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Cell kinetics of growth cartilage of achondroplastic (*cn*) mice

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INTRODUCTION

In a previous paper (Thurston, Johnson, Kember & Moore, 1983), the kinetic and histological changes which occur in the stumpy (*stm*) chondrodystrophy in the mouse were described. The present paper extends this work to another mouse mutant condition, achondroplasia (*cn*, Lane & Dickie, 1968). Although the dwarf phenotype is common to both mutants, their underlying lesion and mode of growth differ. Achondroplasia is a chondrodystrophy which retards growth from the time when homozygous abnormals can first be classified; in stumpy mice, growth is sharply arrested for a time at around 16 days of age with a later partial recovery. This paper examines the cell kinetics and histology of achondroplasia in an attempt to assess the effect of the gene on the cell proliferation rate, the size of the proliferating cell population and the structure of the growth plate.

MATERIALS AND METHODS

All mice used (Table 1) were littermates derived from matings between known heterozygotes kept in the animal house of the School of Medicine in Leeds. These mice were originally supplied by the Jackson Laboratory, Bar Harbor, Maine.

Homozygous abnormal mice together with normal littermates (+/+ or +/*cn*), aged 16–34 days, were injected intraperitoneally with tritiated [6-H^3]thymidine (Radiochemical Centre, Amersham, specific activity 5·0 Ci/mmol) at a concentration of 0·5 μCi/g body weight. One hour later they were killed by ether overdose. Knee regions were excised and fixed in buffered formol saline. Bones were stripped of musculature and decalcified in a 5·5% solution of EDTA, buffered to pH 7·4 with a phosphate buffer, for a minimum of seven days. When decalcification was complete the bones were returned to formol saline for 24 hours, dehydrated and embedded in paraffin wax. Sagittal sections, 7 μm thick, were cut from near the midline of the knee joint. Autoradiographs were prepared by dipping slides in Ilford K5 emulsion, diluted with an equal volume of distilled water. The slides were then exposed for four weeks in light tight boxes at 4 °C. The autoradiographs were developed in Kodak D19 and fixed in Kodafix, washed, stained with haematoxylin and eosin and mounted in DPX.

Some sections from each mouse were treated with histochemical stains to demonstrate specific cartilage constituents. These were Heidenhain's Azan variant and

* Reprint requests to Dr Johnson.

Table 1. *Animals used in the study of* cn *cell kinetics*

Age (days)	Normal (+/+ or +/*cn*)	Abnormal (*cn*/*cn*)
16	2	2
17	2	2
22	6	6
26	2	2
34	2	2

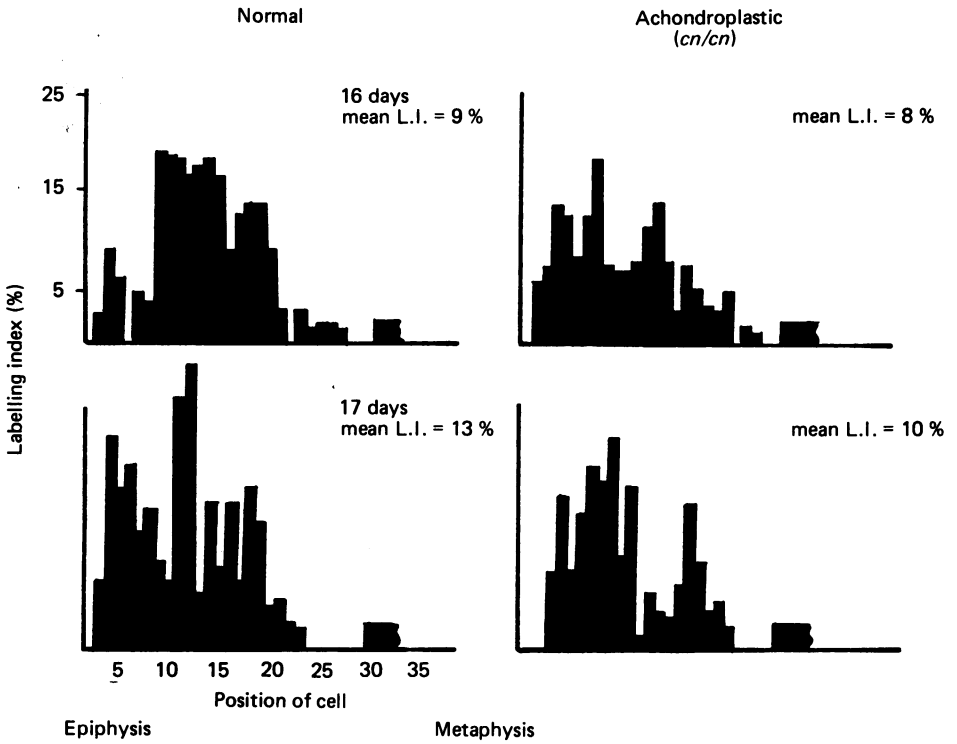


Fig. 1. Labelling profiles of the growth plate of the proximal end of the tibia in normal and achondroplastic mice aged 16 and 17 days. Each profile is the mean of at least two animals and each cell profile based on not less than 100 labelled cells. L.I., labelling index.

Lillie's Allochrome for collagen, periodic acid–Schiff (PAS) for glycogen, Von Kossa's stain for calcium and toluidine blue for sulphated glycosaminoglycans.

Measurements

Labelling profiles were constructed for the proximal growth plate of the tibia and the heights of 100 hypertrophic cells per section measured, as detailed in Thurston *et al.* (1983).

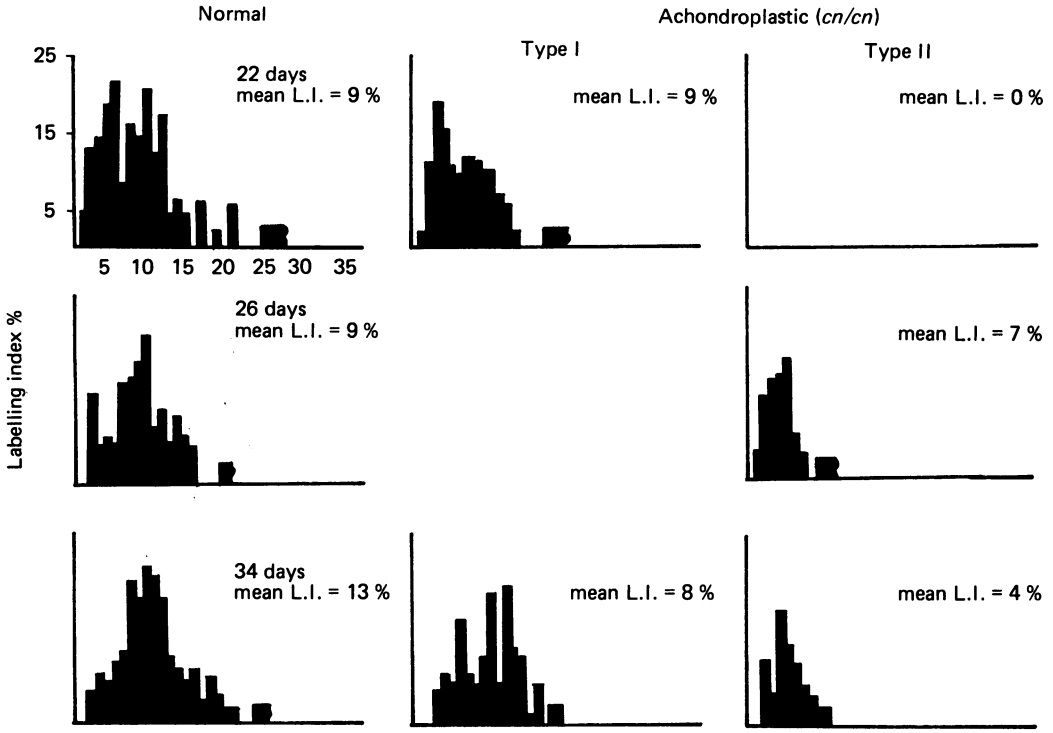


Fig. 2. Labelling profiles in normal and achondroplastic mice aged 22–34 days. Details as in Figure 1. L.I., labelling index.

RESULTS

Observations on mice homozygous for *cn* showed that they could be divided into two distinct, non-overlapping, categories on the basis of their growth plate kinetics and histology from 22 days onwards. Homozygous abnormal mice younger than this appeared uniform in type. The samples at 22, 26 and 34 days of age sometimes included both types of individual, sometimes only one, but never an intermediate form. The least severely affected individuals were called Type I and their more severely affected sibs Type II.

Figures 1 and 2 show the labelling profiles for the proximal growth plate of the tibia of normal and achondroplastic mice aged 16–34 days. Table 2 shows the cell parameters calculated from the data in Figures 1 and 2 and the results of hypertrophic cell measurements.

At 16 and 17 days, hypertrophic cell height was significantly reduced in *cn* homozygotes, but the number of cells in the effective proliferation zone was hardly reduced and the mitotic rate was normal. Type I dwarves at 22, 26 and 34 days were affected only with respect to their hypertrophic cell height. Type II dwarves showed a much reduced cell size in the proliferation zone, a reduced mitotic index and reduced hypertrophic cell height.

Figure 3 shows the calculated growth rates of normal and *cn* mice, using the formula (Sissons, 1955): Growth rate = rate of production of new cells per cartilage column × maximum height of hypertrophied cell.

Type I individuals (plus the samples at 16 and 17 days, which seemed to accord better with Type I than Type II) had a growth curve of the same shape as did normal

Table 2. *Cell kinetic parameters for proximal growth plate of the tibia for normal and achondroplastic mice aged* 16 *to* 34 *days*

Age (days)	Number of cells in effective proliferation zone	Labelling index (%)	Hypertrophic cell height (μm)
16 normal	19	9	30±1·1
cn dwarf	19	8	16±0·5*
17 normal	18	13	30±2·3
cn dwarf	17	10	18±1·3*
22 normal	13	9	30±1·0
cn dwarf I	11	9	16±1·1*†
cn dwarf II	0	0	9±0·7*†
26 normal	13	9	25±2·1
cn dwarf II	6	7	14±0·7*
34 normal	13	9	26±1·4
cn dwarf I	12	8	18±0·7*
cn dwarf II	8	4	11±1·4*

Cell heights marked * are significantly different from normal, $P < 0{\cdot}05$. Those marked † are significantly different from each other, $P < 0{\cdot}05$.

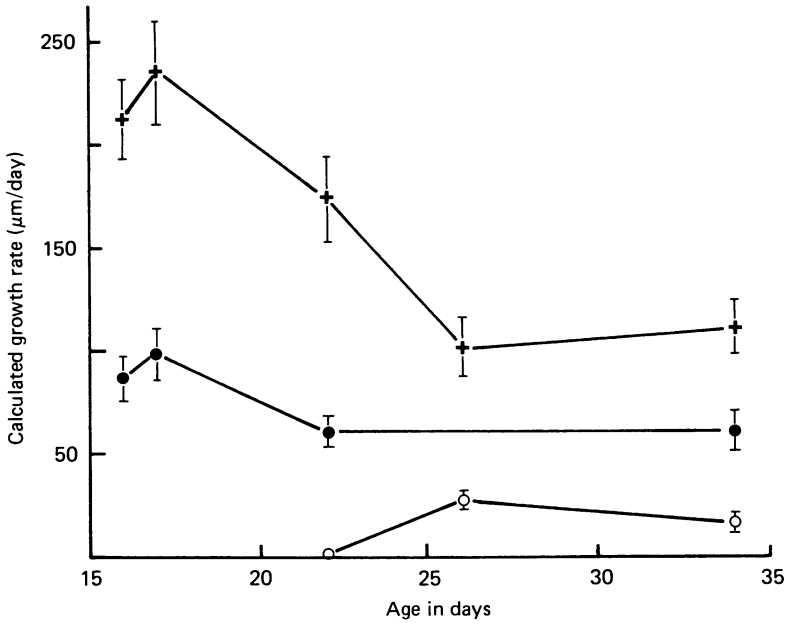
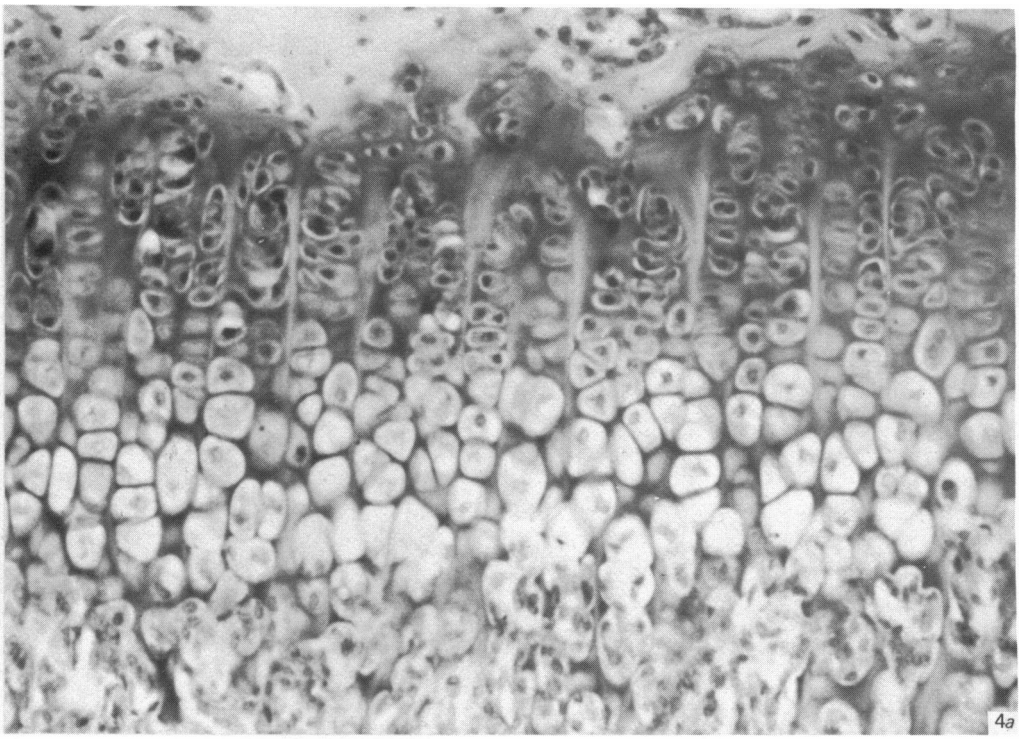


Fig. 3. Calculated growth rate values (±standard error) for normal and achondroplastic mice aged 16 to 34 days. Crosses, normal; closed circles, achondroplasia type I; open circles, achondroplasia Type II.

mice, although growth was much reduced at all points measured. Type II achondroplastic mice had a calculated growth rate of zero at 22 days due to a total absence of labelled cells in their cartilage, although label was present in metaphyseal cells (Fig. 5). At 26 and 34 days, a little growth was present.

The total amount of growth made by the proximal tibial growth plate was calcu-



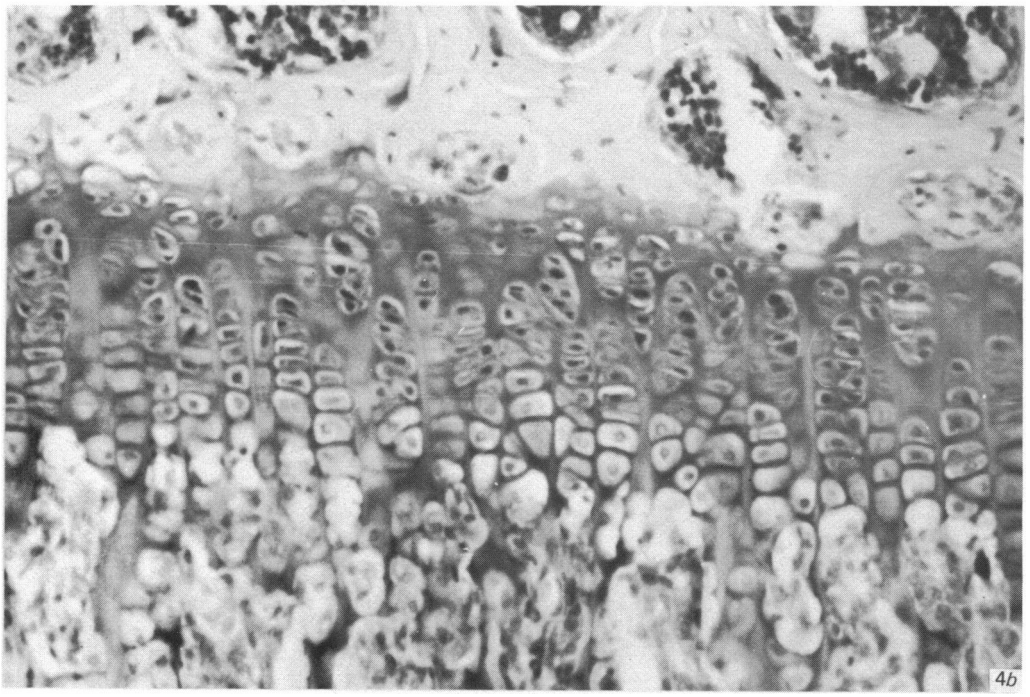
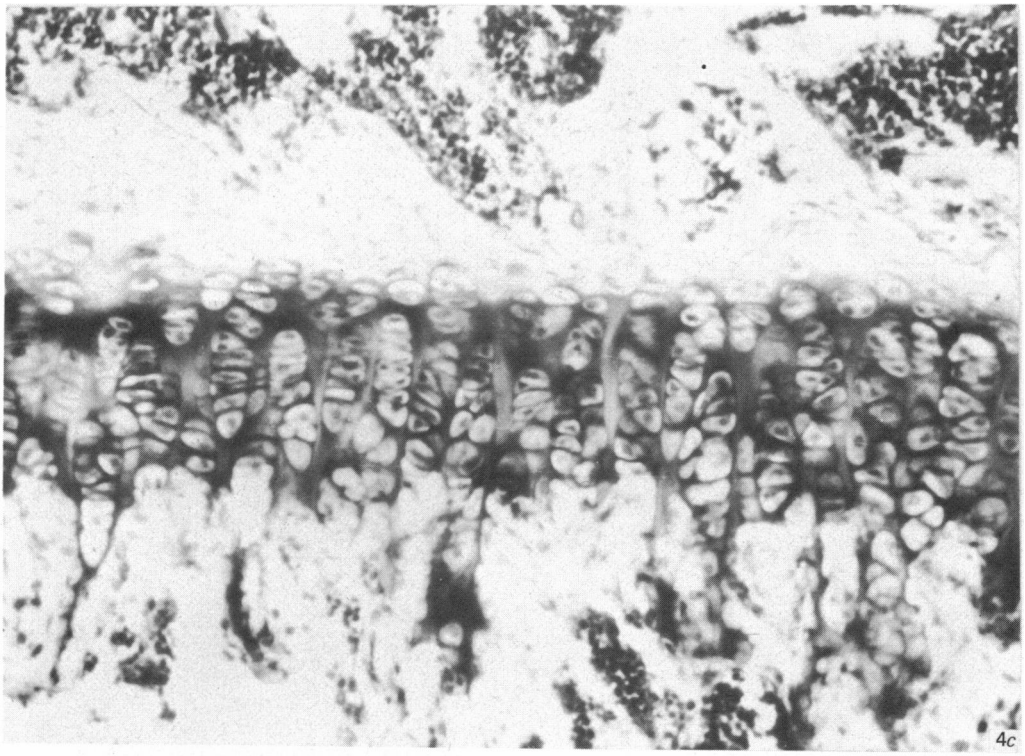


Fig. 4 (*a–d*). Transverse sections through the upper growth plate of the tibia of (*a*) normal, (*b*) *cn/cn* Type I, (*c*) and (*d*) *cn/cn* Type II mice aged 34 days. × 120.



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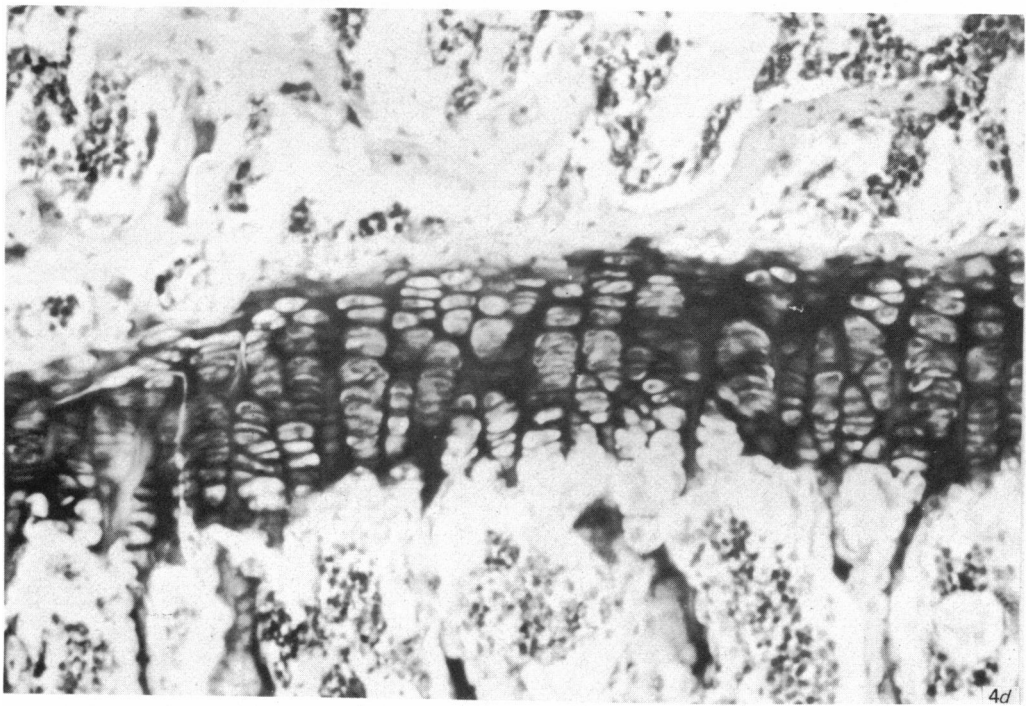

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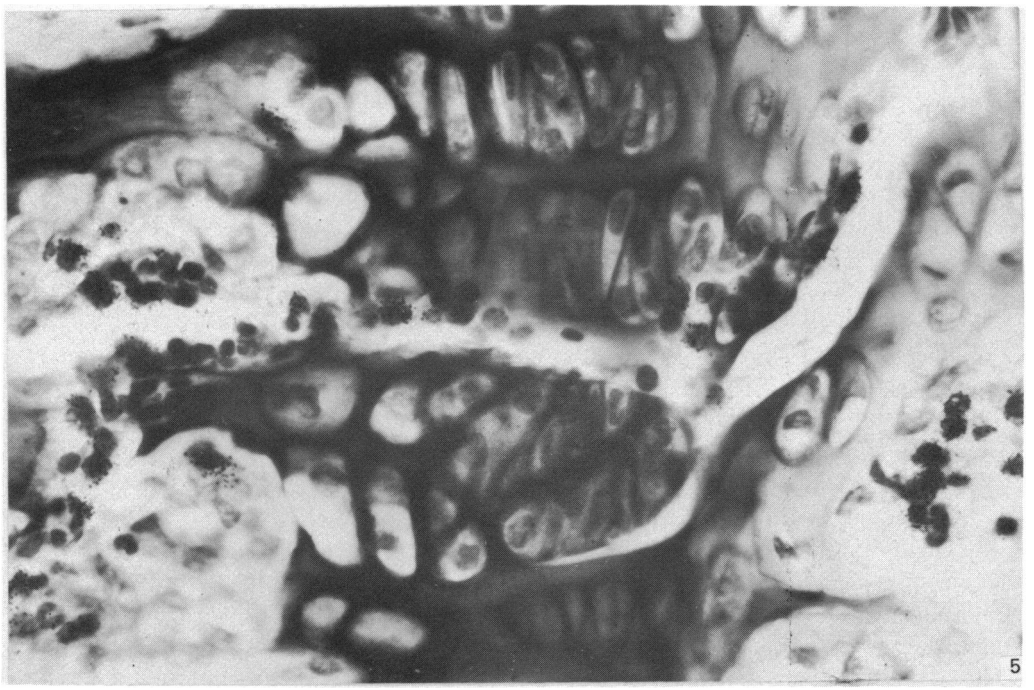

Fig. 5. Piercing of the growth plate by capillaries at 22 days in a Type II *cn/cn* homozygote. Metaphyseal cells are heavily labelled with tritiated thymidine but no grains are seen in the growth plate. ×240.

lated for normal mice and for type I achondroplastic littermates. Normal mice grew 2·8 mm in this period of 18 days, a mean rate of 155 μm per day. Type I *cn* homozygotes grew 1·2 mm, or 66 μm per day. Normals thus grew 2·3 times as fast as *cn* over the period measured.

Histology

Types I and II achondroplastic mice (Fig. 4) could first be distinguished at 22 days.

In Type I, which was the less severe form of the condition, the growth plate was narrowed, due to reduced cell hypertrophy: trabecular formation was somewhat deranged. In Type II, there was a very narrow growth plate due to the reduced number of cells in every cartilaginous zone. This was particularly evident in the hypertrophic zone due to the limited increase in hypertrophic cell height. Often, the bottom cell in a column was a normal nucleated chondrocyte which showed none of the usual degenerative changes of hypertrophy. The chondrocytes were arranged in quite regular columns, but tightly packed together and often with hyperchromatic nuclei. Trabecular formation was limited to the presence of stout longitudinal septa. As early as 22 days (Fig. 5) the growth plate was seen to be pierced by capillaries.

Abnormal distribution of PAS-positive glycogen was seen in both types of homozygote. In each case, glycogen was still present in the lowest cell in each column. Clearly these cells were being invaded on the metaphyseal side before glycogen stores had been depleted as part of the process of normal hypertrophy.

DISCUSSION

Perhaps the most interesting aspect of the present work is the clear demonstration of two types of abnormal *cn* homozygote mice at 22 days and their subsequent fate.

Lane & Dickie's (1968) original description of *cn* was based on gross appearance and no heterogeneity was noted amongst homozygotes. Konyukhov & Paschin (1970), who were the first to study the mutant histologically, report reserve and columnar zones of normal appearance in epiphyseal growth plates but a reduced zone of hypertrophy, with individual cells less hypertrophied than normal; chondrocyte columns were regular. Much the same appearance is presented by the Type I homozygote in the present study.

Silberberg & Lesker (1975) note short, rather deranged, chondrocyte columns at two weeks, and two distinct types of homozygotes at four weeks of age. In the less affected type, growth zones were comparatively regular with reduced hypertrophy, but in the second, more abnormal group, growth zones were strikingly abnormal with short columns of small chondrocytes, no hypertrophic cells and no primary spongiosa: growth plates were frequently perforated. Interestingly, the worst affected individuals were not the smallest, having a mean weight of 14 g against 12 g in the less affected type. The results of the present study agree with these findings; it is not possible to classify individual *cn/cn* mice as Type I or II on the basis of size or appearance. Silberberg & Lesker (1975) use these data to suggest that the more severe defect is not due to trauma, for example at weaning, or due to malocclusion.

Silberberg, Hasler & Lesker (1976) examined the ultrastructure of cartilage from *cn* mice aged 3–7 weeks. Possibly in one 3 weeks old mouse, and certainly in some older individuals, there were chondrocytes with scanty, short, cytoplasmic footlets, sparse endoplasmic reticulum with collapsed cisterns, a minute Golgi apparatus and lipid droplets. It is tempting to equate this extreme phenotype with the more serious type of *cn* abnormality seen in light microscope sections. However, Silberberg *et al.* also observed similar defects in three out of ten normal littermates, which they ascribe to the presence of the *cn* gene in heterozygous form.

Bonucci *et al.* (1976) have found shortened cartilage columns and a compressed hypertrophic zone and suggest that the phenotype is based upon premature ageing of the cartilage. No mention is made of two types of abnormal mouse.

Bonucci *et al.* (1977) have also investigated *cn* ultrastructure. They find only that the growth columns have fewer cells, that the hypertrophic cells do not seem to be degenerating and that deposits of glycogen are abundant. Again, there is no mention of heterogeneity.

Biochemical studies by Kleinman, Pennypacker & Brown (1977) showed no consistent abnormality, and no heterogeneity. Kleinman *et al.* were unable to repeat the findings of Silberberg & Lesker (1975) of increased hydroxyproline content in *cn*. Kleinman's strain, assessed by Silberberg & Lester, had normal hydroxyproline levels.

How can these apparently contradictory findings be reconciled? It seems clear that two types of homozygotes are present in the stocks held by Silberberg and the present authors, absent in the stocks of Bonucci and Kleinman, and not reported in the stocks of Konyukhov. The two types of homozygote have not been described earlier than 22 days (present authors' stocks), although *cn* homozygotes can be distinguished from normal littermates at 5–6 days after birth. The fact that severely

affected individuals are not significantly smaller than those less severely affected (a) mediates against trauma, such as the shock of weaning, which affects growth rate in normal and chondrodystrophic mice (Johnson, 1978) and (b) suggests that the difference between extreme and less extreme phenotypes occurs later in life than the primary effects of the *cn* gene.

Silberberg and colleagues' (1975) finding that abnormal chondrocytes also occur in the normal littermates of *cn* homozygote mice is unlikely to be based on the heterozygous manifestations of *cn*, which, as far as is known, is totally recessive. It seems more probable that a second factor, hormonal, nutritional or genetic, is present in some stocks of *cn*, but not in others, and is seen in mice of the genotype $+/cn$ or $+/+$ merely as a slight derangement of adult chondrocytes. In *cn/cn*, it has an additive effect which renders the manifestation of *cn* in homozygous form much more severe.

Is a genetic factor a possibility? All mice carrying *cn* are descended from the original Bar Harbor stock. Konyukhov & Paschin's (1970) mice, from the date of their paper, cannot have been too dissimilar from Lane & Dickie's (1968) original stock. By the time that mice were passed to Silberberg in 1968 outcrosses had been performed, and Silberberg's stock could have come from one of three lines (1) a line outcrossed to *C3H/HeDi*, (2) a line outcrossed to both *C3H/HeDi* and *AKR/J* or (3) a line outcrossed to *C3H/HeDi*, *AKR/J* and *LG/J*. The mice which were exported to the United Kingdom were from line 2. By the time Kleinman received mice in 1975, line 3 had been further outcrossed to the F_1 of *C57BL/6J* × *C3HB/J-a/a* (P. W. Lane, personal communication). Clearly, the *cn* mice investigated by different investigators do not have a uniform genetic background and modifier genes could have been introduced.

The present results from $+/cn$ and $+/+$ mice are not unduly heterogeneous and show great similarity to those of normal mice from *stm* and *smc* stocks which were processed at the same time, and came from the same animal house.

It is shown in this paper that the effect of the *cn* gene acting alone in the Type I homozygote is merely to reduce the hypertrophic height of mutant chondrocytes without affecting the size of the effective proliferation zone or the labelling index. These findings are in accord with previous work on *cn*.

Furthermore, in the authors' stock, a more extreme phenotype of *cn/cn* can be demonstrated as early as 22 days: in the more severely affected mice, hypertrophic cell height is reduced as is the size of the proliferative zone and the labelling index. The results for older mice are less clear-cut, although suggestive. Do the two types of homozygotes persist, or do older examples really represent parts of a continuum sampled in small numbers? It is hoped to extend the work in future to clarify this particular point.

SUMMARY

Mice homozygous for the recessive gene achondroplasia (*cn*) aged 16 and 17 days and some homozygotes aged 22–34 days have disruptions in the growth of the proximal tibial growth plate which are due solely to reduced hypertrophic cell height. A second class of homozygote, distinguishable at 22 days, has a greater disruption due to much reduced hypertrophic cell height, reduced labelling index and reduction of the number of cells in the effective proliferative zone.

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REFERENCES

BONUCCI, E., DEL MARCO, A., NICOLETTI, B., PETRINELLI, P. & POZZ, L. (1976). Histological and histochemical investigations of achondroplastic mice: a possible model of human achondroplasia. *Growth* **40**, 241–251.

BONUCCI, E., GHERARDI, G., DEL MARCO, A., NICOLETTI, B. & PETRINELLI, P. (1977). An electron microscopic investigation of cartilage and bone in achondroplastic (*cn*) mice. *Journal of Submicroscopical Cytology* **9**, 299–306.

JOHNSON, D. R. (1978). The growth of femur and tibia in three genetically distinct chondrodystrophic mutants of the house mouse. *Journal of Anatomy* **125**, 267–275.

KLEINMAN, H. K., PENNYPACKER, J. P. & BROWN, K. S. (1977). Proteoglycan and collagen of achondroplastic (*cn/cn*) neonatal mouse cartilage. *Growth* **41**, 171–177.

KONYUKHOV, B. V. & PASCHIN, Y. V. (1970). Abnormal growth of the body, internal organs and skeleton in the achondroplastic mice. *Acta biologica hungarica* **21**, 347–354.

LANE, P. W. & DICKIE, M. (1968). Three recessive mutations producing disproportionate dwarfing in mice. *Journal of Heredity* **59**, 300–308.

SILBERBERG, R. & LESKER, P. (1975). Skeletal growth and development of achondroplastic mice. *Growth* **39**, 17–33.

SILBERBERG, R., HASLER, M. & LESKER, P. (1976). Ultrastructure of articular cartilage of achondroplastic mice. *Acta anatomica* **96**, 162–175.

SISSONS, H. A. (1955). Experimental study on the effect of local irradiation on bone growth. In *Progress in Radiobiology, Proceedings of the Fourth International Conference on Radiology* (ed. S. J. Mitchell, B. E. Holmes & C. L. Smith), pp. 436–448. Edinburgh: Oliver & Boyd.

THURSTON, M. N., JOHNSON, D. R., KEMBER, N. F. & MOORE, W. J. (1983). Cell kinetics of growth cartilage in stumpy: a new chondrodystrophic mutant in the mouse. *Journal of Anatomy* **136**, 407–415.