Independent Differentiation in Components of the Pituitary Complex in the Wood Frog.* (23751)

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Blount(1) reported that the epithelial pituitary primordium in amblystoma does not differentiate after transplantation unless some brain tissue is transplanted with it. This concept of dependence of the buccal pituitary upon nervous tissue in differentiation has been supported by numerous investigators working with a variety of species and technics(2-9). Although these authors differ among themselves as to the degree of dependence of the pars anterior, they agree in finding that the pars intermedia does not differentiate nor does it secrete its pigment-regulating hormone if the buccal primordium is separated from its normal contact with the brain. Most investigators indicate that the infundibulum is the specific area of the brain which has this inductive influence over the pituitary primordium although Eakin(7) who, with his collaborators has explored this problem with the most varied approaches, has concluded that other neural tissue may substitute adequately for infundibulum.

On the other hand, working with various species of Rana not used by the other workers, Atwell and the present author have published several studies since 1935 in which independent differentiation of the isolated primordium into both pars anterior and intermedia was secured (for literature see 10,11). In Etkin's studies the intermedia showed great overgrowth and hyperfunction when transplanted heterotopically and he has provided other evidence(12) that the secretory activity of the pars intermedia is controlled by inhibitory influences from the hypothalamus. The differences in results were considered by various authors in the early 1940's to arise chiefly from the use of slightly more advanced primordia by Atwell and Etkin than by the other workers and perhaps also because of some species differences in time of embryonic determination (see discussion in Blount(3)). The general picture which emerged at that time was that the hypothalamus has a stimulating or inducing influence on the differentiation of the pars intermedia in the embryo. Subsequently in the definitive tadpole and frog, this influence is replaced by an inhibitory control over growth and function of this lobe by the brain (Etkin(12)).

The present investigation is part of an attempt to ascertain at what time the presumptive pituitary becomes embryonically determined to form pars intermedia. This involves the verification of the independent differentiation of the definitive primordium in Ranidae by experiments with more precisely defined stages and by more critical evaluation of the absence of neural tissue in the graft than was attempted in any previously reported work. A separate question is that of the differentiation of the pars nervosa when the epithelial primordium is removed. Smith(13) in his classic study of hypophysectomy in the frog reported that the pars nervosa is reduced and abnormal in development in such animals. Our present concept of the nervosa as essentially a storage organ for neurosecretion(14) raises the question as to whether in the absence of the epithelial lobe the neurosecretory mechanism develops at all.

Methods. Tail bud embryos (Shumway Stage 17) of *Rana sylvatica* were used. The primordium at this stage is a small broadly-based projection of the deeper layer of the ectoderm ventral to the prosencephalon (Fig. 1). After removing the superficial layer of ectoderm a cut was made into the foregut cavity ventral to the primordium. The primordium was stripped from its contact with the brain from which it separates readily and cleanly except at the anterior dorsal aspect where the deep ectoderm fuses with the brain

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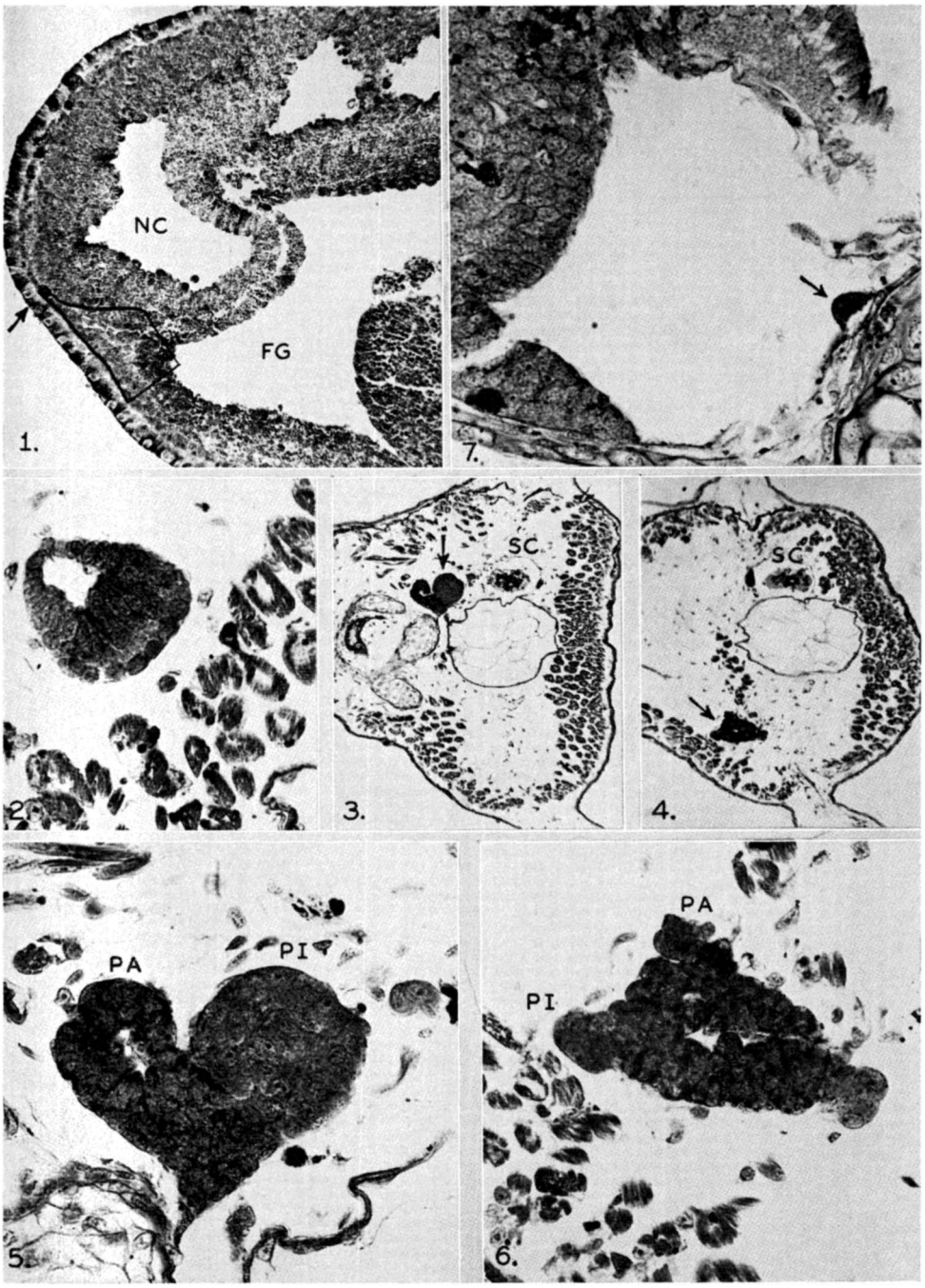

FIG. 1. Median sagittal section of Stage 17 embryo. Area taken in the graft is enclosed by the inked line. NC = neurocoel; FG = foregut. Arrow indicates region where deep ectoderm fuses with brain wall. Photograph 60 ×.

FIG. 2. Vesicle of neural tissue differentiated in one graft. Photograph 390 ×.

FIG. 3. Section through tail showing host spinal cord (SC) with graft (arrow) located nearby. Photograph 60 ×.

FIG. 4. Section through tail showing spinal cord (SC) and graft (arrow) located at considerable distance. Photograph 60 ×.

FIG. 5. Same graft as Fig. 3 at high power. PA = pars anterior; PI = pars intermedia. Photograph 390 ×.

FIG. 6. Same graft as Fig. 4 at high power. Photograph 390 ×.

FIG. 7. Sagittal section through infundibular region of donor animal showing neurosecretory mass at arrow. Photograph 390 ×.

TABLE I. Summary of Findings on Hosts to Grafts of Buccal Pituitary Primordium.

Survivors to Stage 25 = 9	Fixed at Stage 25 = 6	Fixed in late tadpole stage = 3
Pigmentation at Stage 25—	Normal = 8; Intermediate = 1;	Silver = 0
Orthotopic pituitary—	Absent = 6; Present = 2;	Undetermined = 1
Graft pituitary		
6 fixed at Stage 25	P. ant. = 6; P. intermed. = 6;	Degenerating tissue = 0
3 fixed tadpole stage	" = 1; " = 1;	*Idem* = 2
Graft nervous tissue		
6 fixed Stage 25	Absent = 5; Present = 1	
3 fixed tadpole stage	" = 3?; " = 0?	
Graft position respecting host nervous system		
6 fixed Stage 25	Close = 1; Distant = 5	
3 fixed tadpole stage	" = 0 " = 3	

from which it had to be separated by cutting. (Arrow in Fig. 1). Unless the separation seemed clean-cut the specimen was discarded. The innermost cells of the primordium are fused with the endoderm. This endoderm was taken with the graft to avoid injury to the latter. For the same reason some of the lateral ectoderm was also taken. The primordium thus dissected was quickly inserted into a prepared opening in the tailbud of a previously hypophysectomized (*i.e.* epithelial primordium removed) host embryo of like size. The choice of the tailbud as the site of implantation was based on author's recent experience indicating that this region yields a high percentage of "takes." The dissection and transplantation were accomplished in a minute or less. The donor was then examined. If the brain floor appeared to have been damaged both donor and host were discarded. A further check on the accuracy of the dissection was afforded by keeping and studying the donors as well as hosts. Ten pairs of animals were prepared. Experimental animals were raised to the end of the embryonic period and beginning of feeding (Shumway Stage 25). The presence or absence of the pigmentary hormone of the pars intermedia can be definitely ascertained at this time by the state of dispersion of the melanophores and xantholeucophores. A silvery appearance of the animal, expanded xantholeucophores and contracted melanophores, indicates the absence of the pars intermedia in its entirety and of its hormone. By sacrificing all but 3 of the hosts at Stage 25 rather than in late tadpole stages as in previous work it was hoped to avoid the possibility that some brain or gland differentiation had occurred but had been lost by degenerative changes before fixation. The hosts were killed in Helly's fixative. Heads and tails of hosts were sectioned and stained with Mallory's triple stain or with cresyl fast violet. The heads of donors were sectioned and stained with aldehyde fuchsin to demonstrate neurosecretory material.

Results. The pigmentary and histological findings on the host animals are summarized in Table I. It can be seen that all 9 surviving animals showed pigmentary evidence of the presence of pars intermedia secretion at normal or near normal levels. Since in 6 of these the animal's own pituitary was proved in sections to be absent, the pigmentary hormone must have been produced by the grafts. The histological findings showed that in all six animals fixed at Stage 25 both pars anterior and intermedia were differentiated in the graft. Five of these showed no nervous tissue present in the graft. In one a small vesicle of nervous nature (Fig. 2) was found in the graft area, but even here the vesicle was entirely separated from the pituitary tissue. One graft was found to lie close to a spinal root ganglion of the host (Fig. 3) but the 5 others were clearly and widely separated from cell bodies of the host nervous system (Fig. 4). If we exclude the animal showing the small neural vesicle, 4 of these six grafts must then be presumed to have developed without association with any nervous tissue

of graft or host. In 2 of the 3 animals fixed in late tadpole stages some degenerating tissue of uncertain nature was found. These animals do not therefore provide critical evidence on graft differentiation and need not be considered here in detail.

In sections the pars anterior of each of the Stage 25 grafts clearly shows characteristic tissue differentiation (Fig. 5, 6). The cells are small, arranged in compact cords and contain much embryonic pigment. No chromophilia is yet seen. In all respects, size, form and histological differentiation, the grafted pars anterior is entirely comparable to that of the normal gland at this stage of development. The pars intermedia is likewise readily identified in the grafts. The cells are large, with ample slightly basophilic cytoplasm (Fig. 5, 6). In 3 instances the size of the organ was very small though the tissue was cytologically well differentiated (Fig. 6). The microscopic structure of pars intermedia, except for size, was like that of the normal gland of the same stage. However, it suffered more of a loss in size on transplantation than did the pars anterior. This is the opposite of the condition found previously in long term grafts(11) where the pars intermedia was found to be hypertrophied and the pars anterior poorly developed. Two of the 3 animals kept to late tadpole stages showed excess body pigmentation. One of these subsequently became light in color and in section showed degenerating graft tissue. The other showed pars intermedia hypertrophy as previously reported.

The graft pituitaries generally lie in loose connective tissue usually in contact with host muscle fibers at some point. Other structures were found in the graft, such as cartilage, horny teeth, etc. as Eakin and others have reported. No consistent association of these tissues with the pituitary was noted but contact between pituitary and cartilage (Fig. 5) was most common.

The 9 surviving donor animals were all silver in color and upon sectioning were seen to lack entirely the epithelial pituitary. This indicates that the entire presumptive pituitary had been adequately removed in making the grafts.

In the normal animal at this stage the posterior tip of the infundibulum is formed by a very thin-walled vesicle continuous with the thin-walled roof of the infundibulum. A slight thickening in this vesicle in the region of its contact with the pars intermedia constitutes the early pars nervosa. With the aldehyde fuchsin technic a spot or a small diffuse area which stains intensely can be observed in this pars nervosa. This stained material presumably represents accumulated neurosecretory granules and serves to identify the pars nervosa. In the donor animals of this experiment the thin vesicular tip of the infundibulum is larger and more "blown-out" than normal. In 8 of the 9 specimens one to three spots of intensely staining material could be found at the tip of the infundibulum (Fig. 7). These are more concentrated than in the normal animal but clearly represent the accumulation of neurosecretory material as in the normal pars nervosa. The rest of the infundibular lobe of the brain is as large as in the normal animal but is abnormal in shape, and is lacking the clearly developed bilobed character found in the normal animal. This abnormality in shape has been known to characterize the infundibulum of the hypophysectomized animal since the original work of Smith(14).

Discussion. We conclude that in *Rana sylvatica* the epithelial primordium at the stage in which it first forms a recognizable thickening (Stage 17) is able to differentiate when transplanted to the tail to form both pars anterior and pars intermedia without further contact with the brain cells with which it normally is associated. It can so differentiate while lying in an area of loose connective tissue and muscle in which no host nerve cells are found. This conclusion is in agreement with most recent work(7,8) with respect to the pars anterior but not with respect to the pars intermedia. Since the other workers used different species it is possible that this divergence in results is due to a species difference in the time of embryonic determination in the primordium. In view of the use of Stage 18 animals of *Hyla regilla* by both Burch(4) and Eakin(7) which, as figured by Burch, have primordia even more advanced

than those used here, the differences in our results cannot be explained by my use of an earlier morphological stage. It is possible, therefore, that there is some difference between species in regard to differentiating capacities at the same stage, (stage 17-18).

In addition to species differences, however, there are differences in technic between the work reported here and that of others who have made transplants of primordia. This difference is most conspicuous regarding the degree to which the primordia are manipulated and regarding the site of implantation. I am inclined to view these differences as very important in determining the extent of development obtained although we have no way now of evaluating the relative importance of species as compared to technic differences. The point of view that I have adopted is that the graft should receive a minimum of handling and a maximum of protection by surrounding tissues. In the present series some tissue surrounding the primordium was taken on all sides except, of course, on the side in contact with brain. The small size of the surviving pars intermedia in 3 of the present grafts suggests that this tissue is especially vulnerable to injury in the course of the operation. The pars intermedia arises from the deepest cells of the invaginating primordium (15) and therefore would be most exposed to damage with resulting loss of cells. No attempt was made here to clean off neural cells from the transplant since such, if present, could not be distinguished from primordial cells. Manipulation was, therefore, as likely to remove cells of the primordium as it was to remove possible adherent nerve cells. The most satisfactory criterion of the presence or absence of nerve cells in the graft is the differentiation to be seen at the end of the embryonic period. The excellent differentiation attained by pituitary as well as other tissues in the tail bud location gives confidence in the use of this criterion. As indicated above, neural differentiation was absent in 5 of 6 animals fixed at Stage 25.

It should be noted that the presumptive pituitary cells originate in close association with the neural plate. The primordium from the time of its first appearance is in contact with neural tissue. It has been isolated in the present experiment at a stage before it has sunken in deeply enough to reach the infundibular region of the embryonic brain (Fig. 1). It is possible, therefore, that inductive influences from the brain might have acted upon presumptive pituitary cells before Stage 17. If so, they must come from regions anterior to the infundibulum and act before the definitive pituitary primordium is formed in this species. The present experiment, therefore, does not exclude the possibility of an inductive influence of neural upon hypophyseal tissue. However, it does show that such influence, if present in this species, must come from the neural plate anterior to the infundibulum and act before the morphologically distinct primordium is formed.

The presence of typical masses of neurosecretory material at the tip of the infundibulum of the donor animals shows that even in the absence of the epithelial lobes the neurosecretory mechanism of the hypothalamus does differentiate. There is abnormality in the form of the infundibulum the significance of which remains to be elucidated. It should be noted that these animals were checked at operation and none showing injury to the brain were used. Furthermore, the grafts indicate that no nerve cells were taken with the primordium except in one case. The abnormality of the infundibulum cannot be ascribed to incidental injury to the brain, therefore, but is more likely to be related to the absence of the epithelial lobes.

Summary. The epithelial primordium of the pituitary in Stage 17 embryos of *Rana sylvatica* was removed without including or injuring adjacent brain and transplanted into the tail bud of hypophysectomized host embryos of the same stage. Donors and hosts were studied for pigmentary reaction and for histological differentiation of appropriate tissues. It was concluded that the epithelial primordium when it first appears as a morphologically distinct entity is capable of differentiating into both pars anterior and pars intermedia without further contact with brain or other nerve cells. It is likewise concluded that the neurosecretory mechanism of the hypothalamus differentiates normally in the ab-

sence of the epithelial pituitary.

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Interactions Between Mononuclear Phagocytes and *Brucella abortus* Strains of Different Virulence.* (23752)

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In prior studies on the multiplication of *Brucella abortus* in mononuclear phagocytes (monocytes) of guinea pigs, maintained *in vitro,* it was noted that after different periods of incubation some monocytes always tended to contain considerably more bacteria than others(1). It was suspected that such heterogeneity may have been due in part to a genetic heterogeneity among members of the bacterial population in regard to characteristics influencing ingestion by phagocytes and intracellular multiplication. It is well known that virulent smooth (S) and relatively avirulent rough (R) types are phagocytosed at different rates by polymorphs. In addition, it has been shown that nonsmooth *B. abortus* mutants fail to multiply within chick embryo fibroblasts capable of supporting multiplication of smooth type cells(2). Furthermore, when the culture used in the prior quantitative studies(1) was examined 2 years later, appropriate tests(3) revealed that it contained approximately 20% R types in addition to S cells. Therefore, additional studies were initiated to determine the extent to which differences in antigenic and virulence characteristics of the bacteria may influence their interactions with monocytes from normal or immune guinea pigs.

Methods. Tissue culture technics followed essentially those detailed previously(1). Monocytes were harvested from guinea pigs 3-4 days following intraperitoneal injection of 2 ml sterile mineral oil. They were washed, counted and suspended in Hanks solution containing 30% homologous serum to yield a concentration of approximately 1 x 10^6 cells/ml. Bacteria harvested from 18-hour-old tryptose agar cultures were added to yield concentrations approximating either 2 x 10^8/ml (*high* inoculum) or 2 x 10^7/ml (*low* inoculum). 1.5 ml of such suspensions were placed into rubber-stoppered Porter flasks containing flying cover slips, and incubated at 37°C. Two hours later the liquid was replaced by serum-Hanks' containing 10 μg of streptomycin per ml. It was established that this concentration of antibiotic will kill all except $<0.001\%$ of the extra-cellular bacteria

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