Cell proliferation in the developing wing-bud of normal and talpid\(^3\) mutant chick embryos

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SUMMARY

Previous measurements on mitotic division rate or cell cycle time have been made on samples from a few discrete limb regions or by continuous sampling, but only down a unidimensional limb axis, disregarding morphological discontinuities such as the presence or absence of cartilage. This study presents a new analysis on normal and talpid\(^3\) mutant chick embryos, measuring mitotic rate and also cell density through the central proximo-distal axis and at the limb periphery, taking into account the development of cartilage regions. Differentiation of cartilage is correlated with a marked drop in mitotic rate, accounting for a proximo-distal gradient of mitosis in central counts which was not observed at the limb periphery. Talpid\(^3\) limbs at an early stage show a central mitotic gradient, but the reverse of that observed in normal limbs.

INTRODUCTION

The vertebrate limb-bud exemplifies the two major problems of morphogenesis: (1) the production of a structure of complex and characteristic form by the activity of its constituent cells and (2) the mechanisms at genetic and other levels, e.g. of cell interaction, which control this activity. The parameters which are significant for the first problem have been listed by Ede & Law (1969): cell number and rate of proliferation; cell size, shape and packing density; cell position and movement; and constraints imposed by boundary membranes, e.g. on the mesoderm by the overlying ectoderm. Of these, the most important in producing the increasing mass of the limb is cell proliferation by mitosis, though changes in cell size and packing density may each play a minor role. What produces the characteristic developing shape of the limb-bud is more problematical, and the role of cell proliferation is not entirely clear in spite of a number of investigations on mitosis in the chick limb-bud by, for example, Cairns (1966), Janners & Searls (1970), Hornbruch & Wolpert (1970) and Thorogood & Hinchliffe (1975).

The purpose of the present investigation has been to supplement the informa-
tion obtained by these authors on the normal limb-bud, and to obtain insight by a corresponding study in the talpid* mutant, where the shape of the outgrowing bud is drastically altered. The information obtained will be incorporated into a computer simulation model of limb development currently being developed by O. K. Wilby in this laboratory, based partly on the early model developed by Ede & Law (1969). Once a satisfactory model for the production of limb development is achieved, the way is open for an attack on the other major problem – the analysis of its underlying control mechanisms.

MATERIALS AND METHODS

Eggs were incubated from an inbred Shaver line producing normal (+/+), ta3/+ and talpid3 (ta3/ta3) mutant embryos. Sixteen right and left wing-buds were amputated from mutant and normal at stages 24 (2N, 2T), 25 (3N, 3T), and 27 (3N, 3T) (Hamburger and Hamilton). The limb-buds were fixed in Bouin, wax-embedded, and cut transversely in 7 \( \mu m \) sections, which were stained with haematoxylin and eosin. Counts of mitotic figures and all nuclei were made in every tenth section at a central and at an anterior peripheral position, within the area covered by a square eyepiece graticule of 2.5 \( \times 10^4 \) \( \mu m^2 \). In order to calculate the mitotic index blood vessels were never included in the areas chosen for counting, which comprised between 200 and 600 cells, and myogenesis does not occur in these regions. An attempt was made never to include cartilage cells in peripheral counts. This was not always possible with talpid3 limb-buds. The complete section was also drawn at a lower power with a camera lucida attachment. The limb-bud outline of each limb was then reconstructed from these drawings with its distoproximal parameters altered to fit an arbitrary length of 100 units. Limb width was changed by an arbitrary but constant amount for each stage to compensate for any distortion produced by the change of proximo-distal length. The figures (Figs. 1, 2, 3) show the plotted distribution of mitosis and the silhouette of the corresponding limb-bud. Cartilage silhouettes, also reconstructed from the drawings, are superimposed. The path (width accurately reconstructed) along which mitoses were measured is also shown. This path was used as a base line for the reconstruction of both centrally and peripherally counted limb-buds. The limb is distorted symmetrically about this path. Thus the points where counts leave or enter regions of cartilage is shown as accurately as possible. No estimation of cell cycle time was made.

RESULTS

Statistical analysis. Regressions were calculated of the number of mitotic figures per 100 nuclei for each limb, \( p_1, p_2, \ldots, p_n \) upon \( N_1, \ldots, N_n \), the positions in the limb as a fraction of total length of axis. A quadratic polynomial was fitted to \( p \) and to its square root for each limb separately. The square root
of \( p \) gave an estimate of sampling variance which was homogeneous between limbs after allowing for quadratic regression (Bartlett's test gave \( \chi^2_{16} = 35.0 \) for \( p \) and \( \chi^2_{16} = 1.4 \) for \( \sqrt{p} \)).

Analysis of variance was used to test the differences between limbs for the mean, linear and quadratic coefficients \( (B_0, B_1, B_2) \) respectively. In the case of the \( B_0 \) and \( B_1 \), the component of variation between limbs was significant. Significant differences between mean levels of \( p \) were not apparent for \( B_0 \). Only the mean differences between days were apparent for \( B_1 \) \( (P < 0.05) \). For \( B_2 \), however, differences for the main effects of time, embryo type and whether counted centrally or peripherally were apparent \( (P < 0.05) \).

Analysis of the mitotic distributions. The plotted mitotic counts for single representative limb-buds are presented in Figs. 1, 3, 5 and these must be assessed in conjunction with the statistical analysis based on the summed data. Some of the conclusions to be drawn from them have firm statistical significance, but others must be only inferences which have more or less plausibility according to whether the trends on which they are based show a consistency with those observed in other limb-buds, of the same stage and also in successive stages. In each case, there are some sharp irregularities at various levels of the limb-bud which must be accepted as arising from sampling error, but in the main irregularities can be plausibly related to the limb-bud region, either cartilage or non-cartilage, through which counts are passing. However, to achieve a completely satisfactory analysis it will be necessary to undertake an investigation on a much more extensive scale than this or than any previously reported elsewhere.

The statistically validated conclusions are as follows: in normal embryos in the central region there is a gradient of mitosis from a high level distally at the tip to a lower level proximally at the base of the bud at all stages, 24, 25 and 27. Within the gradient at each stage the counts drop off sharply distally as we move back from the tip, then more gradually proximally. There are fewer mitoses overall at stage 27 than at stage 25. In the peripheral regions (there is no peripheral count at stage 24) there is again a gradient from high distal to low proximal at stage 25, but there is no sharp distal drop; at stage 27 the gradient has disappeared, with no significant differences between counts along the length of the bud. As in the central region, but more dramatically, there is a drop in the overall number of mitoses between stages 25 and 27.

In talpid embryos there are certain similarities but also some interesting differences from normal. In the central region there is a mitotic gradient at stage 24, but with reverse direction, with low distal and high proximal counts. At stage 25 there is a gradient with the normal direction, but with a very steep decline distally, as we move back from the tip, and thereafter an almost constant level to the base. At stage 27 there is a proximo-distal gradient as in normals and again with a smooth decline along the gradient from tip to base. In the peripheral regions there is a gradient from high distal to low proximal in stage 25 as in normal embryos but with a sharp rise between the two cartilage complexes,
followed by a steep fall towards the base of the limb. At stage 27, again as in normals, the gradient has disappeared, though counts appear to be lowest at the distal and proximal limits of the limb-bud, but – what is very striking – in this case there is no fall in the overall number of mitoses.
Correlation of mitosis with position in limb-bud

*Normal.* At stage 24 in both normal limb-buds (Fig. 1), there is a dip in mitotic level corresponding to the non-cartilage contained by the forking of radius and ulna. This dip can again be seen in stage-25 limb-buds (Fig. 3).

![Graphs showing changes in cell densities and mitotic indices at stage 24.](image)

Fig. 2. Changes in cell densities (●) compared with mitotic indices (○) at stage 24.

There is no corresponding dip in stage-25 peripheral counts. This is followed by a sharp rise and a fall as counts enter and pass through the humerus. At stage 25 counts also start to fall as they enter and pass through the just-initiated metacarpal–carpal–digit complex.

At stage 27 it is clear that whenever counts pass through cartilage they fall and rise in non-cartilage regions. There are no such corresponding irregularities in the stage-27 peripheral counts. Within the distal 10–20% of normal limbs
Fig. 3. Change of mitotic index with position in limb along the central proximodistal axis and the limb periphery at stage 25 in normal and *talpid* embryos.
there appears to be a distinct fall in mitotic index towards the distal tip which is not related to the presence of cartilage.

*talpid*³. No definitive area of mucopolysaccharide-secreting cells can be detected in stage-24 (Fig. 1) *talpid*³ wing-buds. The two curves obtained show different irregularities. But at stage 25 (Fig. 3) there is a clear sharp fall as soon
Fig. 5. Change of mitotic index with position in limb along the central proximo-distal axis and the limb periphery at stage 27 in normal and talpid\textsuperscript{3} embryos.
Fig. 6. Changes in cell densities (●) compared with mitotic indices (○) at stage 27.
as counts enter and pass through the metacarpal-carpal complex, followed by a rise between this region and the proximal humerus, radius and ulna complex. This peak is repeated in peripheral counts at stage 25, and counts also pass through the periphery of the cartilage-forming areas. The proximal falling off of peripheral counts in stage-25 *talpid*³ limb-buds also repeats that observed in central counts. However, there is no sudden distal drop.

At stage 27 (Fig. 5) the distal drop in mitotic counts on entry into cartilage is seen again but only two other features are seen in both examples. Firstly, the drop is maintained into proximal regions. Secondly, there is a rise observed through the distal 50% of the radius and ulna–humerus complex. This corresponds to a division which appears at this stage in *talpid*³ between radius and ulna. As soon as counts pass into the humerus, they begin to fall again.

Table 1. Average cell densities (cells per 10⁶ μm²) in normal and *talpid*³ cartilage and non-cartilage at stages 24, 25, 27. The error given is the standard deviation

<table>
<thead>
<tr>
<th>Stage</th>
<th>Normal Cartilage/pre-cartilage</th>
<th>Non-cartilage</th>
<th>talpid³ Cartilage/pre-cartilage</th>
<th>Non-cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>235 ± 20-1</td>
<td>208 ± 28-4</td>
<td>214 ± 21-3</td>
<td>208 ± 20-1</td>
</tr>
<tr>
<td>25</td>
<td>246 ± 24-2</td>
<td>233 ± 9-6</td>
<td>211 ± 13-5</td>
<td>212 ± 21-3</td>
</tr>
<tr>
<td>27</td>
<td>194 ± 44-7</td>
<td>320 ± 27-2</td>
<td>242 ± 18-4</td>
<td>249 ± 18-1</td>
</tr>
</tbody>
</table>

*Changes of cell density in developing cartilage and non-cartilage regions*

All values were taken from strictly cartilage or strictly non-cartilage regions except in the case of *talpid*³ stage-24 limb-buds. At this stage in *talpid*³ there is a diffuse area of mucopolysaccharide-secreting cells, most intense proximally, tapering off distally. The anterior 50% of the limb was taken as non-cartilage, the rest pre-cartilage. Table 1 gives the average cell densities per 10⁶ μm² and Table 2 gives the results of an analysis of variance on the data. A square root transformation was used throughout to reduce the variance.

Cell density in normal pre-cartilage areas is similar at stage 24 and stage 25 and is higher than in respective non-cartilage areas; it falls to a value not significantly different from the non-cartilage at stage 27. Cell density increases in the non-cartilage between stage 24 and 25 but does not change from stage 25 to 27.

The cell density remains the same in *talpid*³ pre-cartilage between stage 24 and 25 but rises significantly between stage 25 and 27. At no stage is there any significant difference between non-cartilage and pre-cartilage. The non-cartilage therefore follows the pattern observed in pre-cartilage, increasing between stage 25 and 27.

Normal pre-cartilage is denser than *talpid*³ at stage 24 and stage 25, but less dense at stage 27. *Talpid*³ non-cartilage is significantly less dense than normal non-cartilage at stage 25 but at stage 24 and 27 they are of similar density.
Table 2. Results of an analysis of variance on the cell densities given in Table 1

The comparisons being made are given in the top column. The null hypothesis is that pairs of results are not significantly different. $P =$ probability of finding the $F$ value ($P<0.05$ taken as significant) with the given degrees of freedom (d.f.) within the two groups.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cartilage and non-cartilage</th>
<th>Normal and talpid$^a$</th>
<th>Normal and talpid$^a$</th>
<th>Cartilage and stage</th>
<th>Normal and talpid$^a$</th>
<th>Non-cartilage and stage</th>
<th>Normal and talpid$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td></td>
<td>17</td>
<td>$&lt;0.025$</td>
<td>19</td>
<td>$&gt;0.25$</td>
<td>20</td>
<td>$&lt;0.025$</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>23</td>
<td>$&lt;0.005$</td>
<td>24</td>
<td>$&gt;0.25$</td>
<td>29</td>
<td>$&lt;0.005$</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>46</td>
<td>$&gt;0.10$</td>
<td>22</td>
<td>$&gt;0.25$</td>
<td>41</td>
<td>$&lt;0.005$</td>
</tr>
</tbody>
</table>

Table 3. Regression and correlation analysis of change of cell density with mitotic index in normal and talpid$^a$ limbs

<table>
<thead>
<tr>
<th>Embryo</th>
<th>Where counted in limb</th>
<th>Average mitotic index</th>
<th>Average cell density in $1000 \mu m^2$</th>
<th>Number of values</th>
<th>Regression coefficient (B)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Periphery</td>
<td>4.15</td>
<td>24.13</td>
<td>32</td>
<td>-0.307</td>
<td>-0.268</td>
</tr>
<tr>
<td></td>
<td>Central proximo-distal axis</td>
<td>3.73</td>
<td>24.17</td>
<td>77</td>
<td>-0.529</td>
<td>-0.371</td>
</tr>
<tr>
<td>Talpid$^a$</td>
<td>Periphery</td>
<td>6.47</td>
<td>22.96</td>
<td>25</td>
<td>-0.589</td>
<td>-0.560</td>
</tr>
<tr>
<td></td>
<td>Central proximo-distal axis</td>
<td>3.72</td>
<td>23.19</td>
<td>65</td>
<td>-0.34</td>
<td>-0.246</td>
</tr>
</tbody>
</table>
Comparison of mitotic index with cell density. Mitotic counts from all limbs at all stages were correlated with cell density in talpid³ and normal both in the centre of the limb and at the periphery. The values are plotted in Fig. 7. Table 3 contains the initial analysis of these figures. The correlation coefficients (c) and the $t$-test comparison of observed with expected values of mitotic index, given the calculated regression coefficient (B), agree that there is no significant
correlation of mitotic index with cell density at the periphery of normal limb-buds, but there is a very high correlation along the central proximo-distal axis. Both central and peripheral values of mitotic index and cell density are strongly correlated in talpid³ limb-buds.

A further analysis of covariance revealed that the means for talpid³ and normal central measurements are significantly different ($F = 4.645$, d.f. = 139: $P < 5\%$). An area along the path of central counts having the same cell density in talpid³ and normal would have a higher mitotic index in talpid³. But the means for talpid³ central and talpid³ peripheral do not differ ($F = 1.257$, d.f. = 87: $P > 25\%$). In both cases (the comparison of (1) normal with talpid³ central, and (2) talpid³ central and peripheral) the slope, i.e. the rate of change of mitotic index with cell density, is not significantly different ($P > 25\%$).

The distribution of individual cell density measurements along the limb axis for each case is presented in Figs. 2, 4 and 6, in which the data for mitotic indices is replotted for comparison. No statistical analysis is possible in this case but certain general tendencies emerge. The results for normal central regions reflect the inverse relationship between mitotic index and cell density. So do talpid³ central regions, except at stage 24, where our statistical analysis has masked what is clearly a direct relationship between them. The distributions for peripheral regions also reflect the statistically validated conclusions, i.e. no correlation in normals, but an inverse relation in talpid³. Where mitotic index and cell density are inversely related the pattern of cell density generally follows the pattern of chondrogenesis; in normal central regions at stages 24, 25 and 27 there are rises in cell density on entering chondrogenic regions and falls on leaving them, and corresponding falls and rises in mitotic index. This is also true for central sections in talpid³ in stages 25 and 27; at stage 24 mitotic index and cell density are both highest proximally but here chondrogenesis is not clearly established. There is some indication of an inverse relationship which is not associated with chondrogenesis at the distal tip of normal limb-buds.

**DISCUSSION**

**Mitosis**

*NORMAL WING-BUD* 

The existence of a mitotic gradient, with a higher count distally, was first noted by Amprino (1965). It has been investigated by Hornbruch & Wolpert (1970), who showed that the overall level of mitosis dropped steeply between stages 18 and 23 and more gradually thereafter, and the existence of a gradient which was statistically significant from stage 25. Our investigation shows an overall proximo-distal gradient at stages 24, 25 and 27 but, because the sampling method distinguishes between central and peripheral counts, it is possible to add information about the variation between different regions which affects our interpretation of the gradients.
There is a clear difference between central and peripheral regions which is probably related to the presence of differentiating cartilage cells centrally. The gradient exists at all three stages in the central regions, but there are great irregularities in the slope; in peripheral regions the gradient exists at stage 25, with a much smoother curve, but it has disappeared at stage 27 when there is also a drop in peripheral mitotic index from 4–8 to 1–4. Janners & Searls (1970) have shown that by stage 24, when mucopolysaccharide synthesis is beginning, 75% of cells in the proximal central region have stopped dividing, but only 25% in the subapical region. This is also the stage at which pre-cartilage condensation begins, in which the chondrogenic regions become defined by an increased packing density, 30% over the surrounding mesenchyme according to Gould, Day & Wolpert (1972) and 60% according to Thorogood & Hinchliffe (1975). We may therefore conclude that the process of cartilage differentiation, which commences with the proximal elements and spreads distally, is accompanied by a decrease in the number of dividing cells and an increase in packing density, and that there will consequently be a gradient of each within the limb-bud, with opposite directions and consequently an inverse relationship between the two.

The marked irregularities in the slopes for the central regions will arise from the sequence of sampling regions passing through the complex alternation of chondrogenic and non-chondrogenic areas produced by the developing elements of the cartilage pattern (Figs. 1, 3, 5) and from temporal differences arising from the sequential origin of these skeletal elements. On entry into cartilage there is a sudden fall in mitotic index and on leaving it there is a rise.

As we should expect if the inverse relationship between mitotic index and cell density is predominantly due to the events of chondrogenic differentiation, we find no correlation between them in the peripheral regions. There is however a gradient of mitosis at stage 25, though not a steep one, and this is clearly not related to chondrogenesis. The peripheral mesenchyme is behaving differently from the central mesenchyme, and in stage 27 the mitotic index has dropped along the whole of its length. We have no explanation for this distinct behaviour, but the maintenance of high levels of peripheral mitosis between stages 25 and 27 may be related to the expansion of the distal paddle which occurs at this stage.

Additional irregularities in the slopes will be expected where the count passes through any area of cell death. Thus at stages 24 and 25 there is a fall in the central counts at the point of divergence between radius and ulna, but not at stage 27. This corresponds to the region of the ‘opaque patch’, an area of cell death investigated by Hinchliffe & Thorogood (1974) which disappears by stage 27.

The inverse relationship which is indicated at the distal tip of normal limb-buds in Figs. 2, 4 and 6, with a dip in mitotic index, i.e. reversing the direction
Cell proliferation in chick embryos

of the overall gradient, and a corresponding small rise in cell density, which is clearly not related to chondrogenesis, requires further investigation.

talpid³ wing-bud

In the talpid³ central region there is a gradient with high mitotic index distally at stages 25 and 27, with a drop behind the tip to a low proximal level. As in normals, the central mitotic counts follow the cartilage pattern closely (Figs. 1, 3, 5); at stage 27 there is even a small peak corresponding to the gap between humerus and radius/ulna blocks, though the distinction between blocks is less clear in talpid³ and the gap itself is not shown in the figure. Cell death in the ‘opaque patch’ region has been shown by Hinchliffe & Thorogood (1974) to be absent or markedly reduced, so the absence of a dip in this region is to be expected. As in normals, there is an overall inverse correlation between mitotic index and cell density in the central region which may again be explained as arising from the sequence of events in chondrogenesis.

Earlier, at stage 24, there is a steep gradient with high mitotic index proximally, i.e. the reverse of that found in normals at all our stages and of talpid³ at later stages. Janners & Searls (1970) have shown that at stage 19 proximal cells are dividing rather more rapidly than distal cells (12·1 h cycle against 13·6 h) but that by stage 24 they have equalized at 13·2 h. In talpid³ we have no information about cycle times, but the equalization has clearly not occurred and may have been exaggerated. In normals the more rapid proliferation of proximal cells must be offset by the reduction in number of dividing cells as chondrogenesis begins, but in talpid³ chondrogenesis is somewhat retarded (Hinchliffe & Ede, 1967) and there is no clear evidence of it at stage 24, so we may take it that a high proportion of cells are still dividing and the consequent drop in mitotic index spreading from the base of the limb will not have begun.

In the peripheral regions in talpid³ the picture at stage 25 is similar to that observed in normal limb-buds, with a mitotic gradient with the lowest index at the base. Again, as in normals, at stage 27 the gradient has disappeared and there are no statistically significant differences in the proximo-distal series of sections. There are, however, two differences.

Firstly, in the mutant there is an overall inverse correlation between cell density and mitotic index in the peripheral regions, whereas in the normals this was found only in the central regions where we related it to the chondrogenic activity of some cells. This is not unexpected, since while the chondrogenic regions in normal embryos are clearly defined and were rarely included in the peripheral counts, in talpid³ the limits of the chondrogenic areas, as revealed by acid mucopolysaccharide staining (Hinchliffe & Ede, 1967) and chondroitin sulphate synthesis (Hinchliffe & Thorogood, 1974), are extremely blurred, shading off into the peripheral regions (Fig. 8). We may therefore expect some of the mitotic reduction and the increase in cell density, which accompanies chondrogenesis, to occur in the peripheral regions, giving the inverse correlation.
Secondly, in the mutant there is no drop in peripheral mitosis from stage 25 to stage 27 as there is to a marked degree in normal limb-buds. This effect of the *talpid*³ gene has also been observed by Ede & Flint (1972). In cultured aggregates of limb-bud mesenchyme cells, mitotic activity in the non-cartilaginous periphery declined sharply in normal aggregates over 4 days of culture but continued to increase in *talpid*³ aggregates, so it appears to be an autonomous effect of the gene on non-chondrogenic limb mesenchyme cells. Within the limb-bud, examination of Figs. 3 and 5 suggests that there is a peak of mitosis corresponding to the widest part of the limb paddle, and it may be that this contributes to the production of the broad fan-shaped outgrowth of the mutant limb-bud.

**Cell density**

*Normal wing-bud*

In the cartilage regions, density does not change between stages 24 and 25 and is significantly greater than in non-cartilage regions. These results are in line with the observations of Thorogood & Hinchliffe (1975), who found a rise in cell density between stages 22 and 24 in chondrogenic regions but no corresponding rise in non-chondrogenic regions. They lend support to the hypothesis of pre-cartilage condensation suggested by Ede & Agerbak (1968) and developed by Ede & Flint (1972), namely that cartilage condensations are formed by aggregation of cells to produce a local increase in cell density. Since mitosis is observed to fall in pre-cartilage areas, the condensation effect is not a static increase resulting from mitosis but is more probably produced by a slight but active movement of cells towards an aggregation centre.

talpid³ wing-bud

Hinchliffe & Ede (1967) and Hinchliffe & Thorogood (1974) have shown that chondrogenesis in early *talpid*³ limbs is both diffuse and retarded compared to normal limbs. This is reflected in our results for cell density change in *talpid*³ limbs. In the differentiating cartilage region, cell density does not change between stages 24 and 25 and is lower than in normal limbs of the same ages. But there is a great increase in cartilage cell density between stages 25 and 27 which, when compared with the earlier stage-22–24 increase (Hinchliffe & Thorogood, 1974) in normal limbs, suggests a delay in condensation in *talpid*³ cartilage. The *talpid*³ non-cartilage is of the same density as cartilage through all stages, itself also increasing between stages 25 and 27. Ede & Flint (1975a, b) have shown that *talpid*³ cells adhere to one another more strongly than do normal cells. In work on the development of limb mesenchyme aggregates, Ede & Flint (1972) suggested that there might be a feedback inhibition on chondromucoprotein production in *talpid*³ cartilage due to the very sticky cells not separating, causing the intercellular space to become saturated with product. This would explain the poor and diffuse staining for acid mucopoly-
Fig. 8. A and B are outlines of normal and talpid3 limbs at stage 27 with areas of cartilage given in stipple. The bar across each limb represents the level of the section shown in C (normal) and D (talpid3); E and F are higher power photographs of C and D respectively, showing the transition from cartilage to non-cartilage.
saccharides in early talpid3 limbs compared to normal. The lack of difference between cell density in cartilage and non-cartilage also suggests that normal condensation is retarded in regions that are in fact staining for acid mucopolysaccharides. This would contribute to the observed absence of pattern, i.e. radius/ulna and digital regions in the talpid3 limb skeleton, up to stage 25. The increase in cell density of cartilage between stages 25 and 27 in talpid3 is not accompanied by any increase in mitosis between these stages and probably represents a delayed condensation process. In the non-cartilage regions there is a definite decrease in mitotic index between these stages, but in talpid3 through some direct or indirect effect of the gene, it remains at a constant and much higher level which may account for the observed increase in cell density.

The talpid3 gene has been shown to increase cell adhesion and reduce cell movement (Ede & Flint, 1975a, b) and to decrease cell death in limb mesenchyme both in vivo (Hinchliffe & Thorogood, 1974) and in reaggregation cultures (Ede & Flint, 1972). The data presented here necessitate adding an effect on cell proliferation.

It is not possible to say which is the primary effect of the gene, and all could be consequences of some effect at the cell surface. How these effects are related to the abnormal morphogenesis of the limb is under investigation.

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