On the Mechanism of Distal Twinning of the Chick Embryo Limb Bud

Partial or total twinning of the hand takes place after reversal of the ap-axis of the wing bud apex in chick embryos of stages 18–23[1,2]. Exchange of the ap-reoriented apex between wing and leg bud leads to the same result[3]. Distal twinning was observed even in embryos operated at the stages 25–26 when the ap-reoriented apex was grafted to the amputation surface of the proximal third of the wing bud[4].

Apparently, the pre-axial mesoderm of the apex, which normally contributes little to the formation of the distal parts of the wing, undergoes enhanced growth and forms digits in excess when brought into association with proximal post-axial mesoderm. This seems to indicate that the development of the apical territories is governed by the proximal mesodermal districts of the limb bud[4]. The proximal territories would exert a direct organogenetic influence on the distal mesoderm[1,3–5]. Alternatively, it has been supposed that the limb bud mesoderm would evoke in the apical ridge of the ectoderm the ability to induce the outgrowth of the subjacent mesoderm[2]. According to the latter view, an uninterrupted activity of the ectodermal ridge maintained by a 'mesodermal factor' is required for the continued elaboration of distal limb elements from the reoriented limb apex. Evidence has been offered of the proximodistal transmission of 'structured material' through millipore filters interposed between the post-axial portion of the proximal mesoderm and the post-axial (originally pre-axial) region of the ap-reoriented wing apex from which supernumerary digits form[6]. 12 h or more of apex reversal would be required for the transmission of the 'maintenance factor' from the stump to the pre-axial tissues of the apex[6]. Whether the 'material' mentioned attains and activates the ectodermal ridge or whether it exerts a direct influence on the apical mesoderm, is still a matter of speculation[5].

If the inductor activity of the apical ridge of the reoriented limb apex were a prerequisite for distal twinning, the latter process would be prevented by removing the ridge. The following experiment shows that this is not the case.

The distal third, or fourth, of the wing bud was excised in chick embryos of stages 23–25, and grafted in ap-reversed orientation to the proximal stump of the same bud whose intermediate proximo-distal third had been discarded. From 5–10 h after, the apical ridge and a narrow band of the ectoderm of the adjacent ventral and dorsal surfaces were removed from the entire border of the reoriented apex, which was already firmly adherent to the bud stump and partially or completely revascularized. Embryos of stage 24 and 25 were used preferably, because the individuation of the distal wing territories is then nearly completed and therefore apical deficiencies consequent to removal of the ectodermal ridge are less severe than in earlier embryos[7,8]. The intermediate third of the wing bud was removed to prevent development of supernumerary pre-axial digits which have been shown to form from the bud segment mentioned under conditions similar to those of the present experiment[3,4].

The histological study of limb buds fixed from 10–90 h after the operation showed that the distal border of the graft was covered by regenerated commun thin ectoderm within 16–20 h. No indication of regeneration of the ectodermal ridge was observed. Numerous cells underwent regression in the uncovered mesoderm of the graft during the first 20–24 h. Secondary alterations of the superficial venous network became apparent. There occurred a relative developmental reduction of a part of the apical material; the reduction was less marked pre-axially and post-axially. Consequently, the originally convex profile of the apex became gradually flat, then concave distally: 2 divergent outgrowths developed. In general, the proximal part only of the hand elements forming in the outgrowths reached the cartilaginous stage; the more distal elements underwent a variously extensive reduction at the stage of mesenchymal blastemes or they failed to develop altogether.

Indications of symmetrical twinning of the grafted autopode were apparent, however, in the skeletal and in the cutaneous components in embryos fixed between the 11th and the 15th day. The number and the size of the

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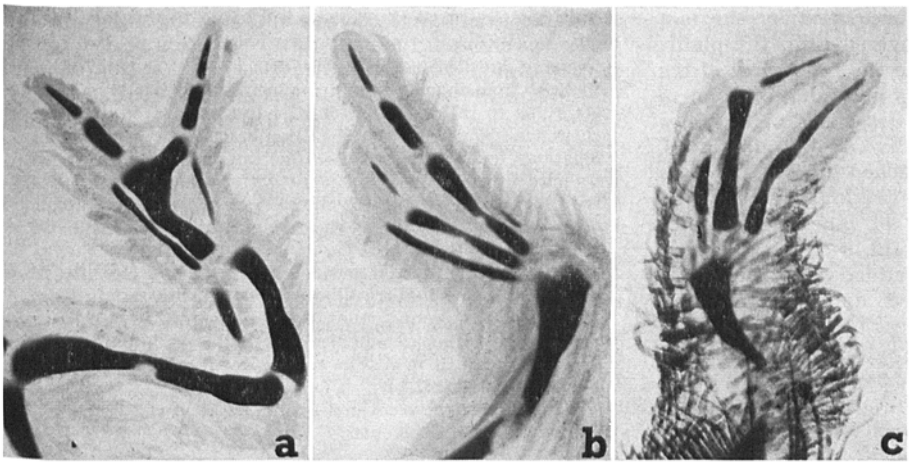

3 examples of twinning of the ap-reorientated apex of the right wing bud in embryos of the stages 24–25. In (a) the apical ridge was preserved, in (b) and (c) it was removed 5 and 6 h respectively after apex reversal. The wings are seen from the dorsal aspect.

skeletal pieces in the post-axial outgrowth were steadily greater than those of the 2nd digit of a merely ap-reversed hand. In several embryos a well recognizable 3rd digit, or the proximal part of the 3rd and 4th digit, developed post-axially and pre-axially. The feather pattern of the surfaces of the twinned hand was dorso-ventrally reversed; the feathers of the post-axial (originally pre-axial) border of the wing apex appeared symmetrical to those of the pre-axial border.

In the Figure 3 examples of twinning of the ap-reoriented apex of the right wing bud in embryos of the stages 24–25 are reported. In (a) the apical ridge was preserved, in (b) and (c) the apical ridge was removed 5 and 6 h respectively after apex reversal. The wings are seen from the dorsal aspect.

These findings indicate that twinning of the hand takes place under the conditions of the present experiment, although variously severe deficiencies of the terminal skeletal and cutaneous structures occur in the ap-reoriented (present experiment) as in the normally oriented apex of the wing bud deprived of its ectodermal thickening[7,8]. It can be concluded that twinning of the ap-reoriented prospective autopode does not depend on the presence and on the inductor activity of the apical ridge of the ectoderm. Apparently, it is the consequence of direct influences exerted by the proximal territories of the limb bud on the growth and the organogenesis of the apical mesoderm.

Résumé. L'excision de la crête apicale 5–10 h après l'inversion de l'axe antéro-postérieur de la partie distale du bourgeon de l'aile n'empêche pas le développement de duplications terminales. Ces duplications seraient dues au jeu d'influences organogénétiques des régions proximales de l'ébauche s'exerçant directement sur le mésoderme apical.

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A Metabolic Pathway of Gramine in Barley

In the last 20 years a great deal has been learned about the biogenesis and metabolism of alkaloids. Unfortunately the function of alkaloids in plants still remains a mystery.

Some interesting approaches to this problem deal with the translocation, distribution, degradation and the relationship between alkaloid content and age of the plant. An excellent account on these aspects of alkaloid biochemistry is that of MOTHES[1].

We wish to report here our recent findings on gramine, which may perhaps throw more light on the metabolism of this substance in barley.

The alkaloid gramine, which is formed in germinating barley from tryptophan[2–5], appears in barley shoots on the 3rd day after germination and remains there in detectable quantities until the 50th day[6]. We have experienced this phenomenon while experimenting with a native strain of Lebanese barley (designated as Baladi 25, Telamara) attributed to *Hordeum distichon* L. When we administered gramine (labelled in the carbon attached to the ring) to the above barley (60 days old), 0.84% of the alkaloid was converted into tryptophan[7]. In the course of this biosynthesis study we were impressed by the fact that no labelled gramine was recovered from the plant; we therefore undertook to examine the possibility of the biological degradation of gramine's methylene carbon to carbon dioxide.

In a representative experiment, five 60-day-old shoots, cut very close to their grain and washed thoroughly, were allowed to stand in a cup within a bottle (covered with a black cloth) containing 1.98 mg of labelled gramine[8] ($10.5 \cdot 10^6$ dpm/mg) dissolved in 2 ml of $5 \cdot 10^{-3}$ *N*-acetic acid[9]. 5 ml of distilled water were added daily to the cup and, when nearly all was absorbed, another 5 ml were added in order to attain the greatest possible absorption of the radioactive material. Carbondioxide-free air was allowed to carry the carbon dioxide expired by the plants into 2 barium hydroxide traps. The precipitated barium carbonate was collected every 6 h for radioactivity counting[10]. Since the carbonate was found to be highly radioactive, samples were collected for 8 days. The plants were then removed from the bottle, washed thoroughly, dried (at 50 °C for 24 h) and subsequently cut into small pieces to produce 2.4 g of herb.

Very little radioactive gramine[11] ($1.0 \cdot 10^4$ dpm) was recovered when a sample (1.2 g) of the herb was analysed by a method similar to that of GOWER and LEETE[5]. When the protein fraction of another plant sample (1.2 g) was examined, according to our previous method[7], it was found that 0.4% of the gramine was incorporated into tryptophan. An aliquot from the total $BaCO_3$ (collected during 8 days) was converted to CO_2, which was trapped

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[8] Gramine, labelled in the carbon attached to the ring, was synthesized from indole, dimethylamine and C^{14} paraformaldehyde according to the procedure of H. KUHN and O. STEIN, Ber. dt. chem. Ges. *70*, 567 (1937). The C^{14} gramine was purified by 2 sublimations, and after 1 crystallization from *n*-hexane was shown to be pure by mixed melting point with authentic gramine, UV-spectroscopy and thin-layer chromatography in 2 solvents. Subsequent radioautography of the chromatograms revealed only one radioactive spot.
[9] All radioactivity measurements unless otherwise specified were performed in a liquid-scintillation counter with an efficiency of 70% and a background count of 12 counts/min.
[10] The barium carbonate samples were plated at infinite thickness and were counted in a thin-window gas-flow counter (Hewlett-Packard Model 5202 L).
[11] The identity of the recovered gramine was proved by chromatography (TLC) in 2 solvents.