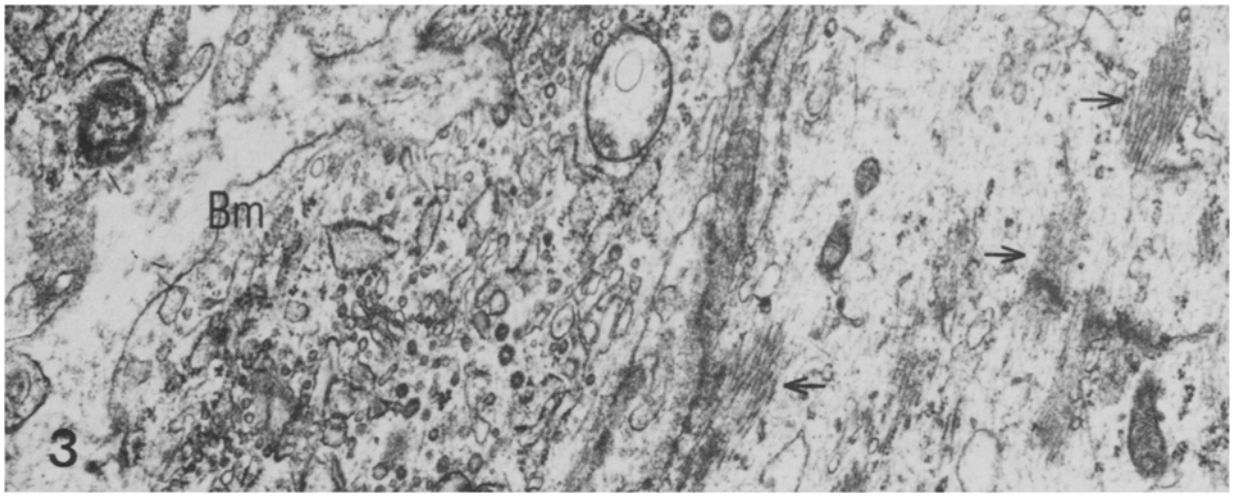

Figure 3. Electronmicrograph from same specimen as in figure 2 showing that the polar cells in the transplant are myogenic, containing organizing myofilaments (arrows). Basement membrane (Bm) of the new cell can be seen; × 16,000.

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Surface morphology of the subfornical organ: Effects of low and high sodium chloride diet

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Summary. Ependymal cells found in the subfornical organ of the rat were counted. Cells covered by small microvilli, small protrusions and smooth cells were frequently found. Also present were cells with long or short cilia, cels with large protrusions and supraependymal cells. High and low sodium diets reduced the number of cells with large protrusions. Microvilli-covered cells increased after a low sodium diet.

The subfornical organ (SFO) has been described as a circumventricular structure placed between the columns of the fornix at the rostral wall of the third ventricle, near the point where the choroid plexuses of the lateral and third ventricles converge[2]. In the rat the surface morphology of this midline elevation shows different types of cells which characterize 3 zones[3]. The structure of some of these cells is modified by water deprivation or hypovolemia[4]. Moreover, factors capable of altering sodium metabolism such as adrenalectomy or sodium-deficient diets affect the size of the neuronal nuclei and produce vacuolization of cytoplasm[5]. Lesion of the SFO increases sodium intake[6]. Sodium excretion after intracarotid infusion of hypertonic saline is prevented by this lesion[7]. This supports a participation of SFO in salt-water homeostasis. To provide more information about SFO as a target of changes in salt intake and especially the participation of the ependymal cells, the surface morphology was studied in rats on a salt-free diet or a hypertonic sodium chloride solution. In order to provide evidences of such changes a quantification of the different types of ependymal cells was made in the above groups and compared to normal animals.

Material and methods. Male Wistar Chbb rats (300–400 g) were housed individually. Control animals (n = 6) were maintained on rat chow (200 mEq Na + /kg) and tap water. High sodium intake rats (8) had 1% sodium chloride solution as drinking fluid. The 'salt free' group (7) had access to a rat chow with less than 4.1 mEq Na + /kg and distilled water. A week after, animals were beheaded, the SFO removed, fixed in 2% glutaraldehyde in Millonig buffer for 2.5 h and post fixed in OsO_4 for 1.5 h in the same buffer. The tissues were dehydrated through increasing concentrations of acetone and finally critical-point dried from CO_2. Tissue blocks were coated with gold-palladium. A picture of each SFO at 150 X and 10–20 pictures at 3000 X were taken of zones 2 and 3 as described by Phillips et al.[2]. The sagittal and medial axes were measured. A reticule containing 20 points was used to count the different types of cells. The point distribution avoided counting the same cells twice. The number of long protrusions per picture was also counted. The means ± SEM of points over each type of cell per 100 cells in each group of animals was calculated but this value was not taken into consideration for the statistical analysis. The results were analyzed by the Kruskal-Wallis one way analysis of variance. The Mann-Whitney U Tests to compare 2 groups were used when the above procedure gave a significant result.

Results. The SFO sagittal axis measured 345 ± 44 μm and the

medial axis 398 ± 36 μm. These axes did not change after different salt intakes. The SFO were encircled by ciliated cells of the third ventricle. The number of these cells decreased in the external zone of the SFO (fig. 1). Cells showing a bunch of cilia-like structures about 15 μm long were occasionally found in the main body. These cilia rose from microvilli cells (fig. 2). The number of cilia per cell varied from more than 20 to, occasionally, only 3 or 4 (fig. 3). Ependymal cells characterized by a smooth surface were evenly distributed (fig. 4). The cells more often found showed small microvilli. Their number varied from cells with only scattered microvilli to cells with a densely-covered microvilli surface (fig. 4). The high magnification study of some of these cells showed long folds (1 μm) of apical membrane with a height of 0.1 μm (fig. 6). Short cilia (2–6 μm) cells were found in the central area of the SFO as well as in the anterior or posterior area (fig. 4). Also occasionally 2 or 4 short cilia were seen emerging from the same cell (fig. 5). No globules on the tips of the cilia were found.

A special structure that can be called a large protrusion was found in the SFO. They took different shapes; they could be seen as blebs of about 2 μm of diameter (fig. 6) or as long protrusion (fig. 7) attached directly to the cell surface by a narrow (0.5 μm) or wide (2 μm) neck. Some large protrusion had a long pedicle that could reach a length of 3 μm with a width of 0.3–1.2 μm. The main body reached a length of 8 μm and showed a rough surface covered with small 0.05–0.40 μm round excrescences (fig. 8). Cells with small round protrusions of 0.35–0.75 μm were found (fig. 7). Supraependymal cells and neuronal bodies with their network-forming process were always present in the SFO surface.

The study of SFO taken from animals on a high or low sodium diet showed the presence of all the cells described above. In spite of the difficulty of classifying any single cell based on specialized surface characteristics, the following marks were taken in order to count the cell population; the presence of a bunch of cilia or a single cilium; the surface covered only by microvilli; the presence of small protrusions or large protrusions; not any of the above elements ('smooth cells') and finally, cells that were difficult to classify ('others'). The table shows the relative number of cells found in control and in different salt intake animals. The number of microvilli-covered cells were increased in the SFO of animals submitted to a low sodium diet. Cells with large protrusions decreased in number after a high or low sodium diet. The number of large protrusions per picture decreased in the groups with a modified sodium diet (table).

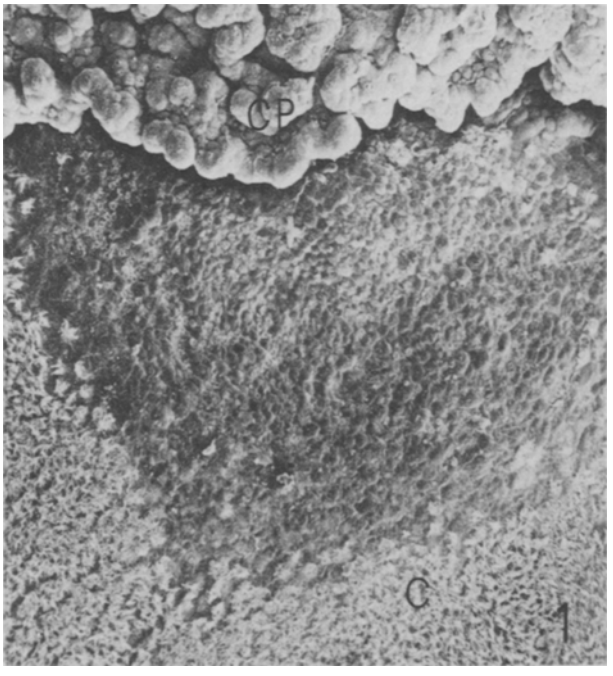

Figure 1. Low-magnification micrograph of subfornical organ. Choroid plexus (CP), cilia of the ventricular wall (C). ×200.

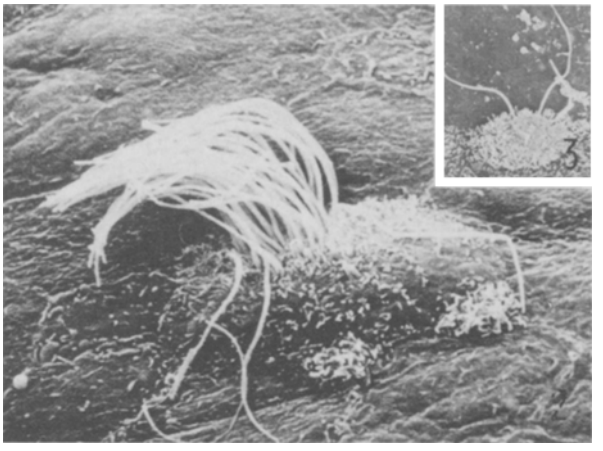

Figure 2. SFO cell with microvilli and a bunch of cilia. ×3000.

Figure 3. Cell covered with microvilli and with only 3 cilia. ×1500.

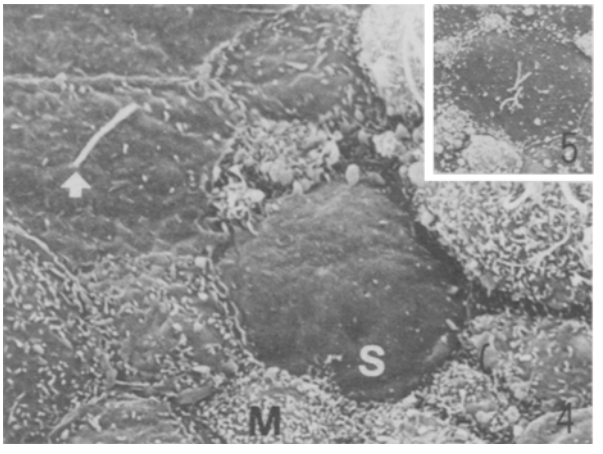

Figure 4. Cell classified as 'smooth cell' (S). Cell with a short cilia (arrow) microvilli cell (M). ×3000.

Figure 5. 4 short cilia arising from the same cell. ×1500.

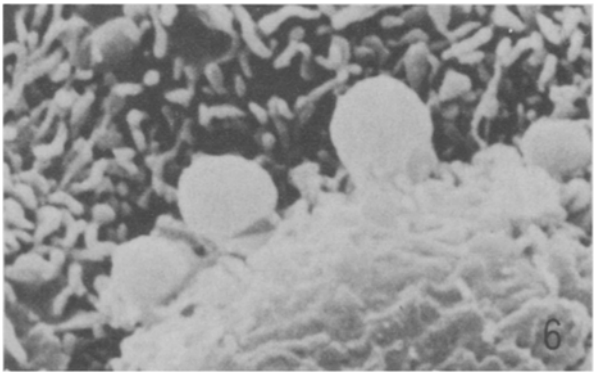

Figure 6. A cell with 3 blebs attached by a thin neck. In the same cell folds of apical membrane. Another cell with small microvilli. ×10000.

Discussion. The above results show the relative participation of each type of cell in the surface morphology of the SFO. We have found all the types of cells previously described in the rat

Relative number of cells found in control and in different salt intake animals

Cells with:	Cilia	Short cilia	Microvilli only	Small protrusions	Large protrusions	Smooth surface	Others	Total No. of cells analyzed	No. of large protrusions per picture	Total No. of pictures
Control	3.6 ± 0.9*	2.3 ± 0.7	30.9 ± 5.8	23.4 ± 6.9	9.9 ± 2.7	28.1 ± 6.9	1.4 ± 0.2	1540	3.5 ± 0.7	77
High sodium	2.9 ± 0.8	2.9 ± 0.8	38.5 ± 4	13.7 ± 3.1	4.8 ± 3.1[a]	25.7 ± 4.3	0.9 ± 0.5	2540	1.93 ± 0.38[a]	127
Low sodium	2.1 ± 0.9	1.3 ± 0.2	59.4 ± 4.9[b]	10.5 ± 2.6	2.4 ± 0.8[a]	22.7 ± 3.9	1.1 ± 0.6	2160	1.17 ± 0.29[b]	108

* Means ± SEM of each type, per 100 cells; [a] $p < 0.05$ compared to control; [b] $p < 0.01$ compared to control.

SFO[3,4]. But some differences can be pointed out. The small protrusions found in all the cells[2] of zone 1 and 2 were present in our animals in about 25% of the cells, especially in zone 2. This may be explained by the different method of fixation used. This also may account for the bigger diameter fo our small protrusion. The short cilia found were shorter than the cilia described in rat[3]. They had the same length as the kinocilia described in rabbit[8] but they were devoid of any secretory covering material. Short cilia, although scarce, were found always in zone 2. Exceptionally 2 to 4 short cilia were found. We may also add to the description of cilia distribution that, although this was rare, only 2 or 3 long cilia were present in some cells. Microvilli have been described[9], though it may be possible that some of them represent membrane foldings with a length larger than the height, which increase the cell surface. This may be supported by the fact that SEM allow the complete view of this structure instead of a thin section as seen in TEM. The increase of cells with microvilli after submitting rats to a low sodium diet would increase the total area of interchange between SFO and cerebrospinal fluid. The large protrusions described could be considered as one type of structure, seen in different functional stages, or different structures which could take a similar shape. Their physiological function is unknown but we can speculate that they may have some secretory substance to be discharged into the cerebrospinal fluid or may have a receptor function. It seems improbable that a receptor of this magnitude can be atrophied after a stimulus of relatively short duration but if low and high sodium diets produce a common stimulus, the release of some substance by the SFO could explain the reduction in number of the large protrusions. The secretory function of the SFO has been considered[8]. Nevertheless, the possibility that some of them, the large protrusion with a long pedicle, are axons growing right through ependymal cells cannot be disregarded.

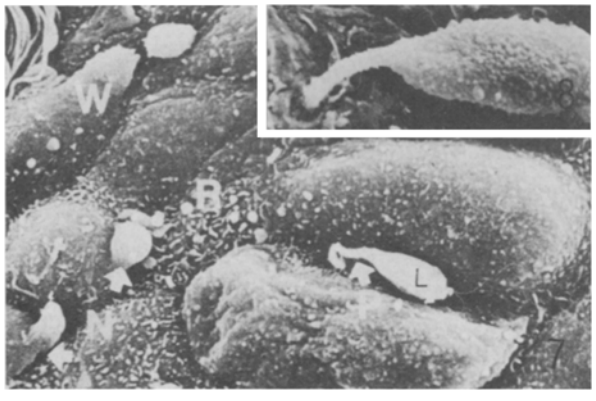

Figure 7. Large protrusions with narrow (N) or width (W) neck. Large protrusion (L) with a long pedicle (P). Small round protrusion (R). × 3000.

Figure 8. High magnification of a large protrusion covered by round excrescences. ×5000.

1 Acknowledgments. We thank Dr A.C. Taquini for his helpful discussions and Servicio de Microscopía Electrónica de Barrido, Conicet, and Facultad de Odontología for technical assistance.
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3-OH-Kynurenine content and ommochrome formation in the developing compound eye of *Ephestia kühniella*

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Summary. The eye color of *Ephestia kühniella* is primarily determined by the ommochromes xanthommatin and ommin. The pigmentation, an important part of eye differentiation, occurs mainly during the pupal stage. Comparative studies on eye colour mutants indicate that a first step in ommochrome synthesis is the binding of the precursor 3-OH-kynurenine to developing pigment granules. Both xanthommatin and ommin are present from the early beginning of eye differentiation, and exhibit different developmental profiles.

The pigmentation of plants and animals is a favored subject in developmental biology and genetics. A classical example is the eye color of insects. The screening pigment in the compound eyes of these animals consists mainly of ommochromes and only to a very small extent of pteridines. The direct precursor of ommochromes is 3-OH-kynurenine[1]. The synthesis of this metabolite, which is derived from tryptophane through a well-characterized series of enzymatically catalyzed oxidation steps, is well understood. In contrast, studies of the processes governing the formation of ommochromes are still at best preliminary