period of vascular refractoriness does not seem to be a prerequisite.

Although hyperresponsiveness seems to develop whenever renin or angiotensin is administered chronically, its demonstration has been possible only under certain conditions: by showing that subpressor doses of angiotensin caused a sustained rise in pressure[3], or that the hypertension resulting from infusion of pressor doses of angiotensin remains unchanged when infusion rate is reduced[4]. In our experiment hyperresponsiveness was demonstrable when the rise in pressure caused by the renin contained in kidney extracts had subsided. Whenever angiotensin was injected during the response to an injection of renin, pressor responses instead of being increased were decreased thus suggesting that elevated amounts of circulating renin and angiotensin were masking the hyperresponsiveness.

As observed in dogs[5], we found that responses to another polypeptide vasopressin were decreased when superimposed on the response from kidney extracts. Results were however different concerning tyramine. The enhancement of tyramine responses by angiotensin or renin in dogs[5,6] contrasts with its absence in the rat. Responses to norepinephrine in rats and dogs were not affected by the administration of kidney extracts or angiotensin[5].

The fact that renal hypertension at least during its chronic phase is not consistently associated with an increase in plasma renin or angiotensin has been considered against the sustained participation of the renal pressor system. Presently available analytical procedures may not be sufficiently sensitive. Indirect evidence suggests that chronic renal hypertension is associated with small elevations in plasma angiotensin[5], which may be sufficient to maintain hypertension because of hyperresponsiveness.

Résumé. La réponse hypertensive à des injections s.c. d'extrait de rein n'ayant initialement que peu ou pas d'effet sur la tension artérielle s'accroit progressivement avec la durée du traitement.

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An Analysis of Distal Dominance in the Regenerating Limb of the Axolotl

The concepts developed to explain morphogenetic control of axial polarity of regenerating invertebrate organs, have recently been used in an attempt to explain the development of polarity during urodele limb regeneration[1]. It has been proposed that the distal-most regenerating organs in a linear system permit the morphogenesis of only those organs proximal to them. For example, in coelenterates the distal portion of a regenerating hydranth, when transplanted homoplastically to the distal end of a regenerating host hydranth, suppresses the morphogenesis of a similar distal organ but not of more proximal ones[2]. This inhibitory action is thought to be mediated by a proximally flowing substance which originates in the distal organ. Since, however, specific evidence for distal inhibition in urodele limb regeneration has not been reported, the purpose of this communication is to describe experiments designed to determine what, if any, influence a grafted distal organ has on the development of an homologous distal organ during urodele limb regeneration.

Materials and methods. The axolotl *Ambystoma mexicanum*, measuring 14–16 cm in overall length, was used exclusively. The animals were divided into 6 groups. Following anesthesia with MS222 (Sandoz), the right hind limbs of the axolotyles were amputated through the midfemoral region. In all but 1 of the 6 groups, the ventral $^2/_3$ of the wound surface was closed with sutures to prevent the initiation of host stump regeneration. In groups I, II, IV and V a distal organ (a small differentially pigmented donor foot amputated through the tarsal region) was homoplastically transplanted to the remaining wound surface. In group III, a donor limb segment, amputated through the distal end of the femur and again midway through the femur (a level similar to that of the host stump amputation surface), was transplanted in normal axial orientation, to the open dorsal wound surface as in groups I, II, IV and V and served as a control. To aid healing of the graft to the stump, the operated larvae were placed for 48 h in a refrigerator at 6 °C, after which they were placed in 20 °C incubators for the duration of the experiment. By 10 days all grafts were reinnervated and revascularized, and the sutured amputation surfaces of all groups but V were reopened at this time. To determine the effect of a fully differentiated distal organ on a freshly reamputated proximal limb stump (group I), the unobstructed area of the host limb's amputation surface was reopened by excising the sutured skin, but the foot graft was not amputated. To determine the effect of a freshly amputated distal organ on a freshly reamputated proximal limb stump (group II), the foot graft was amputated through the metatarsals at the same time that the unobstructed wound surface of the host stump was reopened. In the controls (group III), the host amputation surface was reopened at the same time that the distal surface of the limb segment graft was reopened. To determine the effect of a freshly amputated distal organ on a regenerating proximal limb stump (group IV), a mound blastema was allowed to form on the unobstructed wound surface of the host stump before the grafted foot was amputated through the metatarsals. To determine the effect of a regenerating distal organ on a freshly reamputated proximal limb stump (group V), the

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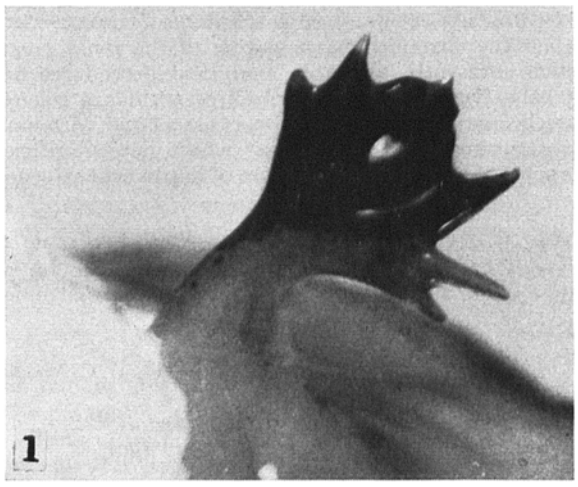

Fig. 1. A ventral view of a lightly pigmented host limb regenerate and a darkly pigmented foot graft. The proximal end of the foot graft (tarsal region) articulates at an homologous level (tarsal region) with the host limb regenerate. × 3.

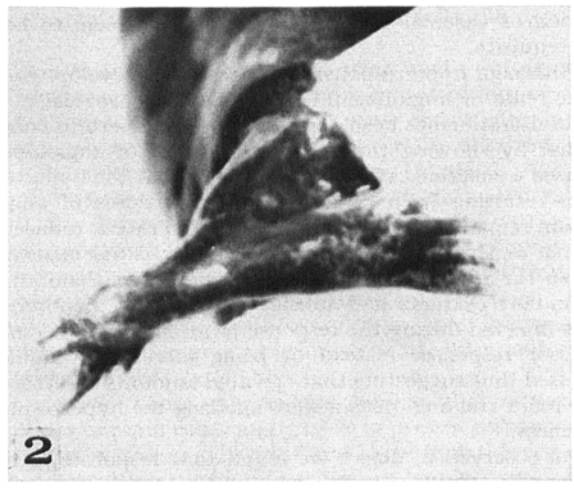

Fig. 2. A distal view of a regenerated proximal limb graft (left) and a host limb regenerate (right) from the same stump. The proxima limb segment articulates with the host stump regenerate at the mid-femur level. × 2.

Influence of a foot graft on the regeneration of a proximal limb stump

Group	No. of cases	No. of host stumps regenerating with viable grafts	No. of grafts regressing
I	10	7 (70%)	3 (30%)
II	8	6 (75%)	2 (25%)
III	13	8 (62%)	5 (38%)
IV	9	7 (78%)	2 (22%)
V	8	5 (63%)	3 (37%)
VI	7	7 (100%)	
Total	55	40 (73%)	15 (27%)

foot transplant was amputated through the metatarsals and allowed to form a mound blastema before reopening the unobstructed wound surface of the host stump. Finally, to determine the effect of a whole distal organ on a normal host blastema (group VI), a foot was transplanted to the dorsal side of the base of a normal mound blastema with no further surgical interventions.

Results and discussion. As shown in the Table, 27% of the transplants totally regressed. In all these cases the host stump regenerated a complete limb. In 73% of the cases, with no significant variation between groups, the host limb regenerated its missing distal structures despite the continued presence of the foot graft. Those grafts which were also amputated (groups II, IV, V) regenerated distal structures. Regeneration also occurred in both host and graft in the control group (III).

Under the experimental conditions described above, therefore, the presence of a grafted distal organ (a foot), whether regenerating or not, in no way prevented the regeneration of like distal structures in the host limb stump. It should be pointed out, however, that the data presented here apply only for 2 closely apposed separate organ systems. Experiments are now underway to determine if distal inhibitory influences are effective within a single limb blastema system.

An unexpected but highly interesting result of this study was the discovery of a precise relationship between the levels of a graft's proximal amputation and its articulation along the host regenerate's longitudinal axis. All limb grafts, except in group III, consisted of hind feet removed from the donor limbs by amputation through the tarsal cartilages. At the blastema formed on the unobstructed area of the wound surface of the host limb stump, it gradually enlarged until it incorporated the area of healing between the soft tissues of the graft and the host stump. As the regenerate of the host stump continued to grow and differentiate, the foot graft, now an appendage of the host's regenerate, was carried distally by the outgrowing blastema. All the 32 surviving foot grafts established permanent articulation at the tarsal level of the host regenerate – a level homologous with their own most proximal level (Figure 1). In none of the 8 surviving control proximal limb grafts (group III), on the other hand, did the developing host blastema carry the graft distad. These proximal grafts developed final articulation at the mid-section of the regenerated host femur – a level homologous with their own most proximal level (Figure 2). The mechanism of this 'homologous level articulation' is unknown but it deserves further study. The fact that a graft can apparently 'recognize' its homologous level along a host regenerate would seem to indicate that a disto-proximal gradient of morphogenetic character does exist in the axolotl limb regeneration system[3].

Résumé. La régénération d'une patte (*Ambystoma mexicanum*) n'est pas empêchée par la présence d'une greffe d'une patte sur la surface d'amputation d'un membre hôte. La greffe d'une patte cependant articule avec le régénérat hôte à un niveau homologue le long de l'axe du membre.

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