POLARIZING ACTIVITY IN THE DEVELOPING LIMB OF THE SYRIAN HAMSTER (1)

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ABSTRACT The transplantation of small pieces of tissue from the limb buds of 9 1/2 - 10 day hamster embryos to the wing bud of the chick results in the induction of supernumerary wing structures. The anteroposterior polarity of these induced structures is under the control of the transplanted hamster tissue. The developing hamster limb thus has limb polarizing activity similar to that found in avian species and, as in the chick, the activity is found primarily in the posterior region of the limb bud.

A region in the developing chick wing has been described as a zone of polarizing activity (Saunders and Gasseling, '68). This polarizing activity is confined to the mesoderm and the posterior region of the wing (A. MacCabe, Gasseling and Saunders, '73), though recent evidence suggests a gradient of activity exists (J. MacCabe and Parker, '75). The activity is defined by its ability to induce polarized supernumerary limb structures when tissue possessing the activity is transplanted to the apex or preaxial border of a host limb. The induced limb structures are polarized along their anteroposterior axis and the posterior border of the supernumerary growth develops facing the polarizing tissue. Polarizing activity has been found in several avian species (Balcuns et al., '70) but has not been reported in mammals. The results reported here show limb bud polarizing activity is present in the Syrian hamster in approximately the same location as that of the chick.

MATERIALS AND METHODS Pregnant LVG-LAK hamsters were purchased from

Lakeview Hamster Colony, Newfield, New Jersey and sacrificed on day 9 1/2 - 10 of gestation. The uterus was immediately removed, rinsed in sterile Tyrode's saline and the embryos were removed. The limb buds were cut off and small pieces (approx. .15 x .15 x .15 mm) of tissue excised to be assayed for polarizing activity.

Fertile White Leghorn chicken eggs were obtained from the University of Tennessee Department of Animal Science. The hamster tissue was assayed for polarizing activity by transplanting it to a four day (stgs. 21-22, Hamburger and Hamilton, '51) chick wing. Camera-lucida drawings were made of both donor and host limbs to record the site of the tissue donated and the transplant site. Host embryos were examined on the day following the operation to be sure the transplant remained in place. The hosts were allowed to develop for an additional 6-8 days and then were sacrificed, fixed in formalin and stained with methylene blue to reveal their cartilaginous skeletal elements. Several hosts were sacrificed one, two and three days after receiving the hamster transplant and fixed in Carnoy's fixative for histological examination.

RESULTS Four regions of the 9 1/2 - 10 day hamster hindlimb were assayed for polarizing activity: the posterior border, the anterior border, the distal tip and the mid-dorsal region. Tissues from these sites were transplanted to the anterior borders of right chick wings after removal of similar sized pieces from the hosts. Histological examination after one, two and three days revealed that the donor tissue remained healthy with little necrosis (fig. 1), though no outgrowth of the donor tissue was observed. After the hosts were sacrificed, supernumerary host wing structures were classified as "good" if sufficiently developed that anteroposterior polarity could be diagnosed, "poor" if polarity could not be diagnosed or "none" if the host wing failed to duplicate. A summary of these results is presented in table 1.

TABLE 1

Results of Transplanting Hamster Hindlimb Tissue to the Anterior Border of Chick Wing Buds

Origin of Donor Tissue	Number of Transplants	Host Limb Duplications		
		Good	Poor	None
Posterior border	37	20 (54%)	7 (19%)	10 (27%)
Anterior border	34	0	0	34 (100%)
Distal tip	35	0	3 (9%)	32 (91%)
Mid-dorsal region	37	1 (3%)	5 (14%)	31 (84%)

In nine of the 20 transplants from the posterior border resulting in "good" duplications, digits II and III were duplicated and anteroposterior polarity was recognizable on the basis of digit order and feather follicle distribution (fig. 2). The remaining eleven "good" duplications were supernumerary IIIs and the polarity was diagnosed by the distribution of follicles. All twenty of the polarized supernumerary structures had reversed anteroposterior polarity and were left supernumerary structures on a right wings. Thus their posterior borders developed adjacent to the transplanted hamster tissue. In each of the seven cases where transplantation of the posterior border tissue gave "poor" duplications the supernumerary structure was a digit II.

Tissue transplanted from the anterior border of the hamster hindlimb failed to induce supernumerary host wing structures (fig. 3). In most cases this was also true of donor tissue taken from the distal tip of the limb, however in three cases host wing duplication did result. In all three of these the supernumerary structure was a small digit II and anteroposterior polarity could not

be diagnosed. Tissue from the mid-dorsal region of the hamster hindlimb gave small wing duplications in a few cases, one of which had anteroposterior polarity indicated by the distribution of follicles. This supernumerary structure was an oversized digit II. The remaining five duplications induced by mid-dorsal tissue were IIs without recognizable polarity (fig. 4).

In addition to the results summarized in table 1, nine transplants from the posterior border of hamster hindlimbs were implanted into the tip, rather than the preaxial border, of host chick wings. Six of these induced supernumerary wing structures, three of which had recognizable polarity along the anteroposterior axis. In all three, the polarity was the same as that of the normal host wing structures and they were therefore right supernumerary structures on a right wing. Thus in these transplants the posterior border of the supernumerary structures again developed adjacent to the site of the donor tissue.

In a few cases tissue fragments from hamster forelimbs were transplanted to the anterior border of chick wings. Eight out of 10 fragments from the posterior border of the donor limb induced supernumerary structures in host

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FIGURE LEGENDS

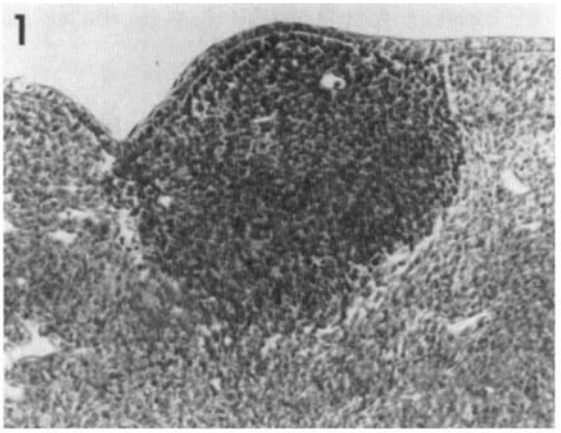
1 A sagittal section through the preaxial border of a 5 day chick wing 24 hours after receiving a transplant of hamster polarizing tissue (dark staining region). The sections are 5μ thick and stained with hematoxylin and eosin (200 X).

2a Supernumerary wing structures (arrows) induced by the transplantation of hamster polarizing tissue to the anterior border of a four day chick wing. The position of the small digit II and larger III and the distribution of follicles on the III indicate reversed anteroposterior polarity.

2b The same limb as shown in 2a after staining the cartilaginous skeleton with methylene blue and clearing in methyl salicylate (6 X).

3 An essentially normal wing that developed after transplanting anterior hamster limb tissue to the anterior border of the wing bud at four days of incubation (6 X).

4 A supernumerary digit II (arrow) that developed after transplanting tissue from the mid-dorsal region of a hamster hindlimb to the anterior border of the wing at four days of incubation (6 X).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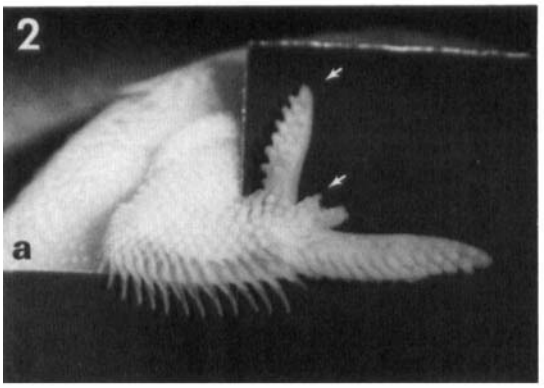
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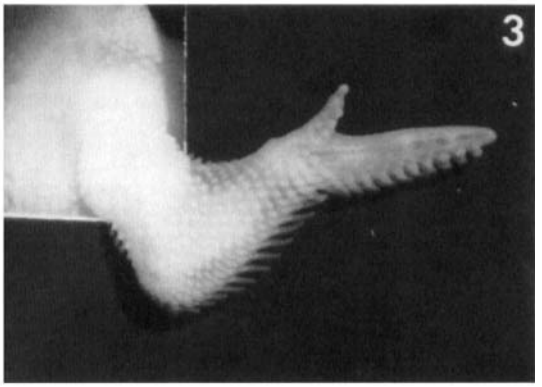
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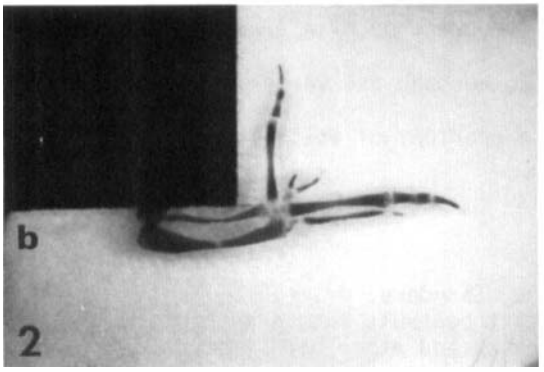
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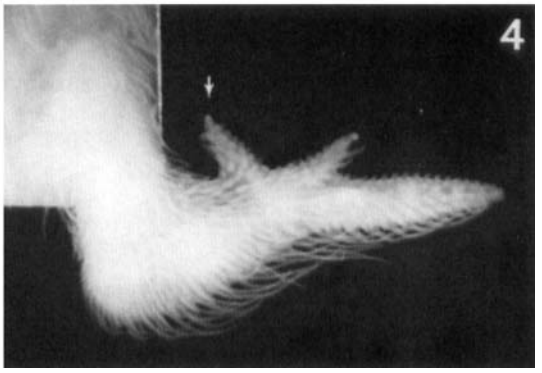
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limbs. Five of these had recognizable anteroposterior polarity and all were reversed with respect to normal host structures. Thirteen transplants from the anterior border of hamster forelimbs failed to induce supernumerary host wing structures.

DISCUSSION It is apparent that there is limb bud polarizing activity in the posterior region of the developing hamster limb similar to that found in the chick. The transplantation of tissue from this region to four day host chick wings frequently results in the development of supernumerary structures which are clearly of host type. In addition, regardless of the site of transplantation, the posterior border of the induced outgrowth always develops adjacent to the donor tissue. The transplanted hamster tissue thus controls the anteroposterior polarity of the outgrowth. The hamster polarizing activity appears by these criteria to be essentially indistinguishable from the polarizing activity of the chick.

Hamster polarizing activity seems to extend into the middle of the limb bud, though the activity there is weak as indicated by both the frequency and type of duplication obtained. Though no activity has been found in the middle of the chick limb using this in vivo assay, an in vitro assay has detected middorsal activity in the chick, suggesting the presence of a gradient of polarizing activity from posterior to anterior (J. MacCabe and Parker, '75). The evidence presented here suggests that such a gradient of activity may also exist in the hamster hindlimb.

LITERATURE CITED

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