# Kinetic parameters in the growth plate of normal and achondroplastic (cn/cn) mice

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## INTRODUCTION

Achondroplasia is a well known genetically mediated disease in man and domestic animals producing a familiar short-legged phenotype. Investigations of discrete achondroplasias (or, more properly) chondrodystrophies due to different genes, mainly in laboratory animals, have shown that the reduced growth of cartilage is in fact a symptom which may have many first causes. Amongst these (review by Johnson, 1986) are a generalised defect in oxidative phosphorylation (achondroplastic [ac] rabbit), APS (adenosine 5′-phosphate) and ATP (adenosine triphosphate) kinase (brachymorphic [bm] mouse), and localised defects in proteoglycan core protein (nanomelic [nm] chick). Such biochemical lesions are often accompanied by a distinctive ultrastructural appearance of the cartilage matrix or cells. In yet other conditions (eg the stumpy [stm] mouse) there are major defects in the process of cell division.

The mutant we have studied, achondroplasia ([cn], Lane & Dickie, 1968) in the mouse remains enigmatic. Although the symptoms of classic chondrodystrophy are plain to see, no biochemical, ultrastructural nor gross mitotic abnormality has been described. In a previous paper (Thurston, Johnson & Kember, 1985) a limited study of the cell kinetics of normal and cn growth plates was performed in order to try to localise the site of the defect in cn. In this paper, we extend this study to cover a larger sample, a greater age range and to include more detailed measurements of cartilage cells.

Achondroplasia [cn] is also unique in having, apparently, two distinct phenotypes associated with the cn/cn genotype (Thurston *et al.* 1985). We have taken the opportunity presented by this further study to reinvestigate this phenomenon.

## MATERIALS AND METHODS
### Animals

Achondroplastic mice were bred by crossing known or suspected heterozygotes from the stock kept in the animal house of Leeds University. Sixty six test matings were set up in all.

### Light microscopy

32 pairs of abnormal mice and normal littermates of the same age were killed by chloroform overdose (Table 1). Knee regions were excised and immediately fixed in 10% formal saline (buffered to pH 7) for a minimum period of 72 hours. The bones were then stripped of musculature and returned to formal saline for a further 24 hours. They were decalcified in 20 volumes 5·5% EDTA buffered to pH 7·4, replaced each 5th

Table 1. *Numbers of pairs of mice studied at each age*

| Age in days | No of littermate pairs |
|---|---|
| 7 | 2 |
| 14 | 2 |
| 17 | 2 |
| 22 | 4 |
| 23 | 4 |
| 24 | 5 |
| 25 | 5 |
| 28 | 2 |
| 39 | 2 |
| 41 | 2 |
| 55 | 2 |
| Total | 32 |

day. Decalcification was found to be adequate for the preparation of sections on a rotary microtome when a mounted needle was seen and felt to pass through the femoral shaft with ease. No attempt was therefore made to find the end point of decalcification by radiographic or chemical means. Adequate decalcification usually occurred in 10–14 days.

Decalcified left knee regions were dehydrated, embedded in paraffin wax and sectioned longitudinally at 8 $\mu$m in a sagittal plane. Sections were mounted on albumin coated slides and stained with haematoxylin and eosin.

## Histomorphometry

The reserve zone, the zone of proliferation, and the zone of hypertrophy were defined following the criteria of Buckwalter *et al.* (1985). Images of sections were projected at known magnification via the drawing tube of a Leitz microscope onto the digitising tablet of a Kontron Videoplan computer (Kontron Messgerate GMBH) and the extent of each zone measured semi-automatically. The total growth plate height and the heights of reserve, proliferative and hypertrophic zones were measured in at least 15 sections from each growth plate. In 7 days old, and some 14 days old animals the secondary centre of ossification was absent, making it impossible to define a reserve zone.

Individual cells within each zone were measured by means of an eyepiece graticule to ascertain cell height (parallel to the long axis of the bone) and cell width (perpendicular to the long axis of the bone). The hypertrophic zone was subdivided into equal upper and lower portions (after Buckwalter *et al.* 1985) and the uppermost cell of the upper zone and the lowest intact cell in the lower zone measured. At least 100 cells from each zone were measured, usually from one section.

## Autoradiography

Some of our pairs of littermates were weighed and injected intraperitoneally with tritiated thymidine-6-T (Radiochemical Centre Amersham International PLC) of specific activity 37 MBq mol$^{-1}$ at a dose of 1·85 MBq mol$^{-1}$ per gram body weight one hour before killing. Right knee regions were decalcified and sectioned as described above, dewaxed, hydrated and coated with Ilford K5 emulsion diluted 2:1 in distilled water. They were then exposed in light-tight boxes at 4 °C for 4–6 weeks, developed in Kodak D19, fixed in Ilford Hypam, washed and stained as before.

A labelling profile was constructed from the flat tibial epiphysis according to the

Table 2. *Mean heights of growth plate zones in normal and achondroplastic mice aged 7–55 days. Dimensions are given as* $\mu m \pm$ *S.E.M.*

| Age | Genotype | Reserve zone | Proliferative zone | Hypertrophic zone | Total height |
|---|---|---|---|---|---|
| 7 | + | — | 314·7±11·8 | 236·6±12·9 | 551·3±9·8 |
|  | cn/cn | — | 312·5±8·1 | 138·5±4·3** | 451·0±7·35** |
| 14 | + | — | 242·8±9·2 | 421·7±8·5 | 664·5±8·2 |
|  | cn/cn | — | 171·9±14·8** | 104·5±6·6** | 276·4±12·8** |
| 17 | + | 31·1±2·6 | 234·4±12·1 | 284·3±11·1 | 549·8±10·1 |
|  | cn/cn | 27·1±1·8 | 110·85±3·9** | 68·13±4·05** | 206·0±5·8** |
| 22 | + | 32·0±1·6 | 159·1±5·0 | 175·3±10·5 | 366·5±11·7 |
|  | cn/cn | 28·5±1·3 | 119·28±3·5** | 45·5±2·1** | 193·3±4·13** |
| 23 | + | 33·8±1·7 | 151·4±3·1 | 164·3±6·8 | 349·2±8·0 |
|  | cn/cn | 33·5±3·0 | 98·4±3·6** | 38·7±2·1** | 170·6±6·4** |
| 24 | + | 30·2±1·7 | 159·2±168·2 | 168·2±6·6 | 357·4±9·8 |
|  | cn/cn | 24·9±1·7* | 99·1±2·1** | 42·1±1·5** | 166·1±2·2** |
| 25 | + | 27·1±1·9 | 146·5±5·8 | 144·5±5·9 | 318·1±10·8 |
|  | cn/cn | 28·8±1·3 | 102·5±1·4** | 50·3±1·6** | 181·6±0·3** |
| 28 | + | 26·5±3·4 | 116·4±6·0 | 134·2±2·5 | 277·1±3·6 |
|  | cn/cn | 28·2±2·6 | 108·9±2·7 | 39·0±3·4** | 176·1±2·8** |
| 30 | + | 27·8±1·6 | 143·2±8·7 | 122·4±4·3 | 293·3±12·5 |
|  | cn/cn | 26·0±1·6 | 76·8±3·2** | 62·4±3·0** | 165·2±4·3** |
| 39 | + | 21·7±0·5 | 85·0±1·2 | 85·8±0·8 | 192·5±1·4 |
|  | cn/cn | 24·6±0·4* | 57·5±1·1** | 55·9±0·9** | 138·0±1·2** |
| 41 | + | 18·4±0·45 | 91·3±0·93 | 61·9±1·1 | 171·6±1·4 |
|  | cn/cn | 19·7±1·3 | 87·4±2·1 | 50·4±2·23** | 157·5±3·8* |
| 55 | + | 17·9±0·8 | 53·3±2·4 | 64·9±3·4 | 136·1±4·5 |
|  | cn/cn | 19·1±1·7 | 61·1±2·9 | 55·5±4·2 | 135·7±7·2 |

$* P < 0.05; ** P < 0.001.$

In Tables 2–6, results are based on 15 sections taken from each growth plate. The number of growth plates is identical to the number of mice at any given age in Table 1.

protocol of Kember (1960, 1971) and based upon a minimum of 50 labelled cells, usually from one section. Mean labelling profiles presented here are based upon results from two mice. The number of cells in the maturation, proliferation and hypertrophic zone was also recorded.

Longitudinal growth rate was calculated according to the formula of Sissons (1953): growth rate = rate of production of new cells per cartilage column × maximum height of hypertrophied cell. The mean cell cycle time was calculated according to the methods of Thurston *et al.* (1985) on grounds of comparability. The number of proliferating cells per column was calculated using the methods of Kember (1972), who defined a main proliferation zone (where the labelling index exceeds the mean) and an effective proliferation zone which is rather larger and weights labelling index according to the position of a dividing cell in the cartilage column.

## RESULTS

### *Histology of the growth plates*

At 7 days the chondrocytes in the proximal growth plate of the tibia of normal mice were not strictly aligned into columns and the secondary centre of ossification had not

Table 3. *Reserve zone cell height, width and height:width ratio. Dimensions are* $\mu m \pm S.E.M.$

| Age (days) | Genotype | Cell height | Cell width | Height:width ratio |
|---|---|---|---|---|
| 17 | + | 5·4 ± 0·3 | 13·4 ± 0·6 | 0·40 |
|    | cn/cn | 4·9 ± 0·3 | 13·1 ± 0·8 | 0·37 |
| 22 | + | 7·2 ± 0·2 | 15·8 ± 0·3 | 0·46 |
|    | cn/cn | 7·2 ± 0·3 | 15·4 ± 0·3 | 0·47 |
| 23 | + | 6·2 ± 0·2 | 16·8 ± 0·3 | 0·37 |
|    | cn/cn | 5·6 ± 0·2 | 15·8 ± 0·3 | 0·35 |
| 24 | + | 6·8 ± 0·2 | 16·5 ± 0·4 | 0·41 |
|    | cn/cn | 6·5 ± 0·2 | 15·1 ± 0·4 | 0·43 |
| 25 | + | 7·4 ± 0·3 | 15·6 ± 0·4 | 0·47 |
|    | cn/cn | 7·0 ± 0·2 | 15·7 ± 0·4 | 0·46 |
| 28 | + | 7·3 ± 0·4 | 14·5 ± 0·5 | 0·50 |
|    | cn/cn | 7·0 ± 0·2 | 15·2 ± 0·5 | 0·46 |
| 30 | + | 6·5 ± 0·2 | 16·0 ± 0·5 | 0·41 |
|    | cn/cn | 6·5 ± 0·2 | 15·0 ± 0·5 | 0·43 |
| 39 | + | 5·8 ± 0·4 | 12·0 ± 0·5 | 0·48 |
|    | cn/cn | 5·9 ± 0·3 | 12·5 ± 0·4 | 0·47 |
| 41 | + | 4·8 ± 0·2 | 12·5 ± 0·4 | 0·38 |
|    | cn/cn | 5·7 ± 0·3 | 13·3 ± 0·5 | 0·42 |
| 55 | + | 5·3 ± 0·4 | 10·7 ± 0·4 | 0·49 |
|    | cn/cn | 7·8 ± 0·4* | 13·5 ± 0·7* | 0·57 |

\* $P < 0.05$.

yet appeared. By 14 days the columns were more ordered and the growth plate appeared well organised. The secondary centre of ossification was sometimes present, but there was no bony epiphysis. By 17 days the secondary centre of ossification had reached maximum size and the bony epiphysis could be seen. By 22 days the growth plate was judged to be fully formed. At 30 days the growth plate was thinner, due to resorption on the epiphyseal aspect and reduction in the hypertrophic zone. At 41 days there was a reduction in all zones of the growth plate and by 55 days there was evidence of the beginnings of epiphyseal closure: the chondrocytes were separated by more matrix and column organisation was beginning to fail.

The growth plates of achondroplastic mice can be readily distinguished from those of normal littermates from Day 7 to Day 39. They are thinner, with a reduction in each zone. This was particularly obvious in the hypertrophic zone. Chondrocytes were aligned into regular columns although they seemed close-packed in some areas. The primary spongiosa was scanty and individual trabeculae were often composed of short thick longitudinal septa. The formation of the secondary centre of ossification and the bony epiphysis was not noticably retarded.

At Days 41 and 55 the growth plates of achondroplastic mice were difficult to distinguish from those of their normal littermates. The growth plate and each zone within it appeared to be of equal height in both cn/cn and normal mice. Spongiosa was scanty in both genotypes. The histological appearance of the cn/cn epiphysis has been amply illustrated previously (See eg. Konyukhov & Paschin, 1970; Thurston *et al.* 1985).

Table 4. *Proliferative zone cell height, width and height:width ratio. Dimensions are*
$\mu m \pm$ *S.E.M.*

| Age (days) | Genotype | Cell height | Cell width | Height:width ratio |
|---|---|---|---|---|
| 7 | + | 8·0±0·4 | 18·0±0·4 | 0·44 |
|   | cn/cn | 5·1±0·2** | 16·7±0·43* | 0·30 |
| 14 | + | 5·4±0·2 | 17·4±0·6 | 0·31 |
|   | cn/cn | 4·6±0·2* | 14·7±0·4* | 0·31 |
| 17 | + | 6·8±0·5 | 13·9±0·8 | 0·49 |
|   | cn/cn | 4·0±0·1** | 14·8±0·5 | 0·27 |
| 22 | + | 5·6±0·2 | 16·1±0·3 | 0·34 |
|   | cn/cn | 3·9±0·1** | 16·1±0·3 | 0·24 |
| 23 | + | 5·3±0·2 | 14·4±0·3 | 0·36 |
|   | cn/cn | 4·8±0·1* | 15·2±0·3 | 0·31 |
| 24 | + | 6·3±0·3 | 14·1±0·5 | 0·45 |
|   | cn/cn | 5·1±0·1* | 15·2±0·3 | 0·33 |
| 25 | + | 5·7±0·2 | 14·7±0·3 | 0·38 |
|   | cn/cn | 5·1±0·13* | 17·1±1·2* | 0·30 |
| 28 | + | 6·0±0·2 | 15·8±0·7 | 0·38 |
|   | cn/cn | 5·2±0·2* | 12·7±0·7** | 0·41 |
| 30 | + | 6·6±0·3 | 13·9±0·4 | 0·43 |
|   | cn/cn | 7·1±1·9 | 13·9±0·4 | 0·51 |
| 39 | + | 5·1±0·2 | 11·9±0·4 | 0·42 |
|   | cn/cn | 3·5±0·2** | 12·8±0·5 | 0·27 |
| 41 | + | 5·1±0·2 | 12·9±0·5 | 0·39 |
|   | cn/cn | 5·4±0·2 | 14·4±0·4 | 0·38 |
| 55 | + | 5·7±0·1 | 12·6±0·4 | 0·45 |
|   | cn/cn | 6·7±0·5 | 15·6±0·5** | 0·43 |

$* P < 0.05; ** P\ 0.001.$

### Histomorphometry

Table 2 shows the heights of growth plates and growth plate zones in normal and cn/cn mice aged 7–55 days. The total height of the normal growth plate peaks at 14 days (664 $\mu$m) then declines gradually to 136 $\mu$m at 55 days. The growth plate is made up of a reserve zone, definable from 17 days onwards, a proliferative zone and a hypertrophic zone. The reserve zone declines slowly from about 30 $\mu$m in height over the period 17–30 days to 18 $\mu$m at 55 days. The proliferative zone is largest at 7 days (315 $\mu$m) then gradually declines to occupy only 53 $\mu$m at 55 days. The hypertrophic zone increases sharply in height from 236 $\mu$m at 7 days to 420 $\mu$m at 14 days, then declines to 65 $\mu$m by 55 days.

In achondroplastic mice the overall height of the growth plate is less than normal at 7 days (451 vs. 551 $\mu$m), then decreases further, not showing the 14 days peak seen in normal mice. Examination of the height of constituent zones shows that this is due to a decrease in the height of the proliferative zone from Days 14–39 and an even greater decrease in the hypertrophic zone from Days 7–39.

### Cell size

Tables 3–6 show the cell height and width in each zone of the growth plate. In the reserve zone (Table 3) there is little systematic change in cell size with age, apart from

Table 5. *Upper hypertrophic zone cell height, width and height:width ratio.*
*Dimensions are* $\mu m \pm$ S.E.M.

| Age (days) | Genotype | Cell height | Cell width | Height:width ratio |
|---|---|---|---|---|
| 7 | + | 21·8 ± 0·8 | 21·5 ± 0·8 | 1·00 |
| | cn/cn | 21·3 ± 0·5 | 19·8 ± 0·6 | 1·07 |
| 14 | + | 18·6 ± 0·6 | 18·0 ± 0·7 | 1·03 |
| | cn/cn | 15·1 ± 0·6** | 15·5 ± 0·7 | 0·97 |
| 17 | + | 18·9 ± 0·7 | 18·4 ± 0·7 | 1·02 |
| | cn/cn | 13·3 ± 0·8** | 14·9 ± 0·9* | 0·89 |
| 22 | + | 17·1 ± 0·4 | 18·2 ± 0·4 | 0·93 |
| | cn/cn | 11·8 ± 0·5** | 15·5 ± 0·4** | 0·76 |
| 23 | + | 13·55 ± 0·4 | 17·1 ± 0·4 | 0·79 |
| | cn/cn | 9·4 ± 0·3** | 16·6 ± 0·4 | 0·56 |
| 24 | + | 18·0 ± 0·7 | 17·7 ± 0·4 | 1·01 |
| | cn/cn | 11·9 ± 0·5** | 16·0 ± 0·43* | 0·74 |
| 25 | + | 17·0 ± 0·6 | 18·5 ± 0·4 | 0·91 |
| | cn/cn | 11·5 ± 0·4** | 16·7 ± 0·4 | 0·68 |
| 28 | + | 15·9 ± 0·6 | 15·9 ± 0·7 | 1·00 |
| | cn/cn | 11·3 ± 0·6** | 12·9 ± 0·43* | 0·87 |
| 30 | + | 16·6 ± 0·5 | 17·4 ± 0·5 | 0·95 |
| | cn/cn | 11·0 ± 0·4** | 14·3 ± 0·5** | 0·76 |
| 39 | + | 20·9 ± 0·8 | 17·2 ± 0·5 | 1·21 |
| | cn/cn | 11·8 ± 0·6** | 13·7 ± 0·5** | 0·86 |
| 41 | + | 12·7 ± 0·5 | 14·8 ± 0·6 | 0·85 |
| | cn/cn | 12·2 ± 0·5 | 13·6 ± 0·5 | 0·89 |
| 55 | + | 12·6 ± 0·5 | 15·8 ± 0·6 | 0·79 |
| | cn/cn | 10·9 ± 0·5* | 14·6 ± 0·5* | 0·74 |

* $P < 0.05$; ** $P < 0.001$.

a tendency for cells from mice over 30 days to be proportionately smaller in height and width, with a cell height of about 6 $\mu$m and width of about 15 $\mu$m, giving a height:width ratio of 0·4. Figures for achondroplastics are very similar throughout. In the normal proliferative zone (Table 4) there seems to be no trend in cell size with age up to 39 days followed by a slight decrease; the height:width ratio is rather variable, rising to 0·49 at 17 days, reflecting variability in both height and width measurements. In cn/cn, cell dimensions were significantly smaller than normal in 12 out of 24 comparisons and significantly larger twice.

In the upper hypertrophic zone (Table 5), where we measured the highest hypertrophic cell in a cartilage column, cells were essentially equidimensional in section with a height:width ratio of 1, although this tended to fall in older mice. In cn/cn the height:width ratio was lower, accounted for by a significant decrease in cell height in 10 out of 12 comparisons. Cell width was also decreased in 7 out of 12 comparisons, but to a smaller extent.

In the lower hypertrophic zone, where we measured the last intact hypertrophic cell in a column, cell height regularly (but not invariably) exceeded cell width in normal mice (Table 6). Cell height was roughly constant at around 24–31 $\mu$m over the period 7–28 days, then declined to 15 $\mu$m. Cell width also declined in older mice. In cn/cn cell

Table 6. *Lower hypertrophic zone cell height, width and height:width ratio.*
*Dimensions are μm ± S.E.M.*

| Age (days) | Genotype | Cell height | Cell width | Height:width ratio |
|---|---|---|---|---|
| 7 | + | 26·8±1·1 | 26·3±1·2 | 1·02 |
|   | cn/cn | 21·3±0·8* | 21·6±0·93* | 0·99 |
| 14 | + | 28·7±1·5 | 25·3±1·8 | 1·13 |
|   | cn/cn | 16·7±0·8** | 17·5±0·8** | 0·95 |
| 17 | + | 23·7±0·9 | 20·5±0·6 | 1·15 |
|   | cn/cn | 17·4±0·9** | 14·4±0·7** | 1·20 |
| 22 | + | 28·6±0·7 | 21·6±0·7 | 1·32 |
|   | cn/cn | 14·8±0·5** | 18·1±0·4** | 0·82 |
| 23 | + | 25·9±0·9 | 19·5±0·6 | 1·32 |
|   | cn/cn | 13·7±0·5** | 16·9±0·5** | 0·81 |
| 24 | + | 30·6±0·7 | 19·5±0·4 | 1·56 |
|   | cn/cn | 13·8±0·5** | 17·93±0·6* | 0·77 |
| 25 | + | 26·4±0·8 | 20·0±0·6 | 1·32 |
|   | cn/cn | 15·6±0·6** | 16·8±0·6** | 0·92 |
| 28 | + | 30·7±1·2 | 22·7±1·2 | 1·35 |
|   | cn/cn | 13·8±0·6** | 16·2±0·7** | 0·85 |
| 30 | + | 27·0±0·7 | 20·0±0·5 | 1·35 |
|   | cn/cn | 14·1±0·6** | 15·7±0·5** | 0·89 |
| 39 | + | 24·5±0·9 | 17·9±0·6 | 1·36 |
|   | cn/cn | 14·2±0·5** | 15·0±0·5** | 0·94 |
| 41 | + | 14·8±0·7 | 15·9±0·5 | 0·93 |
|   | cn/cn | 15·1±0·7 | 13·1±0·4* | 1·15 |
| 55 | + | 16·2±0·8 | 16·2±0·4 | 1·00 |
|   | cn/cn | 12·8±0·5* | 16·0±0·5 | 0·80 |

\* $P < 0.05$; \*\* $P < 0.001$.

height was significantly less than normal in 11 out of 12 comparisons, as was cell width.

Dividing the mean cell height into the mean zone height will give an indication of the number of cells per zone (Table 7). The figure so obtained for the proliferative zone (as defined histologically) shows a peak at 14 days in normal mice followed by a gradual decline from around 30 cells at three weeks to 9 at 55 days. In the achondroplastic mouse the proliferative cell number is greatest at 7 days (when it is abnormally high) then follows the normal pattern of decline at a slightly subnormal level.

In the hypertrophic zone the normal cell number again peaks at 14 days then declines: in abnormals it falls away from 7 days at subnormal levels.

*Labelling profiles*

Figure 1 shows the labelling profiles from proximal tibial growth plates of mice aged 7, 14, 22 and 28 days. The extent of the hypertrophic zone in mice given the label is also marked on these histograms. In normal mice the mitotic profile is at its greatest extent at 7 and 14 days where it includes about 30 cells. In older mice the number

Table 7. *Number of cells in proliferative and hypertrophic zones. The cell number is obtained by dividing the mean cell height* (Tables 4, 6) *into the mean zone height* (Table 2)

| Age in days | Genotype | Cell number | |
|---|---|---|---|
| | | Proliferative zone | Hypertrophic zone |
| 7 | + | 39 | 9·0 |
| | cn/cn | 62 | 6·5 |
| 14 | + | 45 | 14·6 |
| | cn/cn | 37 | 6·3 |
| 17 | + | 34 | 11·9 |
| | cn/cn | 28 | 3·9 |
| 22 | + | 28 | 6·1 |
| | cn/cn | 30 | 3·0 |
| 23 | + | 28 | 6·3 |
| | cn/cn | 21 | 2·7 |
| 24 | + | 25 | 5·5 |
| | cn/cn | 19 | 3·4 |
| 25 | + | 26 | 5·5 |
| | cn/cn | 20 | 3·2 |
| 28 | + | 19 | 4·3 |
| | cn/cn | 21 | 2·8 |
| 30 | + | 22 | 4·5 |
| | cn/cn | 11 | 4·4 |
| 39 | + | 17 | 3·5 |
| | cn/cn | 16 | 3·8 |
| 41 | + | 18 | 4·1 |
| | cn/cn | 16 | 3·3 |
| 55 | + | 9 | 4·0 |
| | cn/cn | 9 | 3·3 |
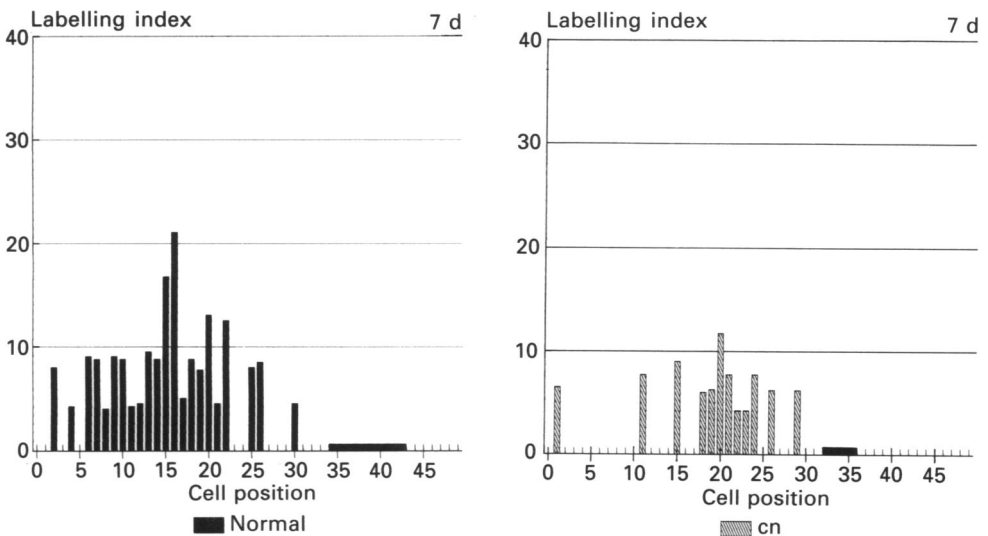


Fig. 1. Labelling profiles for normal and cn/cn mice aged 7, 14, 22 and 28 days. Labelling index is given in % cells labelled. The size and position of the hypertrophic zone is marked in each graph by the horizontal black bar.
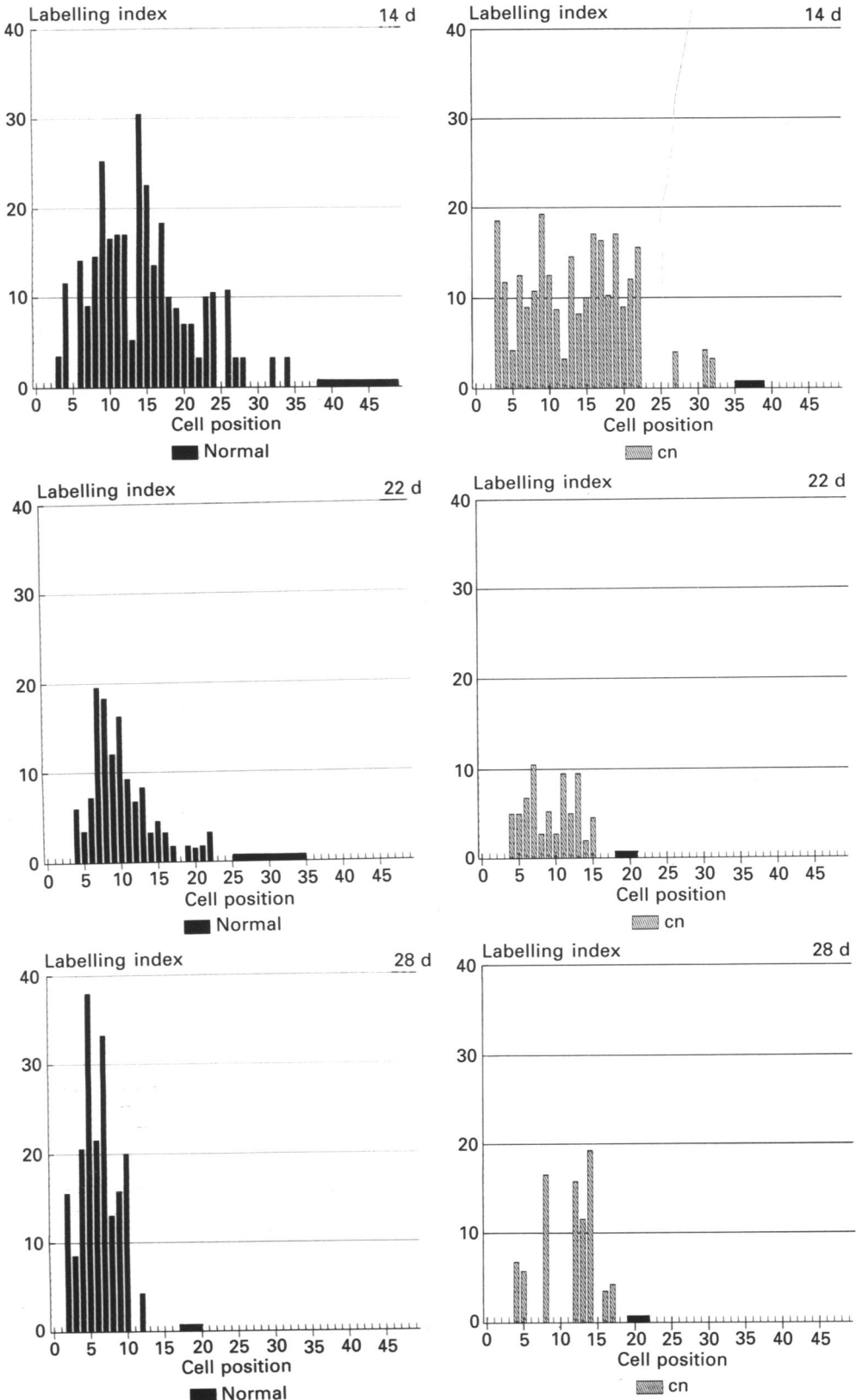
Fig. 1.

Table 8. *Cell kinetic parameters for proximal growth plate of the tibia for normal and achondroplastic mice aged 7–28 days*

| Age (days) | | No. of cells in main proliferation zone | No. of cells in effective proliferation zone | Labelling index (%) | Longitudinal growth rate ($\mu$m/d) |
|---|---|---|---|---|---|
| 7 | + | 21 | 25 | 6·5 | 107·0 |
| | cn | 28 | 29 | 3·3 | 49·5 |
| 14 | + | 12 | 19 | 12·9 | 162·1 |
| | cn | 20 | 21 | 9·3 | 83·6 |
| 22 | + | 7 | 11 | 8·7 | 65·6 |
| | cn | 9 | 10 | 7·2 | 25·6 |
| 23 | + | 7 | 11 | 10·7 | 72·9 |
| | cn | 11 | 13 | 4·4 | 18·9 |
| 28 | + | 8 | 10 | 15·1 | 110·4 |
| | cn | 7 | 13 | 6·0 | 15·8 |
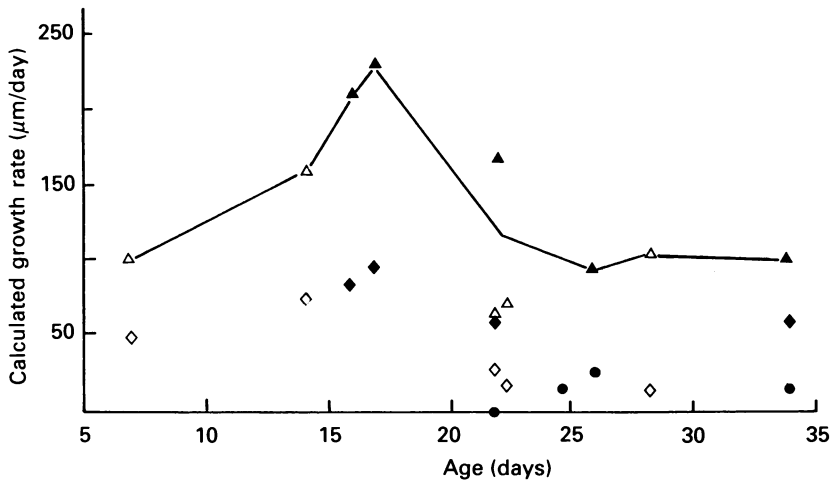


Fig. 2. Calculated growth rate values for normal and cn/cn mice aged 7–34 days. This Figure combines data from this paper and from Thurston *et al.* (1985). Key: solid triangles, normal (Thurston); open triangles, normal (this paper); solid diamonds, cn/cn (Thurston Type I); solid circles, cn/cn (Thurston Type II); open diamonds, cn/cn (this paper).

of cells in the proliferative zone (defined here by mitoses, not histological appearance and so rather smaller than the cell number obtained by dividing zone height by cell height as it does not include a number of maturing cells) is reduced to around 20 at 22 days and 12 at 28 days. Mitotic rate varies between 6·5 and 17·6%. Mitotic activity in all cases shows a peak towards the centre of the proliferative zone.

In cn/cn mice the mitotic profile also extends over about 30 cells in 7 and 14 days old mice, then declines to 14–17 cells. The mitotic rate is lower at 3·3–7·2%. Mitoses seem to be spread rather evenly throughout the proliferative zone rather than showing a definite peak.

Between the last cell undergoing mitosis and the first seen to be entering hypertrophy is a maturation zone of 3–5 cells which seems to vary neither with age nor genotype. The hypertrophic zone is 9 cells long in normal 7 days old mice, increases a little at 14 days and then declines. In cn/cn mice at all ages except 28 days the number of hypertrophic cells per column is about half the normal value.
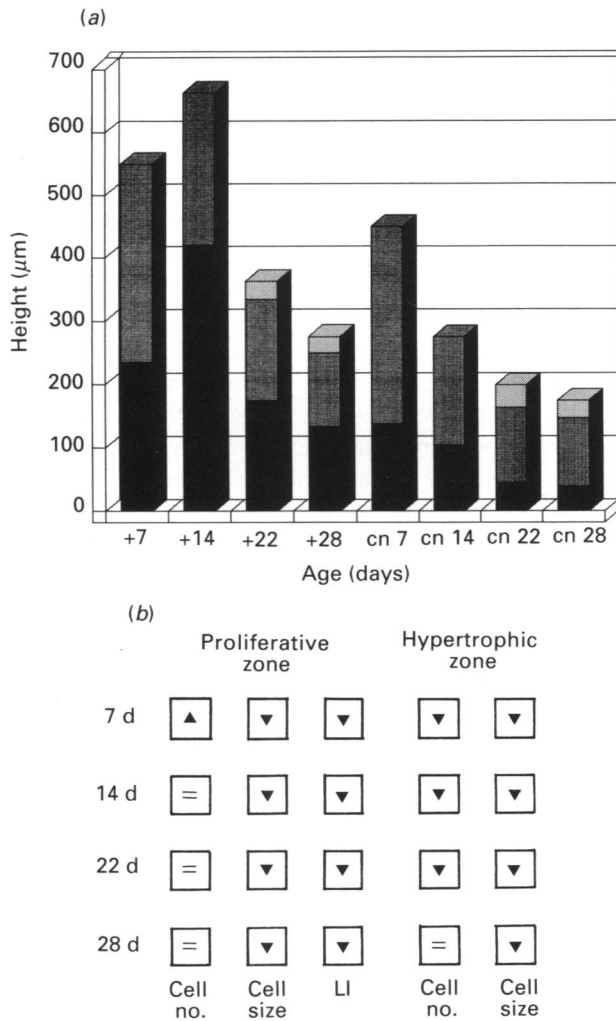
(a)



(b)



Fig. 3. (*a–b*). Summary Figure of the main findings of this paper in mice aged 7–28 d. Figure 3(*a*) represents the height of the epiphyseal plate and its constituent zones. ■, Hypertrophic; ▨, proliferative; ▨, reserve. Figure 3(*b*) summarises the data on cell number, size and division rate.

Calculated cell kinetic parameters are given in Table 8. The effective proliferation zone is, of course, slightly larger than the main proliferation zone but the correction applied makes little difference to the pattern of the proliferation zone described above. The calculated daily growth rate in normal mice is a little over 100 $\mu$m at 7 days, rises to a maximum of 162 $\mu$m at Day 14 and then falls back to 65–100 $\mu$m. In cn/cn growth rate also rises between Day 7 and 14, then declines to a much lower level. At no stage is the cn/cn growth rate equal to that seen in normal littermates. Figure 2 combines data from the present study with that from Thurston *et al.* (1985). The general agreement is good, except that our present figures for 22 days seem to be rather low.

### Summary figure

The behaviour of the growth plate over the period of study is rather complex since overall size, relative size of constituent zones, cell size, cell number and mitotic rate all vary with age. Figure 3 presents four 'snapshots' of the normal and cn/cn growth

plate at the ages where we have figures of all these parameters and summarises the significant changes between normal and achondroplastic.

## DISCUSSION

Achondroplasia in the mouse was described by Lane & Dickie (1968) as a typical chondrodystrophy with a domed skull, shortened limbs and tail and reduced birth weight. Abnormals became progressively more retarded and never reached normal adult weight. The standard measures of body length (skull, body, tail, long bones) are all around 70% of their normal values, with bone widths a little less than normal.

The epiphyseal plates were first studied by Konyukhov & Paschin (1970) who reported reserve and proliferative zones of normal size but a reduction in the zone of hypertrophy; individual chondrocytes were less hypertrophied than normal. Chondrocyte columns were regular. Bonucci et al. (1976) confirmed the shortening of the cartilage columns and compression of the hypertrophic zone with a recognisable normal series of proliferation maturation and hypertrophy and suggested premature ageing of the chondrocytes as a cause of the abnormality. Miller & Flynn-Miller (1976) found fairly regular cartilage columns in the synchondroses of the skull, but irregular organisation of the collagen matrix.

On the ultrastructural level Silberberg, Hasler & Lesker (1976) found a large number of dense lysosome-like bodies within chondrocytes and abundant glycogen deposits. The matrix had rather densely packed collagen fibres. Bonucci et al. (1977) found no ultrastructural abnormalities apart from extensive glycogen deposits.

Biochemical parameters have also been investigated by several authors, but with inconsistent results (review by Johnson, 1986). It seems that no known biochemical defect is regularly present in cn mice.

This apparent anomaly between various cn strains also embraces histological appearance. Silberberg & Lesker (1975) described two types of homozygote at four weeks of age. In less affected individuals growth zones were fairly regular with small chondrocytes, decreased hypertrophy and increasing numbers of necrotic cells (thus corresponding well to Khonyukhov's original description). The second homozygous phenotype was more affected, with short cartilage columns and small cells separated by large amounts of matrix. There were no hypertrophic cells and no spongiosa; instead a bony lamella was present beneath the abnormal cartilage. Thurston et al. (1985) also found two classes of homozygote, which could be distinguished on both their histology and growth potential. A possible explanation of the absence of these two types of homozygote in the stocks of Kleinman & Bonucci is given by Thurston et al. (1985) and Johnson (1986).

One of the reasons for the present investigation was to look again at the problem of the two types of homozygotes in our cn stock. The stock held in the Leeds animal house is linearly descended from that used by Thurston et al. (1985). Our sample of 66 test matings however produced no Type II (extreme) heterozygotes and we must conclude that whatever factor was present in 1984 has now disappeared. Our homozygous abnormals seem to be rather more extreme than the Type I we described in 1985, but less extreme than Type II. We saw no great variability in homozygotes in the present sample and are disinclined to think that our earlier work was based on the extreme ends of the distribution inadequately sampled. We can make no further useful comment on this very odd situation.

Our previous findings on Type I homozygotes (the less extreme, Thurston et al. 1975) were simply that the gene tended to reduce the height of hypertrophic cells without affecting the size of the effective proliferative zone or the mitotic index.

Repetition of this work on larger samples and different ages and the addition of zone height and cell height to our measurements leads us to slightly different conclusions. Our fullest data, summarised in Figure 3, from mice aged 7, 14, 22 and 28 days, give us weekly snapshots of the growth plate.

Essentially the growth plate can be regarded as composed of two zones, the proliferative and the hypertrophic. The reserve zone is not clearly distinguishable at 7 and 14 days, is small, and is apparently unaffected by the gene in respect of overall height, cell height or cell number. The proliferative zone, which by convention contains cells undergoing mitosis, can be measured in various ways. In terms of size the normal proliferation zone is largest at 7 days, declines a little over Days 14–21, then rather more by Day 28. If we estimate proliferative zone size by two slightly different and more sophisticated methods, which rely on the presence of cell division, we find that main and effective proliferation zones have in fact decreased in size between Days 14 and 21, and do not decrease further by Day 28.

In achondroplastic mice the proliferation zone is histologically of normal size on Day 7, but apparently made up of many flattened cells. At 14 and 21 days it is smaller than in normal littermates and, at 28 days is the same size.

The normal hypertrophic zone enlarges dramatically between Days 7 and 14, then declines. In achondroplastic mice this zone is smaller on Day 7 and does not show an increase at Day 14.

It is clear from these results that there are disturbances in both proliferative and hypertrophic zones from Day 7 onwards (which fits with the fact that cn/cn can be distinguished from normal littermates from birth). The number of cells in the proliferative zone (estimated by division of mean cell height into zone height, Table 7) appears to be elevated at 7 days and normal thereafter. If this effect is real and there is an excess of cells in the 7 days cn proliferative cell zone we must ask what happens to them subsequently. Silberberg *et al.* (1976) mention an excess of degenerating and dead cells in 3 weeks old cartilage from cn/cn electron micrographs but there seems to be no other mention of excessive cell death in the literature. We did not see any sign of increased cell death at the next age studied, 14 days, but it may have occurred unnoticed in the intervening period.

The height of the cells is decreased throughout in the abnormal, as are the size of the zone (measured from sections), the main and effective proliferation zones (calculated from the pattern of mitoses) and the labelling index for mitoses. In the hypertrophic zone both the number of cells per column and the height which they reach on hypertrophy is decreased.

In the absence of positive data on biochemical or ultrastructural lesions it seems that we must ask if the changes we have described are sufficient to account for the chondrodystrophy which we see. The main size decrease in cn growth plates is along the length of the bones, where growth is greatest. But we have also been able to show decreased cell widths, albeit with less consistency. It seems quite possible that the underlying defect in cn is one of decreased cell growth, coupled with decreased mitotic rate and that a generalised metabolic defect, such as those described in the achondroplastic rabbit or brachymorphic mouse (see Johnson, 1986, for details), and which has not been sought out in cn may once again be selectively producing an achondroplastic phenotype.

## SUMMARY

We have reinvestigated the overall size, zone size, mitotic rate and growth rate in the tibial epiphysis of normal and achondroplastic (cn/cn) mice aged 7–55 days. Our

present sample did not contain the two phenotypes characteristic of some stocks carrying the gene: cn/cn mice showed smaller zone sizes within the growth plates, smaller cells, decreased mitotic indices and growth rates.

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