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Observations of growth plate development in achondroplastic (cn/cn) mice

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Summary. The autosomal recessive gene for achondroplasia (cn) in the homozygous condition inhibits the growth of the cartilaginous skeleton in mice. Changes in the width of the proximal growth plate of the tibia and the caudal vertebrae and in the number of proliferative and hypertrophic cells were recorded in normal and achondroplastic (cn/cn) mice during the first 30 neonatal days.

In normal mice as in achondroplastics, the width of the cartilaginous growth plate decreased progressively with increasing age. The proliferative zone of cn/cn mice was similar in structure to that of normal mice and showed no significant difference. Hypertrophied cartilage was less wide and there were fewer hypertrophic cells at the epiphyseal plates than found in normal mice.

The data of the present study show that retarded longitudinal bone growth in cn/cn mice is due to reduced hypertrophy of the chondrocytes.

Introduction.

Inherited achondroplasia has been reported in a wide variety of vertebrates, including man (Rimoin, 1970, 1975 ; Maroteaux and Lamy, 1964). While the bases of dwarfism differ from mutant to mutant, the gross morphological manifestation is similar (Hall, 1978).

Achondroplasia in mice is characterized by a form of disharmonic dwarfism due to an alteration of the growth process of the long bones. This defect is hereditary, manifesting itself in homozygous recessive conditions due to the cn allele (Lane and Dickie, 1968).

Morphological and histological investigations have shown that, at the homozygous stage, the cn allele acts mainly on long bone growth (Jolly and Moore, 1975), particularly on the growth cartilage (Konyukhov and Paschin, 1970) determining the skeleton's longitudinal growth capacity.

Although reduced in size, the growth cartilage retains its columnar organization (Bonucci *et al.*, 1976) characterized by a series of four layers : (i) the reserve zone, (ii) the proliferative zone where the chondrocytes, arranged in parallel columns, multiply, (iii) the maturation zone where the chondrocytes increase in volume, (iv) the hypertrophic zone where the chondrocytes lyse and the temporary calcification line begins.

Electron microscope observation of the four zones and the cartilage matrix in cn/cn mice has revealed no significant alterations. Occasionally, calcification was found in the maturation zone together with glycogen accumulation in the hypertrophic chondrocytes (Bonucci *et al.*, 1977). The levels of both hydroxyproline and sialic acid are reported to increase (Silberberg and Lesker, 1975) and to lie within normal limits (Kleinman *et al.*, 1977).

Biochemical analysis of glycoproteins and collagen shows that they do not alter in composition or content (Kleinman *et al.*, 1977). Nevertheless, reduced growth of the long bones is certainly due to an alteration of cartilage growth and differentiation.

A correlation has been found between the rate of long bone longitudinal growth and variations in growth cartilage width in mouse (Silbermann and Kadar, 1977*a*) and rat (Moss-Salentijn, 1974).

The aim of the present study was to evaluate the development of the growth plate by measuring its thickness and by comparing the respective cellularity of the proliferative and hypertrophic zones.

Material and methods.

Achondroplastic (cn/cn) and normal (cn/+ ; +/+) mice were obtained by mating heterozygous pairs donated by the Jackson Laboratory, Bar Harbor. The cn/cn animals could be identified by weight reduction, shortness of limb and a dome-shaped head.

Twenty-four cn/cn and 24 normal mice from the same litter, aged 24 h and 5, 10, 15, 20 and 30 days, were used. After sacrifice, we dissected the knee joints with parts of femur and proximal epiphysis of the tibia, together with segments of spinal column, including caudal vertebrae. The specimens were fixed for 24 h in buffered paraformaldehyde, decalcified in EDTA solution (10 p. 100) and embedded in Histowax. Serial sections 8-μm thick were cut through a frontal plane and stained with hematoxylin and eosin.

To evaluate the longitudinal thickness of the growth cartilage and its zones, we took five measurements for each of the 5 sections for each slide, using a micrometer eyepiece attached to the microscope.

Accurate differentiation of the most sacral zone of the plate was difficult in the younger mice. The proliferative zone was taken to be that characterized by aligned chondrocytes ; the hypertrophic zone, running from the end of the columns to the calcification zone, included the intermediate maturation stage.

We also assessed the mean number of cells on the longitudinal axes of the above zones as the mean of three counts per section. Means and standard errors were calculated from all numerical data and the means were compared using Student's t-test.

Results.

Growth cartilage of the caudal vertebrae. — At all the ages considered, the growth cartilage of cn/cn mice was thinner than that of normal mice (fig. 1) ; we obtained the following results concerning its component layers in cn/cn mice : (i) the proliferative zone did not differ significantly in either thickness or rate of reduction from that of normal mice at 1, 5, 15, 20 and 30 neonatal days (fig. 3). In both cn/cn mice and the

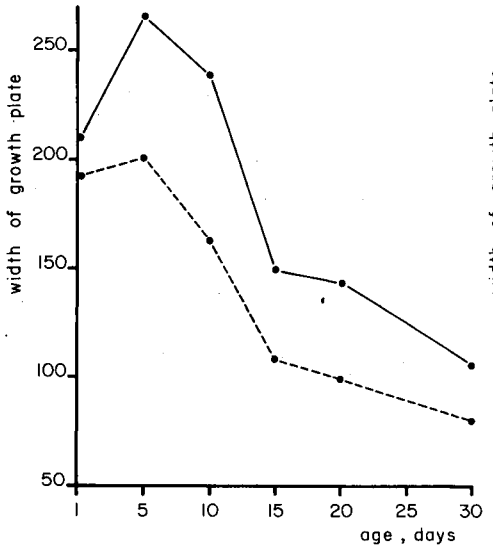

FIG. 1. — *Total width of caudal vertebra growth plate in normal (——) and achondroplastic (------) mice.*

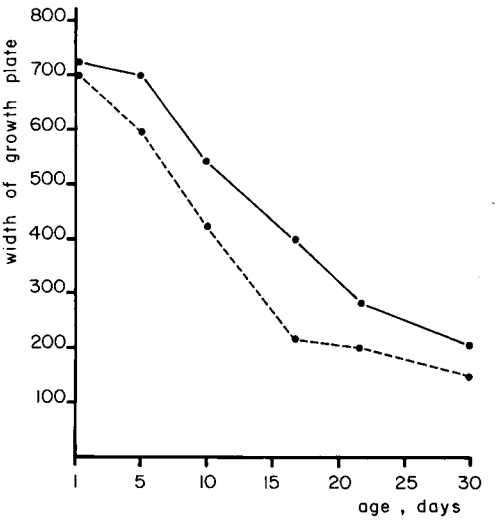

FIG. 2. — *Total width of proximal tibia growth plate in normal (——) and achondroplastic (------) mice.*

controls, the mean number of cells in the proliferative zone diminished between neonatal days 1 and 30. The slight difference in cell number had no significant effect on thickness (table 1) ; (ii) the hypertrophic zone was constantly and clearly reduced (graph 4) as was the number of cells observed in that zone (table 1). The increase in the thickness of this zone between neonatal days 1 and 5 corresponded to the formation of the center of ossification. It is interesting to note that there was a smaller increase (58.5 p. 100 in cn/cn *vs* 105.7 p. 100 in controls) in the width of the hypertrophic zone in

TABLE 1

Number of cells in the proliferative and hypertrophic zones of the caudal vertebra growth cartilage in achondroplastic and control mice

Days	Control mice		Achondroplastic mice	
	Prolifer. zone	Hypertroph. zone	Prolifer. zone	Hypertroph. zone
1	26.25 ± 0.96	3.41 ± 0.37	26.80 ± 0.3	2.4 ± 0.16
5	23.74 ± 0.50	6.19 ± 0.23	23.24 ± 0.52	4.91 ± 0.20
10	23.32 ± 0.96	4.96 ± 0.20	23.22 ± 1.28	4.28 ± 0.13
15	12.13 ± 0.82	5.20 ± 0.27	10.28 ± 0.47	3.42 ± 0.16
20	15.83 ± 3.09	6.38 ± 0.28	13.12 ± 0.90	4.12 ± 0.19
30	11.10 ± 0.69	5.00 ± 0.29	7.53 ± 0.35	3.13 ± 0.35

The differences are always significant in the hypertrophic zone. ($P < 0.001$ at neonatal days 1, 5, 15, 20 and 30 ; $0.01 > P > 0.001$ at neonatal day 10.)

cn/cn animals which thus remains smaller right up to day 30 (fig. 3). The ratio between the thicknesses of the proliferative and the hypertrophic zones was always higher in achondroplastics, indicating a constant reduction of the hypertrophic zone.

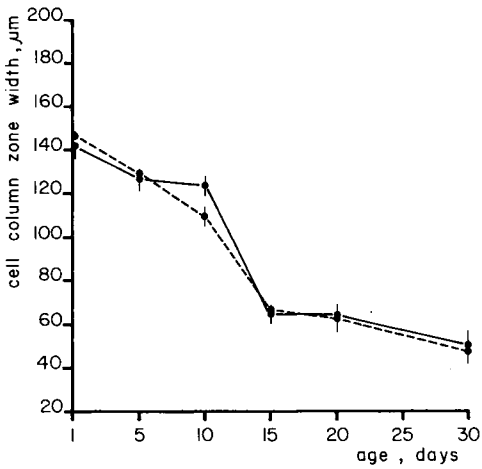

FIG. 3. — *Width of proliferative zone of caudal vertebra growth cartilage in normal* (——) *and achondroplastic* (------) *mice.* The differences are not significant (mean ± SD).

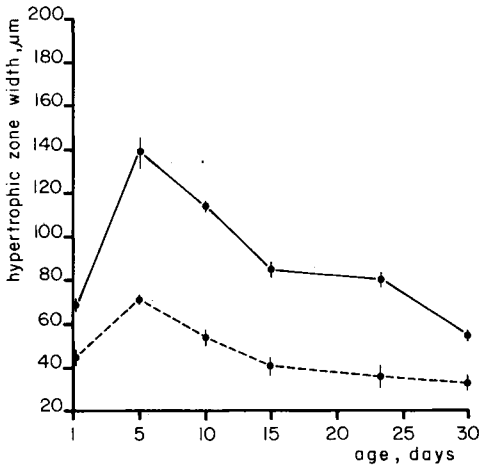

FIG. 4. — *Width of hypertrophic zone of caudal vertebra growth cartilage in normal* (——) *and achondroplastic* (------) *mice.* The differences are always significant ($P < 0.001$) mean ± SD).

Growth cartilage of the proximal epiphysis of the tibia. — The growth cartilage of the tibia had a faster longitudinal growth rate than that of vertebrae, raching its maximum length as early as neonatal day 1. The longitudinal thickness in normal mice was always greater than in achondroplastic mice and diminished from neonatal days 1 to 30. The two thickness curves show parallel behaviour (fig. 2). From neonatal days 1 to 20, the proliferative zone of the control mice and the achondroplastics diminished rapidly in respect to cell thickness and number (fig. 5 ; table 2). The proliferative zone was thinner in the cn/cn animals between days 10 and 20. This reduction was morphologically concomitant with early formation of the secondary center of ossification of the epiphysis.

In the achondroplastics, the hypertrophic zone was always less thick and had a smaller number of cells (fig. 6, table 2). This difference increased from neonatal days 1 to 20.

The ratio between the thickness of the proliferative zone and that of the hypertrophic zone was always higher in the cn/cn mice.

Discussion.

The results confirmed the correlation established between growth cartilage thickness and long bone growth (Silbermann and Kadar, 1977*a* ; Moss-Salentijn, 1974 ; Thorngren and Hansson, 1973). Achondroplastic mice were characterized by thin

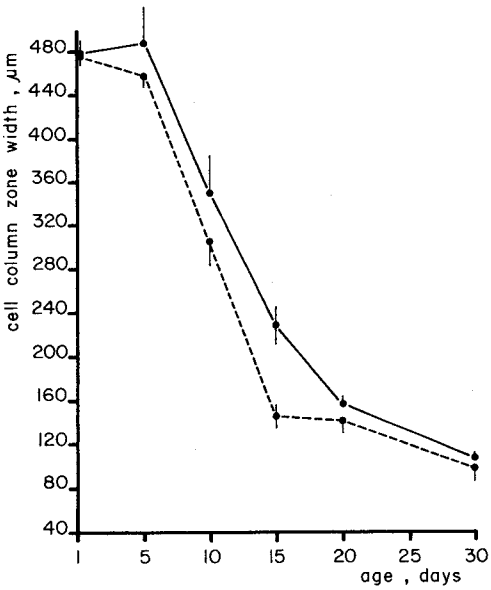

FIG. 5. — *Width of proliferative zone of proximal tibia growth cartilage in normal (——) and achondroplastic (------) mice.* The differences are significant at 10 and 15 neonatal days ($P < 0.01$) (mean $\pm$ SD).

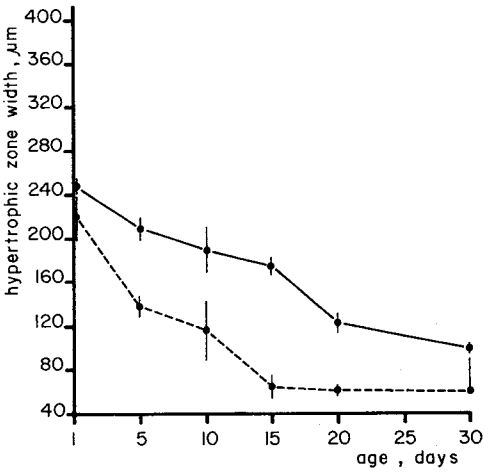

FIG. 6. — *Width of hypertrophic zone of the growth cartilage of normal (——) and achondroplastic (------) mice.* The differences are always significant ($P < 0.001$) (mean $\pm$ SD).

TABLE 2

Number of cells in the proliferative and hypertrophic zones of the tibia growth cartilage in achondroplastic and control mice

Days	Control mice		Achondroplastic mice	
	Prolifer. zone	Hypertroph. zone	Prolifer. zone	Hypertroph. zone
1	95.75 $\pm$ 1.74	15.0 $\pm$ 0.86	87.3 $\pm$ 5.24	14.25 $\pm$ 0.85
5	94.37 $\pm$ 4.50	12.0 $\pm$ 0.46	80.2 $\pm$ 2.65	11.0 $\pm$ 0.91
10	55.60 $\pm$ 4.84	10.6 $\pm$ 0.64	54.6 $\pm$ 5.24	8.8 $\pm$ 0.58
15	43.0 $\pm$ 4.93	12.3 $\pm$ 0.34	23.5 $\pm$ 2.18	4.16 $\pm$ 0.48
20	20.3 $\pm$ 3.29	10.0 $\pm$ 0.58	17.7 $\pm$ 0.59	5.25 $\pm$ 1.31
30	16.0 $\pm$ 2.08	6.3 $\pm$ 0.66	12.7 $\pm$ 3.35	5.00 $\pm$ 0.57

The differences are significant in the hypertrophic zone. ($0.1 > P > 0.01$ at neonatal days 1 and 5; $P < 0.001$ at neonatal days 10, 15, 20, 30.)

growth cartilage in both the caudal vertebrae and the proximal epiphysis of the tibia, corresponding to reduced growth.

Generally, the reduction in total growth cartilage is considered to be related to proliferative zone reduction (Thorngren and Hansson, 1973) and thus to the reduced rate of new chondrocyte production which depends on the number of cells in the proliferating pool and their mean rate of division (Kember, 1971).

The caudal vertebrae of achondroplastic mice displayed no significant differences in proliferative zone thickness with respect to the controls of the same age. Likewise, there were no significant differences in the proximal epiphysis of the tibia, except at 10 and 15 days of age. The differences in proliferative zone thickness were concomitant with the early disappearance of the secondary center of ossification in achondroplastic mice.

At later ages, the differences in proliferative zone thickness tended to disappear with the formation of the secondary center of ossification in the control mice. There are no available data suggesting that proliferative zone thickness is directly correlated with the formation of the ossification center.

On the other hand, data in the literature have constantly reported hypertrophic zone thickness. At all the ages studied, hypertrophic zone thickness, in both the caudal vertebrae and the proximal epiphysis of the tibia, was reduced in cn/cn mice. This reduction was generally due to a reduction in the number of cells and probably to their smaller diameter, as indicated by the lower ratio between zone thickness and the number of hypertrophic cells.

The latter data suggest that the reduction ih the growth cartilage of achondroplastic mice can be mainly attributed to a reduction in hypertrophic zone thickness, although the mechanism by which this occurs remains to be defined. The opposite situation has been found in mice treated with triamcinolone, a cortisone analogue (Silbermann and Kadar, 1977*b*, *c*). In these mice, proliferative zone thickness and cell number are reduced and, in the hypertrophic zone, cell number and thickness are increased.

The growth cartilage in rachitic rats is thicker than in normal rats, and the zone of hypertrophy and temporary calcification are larger. When vitamin D is administered, chondrocyte maturation occurs and the hypertrophic zone is reduced (Mankin and Lippiello, 1968). In the case of rachitic chickens with vitamin D and plasma calcium deficiencies, the growth cartilage is thicker and the size of the matrix proteoglycans is altered (Dickson *et al.*, 1979).

It must also be pointed out that hypertrophy is usually related to mineralization of the extracellular matrix. Mineralization is accompanied by reduced resistance to vascular invasion. Furthermore, the penetration of the vessels through the perichonrium may be related to the death of the chondrocytes (Hall, 1978).

These data could mean that in achondroplastic mice the process of chondrocyte differentiation, occurring during chondrocyte hypertrophy, is disturbed. What regulation factor is affected by the cn allele at the homozygous stage still remains to be determined.

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Résumé. L'allèle récessif autosomique (cn) à l'état homozygote réduit la croissance des os longs dans les souris cn. Nous avons évalué l'épaisseur longitudinale du cartilage de croissance de l'épiphyse proximale du tibia et des vertèbres et le nombre des cellules de la zone de prolifération et d'hypertrophie dans les souris normales et achondroplasiques (cn/cn) pendant les 30 premiers jours de vie. L'épaisseur longitudinale du cartilage de croissance des souris achondroplasiques est toujours inférieure à celle des souris normales. En considérant les zones composantes on observe que la zone de prolifération dans les souris cn/cn

n'est généralement pas différente de façon significative de celle des souris normales. La zone d'hypertrophie est réduite de façon significative, constamment chez l'achondroplasique ; en même temps on observe une réduction du nombre des cellules de cette zone.

Les résultats de ce travail montrent que la réduction de l'accroissement des souris cn/cn est en rapport avec la réduction de la zone hypertrophique.

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