Regeneration in Hypophysectomized Anuran Tadpoles.* (25630)

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The concept that normal regeneration in Amphibia depends in part upon the pituitary gland derives largely from experiments on the urodele limb(1-6). Lens regeneration, however, takes place in absence of the pituitary in *Triturus v. viridescens*(7-9) and retina regeneration(8) as well as regeneration of iris (9) occur in hypophysectomized newts. To account for the contrasting effects of hypophysectomy on limb regeneration and retina-iris-lens regeneration it may be useful to consider the effects of hypophysectomy on regeneration of other parts. It is well known that the tail-tip of the anuran tadpole regenerates, and that, as in the limb, the stimulus is amputation. There is much more similarity in mode of regeneration between limb and tail than between limb and lens. But, in contrast to the limb response in Triturus, the anuran tail-tip regenerates in hypophysectomized animals. This information was obtained incidentally in experiments on regeneration of denervated lateral-line organs(10) and, more recently, in other somewhat similar experiments to be reported later. Comparison of normal and hypophysectomized *Rana pipiens* tadpoles from these experiments with respect to tail-tip regeneration only is presented here.

Materials and methods. The study is based on 113 tail-tip regenerations from repeated amputation in 29 *Rana pipiens* tadpoles. Sixteen of these animals were normal controls for 13 which had been hypophysectomized in the late tail-bud stage of the embryo by the standard procedure of Smith(11). Criteria used for successful hypophysectomy were the characteristic alteration in pigmentation and persistence of the larval state. Ages of both hypophysectomized and normal animals ranged from 3½ to 5 months at start of the series of amputations. Additional data were obtained from tail-tip amputation on 4 tadpoles which had been hypophysectomized one year and 9 months previously. Tadpoles were kept individually in finger bowls and fed maximally on desiccated liver and spinach. Tricaine methanesulfonate (MS222) anesthetization was used during each amputation and while the animals were in the microchamber for observation of the regenerating system. Tail-tips were measured by use of an ocular grid in the compound microscope. Serial sections of 12 regenerated tail-tips from control animals and 22 from hypophysectomized ones were prepared by standard Zenker-haematoxylin-tetrabromfluorescic acid technics.

Results. Tail-tips regenerated in all cases regardless of presence or absence of the epithelial hypophysis. The data from the first of the series of amputations are summarized in Table I. Not only did all tail-tips regenerate, but there is no significant difference in amount of regenerated tail-tip per unit time related to presence or absence of pituitary influence. The calculated index (mm/days) is not intended to imply a uniform *rate* of regeneration, but is a correction for the possibly misleading figure giving percentage of tail-tip regeneration by recognition of the various times allowed for regeneration. The timing was dictated, of course, by factors related to the primary purpose of the experiment–study of lateral-line organ regeneration.

Data from the second through the seventh set of amputations are summarized in Table II. There is again no essential difference between experimentals and controls. Furthermore, there seems to be no significant diminution in ability to regenerate tail-tip with increased age in the hypophysectomized animals or up to time of resorption of the tail at metamorphosis in the controls. Since constant temperature was not maintained during the course of the experiment which ran from time of hypophysectomy in October to the following September, effects of different seasonal temperatures are undoubtedly reflected in the data. However, at any given time during the experiment all animals were under

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TABLE I. Data from First Set of Regenerations.

Tail-tip amputated (mm)*		Time of regeneration (days)	Tail-tip regenerated (mm)*		% of Tail-tip regenerated		Index (mm/days)	
H†	C†		H	C	H	C	H	C
6.		51	5.		83.3		.10	
8.	6.5	47	5.5	7.	68.8	107.	.12	.15
	3.	47		5.5		183.		.12
	8.	43		4.		50.		.09
	8.	38		4.5		56.3		.12
4.	9.	36	4.	7.	100.0	77.8	.11	.19
6.	7.	36	5.	5.	83.3	71.4	.14	.14
4.	10.	36	4.	5.	100.0	50.0	.11	"
7.	8.	35	3.5	5.	50.0	62.5	.10	"
5.5	7.5	35	4.	5.	72.7	66.7	.11	"
7.	5.5	35	5.	5.	71.4	91.0	.14	"
5.	11.	35	4.	4.	80.0	36.4	.11	.11
	7.	35		5.		71.4		.14
	7.	35		4.		57.1		.11
	5.5	35		4.		72.7		.11
7.		34	5.		71.4		.15	
5.		34	3.		60.0		.09	
6.		34	4.		66.7		.12	
5.		34	4.		80.0		.12	
	5.	33		4.		80.0		.12
	8.	24		2.5		31.3		.10
					Avg index (mm/days)		.12	.13

* mm to nearest 0.5. † H = hypophysectomized tadpole; C = control.

similar conditions and may be reasonably compared.

The 4 animals hypophysectomized one year and 9 months prior to amputation also regenerated tail-tips. Approximately one-third of the amount removed was replaced in 20 days. One of the animals died before measurement, but the 3 others regenerated 5 mm, 4 mm, and 4 mm, respectively giving an average index (mm/days) of .22. These tail-tips regenerated in June-July under temperature conditions similar to those for series 5 listed in Table II. Histologically no essential differences could be determined in the characteristics of the regenerates between control and hypophysectomized animals.

TABLE II. Summary of Data from 2nd through 7th Set of Regenerations.

Amputation No.								
2		3		4		5	6	7
mm regenerated/days								
H*	C*	H	C	H	C	H	H	H
.15	.20	.16	.22	.23	.31	.17	.23	
.17	.23	.21	.14	.23	.16			
.12	.17	.14	.18	.13	.16			
.15	.25							
.15	.21	.18	.19	.16	.19	.22	.12	
.17	.27	.14	.30	.10	.22			
.15	.14		.15		.19			
.14	.24	.21	.18	.23	.09	.35	.18	.13
.19	.19	.15	.24	.16	.31	.23	.23	.11
.18	.14	.16	.15	.16	.09	.25	.18	
.20	.25	.16	.24	.16	.13	.23	.18	.09
.15	.15	.20	.14	.26		.35	.20	.16
Avg index (mm/days)								
.16	.20	.17	.19	.18	.19	.26	.19	.12

* H = hypophysectomized tadpole; C = control.

Discussion. It appears obvious from the foregoing data that removal of the epithelial hypophysis in the embryo does not prevent regeneration of the tail-tip in the *Rana pipiens* tadpole. Twenty-nine cases from earlier experiments on regeneration of lateral-line organs in regenerating tail-tips of hypophysectomized tadpoles may be cited as additional evidence(10). Of these, 14 cases were *Rana pipiens*, 11 *R. sylvatica* and 4 *R. palustris*. The tadpole tail, therefore, differs strikingly from the Triturus limb in its independence of pituitary influence in regeneration.

Whether the cells of the blastema in urodele limb regeneration are dedifferentiated specialized cell types(4,5) or mesenchymatous fibroblasts(12) there is an aggregation of indifferent cells from which the new limb

develops epigenetically. Holtzer(13) has presented evidence that the urodele tail also forms a blastema and that tail and limb regeneration may be more similar than formerly thought. If this should be true for the anuran tail, the well-supported hypothesis(6) that it is amputational stress which brings into operation a synergistic pituitary-adrenal action necessary for wound healing in preparation for blastema formation in the limb would not hold for the anuran tadpole.

Schotté and Murphy(7) suggest that the essential difference between limb and lens regeneration with respect to their differing responses to hypophysectomy is that, in contrast to limb amputation, there is no trauma to the iris in lensectomy. Since no wound healing in the dorsal iris precedes lens regeneration, presence of the pituitary is unnecessary. However, Stone(9) excised a dorsal segment of the iris concomitantly with lensectomy and obtained regeneration of lens from the wounded iris. Rate of regeneration was slower in hypophysectomized newts than in the controls but regeneration was completed.

Stone and Steinitz(8) attribute the fundamental difference between limb and lens regeneration to the different modes of origin of the cells forming the source of the regenerate. It is clear that the new lens has its origin in cells which result from the " 'dedifferentiation' of highly organized cells to a level at which they can be subjected to the inducing influence of factors directing them to a different aim"(8,p.493). Visual retina and iris also regenerate from retina pigment cells by a similar type of "dedifferentiation"(9). There is no true blastema formation.

It may be that the difference in response to pituitary absence in Triturus limb regeneration and anuran tail regeneration is genetic, or it may depend upon differences in the processes of wound-healing and blastema formation between limb and tail. In addition, it may be that different mechanisms are operating in adult and immature forms. A series of experiments to test the latter is in preparation.

Summary. 1. A comparison was made between regenerating tail-tips in normal and hypophysectomized *Rana pipiens* tadpoles. 2. No significant differences were noted in amount of regeneration/unit time or in the histology of regenerates. 3. Age of the hypophysectomized animal had no discernible effect on tail-tip regeneration. 4. Independence of pituitary influence in regeneration exhibited by the anuran tail and Triturus retina-iris-lens system is discussed in relation to the dependence of limb regeneration in Triturus.

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