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A STUDY OF A RECONSTITUTED HYPOPHYSIS AND ITS FUNCTION IN CHIMAERIC BIRD EMBRYOS

BY

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ABSTRACT

A study of a reconstituted hypophysis and its function in chimaeric bird embryos. - Experiments consisting in replacing the encephalon of a chick embryo by that of a quail embryo at the same stage of development were realized on 42 hour-old (12-15 somites) embryos. They led to the development of chimaeras. The reconstitution of the embryonic head concerned all territories. More peculiarly, the hypophysis is composed of two parts belonging to the host (adenohypophysis) and to the grafted tissue (neurohypophysis). Immunocytological reactions displayed some hormones, like ACTH and oxytocin which are normally secreted. It may be assumed that a reconstituted hypophysis is able to secrete hormones as does a normal one. On the other hand, some hormones, like vasopressin, are not secreted.

Key-words: Chick-quail graft, adenohypophysis, neurohypophysis, hormone secretion, ACTH, oxytocin.

INTRODUCTION

The hypophysis of birds differentiates from two main elements: i) the infundibulum, which develops into neurohypophysis and ii) the Rathke's pouch, from ectodermal origin, which differentiates into adenohypophysis (Fig. 1). A third endodermal anlage, the Seessel's pouch, begins to develop, but throws back rapidly (Doskocil, 1970; Ferrand, 1970; Jacobson *et al.*, 1979)

Different inductive processes lead to the development of the definitive hypophysis. The encephalic floor acts upon the stomodeal epithelium, which gives rise to the adenohypophyseal bud. This induction takes place between stages 17 to 24 somites [stages 12-15 of Hamburger and Hamilton (1951)]. However, the adenohypophyseal anlage, when separated from the encephalic floor, never develops into adenohypophyseal tissue. After 25 somites-stage, the anlage is completely determined and becomes able to differentiate independently of the encephalic floor (Ferrand, 1969a). It acquires its definitive developing potency at three days (Ferrand, 1970).

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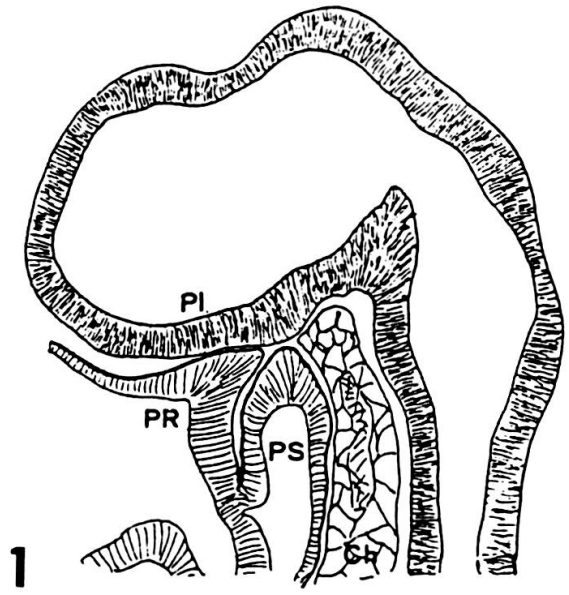

FIG. 1.

A sagittal section of the head of a 19 somite-stage chick embryo. PR: Rathke's pouch; PS: Seessel's pouch; Pl: encephalic floor; Ch: notochord;

The adenohypophyseal bud develops into an epithelial stalk, which comes in contact with the infundibulum. The latter undergoes an inductive influence from the adenohypophysis and develops into neurohypophysis. This process is relatively long and occurs between 5 to 10 days of incubation (Doskocil, 1970).

It was demonstrated that the different parts of the embryonic encephalon are able to induce the formation of cranial bones (Schowing, 1968). On the other hand, the replacement of the embryonic chick encephalon by that of a quail at the same stage of development leads to the reconstitution of a complete skull, the size of which being exactly adapted to that of the grafted territories. It was suggested that the quail encephalon induces cranial bones, as does the chick encephalon, into the encephalic mesenchyme of the chick embryo and acts as site-specific inducer of the various components of the skull (Schowing and Robadey, 1971; Schowing, 1974).

If the epithelial anlage of the hypophysis is isolated after 25 somites-stage, it does not request the presence of neural tissue to differentiate (Le Douarin *et al.*, 1967a). However, the differentiation of the adenohypophyseal tissue is obtained in the more cases as the anlage is removed at later stages of development. Experiments of associations between the future adenohypophysis and different tissues showed that neither the notochord nor the cephalic mesenchyme are able to influence the development of the adenohypophysis (Ferrand, 1970). It was also demonstrated that the hypophysis development is never influenced by other endocrine glands, such as hypothalamus (Ferrand *et al.*, 1980) or pineal body (Doskocil, 1978).

The adenohypophysis of birds is constituted of a *pars distalis* and a *pars tuberalis*. The *pars distalis* is itself subdivided in cephalic and caudal lobes. According to Brasch and Betz (1971), the cephalic lobe secretes probably the adenocorticotropic hormone (ACTH), the thyreotropic hormone (TSH) and the gonadotropic hormone (FSH), while the caudal lobe produces the luteinizing hormone (LH). The growth hormone (GH) may be secreted either by the caudal lobe (Enemar, 1967), or by both lobes (Brasch and Betz, 1971). It seems that both melanotropic hormones (MSH) may not be produced by the *pars distalis*, according to the works of Fellmann *et al.* (1975) and Ferrand *et al.* (1980) concerning the immunodetection of MSH.

Few is known about the *pars tuberalis*, which generally encircles the pituitary stalk and covers a part of the diencephalon.

Secretion granules appear in the adenohypophysis after the 6th day of incubation, as well in chick as in quail embryo (Grignon *et al.*, 1966; Guedenet *et al.*, 1970; Betz and Jarskar, 1974; Daikoku *et al.*, 1974). The first hormone to be secreted is the ACTH, which appears at the 8th day of incubation in the chick (Szekely *et al.*, 1958; Pedernera, 1972). At the 10th day, TSH can be found (Trunnel and Brayer, 1953; Trunnel and Wade, 1955), while gonadotrophins LH and FSH appear later, after about 13 days of incubation (Kinyon and Watterson, 1958; Betz, 1967; Woods and Weeks, 1969). The growth hormone (GH) is secreted after 15 days of development (Enemar, 1967).

The neurohypophysis contains neurosecretory fibers which extend to the *pars eminens* and hypothalamus. Histological data indicate the presence of neurosecretory granules in the neural lobe from the 13th day onwards (Guedenet *et al.*, 1967; Enemar and Ljunngreen, 1968; von Lawzewitsch, 1969), on day 14 in the *pars eminens* and on day 15 in the hypothalamus (von Lawzewitsch, 1969). Other data reveal the presence of oxytocin in chicken (Munsick *et al.*, 1960; Acher *et al.*, 1960; Heller and Pickering, 1961), while controversy arose when Munsick (1964) expressed some doubt concerning the presence of vasopressin in pituitary extracts, as described by Chauvet *et al.* (1960). Different hormones, like vasotocin and mesotocin, have been then chemically identified as neurohypophyseal hormones of birds (Acher *et al.*, 1970; Nouwen *et al.*, 1983). Little is known about the role of these hormones in avian embryo. All results concerning oxytocin and vasopressin were obtained from adult birds, no data are related to embryonic stages.

Chick-quail chimaera have external characteristics of both species. Data relative to the head morphogenesis let suppose that other organs than bones could be restored and develop after brain transplantation (Schowing *et al.*, 1992). To determine whether such a reconstituted hypophysis could be functional or not, we proceeded as for earlier experiments and completed our investigations by using immunocytological techniques at different stages of development to elicit hormonal secretions of the pituitary gland. Unfortunately, the mortality rate was so high over 14 days that we limited our observations at earlier stages.

MATERIAL AND METHODS

The encephalon of 42 hours-old chick embryo was removed by using micro-scalpels forged from stainless steel acupuncture needles. It was then replaced by the encephalon of a quail embryo at the same stage of development (Fig. 2). The chimaeric embryos were incubated until 14 days, and then prepared for histology using Bouin's fixative. Control embryo slides were stained with Toluidine blue, while experimental ones were stained one out of two by a multiple colouration composed with PAS-Weigert's haematoxylin-Alcian blue-Orange G. This technique allowed to distinguish the differences between host and grafted tissues.

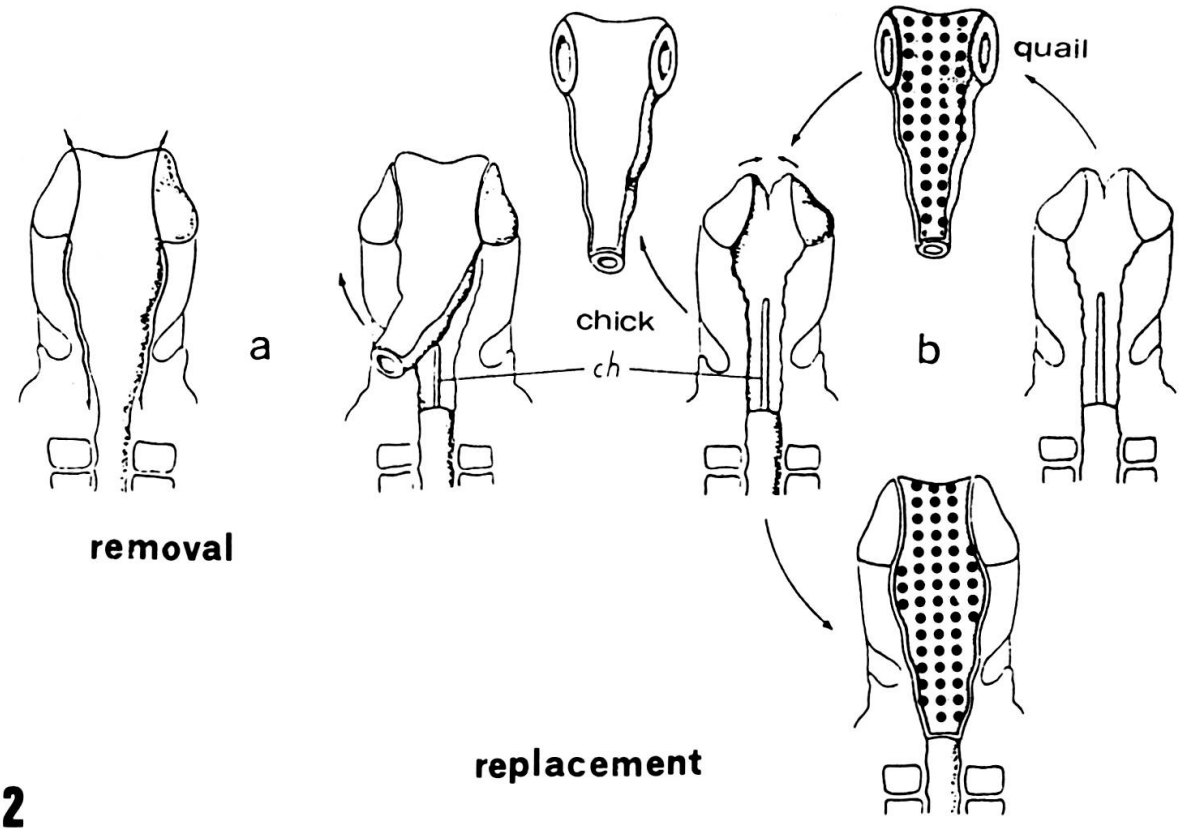

FIG. 2.

Drawing of the transplantation experiment. The encephalon of the chick is removed, then replaced by the encephalon of a quail embryo.

Uneven slides were kept for immunocytological reactions. They were exposed after rehydratation to antibodies at different dilutions. The ABC method was used as follows: i) slides were successively exposed to a first antibody (A, rabbit antiserum), then to a second (B, anti-rabbit goat antiserum), and finally to (C) peroxidase-avidin-biotin complex. Marked cells were then revealed by diaminobenzidin tetrahydrochloride (DAB).

RESULTS

Morphological aspects of grafted embryos

The upper part of the head is well reconstituted. All bones of the cranial vault develop normally, but are slightly reduced in comparison with controls at the same stage. The cranial floor retains its normal morphology. The *sella turcica* region is characterized by the presence of the hypophysis in which two parts are recognizable. The adenohypophysis belongs to the host and derives from a long epithelial stalk (Fig. 3).

The neurohypophysis appears well differentiated. Both parts are well connected and build a complete hypophysis (Fig. 4). The optic chiasma is apparently normal. Both chick and quail cells are recognizable and connected together.

Immunocytological results

Tests made with vasopressin and HGH hormone were negative for all cases we studied until 14 days. After these stages, positive reactions were given for HGH by one 20 day-old chick embryo. Among the other tests, positive results were given by ACTH and oxytocin (Table 1). ACTH is localized in the adenohypophysis, while oxytocin appears in the neurohypophysis (Figs. 5-7). We observed identical results in both chick and quail embryos.

TABLE 1.

Antibody reactions to different hormones in older embryos.

antibody	20 day-old chick embryo	15 day-old quail embryo
ACTH	+	+
endorphin	–	–
FSH	–	–
MSH	–	–
oxytocin	+	+
prolactin	–	–
TSH	–	–
HGH	+	–
vasopressin	–	–

Immunocytological tests applied to experimental embryos gave positive results at different stages of development. ACTH label was revealed after the 9th day of incubation, while oxytocin was visible at the 14th day (Figs 6-8). Table 2 summarizes these results.

TABLE 2.

Reactions of chimaeric embryos to ACTH and oxytocin at early stages of development.

Stage	ACTH	oxytocin
9 days	+	–
11 days	+	–
12 days	+	–
13 days	+	–
14 days	+	+

CONCLUSIONS

Our experiments show that the hypophysis of chimaeras is perfectly reconstituted. According to the work of Ferrand (1969a, 1969b, 1970), the encephalic floor exerts a specific inductive influence upon the epithelium of the Rathke's pouch. This induction is required for further development of the adenohypophyseal anlage, which cannot differentiate when separated from the encephalic floor before 25 somites-stage.

After removal of the embryonic encephalon at early stages (10-15 somites), the adenohypophysis fails to develop. On the contrary, when the chick embryonic encephalon is replaced by that of a quail embryo at the same stages, the adenohypophysis differentiates and becomes functional as a normal one. This demonstrates that the grafted quail encephalon enables the development of the Rathke's pouch and plays at its level the same site-specific inductive role as at the level of the cranial vault (Schowing and Robadey, 1971).

Cells which secrete ACTH are localized in the cephalic part of the adenohypophysis. According to Pedernera (1972) and Fellmann *et al.* (1975), ACTH secretion occurs at the 8th day of development. We observed that corticotropic cells are labelled in 9 day-old chimaeras. In earlier stages, only few cells are labelled. Then, during development, ACTH secreting cells become more and more numerous. This observation confirms the findings of Ferrand *et al.* (1974, 1980), who i) described the same phenomenon and ii) demonstrated that the differentiating adenohypophysis, when isolated from infundibulum at later stages, functions without hypothalamic stimuli.

Later, the infundibulum undergoes an adenohypophyseal induction and develops into functional neurohypophysis. Our chimaeras show that this process corresponds effectively with that described by Doskocil (1970) in normal developing chick embryo.

A peptide resembling oxytocin is widely distributed throughout the vertebrate phylum. Pharmacological data suggested the presence of oxytocin in the chicken (Acher *et al.*, 1960; Munsick *et al.*, 1960; Heller and Pickering, 1961), the pigeon (Heller and Pickering, 1961), and the turkey (Munsick, 1964). Qualitative aminoacid composition of purified material from chick pituitary glands was in agreement (Acher *et al.*, 1960, 1970; Chauvet *et al.*, 1960). In our experiments, oxytocin was displayed in the neurohypophysis at the 14th day of incubation, as well in controls as in chimaeric

FIGS 3-4.

3. A total view of the hypophysis of a 11 day-old chimaeric embryo, showing the epithelial stalk of the earlier Rathke's pouch (arrow) and the correlations between adenohypophysis (A) and neurohypophysis (N). I: infundibular region; O, optic chiasma. l00x; 4. 14 day-old chimaeric embryo, showing the whole reconstituted hypophysis. A, adenohypophysis (chick); N, neurohypophysis (quail). Both parts are not yet joined together, so that a space may temporarily subsist between them. 125x.

embryos. No positive reaction did occur before this stage. These results demonstrate that i) after grafting a quail encephalon into the head of a chick embryo, grafted territories having undergone an induction can normally develop and become functional, and ii) the secretion takes place at normal stages of development.

Oxytocin has a weak and inconsistent antidiuretic effect in high doses (Munsick *et al.*, 1960). No more is known, so that the function of oxytocin in birds remains still obscure.

According to Chauvet *et al.* (1960), a significant quantity of vasopressin was isolated from chick posterior lobe extracts. This result, which concerns chicken more than 10 weeks of age, was however negated by Munsick (1964). Vasopressin was not found in our samples. If vasopressin does exist, it could appear between the 14th day of incubation and the hatching period. Further experiments in this way must be made to bring some light about this problem. In parallel with the question of vasopressin, HGH could be also displayed by the same experimental protocol. According to Enemar (1967), the first appearance of HGH occurred in the caudal lobe of the pars *distalis* in 15 day-old chick embryo. We just found HGH in one 20 day-old chick embryo, i.e. one day before hatching. Consequently, we can suppose that this hormone is not synthetized at earlier stages.

To conclude, the inductive processes between the Rathke's pouch of a chick embryo and a grafted quail encephalon occur as in normal embryos. They lead to i) the differentiation of the adenohypophysis which ii) acts upon the infundibulum, the latter developing later into neurohypophysis. Hormonal secretions begin in both parts approximatively at the same stages of development as in chick and quail control embryos.

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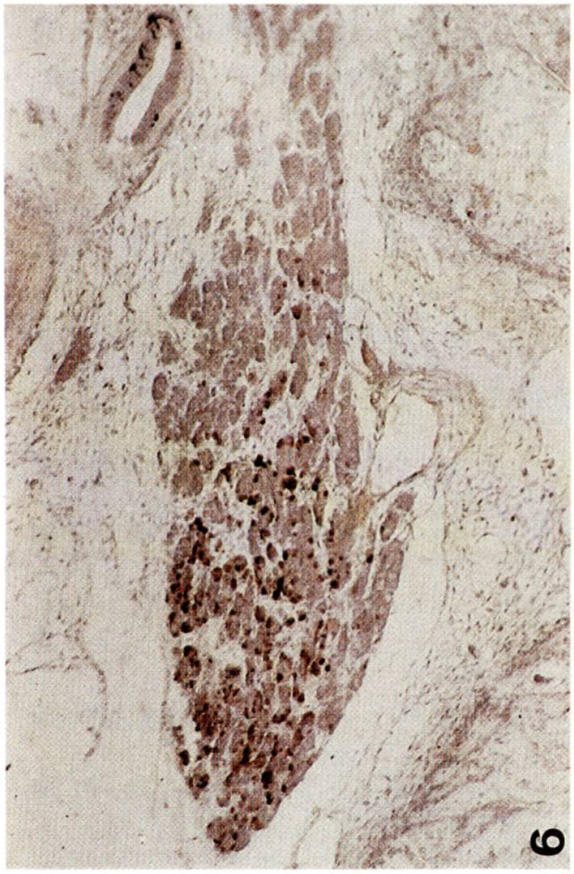
RÉSUMÉ

Des expériences consistant à remplacer l'encéphale d'un embryon de poulet par celui d'un embryon de caille de même stade ont été réalisées sur des embryons de 42 heures d'incubation (12-15 somites), et conduisent à l'apparition de chimères. La reconstitution de la tête embryonnaire touche tous les territoires. L'hypophyse est

FIG. 5-8.

5. Positive reaction to ACTH in the adenohypophysis of a 14 day-old quail embryo. Granules are distributed in the anterior part of the adenohypophysis. 250x; 6. Positive reaction to ACTH in the adenohypophysis of a 14 day-old chimaeric embryo. Same distribution of granules as in control embryos. 125x; 7. Positive reaction to oxytocin in the neurohypophysis of a 20 day-old chick embryo (arrow). 250x; 8. Positive reaction to oxytocin in the neurohypophysis of a 14 day-old chimaeric embryo (arrow). 500x.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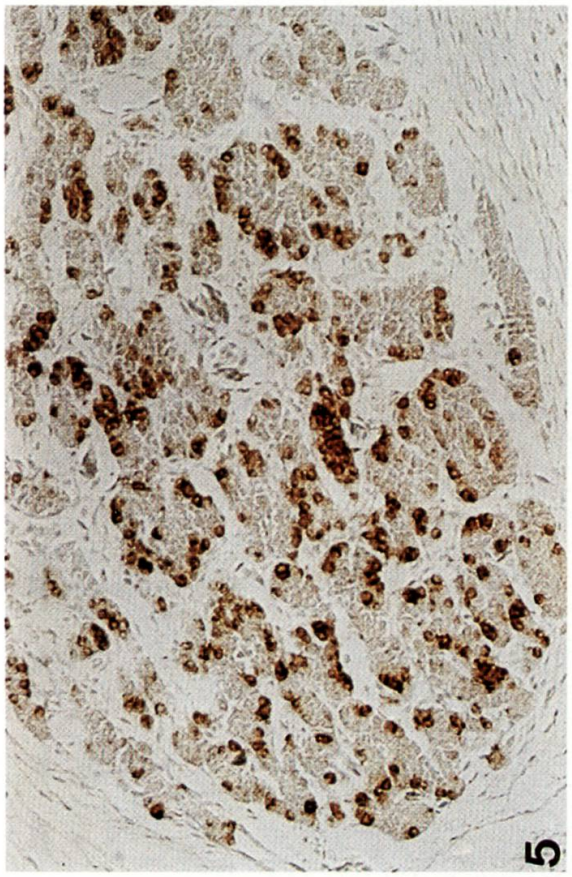
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constituée de deux parties appartenant à l'hôte (adénohypophyse) et au tissu greffé (neurohypophyse). Des réactions immunocytologiques montrent que certaines hormones, telles que l'ACTH et l'oxytocine, sont normalement sécrétées. On peut admettre qu'une hypophyse ainsi reconstituée est capable de sécrétion au même titre qu'une hypophyse normale. En revanche, d'autres hormones, comme la vasopressine, ne sont pas sécrétées.

LITTERATURE CITED

ACHER R., CHAUVET J., LENCI M. T. - Isolement de l'ocytocine du poulet. *Biochim. Biophys. Acta, 38:* 344-345, 1960.

ACHER R., CHAUVET J., CHAUVET M. T. - Phylogeny of the neurohypophysial hormones. *Eur. J. Biochem., 17:* 509-513, 1970.

BETZ T. W. - The effects of embryonic *pars distalis* grafts on the development of hypophysectomized chick embryos. *Gen. comp. Endocr., 9:* 172-186, 1967.

BETZ T. W., JARSKAR R. - Chicken pars distalis development. *Cell. Tissue Res., 155:* 291-320,1974.

BRASCH M., BETZ T. W. - The hormonal activities associated with the cephalic and caudal regions of the cockerel *pars distalis. Gen. comp. Endocr., 16:* 241-256, 1971.

CHAUVET J., LENCI M. T., ACHER R. - Présence de deux vasopressines dans la neurohypophyse du poulet. *Biochim. Biophys. Acta, 38:* 571-573, 1960.

DAIKOKU S., KEUCHI C., NAKAGAWA H. - Development of the hypothalamohypophyseal unit in the chick. *Gen. comp. Endocr., 23:* 256-275,1974.

DOSKOCIL M. - The mutual morphogenetic influence of the stomodeal ceiling and of the brain anlage. *Folia Morphol., XVIII:*149-160, 1970.

DOSKOCIL M. - Relationship between the pineal body and the pituitary in early stages of development of the chick embryo: an experimental study. *Folia Morphologica, XXVI:* 160-169,1978.

ENEMAR A. - Ontogeny of the hypophysial growth-promoting activity in the chick. *J. Endocrinol., 37:* 9-15, 1967.

ENEMAR A., LJUNNGREN L. - The appearance of monoamines in the adult and developing neurohypophysis of *Gallus domesticus. Z. Zellforsch. Mikrosk. Anat., 91:* 496-506,1968.

FELLMANN D., FRANCO N., BLOCH B., HATIER R. - Etude immunocytochimique des cellules corticotropes de l'adénohypophyse de l'embryon de Poulet. *Bull. Ass. Anat., 59:* 631-637,1975.

FERRAND R. - Influence inductive exercée par le plancher encéphalique sur l'ébauche adénohypophysaire aux jeunes stades de développement de l'embryon de Poulet. *C. R. Acad. Sci. Paris, 268:* 550-553, 1969a.

FERRAND R. - Différenciation en greffe chorio-allantoïdienne de l'épithélium de la poche de Rathke d'embryons de caille et de poulet. *C. R. Soc. Biol., 163:* 2669-2672, 1969b.

FERRAND R. - Différenciation de tissu adénohypophysaire à partir de la poche de Rathke greffée chez des embryons de Poulet privés d'hypothalamus. *C. R. Acad. Sci. Paris, 270:* 1480-1481, 1970.

FERRAND R., PEARSE A. G. E., POLAK J. M., LE DOUARIN N. M. - Immunohistological studies on the development of avian embryo pituitary corticotrophs under normal and experimental conditions. *Histochem., 38:* 133-141,1974.

FERRAND R., FREMONT P. H., DUBOIS M. P. - Immunocytological study on the differentiation of chick and quail adenohypophysis epithelial rudiments, grafted or cultivated in vitro: evidence for polypeptidic hormones. *The American Journal of Anatomy, 158:* 411-431, 1980.

GRIGNON G., GUEDENET J. C., HATIER R. - Sur la cytologie de l'adénohypophyse de l'embryon de Poulet étudiée au microscope électronique. *Bull. Ass. Anat., 5le réunion,* 466-470, 1966.

GUEDENET J. C., GRIGNON G., HATIER R. - Aspects ultrastructuraux du développement de la neurohypophyse chez l'embryon de Poulet. *C. R. Soc. Biol., 161:* 1334-1335, 1967.

GUEDENET J. C., GRIGNON G., FRANCO N. - Etude critique de la mise en évidence des cellules à grains glycoprotidiques chez le Poulet au cours de la vie embryonnaire et de la période post-natale. 7e congrès international de microscopie, Grenoble, *3:* 565 (Abstract), 1970.

HAMBURGER V., HAMILTON H. L. - A series of normal stages in the development of the chick embryo. *J. Morphol., 88:* 49-92,1951.

HELLER H., PICKERING B. T. - Neurohypophysial hormones of non-mammalian vertebrates. *J. Physiol., 155:* 98-114,1961.

JACOBSON A. G., MIYAMOTO D. M., MAI S. H. - Rathke's pouch morphogenesis in the chick embryo. *J. exp. Zool, 207:* 351-366, 1979.

KINYON N., WATTERSON R. L. - Reduced endocrine activity of ovaries of hypophysectomised duck embryos as indicated by modified development of genital tubercle and syrinx. *Physiol. Zool., 31:* 60-72, 1958.

LAWZEWITSCH I. VON.- Development of the neurosecretory hypothalamic-hypophyseal system of the chick embryo as evidenced in the total preparation. *Acta Anat., 72:* 83-93, 1969.

LE DOUARIN N., FERRAND R., LE DOUARIN G. - Evolution de l'adénophypophyse isolée du plancher encéphalique aux jeunes stades de développement. *C. R. Soc. Biol., 161:* 1807-1811, 1967a.

LE DOUARIN N., FERRAND R., LE DOUARIN G. - La différenciation de l'ébauche épithéliale de l'hypophyse séparée du plancher encéphalique et placée dans des mésenchymes hétérologues. *C. R. Acad. Sci. Paris, 264:* 3027-3029, 1967b.

MUNSICK R. A. - Neurohypophysial hormones of chickens and turkeys. *Endocrinology, 75:* 104-112, 1964.

MUNSICK R. A., SAWYER W. H., VAN DYKE B. T. - Avian hypophysial hormones: pharmacological properties and tentative identification. *Endocrinology, 66:* 860-871, 1960.

NOUWEN E. J., DECUYPERE E., MICHELS H., KUHN E. R. - The presence of vasotocin and mesotocin in serum and the hypophalamo-neurohypophyseal axis of chick embryos before hatching and in posthatch chicks. *Gen. comp. Endocrinol., 50*, 445-451, 1983.

PEDERNERA E. A. - Adrenocorticotropic activity in vitro of the chick embryo pituitary gland. *Gen. comp. Endocr., 19:* 589-591,1972.

SCHOWING J. - Mise en évidence du rôle inducteur de l'encéphale dans l'ostéogenèse du crâne embryonnaire du Poulet. *J. Embryol. exp. Morphol., 19:* 83-93, 1968.

SCHOWING J. - Rôle morphogène de l'encéphale embryonnaire dans l'organogenèse du crâne chez l'Oiseau. *Ann. Biol., 13:* 69-76. 1974.

SCHOWING J., ROBADEY M. - Substitution à l'encéphale embryonnaire de Poulet (Gallus gallus) d'un encéphale embryonnaire de Caille (Coturnix coturnix japonica) de même stade. *C. R. Acad. Sci. Paris, 272:* 2382-2384, 1971.

SCHOWING J., REPETTO M., LENGELE B., DHEM A. - Reconstitution de la tête embryonnaire de poulet après greffe d'un encéphale de caille de même stade, observée en microscopie à balayage. *Acta Anat., 143*, 167, 1992.

SZEKELY G., ENDROECZI E., SZENTAGOTHAL J. - Erscheinen der argentaffinen Substanz und Beginn der Sekretion adrenocorticotrophen Hormonen in der Hypophyse von Hühnerembryonen. *Acta Biol. Hung., 8:* 283-287, 1958.

TRUNNELL J. B., BRAYER F. T. - Factors governing the development of the chick embryo thyroid. I. Determination of the time at which I^{131} collection begins. *J. clin. Endocr. Metab., 13:* 88-94, 1953.

TRUNNEL J. B., WADE P. - Factors governing the development of the chick embryo thyroid. II. Chronology of the synthesis of iodinated compounds studied by chromatographic analysis. *J. clin. Endocr. Metab., 15:* 107-117, 1955.

WOODS J. E., WEEKS R. L. - Ontogenesis of the pituitary-gonadal axis in the chick embryo. *Gen. comp. Endocr., 13:* 242-254,1969.