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In vitro and in vivo Studies on Cytodifferentiation of Pituitary Clonal Cells Derived from the Epithelium of Rathke's Pouch*

Masataka Shiino, Hiroshi Ishikawa, and Edward G. Rennels**
Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

Summary. A clonal strain of anterior pituitary cells was derived from Rathke's pouch of the rat. These cells were shown to secrete ACTH, growth hormone and prolactin but no glycoprotein hormones, when grown in vitro. Cells from the 2A8 clone were implanted for one month under kidney capsules or into hypothalami of hypophysectomized female rats. Under the kidney capsule, prominent prolactin cells and poorly developed cells of other types were differentiated as seen in usual pituitary grafts. In hypophysiotrophic areas of the hypothalamus, the grafts were cytodifferentiated into various types of anterior pituitary cells with rich vascularization. These cells had the ultrastructural features indicative of hormone secretion. Increases in body and ovarian weights reflected the secretion of somatotrophic and gonadotrophic hormones. The results obtained indicate that implants of 2A8 clonal cells may differentiate into all types of anterior pituitary cells under the influence of hypothalamic hormones or perhaps some unknown factors present in the general systemic circulation of the rat.

Key words: Pituitary cell clone – Implantation – Cell differentiation – Kidney – Hypothalamus.

Introduction

Previously we have successfully established 65 clonal strains of pituitary anlage cells derived originally from epithelial cells of Rathke's pouch (Ishikawa et al., 1977). We determined by radioimmunoassay that one of these clones (2A8) secreted simple protein hormones (ACTH, GH and prolactin) but no glycoprotein

Send offprint requests to: Dr. Masataka Shiino, Department of Anatomy, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284, USA

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hormones (FSH, LH and TSH) in vitro. Early stages of fetal pituitary glands before the 15th day of fetal life may contain only small concentrations of hormones (Birge et al., 1967) and either no basophils (Sétáló et al., 1976) or a few basophils (Phillips and Schmidt, 1958). In our previous work, we established that unknown factor(s) present in fetal brain extracts may be necessary for the differentiation of pituitary anlage cells (unpublished data). After establishment of several NRR (normal rat Rathke) cell lines, we found that adult median eminence extract was required for the cytodifferentiation of cells and for the production of hormones. Fetal brain extract and adult median eminence extract, however, were not effective in promoting the cytodifferentiation of glycoprotein hormone secreting cells in vitro.

Consequently, we transplanted one of our multipotential pituitary cell clones (2A8 clone) under the kidney capsule or into hypothalamic hypophysiotrophic areas of hypophysectomized female rats in order to observe by light and electron microscopy if further cytodifferentiation of multipotential cells might take place.

Materials and Methods

One of our multipotential pituitary cell clones (2A8) was transplanted under the kidney capsule or into the hypophysiotrophic areas (i.e., median eminence or preoptic area) of hypophysectomized female rats (60 day-old Sprague-Dawley, 140–150 g body weight). Hypophysectomy was performed by the intra-aural method 10 days before the transplantation. The rats were fed Purina rat chow and 5% sugar solution ad libitum and kept in temperature controlled (23° C ± 1°) quarters with a 14:10 light-dark cycle. Only animals which failed to gain weight or lost weight during this 10 day period were used in the transplantation experiments.

Prior to implantation, dissociated culture cells were mixed in 12% gelatin solution dissolved in Ham's F 10 medium (1:1). Transplantation of cells (about 1×10^6 cells/0.01 ml/rat) under the kidney capsule was carried out using a 20-gauge needle affixed to a tuberculin syringe. The site of the capsule where the needle was inserted was burned with a heated glass rod to prevent the leakage of cells. One month after the implantation the grafts were removed from the kidney capsules for examination by light and electron microscopy. Transplantation of cells (about 1×10^6 cells/0.01 ml/rat) into the hypothalamus was performed using a stereotaxic instrument and following an atlas of the rat brain (Sherwood and Timiras, 1970). Thirty days after the implantation, the host animals were autopsied following decapitation and the grafts were fixed and prepared for light and electron microscopic examinations. The areas of the hypothalamus into which cells were implanted were the preoptic area and the median eminence. The body weights of host animals were checked every day and ovaries of hypophysectomized animals bearing grafts in the hypothalamus were weighed at sacrifice. The hypophyseal area was carefully checked for remnants of pituitary tissue but none were recognized. For preparation of the cells for electron microscopy the culture cells were fixed in equal parts of 2% osmium tetroxide solution and 6% paraformaldehyde in 0.2 M cacodylate buffer solution (pH 7.4) for one hour at 0° C. After fixation the cells were gently centrifuged to concentrate the cells and the subsequent procedures were according to our previous method (Shiino et al., 1972). Dehydration was carried out in ethanol and the cells were embedded in the plastic mixture recommended by Spurr (1969). Each time the solutions were changes it was necessary to employ light centrifugation in order to retain the cells. Grafted cells were fixed for 6 h in the above fixing solution. For light microscopy (LM) the culture cells and grafted cells were fixed in Bouin's solution and stained by hematoxylin-eosin or the PAS stain.

Results

When 2A8 clonal cells grown in vitro were examined by electron microscopy they were found to be essentially agranular. However, these cells contained numerous

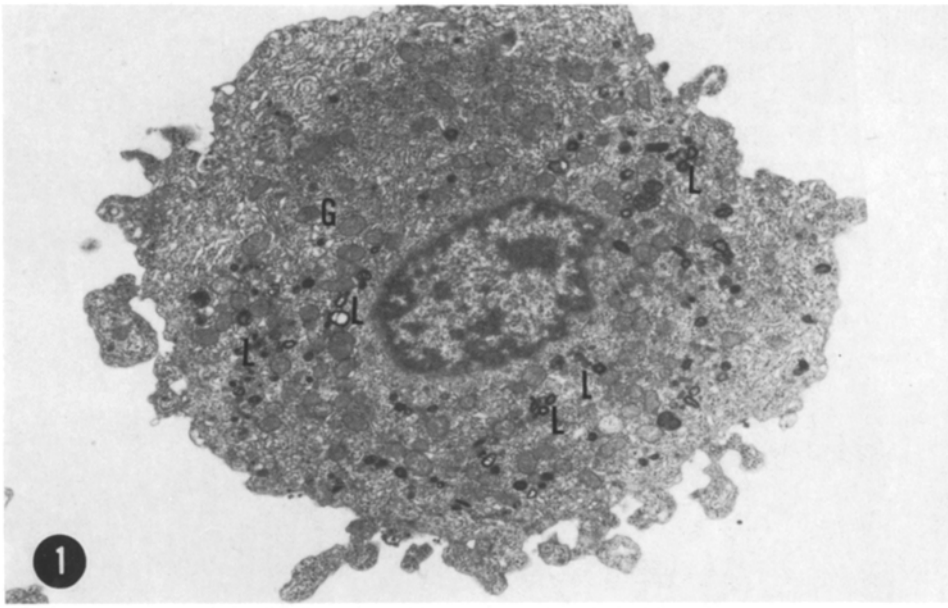


Fig. 1. An example of isolated 2A8 cultured cell. Well developed endoplasmic reticula, numerous mitochondria and lysosomes, and surface microvilli are characteristic of this clonal cell. *L* lysosome; *G* Golgi complex. × 6000

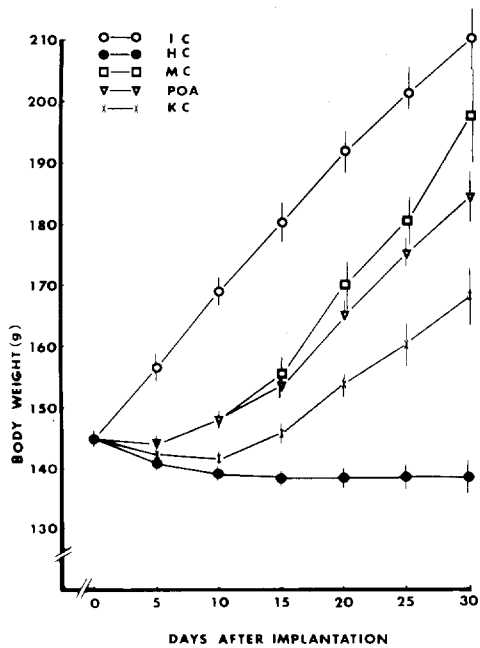
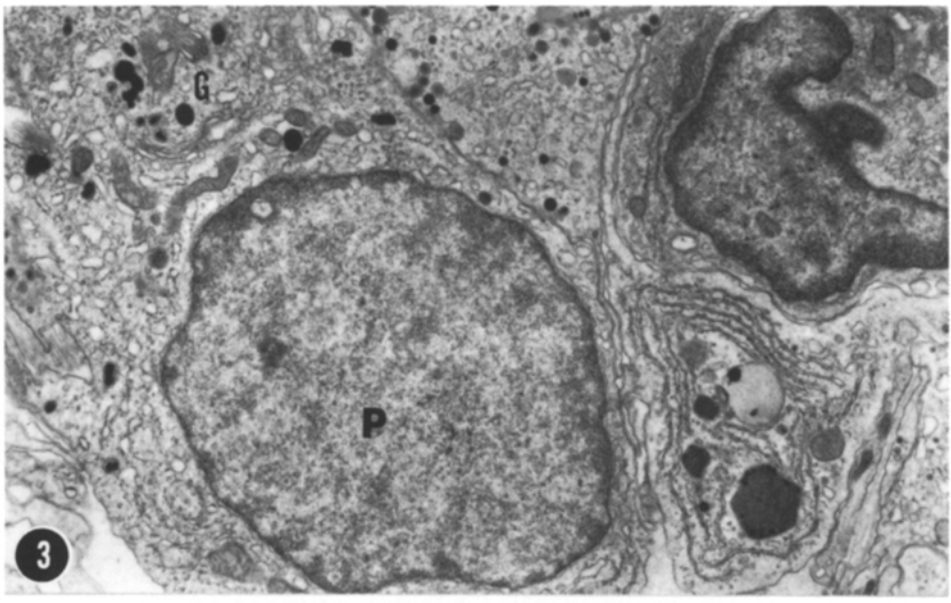


Fig. 2. Body weights of host (hypophysectomized) animals bearing multipotential clone (2A8) under kidney capsule or in hypothalamus. *IC* Intact control (7 rats); *HC* Hypex control (7 rats); *POA* Preoptic area (5 rats); *MC* Median eminence (5 rats); *KC* Kidney capsule (5 rats)



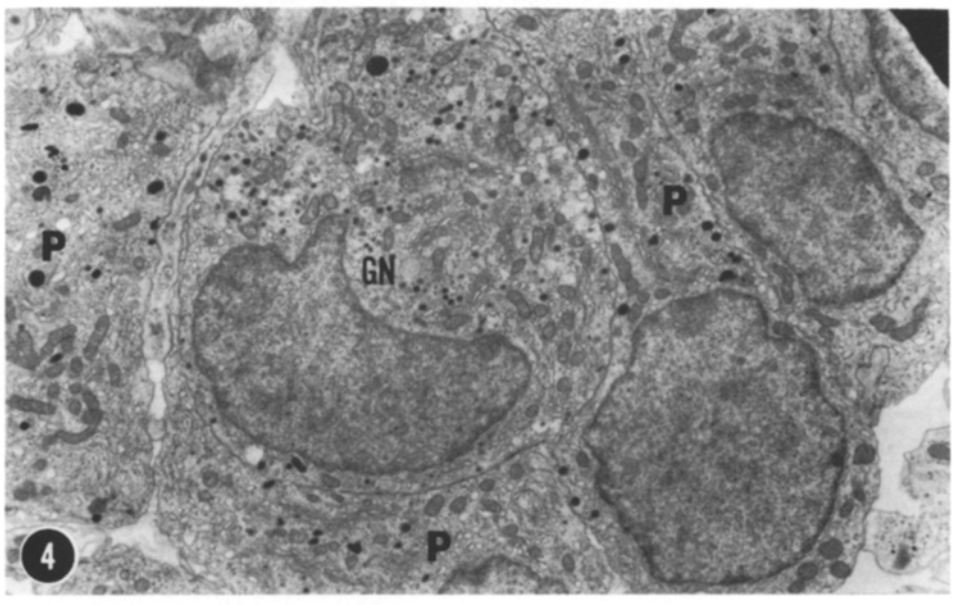


Fig. 3. A prolactin cell derived from 2A8 clonal cells implanted under the kidney capsule. A well developed Golgi zone (*G*) is seen. ×9000

Fig. 4. An example of a gonadotroph (*GN*) developed from 2A8 clone under the kidney capsule. A clear Golgi complex, mitochondria and secretory granules may be seen. *P* prolactin cell; *GN* gonadotroph. ×6000

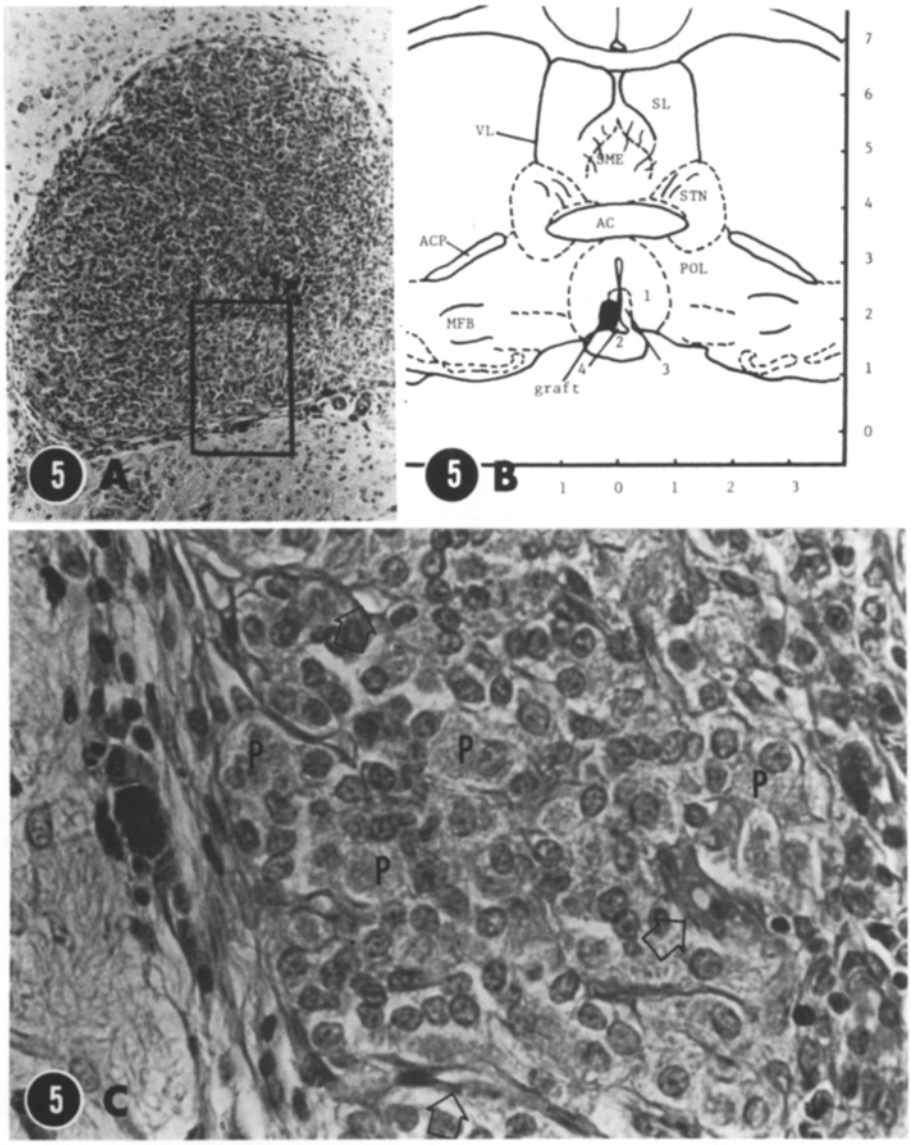


Fig. 5A–C. Implanted region of pituitary clonal cells (2A8) after grafting in preoptic area (POA). (The diagrams in Figures 5 **B** and 6 **B** are from the atlas by Sherwood and Timiras.) *1* Area preoptica medialis; *2* Chiasma opticum; *3* Area preoptica periventricularis; *4* Ventriculus tertius. **C** Higher magnification from A. Notice many large PAS positive cells (*P*) and capillaries (arrows). × about 350

mitochondrial and lysosomal organelles in the cytoplasm. Their nuclei were spherical in shape and the Golgi zone was moderately developed, and granular endoplasmic reticulum and polysomes were prominent in most of the cells. Cytoplasmic projections (microvilli) were a characteristic of these cells (Fig. 1).

Body weights of host animals bearing grafted pituitary cells beneath kidney

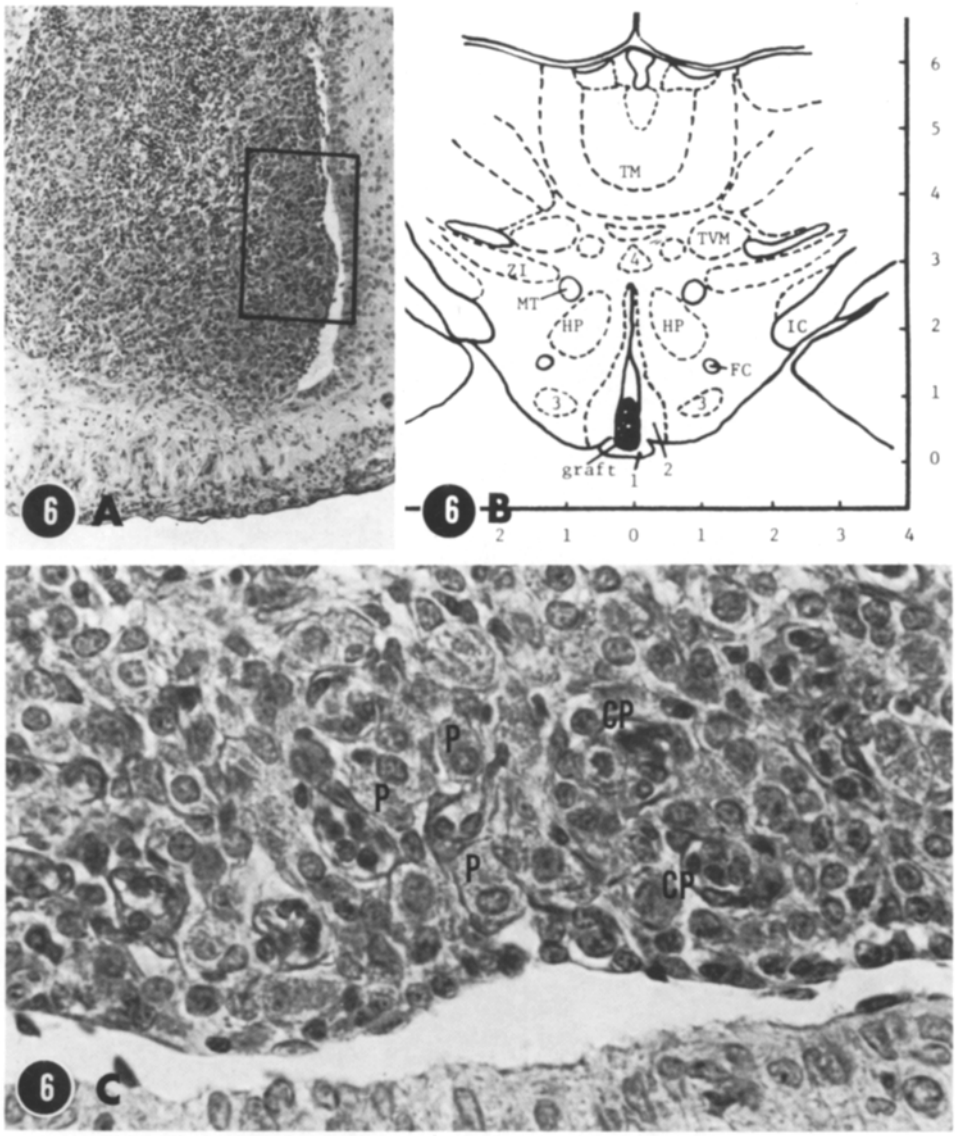


Fig. 6A–C. Implanted region of pituitary clonal cells (2A8) and grafted cells implanted in median eminence (*ME*). **A** and **B** Grafted cells implanted in ME. *1* Infundibulum; *2* Nucleus periventricularis arcuatus; *3* Nucleus premamillaris; *4* Nucleus reuniens. **C** Higher magnification from B. *P* PAS positive cells, *CP* Capillary. × about 350

capsule or in hypothalami were definitely increased compared with controls (Fig. 2).

Following transplantation beneath the kidney capsules of hypophysectomized female rats, these cells developed into many typical prolactin cells, some clearly defined gonadotrophs and some other poorly defined cells containing small

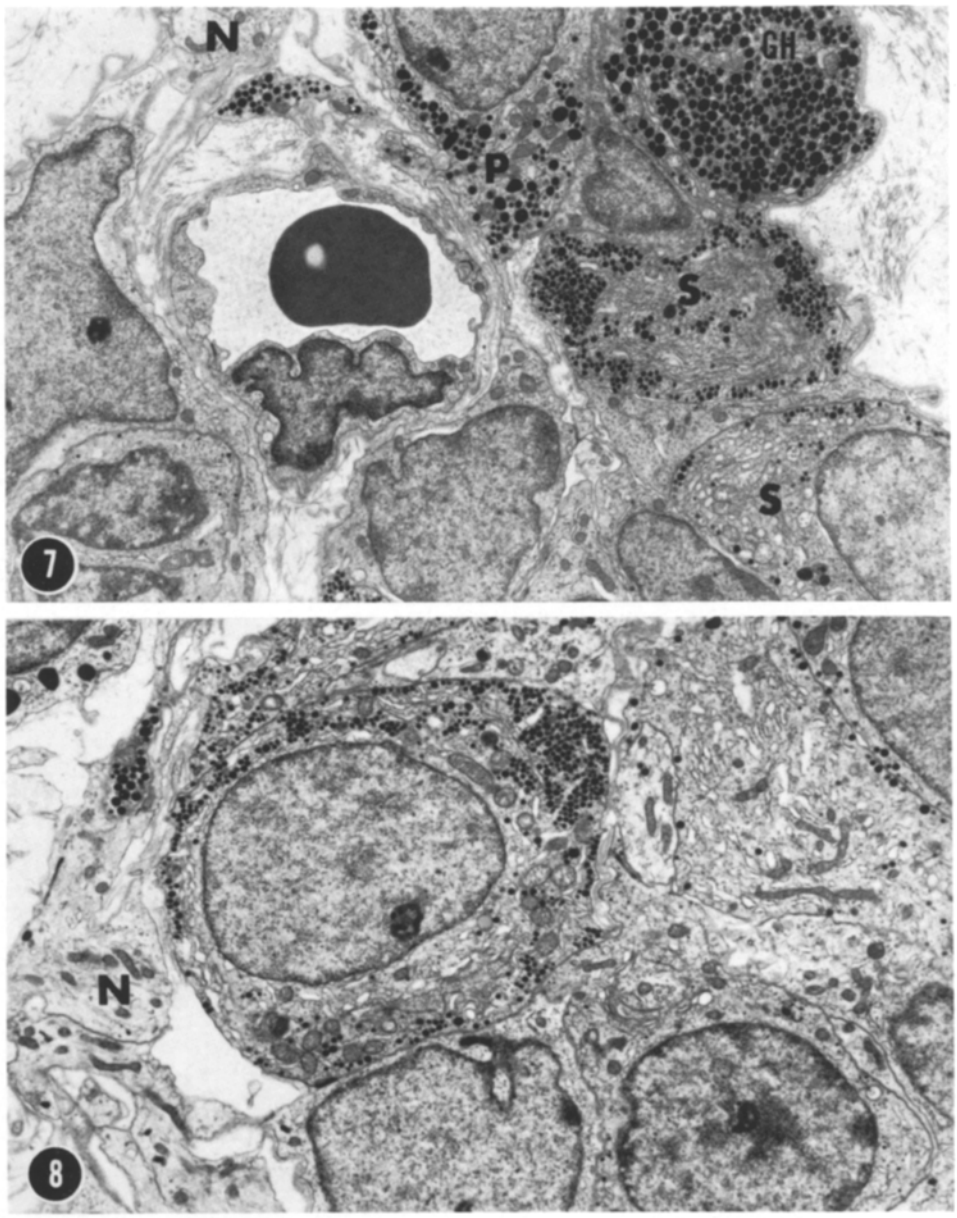
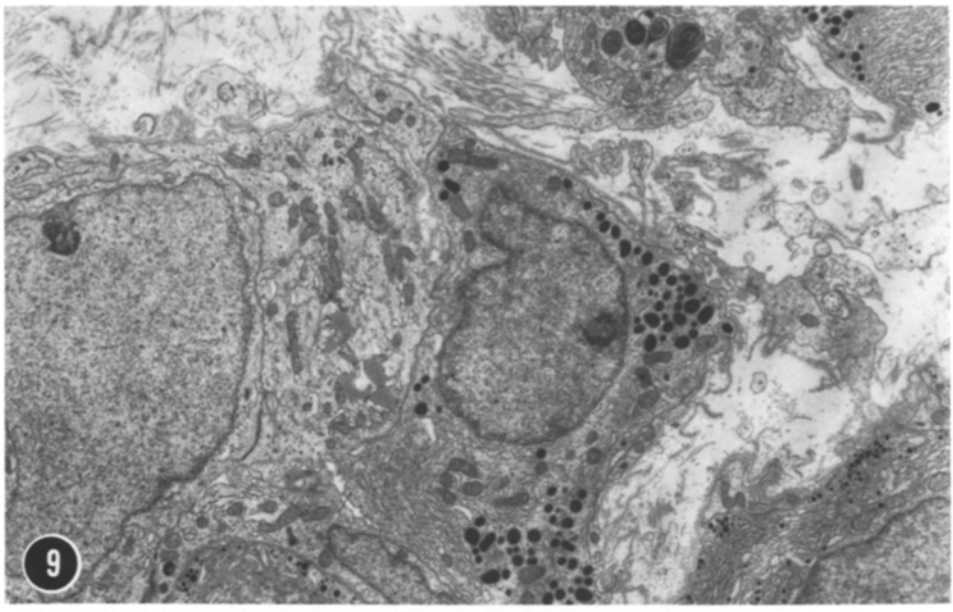


Fig. 7. Pituitary cells from 2A8 clone after transplantation to the median eminence. Three typical granular cells and a capillary may be observed. *P* Prolactin cell; *GH* Somatotroph; *S* Small granule-containing cell (gonadotroph); *N* nerve fiber. × 3600

Fig. 8. An example of a small granule-containing cell developed from 2A8 clonal cell (thyrotroph) situated near nerve fibers of the hypothalamus (POA). *N* nerve fiber. × 6000



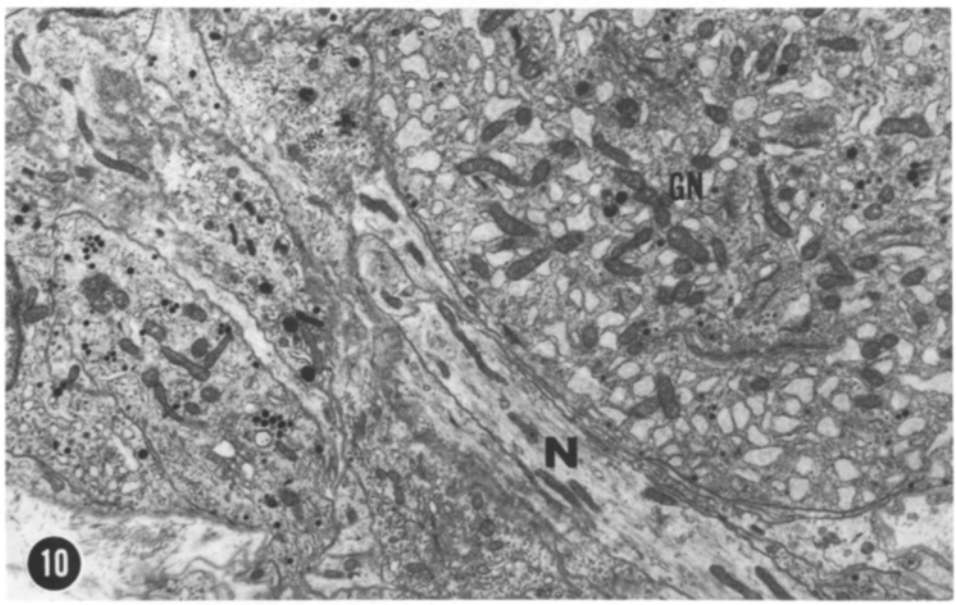


Fig. 9. A prolactin cell developed from 2A8 clonal cell located in preoptic area of hypothalamus (POA). × 6000

Fig. 10. A gonadotroph (*GN*) differentiated from 2A8 clonal cell observed in median eminence. × 12,000. Nerve fibers are closely associated with the gonadotroph. *N* nerve fiber

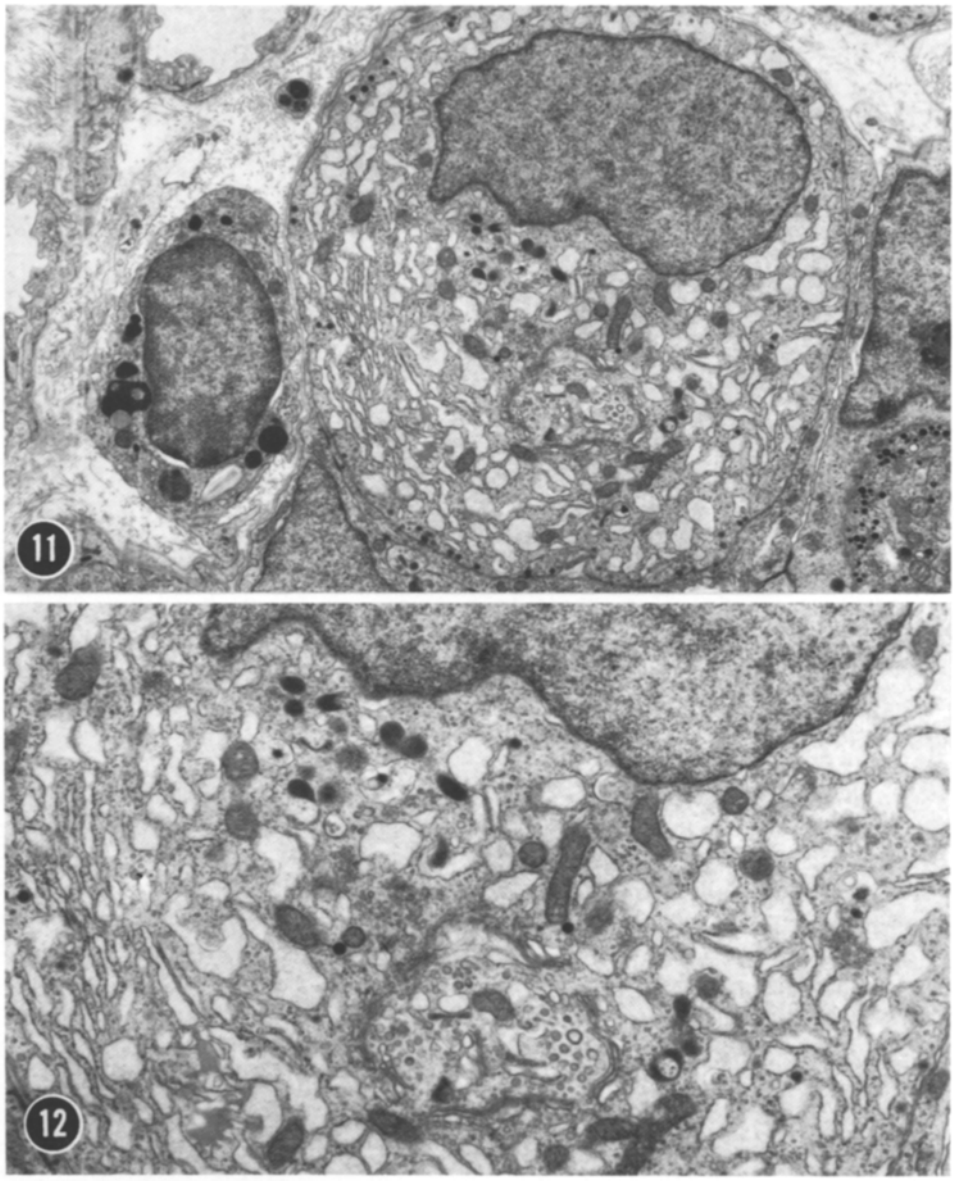


Fig. 11. An example of activated gonadotroph differentiated in median eminence. × 9000. Dilated endoplasmic reticula, scarce secretory granules and a well developed Golgi complex indicate active secretion of hormone

Fig. 12. High magnification of Golgi area of Figure 11. Note small Golgi vesicles and well developed ER. × 28,000

Table 1. Effect of transplantation of multipotential clone (2A8) on the body and ovarian weight

Host animal	Animal no.	Body weight (g)	Ovary weight (mg)
Hypex[a]	7	138 ± 3.07[b]	12.0 ± 0.56[b]
Implantation			
KC	5	168 ± 5.11[c]	16.5 ± 1.73[g]
ME	5	197 ± 8.22[d]	43.7 ± 3.76[h]
POA	5	186 ± 4.52[e]	36.4 ± 3.42[i]
Intact control	7	210 ± 5.26[f]	52.2 ± 2.35[j]

Group means were compared with the means of the Hypex groups by Student's t test: [c] $P < 0.01$; [d,e,f] $P < 0.001$; [g] $P < 0.1$; [h] $P < 0.001$; [i] $P < 0.01$; [j] $P < 0.001$

[a] Hypex = Hypophysectomized rat; KC = Kidney capsule; ME = median eminence; POA = Preoptic area

[b] Mean ± S.E.

granules. Especially prominent were prolactin cells containing a well developed Golgi zone in which many immature secretory granules were seen (Figs. 3 and 4). When sections were stained with PAS and examined by light microscopy a few stained cells were seen.

Cells of the 2A8 clone implanted into the preoptic area or median eminence of the hypothalamus revealed various types of anterior pituitary cells. A rich vascularization was observed in the grafts. After using the PAS stain, many cells were positively stained (Figs. 5 and 6). They were clearly distinguished into prolactin cells, somatotrophs, thyrotrophs, gonadotrophs, and follicular cells which were similar to those observed in normal adult rat anterior pituitary glands (Fig. 7–10). All of the granular cells observed appeared to be functionally active cells, especially the gonadotrophs which were comparatively large in size and contained well developed Golgi complexes and abundant granular endoplasmic reticulum. Moreover, secretory granules and lysosomes were scarce in these types of cells, indicating an active hormone secretion as shown in Figs. 11 and 12. The cells seemed to be closely associated with nerve fibers of the hypothalamus in many cases.

Ovaries of host animals bearing grafts in the hypothalamus were significantly heavier than those of controls (Table 1).

Discussion

By electron microscopic examination the 2A8 clonal cells were found to contain very few granules. However, the fact that many cells contained a well developed endoplasmic reticulum, many mitochondria and a prominent Golgi zone may indicate a good production of hormones in vitro. The small accumulation of secretory granules in these cells may suggest that the turnover of hormone is so rapid that mature granules are not stored in the cytoplasm. The significance of

numerous lysosomes in many of these cells in vitro is not clear, but these structures may be concerned with the digestion of intracellular products.

After these cells were transplanted under the kidney capsule or into hypophysiotrophic areas of the hypothalamus they were observed as normal mature cells. Under the kidney capsule the majority of granular cells were prolactin cells, but some cells were recognized as poorly developed gonadotrophs or other small-granule containing cells as are usually seen in grafted anterior pituitary glands (Nikitivitch-Winer and Everett, 1959; Rennels, 1962; Shiino and Rennels, 1975).

From the increase of body weights in host animals bearing pituitary grafts under the kidney capsule, it is clear that the graft secreted some growth hormone but growth hormone cells were morphologically not equal to those of the normal mature type. In the grafts, one of the interesting findings was the appearance of cells not seen in the vitro clone, especially gonadotrophs of the type Kurosumi (1968) designated as FSH gonadotrophs. Although this type of cell was not exactly equivalent in ultrastructure to the FSH gonadotroph of the normal rat pituitary, it was clearly an example of cytodifferentiation of cells of the 2A8 clone into gonadotrophs after transplantation. Presumably basophils are induced to differentiate by unknown factors existing in the circulating blood which were not contained in the culture medium since median eminence extract, TRH or LH-RH have not been effective in inducing the cytodifferentiation of gonadotrophs from 2A8 cells in vitro (unpublished observations).

The appearance of prolactin cells, gonadotrophs and other small-granule containing cells from 2A8 clone under the kidney capsule of hypophysectomized rats seems not to support the conclusions of Schechter (1971), Szentagothái et al. (1972), and Daikoku et al. (1973) that the cytodifferentiation of anterior pituitary cells is initiated in the absence of hypothalamic control. Hypothalamic hormones in sufficient amounts to stimulate the cytodifferentiation of grafted cells placed under the kidney capsule of hypophysectomized rats may be present in the systemic circulation and thus reach the kidney grafts (Nallar and McCann, 1965; Schneider and McCann, 1970; Wheaton and Fawcett, 1974). We assume that our 2A8 clonal cells could include uncommitted cells which could differentiate into the various glycoprotein hormone producing anterior pituitary cells under the influence of stimulating factors in the circulating blood.

On the other hand, the grafted cells transplanted into the hypothalamus differentiated into the several types of typical anterior pituitary cells. From the increasing body weights of host animals bearing grafts the growth hormone producing cells were apparently active in synthesizing and releasing their hormone. The gonadotrophs especially, appeared to exhibit all of the morphologic criteria of cells which were secretorily active. Higher ovary weights of host animals support this conclusion. This fact indicates that the hypophysiotrophic areas of the hypothalamus are the best environment for the cytodifferentiation of implanted 2A8 clonal cells. Implanted cells appeared as active cells, especially in the median eminence, from the viewpoint of ultrastructural features. The cells were closely associated with nerve fibers but this need not imply a functional relationship since the implantation sites were replete with nerve processes. A rich vascularization of the grafts indicated that active secretion of hormones into the capillaries would be possible.

Implants of neonatal rat anterior pituitary glands or isolated pituitary chromophobes into the hypophysiotrophic region of the hypothalamus showed evidence of stimulation of thyroid glands and gonads, i.e. production of glycoprotein hormones and perhaps the stimulation of cytodifferentiation (Knigge, 1962; Flament-Durand and Desclin, 1968; Averill, 1969; Yoshimura et al., 1969). Gash et al. (1975) transplanted fetal Rathke's pouch epithelial tissues of rat embryos of 11–15 days gestation into the hypothalamus of hypophysectomized rats. They observed that undifferentiated primitive cell implants formed massive growths in the brain, and that fetal pituitary tissue removed on the 13th day was the youngest age capable of developing good histology characteristic of the adult gland.

To our knowledge, this is the first demonstration of the cytodifferentiation of multipotential clonal cells derived from rat anterior pituitary anlage after implantation of the cells into the hypothalamus. The implants of our 2A8 clone were clearly reconstructed into all types of anterior pituitary cells in the hypothalamus with rich vascularization, and the histology of the grafts was similar to that observed by Gash et al. (1975). The results obtained in these studies indicate that our 2A8 clone has a high potency to develop into all types of mature anterior pituitary cells when placed in an in vivo environment. It must be remembered that the 2A8 clone contains no basophils (thyrotrophs or gonadotrophs) when examined by routine LM examinations after growth in vitro. Furthermore, this clone when grown in control medium or in median eminence extract-supplemented medium produces no glycoprotein hormones that can be detected by radioimmunoassay. It follows, therefore, that some factors must be present in the general systemic circulation of the hypophysectomized rat which can induce the differentiation of gonadotrophs and thyrotrophs since both cell types were recognized in the clonal cells after transplantation.

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