
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between the rostral and caudal ends of the brain. The tissue blocks were further fixed in the similar fixative overnight at 4°C. Following the buffer wash, the tissue blocks were post-fixed in 2% osmium tetroxide in 0.1 M cacodylate buffer pH 7.4. They were dehydrated in a graded series of ethanol and critical point dried in liquid carbon dioxide. They were then mounted on a specimen stub, sputter coated with gold for 3 min and viewed in a Philips 505 scanning electron microscope operated at 20 kV.

For transmission electron microscopy, tissue blocks containing the choroid plexuses were post-fixed in ice-cold 1% osmium tetroxide containing 1.5% potassium ferrocyanide. Dehydration was in an ascending series of ethanol. The blocks were embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400T electron microscope.

OBSERVATIONS

The choroid plexus of the embryonic turtles consisted of a tuft of finger-like projections which extended into the cerebral ventricle. In the scanning electron microscope, the plexus almost filled up the ventricular lumen (Fig. 1). It has a furry covering with widely scattered epiplexus cells (Fig. 1). In some areas, the epiplexus cells were heavily populated amongst the palisade of microvilli and cilia from the epithelial cells (Fig. 2).

The epiplexus cells on the luminal surface of the epithelium were pleomorphic. There were no structural differences of the cells in the various age groups examined. In the scanning electron microscope, the commonest form was the cell with a stellate body with 3 to 5 radiating long cytoplasmic processes which often terminated at expansions over the epithelial cells (Fig. 3). The surface of the cell body was smooth (Fig. 3, 4). Arising from the main process were secondary fine processes implanted into the microvilli palisade of the epithelial cells (Fig. 4). Sometimes, the main processes displayed numerous blebs (Fig. 5). Rarely, the cell body displayed a verrucous appearance (Fig. 6).

In the transmission electron microscope, the epiplexus cells showed an irregular nucleus with prominent margination of chromatin masses (Fig. 8). The cytoplasm contained profiles of rough endoplasmic reticulum and a variable number of large dense bodies (Fig. 8). At the periphery in the subplasmalemmal region were present some microvesicles (Fig. 8). Cytoplasmic vacuoles were rarely seen.

So far, supraependymal neurons as described by Mitchell and Card (1978) in the guinea pig have not been identified in the present materials using the combined scanning and transmission electron microscopy.

The choroid epithelial cells showed well-developed microvilli and tufts of cilia (Fig. 7, 8). Sometimes, the cilia terminated as a swelling (Fig. 5). One salient feature of the epithelium was the presence of numerous spherical blebs on its luminal surface on some of the cells (Fig. 7).

DISCUSSION

The present study on the choroid plexus of the embryos of the turtle Trionyx sinensis showed the presence of epiplexus cells as early as 27 embryonic days. The distribution of the cells on the choroid plexus was extremely variable. The heavy concentration
Fig. 3. A star-shaped epiplexus cell resting on the microvilli palisade of choroid epithelial cells. The cell shows four long cytoplasmic processes, one of which terminates as an expansion (E). The cell body is smooth. A variable number of long cilia project from the epithelial cells and some of them are entangled over the epiplexus cell. Cytoplasmic protrusions (P) of different sizes are seen on several of the epithelial cells. 52 days embryo. ×3,400

Fig. 4. A portion of an epiplexus cell showing slender cytoplasmic processes extending from a main process (P) and are deeply implanted into the microvilli palisade from the epithelium. 52 days embryo. ×6,800

Fig. 5. An epiplexus cell displaying an extremely blebbed cytoplasmic process. Note that some of the cilia terminate at swellings (arrows). 52 days embryo. ×4,000
of the cells at certain sites is difficult to explain since there was no sign of activation of the cells, e.g. phagocytosis, which is known to be the main function of the cell type (Carpenter, McCarthy and Borison, 1970). The majority of cells observed exhibited long filopodial processes with a terminal expansion resting on the ventricular surface of the epithelium. Our earlier study (Ling, Tseng and Wong, 1984) on the epiplexus

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**Fig. 6.** A blebbed epiplexus cell. 52 days embryo. ×6,800

**Fig. 7.** Cytoplasmic protrusions from choroid epithelial cells. 52 days embryo. ×6,800

**Fig. 8.** An epiplexus cell resting on the microvilli (Mv) of the choroid epithelium (E). The cell contains a few dense bodies (d) and a few subsurface microvesicles (arrows). 52 days embryo. ×9,750
cells in the prenatal rats had shown that long filopodial processes are mainly employed by the cells for anchoring or structural support. On the other hand, these processes may be utilized as probes for detection of foreign bodies which may be ingested (Hosoya and Fujita, 1973; Tseng, Ling and Wong, 1984).

Some occasional epiplexus cells observed in the present study however displayed 'blebs' both on the soma as well as on the cytoplasmic processes. It has been suggested that the pleomorphic microappendages on the intraventricular macrophages including epiplexus—and supraependymal cells probably reflect different degrees of activation or stages of development (Sturrock, 1979; Bleier and Albrecht, 1980). In our study of the epiplexus cells in prenatal rats treated with 6-amino-nicotinamide, it has been demonstrated that blebbing is an immediate expression of these cells in the initial stage of phagocytosis (Ling, Tseng and Wong, 1985). In the transmission electron microscope, the epiplexus cells in turtle embryos showed structural resemblances to cells in higher vertebrates (Carpenter, McCarthy and Borison, 1970; Ling, 1979, 1981; Sturrock, 1979) in that they contained dense bodies which were probably lysosomes. Furthermore, just as in the rat epiplexus cells, small pit-like coated vesicles were present in the subplasmalemmal region (Ling, 1979). From their ultrastructural features it is postulated the epiplexus cells in the turtle embryos are phagocytic although the majority of the cells are probably at their resting stage. The small coated vesicles as displayed by these cells near to the cell surface probably represent pinocytotic vesicles from the cerebrospinal fluid.

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