μEq/100 g/hr during the control period (n=8), 1359 ± 579 during the first 30 minutes and 1104 ± 329 during the second 30 minutes after theophylline. Theophylline did not, therefore, appear to stimulate the extrusion of chloride by the gill under circumstances that eliminated exchange diffusion of chloride.

MORPHOGENETIC CONTROL DURING TAIL REGENERATION IN *Plethodon cinereus*: THE ROLE OF WHOLE SKIN

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Stimulating tissues to grow or proliferate may be accomplished by a wide variety of traumatic insults, but it requires a delicate system of regulatory influences and interactions to restore or regenerate the complex internal architecture of a limb or a tail. One approach to elucidating the morphogenetic mechanism in urodele limb regeneration has been to alter the morphogenetic interactions through specific manipulations of the tissues contributing to, or possibly influencing the expression of, the epimorphic process.

Several previous reports have indicated that various rotations of limb skin around the limb stump effect morphogenesis resulting in abnormal regenerates (Settles, Anat. Rec. 166:375, 1970; Lheureux, Ann. Embryol. Morphog. 5:165-178, 1972 and Wilh. Roux Arch. 176:285-327, 1975; Carlson, Devel. Biol. 39:263-285, 1974). In a more detailed analysis, Carlson (Devel. Biol. 47:269-291, 1975) has shown that the sites of active morphogenetic control in the limb system reside in the skeletal muscle and dermal component of the skin. Disharmonies in the relative axial positioning of these tissues at the stump surface frequently lead to the production of hypermorphic or complete supernumerary limbs.

These results have prompted the formulation of a widely publicized model for pattern regulation in epimorphic fields (French et al., Sci. 193:969-981, 1976; Bryant, et al., Sci. Am. 237:67-77, Bryant and Iten, Devel. Biol. 50:212-234, 1976). The model rests upon data derived from research on limb systems. Since the urodele tail is also an epimorphic field, the following experiments were performed to test the validity of this generalized model. The organisms of choice in this protocol was the adult red-backed salamander, *Plethodon cinereus*.

Method

All animals were collected from the woods adjacent to the MDIBL. Preceding any operative procedure, animals were anesthetized by total immersion in 1-2% MS 222 (ethyl-m-aminobenzoate, Eastman) and then transferred to amphibian saline-moistened paper towelling on the stage of a dissecting microscope. Two series of skin-grafting experiments were performed as follows.

In the first series, a midventral incision through the skin was made traversing 6-8 segments midway between cloaca and tail tip. Circumferential incisions were then made at each end of the longitudinal incision such that a cuff of whole skin could be removed from the tail and cleaned of any adhering tissue fragments. The skin was then either replaced in its original orientation (control) or rotated 180° about the long axis of the tail and replaced such that dorsal skin was now ventral (experimental). Grafted cuffs were sutured closed with 7-0 suture silks (Ethicon) and the animals returned to a shallow dish of Holtfreter's saline where recovery occurred quite rapidly.

In the second series, a cuff of tail skin was removed and cleaned as described above. However, the skin was subsequently rotated 90° while flat and then replaced on the denuded graft bed. The red dorsal stripe at the graft locus now made a complete circuit around the tail. Closure and recovery were as in series one.

Following bath grafting procedures, a ten-day period was allowed for healing and monitoring of grafting success. All tails were subsequently amputated through the middle of the skin graft and observed superficially for (1) initiation of regeneration and (2) gross morphogenesis of any regenerate. As

the tails were amputated, each distal segment was fixed in Bouin's solution and processed for light microscopy to determine the stability and viability of the skin graft at the time of amputation (10 days post-rotation). The size and general morphology of the skin glands allowed easy distinction between dorsal and ventral skin thereby permitting determination of whether a graft remained in its rotated position.

Animals were "sacrificed" at 24 hours and at subsequent five-day intervals. As tail tissues were harvested from groups of 3-5 animals at the specific stages indicated, the desired caudal segments were fixed in Bouin's solution for histological analysis and the animals returned to the area from which they had been collected. During the progress of this experiment, animals were maintained in containers provided with wet paper towelling and fed live fruit-flies (*Drosophila melanogaster*) which were kindly provided by Dr. John Ringo, Department of Zoology, University of Maine-Orono.

Results

In all cases where the tail did not autotomize, the skin remained as grafted. Furthermore, there were no instances where regeneration was not normally initiated.

Rotation of the skin cuff 180° around the long axis of the tail appeared to have no effect on regenerative mechanisms. This confirms preliminary observations from last year's pilot study (Dinsmore, Bull. MDIBL 16:22, 1976). Events occurred in the sequence previously described for amputated tails (Dinsmore, J. Exp. Zool. 199:163-176, 1977). Although the skin of the stump retained its "upside-down" configuration, the skin of the regenerate differentiated in a normal orientation.

The second series produced a stump surface whose skin represented an exclusively dorsal aspect around the entire circumference of the amputation. Nevertheless, regeneration proceeded quite normally, the internal architecture as well as the skin morphology being restored in normal relationships.

Discussion

In approximately 150 cases where tail skin has been rotated as described above, none has been regeneratively repressed and all have produced morphologically normal regenerates considering both integument and internal tissue organization as determining criteria. It would therefore appear that previous experiences with the limb system are unique to that system and that the proposed model for pattern regulation in epimorphic fields (French, et al., ibid.) does not apply to the tail field.

The model is, however, quite valuable in underlining the differences between the two epimorphic fields and may further allow detailed analysis of the trophic interactions between tissues. The limb has at least two tissues actively engaged in morphogenetic control of the regenerate (Carlson, ibid., 1975). Normal harmonious interactions result in complete restoration of a lost limb. If, however, the normal relationships between these two components are disrupted, each appears to have the capability of becoming an autonomous organization center resulting in hypermorphic or duplicated regenerates. Although the tail skin is proportionately thicker than limb skin and the same type of manipulation and criteria for evaluating the regenerates were used, the data indicate no morphogenetic activity in this tail tissue. In addition, previous work (Dinsmore, ibid.) and current results on tail muscle morphogenesis also preclude this tissue from control of tail regeneration.

Considering that the spinal cord is essential for initiating and promoting tail regeneration while neither whole skin nor muscle appear to be more than passively involved in morphogenesis of the regenerate, it is concluded that the regenerating spinal cord is the major if not exclusive source of morphogenetic control in this system. Current investigations are aimed at determining whether or not this control may be expressed through segmental ganglion neogenesis resulting in the observed segmental differentiation of the internal tail tissues.