J. Anat. (1983), 136, 2, pp. 407–415
With 4 figures
Printed in Great Britain

Cell kinetics of growth cartilage in stumpy: a new chondrodystrophic mutant in the mouse

M. N. THURSTON,* D. R. JOHNSON,† N. F. KEMBER* AND W. J. MOORE†

* *Department of Physics, Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ and*
† *Department of Anatomy, School of Medicine, Medical and Dental Building, University of Leeds, Leeds LS2 9JT*

(*Accepted* 2 *July* 1982)

INTRODUCTION

In recent years the various hereditary chondrodystrophies which occur in both man and many laboratory animals, have received attention. Stumpy, the object of study in this paper, is a relatively new chondrodystrophic mutant which has a distinctive cartilage phenotype. It is of particular interest because it appears to resemble human achondroplasia and Dexter cattle (Ferguson, Wallace & Johnson, 1978) in that the limbs are shortened to a greater degree proximally than distally.

Recent work has shown that almost every bone in the stumpy skeleton differs from normal litter mates to a greater or lesser degree (Ferguson *et al.* 1978). Furthermore, there is evidence that stumpy undergoes a sudden check in the growth in the 16–21 day period (Johnson, 1978*a*), and ultrastructural observations on the epiphyseal cartilage at that time reveal that there is a failure of chondrocytes to move apart after mitosis.

This paper describes some of the kinetic and histological changes which occur with time in the stumpy mutant, comparing them with their normal litter mates. The main aim of the study was to examine the patterns of labelling in stumpy growth cartilage, to determine whether the reduced growth and sudden check in growth were reflected in changes in the cell proliferation rate or in the size of the proliferating cell population.

MATERIALS AND METHOD

All mice used were litter mates derived from matings between two known heterozygotes. Normal and stumpy mice, age 10–41 days, were injected intraperitoneally with tritiated thymidine-6-T (Radiochemical Centre, Amersham, specific activity 5·0 Ci/mmol) at a concentration of 0·5 μCi/g body weight, 1 hour before they were killed with an overdose of ether. After death the knee regions were excised and fixed in buffered formol saline. The bones were stripped of musculature and decalcified in a 5·5 % solution of EDTA, buffered to pH 7·4 with phosphate buffer, for a minimum of 7 days. When decalcification was complete the bones were returned to formol saline for 24 hours, dehydrated, and embedded in paraffin wax. Sagittal sections were cut at 7 μm, the sections being taken from the mid-line of the knee joint. Sections were de-waxed, hydrated and air-dried. Autoradiographs were prepared by dipping the slides in Ilford K5 emulsion, diluted 1 part emulsion to

1 part water. The slides were exposed for 4 weeks in light-tight boxes and stored at 4 °C. The autoradiographs were developed in Kodak D19 and fixed in Kodafix, washed, stained with Ehrlich's haematoxylin and eosin, dehydrated, cleared and mounted in DPX.

Measurements

After 4 weeks exposure, all labelled cells showed a high grain count (greater than 40 grains/nucleus) so that there was little chance of false positives being scored. Only sections which had a low background (maximum number of grains per nucleus = 2) were used.

Observations were concentrated on the growth plate of the proximal end of the tibia only. For each age group, (10, 16, 18, 21, 26, 34, 41 days), two animals of each phenotype were studied.

The following observations were undertaken:

(1) A labelling profile was constructed. This involved counting down the cartilage columns, cell no. 1 being the cell nearest the epiphysis, and recording the positions of all labelled and unlabelled cells within the column (Walker & Kember, 1972). This enables the percentage of labelled nuclei at any position down the column to be calculated. Counts were based on not less than 100 labelled nuclei. From the labelling profile, the size of the proliferation zone may be defined and also a mean labelling index for the proliferation zone. The extent and position of the four recognisable zones (resting, proliferating, maturing, hypertrophying) were noted.

(2) The heights of 100 hypertrophic cells were measured, using a calibrated eye-piece graticule, the height being the dimension of the cell in the direction of growth of the bone. These were adjacent cells along the metaphyseal border of the plate, the last whole hypertrophic cell which had not yet been invaded by a metaphyseal loop being measured.

(3) The following stains were employed to observe matrix constituents and cellular detail: (i) Heidenhain's azan variant – for collagen; (ii) Toluidine blue – for mucopolysaccharides; (iii) Von Kossa – for calcium; (iv) Feulgen reaction – for DNA.

RESULTS

The results of the analyses of the bones are recorded in Figure 1 and in Table 1. Figure 1 shows the labelling profiles for the proximal growth plate of the tibia of stumpy mice and their normal litter mates, age 10–41 days. The profiles show the frequency with which labelled cells were found at each position down the cartilage column. They also show the average length of the cartilage columns and the approximate length and position of the hypertrophic zone.

The profiles for all normal mice were roughly similar in shape, the frequency of labelling being lower in the first few cell positions (which may correspond to a reserve or stem cell zone), highest in the middle cell positions and falling off on the metaphyseal side. Maturation of chondrocytes occurred in the cell positions which lay in between the end of the proliferation zone and the hypertrophic zone.

Stumpy profiles were somewhat more variable. The profiles up to and including day 21 were most obviously different from normal. At 16, 18 and 21 days the hypertrophic zone abutted directly on to the proliferation zone, leaving no room for differentiation and maturation of chondrocytes. At day 10 the bulk of proliferation appeared to be concentrated in the first 14/15 cell positions. After 21 days the stumpy

Table 1. *Cell kinetics parameters for proximal growth plate of the tibia for normal and stumpy mice aged* 10–41 *days*

Age (days)		Number of cells in 'effective' proliferation zone	Labelling index (%)	Hypertrophic cell height (μm)
10	Normal	18	~15	27±1·4
	Stumpy	15	~13	20±0·7
16	Normal	18	~11	27±2·0
	Stumpy	19	~6	17±1·0
18	Normal	14	~8	26±2·1
	Stumpy	14	~11	17±0·6
21	Normal	13	~11	23±2·2
	Stumpy	12	~8	15±0·7
26	Normal	15	~8	23±1·0
	Stumpy	15	~11	15±0·7
34	Normal	13	~9	22±0·8
	Stumpy	17	~10	17±1·6
41	Normal	8	~11	19±0·7
	Stumpy	7	~12	17±1·4

growth plate made some kind of recovery and the profiles and mean labelling indices resembled more closely those of normal mice.

Calculation of the growth rate

Sissons (1955) was the first to show that the growth rate of a long bone may be calculated thus:

$$\text{Growth rate} = \text{rate of production of new cells per cartilage column} \times \text{maximum size of hypertrophied cell},$$

where the rate of production of new cells = number of cells in proliferating pool × rate of division. The growth contribution of each position in the column is calculated and the values summed to give a total growth rate for the proximal growth plate of the tibia.

'Effective' proliferation zone size

The number of cells in the proliferation pool was indicated by the extent of the labelling profile. However, the bulk of proliferation occurred in a central zone, with lower frequencies of labelling found in the cell positions on either side. These lower frequencies may be taken fairly into account by using an arbitrary method (Kember, 1972) to calculate, the so called 'effective' proliferation zone, which serves as a useful basis in the comparison of proliferation zone sizes. For each profile the mean labelling index was taken as a standard and the main proliferation zone was taken to lie between the two extreme cell positions with frequencies exceeding the mean. For the lower frequencies a fractional weighting factor equal to the frequency at that position divided by the mean frequency was added to the proliferation zone total. Thus, for the top left profile in Figure 1 the mean frequency = 14·5%. Positions 5–17 defined the main proliferation zone, i.e. 13 cells long, to which was added 5·9/14·5 for position 2, 10·0/14·5 for position 3, and so on, to arrive at the 'effective' proliferation zone size.

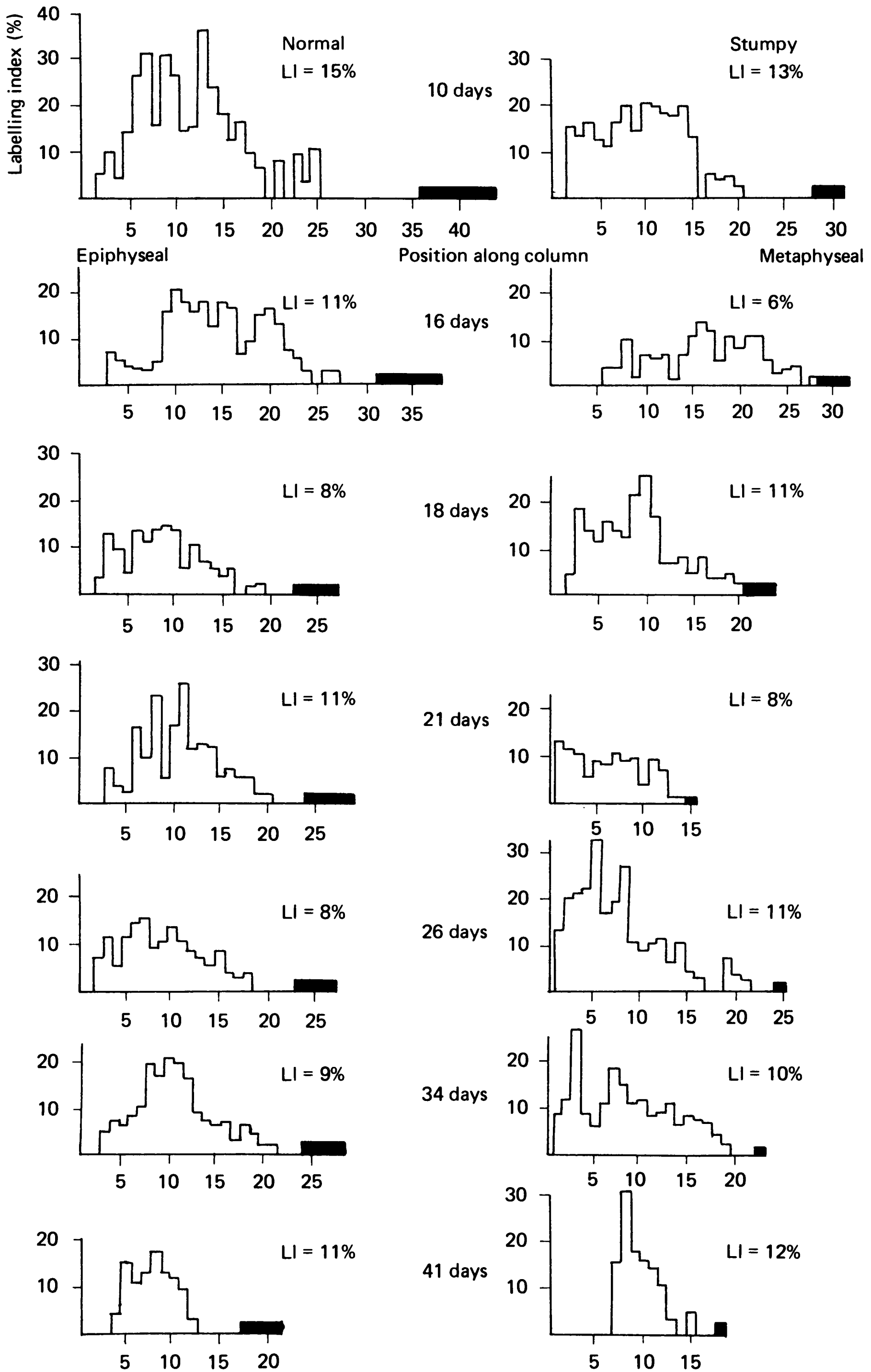

Fig. 1. Profiles of percentage labelled nuclei at each position down the cartilage columns of the proximal growth plate of the tibia in normal and stumpy mice, aged 10–41 days. The size and position of the hypertrophic zone are indicated by the horizontal bar on the right of each profile. The mean labelling index for each growth plate is also given.

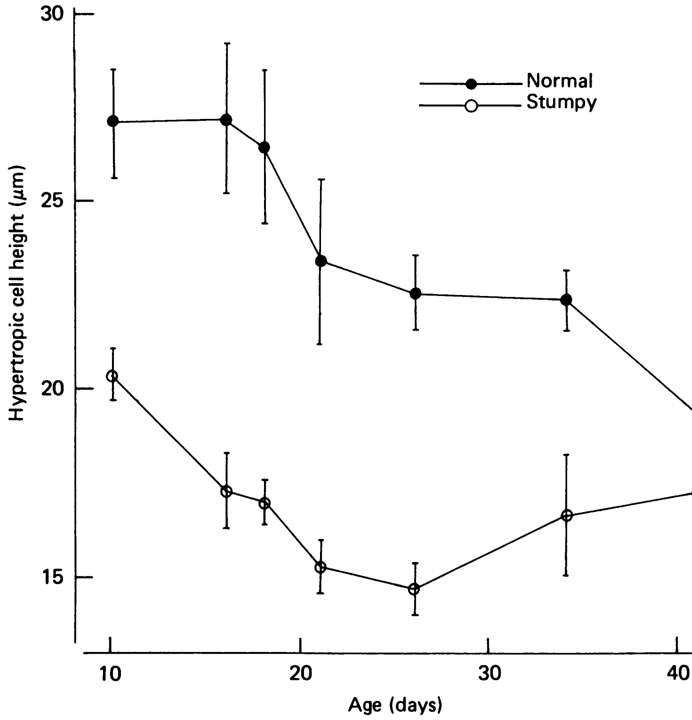

Fig. 2. Hypertrophic cell height measurements (±s.e.) in normal and stumpy mice, aged 10–41 days.

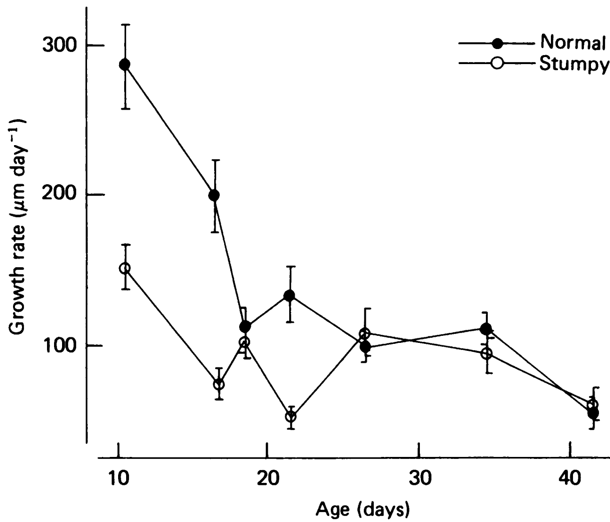

Fig. 3. Growth rate (±s.e.) for the growth plate of the proximal tibia in normal and stumpy mice, aged 10–41 days.

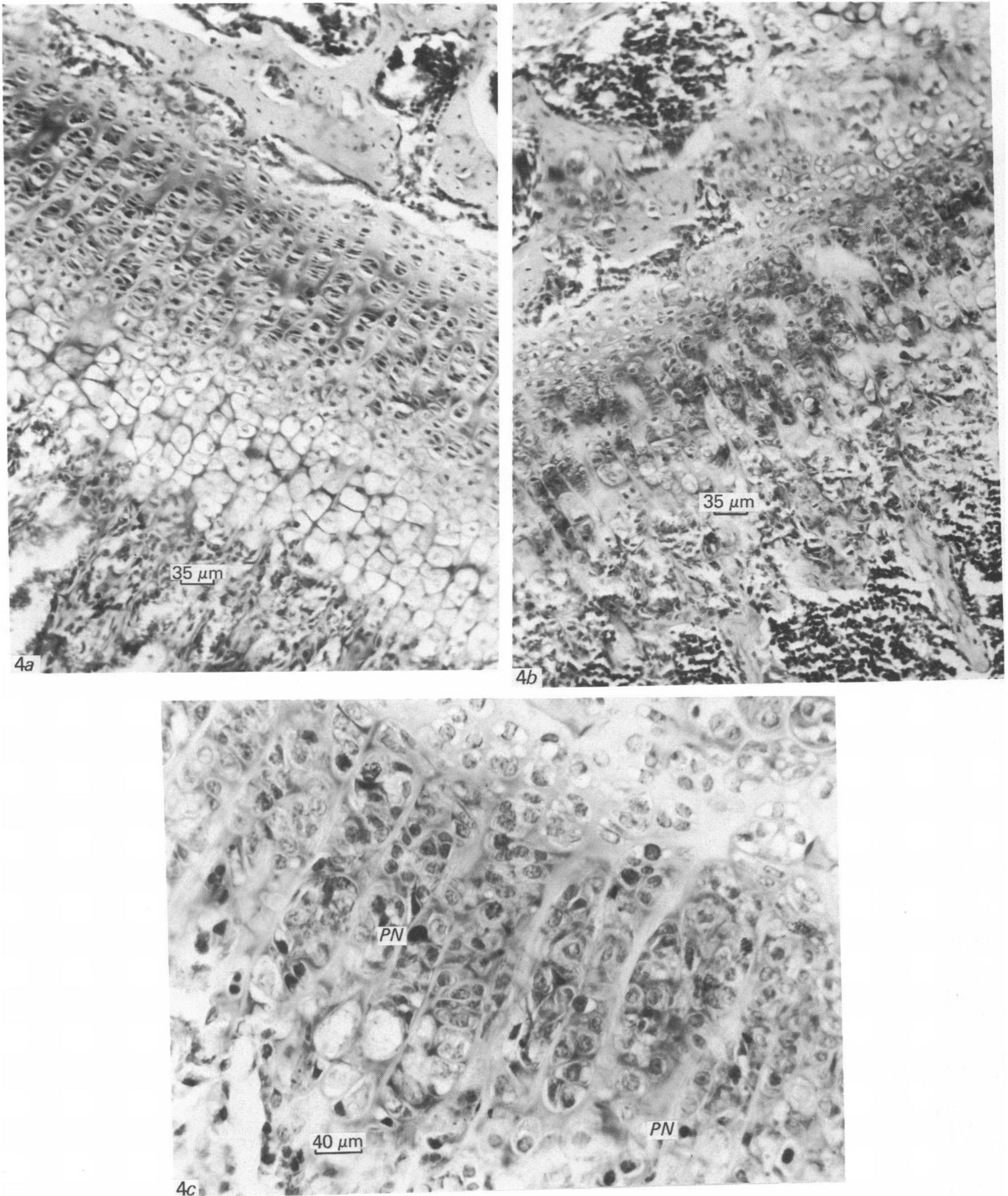

Fig. 4 (*a–c*). Typical appearance of the proximal growth plate of the tibia at 21 days in: (*a*) Normal mouse; (*b*) Stumpy litter mate; (*c*) Stumpy growth plate under higher magnification showing generalised disorganisation and lack of hypertrophy. The pyknotic nuclei (*PN*) can be seen as densely staining bodies scattered throughout the growth plate. Erhlich's haematoxylin and eosin.

Figure 2 shows the results of the measurements of hypertrophic cell height. On these data an analysis of covariance was carried out and it was found that at each age normal hypertrophic cell height was significantly higher than stumpy hypertrophic cell height at the 5% probability level, except at day 41.

The growth rates for normal and stumpy mice of various ages are illustrated in Figure 3. The salient points to emerge were that in the 10–21 day period stumpy mice exhibited a reduced growth rate in comparison to normal mice. Thereafter, the growth rates in normal and stumpy were remarkably similar, as stumpy mice appeared to recover.

It was felt that no statistical analysis should be carried out on labelling indices and proliferation zone sizes because these are based entirely on subjective data.

The histology of the growth plate of normal and stumpy mice was observed throughout the period of 10–41 days. The typical appearance of the growth plate in both stocks at 21 days is shown in Figure 4. The normal mouse of this age (Fig. 4*a*) revealed neatly aligned chondrocytes in regular columns. The wedge-shaped chondrocytes of the proliferation zone were distinguished from the rounder maturing chondrocytes, which in turn swelled and became the cells of the hypertrophic zone. The stem cell zone was also distinguished at the top of the cartilage plate where the chondrocytes appeared to be arranged more in groups scattered throughout the matrix rather than attached to specific columns.

This picture of well ordered endochondral ossification contrasts strongly with the condition found in the stumpy growth plate (Fig. 4*b*). The columnar structure was still evident to a certain extent although the chondrocytes in places appeared more in groups than in columns. Most obvious in the same stumpy growth plate at a higher magnification (Fig. 4*c*) was the complete lack of differentiation into zones: the chondrocytes of the proliferation zone were not typically wedge-shaped but more rounded, and did not significantly differ from maturing chondrocytes. Hypertrophic cells were sometimes isolated, and there was a complete lack of any form of hypertrophic zone. Often the bottom-most cell of a cartilage column being invaded was a 'normal' flattened chondrocyte, showing no sign of hypertrophy. Chondrocytes often appeared to be 'stuck' together with neighbouring chondrocytes.

The four stains which were employed to demonstrate matrix constituents and cellular detail did not reveal any gross deviations in stumpy from the normal appearance at any age. The Feulgen reaction for DNA did, however, reveal a sudden appearance at 21 days of many pyknotic cells in some columns and a certain amount of nuclear debris in some chondrocytes in the stumpy growth plate, which were rarely observed in normal mice.

DISCUSSION

Measurements on papain preparations of bones in stumpy (Johnson, 1978*b*) show that femora and humeri in stumpy are smaller than those of normal litter mates at all ages from 6 to 60 days, but that the shortened bones grow strongly during the period 12–16 days. Histological and ultrastructural studies on mice aged 5–14 days suggested a slight increase in mitotic index and a reduced staining of the cartilaginous matrix. Johnson (1977) had also noted an increased number of chondrocytes per lacuna and that adjacent cells were often atypically united by a narrow intercellular gap between much convoluted membranes or by tight junctions.

The present more detailed study adds to this picture. The day 10 cartilage plate in the tibia shows slight deviation in structure from normal: smaller hypertrophic cell

height, columns not strictly regular, chondrocytes not wedge-shaped. The disorganisation worsens and at day 21 the plate shows extreme irregularities, most noticeably with the appearance of many pyknotic cells in some columns and nuclear debris in some chondrocytes: these could be sequelae of the abnormal cell division reported by Johnson. There is also a complete lack of hypertrophy at this time.

Kinetically the picture is not so clear, and caution should be exercised in interpreting these results because of the subjective factors involved in quantitative histology. The labelling index is variable in normal mice and even more so in stumpy. The size of the proliferation zone remains roughly the same in normal and stumpy mice up to the day 34 mark. The hypertrophic cell height, however, is significantly lower in stumpy than in the normal, until 34 days. It appears, therefore, that of the three parameters contributing to overall bone growth, size of proliferation pool labelling index and hypertrophic cell height, it is the latter two which differ and are responsible for the dwarfing seen in stumpy mice. It is believed that the regulation of growth is controlled through cell division rate and not through changes in the size of the proliferation pool which remains fairly constant during active growth (Walker & Kember, 1972) and this certainly accords with stumpy observations.

The chondrodystrophies have been categorised as quantitative or qualitative (Rimoin, 1975). Stumpy does not fit into either category but shows features of both. It seems likely that the abnormal architecture seen at 10 days, and which progressively worsens, is linked to the qualitatively abnormal cell division previously reported. This quantitatively affects the ordered pattern of column formation necessary for mammalian endochondral ossification. Differentiation also appears defective because the process of maturation and subsequent hypertrophy is lacking. The number of hypertrophic cells produced and their heights are always well below normal values, indicating poor attempts at hypertrophy.

This study has shown that of the many factors involved in determining overall bone growth, in the stumpy mutant it appears that the reduced hypertrophic cell height is of major importance in producing this phenotype, whilst the size of the proliferating cell population and its division rate are of minor importance.

SUMMARY

The proximal growth plates of the tibiae in normal and stumpy mice aged 10–41 days were studied. Autoradiographic studies using tritiated thymidine enabled the size of the proliferating cell population and the labelling indices of the growth plates to be determined. Hypertrophic cell heights were also measured.

From these data the overall growth rates for the proximal growth plate of the tibia in normal and stumpy mice were calculated. It was found that the major factor responsible for the reduced growth rate in stumpy up to 21 days was the small hypertrophic cell height, while cell proliferation zone size and labelling indices were of minor importance. Histological observations also revealed a lack of organised endochondral ossification, which worsens with age.

REFERENCES

Ferguson, J. M., Wallace, M. E. & Johnson, D. R. (1978). A new type of chondrodystrophic mutant in the mouse. *Journal of Medical Genetics* **15**, 128–131.

Johnson, D. R. (1977). Ultrastructural observations on stumpy (stm), a new chondrodystrophic mutant in the mouse. *Journal of Embryology and Experimental Morphology* **39**, 279–284.

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

JOHNSON, D. R. (1978*b*). The effect of a single injection of papain on growth of the limb bones of the mouse. *Growth* **42**, 27–30.

KEMBER, N. F. (1972). Comparative patterns of cell division in epiphyseal cartilage plates in the rat. *Journal of Anatomy* **111**, 137–142.

RIMOIN, D. L. (1975). The chondrodystrophies. *Advances in Human Genetics* **5**, 1–118.

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 Radiobiology* (ed. J. S. Mitchell, B. E. Holmes & C. L. Smith), pp. 436–448. Edinburgh: Oliver & Boyd.

WALKER, K. V. R. & KEMBER, N. F. (1972). Cell kinetics of growth cartilage in the rat tibia. I. Measurements during ageing. *Cell and Tissue Kinetics* **5**, 409–419.