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The proteinaceous content and possible physiological significance of dense-cored vesicles in hamster and mouse pinealocytes

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Summary. Electron dense-cored vesicles (DCV), originating in the Golgi apparatus and migrating into the perivascular processes, constitute a characteristic feature of the pinealocytes in the pineal gland of mice and hamsters. This report presents the results of ultracytochemical studies carried out to clarify the nature and physiological significance of these vesicles. Using proteases and the PA-TCH-silver technique on ultrathin sections, it was concluded that the dense core of DCV was proteinaceous in nature. These data, correlated with previous pharmacological and cytophysiological studies, showed the important role of DCV in the storage and intracellular migration of a proteinaceous compound of unknown significance. However, in agreement with authors who hypothesized the elaboration of active peptidergic compounds in the mammalian pineal gland to explain some effects that could not be ascribed to indoleamines, it was proposed that DCV might store a carrier-neurohormone complex. The presence in the DCV of one indoleamine, such as serotonin, or of several indoleamines, has still not been established in mammals.

Introduction.

The mammalian pineal is essentially composed of two types of intrinsic cells, pinealocytes and interstitial cells (glial-like cells or astrocyte-like cells). These two types are unique due to their peculiar phylogeny. Pinealocytes are derived from photoreceptor cells and interstitial cells from the so-called supportive cells of the pineal of lower vertebrates (Collin, 1969, 1971, 1979).

A number of cytophysiological investigations have demonstrated that pinealocytes possess neuroendocrine properties, but our knowledge of their secretory processes is still in its infancy ; there are fewer data on these cells than on their precursors, the rudimentary photoreceptor cells, which are intermediate between pinealocytes and photoreceptor cells in the scale of phylogenetic and ontogenetic differentiation (Collin, 1969, 1971, 1979 ; Oksche, 1971 ; Oksche *et al.*, 1971).

One of the certain common functions of rudimentary photoreceptor cells (RP) and pinealocytes (Pi) is the genesis of dense-cored vesicles (DCV) from the Golgi complex and their migration into the perivascular processes or the polar secretory

terminals (Collin, 1969-1979). Most authors consider that the DCV in Pi are the storage sites of one or more active compounds. It has been concluded previously in some reptiles and birds (Collin, 1969 ; Collin and Meiniel, 1971, 1973*a*, *b* ; Collin *et al.*, 1976, 1977*a*, *b* ; Juillard and Collin, 1976, 1978 ; Juillard *et al.*, 1977) that a proteinaceous compound is regularly present in the DCV and that serotonin (5-HT), probably bound to this proteinaceous compound, may be also stored in the DCV of some species and/or under certain circumstances.

This study was undertaken to clarify the ultracytochemical properties of DCV in mammalian pinealocytes since (i) it had been determined that active compounds were stored in the DCV or secretory granules of peptidergic neurons and of a number of endocrine cells engaged in the elaboration of polypeptidic or glycoproteic hormones and (ii) Quay (1974), Ebels (1976) and Benson *et al.* (1976) suggested that peptidergic compounds of unknown chemical structure were present in the pineal.

This preliminary study attempts to contribute to a better understanding of the secretory processes in mammals by defining the proteinaceous content of the DCV in the pinealocytes of mice and hamsters, as observed by Collin (Pevet and Saboureau, 1973). The present data have been briefly summarized previously by Collin, Juillard and Brisson (1977*b*). Electron microscopic studies of the cell types studied in the present report have been carried out previously in mice (Ito and Matsushima, 1967, 1968 ; Pellegrino de Iraldi, 1969 ; Matsushima and Reiter, 1975 ; Upson *et al.*, 1976) and hamsters (Sheridan, 1967, 1969, 1975 ; Sheridan and Reiter, 1968, 1970 ; Clabough 1971 ; Collin, 1969 ; Lin *et al.*, 1975).

Material and methods.

Pineal localization of proteinaceous and/or glycoproteinaceous compounds have been investigated in two species of mammals : the mouse, *Mus musculus*, L. and the golden hamster, *Mesocricetus auratus*, Waterhouse. The animals were kept in normal laboratory conditions under a light/dark cycle (14 hrs. light/10 hrs. darkness)

TABLE 1

Number of animals	Time killed	Fixative : glutaraldehyde solution 4 °C	Washing	Postfixation 4 °C	Embedding
4 mice 3 hamsters	11 : 00 a.m.	2,5 p. 100 ; 25 min.	30 min.	OsO_4 2 p. 100 45 min.	Epon
3 mice	7 : 30 p.m.	2 p. 100 ; 25 min. 2,5 p. 100 ; 25 min. 2 p. 100 ; 25 min.	18 hrs.	OsO_4 2 p. 100 1 hr.	
1 hamster		2 p. 100 ; 20 min.			
1 mouse 1 hamster	7 : 30 p.m.	2 p. 100 ; 20 min.	18 hrs.	no	GMA

in a temperature-controlled room (25 °C). Water and standard laboratory chow were provided *ad libitum*. They were killed by decapitation at 11 : 00 a.m. and 7:30 p.m. in December and March. The pineal gland was immediately flooded with cold fixative : glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (table 1).

After fixation, some pineal glands were washed for 18 hrs. in phosphate buffer to eliminate the glutaraldehyde, which might inhibit proteolytic action or produce a background reaction with the PA-TCH-silver technique used.

Protein cytochemistry. — Proteolytic extraction was performed according to Monneron (1966) and Monneron and Bernhard (1966). The ultrathin sections were collected in rings and oxidized in a 10 p. 100 periodic acid solution for 20 to 30 min. ; after several washings they were transferred into enzymatic solutions, as shown in table 2. Two proteases were used : Pronase (Sigma Chemical Company, Saint Louis, Mo.) and Pepsin (Worthington Biochemical Corp., New Jersey).

TABLE 2

Number of animals	Enzyme	Concentration of enzymatic solution (p. 100)	Solvent	Lenght and temperature of incubation
8 mice and 5 hamsters	Pronase	0,1 0,2 0,3 0,5	Maleate buffer pH 7.4	20 min. to 10 hrs. (40 °C)
	Pepsin	0,3 0,4 0,5	HCl N/10 pH 1.5	20 min. to 9 hrs. (38 °C)

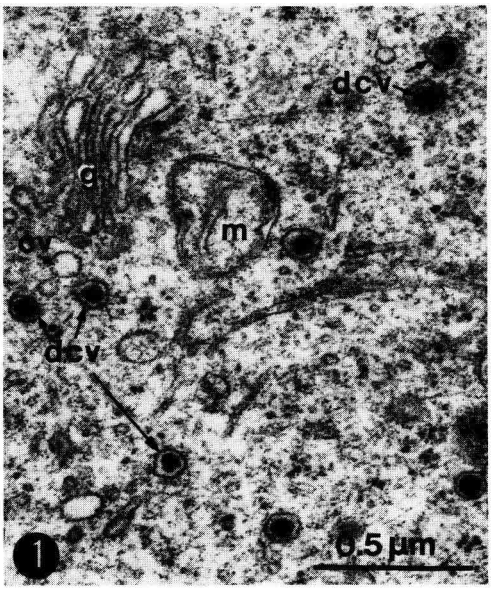
Control ultrathin sections were treated as previously, but were incubated in maleate buffer (pH 7.4) or HCl N/10 (pH 1.5) without protease. After incubation and washings, the sections were collected for electron microscopic examination on formvar-coated copper grids stained with uranyl acetate and lead citrate.

Cytochemistry of complex carbohydrates. — We used the periodic acid-thiocarbohydrazide-silver proteinate technique (PA-TCH-silver technique). The pineal sections, mounted on golden grids, were floated 40 min., 24, 48 and 72 hrs. on thiocarbohydrazide (TCH) ; they were then processed (Thiéry, 1967 ; Juillard and Collin, 1978) and examined without staining in a Hitachi Hu 11 Cs electron microscope.

Results.

Ultrastructure and localization of dense-cored vesicles (figs. 1, 2).

A characteristic feature of the pinealocytes of both the mammals studied was the existence of clear and dense-cored vesicles (DCV) in the perikaryon, which were originating in the Golgi complex. The vesicles had a mean diameter of 110 nm and a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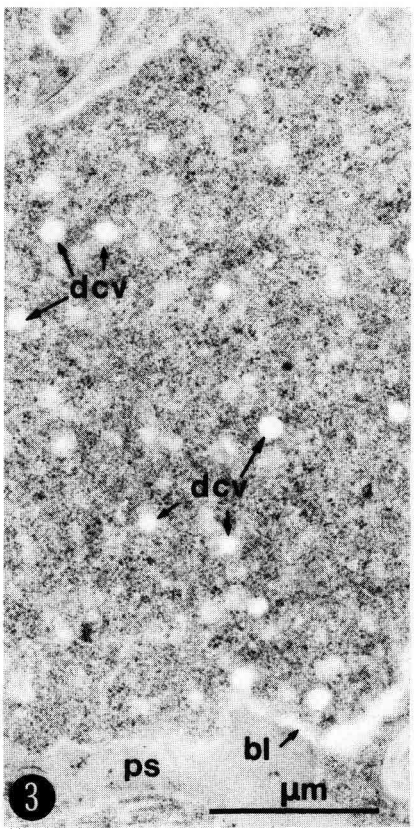
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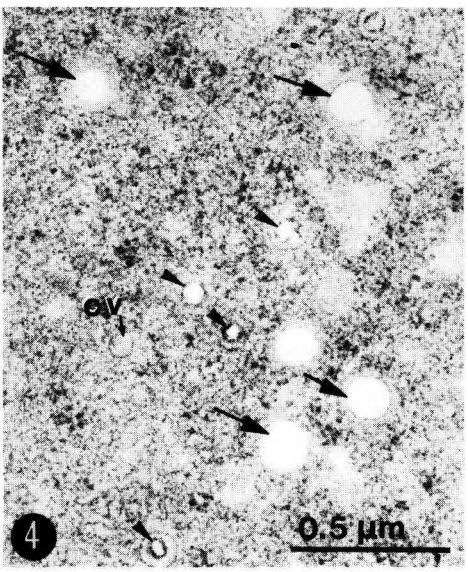
μm
3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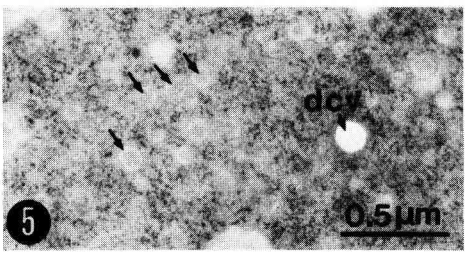
4
0.5 μm


dcv
5
0.5μm

dense core of about 80 nm in mice (Pellegrino de Iraldi, 1969) and a diameter ranging between 65 and 170 nm (Collin, 1969) or from 50 to 120 nm (Sheridan and Reiter, 1970 ; Sheridan, 1975) in hamsters. These vesicles migrated into the perivascular terminal processes where they accumulated, at least provisionally. The electron-dense core filled the DCV more or less, leaving a rim between it and the limiting membrane. The number of DCV varied in the pinealocyte endings and all intermediates between DCV with a large core and clear vesicles were observed, suggesting a release mechanism.

Protein cytochemistry.

Control sections. — No visible changes occurred in the fine structure of non-enzymatically treated sections (compare figs. 1, 2 with 7).

Enzymatically treated sections. — The same observations were made in both mammalian species :

— *Pronase hydrolysis.* After oxidation and 1 hr. of enzymatic treatment in 0.2 p. 100 pronase solution, the DCV dense cores were digested ; they resembled clear holes (fig. 3). The collagenous fibrils, membranes and matrix of mitochondria and triplets of centrioles were also attacked. On thicker sections, a few DCV were only partly hydrolyzed, i.e. the dense core was attacked first, leaving the rim.

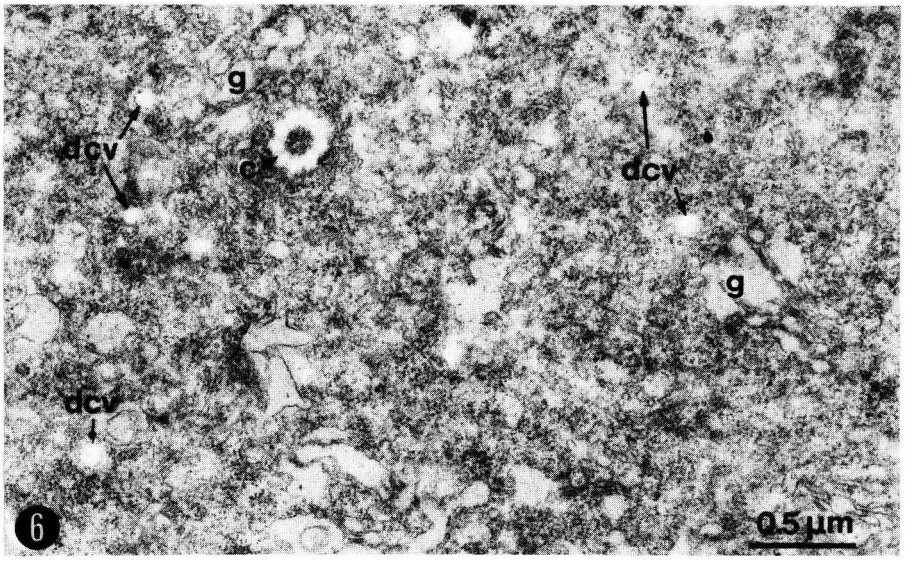
When a 0.1 p. 100 pronase solution was used for 1 hr., or a 0.2 p. 100 one for 30 min. (fig. 4), some DCV remained intact, but the dense core in most of them was replaced by a clear hole. Different stages of proteolysis were seen, probably depending on section thickness, the concentration of the enzymatic solution and incubation time (Monneron and Bernhard, 1966). The differences observed among DCV also depended upon the qualitative and quantitative properties of the proteinaceous compounds stored in them, perhaps due to their different stages of maturation.

On the contrary, with the enzymatic treatments used, pronase did not seem to either affect clear vesicles (fig. 5, 8) located in the DCV population, or the microfilaments and microtubules.

FIG. 1. — *Mouse pineal* fixed in glutaraldehyde, embedded in epon. Normal section staining with uranyl acetate and lead citrate in the perikaryon of a pinealocyte : a Golgi complex (g), from which clear vesicles (cv) and dense-cored vesicles (dcv) originate may be observed. m : mitochondria (× 42 000).

FIG. 2. — *Mouse pineal* fixed as previously. Normal section of a pinealocyte perivascular process, showing numerous dense-cored vesicles (dcv) and clear vesicles (cv). bl : basal lamina ; m : mitochondria ; ps : perivascular space (× 28 000).

FIG. 3-5. — *Mouse pineal.* Ultrathin sections floated 30 min. on periodic acid, then incubated in 0,2 p. 100 pronase solution. (3) during 1 hr : dense-cored vesicles (dcv) located in a perivascular process appear like clear holes (see text). Basal lamina (bl) is also digested. ps : perivascular space (× 22 400). (4) during 30 min : different stages of extraction are seen in dense-cored vesicles (dcv). In some dcv the dense core is partly digested (arrowheads) ; in others the dense core and the rim have disappeared (arrows). cv : clear vesicle (× 42 000). (5) during 30 min : one dense-cored vesicle (dcv) is totally digested as are membranes of clear vesicles ; the content of the latter (arrows) remains intact (× 28 000).


g
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g
6
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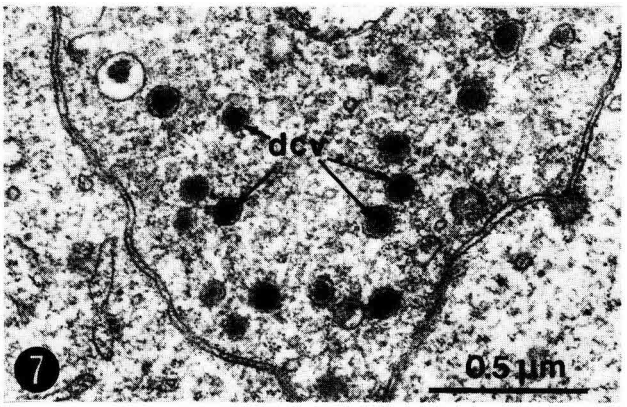
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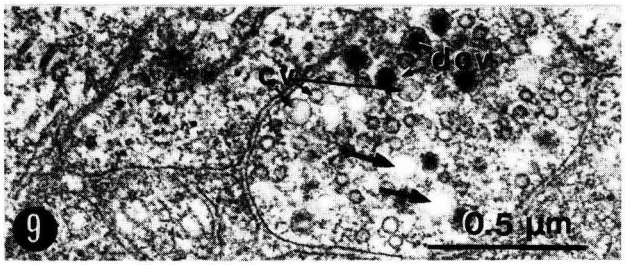
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9
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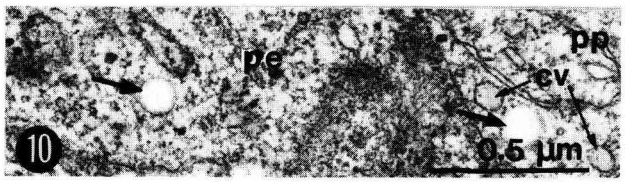
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pp
cv
10
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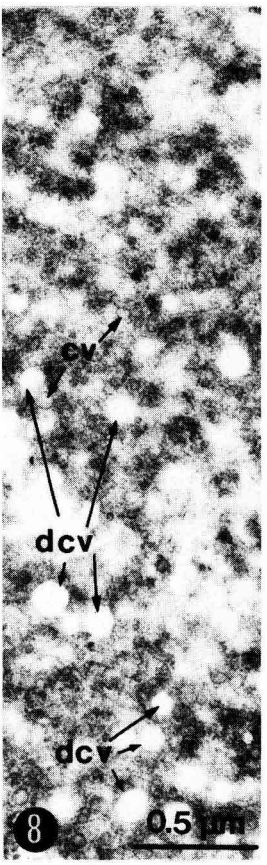

cv
dcv
dcv
8
0.5 µm

— *Pepsin hydrolysis.* After oxidation, pepsin action was effective with a 0.3 p. 100 enzymatic concentration. After 1 hr. of treatment, the content of some DCV completely disappeared ; other DCV were partially attacked or not. Collagenous fibrils were always quickly digested. Clear vesicles did not seem to be modified (fig. 9). When incubation time was prolonged from 2 to 9 hrs., more vesicle dense cores were digested (fig. 6, 10). Mitochondria and microtubule triplets of centrioles were also attacked, but the clear vesicles were not. When the concentration of the enzymatic solution was increased, DCV extraction was more pronounced and more DCV were digested. However, a few DCV appeared intact, indicating that their dense cores were very resistant to the proteolytic action of pepsin ; clear vesicles, microfilaments and microtubules were unmodified.

In conclusion, the dense cores of DCV were the first cell features to be attacked, after the collagenous fibrils, by both proteases, thus clearly suggesting the proteinaceous nature of the granules.

Cytochemistry of complex carbohydrates.

Control sections. When the periodic acid treatment was omitted, no silver precipitates were observed, even after 72 hrs. of exposure on TCH (fig. 11).

Treated sections.

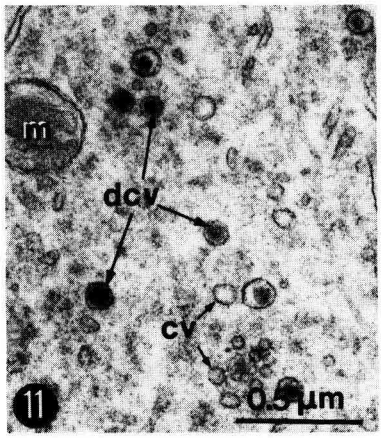
A) 40 *min. of exposure on TCH solution* demonstrated the presence of glycogen particles in most pineal cells, as evidenced by silver precipitates. The quantity varied from one cell to another. Some cells contained no glycogen. Lysosome-like bodies might also be reactive. There was a difference in the two species studied :

a) *Mice* : Small clusters of α-type glycogen were generally found scattered in the cytoplasm of pinealocytes. In our material, interstitial cells were characterized by the presence of β-type glycogen particles, freely dispersed in the cytoplasma (fig. 12).

b) *Hamsters* : Only clusters of α-type glycogen were found scattered in both types of pineal cells.

FIG. 6. — *Mouse pineal.* Section in the pinealocyte perikaryon, oxidized by periodic acid, then floated for 4 hrs on 0.3 p. 100 pepsin. The dense cores of dense-cored vesicles (dcv), originating in Golgi complex (g), and triplets of a centriole (c) are completely extracted (× 28 000).

FIG. 7-10. — *Hamster pineal.* (7) Control section, oxidized by periodic acid then floated on HCl N/10 free of protease : in the pinealocyte process the dense-cored vesicles (dvc) are intact (× 4 2000). (8) Section oxidized then floated 30 min. on 0.4 p. 100 pronase in maleate buffer. The dense-cored vesicles (dcv) are totally digested. The content of clear vesicles is persistent (× 33 600). (9) Section treated on 0.3 p. 100 pepsin for 1 hr 30. Some dense-cored vesicles (dcv) are intact while the dense cores of others (arrowheads) are totally digested. Clear vesicles (cv) are not modified (× 42 000). (10) Section treated as in fig. 6. The dense cores of dense-cored vesicles, located in a pinealocyte process (pp) and in a pinealocyte perikaryon (pe), are totally digested. A thin rim (arrow) is present. The content of clear vesicles (cv) seem to be unattacked by pepsin (× 42 000).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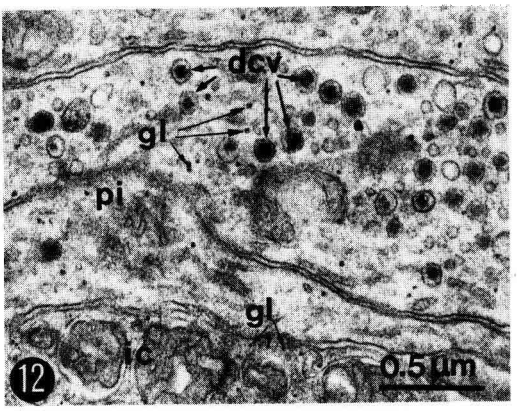
11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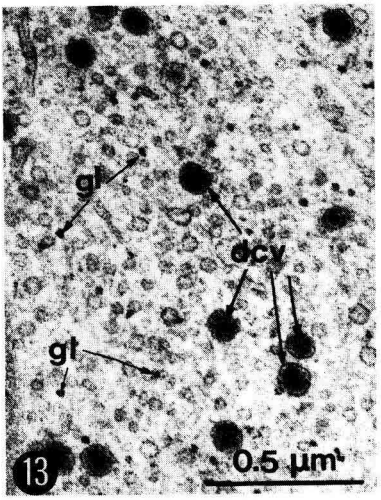
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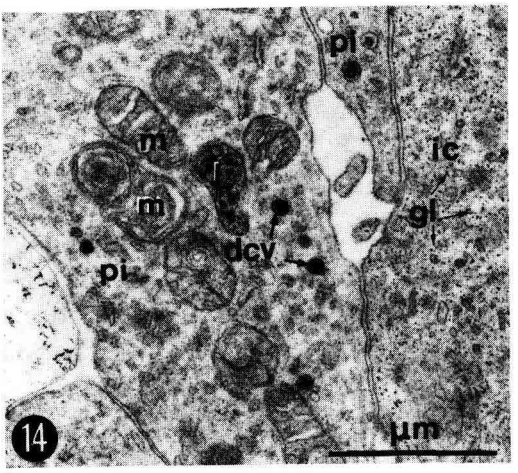
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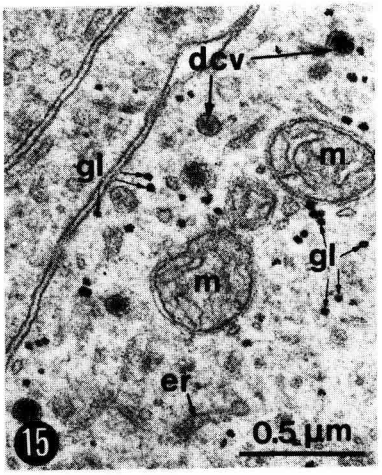
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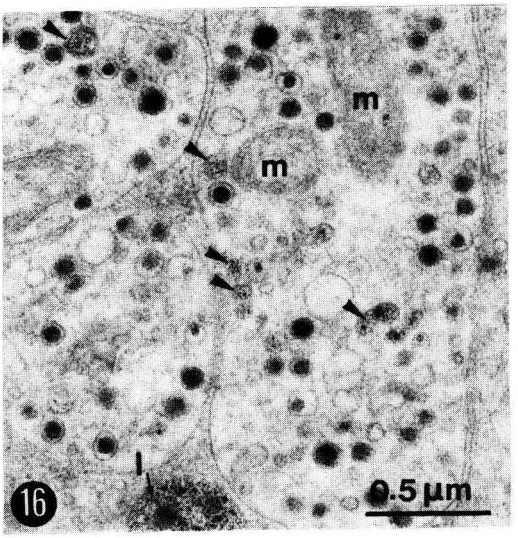
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16

B) *After 24 or 48 hrs. of TCH treatment* (revealing mucopolysaccharides, figs. 13, 14), *or after 72 hrs.* (indicating the presence of glycoproteins, figs. 15, 16), the ultrathin sections were not very different from those observed previously, except that positive reactions occurred in some clear vesicles, mostly localized in the pinealocyte regions containing dense-cored vesicles. The core and the rim of the DCV were never labelled by silver precipitates, whatever the length of incubation. Glycoproteins were not observed (see Discussion) in our conditions.

Discussion.

Pinealocyte secretory processes.

In studies on laboratory rats or wild mammals, Pevet (1977*a*), Pevet and Karasek (1977), Karasek and Marek (1978) considered two types of secretory processes in pinealocytes. One was first proposed by Vivien (1964) in the ringed-snake, then generalized to other Amniota by Collin (1969, 1971, 1976, 1979) and Oksche *et al.* (1971) ; it was characterized by the formation of dense-cored vesicles (DCV) in the Golgi complex ; the other type was recently proposed by Pevet and Karasek and corresponds to the elaboration of material directly from the cisternae of the rough endoplasmic reticulum. Pevet (1977*b*) studying moles, demonstrated the proteinaceous nature only of the material present in cisternae.

On the basis of ultracytochemical, cytophysiological and biochemical data, Collin (1969-1979) distinguished the elaboration of proteinaceous compounds-struc-

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FIG. 11. — *Mouse pineal.* Pinealocyte control section treated according to PA-TCH-silver technique except periodic acid treatment. After 72 hrs on thiocarbohydrazide, no silver precipitate is observed. cv : clear vesicle ; dcv : dense-cored vesicles ; m : mitochondria (× 33 600).

FIG. 12. — *Mouse pineal.* Section treated according to PA-TCH-silver technique. After 40 min. of thiocarbohydrazide treatment, the silver precipitates indicate the presence of glycogen (gl) particles in pinealocytes (pi) and interstitial cell (ic) processes. dcv : dense-cored vesicles (× 28 000).

FIG. 13. — *Hamster pineal.* Section stained according to PA-TCH-silver technique, with 24 hrs on thiocarbohydrazide. Electron dense particles of glycogen (gl) are scattered in cytoplasm. No reaction on dense-cored vesicles (dcv) (× 42 000).

FIG. 14. — *Mouse pineal.* Section stained as previously. Very fine, highly electron-dense β-particles of glycogen (gl) are scattered in the hyaloplasm of an interstitial cell (ic). In two pinealocytes (pi), no glycogen particles are detected in the hyaloplasm. The dense-cored vesicles (dcv) are not reactive. Only a lysosomal-like body (l) is filled with silver precipitates. m : mitochondria (× 22 400).

FIG. 15. — *Mouse pineal.* Section stained as previously but treated on thiocarbohydrazide during 72 hrs. Numerous clusters of α-particles of glycogen (gl) are observed in the pinealocyte hyaloplasm. The dense-cored vesicles (dcv) are not reactive. er : endoplasmic reticulum ; m : mitochondria (× 33 600).

FIG. 16. — *Mouse pineal.* Section stained as previously. In these pinealocyte processes, precipitates are absent in hyaloplasm. Only some scarce clear vesicles are filled with electron-dense particles (arrowheads). l : reactive lysosomal-like body ; m : mitochondria (× 33 600).

tural proteins, enzymes, neurohormone (?) — from that of indoleamines in the same cell type (pinealocytes or rudimentary photoreceptor cells). The usual sequence of events in the protein secretion of rudimentary photoreceptor cells (RP) and pinealocytes (Pi) was compared to that of peptidergic neurons : synthesis by a ribosomal mechanism (ribosomes attached to the rough endoplasmic reticulum membrane), segregation and intracellular transport into the endoplasmic reticulum, concentration in the Golgi complex, intracellular storage and migration of some proteins (a peptidergic neurohormone ?) into the DCV, and release. However, the mechanisms of release of DCV content by diffusion and/or exocytosis were not definitively established, and whether the production of DCV and that of material originating directly from the cisternae of the rough endoplasmic reticulum « are in some way coupled or indeed function independently remains to be elucidated » (Pevet and Karasek, 1977 ; Karasek and Marek, 1978). However, it must also be noted that the material originating directly from the cisternae of the rough endoplasmic reticulum is not specific to pinealocytes, the chief cells of the pineal gland (Collin, 1979). According to Collin (1979), such a phenomenon was observed in the interstitial (supportive) cells of several lower vertebrates and mammals, i.e. the so-called pinealocytes of population II (Pevet, 1977*a*) and more recently in the receptor cells of pike (Falcon, unpublished data).

The other secretory process (Collin, 1979) present in RP, as in Pi, concerns the elaboration of indoleamines and is quite different from that of protein secretion. This second process, apparently absent in peptidergic neurons, is well known in the endocrine cells of the APUD series (Pearse, 1969). In the biosynthesis of indoleamines « an enzymatic process replaces the ribosomal mechanism. In this case, a transport mechanism would be also involved, because biosynthetic enzymes have to be transported from their sites of formation to their sites of activity » (Collin, 1979). These sites of activity were also analyzed by the same author.

Ultracytochemical properties of DCV.

In this report, the presence of a proteinaceous compound in the pinealocyte DCV of two mammals is determined, using two proteolytic enzymes, pronase and pepsin. The action mechanisms of these proteases have been extensively discussed (Monneron, 1966 ; Monneron and Bernhard, 1966 ; Collin and Meiniel, 1972 ; Juillard and Collin, 1978). The data of the present ultracytochemical study using proteases, and that of previous studies in our laboratory, confirm the results of some physiologists and biochemists showing that the mammalian pineal produces active low molecular weight compounds (peptides or polypeptides), mainly studied in terms of their reproductive effects. *Pi, as well as RP, are protein-secreting cells.*

The absence of positive reactions after the use of the PA-TCH-silver technique (Thiéry, 1967) does not negate the presence of glycoproteins in DCV ; Krstić (1975, 1976, and personal communication to Collin) found them in the DCV of rats. The apparent contradiction in DCV glycoprotein content in different rodents may be provisionally explained by a possible circadian rhythm of this substance, similar to the rhythm of glycogen in mice (Kachi *et al.*, 1971*a*, *b*, 1975). Furthermore, in the present work, glycogen has been localized in mice and hamsters, although only in special conditions (see Material and methods).

Since methoxyindoles (melatonin and methoxytryptophol) have been considered as active compounds of the mammalian pineal (Minneman and Wurtman, 1975), several authors have suggested the presence of such substances (or that of the precursor, 5-HT) in DCV. Some monoamines were found in association with a proteinaceous compound in the DCV or secretory granules of monoaminergic neurons or of APUD cells. The supposition that one or more indoleamines might be present in pinealocyte DCV incited a number of pharmacological investigations ; several drugs believed to change indoleamine storage and metabolism were used mainly *in vivo* (Collin, 1979). For example, in hamsters, mice, rats and rabbits (Pellegrino de Iraldi, 1966, 1969 ; Arstila, 1967 ; Romijn, 1976), the DCV were not depleted by reserpine. When Romijn (1972, 1976) used p-CPA, an inhibitor of the biosynthesis of 5-HT and p-chloroamphetamine lowering the activity of tryptophan-5-mono-oxygenase, no apparent changes were observed in the DCV of rabbits. Similar results were obtained recently using complementary techniques in a very complete study of the parakeet (Collin *et al.*, 1976 ; Juillard *et al.*, 1977 ; Juillard and Collin, 1978). In Amniota, variations observed after drug treatments mainly concern the number and the diameter of DCV. These results are difficult to interpret (Collin, 1979) owing to present insufficient knowledge of the chemical structure of DCV and to the fact that it is impossible to dissociate the well known role of the drugs from their less specific action (von Euler, 1973). Thus, since electron microscopic studies of the DCV dense core in Amniota showed hardly any or no qualitative modification (as contrasted with the granulated vesicles of pineal sympathetic endings under reserpine treatment), Collin (1979) suggested that indoleamines, when present in DCV, could not be directly visualized on the classical ultrathin sections because they were masked by another proteinaceous compound, as shown previously by Collin and Meiniel (1972), Petit (1976), Vivien-Roels (1976) studying reptiles, Juillard and Collin (1978) studying birds and Juillard studying mammals here (Collin *et al.*, 1977 *a*).

Apparently only one preliminary observation in the literature concerns the presence of 5-HT in the DCV of hamster pinealocytes (Lin *et al.*, 1975). New studies must be carried out in this direction.

However, considering the variations of 5-HT content — as shown by the technique of Falck *et al.* (1962) in mammals and in other groups of vertebrates (Collin, 1979) — and the pharmacological data, it is definitively demonstrated that a proteinaceous compound is regularly present in the DCV of RP cells and Pi. The presence of 5-HT in DCV varies in different species, depending upon the quantity of remaining 5-HT (which is not rapidly metabolized) and probably also, in a given species, upon daily changes ; the circadian rhythm of 5-HT and its related indoleamines is well known (Quay, 1974 ; Wurtman *et al.*, 1968 ; Axelrod, 1974, 1977 ; Axelrod and Zatz, 1977). Furthermore, many other sites in pinealocytes, interstitial cells and sympathetic fibers could store 5-HT (Collin, 1979).

Physiological significance of DCV proteinaceous content.

From the many experiments on mammals, it appears that pinealocytes are very sensitive cells and that several kinds of inputs control their secretory processes (Collin, 1979). From a cytophysiological point of view, a number of pinealocyte organelles,

including DCV, have been experimentally modified qualitatively and/or quantitatively. DCV decrease or increase was obtained in a seasonal breeder (Roux *et al.*, 1977) and after blinding and continuous darkness in hamsters (Sheridan, 1975) and mice (Upson and Benson, 1977) ; continuous illumination in mice (Upson *et al.*, 1976); sympathectomy in rabbits (Romijn, 1975), hamsters (Lin *et al.*, 1975 ; Sheridan, 1975) and mice (Pellegrino de Iraldi, 1969) ; administration of parasympatholytic drugs in rabbits (Romijn, 1976) ; surgical castration in rats (Karasek *et al.*, 1976) ; injection of human chorionic gonadotropin (HCG) and pregnant mare serum gonadotropin (PMSG) in rats (Karasek and Marek, 1978) ; in the presence of norepinephrine (NE) or dibutyryl-cyclic-adenosine 3'-5'-monophosphate (db-c-AMP) in rat pineals cultured *in vitro* (Karasek, 1974) or in rabbits (Romijn and Gelsema, 1976). It has also been shown that a mean number of DCV in rabbits (Romijn *et al.*, 1976) and mice (Benson and Krasovich, 1977) have a circadian rhythm. From experiments with melatonin administration or with sympathectomy, Benson and Krasovich (1977) concluded that DCV rhythms depended upon intact innervation.

All these quantitative variations of DCV, as the qualitative and quantitative variations of other organelles implied in the process of protein secretion (Collin, 1979), suggest (as in peptidergic neurons and some endocrine cells) that DCV may play an important role in the mechanisms of storage, intracellular transport and release of an active proteinic compound. The cytophysiological and ultracytochemical data of this study agree with those of authors who, from biochemical and physiological data, have suggested the presence of specific peptide or protein hormones in mammalian pineals (Quay, 1974 ; Ebels, 1976 ; Benson *et al.*, 1976).

Upson *et al.* (1976), and Benson and Krasovich (1977) in particular, proposed that DCV might contain an antigonadotropic principle. Naturally, if new experiments are necessary to check or to discard this hypothesis, it is also to be supposed — on the basis of the various effects of the pineal (Wurtman *et al.*, 1968 ; Quay, 1974 ; Relkin, 1976) — that the presumed active protein principle, stored in DCV, plays a more general role, possibly acting on gonadic target cells (via the hypothalamus), but indirectly or directly on other target cells.

Finally, taking into account the detailed discussions of previous papers on reptiles, birds and mammals (Collin and Meiniel, 1971 ; Collin, 1976, 1979 ; Collin *et al.*, 1977*a*, *b* ; Juillard and Collin, 1978), this report suggests that the usual proteinaceous component of DCV may be :

1) a carrier for serotonin and perhaps some derivatives, but that it is probably

2) a specific complex peptidergic neurohormone-carrier to which a more or less important part of the indoleaminergic pool may be bound. Such a peptidergic neurohormone would contribute to explain the pineal effects that cannot be ascribed to active indoleamines.

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Résumé. Les pinéalocytes de la Souris et du Hamster sont caractérisés par la présence de vésicules à cœur dense d'origine golgienne, qui émigrent dans les prolongements à polarité vasculaire.

De l'étude ultracytochimique, mettant à profit l'action de protéases et la technique à l'acide périodique-thiocarbohydrazide-protéinate d'argent, il ressort que le cœur dense des vésicules est de nature protéique.

Ces résultats, de même que les études pharmacologiques et cytophysiologiques antérieures, soulignent le rôle important que peuvent jouer les vésicules à cœur dense dans le stockage et la migration intracellulaire d'un composé protéique dont la signification est encore inconnue. Compte tenu de l'existence de principes actifs de nature vraisemblablement peptidique dans la pinéale des Mammifères, on suggère que les vésicules à cœur dense pourraient stocker un complexe protéique vecteur-neurohormone.

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