THE MANNER OF REGENERATION OF THE PANCREAS IN AMPHIBIA

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L. D. Liozner A. G. Babaeva, and V. F. Sidorova

Laboratory of Growth and Development (Head—Professor L. D. Liozner),
Institute of Experimental Biology (Director—Professor I. N. Maiskii)
Academy of Medical Sciences of the USSR, Moscow
(Presented by Active Member of the Academy of Medical Sciences
of the USSR, N. N. Zhukov-Verezhnikov)
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The regeneration of the mammalian pancreas has been studied by several authors [2], but the manner in which the regeneration takes place has received little attention. Only within recent years has it proved possible to demonstrate, by means of experiments on guinea pigs and monkeys, that regeneration of the pancreas takes place as a result of regenerative hypertrophy, i. e., growth of the remains of the organ without restoration at the site of removal. Because of this discovery concerning the mammalian pancreas, the nature of pancreatic regeneration in lower vertebrates, particularly in Amphibia, has acquired a new importance and is of great interest. The authors of the only two papers dealing with the regeneration of amphibian pancreas [1, 14] both agree that the pancreas possesses the ability to regenerate itself by epimorphosis, i. e., by an outgrowth of tissue from the wounded surface, and this same conclusion is arrived at despite the considerable interval of time between the publication of the two articles. In our opinion, these findings must be reconsidered, for neither of these authors considered the possibility that pancreatic regeneration in Amphibia is accompanied by regenerative hypertrophy because of this both researches were at fault. The aim of our own research was to study the regeneration of the pancreas in tadpoles and adults of several amphibian species following resection of one third to three-quarters of the organ.

EXPERIMENTAL

Different amounts of pancreatic tissue were removed from tadpoles of 3 species — Rana temporaria, Rana ridibunda, and Pelobates fuscus — in developmental stages 1-3a (according to Blacker's scheme). The site of removal varied in the different experimental series. Tadpoles were examined 3-11 days after the operation. We removed one third of the pancreas from fire-bellied toads (Bombina bombina), trying to prevent any damage of the pancreatic duct. The toads were examined 8-60 days after the operation. The pancreatic glands of tadpoles and of adult Bombina were weighed on a torsion balance, fixed in Carnoy's fluid, embeded in paraffin wax, and sectioned at 8μ thick. The number of mitoses in 200 field (ocular 10 ×, objective — immersion, MBI-3 microscope) were counted from these sections. Using an Abbe drawing apparatus we sketched the cell outlines (magnification 760 ×) and the nucleus outlines (3,100 ×) onto paper, cut out the figures produced in this way, and weighed them on torsion balances in order to determine the areas of the cells and nuclei.

RESULTS

The weight and dimensions of the pancreas undergo a marked reduction at the time when the tadpole metamorphoses into a young frog [1]. For this reason, some of the experimental and control tadpoles were examined in early stages of development and others in later stages. The changes associated with the regenerative process were similar for all species of Amphibia, the only variation which occurred was in the speed of the process and its degree of completion.

In no single case was it possible to observe any covering over of the defect in the gland tissue caused by the operation. The defect occasioned by the operation persisted to the end of the experiment irrespective of the stage

of development at which the latter had been performed. Thus, there was no regeneration of the part from the pancreas. This finding was confirmed on hundreds of tadpoles, the result in every case being the same. Despite the absence of any true regeneration of the gland tissue removed by operation, there was a restoration of the weight of the gland.

Eight series of experiments were conducted with R. temporaria tadpoles (using 15-39 tadpoles per experimental series and the same number for the control series). In all 340 tadpoles were used. When one-third of the pancreas was resected and the reexamination occurred after 5-11 days, the weight of the gland in the experimental animals relative to that in the controls varied within limits of 76-91%. When one-half of the gland had been resected the relationship was 68-75%. Thus, if we take complete restoration as 100, the amount of restoration occurring after resection of one third of the gland is approximately 50 and after one-half about 40. It is obvious, therefore, that the restoration is not complete; at least, not in the period of time covered by our own observations.

Eight series of experiments were carried out on R. ridibunda tadpoles. There were killed 1-9 days after operating. A total of 310 tadpoles were used. After resection of one-half of the pancreas, the weight of the gland in the experimental series was 61 to 84% that in the controls at the time of observation. After resection of three-quarters of the gland the relative weights varied between 41 and 58% at the end of 5-8 days. In this case, the percentage restoration was no greater than 45.

We conducted 6 series of experiments on P. fuscus, using a total of 110 tadpoles (6-10 for each series). After resection of one-half of the gland, the weight of the pancreas in the experimental animals was 93-120% that of the weight in the controls at the end of 5-10 days. The regenerative capacity of the pancreas in this species therefore appears to be greater than that of the other two species of Amphibia restoration of the weight of the gland was complete or almost complete. In an experimental series involving tadpoles examined 3 days after operation, the weight of the pancreas in the experimental animals equalled 75% of the weight of the controls.

Histological sections of the gland prepared one day after resection showed changes characteristic of most damaged organs. Blood clots forming at the site of the operation persisted for several days. The region of the gland adjacent to the actual wound exhibited leukocytic infiltration. Furthermore, the typical structure of the gland in this region was destroyed. Nevertheless, all these changes were restricted to tissues lying close to the wound. We were never able to detect any zone of newly formed tissue, an observation which was in accord with the preservation of the defect. All that could be seen was a small amount of connective tissue growth and slight growth of the ducts over the wound surface in the last stages of the experiment. Thus, regeneration of the pancreas in tadpoles takes place by means of regenerative hypertrophy.

We did not discover any increase in the number of mitoses in the regenerating pancreas of R. ridibunda, although determination of mitotic activity was carried out for 3-9 days after the operation, i. e., the period when an increase might be expected. The mitotic activity varied within limits of 0.31-0.88% and activity was the same over the whole gland. In the later stages of observation the number of mitoses fell sharply both in the experimental and control series after the operation.

In P. fuscus tadpoles the mitotic activity of cells near the wound surface (3-4 fields of vision away from the wound) reached 1.1 % 3 days after the operation; throughout the whole of the parenchyma the mitotic activity was 1.15%. No mitoses were observed in the control series.

On the fifth day of the experiment the mitotic activity among the controls was 0.03% and in the experimental series 0.05%. After the fifth day, no difference was observable between the mitotic activity of the controls and of the experimental series, and in both the activity was very low indeed.

Tadpoles of R. ridibunda exhibited a definite hypertrophy of both cells and nuclei in the reganerating pancreas. Three days after the operation the area of the cells had increased by 24% compared with the area in the controls and the area of their nuclei by 16% (equivalent to $P = 0.01$ and $P = 0.015$). Nine days after the operation the area of the experimental cells was 33% greater than that of the controls ($P = 0.001$) but hypertrophy of the nucleus was not observed.

The obvious conclusion to be drawn from these results is that regeneration of the pancreas in tadpoles takes place by the proliferation of cells or by their hypertrophy and that differences occur between individual species. It should be noted that the regenerative ability of some species is not high and the gland is not completely restored.

In adult Bombina, as in tadpoles of the same species, there was no tissue regeneration at the wound surface and the site of the excision remained clearly visible throughout the experiment. At the same time, however, the weight of the gland was restored. Eight days after the operation, the weight of the pancreas was 73% of that in the control animals which had suffered on operation, i. e., restoration of the weight had only just begun. After 14-60 days the weight of the pancreas equalled 82-91% that of the controls. Hence, by this time restoration of the weight was almost complete. Mitotic activity in the pancreas of Bombina sharply increased after excision of some of its tissue, but this increase did not occur until 14 days after the operation. At this time mitotic activity was 1.68% in the experimental animals compared with almost zero in the controls. Thirty and 60 days after the operation the mitotic activity of the secretory epithelium was even greater, at 0.85% (P = 0.004) and 0.38% (P = 0.02), compared with 0.08% in the controls. Hypertrophy of the cells and nuclei was not observed either at 14 days or 30 days after the operation. It would appear that, in contrast to the situation in tadpoles, regeneration of the pancreas in the adult Bombina occurs mainly as a result of cell proliferation.

These results suggest that in the larvae and adult Salientia studied by the author, regeneration of the pancreas takes place by means of regenerative hypertrophy. This is definitely implied by the presence of such decisive features as the retention of the wound defect, the distribution of the regenerative changes (mitoses, hypertrophy of the cells) throughout the whole of the parenchyma of the gland, and the complete or partial restoration of the pancreatic weight.

In view of these findings, the opinion that regeneration of the pancreas in the Salientia takes place by means of epimorphosis [1, 4] appears inconclusive. The authors concerned did not establish the size of the "regenerated material" in their experiments. This leads us to think that no "regenerated material" was actually formed and the authors were merely dealing with normal reaction changes or with the formation of a very small amount of new material in the vicinity of the wound. It is of some interest that in the experiments of A. A. Voitkevich and L. N. Kuleshova [1] even the weight of the gland was not restored after operation and hence it is difficult to speak of its actual regeneration. The large number of animals used in our experiments and the fact that they belonged to different species has enabled us to present a clear picture of pancreatic regeneration in the Salientia and allows us to assert quite definitely that it takes place by the method decribed above. In passing, it may be said that experiments on mammals have not yielded any definite information as to whether regeneration of the pancreas following resection takes place, to any significant degree, by means of epimorphosis. It would seem therefore, that regeneration of the pancreas takes place similarly in both the lower and higher vertebrates.

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