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Polarizing and maintenance activities in two polydactylous mutants of the fowl: *diplopodia*[1] and *talpid*[2]

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SUMMARY

In two polydactylous mutants, *talpid*[2] and *diplopodia*[1], polarizing activity appears to be present only in the normal location in the posterior corner of the limb-bud. There is, thus, a preaxial extension of maintenance activity without an accompanying extension of demonstrable polarizing activity. Experiments involving apex exchanges between normal and *talpid*[2] wing-buds suggest that the movement of 'maintenance factor' *per se* from preaxial *talpid*[2] limb tissue into a normal apex does not occur.

INTRODUCTION

Gasseling & Saunders (1964) reported that tissue from the posterior border of the early chick wing-bud has an important role in determining limb symmetry. When tissue from this area was grafted to the anterior border or to the apical tip of a host wing a second outgrowth formed from preaxial limb tissue, a region which normally contributes little to limb outgrowth. The grafted tissue controlled the polarity of the anteroposterior (a–p) axis since the posterior border of the induced outgrowth always faced the graft site. This region thus functions both to induce the outgrowth of supernumerary limb structures from preaxial tissue and to determine the polarity of the newly induced outgrowth. The active area has been referred to as the zone of polarizing activity by Balcuns, Gasseling & Saunders (1970). Other means of bringing tissue from the polarizing zone into contact with preaxial (uninduced) limb tissue also result in duplications. If a wing apex is excised, reoriented 180° and replaced on its own cut stump, duplicate wing tips form (Saunders, Gasseling & Gfeller, 1958; Amprino & Camosso, 1958). There is evidence that these duplications are due to the transmission of an influence from the limb stump into the reoriented

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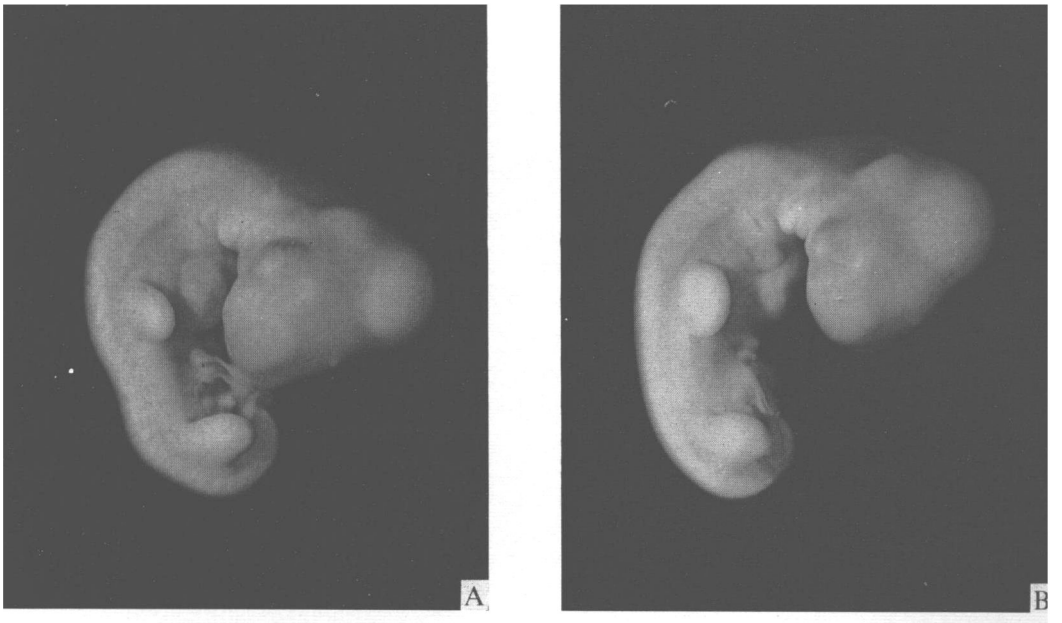

Fig. 1. Normal (A) and *talpid*^{2} (B) embryos of 4 days incubation. The broader wing-bud is evident in the mutant while the leg-bud is still very similar to that of the normal sib.

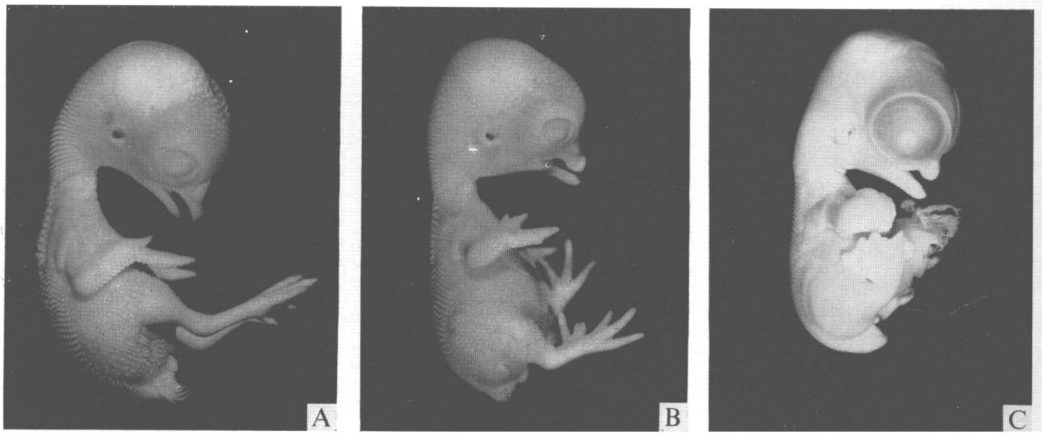

Fig. 2. Normal (A), *diplopodia*^{1} (B) and *talpid*^{2} (C) embryos at 11 days incubation. The *diplopodia*^{1} embryo has the normal digits plus additional digits preaxially. In the *talpid*^{2} limbs normal digits cannot be recognized. Both limbs have eight or nine syndactylous digits which are symmetrical in the anteroposterior axis.

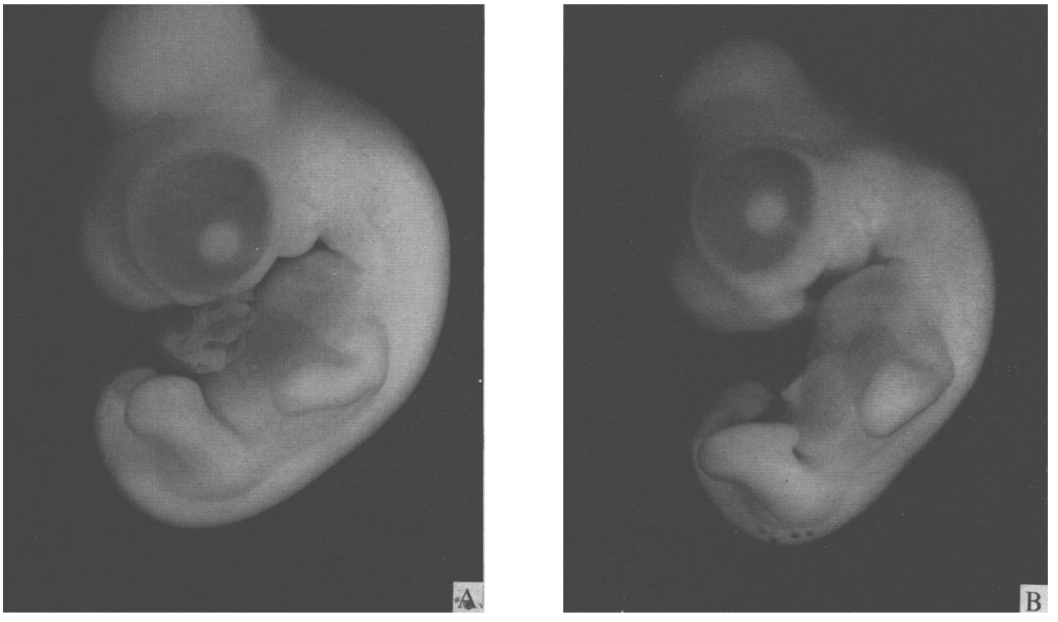

Fig. 3. Normal (A) and *diplopodia*[1] (B) embryos of 5½ days incubation. Note the extra preaxial thickening on both of the mutant limb-buds.

apex (Saunders & Gasseling, 1963). Several experiments have indicated that it is the polarizing zone that is the source of the transmissible agent that accounts for such duplications (MacCabe & Abbott, in preparation).

In the studies reported here two polydactylous mutants have been used to further explore the roles of maintenance and polarizing activities in the developing avian limb. Several polydactylous mutants affect the pattern of asymmetry in the a–p axis of the limb (Abbott, 1967, for review). In *talpid*[2] (Abbott, Taylor & Abplanalp, 1960) the normal asymmetry in the a–p axis is never established and the embryos develop broad limb-buds which retain their symmetrical shape (Fig. 1). The *talpid*[2] wing-bud develops from a more extensive base than does the normal and can be distinguished by stage 18; the mutant leg bud can be recognized later, at approximately stage 20. At 11 days (Fig. 2), both appendages are broad and short with 9–10 syndactylous digits, none of which are specifically recognizable as members of the normal digit complement. All the long bones are reduced, with the most proximal being most affected. The defect in *talpid*[2] has been shown to be localized in the mesodermal component of the limb (Goetinck & Abbott, 1964; Fraser & Abbott, 1971).

In the *diplopodia*[1] mutant, which is also mesodermal (Abbott, 1959, 1967), extra digits are present preaxially in addition to the normal digits that develop from largely postaxial limb-bud tissue. In this respect the *diplopodia*[1] limb

resembles duplications obtained by limb apex reorientation (Amprino & Camosso, 1958, 1959; Saunders *et al.* 1958; Saunders & Gasseling, 1959). In *diplopodia*[1] embryos both limb-buds appear normal until about stage 20–21. At this time an area of preaxial outgrowth becomes evident on both wing- and leg-buds. This can be seen clearly in the 5½-day *diplopodia*[1] embryo illustrated in Fig. 3. In older *diplopodia*[1] embryos the wings typically have the three normal digits, II, III and IV, present in addition to three supernumerary digits located proximal to the position of the pollex (Fig. 2). The legs usually have three supernumerary digits anterior to the position of the normal hallux and the most proximal of these is usually the largest.

MATERIALS AND METHODS

Both *diplopodia*[1] and *talpid*[2] are autosomal recessive genes and are lethal in late incubation stages. *Talpid*[2] embryos usually die between 14 and 16 days of incubation and *diplopodia*[1] embryos between 18 days and hatching. Since mutant embryos for experimental studies must be obtained from matings between heterozygous carriers, only about one quarter of the fertile eggs are expected to contain mutants. The proportion available is usually somewhat less because there is, under experimental conditions, a slightly higher mortality of mutants than of normal segregants. In this study a large proportion of the experimental operations were performed at stages before either of the mutants could be distinguished from their normal siblings and, in these cases, it was necessary to remove the donor tissue *in ovo* and return the donors to the incubator until they reached a stage when the phenotype was clearly evident. In most cases *camera lucida* drawings were made of the operation at the time it was performed and on the following day. Embryos were sacrificed after a total of 11–12 days of incubation. Some were stained in methylene blue to reveal cartilage structures. Normal segregants served as one source and incrossbred White Leghorn embryos served as an additional source of normal controls.

Several areas of both mutant wings were tested for polarizing activity. In each case a small piece of tissue was transplanted from a donor limb to a normal host wing-bud in which a graft site had been prepared by the removal of a piece of tissue of equivalent size. The donor tissue to be tested was transplanted to either the apex or the preaxial border of the host wing. The development of duplicate host structures in the preaxial limb tissue was taken as positive evidence for polarizing activity in the donor tissue.

The apices of wing-buds were reoriented 180° in *diplopodia*[1], *talpid*[2] and normal embryos. In each case, the apical $\frac{1}{4}$–$\frac{1}{3}$ of stage 20–23 embryo wing-buds was excised either with a glass needle or with scissors made from watchmaker's forceps. Small glass tacks were used to hold the apex in place until attachment occurred (after Saunders *et al.* 1958).

Apex exchanges between normal and mutant limbs were performed with the

Table 1. *Tests for polarizing activity (stages 20–23)**

	No. of grafts	No. with polarizing activity	No. with outgrowth of donor tissue†
A. Posterior corner of *talpid*^{2} wing	12	12	0
B. Anterior corner of *talpid*^{2} wing	18	0	8+1?
C. Distal tip of *talpid*^{2} wing	17	0	9
D. Central mesoderm of *talpid*^{2} wing	13	0	0
E. Posterior corner of *diplopodia*^{1} wing	12	11	0
F. Anterior corner of *diplopodia*^{1} wing	15	0	5
G. Distal tip of *diplopodia*^{1} wing	8	0	2

* All stages according to Hamburger & Hamilton, 1951.

† Slight outgrowth of donor tissue occurred in a proportion of grafts taken from regions other than the posterior corner of the wing.

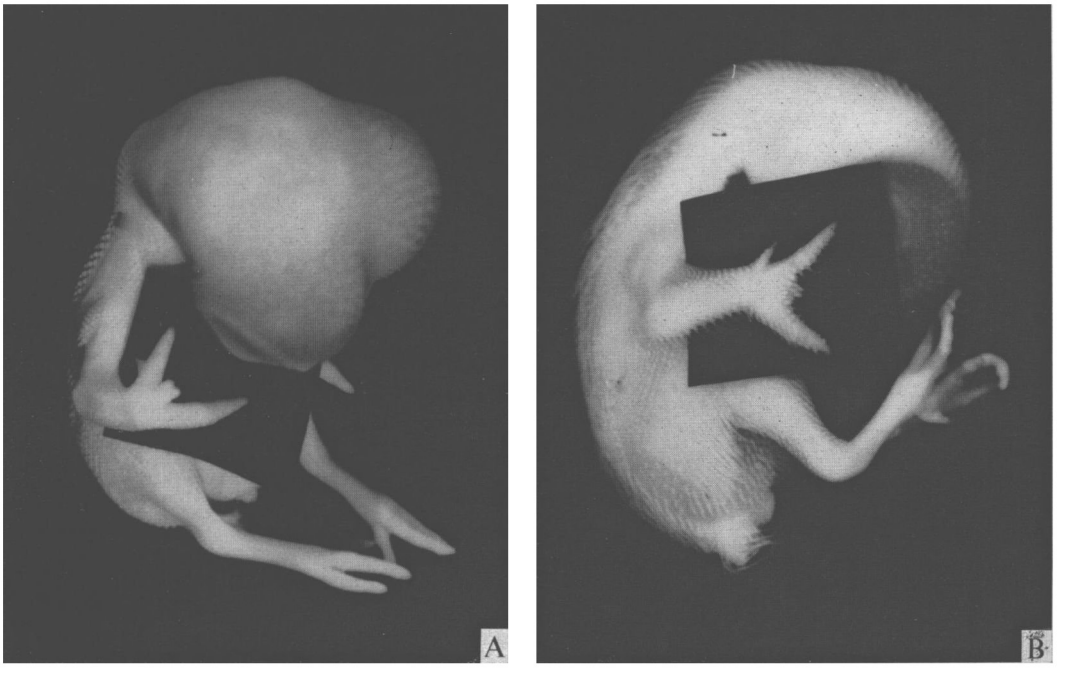

Fig. 4. Wing duplications resulting from grafting polarizing tissue from (A) a 4-day *talpid*^{2} wing-bud to the anterior border of a normal 4-day host wing-bud and (B) a 4-day *diplopodia*^{1} wing-bud to the apex of a 4-day normal host wing-bud.

Table 2. *Wing apex reorientation (Hamburger–Hamilton stages 20–23)*

	No. of grafts	No. with duplicate wing tips
Talpid^2 wing	14	0
Diplopodia^1 wing	9	8
Normal wing	68	64

Table 3. *Wing apex exchanges (Hamburger–Hamilton stages 20–23)*

	No. of grafts	No. with duplicate wing tips
Talpid^2 apex on normal stump		
Normal orientation	10	0
Reversed a–p orientation	18	0
Normal apex on *talpid*^{2} stump		
Normal orientation	13	0
Reversed a–p orientation	9	9

a–p axis of the apex either normal or reversed with respect to the stump. Host embryos were examined for duplications of the transplanted apex on the second and third days following the operation and again at sacrifice after 11–12 days of incubation.

RESULTS

Grafts of tissue from the polarizing zone of both *talpid*^{2} and *diplopodia*^{1} embryos to the apex or to the anterior border of a normal host wing produced duplications indicating that both had polarizing activity in the normal location (Table 1; Fig. 4A, B). These duplicate limb structures were identical to those which are induced by polarizing tissue from normal limbs. In most cases, the a–p polarity of the duplicate structure could be determined and in all of these the posterior border faced the graft site. Grafts from other areas of mutant wings did not produce duplications, although in a number of cases some outgrowth of donor tissue occurred. This was most frequent in grafts from *talpid*^{2} donors. In all but one case the extra digit produced was identified as being of donor origin by examination 1 or 2 days following the operation. In a single case, taken from the anterior corner of a talpid wing, there was no evidence of donor outgrowth 48 h after the operation and a single digit formed. It did not resemble the structures obtained from grafts of polarizing tissue (Table 1 B). Our results thus showed that neither the anterior corner nor the distal tip of *talpid*^{2} and *diplopodia*^{1} wings had polarizing activity nor did grafts taken from

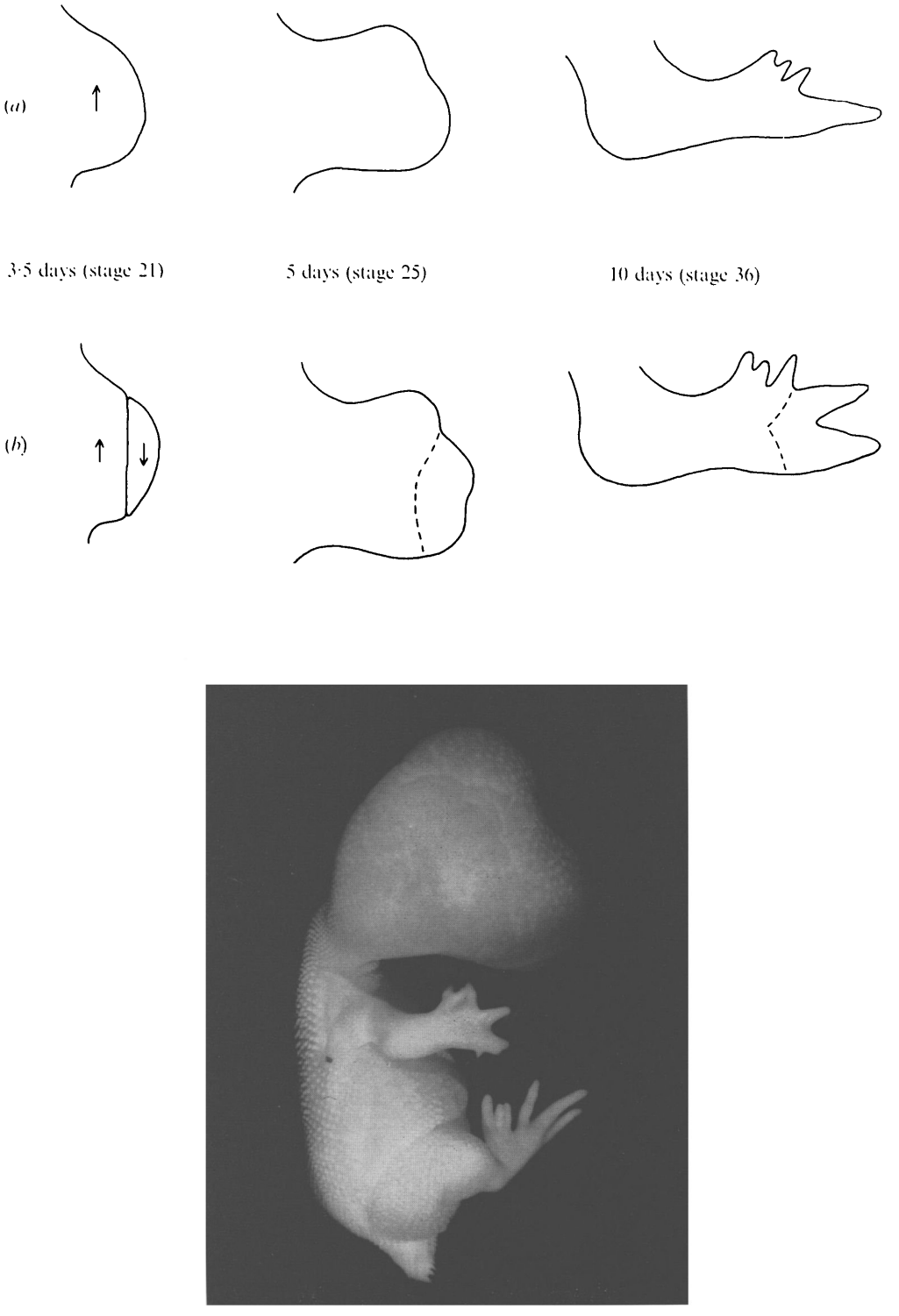

FIGURE 5

Diagram of three stages of wing outgrowth in *diplopodia*[1] (*a*) and outgrowth following wing apex reversal at 4 days (*b*). The photograph illustrates the duplication obtained in this operation. Accessory digits are located proximal to the duplicated apex.

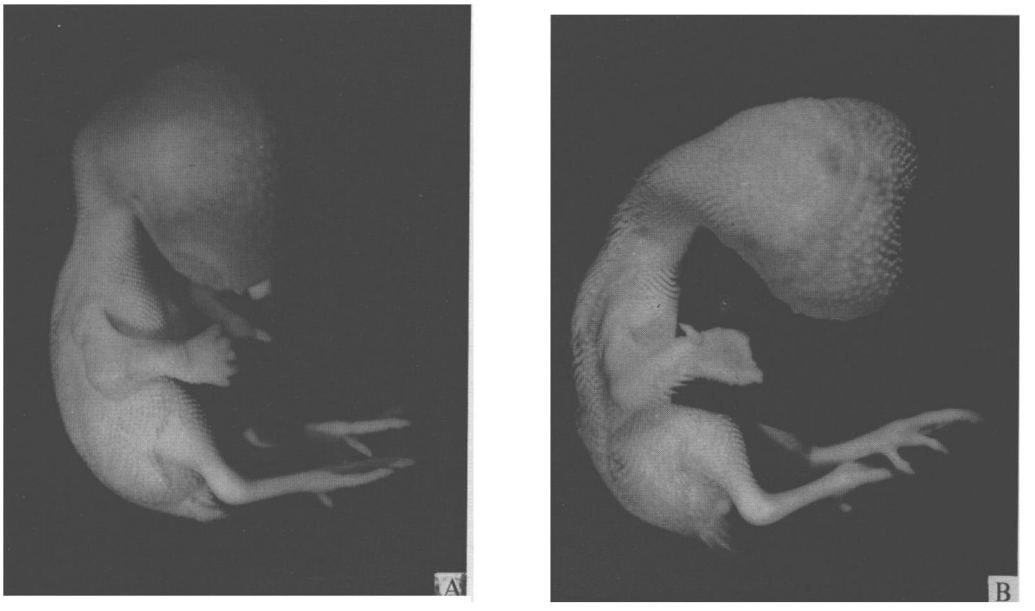

Fig. 6. Wings resulting from grafting a *talpid*^{2} apex on to a normal stump in normal orientation (A) and reversed a–p orientation (B). No duplication of the *talpid*^{2} apex occurs.

central mesoderm in *talpid*^{2}. In this respect the results obtained with both mutants resembled those obtained with grafts from normal control wings (MacCabe, 1971).

The series of grafts in which the apex of the wing was excised and replaced in reverse orientation showed that normal and *diplopodia*^{1} wings responded in the same manner, i.e. duplicate wing tips formed but *talpid*^{2} wings did not duplicate (Table 2). In *diplopodia*^{1} extra preaxial structures developed in addition to the duplicate wing tips (Fig. 5).

The results of the series of apex exchanges between *talpid*^{2} and normal wings in normal and reversed a–p orientation are shown in Table 3. When a *talpid*^{2} apex was placed on a normal stump, only a single wing tip formed irrespective of whether the graft was placed in normal orientation or in reversed a–p orientation (Fig. 6). In contrast, a normal apex on a *talpid*^{2} stump duplicated in reverse orientation, but did not duplicate in normal orientation (Fig. 7). In general these unduplicated apices were somewhat larger than the duplicate apices induced by placing the apex on the *talpid*^{2} stump in reversed antero-posterior orientation. In addition, three grafts of a *diplopodia*^{1} apex on to a normal stump and three of a normal apex on a *diplopodia*^{1} stump in normal orientation all failed to duplicate.

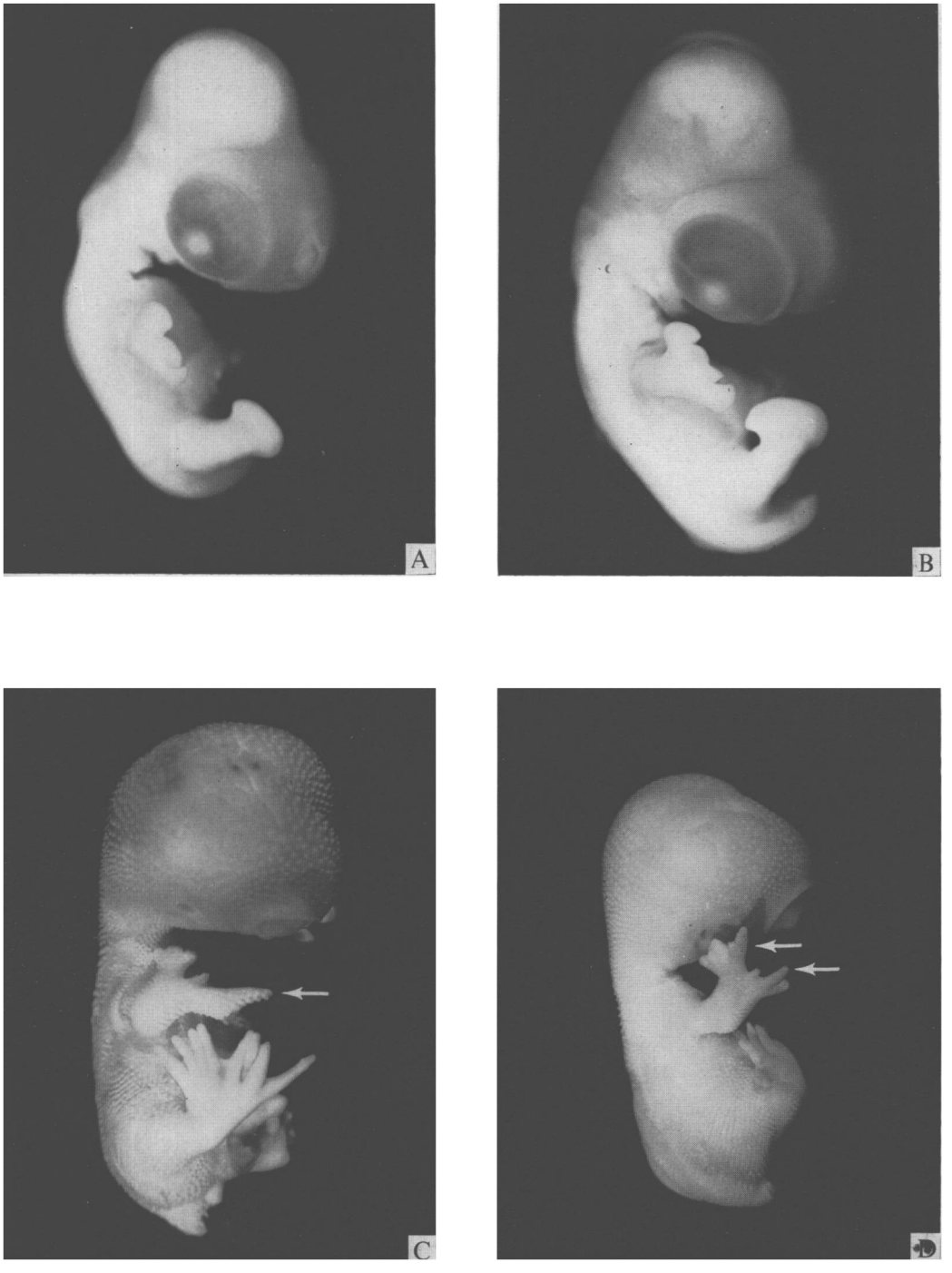

Fig. 7. Two stages in the development of a normal apex grafted to a *talpid*^{2} stump in normal (A and C) and reversed (B and D) orientation. Duplication of the apex occurs in reversed but not in normal orientation.

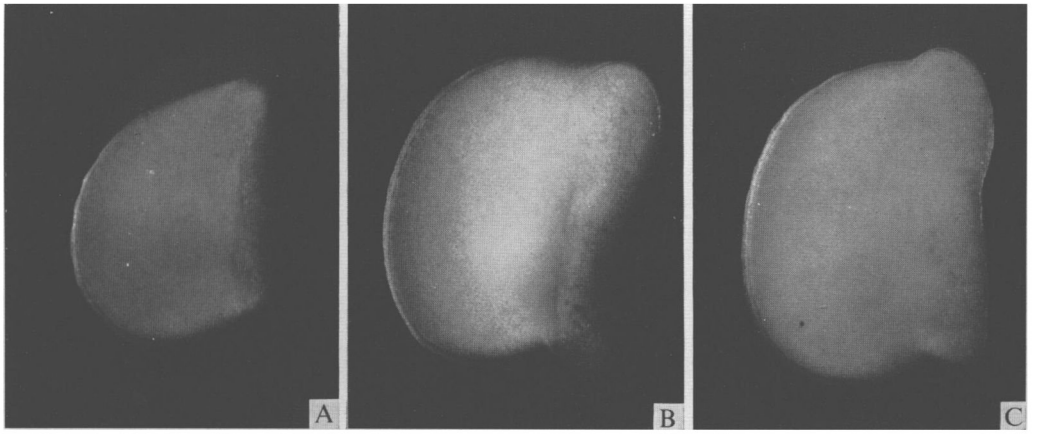

Fig. 8. Four-day right wing-buds from normal (A), *diplopodia*[1] (B), and *talpid*[2] (C) embryos. This view of the inner limb surface shows the preaxial extension of the apical ectodermal ridge in both mutant limbs. × 160.

DISCUSSION

Grafts of polarizing tissue from the avian limb will induce duplicate limb structures from preaxial limb-bud tissue. These supernumerary structures have a specific anteroposterior polarity which is determined by the position of the grafted polarizing tissue. Previous studies have suggested that a transmissible agent from the polarizing tissue is responsible for duplications resulting from apex reorientation and that the ridge maintenance property of the mesoderm is not transmissible (MacCabe & Abbott, in preparation). The results obtained in these experiments support the suggestion that 'maintenance factor' (Zwilling & Hansborough, 1956) is not transmitted freely through limb mesoderm. The exact relationship between polarizing and maintenance activities is not clear. However, grafts of polarizing tissue to the preaxial region of the limb result in ridge thickening and subsequently, additional outgrowth (Saunders & Gasseling, 1968), and therefore presumably in the development of ridge maintenance activity by the underlying mesoderm.

Both *talpid*[2] and *diplopodia*[1] have polarizing activity in the normal location and none in the central or anterior regions of the wing. Preaxial limb outgrowth occurs in both of these mutants, accompanying preaxial ridge thickening (Fig. 8) and therefore underlying ridge maintenance activity. In *diplopodia*[1] the normal asymmetry appears to be present in the postaxial region of the limb, but there is additional thickening of the ridge preaxially, and thus maintenance activity preaxially as well. This maintenance of preaxial ridge may be independent of polarizing activity since there is no additional source of polarizing activity in this location. In *talpid*[2] the thickened ridge extends continuously along the entire periphery of the limb-bud. Presumably, therefore, maintenance activity is distributed more or less uniformly throughout the *talpid*[2] limb.

This situation offers an opportunity to test the concept of a transmissible maintenance activity. Can maintenance activity be transmitted from the *talpid*^{2} preaxial mesoderm to the preaxial region of a normal limb apex, and induce preaxial outgrowth? This was tested by excising a *talpid*^{2} wing apex and replacing it with a normal apex in normal orientation. No duplications of the apex occurred. Apparently maintenance activity is not capable of moving from the *talpid*^{2} stump into the preaxial region of the apex. Duplications do occur if the normal apex is placed on a *talpid*^{2} stump in reversed a–p orientation, presumably as a result of the normally located polarizing tissue along the posterior border of the limb. If a *talpid*^{2} apex is reoriented 180° on its own stump, no observable duplications occur. This is apparently due to the inability of the *talpid*^{2} apex to respond to the polarizing influence since the *talpid*^{2} apex also fails to duplicate when reversed on a normal limb stump. This is not unexpected since the entire distal border of the *talpid*^{2} limb is already under the influence of a thickened ectodermal ridge, i.e. limb digits form from preaxial as well as postaxial limb tissue. Apparently tissue which is already committed to form digits is not capable of responding to polarizing activity. This not only includes preaxial *talpid*^{2} tissue but also postaxial limb tissue of *talpid*^{2} and normal limbs.

Though this study suggests that mesodermal maintenance activity is not transmissible, it tells us little about the origin of the *talpid*^{2} defect. Possibly, either the preaxial extension of the ridge is unrelated to polarizing activity or alternatively, the polarizing influence is more readily transmitted through *talpid*^{2} mesoderm. Work with a similar mutant, *talpid*^{3} (Ede & Kelly, 1964; Ede & Agerbak, 1968) suggested that the morphological abnormalities reflected an increased mesodermal cell adhesiveness and a reduced cell motility. Niederman & Armstrong (1972), however, were unable to demonstrate sorting between normal and *talpid*^{2} cells *in vitro*.

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