Extirpation and Transplantation of the Pancreatic Rudiments of the Salamanders, *Amblystoma punctatum* and *Eurycea bislineata*

B. E. FRYE [1]

Department of Zoology, University of Michigan, Ann Arbor, Michigan

In tetrapod vertebrates the pancreas originates at two or three diverticula from the posterior level of the foregut. These include a dorsal rudiment, which arises from the roof of the embryonic duodenum, opposite the liver diverticulum, and one or two ventral rudiments. The latter may arise either from the ventro-lateral duodenal walls, near the liver, or, perhaps more commonly, directly from the base of the ductus choledochus itself. The diverticula generally fuse so early and completely that it is difficult to say exactly what contribution each of the original rudiments makes to the definitive gland. Even so, numerous investigators have concluded, on the basis of the histological distribution of the islets, that most or all of them originate from the dorsal lobe. Important experimental support of this was provided by the work of Wolf-Heidegger ('36) who transplanted dorsal and ventral pancreatic rudiments into the posterior abdominal region and found that islets arose only in dorsal pancreas thus isolated, never in the ventral pancreas. This work was done with the toad, *Bombinator pachypus*.

The purpose of this report is to (1) corroborate and extend these results with regard to the prospective significance of the separate pancreatic rudiments of the salamanders, *Amblystoma punctatum* and *Eurycea bislineata*, (2) describe some histological features of transplanted rudiments, and (3) provide some information about the development of depancreatized larvae.

The work includes two main groups of experiments: (1) extirpation experiments, in which one or another of the rudiments was deleted from any contribution to the definitive gland, and (2) transplantation experiments, in which the pancreatic rudi-ments were placed singly onto the posterior yolk mass of host embryos for further development.

MATERIALS AND METHODS

Eggs of *Amblystoma punctatum* and *Eurycea bislineata* were collected in the field and reared in the laboratory. The eggs were kept in filtered pond water at room temperature. After hatching the larvae were placed one to a "cube" in plastic ice cube trays. Using these containers one can keep large numbers of individual experimental animals, or stock cultures of the cannibalistic Amblystoma larvae, with a minimum of space, effort and expense.[2] The larvae were reared on a diet of Enchytrea worms.

Operations were carried out under MS 222 anesthesia (Sandoz Pharmaceutical) in permoplast base operating dishes, in either urodele operating medium (Rugh, '48) or in 0.4% sodium chloride made up in pasteurized pond water. Sodium sulfadiazine was added to the operating medium and to the post operative culture dishes until healing was complete. All operations were done on late embryos or prefeeding larvae; for specific stages see table 1.

In the extirpation experiments a small incision was made through the body wall overlying the rudiment to be removed. The rudiment was held with a watchmakers forceps and cut loose from the gut or ductus choledochus with a scissor-like action of a second forceps. The area was

checked for adhering bits of pancreatic tissue which were cleaned out with the forceps and a hair loop. No special post operative care was necessary, and complete healing occurred within about 12 hours.

In the transplantation experiments a donor animal was cut apart to expose the desired rudiment. This was cut loose and dissected free of most of the adhering yolk and mesenchyme. With a mouth controlled pipette it was transferred to a previously prepared recipient. The pipette was inserted into the coelom through a hole cut in the lateral body wall, and the graft deposited onto the posterior yolk mass.

Tissues were fixed in Bouin's fixative, sectioned at 8 or 10 μ and stained with Gomori's modification of Heidenhain's azan ('39) or with the stain of Rona and Morvay ('56).

OBSERVATIONS AND RESULTS

A summary of the experimental groups, with data on the survival of operated animals and recovery of transplants is contained in tables 1 and 2.

A. Extirpation experiments

1. *Eurycea bislineata*. Extirpation of the pancreatic rudiments was performed at stage 39 or 40.[3] A brief summary of the early development of the pancreas in

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[3] The stage numbers for Eurycea refer to approximately equivalent stages of the Harrison series for Amblystoma (see Rugh, '48) as extended by the author (Frye, '58). No staging series was available for Eurycea.

TABLE 1

Summary of experimental groups

Type of operation	Stage of operation[1]	Number of cases	Successful cases
1. Extirpation experiments			
Amblystoma punctatum			(survival)
Dorsal lobe	45–46	38	33
Sham controls	45–46	19	19
Eurycea bislineata			
Right ventral lobe	39–40	15	15
Dorsal lobe	39–40	15	14
Sham controls	39–40	10	9
2. Transplantation experiments			
Amblystoma punctatum			(grafts recovered)
Dorsal lobe	41–43	20	15
Ventral lobe	42–43	18	11
Eurycea bislineata			
Dorsal lobe	39–40	14	6
Ventral lobe	39–40	9	1

[1] Based on the Harrison series for Amblystoma

TABLE 2

Larval growth and stage after dorsal pancreatectomy (Amblystoma punctatum)

Post operative interval	Experimental			Controls		
	No.[2]	Length	Stage[1]	No.[2]	Length	Stage[1]
days		*mm*			*mm*	
10	24	17.2	48.9	19	17.2	49.1
30	22	21.0	51.4	18	20.8	51.3
50	21	24.4	52.7	17	24.9	52.7
70	15	28.7	54.2	14	28.4	54.2
90–95	9	30.1	55.0	4	30.3	55.0
120–125	8	31.4	56.0	3	31.0	56.0
Av. increment		15.4	10		15.0	10

[1] Harrison stages.
[2] Number of specimens decreases mainly due to fixation at intervals.

this species is presented here for use in evaluating the experiments.

The ventral pancreatic rudiments arise at stage 38 as outgrowths from the walls of the ductus choledochus at the lateral margins of the angle formed by the junction of the latter with the presumptive duodenum. The dorsal rudiment appears slightly later (stage 39) and arises from the roof of the duodenum 20–30 μ anterior to the ductus choledochus. As it develops the dorsal rudiment is moved relatively posteriorly by the formation of the duodenal loop, but itself grows anteriorly and mesially toward the right ventral pancreatic rudiment. At the same time the latter is rotated by the growth of the duodenum so that it comes to lie dorsal rather than lateral to the gall bladder rudiment, and extends backward along the gut in the direction of the dorsal lobe. In this manner the two meet and begin to fuse by stage 45 and are indistinguishably fused by stage 46 +. The left ventral rudiment never contacts the dorsal lobe and is separated from the right ventral rudiment by the gall bladder and the ductus choledochus. There is superficial fusion of the two ventral lobes as they grow and come in contact around the bile duct, but this is a relatively narrow contact and the borders of the left ventral lobe remain fairly sharp, even in the mature larva.

Histogenesis follows a similar pattern to that described for *Amblystoma opacum* (Frye, '58). Because of the intimate fusion of the dorsal and right ventral rudiments one cannot be sure from descriptive studies, but it appears that islets do not originate from the ventral lobe material, at least during the period under consideration.

The right ventral pancreatic rudiment was removed in 15 specimens, all of which survived and were examined histologically. Absence of this lobe in no apparent way alters the rate or pattern of histogenesis of the left ventral lobe or the dorsal lobe as compared with the normal or sham operated controls. Islet potent cells are present in the dorsal lobe by stage 47 or 48 and small islets appear by stage 49. Since the dorsal and left ventral rudiments are completely separated after removal of the right ventral rudiment it is possible to assert definitely that the left produces no islet tissue.

There is no regeneration of the right ventral rudiment; however, in two of the 15 specimens there are small pieces of this lobe, which, for the following reasons, I judge to have developed from fragments left at the time of extirpation: (1) the remnants show no delay in histogenesis, such as one might expect if regeneration were the source of the tissue, (2) there is no noticeable increase in mitotic activity, and (3) in none of our experiments is there any appreciable regenerative activity when removal is complete to the duct stump.

The dorsal lobe was extirpated in 15 specimens, 14 of which survived and have been examined histologically. The ventral lobes develop normally and do not contain islets.

There is no regeneration of the dorsal lobe from the duct stump, but in three specimens there are small remnants of dorsal lobe. That these have developed from fragments left at the time of excision is certain for the reasons given in the discussion of the ventral lobe experiments, and for the additional reason that in each case the fragment is suspended in the dorsal mesentery some distance from the duodenal wall, from which regeneration would be expected to occur. In two specimens the remnants have no duct and have developed after the pattern of ductless transplants described below. Islet tissue is present in two of the three fragments.

Extirpation of either the right ventral or the dorsal pancreatic rudiment had no apparent effect upon the rate of development and growth of the Eurycea larvae, as compared to intact and sham-operated controls, for as long as six weeks after the operation.

2. *Amblystoma punctatum.* The normal pattern of histogenesis of the pancreas has already been published for the closely related species, *A. opacum* (Frye, '58). Since the picture is seemingly identical in the two, no further account of the normal situation will be given here.

The dorsal rudiment was extirpated in 38 specimens, 33 of which survived. The operation was performed at stage 45 or

46, at which time the dorsal and ventral lobes are still histologically undifferentiated and unfused. Eighteen specimens have been examined and in all of these the ventral lobe is normal and comparable to normal or sham-operated controls of an equivalent stage. Islet tissue does not appear in the ventral lobe under these circumstances.

In 5 of the 18 specimens examined there are present small pieces of dorsal lobe tissue. According to the sort of evidence mentioned above, three of these have originated from fragments of the dorsal rudiment which were left at the time of excision. All three of these lack duct drainage and are similar in appearance to certain grafts which fail to establish duct drainage, as described below. There is no islet tissue present in any of these specimens. In the remaining two cases there are present minute regenerates of pancreatic tissue in close association with the original duct stump and the duodenal wall. One of these regenerates is represented by a short duct-like outgrowth from the gut, without any acinar or islet tissue associated. The other consists of a spherical cluster of undifferentiated cells embedded between the outer muscularis and the serosa of the gut wall, and appears to have proliferated from the stump of the duct of the excised gland.

The rate of growth and differentiation of larvae subjected to dorsal pancreatectomy is not appreciably altered as compared to sham (or normal) controls. Data covering the larval period up to about the time of metamorphosis are presented in table 2. As was noted above, these specimens lack any islet tissue.

B. Transplantation experiments

The experiments to be reported here include only those done with Amblystoma since attempts to transplant the pancreatic rudiments of Eurycea larvae were generally unsuccessful. All of the transplants described are homografts in which the host and the donor were of the same developmental stage.

1. *Dorsal lobe transplants.* Of 20 specimens examined histologically, 15 grafts were recovered. The remaining five were presumably either resorbed or were expelled from the host before the incision healed.

With one exception the grafts lie on the right side of the colon within the coelom. All of the grafts are well vascularized by vessels arising in the dorsal mesentery, the visceral or parietal peritoneum and, in one instance, directly from the dorsal aorta. In 8 of the 15 grafts duct drainage has been established, leading to the colon.

The grafts are inhibited in their growth relative to the intact dorsal pancreatic tissue of the host (or of controls). Restriction of growth is reflected in failure of outgrowth of the graft tissue into the coelom and along the mesentery and gut surface as normally occurs. Rather, the grafts retain a compact, generally ellipsoid form. This failure of normal outgrowth has certain consequences on the histology of the islets of Langerhans (see below).

Because of the small number of grafts studied at each stage I have not attempted a precise quantitative study of the growth of the islets. Estimates, based on the linear dimensions of the grafts and on areas of projected sections, indicate that whereas the volume of the intact dorsal pancreas increases approximately 50–75 times between stages 43 and 55, the grafts increased by a factor of only eight to ten times during the same period. The numerical basis for these estimates is given in table 3.

Histologically all of the grafts in this series are recognizable as pancreatic tissue; that is, they contain ductular, acinar and insular components. However, they are variable with regard to details, and the following types can be recognized:

(1) Grafts in which duct drainage into the colon is inadequate or absent, with the consequence that the ducts and acini are more or less distended and in the most severe cases tend to take on a highly "follicular" appearance (figs. 7, 9, 11). Islet tissue is present in these grafts, but is usually not of normal histology. Rather, the islet cells form layers around the smaller ducts and compact masses filling the crevices between the larger distended ducts.

(2) Grafts in which the proportion of the ductular and insular components are markedly greater than normal relative to

TABLE 3

Growth of pancreatic grafts, (Amblystoma punctatum)

Stage[1]	Linear dimensions[2]	Estimated volume[3]
Initial (43)	300 × 200 × 200	3,– 4,000,000
47		
Graft	400 × 250 × 250	12,– 15,000,000
Host	500 × 300 × 275	20,– 25,000,000
55–56		
Graft	600 × 500 × 300	35,– 40,000,000
Host	1500 × 1200 × 250	234,–240,000,000

[1] Three specimens at each stage presented. Additional specimens at intermediate stages support this pattern.
[2] Length × greatest cross sectional height and width.
[3] In cubic micra, estimated by assuming the shape of an ellipsoid. This probably tends to give minimal values for the host dorsal pancreas, which tends toward a rectangular parallelepiped in shape, except at ends.

the acinar component. The ductular-insular component is generally sharply demarcated from the acinar part and may form 50% or more of the gland. This tissue may form a "medulla" surrounded by an acinar "cortex," or there may be more complete separation, with the acini forming a cap on one side of the duct mass (fig. 9). In either case the islet tissue is intimately associated with the ductular component and varies in histology from clusters of cells interspersed among or around the ducts to islets which differ from normal only in the compact arrangement of the cells.

(3) Grafts of normal or nearly normal histology (figs. 3, 4, 6, 10). The only visible defect here is an excessive compactness of histological construction. This I presume to relate to failure of graft outgrowth, as mentioned above, and to be a consequence of lack of space for the various histological components to expand freely. The islet tissue is especially affected in this regard, in two particular respects: (a) the islets are often compactly arranged, lacking the typical cordlike arrangement of the cells, and (b) islet tissue is restricted to one large islet or mass of islet-potent cells near the center of the gland.

Grafts of group three and usually of group two have well formed ducts draining into the colon. Zymogen is present and the tissue appears to be functional.

2. *Ventral lobe transplants.* Eighteen animals were sectioned and examined, from which 11 grafts were recovered. All of these contain recognizable acinar and ductular components, but *none contain islet tissue.* Otherwise they are histologically similar to the dorsal pancreatic grafts. A ventral rudiment graft is pictured in figure 2.
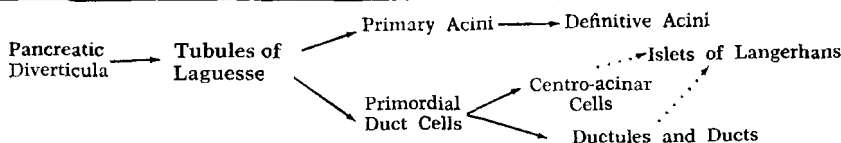
DISCUSSION

1. *Islet origin.* This work supports the proposition that the genesis of islet tissue is a special property of the dorsal lobe of the pancreas. This has been suggested many times, for most vertebrate groups, on the basis of the relatively greater abundance of islet tissue in the body and tail of the gland, which are supposed to originate mainly from the dorsal rudiment, as compared to the relative paucity of islets in the head of the gland, said to be derived from ventral lobe. (See Wolf-Heidegger, '36, for an extensive discussion of the older literature. Some key references are cited in Frye, '58.)

The experiments of Wolf-Heidegger ('36) (in which wedges of the body wall overlying and including either the dorsal or the ventral rudiments were transplanted to the posterior abdomen) and the present work provide strong experimental proof for this contention so far as amphibia are concerned. In fish it is quite certain, from the anatomical relationships of the pancreatic lobes, that islets appear only in the dorsal lobe of some species (Siwe, '26; Vorstman, '39). No experiments have been done to confirm this in the higher tetrapods. However, Weber ('20) cites a case of a young diabetic with an apparent congenital absence of the dorsal pancreatic rudiment (only the head was present) and

a total absence of islet tissue. Normally it is presumed that islets form in the head region of the pancreas of birds, reptiles and mammals only to the extent that dorsal lobe tissue invades the head during fusion of the lobes (*op. cit.*). Bencosme and Liepa ('55) have reported that the uncinate process of the dog pancreas, which is of purely ventral lobe origin, contains no islets of Langerhans. On the basis of such evidence it seems likely that insulagenesis is a special property of the dorsal rudiment in all vertebrate classes.

Except with regard to the origin of the islets of Langerhans, the cellular pattern of differentiation of the two lobes is alike (Frye, '58) and can be represented by the following scheme (The path of islet formation, present only in the dorsal lobe, is indicated by dotted lines):

Pancreatic Diverticula ⟶ Tubules of Laguesse ⟶ Primary Acini ⟶ Definitive Acini
⟶ Islets of Langerhans
Primordial Duct Cells ⟶ Centro-acinar Cells
⟶ Ductules and Ducts

This pattern persists beyond embryogenesis, as indicated by the capacity of the pancreas to form new islets throughout life (Hellman, '59) or during regeneration (Johnson, '50), and by the fact that the ratio of endocrine to exocrine pancreas can be changed almost at will by manipulating the blood glucose level, or conditions which modify blood glucose (Haist et al., '49). In considering the geometric relationship of the islets to the exocrine tissue, it is apparent that the ability to undergo such changes must be an intrinsic property of the pancreatic tissue and could not be continuously imposed by an organizer, in the usual embryonic sense. This observation suggests, however, that external influences, such as blood sugar level, may effect such changes by modifying the propensity of a pancreatic cell to differentiate along one route or another.

Presently no explanation for the deficiency of the islet forming steps in the ventral pancreas is forthcoming. Cogent experiments to determine the basis of this morphogenetic "defect" have not been done, nor is it apparent what should be done. If conditions could be devised to induce islet formation in the ventral lobe, or even to suppress islet formation in the dorsal lobe, we would be on a firmer basis for speculation. As yet pancreatic rudiments have not been isolated or transplanted early enough to determine when the rudiments are "determined" with respect to islet formation. Wolf-Heidegger's experiments involved transplantation of the rudiments at the tail bud stage. However, since he transplanted entire transects from the body wall, his transplants were not truly isolated in the sense of being removed from possible local inductive influences emanating from overlying mesenchyme, or other tissues. The role of mesenchyme in the regional differentiation of the gut (Okada, '60) and in the morphogenesis of specific rudiments (Grobstein, '53a, b) has been demonstrated.

Although we are not prepared to discuss causal morphogenetic mechanisms responsible for the absence of islets from the ventral lobe, the present experiments do appear to have eliminated one possibility: namely, local inhibition of this lobe by the dorsal lobe. Perhaps by virtue of a greater propensity for insulagenesis the dorsal lobe releases specific inhibitors or competes for some essential factor. This possibility is untenable since neither removal of the dorsal lobe nor transplantation of the ventral has any effect upon differentiation of the ventral rudiment.

2. *Larval survival and growth.* Removal of the pancreatic rudiments does not impair survival or growth of the larvae, so long as one lobe of the pancreas is left to carry on the digestive functions. Although no total pancreatectomies were done in this investigation, unpublished experiments by the author with *Rana pipiens* larvae indicate that after total removal of the pancreas growth is completely stopped and survival reduced to a

few days, because of digestive failure. More important, perhaps, is the observation in the present experiments that total absence of the endocrine portion of the pancreas is without consequence during the larval period, at least up to the beginning of metamorphosis. This substantiates the belief (Frye, '58) that the islet tissue of amphibian larvae does not function during the larval period. Some indirect evidence has been produced indicating that function in fact first appears in the islets at the time of metamorphosis (Aron, '28a, b; Janes, '38) and is presently being corroborated by the author through physiological studies of metamorphosing larvae.

3. *Graft growth.* The observation that the transplanted pancreatic rudiments grow to only about 15–25% of the size of the intact gland over the same period needs to be substantiated and quantitated through more extensive experiments. We have currently in progress some experiments to determine whether this might be a result of competitive inhibition of the graft by the host homologous tissue, according to the theory of specific inhibition (Rose, '52). Other possibilities include (1) poor vascularity of the grafts, (2) damage during transplantation and (3) disturbance of normal epithelio-mesenchymal relationships. The histological appearance of the grafts and their state of differentiation does not support the first two suggestions. The latter possibility is suggested by the fact that in normal growth the pancreas spreads out through the dorsal mesenteries along the gut. The grafts, on the other hand usually lie nearly free within the coelom and tend to assume a compact spherical shape. Grobstein ('53a, b) has described a specific interaction between the mesenchymal and epithelial components in the morphogenesis of the mouse submandibular gland. This possibility can be checked in the present situation by leaving the mesenchyme intact on the transplants, by attempting to transplant into the dorsal mesentery or retroperitoneally, or by transplanting anteriorly in the region of the duodenum, thus providing "specific" mesenchyme.

SUMMARY

Differentiation of the dorsal and ventral pancreatic rudiments was studied (a) after extirpation of one or another of the rudiments, or (b) after homografting singly onto the posterior yolk mass. Extirpation was done at a stage prior to fusion of the dorsal and ventral lobes and before islet differentiation had occurred. Transplantation was done before any histological differentiation of the rudiments had occurred. Tissues were prepared for histological study at intervals from Harrison stage 41 up to metamorphosis. The capacity for differentiation of islets of Langerhans is restricted to the dorsal rudiment, since islets always arise in the dorsal lobe, but never differentiate in the ventral lobe under these conditions. Dorsally depancreatized larvae, which are consequently completely lacking in islet tissue, survive, grow and differentiate normally up to the time of metamorphosis. This corroborates other data indicating that the islets of amphibian larvae are not functional prior to metamorphosis.

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PLATE 1

EXPLANATION OF FIGURES

All figures are of pancreas of *Amblystoma punctatum.*

1 Graft of a dorsal pancreatic rudiment at stage 43, a few days after transplanting. The rudiment (arrow) lies in the coelom to the left of the yolk-laden presumptive colon, into which a cord of pancreatic cells is pushing. This illustrates the manner in which duct drainage is established between the graft and the gut. × 120.

2 Graft of a ventral pancreatic rudiment showing normal acinar differentiation and duct drainage (d) into the colon (g), but no islet tissue. × 120.

3 Part of a dorsal pancreas graft showing completely normal histology of the acini and a well formed duct (d) which drains into the colon. × 225.

4 Same specimen as figure 3, but in a different section, showing completely normal islets (i) in the graft. The histology of the exocrine and endocrine tissue in this graft is indistinguishable from the normal host pancreas. × 225.

5 Same specimen as figure 3, showing a section of the host dorsal pancreas, with an islet (i), for comparison with figure 4. × 225.
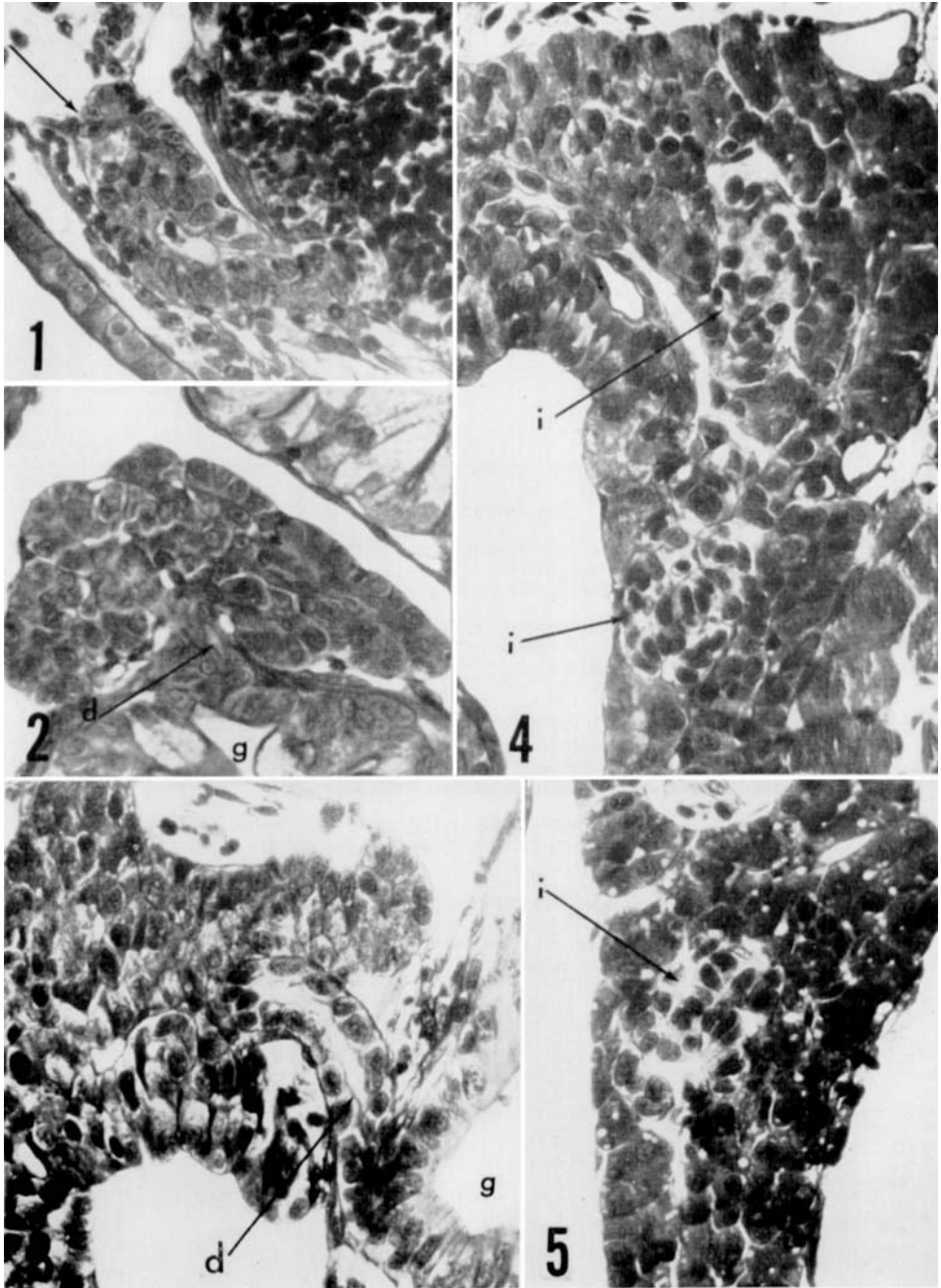
Figures 6–11 are all of dorsal pancreas transplants in Amblystoma, fixed at stage 52–55.

6   Graft of dorsal pancreatic rudiment showing near-normal formation of the acini, but with excessive compactness. The islet tissue forms a compact layer (i) around a small central duct (d). × 225.

7   Dorsal pancreatic graft of compact formation, but with a well formed islet in the center (i). This graft has no duct opening into the gut and some vesiculation of the acini and ducts is seen (v). × 225.

8   This small graft grew less than usual and is poorly differentiated histologically. Even so, acini (a) and islet cells (i) can be recognized. The acini are distended due to absence of drainage into the colon. × 225.

9   Large graft with a large mass of ductular-insular cells (d–i) and a cap of acinar tissue on one side. One questions how this segregation of morphogenetic potencies, and the relative exaggeration of the insularductular component can occur. × 120.

10 The acini of this graft are large and strongly basophilic, and slightly distended, although a small duct opens into the colon (g) in another section. × 120.

11 A graft which lacks duct drainage into the colon and shows the consequent highly follicular (f) appearance of the distended ducts and acini. At higher magnification the cells marked (i) can be identified as probably islet tissue. × 120.