# Development of intratesticular implants of rat pituitary primordia[1]

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*Summary.* Intratesticular grafts of pituitary primordia from 11–13-day-old rat embryos developed in 6 weeks into round nodules approximately 1 mm in diameter. The nodules were principally composed of well differentiated glandular and nervous tissues.

A previous study[3] demonstrated that allografts of rat pituitary anlagen (PA) in the hypothalamus of hypophysectomized female hosts could develop into large tumors consisting of glandular, nervous, and connective tissues associated with variable quantities of bone. Biological and immunological assays indicated that these tumors secreted luteinizing hormone, follicle-stimulating hormone, thyrotropin, and corticotropin. The tendency to form large growths or tumors was significantly related to the age of the tissue donor. Tumors developed primarily from grafts of 12-day PA while grafts from 13-day and older embryos usually formed small growths of anterior pituitary-like tissue.

The pronounced tendency of 12-day PA to develop into large tumors is of great interest as it seems to offer a model system to investigate mechanisms involved in the neoplasia of embryonic glandular tissue. To further study the developmental potential of pituitary primordia, we decided to examine its growth responses in sites outside of the brain. This paper reports one such experiment in which PA from 11–13-day-old embryos was grafted into the testes of adult male rats. The testes were chosen because they will, in mice, support the development of large tumors (i.e. teratocarcinomas) from grafts of 1–7$^1/_2$-day-old embryos[4,5].

Charles River outbred albino CD rats were maintained in our colony at 22 °C on a 14/10 h light/dark cycle. Food and water were provided ad libitum. For control and host animals, 32 adult male rats, 4–8 months old and weighing from 300–550 g were divided by use of a random number table into 4 equal test groups. Embryos were removed from timed-pregnant female rats of 11-, 12-, or 13-day gestation. Using procedures described elsewhere[3], PA was microsurgically isolated and injected into the right testis of a pentobarbital anesthesized (30 mg/kg i.p.) male host. For controls, 8 males were sham-operated. The hosts were sacrificed by decapitation 6 weeks after implantation and the implanted testes were removed, fixed for 12 h in Helly's solution and processed for light microscopy. Every 10th section was stained with hematoxylin and eosin for implant localization. Adjacent sections of the implant growth were stained to differentiate adenohypophyseal cytology[6]. For statistical analysis, M × N contingency tables were evaluated by the chi-square approximation of probabilities.

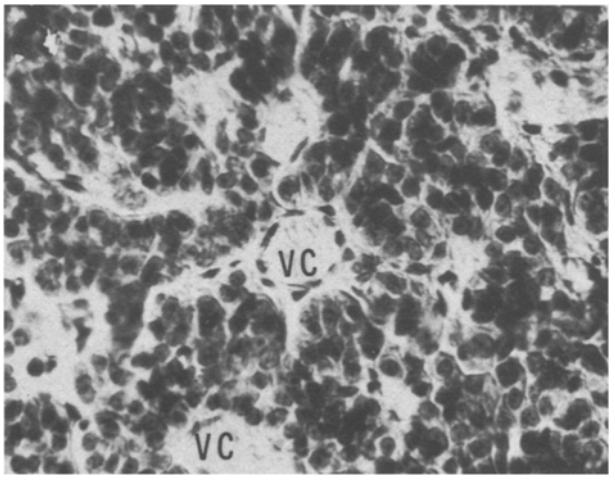
Tissue graft development was identified in the testes of 16 host animals with 2 different kinds of growths being distinguished by their morphology. Distribution was not random ($p < 0.01$, table), but a sharp demarcation in implant development was apparent between the grafts from 12- and 13-day-old donors.

Most implants grew into round nodules, about 1 mm in diameter, that consisted of nervous and glandular tissues associated with lesser quantities of smooth muscle and connective tissue. The glandular cells formed small domains of well-vascularized, anastomosing cords of cells (figure) morphologically like the anterior pituitary. The individual cells had a moderate nucleus/cytoplasm ratio. Granules were not evident in the uniformly basophilic cytoplasm. Some cells did lightly react with periodic acid-Schiffs (PAS) and orange G stains, but the staining reactions were close to background levels. Cysts lined with ciliated and PAS positive cells were associated with the glandular cords of cells.

Tissue graft development correlated with age of donor

| Embryonic donor age | Growth type Nervous and glandular tissue | Connective tissue and bone |
|---|---|---|
| 11 days | 4 | – |
| 12 days | 5 | 1 |
| 13 days | 1 | 5 |

The number of host animals showing different types of graft development are compared with the age of the embryonic donors. The distribution is nonrandom ($p < 0.01$).




Cords of glandular cells developing from an implant of pituitary primordia from an 11-day-embryo. Note the extensive vascular channels (light areas VC, with outlines of red blood cells). Nomarski optics, ×475.

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In 6 hosts, 5 of which had received implants from 13-day donors, the PA developed into small growths (less than 1 mm in diameter) consisting of connective tissue, smooth muscle, and bone. Various degrees of lymphocytic infiltration were evident.

The major finding of this experiment was that 12-day PA grafts can exhibit different growth responses. 6 weeks after implantation in the testes, grafts of 12-day PA were less than $^1/_{10}$ the size of the same age donor tissue growing for a month in the hypothalamus. Distinct histological differences also separated the types of growths found in the 2 sites. Adenomas and undifferentiated cells, characteristic constituents of tumors from brain implants, were not evident in the testicular growths. Nervous tissue commonly developed from PA grafts to both sites.

At this time it is difficult to explain why grafts from 13-day-old donors showed signs of tissue rejection that were not generally evident in the grafts from 11- and 12-day-old donors. The reason may simply lie in the fact that outbred animals were used in this experiment and there were possibly major histocompatibility differences between the hosts and the 13-day-old embryonic donors. Whether the observed tissue rejection was a result of histocompatibility factors or of more fundamental differences between 12- and 13-day PA could be tested by studies on isogeneic animals.

## A procedure for the rapid freezing of whole embryos

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*Summary.* A procedure for the rapid freezing of whole chick embryos for histochemical treatment is described. The problems of deformation during preparation for quenching and orientation for sectioning have been largely overcome by placing embryos inside lengths of chicken trachea. The subsequenct disorientation of tissues that follows cracking and shattering due to the rapid freezing of whole embryos is avoided. The method permitted a more precise identification of the position and time of appearance of formaldehyde-induced fluorescence and myosin antibody immunofluorescence in serially sectioned embryos.

Studies of the early vertebrate embryo by histological and histochemical methods often require an examination of the position and the time of appearance of developing tissues. This is normally accomplished through techniques of fixation which retain the whole embryo intact for subsequent serial sectioning in any desired plane, thus enabling an understanding of the morphological changes taking place. However, the retention of the form and orientation of the whole embryo necessary for such an analysis is much more difficult for histochemical techniques requiring quenching with, for example, liquid propane.

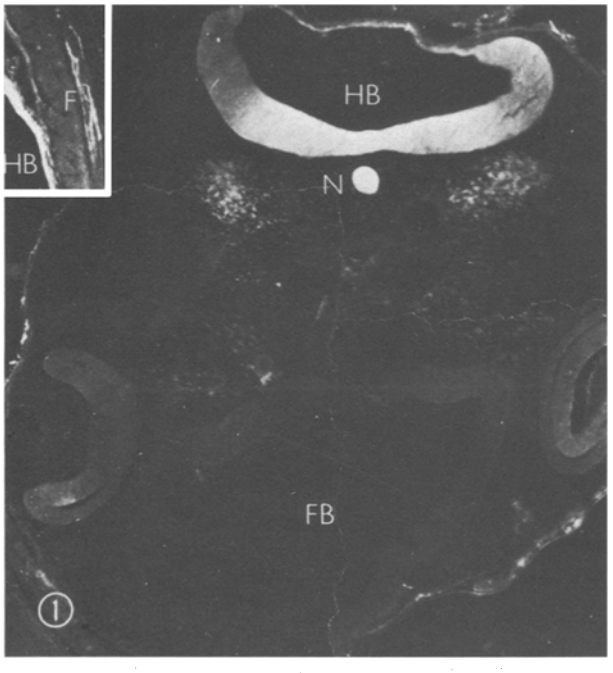


Fig. 1. Transverse section of a stage 16 embryo (FIF technique after α-methylnoradrenaline loading). Note preservation of form of forebrain (FB) and hindbrain (HB). Notochord (N) is intensely fluorescent. Inset: control fluorescent fibres (F) in tracheal housing. ×90.

The normal procedure of placing tissue on small pieces of paper before quenching results in gross deformation of soft embryonic structures when embryos are removed from a supporting liquid medium, making it impossible to cut transverse cryostat sections. An even more serious problem occurs due to cracking of the tissue which often leads to the disintegration of the embryo. Although quenching of small pieces of special interest partially overcomes the latter problem, serial or semiserial sections are generally more informative when examining developing tissues.

We have obtained good serial transverse sections from chick embryos of stage 11–27[2] from both freeze-dried preparations for formaldehyde induced fluorescence (FIF) of catecholamines[3] and fresh frozen preparations for myosin-antibody immunofluorescence[4] by placing the embryo in an older bird's trachea prior to quenching. This housing preserves the form of the embryo and enables precise orientation for later sectioning as well as retaining in a normal location any pieces that might otherwise be lost due to cracking. The trachea also acts as a control through fluorescence of the adrenergic perivascular innervation when using the FIF technique (figure 1) and of the perivascular smooth muscle and tracheal skeletal muscle for myosin-antibody immunofluorescence.

1 We gratefully thank Dr Ute Gröschel-Stewart for preparing sections for immunofluorescence.

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