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The growth of femur and tibia in three genetically distinct chondrodystrophic mutants of the house mouse

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INTRODUCTION

During the last few years there has been a revival of interest in genetically determined disproportionate dwarfing (chondrodystrophy, formerly achondroplasia) in both man and laboratory animals (Rimoin, 1975).

The use of biochemical and ultrastructural techniques to supplement the findings of classical histology and histochemistry has allowed us to see that chondrodystrophy, i.e. the reduction or cessation of growth of cartilage, is a symptom of a more fundamental abnormality rather than a disease in its own right. Disturbances in metabolism which produce a chondrodystrophic phenotype in laboratory animals have implicated oxidative phosphorylation in mitochondria (Bargman, Mackler & Shepard, 1972), the mucopolysaccharide content of the matrix (Seegmiller, Fraser & Sheldon, 1971; Seegmiller, Ferguson & Sheldon, 1972; Fraser & Goetinck, 1971; Pennypacker & Goetinck, 1976), sulphation of the matrix (Orkin, Pratt & Gill, 1976) and synthesis of protein, probably collagen (Johnson & Hunt, 1974).

Evidently cartilage is a tissue at risk: something about its structure and metabolism (we may hazard a guess that avascular nutrition and consequent low oxygen tension are significant) seems to render it particularly sensitive to metabolic upsets. Biochemical studies (Johnson & Hunt, 1974) have also shown that the causal factors in chondrodystrophy may be transitory, operating for only a few days in the early life of the animal when the rate of bone growth is maximal.

One would expect the growth curves of long bones to be different in chondrodystrophies resulting from different combinations and times of operation of genetic and epigenetic factors. If the growth potential of mutant cartilage is lowered by different amounts one might expect a family of parallel curves; differences in the times of action of the genes should also be reflected in the growth curves.

Three genetically distinct chondrodystrophic mutations were available for study. Two of these (achondroplasia, *cn*, and brachymorphic, *bm*) affect proximal and distal limb elements almost equally (Lane & Dickie, 1968) while the third (stumpy, *stm*) shortens proximal elements more than distal ones (Johnson, unpublished; Table 1).

MATERIALS AND METHODS

Papain digestion preparations were made of the skeletons of 191 mice (+/+ or +/*cn* 23, *cn*/*cn* 30; +/+ or +/*bm* 41; *bm*/*bm* 44; +/+ or +/*stm* 26, *stm*/*stm* 27) aged between 6 and 128 days. Mice used were litter-mates derived from matings between two known heterozygotes. Stocks were not co-isogenic, but were reared under the same environmental conditions. Femora and tibiae were isolated and measured under a dissecting microscope. The following measurements were made:

overall length of bone minus epiphyses, most proximal point to foramen of nutrient artery, foramen of nutrient artery to most distal point. The foramen used was, in all cases, that of the principal nutrient artery; this was easily found in most bones, and its position corresponded to that in the illustrations given by Brookes (1971) for the rat. In some cases, usually in older mice, the foramen was obliterated, and in such bones only the overall length could be measured.

Table 1. *Ratio of mutant:normal limb bone length in four strains of adult chondrodystrophic mice*

	cn/cn:+*	*bm/bm*:+*	*stm/stm*:+†
Humerus	0·66	0·72	0·58
Radius	0·63	0·69	0·67
Ulna	0·62	0·71	0·73
Femur	0·61	0·69	0·76
Tibia	0·60	0·63	0·73

* From Lane & Dickie (1968).
† Unpublished observations.

Table 2. *Length of femur (in mm) in chondrodystrophic mice and normal litter-mates at various ages*

Age (days)	+	*cn/cn*	+	*bm/bm*	+	*stm/stm*
6	4·46±0·05 (5)	3·65±0·06 (4)	—	—	4·38±0·03 (6)	3·55±0·17 (6)
12	—	4·50±0·14 (7)	6·16±0·07 (10)	5·30±0·14 (16)	6·26±0·16 (10)	4·72±0·08 (8)
16	7·53±0·22 (6)	5·37±0·16 (6)	7·70±0·18 (8)	5·26±0·10 (12)	7·35±0·07 (8)	6·12±0·32 (10)
21	9·30±0·15 (6)	6·20±0·34 (8)	8·60 (6)	6·25±0·56 (5)	8·34±0·17 (8)	5·88±0·15 (10)
32	10·40±0·28 (6)	6·24±0·29 (5)	10·00±0·16 (16)	6·03±0·14 (16)	9·25±0·07 (6)	6·25±0·09 (8)
42	11·70±0·22 (4)	6·95±0·15 (4)	12·13±0·08 (6)	7·10±0·38 (6)	—	—
60	12·20±0·58 (8)	7·16±0·14 (9)	13·27±0·03 (20)	8·00±0·13 (16)	13·09±0·18 (15)	8·35±0·16 (12)
75	12·36±0·10 (6)	6·82±0·19 (8)	13·65±0·09 (10)	8·80±0·21 (12)	—	—
100+	13·90±0·14 (2)	8·15±0·13 (8)	—	10·00 (1)		

Results are given as mean±S.E.M. in mm. Numbers in parentheses indicate number of bones measured.

RESULTS

The growth patterns of femora and tibiae were very similar in the three normal control groups studied, the slight variations seen being attributable to sampling error or to the varying genetic background of the stocks (Tables 2 and 3, Fig. 1).

All three mutant strains differed from normal litter-mates in showing marked interruptions in growth. This is seen even more clearly if the rate of growth per day is calculated from the mean lengths of bones sampled at various ages (Fig. 2). It is then

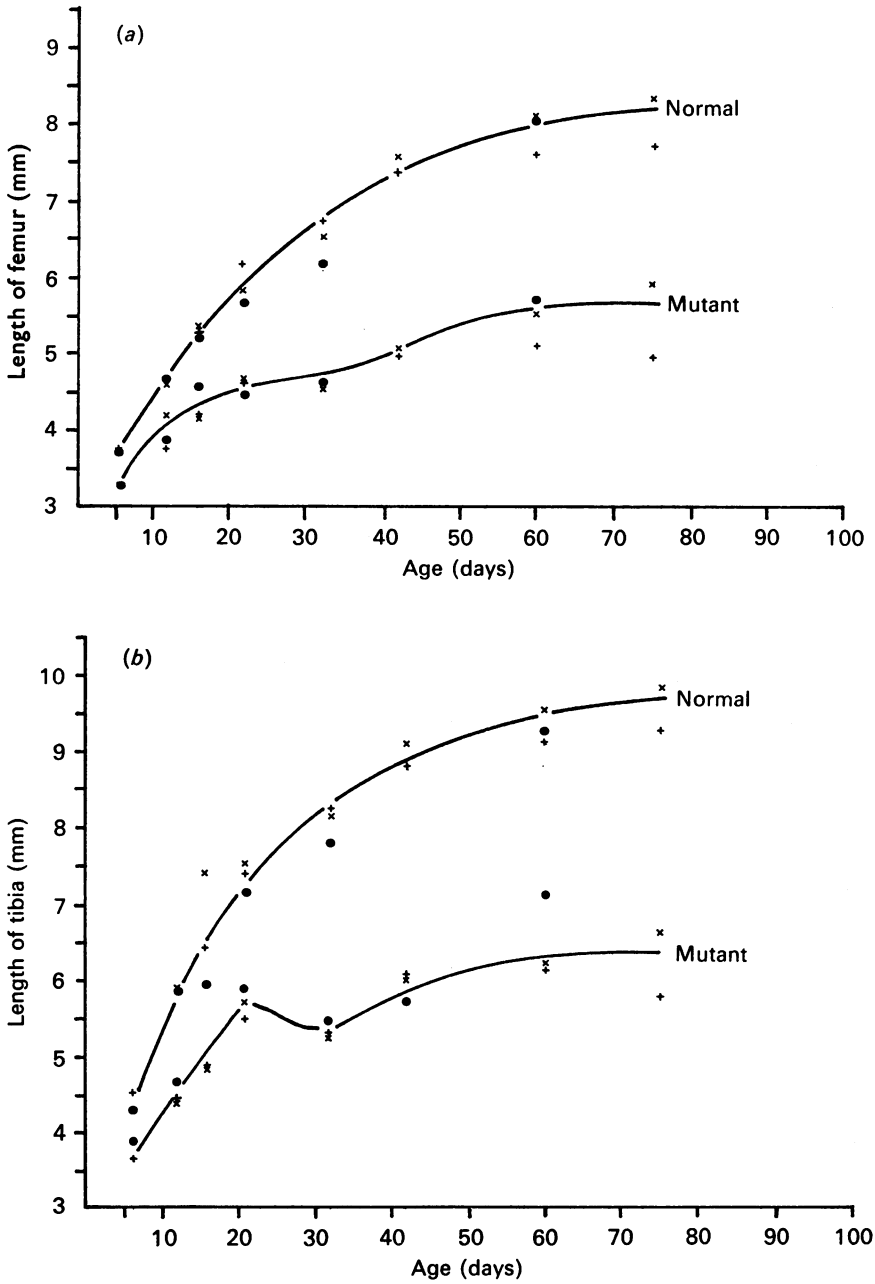

Fig. 1. Growth of (*a*) femur, (*b*) tibia in normal and chondrodystrophic mice. +, *cn* stock; x, *bm* stock, ●, *stm* stock.

apparent that normal, *cn* and *bm* bones suffer a check in growth at the time of weaning (the mice were weaned between 21 and 28 days), but that this check is much more severe in mutant mice. Growth curves for *cn* and *bm* femora and tibiae are very similar: stumpy behaves in a different manner, with a strong growth peak in the 12–16 day interval (increase in length over the period 12–16 days: *stm*/*stm* femur 0·35, + femur

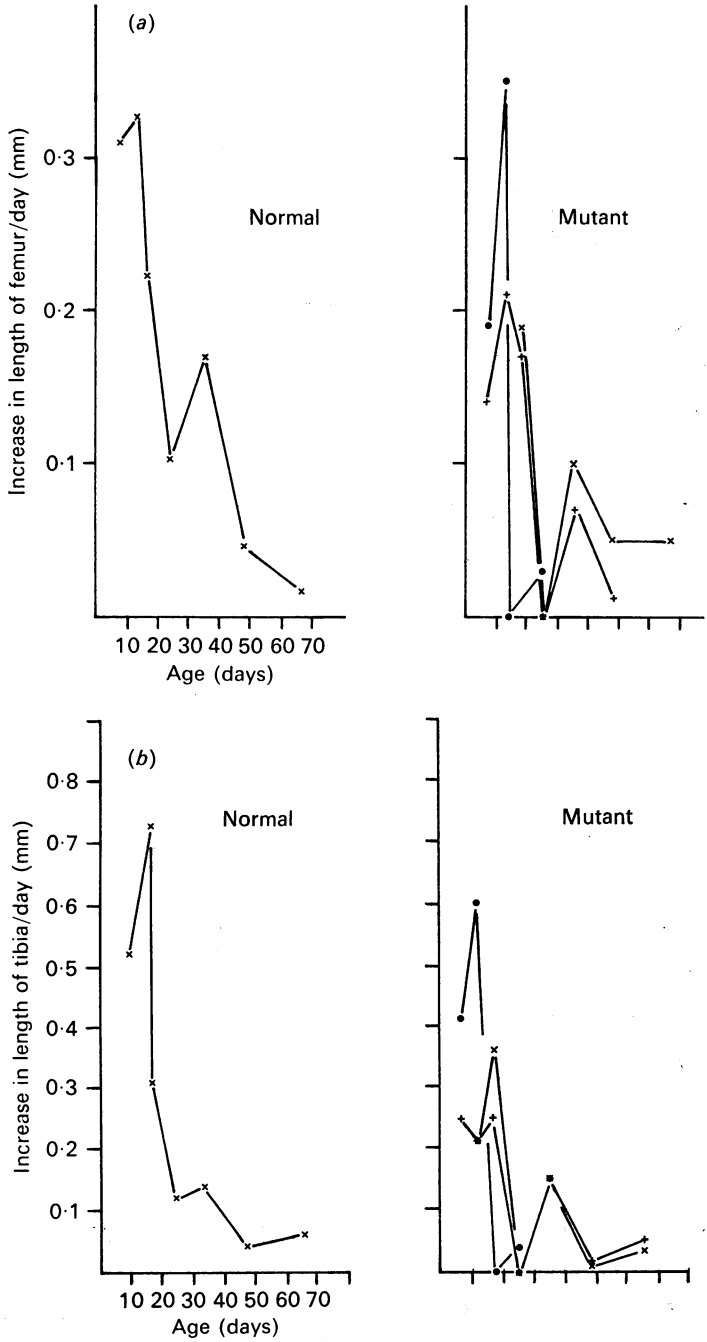

Fig. 2. Growth per day of (*a*) femur, (*b*) tibia in normal and chondrodystrophic stock. Left hand graph shows mean of normal controls, right hand graph individual genotypes. +, *cn*/*cn*; x, *bm*/*bm*; ●, *stm*/*stm*.

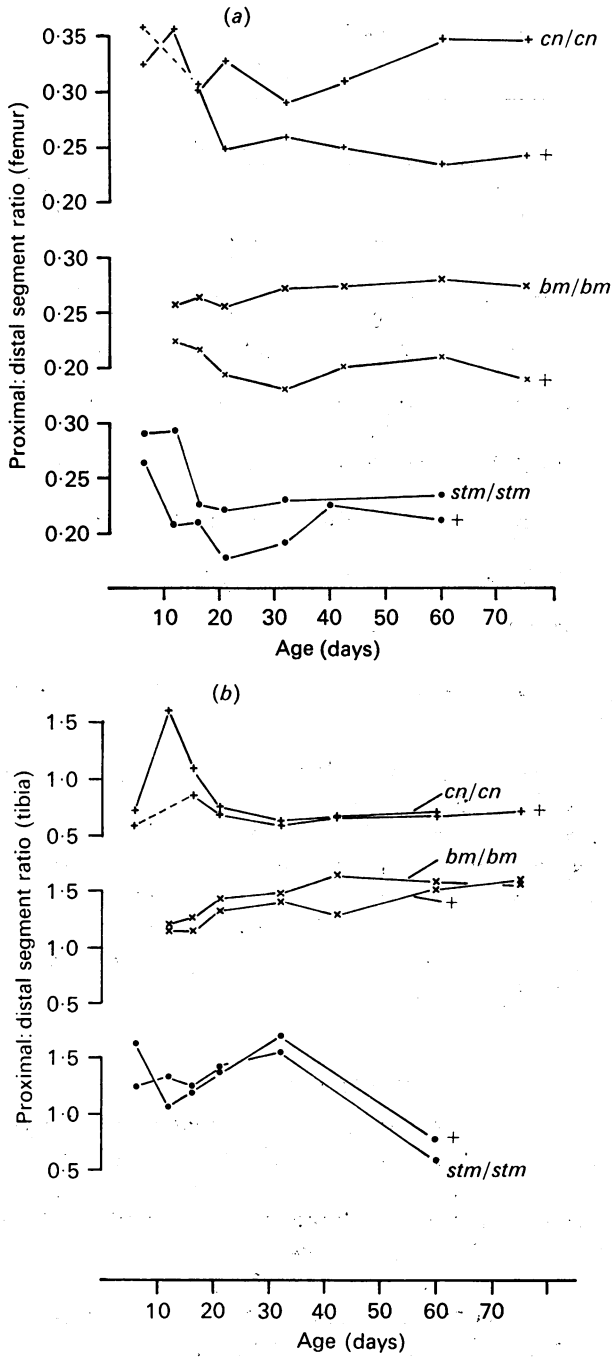

Fig. 3. Ratio of proximal:distal segment of (*a*) femur, (*b*) tibia. Symbols as for Fig. 1.

Table 3. *Length of tibia (in mm) in chondrodrystrophic mice and normal litter-mates at various ages*

Age (days)	+	*cn/cn*	+	*bm/bm*	+	*stm/stm*
6	6·04±0·16 (5)	4·25±0·11 (4)	—	—	5·54±0·37 (5)	4·66±0·20 (5)
12	—	5·80 (7)	8·75±0·06 (14)	5·70±0·07 (16)	8·66±0·27 (10)	6·30±0·09 (7)
16	9·87±0·23 (6)	6·66±0·21 (6)	11·7±0·61 (10)	6·55±0·14 (12)	8·80±0·21 (8)	8·70±0·40 (8)
21	11·87±0·14 (6)	7·89±0·63 (7)	12·0 (6)	8·35±0·23 (5)	11·23±0·22 (8)	7·90±0·16 (10)
32	13·40±0·28 (6)	7·50±0·49 (6)	13·22±0·22 (16)	7·39±0·25 (18)	12·5 (6)	8·38±0·16 (8)
42	14·50±0·38 (4)	9·10±0·07 (4)	15·10±0·18 (6)	8·95±0·51 (6)	—	—
60	15·10±0·55 (8)	9·18±0·32 (10)	16·00±0·16 (20)	9·36±0·20 (16)	15·37±0·27 (15)	11·20±0·08 (11)
75	15·45±0·45 (8)	8·50±0·28 (8)	16·61±0·11 (10)	10·21±0·19 (14)	—	—
100+	16·0±0·28 (2)	9·77±0·06 (8)	—	11·9 (1)		

Results are given as mean ±S.E.M. in mm. Numbers in parentheses indicate number of bones measured.

0·33, *stm/stm* tibia 0·60, +tibia 0·73 mm day^{-1}) followed by a cessation of growth in the 16–21 day period, i.e. before weaning.

Figure 3 shows the ratios of proximal:distal bone length in normal and chondrodystrophic litter-mates, the foramen of the principal nutrient artery being used as a 'fixed' intermediate reference point. It can be seen that, although the shape of the curve varies between stocks, the values obtained for mutant individuals follow those of normal litter-mates. In the femur the ratio is usually higher in abnormals; in the tibia, the curves are virtually identical.

DISCUSSION

It is not surprising that adequate samples of normal bones, large enough to be measured with a fair degree of accuracy, should conform to the classical growth curve which we have come to expect. It is also not surprising that the process of weaning imposes a check on increase in bone length. The effect of weaning on the increase in weight of young mice is well known.

What is perhaps surprising is that the effect of weaning on *cn* and *bm* mice is so great that bone growth stops completely for a time. This is unlikely to be an artefact as it was seen in femora and tibiae from both strains.

Stumpy appears to differ. While *cn* and *bm* are growing at subnormal rates in the pre-weaning period, stumpy grows at normal speed. However, between 16 and 21 days, whilst *cn* and *bm* are still growing, neither *stm* femur nor tibia increase in length. During the 21–32 day interval, when both *cn* and *bm* have stopped growing, *stm* bones are increasing a little in length. The growth of long bones in mammals takes place at their proximal and distal epiphyses, and has been known to be unequal

Table 4. *Ratio of mutant:normal limb bone length in three strains of chondrodystrophic mice at various ages*

	Femur			Tibia		
Age (days)	*cn/cn*: +	*bm/bm*: +	*stm/stm*: +	*cn/cn*: +	*bm/bm*: +	*stm/stm*: +
6	0·81	—	0·81	0·70	—	0·84
12	—	0·86	0·75	—	0·65	0·73
16	0·71	0·68	0·83	0·67	0·56	0·73
21	0·67	0·72	0·70	0·66	0·69	0·70
32	0·60	0·60	0·68	0·56	0·56	0·67
42	0·59	0·59	—	0·63	0·59	—
60	0·59	0·60	0·63	0·61	0·58	0·73
75	0·55	0·64	—	0·55	0·61	—
100+	0·59	—	—	0·61	—	—

postnatally since the time of Hales (1727). In the hind limb the epiphyses nearest the knee (i.e. distal end of femur and proximal end of tibia) grow most. The relative growth of the two ends can be investigated by reference to a fixed point somewhere between them. The best way to achieve this is to introduce a radio-opaque marker into the bone and then to take serial radiographs (Brodin, 1955; Heikel, 1960; McCormick, Lowe & Ashworth, 1972). As this method was not feasible in this study, it was decided to use a naturally occurring fixed point, the foramen of the principal nutrient artery. The use of this foramen may be objected to on several counts. First because there are several nutrient foramina, and they may lead to misidentification. This was not a problem in the present study as the principal foramen was by far the largest in the young mice used. Secondly because the foramen may become occluded in later life. This was so in the mice used, where some measurements could not be made on the oldest bones because the foramen could not be seen. Thirdly because differential surface bone accretion causes movement of the foramen. This objection is based on the work of Payton (1932, 1934) on madder-fed pigs. Payton showed, by utilising other bony fixed points, that the foramen does, in fact, move by differential growth of surface bone. Using Payton's figures for the ulnae of pigs no. 14 and 6, the foramen moved 10 mm in 282 days, or 0·03 mm/day. This movement becomes less important, however, when we are comparing normal with abnormal rather than when making absolute measurements. Differential surface growth is not known to be affected in chondrodystrophic mice.

If we accept the foramen of the nutrient artery as a valid reference point, we may compare proximal:distal segment lengths in bones from normal and mutant mice. For femora, the curves are nearly parallel (Fig. 3), with the mutant values higher throughout, indicating a relative shortening of the distal, fastest growing, end of the bone. In the tibiae, the curves are virtually identical. Perhaps the most interesting feature of these results is not the difference between normal and mutant individuals, but the differences between stocks. Differential growth of the ends of long bones has not been observed in fetal mammals (Bisgard & Bisgard, 1935; Felts, 1954; Brookes, 1963) and the time and mode of origin of the differential is, therefore, unknown. It seems likely, however, from the instability of the proximal:distal ratio in young animals, that the differential is established early in postnatal life, and that the mode of establishment may have a genetic component.

The findings of this paper are in general agreement with those of Konyukhov &

Paschin (1970), who found that *cn* long bones grow less than those of normal littermates during the first four weeks after birth, and with those of Silberberg & Lesker (1975), who found an abnormal:normal ratio of 0·6 between *cn* and normal bones (Table 4).

It seems that the *cn* and *bm* mutants resemble each other closely in the way bone growth is affected, although the biochemical bases of the conditions may well be quite different. Brachymorphic mice are known to show reduced sulphation of the cartilage matrix (Orkin *et al.* 1976): the aetiology of *cn* is at present unreported, although the ultrastructure seems unremarkable (Silberberg, Hasler & Lesker, 1976). Both these mutant phenotypes show a reduction in growth and an exaggerated response to weaning. Stumpy, which produces a slightly different phenotype, with more reduction in proximal than in distal bones (in which it resembles Dexter cattle; Crew, 1924), shows a different pattern of growth. The significance of this fact may emerge from the biochemical and ultrastructural studies on *stm* which are in progress.

SUMMARY

Growth of femora and tibiae has been measured in mice carrying three distinct chondrodystrophic mutants (achondroplasia *cn*, brachymorphic *bm* and stumpy *stm*) aged 6–128 days, and in normal litter-mates.

cn and *bm* resemble each other in growing slowly until the time of weaning, when growth is interrupted. *stm* grows strongly at first, but stops at around 14 days. The significance of these findings is discussed.

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