Chick and duck blastoderms can expand on each others vitelline membranes

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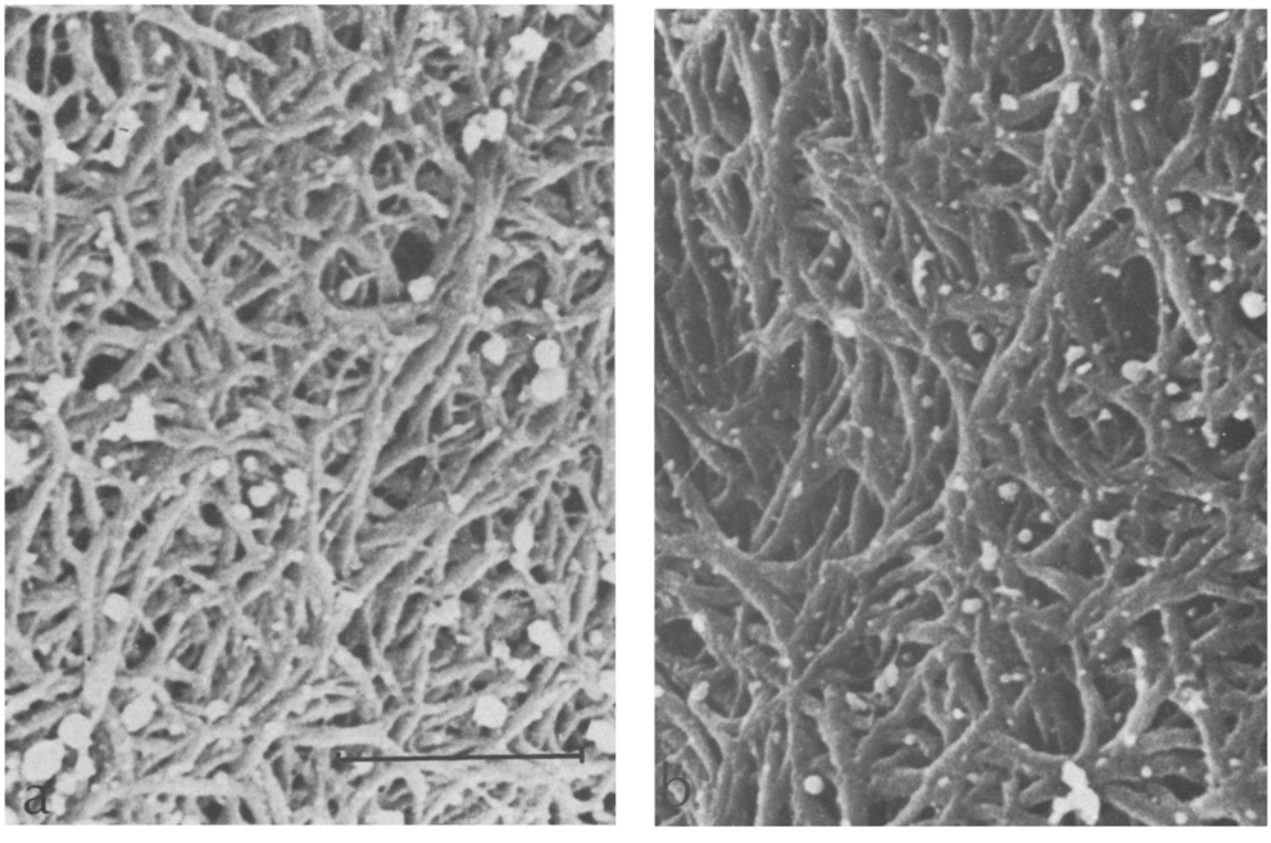
Summary. The expansion rates *in vitro* of chick and duck blastoderms transferred to each others vitelline membranes were compared with blastoderms left in place. Duck blastoderms expanded slightly faster than chick, irrespective of membrane type. There was no specificity.

Since cells can move on surfaces they do not normally encounter, the exact chemical nature of the surface is unlikely to be vital. However, studies with cultured cells on non-biological surfaces have shown that changes in surface quality can alter cell motility[2,3]. In addition, 2 recent reports[4,5] have claimed a high degree of surface specificity in the case of avian blastoderm expansion, one of the few cases where a surface normally used for cell migration can be obtained in quantity. Some unsatisfactory features of these reports suggested a re-investigation would be useful.

Materials and methods. The basic experiment used the explantation method of New[6,7] to compare the rates of expansion of chick blastoderms on duck vitelline membranes (and vice versa) with their normal rates. Blastoderms of approximately equal diameters, well attached by their edge cells to the vitelline membrane inner surface[8,9], were obtained by incubating fertile domestic fowl (*Gallus gallus,* De Kaalb strain) and domestic duck (*Anas platyrhynchos,* an Aylesbury/Pekin hybrid) eggs 20–24 h and 34–36 h respectively[10]. Since detachment, using fine forceps, can inflict some damage, it is necessary for both control embryos (on their own vitelline membranes) and transfers to be detached. Re-attachment of the edge is usually complete within 2 h incubation[8]. Cultures once set up were therefore left a minimum of 2 h before measuring. Exactly superimposed outline drawings were made, using a Wild M5 and camera lucida, at 0 h and after an expansion period of 5 h. Expansion cannot reliably be measured much longer because the blastoderm reaches the culture ring. Some blastoderms were examined after a further 12 h to assess longer term effects. Expansion rate for a blastoderm was assessed by placing a grid over the drawings which picked 10 random points round the circumference. Migration distance at each point was measured, the mean for the whole blastoderm calculated and then converted to $\mu m\ h^{-1}$. Occasional points where re-attachment failed were excluded.

Some vitelline membranes were fixed in glutaraldehyde and osmium tetroxide, then critical point dried for scanning electron microscopy in a search for structural differences.

Results. Expansion rates are shown in the table. The discussion explains the different controls. On overnight incubation, both control and transfer blastoderms developed normally. Figure 1 shows how the inner surface of the vitelline membrane – the surface used for blastoderm expansion – appears in SEM. The chick membrane shows a network of fibrils of variable dimension, similar to those found previously[4,11]. The duck membrane does not appear to differ materially.



Scanning electron micrographs of the fibrillar network forming the inner surface of the vitelline membrane. *a* Chick; *b* duck. Scale 5 µm.

Discussion. The main result of these experiments is that there is no specificity: duck blastoderms expand as well on chick vitelline membranes as on their own, and vice versa (table, a). The controls require some comment. Transfer cultures required both detachment and removal to a new vitelline membrane. Since both procedures could conceivably affect subsequent expansion 2 sets of controls were used for chick embryos with chick albumen: series 1: detached but left on the original membrane; series 2: detached and removed to a new chick membrane. The results vindicated this caution: the control chick embryos of series 1 expanded significantly faster than the transfers to duck, but the difference using series 2 was not significant. This probably explains the significant difference in the same comparison using duck albumen where the controls were only of the series 1 type. A similar precaution was not necessary for the duck blastoderms since these usually detached spontaneously from their vitelline membranes, avoiding the need for possibly damaging manipulation.

I originally felt that the source of the culture medium (thin egg albumen) might matter, but the results in the table, b, show no specificity here either (only series 2 controls used in this comparison). The main protein of egg white, ovalbumin varies enough from species to species to be of taxonomic value[12,13] but even the large difference between chick and duck ovalbumin has no effect on blastoderm expansion.

The only clear cut difference was that the duck blastoderms expanded faster than the chick ones, whatever the circumstances (table, c: only series 2 controls used in this comparison). Since the rate of expansion of chick blastoderms varies at different stages[14], a small difference in rate between these 2 species is not unexpected.

Inspection of the table, a, shows that blastoderms expand more quickly on average on chick than on duck membranes. However, pooling of the comparable data (table, d) shows that this difference is not statistically significant.

It seems to me unsurprising that 2 surfaces evolved for essentially the same purpose, supporting rapid epithelial cell migration, and belonging to members of the same class, should show such similar properties and appearance (figure). Indeed the chick vitelline membrane (and presumably also the duck vitelline membrane) provides a good surface for several sorts of cells to move on[15]. It would be interesting to look at the inner surface of reptile vitelline membranes.

What then of the previous reports of specificity? One seems to be simply an error. Chernoff and Overton[4] cite Jensen[16] as finding that chick blastoderms expand hardly at all on duck vitelline membranes; but Jensen's paper contains no mention of such an experiment. Haas and Spratt[5] found considerable specificity in respect of both membrane and medium type in comparisons of chick, duck and turkey blastoderm expansion. Their experiments are, however, open to several criticisms: it is unclear how they measured expansion; they used unincubated blastoderms which are more difficult to work with successfully; they give no statistical analysis of their results; there are controls only for the chick blastoderms; and their culture method involves laying the vitelline membrane on an agar surface, with no culture ring, whereas it is known that normal expansion requires a fairly taut membrane[17].

Combination (G = *Gallus;* A = *Anas*)

Blastoderm	Membrane	Medium	Number of cultures	Expansion rate μm h^{-1} (mean±SD)	Probability (Student's t-test)
a) For any blastoderm and albumen type, does the vitelline membrane type affect the rate of expansion?					
A	A	A	10	368±68	
					$p > 0.10$
A	G	A	12	416±70	
G	A	A	15	304±43	
					$0.05 > p > 0.02$
G	G	A	10	349±47	
A	A	G	12	391±72	
					$p > 0.10$
A	G	G	10	407±57	
G	A	G	10	277±38	
					$p < 0.001$
G	G	G(1)	10	377±69	
					$0.10 > p > 0.05$
G	G	G(2)	12	311±48	
b) Does albumen type affect expansion rate?					
A	A&G	A	22	394±72	
					$p > 0.10$
A	A&G	G	22	398±65	
G	A&G	G	22	295±46	
					$0.10 > p > 0.05$
G	A&G	A	25	322±47	
c) Do *Anas* and *Gallus* blastoderms expand at the same rate?					
A	A&G	A&G	44	396±67	
					$p < 0.001$
G	A&G	A&G	47	309±49	
d) Do *Gallus* membranes allow faster expansion than *Anas* membranes?					
A	A	A&G	22	381±70	
					$p > 0.10$
A	G	A&G	22	412±63	
G	A	A&G	25	293±43	
					$p > 0.10$
G	G	G	12	311±48	

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Axoplasmic transport in regenerating limbs of *Ambystoma maculatum* larvae[1]

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Summary. Axoplasmic transport of ^{3}H-leucine labelled molecules from spinal cord segments into regenerating larval salamander limbs was observed. However, labelled molecules were not observed in cells of the regeneration blastema.

Although limb regeneration in urodele amphibians is nerve-dependent, the precise nature of the neural influence is not known. It is thought, however, that nerve cell bodies produce a trophic substance, likely a peptide or protein, which is transported intracellularly, by axons, to the regeneration areas. Presumably, this substance(s) then initiates and/or controls cellular activities[2,3]. Indeed, it has been established that nerves play a major role in the regenerative process by stimulating mesenchymatous cell proliferation[4–7]. Nerve-to-nerve cell and nerve-to-muscle cell transport has been reported in other systems[8,9], but whether or not this type of intercellular transport occurs during urodele limb regeneration is unknown. The present investigation was undertaken to determine whether or not some substance(s), synthesized in the spinal cord of larval *Ambystoma*, is transported via axons to the regeneration area of the forelimb and, possibly, into the blastema cells.

Materials and methods. Our methods involved labelling spinal cord segments, which innervated regenerating forelimbs, and studying the subsequent distribution of the label. *Ambystoma maculatum* larvae (20–25 mm) were collected locally, kept at 20 (±1) °C in individual dishes and fed *Tubifex* worms twice weekly. Donor larvae were anaesthetized in 0.2 g/l tricaine methanesulfonate and injected i.p. with 15 µCi (30 µl volume) of ^{3}H-L-leucine, ^{3}H-uridine, or ^{3}H-D-glucose using a No. 34 gauge needle (Hamilton). 24 h later these larvae were reanaesthetized. Next, their ^{3}H-labelled spinal cords were surgically removed in one piece from the cervical to pelvic regions, placed in amphibian Ringer's solution and cut into 2 or 3 segments. Using fine forceps as a probe, a tunnel was made mid-way in the dorsal tail-fin of an anaesthetized unlabelled host larva (figure 1), and a labelled donor spinal cord segment was transplanted (allografted) through the posterior tunnel opening. Next, the host animal's right forelimb was disarticulated at the shoulder and the skin removed from the upper-arm. Then, the denuded part of the limb was inserted into the anterior tunnel opening so that the head of the humerus abutted the labelled donor spinal cord segment[7,10] (figure 1).

Within 2 days the limb transplants (autografts) became vascularized and subsequently well-innervated by nerves from the allografted spinal cord segments (figure 1). Spasmodic movements of the autografted limbs indicated when innervation had occurred[7,10]. Animals were than anaesthetized and the transplanted limbs amputated through the radius-ulna or carpal regions (figure 1). After 2–14 days of regeneration, graft-bearing tails were fixed in G-Bouin's[11], embedded in paraffin, and serially sectioned at 5 µm. Routine liquid emulsion (Kodak NTB-2) autoradiography was employed, followed by staining with haematoxylin and orange-G-eosin[12]. Free, unincorporated ^{3}H-leucine was apparently washed-out during fixation and dehydration of tissues[13].

Results and discussion. In all 23 cases, the transplanted spinal cord segments were ^{3}H-labelled uniformly and heavily with 200–400 grains/1000 µm^2. No other tissues were labelled above background levels. In the 8 cases of the ^{3}H-leucine series, the nerve trunks that grew-out from the spinal cord transplants and innervated the unlabelled regenerating limbs were also heavily labelled (figures 2 and 3). Furthermore, labelled nerve fibres can also be traced out into the regeneration area (figures 4 and 5). In the 9 ^{3}H-uridine and 6 ^{3}H-glucose cases, however, there was no isotope found in the nerve trunks or nerves innervating the

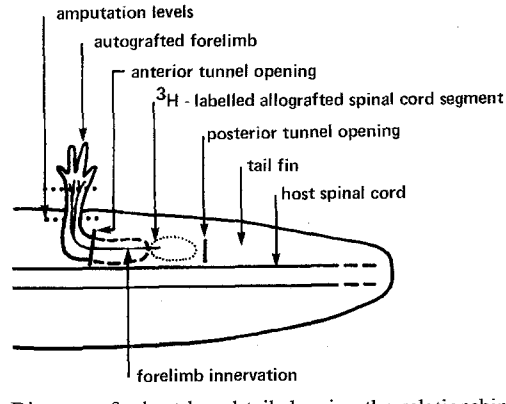

Fig. 1. Diagram of a host larval tail showing the relationship of an autografted forelimb and a ^{3}H-labelled, allografted, spinal cord segment in the tunnel of the dorsal tail fin of a non-labelled host larva. Amputation levels are indicated.