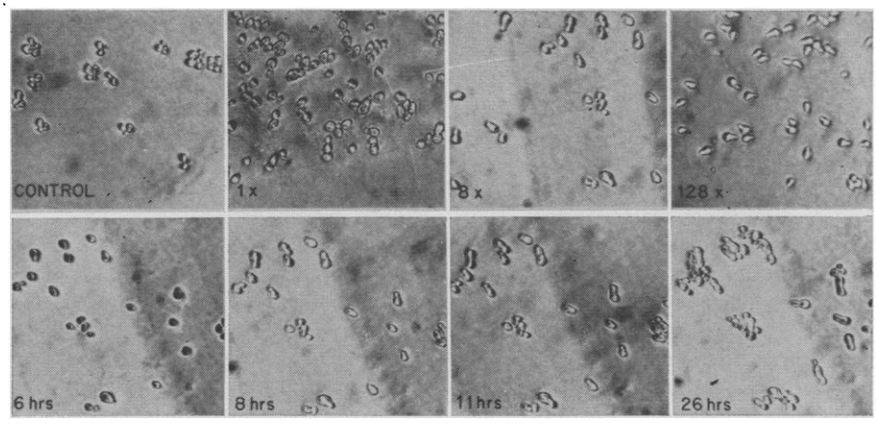

Fig. 2. (Top) Response of *a* cells to a dilution series of a concentrated and partially purified filtrate of α cells. The cells were streaked on minimum agar medium near wells containing the dilutions of the active fractions and incubated at 30°C. Photographs were taken after 8 hours. The control shows cells from the same plate streaked at some distance from the wells containing the active dilutions. (Bottom) Response of *a* cells to a single concentration of sex factor (8 units per milliliter). Photographs of the same group of cells were taken over a period of 24 hours.

isolation, and characterizing the factor. The activity is tested by exposing *a* cells on minimum agar medium to fractions placed in wells in the agar and observing the response directly under the microscope (Fig. 2). The photomicrographs were taken after the plate had been incubated for 8 hours at 30°C. The extreme elongation is especially impressive at higher concentrations, but even at a concentration of 1 unit per milliliter (defined as the lowest concentration achieved by twofold dilutions which will cause a detectable response) only a few buds are observed, whereas the control cells from the same plate have budded normally.

The sex factor can be concentrated from culture filtrates by adsorption to the weakly acidic cation exchanger, Amberlite CG-50, and elution with acid ethanol. After concentration of the eluate the active fraction is again adsorbed on a small column of the same resin. Impurities were removed by elution with 4.3*M* acetic acid. Elution with 8.6*M* acetic acid followed by concentration in a rotary evaporator yielded a concentrated solution of the factor. Further purification can be achieved by paper chromatography in a butanol, acetic acid, and water (4 : 1 : 5) system.

Physical and chemical properties of the factor have been inferred from studies of the influence of various conditions on its capacity to stimulate the elongation response. It has a molecular weight between 1000 and 2000, estimated by gel filtration on Sephadex G25. It resists boiling at slightly acid *p*H, but is unstable at alkaline *p*H even at room temperature. It is destroyed by strong acid hydrolysis or by proteolytic enzymes such as pronase or pepsin. It is not extractable by lipid solvents such as ether or chloroform and methanol, and it is strongly bound to cation-exchange resins. Preparations purified by paper chromatography give a weak or negative ninhydrin reaction but yield ninhydrin-positive material upon hydrolysis.

The properties described can be explained by the assumption that the factor contains several amino acids in peptide linkage that are necessary for its activity. They are not consistent with those of a steroid and, therefore, it is undoubtedly not the same as the steroids that cause expansion of cells (*7*).

The fact that this sex factor and the ability of α cells to mate can be affected by the same mutation implies that it plays an important role in the physiology of conjugation. The occurrence of sterile mutants that still produce the factor indicates that the ability of α cells to mate depends on additional gene products.

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Limb Movements in a Monotreme (Tachyglossus aculeatus): A Cineradiographic Analysis

Abstract. *In a walking echidna the principal movement of the humerus is long-axis rotation. The humerus remains approximately perpendicular to the sagittal plane, but the femur is directed anterolaterally at angles from 35° to 50°. In addition to long-axis rotation, the femur elevates and depresses in an arc which usually varies between 40° and 90°. The femoral angle, the femoral elevation and depression, and the plantar contact of the manus beneath the glenoid are features found also in generalized therians.*

Monotremes are survivors of a nontherian group of Mesozoic mammals (*1*) and retain many anatomical and physiological features indicative of a relatively primitive level of mammalian organization (*2*). Despite their numerous dietary and ecological specializations, monotremes are potentially useful as analogs in investigations of early mammalian evolution. Monotreme limb posture and movements are commonly cited as representing a primitive mammalian or even reptilian pattern (*3, 4*), although in fact no detailed functional analysis of monotreme limbs in vivo has been published. I have used cineradiography to analyze limb posture and movements in an Australian echidna (*Tachyglossus aculeatus*), which represents one of three extant monotreme genera.

The single adult echidna used in this

study walked freely on a radiological examining table, constrained only by an enclosure measuring 20.3 by 121.8 cm. The enclosure was narrow enough to ensure that the echidna walked in a more or less straight line but wide enough to permit complete freedom for the limbs. Heavy paper on the table prevented the echidna's feet from slipping; a 5-cm wire grid over the pen provided reference points for motion analysis. Films were taken with a Bell and Howell 70 DR cine camera mounted on a 9-inch (1 inch = 2.54 cm) Philips image intensifier and run at 32 and 64 frames per second with Kodak Linagraph Shellburst 16-mm film. X-ray factors were 95 kv and 3.5 Ma. The x-ray source mounted beneath the table and the image intensifier mounted above the table yielded dorsoventral projections of the walking echidna. Films viewed on a rear projection screen by means of a Traid 16 N stop-motion projector permitted tracings of individual frames. All frames representing a particular phase in a locomotory cycle were compared; thus the principal findings summarized here have been verified many times over.

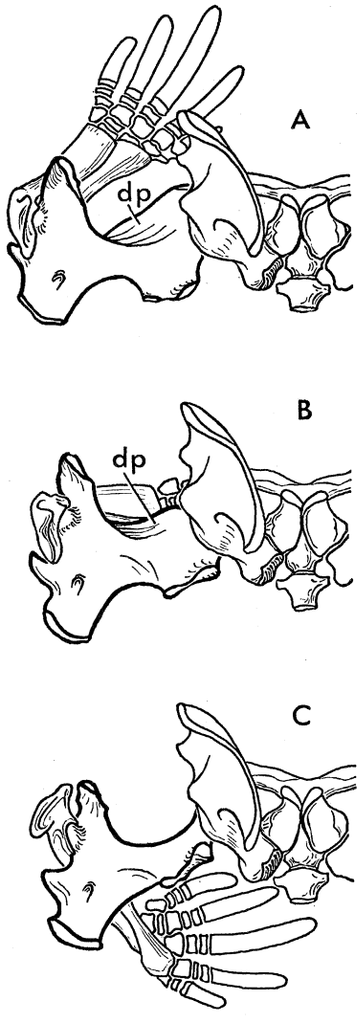

Fig. 1. Left forelimb and shoulder girdle of a walking echidna (*Tachyglossus aculeatus*) in dorsal view interpreted from cineradiography. (A) phase I, incipient propulsion; (B) phase II, middle of propulsion; (C) phase III, terminal propulsion *dp*, deltopectoral crest. Approximately ×½.

For descriptive convenience, locomotory movements are divided into four phases. Phase I is the start of the limb's propulsive movement; the foot has just regained surface contact, and the body begins to move forward relative to the foot. Phase III is the completion of the propulsive movement; the foot is about to lose plantar contact and move forward relative to the body. Phase II, halfway between I and III, represents a midpoint in the propulsive movement. In phase IV, halfway between III and I, the limb is being protracted forward to begin another step.

The proximodistal axis of the humerus (running through the head and radioulnar articulation) remains more or less perpendicular to the sagittal plane throughout all phases. The degree of anteroposterior movement of the humerus is small; in some sequences, the distal end is more anterior than the proximal in phase I compared to phase III; in other sequences, the reverse is true (see Fig. 1, A and C). The latter shift relates to the peculiar posteromedial orientation of the antebrachium during phase III. The principal movement of the humerus is rotation about its proximodistal axis. From phase I to III, the total rotation is estimated to be about 40° to 50° (in a counterclockwise direction seen on the left side, clockwise on the right). As evidence of this rotation, for example, the deltopectoral crest (*dp*, Fig. 1, A and B) is positioned anterior to the shaft in phase I but ventral to the shaft in phase III. The successive shortening of the humerus in dorsoventral projection (Fig. 1, A–C) indicates elevation of the proximal relative to the distal ends from phases I to III, but this movement is probably no more than 10° to 15°. The long axes of the antebrachium and manus in phase I are directed anteromedially at angles of approximately 50° to 55° to the sagittal plane (Fig. 1A). Humeral rotation subsequently moves the antebrachial axis parallel to the humeral shaft (phase II) and then behind the shaft (phase III). During phase III the antebrachial axis is oriented posteromedially at about 35° to the sagittal plane. The long axis of the manus is perpendicular to the body. Except for the direction of movement and for the fact that the limb is lifted, phase IV differs little from phase II.

The principal locomotory movements of the femur are rotation about its proximodistal axis (from the head through the patellar groove) and elevation and depression. Rotation is demonstrated by a shift in the position of the greater trochanter from posterior to the femoral axis in phase I (*gt*, Fig. 2A) to nearly above the axis in phase III (*gt*, Fig. 2C). Total rotation is estimated to be about 45° (in a counterclockwise direction seen on the left

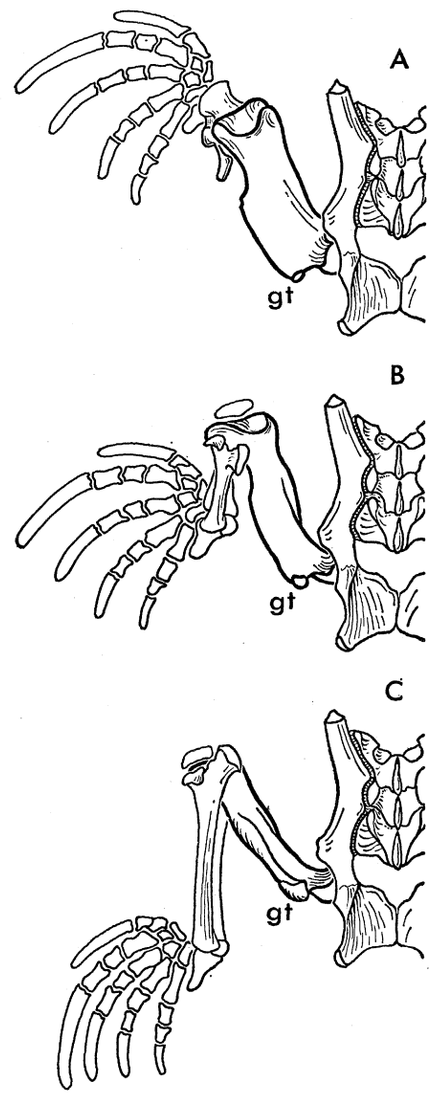

Fig. 2. Left hind limb and pelvis of a walking echidna (*Tachyglossus aculeatus*) in dorsal view interpreted from cineradiography. (A) phase I, incipient propulsion; (B) phase II, middle of propulsion; (C) phase III, terminal propulsion. *gt*, greater trochanter. Approximately ×½.

side, clockwise on the right). Shortening of the femoral axis in dorsoventral projection (not shown in Fig. 2) indicates that the proximal end elevates relative to the distal end in phases II and III; this movement may vary from as much as 90° to as little as 40°. The long axis of the femur also sweeps posteriorly during propulsion, starting at about 35° to 40° to the sagittal plane at phase I and ending at about 50° or more at phase III (Fig. 2A, C). At phase I the crural axis is nearly vertical although directed somewhat anterolaterally; the foot is directed laterally (Fig. 2A). In phases II and III the crural axis is approximately parallel to the sagittal plane, and the foot is rotated to point more posteriorly (Fig. 2, B and C). At the termination of phase III, the foot is directed posteriorly in a final thrusting movement (not illustrated). Except for the direction of movement and for the fact that the limb is lifted, phase IV is little different from phase II.

This analysis emphasizes some previously overlooked functional aspects of monotreme limbs and contradicts some common assumptions. Rotation is the principal locomotory movement of the humerus (*5*), not anteroposterior protraction as has been suggested (*3*). The antebrachium is directed ventromedially so that the manus, positioned approximately beneath the glenoid in phase II (Fig. 1B), supports the body in a more typically mammal-like stance than would be the case were the antebrachium perpendicular to the humerus as has been assumed (*6*). Femoral orientation, usually regarded as slightly anterior to the transverse plane (*6*), varies from approximately 35° to 50° from the sagittal plane and is thus similar to that in nonspecialized therians. Femoral elevation and depression in a 40° to 90° arc is likewise similar to a therian pattern. Statements that monotreme limbs are reptilian in posture (*4*) or that they sprawl in a manner comparable to that of lizards (*7*) are imprecise and with regard to the echidna, at least, are inaccurate. The limbs of the echidna support the body well off the ground, even when the animal is stationary or walking slowly. During propulsion, the femoral orientation and movement and the plantar contact of the manus beneath the glenoid are features found also in generalized therians that I have studied (for example, *Didelphis*). In contrast, the "sprawling" posture of most lizards (for example, *Iguana*) involves femoral angles that may vary from 50° (phase I) to 120° (phase III) and plantar contact of the manus lateral to the glenoid and elbow.

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Cones of Living Amphibian Eye: Selective Staining

Abstract. *The outer segments of cones are selectively stained by the chlortriazinyl dye Procion Yellow injected into the vitreous humor. Since the dye does not cross nerve cell membranes, the selective staining of cones is further evidence for structural differences between rod and cone outer segments. Specifically, it is believed that cone saccules are open to extracellular space whereas rod saccules are not.*

The terms rod and cone aptly describe the morphology of amphibian photoreceptor outer segments. In recent years evidence has gradually accumulated that these differences in form are accompanied by other important distinctions between the two. Specifically, the repeating lamellar membranes of cones appear to be contiguous with the cell membrane whereas those of rods do not (*1*); the rod basal lamellae regenerate continuously and thus migrate toward the pigment epithelium whereas those of the cones do not (*2*), and cones are the chief source of the early receptor potential (*3*) of the frog retina despite the preponderance of rod pigment (20:1) (*4*). To these distinctions we now add a striking disparity in staining reaction between rod and cone outer segments in the living animal, a disparity which we believe derives from the above-mentioned structural differences in outer segment membrane topography of the two types of photoreceptor.

We used the fluorescent dichlortriazinyl dye Procion Yellow (M-4RS, ICI America, Inc.) recently introduced by Stretton and Kravitz (*5*) as a single-cell marker in neurophysiology. Since it does not cross the cell membrane to leave a neuron once injected (*6*) and does not enter neural cells from extracellular space (*7*), it is virtually an ideal marker. Twenty-four hours after injection of 25 μl of a freshly made 0.5 to 4 percent aqueous solution of the dye into the vitreous humor, mudpuppies (*Necturus maculosus*) or frogs (*Rana pipiens*) were decapitated, and their eyes were enucleated and quenched in isopentane cooled to −130°C in a liquid nitrogen bath. Thereafter, the eyes were freeze-dried at −35°C for 3 days and then embedded in paraffin at 60°C. Sections were cut at either 5 or 12 μm, mounted in xylene and viewed in a Zeiss microscope equipped for fluorescence darkfield, fluorescence polarization, and birefringence observations.

When viewed either by darkfield (exciting filter BG 12, barrier filter Zeiss 50) or between crossed polarizers (exciting filters BG 12 and BG 3 in series, no barrier filter) the cone outer segments appeared brilliant yellow-orange whereas the rod outer segments were a dim green outlined by a fine yellow margin (Fig. 1). The cones were several orders of magnitude brighter than the rods. In control preparations (no Procion Yellow) there was little difference in the intensity of the dim fluorescence emitted by rods and cones and no orange color whatever (Fig. 2).

In an attempt to clarify what appeared to be a selective binding of Procion Yellow to cone outer segments, polarization studies were carried out. Visual receptor outer segments are anisotropic, characteristically showing transversely oriented lamellar membranes, visual pigment dichroism, and positive uniaxial birefringence. Procion Yellow contains several planar aromatic rings and if binding to outer segment membranes involves adsorptive (Van