Electron microscopic studies of the blood-pituitary barrier in the newt

By

Masao Sano

Department of Anatomy, Aichi-Gakuin University
School of Dentistry, Nagoya, Japan

Introduction

Fine structure of capillaries in the anterior pituitary gland has been repeatedly reported by Rinehart and Farquhar (1955), Ichikawa (1959) and Farquhar (1961) in the rat, Yamada and Sano (1960) in the mouse and Cardell (1963) in the human. According to these reports, capillary lumen is separated from the parenchyma by the fenestrated endothelium, endothelial basement membrane, pericapillary space and parenchymal basement membrane from luminal to parenchymal sides. Such an architectural organization has been observed not only in the anterior pituitary but also in the other endocrine organs (Monroe 1953, Ekholm 1957, Wissig 1960 in the thyroid, Bencosme and Pease 1958 in the pancreatic islets, Trier 1958 in the parathyroid, Zelander 1957 and 1959 in the adrenal cortex, Palay 1957, Hartmann 1958 in the posterior pituitary). At present, it is generally accepted in a variety of endocrine glands that these structural elements function as barriers to material transport between the blood and the parenchyma.

These reports are concerned with mammals and the present author has not yet encountered with those of lower vertebrates. The present author preliminarily reported that the capillary endothelium has no fenestration in the newt anterior pituitary (Sano 1963). At the present report, some unique observations about pericapillary structures will be added.

Material and Methods

Material used is newts (*Diemyctylus pyrrhogaster* Boie) which were captured in summer (July), fall (October) and winter (February).
Pituitaries were obtained after decapitation and fixed with 2% osmium tetroxide in veronal-acetate buffer pH 7.4 (Pala de 1952) with sucrose (Caulfield 1957) at about 0°C for 2 to 3 hours. Then they were washed and rapidly dehydrated with graded acetone solutions. For embedding Epon 812 was used. Sectioning was done with JEOL JUM-5 ultramicrotome on glass knives. Thin sections were mounted on collodion coated copper grids and stained with uranyl acetate (Wat son 1958) or lead acetate (Millonig 1961). JEOL JEM-5 and JEM-7 electron microscopes were used.

Observations

Endothelium

Capillary lumen which varies in width and contains here and there solitary or grouped red blood corpuscles as in higher vertebrates is completely lined by the continuous endothelial cytoplasm (Fig. 1). The endothelium contains a moderate amount of cytoplasm in perikaryal region which appears to protrude into the capillary lumen (Figs. 5, 6). Away from the nucleus the cytoplasm becomes thinner and attenuated cytoplasmic processes extend for long distance. In the thinnest portions they measure about 40 to 50 m. Even in these thinnest areas they have not such diaphragmed pores as reported in higher vertebrates (Figs. 1, 5). Occasionally one can encounter figures which suggest fenestration of the endothelium, but they represent undoubtedly vesicle-containing parts of the cytoplasm cut obliquely. In the present data, after all, the capillary endothelium has no fenestrations throughout the year. Contiguous cells are complicatedly interdigitating like the suture of skull bones and are sometimes connected each other by desmosomes (Fig. 6).

The endothelial sheath of capillaries consists of a single layer of the endothelium, but the endothelial cytoplasm is arranged frequently in two or three layers in some places. In areas showing stratified arrangement of the endothelial cytoplasm, the innermost attenuated one is backed up outside by other cytoplasmic process(es) of the endothelium (Fig. 5). Although between these layers there is a space containing amorphous substance similar to that in the capillary lumen, the space is completely surrounded by the cytoplasmic processes and there is no area where the space freely opens into the pericapillary space.

In the vicinity of the nucleus the endothelial cytoplasm contains mitochondria, Golgi-substance, rough-surfaced endoplasmic reticulum
and free ribosomes, while in the distal portions it scarsely contains these cell organells (Figs. 5, 6). On the other hand, small vesicles are rich throughout the cytoplasm, which are considered of pinocytotic (Figs. 2, 3, 5, 6). Furthermore, rounded or elongated vacuoles various in size are numerous observed in the cytoplasm (Figs. 2, 3). Many of them contain material similar to that in the capillary lumen and some contain rather dense homogeneous substance. These vacuoles occur numerous in summer, few in winter and moderately in fall.

**Basement membrane and pericapillary space**

The pericapillary space varies widely in width and is filled with relatively electron dense amorphous material showing a finely mottled appearance (Figs. 2, 3, 5, 6). Thus, in newt pituitaries one cannot find such a clear pericapillary space as seen in mammals. Apposing to the parenchyma, the pericapillary space is delineated by an electron dense line which corresponds to the parenchymal basement membrane in mammals. The line measures about 30 mμ in thickness and courses in parallel to outer limit of the parenchyma. A clear zone about 70 to 100 mμ in width can be traced separating the parenchymal basement membrane from the parenchyma. On the endothelial side of the pericapillary space, there is usually no similar dense line to the parenchymal basement membrane. Thus, dense substance filling the pericapillary space is directly adjoining to the endothelial plasma membrane.

In the pericapillary space pericytes are frequently observed. There are, however, no secretory granules and no cytoplasmic processes or fragments of parenchymal cells containing secretory granules.

**Intercalated cell**

Although in some areas of the parenchyma facing to the pericapillary space secretory cells are directly apposed to the parenchymal basement membrane, in greater parts slender cytoplasmic processes of cells different from usual secretory cells are interposed between secretory cells and the parenchymal basement membrane (Figs. 1, 2, 3, 5). For the sake of description these cells are named "(pituitary) intercalated cells", because such cells have not yet been reported. Nuclei of these cells are located in the vicinity of the pericapillary space and their cytoplasm is not so rich as usual secretory cells (Figs. 1, 3, 5). From the perikaryon they directly extend long thin cytoplasmic processes under the parenchymal base-
ment membrane (Figs. 3, 5). Of course, these processes are separated about 70 to 100 \( \mu \) from the basement membrane by a clear zone mentioned above. Since intercalated cell processes can be traced for long distance in many sections, it can be thought that they are not tubular or band-like but sheet-like. These processes do not entirely invest the capillary bed and areas devoid of the cover of intercalated cells are observed here and there (Fig. 1). After all, several intercalated cells constitute a cytoplasmic sheet which surrounds, if not completely, the capillary bed. The capillary investment develops in high extent in summer, low in winter and moderately in fall. In other words, cytoplasmic processes of intercalated cells are longer and thinner in summer and shorter and thicker in winter.

Interleaved cells abundantly have small vesicles similar to pinocytotic ones in the endothelial cytoplasm. They contain relatively rich free ribosomes although the rough-surfaced endoplasmic reticulum is not well developed. Frequently there can be encountered lipid inclusions in the cytoplasm (Figs. 3, 4). Creneated contured electron dense lipid bodies are usually observed in the vicinity of the nucleus. They appear rich in fall.

Besides, intercalated cells put cytoplasmic processes into narrow intercellular spaces among secretory cells and fill them. Infrequently intercalated cells are connected one another and with secretory cells by desmosomes.

**Discussion**

It is generally believed in the anterior pituitary and other endocrine organs of mammalian species that there are interposed fenestrated endothelium, endothelial basement membrane, pericapillary space and parenchymal basement membrane between the capillary lumen and the parenchyma and that these structures making up the capillary bed function as barriers for material transportation between the blood and the parenchyma. In the newt anterior pituitary, except for perikaryal region the endothelial cytoplasm attenuates and measures about 40 to 50 \( \mu \) in thickness in the thinnest portions. Even in these thin areas the endothelial cytoplasm is continuous and shows no fenestrations. In cold-blooded vertebrates like newts, it has to be noted that capillaries may show seasonal changes according to pituitary function. However, the present data showed no fenestration of the cytoplasm throughout the year.

On the other hand, the endothelial cytoplasm contains numbers
of pinocytotic vesicles. This suggests active transport of substance from capillary lumen to the parenchyma or vice versa.

The endothelium has a number of vacuoles or large vesicles which contain similar material to that in the capillary lumen. Further, cytoplasmic flaps of the endothelium which are protruding into capillary lumen occur frequently. These data suggest active intake of material by the endothelium as being considered by Uchino and Hosokawa (1963) and Donahue (1964). The present data clearly show the direction of material transport, namely, from the blood to the parenchyma.

The pericapillary space appears as a clear space in mammalian pituitaries, while in newts it is filled with relatively dense amorphous material and shows mottled appearance. Such appearance does not change through the year. From this, it is reasonable to think that the pericapillary space in newts may function as a denser filter than in mammals against material transport between the blood and the parenchyma. Although pericytes are frequently observed in the pericapillary space, intact secretory granules and secretory cell fragments or extensions (Rinehart and Farquhar 1955 in rats and Salazar and Peterson 1964 in rabbits) were not detected in newts as in mice (Yamada and Sano 1960) and rats (Ichikawa 1959). The present author thinks that such phenomena as observed by Salazar and Peterson (1964) and Rinehart and Farquhar (1955) may be manifestations of a transient reaction under some specific conditions.

In the parenchyma of the newt anterior pituitary, although in some areas secretory cells are directly apposed to the parenchymal basement membrane, in many places slender long cytoplasmic processes of cells differing from usual secretory cells are interposed between the parenchymal basement membrane and secretory cells. Of course, these cytoplasmic processes are separated by a clear zone 70 to 100 mμ in thickness from the basement membrane. These cells are named "(pituitary) intercalated cells" for the descriptive purpose. Several processes of intercalated cells constitute a sheath which surrounds, if not completely, the capillary bed. Similar investment by epithelial reticular cells was reported in capillaries of the thymus (Weiss 1963, Hoshino 1965), but there is no report in the anterior pituitary. It requires further studies to clarify whether the investment in the present data is homologous with that in the thymus or not.

Intercalated cells contain abundant pinocytotic vesicles through-
out the cytoplasm. It is presumed that these cells engaged in active transport of material from the capillary lumen to the parenchyma or vice versa. Since cytoplasmic processes of these cells show seasonal change in shape, it can be thought that these cells react on environmental conditions. Intercalated cells have frequently lipid inclusions in the vicinity of the nucleus. These inclusion bodies were most abundant in fall. Thus, it is suggested that intercalated cells may have lipid storing function as a source of nutrient. Since intercalated cells are infrequently connected one another and with secretory cells by desmosomes, these cells may function as a supporting element for secretory cells. However, connections by desmosomes were not encountered so frequently as suggesting a firm network throughout the parenchyma. Similar cells in shape to intercalated cells have been reported by some investigators, that is, “stellate cell” in the rat (Rinehart and Farquhar 1955), “no. 5 type cell” in the salamander (Cardelli 1964) and cells suggesting supporting function in the monkey (Yamashita 1965). These reports unfortunately do not refer to precise interrelationship between these cells and the capillary bed.

**Summary**

Fine structure of capillaries of the newt anterior pituitary throughout the year was investigated by electron microscopy. The data are represented with a semischematic micrograph (Fig. 1) and summarized as follows:

1. The capillary lumen is continuously lined by the endothelium. The endothelial cytoplasm attenuates about 40 to 50 μm in thickness in the thinnest portions and shows no fenestrations anywhere.

2. The pericapillary space is filled with amorphous dense substance and shows mottled appearance. Although the endothelial basement membrane is not clear in general, outer limit of the pericapillary space is delineated by the parenchymal basement membrane about 30 μm in thickness which is separated from the parenchyma by a clear zone 70 to 100 μm in width.

3. In the parenchyma there are “(pituitary) intercalated cells” around the pericapillary space. They have long slender sheet-like cytoplasmic processes and several of them constitute a sheeth by which the capillary bed is, if not completely, invested.

4. It is suggested that intercalated cells function as a barrier together with the endothelium, the basement membrane and the
Blood-pituitary barrier in newt pericapillary space. Furthermore, intercalated cells may also serve to store lipid and to support secretory cells.

**Literatures**


Ekholm, R.: The ultrastructure of the blood capillaries in the mouse thyroid gland. Z. Zellforsch., 46, 139-146 (1957).


**Zelander, T.:** The ultrastructure of the adrenal cortex of the mouse. *Z. Zellforsch.,* 48, 710-716 (1957).

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**Interpretation of figures**

**Fig. 1.** Semischematic electron micrograph showing barriers between blood (CL) and parenchyma. Outline of the endothelium (END) and intercalated cells (IC) is sharply drawn. Parenchymal basement membrane (PBM) and pericapillary space (PS) are represented by dotted line and stippled area respectively. For details see the text. From a pituitary obtained in July. x10000.

**Fig. 2.** Endothelium (END) has many vacuoles or large vesicles containing material similar to that in capillary lumen (CL). Small vesicles are also abundant. Pericapillary space (PS) show finely mottled appearance and parenchymal basement membrane (PBM) is clear. Intercalated cell processes (arrows) are very slender and show wavy course. From a pituitary obtained in July. x20000.

**Fig. 3.** An intercalated cell (IC) is shown. A nucleus is located apposing to pericapillary space (PS). Cytoplasmic processes (arrows) are extending towards upper right and lower left. In the cytoplasm small vesicles are abundant and two bizarre-shaped lipid bodies (LB) are seen. In the lower middle a pericyte (PC) is recognized. From a pituitary obtained in July. x20000.

**Fig. 4.** Two lipid inclusions (LB) are shown. They are electron dense and crenated in profile. At the left a lysosome-like body is seen. In the cytoplasm small vesicles are abundant. From a pituitary obtained in February. x15000.

**Fig. 5.** A slender cytoplasmic process of an endothelium (END) is backed up outside by the other cytoplasmic processes (arrows) and it shows no fenestrations. An intercalated cell (IC) extends a cytoplasmic process to the right (double arrow). A desmosome (D) is seen. From a pituitary obtained in October. x15000.

**Fig. 6.** Perikaryal region of an endothelium (END) is shown. Small vesicles are abundant in the cytoplasm. Cytoplasmic flaps of the endothelium are bridged (arrow). Interdigitation of adjoining endothelial cells is recognized. A part of a red blood corpuscle (RBC) is seen. From a pituitary obtained in February. x15000.
Fig. 2.

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Fig. 3.

Fig. 4.

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Plate IV

Fig. 5.

Fig. 6.

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