REACTION OF THE PANCREAS TO INJURY IN TADPOLES IN RELATION TO METAMORPHOSIS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 6, pp. 84-87, June 1962
Original article submitted June 1961

The regenerating power of the pancreas in response to the action of various hormones and during maintenance on a uniform carbohydrate or protein diet has been studied in mammals by L. N. Kuleshova [3, 4]. Her results showed that in experimental conditions a carbohydrate diet favors the repair and, in particular, the regeneration of the islet tissue.

We have conducted an analogous investigation in animals in which the character of the diet undergoes essential changes in ontogenesis. This state of affairs is observed notably in the larvae of the tailless amphibians, in which the character of the diet before metamorphosis (vegetarian) is replaced after metamorphosis by a different type of diet (animal objects). We studied the reaction of the pancreas to injury at different stages of development – long before metamorphosis and immediately before its onset.

EXPERIMENTAL METHOD

Experiments were conducted on the larvae of the frog Pelobates fuscus. In the tadpoles of this species, long before metamorphosis (stage II in L. Ya. Blyakher's classification [1]), and also in the larvae as metamorphosis commenced, either $^1/_4$ or $^1/_2$ the pancreas was removed. The number of tadpoles in the different series varied from 9 to 12. The observations lasted for 5 and 10 days. The animals were killed and the pancreas weighed and taken for histological examination. At the time the tadpoles were sacrificed, the body, tail, limbs, and gut were weighed and measured; these figures enabled the character of development of the tadpoles to be judged objectively.

The pancreases were fixed in Bouin's fluid and embedded in paraffin wax. Serial sections were stained with hematoxylin and eosin, and also with aldehyde-fuchsin by Halmi's method; the presence of RNA was shown by Brachet's method. During study of the sections we measured the height of the undifferentiated translucent cells, the height of the acinar cells and the zymogen layer, the diameter of the nuclei, and we also counted the mitoses in the zone of regeneration and in the residual part of the pancreas (in 10 fields of vision under the immersion lens). The area of the zone of regeneration was also determined in the histological sections. For this purpose the outline of the zone of regeneration was superimposed on the outline of the whole histological section on paper. Knowing the true magnification of the microscope, the area could then be calculated in absolute values (in mm^2).

EXPERIMENTAL RESULTS

The results show that with the onset of metamorphosis and with its completion the weight of the pancreas falls appreciably (see Table 1). The weight of the organ in the stage II tadpoles was 24 mg, and in the young frogs 6.5 mg.

The microstructure of the pancreas in the young larvae, feeding mainly on plants, is characterized by the presence of large numbers of undifferentiated, proliferating epithelial cells, arranged in groups, and containing very little RNA. These structures have small lumina and resemble small proliferating efferent ducts (see Fig. 1, a). They are formed of translucent prismatic cells with large nuclei (average diameter 6.6 μ). Closely connected with these proliferating structures are small groups of cells which are subsequently transformed into islet cells. These future islet cells are polygonal in shape, but in their early stage of development they show little difference structurally from the slightly differentiated cells bordering the walls of the small ducts.

The acini around the undifferentiated epithelial structures consist of conical cells very similar in height to the proliferating cells, but containing nuclei of a smaller diameter (4.9 μ). Few secretory granules are present in the cells

TABLE 1. Indices of Metamorphosis and Data of the Weight of the Intact and Injured Pancreas in Larvae of the Frog Pelobates fuscus

Group of animals	Stage of development	Length (in mm)				Weight of pancreas	
		of the whole tadpole	of the tail	of the limb	of the gut	in mg	ratio to body wt.
Control	Before metamorphosis (stage II)	71	40	16	545	24.0	0.37
	Onset of metamorphosis (stage III)	79	55	18	582	23.2	0.35
	Before completion of metamorphosis (stage V)	52	23	37	56	15.0	0.44
	Young frogs	–	0	39	51	6.5	0.28
Experimental, 10 days after operation	Before metamorphosis (stage II)	69	44	14	366	14.0	0.29
	Onset of metamorphosis (stage III)	73	49	25	261	8.5	0.18
	Before completion of metamorphosis (stabe V)	48	9	35	55	To avoid injury the tissue of the pancreas was taken with an area of the gut, and weighing was omitted in these experiments	

of the terminal divisions of the gland, and they do not appear in all the cells at the same time. The cells of the mature acini contain more RNA than the undifferentiated epithelial cells, and it is distributed more or less uniformly throughout the cytoplasm.

The pancreas of the young frogs formed as a result of metamorphosis has acquired a new structure. The proliferating undifferentiated epithelial structures in the gland, typical of the larvae, are no longer found. Acinar tissue predominates, its cells rich in zymogen (see Fig. 1, b). Whereas the height of the zymogen layer in the cells of the terminal divisions in the tadpoles averages 0.9 μ, in the young frogs it is increased to 8.7 μ. The RNA in the acinar cells of the young frogs is not distributed throughout the cytoplasm, but is localized principally in its basal parts. Few efferent ducts are seen, and they are small in caliber. The islets are few in number, situated among the zymogen-rich acini, and they consist of bands of oxyphilic cells with intensively stained nuclei. In the pancreas of the young frog areas of gland tissue destroyed in the process of metamorphosis can still be seen.

Five days after removal of half the pancreas, numerous mitoses can be observed in the young tadpoles, scattered throughout the remaining parenchyma of the organ. In the region of the injury the very active proliferation of the undifferentiated epithelial structures leads to the formation of numerous epithelial bands and tubes (see Fig. 1, c, d), the average diameter of which is 29 μ, while the diameter of the nuclei of the cells forming these tubes is 8 μ. Counts of the mitoses showed that in 10 fields of vision in the zone of regeneration there are on the average 25 dividing cells. Ten days after operation the mitotic activity of the epithelial cells diminishes; at this period the average number of mitoses in 10 fields of vision is 8, and these are present only in the epithelial bands and tubes. No mitoses are observed in the functionally restored area of the parenchyma.

Thus 10 days after removal of half the pancreas from young tadpoles not yet starting on metamorphosis, areas of new tissue are formed in the region of injury, consisting of newly developing secretory divisions and zones of continuing proliferation of the epithelial bands and tubes (see Fig. 1, e). The zone of regeneration in these conditions comprises about $^1/_3$ the total area of the glandular parenchyma of the organ.

After removal of one quarter of the pancreas the proliferation of the epithelial cells in the residual organ and in the region of injury is less intensive than after removal of half the pancreas. Consequently, fewer new epithelial structures are formed. It must be noted that in both variants of the experiment the connective tissue is relatively weakly represented in the region of injury, although its reaction was intensive immediately after the operation. A few cells of the fibroblastic series are present in the region of the defect.

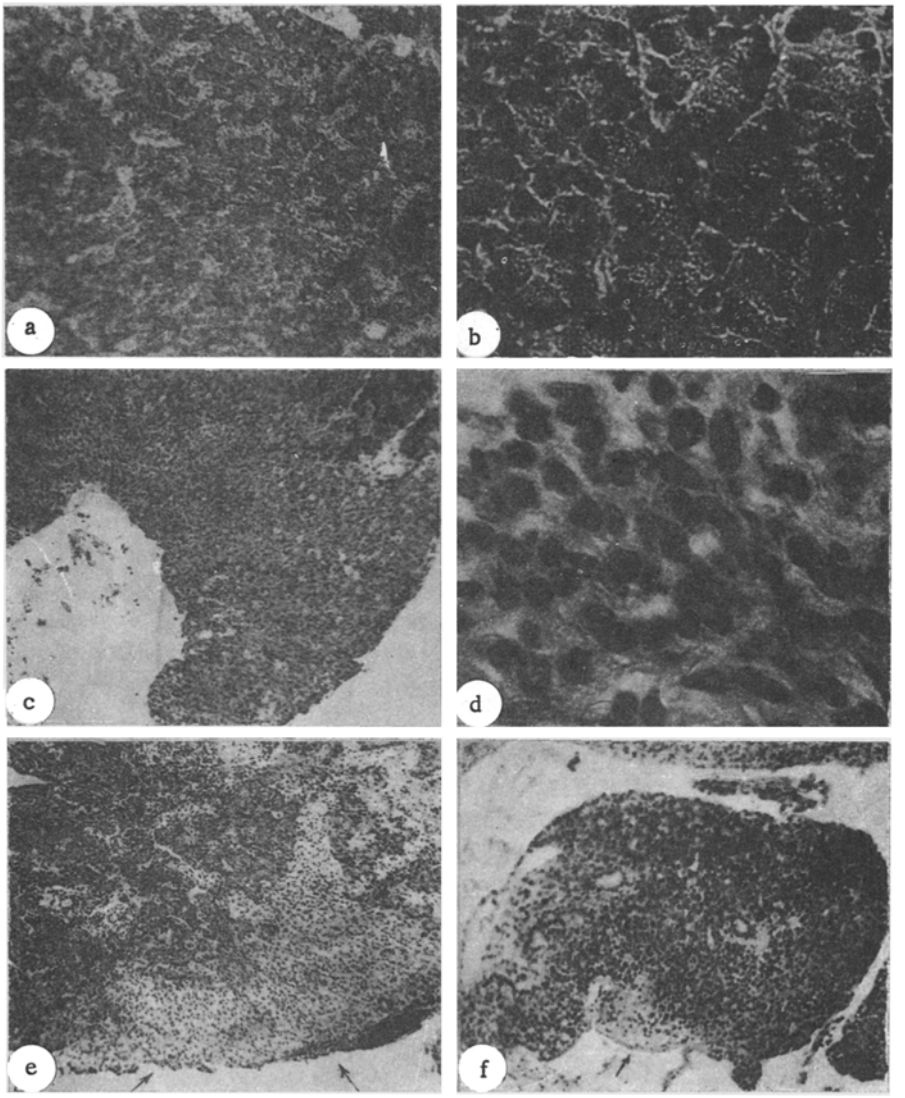

Fig. 1. Changes in the pancreas in response to its injury in tadpoles in the process of metamorphosis. a) Pancreas of an intact tadpole before metamorphosis. Many undifferentiated epithelial structures among the acinar tissue. b) Pancreas of a young frog after metamorphosis. The acini contain numerous zymogen granules. Magnification 480 ×. c) Pancreas of a tadpole before metamorphosis 5 days after removal of half the organ. Many epithelial tubes and bands of proliferating cells can be seen in the zone of regeneration. Magnification 108 ×. d) In the same zone of regeneration mitoses can be seen. Magnification 960 ×. e) An area of the regenerating pancreas (indicated by arrows) in a tadpole before metamorphosis, 10 days after removal of half the organ. Regenerated acini can be seen along with continued proliferation of undifferentiated epithelial structures in the boundary zone. Magnification 108 ×. f) Pancreas of a tadpole at the end of metamorphosis, 10 days after removal of half the organ. In the reconstructed pancreas there is a connective tissue scar (arrow) in the region of the injury. Stained with hematoxylin and eosin. Magnification 108 ×.

The removal of parts of the pancreas, differing in their volume, from tadpoles at a later stage, starting to undergo metamorphosis, does not lead to the formation of new structures in the region of injury; a connective-tissue scar develops at the site of injury (see Fig. 1,f). The mitotic activity in the residual part of the organ is slight (5 days after the operation only 2 or 3 mitoses can be counted on the average in 10 fields of vision).

Hence the regenerative power of the pancreas in amphibians varies according to the stage of development of the larva and also according to the volume of the part of the organ removed. With the onset of metamorphosis no regeneration takes place as a result of the ontogenetic reconstruction of the organ.

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