Methylated xanthines are reported to raise cAMP levels by inhibiting phosphodiesterases. In fact, these compounds in both regular and Ca^{2+}-free sea water + EGTA accelerated the normal starting time for polar lobe formation and cytokinesis: 0.26 mM xanthine and 1 mM theophyline caused a 2-4 min acceleration, 1 mM theobromine caused a 5-10 min acceleration, and 1 mM caffeine caused as much as a 15 min acceleration in the starting time.

A number of phospholipid-interacting drugs are reported to inhibit Ca^{2+}-activated, phospholipid-dependent protein kinases. Chlorpromazine at 25 μM delays both polar lobe formation and cytokinesis, and at 100 μM stops both processes. Verapamil, D600, and nifedipine are Ca^{2+}-uptake inhibitors and verapamil, at least, also is an inhibitor of the protein kinase type above. Both verapamil and D600 at 100 μM cause a substantial delay in the normal starting times for polar lobe formation and cytokinesis, especially in Ca^{2+}-free sea water + EGTA. Nifedipine, even at 20 μM, causes a substantial delay in both the presence and absence of exogenous free Ca^{2+}.

Ouabain (10^{-3}M), veratridine (10^{-4}M), and sodium orthovanadate (2.5×10^{-3}M) had no effect on lobe formation and cytokinesis of Ilyanassa eggs in the presence or absence of exogenous Ca^{2+}. In addition, double-barrel microelectrodes were constructed with one barrel filled with 3 M KCl for measurement of membrane potential and the other barrel filled with a continuous column of antimony for measurement of pH. The final tips on these electrodes (3-5μ) were generously pulled by Dr. Klaus Beyenbach. When Ilyanassa eggs were impaled with the tips of such electrodes, the recordings showed that no detectable changes in membrane potential or intracellular pH occurred during polar lobe formation and cytokinesis.

From the inhibitor experiments, we conclude that both cyclic nucleotide activated-, and Ca^{2+}-activated protein kinases should be measured directly in these eggs during cell shape changes. Supported by NIH HDO7193.

7 REGENERATION FROM LONGITUDINALLY SPLIT FORELIMBS IN PLETHODON CINEREUS.

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When a urodele amphibian loses a limb or its tail, it grows a new one which is structurally and functionally identiaal to the one lost, a process known as epimorphic regeneration. Current studies are aimed at identifying 1) the locus of the pattern which regulates the genesis of new form during regeneration and 2) the mechanisms by which positional information required for pattern regulation is transmitted. Limb skin has been shown to be morphogenetically active during limb regeneration and, in the absence of the other morphogenetically active tissue (skeletal muscle), sufficient to regulate pattern for a complete limb regenerate. The converse also applies. A recent model (French, et al., Sci., 193: 969-981, 1976; Bryant, et al., Sci., 212: 993-1002, 1981) proposes that a complete circumference ("complete circle") of positional information (e.g., a normal circumference of skin) is necessary for limb regeneration to occur. Thus, if a longitudinally split limb were amputated, regeneration would not proceed because there is a gap in the normal "circle" of positional information. It was suggested that halved limbs would instead regenerate the circumferential base of the pattern and then produce normal distally complete regenerates (Bryant, Nature 263: 676-679, 1976). This contradicts the earlier works of Weiss (Arch. f. Entw.-mech. 107: 1-53, 1926) and Goss (J. Morph. 100: 547-564, 1957) who found that while split limbs could produce normal, distal regenerates they also regenerated half-limbs resembling the distal structure which had been amputated.

The split-limb experiments have been repeated in an attempt to resolve this apparent conflict in results. Red-backed salamanders, Plethodon cinereus, were collected and maintained as in earlier studies (Bull. MDIBL 20: 23-24, 1980). On the stage of a dissecting microscope, both the forelimbs of anesthetized animals were split longitudinally from between the second and third digits proximally to the elbow. The entire post-axial halves of the forelimbs, which included ulna, digits 3 and 4 and associated soft tissues, were discarded. Animals were placed in dilute amphibian saline for recovery and then returned to their covered fingerbowls. Following a 2-4 day period of

wound healing, the animals were again anesthetized and their forelimbs amputated through mid-radius. The stump created presents a "half surface" from which regeneration might proceed. The animals were subsequently observed for at least 60 days during which time progress of regenerates was observed.

The results confirm the earlier observations that although split-limbs can regulate to produce distally complete normal regenerates, a majority of the amputated half-limbs produced deficient regenerates. The range of regenerate expression was from completely arrested outgrowth (no regeneration) to a normal, 4-digit hand. Most of the reduced regenerates reflected their origins from the pre-axial half of a split forelimb. These were seen to arise directly from the apical end of the amputated limb. The record for distally complete regenerates indicated an oblique origin reflecting a base of outgrowth other than the intended "half-stump". By employing an oblique base, the regenerate has access to complete circumferential "positional information" and is thus able to regulate completely. Allometric growth also enables the oblique outgrowth to subsequently rectify its relationship with the axis of the limb. This latter phenomenon has been described during urodele tail regeneration from oblique amputation surfaces (Spallanzani, 1768; Todd, 1823 and personal observations). From the description offered by Bryant (vide supra), the complete regulation she observed is related both to the operative technique employed and the consequent oblique outgrowth induced. While the explanation offered was that "intercalation" of positional values occurred restoring a complete circle of information, the present study has shown that 1) complete regulation of distal limb regeneration from a split forelimb employs the oblique mode of outgrowth to provide a normal base of positional information and 2) regeneration of a half-limb from the apex of an amputated split-limb can indeed occur in the absence of a "complete-circle" of positional information.
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HEMODYNAMICS OF THE ISOLATED, PERFUSED HEAD OF THE DOGFISH "PUP"

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In recent years various laboratories have utilized perfused head preparations to investigate both hemodynamic and osmoregulatory parameters of marine and freshwater teleost fish (see Claiborne and Evans, J. Comp. Physiol., 138: 79-85, 1980, for relevant citations). Similar techniques have not been utilized with elasmobranchs. However, at least the hemodynamics of the elasmobranch gill have been studied using both isolated, perfused gill arches (Davies and Rankin, Comp. Gen. Pharmacol., 4: 139-147, 1973) and perfused intact fish (Kent and Peirce, Comp. Bioch. Physiol. 60c: 37-44, 1978). Unfortunately, these studies disagreed about the effect of epinephrine on gill resistance, with the former showing that the drug produced a transient fall in gill resistance, and the latter study finding no effect on gill resistance. Importantly, no studies of ionic transport across the elasmobranch gill have utilized perfused heads. This would be of great interest since it has recently been shown that the gill of Squalus acanthias excretes both NH_4 and H in exchange for sea water Na and possibly also functions in extrusion of both Na and Cl (Evans, J. Exp. Biol., 1982, in press; Evans and Mansberger, Bull. MDIBL 19: 101-103, 1979). The present study was initiated to examine the hemodynamics of the isolated, perfused head of S. acanthias "pups" in an effort to test this preparation for further use in investigations of elasmobranch gill transport, as well to test directly the effect of epinephrine, phentolamine, propranolol, carbachol and atropine on gill resistance and pattern of blood flow.

Perfused heads were prepared as described previously (Claiborne and Evans, 1980, op. cit.) with the exception that the cannula into the conus arteriosus was a short (1-2 cm) length of flared PE 50 tubing. Irrigation (sea water) flow rates were usually 175 ml. min^{-1}, but in some experiments were varied between zero and 350 ml min^{-1}. Perfusion flow rates were usually 0.75 ml·min^{-1}, but were varied between 0.4 and 1.75 ml·min^{-1} in