

Myelinated and non-myelinated nerve fibres of the posterior commissure are in close contact with the base of the SCO cells.

The distal region of the SCO cells extends from the supranuclear zone to the cell surface which protudes into the third ventricle (Figure 2). In the area immediately above the nucleus, there are cisternae of rough reticulum arranged in rows which are parallel to the long axis of the cells, and ribosomes both free and organized into polyosomes. There are also granules of medium electron density with a central electron dense core surrounded by a clear halo (type B, Figure 3c), and a small Golgi apparatus made up of vacuoles, cisternae and vesicles; coated vesicles are present near the Golgi apparatus (Figure 3). In the apical zone, there are vacuolated and non-vacuolated electron dense granules (type A, Figure 3a and b), and granules of medium electron density with a central electron dense core surrounded by a clear halo (type B). Also present in this zone are numerous mitochondria with a dense matrix. At the free surface there are numerous microvilli containing 2-3 filaments and cilia of the 9+2 type (Figure 2). In the zone of contact between ependymal cells, typical terminal bars are observed and at the cellular apex there are few cytoplasmic protrusions filled with small granules.

These observations, when compared with those made on the summer SCO cells, demonstrate that in the winter there is a reduction in the development of the Golgi apparatus and the number of the type A and B granules. Moreover, also the type C secretion is strongly reduced and confined in the basal region, while in the summer this type of secretion is well represented also in the RER cisternae parallel to the long axis of the cells that are found in the supranuclear zone. The reduction of the Golgi apparatus is probably related to the lower secretory activity, and so is the disappearance of the ample sacks of secretory material from the SCO basal region, and the appearance in their place of large vacuoles. The secretory material (type C) of low electron density present in the basal region of the SCO, very abundant in the summer and scarce in the winter, is probably to be identified with the material Gomori and PAS positive described with the light microscope, which during the winter decreases and disappears. At the cellular apex, during strong apocrine-like secretory activity in the summer, several cytoplasmic protrusions are present. In the winter, the reduced apocrine-like secretory activity is also shown by the decrease of cytoplasmic protrusions; the increase of microvilli in this period would be related to this. No sexual difference has been found.

## The Supraependymal Cells of the Rat Hypothalamus: Changes in their Morphology and Cell Number During the Ovarian Cycle

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**Summary.** The number of SEC in the hypothalamus of the rat change during the ovarian cycle (5-8 cells in oestrus, 100 cells in dioestrus per ventricular surface). The changes in the number as well the morphology of the SEC support the hypothesis that they are of mesenchymal nature.

The existence of free cells on the surface of the ependyma (supraependymal cells) and the choroid plexus (epiplexus cells) has been known for some time<sup>2</sup>. Recently with the application of SEM (scanning electron microscopy) techniques, the question of the distribution, morphology and function of the supraependymal cells has become actual again<sup>3-6</sup>. In the hypothalamus, these cells have been found on the surface of the nonciliated areas of the ependyma, especially in the proximity of the recessus infundibularis<sup>7-9</sup>. In this area the hypothalamic ependyma shows cyclic changes at the apical cell poles, i.e., variations in the quantity of microvilli and size of protrusions, as well as release of cellular material. These changes can be correlated with the phases of the ovarian cycle<sup>8,10,11</sup>. The nature of the supraependymal cells in the hypothalamus is an intensively discussed topic. On the basis of their morphology and cytological characteristics and distribution, it was suggested that they may be mesenchymal cells with phagocytic activity involved in the renewal of this region of the ependyma and generally active in the transport of cell debris<sup>12</sup>. In connection with this hypothesis, the question which was investigated in this study is whether the cell number and the surface morphology of the supraependymal cells in the hypothalamus show variations which correlate with the cyclic changes of the ependyma during the ovarian cycle in the rat.

**Methods.** Subjects were 24 females and 6 males Sprague-Dawley rats. The animals were maintained on a reversed lighting schedule (12 h light, 12 h darkness). The estrous cycle was controlled daily by studying of the vaginal cytology<sup>13</sup>. The females were killed in groups of 6 animals in different phases of the estrous cycle. The males were sacrificed in 2 groups, one after the 'dark' hours, the other after the 'light' hours.

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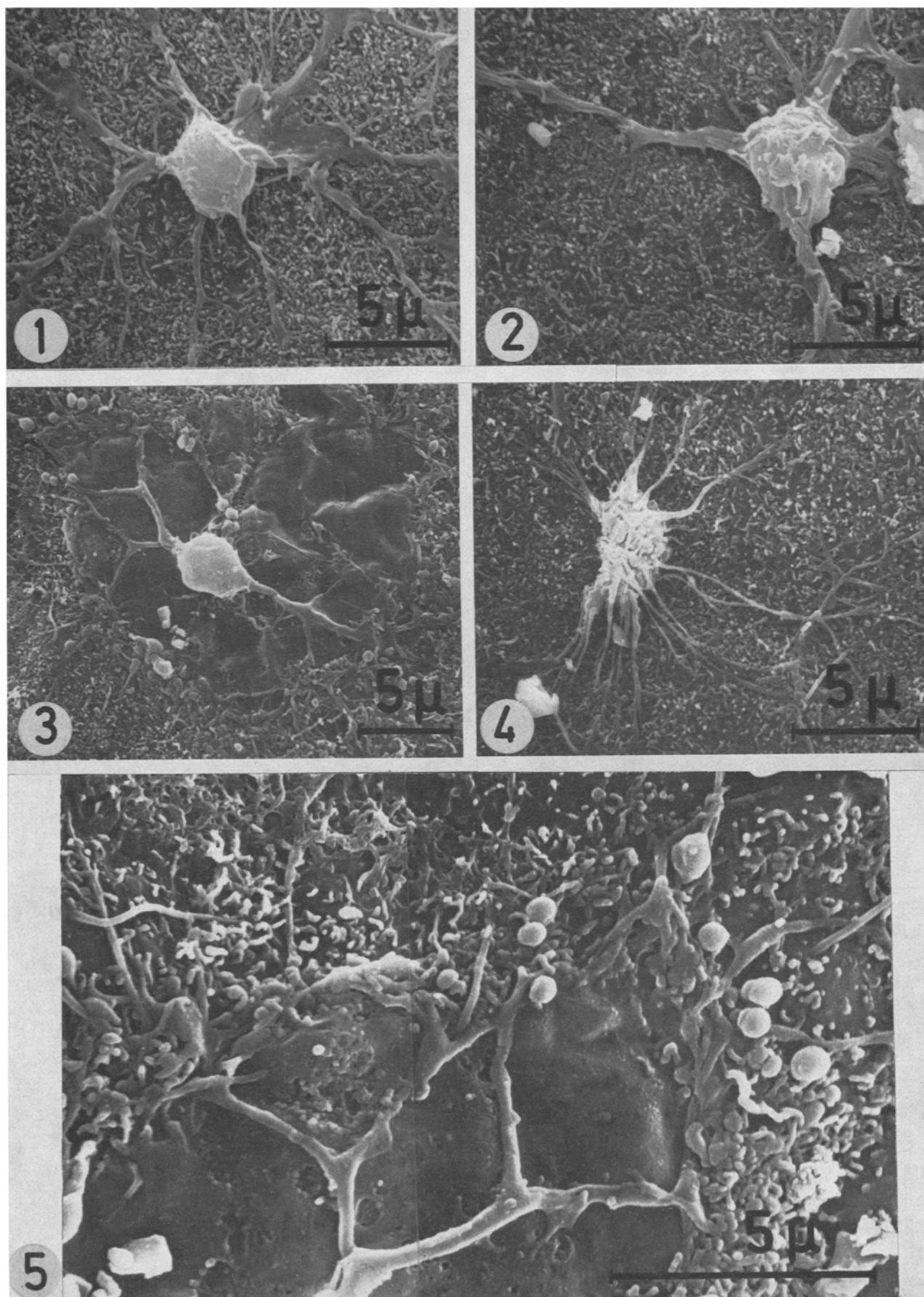


Fig. 1. Supraependymal cell (SEC) with some processes and cytoplasmic laminar folds. Fig. 2. SEC without laminar folds. Fig. 3. SEC with conspicuous cytoplasmic laminar folds. Fig. 4. SEC with a form similar to the macrophages in culture. Fig. 5. Outer border of a laminar fold (see Figure 3).

The fixation and preparation for the SEM was made as previously reported<sup>7</sup>. The specimens were observed with a Jeol Scanning Microscope JMS 35 at 25 KV after being coated with gold in a sputtering apparatus LWU (München).

**Results and discussion.** The supraependymal cells (SEC) in both sexes were localized on the nonciliated ependymal surface of the hypothalamus. This area corresponds to the projection of the arcuate nucleus and to the major part of the hypophyseotropic area<sup>7</sup>. The greatest concentration of these cells was found on the anterior part of the area, near the retrochiasmatic region and the transition zone between ciliated and non-ciliated areas. The restriction of the SEC to this area was observed during all phases of the ovarian cycle. The cell number, however, varied from phase to phase as follows: Oestrus: between 5 and 8 supraependymal cells per ventricular surface; metoestrus: between 70 to 80 cells; dioestrus: between 95 and 100 cells; pro-oestrus: 35 to 45 cells per ventricular surface. These variations were not observed in the males, which showed an average of 25 to 35 cells.

Four basic morphological types of SEC could be determined according to the number and shape of their processes, which take up differing degrees of contact with the ependyma. On some occasions the processes even protrude between the ependymal cells. These forms or types have been found in all phases of the ovarian cycle, although no phase shows exclusively one type. The type which has 2 or 3 processes and some cytoplasmic laminar folds (Figure 1) is the most common, followed by the type without the laminar folds (Figure 2). The type with enormous laminar folds that cover several ependymal cells was found more frequently during oestrus than in any other phase (Figure 3), whereas the one with many

thin processes is more frequent during di-oestrus (Figure 4). This latter form is similar to the macrophages in vitro<sup>14</sup>.

The outer borders of the laminar type (Figure 5) possess many thin finger-like as well as spherical protrusions which may be related to functions of particle uptake, cell locomotion and/or renewal of the cell membrane<sup>15</sup>. Although these laminar extensions are morphologically not similar to the ruffled borders of the macrophages in vitro, this cannot be used as an argument against the interpretation of the SEC as macrophages, because the formation of ruffled borders depends on the nature of the underlying substrate<sup>16</sup>. These four morphological types probably correspond to different behavioral expressions which each cell shows as an answer to environmental necessities by altering its shape and its processes. This assumption points to a similarity of the SEC to mesenchymal cells. Without experimental data to prove the contrary, the variations in cell number must be interpreted as being dependent upon such environmental factors as cyclic changes in the ependyma itself. On the basis of previous studies in vitro, the above-mentioned behavioral aspects are not comparable to those of the neurons and neuroglia<sup>15</sup>. It is also difficult to imagine the migration of neurons and neuroglia cells across the ependyma into the ventricle in adult animals. For these reasons, and according to the hypothesis of BLEIER et al<sup>12</sup>, the SEC could be considered to be a specialized population of mesenchymal cells involved in the renewal of the ependyma as well as the transport of cell debris and foreign particles.

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## On the Mechanism of Water Uptake by the Developing Eggs of *Calotes versicolor*

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**Summary.** Experiments show that osmotic gradient has a role in the absorption of water by the semi-cleidoic egg of *Calotes versicolor*. The observations also indicate that in nature the eggs need to be laid in water-saturated soil and can easily withstand flooding but not dryness.

The absorption of copious amounts of water (5 times the amount in freshly laid eggs) by the eggs of the lizard, *Calotes versicolor* during the entire period of incubation is a physiological necessity<sup>1</sup>. The present communication reports on the role of the osmotic gradient in the absorption of water, and also on some ecological parameters necessary for the development of the eggs of *Calotes*.

**Materials and methods.** Eggs, obtained from the uteri of gravid females, were incubated on cotton beds flooded with glass-distilled water<sup>1</sup> and embryos staged<sup>2</sup>. The surface area of eggs was calculated according to the formula for a prolate spheroid<sup>3</sup>. To study the role of osmotic pressure in water uptake, the eggs were incubated on cotton beds soaked with different concentrations of sodium chloride. Water flooding – a normal possibility in nature since the eggs are usually laid in the rainy season and deposited underground – was simulated by complete immersion of the eggs in water. The eggs were incubated on dry cotton beds in a glass chamber containing a beaker filled with water (environment of 100% humidity) to test

whether the eggs laid after the end of the rainy season<sup>2</sup> can survive conditions where presumably only water vapour and no water is available in soil interstices. The incubation of egg in a desiccator (environment of 0% humidity) was made to ascertain the rate of water evaporation from the egg to elucidate the nature of egg shell. During incubation, the surface area of the egg increases from about 3.0 cm<sup>2</sup> to about 8.0 cm<sup>2</sup>, and consequently eggs of different sizes were used in these experiments.

**Results and discussion.** The mechanism of water uptake by the eggs of *Calotes versicolor* appears to be passive, and mediated by an osmotic gradient, because a) the higher the osmotic pressure exerted by the environmental liquid, the less was the uptake of water (Figure, Table I); b) the eggs completely immersed in water did not differ

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