AN ULTRASTRUCTURAL COMPARATIVE STUDY ON THE CHOROID PLEXUS IN VERTEBRATES

LILIANA BABEŞ and LOTUS MEŞTER

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Les auteurs ont examiné l'évolution des cellules épithéliales du plexus chorodien chez quelque espèces des vertébrés.

Ils ont montré que, au fur et à mesure que la spécialisation fonctionnelle s'opère, on peut rencontrer des modifications dans la structure fine des cellules épithéliales. Dans l'échelle des vertébrés on peut remarquer un perfectionnement continu de ces structures — responsables de l'élaboration du liquide céphalorachidien — depuis les cyclostomes jusqu'aux mammifères.

Choroid plexuses are cerebral structures which can be found in the lateral ventricles of the telencephalon and they form the roof of the third (diencephalon) and fourth (myelencephalon) ventricles. They consist in a monostratified epithelium, abundantly branched, on a basal membrane under which there are the connective tissue and the blood vessels.

We have focused our attention on their structure, owing to the fact that within these structures, some interesting physiological processes occur: dialysis, secretion, resorption, the active transport of salts and water, processes which result in the elaboration of the cerebrospinal fluid.

The study of the choroid plexus was the object of some important morphological investigations (by light and electron-microscopy) and of histochemical, biochemical, physiological and pathological studies, which Dohrmann [22], has surveyed in a remarkable synthesis on the history of the plexus.

The present study aims at treating the epithelial cells of the choroid plexus from the point of view of compared morphology, in order to follow their structural progress and to point to their functional specialization occurring during their evolution.

Undoubtedly, the choroid plexus is the main structure producing the cerebrospinal fluid. What is still to be discussed is the way in which choroid plexus cells take part in the formation of this fluid. We have found it useful to deal with a comparative study of the fine structure of the choroid plexus, which should make its contribution to enriching the morphological features necessary to the experimental studies of normal and pathological morphology and physiology.

MATERIALS AND METHODS

For the study of the choroid plexus, the material was collected from the following vertebrate classes, orders and species, which have never been studied before: Cl. Cyclostomata, Ord. Petromyzones: *Eudonto-*

myzon danfordi Regan., Cl. Osteichthyes, Ord. Cypriniformes : *Carassius auratus gibelio* (Bloch.), *Cyprinus carpio* L.; Cl. Amphibia; Ord. Anura : *Rana ridibunda* Pallas, Ord. Urodela : *Triturus cristatus* (Laur.); Cl. Reptilia, Ord. Cryptodira; *Emys orbicularis* (L), Ord. Squamata : *Ablepharus kitaibelii fitzingeri* Mertens; Cl. Aves, Ord. Galliformes : *Coturnix coturnix* (L), Ord. Passeriformes : *Passer domesticus* (L) and *Corvus monedula* (L); Cl. Mammalia, Ord. Insectivora : *Talpa europaea* L. and *Erinaceus europaeus* L; Ord. Fissipedia = Carnivora : *Vulpes vulpes* L.

The choroid plexus was removed from the lateral ventricles, the third and fourth ventricles. The place from which the fragments of choroid plexus were collected is indicated in diagrams which represent sagittal sections through the brain of five vertebrate classes (Pl. I, Fig. 1) [29], (Pl. I, fig. 2) [30], (Pl. I, fig. 3) [31], Pl. I, fig. 4,5) [18]. The choroid plexus was removed by dissection after skull trepanning. Sometimes the dissection was made in fixation fluid. For examination with the light microscope, the choroid plexus fragments were fixed in Bouin's fluid and embedded in paraffin. The sections of various thicknesses (6 to 10μ) were stained with hemalaun — eosine.

The choroid plexus fragments were prepared for examination in the electron microscope by routine methods, including prefixation for 12 hours in glutaraldehyde, cold fixation for 1 hour in 1% osmium tetraoxide buffered with Millonig's buffer phosphate at pH 7.2, followed by dehydration in graded acetone and embedding in Vestopal W, Epon 812 and Durcupan ACM. Thin sections were cut with LKB, Tesla and Porter-Bloom microtomes. The sections were placed on coated grids and stained with uranyl acetate and then with lead citrate and were examined in a Hitachi H.U. 11 and Jem 7 electron microscope.

RESULTS AND DISCUSSION

The choroid plexus structure in the cyclostomes: With a view to noticing the structural evolution of the choroid plexus, the main structures producing the cerebrospinal fluid, we proceeded to investigate this structure in the Agnate. These lower vertebrates, which are represented by the two orders : Mixini and Petromyzones, are, from this point of view, extremely different from each other. Mixine as well as Amphioxus (Cephalochordata) are completely deprived of any choroid plexus [1], although their cerebral cavities are full of cerebrospinal fluid. Because of this important conclusion, some authors think that choroid plexuses are not the only cerebrospinal fluid producing structures. This idea is also supported by the fact that there are embryonic stages in which the choroid plexus cannot yet be found, but which possess the cerebrospinal fluid within the brain cavities and in the ependymal channel [54]. These observations have led to the opinion that there are also other cerebral and circumventricular structures, such as ependymal epithelium, and a series of chemoreceptors which take part in the cerebrospinal fluid formation [71].

As a defined structure, the choroid plexus appears only in Petromyzontidae. A detailed description of these structures in adults is Ladman

and Roth's study [40], whose research is based on the *Petromyzon marinus*.

To these structural data, we shall add our observations on the embryonic development of the choroid plexus in Petromyzontidae *(Eudontomyzon danfordi)*. In the very small larvae of this species, when the brain is not well differentiated having a tubular aspect, a real choroid plexus is growing on the median line from the monostratified roof of this brain (Pl. II, Fig. 6). Connective elements, blood vessels and chromatophores pervade the choroid plexus. This plexus is very abundantly branched at the level of the myelencephalon, where it forms a rich roof to the fourth ventricle. Its further development consists in increasing the number of the folds within this structure.

We would mention a major aspect, namely, at larval stages, when from the epithelium of the primitive nervous tube, the nervous organs of the brain develop through numerous divisions (Pl. II, Fig. 7) we could never notice any cell divisions in the choroid plexus, which develops tremendously. (This is in full concordance with observations in the literature [2], [24] about the mammalian choroid plexus). Therefore we can advance two hypotheses: either the plexus cells divide themselves by amitose (this can be hardly accepted because aspects of this kind of division have not been noticed), or they result from multiplication of the cells in the ependymal epithelium, which is more plausible. Starting from this point, we think that as a result of the numerous mitotic divisions which take place in the epithelium of the primitive neural tube, a certain part of the resulting cells move behind this epithelium and form the walls of the nervous organs, whereas another part pushes this epithelium towards the dorso-median line, where, for lack of space, choroid plexuses are formed by continuous foldings in these regions. The fact may be explained by the different position of the division spindle in the ependymal cells [44].

The choroid plexus structure in fishes. The material *(Cyprinus carpio* and *Carassius auratus gibelio)* we study is part of the Teleosteans; in this case, the choroid plexus has a particular embryonic development as far as the encephal is concerned. The plexus can be found in the diencephalon, where it gives birth to a series of structures bearing the generic name of epiphyseal organs (epithalamus). It can be found both at the level of the mesencephalon and at the level of the myelencephalon (fourth ventricle). It is absent in the telencephalon due to the fact that the telencephalon has a characteristic way of formation (eversion), because of which the roof does not consists of a nervous substance but of a monostratified epithelium (membrana tectoria) to which the vascular connective tissue (leptomeninges) is added, forming the choroidal toil. That is why the telencephalon exhibits, in fact, only a ventricular cavity and is deprived of choroid plexus.

A well-developed choroid plexus lies at the level of the third ventricle, forming the roof of the diencephalon (epithalamus). This choroid plexus forms the vellum transversum, which marks the limit between diencephalon and telencephalon, the dorsal sac, the parapineal body and the pineal body or epiphysis. The detailed development of these structures

in *Cyprinus carpio* was described in a recent paper by Dornescu *et al.* [29].

In *Cyprinus carpio* and in *Carassius auratus gibelio* as well, there are only the dorsal sac and the epiphysis in the roof of the diencephalon. The parapineal body appears only during the first stages of embryonic development, afterwards regressing until its complete disappearance. The dorsal sac develops remarkably forming numerous folds which in turn form second and third order branches. It includes the epiphyseal stalk and represents in fact the whole choroid plexus of the third ventricle, out of which the material of our study was collected. This dorsal sac consists of a monostratified epithelium formed of prismatic cells lying on a continuous basal membrane.

The structure of the choroidal toil, which we have studied in *Carassius auratus gibelio* in the electron microscope is simpler. It consists of a monostratified, flat epithelium, without cilia and microvilia [5].

The choroid plexus in *Carassius auratus gibelio* and *Cyprinus carpio* consists of a monostratified epithelium with tall, prismatic cells, lying on a continuous basal membrane uniformly thick. The apical pole of these cells exhibits cytoplasmic expansions with an irregular form, which do not form a real brush border, as in Amphibians and Reptilia, but rather suggest an unstable, always changing disposition (Pl. II, Fig. 10). Undoubtedly, as a real brush border, these expansions increase the surface of contact between the choroid plexus and the cerebrospinal fluid. This fact also supports the idea that choroid plexus cells have in a certain way, a resorption function.

In certain cells, a remarkable protrusion of the cytoplasm in the ventricular space filled with cerebrospinal fluid can be noticed among the thin and irregular cytoplasmic expansions.

At the apical pole of the cells, we can also notice many cilia having a typical structure (2 + 9). Their disposition is not uniform; there are zones in which the cells are completely deprived of cilia and zones in which the cilia overcrowd.

The basal pole of the choroid plexus cells is doubled by a very thin basal membrane. Plasmalemma in the basal pole forms only very few and very short infoldings, so that we cannot speak of a basal labyrinth. This one can be found only starting with Reptilia.

Inside each cell, nearer to the basal pole, there is an elongate nucleus with a double and pored membrane, with uniformly distributed chromatin.

The cell cytoplasm is very rich in organelles. Mitochondria are small and very numerous and spread over the whole cell. Another important feature is that most of these mitochondria exhibit some tubular cristae (Pl. II, Fig. 11). This fact, cofirmed by the images given by Obermüller-Wilen [50], who studied *Leuciscus rutilus* (actually *Rutilus rutilus)*, added to the presence of a smooth, well-developed endoplasmic reticulum, makes us support the idea of a possible synthesis of steroid substances that takes place in the choroid plexus epithelial cells.

It is worth mentioning that these features of the steroidogenic cells, which seldom appear in fishes, can be found all up through Reptilia, being absent in birds and mammals.

In these cells there are also vesicles of the Golgi complex and free ribosomes, lysosomes and multivesicular bodies. In the cytoplasm, there are numerous vesicles, lying especially at the basal pole; they are also frequent in the apical pole and even in the cytoplasmic expansions of the latter (Pl. II, Fig. 10). We suppose that they are pinocytotic vesicles.

The choroid plexus structure in amphibians. The choroid plexus has been studied in Amphibians more than in other vertebrate classes, apart from mammals, perhaps. Both, the Anura *(Rana esculenta, Rana fusca* [61], *Rana temporaria;* [56], [57], [59] and the Urodela *(Necturus maculosus);* [13], *Ambystoma mexicanum* [32], [33], [35], [37] were the object of some structural investigations to the results of which our researches on *Rana ridibunda* and *Triturus cristatus* are added. Also, using diverse species of *Rana*, Wright [73], [74] made a series of important physiological experiments regarding the transport of substances and ions through the choroid plexus.

In *Rana ridibunda* like in the other vertebrate classes, the choroid plexus structure is involved in the process of elaboration of the cerebrospinal fluid. The choroid plexus in *R. ridibunda* generally exhibits the same structure (Pl. III, Fig. 12). In the choroid plexus epithelial cells, which are isodiametric, as we have mentioned before, are uniformly tall, presenting a central nucleus, sometimes deeply incised.

The organelles are represented by the Golgi complex, vesicles and tubules of the smooth endoplasmic reticulum, lysosomes with fine granular content, and sometimes smaller or bigger lipid droplets (Pl. IV, Fig. 18), having an irregular border and probably playing an energetic role [58], [59], [43]. A very typical feature is the arrangement of the mitochondria in a strictly perinuclear zone (Pl. IV, Fig. 17). We underline the presence of the mitochondria with tubular cristae (Pl. IV, Fig. 17, 18). Ergastoplasma is missing.

The apical pole of the cells presents cytoplasmic expansions with an extremely irregular border (Pl. III, Fig. 14). The irregular border of these expansions, as well as the abundance of the pinocytotic vesicles, at this level, suggest that, like in fishes and reptiles, this apical structure is not stable, but rather in a continuous change.

Plasmalemma forms some intermingled folds on the lateral borders of the cells (Pl. IV, Fig. 17). At the basal pole of the cell, the infoldings of the basal plasmalemma are quite rare and less deep; sometimes they are absent (Pl. IV, Fig. 19).

The other species we have studied, *Triturus cristatus*, has a choroid plexus, slightly developed in the lateral ventricle (Pl. III, Fig. 13), and a well-represented plexus in the third and fourth ventricles. Unlike *Rana*, the cells of the choroid epithelium forming the plexus have not the same size. There are zones in which this epithelium is very thin and that is why the nucleus in these regions is very flat and has an ellipsoidal form. In other regions, the epithelial cells are higher and their basal pole is prominent within the choroid vilosity (Pl. III, Fig. 16).

The apical pole of the epithelial cells in *T. cristatus* resembles the one belonging to Rana. The cytoplasmic expansions have an irregular border, with their distal end markedly swollen; within these cells, there are numerous vesicles of different sizes. At the basal part of these expansions

there are also numerous vesicles. Some of them open towards the ventricular lumen. The whole apical pole of the cells is full of such vesicles. One can see for example, that some of them are formed by the successive obstruction of these tubuli (Pl. III, Fig. 15).

Mitochondria are spread over the whole cytoplasm of the epithelial cell, but in most cases their greatest density can be noticed in the basal pole (Pl. III, Fig. 16). They might be mitochondrial pumps, although the tubular infoldings of basal plasmalemma are absent. Like *R. ridibunda*, such little sinuous infoldings of the plasmalemma can be seen sometimes.

The choroid plexus structure in reptiles. From literature, we have noticed that besides fishes, reptiles are the least studied from the viewpoint of the choroid plexus ultrastructure. The only paper regarding the structure of the choroid plexus in Reptilia belongs to Murakami [49], who studied this structure in the lizard *Geko japonicus*. That is why we have considered that the description of the plexus in other reptiles might complete the comparative study in vertebrates, insofar as its structure and functions are concerned. The species we have chosen are *Ablepharus kitaibelii fitzingeri* (Pl. V, Fig. 20) and *Emys orbicularis* [42].

The epithelial cells, which appear isodiametric in the light microscope, have a remarkably irregular border when studied by means of the electron microscope. Unlike the cells in the plexus belonging to the other vertebrates, they present cytoplasmic expansions, sometimes quite long; these expansions protrude from the basal pole in the lumen of the choroid vilosities. The density of the mitochondria within these expansions is remarkable. (Pl. V, Fig. 22).

In the species we studied we cannot speak of a brush border, if we consider that a brush border consists of the same sized microvilia having a regular arrangement. The apical pole of the epithelial cells in *E. orbicularis* and *A. kitaibelii*'s choroid plexus presents cytoplasmic expansions with an extremely irregular form (Pl. V, Fig. 21). In all these expansions there are a lot of pinocytotic vesicles of different size. Together with Maxwell and Pease [45], we consider that this aspect of the apical pole does not represent a constant structure owing to the intensity of the pinocytotic process taking place at this level.

The basal pole, as we have mentioned before, presents characteristic features. The types of cells characteristic of mammals are announced by the two types of cells this basal pole determines. Among all these, the type of cells deprived of plasmalemmal tubular infoldings or with very few such infoldings can be met in all vertebrates and this is the only one present in fishes and amphibians (Pl. V, Fig. 23). The other type represented by cells with fine tubular infoldings of the basal plasmalemma and with mitochondrial pumps, appears for the first time in reptiles (Pl. V, Fig. 22; Pl. X, Fig. 40) and is very well defined in mammals. This type of cell is adequate to the active transport processes taking place as follows: from the blood vessels to the epithelial cells and then to the ventricular lumen with cerebrospinal fluid.

The cellular organelles are represented by: mitochondria, smooth endoplasmic reticulum, free ribosomes, different sized vesicles, multivesicular bodies, Golgi complex (Pl. V, Figs 22, 23; Pl. VI, Fig. 24), lysosomes. As far as the position of the mitochondria within the epithelial

cells is concerned, it is worth mentioning that they present a great density in the apical pole of the cells and at the level of the basal labyrinth, and especially in the cytoplasmic expansions of the basal pole. This polar distribution of mitochondria suggests the possibility of the fluid and ion transit, both from the basal to the apical pole of the cell and the other way round; all this supports the idea of a two-way transport [65].

It is interesting that in many of the epithelial cells belonging to *Emys*' choroid plexus, we have noticed the presence of concentric lamellar structure with considerable dimensions (Pl. VI, Fig. 26). Within these lamellar bodies, as well as among the lamellae they are made of, one can notice vesicles of different dimensions. Such vesicles are also in great number outside the lamellar structure. Such lamellar structure is to be found in numerous types of normal adult or embryonic cells, in cells that regenerate themselves and in cells that suffered a treatment with chemical substance and X-radiations [41], [38].

In most authors' opinion this structure represents centres forming the membrane of the smooth or rough endoplasmic reticulum. In the cells we have studied these structures seem to have some link with the Golgi complexes (Pl. VI, Fig. 25) which sometimes are extremely abundant (Pl. VI, Fig. 24).

The nucleus of the epithelial cells is voluminous, ovoid, and, within the chromatic material, uniformly distributed. The nucleus has central or eccentric positions (sometimes very near the basal pole of the cell) and quite often it presents deep incisions. This incised aspect of the nucleus proves the existence of some remarkable nucleo-plasmatic interactions which support the idea of active participation of these structures in forming the cerebrospinal fluid.

The choroid plexus structure in birds. The study of the choroid plexus in birds is known especially from Doolin and Birge's works [26], [28], who undertook histological and histochemical researches with the light and the electron microscope in hens, on adults and in the embryonic development. We can also mention Doolin [27], who studied the detailed structure of the cilia presented by the choroid plexus and ependymal epithelium in hens, cilia having a particular structure in certain zones.

In order to cover the evolutional series of the epithelial plexus cells in Vertebrates, we have chosen the species *Corvus monedula*, *Passer domesticus* and *Coturnix coturnix*, which we have observed in the light (Pl. VII, Fig. 27) and the electron microscope.

Studying these species and on the basis of certain papers on the choroid plexus in birds, we have distinguished a series of general features and other typical features of the birds' choroid plexus.

The image of the apical pole of these cells suggests, unlike the one of the fishes, amphibians and reptiles, the presence of some microvillosities, more regular in form and dimension, and therefore the presence of a more stable structure. At the basis of the microvilli there are sometimes open pinocytotic vesicles (Pl. VII, Fig. 28).

These epithelial cells are very rich in organelles. The mitochondria are very abundant, spread over the whole cell, but their greatest density is in the apical pole of the cell. These mitochondria have a dense matrix

and transversal cristae. They are, therefore, variable and the very interesting fact is that their most frequent association is with the rough endoplasmic reticulum, tubuli which come to surround the mitochondria (Pl. VII, Fig. 31). This association probably proves the synergic action of the two constituents in various energetic processes, linked to the so much discussed functions of these cells.

Unlike Reptilia in which the epithelial cells of the plexus presented an incised nucleus, the nucleus of the epithelial cells in the choroid plexus of birds never presents incisions. In the species we studied, we did not meet binucleate cells either — although Doolin and Birge [28] encountered frequently cases of such binucleate cells in hens.

When taking into account the basal pole of the cell, the same two types of cells present in Reptilia can also be found in birds : the cells with the basal pole deprived of fine tubular infoldings (Pl. VII, Fig. 29) and the cells with numerous such sinuous infoldings, which prove an active participation in the fluid transport, as well as in the active ion transport (Pl. VII, Fig. 30).

The choroid plexus structure in mammals. In the most advanced class of Vertebrates, *the choroid plexuses*, although consisting of the same elements, are more complex and this undoubtedly proves a remarkable functional improvement. From a physiological point of view, they are also the main cerebrospinal fluid producers, though they might be involved, as in other vertebrates, in diverse physiological processes, for instance, in the regulation of the hydric metabolism [63].

Among all classes of Vertebrates, the Mammals were studied mostly from the viewpoint of the structure of the choroid plexus epithelial cells. In this respect we mention the following works done on : opossum [72], rabbit [45], [48], [55], [66], [67], [68], mouse, rat [72], guinea-pig [15], hamster [11], dog [64], [72], cat [45], [14], monkey [79], [47] and man [6]; [7]; [8]; [25],[51], [52], [53]; [34], [46].

To this we must add the extremely complete and systematic study of Lucy Arvy [2] on the choroid plexus in Mammals.

All these studies represent a basis for our descriptions on the species we have studied : *Talpa europaea* [3], *Erinaceus europaeus* [4] and *Vulpes vulpes*.

Although the component elements of the choroid plexus are the same in the whole series of Vertebrates, we must underline the fact that the epithelium component presents differentiated cells. This aspect might be slightly present in Reptiles, but is very well marked in Mammals.

Thus, within the monostratified choroid epithelium there are two distinct types of cells, present in various proportions in all the species of Mammals we have studied. The morphologic criterion used for this classification was represented by the structures determined by the basal plasmalemma. This structure has a precise physiological significance established by comparison with other types of cells characterized by the presence of these structures whose functional role is well known. The two cell types are very much alike, the cells forming the proximal tubule of the nephron [69]. In one of these cell types, the basal plasmalemma presents fine tubular infoldings with a very sinuous aspect. These fine infoldings advance towards the nucleus and sometimes they by pass it.

In the cytoplasm, among these infoldings, there are numerous mitochondria (Pl. VIII, Fig. 34, Pl. X, Fig. 42). This type of cells resemble the cells from the initial part (near Bowmann's capsule) of the proximal renal tubule. In certain species of Mammals (*T. europaea* and *V. vulpes*), there is an epithelial cell type which we consider more advanced may present a morphologic variant which we have already described in a previous work [3]. It deals with the choroidal epithelial cells in which the basal plasmalemma forms large, sinuous infoldings. When longitudinally crossed, these infoldings resemble the end feet of the renal podocyte. Nevertheless, it is hard to assume, only on the basis of the morphologic data, that these cell variants represent also different functional specialisations (Pl. VIII, Figs 35, 36; Pl. X, Fig. 41).

In the other type of cells which are less high than the former, the basal plasmalemma does not quite form any infoldings. With the exception of some shallow folds, the plasmalemma is smooth and parallel to the basal membrane. This cell type is similar to the cells from the end part (in front Henle's ansa) of the proximal renal tubule (Pl. IX, Fig. 37, Pl. X, Fig. 39).

Following these two types of cells in the vertebrate series, we think that the former type represents the results of the functional specialization of the latter type, and therefore, the two cell types have no different functions.

The phylogenetic evolution of these cell types is similar to the ontogenetic evolution in Mammals [67], [68].

This structural similarity noticed under normal conditions can support the idea that the cerebrospinal fluid elaboration is similar to the urine elaboration by the nephron and therefore, dialysis, secretion and resorption processes are involved.

Dialysis as a way of forming the cerebrospinal fluid was one of the first hypotheses about the choroid plexus way of functioning. Undoubtedly, at least some of the cerebrospinal fluid components result from the blood; they only pass through the epithelial cells of the plexus. The comparison between the cerebrospinal fluid and the blood plasma has proved that the dialysis process is not sufficient and the secretion and resorption idea was advanced. This idea is supported by the morphologic characteristics of the choroid plexus epithelial cells.

Resorption is morphologically confirmed by the presence of microvilli and of numerous pinocytotic vesicles in the apical pole of the cells. As far as secretion is concerned, things are more difficult and a common agreement has not yet been reached, because it does not appear as a figurative process. It is true that the ergastoplasma is abundant in many cells of the choroid plexus, but it is at this level that proteins are synthetized for the inner necessities of the cells. It is probable that many of the cerebrospinal fluid components are due to an active transport. This process finds its morphologic expression in the presence of the mitochondria pumps [60], [16], [17], [39], [61].

From the study of these three species and from the data oferred by the literature about Mammals, we found a series of structural characteristics distinguishing the choroid plexus in this class from the same structure belonging to other vertebrate classes. Thus, in Mammals, we

have never noticed any polar or perinuclear distribution of the mitochondria. These organelles form mitochondrial pumps by their arrangement among the basal plasmalemma infoldings (Pl. VIII, Fig. 34), but, at the same time, they are spread over the whole cellular cytoplasm and have a uniform density. The nuclei of the epithelial cells are spherical or ovoid (Pl. VIII, 33, Pl. IX, Fig. 37); they have an irregular border, and are completely deprived of incisions, at least in adults. Dohrmann [20] underlines that in the mouse embryos there are lobated nuclei which cannot be found soon after birth and in adults.

Biondi bodies, ring structures consisting also in fibrillar elements and lipoid pigments, described by Biondi and confirmed by other authors [51], [53],[19], have not appeared in any of our slides, because the adults were not so old. As it is known, the appearance of the Biondi bodies is a phenomenon linked to aging.

As for the structure of the blood capillaries in the connective stroma from the choroidal villosities in Mammals, we can say that these capillaries are made of an endothelium deprived of pores or with a very little number of pores, and they are almost completely surrounded by pericyte (Pl. IX, Fig. 38).

GENERAL CONCLUSIONS

Although the evolution of the choroid plexus in Vertebrates does not exhibit spectacular structural changes, the plexus preserving on the whole the same features, we must underline the fact that in the details of the fine structure the plexus cells present several characteristic aspects, at least as far as classes are concerned. This proves a continuous specialization with a view to carrying out the essential function, namely the cerebrospinal fluid elaboration. Therefore :

1. In lower vertebrates, fishes, amphibians and reptiles (to a certain extent), the cytoplasmic expansions in the apical pole have extremely irregular forms and their dimensions are very different within these expansions, and at their basis, there are numerous vesicles, sometimes arranged in rows. At times, these vesicles open to the basis of cytoplasmic expansions. Yet, beginning with birds and mammals, the apical expansions get quite a regular form and arrangement.

2. Cilia, which are also present in the apical pole of the cells have a typical structure (2 + 9). They are abundant in lower vertebrates and decrease progressively towards mammals, where they are rare in adults and more numerous in embryos.

3. The basal pole of the cells presents very interesting positions linked to the plasmalemma in this zone of the cells. Plasmalemma forms fine tubular infoldings among which mitochondria are disposed. In this way the structure called basal labyrinth is formed. In lower vertebrates (cyclostomes, fishes and amphibians) this basal plasmalemma forms very few and short infoldings (Pl. IX, Fig. 39). They are not always accompanied by mitochondria though in certain cases we have noticed mitochondria clumps in the basal pole of the cell, without any plasmalemma infoldings *(Triturus cristatus)*. We call these cells primitive type cells. They are to be found in all vertebrates we have studied, from cyclostomes

to mammals, but beginning with the reptiles they considerably decrease. Beginning with the reptiles the continuous improvement of this basal labyrinth can be noticed. The basal plasmalemma infoldings are long and they come near the nucleus, sometimes by-passing it. In cytoplasmic spaces among the infoldings there are mitochondria (Pl. X, Fig. 40). These cells are well represented in reptiles and birds and very abundant in mammals. We have called them advanced type cells (Pl. X, Fig. 42). In some mammals there are also certain cells whose plasmalemma forms large infoldings (and feet); these cells resemble the podocyte from the renal capsule and they probably play a role in dialysis (Pl. X, Fig. 41).

4. *Epithelial cells of the cytoplasm* contain numerous cellular organelles whose distribution is typical sometimes. Thus, the rough endoplasmic reticulum, whose presence proves an intense protein synthesis is present in the choroid plexus epithelial cells in almost all the vertebrates we have studied. The R.E.R. is less abundant in the apical pole of the epithelial cells in fishes; it is less represented in amphibians, both in Anura and in Urodela; it is represented by a few sacculi spread over the cell, in reptiles; it is well represented in birds where each R.E.R. sacculi surrounds mitochondria, and it is very well developed in mammals, being situated in the apical pole of the cells, in most cases, and sometimes it presents the same relationships with mitochondria.

5. The Golgi complex is also present in all Vertebrate species we have studied and is formed of its characteristic components. We mention the fact it is very well represented in reptiles (3 to 5 such Golgi complexes in a cell).

We could not notice the presence of any granular secretion, either in R.E.R. or in the Golgi complexes — and our data are also confirmed by literature.

6. Mitochondria are present in epithelial cells and they are always in great numer in all vertebrates. Within the cell, they have variable distribution depending on the species studied. In amphibians mitochondria have a characteristic distribution; in Anura they can be recognized in a strictly perinuclear zone; in Urodela they are in clumps, especially in the basal pole of the cells. In Reptilia they have a polar distribution, their greatest density is visible in a zone in the apical and basal pole of the cells. In birds, mitochondria are surrounded by a rough endoplasmic reticulum. These relationships can be noticed in mammals, too, where mitochondria are large, numerous and spread over the whole cellular cytoplasm forming in the basal pole a distribution known under the name of basal labyrinth.

A quite interesting observation is that in fishes, amphibians and reptiles, mitochondria have numerous tubular cristae, whereas in birds and mammals, these cristae are lamellar, without any exception.

7. Besides the organelles mentioned before, in the epithelial cells of the choroid plexus we often meet multivesicular bodies, lysosomes with fine granular content, dense bodies or with an inner vacuole, lipidic droplets with an irregular border.

8. The nucleus of the epithelial cells is voluminous, spherical or ovoid, with a pored doubled membrane, with a relatively uniformly

distributed chromatin. The position of the nucleus in the choroid plexus cells is variable. It has a central position, but it can be often eccentric and in most cases near the basal pole of cells. It is incised in fishes, amphibians and reptiles and nonincised in birds and adult mammals.

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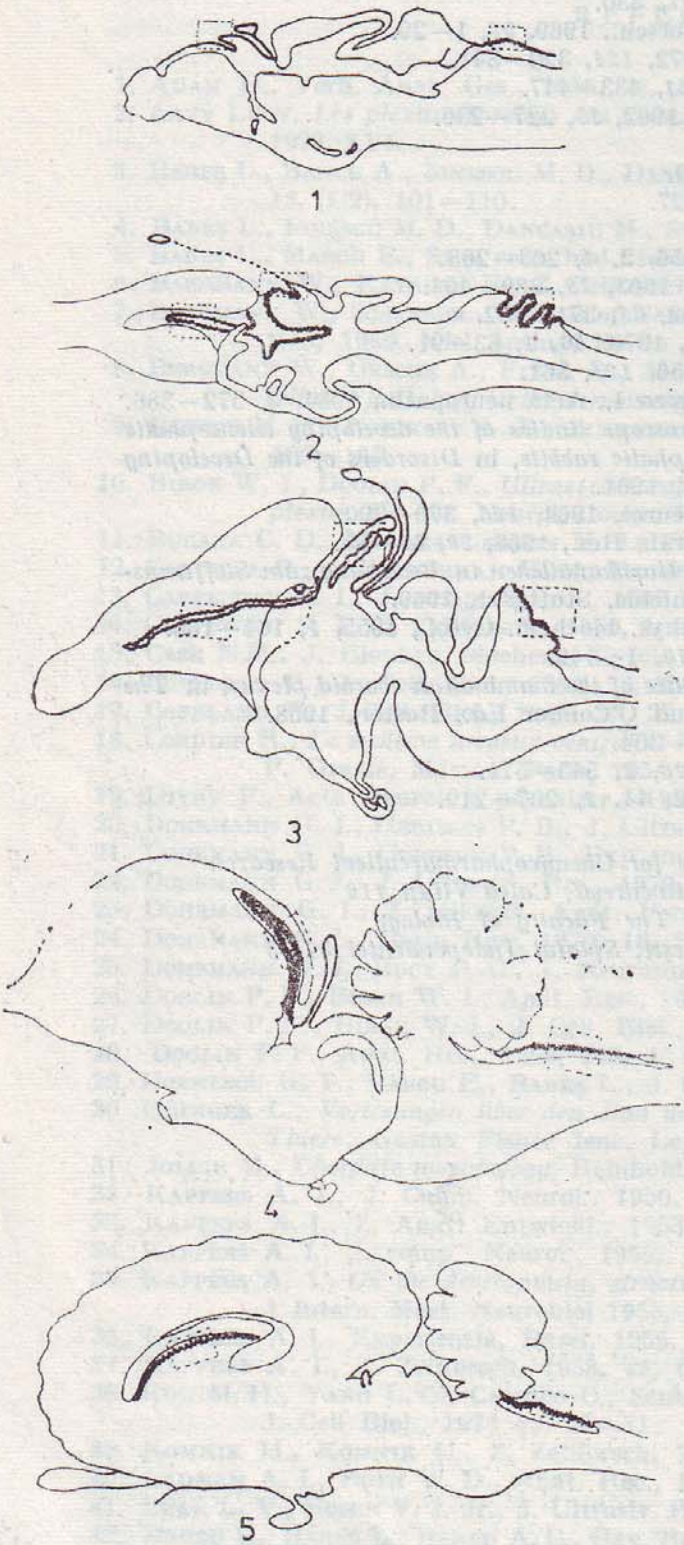


PLATE I. ✧ Schematic representation of the place from which the choroid plexus was removed: Fig. 1 — fishes; Fig. 2 — amphibians; Fig. 3 — reptiles; Fig. 4 — birds; Fig. 5 — mammals (brain sagittal sections).

PLATE II. ✧ Fig. 6. — *Eudontomyzon danfordi* (larva) — Telencephalic choroid plexus at the beginning of the development;

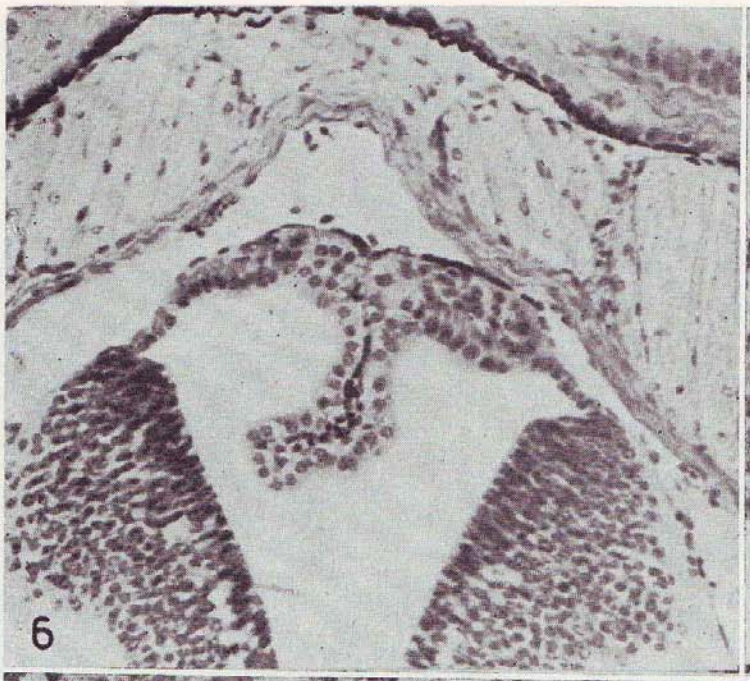
✧ Fig. 7. — *Eudontomyzon danfordi* (larva) — — Numerous mitotic divisions in ependymal cells (arrows);

✧ Fig. 8. — *Carassius auratus gibelio* — Choroid epithelium forming the dorsal sac which includes the epiphysis stalk;

✧ Fig. 9. — *Carassius auratus gibelio* — The basal pole of cells from the choroid plexus epithelium 5800×;

✧ Fig. 10. — *Cyprinus carpio* — The apical pole of the epithelial cell from the choroid plexus 16,100×;

✧ Fig. 11. — *Cyprinus carpio* — Mitochondria presenting tubular cristae 41,500×;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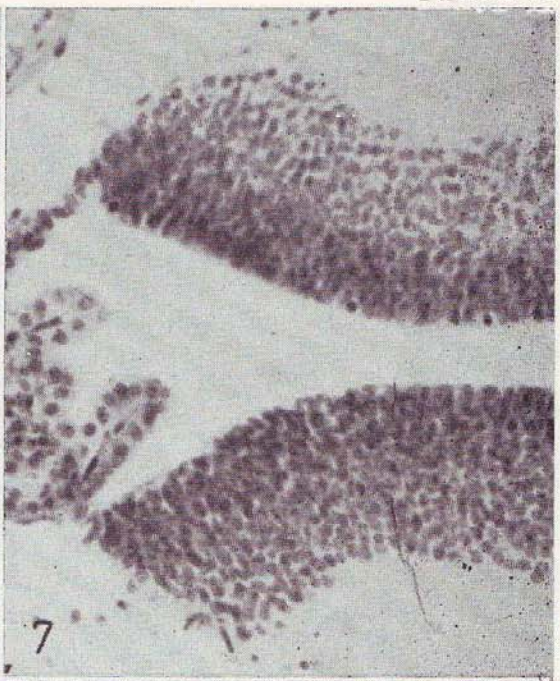
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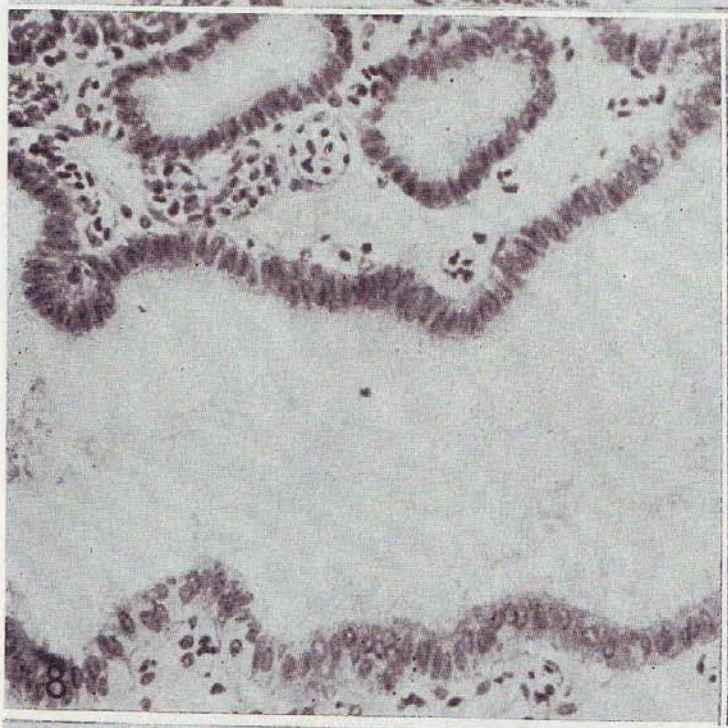
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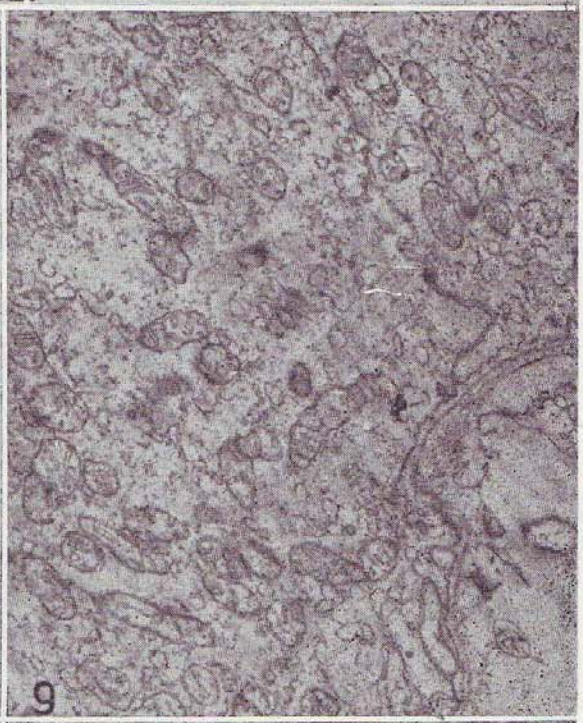
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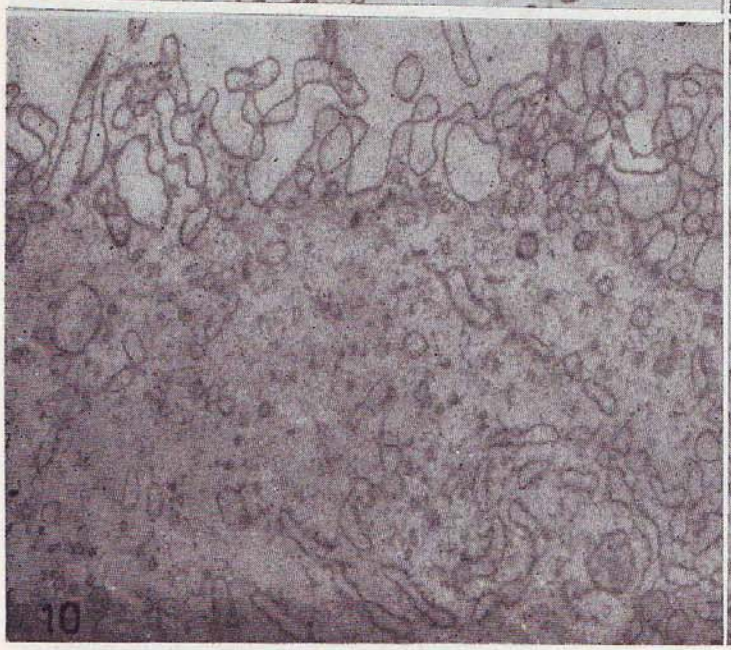
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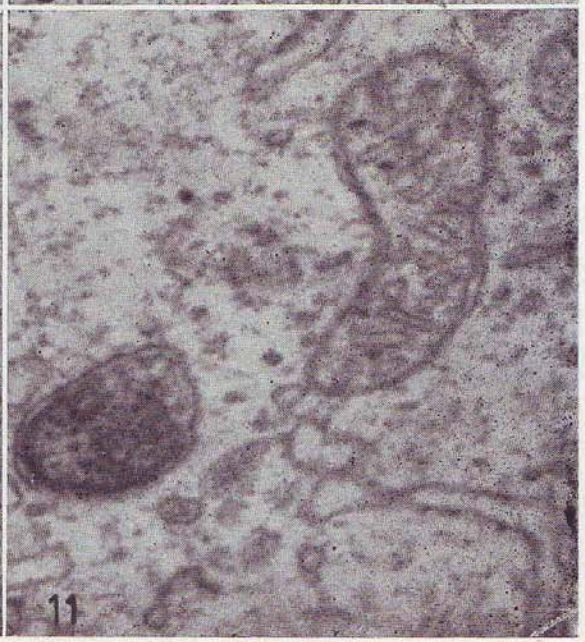
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PLATE III.

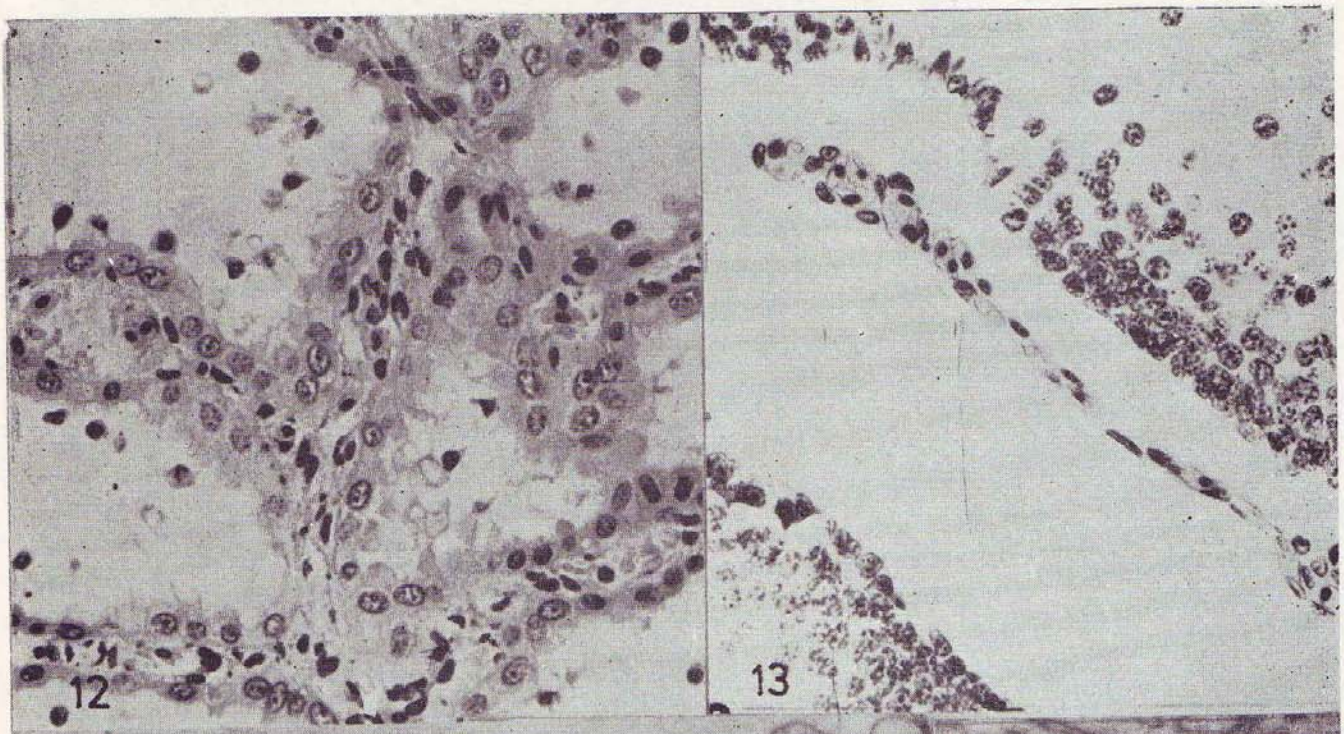
✧ Fig. 12. — *Rana ridibunda* — The myelencephalic choroid plexus folds.

✧ Fig. 13. — *Triturus cristatus* — Telencephalic choroid plexus.

✧ Fig. 14. — *Rana ridibunda* — The apical pole of an epithelial plexus cell. Microvilli dilated at their distal end and very numerous vesicles 18,000×.

✧ Fig. 15. — *Triturus cristatus* — A zone from the apical pole of an epithelial plexus cell in which there are numerous vesicles 20,000×.

✧ Fig. 16. — *Triturus cristatus* — The basal pole of an epithelial cell doubled by the basal membrane and deprived of plasmalemmal infoldings 20,000×.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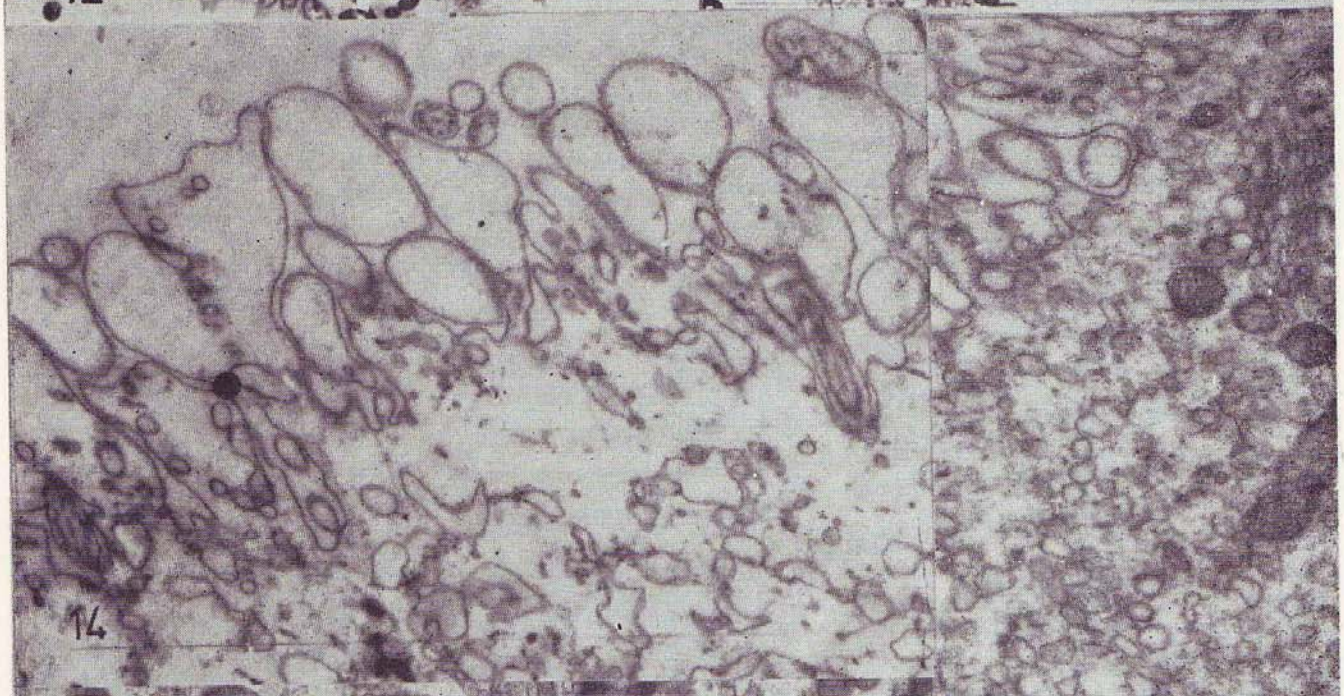
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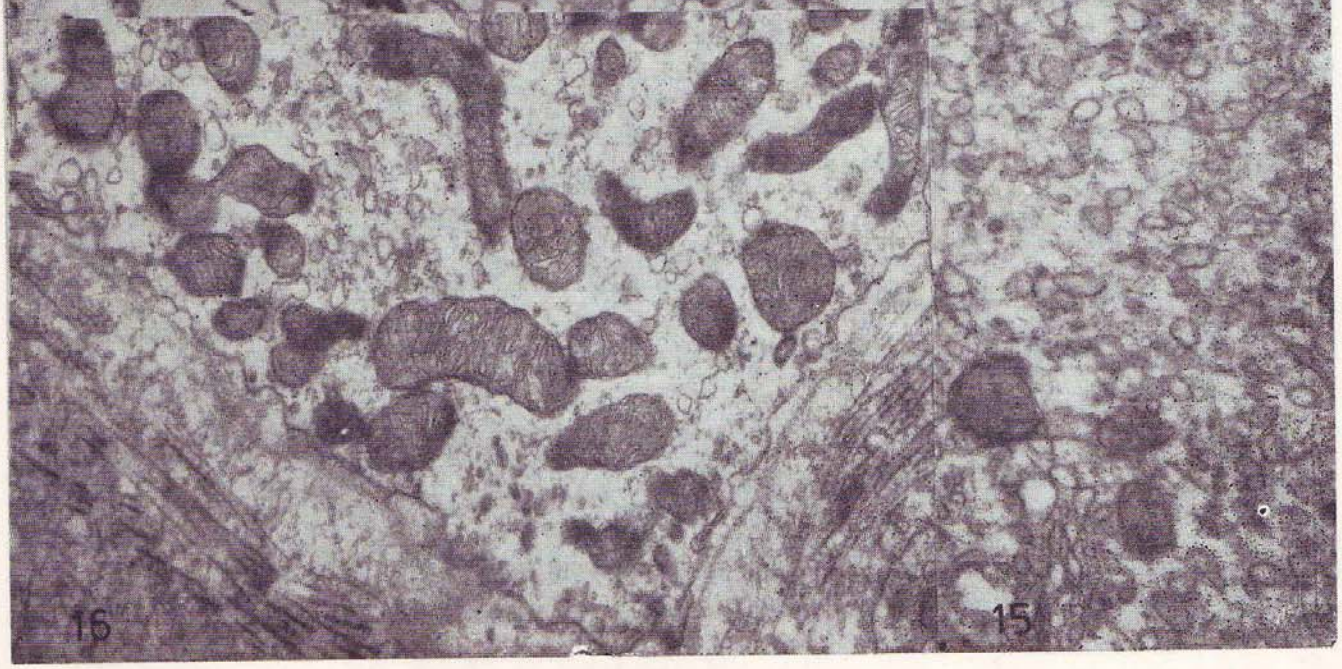
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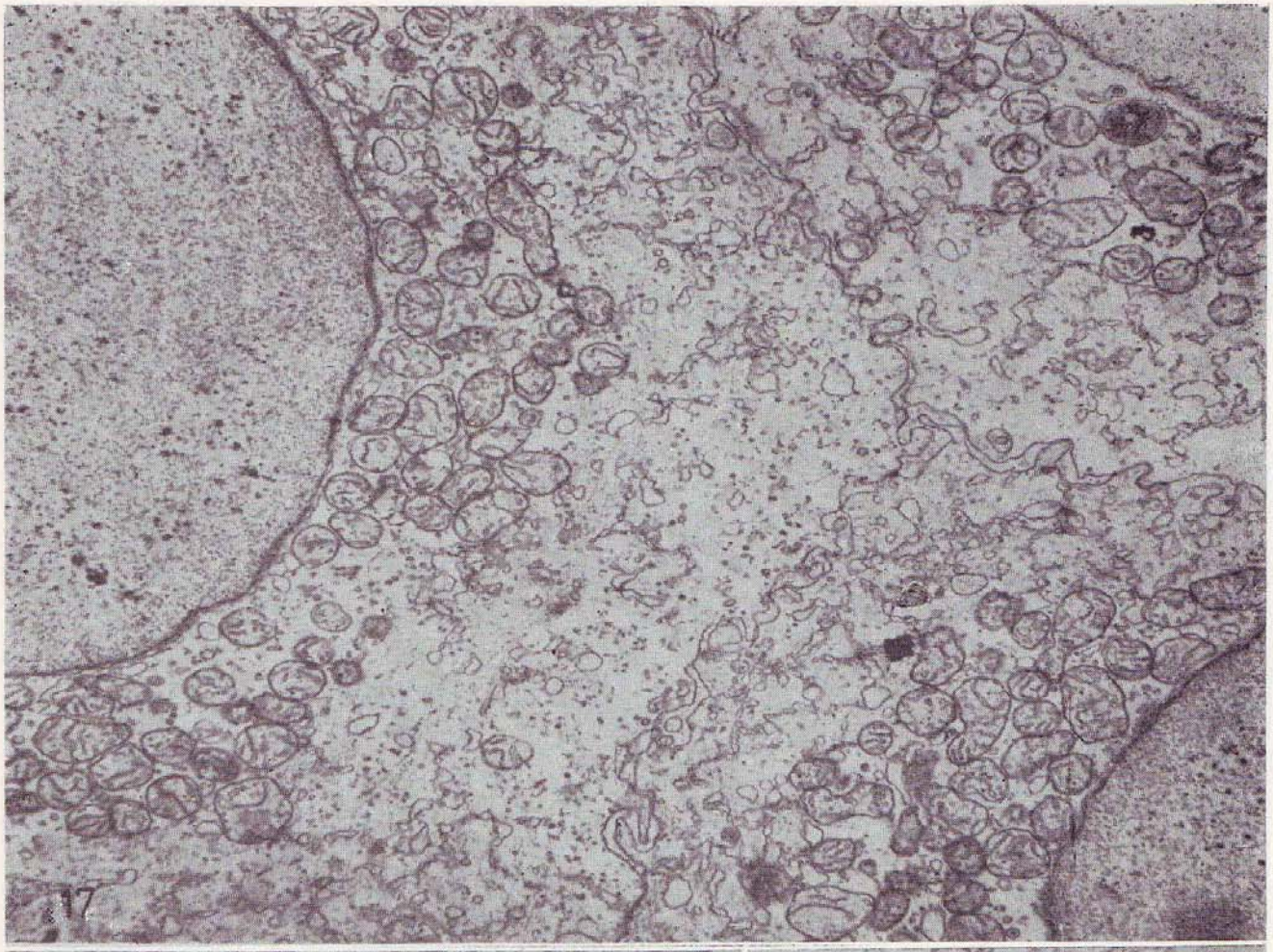
PLATE IV.

✧ Fig. 17. — *Rana ridibunda* — The perinuclear disposition of the mitochondria 10,900×.

✧ Fig. 18. *Rana ridibunda* — Cytoplasmic region from an epithelial plexus cell with mitochondria, Golgi complexes, lysosomes and lipid droplets 18,900×.

✧ Fig. 19. — *Rana ridibunda* — The basal pole of an epithelial cell doubled by a basal membrane and deprived of plasmalemmal infoldings 34,000×.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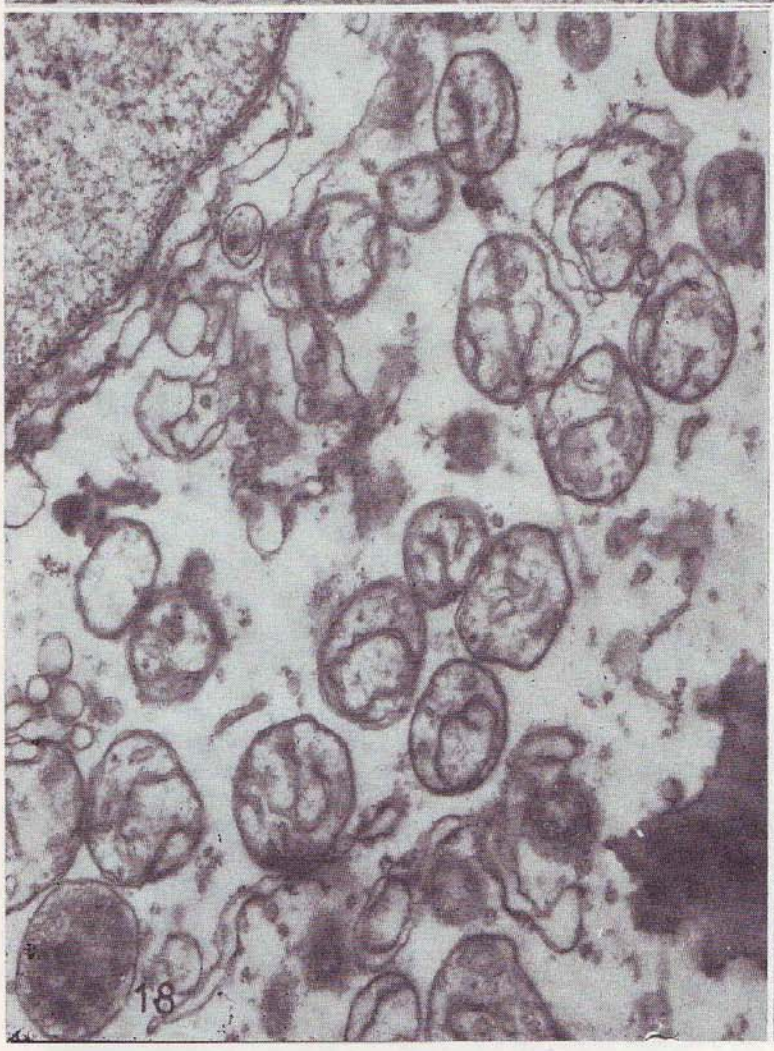
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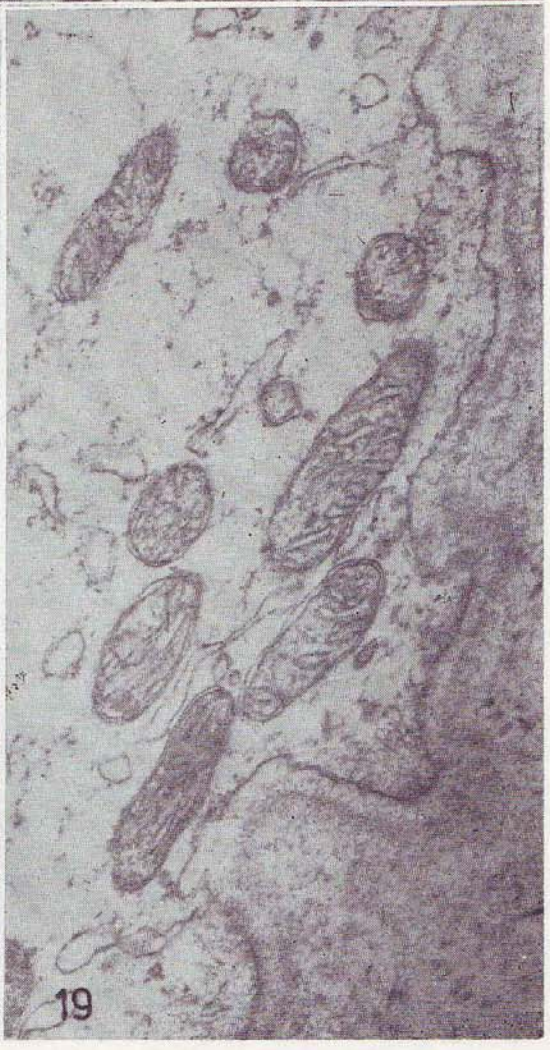
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PLATE V.

✧ Fig. 20. — *Ablepharus kitaibelii fitzingeri* — Choroid plexus from the third ventricle.

✧ Fig. 21. — *Emys orbicularis* — The apical pole of an epithelial plexus cell with irregular cytoplasmic expansions and numerous vesicles 34,000×.

✧ Fig. 22. — *Emys orbicularis* — The basal pole of an epithelial plexus cell with plasmalemmal infoldings, basal membrane and mitochondria 17,900×.

✧ 23. — *Emys orbicularis* — A cytoplasmic region of an epithelial cell, near the nucleus. There are mitochondria with lamellar and tubular cristae and lipid droplets with irregular border 20,300×.

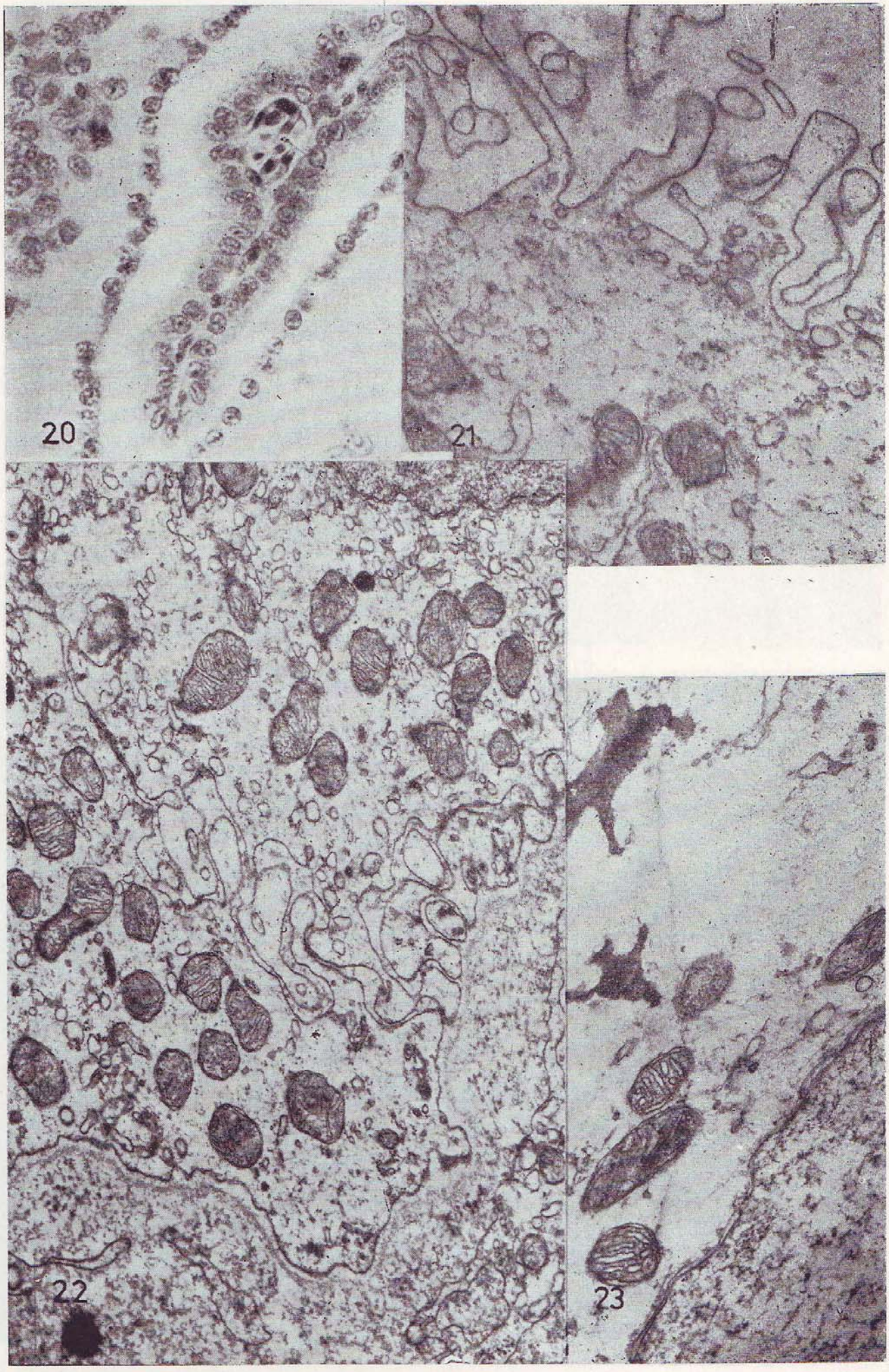
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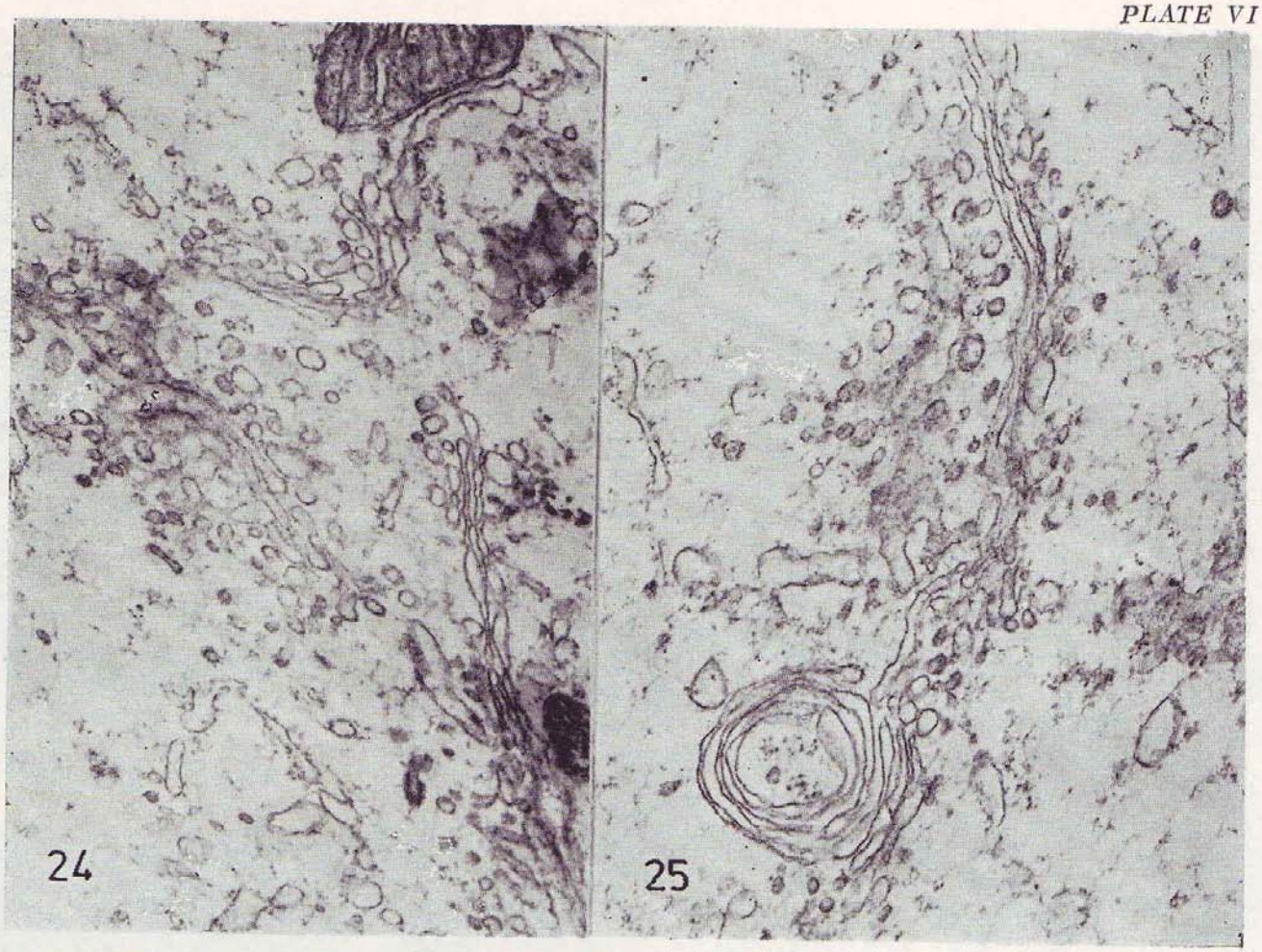
PLATE VI. ✧ Fig. 24. — *Emys orbicularis* — Numerous Golgi complexes 20,200×.

✧ Fig. 25. — *Emys orbicularis* — The Golgi complex with concentric dispositions of the sacculi 21,400×.

✧ Fig. 26. — *Emys orbicularis* — Concentric lamellar body with remarkable dimensions 10,000×.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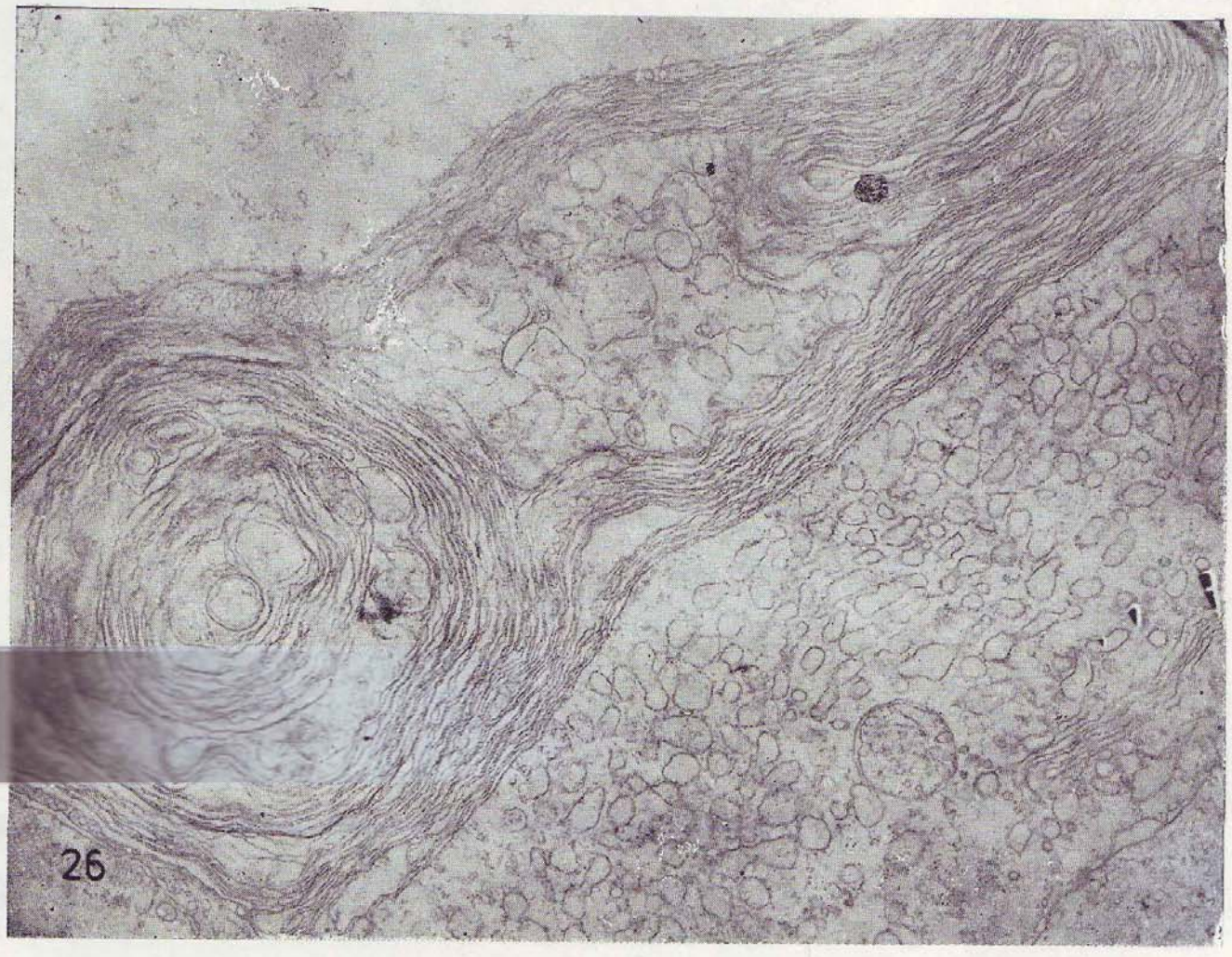
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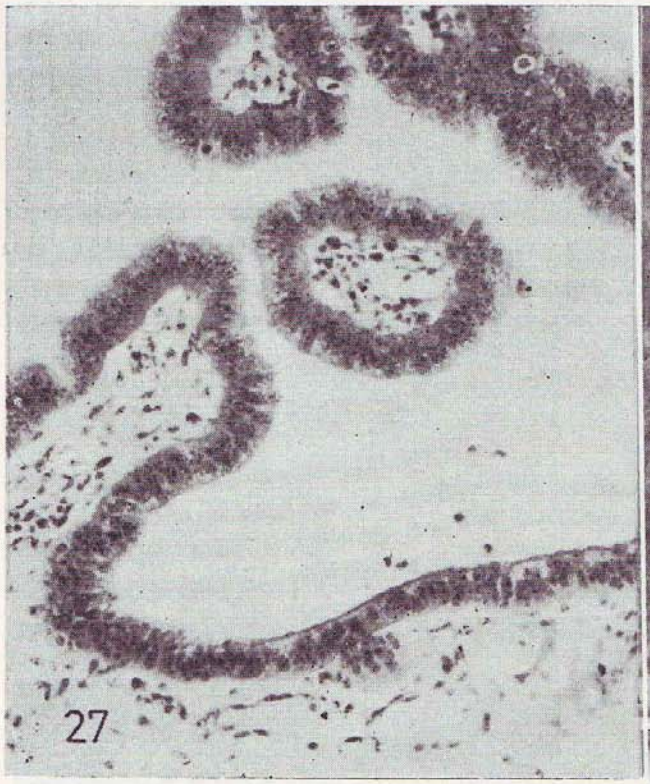
PLATE VII. ✧ Fig. 27. — *Corvus monedula* — The telencephalic choroid plexus.
✧ Fig. 28. — *Coturnix coturnix* — The apical pole of an epithelial cell. The microvilli have a more regular disposition and very numerous pinocytotic vesicles at their basis (arrow) 14,430 ×.
✧ Fig. 29. — *Coturnix coturnix* — The basal pole of an epithelial cell deprived of plasmalemmal infoldings and doubled by a thick basal membrane 14,430 ×.
✧ Fig. 30. — *Coturnix coturnix* — The basal pole of an epithelial cell with sinuous plasmalemmal infoldings 11,000 ×.
✧ Fig. 31. — *Coturnix coturnix* — Mitochondria surrounded by R.E.R. saculi 17,600 ×.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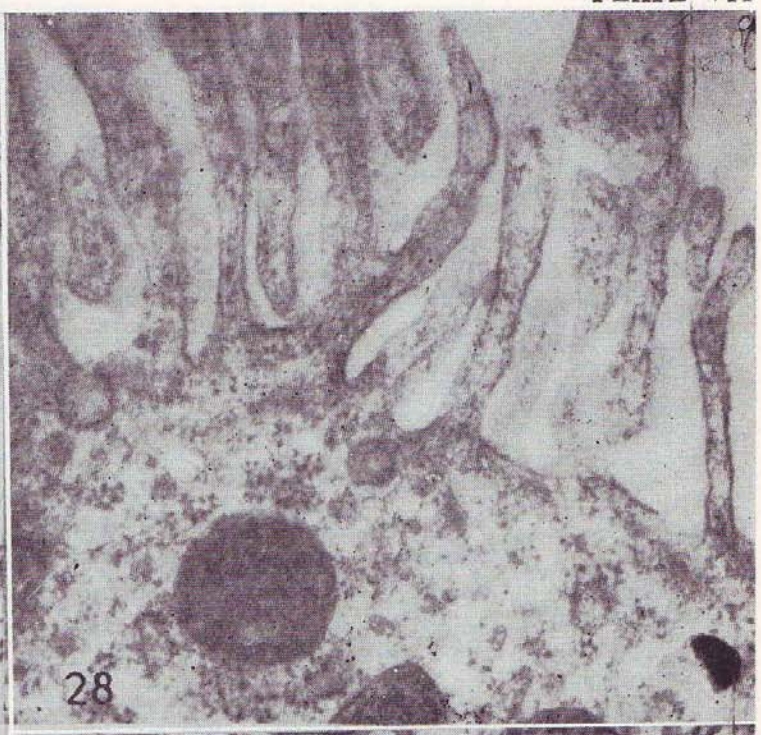
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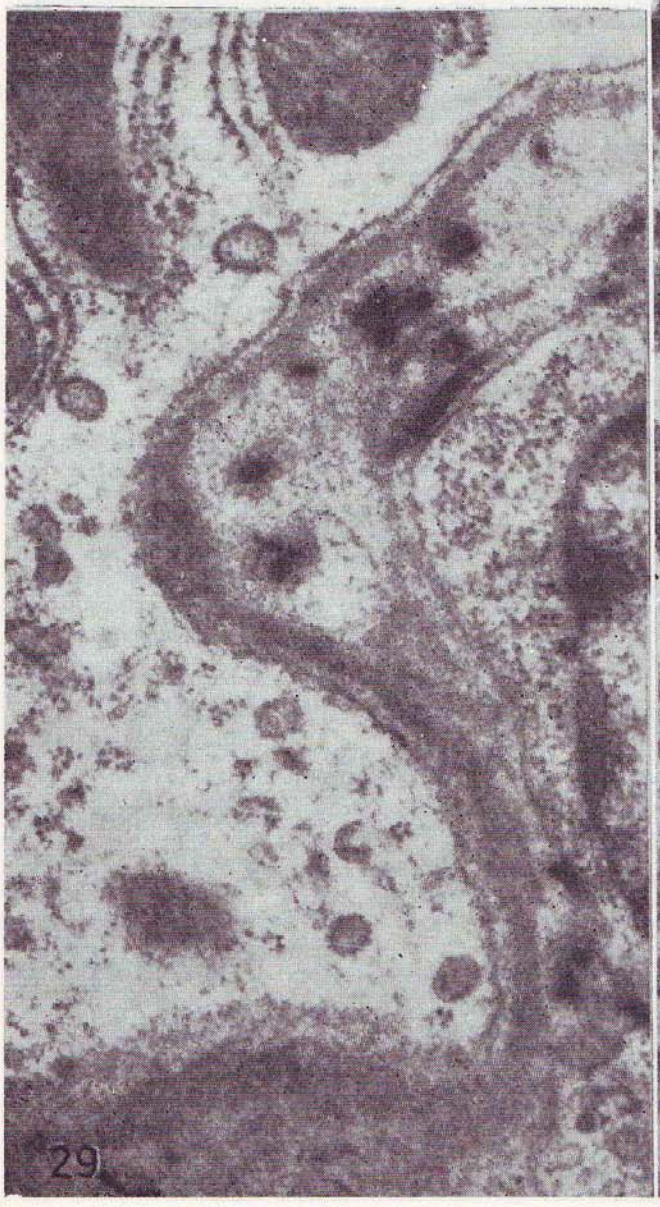
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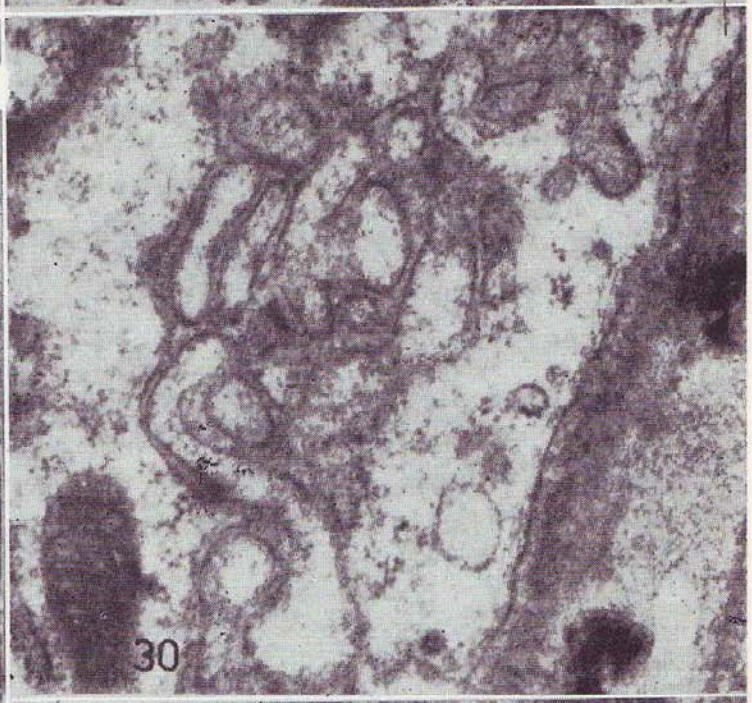
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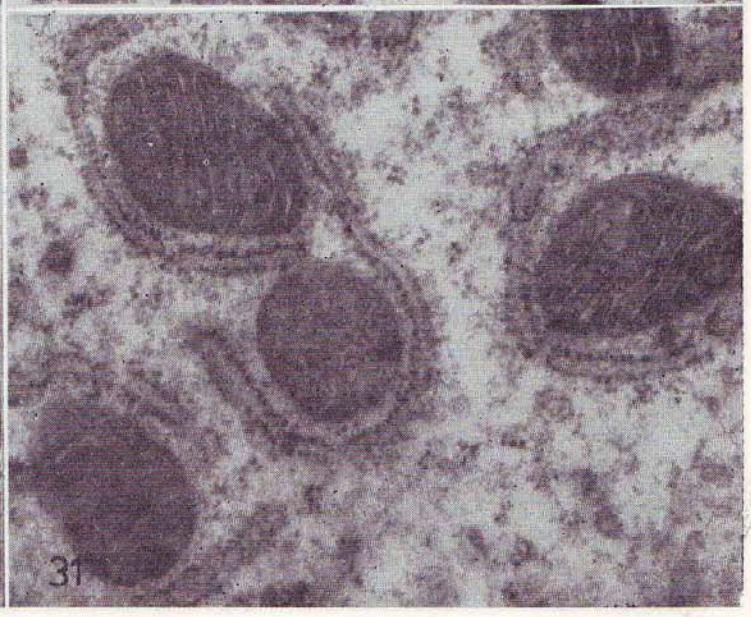
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PLATE VIII. ✧ Fig. 32. *Talpa europaea* — The choroid plexus from the fourth ventricle.

✧ Fig. 33. — *Talpa europaea* — The epithelial plexus cell in which there are a spherical nucleus, mitochondria with lamellar cristae, R.E.R. and the apical pole with microvilli 10,000×.

✧ Fig. 34. — *Talpa europaea* — The basal pole of a cell with thin sinuous plasmalemmal infoldings 20,400 ×.

✧ Fig. 35. — *Talpa europaea* — The basal pole of a cell with large plasmalemmal infoldings 18,200 ×.

✧ Fig. 36. — *Vupes vulpes* — The basal pole of a cell with large plasmalemmal infoldings 14,900 ×.

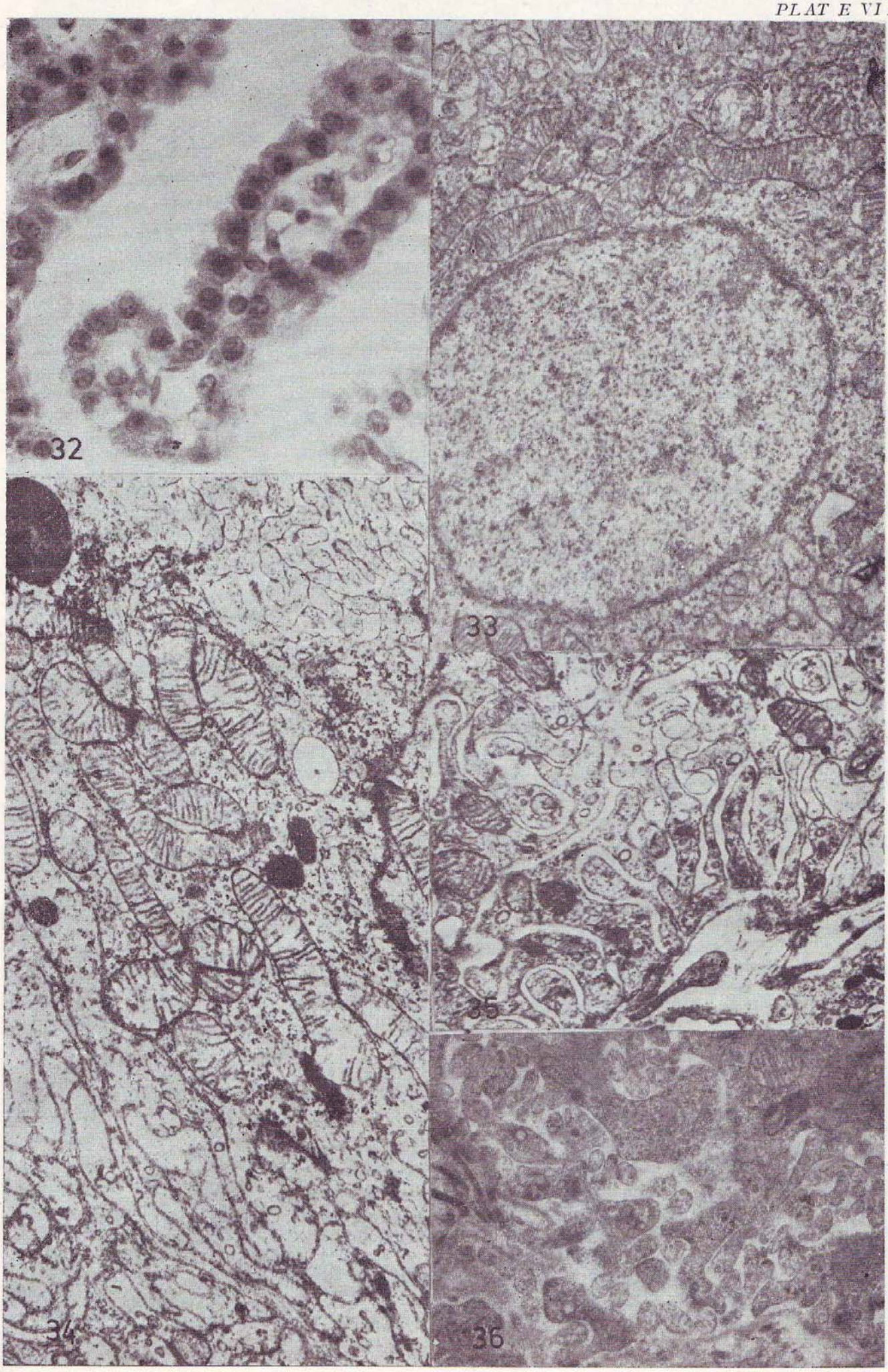
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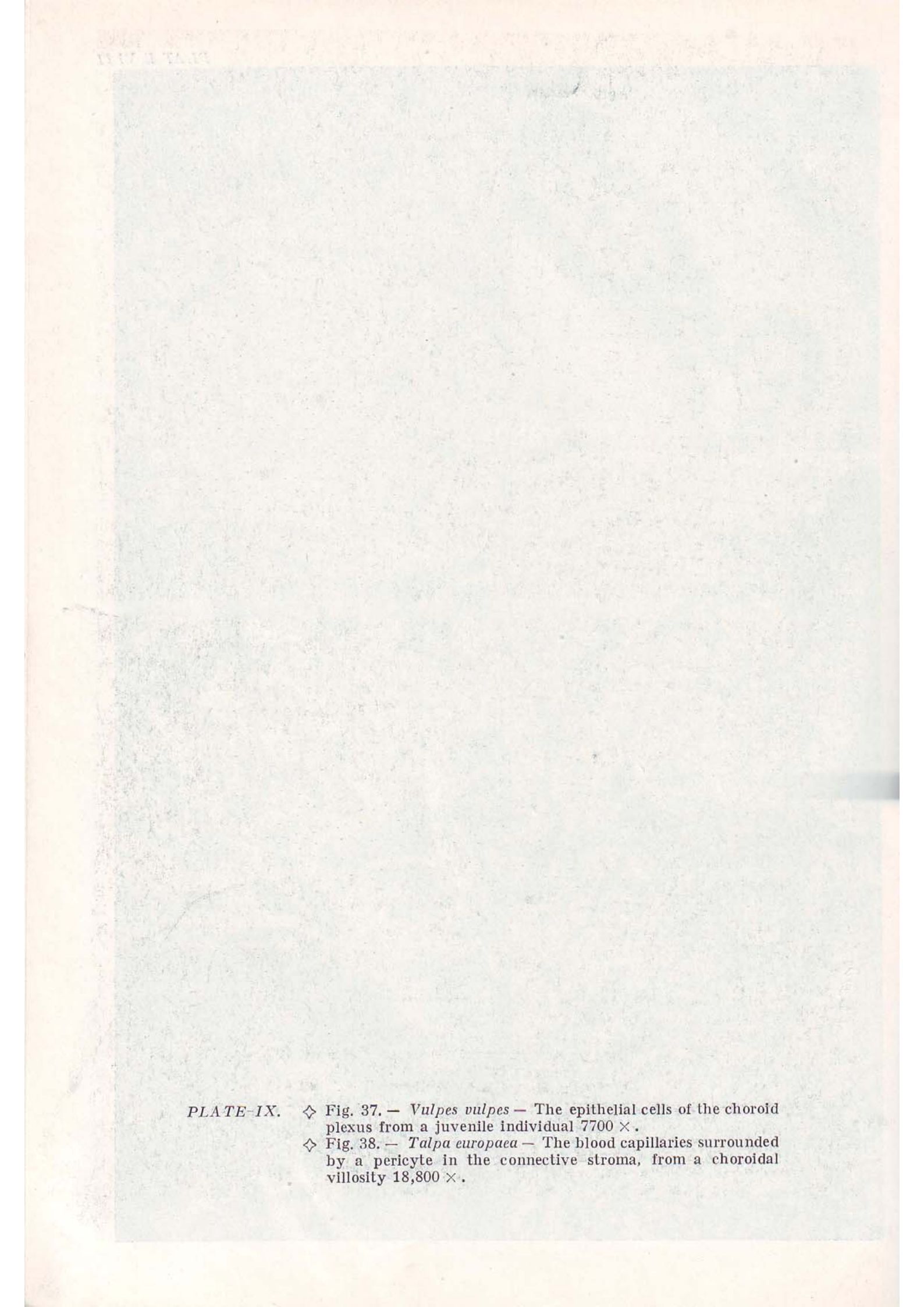
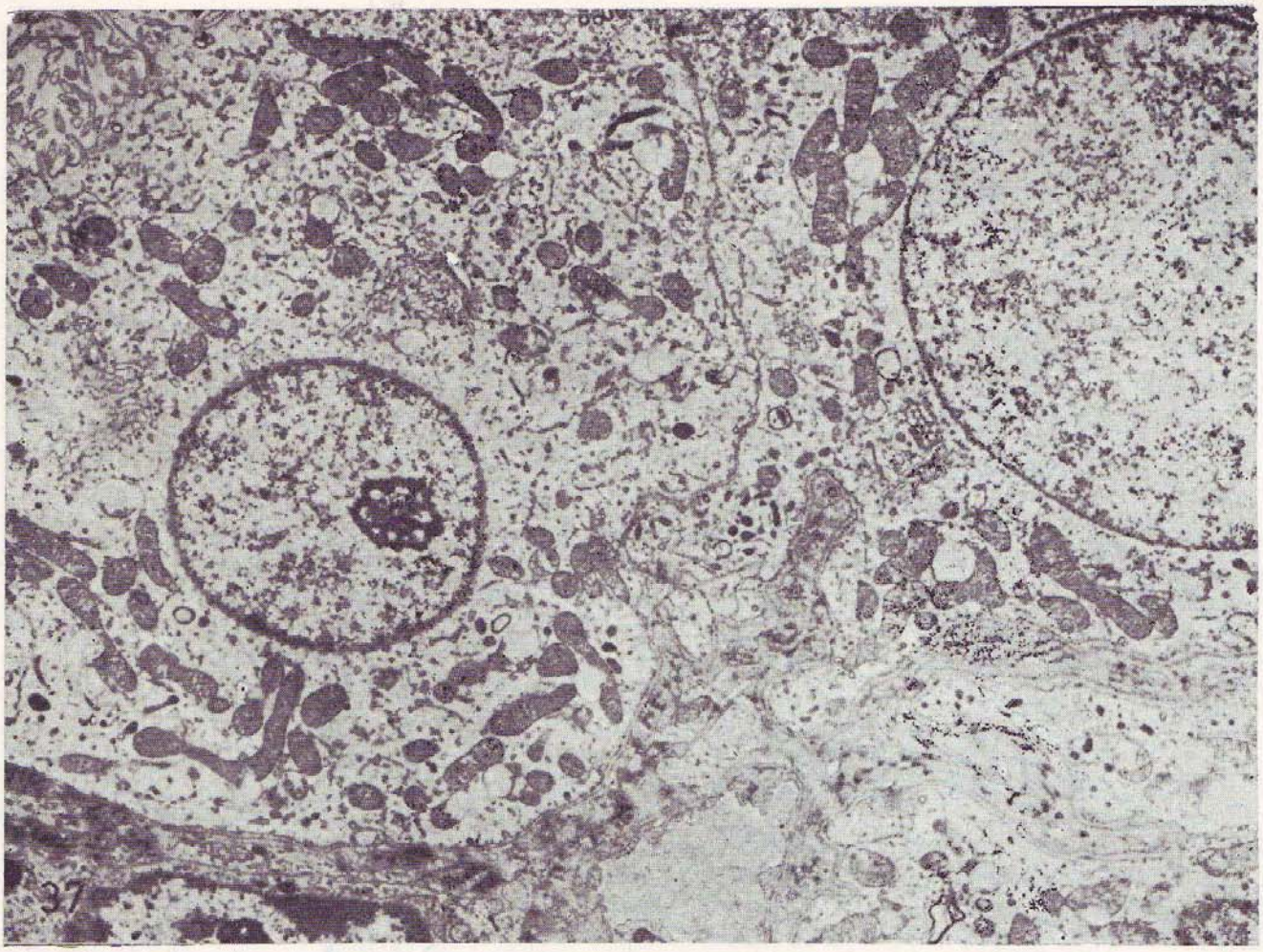
PLATE IX.



◇ Fig. 37. — *Vulpes vulpes* — The epithelial cells of the choroid plexus from a juvenile individual 7700 ×.

◇ Fig. 38. — *Talpa europaea* — The blood capillaries surrounded by a pericyte in the connective stroma, from a choroidal villosity 18,800 ×.



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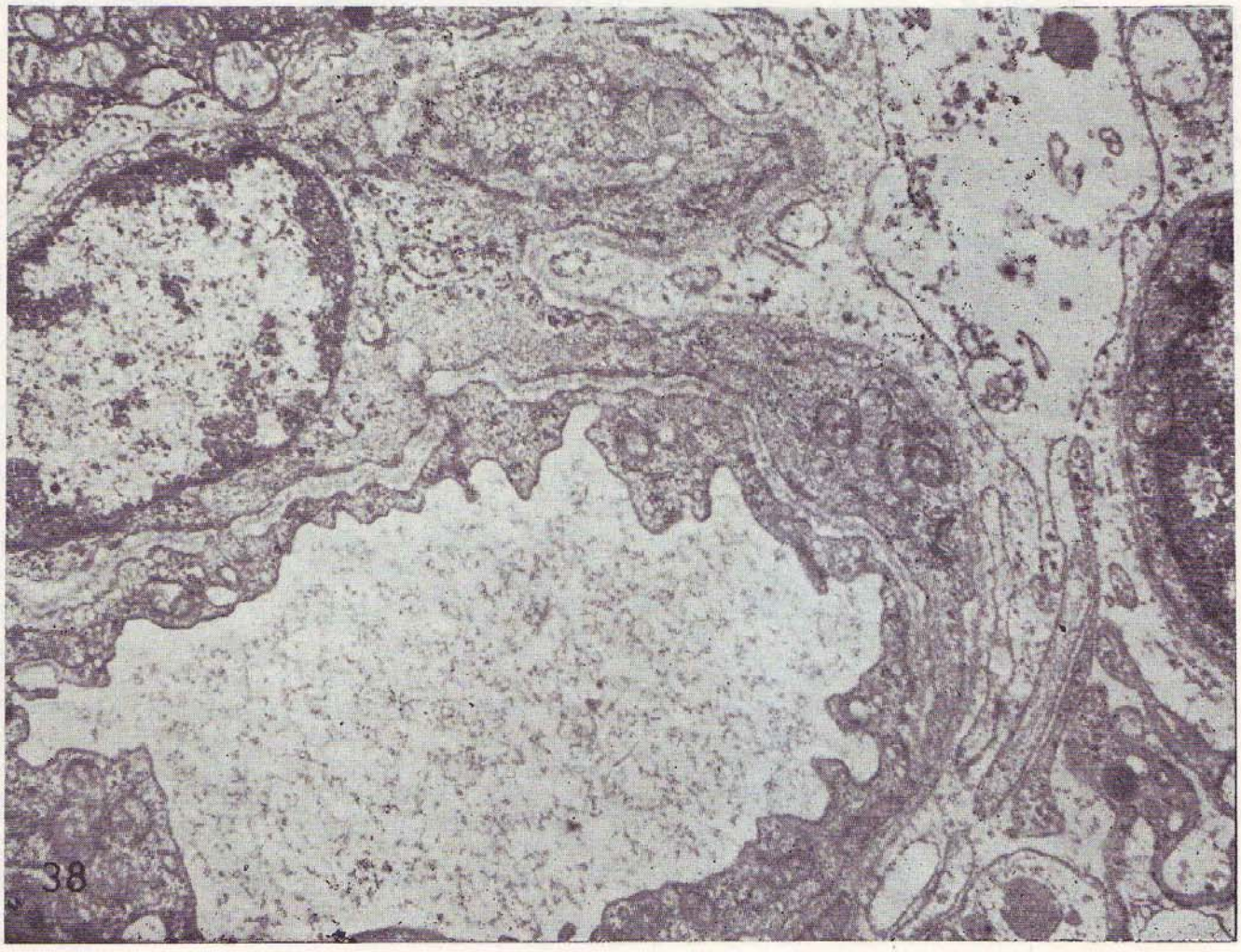

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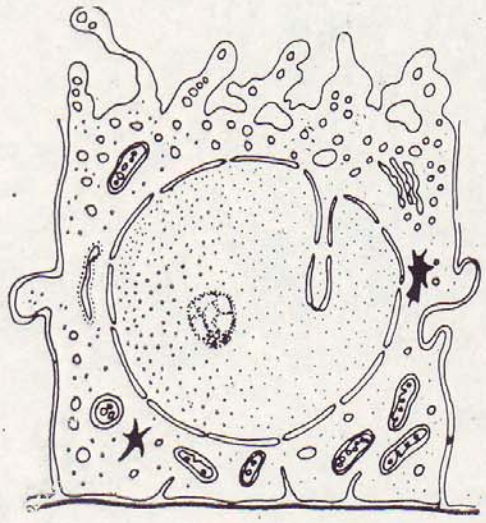

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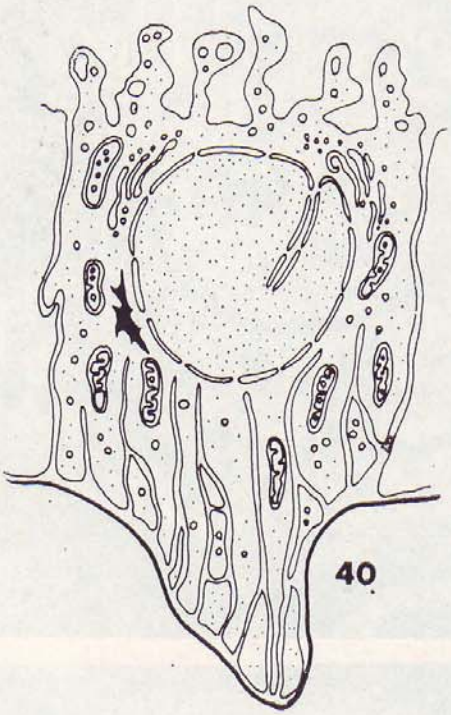

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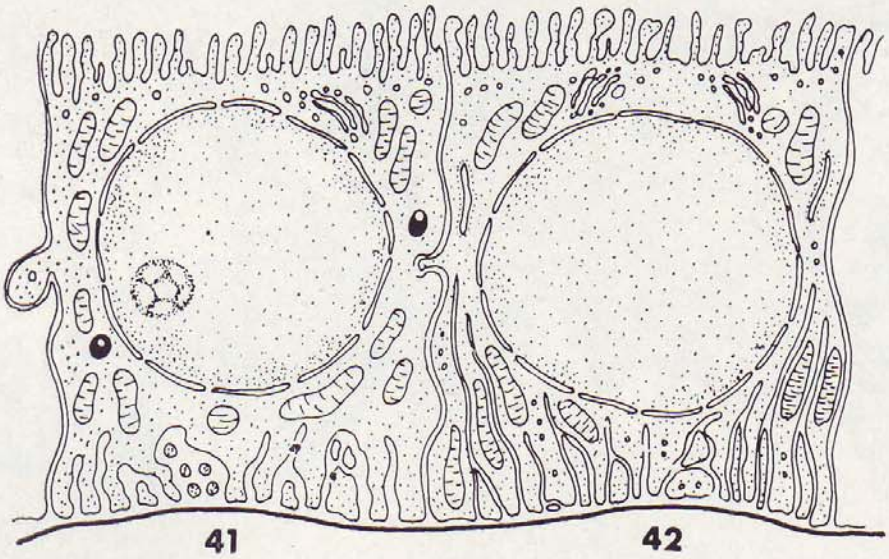

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PLATE X. ✧ Schematic representation of the epithelial cell types in vertebrate phylogenetic development :

✧ Fig. 39. — The primitive cell type from fishes and amphibians.

✧ Fig. 40. — The evolved cell type from reptiles and birds.

✧ Fig. 41. — The cell type with large plasmalemmal infoldings in mammals.

✧ Fig. 42. — The cell type with thin plasmalemmal infoldings in mammals.