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Invited Review

Comparative histology of pineal calcification

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Summary. The pineal organ (pineal gland, epiphysis cerebri) contains several calcified concretions called "brain sand" or acervuli (corpora arenacea). These concretions are conspicuous with imaging techniques and provide a useful landmark for orientation in the diagnosis of intracranial diseases. Predominantly composed of calcium and magnesium salts, corpora arenacea are numerous in old patients. In smaller number they can be present in children as well. The degree of calcification was associated to various diseases. However, the presence of calcified concretions seems not to reflect a specific pathological state. Corpora arenacea occur not only in the actual pineal tissue but also in the leptomeninges, in the habenular commissure and in the choroid plexus.

Studies with the potassium pyroantimonate (PPA) method on the ultrastructural localization of free calcium ions in the *human pineal*, revealed the presence of calcium alongside the cell membanes, a finding that underlines the importance of membrane functions in the production of calcium deposits. Intrapineal corpora arenacea are characterized by a surface with globular structures. Meningeal acervuli that are present in the arachnoid cover of the organ, differ in structure from intrapineal ones and show a prominent concentric lamination of alternating dark and light lines. The electron-lucent lines contain more calcium than the dark ones. There is a correlation between the age of the subject and the number of layers in the largest acervuli. This suggests that the formation of these layers is connected to circannual changes in the calcium level of the organ. The histological organization of the human pineal is basically the same as that of mammalian experimental animals.

Pineal concretions present in *mammalian animal species* are mainly of the meningeal type. Meningeal cells around acervuli contain active cytoplasmic organelles and exhibit alkaline phosphatase reaction in the rat and mink, an indication of a presumable osteoblast-like activity. Using Kossa's method for the staining of calcium deposits, a higher calcium concentration was detected in the rat pineal than in the surrounding brain tissue. Since in parathyroidectomised rats calcified deposits are larger and more numerous than in controls, the regulation of the production of acervuli by the parathyroid gland has also been postulated.

In most of *submammalian species,* the pineal organs (pineal-, parapineal organ, frontal organ, parietal eye) are photoreceptive and organized similarly to the retina. Acervuli were found in the pineal of some birds. The pineal organs of lower vertebrates (fish, amphibians, reptiles) exhibit a high calcium content by ultrastructural calcium histochemistry (PPA-method). However, concrements are not formed. The accumulation of Ca^{2+} seems to depend on the receptor function of the organ. Comparing pineal and retinal photoreceptors in the frog, the photoreceptor outer segments of pinealocytes as well as retinal cones and rods show a large amount of Ca-pyroantimonate deposits. In dark adapted animals calcium ions are present in both sides of the photoreceptor membranes of the outer segment, whereas calcium is shifted extra-cellularly following light adaptation.

Overviewing the data available about the pineal calcification, we can conclude that a multifactorial mechanism may be responsible for the calcification. The pineal of higher vertebrates is not just a simple endocrine gland, rather, its histological organization resembles a folded retina having both hormonal and neural efferentation. Mammalian pinealocytes preserve several characteristics of submammalian receptor cells and accumulate free Ca^{2+} on their membranes (1). In the thin walled retina and in the similarly organized pineal of submammalian species, the diffusion of extracellular calcium is probably easy and there is a lesser tendency to form concrements. In the larger mammalian pineal the compaction of a high amount of pineal cells actively exchanging calcium ions is supposed to increase the local concentration of calcium (2). Further, neural

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elements of more developed species exhibit a higher rate of calcium exchange and, consequently, a higher number of calcium deposits (3). Finally, the barrier effect of the multilayered pineal arachnoid and the tight-junctions among their cells in mammals presumably promote the intrapineal concentration of calcium ions. The formation of acervuli may be regulated by calcitonin, and the periacervular arachnoid cells ("acervuloblasts") act like osteoblasts (4).

Key words: Calcification, Pineal organs, Photoreception, Retina, Electron microscopy

Contents

1. Introduction

The pineal organ contains calcified concretions (corpora arenacea, acervuli or brain sand) measuring from some microns to several millimeters in diameter. The larger ones are easily identifiable on X-ray, CT- and MR-pictures. Pineal acervuli are also present in several mammals and in some birds (Naffzinger, 1925; Vastine and Kinney, 1927; Moreau, 1966; Japha et al., 1976; Krstic and Golaz, 1977; Welsh and Reiter, 1978; Hurbut et al., 1987; Vígh et al., 1989a,b; Vígh and Vígh-Teichmann, 1988, 1989a, 1992).

There are numerous hypotheses concerning the formation of brain sand. Most of them state that calcifying organic material, cells or fibers, forms the core of the acervuli. Krstic (1986) and Ueck and coworkers (1987) found calcium ATPase activity in pinealocytes. The Ca^{2+}ATPase is an enzyme that uses the hydrolysis of ATP (adenosine triphosphate) to pump calcium out of the cell. The free intercellular calcium precipitating on various structures may form the acervuli. On the other hand concrements can appeare intracellularly as well. Pochet and coworkers (1987) demonstrated calcium binding protein in the pineal and retina of several vertebrates. Calbindin and Calretinin were found in the pineal of different mammals and man (Krstic, 1988; Krstic and Nicolas, 1994; Novier et al. 1996). The role of these proteins is to bind and transport calcium and regulate its intracellular level. According to Krstic (1985) an increased cytoplasmic Ca^{2+} concentration presents histochemichal evidence of cell degeneration.

Histochemical demonstration of free calcium ions revealed that the pineal contains more calcium than the surrounding brain tissue in the rat (Vígh et al., 1989b). This observation highlights the fundamental question of pineal calcification: why does the pineal contain more calcium than other tissues? The mammalian pineal organ that derives from the photoreceptive pineal organs of lower vertebrates is different in structure from the surrounding brain tissue.

In contrast to the common view, comparative analysis of the photoreceptive pineal organs (parietal eye, frontal organ, pineal and parapineal organs) in various vertebrates shows that rather than simple glandular cells, pinealocytes are actually neuronal sensory cells having both hormonal and neural efferentation. For this reason, we prefer the term "pineal organ" to "pineal gland". Histologically, the pineal organs are composed of ependymal, glial cells, neurons and pinealocytes being cytologically similar to photoreceptor cells (Vígh and Vígh-Teichmann, 1974, 1975, 1981, 1986, 1988; Vígh-Teichmann et al., 1980, 1986, 1988, 1991, 1993; Vígh-Teichmann and Vígh, 1986, 1992, 1994; Vígh et al., 1986a,b, 1995a,b).

The investigation of pinealocytes as specialized neurosensory cells furnished new evidence for the understanding of the high calcium content of the pineal tissue. In the present review, we summarize the data about the localization of Ca-ions, with special reference to the receptor and effector endings of the pinealocytes as well as to putative structures involved in the circulation and accumulation of this cation during pineal calcification. The electronmicroscopic localization of calcium in the photoreceptor pinealocytes of lower vertebrates is also compared with that in retinal rods and cones.

2. Calcification of the human pineal

The calcified concretions of the human pineal have been known for several hundred years (for review of earlier literature see in: Rio-Hortega, 1932; Bargmann, 1943). Larger corpora arenacea are detectable with imaging examinations and are used as natural orientation landmarks in the diagnosis of intracranial diseases. The grade of dislocation of the pineal from the midline correlates with the grade of neurological symptoms. However, asymmetrical calcification may mimic a pathological shifting of the pineal (Dyke, 1930; Pilling and Hawkins, 1977; Michotte et al., 1977; Macpherson and Matheson, 1979; Moller et al., 1979; Van-Woerden and Vencken, 1979; Ketonen, 1980; Chang et al., 1981).

The acervular minerals morphologically correspond to hydroxyapatite and carbonate apatite. Electron probe microanalysis reveals the existence of calcium and phosphorus as main components of the acervuli. Small quantities of magnesium strontium and sulphur were also detected (Angervall et al., 1958; Earle 1965; Palladini et al., 1965; Mabie and Wallace, 1974; Krstic, 1976; Michotte et al., 1977; Moller et al., 1979).

Pineal concrements are numerous in older patients, but they are present in children as well (Kerényi and Sarkar, 1968; Pillai, 1968; Tapp and Huxley, 1971; Macpherson and Matheson, 1979; Reyes, 1982; Hasegawa et al., 1987; Winkler and Helmke, 1987;

Schmid, 1993; Schmid and Raykhtsaum, 1995). Examined by computer tomography, calcified concretions were found in 2% of 0-3-year-old patients, in 32% of 10-18-year-old patients, in 53% of 20-29-year-old patients and in 83% of patients above 30 years of age (Macpherson and Matheson, 1979). According to Zimmermann and Bilaniuk (1982), the number of concretions increases significantly in puberty. Preceding the menopause, pineal calcification was found in a higher degree in women than in men. Osteoporosis dimininshes the number of concrements (Tapp and Huxley, 1971, 1972).

The occurance of pineal concrements varies among different populations and nations (Chiba and Yamada, 1948; Daramola and Olowu, 1972; McKay, 1973; Adeloy and Felson, 1974; Oon, 1975;

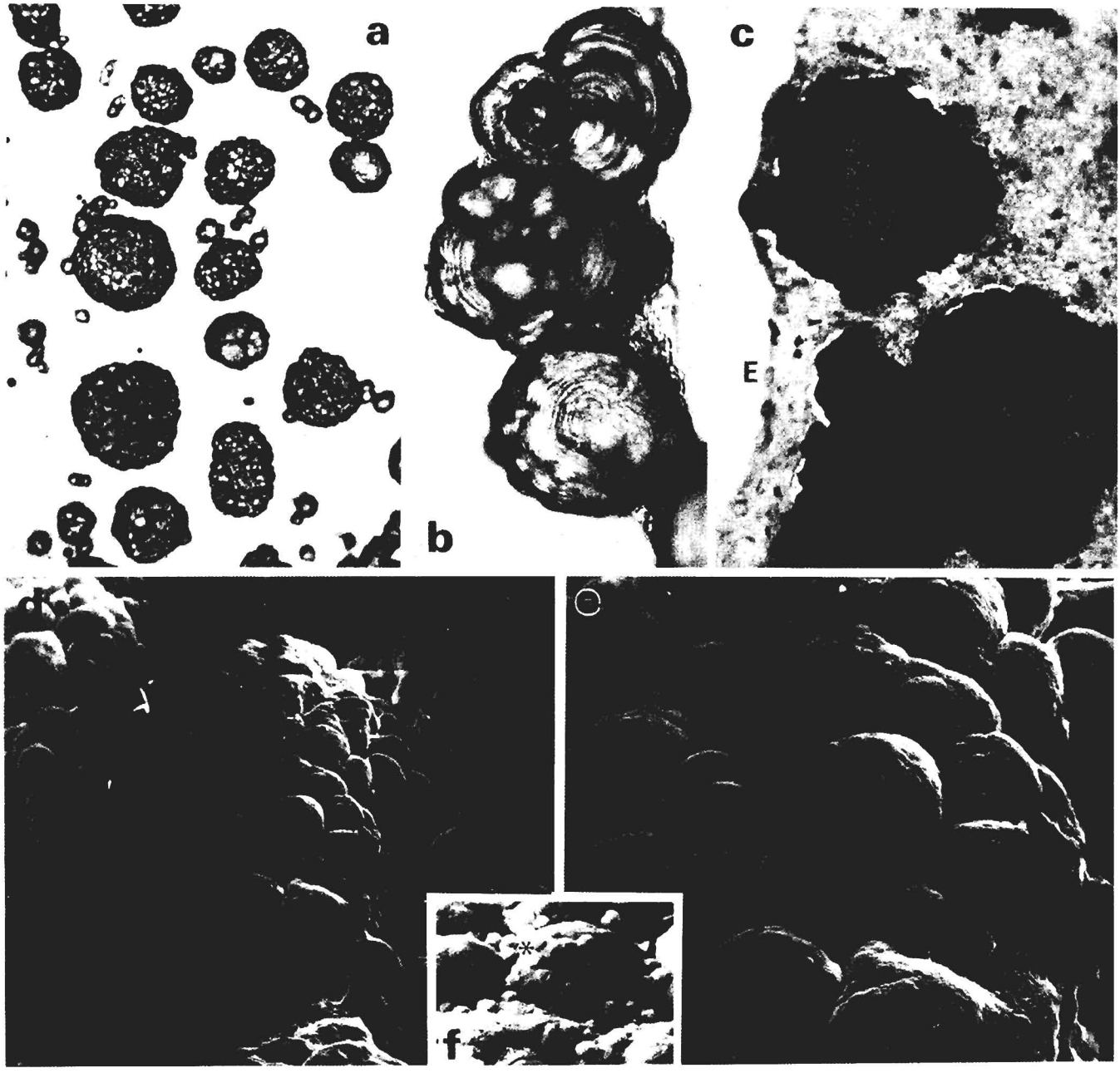

Fig. 1. Concrements in the human pineal. **a.** Isolated intrapineal corpora arenacea of different sizes. Note the globular surface of the concrements. x 45. **b.** Laminated concrements from the meningeal capsule of the pineal. x 80. **c.** Calcified concrements of the habenular commissure. E: ependymal surface. Kossa's method. x 120. **d and e.** Scanning electron microscopy of human intrapineal concrements. d, x 200; e, x 500. **f.** Secondary microglobules (asterisks) on the surface of the concrement x 650

Mugondi and Poltera, 1976; Bhatti and Khan, 1977; Fan, 1983; Abbassioun et al., 1987). The differences found in calcification are attributed to different nutritional or environmental conditions. The highest incidence of pineal calcification was found among Ugandans (Mugondi and Poltera, 1976) indicating the role of high circannual light intensity near the equator.

Pineal, meningeal and choroid calcification

Calcification is not confined to the pineal tissue, it also occurs in other parts of the brain such as the habenular region, choroid plexus and leptomeningeal tissue (Bargmann, 1943; Voss, 1957; Wildi and Frauchinger, 1965; Heindrich et al., 1970; Heil, 1972;

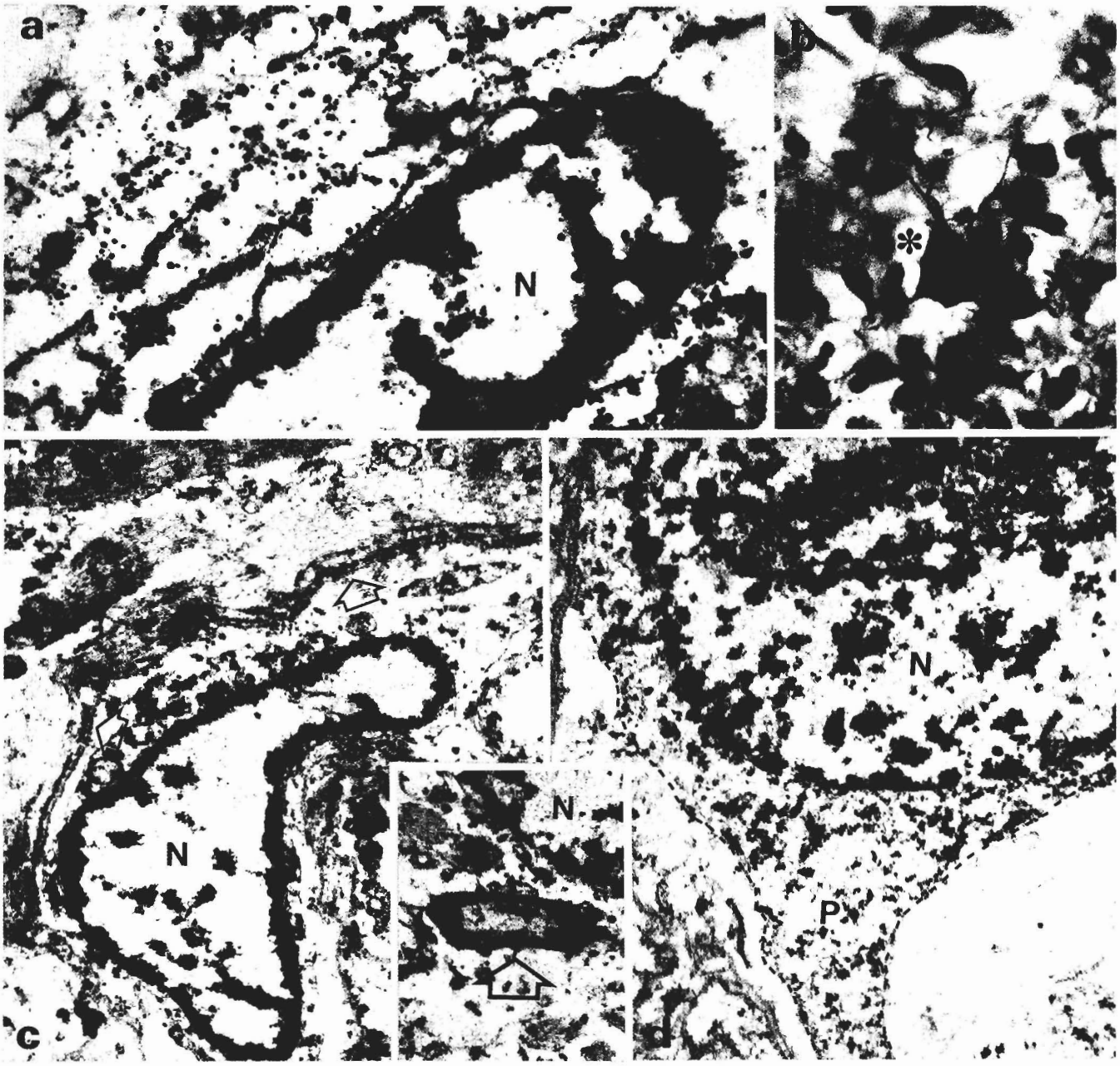

Fig. 2. Structure of the human pineal. **a.** Electron microscopic localization of Ca^{++} ions by pyroantimonate reaction (black dots) on cell membranes and in the nucleus (N) of the pineal arachnoid. x 16,500. **b.** Multipolar neuron (asterisk) in the proximal part of the pineal organ. Golgi impregnation. x 400. **c.** Marginal glial cell enclosing the surface of the pineal neural tissue. Arrows: basal lamina; N: nucleus. x 12900. **d.** Pinealocyte (cell membrane is marked with dotted line) with invaginated nucleus (N) and basal process (P). x 9,600. Inset: myelinated nerve fiber (arrow) among pinealocytes. N: nucleus of pinealocyte. x 9,600

Abbassioun et al., 1987; Macpherson and Matheson, 1979; Allen et al., 1982; Yamagami and Handa, 1985; Alcolado et al., 1986; Kendall and Cavanagh, 1986; Okudera et al., 1986; Vígh and Vígh-Teichmann, 1989a, 1992; Sandyk, 1992a,b). As the "capsule" and septa of the pineal organ are formed by the arachnoid and pia mater sheats of the meninges, microscopical meningeal concrements are seen "inside" of the pineal as well. Electron microscopy shows that menigeal acervuli are always separated from the pineal nervous tissue by glial limiting membrane and basal lamina.

Meningeal acervuli differ in structure from those of the pineal nervous tissue by showing a clear concentric lamination. In contrast, intrapineal corpora areanacea are chacterized by a surface with globular structures (Fig. 1). There is a positive correlation between the number of layers of the largest acervuli and the age of human pineals (Vígh-Teichmann and Vígh, 1992), suggesting that the formation of layers is connected to circannual changes in the calcium level of the organ (see also Trentini et al., 1987).

Using Kossa's method to stain calcium ions, calcium was found to be abundant in or around corpora arenacea of human pineal at the light microscopical level. Studies on the ultrastructural localization of calcium ions with the potassium pyroantimonate (PPA) method (Vígh and Vígh-Teichmann, 1989a) revealed the presence of cell membrane-bound calcium pyroantimonate deposits in the human pineal arachnoid (Fig. 2a). This finding underlines the role of the cell membrane in the accumulation of calcium ions.

The relation of calcification to different pathological conditions

There is a large body of information in the literature about the connection of pineal calcification and pathological conditions such as the formation of pineal cysts (Kohli et al., 1992; Momozaki et al., 1992; Jinkins et al., 1995) or pineal tumors (Lin et al., 1978; Moller et al., 1979; Ganti et al., 1986; Chang et al., 1989; Momozaki et al., 1992). Magnetic resonance imaging enables the recognition of medium and large-sized pineal concrements. However, computed tomography is needed when small sized calcifications are to distinguished from pineal cysts (Kohli et al., 1992; Jinkins et al., 1995).

Several studies relate the degree of calcification to pineal melatonin secretion (Cohen et al., 1978; Sandyk and Kay, 1991a,b; Sandyk et al., 1992a,b; Schmid, 1993). In normal human subject no relationships were found between plasma melatonin and urinary 6-sulphoxymelatonin - a major melatonin metabolite - and pineal calcification (Bojkowski and Arendt, 1990). Hyperparathyroidism (Irnell et al., 1970) and oosteoporosis (Sandyk et al., 1992a), seem to influence the pineal calcification.

Some papers correlate the number of the concrements to symptoms of diseases such as hypogenitalism (Moreau and Cohen, 1965), Alzheimer disease (Friedland et al., 1990), brest cancer and endometrial carcinoma (Cohen et al., 1978; Sandyk et al., 1992b), epileptic seizures (Sandyk, 1992b), Down syndrome (Ieshima et al., 1984; Arai et al., 1995), cerebral atrophy (Sandyk and Awerbuch, 1994a), multiple sclerosis (Sandyk and Awerbuch, 1994a,b), schizophrenia (Sandyk, 1993) and orientation disorders ("defective sense of direction", Bayliss et al., 1985). Whereas a low rate of calcification was found in pineal teratoma, high incidence was detected in cases of pineal germinoma (Chang et al., 1981, 1989) and in several other diseases. Nevertheless, the appearance of pineal concrements do not seem to be a specific complication of any disease studied.

The histological organization of the human pineal gland

As already mentioned, most of the authors investigating the function of the pineal consider the organ a melatonin secreting gland. Even the pineal calcification was mainly studied from this point of view. Recent ultrastructural studies, however, demonstrate the neural character of the pineal organ not only in lower vertebrates but also in human. Its hormonal function appears to be similar to the neurosecretory activity of hypothalamic nuclei, where hormones are produced by neural tissue and released via axon terminals in the neurohypophysis or median eminence (Vígh and Vígh-Teichmann, 1989a;Vígh-Teichmann and Vígh, 1992).

Besides of pinealocytes that are analogous to photoreceptor cells of lower vertebrates, the human pineal constains neurons, nerve fibers and glial cells in an organization characteristic for neural tissues (Fig. 2b-d). The "capsule" of the organ is the continuation of the diencephalic meninges and is comprised of arachnoid and pia mater. These sheats penetrate the organ and separate the lobular foldings of the wall of the pineal gland. Like other parts of the central nervous system, the pineal tissue is also isolated from the meningeal septa by the external limiting glial membrane and basal lamina. This histological organization of the human pineal gland is basically identical with that of mammals, permitting the comparison of pineal calcification of humans and mammalian experimental animals.

3. Pineal and meningeal calcification in mammals

Pineal concretions have been reported to be present in some mammalian species as the cattle, the horse, the sheep, the donkey, the monkey, the guinea pig, the Mongolian gerbil, the mink, the bat and the rat (Rio Hortega, 1932; Lukaszyk and Reiter, 1975; Erdinc, 1977; Krstic and Golaz, 1977; Diehl, 1978; Vollrath, 1981; Allen et al., 1982; Jung and Vollrath, 1982; Welsh, 1984, 1985; Humbert and Pévet, 1991; Lewczuk et al., 1994). Both intrapineal and meningeal calcifications occur is some of these animals.

Meningeal calcification in the pineal of mammals

In the *rat pineal* (Erdinc, 1977; Diehl, 1978; Vígh and Vígh-Teichmann, 1989a; Vígh et al., 1989b; Humbert and Pévet, 1991) corpora arenacea of various size and density occur mainly among the cells of the

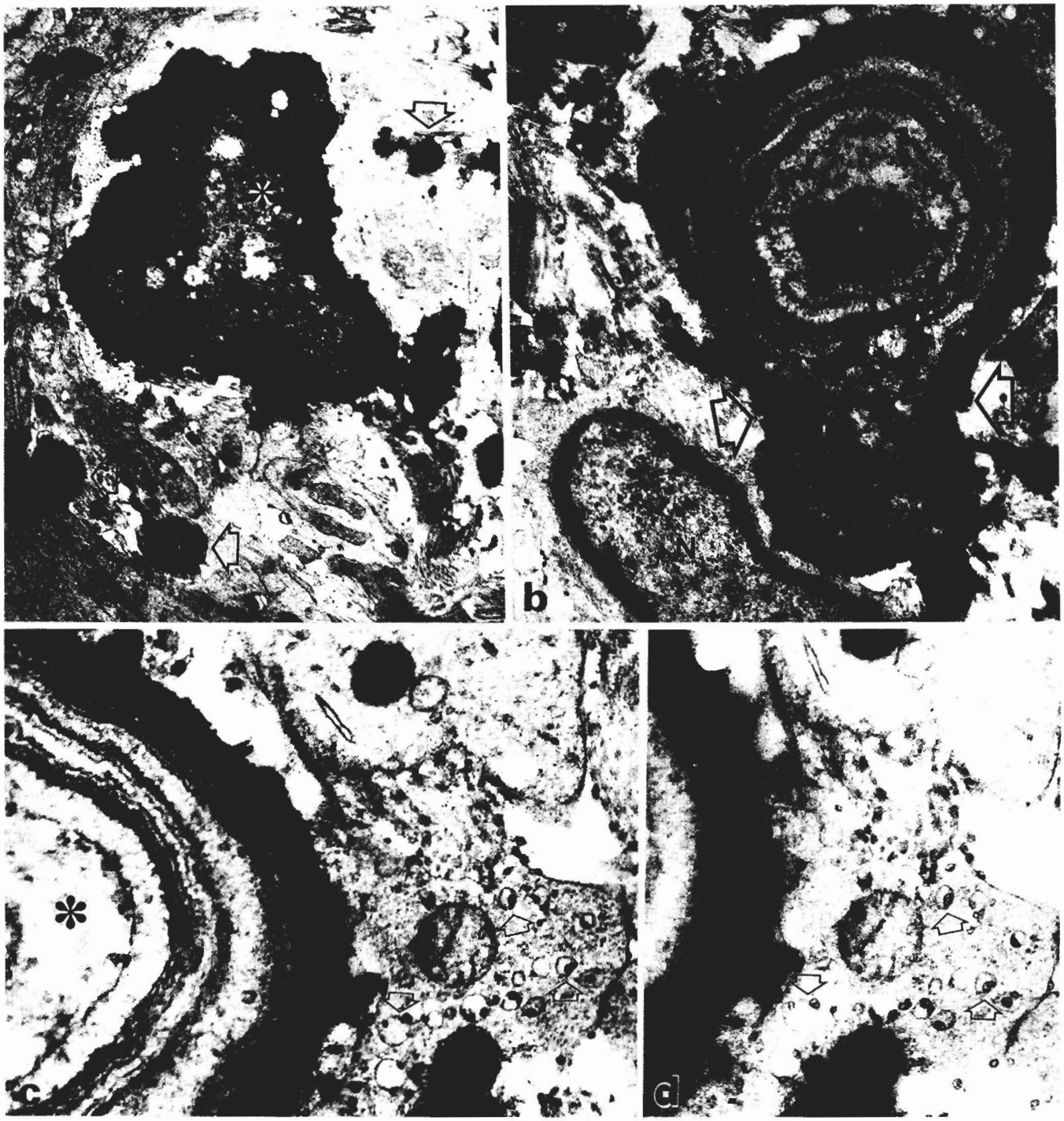

Fig. 3. Pineal calcification in the rat. **a.** Small (open arrows) and large (asterisk) meningeal concrements in the pineal arachnoid. x 10,400. **b.** Fused corpora arenacea in the pineal leptomeninx (marked with open arrows). N: nucleus containing Ca-pyroantimonate deposits of an arachnoid cell. x 15,200. **c.** Ca-pyroantimonate deposits (arrows) in cytoplasmic vesicles of a meningeal cell near a concentric concrement (asterisk). x 24,000. **d.** The same section after decalcination, note the loss of density of Ca-pyroantimonate deposits in cytoplasmic vacuoles (arrows). x 25,000

arachnoid covering the organ around the pia mater tissue. Concrements are absent or rare in 1-to-2-month-old animals, while they are usually present in the 4-to-6-month-old rats (Fig. 3a). In the 4-month-old animals, arachnoid cells contain a varying amount of Ca-pyroantimonate deposits (Fig. 3b). Following decalcination by means of EDTA the sections reacted with pyroantimonate exhibit a smaller density of deposits, indicating their lower Ca-content (Fig. 4c,d). Most of the precipitates occur in locally enlarged intercellular spaces. Here, microacervuli were found (Fig. 4a) suggesting that calcium-rich microenvironment was responsible for the appearance of the concrements (Vígh et al., 1989b).

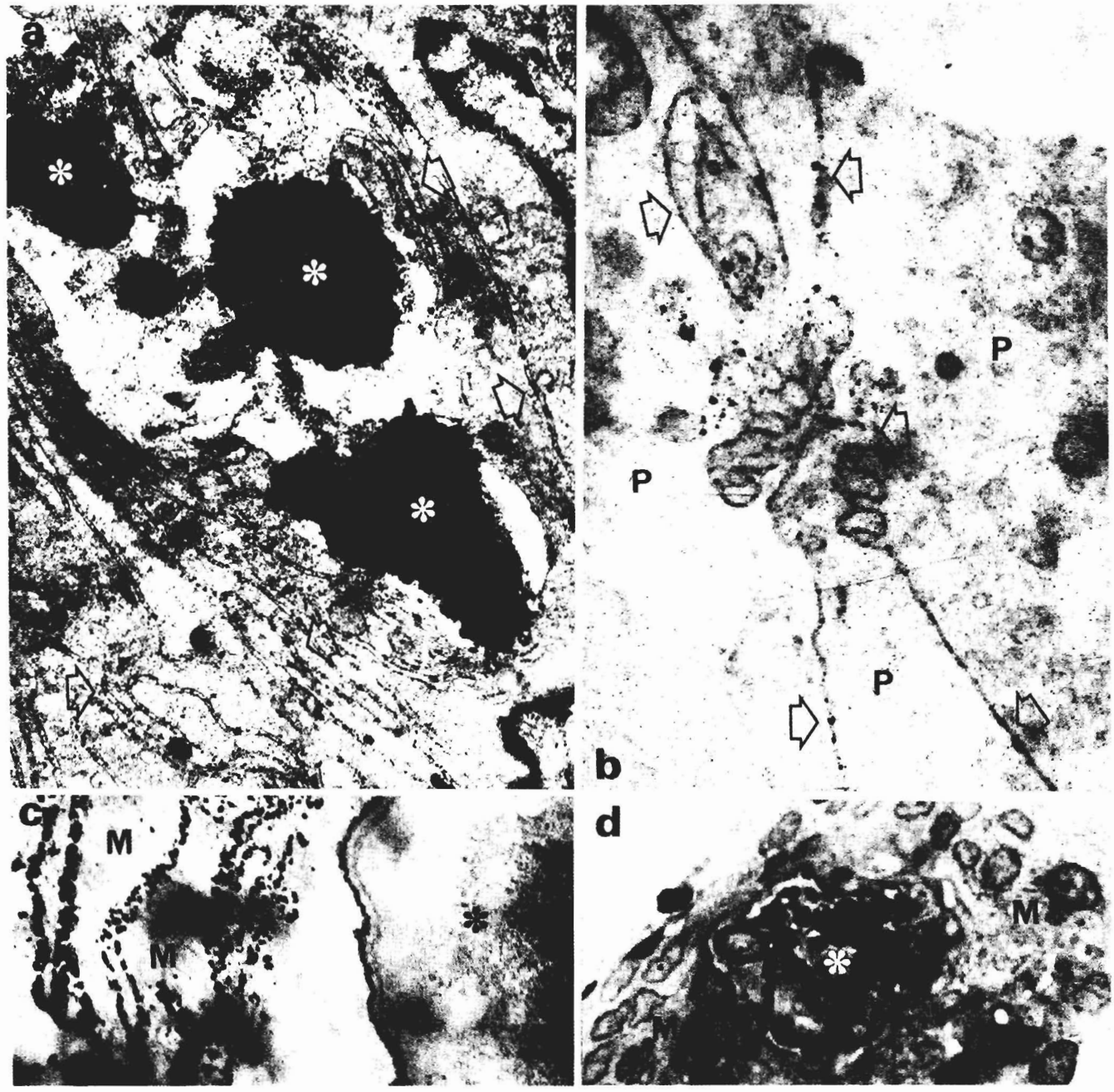

Fig. 4. Meningeal and intrapineal Ca-deposits in the rat. **a.** Micoacervuli (asterisks) in enlarged intercellular spaces of the pineal arachnoid. Arrows: Ca-pyroantimonate deposits alongside the cell membranes. x 21,000. **b.** Ca-pyroantimonate deposits (arrows) alongside the cell membrane of pinealocytes (P). x 26,100. **c.** Alkaline phosphatase reaction alongside the cell membrane of periacervular meningeal cells (M) of the 5-month-old rat. Asterisk: border of an acervulus. x 21,400. **d.** Large concrements (asterisk) in the pineal meningeal tissue of a parathyroidectomised rat. x 1,120

In the actual pineal nervous tissue proper, corpora arenacea are rare in young animals. Small Ca-pyroantimonate deposits are localized on the cell membrane of pinealocytes (Fig. 4b) but there are no signs of acervulus formation in the pinealocytes or elsewhere in the pineal nervous tissue proper, between 1 and 6

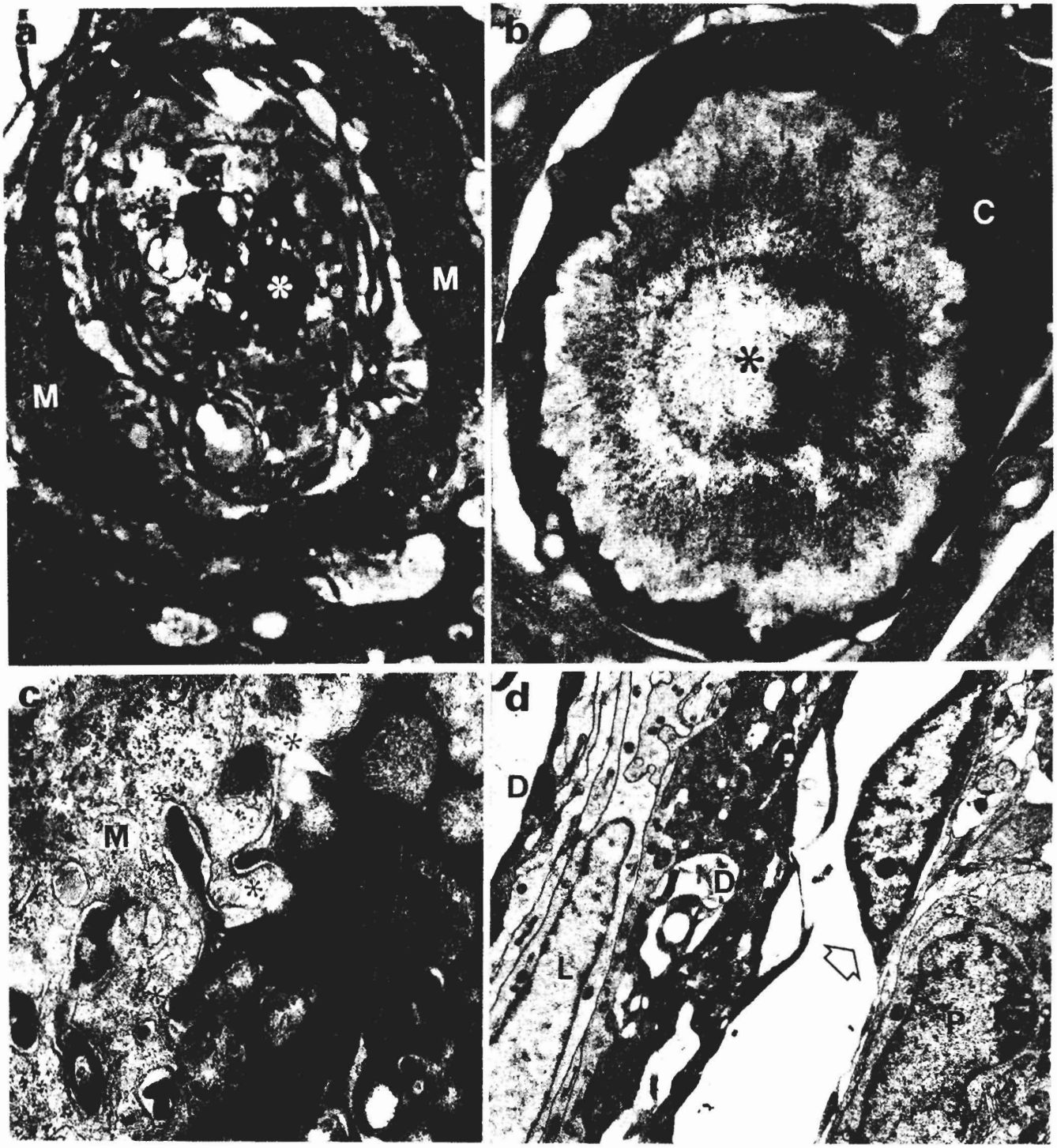

Fig. 5. Meningeal concrements in the pineal organ of the bat. **a.** Calcified area (asterisk) insulated by meningeal cells (M). x 6,300. **b.** Intracellular concentric acervulus (asterisk) in an arachnoid cell (C) of the pineal. x 14,600. **c.** Superficial processes (asterisk) of an acervulus penetrating the cytoplasm of the neighboring meningeal cell (M). x 29,500. **d.** Dark (D) and light (L) cells of the pineal arachnoid. P: pinealocyte; arrow: cell of the pia mater. x 7,000

months of age. These findings demonstrate that corpora arenacea first appear in the leptomeninx of the rat. Similarly to osteoblasts in the bone tissue, periacervular meningeal cells show a distinct alkaline phosphatase reaction on their cell membrane as detected by electron microscopic cytochemical method of Hugon and Borgers (1966) in the rat and mink (Fig. 4c). There is a similarity between arachnoid cells insulating acervuli and ameloblast cells of the rat incisor (Eisenmann et al., 1979; Vígh et al., 1989b) a pattern suggesting that meningeal cells may act like ameloblasts or osteoblasts ("acervuloblasts").

The laboratory rat seems to be especially usefull in studying pineal calcification of meningeal type. Pineals of parathyroidectomised rats contained larger meningeal concrements, than the control animals (Fig. 4c,d), a reasult showing a possible regulation mechanism of the production of acervuli by parathormon and/or calcitonin.

Corpora arenacea were found among the cell layers of the arachnoid of *the pineal organ of the bat* (*Myotis blythi oxignathus*) as well (Vígh et al., 1989a). Concrements occur in lacunar enlargements of the arachnoid, mainly in the thickened dorsal portion of the pineal leptomeninx (Fig. 5a). There is no sign of acervulus formation in the pinealocytes or elsewhere in the pineal nervous tissue proper. These findings confirm the view that corpora arenacea can be produced by the pineal arachnoid. Sometimes, concrements develop inside of meningeal cells (Fig. 5b). Thus, in addition to secretory and resorptive functions, arachnoid cells are

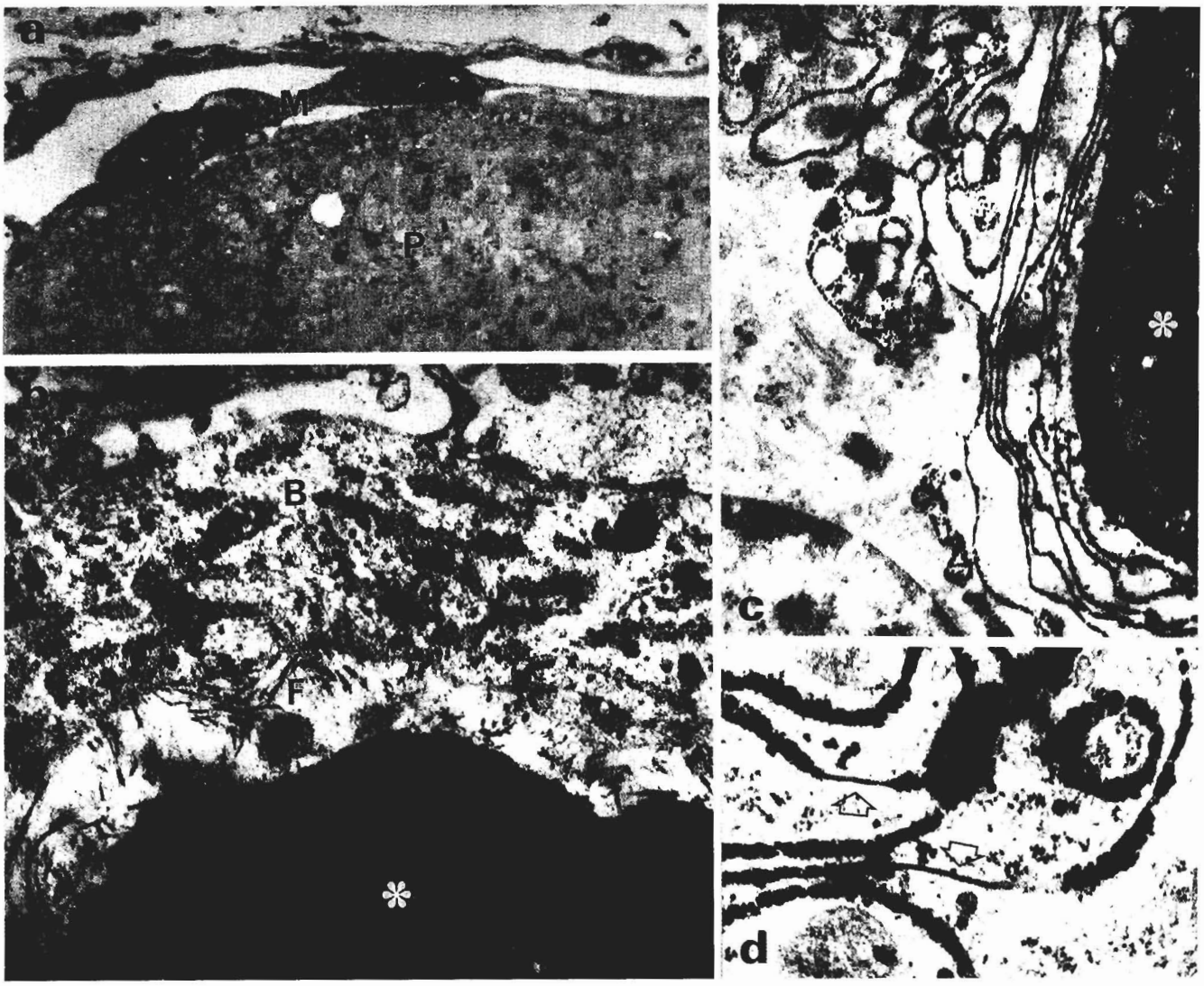

Fig. 6. Meningeal corpora arenacea in the pineal organ of the mink. **a.** Meningeal acervuli in the dorsal arachnoid (A) of the pineal (P). x 420. **b.** Collagen microfibrills (F) and fibroblast (B) bordering the meningeal concrement (asterisk). x 18,800. **c.** Intercellular Ca-pyroantimonate deposits (black dots and lines) around a pineal concrement (asterisk). x 13,500. **d.** Ca-pyroantimonate deposits are missing at the site of tight junctions (arrows) between arachnoid cells surrounding concrements. x 33,000

also capable of acervulus formation (Fig. 5c).

The pineal arachnoid of the bat consists of electron-lucent cells connected by cell junctions to flat sheats and sandwiched on both sides by electron-dense cell rows (Fig. 5d). Several tight junctions connect the neighbouring meningeal cells. Among the superficial cell layers, collagen fibrills form loose bundles. Meningeal acervuli are insulated by collagen fibrills and exhibit concentric layers of various density, similar to those observed in human meningeal concrements of the pineal organ.

Meningeal and intrapineal calcification in the mink

Similarly to the human pineal, the mink (*Mustela*

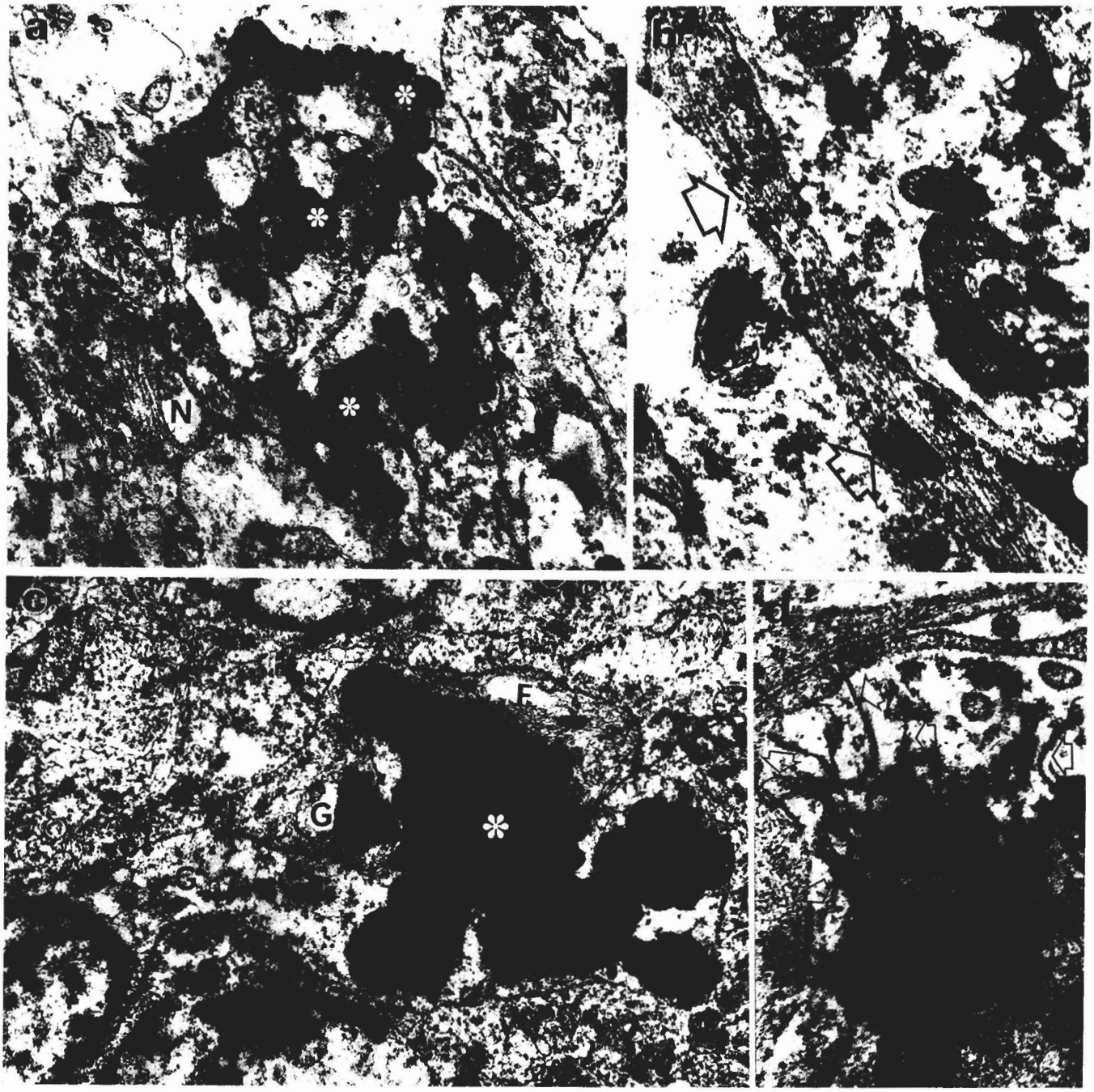

Fig. 7. Intrapineal corpora arenacea in the pineal organ of the mink. **a.** Microacervuli (asterisk) among nerve fibers (N) of the pineal nervous tissue. x 18,000. **b.** Ca-pyroantimonate deposits (black dots and lines) alongside the cell membrane and inside of a pineal axon (arrows). x 21,400. **c.** Microacervuli (asterisk) inside glial cells (G). F: glial microfibrils. x 36,000. **d.** Pin-shaped structures resembling hyroxylapatite crystalls (arrow) in and around a pineal microacervulus (A). x 37,500

vison) has both intrapineal and meningeal concrements (Vígh and Vígh-Teichmann, 1992). The pineal complex in the mink consists of two parts: a larger ventral and a smaller dorsal pineal. Two pineal organ are know in most of the lower vertebrates (see in the next chapter). Both pineals are encapsulated by the meningeal tissue of the brain stem. Acervuli were found primarily in the larger ventral pineal (Fig. 6a). Meningeal cells around

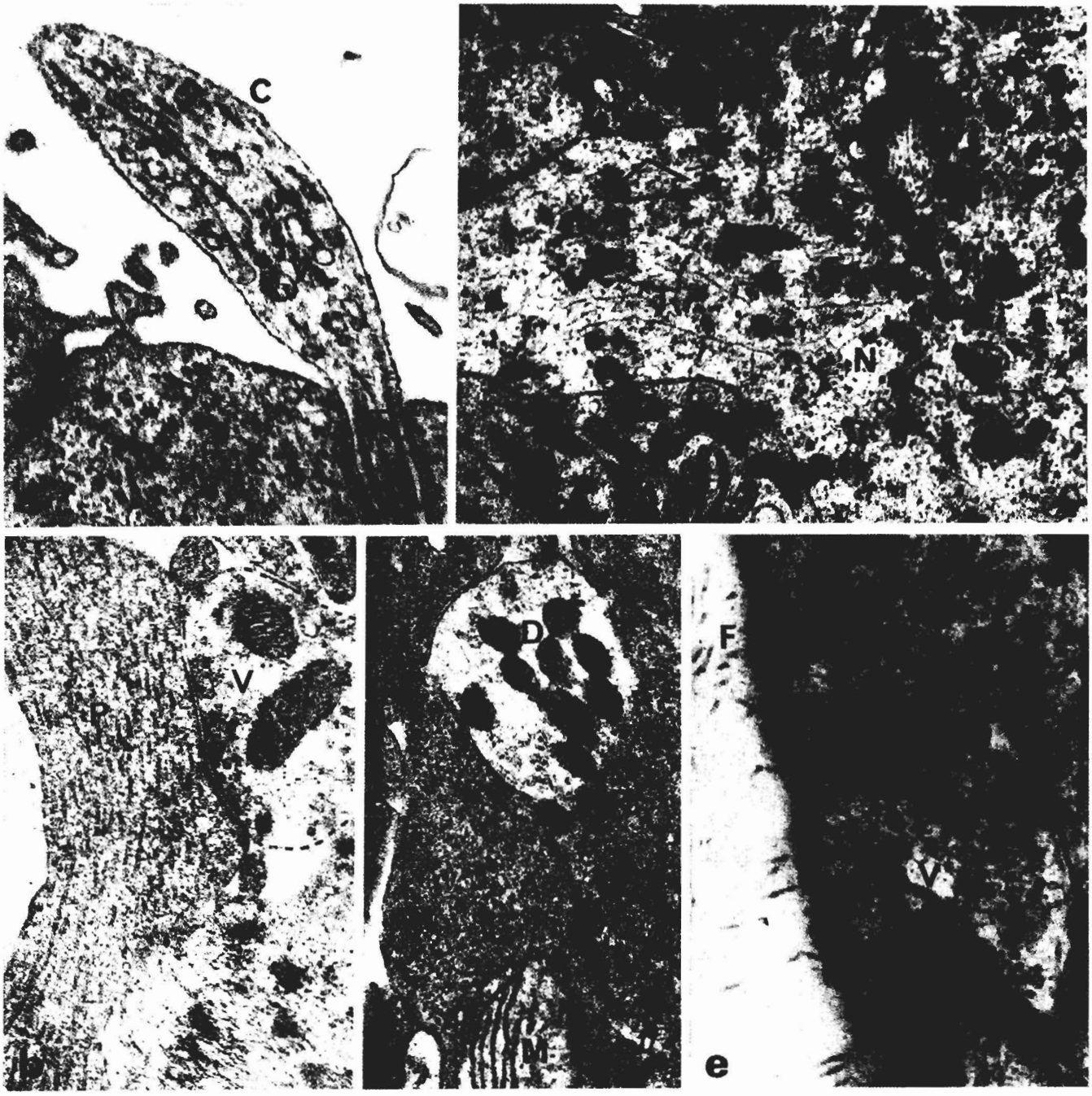

Fig. 8. Histological organization of the mammalian pineal organ. **a.** Photoreceptor outer segment-like cilium of a CSF-contacting pinealocyte (P) of the ferret (Putorius furo). Note the vesicles (V) in the enlarged cilium (C). T: microtubules. x 59,000. **b.** GABA immunoreaction (black dots of immunogold particles) of an axon (cell membrane marked with dotted line) terminating on a pinealocytic process (P) of the cat.V: synaptic vesicles. x 47,000. **c.** Part of a pineal neuron (N). Note the ribbon (R) containing pinealocytic terminal (arrow) on the neuron. T: microtubules. x 8,800. **d.** Glutamate immunoreaction (black dots of immunogold particles) of pinealocytic axons (AX) terminating on the dendrite (D) of a pineal neuron. M: large mitochondrium of the pinealocyte; R: synaptic ribbons. Cat. x 10,000. **e.** Neurohormonal terminals containing synaptic and granular vesicles (V) on the surface of the pineal of the bat (*Myotis myotis*). F collagen fibrils. x 26,000

the concrements often contain an aboundant rough endoplasmic reticulum. There are bundles of collagen fibrills between these cells and the meningeal acervuli (Fig. 6b). The localization of calcium ions detected alongside the membrane of periacervular cells by pyroantimonate cytochemistry suggests a membrane activity as the source of the calcium ions (Fig. 6c). There are no calcium ions at the level of tight junctions of the meningeal cells. These junctions may have a barrier effect for the extracellular diffusion of calcium ions (Fig. 6d).

Intrapineal microacervuli often occur among nerve fibers of the organ (Fig. 7a). Calcium pyroantimonate deposits were found alongside the cell membrane of axons and inside of their cytoplasm (Fig. 7b). Occasionally, concrements are located inside glial cells (Fig. 7c). In the substance of microacervuli, pin-shaped structures resembling hydroxyapatite crystals were found (Fig. 7d). Similar crystals were described in other species as well (Mabie and Wallace, 1974; Krstic, 1976, 1986; Vígh and Vígh-Teichmann, 1992)

The histological organization of mammalian pineal

Similar to the human pineal organ, the pineal of mammalian animals contains pinealocytes, neurons, glial cells, nerve fibers and synapses (Vígh and Vígh-Teichmann 1975, 1988, 1992). In the pineal lobules, pinealocytes are arranged around strait lumens corresponding to the photoreceptor spaces of the photosensitive submammalian pineals. A 9+0-type cilium marks the receptory pole of the pinealocytes which may form an inner-segment-like dendrite terminal in the pineal lumens. The cilia correspond to the outer segments forming photoreceptor membrane multiplications in the pineal of submammalians as well as in certain insectivorous and mustelid mammals (Fig. 8a).

Pinealocytes receive GABA-ergic axon terminals originated from intrapineal GABA-ergic neurons or from extrapineal sources (Fig. 8b). Axonal processes of pinealocytes contain synaptic ribbons and terminate on intrapineal neurons (Fig. 8c,d). Presynaptically, immunoreactive excitatory amino acids accumulate in the pinealocytic axons as well as in the axons of pineal neurons. The latter enter the pineal tract terminating among others in the medial habanular nucleus. These connections represent the neural efferentation of the organ (Vígh-Teichmann et al., 1991; Vígh et al., 1995a,b). The structural basis of hormonal efferentation (melatonin release) is represented by axonal processes (collaterals?) of pinealocytes which form neurohormonal endings. The neurohormonal endings - like those of the neurohypophysis - pierce the perivascular limiting glial membrane to terminate on the superficial basal lamina of the organ in front of vessels (Fig. 8e).

Some pinealocytes of mammals are located at the ventricular surface of the pineal recess (Welsh, 1983). These cerebrospinal fluid (CSF)-contacting pinealocytes in young mammals develop vesiculated outer segments as seen in the ferret (Vígh and Vígh-Teichmann, 1993). Similar ciliary outer segments were found in adult bats (Pévet et al., 1977). These cilia are not opsin immunoreactive with the known antisera but in some species, rod opsin immunoreactivity was observed alongside of the cell membrane of pinealocytic perikarya. These results indicate that, originating from the photoreceptor pinealocytes of submammalians during evolution, the pinealocytes of mammals preserved some photoreceptor traits of these ancestors (Vígh-Teichmann et al., 1993; Vígh-Teichmann and Vígh, 1994). This heritage may be involved in the pronounced calcium storage of the organ. Seasonal changes of the acervuli and the effect of darkness on the number of concretions suggest a connection between fluctuations of ambient light intensity and pineal calcification (Lewinsky et al., 1983; Trentini et al., 1987). Therefore, the investigation of the free calcium ions in the photoreceptor pineal complex and the retina of submammalian species can serve a better understanding of the significance of high intrapineal calcium level.

4. Calcium ions in the submammalian pineal and retina

In most *submammalian species*, the pineal is a light percepting organ containing different types of photoreceptor pinealocytes. Responding to circadian and circannual changes of environmental light, these cells initiate quick and slow behavioral reactions by neural and a hormonal efferentation. In these animals, i.e. in birds, reptiles, amphibians, fishes and cyclostomes, the pineal organs have an organization and chemical composition similar to that of the retina (Vollrath, 1981; Ekström, 1984; Vígh et al., 1985; Vígh and Vígh-Teichmann 1988; Ueck et al., 1989).

The structure of submammalian pineal organs

In most submammalian vertebrates the so-called pineal complex derives from the diencephalic roof in form of two separate organs: the pineal organ and parietal eye in reptiles, the pineal organ and frontal organ in frogs, the pineal organ and parapineal organ in bony fishes and lampreys, respectively (Vollrath, 1981). In addition to neurons and ependymal-glial cells, these organs contain rod- and cone-like pineal photoreceptor cells as shown by morphologic characteristics and immunocytochemical staining pattern (Vígh and Vígh-Teichmann 1981, 1988).

The pineal photoreceptors are polarized cells, with a receptor pole represented by a dendrite-like process forming an inner and outer segment that protrude into the pineal lumen (Fig. 9a). The photoreceptor cilium contains microtubuli in a 9x2+0-type organization. In most species examined so far, the cilia develop a varying amount of photoreceptor membrane multiplications.

In 1980 immunoreactive opsins were demonstrated in distinct photoreceptor cell types of various sub-

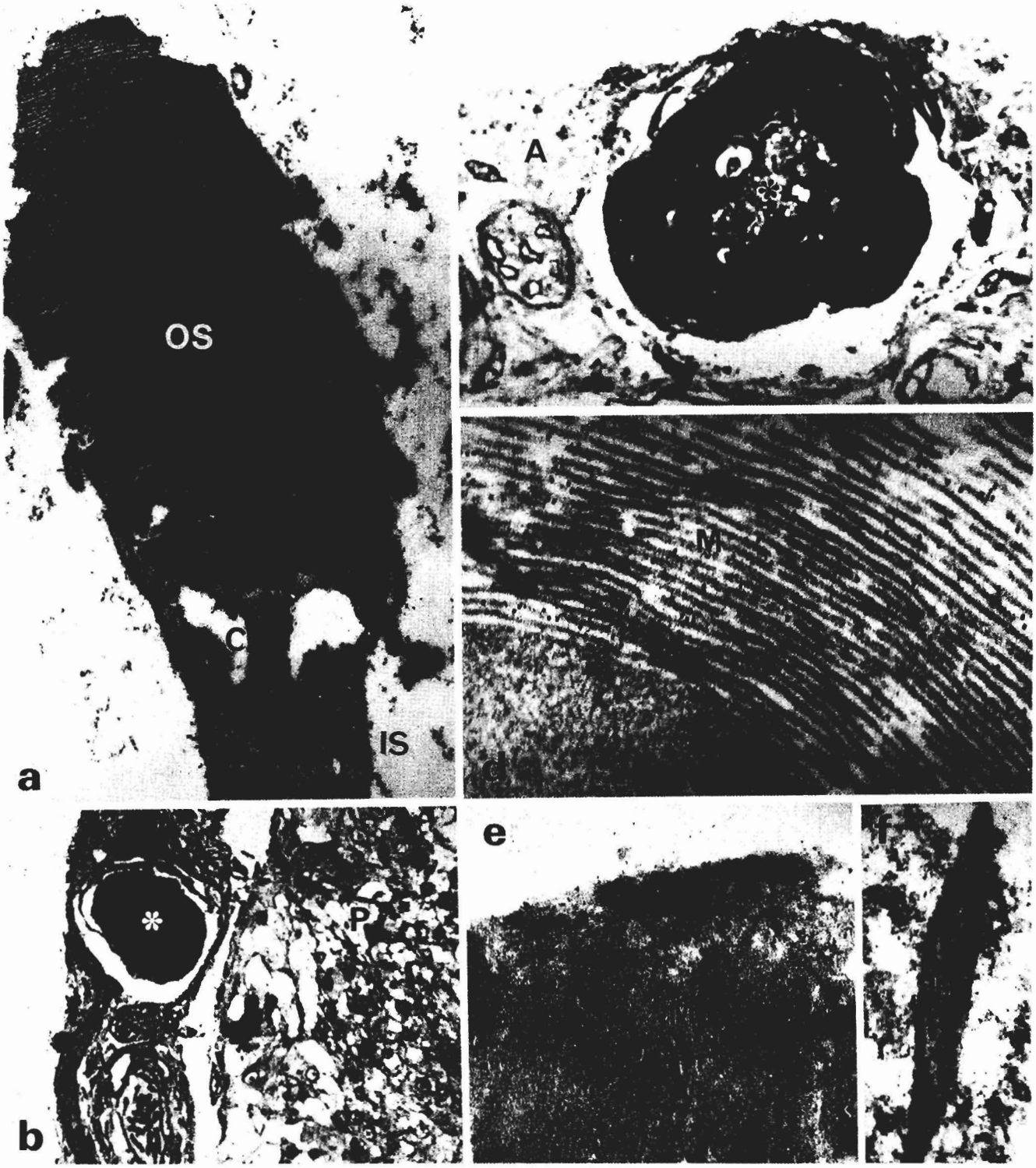

Fig. 9. Structure of the submamalian pineal organ. **a.** Photoreceptor pienalocyte of the pineal organ of the frog (*Rana esculenta*). C: connecting cilium; IS: inner segment, OS: outer segment. x 11,000. **b and c.** Meningeal concrements (asterisks) in the pienal organ of goose. A: pineal arachnoid; P: pineal tissue. b, x 128; c, x 360. **d.** Pinopsin immunoreaction (black immunogold particles) on the photoreceptor membranes (M) of the outer segment of the pinealocyte of the pigeon. C: central matrix of the outer segment. x 56,000. **e.** Ca-pyroantimonate reaction (black dots) of the photoreceptor membranes (M) of the outer segment of frog pinealocyte. x 11,000. **f.** Ca-pyroantimonate reaction (black dots) on the myeloid body (B) of the pineal ependymal cells of the frog (*Rana esculenta*). x 15,000

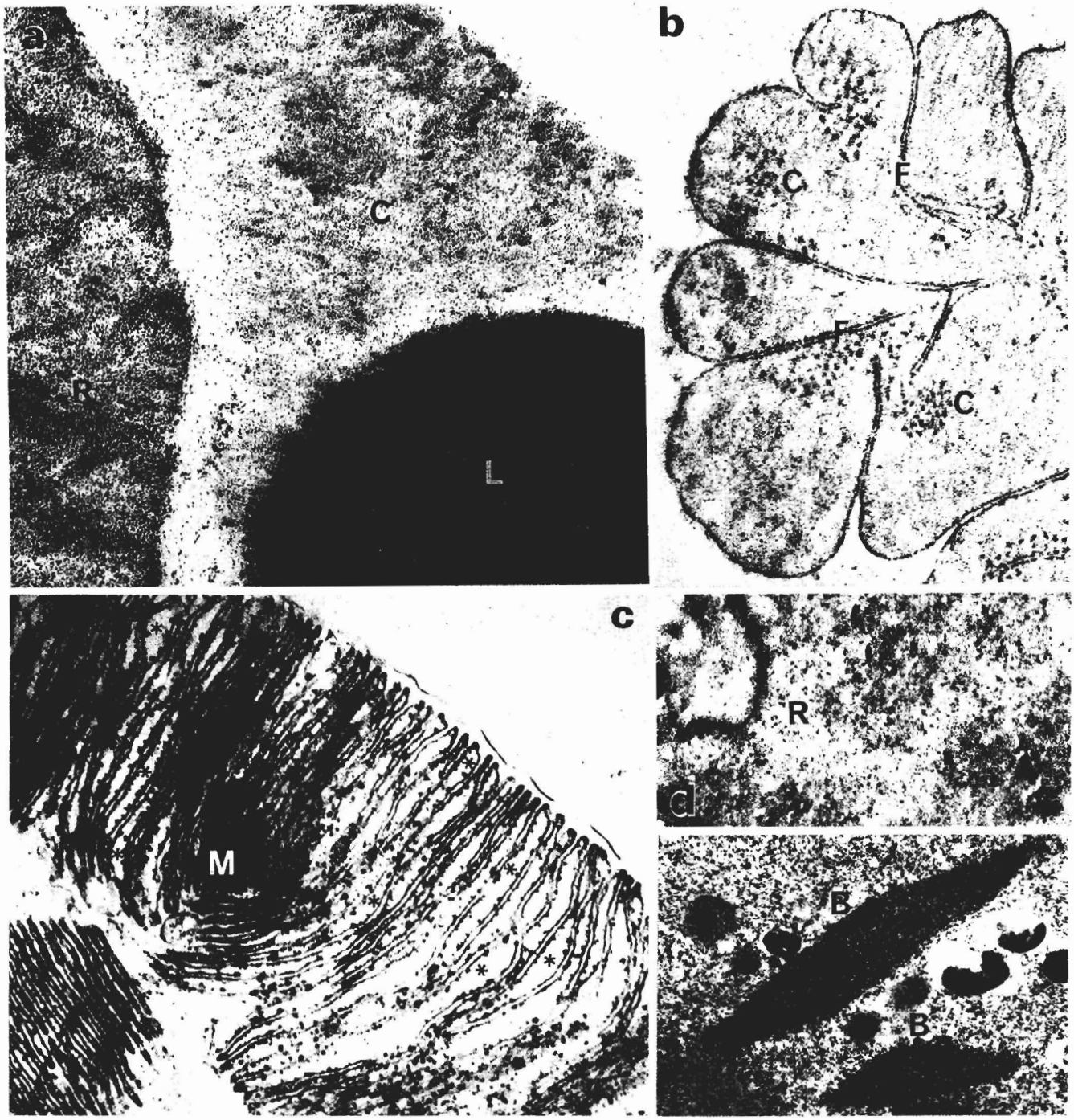

Fig. 10. Ca-pyroantiomonate reaction (black dots) of the retina. **a.** Strong reaction in the retinal rod outer segment (R) and weak reaction in the cone outer segment (C) of the frog retina. L: lipid droplet of the cone inner segment. P: pigment granules of the pigment epithelium. x 27,300. **b.** Cross-section of a photoreceptor disk of the retinal rod of the frog (*Rana esculenta*) Note the Ca-pyroantimonate deposits on the cell membrane fissures (F) and in the cytoplasm (C). x 11,300. **c.** In the retina of the light-adapted rabbit, the Ca-pyroantimonate deposits are concentrated on the extracellular side (asterisk) of the photoreceptor membranes (M). x 57,1000. **d.** Presynaptic accumulation of calcium in the rod-bipolar synapse of the frog. R: synaptic ribbon. x 33,88. **e.** Strong pyroantimonate reaction in the myeloid bodies (M) of the retinal pigment epithelium of the frog. x11,500

mammalian species. These findings initiated an extended search in the pineal cells for substances that are known for playing a role in the phototransduction mechanism of the retina. The presence of several synaptic mediators known in the retina was also demonstrated in the pineal organ. These results indicate a photoreceptive function, not only for the pineal cells of fishes and amphibians but also for those of higher vertebrates like reptilians and birds (Vígh-Teichmann et al., 1980, 1983a,b, 1986, 1988, 1989, 1991, 1993; Vígh and Vígh-Teichmann, 1981, 1986; Korf and Vígh-Teichmann, 1984; Ohshima and Matsuo, 1991; Vígh-Teichmann and Vígh, 1990, 1992, 1994; Okano et al., 1994; Vígh et al., 1982, 1995a,b; Max et al., 1995; Hirunagi et al., 1997).

Calcium histochemistry of pineal photoreceptors

Of submammalian species, *some birds* were found to develop meningeal type concrements in their pineal organ (Fig. 9b). The pineal arachnoid is a multilayered sheath in birds. The pinealocytes are arranged around photoreceptorial lumens with their inner and outer segments protruding into the lumen. The outer segments are pear-shaped, and the photoreceptor lamellae immunoreact with antibodies raised against retinal rodopsins and the pineal specific pinopsin, respectively (Fig. 9d, Hirunagi et al., 1997; Vígh et al., 1997). There are no acervuli in reptiles, amphibians, fishes and cyclostomes, but calcium cytochemistry shows a high intracellular calcium content. The meninges are thin in these animals and do not show any sign of calcification.

Studying the ultrastructural localization of calcium ions in the *frog pineal* organ, several Ca-pyroantimonate deposits were found in the photoreceptor outer segments (Fig. 9e). The outer segments of the immunocytochemically rod-like pinealocytes contained more deposits than cones. A considerable amount of calcium was concentrated in the photoreceptor effector terminals, especially around the synaptic ribbons in the synapses between photoreceptors and secondary pineal neurons. Calcium was also concentrated in myeloid bodies of the pineal ependyma (Fig. 9f). The structural similarity between pineal and retinal photoreceptors is underlined by the similar localization of calcium ions as detected by the PPA method.

Localization of calcium ions in the retina

Retinal outer segments are aboundant in Ca-pyroantimonate deposits. Similarly to the pineal, retinal rods contain more deposits than cones (Fig. 10a). On cross-sectios of outer segments the calcium is localized alongside and beneath the fissures of the photoreceptor disks (Fig. 10b). In dark adapted animals the presence of calcium was detected on both the cytoplasmic and intradiscal side of the photoreceptor membranes. In light adapted animals (frogs and rabbits), Ca-pyroantimonate deposits were concentrated on the extracellular (intradiscal) side of the disks (Fig. 10c). This result demonstrates a shifting of calcium ions to the intradiscal space during photoreception (Athanassious et al., 1984; Vígh and Vígh-Teichmann, 1989a; Vígh-Teichmann and Vígh, 1992).

Calcium is also accumulated in the presynaptic part of synapses between photoreceptors and bipolar neurons, preferentially around synaptic ribbons (Fig. 10d). The retinal pigment epithelium contains a considerable amount of calcium as well. The highest number of Ca-pyroantimonate deposits were found alongside of the membranes in the myeloid bodies of the pigment epithelium (Fig. 10e), suggesting a role in the calcium accumulation for the myeloid bodies. It is worth mentioning that myelin bodies are present in the glial cells of the pineal organ of lower vertebrates as well, and show a pronounced accumulation of calcium pyroantimonate deposits (see: Fig. 9f). The pineal ependyma and the retinal pigment epithelium seem to have a similar role regarding its calcium accumulation by their myelin bodies (Vígh and Vígh-Teichmann, 1989a).

5. General conclusions

As mentioned in the Introduction, the fundamental question regarding the pineal calcification is: why the pineal contains more calcium than other tissues? Comparing the structure and calcium content of the pineal organs of various vertebrates, we can get an insight into its specific organization, and thus, the possible source of the high pineal calcium level can be unravelled.

The most simple pineal complex, the pineal and parapineal organs of lampreys (cyclostomes) are thin-walled, saccular evaginations of the diencephalic roof. They have a retina-like structure composed of photoreceptor pinelocytes, secondary neurons, synaptic neuropil and ependymal-glial cells. Calcium ions were found to be concentrated in the photoreceptor outer segments of pineal cells and in myeloid bodies of pineal ependymal cells. During the course of the pineal evolution from amphibians through mammals, the gradual increase of the cell number is accompanied by a reduction of the luminal volume of the organ. Contrary to the general belief about the glandular transformation of the pineal during evolution, the histological organization conserves many similarities to that of the retina and still have both hormonal and neural efferentation already present in lampreys (Vígh and Vígh-Teichmann, 1988, 1992).

Since the pineal develops by folding of its wall, we can consider the pineal organ of higher vertebrates, including the human as a "folded retina", having both neural and hormonal outputs. Similar condensation of the wall of the retina during evolution would not have been possible because of its function as a planar screen adjusted in the focal plane of the ophthalmic refractive tissues (Vígh et al., 1997). The comparison of pineal and retinal photoreceptors of submammalians with calcium histochemistry demonstrate a striking similarity between

the two organs. It is known that calcium plays an important role in the photochemical transduction process (Yau and Nakatani, 1985; Fain and Matthews, 1990; Kroeber et al., 1997). The localization of calcium ions depend upon whether the photoreceptors are light- or dark adapted. In dark adapted state free calcium ions are localized on both sides of the photoreceptor membranes of the outer segment, while in light adapted animals they are accumulated on the extracellular side of the membranes (Athanassious et al., 1984; Vígh and Vígh-Teichmann, 1989a,b; Vígh-Teichmann and Vígh, 1992; Vígh et al., 1997).

Corpora arenacea show a concentric lamination of dark, protein-rich and light, calcium rich layers (Mabie and Wallace, 1974). The lamination may correspond to fluctuating pineal activity following the circannual changes of the sunlight intensity (Vígh and Vígh-Teichmann, 1992). A relatively high incidence of pineal calcification was found in Uganda (Mugondi and Poltera, 1976) probably due to the significantly higher light radiation intensity near the equator.

In the evolution, the differentiation of neural elements is accompanied by a higher level of cytological calcium exchange, due to higher electrical activity. The pineal organ of mammals is larger in size than that of fish and amphibia, the pineal lumen is narrow, and its wall is thickened. A high amount of pineal cells exhibiting Ca-pyroantimonate deposits are condensed in the organ. In the small-sized pineal of submmammalians the calcium ions can presumably diffuse away easier from the thin walled organ and do not form concrements.

The study aiming at the ultrastructural localization of calcium ions around the meningeal concrements of mammals, including human, revealed a barrier function of tight junctions among cells of the pineal arachnoid. In higher vertebrates the meningeal cover of the pineal consists of several cell layers and contains calcified concretions. There are no calcified deposits in the thin layered meninges of the pineal of lower vertebrates. In some mamals meningeal calcification of the pineal precedes the appearance of intrapineal concrements. In other mammals, only meningeal acervuli are present in the pineal organ, underlining the role played by the multilayered mammalian arachnoid in the calcification process (Vígh et al., 1989a,b; Vígh and Vígh-Teichmann, 1989a; Lewczuk et al., 1994).

Finally, summarising the data available at present about pineal calcification, we can conclude that the acervular formation depends on a *multifactorial mechanism*.

1. It was suggested, that the high rate of Ca-exchange in pinealocytes connected to their receptor and effector membrane functions may be the source of free Ca^{++} ions. Pineal of higher vertebrates is histologically organized like a folded retina having both hormonal and neural efferentation. Mammalian pinealocytes seem to preserve several ancestral traits of submammalian receptor cells and accumulate free Ca^{2+} on their membranes.

2. In the thin walled retina and in the similarly organized pineal of submmammalian species, calcium probably diffuses easier, thereby preventing the formation of concrements. Myeloid bodies of the pineal glial cells and retinal pigment epithelium of lower vertebrates may take part in calcium binding, inhibiting the formation of acervuli. In the larger pineal of mammals the condensation of a higher amount of pineal cells might contribute to increase the local calcium concentration.

3. The rate of concrement formation is probably proportionate to the phylogenetic state of development: more developed vertebrates exhibit higher calcium exchange rate due to its higher membrane activity.

4. In addition, the barrier function of the multi-layered pineal arachnoid and the tight-junctions among their cells in mammals seem to promote the intrapineal concentration of calcium ions. Presumably acting under the influence of calcitonin, some alkaline phosphatase containing arachnoid cells may function like osteoblasts ("acervuloblasts") in the formation of acervuli.

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