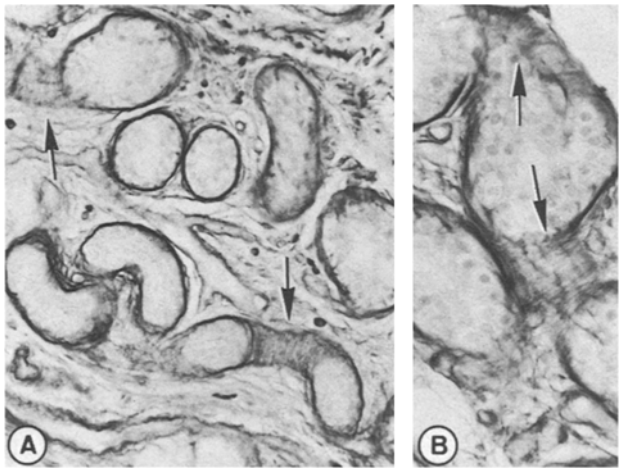

Aldehyde-fuchsin staining after thiosulfation. Basement membranes of sweat gland secretory coils are strongly reactive. In tangentially sectioned regions, positively stained material is seen to form circularly running fibres (arrows). A: × 120; B: × 480.

The basement membrane of secretory coils of sweat glands stains with resorcin-fuchsin or with aldehyde-fuchsin after permanganate oxidation. The thickness of elastica-positive layers, as well as its staining intensity, decreases where the secretory coil transforms to excretory duct. Elastica-positive fibres are seen to form parallel, tightly packed rings which are filed along the tubular gland. The basement membrane does not stain with Verhoeff's hematoxylin. Aldehyde-fuchsin does not tinge this region in untreated sections, whereas resorcin-fuchsin does.

Alcian Blue evinces only pale colouration of basement membranes of sweat gland secretory coils when used in the presence of 0.8 M $MgCl_2$. Mast cell granules in the same section are stained under these conditions. After thiosulfation, basement membranes are intensively stained both with Alcian Blue + 0.8 M $MgCl_2$ and with aldehyde-fuchsin (figure). Positively reacting fibres form parallel rings filed along the secretory coils. Methylation after thiosulfation abolished staining with Alcian Blue or with aldehyde-fuchsin.

SH-groups in sweat gland basement membranes can be visualized with ferric-ferricyanide reagent. In untreated sections, myoepithelial cells are faintly reactive. These cells are seen to be wedged into secretory cells and to rest on an almost unreactive basement membrane. Pretreatment with N-ethylmaleimide abolishes the reactivity of the basement membrane and decreases staining of myoepithelial cells. Basement membranes become strongly reactive after reduction with sodium thioglycollate.

Fine structural studies[4] have shown that elaunin fibres can be aligned between oxytalan fibres and elastic fibres. As a result of studies on elastogenesis, Gawlik[2] has suggested that primordial oxytalan fibres are replaced by elaunin fibres and finally by elastic fibres. In the EM this series is initially characterized by bundles of fibrotubules, 10–12 nm in diameter (oxytalan fibres). After this, increasing amounts of homogeneous amorphous material (elastin) are observed within these bundles and centrally located fibrotubules are encemented: gradual transformation from elaunin fibres (small amorphous cores within fibrotubular bundles) to elastic fibres (amorphous material predominates) takes place. During the course of aging, the amount of fibrotubules at the surfaces of elastic fibres decreases and fibrotubules finally completely disappear[5]. Oxytalan fibres, fibrotubules of elaunin fibres and 'elastic fibre microfibrils' of elastic fibres morphologically correspond. These microfibrils are also closely related concerning their high histochemically demonstrable content of disulfide-groups.

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Uptake of exogenous protein by supraependymal cells of the feline area postrema

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Summary. Supraependymal cells occurring on the surface of the feline area postrema were examined for phagocytic ability. It was shown that they could ingest exogenous horseradish peroxidase that was experimentally introduced into the brain ventricular system. The cells thus bear functional as well as ultrastructural attributes of macrophages, similar to those found in the third ventricle and subarachnoid space.

Macrophage-like cells have been found in various spaces which contain cerebrospinal fluid (CSF) both within and around the vertebrate brain. These include cells lying in the subarachnoid space[3,4], on the ventricular surface of the choroid plexus[5,6], and on the ependymal surface of the 3rd ventricle[7–11,23]. Recent electron microscopic studies[8,9,12–15] have shown that certain subarachnoid, supraplexus and supraependymal (SE) cells have similar ultrastructural characteristics typical of macrophages. Tracer studies using latex beads[9] and India ink, Thorotrast and ferritin[16], and studies challenging macrophage-like cells with live Bacillus Calmette-Guerin[17,18] have confirmed that the cells are phagocytic in nature.

In studies of the ependymal surface of the feline area postrema[19] (AP) a population of SE cells was described on the caudal part of the organ. The cells resembled SE cells of the mammalian third ventricle[7–11], cells lying on the surface of the choroid plexus[12,16], and macrophages of the subarachnoid space[13,17]. To examine the phagocytic ability of the SE cells of the cat AP, we presented an exogenous

protein, horseradish peroxidase (HRP), to these cells via the CSF. Transmission electron microscopy was used to assess the ability of the cells to ingest and phagocytose the foreign protein.

Experimental animals, anaesthetised with i.v. injections of sodium pentobarbital, were placed in a sterotaxic frame. A burr hole was made in the dorsal surface of the skull and a 16-ga needle, in line with a Harvard perfusion pump and injection syringe, was inserted into the left ventricle[20]. 100 mg horseradish peroxidase (Sigma, Type II) was dissolved in 1 ml artificial mammalian CSF and was introduced into the perfusion line and pumped into the ventricles of 3 cats at the rate of 35 $\mu l \cdot min^{-1}$. Control experiments omitted the peroxidase from the ventricular perfusate. Approximately 1 h after the beginning of the protein perfusion, the animals were perfused via the ascending aorta with a mixture of 0.5% glutaraldehyde and 4% formaldehyde in phosphate buffer. The cats were maintained under sodium pentobarbital anaesthesia throughout the procedure.

The region of the AP was excised and incubated in 0.05% diaminobenzidene[21]. Post-fixation was carried out in buffered 1% OsO_4, and tissues were dehydrated and embedded for electron microscopy. En bloc staining with 0.5% aqueous uranyl acetate was employed. Semi-thin sections were stained with 1% Toluidine blue in 1% sodium borate, and ultra-thin sections were treated with lead citrate before examination in the electron microscope.

Supraependymal cells were found dispersed over the caudal portion of the AP. Cells ranged from 13 to 40 μm in diameter, and were usually ovoid in cross section (figure 1). No change from non-experimental tissues fixed for electron microscopy[19] was noted in the location or relative frequency of SE cells. Only a few such cells were found in any of the experimental or control animals, always on the most caudal aspects of the paired lobes of the AP.

Horseradish peroxidase reaction product was seen coating the ependymal surface of the floor of the 4th ventricle as well as the apical surfaces of the SE cells of experimental animals (figure 1). A variable amount of peroxidase reaction product was seen within many of the vacuoles of these cells (figure 2). The reaction product was generally found coating the luminal surface of the vacuoles, and did not completely fill the lumina (figure 2). All of the SE cells examined, in all of the experimental animals displayed a number of vacuoles containing the reaction product. In control experiments, no reaction product was seen coating the ependymal surfaces, the surfaces of the SE cells, or contained within the vacuoles of these cells.

The results of this study support the suggestion[9] that a resident population of macrophages is to be found within the ventricular system of the vertebrate brain. Supraepen-

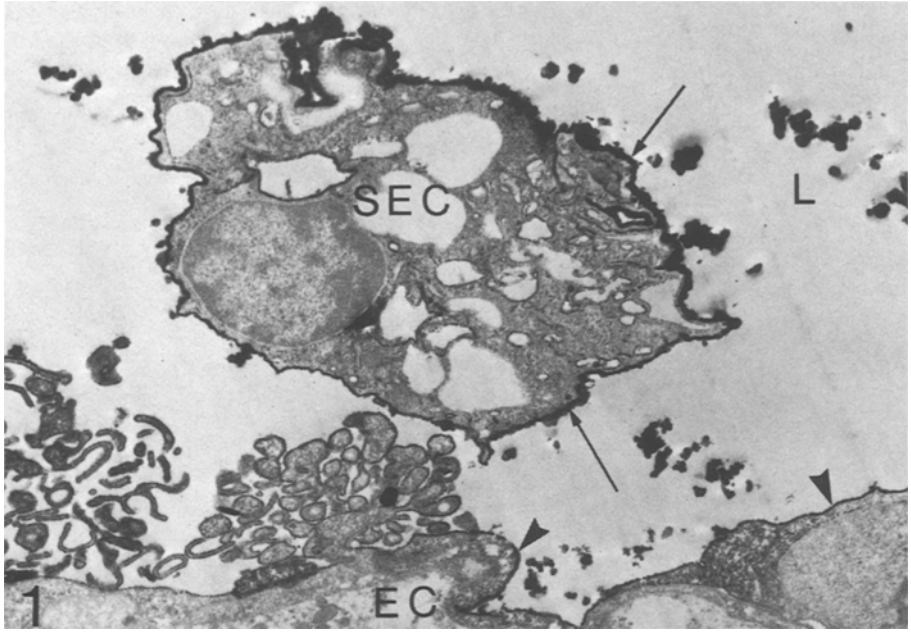

Fig. 1. Electron micrograph of the surface of the cat area postrema which had been exposed to exogenous horseradish peroxidase injected into the cerebrospinal fluid. Note the coating of reaction product over the surface of the supraependymal cell (SEC) (arrows) and covering the apical surfaces of the ependyma (EC) (arrowheads). Reaction product can also be seen as electron-dense material lying free within the lumen (L) of the 4th ventricle. × 6000.

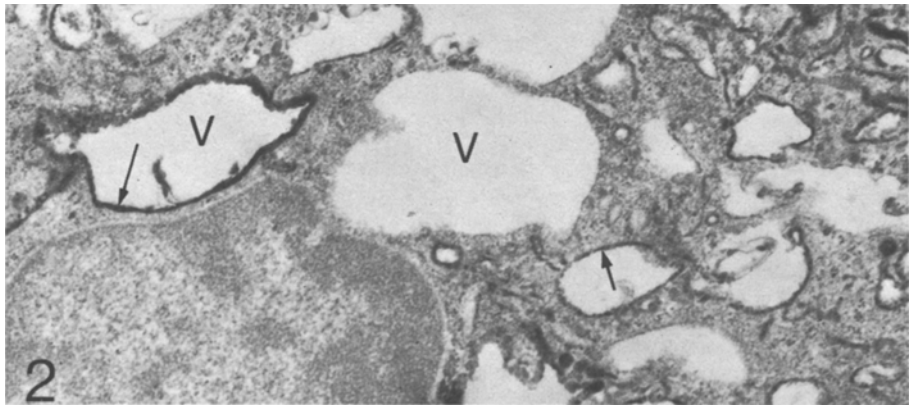

Fig. 2. Higher power micrograph of supraependymal cell seen in figure 1. Note the peroxidase reaction product (arrows) coating the luminal surfaces of some of the vacuoles (V) within the cytoplasm of the cell. × 19,000.

dymal cells of the cat described here bear a striking resemblance to cells found in various other CSF-containing compartments in and around the central nervous system[8,11-18,22]. Certain vacuoles of the 'pial free cells' of the dog subarachnoid space[14] contained quantities of HRP reaction product either almost filling the lumina or simply adhering to the inner surfaces of the vacuolar membranes when the protein was introduced into the CSF. This occurred in a similar fashion in the SE cells of the AP in the present study, and, in fact, the appearances of the cells in electron micrographs of the 2 studies are remarkably similar. In all of the investigations referred to above, it has been suggested that systems of macrophages or macrophage-like cells regularly occur in the various CSF-containing spaces.

Supraependymal cells consistently occur on the surface of the cat AP at the caudal end of the 4th ventricle surrounding the entrance to the central canal of the medulla oblongata[19]. These cells have now been shown to be capable of phagocytosis of a foreign protein and to display all the characteristics of macrophages found, for example, in the 4th ventricle and subarachnoid space of dogs[12,18]. It therefore seems reasonable to suggest that the SE cells of the AP of the cat are macrophages, and further, that they form part of an extensive system of macrophages in the CSF-containing compartments both within and around the central nervous system.

The origin of the cells seen in the present study and in the studies referred to above is still unknown. It has been suggested that they may arise from monocytes, and may enter the CSF through the choroid plexus[22]; they may arise from ependymal cells or microglia-like cells of the brain parenchyma[15]. Similarly, the function of such macrophages has not been well established. They may remove normal metabolic waste from the underlying surface[22]. They may also act as a first line of defence against foreign organisms that invade the ventricular system since it has been shown[24] that membrane-limited viral nucleocapsids are incorporated into SE macrophages after experimental intracerebral inoculation of mumps virus into hamsters. Further experimental studies of this latter type are, however, necessary in order to establish with certainty the functional significance of macrophages on the ventricular surfaces of the mammalian brain.

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X-irradiation of mice in early fetal period influences dose-dependently sex ratio of offspring until weaning

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Summary. Fractionated X-irradiation of mouse fetuses on gestation days 11-13 resulted in a significantly increased postnatal mortality of female litters. This occurred only at 3 × 110 rad, which was the threshold for the formation of typical neuroepithelial, rosette-like malformations.

X-irradiation in the early fetal period, which is the most sensitive period (between gestational days 10 and 13) in the mouse for distinct histological effects on the central nervous system (CNS), results in a variety of malformations[2,3]. These anomalies (e.g. porencephaly[4]) partially originate from extensive perinatal repair processes and are therefore only lethal in the suckling period. In a previous report[3], we quantified the CNS lesion pattern at term and decided to look for correlations between these morphological criteria and the incidence of stillbirths or postnatal deaths.

Materials and methods. X-irradiation of pregnant mice was performed on gestation days 11, 12 and 13, as reported previously (180 kV, 10 mA, 0.3 mm copper plate filter, focus target distance 40 cm; dose rate 1 rad/sec)[3,4]. Originally we used irradiation doses of 3 × 100, 3 × 120 and 3 × 140 rad, but in our search for the threshold dose for clear-cut malformations, so-called rosettes[3], we concentrated mainly on a dose of 3 × 110 rad. The dams were born at the time scheduled. The stillbirths were collected immediately, and postnatal deaths were collected 3 times a day. The percentage of wasted litters was thus very low. The sex of these dead animals was determined by abdominal inspection. Another 37 control dams and 55 dams irradiated with 3 × 110 rad on days 11-13 were sectioned on day 18 post conception (p.c.). The individual positions of the fetuses in utero were recorded, and these fetuses were weighed and consequently inspected for their gonadal sex. The placental weights were also determined.

Results. The number of dams used in the various groups and the subsequent sex ratios of the offspring at weaning are listed in table 1. In the low dose range between 3 × 100 and 3 × 110 rad, the sex ratio (♂ : ♀) abruptly increased, which was caused by a high mortality of the females. This was most extreme in group III 2 with a male : female index