Epithelial–mesenchymal interactions in the development of chick facial primordia and the target of retinoid action

S. E. WEDDEN*

Department of Anatomy and Biology as applied to Medicine, The Middlesex Hospital Medical School, Cleveland St, London W1P 6DB, UK

* Present address: Department of Physiology and Biophysics, Harvard Medical School, 25 Shattuck St, Boston, Massachusetts 02115, USA

Summary

The development of the chick face involves outgrowth of buds of tissue, accompanied by the differentiation of cartilage and bone in spatially defined patterns. To investigate the role of epithelial–mesenchymal interactions in facial morphogenesis, small fragments of facial tissue have been grafted to host chick wing buds to continue their development in isolation. Fragments of the frontonasal mass give rise to typical upper-beak-like structures: a long central rod of cartilage, the prenasal cartilage and an egg tooth. Meckel's cartilage, characteristic of the lower beak, develops from fragments of the mandible. Removal of the ectoderm prior to grafting leads to truncated development. In fragments of frontonasal mass mesenchyme only a small spur of cartilage differentiates and there is no outgrowth. The mandible is less affected; a rod of cartilage still forms but the amount of outgrowth is reduced.

Retinoid treatment of chick embryos specifically affects the development of the upper beak and outgrowth and cartilage differentiation in the frontonasal mass are inhibited. The mandibles, however, are unaffected and develop normally. In order to investigate whether the epithelium or the mesenchyme of the frontonasal mass is the target of retinoid action, recombinations of retinoid-treated and untreated facial tissue have been grafted to host wing buds. Recombinations of retinoid-treated frontonasal mass ectoderm with untreated mesenchyme develop normally whereas recombinations of untreated ectoderm with retinoid-treated mesenchyme lead to truncations. The amount of outgrowth in fragments of mandibular tissue is slightly reduced when either the ectoderm or the mesenchyme has been treated with retinoids. These recombination experiments demonstrate that the mesenchyme of the frontonasal mass is the target of retinoid action. This suggests that retinoids interfere with the reciprocal epithelial–mesenchymal interactions necessary for outgrowth and normal upper beak development.

Key words: epithelial–mesenchymal interactions, retinoids, facial morphogenesis, chick embryos.

Introduction

The chick face develops from populations of cells derived mainly from the neural crest (Noden, 1975; Le Lievre, 1978). The frontonasal mass, a bud of tissue covered by ectoderm, forms most of the upper beak and contains neural crest cells that have migrated from the level of the prosencephalon. The lower beak develops from paired buds of tissue, the mandibular primordia, and contains both mesencephalic and rhombencephalic neural crest cells. The development of both upper and lower beaks involves considerable outgrowth, accompanied by the differ-

entiation of cartilage and bone in spatially defined patterns. In the upper beak a central rod of cartilage, the prenasal cartilage develops. In the lower beak, paired rods of cartilage, Meckel's cartilages, form.

The development of limbs appears fundamentally similar to that of the beaks. Small buds of tissue grow out with the accompanying laying down of patterns of tissues. It has been well established that epithelial–mesenchymal interactions are involved in the development of the chick limb. The apical ectodermal ridge, a thickened rim of epithelium at the tip of the bud is required for outgrowth (Saunders, 1948; Summerbell, 1974). The apical ectodermal ridge also

maintains the progress zone, a region of undifferentiated cells at the tip of the limb that is involved in pattern formation (Summerbell, Lewis & Wolpert, 1973). In addition to the interaction between the apical ectodermal ridge and the mesenchyme, a reciprocal interaction involving a signal from the mesenchyme is required to maintain the apical ridge (Zwilling & Hansborough, 1956).

Interactions with epithelia are required for chondrogenic and osteogenic differentiation of avian cephalic neural crest cells (Tyler & Hall, 1977; Hall, 1980; Bee & Thorogood, 1980). However, it is not known whether epithelial–mesenchymal interactions are involved in the outgrowth of the facial primordia and in the laying down of the spatial pattern of differentiated tissues. A progress zone mechanism may be involved in upper beak development. This is suggested by the 'sheet' of cartilage that forms in micromass cultures of cells from the frontonasal mass (Wedden, Lewin-Smith & Tickle, 1986). This chondrogenic pattern is strikingly similar to the pattern obtained with cells from the progress zone of chick limb buds (Archer, Cottrill & Rooney, 1984). This suggests that the frontonasal mass consists of a homogeneous population of potentially chondrogenic cells which have yet to receive positional cues to direct their differentiation. In mandible cell cultures the pattern of chondrogenesis is more complex.

Following retinoid treatment of embryos, the upper beak does not develop and outgrowth and cartilage differentiation in the frontonasal mass are inhibited (Tamarin, Crawley, Lee & Tickle, 1984). In contrast, the development of the mandible is unaffected, even when retinoids are applied over a wide range of developmental stages (Wedden & Tickle, 1986a). The absence of cartilage in the upper beak does not appear to be due to an inhibition of chondrogenic differentiation (Wedden, Lewin-Smith & Tickle, 1987). Retinoids appear to affect the coupled processes of outgrowth and pattern formation that may be mediated by epithelial–mesenchymal interactions.

To gain insight into the mechanisms involved in the development of the frontonasal mass and mandible, the role of epithelial–mesenchymal interactions has been investigated using a grafting procedure that allows small fragments of facial tissue to develop in isolation (Wedden & Tickle, 1986b). The results show that removal of the ectoderm from facial primordia leads to truncations. The development of the frontonasal mass is more affected than the mandible. Using this approach, the question of whether the epithelium or mesenchyme of the frontonasal mass is the target of retinoid action has been investigated. Recombination experiments show that the mesenchyme of the frontonasal mass is the tissue that is directly affected

by retinoid treatment. This suggests that retinoids interfere with the reciprocal epithelial–mesenchymal interactions necessary for normal upper beak development.

Materials and methods

Grafting of facial tissue

Normal or retinoid-treated stage-24 chick embryos (Hamburger & Hamilton, 1951) and equivalent stage quail embryos were used to provide the tissue for grafting. The frontonasal mass and mandibles were dissected in culture medium (Minimum Essential Medium + 10 % fetal calf serum + 4 mM-L-glutamine + 200 units ml^{-1} penicillin, 200 μg ml^{-1} streptomycin and 0·5 μg ml^{-1} fungizone (antibiotic–antimycotic) (Gibco Biocult)). The frontonasal mass was divided into two or three fragments of approximately 0·5–0·8 mm^3 and each mandibular primordium was either left whole or divided into half (approx. size 0·2–0·4 mm^3) (Fig. 1). Each of these tissue fragments was grafted into the wing bud of a host stage-23 to -24 chick embryo. A small cube of tissue was removed from the dorsal surface of the host right wing bud and the piece of tissue to be grafted was manoeuvered into the hole, ensuring the correct orientation. The ectoderm, if present, was placed uppermost. Once the graft was in place the egg was resealed and reincubated at 38°C. Grafts were made of fragments of facial primordia, facial mesenchyme, facial primordia from embryos treated with retinoids and recombinations of epithelial and mesenchymal tissues of the face.

Retinoid treatment of chick embryos

Embryos were treated with retinoids using the method described in earlier studies (Tamarin *et al.* 1984). This method of application reproducibly affects the development of the face without causing any physical damage to
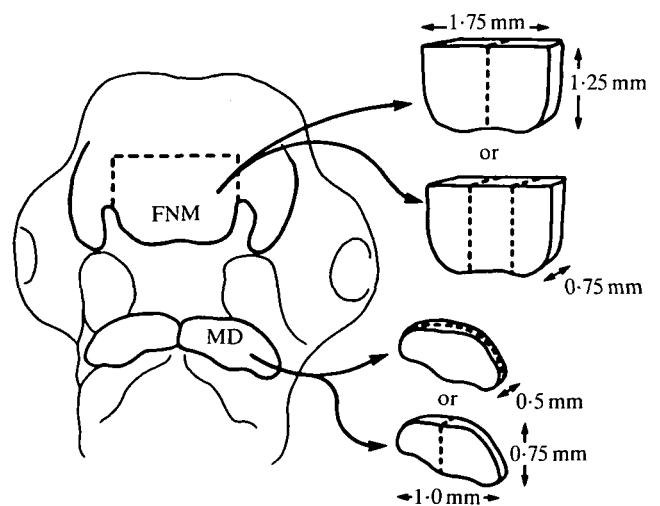

Fig. 1. Diagram illustrating how the face of a stage-24 chick embryo was divided up into the fragments used for grafting to host chick wing buds. Dashed lines (---) represent lines of tissue dissection. FNM, frontonasal mass; MD, mandible.

Table 1. *Summary of the development of grafts of stage-24 intact facial primordia and facial mesenchyme*

Facial tissue	Number of successful experiments	% of grafts containing cartilage	% of grafts with cartilage rods >1 mm	% of grafts with an egg tooth
Intact FNM*	21	100	100	90
FNM mesenchyme	11	100	27	9
Intact MD†	7	100	86	NA‡
MD mesenchyme	13	92	62	NA

* FNM, frontonasal mass.
† MD, mandible.
‡ NA = Not Applicable.

the facial tissue. AG1-X2 beads (in formate form, from Bio-rad), of 200 μm diameter were soaked in a dimethyl sulphoxide (DMSO, Sigma) solution of either $10\,mg\,ml^{-1}$ all-*trans*-retinoic acid (Sigma) or $0\cdot05\,mg\,ml^{-1}$ of a relatively stable retinoid analogue, (E)-4-[2-(5,6,7,8-tetra-hydro-5,5,8,8-tetramethyl-2-napthalenyl)-1-propenyl] benzoic acid, (TTNPB, Ro 13-7410 – a gift from Hoffmann–LaRoche, Basel, Switzerland). The beads were then implanted into the anterior margin of stage-20 chick wing buds. In the main series of experiments, the embryos were treated with either all-*trans*-retinoic acid for 24 h or TTNPB for 12–24 h. These lengths of exposure lead to full beak defects (Wedden & Tickle, 1986a). For treatment times of less than 24 h, the beads were removed from the wing buds at the appropriate times and the embryos returned to the incubator until they had reached stage 24. For each group of treated embryos, at least one embryo, exposed to retinoid for the same length of time as the others, was left to develop to confirm that the retinoid treatment was successful in producing a beak defect. These embryos were fixed on day 10 in 5 % trichloroacetic acid and stained with Alcian green in order to visualize the cartilage elements.

Separation and recombination of ectoderm and mesenchyme

The ectoderm and mesenchyme of the frontonasal mass and mandibles of donor embryos were separated by treatment with 2 % trypsin solution at 4°C for 45 min. The mesenchyme was divided into fragments (Fig. 1) and grafted alone or recombined with ectoderm. Recombinations of separated ectoderm and mesenchyme were carried out in culture medium on ice. Small fragments of mesenchyme were placed on top of the appropriate ectoderm, making sure of the correct orientation of the tissues. To ensure that reannealing took place, a much larger piece of ectoderm than mesenchyme was used – ectoderm from a whole frontonasal mass or mandible was wrapped as closely as possible around a square of mesenchyme. The recombined tissues were left to reanneal at 38°C for at least 2 h, after which time any superfluous ectoderm was removed. The fragments of reannealed tissues were then grafted to host embryos.

Analysis of the grafts

The host embryos were examined the day after the operation and the presence or absence of the graft recorded. Some grafts of isolated quail facial mesenchyme were fixed

after either 24 or 48 h, in order to investigate the healing of the host wing ectoderm (see below). The majority of the grafts was left to develop for a total of 6 days. The right wings bearing the grafts were fixed in 5 % trichloroacetic acid, stained with alcian green and cleared in methyl salicylate. The grafts were scored for the presence of typical 'beak-like' structures; a prenasal cartilage and egg tooth (a transitory, horny structure, which is found at the distal tip of the upper beak and helps the chick break out of the egg) for frontonasal mass fragments or Meckel's cartilage for mandibular fragments. The lengths of the cartilage elements were measured under a dissecting microscope using an eye-piece graticule. The Student's *t*-test was used to analyse the data. In order to examine membrane bone formation, some grafts were left to develop for a total of 10 days. These were then fixed in 95 % ethanol and double stained with alcian blue and alizarin red S (McLeod, 1980).
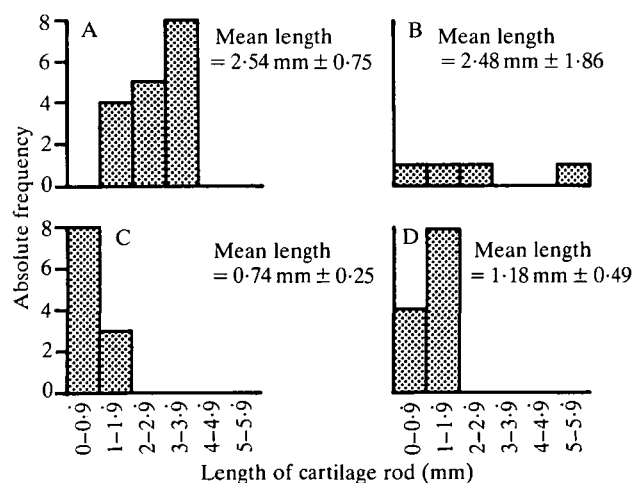

Fig. 2. Histograms showing the absolute frequency of cartilage rod formation against length (in mm) in cartilage-containing grafts of stage-24 chick facial tissue fixed at 6 days. The mean lengths of the cartilage rods and the standard deviations are indicated on each histogram. (A) Intact frontonasal mass; (B) intact mandible; (C) isolated frontonasal mass mesenchyme; (D) isolated mandibular mesenchyme. N.B. The discrepancies between the number of grafts illustrated here and those listed in Table 1 are due to the fact that some of the grafts were used for sectioning before they could be accurately measured.

Sectioning of the grafts

Grafts of either chick or quail facial tissue required for sectioning were fixed in half strength Karnovsky's fixative (Karnovsky, 1965) for at least 2 h at 4°C. The grafts were stained with alcian green to show cartilage and mounted in Araldite. 1 μm sections were cut with glass knives on a Cambridge Huxley ultramicrotome. The sections were either stained with toluidine blue or the Feulgen reaction (Le Douarin, 1973) was used to visualize the quail tissue.

Results

Controls – fragments of facial primordia
   Frontonasal mass

Small fragments from the frontonasal mass of stage-24 chick embryos develop into typical upper-beak-like structures. Such structures develop regardless of whether the tissue fragment is derived from a central or lateral region of the frontonasal mass (Wedden & Tickle, 1986b). The results are summarized in Table 1 and in Fig. 2A. There is considerable outgrowth of the graft and cartilage differentiates to form a long central rod, the prenasal cartilage. An egg tooth develops at the distal end (Fig. 3A). On three occasions more than one prenasal cartilage and associated egg tooth developed from a single graft. Fragments taken from either a third or a half of a frontonasal mass grow to the same extent ($P > 0.5$, Student's t-test). By 6 days the prenasal cartilage is approximately 2·5 mm long. Membrane bone (premaxillary bone) has differentiated in grafts fixed at 10 days. Fig. 4 illustrates a section through a graft that has developed from a fragment of quail frontonasal mass tissue. This shows that the outgrowth is derived exclusively from the grafted tissue.
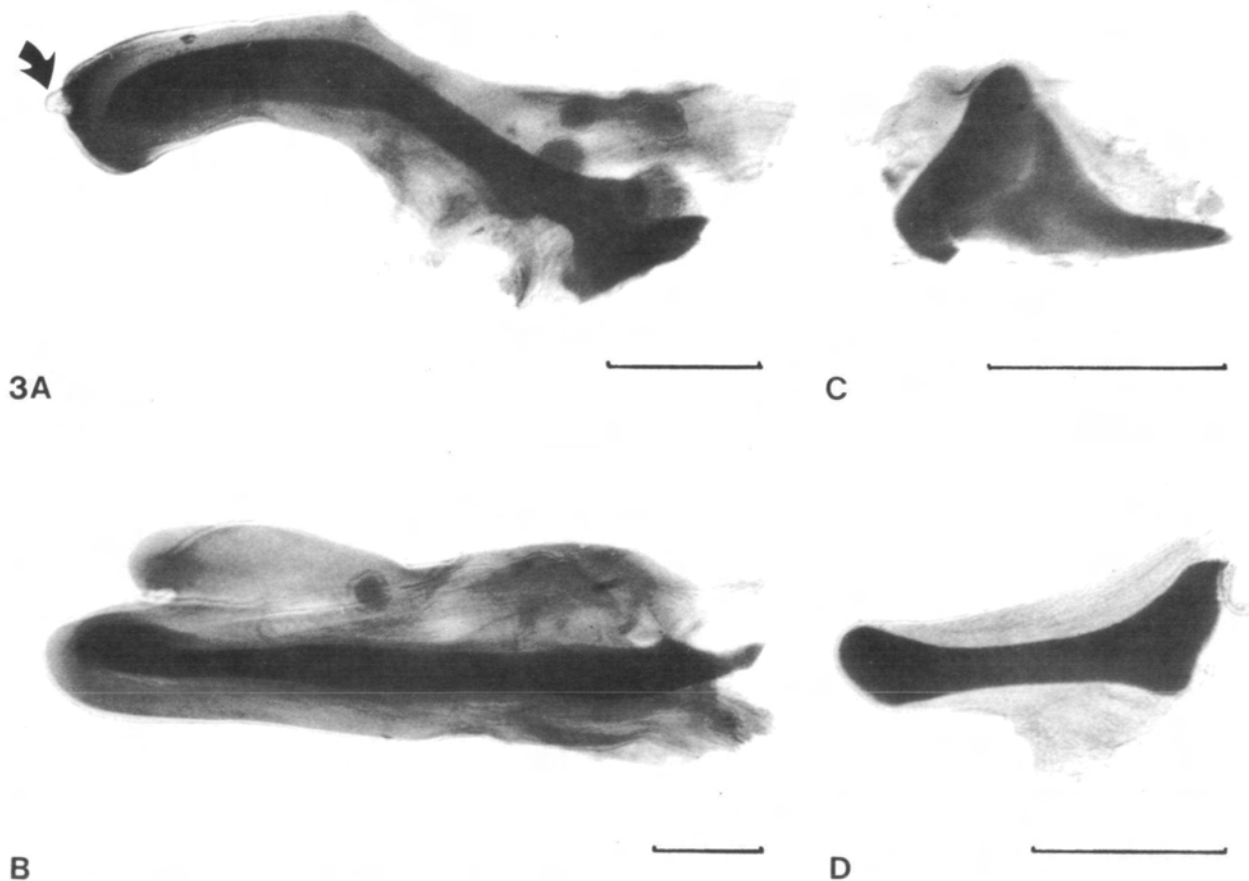

Fig. 3. Wholemounts of cleared grafts of stage-24 chick facial tissue that have been fixed at 6 days and stained with Alcian green to show cartilage. Bars, 1 mm. (A) Graft derived from the central third of an intact frontonasal mass. Note the central rod of cartilage, the prenasal cartilage. Arrow indicates egg tooth. (B) Graft derived from half of an intact mandible. Note the central long rod of cartilage, Meckel's cartilage. (C) Graft derived from a fragment of frontonasal mass mesenchyme. Only a small spur of cartilage has differentiated. There is very little outgrowth and an egg tooth is not present (compare with A). (D) Graft derived from isolated mandibular mesenchyme. A short rod of cartilage has differentiated. Note that the amount of outgrowth is reduced compared with the graft of intact mandible in Fig. 3B.

Mandible

Characteristic lower-beak-like structures develop when either a whole or half of a stage-24 chick mandible is grafted. The results are summarized in Table 1 and in Fig. 2B. As in fragments of the frontonasal mass, there is considerable outgrowth of
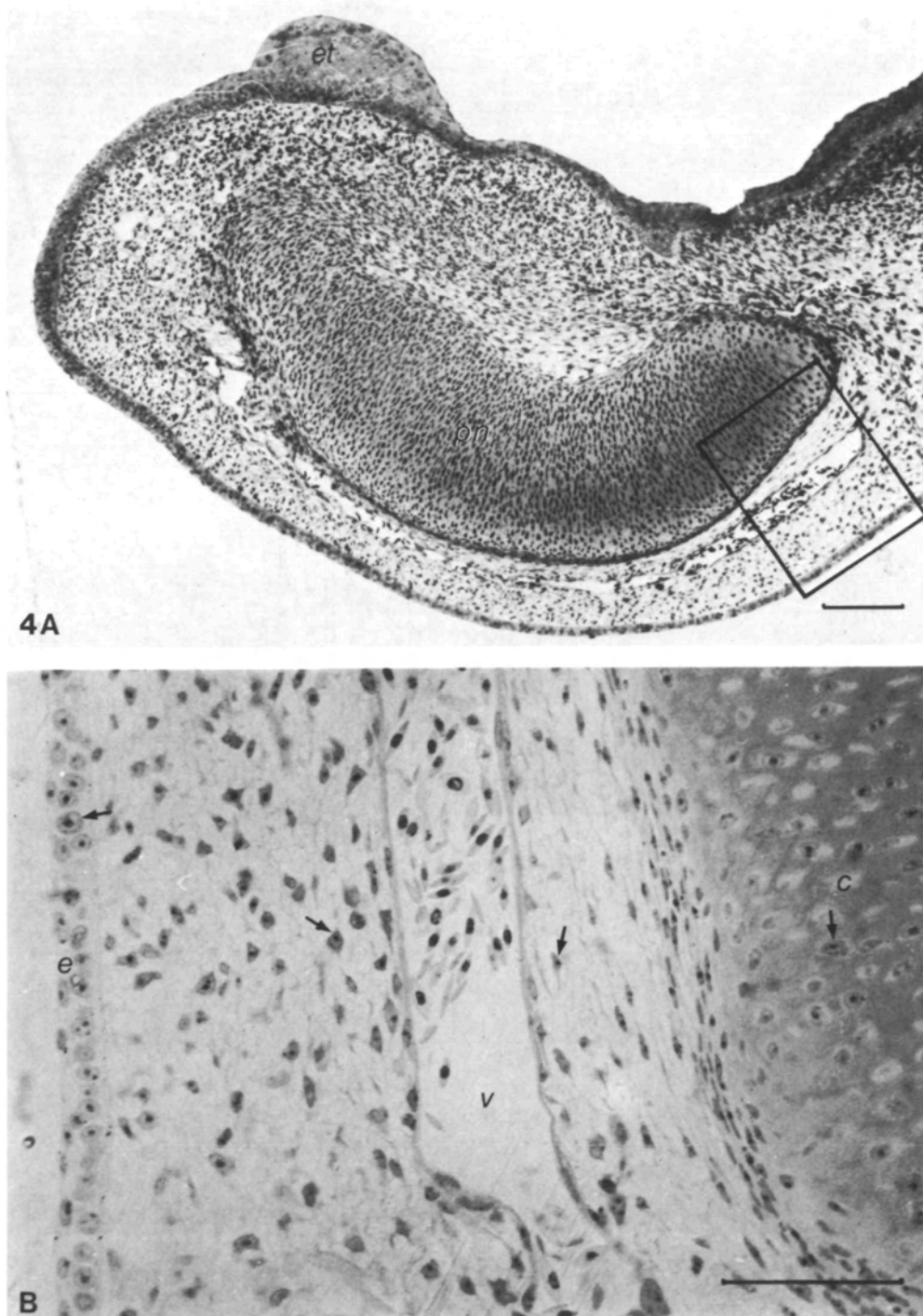

Fig. 4. (A) 1 μm section through a graft derived from the central third of the frontonasal mass of a stage-22 quail embryo. Graft fixed at 4 days. Section stained with toluidine blue. Bar, 100 μm. Note the central prenasal cartilage (*pn*) and egg tooth (*et*). (B) High-power magnification of the region indicated in A. Bar, 50 μm. Section stained with Feulgen. Examples of quail cells, visible by their intensely staining nuclei, are arrowed. Note that the host, chick tissue does not participate in the formation of the graft and that quail cells are visible throughout. *e*, ectoderm; *c*, cartilage; *v*, blood vessel.

tissue and the differentiation of a central rod of cartilage, in this case Meckel's cartilage (Fig. 3B). The mean length of Meckel's cartilage, after 6 days of growth, is approximately 2·5 mm. In grafts fixed at 10 days, membrane bone has differentiated.

Development of fragments of facial mesenchyme

To investigate the role of epithelial–mesenchymal interactions in facial morphogenesis, the ectoderm was removed from fragments of facial primordia prior to grafting to host embryos. The results are summarized in Table 1 and in Fig. 2C,D.
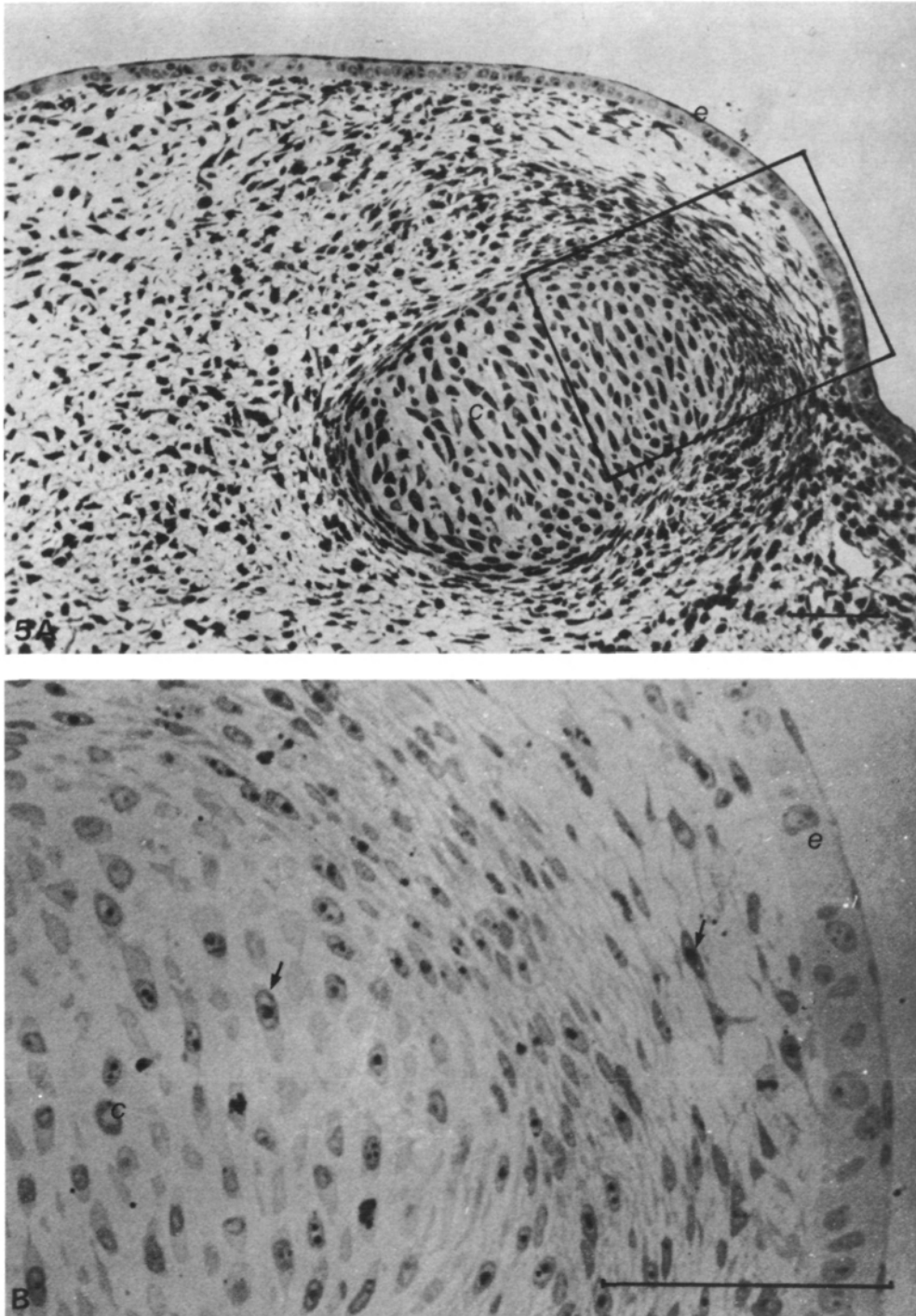

Fig. 5. (A) 1 μm section through a graft of stage-24 quail mandibular mesenchyme fixed at 48 h. Section stained with toluidine blue. Bar, 50 μm. Note that ectoderm (*e*).from the dorsal surface of the host chick wing bud has grown over the exposed surface of the graft. Cartilage (*c*) has differentiated in the centre of the graft. (B) High-power magnification of the region indicated in A. Bar, 50 μm. Section stained with Feulgen. Quail cells are indicated by arrows. The ectoderm is host in origin and contains no quail cells.

In grafts of frontonasal mass mesenchyme, only small fragments of cartilage differentiate (Fig. 3C – mean length of 0·74 mm). The amount of outgrowth is significantly reduced compared with intact tissue ($P < 0.001$). An egg tooth is present in only one graft (out of eleven). Some outgrowth occurs in grafts of mandibular mesenchyme and after 6 days, 62 % contain a structure that is similar to Meckel's cartilage and longer than 1 mm (Fig. 3D). However, the mean length of the cartilage rods is only 1·18 mm, compared with 2·48 mm in grafts of intact mandibular tissue ($P < 0.05$).

Analysis of sections of grafted mesenchyme from quail facial primordia show that by 48 h the dorsal ectoderm of the host chick wing bud has healed over the exposed surface of the graft (Fig. 5).

Development of fragments of facial primordia treated with retinoids

To investigate how retinoid-treated facial primordia develop in isolation, fragments of facial tissue from embryos that had been treated with a sufficient dose of retinoid to lead to a severe beak defect were grafted to host embryos. The results are summarized in Table 2 and in the histograms in Fig. 6A,B.

The development of grafts of treated frontonasal mass is inhibited. Cartilage differentiates, but only in the form of a small spur (Fig. 7A). There is significantly less outgrowth in comparison with untreated tissue ($P < 0.001$) and egg-tooth formation is completely inhibited. In grafts of retinoid-treated mandibles, cartilage differentiates to give a well-defined Meckel's cartilage (Fig. 7B). By comparison with untreated tissue, slightly fewer of the grafts give rise to a Meckel's cartilage longer than 1 mm. However, after 6 days the mean length of the cartilage rods is 2·29 mm and this is not significantly different from the

growth of untreated mandibular fragments ($P > 0.5$). These results confirm that the behaviour of isolated fragments of treated facial primordia parallels the *in vivo* development of the primordia of retinoid-treated embryos.

Recombinations of retinoid-treated and untreated facial tissues

To investigate the target of retinoid action in the frontonasal mass, the ectoderm and mesenchyme of retinoid-treated embryos were separated and recombined with the appropriate tissue from untreated embryos. As controls, grafts were made of re-annealed, untreated tissues. The results are summarized in Table 2 and Fig. 6.

The development of grafted recombinations of retinoid-treated frontonasal mass ectoderm with untreated mesenchyme is similar to that of untreated grafts ($P > 0.5$) and a well-defined prenasal cartilage is formed (Fig. 8A). There is, however, a slight reduction in the number of grafts showing egg-tooth formation (56 %, compared with 75 % in control recombinations). In contrast, when retinoid-treated frontonasal mass mesenchyme is recombined with untreated ectoderm, the development of the graft is inhibited. Cartilage differentiates, but only in the form of short fragments (Fig. 8B). More than one cartilage fragment may be present in a graft but in comparison with untreated tissue, the amount of outgrowth is drastically reduced ($P < 0.001$) and the lengths of cartilage never exceed 0·6 mm. Furthermore, egg-tooth formation is completely inhibited.

Recombinations of mandibular tissue

The results of recombinations of retinoid-treated and untreated mandibular tissues are included in Table 2 and in the histograms in Fig. 6. Outgrowth in recombined mandibular tissue is reduced when either the

Table 2. *Summary of the development of grafts of retinoid-treated stage-24 facial primordia and grafts of recombinations of retinoid-treated and untreated facial ectoderm and mesenchyme*

| Facial tissue	Number of successful experiments	% of grafts containing cartilage	% of grafts with cartilage rods >1 mm	% of grafts with an egg tooth
Frontonasal mass				
Retinoid-treated	7	100	43	0
Treated ectoderm with untreated mesenchyme	9	100	89	56
Untreated ectoderm with treated mesenchyme	6	100	0	0
Reannealed untreated tissue	12	100	92	75
Mandible				
Retinoid-treated	11	91	63	NA*
Treated ectoderm with untreated mesenchyme	14	100	71	NA
Untreated ectoderm with treated mesenchyme	12	100	58	NA
Reannealed untreated tissue	11	100	64	NA

*NA = Not Applicable.
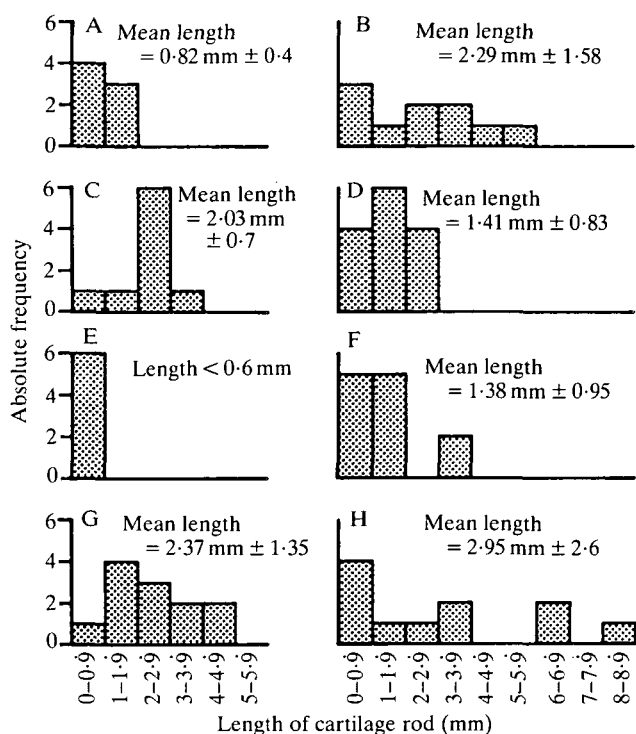
Fig. 6. Histograms showing the absolute frequency of cartilage rod formation against length (in mm) in cartilage-containing grafts of stage-24 chick facial tissue fixed at 6 days. The mean lengths of the cartilage rods and the standard deviations are indicated on each histogram. (A) Intact frontonasal mass from retinoid-treated embryos. (B) Intact mandibular primordia from retinoid-treated embryos. (C) Recombinations of frontonasal mass tissue; retinoid-treated ectoderm with untreated mesenchyme. (D) Recombinations of mandibular tissue; retinoid-treated ectoderm with untreated mesenchyme. (E) Recombinations of frontonasal mass tissue; untreated ectoderm with retinoid-treated mesenchyme. (F) Recombinations of mandibular tissue; untreated ectoderm with retinoid-treated mesenchyme. (G) Control recombinations of untreated frontonasal mass ectoderm and mesenchyme. (H) Control recombinations of untreated mandibular ectoderm and mesenchyme.

ectoderm or mesenchyme has been treated with retinoids (Fig. 6D,F – compare with H). Structures similar to Meckel's cartilage still form in both types of recombinations but they are only about half the length of those formed in control reannealed fragments.

Discussion

Grafts of fragments of frontonasal mass and mandibular primordia develop into characteristic beak-like structures. When the ectoderm is removed from the frontonasal mass, only small fragments of cartilage differentiate and outgrowth is almost completely inhibited. In the mandible, removal of the ectoderm substantially reduces the amount of outgrowth but cartilage still differentiates to give a rod-like structure. Retinoids specifically inhibit outgrowth and pattern formation in the frontonasal mass. Recombination experiments show that retinoids affect the mesenchyme of this primordium.

The role of ectoderm in outgrowth and pattern formation in facial primordia

Removal of facial ectoderm effectively truncates the development of fragments of the facial primordia. The development of the frontonasal mass is much more affected than the mandible ($P < 0.02$, Student's t-test). The effect is analogous to that obtained when the apical ectodermal ridge is removed from developing limb buds and truncated limbs develop (Summerbell, 1974). In developing limbs, the degree of truncation depends on the time at which the ridge is removed. When the ridge is removed early in development, the limbs are more severely truncated. The difference in the degree of truncation between the frontonasal mass and mandible could be related to the relatively advanced development of the mandibles (Parker, 1869).

The finding that the facial ectoderm has similar properties to the apical ectodermal ridge of limb buds is consistent with the suggestion that pattern formation along the proximodistal axis of the beaks involves a progress zone mechanism (Wedden *et al.* 1986). If a progress zone is involved in upper beak development, then a prediction would be that the mitotic index of the mesenchyme cells at the distal tip of the frontonasal mass would be high. Such a progress zone mechanism may specify not only the pattern of cartilage differentiation but also that of membrane bone.

These results show for the first time, that facial ectoderm is required for outgrowth and the accompanying differentiation of cartilage to form rod-like structures within the facial primordia. Using organ culture and chorioallantoic membrane (CAM) grafting, it has been shown that the growth and survival of intact mandibular primordia is enhanced compared with that of isolated mandibular mesenchyme (Tyler & Hall, 1977). Furthermore, chick frontonasal mass mesenchyme, when grafted to the CAM, only shows signs of 'beak organization' when recombined with ectoderm (cephalic skin or chorionic ectoderm) (Tonegawa, 1973). It should be noted that the dorsal wing ectoderm that grows over the exposed mesenchyme in the experiments here, appears to be incapable of supporting normal outgrowth or the development of typical beak-like structures from stage-24 facial tissue. In only one graft, the
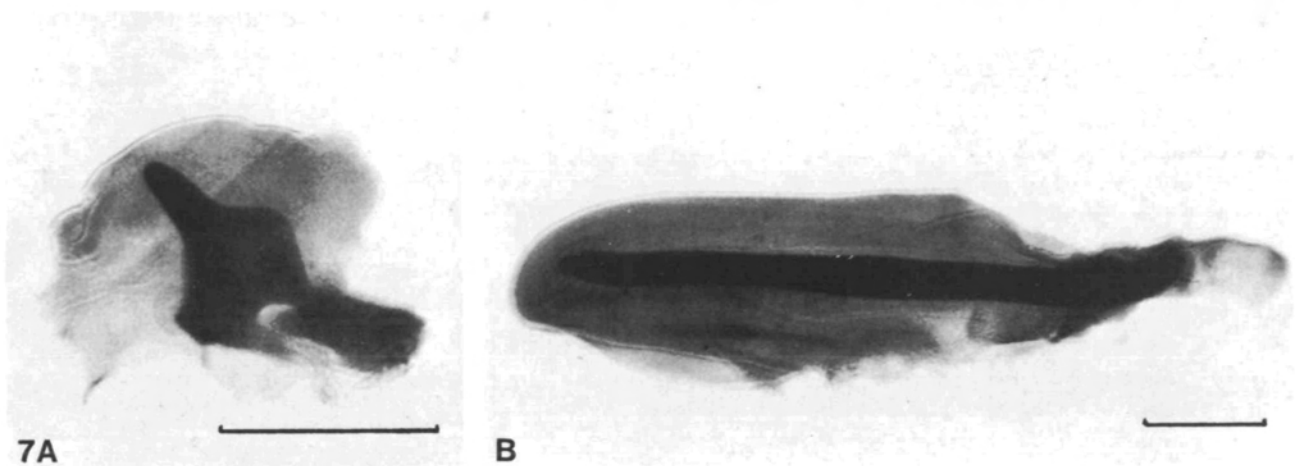
Fig. 7. Wholemounts of cleared grafts of facial primordia from retinoid-treated stage-24 chick embryos. Grafts fixed at 6 days and stained with alcian green to show cartilage. Bars, 1 mm. (A) Frontonasal mass. Note that only a small spur of cartilage has differentiated, similar to that formed in grafts of isolated frontonasal mass mesenchyme (Fig. 3C). There is very little outgrowth and an egg tooth is not present (compare with control, untreated tissue, Fig. 3A). (B) Mandible. Note that, as in untreated tissue (Fig. 3B), Meckel's cartilage has differentiated in the centre of the graft.
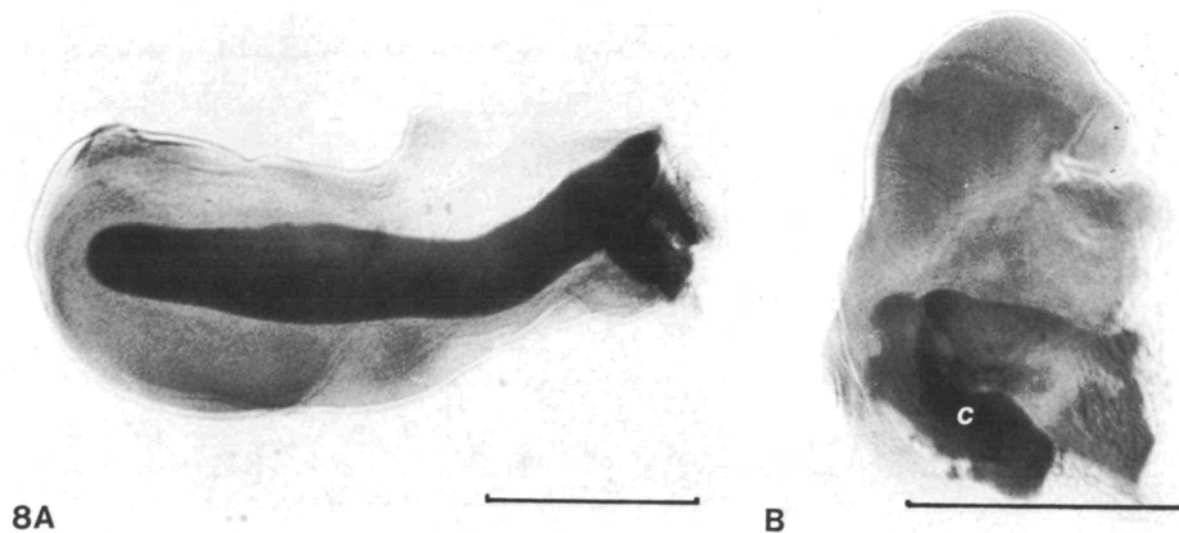

Fig. 8. Wholemounts of cleared grafts of recombinations of retinoid-treated and untreated stage-24 frontonasal mass tissue. Grafts fixed at 6 days and stained with alcian green to show cartilage. Bars, 1 mm. (A) Retinoid-treated ectoderm with untreated mesenchyme. Note the formation of a prenasal cartilage and egg tooth, similar to that formed in control grafts (Fig. 3A). (B) Untreated ectoderm with retinoid-treated mesenchyme. Note that only small fragments of cartilage (c) have differentiated at the base of a mass of soft tissue. The graft is similar to that formed from fragments of intact, retinoid-treated frontonasal mass tissue (Fig. 7A) and a rod of cartilage and egg tooth are not present (compare with 3A and 8A).

dorsal wing ectoderm responded to an inductive signal from the underlying frontonasal mass mesenchyme and gave rise to an egg tooth (Tonegawa, 1973). These findings suggest that only epithelia with particular properties can elicit morphogenesis. The specificity of the epithelia, the stage dependency and the time requirement for such an interaction, however, have yet to be determined.

Effects of retinoids on development of facial primordia

The truncated upper beaks that develop following retinoid treatment mimic the effect of removing the ectoderm. Therefore a simple explanation for the retinoid-induced defect is that the ectoderm of the frontonasal mass is affected. However, recombinations of retinoid-treated ectoderm and untreated mesenchyme do not lead to truncations. This implies

that retinoids do not have a direct effect on the ectoderm of the frontonasal mass. Instead retinoids primarily affect the mesenchyme and recombinations of retinoid-treated mesenchyme and untreated ectoderm result in truncations.

The development of the recombinations of tissues from untreated and treated mandibular primordia is puzzling. When either the ectoderm or the mesenchyme has been treated with retinoids, the outgrowth of the recombined mandibular fragments is reduced. Application of vitamin A to recombinations of cultured mouse tooth germs *in vitro*, inhibits the reformation of an intact basement membrane, necessary for odontoblast differentiation (Humerinta, Thesleff & Saxen, 1981). It is possible that retinoids may be similarly affecting the ability of the dissociated facial tissues to re-establish their normal interface.

Mechanism of retinoid action

Perhaps one of the most striking features of this retinoid-induced facial defect is its specificity. The mandible is completely unaffected, even when retinoids are applied as early as stage 15 (Wedden & Tickle, 1986*a*). Retinoids inhibit chondrogenesis in micromass cultures of facial cells but this cannot explain the specificity of the retinoid-induced facial defect. The cells of both facial primordia are equally sensitive to the effects of retinoids. Furthermore, following a retinoid treatment that leads to the specific facial defect, the same concentration of retinoid is found in both the frontonasal mass and mandibles (Wedden *et al.* 1987). Epithelial–mesenchymal interactions appear to be involved in outgrowth of the mandible, as well as the frontonasal mass. However, these interactions in the mandible are not affected by retinoid treatment.

Retinoids could act in the frontonasal mass by either changing the mesenchymal response to a signal from the ectoderm or indirectly leading to changes in the ectoderm. A similar effect may account for the linked changes in bud morphogenesis and pattern formation that occur when retinoids are applied to developing limbs (Lee & Tickle, 1985). For example, the apical ectodermal ridge increases in length when additional digits are specified and the limb bud broadens. It appears most likely that these are the result of changes induced in the mesenchyme of the treated buds. Although retinoids have well-documented effects on epithelial cell differentiation (Sagami & Kitano, 1970; Dhouailly & Hardy, 1978; Hardy, Sweeny & Bellows, 1978), in amphibian limb regeneration, where retinoids bring about pattern changes, it is also the mesoderm that is affected (Maden, 1984).

References

ARCHER, C. W., COTTRILL, C. P. & ROONEY, P. (1984). Cellular aspects of cartilage differentiation and morphogenesis. In *Matrices and Cell Differentiation* (ed. R. B. Kemp & J. R. Hinchliffe), pp. 409–426. New York: Alan R. Liss, Inc.

BEE, J. & THOROGOOD, P. (1980). The role of tissue interactions in the skeletogenic differentiation of avian neural crest cells. *Devl Biol.* **78**, 47–62.

DHOUAILLY, D. & HARDY, M. H. (1978). Retinoic acid causes the development of feathers in the scale-forming integument of the chick. *Wilhelm Roux' Arch. devl Biol.* **185**, 195–200.

HALL, B. K. (1980). Viability and proliferation of epithelia and the initiation of osteogenesis within mandibular ectomesenchyme in the embryonic chick. *J. Embryol. exp. Morph.* **56**, 71–89.

HAMBURGER, V. & HAMILTON, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.

HARDY, M. H., SWEENY, P. R. & BELLOWS, C. G. (1978). The effects of Vitamin A on the epidermis of the fetal mouse in organ culture – An ultrastructural study. *J. Ultrastruct. Res.* **64**, 246–260.

HUMERINTA, K., THESLEFF, I. & SAXEN, L. (1980). *In vitro* inhibition of mouse odontoblast differentiation by Vitamin A. *Archs Oral Biol.* **25**, 385–393.

KARNOVSKY, M. J. (1965). A formaldehyde glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* **27**, 137a.

LE DOUARIN, N. (1973). A biological cell labelling technique and its use in experimental embryology. *Devl Biol.* **30**, 217–222.

LE LIEVRE, C. S. (1978). Participation of neural-crest derived cells in the genesis of the skull in birds. *J. Embryol. exp. Morph.* **47**, 17–37.

LEE, J. & TICKLE, C. (1985). Retinoic acid and pattern formation in the developing chick wing: SEM and quantitative studies of early effects on the apical ectodermal ridge and bud outgrowth. *J. Embryol. exp. Morph.* **90**, 139–169.

MADEN, M. (1984). Does Vitamin A act on pattern formation via the epidermis or the mesoderm? *J. exp. Zool.* **230**, 387–392.

McLEOD, M. J. (1980). Differential staining of cartilage and bone in whole foetuses by Alcian blue and Alizarin red S. *Teratology* **22**, 299–301.

NODEN, D. M. (1975). An analysis of the migratory behaviour of avian cephalic neural crest cells. *Devl Biol.* **42**, 106–130.

PARKER, W. K. (1869). On the structure and development of the skull of the common fowl (*Gallus domesticus*). *Phil. Trans. Roy. Soc. London* **159**, 755–807.

SAGAMI, S. & KITANO, Y. (1970). Electron microscope study of the effect of Vitamin A on the differentiation of embryonic chick skin. *Br. J. Derm.* **83**, 565–571.

SAUNDERS, J. W. JR (1948). The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. *J. exp. Zool.* **108**, 363–403.

SUMMERBELL, D. (1974). A quantitative analysis of the effect of excision of the AER from the chick limb bud. *J. Embryol. exp. Morph.* **32**, 651–660.

SUMMERBELL, D., LEWIS, J. & WOLPERT, L. (1973). Positional information in chick limb morphogenesis. *Nature, Lond.* **244**, 492–496.

TAMARIN, A., CRAWLEY, A., LEE, J. & TICKLE, C. (1984). Analysis of upper beak defects in chicken embryos following treatment with retinoic acid. *J. Embryol. exp. Morph.* **84**, 105–123.

TONEGAWA, Y. (1973). Inductive interactions in the beak of a chick embryo. *Devl, Growth & Diff.* **15**, 57–71.

TYLER, M. S. & HALL, B. K. (1977). Epithelial influences on skeletogenesis in the mandible of the embryonic chick. *Anat. Rec.* **188**, 229–240.

WEDDEN, S. E. & TICKLE, C. (1986a). Quantitative analysis of the effect of retinoids on facial morphogenesis. *J. Craniofac. Genet. devl Biol.* Supplement 2, 169–178.

WEDDEN, S. E. & TICKLE, C. (1986b). Facial morphogenesis and pattern formation. In *Progress in Developmental Biology, Part A* (ed. H. C. Slavkin), pp. 335–337. New York: Alan R. Liss, Inc.

WEDDEN, S. E., LEWIN-SMITH, M. R. & TICKLE, C. (1986). The patterns of chondrogenesis of cells from facial primordia of chick embryos in micromass culture. *Devl Biol.* **117**, 71–82.

WEDDEN, S. E., LEWIN-SMITH, M. R. & TICKLE, C. (1987). Analysis of the effects of retinoids on cartilage differentiation in micromass cultures of chick facial primordia and the relationship to a specific facial defect. *Devl Biol.* (Submitted for publication).

ZWILLING, E. & HANSBOROUGH, L. (1956). Interaction between limb bud ectoderm and mesoderm in the chick embryo. III. Experiments with polydactylous limbs. *J. exp. Zool.* **133**, 219–239.