Histol Histopath (1991) 6: 387-393

Histology and Histopathology

Circadian and seasonal variations in pineal gland intercellular canaliculi in the white rat

F. Martínez Soriano, C. Cimas García and A. Ruiz Torner
Morphological Sciences Department, Valencia University, Valencia, Spain

Summary. Seventy Wistar rats are used to study the changes in pineal intercellular canaliculi over a 21-hour period and for two different photoperiods (pre-autumn, first week of September, and winter, first week of February). The study considers these changes at pineal body, cortical and medullar level separately, and compares the values obtained. The results show variations in canalicular surface at different point times (10:00, 14:00, 18:00) and for both photoperiods.

The variations are found to favour the cortical layer, and are also observed between nocturnal and diurnal hours. Canalicular surface to greater during the diurnal hours of both photoperiods.

Interesting histological findings are described that suggest an important function of the intercellular canaliculi in pineal gland metabolic exchange.

Key words: Pineal gland, Intercellular canaliculi, Circadian changes, Photoperiod changes

Introduction

The perivascular space of the rat pineal gland is of great importance in the exchange between pineal parenchyma and the capillaries. Morphologically there are well defined zones (for revision see Vollrath, 1981) where variable numbers of pinalocytic processes pass over the discontinuous outer basal lamina (Gusek and Santoro, 1961; Rodin and Turner, 1966; Arstila and Rhinne, 1967) depending on the species under study (Anderson, 1965; Watenberg and Gusek, 1965; Watenberg, 1968).

While the perivascular spaces of the pineal gland are morphologically well defined, in most species a number of them possess extensions situated between the pinealocytes and, in the absence of an inner basal lamina, contact with the cytoplasmic membrane. These extensions or channels were described in the literature as «widened intercellular spaces» (Rhodin and Turner, 1966; Arstila and Rinne, 1967; Lues, 1971), «interfacial lakes» (Wolfe, 1965; Romijn, 1973) «circumluminar arrays» (Wolfe, 1965) or «intracellular canaliculi» (Quai, 1974; Krstic, 1975, 1979).

The channels are ramified primary, secondary and tertiary branches with a three-dimensional distribution, and forming an interglandular labyrinth with frequent anastomoses (Krstic, 1979). The suggested function of this intercellular channel system is the release of pinealocytic substances into the perivascular spaces or the action of humoral substances of the pinealocytes (Quay, 1974; Krstic, 1979).

TEM observations are more common than SEM studies, and with the exception of Wolf's study (1965) descriptions are brief. These studies confirm the general occurrence of these channels in many mammals.

On the other hand, functional variations were reported by Quay (1974), who observed changes in channel width depending on the hour of the day; this suggest a circardian rhythm in channel diameter.

As pineal intercellular channels are presumably of importance in the relations between pineal tissue and perivascular space (Martínez Soriano et al., 1984) this study was made to examine in more detail the normal ultrastructure of the channels in the superficial pineal gland of the ultrastructure of the channels in the superficial pineal gland of the normal adult rat, along with their relation to pineal tissue. Variation in channel surface over a 24-hour period at both cortical and medullar pineal level was also studied for two different seasonal periods.

Materials and methods

Seventy male Wistar rats (divided into sets of 35 each) weighing 275 ± 18 g. were used in the study. All

Offprint requests to: Dr. F. Martínez Soriano, Departamento de Ciencias Morfológicas, Facultad de Medicina, Avda. Blasco Ibáñez 17, 46010 Valencia, Spain

were sacrificed (Nembutal 10% intraperitoneally in groups of five each, every four hours (06:00, 10:00, 14:00, 18:00, 22:00, 02:00, 06:00) and between September, 11 and 12 (pre-autumn period). The process was repeated a second time between February 2 and 3 (winter period) 1985.

All animals underwent intracardiac 5% glutaraldehyde perfusion following saline cleansing. The specimes were refixed with osmium tetroxide. Dehydration followed in graded acetone series and contrasted with 5% uranyl acetate and embedded in Epon.

The canalicular surfaces were determined from photographs fo semithin sections (1 μm) under x 100 magnification and amplified 10 times. Photograph surface area was 10 cm^2.

The procedure employed to calculate surface area was as follows:

Total photo surface area (TPS) = Photo weight (PW).

Canalicular surface area (CS) = canalicular surface weight (CSW).

The weight (in g) was calculated by cutting out each surface and weighing it with a standard balance. This provided the values of (TPS), (PW) and (CSW), whereby (CS) was easily calculated. These values were obtained in cm^2, multiplied by 10 (8) and divided by the number of magnifications; this provided surface area in μm^2.

This procedure was employed with four photographs, each corresponding to a different section per animal, and including both the central and peripheral zone.

Statistical evaluation of the data obtained was carried out by applying the Student t-test for continuous variables and small samples. Data processing was done with the Statworks and Systat statistical programmes, supported by a graphis software package on an Apple McInstosh Plus Computer.

Results

The results obtained are given in the Diagrams. The differences between cortical and medullar canalicular surfaces in both groups were not statistically significative for either period (Diagram 1). On the other hand, the differences in surfaces between the nocturnal and diurnal hours were significative at cortical ($p < 0.0001$) and medullar level ($p < 0.0005$) during the pre-autumn period (Diagram I). The differences canalicular surface were also significative during the winter period ($p < 0.0005$) (Diagram I).

The comparison between cortical and medullar layers during the nocturnal and diurnal hours for both photoperiods is given in Diagram II. There were significative differences in the nocturnal pre-autumn ($p < 0.0005$) and winter period ($p < 0.0001$) and during diurnal hours ($P < 0.0005$) of both periods, always in favour of the cortical canalicular surfaces.

No significative differences were found between either cortical or medullar canalicular surfaces in either period. (Diagram I). Likewise, no significative total differences were observed between either photoperiods.

On the other hand, the differences between cortical and medullar canalicular surfaces at each of the point-times considered were significative at 10:00 ($p < 0.0005$), 14:00 ($p < 0.0001$) and 18:00 ($p < 0.0005$) during the winter period, and, at the same hours ($p < 0.0005$) for the pre-autumn period. In each case, the differences favoured the cortical surfaces (Diagram III).

No significative differences were found between the point-time layers of winter and pre-autumn period (Diagram IV).

The ultrastructural study of intrapineal channels revealed a network of large and small prolongations often found to be continuous with the perivascular

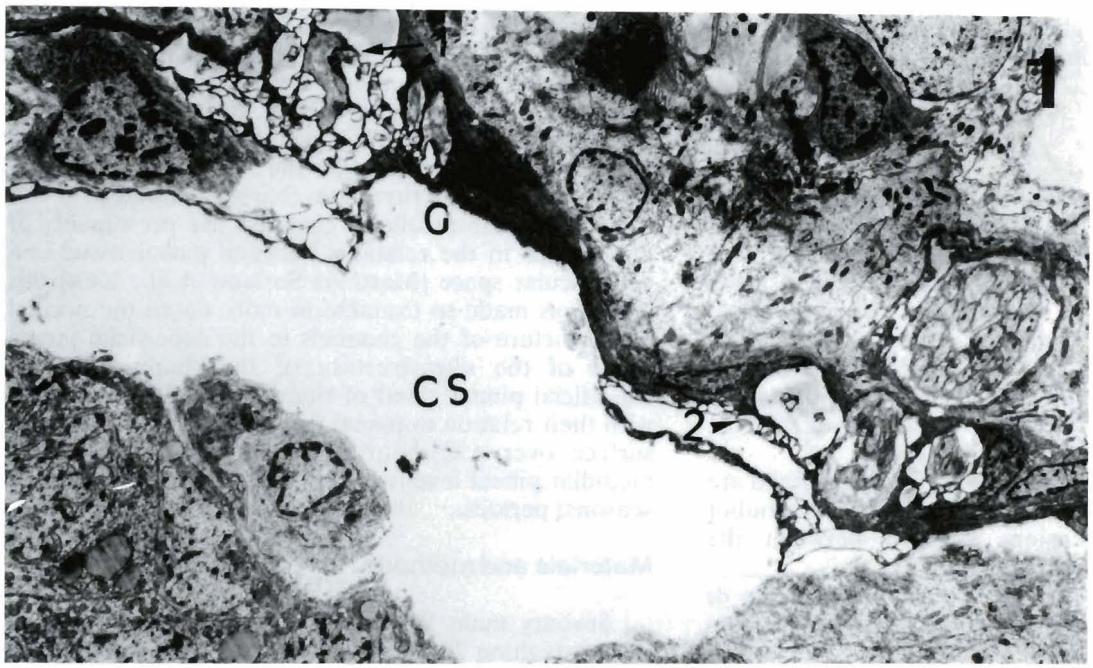


Fig. 1. Panoramic view of "cortical" channels x 7,500 (G) Glial cells (CS) Canalicular space. (1 and 2) Cytoplasmic dilatations of the Glial Cell.

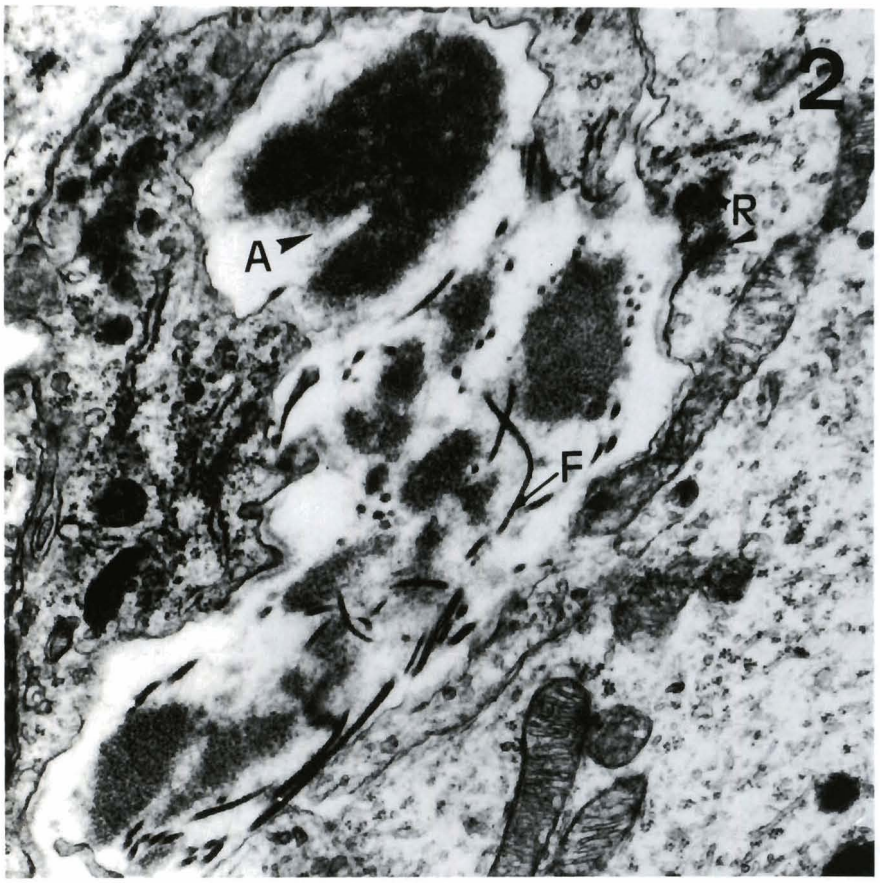


Fig. 2. Intercellular channels with fibrillar and granular material (x 17,000) (A) Amorphous material (F) Fibrilar material (R) "Synaptic" ribbons.

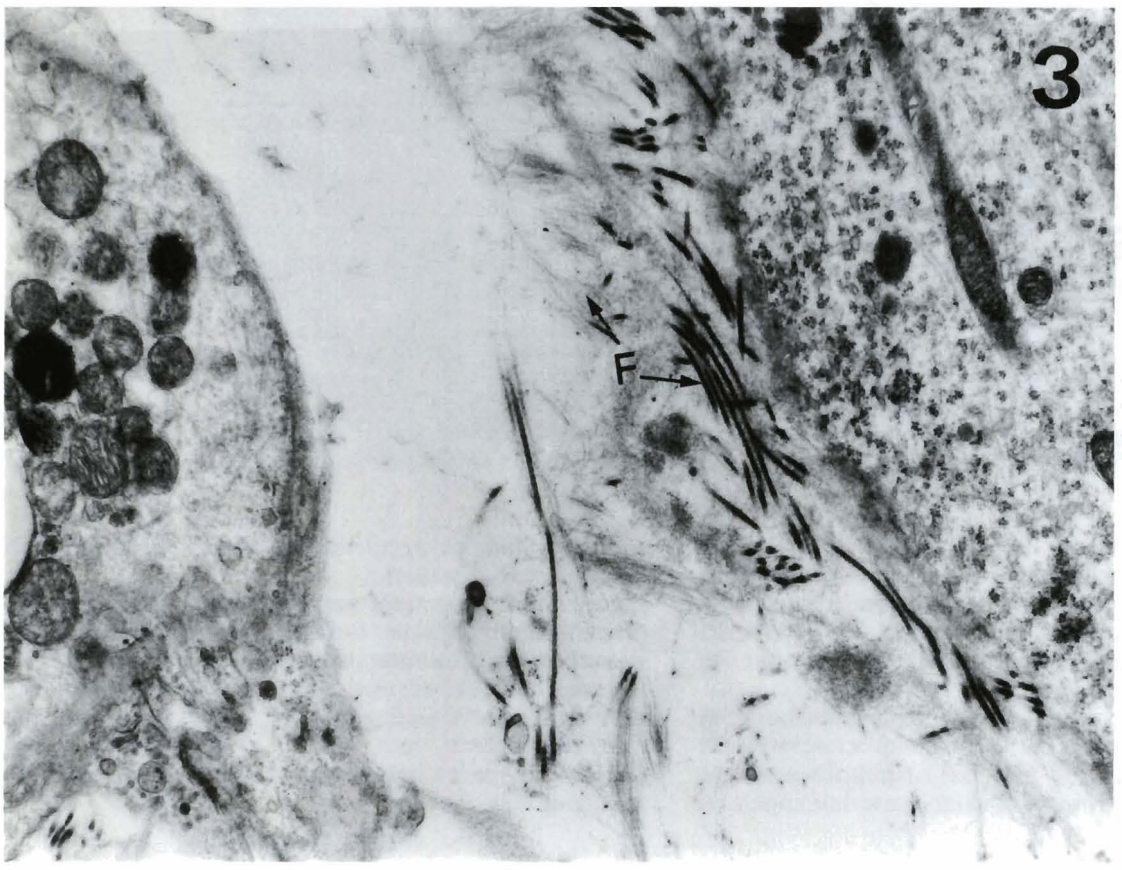


Fig. 3. Intercellular channels with abundant (F) fibrillar contenst (x 17,000).

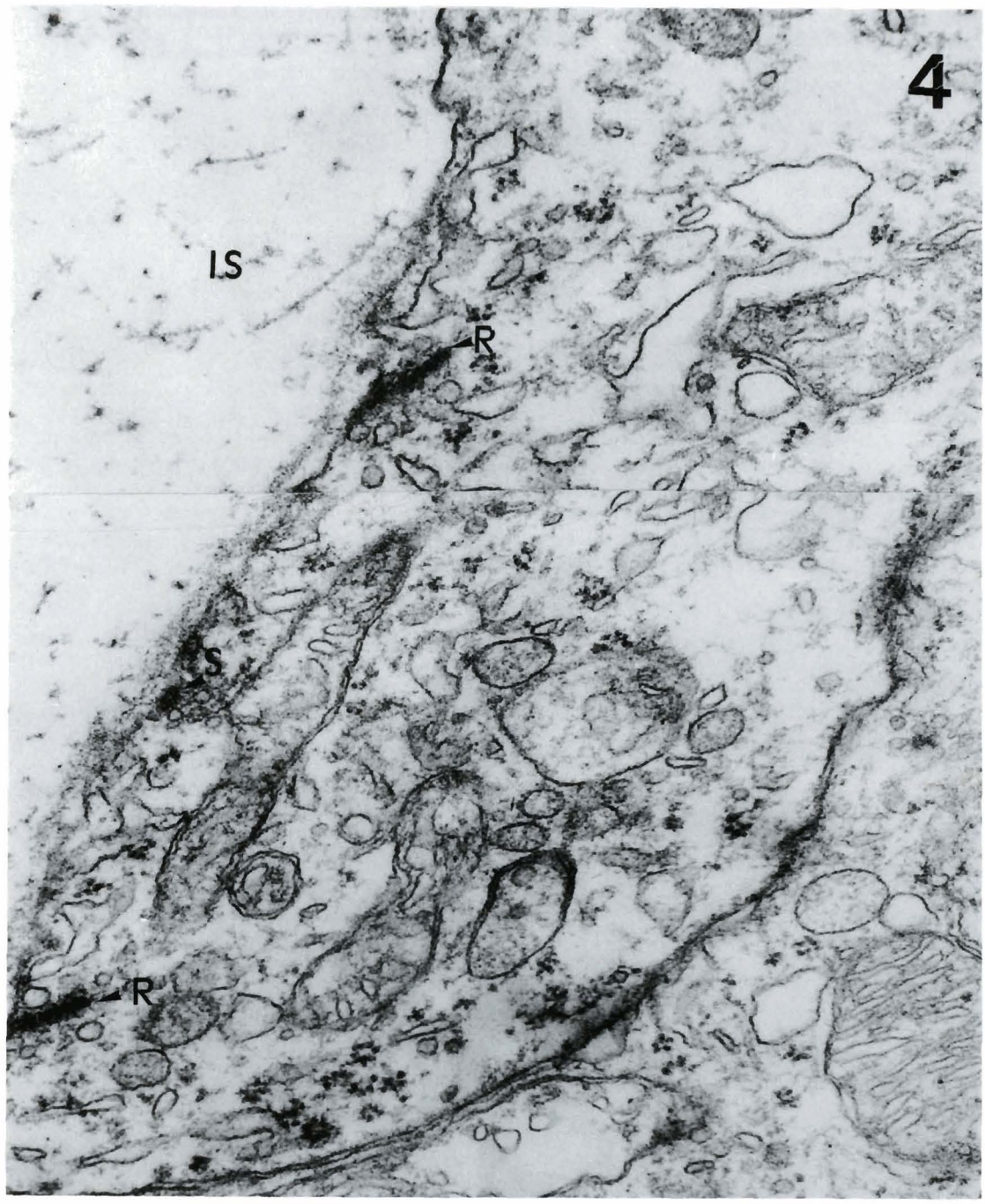


Fig. 4. "Synaptic "ribbons (R) and spherules (S) adhered to pinealocytic membrane (x 36,000) (!S) Intercellular space.

spaces. In the distal or C regions (Vollrath, 1981) analyzed here, the channels presented large dilations particularly apparent in the capsular regions. These channels were frequently surrounded by glial cells presenting prolongations in close relation to the canalicular walls (Fig. 2).

A more detailed study of these spaces throughout the parenchyma showed the presence of an amorphous and filiform intraluminal material of unknown nature (Figs. 2, 3). The granulo-fibrillar material appeared to come from cell structures close to the canalicular margins or within them.

We have observed «synaptic» ribbons along the pinealocyte membranes delimiting the canalicular walls, though never within the canalicular spaces (Fig. 4).

Long glial cell prolongations of different types with dilations and vacuolizations containing granular and filiform material very similar to that found in the canalicular spaces have been observed in proximity to the latter (Fig. 5).

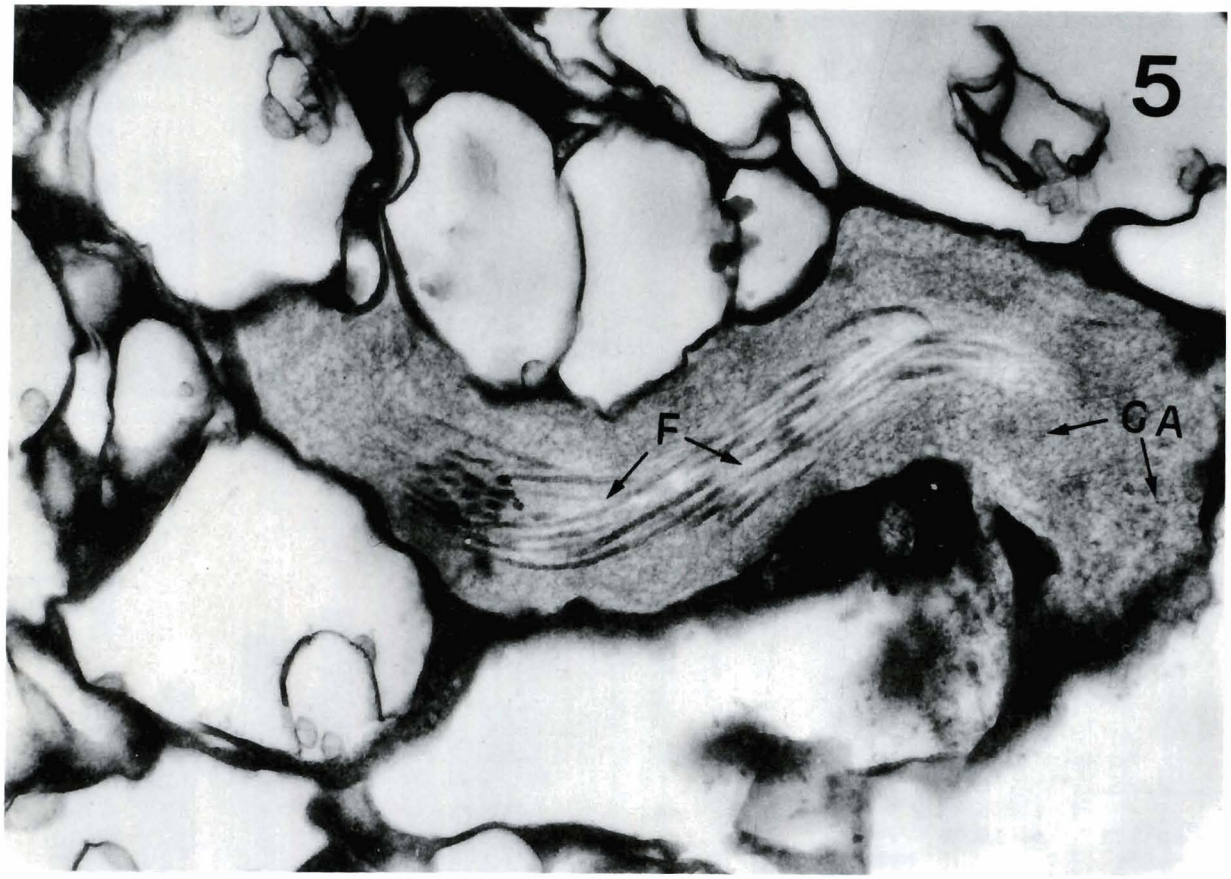


Fig. 5. "Detail of figure 2. (x 36,000). Vacuolar dilatations of the Glial cells with (F) fibrillar and granular (GA) content.

Discussion

Our observations in relation to the intercellular channels confirm the circardian rhytmiticity in the opening and closing of these structures (Quay, 1973, 1974). No global, nor seasonal point-time cortico-medullar differences are found in channel surface.

However, the nocturnal and diurnal differences at cortical and medullar level for both groups of animals were statistically significative.

Likewise, significative differences were observed between the cortical and medullar levels during the nocturnal as well as diurnal hours, and for both periods. (Diagram II). In each case, the larger canalicular amplitude corresponded to the cortical zone. This may be explained by the fact that the pineal vessels hardly penetrated beyond the peripheral zone.

Consequently, if the pineal channels are true metabolic transport pathways, the greatest variation in channel surface areas should logically occur in the region richest in blood vessels.

On the other hand, this function as a metabolic transport mechanism found in the intercellular pineal spaces has also been observed in other glands, e.g. the thyroid gland (Martínez Soriano, 1972) where the coloid material has access to the perivascular space

Diagram I. Cortico-medullar differences

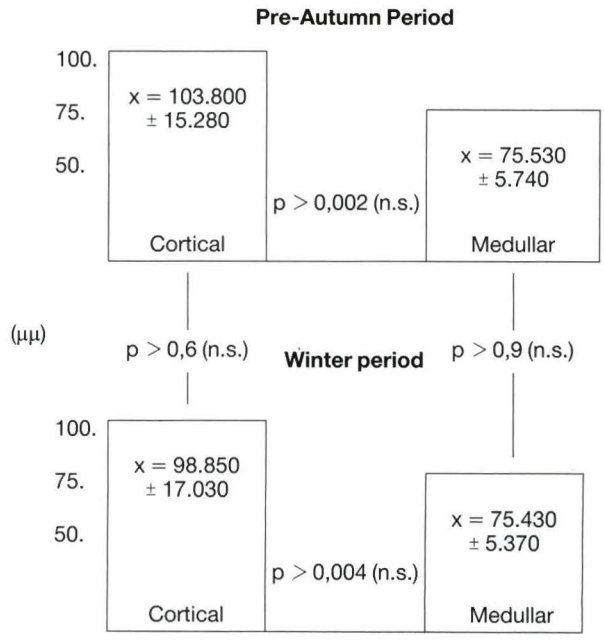


Diagram II. Light-Dark relative values

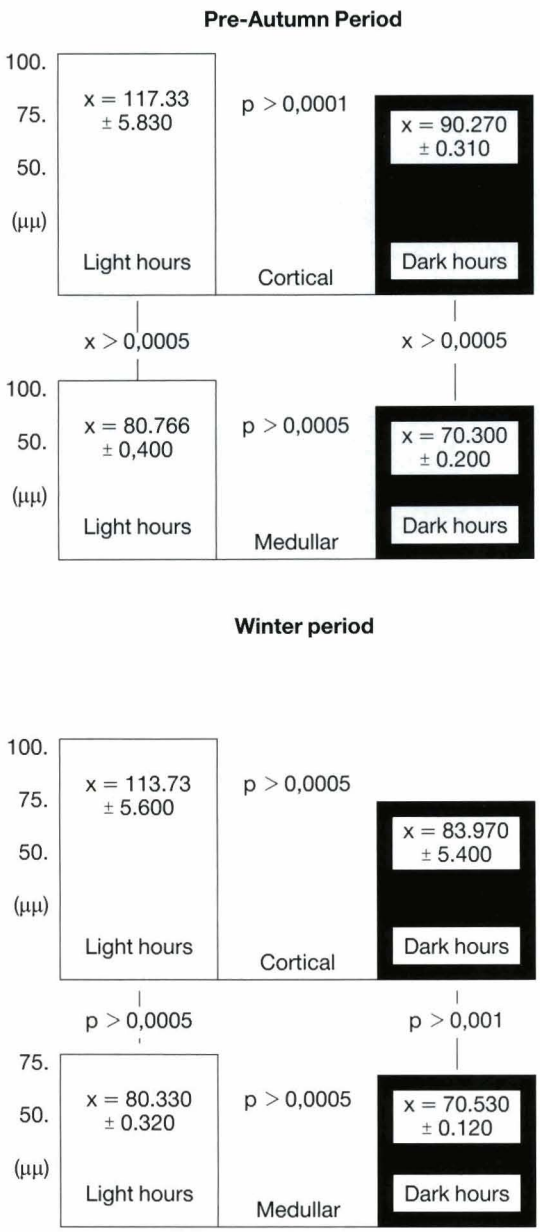
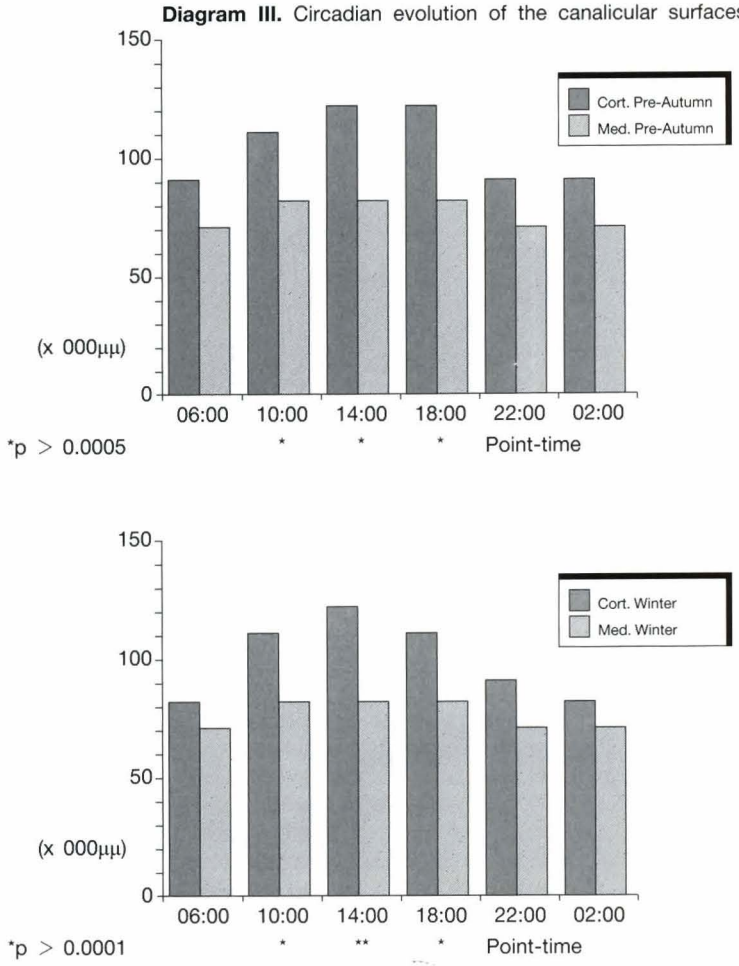


Diagram III. Circadian evolution of the canalicular surfaces



in periods of high glandular activity by means of the dilations found in the intercellular follicular spaces.

Similar results have also been reported for the hypophysis following various stimuli (Lloret, 1976; Escriba and Martínez Soriano, 1976).

Consequently, these variations may well be a mechanism characteristic of different functional states of the gland.

In agreement with other authors, we have found intracanalicular lipids, grannean material, vesicles and fibrillar elements, as well as signs of secretion of this material into the canalicular spaces.

These observations and the light- and hour-dependent surface variations agree with the suggestion made by Quay (1974) as to their functional role in pineal metabolism.

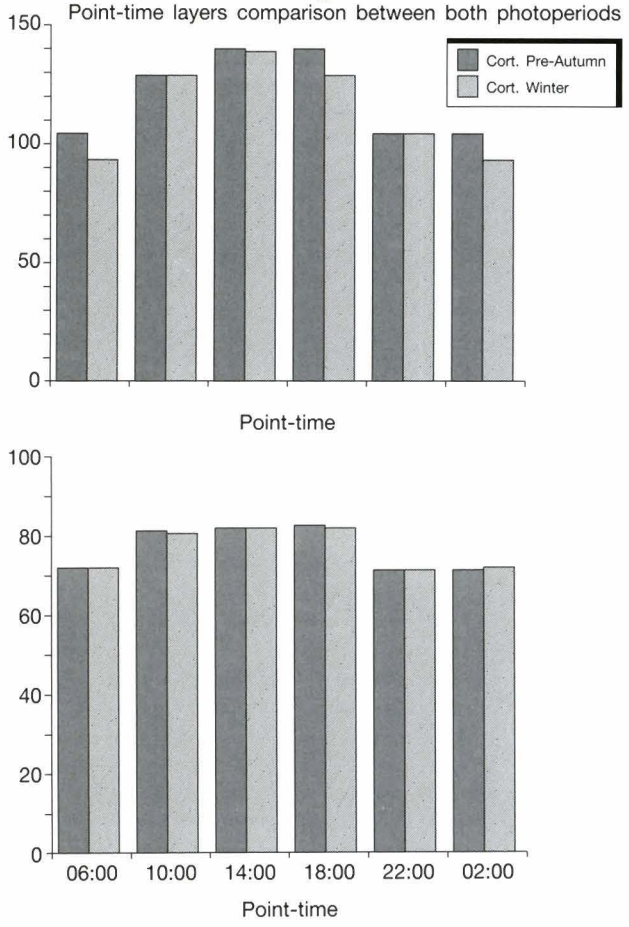
Another interesting aspect is the appearance of fibrillar material within the channels (Gusek and Santoro, 1961; Wolfe, 1965; Krstic, 1965; Arstila and Rinne, 1967). A number of authors consider these elements to be collagen fibres; though most believe their origin to be unknown. We have found this material to be similar to that observed within the neighbouring glial cells (Fig. 2). Moreover, most of it is situated in areas in which glial elements predominate.

Recently, a number of authors (Sozo and Papasomenos, 1983; Highley et al., 1984; Huang et al., 1984; Zang et al., 1985) have isolated fibrillar proteins of glial origin in the pineal glands of various mammals.

All this suggests that these fibrillar elements are related to the fibrillar proteins of pineal glial. In any case, there is enough evidence to suggest that there indeed exist functional differences in the intrapineal channels, and that these differences occur during the circadian cycles.

Moreover, these variations in the channel

Diagram IV.
Point-time layers comparison between both photoperiods



amplitude may be related to the intraglandular tranport of metabolic material from «medullar» to «cortical» layer. This, however, is conditioned by an additional regionalized topographic analysis of the pineal gland.

References

Anderson E. (1965). The anatomy of bovine and ovine pineals. Light and electron microscopic studies. J. Ultrastruct. Res. Supl. 8, 1-80.

Arstila A.U. and Rinne U.K. (1967). Electron microscopic studies on the perivascular secretory processes in the pineal gland of the rat. Acta. Neurol. Scand. 43, 211.

Escriba I. and Martínez Soriano F. (1976). Análisis de las variaciones ultraestructurales de las células adenohipofisarias tras la estimulación con rayos ultravioleta. An. Anat. 25, 115-127.

Gusek W. and Santoro A. (1961). Zur ultrastruktur der Epiphysis cerebri der Ratte. Endokrinologie. 41, 105-129.

Highley H.R., McNulty J.A. and Rowden G. (1984). Glial fibrillary acidic protein and S-100 protein in pineal supportive cells: An electron microscopic study. Brain Res. 304, 117-120.

Huang S.K., Nobiling R., Schachner M. and Taugner R. (1984). Interstitial and parenchymal cells in the pineal gland of the golden hamster. Cell Tissue Res. 235, 327-337.

Krstic R. (1975). Scanning electron microscopic observations in the canaliculi in the rat pineal gland. Experientia. 31, 1072-1073.

Krstic R. (1979). Scanning electron microscopic study of the freeze-fractured pineal body of the rat. Cell Tissue Res. 201, 129-135.

Lues G. (1971). Die Freinstruktur der Zirbeldrusse normaler, trächtiger und experimentell beeninflusster Meerschweinchem. Z. Zellforsch. 114, 38-60.

Lloret García J. (1976). Transformaciones experimentales del substrato entero-hipofisario tras la estimulación adenal de la rata y su repercusión en el Timo. An. Anat. 239-256.

Martínez Soriano F. (1972). Aportaciones al conocimiento del substrato morfológico del Tiroides de la rata blanca tras la pinealectomía. An. Anat. 21, 527-555.

Martínez Soriano F., Herrera M., Pardos C., Smith V., Cimas C. and Botella V. (1984). Normale und experimentelle aspekte von Zirbeldrüssenkanälen. Verh. Anat. Ges. 78, 583-585.

Quay W.B. (1973). Twenty-four hours rhythmicity of pineal canaliculi and evidence for their intrinsic humoral regulation. Physiology 16, 427.

Quay W.B. (1974). Pineal canaliculi: demonstration, twenty four hours rhytmicity and experimental modification. Amer. J. Anat. 139, 81-94.

Rodin A.E. and Turner R.A. (1966). The perivascular space of the pineal gland. Tex. Rep. Biol. Med. 24, 153-163.

Romijn H.J. (1973). Structure and innervation of the pineal gland of the rabbit, *Oryctolagus cuniculis* (L) II. An electron microscopic investigation of the pinealocytes. Z. Zellforsch. 141, 545-560.

Sozos C.H. and Papasozomenos M.D. (1963). Glial fibrillary (GFA) protein containing cells in the human pineal gland. J. Neuropath. Exp. Neurol. 42, (4) 391-408.

Vollrath L. (1981). The pineal organ. In: Hdb. Mikr. Anat. Mensch. Edits. A. Oksche, L. Vollrath. Vol VI/7. Springer-Verlag. Berlin. Heidelberg. New York. pp 173-174.

Watenberg H. and Gusek W. (1965). Licht und elektronenmikroskopische Beobachtungen uber die Epiphysis cerebri des Kaninches. Progr. Brain Res. 10, 296-316.

Watenberg H. (1968). The mammalian pineal organ: electron microscopic studies on the fine structure of pinealocytes, glial cells and on the perivascular compartment. Z. Zellforsch. 86, 74-97.

Wolfe D.E. (1965). The epiphyseal cell: an electron microscopic study of its inter cellular relationship and intracellular morphology in the pineal body of the albino rat. Prog. Brain. Res. 10, 332-386.

Zang X., Nilaver G., Stein B.M., Fetell M.R. and Duffy P.H. (1985). Immunocytochemistry of pineal astrocytes: species differences and functional implications. J. Neuropathol. Exp. Neurol. 44, 486-495.

Accepted February 2, 1991