CHLORIDE EFFLUX ACROSS THE GILL OF *Anguilla rostrata*: EFFECTS OF ELIMINATING EXCHANGE DIFFUSION

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A major portion of the efflux of chloride across the gills of some teleosts adapted to seawater is eliminated if chloride is removed from the external bathing medium, by immersing the fish either in distilled water or in seawater in which another ion has been substituted for chloride. This component of chloride efflux, balanced by an equal influx of chloride and usually thought of as exchange diffusion, may be so large as to dwarf the net extrusion of chloride that maintains osmotic homeostasis For example, exchange diffusion is reported to account for 80-90% of chloride and sodium efflux in *Anguilla rostrata* and *Mugil capito* (J. Gen. Physiol. 50:391-422, 1966). In order to study the process of active chloride secretion by the gill more precisely, we examined chloride efflux in *Anguilla rostrata* in artificial seawater in which chloride was completely replaced by nitrate.

American eels (*Anguilla rostrata*) adapted to seawater for 3 to 10 weeks or freshwater for 3 to 6 weeks were used in these experiments. Chloride efflux was measured over 30-minute periods by injecting 2-3 *m*Ci of ^{36}Cl intraperitoneally, allowing it to equilibrate for 40-60 minutes, monitoring the appearance of ^{36}Cl in an aerated bath of selected composition at 16°C, and measuring the specific activity of ^{36}Cl in plasma at the conclusion of the experiment. Artificial seawater was prepared with the following composition: NaCl, 480 mM; KCl, 10 mM; $CaCl_2$, 10 mM; $MgCl_2$, 25 mM; $MgSO_4$, 25 mM. Seawater free of chloride was prepared as follows: $NaNO_3$, 480 mM; KNO_3, 10 mM; $Ca(NO_3)_2$, 10 mM; $Mg(NO_3)_2$, 25 mM; $MgSO_4$, 25 mM. In preliminary experiments the substitution of sulfate or acetate for chloride in the seawater bath did not give results significantly different from those obtained when nitrate was used as the substitute ion.

Elimination of exchange diffusion reduced chloride efflux by 40-60%. Chloride efflux of 6 seawater-adapted eels was 1317 ± 597 (mean ± s.d.) μEq/100 g/hr in chloride seawater and only 40% of that, or 536 ± 261, in nitrate seawater. Five eels adapted to freshwater for 3 weeks had a chloride efflux of 238 ± 153 in chloride seawater that was reduced to 135 ± 71 in nitrate seawater. Further studies of 9 seawater eels showed a chloride efflux in nitrate seawater of 566 ± 341, whereas 10 freshwater eels (adapted for 6 weeks) had an efflux of 59 ± 43. The marked and well-known difference in chloride efflux induced by adaptation to fresh or salt water was therefore maintained when exchange diffusion was eliminated.

The effect of furosemide on chloride efflux was next studied because of the inhibition of active chloride transport in other tissues produced by this compound. Furosemide, 3 mg/100 gm, was injected intraperitoneally in 4 seawater-adapted eels after an initial control period in nitrate seawater, and two more 30-minute periods were subsequently obtained. Surprisingly, no effect of the drug on chloride transport was discerned. Chloride efflux before furosemide was 569 ± 555 uEq/100 g/hr and afterwards 747 ± 479 and 634 ± 173. It is possible that an inhibitory effect on gill extrusion of chloride was balanced by the diuretic action of furosemide.

Theophylline increases the secretion of chloride by the perfused rectal gland and is reported to stimulate short-circuit current in the opercular skin of *Fundulus heteroclitus*. Theophylline, 12.5 μM/100 gm (0.5 ml of a 25 mM solution per 100 g) was given intraperitoneally to 8 seawater-adapted eels immersed in nitrate seawater. These fish had been adapted to seawater for 10 weeks and their baseline chloride efflux was higher than those discussed previously. Chloride efflux was 1236 ± 519

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