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An experimental and ultrastructural study on the development of the avian choroid plexus*

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Summary. The choroid plexus consists of the choroidal epithelium, a derivative of the neural tube, and the choroidal stroma, which originates from the embryonic head mesenchyme. This study deals with epithelio-mesenchymal interactions of these two components leading to the formation of the organ. Grafting experiments of the prospective components have been performed using the quail-chicken marker technique. Prospective choroidal epithelium of quail embryos, forced to interact with mesenchyme of the body wall of chicken embryos, gives rise to a choroid plexus showing normal morphogenesis and differentiation. The choroidal epithelium induces the differentiation of organ-typical fenestrated capillaries, which are highly permeable to intravenously injected horseradish peroxidase. The choroidal epithelium of the grafts constitutes a blood-cerebrospinal fluid barrier. On top of the choroidal epithelium, there are epiplexus cells displaying a typical ultrastructure. The experimental results show that these cells do not originate from the transplanted neural epithelium. Prospective choroidal stroma of chicken embryos does not exert a choroid plexus-inducing influence upon a quail embryo's neural epithelium isolated from parts of the brain that normally do not develop a choroid plexus. The experiments show that the choroidal epithelial cells are determined at least three days before the first organ *anlage* is detectable.

Key words: Choroid plexus – Epithelio-mesenchymal interactions – Blood-cerebrospinal fluid barrier – Epiplexus cells – Endothelial cells – Differentiation – Quail-chicken chimeras

In birds, as in most vertebrates, each of the four ventricles of the brain contains a choroid plexus (CP) (Schmid 1929; Leonhardt 1980). The CP consists of two different components: (i) a monolayered epithelium, the lamina choroidea epithelialis (choroidal epithelium), which is continuous with the ependymal lining of the brain, and (ii) a well-vascularized stroma (choroidal stroma). The choroidal epithelium is derived from the neural tube and retains its epithelial structure throughout development. The choroidal stroma is part of the vascularized leptomeningeal connective tissue that originates from the embryonic head mesenchyme (Meller and Wechsler 1965; Leonhardt 1980; Bargmann et al. 1982).

To the best of our knowledge, there is only one study concerned with the epithelio-mesenchymal interactions of these two components leading to the formation of the CP; Birge (1962) states that "the development of the choroid plexus tissue is contingent upon some causative interaction between the presumptive choroidal epithelium and vascularized leptomeningeal elements". Since the origin of the interacting components could not be determined in the assay employed by Birge (1962), we investigated this problem using the quail-chicken marker-technique (Le Douarin 1969). Cells of these two species can easily be distinguished, because of the different heterochromatin structures of the nuclei. The quail nuclei possess huge perinucleolar heterochromatin condensations. Briefly, we constructed new combinations of the components of the CP. Additionally, intravenous horseradish peroxidase (HRP)-injections were carried out to obtain information about the differentiation of the capillary system and the development of the blood-cerebrospinal fluid barrier (BCSFB) in the grafts.

Materials and methods

Eggs of the Japanese quail (*Coturnix coturnix japonica*) and the white leghorn chicken (*Gallus domesticus*) were incubated at 37.8° C and 80% humidity. Embryos were staged according to the criteria of Hamburger and Hamilton (1951).

Two series of experiments were performed:

(A) Isolated prospective telencephalic choroidal epithelium from the velum transversum (telencephalon medium) of 2- to 3-day quail embryos (stage 14–18) was grafted into the coelomic cavity of chicken embryos (stage 14–18) in order to follow interactions between the epithelium and the mesenchyme of the body wall (Fig. 1). Thirty-eight experiments of this type were evaluated.

(B) Neural epithelium from parts of the brain that normally do not develop a CP (mesencephalon, telencephalic hemispheres) was isolated from quail embryos (stage 13–18) and placed on top of the prospective choroidal stroma of

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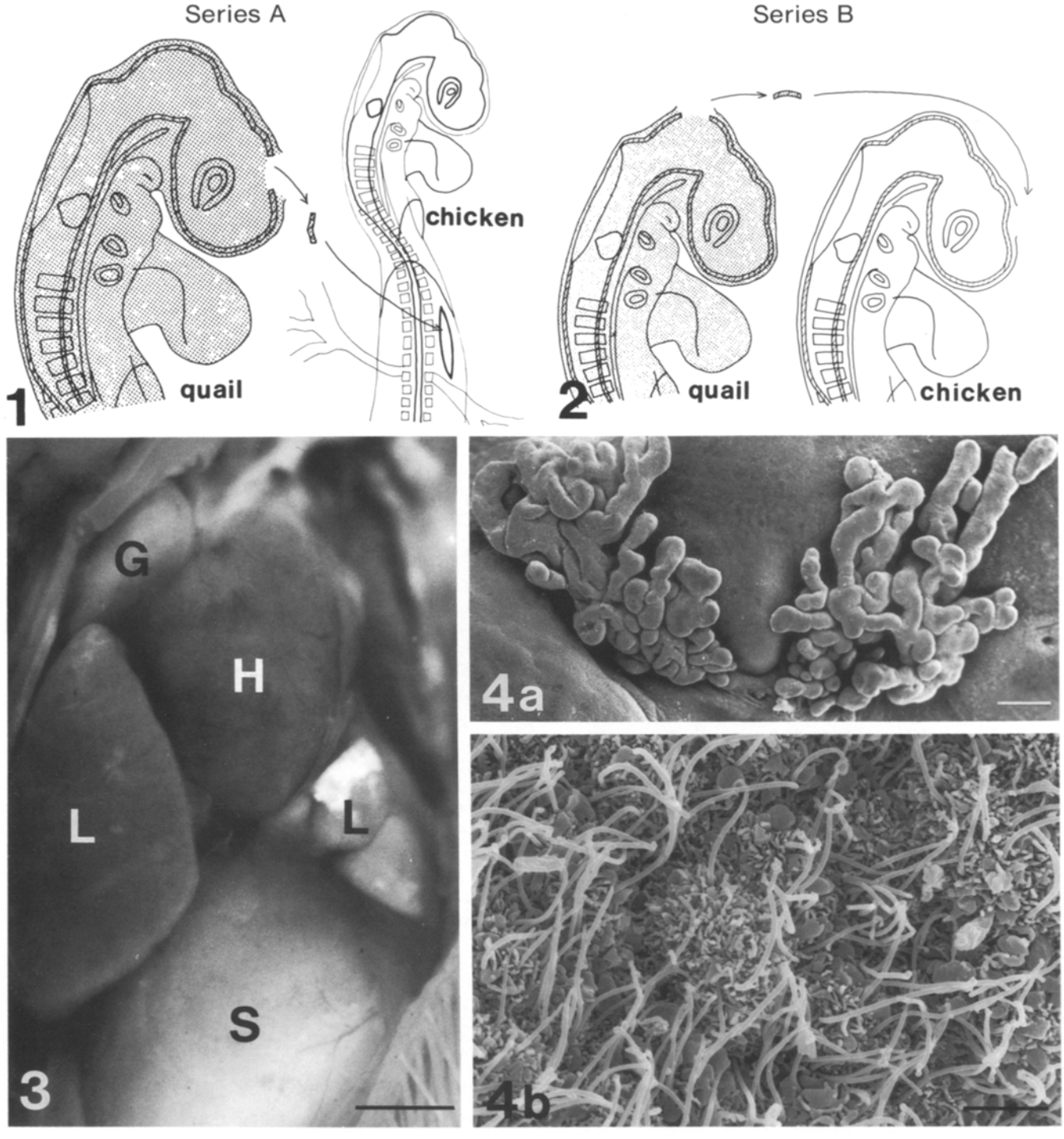


Fig. 1. Scheme showing the combination of isolated prospective choroidal epithelium from the velum transversum of a quail embryo with body-wall mesenchyme of a chicken embryo (Series A)

Fig. 2. Scheme showing the combination of isolated neural epithelium from non-CP-forming areas of the brain of a quail embryo with the prospective choroidal stroma of a chicken embryo (Series B)

Fig. 3. Graft (series A; see Fig. 1) in situ, after 14 days of reincubation. *G* graft; *H* heart; *L* liver; *S* stomach. × 3.5; bar = 5 mm

Fig. 4a, b. Graft (series A) after 14 days of reincubation. **a** Telencephalic CP showing normal morphogenesis. × 40; bar = 200 μm. **b** Higher magnification showing choroidal epithelial cells with cilia, microvilli, and various protrusions. × 4300; bar = 3 μm

chicken embryos (stage 15–18) with the former superficial surface facing the prospective stroma (Fig. 2). Eight experiments of this type were evaluated.

In both series the postoperative incubation period lasted 14 to 15 days. The grafts were then fixed, dehydrated, embedded, serially sectioned, stained according to Feulgen and Rossenbeck (1924), and examined with the light microscope. Grafts of the first series of experiments were also examined with the scanning electron microscope (SEM) and transmission electron microscope (TEM) using conventional methods.

In order to gain insight into the permeability of the blood vessels and the choroidal epithelium, in six cases of the first series, 15 mg HRP type II (Sigma) in 300 μl Locke-

solution was injected into the venous system 10–30 min before fixation with 4% glutaraldehyde in 0.12 M sodium cacodylate buffer. Incubation for HRP-reaction was performed according to Reese and Karnovsky (1967). The grafts were examined by light microscopy and TEM.

Results

A. First series of experiments

The grafts (series A) are attached to the inner body wall of the host embryos as can be seen in Fig. 3. The grafted prospective choroidal epithelium participates in the formation of a CP. The morphology of these CPs is indistinguishable from the telencephalic CP of a normal quail of comparable age. The CP grows in a longitudinal direction and possesses many deep furrows (Fig. 4a). The choroidal epithelial cells bear numerous cilia and many finger-shaped and irregular microvilli (Fig. 4b). The cells clearly have an apicobasal polarity (Fig. 5a–c) with apical cilia, microvilli, and junctional complexes interconnecting the cells (Fig. 5b). The Golgi apparatus (GA) also lies in the apical compartment of the cells. Basally, there are a well-developed rough endoplasmic reticulum (RER), basolateral interdigitations, and a basal lamina (Fig. 5c). Large numbers of mitochondria can be seen within the cells. The nuclei contain up to two nucleoli and the quail-typical perinucleolar heterochromatin condensation, so that chicken and quail nuclei can be distinguished with the TEM (Fig. 5a).

The choroidal stroma of the transplants is mainly made up of fibroblasts, collagen fibrils, and blood vessels (Fig. 5d). The capillary endothelium is very thin and shows fenestrations (Fig. 5e); this suggests that foreign endothelial cells differentiate in a CP-typical manner.

The Feulgen and Rossenbeck-stain shows that the cells of the grafts giving rise to the choroidal epithelium contain nuclei of quail origin. In contrast, the choroidal stroma and the blood vessels are made up of cells with chicken nuclei (Fig. 6). These cells originate from the body wall of the host embryo.

After injecting HRP into a venule of the chorioallantoic membrane of the host embryo, the enzyme can be detected within the choroidal stroma, in the intercellular clefts between the choroidal epithelial cells, and in vacuoles within these cells (Fig. 7a). When the choroidal epithelium has grown in direct contact with the neural part of the graft, HRP is restricted to the close neighbourhood of the choroidal epithelium (Fig. 7b). From this, it can be seen that only the capillaries at the basis of the epithelium are highly permeable to HRP.

The choroidal epithelium of the grafts constitutes a BCSFB. The epithelial cells take up HRP by means of micropinocytotic vesicles (Fig. 7c) and probably digest the enzyme within lysosomes in the apical compartment of the cells (Fig. 7d). The paracellular passage of HRP is blocked in the region of the junctional complex (Fig. 7d).

Epiplexus (Kolmer) cells showing their typical ultrastructure lie on top of the choroidal epithelium of the grafts (Fig. 8a, b). The cells are spherical and possess protrusions of different shapes. The nucleus is indented and located in the compartment of the cell that is averted from the choroidal epithelium. Within the cytoplasm, there are many vacuoles but only a few mitochondria. Filopodia of the cells are in contact with the surface of the choroidal epithelium. The epiplexus cells of the grafts always have nuclei of chicken origin (Fig. 8c), indicating that the cells do not originate from the transplanted neural epithelium of the quail but from the host embryo.

B. Second series of experiments

The grafts of series B do not give rise to a CP. The grafts are attached to the region of the fissura longitudinalis cerebri, rostrally to the pineal organ (Fig. 9a). During the 14 days of reincubation, the grafts develop close to the normal CP of the host embryo (Fig. 9b). The host's choroidal stroma is found adjacent to the graft, which is mainly made up of quail cells (Fig. 9c). These results show that the prospective choroidal stroma does not exert a CP-inducing influence upon the neural epithelium.

Discussion

1. Epithelio-mesenchymal interactions

In the quail and the chicken (El-Gammal 1983), the first *anlagen* of the telencephalic CP cannot be detected before the 5th day of development. Nevertheless, prospective choroidal epithelium of the quail isolated as early as the second day of development (stage 14) gives rise to a CP when it interacts with mesenchyme of the body wall of a chicken embryo. The choroidal epithelial cells of the grafts are of donor (quail) origin. The endothelial cells, like the other cells of the choroidal stroma, originate from the host (chicken) body wall.

These CPs show a normal morphogenesis and differentiation. The choroidal epithelial cells possess the usual set of organelles and are apically linked by junctional complexes. The capillary endothelial cells adjacent to the choroidal epithelium have a thin wall and are fenestrated. These capillaries are readily permeable to intravenously injected HRP. From this, it can be seen that the choroidal epithelium induces the differentiation of organ-typical capillaries in the graft.

Neural epithelium isolated from the mesencephalon and the telencephalic hemispheres of 2–3-day quail embryos, and allowed to interact with the prospective telencephalic choroidal stroma of 2–3-day chicken embryos, does not develop a CP.

The experiments show that the prospective choroidal stroma does not exert an inductive influence upon the neural epithelium leading to the formation of a CP. The development of the organ is dependent upon the existence of prospective choroidal epithelial cells. These are determined at least three days before the development of the first CP-*anlagen*.

Presumptive metencephalic ependymal cells appear to give rise to a CP (Birge 1962). After ablation of the right presumptive metencephalic alar plate of stage-10 chicken embryos, Birge found that a simple cuboidal to columnar epithelium, regenerating from the adjacent ependymal zone, closes the lesion area by the 10th day of development. Following further development, this epithelium comes into

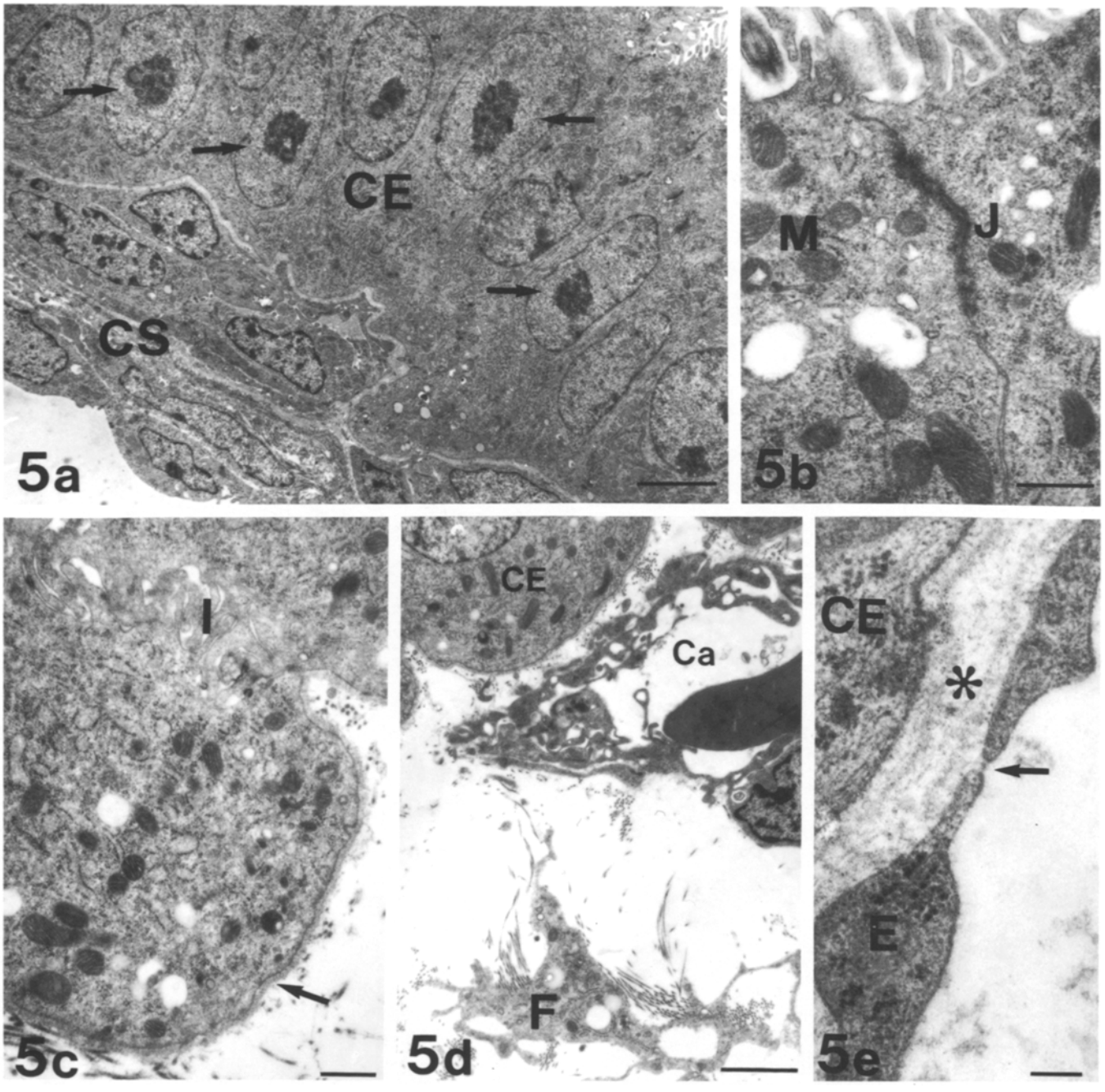
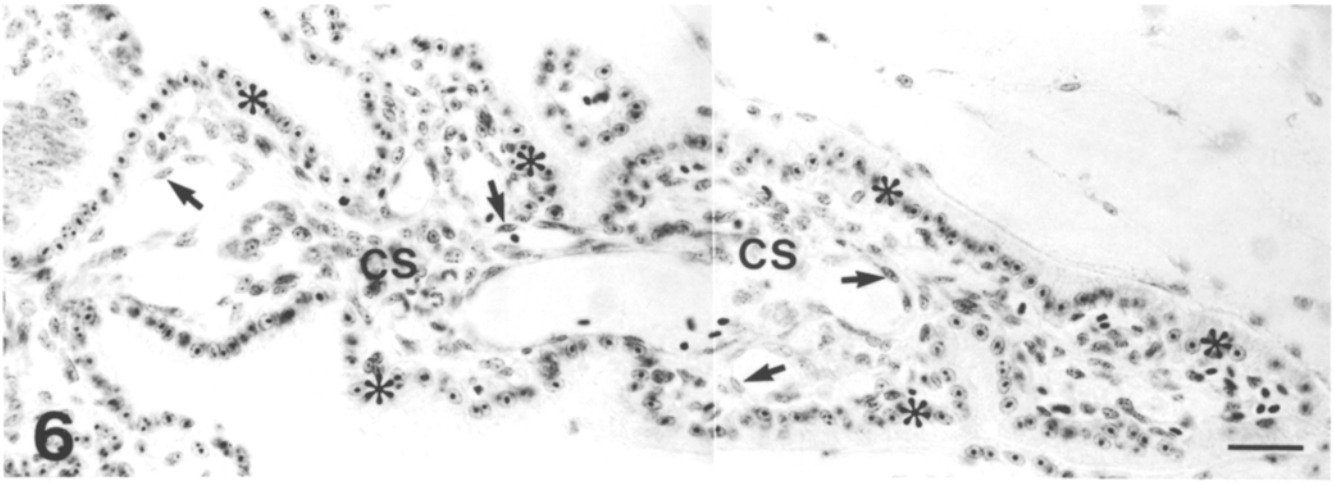
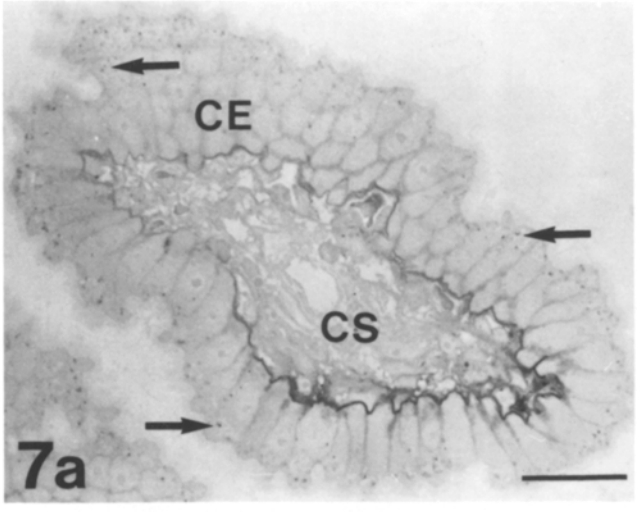


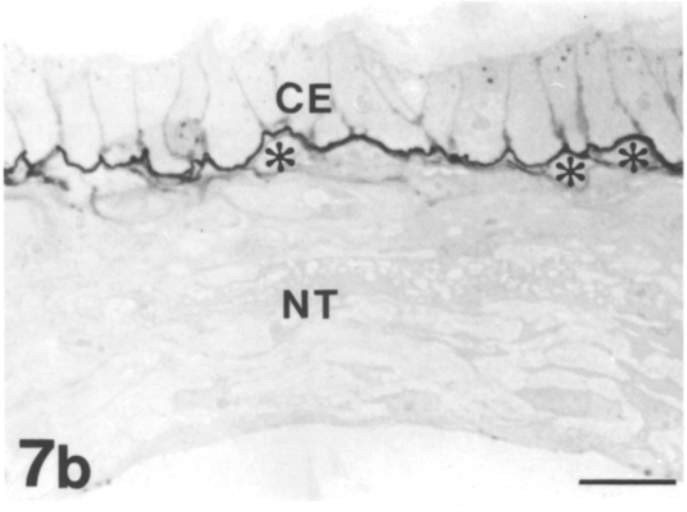
Fig. 5a–e. Grafts (series A) after 14 days of reincubation. **a** Normally structured choroidal epithelial cells (*CE*) with nuclei of the quail type (*arrows*) and choroidal stroma cells (*CS*) with nuclei of the chicken type. ×3000; bar = 4 μm. **b** Choroidal epithelial cells connected by a junctional complex (*J*); mitochondria (*M*). ×24400; bar = 0.5 μm. **c** Choroidal epithelial cells with basal lamina (*arrow*) and basolateral interdigitations (*I*). ×17000; bar = 0.5 μm. **d** Choroidal epithelium (*CE*) with adjacent capillary (*Ca*) and fibrocyte (*F*). ×6000; bar = 2 μm. **e** Endothelial cell (*E*) with fenestration (*arrow*). Choroidal epithelial cell (*CE*), basal laminae (*star*). ×80000; bar = 0.1 μm

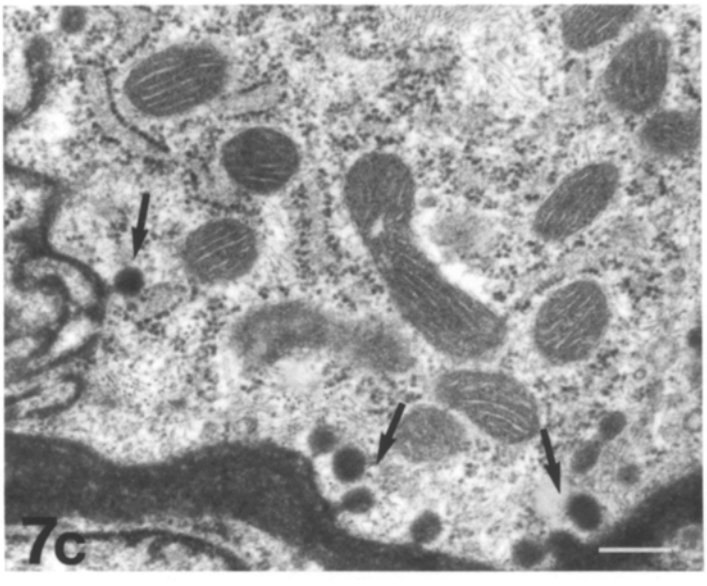
contact with the leptomeningeal tissue and gives rise to a CP. Two important points in the studies of Birge (1962) remain unsettled: (1) regeneration does not necessarily reflect normal development, and: (2) the source of the regenerating epithelium cannot clearly be determined. It is thus probable that cells of the adjacent myelencephalic choroidal epithelium contribute to the regenerating epithelium. As to their prospective fate, these cells develop a CP. This possibility is not only supported by our studies but also by a statement of Birge (1962) himself, who observed that the supernumerary metencephalic CP is "indistinguishable in structure and form from that associated with the roof of the fourth ventricle".

In the normal brain, there are areas such as the posterior part of the roof of the fourth ventricle that remain epithelial during development and that come into contact with the leptomeningeal tissue without developing a CP (Cohen and Davies 1938). Again, this shows that the leptomeningeal









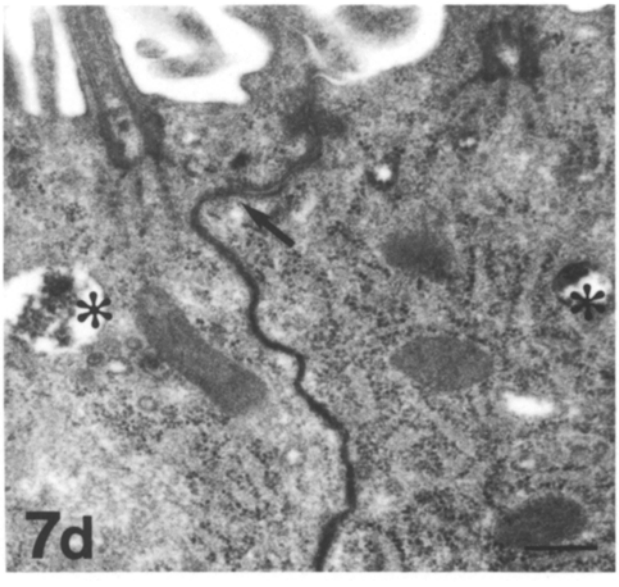


Fig. 6. Graft (series A) after 15 days of reincubation. The choroidal epithelium (*stars*) possesses quail-type nuclei. The choroidal stroma (*CS*) and the endothelial cells (*arrows*) are chicken cells. ×480; bar = 20 μm

Fig. 7a–d. Grafts (series A) after 14 days of reincubation and intravenous injection of HRP 10–30 min before fixation. **a** CP showing HRP within the choroidal stroma (*CS*), between the choroidal epithelial cells (*CE*) and in vacuoles (*arrows*) within these cells. Unstained semithin section. ×660; bar = 20 μm. **b** Choroidal epithelium (*CE*) grown adjacent to the nervous tissue (*NT*) of the graft. HRP can only be found in direct proximity to the CE; capillaries (*stars*). Unstained semithin section. ×1240; bar = 10 μm. **c** The choroidal epithelial cells take up HRP by means of micropinocytotic vesicles (*arrows*). ×45000; bar = 0.2 μm. **d** The paracellular passage of HRP is impeded in the region of the junctional complex (*arrow*) of the choroidal epithelial cells. HRP within apical vesicles (*stars*). ×31900; bar = 0.3 μm

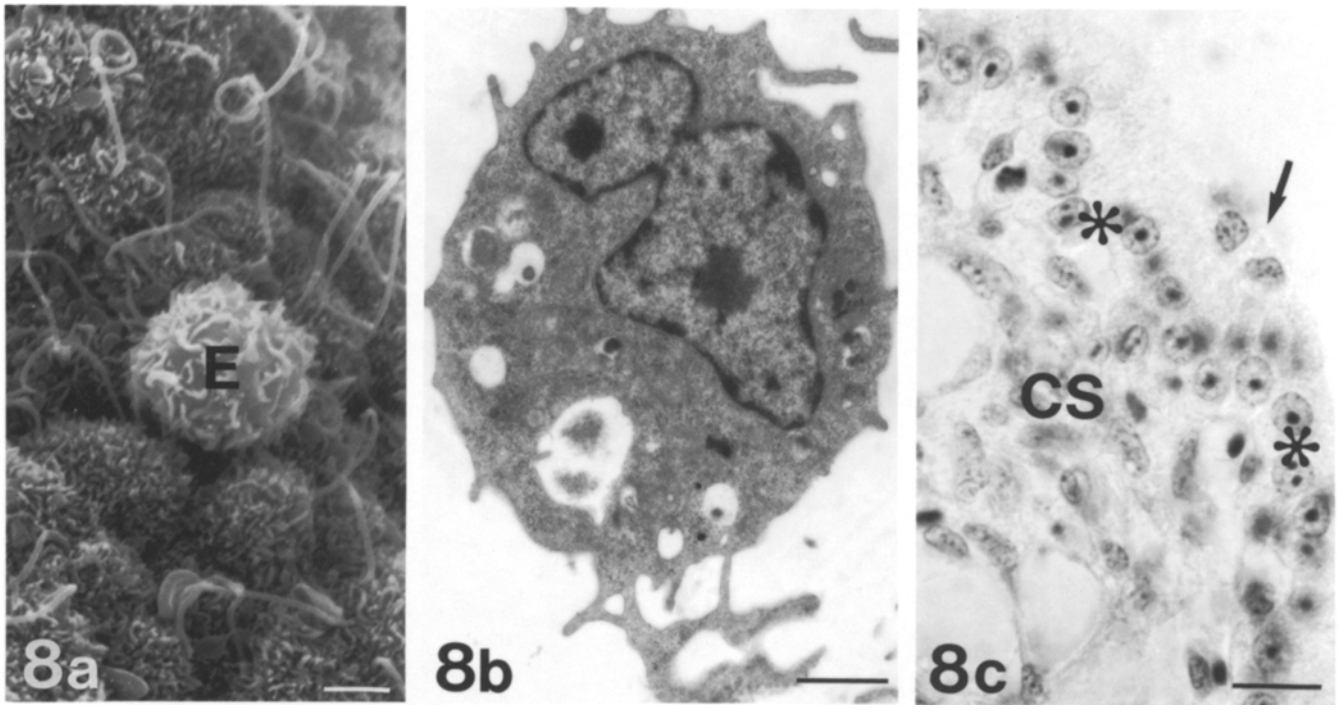


Fig. 8a–c. Grafts (series A) after 14 days of reincubation. **a** Round epiplexus cell (*E*) with various protrusions of the cell surface. ×4400; bar = 2 μm. **b** Round epiplexus cell with eccentric nucleus, few mitochondria, and numerous vesicles and vacuoles. ×11800; bar = 1 μm. **c** Epiplexus cells (*arrow*) with nuclei of chicken type. Choroidal epithelium (*stars*); choroidal stroma (*CS*). ×1150; bar = 10 μm

tissue does not exert a CP-inducing influence on the neural epithelium.

Various standard texts state that the CP develops in a way that the vascularized leptomeningeal connective tissue pushes the choroidal epithelium into the ventricle (Benninghoff 1985; Kahle et al. 1986). This seems improbable, considering that normal development of the CP takes place when the leptomeningeal tissue is substituted by connective tissue of the body wall. Other developmental mechanisms seem to be more important. Among these are the proliferation of the epithelial cells (Soenarjo 1972) and the characteristic development of the epithelium, which changes from a pseudostratified to a monolayered prismatic and finally cuboidal epithelium (Meller and Wechsler 1965).

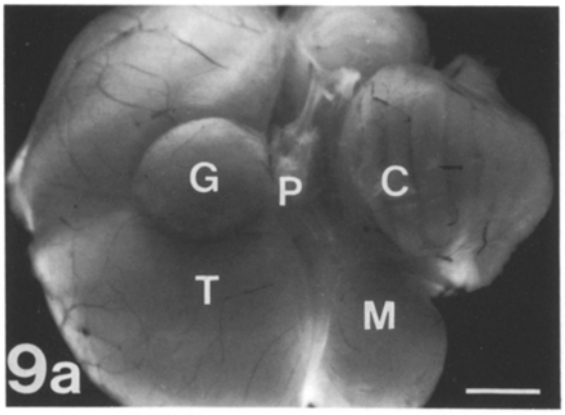
In vitro studies of Meller et al. (1969) have shown that isolated choroidal epithelial cells reaggregate and form rosettes. Therefore, the normal morphogenesis of the CP is dependent on underlying vascularized connective tissue. However, as we have shown, this morphogenetic function is not restricted to the leptomeningeal connective tissue. The importance of the extracellular matrix for the morphogenesis of the CP needs further investigation. The basal lamina of the choroidal epithelium appears to be produced by isolated epithelial cells themselves (Meller et al. 1969); these cells, nevertheless, fail to undergo normal morphogenesis.

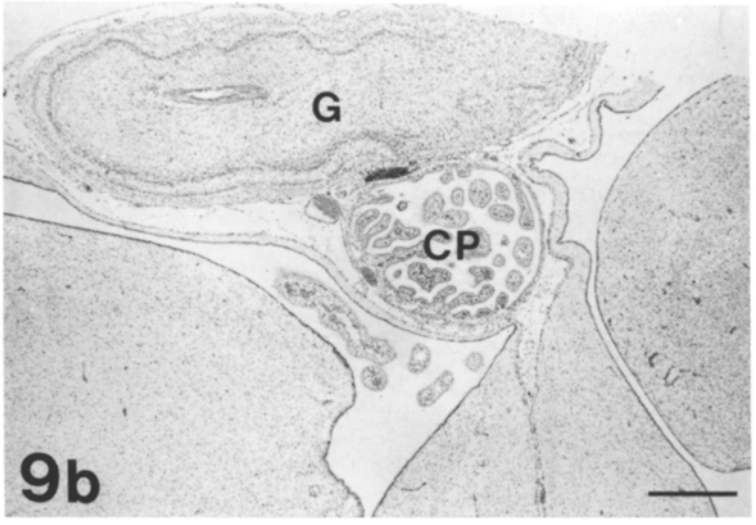
2. Differentiation of blood vessels

The blood vessels in the grafts of series A originate from the body wall of the host embryo. The body wall does not possess fenestrated capillaries. As our studies have shown, the choroidal epithelium representing a particular population of ependymo-glial elements, induces the formation of CP-typical fenestrated capillaries. This induction cannot depend on direct cell contacts since these do not exist in the normal development of the chicken CP (Meller and Wechsler 1965).

Endothelial cells can be detected in very early stages of embryonic development (Pardanaud et al. 1987). In the quail, they are first visible in the area opaca of the head-fold stage (1 day). At the 1-somite stage, they reach the area pellucida and later make up the lining of the heart and the aorta dorsalis. The organ-typical differentiation of the capillary endothelial cells is dependent upon certain interactions within the developing organ. Stewart and Wiley (1981) have shown that abdominal vessels vascularizing grafted prospective brain tissue establish structural, functional and histochemical features of a blood-brain barrier. There are indications that glial cells, not neurons, exert such an influence upon the endothelial cells. Cancilla and De Bault (1983) have shown that, in vitro, glial cells or glial-conditioned medium enhance neural amino-acid transport properties of cerebral endothelial cells. Glial cells co-cultured with endothelial cells induce the formation of γ-glutamyl transpeptidase, an enzyme typical of brain endothelial cells (De Bault and Cancilla 1980).

The induction of CP-typical capillaries by the choroidal epithelial cells stresses the importance of glial cells for organ-typical differentiation of vessels in various parts of the brain. Therefore, when performing grafting experiments in the central nervous system, the effect on the blood-brain barrier of the existence or non-existence of glial cells in the graft should be considered.





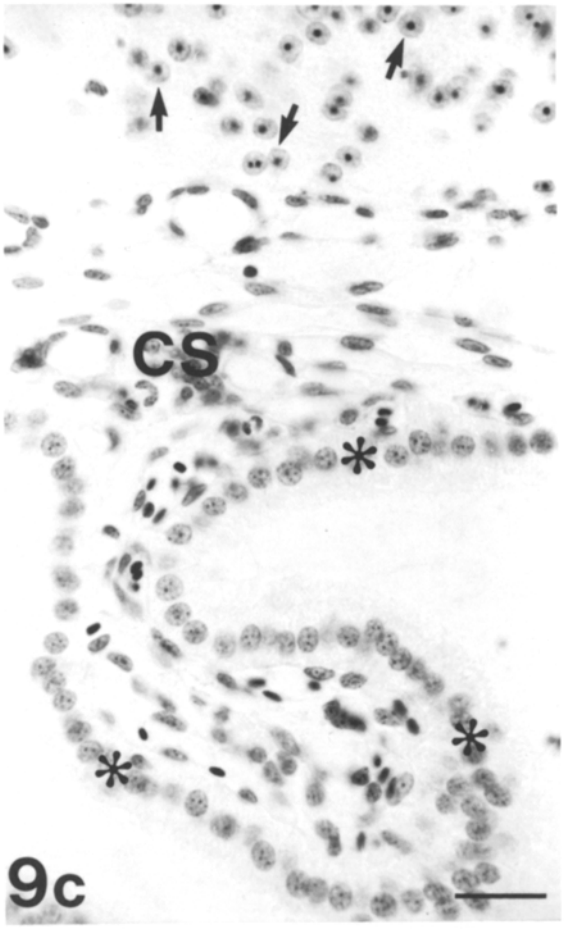


Fig. 9a–c. Graft (series B; see Fig. 2) after 14 days of reincubation. **a** Graft (*G*) in situ, attached to the fissura longitudinalis cerebri rostrally to the pineal organ (*P*). *C* cerebellum; *M* mesencephalon; *T* telencephalon. ×3; bar = 5 mm. **b** The graft (*G*) develops in proximity to the CP of the host embryo. ×28; bar = 400 μm. **c** Higher magnification of Fig. 9b showing quail-type nuclei of the graft (*arrows*) and chicken-type nuclei of the host embryo's choroidal epithelium (*stars*) and choroidal stroma (*CS*). ×580; bar = 20 μm

3. Blood-cerebrospinal fluid barrier

Whereas, in most parts of the central nervous system, the endothelial cells constitute a blood-brain barrier (Reese and Karnovsky 1967), in the CP they are permeable even to macromolecules. The capillary endothelial cells of the CP are fenestrated (Tennyson and Pappas 1964; Meller and Wechsler 1965; Brightman 1967; Castel et al. 1974) and linked by discontinuous fasciae occludentes (Dermietzel 1976).

The CP constitutes a second barrier, the BCSFB. The paracellular passage of intravenously injected HRP and cytochrome c is impeded by the zonula occludens of the choroidal epithelial cells (Becker et al. 1976; Brightman and Reese 1969; Milhorat et al. 1973). On the other hand, small molecules like lanthanum pass this zonula occludens (Castel et al. 1974; Bouldin and Krigman 1975). Heterotopically developed CPs also constitute a BCSFB. Neither a paracellular, nor a transcellular passage of HRP could be detected. Grafted choroidal epithelial cells take up HRP by micropinocytotic vesicles basolaterally and digest the enzyme within apical lysosomes, a process also described for the normal organ (Milhorat et al. 1973; Brightman 1967; Becker et al. 1967; Wakai and Hirokawa 1981).

4. Epiplexus cells

The relationship between amoeboid cells and the choroidal epithelium was originally described by Kolmer (1921). Whereas reptiles and mammals show numerous epiplexus cells (Kolmer 1921), in birds, such as the chicken (El-Gammal 1983) and the quail, there are only a few.

We have found epiplexus cells displaying characteristic ultrastructural features on the choroidal epithelium of the grafts. They occur as round cells with various protrusions and short filopodia, and contain many vacuoles within their cytoplasm. The epiplexus cells of the grafts always possess nuclei of the chicken type. Thus, these cells cannot have originated from the transplanted neural epithelium of the quail. On the other hand, a neuroectodermal origin of epiplexus cells has been proposed by Netsky and Shuangshoti (1970).

Although Kappers (1953) indicates that the epiplexus cells originate from the choroidal stroma, Kolmer (1921), Carpenter et al. (1970), and Sturrock (1978) suggest that they are of hematogenic origin. From the histochemical studies of Schwarze (1975), it is likely that these cells derive from monocytes.

In conclusion, morphogenesis and differentiation of the CP take place when prospective choroidal epithelium is al-

lowed to interact with mesenchyme of the body wall. Apparently, the choroidal epithelial cells induce the development of a CP-typical type of capillaries.

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