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A descriptive study of the rate of elongation and differentiation of the skeleton of the developing chick wing

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SUMMARY

Differentiation of the wing skeleton is clearly visible in whole mounts from stage 24. It proceeds in proximo-distal and postero-anterior sequence. It is possible to map the part of the limb giving rise to each skeletal element as it appears and subsequently. This enables one to estimate: (*a*) a rate of elongation curve for each segmental level, (*b*) the intrinsic rate of change of elongation of the cartilage, (*c*) a definitive extrapolation back to classical presumptive fate maps, (*d*) the normal range of variation in the proportion of the limb occupied by the three main segmental levels (stylopod, autopod, zeugopod).

INTRODUCTION

The developing chick limb has been the subject of numerous descriptive studies. The simplest, yet perhaps the most important for the contemporary research worker, is the series of normal stages described by Hamburger & Hamilton (1951). The outward morphology is by no means the only feature of interest and a number of other important events in early development have been described. For example, the first detectable sign of approaching differentiation in the mesenchyme is said to be the uptake of radioactive labelled sulphate into mucopolysaccharides at proximal levels of stage 22 (Searls, 1965). Slightly later at stage 23, Gould, Day & Wolpert (1972) have reported an increase in cell contact in the dorsal and ventral proximal regions of presumptive myogenesis, while by stage 24 precartilaginous cells may be discerned as areas of high cell density in proximal central regions (Fell & Canti, 1934). This last event is an important one for it marks the first overt step in the formation of the skeletal elements, which at later stages become the main morphological criteria of development.

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An important class of descriptive studies comprises attempts to construct a normal 'presumptive fate map' of development. That is, to show which parts of the early limb-bud give rise to which parts of the adult. Saunders (1948), Amprino & Camosso (1958) and Hampé (1959) placed small extracellular markers at various places in the early limb-bud and then determined where the markers could be found during later development. Assuming that the markers remain adjacent to the same cells, or to their progeny, this method demonstrates the presumptive fate of the original marked cells. Despite the inadequacies of the technique, the resulting fate maps have been of immense practical benefit to experimental embryology. More recently Stark & Searls (1973) have applied a more discriminatory if laborious method to this same concept. They transplanted pieces of mesenchyme labelled with tritiated thymidine into a known position in a host limb, then discovered the position of labelled cells at a later stage. By this means they have constructed elegant fate maps without having to take into account the relative movement of marker and cell. The results also have the important spin-off of demonstrating the absence of significant mixing of cells during these crucial stages. Another highly original method originates with Lewis (1975). He first divides the limb (hypothetically) into slices at right angles to the proximo-distal axis. Then using data from Hornbruch & Wolpert (1970) and Summerbell & Wolpert (1972) he calculates the relative expansion of the width of each slice taking into account changes in cell packing, relative rates of cell division, and changes in cross-sectional area of the limb. This produces a form of fate map which, taken with any other fate map at a single stage, allows one to calculate the relative rate of expansion of each slice and reconstruct the fate map at any other stage.

This latter work in particular demonstrates the close interdependence of growth curves and fate maps. A fate map is essentially a set of hypothetical growth curves for the rate of elongation of various levels along the proximo-distal axis for the major skeletal levels of the developing limb. Stark and Searls similarly recognized this when they extrapolated a set of predicted growth curves from their fate maps. This paper is concerned with the converse. The lengths of skeletal elements can be measured very accurately and repeatably at around 10 days of development (Summerbell & Wolpert, 1973). There is no reason in principle why this technique cannot be extended to still earlier stages. In this way one can construct rate of elongation curves not only for the whole limb, but for each individual skeletal element. This will give, at least for the stages at which the lengths of the skeletal elements can be accurately measured, a 'fate map'. One may even be able to estimate the form of the fate map for stages earlier than those at which the cartilage can be stained, by extrapolation opposite to that performed by Stark & Searls.

At the same time the material provides an opportunity to make a detailed description of the stages in the differentiation of the cartilaginous skeleton.

Pautou (1975) has recently presented evidence for a distinct anterior to posterior sequence of differentiation of cartilage elements in the foot. This work will demonstrate that a similar sequence is present in the wing.

METHODS

Fertilized White Leghorn eggs were incubated at 38 °C. The embryos were sacrificed at various times between the 3rd and 20th day of incubation. The embryos were placed in a dish of Earle's B.S.S. and staged according to Hamburger & Hamilton (1951). The length of the wing from its base to the tip, and from about day 6 from the point of the elbow to the tip, were measured, using an eyepiece graticule, to the nearest 50 μm. The wings were then fixed in 5 % T.C.A. for 3 h, stained in alcian green 8GX in 70 % alcohol with 1 % hydrochloric acid for 3 h, and differentiated overnight in the acid alcohol. They were then dehydrated in ethyl alcohol (two changes of 1 h), and finally cleared in methyl salicylate. The limbs were re-examined using a Zeiss Stereo IV dissection microscope, with transmitted light. The distance from the base to the tip and from the point of the elbow to the tip was again measured. Also the lengths of the humerus, radius, ulna, wrist, and the three elements of digit III were measured, as described in Summerbell & Wolpert (1973). In all, 103 embryos were treated in this manner.

In a second series the eggs were windowed on the third day of development and the length of the limb-bud was measured *in ovo* several times over a period of two or three days before being treated as in the first series; 26 embryos were treated in this manner.

RESULTS

The methods described above produce two sets of data, one from still-living material and the other from fixed and stained material. As the latter treatment causes shrinkage of the tissues it is necessary to compensate for this before the data can be grouped together. Fig. 1 shows a calibration curve for shrinkage. The length of the limb before treatment is plotted against the length of the limb after treatment. There is a very high correlation between the two, and the least-square regression coefficient is drawn. In the rest of the paper all measurements and figures have been converted into their equivalent length for living material using this graph.

Rate of elongation of the wing

Fig. 2 shows two curves. The dashed line shows the change in length of the limb measured as the perpendicular distance between the base of the limb and the tip. At later stages this is very difficult to measure repeatably, and the estimate was discontinued. The solid line shows the change in length of the sum of the different levels of the limb, i.e. the lengths of the humerus, ulna,

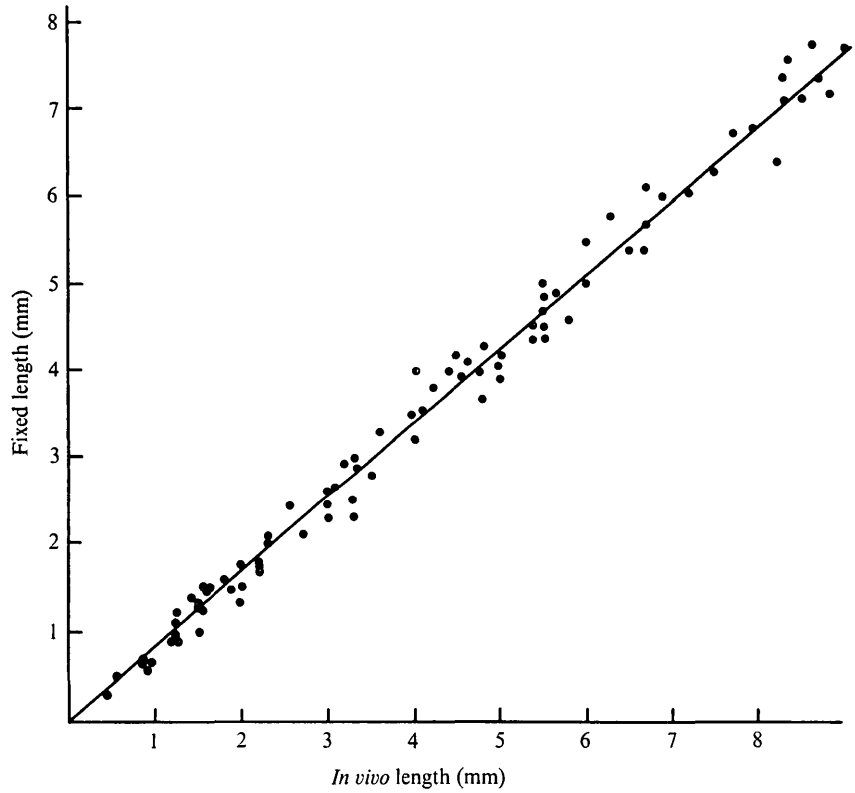

Fig. 1. A graph for converting the observed length of an element after fixing and staining to its original *in vivo* length. Converted *in vivo* lengths were used in all subsequent figures.

wrist region, metacarpal, phalanges I and II of digit III and the undifferentiated tip are added together, and the total is taken as the length of the limb. This length, from about stage 26, is increasingly longer than the direct measurement. This is mainly because of the angle between the humerus, and the main axis of outgrowth. The points on the graph come from two sources. Solid circles are summed skeletal lengths, from the fixed and stained material. The time axis for these points consists of Hamburger & Hamilton (1951) stages estimated to the nearest half stage. The crosses are direct measured lengths for the second series of *in ovo* measurements. The time axis for these points is real time in hours. The fit between real time and 'stage' time is arbitrary, but for the incubation temperature used, and starting from a baseline of stage 18 (=3 days), the fit is quite accurate.

Changes in individual skeletal elements

The values of increase in length of individual skeletal elements are shown in Fig. 3: humerus, ulna and wrist in 3*A*, the three elements of digit III in 3*B*

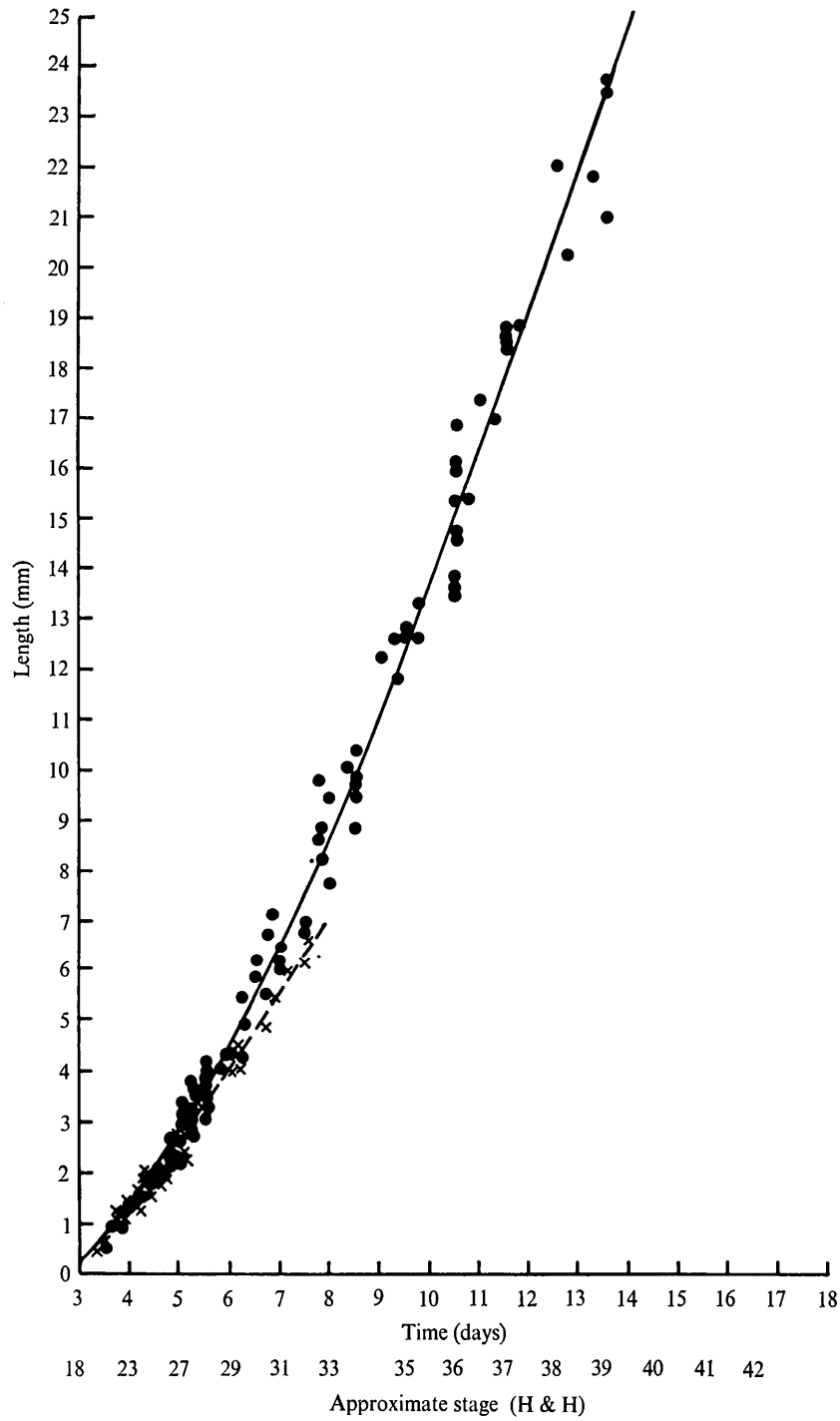

Fig. 2. Rate of elongation of the wing. The dashed line shows the perpendicular distance between the base of the limb and the tip, and the solid line the total summated length of individual skeletal elements. Solid circles are from fixed and stained material and use Hamburger & Hamilton stages for the time axis; crosses are from *in ovo* material and use real time.

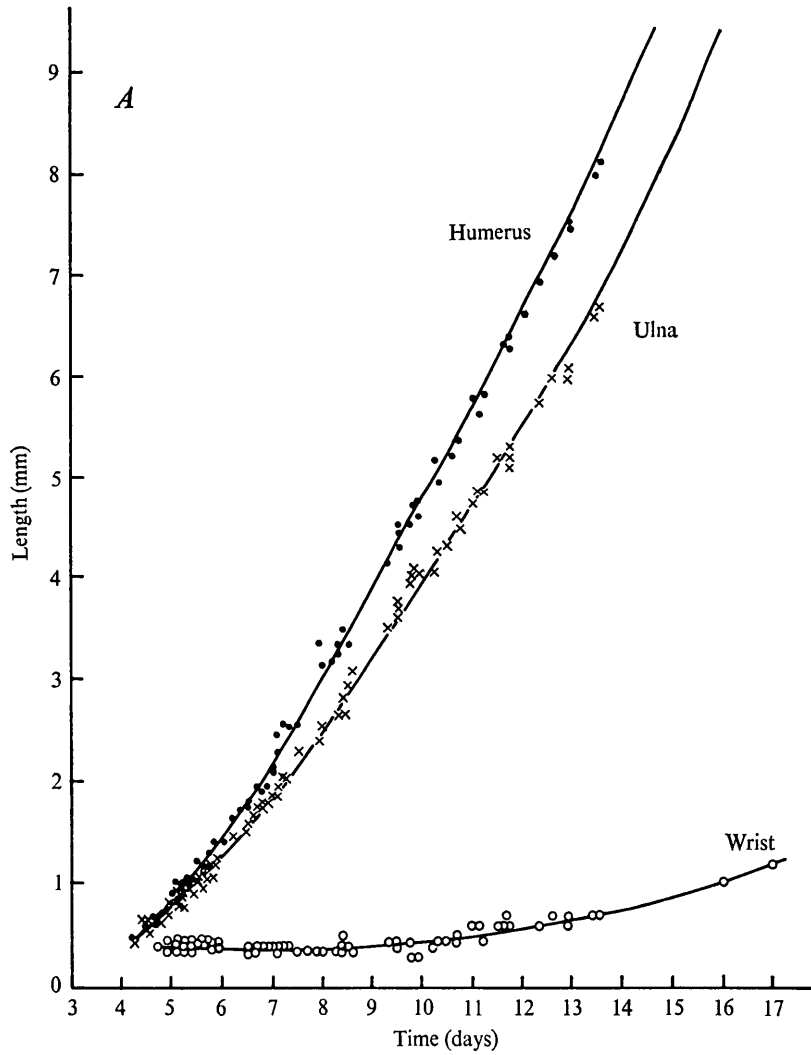

Fig. 3*A*. For legend see opposite.

and the undifferentiated tip region in 3*C*. The position along the time axis for each point is taken as being the intersection of the summated skeletal length on the curve in Fig. 2. Thus the variation in the length of an element is dependent on changes in the proportion of the limb occupied by each level. Although the measurements of individual elements at early stages may seem contentious, the measurement was carried out whenever part of the central mesenchyme appeared distinctly more heavily stained than the surrounding soft tissue. The only interpretation necessary was occasionally, by reference to later stages, to assume an identity for a particular condensation. It is worth noting that measurement of the humerus and the ulna first became

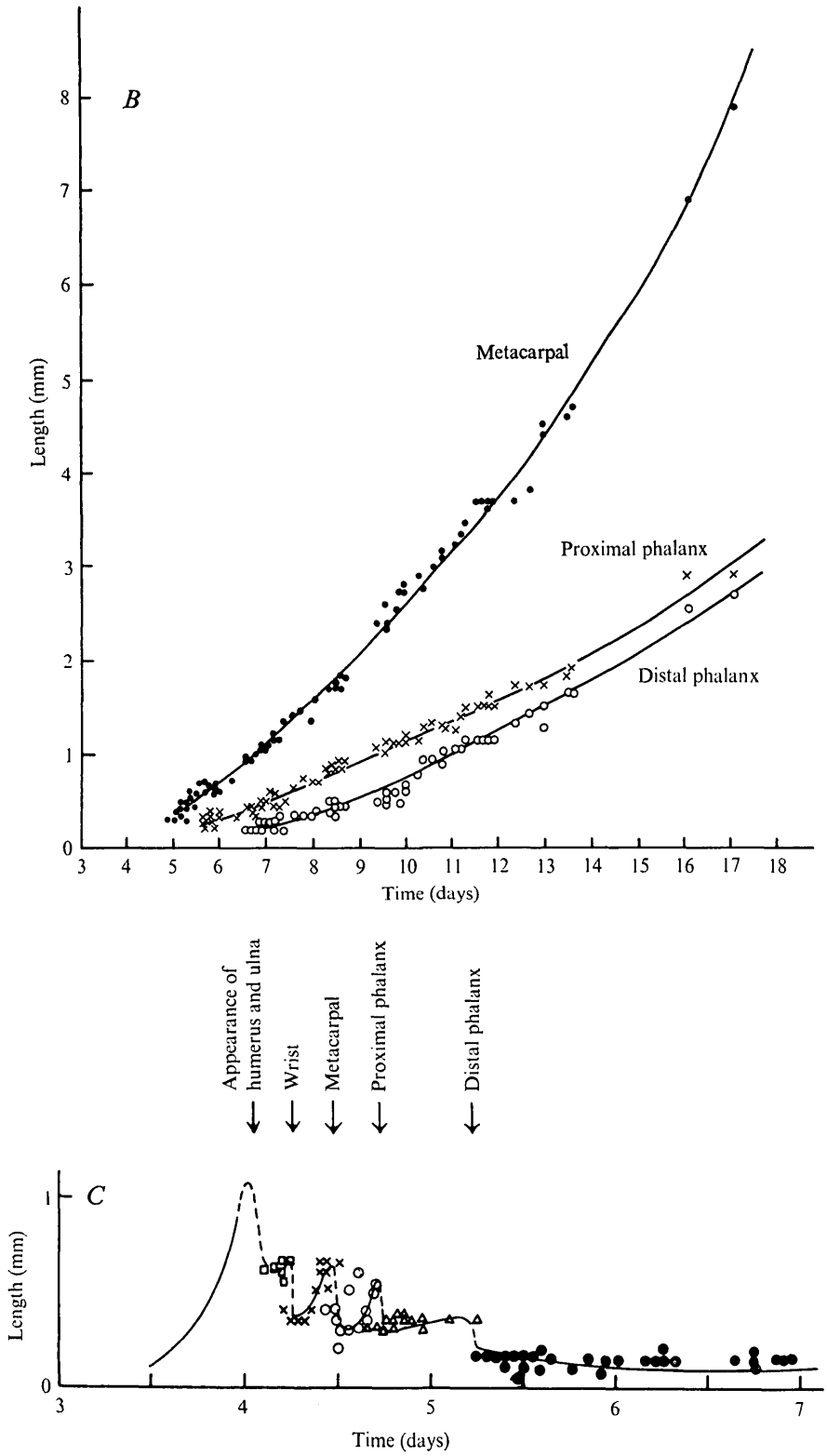

Fig. 3. Rate of elongation of individual skeletal elements, all from fixed and stained material. (*A*) Humerus, ulna, and wrist; (*B*) digit III: metacarpal and phalanges; (*C*) undifferentiated tip.

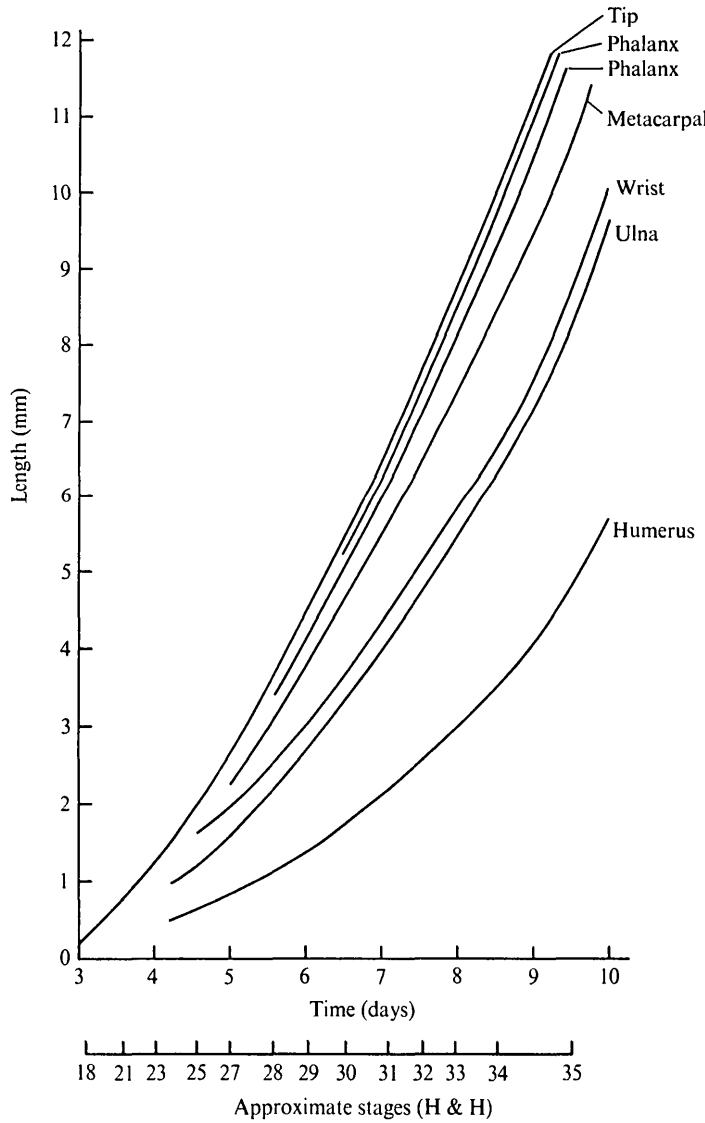

Fig. 4. 'Fate maps' of intermediate stages. Using the data from Fig. 3, mean proportion of the limb occupied by each skeletal level is estimated. The curve of total length should coincide with that of Fig. 2.

possible at the same stage. This corresponds well with the statements of Searls (1965) on early differentiation.

Fate maps of intermediate stages

Using the curves of Fig. 3 one can show the mean contribution of each limb segment to the overall curve of Fig. 2. These form a kind of fate map (Fig. 4). The time axis is calculated as in Fig. 3.

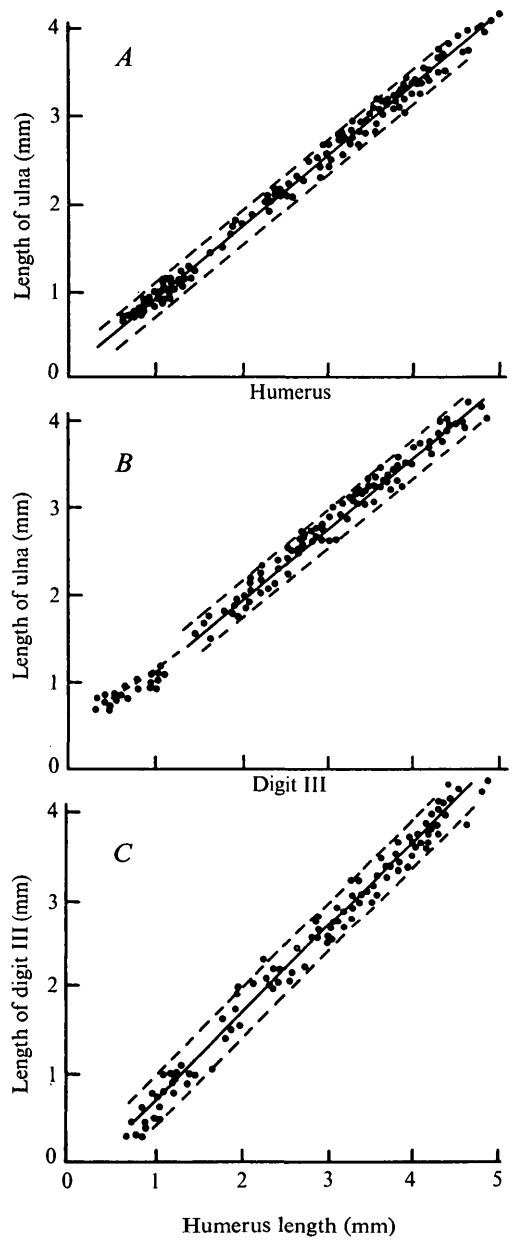

Fig. 5. The normal proportions between the major skeletal levels. (*A*) Ratio between humerus and ulna; (*B*) ratio between ulna and digit III; (*C*) ratio between digit III and humerus. The heavy line shows the least-squared regression line over the arbitrarily chosen linear part of the curve. The dashed line is not a true confidence interval. It represents an equal interval to each side of the regression line within which, by inspection, 95 % of all results ought to lie.

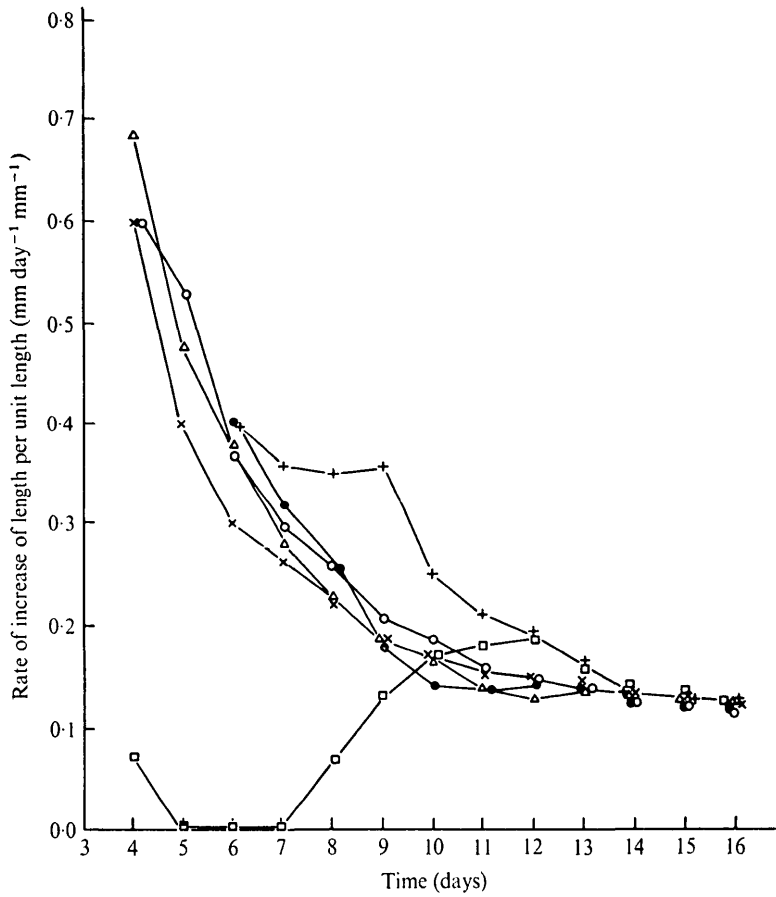

Fig. 6. Intrinsic rate of change of length of tissues at each level: △ = humerus, × = ulna, ○ = metacarpals, ● = proximal phalanx, + = distal phalanx, □ = wrist.

Normal proportions of the limb

By plotting the length of one skeletal element against a second skeletal element one obtains a curve of the normal proportions between these elements. Fig. 5*A* shows the ratio between the lengths of the humerus and the ulna, fig. 5*B* ulna and digit III, and fig. 5*C* humerus and digit III. The dashed lines indicate limits within which one would expect to find 95 % of results.

Intrinsic rate of change of length of cartilage

Figure 6 shows the rate of change of length per unit length of each level. Figure 3 gives a rather distorted picture of the rate of growth of each element. The humerus always appears to be growing faster than the other tissues, but this is because of the head start it obtains in the race. A true picture is given in Fig. 6. The curves here reflect more accurately what must be occurring at

the cellular level, whether the change is caused by growth of cells, division of cells or production of intercellular matrix.

Differentiation of the skeleton

Various stages in the differentiation of the skeleton are shown in Figs. 7 and 8. Nomenclature throughout is taken from Montagna (1945). Particularly at the level of the wrist it was often necessary to identify condensations when they first appeared by reference to later stages. With the exception of digit V no attempt has been made to include a record of transient structures, for example the wrist at stage 28 is a morphological equivalent of the Pleiades. From the photograph – e.g. Fig. 8*B* – one can discern a minimum of five elements; with the microscope, using a little imagination, one can make out several more.

The first inhomogeneity visible with alcian green staining is at stage 24, when the humerus and ulna, more or less simultaneously, become readily apparent (Fig. 7*A*). The ulna has a more obvious rod shape, the humerus is a vague amorphous mass. The radius is only very faintly visible, if at all, but is clearly present by stage 25, Fig. 7*B*. By stage 26 posterior wrist parts are beginning to take up the stain, and it is not always possible to distinguish a clear boundary between wrist and ulna (Fig. 7*C*). The humerus, ulna and radius are well demarcated by stage 27. The posterior wrist, consisting of centrale IV and metacarpal V, is obvious, and metacarpal IV is rapidly becoming clearer. This is followed at stage 28 by metacarpal III then phalanx IV, the anterior wrist parts begin to appear as faint condensations (Fig. 7*E*). By stage 29 the hand plate is still better formed, digit V has started to regress and the proximal parts of digit II are well advanced (Fig. 7*F*). Only the terminal elements of III and II are lacking by stage 30 (Fig. 8*A*). The former appears by stage 32 (Fig. 8*C*) and the latter by stage 34 (Fig. 8*D*). The skeleton has achieved its mature chondrogenic form. The major succeeding changes will be the onset of osteogenesis, at stage 36, Fig. 8*F*.

DISCUSSION

The data contained in this paper provide a detailed description of the changes in length and the differentiation of the skeleton of the wing. It has been usual in research on the limb to carry out experiments during the early stages of development, days 3, 4 and 5, with reference to the presumptive fate map at the time of operating; then to gauge the results at day 10, 11 or 12 in the post-morphogenetic phase. The important events following the perturbation which cause the result occur in this interregnum of days 6, 7, 8 and 9 and are largely ignored. This work is intended to provide a series of normal quantitative controls for this period similar to that provided in later stages by Summerbell & Wolpert (1973). The data provide, coincidentally, a rather

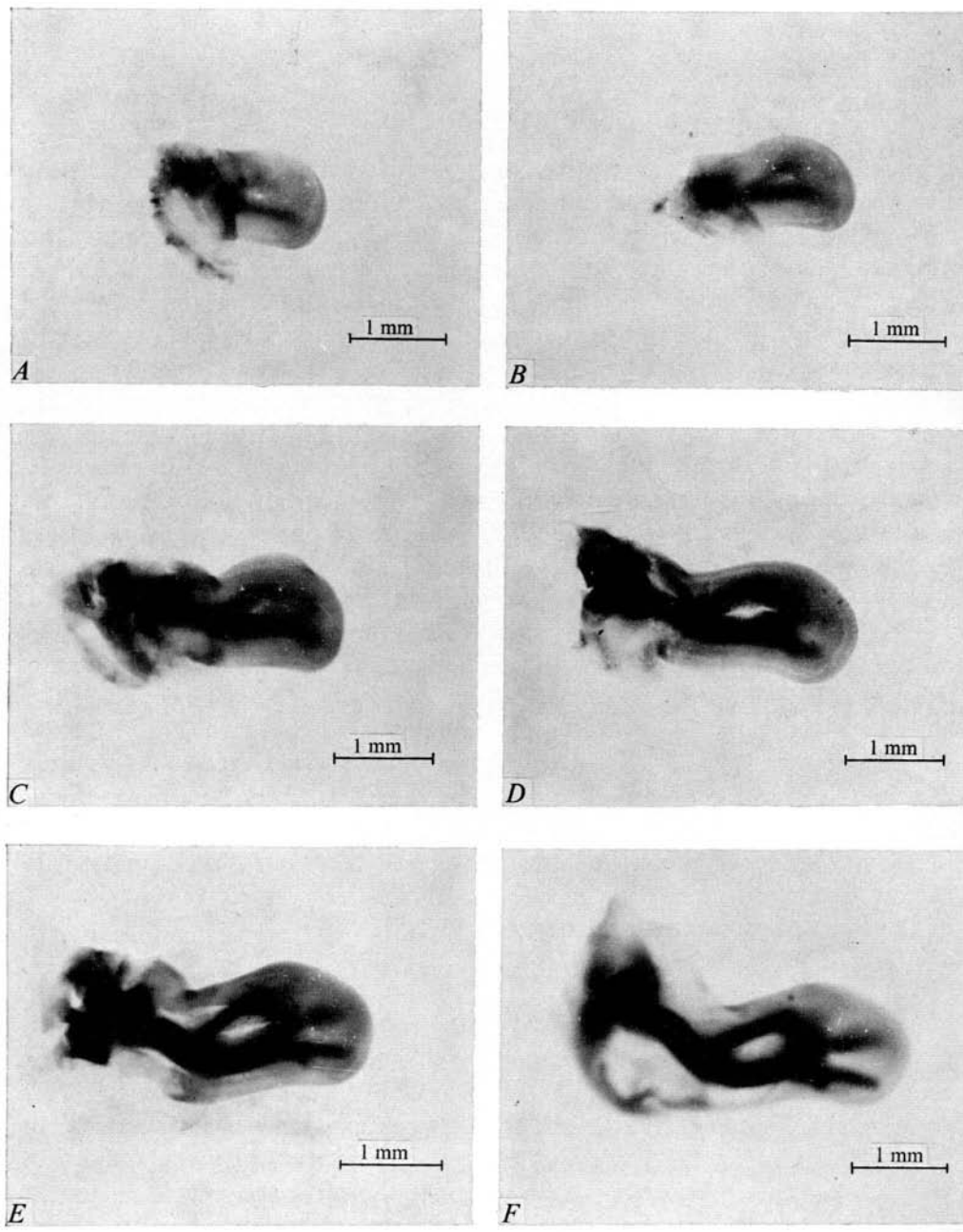

Fig. 7. Photographs of whole mounts. (*A*) Stage 24, (*B*) stage 25, (*C*) stage 26, (*D*) stage 27, (*E*) stage 27/28, (*F*) stage 28.

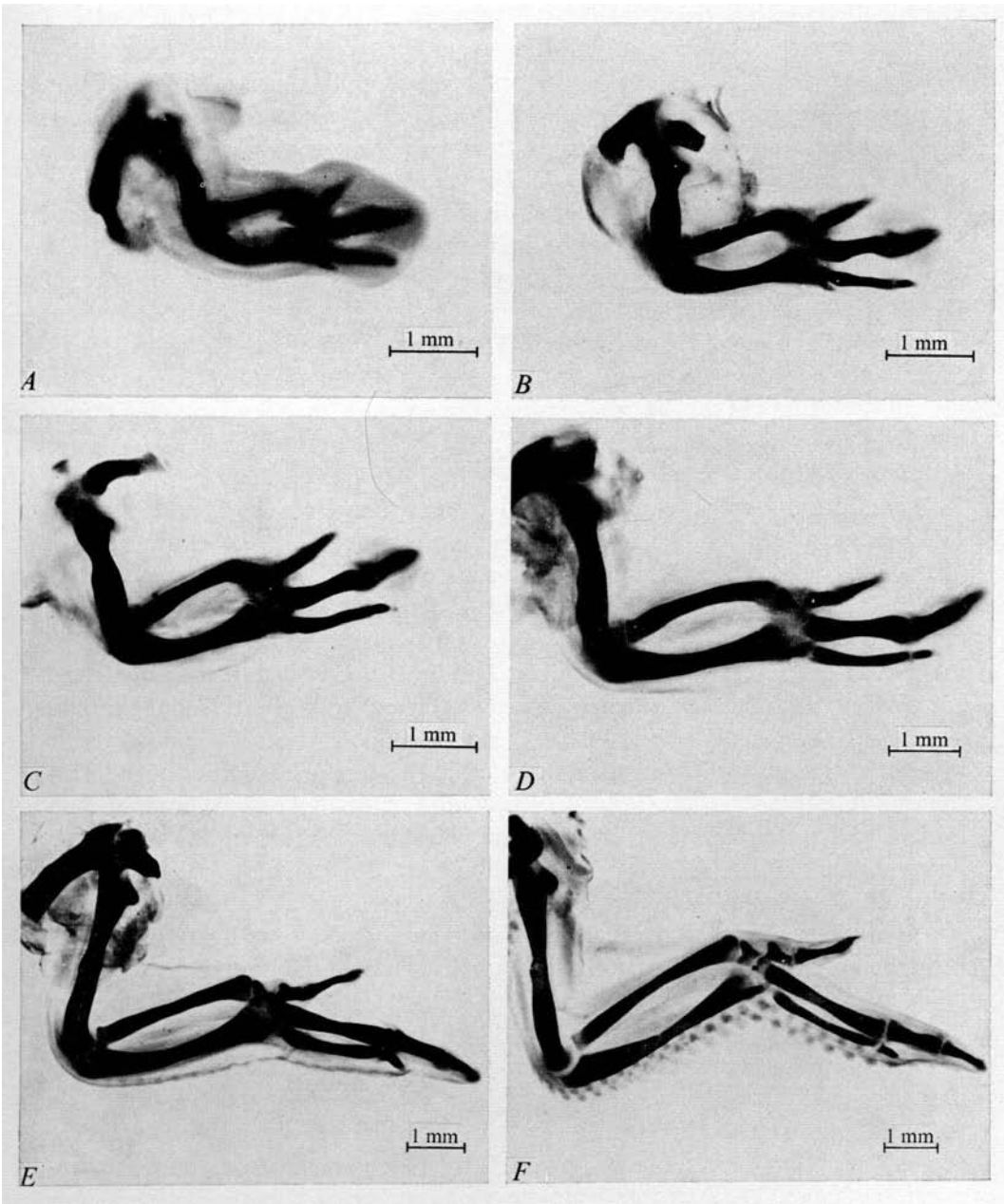

Fig. 8. Photographs of whole mounts. (*A*) stage 29, (*B*) stage 30, (*C*) stage 31, (*D*) stage 33, (*E*) stage 35, (*F*) stage 37.

different method of quantitative analysis of abnormality in the limb. This method is based on the constancy of proportions between different levels of the limb, and the detection of variation from these proportions.

The approach adopted is to first describe the normal course of differentiation of the cartilaginous skeleton of the limb and then measure the components of the skeleton as they appear so as to obtain the rate of elongation for the individual segments of the limb. The segments considered are: (1) stylopod = humerus, (2) zeugopod = ulna, (3) wrist = the interval between the distal epiphysis of the ulna and the proximal end of the metacarpal of digit III, (4) metacarpal of digit III, (5) phalanx I of digit III, (6) phalanx II of digit III, (7) tip = undifferentiated tissue between the most distal cartilage present and the extreme distal tip of the limb. These curves are then used in two ways. First in the analysis of proportion, and second to produce a set of 'fate maps' of normal development for the time at which the skeleton has begun to differentiate.

Differentiation

There is, as one might expect from the work of Saunders (1948) and Amprino & Camosso (1958), a distinct proximo-distal sequence in the appearance of differentiating skeletal elements, similar to that described by Hampé (1959) for the leg. The one major anomaly is, as has already been suggested by Searls (1965), the apparently simultaneous appearance of the humerus and ulna. The earliest that discrete regions of more heavily stained tissue can be detected is stage 24, and the last cartilage element to appear is the tip of digit II at stage 34. This early appearance of alcianophilic condensations in whole mounts correlates well with other reported indications of the onset of differentiation. It is preceded by uptake of sulphate, presumably for incorporation into mucopolysaccharide, (Searls, 1965), and by an increase in density in proximal cells (Fell & Canti, 1934; Gould *et al.* 1972) by about 12 h at stage 22. It is followed by metachromasia in sections at rather later stages as the density of the intercellular matrix increases, the time being rather dependent on the histological technique used. The same approximate sequence of events is well demonstrated in sectioned material, using alcian blue, by Hinchliffe & Ede (1967).

What is perhaps more surprising is the equally distinct posterior–anterior sequence in the appearance of skeletal elements. The analogous sequence has recently been described in the leg by Pautou (1975). The ulna appears before the radius, it remains more heavily stained during early stages, and is comparatively large. The perichondrium develops earlier, so the diaphysis is better delineated. The radius apparently later catches up, for by stage 36 overt ossification, as seen in whole mounts, starts at the same time in both elements. There are similar sequences at the levels of the wrist, the metacarpals and the phalanges. Even the ultimately vestigial digit V maintains its place in the sequence, it being the first of the metacarpals to make an appearance, but it

apparently never forms any more distal structures. It is originally very difficult to distinguish from the centrale IV but is probably present as early as stage 26 at the latest; it reaches its apogee at stage 28 and thereafter noticeably regresses. Digit IV is so much in advance of digit II that its phalanx appears at about the same time as the other's metacarpal. This postero-anterior sequence of development may explain some of the apparent anomalies in experimental work. For example, if the AER is excised at stage 20 one normally obtains more of the ulna than of the radius (Summerbell, 1974). If specification follows the same sequence as differentiation this is readily explained; there is at this stage less of the radius specified.

Rate of change of length curves

As successive elements appear it is noticeable that the original proximo-distal length of the alcianophilic region gradually declines, from about 400 μm for the humerus to about 200 μm for phalanx II of digit III. Initially thereafter the rate of increase of length per unit length is fast, but within two or three days it begins to slow down (see Fig. 6). By day 9 this rate is the same for the stylopod and the zeugopod, i.e. from this point on they maintain constant proportions. By day 11 the two most proximal elements of digit III have also adopted a similar rate and after day 14 the distal element too maintains a constant length ratio to the rest of the limb, at least up until the time of birth. The major anomaly is at the level of the wrist. Although it starts as approximately the same length as all the other levels it does not immediately adopt a fast rate of increase, but rather seems to remain of constant size from day 6 to day 7. One presumes that during this period there must be either virtually no cell division, or else a very high rate of cell death. The work of Hinchliffe & Ede rather suggests the former, as they do not seem to detect abnormally high levels of acid phosphatase activity at the level of the wrist during the early part of this period. On day 7 the wrist suddenly begins to grow again; presumably there is a sudden increase in the rate of cell division. The rate of increase of length per unit length is at first very slow but picks up to a rate exceeding the current rate in other parts of the limb by day 11. By day 14 the rate is the same as for the rest of the limb.

Specification

The initial sequence of appearance of alcianophilic condensations is very interesting. There does not appear to be a gradual advance of the distal boundary of differentiation but rather a series of hops, the hops decreasing from 800 to 200 μm. This observation is rather contrary to a simplistic interpretation of the idea of a monotonic gradient of positional value along the proximo-distal axis, for example as expressed in Summerbell, Lewis & Wolpert (1973) or Summerbell (1974). Then one might expect differentiation to occur as a continuous process, the distal boundary creeping down the limb, perhaps

trailing behind a progress zone by a constant amount. It does however fit well the contrasting ideas of, for instance, Ede & Agerbak (1968), who envisage each element as originally consisting of an aggregation of the precartilaginous cells of a region. These sudden steps also give a rather strange form to the graph of length of undifferentiated tip against time (Fig. 3*C*). There are a series of pulses as the tip grows, then abruptly contracts, as another quantum of tissue differentiates. These data can be related to the progress zone model of Summerbell *et al.* (1973). One would hardly suppose that differentiation would ever extend into the progress zone. These data then set an upper limit on the possible width of the zone at different times: 200 μm at stage 28, 300 μm at stage 26, 350 μm at stage 25 and 600 μm at stage 24. This fits tolerably well with the estimates of Summerbell & Lewis (1975) of 270 ± 80 μm. It also suggests that the width of the progress zone might diminish during development, but no direct evidence could be obtained to support this notion. It is important that differentiation never extends so close to the tip during this crucial period that it does not leave adequate room for a progress zone. After stage 28 the width of the tip remains at about 100–150 μm, where at later stages it probably consists wholly of dermis.

A quantitative analysis of the proportions of the limb

If an operation is carried out on the right wing of an embryo one has an extremely sensitive assay for the effect of the perturbation by comparing it with the contralateral control wing (Summerbell & Wolpert, 1973). One can detect accurately by this method changes in the length of the wing of the order of less than 2 %. The method is of little use if both wings are to be affected, as is the case for example in the effect of drugs on the embryo. One is then able to detect gross physical abnormality with ease, but more subtle modifications are not easily characterized. From the data contained in Fig. 3 it appears that for a later stage the ratio between the lengths of different skeletal elements remains relatively constant. Any variation from the observed control ratio could be used as an assay of abnormality. In Fig. 5 the mean ratios between the lengths of the three major skeletal levels, stylopod, zeugopod and autopod, are drawn for different lengths of each element. The heavy line is the fitted regression line for the straight portion of the graph, and the dashed lines above and below indicate the limits within which 95 % of all normal results ought to lie. Using this method of analysis, it should be possible to detect a deficiency in the length of one element of the order of ± 10–15 % depending on the age of the limb, or equivalent to approximately 5 % of the entire limb. While this is not as sensitive as the direct comparison of right and left limbs, nevertheless it should prove to be a very effective research tool. While it would in principle be possible to extend the method to the proportions within the digits, in fact the sensitivity falls off rather too rapidly due to the shortness of the distal phalanx.

Fate maps

Many factors limit one's ability to draw a fate map. There is the variation between different embryos, the uncertainty as to whether or not all the progeny of a single cell go to form part of the same element, the problem of relative movement between marker and cell or, if the marker is intercellular, the possibility of abnormal interaction between host and graft. Aside from these technical difficulties there are two fundamental prerequisites which must be fulfilled. There must be no large-scale random movements of cells relative to one another, and a cell's fate must be a function of its position in the limb. The former of these fortunately seems to be true for almost any stage of limb development. It seems that normally there is very little mixing of cells even in the very early limb (Stark & Searls, 1973, using radioactively labelled grafts; Summerbell, 1973, using quail grafts; Cioffi, 1975, using quail grafts). Thus perhaps one need not worry about itinerant cells constantly crossing fate-map boundaries and hence changing their presumptive fate. Though even then one should be cautious for there are also experiments demonstrating cell sorting-out, for example Singer (1972) and Cioffi (1975), which show that under rather extraordinary circumstances there is relative movement of cells even at very late stages. The latter is not so easily demonstrated, at least for early stages. One presumes that in the absence of cell mixing the latest at which it must be true is the time of determination; most probably it occurs rather earlier. It is also worth noting that presumptive fate is not the same thing as positional value (Wolpert, 1969). A cell's positional value can and possibly normally does change during development; see for example the models of Summerbell *et al.* (1973), or Summerbell (1975). During normal development a cell's fate cannot by definition change. Fate maps are for ever.

The curves illustrated in Fig. 4 also form a map, a map which shows at each stage the proportion of the proximo-distal axis of the limb occupied by each skeletal level. It cannot strictly speaking be called a fate map, because fate map normally bears a certain connotation of presumptive fate and here the fate is no longer presumptive but apparent. When compared with traditional fate maps it is certainly more accurate and it may prove to be more useful. Its weakness lies in the blank state of the map at the time at which the experimentalist is most interested; its strength is its definitive nature, the exchange of actual for predicted fate. Thus the major difficulties associated with the construction of fate maps are obviated. Morphogenesis is at such an advanced stage at the time of drawing that there is little room for uncertainty as to which cells are going to form which skeletal elements. The appropriate cells are already determined (Searls & Janners, 1967; Cioffi, 1975), and are already differentiating. The inherent difficulties are the interpretation of the form and position of each element as it appears, and the accuracy with which it can be measured.

Although comparisons are odious, the paper would not be complete without setting these maps alongside earlier estimates. It rapidly becomes apparent that they form not an alternative to other fate maps but a complement. Four other fate maps for the wing have been mentioned. Of these those of Saunders can be rapidly passed over; they extend only as far as stage 21 (see Hamburger and Hamilton for this correction of Saunders' stages), and this does not overlap the present work. Similarly the Stark & Searls estimates extend only as far as the least accurate part of Fig. 6, i.e. stage 24; at this stage the fit is far from good, their autopod being considerably shorter. Fig. 3 and 4 will effectively replace their extrapolated growth curves (their fig. 8), for as their estimates become increasingly inaccurate after stage 24 these curves become increasingly more accurate, and the converse. The only fate maps which cover concurrent stages for any length of time are those of Amprino & Camosso. With some modification of their staging it is possible to obtain quite a good fit between the two sets of maps. It appears that this is not the only paper to find Amprino & Camosso's staging a little eccentric. If the outlines of their stages 18 to 24 are compared with those of Stark & Searls there are only small differences during early stages, but by Stark & Searls' stage 24 Amprino & Camosso have only reached stage 23. Similarly Lewis (1975) finds it necessary to call Amprino & Camosso's stage 24 equivalent to his stage 25. This does not alter the validity of the fate maps, it is only a trivial difference in interpretation of Hamburger & Hamilton's stages. If therefore one compares Amprino & Camosso's fig. 15 with Fig. 4 and modifies their staging so that 23 = 24, 24 = 25, 25 = 26, 26 = 27 and 27 = 28, then one obtains quite a good fit between the two maps, the major discrepancy being that Amprino & Camosso, throughout, make the wrist 30% shorter. One cannot of course compare the fate maps of Lewis, which are intended to be used in conjunction with other fate maps. One can compare the rates of growth of total length, and here it seems that the Lewis rate is slower. The difference is within the variation likely to be met within the accuracy of staging. The Lewis fate maps in fact form an excellent complement to these maps. The curves in Figs. 4 and 6 are probably only accurate from about stage 25 – compare the clarity of the staining in Figs. 7A and B. The Lewis fate maps extend up to this stage and allow one to extrapolate back to the earlier stages.

CONCLUSION

These curves provide a simple description of the normal course of development of the wing. It is hoped that succeeding studies will elucidate the changes at a cellular level which produce both the rate of growth and the course of onset of differentiation. It will be particularly interesting to discover if the timing of differentiation is autonomous to the cells, an independently propagating activity, or a factor under the control of one of the major control

centres of the limb, the apical ectodermal ridge or the zone of polarizing activity. The curves are also interesting in that they provide yet another alternative method of producing a fate map. In passing they provide also a new means of detecting abnormality in the limb, the maintenance of normal proportion. This method should be of particular use in assaying the effect of drug action on development.

Finally they provide a set of controls for that very interesting period of development when the skeleton is just beginning to differentiate. This may be of value in indicating the importance of events which occur in between the period when the parts of the limb are specified, and in which experimental perturbations are normally carried out, and the time at which the effect is normally observed.

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REFERENCES

AMPRINO, R. & CAMOSSO, M. (1958). Analisi sperimentale dello sviluppo dell'ala nell'embrione di pollo. *Wilhelm Roux Arch. EntwMech. Org.* **150**, 509–541.

CIOFFI, M. (1975). *Determination and stability during differentiation in the avian limb bud.* Ph.D. Thesis, University of London.

EDE, D. A. & AGERBAK, G. S. (1968). Cell adhesion and movement in relation to the developing limb pattern in normal and *talpid*^{3} mutant chick embryos. *J. Embryol. exp. Morph.* **20**, 81–100.

FELL, H. B. & CANTI, R. G. (1934). Experiments on the development *in vitro* of the avian knee-joint. *Proc. R. Soc.* B **116**, 316–349.

GOULD, R. P., DAY, A. & WOLPERT, L. (1972). Mesenchymal condensation and cell contact in early morphogenesis of the chick limb. *Expl Cell Res.* **72**, 325–336.

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

HAMPÉ, A. (1959). Contribution à l'étude du développement et de la régulation des déficiences et des excédents dans la patte de l'embryon de Poulet. *Archs Anat. microsc. Morph. exp.* **48**, 345–478.

HINCHLIFFE, J. R. & EDE, D. A. (1967). Limb development in the polydactylous *talpid*^{3} mutant of the fowl. *J. Embryol. exp. Morph.* **17**, 385–404.

HORNBRUCH, A. & WOLPERT, L. (1970). Cell division in the early growth and morphogenesis of the chick limb. *Nature, Lond.* **226**, 764–766.

LEWIS, J. H. (1975). Fate maps and the pattern of cell division. *J. Embryol. exp. Morph.* **33**, 419–434.

MONTAGNA, W. (1945). A reinvestigation of the development of the wing of the fowl. *J. Morph.* **13**, 259–295.

PAUTOU, M. P. (1975). Morphogénèse de l'autopode chez l'embryon de Poulet. *J. Embryol. exp. Morph.* **34**, 511–529.

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

SEARLS, R. L. (1965). An autoradiographic study of the uptake of S-35 sulfate during the differentiation of limb bud cartilage. *Devl Biol.* **11**, 155–168.

SEARLS, R. L. & JANNERS, M. Y. (1967). The stabilisation of cartilage properties in the cartilage-forming mesenchyme of the embryonic chick limb. *J. exp. Zool.* **170**, 365–376.

SINGER, R. H. (1972). Analysis of limb morphogenesis in a model system. *Devl Biol.* **28**, 113–122.

STARK, R. J. & SEARLS, R. L. (1973). A description of chick wing bud development and a model of limb morphogenesis. *Devl Biol.* **33**, 138–153.

SUMMERBELL, D. (1973). *Growth and regulation in the development of the chick limb.* Ph.D. Thesis, University of London.

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. H. (1975). Time, place, and positional value in the chick limb bud. *J. Embryol. exp. Morph.* **33**, 621–643.

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

SUMMERBELL, D. & WOLPERT, L. (1972). Cell density and cell division in the early morphogenesis of the chick wing. *Nature New Biol.* **238**, 24–26.

SUMMERBELL, D. & WOLPERT, L. (1973). Precision of development in chick limb morphogenesis. *Nature, Lond.* **244**, 228–229.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1–47.

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