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Diencephalic origin of the pineal gland of the chicken embryo

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Summary. In the present paper, the diencephalic origin of the chick pineal gland was analyzed by a series of experiments: prosencephalic substitution; in vitro culture of isolated diencephalons; and total or partial excission of the diencephalic roof. The results indicate that the differentiation of the chick pineal gland in the rooof of the third ventricle is not influenced by the neighbouring brain vesicles and is of diencephalic origin. Moreover, in order to obtain chick embryos with pineal agenesia, the whole diencephalic roof has to be removed.

Key words: Pineal gland, Origin, Pineal agenesia, Chick embryo

Introduction

The anterior part of the neural tube in the chicken embryo shows, at 40 hours of incubation, three bulbous enlargements called primary brain vesicles. These are craneocaudally located: the prosencephalon or fore brain, the mesencephalon or mid brain and the rombencephalon or hind brain. The prosencephalus undergo subsequent divisions, so two lateral vesicles develop in both sides. These are the telencephalic vesicles that will form the cerebral hemispheres. Between them there is an impaired portion of the primitive prosencephalon, called diencephalon, in which four different parts will develop: the epithelamus, the dorsal thalamus, the ventral thalamus and the hypothalamus (Romanoff, 1960; Nauta and Feitarg, 1986).

According to Krabbe (1955), Boya and Zamorano (1975), Calvo and Boya (1978) and Carpenter and Sutin (1983) the pineal gland in the chick embryo arises as a little hollow sac from the area of the diencephalic roof at 3 days of incubation and will lie between the posterior and habenular commisures. The orifice of communication with the third ventricle closes about the 12th to 14th day of development (Calvo and Boya, 1978) or, according to Romeu and Jullien (1942), Spiroff (1958) and Doskocil (1976), it remains during the first weeks after hatching.

In the 6-day-old embryo, the wall of the pineal outline shows enlargments or mamiliform projections. These are specially located on the anterior extremity of the outline. Central lumens start to appear in these cellular mamiliform projections at about 6.5 days of development (Calvo and Boya, 1978, 1979). Afterwards, due to the formation of the primary follicular structures, the distal part of the pineal gland becomes conspicuous. A primary vascular network surrounding the pineal outline can be seen in 5-day-old chick embryos (Spiroff, 1958; Doskocil, 1976; Bertossi et al., 1983).

There is no previous report in the literature of any study of a possible extradiencephalic participation in the differentiation and development of the pineal gland in the chick embryo. The aim of the present paper is to analyze the diencephalic origin of the pineal gland and to establish a methodology in order to obtain chick embryos with pineal agenesia.

Materials and methods

Eggs of Rhode Island Red strain of domestic fowl were used in this experiment. They were incubated at 38.5° and 75% relative humidity.

Prosencephalic substitution

Dossel's (1958) tungsten needles were used as sharp, thin instruments to manipulate and cut embryo structures under a Wild's stereoscopic microscope.

When the chick embryos reached stage 13 from Hamburger and Hamilton (1951) (48-52 hours of incubation), they were exposed through a window in the

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shell. The prosencephalon was removed and replaced by a quail prosencephalon in a synchronic stage of development (stage 9-10 from Zacchei, 1961). Then, the eggs were resealed with cellophane and returned to the incubator.

From among ten operated embryos only one embryo was alive at 11 days of incubation. At that stage of development, it was sacrificed and its head embedded in wax. Sagital sections of 7 μm thickness were stained according to the Hutson and Donahoe (1984) stain procedure: the sections were hydrated to distilled water, then placed in preheated 3.5 N HCl at 37°C for 40-50 min to produce optimal hydrolysis; after which, they were rinsed in distilled water and then stained with Harri's hematoxylin and eosin. All sections were mounted with DPX. In quail cells, the nucleoli showed a deep staining with the hematoxylin.

In vitro culture of isolated diencephalons

Twelve diencephalons were removed from 2-day-old embryos and isolated from the neighbouring brain vesicles (telencephalic vesicles and mesencephalon). They were cultured in Wolff and Haffen (1952) semisolid media for 2 days and then grafted onto chorioalantoid membrane (MCA) of 7-day-old chick embryos for another 7 days. At the end of this period the diencephalons were removed, embedded in wax and sectioned at 7 μm thickness. Histological sections were stained in haematoxylin-eosin and examined for the presence of pineal gland.

Total or partial excission of the diencephalic roof

Two-day-old embryos were exposed through a window in the equaotorial part of the shell. At that stage of development, the diencephalon is limited craneally by the sulcus telodiencephalicus and caudally by the sulcus mesodiencephalicus. The embryos were stained with the vital dye Neutral Red 1:1000 to allow a better view of the encephalic structures.

Experimental embryos were divided into three groups (Fig. 1): in a first group, the anterior half of the diencephalic roof was removed in 38 embryos and the posterior half in 35 embryos; in a second group, the central area of the diencephalic roof was removed in 35 embryos and, in a third group, the whole diencephalic roof was removed in 44 embryos.

Control embryos were either exposed through a window in the shell at two days of incubation or sham-operated by pricking the diencephalic roof with a tungsten needle.

When the operated embryos reached 8 days of incubation, they were sacrificed and their brains embedded in wax. Histological sections (7 μm) were stained in haematoxylin-eosin and examined under the microscope.

Results

Prosencephalic substitution

At eleven days of incubation, the surviving embryo showed no sign of malformation (Fig. 2). The length of the beak from anterior angle of nostril to tip of bill was 2.4 mm corresponding to a 10-day-old chick embryo (stage 36 from Hamburger and Hamilton). The circumference of the eyelids showed quail characteristics the lateral edge being wider than the medial. In an 11-day-old control quail embryo the circumference of the eyelids was bordered by a row of

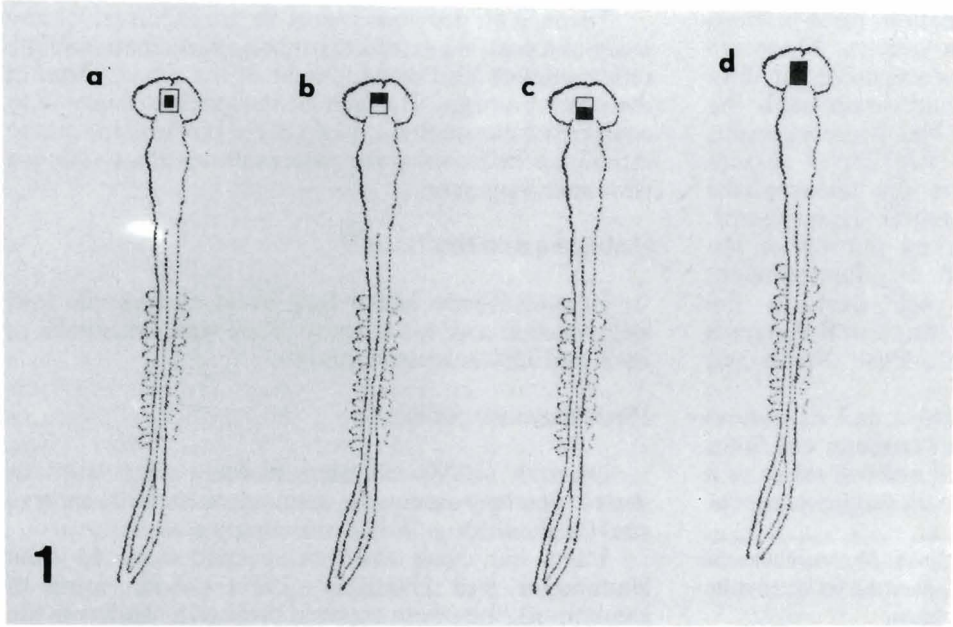


Fig. 1. Schematic representation of the excission of the central area **(a)**, anterior half **(b)**, posterior half, **(c)** and the whole **(d)** diencephalic roof in 2-day-old chick embryo.

Fig. 2. Stage 36 chick embryo in which the prosenphalon is replaced by a quail prosencephalon.

primordia feather germs, but in this particular case this did not happen, as is typical in the chick embryo. The length of the third toe was 4.5 mm and equivalent to a stage 35-36 chick embryo.

The pineal gland, located on the roof of the diencephalon, showed several follicles with central lumen. Two cellular layers could be distinguished in the follicular wall: one near to the lumen and another, thinner than the first one, between the former and the basal lamina, although all the cells seemed to be in contact with the central lumen. All the follicular cells showed a deep staining nucleoli characteristic of the quail (Fig. 3).

In vitro culture of isolated diencephalon

After 7 days in MCA, 50% of the grafted diencephalons were alive. Two of them showed a single small outpocketing of the area of the diencephalic roof (8 μm wide, 8 μm high and 63 μm long) that lay over the third ventricle (Fig. 4a,b). The nuclei of the pineal outline were located towards the basal zone. Mitotic figures were found in the vicinity of the central lumen.

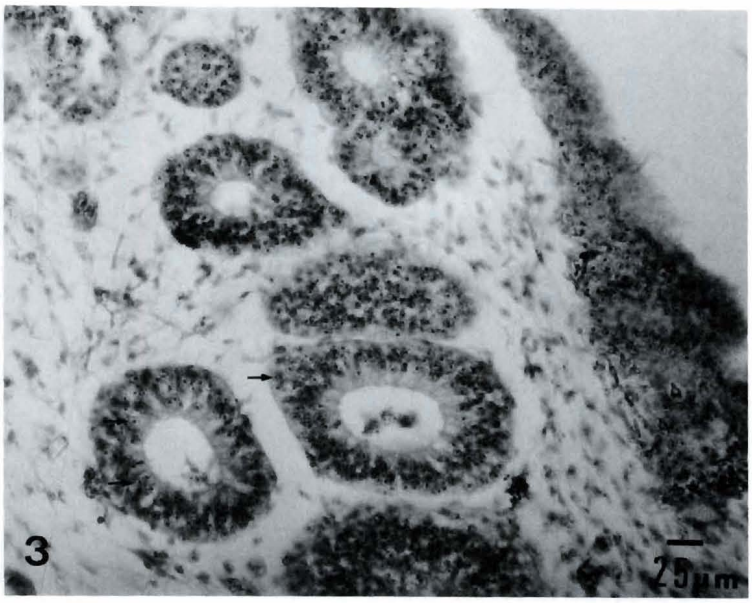


Fig. 3. The cells that form the pineal follicles show a deep staining nucleoli (arrow) characteristic of the quail when the chick prosencephalon is replaced by a quail prosencephalon.

Total or partial excission of the diencephalic roof

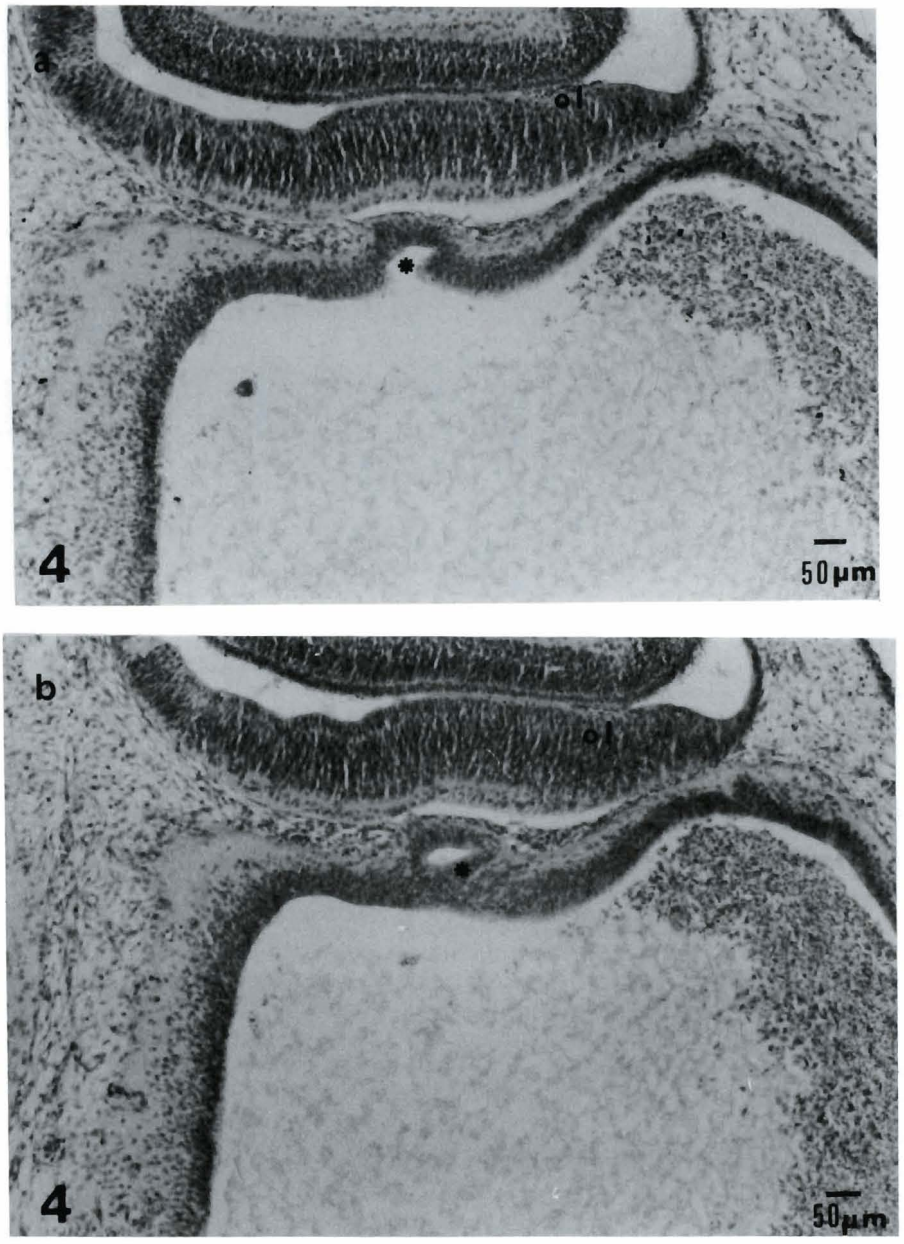
At 8 days of incubation, 46% of the operated embryos were alive showing a complete regeneration of the craneal structures in 14% of the embryos. In controls, 85% of the exposed embryos and 78% of the sham-operated embryos were alive showing no craneal anomalies.

All the embryos in which the anterior or posterior half of the diencephalic roof was removed, showed a well developed pineal gland (Fig. 5a). The same result was found when the central area of the diencephalic roof was removed. However, the excission of the whole diencephalic roof resulted in no differentiation of the pineal gland in any of the operated embryos (Fig. 5b).

Discussion

In the published papers and monographs, the pineal gland has always been considered as a derivative of the roof of the third ventricle. The influence and structural participation from the neighbouring brain vesicles has never been studied. In the present paper, we analyze the pineal origin by carrying out three different experiments.

As shown in Figure 3, when the chick prosencephalon was replaced by a quail prosencephalon, all the cells that form the pineal follicles showed a deep staining nucleoli characteristic of the quail. This proves that no extraprosencephalic cells participate in the follicular structure of the pineal gland. According to Calvo and Boya (1978), the studied pineal gland corresponded to a 7.5 day-old embryo not to an 11-day-old chick embryo. Moreover, as in this case the



Figs. 4a and b. Pineal outline (*) differentiated in the diencephalic roofs after culturing the diencephalons in semisolid media for 48 h and then grafting it onto MCA for 7 days. In this particular case the optic lobe (ol) is displaced and covers the pineal outline.

pineal gland was of quail origin, it should have been in an equivalent stage to a 14-day-old chick embryo.

The Hutson and Donahoe stain technique facilited identification of quail cell types and provided superior histology of the chick tissues by demonstrating cytoplasmatic detail.

In a second experiment, isolated diencephalons were first cultured in semisolid media for 48 hours and then, to avoid the risk of a central necrosis, they were grafted onto MCA of 7-day-old chick embryos. As is shown in Figure 3 the pineal outline developed as a little hollow sac on the roof of the third ventricle. Nevertheless, after 9 days in culture, the pineal gland showed a 4-5 day delay in development when compared with the stages of pineal development established by Calvo and Boya (1978, 1979). This delay could have been caused by simple culturing of the diencephalons.

In a third experiment, the excission of the anterior or posterior half and central area of the diencephalic roof did not stop the normal differentiation and development of the pineal gland in any of the operated embryos. Only by removing the whole diencephalic roof were we able to obtain embryos with pineal agenesia. It can be deduced from these thata that there is not a delimited pineal prospective area in the diencephalic roof of the 2-day-old chick embryo. Further experiments are needed to be able to prove whether the cells that form the roof of the third ventricle have the capacity to differentiate into pineal tissue.

It has also been established, in the present paper, a methodology to obtain embryos with pineal agenesia that can be added to the pinealectomy techniques developed by Santos-Gutiérrez (1965), Stalsberg (1965) and Kobayashi (1968).

Therefore, we can conclude that: first, the pineal gland of the chick embryo is able to differentiate from isolated diencephalons cultured in MCA; second, the differentiation and development of the pineal gland in the roof of the third ventricle is independent from the neighbouring brain vesicles and has a diencephalic origin; and finally, to obtain embryos with pineal agensia, the whole diencephalic roof has to removed in 2-day-old chick embryos.

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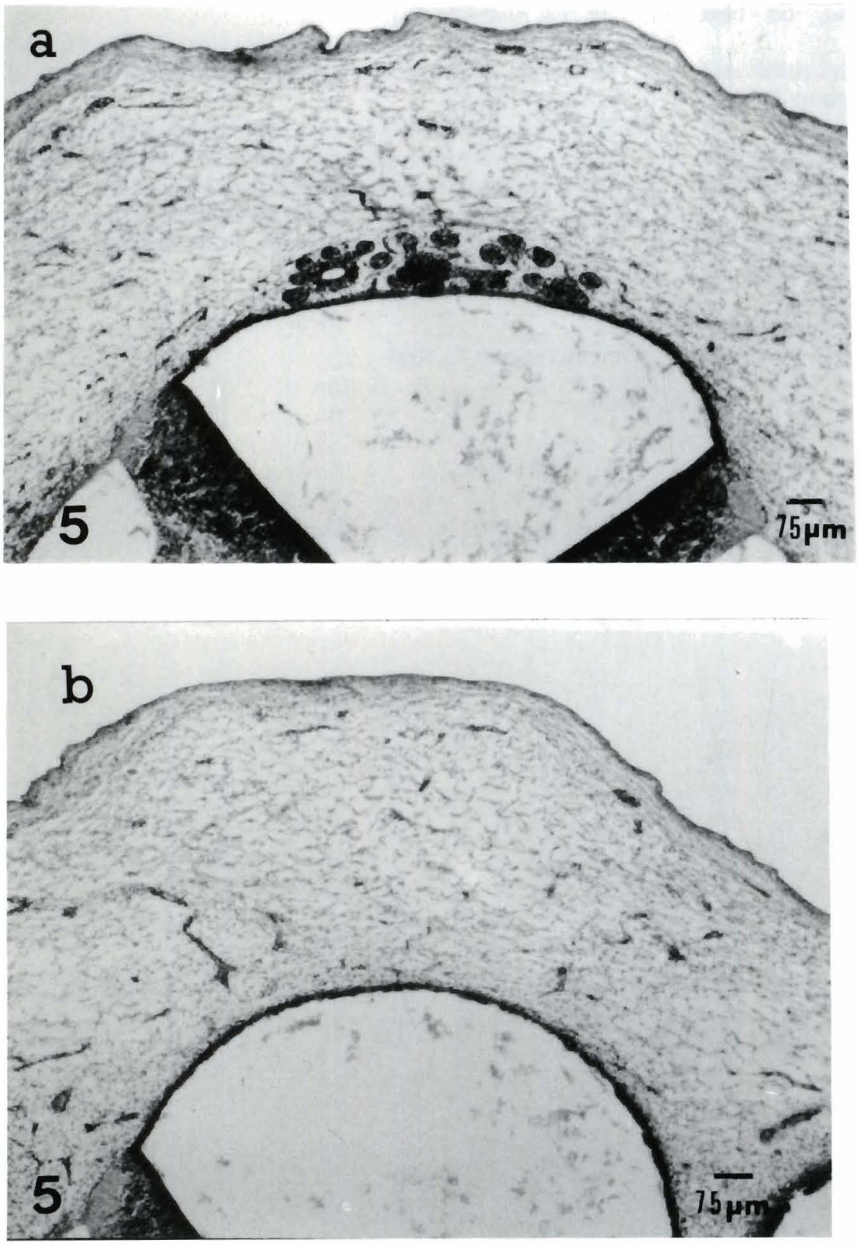

Figs. 5. Brain transversal section of an 8-day-old chick embryo with **(a)** and **(b)** without pineal gland.

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