

Zur Pigmentarchitektonik der Area striata des Menschen

On the Pigmentation Pattern of the Human Area Striata

Die Area striata der Primaten ist durch eine besonders breite Markfaserschicht, den Gennarischen Streifen, gekennzeichnet¹. Die zum Marklager hingewandten Partien des Gennarischen Streifens werden zum grössten Teil von den Endaufsplitterungen der Gratioletschen Sehstrahlung (Projektionsfasern aus dem Corpus geniculatum laterale auf die Sehrinde) aufgebaut². Der Streifen von Gennari teilt die vierte Rindenschicht: beim Menschen liegt er mit einem oberflächlichen Anteil in der Schicht IV_b, – einem Abschnitt der inneren Körnerschicht mit gegenüber den angrenzenden Partien deutlich verminderter Zelldichte –, und mit einem tiefen Anteil in IV_c^{3,4}. IV_c wird hauptsächlich von kleinen bedornten und unbedornten Sternzellen gebildet, die sehr dicht beieinander liegen^{2,5}.

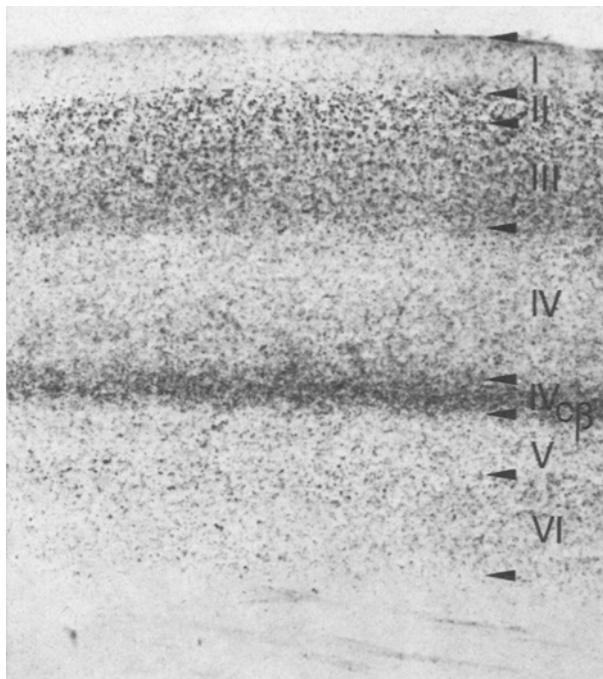
Pigmentarchitektonisch⁶ lässt sich IV_c noch einmal in zwei annähernd gleich breite Zellblätter aufspalten. Das oberflächliche Blatt, IV_{cα}, wird von kleinen Sternzellen bevölkert, die nur wenig Pigment enthalten. In der Mehrzahl der Zellen finden sich feinste Lipofuscin körnchen,

die locker über den Zelleib verteilt sind und sich wenig kräftig mit Aldehydfuchsin anfärben. Der restliche Teil der Zellen bleibt auch im höheren Alter frei von Lipofuscineinlagerungen. IV_{cα} erscheint also bei den für pigmentarchitektonische Arbeiten üblicherweise verwendeten Schnittstärken von 400–800 μm als ein helles Band. Das Bild der Einzelzelle wird hierbei kaum mehr wahrgenommen, vielmehr verschmelzen die als Punktwolken abgebildeten Pigmentablagerungen der zahllosen übereinander liegenden Neurone zu einem mehr oder weniger einheitlichen Grau unterschiedlicher Tönung. IV_{cα} bildet mit den angrenzenden und gleichfalls nur sehr schwach pigmentierten Schichten IV_b und IV_a einen breiten hellen Streifen (Figur). Dagegen besteht eine klare und scharf gezogene Grenze gegen den profunden Teil von IV_c. Diese Schicht, IV_{cβ}, ist im Pigmentgild die auffälligste Komponente der Area striata. Eine entsprechende Schicht findet sich in keinem anderen Feld des Isocortex. IV_{cβ} besteht aus kleinen, überwiegend bedornten Nervenzellen, die noch dichter gepackt sind als die Elemente von IV_{cα}. Die Mehrzahl der Zellen enthält auffällig grobe Pigmentkörner, die sich kräftig mit Aldehydfuchsin anfärben. Die Einzelkörner, die vielfach zentrale Aufhellungen oder Vakuolen aufweisen, sind in geringer Zahl locker im Perikaryon verteilt. Das Erscheinen dieser charakteristischen Pigmentablagerungen hebt die Schicht IV_{cβ} als kräftig getöntes Zellblatt sehr deutlich aus den hellen benachbarten Schichten IV_{cα} und V heraus (Figur).

Summary. Pigment-architecturally, within the area striata of man, IV_{cβ} differs considerably from bright adjacent layers because of the presence of deeply staining lipofuscin granules.

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Area striata des Menschen. Perameisensäure-Aldehydfuchsin, 1000 μm.

¹ F. SANIDES und H. GRÄFIN VITZTHUM, Dt. Z. Nervenheilk. 187, 680 (1965).

² J. S. LUND, J. comp. Neurol. 147, 455 (1973).

³ C. VOGT und O. VOGT, J. Psychol. Neurol. 50, 161 (1942).

⁴ S. POLYAK, *The Vertebrate Visual System* (University Press, Chicago 1957).

⁵ L. J. GAREY, Proc. R. Soc. Lond. B 179, 21 (1971).

⁶ H. BRAAK, Z. Zellforsch. 127, 407 (1972).

Ultrastructural Evidence of a Secretory Process in the Rat Pineal Gland

Despite that the fine structure of the pineal gland has been rather extensively studied by different authors^{1–5}, little is known about the subcellular organelles involved in the biosynthesis and secretion of methoxyindoles, and whether the hormonal products are released to the blood or cerebrospinal fluid. In relation with embryological studies of the human and rat pineal glands, OLSSON⁶ postulated that the secretory products of this organ are released to the blood stream while OWMAN⁷ and FALCK and OWMAN⁸ support the idea that secretory substances

¹ A. MILOFSKY, Anat. Rec. 127, 435 (1957).

² E. DE ROBERTIS and A. PELLEGRINO DE IRALDI, J. biophys. biochem. Cytol. 10, 361 (1961).

³ D. E. WOLFE, Progr. Brain Res. 10, 332 (1965).

⁴ A. U. ARSTILA, Neuroendocrinology suppl. 1, 1 (1967).

⁵ J. A. KAPPERS, J. Neuro-Visceral Relat., suppl. 9, 140 (1969).

⁶ R. OLSSON, Gen. comp. Endocr. 1, 117 (1961).

⁷ CH. OWMAN, Acta morph. Neerl.-Scand. 3, 367 (1961).

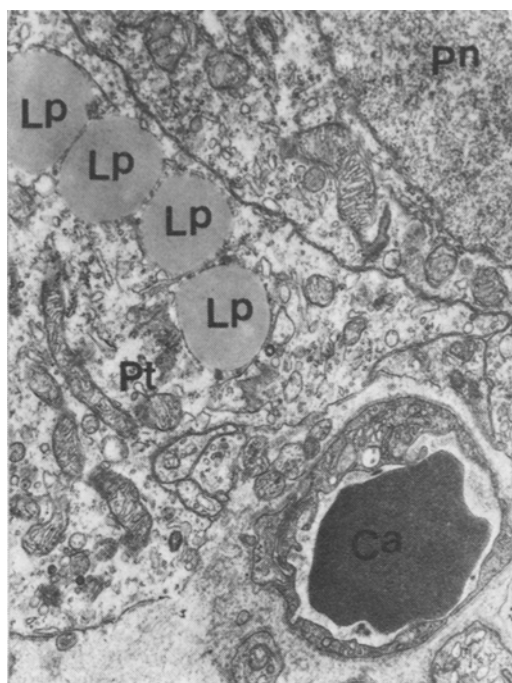
⁸ B. FALCK and CH. OWMAN, Adv. Pharmac. 64, 211 (1968).

are depleted into the cerebrospinal fluid. In regard to the close spatial relationship between the suprapineal recess of the third ventricle and the pineal SHERIDAN and al.⁹ have suggested that these organs could release their secretory products to the ventricular system. Although melatonin has been detected in blood and urine¹⁰ it could be related to a direct extrusion of pineal indole into

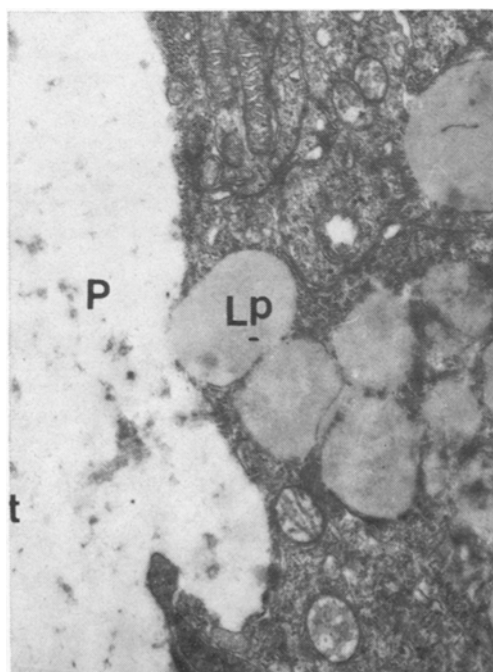
the blood or indirectly by a first secretion into the cerebrospinal fluid. On the basis that this methoxyindole acts directly on the brain tissue¹¹, WURTMAN and ANTON-TAY¹² have suggested that melatonin could be secreted first into the cerebrospinal fluid. Finally KAPPERS¹³ stated that pineal products cannot reach the ventricular cerebrospinal fluid either direct or indirectly, but they are extruded to the pericapillary space. In this paper we have presented ultrastructural evidence that in the rat pineal gland, lipid droplets are secreted into the pericapillary space, and this finding has been discussed in the light that pineal lipids could act as hormonal carriers.

Methods and results. 20 young adult rats (150 g body wt.), males of the Wistar strain, were used in our experiments. Animals were anesthetized briefly with ether and pineal glands rapidly removed, divided in 2 pieces and fixed with somium tetroxide during 1.5 h at 4°C. Osmium fixation was done using 1% osmium tetroxide buffered to pH 7.2 with veronal acetate¹⁴. All the material was dehydrated through graded acetone solutions and were embedded in araldite. At least 20 thin sections were done either in the medullar or cortical portions of each pineal gland with a Reichert ultramicrotome, double stained with uranylacetate and lead citrate, and studied with a Zeiss EM-9A electron microscope. Detailed studies on the ultrastructure of the rat pineal gland have been done by different authors¹⁻⁵, therefore we have focused our attention on the fine structure of the pinealocyte processes and on the secretion of lipid droplets into the pericapillary space. According DEL RIO HORTEGA¹⁵, the pinealocyte is a polymorphic cell with processes ending in polar-shaped enlargements, which are preferentially around the pericapillary (Figure A) and intercellular spaces. Pericapillary polar-shaped enlargements have specific morphological characteristics. They are situated very close to sympathetic nerve ending and contain numerous lipid droplets, which are surrounded by granular points and have a content of homogenous appearance. Around these lipid formations and juxtaposed to them, there are numerous mitochondria, dense-cored vesicles and smooth endoplasmic reticulum (Figure A). Lipid droplets present in the terminal part of the pinealocyte processes migrate towards the pericapillary space and by making protusion on it, are released (Figure B and C). Just after the secretion of the lipid droplet, it can be observed in the pericapillary space, apparently surrounded by a single membrane (Figure D). Lipid secretory activity was more prominent in the pinealocyte than in the interstitial cells. From each 3 capillary areas studied in one of them secretory activity was observed, mainly as migration of lipid droplets through the pinealocyte processes to the pericapillary space.

Discussion. Lipids are present in a great amount in the pineal gland of all mammals¹⁶⁻¹⁹. In the rat, lipids



A) Lipid droplets (Lp) migrating through a pinealocyte terminal pole (Pt) to the pericapillary space. Around the lipid droplets numerous mitochondria, smooth endoplasmic reticulum structures and free ribosomes can be observed. Pn, pinealocyte nucleus; Ca, capillary $\times 12,000$.



B) Lipid droplet (Lp) making protusion in the pericapillary space (P). $\times 16,800$.

⁹ M. N. SHERIDAN, R. J. REITER and J. J. JACOBS, *J. Endocrinol.* **45**, 131 (1969).

¹⁰ J. D. BARCHAS and A. B. LERNER, *J. Neurochem.* **11**, 489 (1964).

¹¹ F. ANTON-TAY and R. J. WURTMAN, *Nature, Lond.* **221**, 474 (1969).

¹² R. J. WURTMAN and F. ANTON-TAY, *Recent. Progr. Horm. Res.* **25**, 493 (1969).

¹³ J. A. KAPPERS, *The Pineal Gland* (Ciba Foundation Symposium, London 1970), p. 3.

¹⁴ G. E. PALADE, *J. exp. Med.* **95**, 285 (1952).

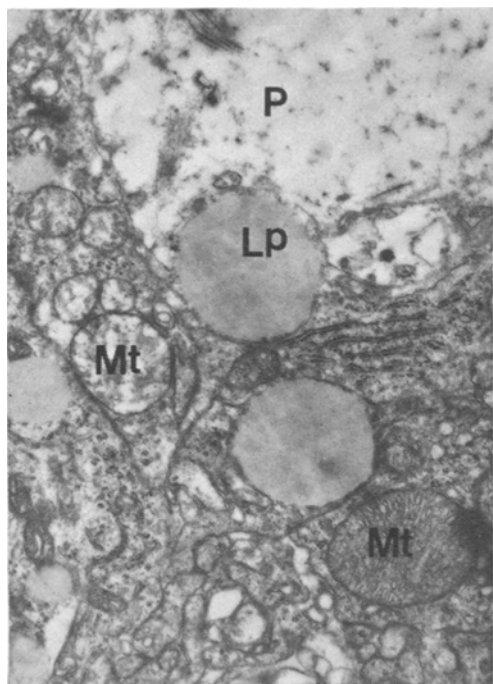
¹⁵ P. DEL RIO HORTEGA, *Archs. Neurol.* **3**, 359 (1922).

¹⁶ N. PROP and A. J. KAPPERS, *Acta anat.* **45**, 90 (1961).

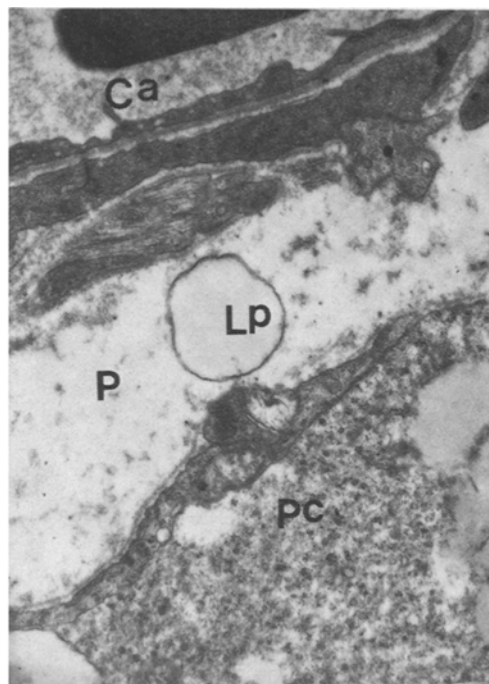
¹⁷ P. JOVAN, T. V. DAI and M. CORMIER, *Bull. Soc. Chim. biol.* **46**, 1121 (1964).

¹⁸ N. PROP, *Progr. Brain Res.* **10**, 454 (1965).

¹⁹ L. ARVY, *C. r. Acad. Sci., Paris*, **253**, 1361 (1961).



C) Secretory phase of a lipid droplet (Lp) into the pericapillar space (P). $\times 12,000$.



D) Lipid droplet (Lp) right in the pericapillar space (P). Ca, capillary; Pc, pineal cell. $\times 12,800$.

represent the 3% of pineal fresh weight²⁰, and related with its great concentrations in phospholipids²¹ and the enzymes which hydrolyze them, PROP¹⁸ has suggested that lipids play an important role in this organ. On the other hand, the frequent association of the lipids with the capillaries seems indicate an endocrine secretory activity²⁰. These suggestions are supported by the fact that hydroxyindole-*o*-methyltransferase (HIOMT)—a rate limiting enzyme in the melatonin biosynthesis— and pineal lipids content fluctuate in a similar way under different experimental and physiological situations^{22–27}. Also other findings seem to support the relationship between lipids and pineal hormones²⁸. When radioactive or unlabeled D-L-5-hydroxytryptophan and nialamide—an inhibitor of the monoamine-oxidase—were added to pineal glands cultures, radioactive grains appeared over the lipid inclusions and the number of lipid droplets increase significantly, which could indicate storage, uptake or secretory phase in the serotonin synthesis. So far, melatonin has not been localized in any subcellular structure, possibly in relation with its small concentration in the pineal gland, rapid secretion rate and its inability to precipitate in form of granules at physiological pH. However melatonin is a very lipid-soluble compound and as cited above there is a relationship between pineal lipid content and HIOMT activity, which could suggest that this substance is secreted with the lipid inclusions. Pericapillar spaces of the pineal gland seem to be one of the most active sites of this organ, as is indicated by the neighborhood between pinealocyte polar processes, nerve endings and capillaries. Intimate relationships of these structures obviously facilitate an efficient system of fast synthesis and secretion of methoxyindoles. Transduction of external or internal messages¹² carried out by the sympathetic nerves stimulate noradrenaline release by the nerve endings which acting on the pinealocyte β -receptors increase the melatonin formation^{29,30}. Although there is no direct proof that methoxyindoles are included in the lipid droplets and further studies have to be done to

demonstrate or discard this suggestion, the release of them is an ultrastructural evidence that the pineal gland secrete, at least part of its products to the blood circulation, and that this process could be influenced by the anatomical and functional relationships of the structures located in the pericapillar space. Also with this finding the question arises if the different components of the lipid droplets can carry other substances outside of cells.

Summary. Pericapillar spaces of the rat pineal gland belong to the most active sites of this organ. Neighborhood of sympathetic nerve endings, capillaries and pinealocyte processes facilitates possibly the synthesis and the secretion of methoxyindoles. Lipid droplets migrate through the pinealocyte cellular processes towards the terminal enlargement or poles, where they are secreted into the pericapillar space, and the possibility that indoleamines are included in the lipid droplets has been discussed.

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²¹ J. ZWEENS, Ph. Dissertation, University Groningen (1964).

²² W. B. QUAY, Gen. comp. Endocr. 1, 211 (1961).

²³ J. AXELROD, R. J. WURTMAN and S. H. SNYDER, J. biol. Chem. 240, 949 (1965).

²⁴ J. ZWEENS, Progr. Brain Res. 10, 540 (1965).

²⁵ R. J. WURTMAN, J. AXELROD, S. H. SNYDER and E. W. CHU, Endocrinology 76, 798 (1965).

²⁶ E. RUGGERI, Riv. Patol. nerv. ment. 19, 649 (1914).

²⁷ J. ZWEENS, Nature, Lond. 197, 1114 (1963).

²⁸ A. U. ARSTILA, H. O. KALIMO and M. HYYPPA, *The Pineal Gland* (Ciba Foundation Symposium, London 1970), p. 147.

²⁹ J. AXELROD, Science 184, 1341 (1974).

³⁰ M. BROWNSTEIN and J. AXELROD, Science 184, 163 (1974).

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