We thank Dr. C. Holliday for teaching us the skate catheterization technique, and Dr. W. G. North for doing the taurine analyses. Supported by NIH HL04457 and NSF PCM-7921476. Dr. H. Schröck was supported by a stipendium from the Deutsche Forschungsgemeinschaft.

THE MORPHOGENETIC INFLUENCE OF GRAFTED TAIL SKIN ON URODELE LIMB REGENERATION

Charles E. Dinsmore, Department of Anatomy, Colleges of Health Sciences and Medicine, Rush University, Chicago, Illinois

Morphogenetic tissue interactions are critical to both the development and the regeneration of complex form and function. It is understood that pattern regulation in these systems must be governed by the communication of positional information within as well as among the tissues of a particular field or territory. Identifying loci of morphogenetic activity is, therefore, fundamental to a more complete understanding of many developmental processes.

Morphogenetic information during urodele limb regeneration has been repeatedly demonstrated to emanate from dermis and skeletal muscle of the normal stump. Positional dislocation of either tissue relative to the other usually results in anomalous limb regeneration. This is, however, not true for the urodele tail. Surgical manipulations which disrupt morphogenetic interactions during limb regeneration have no apparent effect on tail regeneration (Dinsmore, Bull. MDIBL 17:2-3, 1977; Develop. Biol., in press).

The present investigation examines the consequences of superimposing tissue from one regenerative or epimorphic field on a heterotopic field. The model system is the Eastern red-backed salamander, *Plethodon cinereus*, which typically can regenerate both limbs and tail. Autografting tail skin to the limb and subsequently amputating through the graft asks the following questions: Is morphogenetic activity an intrinsic capacity of certain urodele tissues or is it field specific and irreversibly determined during ontogeny? If regenerate morphology indicates graft activity, will it be simply disruptive of normal signalling within the field or will it superimpose morphogenetic information reflecting its origin (i.e., "tailness"), thus providing a chimeric structure?

MATERIAL AND METHODS

Adult specimens of *Plethodon cinereus* ranging from approximately 8.0-10.0 cm total length were collected in the vicinity of the laboratory in Salsbury Cove, maintained in covered 4-inch diameter stacking culture dishes and fed regularly with live fruit flies (*Drosophila melanogaster*). Prior to any of the following surgical procedures, animals were immobilized by immersion in 1% MS 222 (ethyl m-aminobenzoate methanesulfonate, Eastman) and removed to the stage of a dissecting microscope. Two series of experimental skin manipulations were employed to address the questions outlined above. In the first, a circumferential cuff of tail skin was carefully dissected free, isolated in a pool of amphibian saline and cleaned of adhering myofibers. A cuff of skin was then removed from the upper right forelimb creating a graft bed from shoulder to elbow. The graft (tail skin) was then trimmed to appropriate size and introduced onto the graft bed where it was secured in a normal dorsoventral orientation with 2 or 3 interrupted 7-0 silk sutures (Ethicon). Animals in the second series were prepared in the same manner except for the orientation of the graft. The cuff of tail skin was rotated 180° about the long axis of the limb prior to suturing. In this way, the dorsal aspect of the tail skin was on the ventral surface of the limb. Comparable dislocation of limb skin relative to internal tissues almost always results in abnormal limb regeneration.

Following these operative procedures, animals were placed in Petri dishes containing Holfreter's saline where they recovered rapidly. They were then returned to their containers for a 10 day post-operative period to allow graft stabilization and healing. At the end of this period, animals were again anesthetized and both forelimbs amputated at mid-humerus, the left limbs serving as normal controls. The limbs were allowed to regenerate for at least 60 days, an interval sufficient for well-formed four-digit regenerates to develop in the normal sequence for this species. At the time of sacrifice, both the experimental and contralateral control limbs were dissected free at the shoulder and fixed in Bouin's solution. They were evaluated grossly for regenerative success then decalcified and prepared for paraffin embedding, serially sectioned at 8 μm and stained by Mallory's trichrome method. Control and experimental limbs were then compared, evaluating: a) skin morphology as an indicator of graft survival and contribution to the regenerate and b) skeletal composition, especially number of digits and internal cartilaginous elements as an indicator of morphogenetic success.

A total of 41 animals were evaluated providing 22 limbs from the inverted tail skin group and 19 from the normally oriented tail skin group.

RESULTS AND DISCUSSION

Simple amputation of the control left limbs usually resulted in the regeneration of normal 4-digit limbs. Histologically, these limbs were covered by typically thin, sparsely glandular skin and contained the cartilaginous precursors of a normal skeletal complement. Limbs which had received grafts of tail skin prior to amputation yielded a range of responses from no regeneration to a superficially normal 4-digit regenerate. Skin graft orientation, whether normal or inverted, had no apparent influence on the results. Stumps which did not regenerate had histologically obvious, thick glandular skin about their entire circumference. Many examples, where tail-type skin differentiated on the regenerate, had reduction deformations (e.g. fewer digits than normal; distally fused radius and ulna). Those with the most complete regenerates often had an incomplete circumference of tail skin around the stump region. This may have been due to graft resorption or localized gland regression, either of which produces a "wound epithelium" which is more supportive of regenerative events than whole skin. None of the experimental combinations produced chimeric regenerates wherein tail-like structures would appear either grossly or histologically in the limb regenerate. This latter observation is contrary to the earlier studies of Glade (J. Morph. 101:477-522, 1957; Growth 42:253-262, 1978) who, using aquatic species which have tail fins, described fin and tail-like structures on limb regenerates from stumps bearing tail skin grafts. While this may indicate fundamental differences between species, it is a likely reflection of graft contamination with non-skin tissues which express morphogenetic activity or, at least, the capacity to self-differentiate in a heterotopic locus.

The results of the present study indicate that, at best, tail skin may be mildly supportive of limb regeneration causing varying degrees of morphogenetic disruption within the host field. However, in many cases, regeneration was completely inhibited. In those limbs which did regenerate and bore tail skin around the stump at the time of sacrifice, there is no evidence for autonomous morphogenetic contributions of tail structure to the regenerate. It therefore appears that, unlike limb skin, tail skin plays a passive to supportive role in normal epimorphic events. When heterotopically grafted, it interferes with normal morphogenetic signalling in the host field without making an autonomous contribution of morphogenetic information.

A STUDY OF THE KNOWN LIMB TERATOGEN, ACETAZOLAMIDE, AND ITS EFFECT ON LIMB REGENERATION

Charles E. Dinsmore, Thomas H. Maren and Daphne Gardiner, Department of Anatomy, Rush University, Chicago, Illinois, Department of Pharmacology and Therapeutics, University of Florida, College of Medicine, Gainesville, Florida and Georgetown University, Washington, D.C.

Vertebrate limb development and urodele limb regeneration appear to have much in common and because of their structural and sequential similarities, the latter is frequently said to recapitulate the former (Faber Adv. in Morph.,