The relationship between the anterior pituitary gland and the pancreas in tail regeneration of the adult newt

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SUMMARY

Histological examination of the amputated tails of 17 hypophysectomized newts revealed abnormal and extremely retarded regenerates; four of them exhibited total inhibition of regeneration. Thus, under the conditions of hypophysectomy, normal tail regeneration does not ensue in the adult Diemictylus viridescens.

Also, hypophysectomy adversely affects the normal histology of both the endocrine and exocrine parts of the pancreas, as observed by the atrophy of the gland in all hypophysectomized cases. Presumably, the normal function of the gland was altered. This relationship between hypophysectomy and the atrophic pancreas suggests a possible involvement of the pancreas in tail and limb regeneration in the adult newt.

INTRODUCTION

Previous studies have shown that hormones, namely, adrenocorticotropic hormone (ACTH)-stimulated adrenocorticosteroids, growth hormone, thyroxine and prolactin are of prime importance in the regeneration of limb in adult Diemictylus viridescens (see reviews by Schmidt (1968), Schotte (1961) and Thornton (1968)). In addition, Vethamany (1970) has recently reported the involvement of insulin in adult limb and tail regeneration.

Based on their findings Schotte and his associates (1961) postulated that the pituitary-adrenal synergism is a major controlling factor especially on the early stages of limb regeneration, namely, wound healing and dedifferentiation. In 1963, Wilkerson questioned the above hypothesis, since he observed that bovine somatotropin when injected into hypophysectomized animals supported regeneration of the limb.

In addition, several workers have shown that limb regeneration is thyroxine-dependent; if the adult newt is thyroidectomized regeneration is retarded (Walter, 1910; Richardson, 1940; Schotte & Washburn, 1954). Connelly,

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Tassava & Thornton (1968) and Tassava (1969) observed that prolactin alone increased survival and supported limb regeneration in hypophysectomized newts, but less effectively than the combination of prolactin and thyroxine. On the basis of these findings they proposed that a prolactin-thyroxine synergistic control operates in limb regeneration.

Although considerable interest has been directed toward the study of the hormonal control of limb regeneration, little attention has been given to the role of hormones in tail regeneration. Licht & Howe (1969) and Turner & Tipton (1971) showed a hormonal dependence of tail regeneration in the lizard, *Anolis carolinensis*. However, there is a lack of information with regard to hormones in urodele tail regeneration.

Vethamany (1970) showed that insulin insufficiency (due to partial pancreatectomy or alloxan treatment) resulted in an interference with normal limb and tail regeneration and thus demonstrated an insulin-involvement in regeneration. In addition, Vethamany showed that regenerating newt tail blastemata require insulin for growth and differentiation *in vitro* and that a combination of hormones, namely, insulin, growth hormone, hydrocortisone and thyroxine gives optimum results. These findings show that the collective influence of these hormones is greater than the effect of any of them individually. The question now arises whether or not the influence of pituitary gland on regeneration is mediated, at least in part, through the pancreas. The current experiments were designed to determine the effects of hypophysectomy on tail regeneration and the pancreas in the adult newt.

**MATERIALS AND METHODS**

Adult newts, obtained from Central Massachusetts, were kept in dechlorinated tap water at 20 ± 1 °C and fed ground beef twice weekly. All animals were allowed a 2-week period to acclimatize to laboratory conditions prior to experimentation. Medium sized *Diemictylus viridescens* of both sexes, weighing between 1-5 and 2 g, were anesthetized in M.S. 222 (1:1000, Sandoz) and hypophysectomized according to the method of Liversage (1967). The operated animals were kept moist in separate dishes, the water was changed daily and feeding was resumed 1 week post-hypophysectomy. During their post-operative recuperation, all animals were kept at 15 °C for 2 days and then returned to a temperature of 20 ± 1 °C for the duration of the experiment. This procedure minimized the mortality which often occurs during the first few days following hypophysectomy.

Forelimb and tail amputations were performed on the twelfth day following hypophysectomy in order to minimize circulating titres of residual hormones. Right forelimbs were amputated through the region of the mid-humerus and tail amputations were performed approximately 1 cm from the distal tip. Tail regenerates from control as well as hypophysectomized animals, 21–24 days
old, were fixed and examined histologically. In addition, successful removal of the pituitary gland was assessed by the microscopic examinations of serial sections of the heads of the hypophysectomized animals. Also the inhibition of forelimb regeneration in hypophysectomized animals served as an additional indicator of the completeness of hypophysectomy.

Tissues were fixed in Bouins fluid, embedded in paraffin and serially sectioned at 8 μm. Heads and regenerates were decalcified in Jenkins' solution prior to embedding and the sections were stained with hematoxylin and counterstained with orange G-eosin. Pancreatic tissue was stained with aldehyde-fuchsin, Ponceau de xylidine-acid fuchsin and counterstained with fast green (Epple, 1967).

**RESULTS**

Changes in the external appearance of the adult newt following hypophysectomy are well known. The experimental animals become sluggish, eat poorly and die prematurely. Their normal, smooth, moist, olive-green coloured skin takes on a dark coarse granular appearance and molting is inhibited.

The effects of hypophysectomy on tail regeneration and on the structure of the pancreas are summarized in Table 1. Forty-five adult newts were used in this investigation; 25 served as experimental and 20 as controls. Two hypophysectomized animals retained their normal skin conditions (apparently incomplete hypophysectomy) and therefore these two cases were discarded. Six others died 20 days following hypophysectomy. Five of the remaining 17 hypophysectomized newts were fixed for microscopic examination 21 days post-amputation (31 days post-hypophysectomy); six were fixed 24 days post-amputation (34 days post-hypophysectomy); and the remaining six were fixed 27 days post-amputation (37 days post-hypophysectomy).

*Effects of hypophysectomy on tail regeneration*

By 21 days post-amputation, the control regenerates measured 1·9–2·2 mm along the central axis and approximately 2·5 mm wide at their proximal end. Mitoses were observed among the blastema cells; regeneration of the spinal cord and well-defined dorsal and ventral fins were seen at this time. These regenerates also exhibited differentiation of the vertebral cartilages and segmentation of the centre along the cartilage rod (Fig. 1).

Microscopic examination of the seventeen experimental tails revealed considerable retardation or abortive regeneration in thirteen and complete absence of regeneration in four. These tail regenerates were very small (0·6 mm in length) when compared to the size of the controls. Tails that failed to regenerate developed a thick, epidermal covering composed of eight to ten cell layers and the spinal cord failed to extend into the subapical area (Fig. 2). Mitoses were not observed in the epidermis, the spinal cord, or among the blastema cells, and the subapical area itself was greatly reduced. Sparse population of cells in the
Table 1. Effects of hypophysectomy on pancreas and tail regeneration in adult newt

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Figures 1, 2. The arrows indicate the level of amputation and the area to the right of the arrow is the regenerate.

Fig. 1. A mid-sagittal section through a 21-day tail regenerate of a control adult newt. c, Cartilage; s, spinal cord; f, fins. Magnification about ×80.

Fig. 2. A mid-sagittal section through a 21-day tail regenerate of a hypophysectomized adult newt. Note the total lack of regeneration of spinal cord, cartilage differentiation and growth of the blastema, as compared to the control above. Magnification about ×100.
blastema was repeatedly observed although dedifferentiation of bone and sarcoplasmic budding were apparent in the regeneration area.

The procartilage cells were closely packed together and interstitial cartilage matrix was noticeably reduced. Growth of the dorsal and ventral tail fins was also markedly retarded in hypophysectomized animals, and sinuses were evident in the fin region. In some cases, the lack of growth of the fins led to protrusion of the central axis of the regenerate in the form of a spike.

From six to ten remnant anterior pituitary cells were seen in the floor of the cranium in four of the seventeen hypophysectomized animals, nevertheless, tail regeneration was inhibited in all cases. Normal forelimb regeneration failed in the hypophysectomized cases.

Effects of hypophysectomy on the pancreas

The state of the pancreas in fifteen normal and in eight hypophysectomized newts was examined histologically (see Table 1). Microscopic examination of the intact pancreas in the control adult newt revealed an exocrine part, consisting of acini and their related duct system, and also an endocrine component, consisting of the islets of Langerhans. The acinar cells were pyramidal in shape, adhering to a basement membrane and were arranged around a central lumen. The luminal zone of the cells, which varies with the physiological state of the newt, was closely packed with zymogen granules. These granules stain deep purple with aldehyde fuchsin. The acinar cells have spherical nuclei, with conspicuous nucleoli, and darkly stained chromatin material. The ducts of the pancreas are lined with columnar epithelium. The islet cytology of urodele amphibians has been described and reviewed by Epple (1966). Although the gland is predominantly composed of acinar tissue, islets can be readily observed as compact irregular masses of cells; these are distributed randomly among the acinar tissue (Fig. 3). A rich vascular supply is always associated with the islets. The beta cells have large, oval nuclei and granular cytoplasm (Fig. 4). These cells are centrally located in the islets and produce insulin. Glucagon-producing alpha cells are peripherally situated in the islets. Besides their location in the islets, the beta and the alpha cells can be easily distinguished by their blue and orange granules, respectively (using aldehyde fuchsin, Ponceau de xylidine–acid fuchsin and light green staining, following the method of Epple (1967)).

Upon gross examination of the pancreas in all hypophysectomized newts, it became obvious that the gland was considerably smaller and thinner than a normal pancreas. The normal structure of the exocrine and the endocrine components of the gland were greatly altered in the experimental animals. Histological observations revealed extensive atrophy of the entire gland, for example, the acinar cells exhibited considerable shrinking and the cell membranes were poorly defined (Fig. 5). The nuclei were irregular in shape, aggregated in clumps and appeared pycnotic, and lacked well-defined nucleoli and chromatin material,
Adenohypophysis and pancreas in regeneration

Figures 3, 4. The islets lie between arrows.

Fig. 3. A transverse section through the normal adult newt pancreas. An islet (i) is seen among the exocrine acinar tissue. Magnification about ×145.

Fig. 4. Normal adult newt islet; Fig. 3 magnified. The beta cells, b, are seen centrally located. Their nuclei are distinct with prominent chromatin material. The granular cytoplasm of the beta cells is indicative of insulin synthesis. Alpha cells, a, are peripherally located. Magnification about ×590.

and a few cases exhibited cell breakdown. The cytoplasm of the acinar cells was scanty, vacuolated, conspicuously agranular, and devoid of zymogen granules.

In addition to the above observations on the exocrine components, the islets also were markedly affected by hypophysectomy. The number of islets per pancreas in the hypophysectomized animals was two or three, whereas the pancreas in the control animals had an average of seven to ten. In the experi-
Figures 5, 6. The islets lie between arrows.

Fig. 5. A transverse section through the pancreas of an adult hypophysectomized newt (21 days post-hypophysectomy). Note the extensive atrophy in the acinar tissue as well as in the islets. Magnification about x 145.

Fig. 6. Atrophied islet of a hypophysectomized adult newt (21 days post-hypophysectomy). (Fig. 5 magnified.) Nuclei appear pycnotic; vacuolative damage can be seen in several cells. The entire islet shows degeneration. Magnification about x 590.
mental animals, the islets showed considerable shrinking, extensive atrophy, pycnotic nuclei, intra- and intercellular vacuolization and degranulation of the cells (Fig. 6).

**DISCUSSION**

In the current investigation, extirpation of the adenohypophysis resulted in either complete failure of tail regeneration or greatly retarded tail regenerates. With the information presently available, it is difficult to account for this wide individual variation in the rates of tail regeneration in hypophysectomized animals. Similar variations in the rates of tail regeneration have also been reported in the hypophysectomized lizard, *Anolis carolinensis* (Licht & Howe, 1969). In the current study, the primary effect of hypophysectomy on tail regeneration was manifested in a scarcity of cells in the blastema, which ultimately resulted in greatly reduced cartilage formation and fin growth. In some cases, the spinal cord failed to extend into the blastema region although scanty blastema cell accumulation was discernible in the regenerating area. Similar inhibition of tail elongation has been reported by Turner & Tipton (1971) in hypophysectomized lizards. They reported, however, that thyroxine replacement insured the normal development and growth of the ependyma into the blastema.

In order to gain a better understanding of how hypophysectomy affects tail regeneration in adult *Diemictylus*, it is worthwhile to consider the widespread metabolic changes that occur in the whole animal as a result of hypophysectomy. In addition to the numerous external manifestations, namely darkened skin, extreme sluggishness, loss of appetite and weight, hypophysectomy also brings about the following derangements in the metabolism of vertebrates: (a) a lack of ACTH which causes adrenal insufficiency; (b) a lack of TSH which results in hypothyroidism; (c) absence of FSH, LH and prolactin results in reproductive failure in both sexes; and (d) growth hormone deficiency which causes metabolic derangements in protein, fat and carbohydrate syntheses (see Gorbman, 1958; Tepperman, 1965). Furthermore, hypophysectomy causes reductions in: the blood sugar level in dogs, the volume per cent of red blood corpuscles, the weight of the pancreas, and the amount of insulin extractable from the pancreas (Campbell, Chaikoff, Wrenchall & Zemel, 1959).

In our hypophysectomized newts, the pancreas exhibited extensive atrophy of the entire gland, which strongly suggests an alteration in its function. Although similar observations have been made in rats (Haist, 1959) and also in the salamander *Taricha torosa* (Wurster & Miller, 1959), the function of the pancreas has not been correlated with either limb or tail regeneration in adult urodeles, nor has the atrophied pancreas been related to the adverse effects of hypophysectomy on regeneration. The relationship, therefore, between hypophysectomy and the atrophic pancreas on one hand and retardation of limb and tail regeneration on the other, suggests an involvement of the pancreas (directly and/or indirectly) in regeneration.
Although it might be possible to alleviate the effects of insulin deficiency by administering insulin to hypophysectomized animals, replacement therapy was not attempted for the following reasons: hypophysectomized animals (salamander, *Taricha torosa*) frequently develop fasting hypoglycemia and are unusually sensitive to the hypoglycemic effects of injected insulin (Wurster & Miller, 1959). Secondly, the hypoglycemia that develops in hypophysectomized animals is probably due to a combination of diminished glucose output (lack of hyperglycemic agents or hormones) and fasting. Thus, insulin replacement therapy, without an accompanying hyperglycemic factor, only aggravates the hypoglycemic condition and results in even earlier death of the hypophysectomized animal.

Growth hormone, prolactin, TSH-stimulated thyroxine and ACTH-stimulated corticosteroids, are all known to stimulate the production of insulin in mammals and other vertebrates. These hormones, under prolonged stimulatory action on the islets, can induce the exhaustion of the beta cells causing diabetes. Accordingly, these hormones are referred to as diabetogenic hormones (Gorbman, 1958; Haist, 1959; Lazarus & Volk, 1962; Tepperman, 1965). These hormones, when injected into the hypophysectomized salamander (*D. viridescens*), individually or in combinations, restore the normal health of these animals and promote limb regeneration (Richardson, 1945; Schotté, 1961; Wilkerson, 1963; Connelly *et al.* 1968; Liversage & Scadding, 1969; Tassava, 1969). Since each of these hormones directly or indirectly stimulates insulin secretion, the question of an insulin involvement in the regeneration of appendages can justifiably be raised.

In a related study, Vethamany (1970) showed that experimental diabetes due to pancreatectomy or alloxan treatment resulted in a marked interference in limb and tail regeneration in adult newts; animals with an atrophied pancreas always produced stunted and abnormal limb and tail regenerates. However, in the event that the pancreas regenerated or recuperated, normal limb and tail regeneration ensued. Corroborating these findings are the in vitro experiments of Vethamany (1970) which show that the presence of insulin is essential in promoting growth and differentiation of the tail blastemata in culture. This in vitro work further demonstrated that the collective influence of insulin, growth hormone, hydrocortisone and thyroxine is greater than the effect of any of them individually.

The present work shows that following hypophysectomy tail regeneration does not ensue and the exocrine and endocrine parts of the pancreas undergo general atrophy. These findings together with those of Vethamany (1970), suggest that the effects of anterior pituitary hormones on regeneration are mediated at least in part, through the pancreas specifically involving insulin. Furthermore, it becomes clear that the endocrine control in tail or limb regeneration cannot be attributed to any single hormone, rather it must involve the interactions and interdependence of several hormones.
We wish to express our appreciation to Dr M. Globus for his interest and valuable discussions throughout this work and his assistance in the preparation of this manuscript. This paper was prepared from a portion of Ph.D. thesis submitted (by S. V. G.) to the Department of Zoology, School of Graduate Studies, University of Toronto. This investigation was supported by grants from the Province of Ontario (to S. V. G.) and National Research Council of Canada (to R. A. L).

REFERENCES


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