NOTES

A TECHNIQUE FOR MEASURING LINEAR BONE GROWTH DURING SHORT TIME INTERVALS IN CATTLE

A technique is described for meausuring increases in bone length during defined periods before slaughter in cattle. It consists of jugular infusions of oxytetracycline at specified time intervals. Oxytetracycline identifies the sites of ossification in growing bones at the time of infusion by leaving a label which can be seen as a yellow-green fluorescent line under ultraviolet light. By applying a series of labels at known time intervals, the amount and rate of bone growth at any particular site during those time intervals can be determined. In the present experiment the distal epiphyseal growth plate in the radiuses of young cows (five 2-yr-olds, five 3-yr-olds and four 4-yr-olds) were studied. The increase in bone length at that site for the three age groups was found to be 0.0732, 0.0205 and 0.0000 mm/day respectively.

Les auteurs décrivent une technique de mesure de l'accroissement linéaire des os au cours de phase déterminées de la croissance des bovins. On injecte par la jugulaire de l'oxytétracycline à intervalles fixes. Cette substance permet de localiser les sites d'ossification en laissant une marque qui à la lumière ultraviolette apparaît comme une ligne fluorescente jaune-vert. En appliquant ce marqueur en série à des intervalles de temps connus, on peut déterminer le degré et le rythme de croissance aux divers sites. Dans cette expérience, on a étudié la plaque de croissance de l'épiphyse inférieure du radius de jeunes vaches (2, 3 et 4 ans). L'accroissement linéaire à cet endroit était, respectivement, de 0,0732, 0,0205 et 0 mm/jour pour ces tois groupes d'âge.

Bone growth is commonly defined as change in bone weight. In this way, individual bone growth relative to total bone growth has been estimated for cattle by Berg et al. (1978) and Jones et al. (1978a). For some tissues of the body, weight is directly related to the function performed; however, in the case of bones it seems probable that their effectiveness (support and movement) is more closely related to their functional length (Berg and Butterfield 1976) than to their weight. Bone weight may not even accurately reflect the changes that occur in the physical dimensions of the bone (length and circumference) because of changing bone density (Jones et al. 1978b), and medullary cavity contents.

Measurements of bone length taken on the live animal must include a large degree of error, particularly if taken outside the skin, and they are unsuitable when precise measurements are required. Measurements on bones dissected from the carcass can yield accurate estimates on the current bone size but the rate of growth must still be estimated by regression analysis on data from a series of animals. Increases in bone length occur at cartilage plates within the bone. In the method described here, sites of ossification are labelled in vivo so that subsequent increases in bone length can be measured at the time of slaughter.

Fourteen crossbred beef cows were used in this experiment: five 2-yr-olds (mean initial weight 356.2 ± 13.8 (SD)kg), five 3-yr-olds (417.4 ± 40.4 kg) and four 4-yr-olds (472.6 ± 56.4 kg). ''Terramycin'' (Trademark, Pfizer Co. Ltd.), an oxytetracycline solution (100 mg/mL), was used to produce the in vivo label. It was diluted with sterile saline to five times its original volume and administered in a single jugular infusion to provide 15 mg oxytetracycline per kilogram of body weight. This procedure was repeated 5 wk later and the cows were slaughtered after

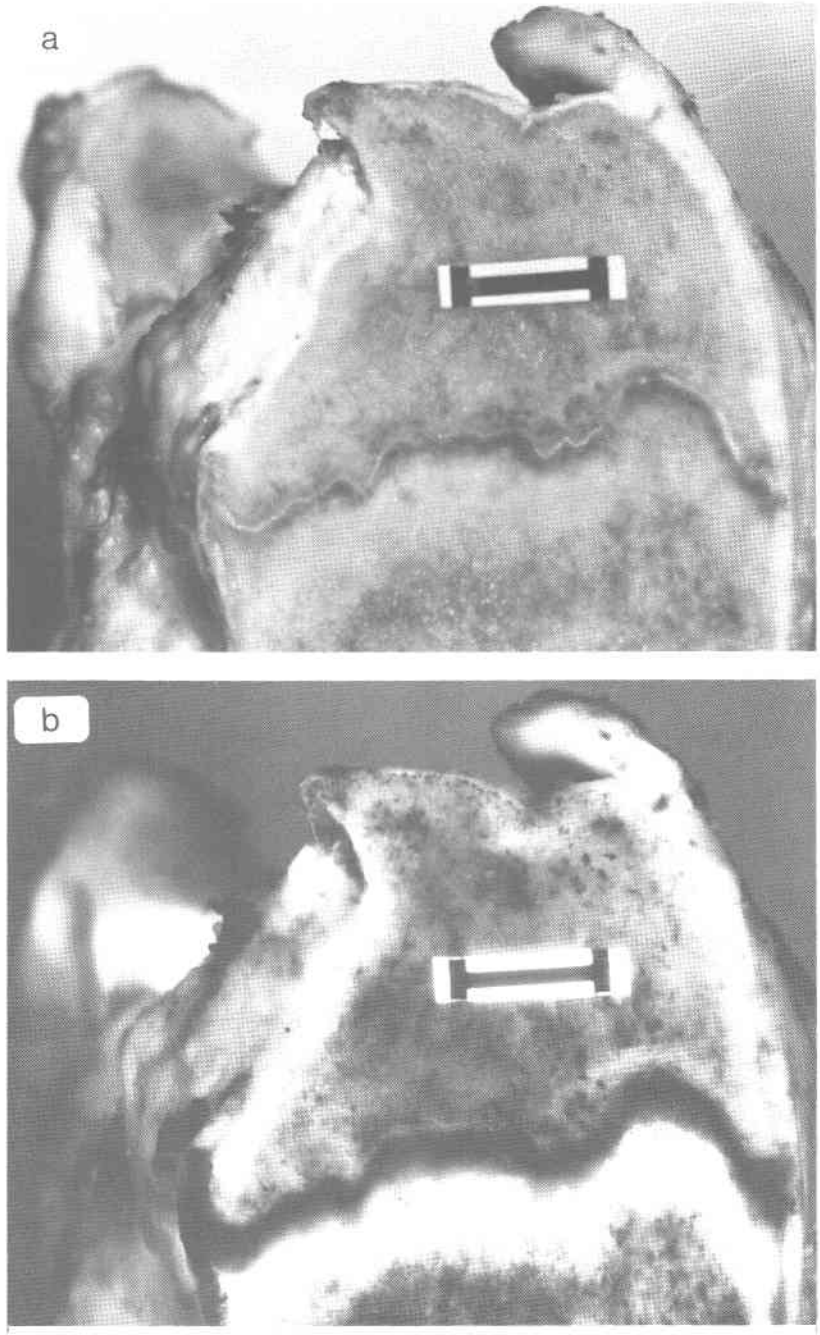
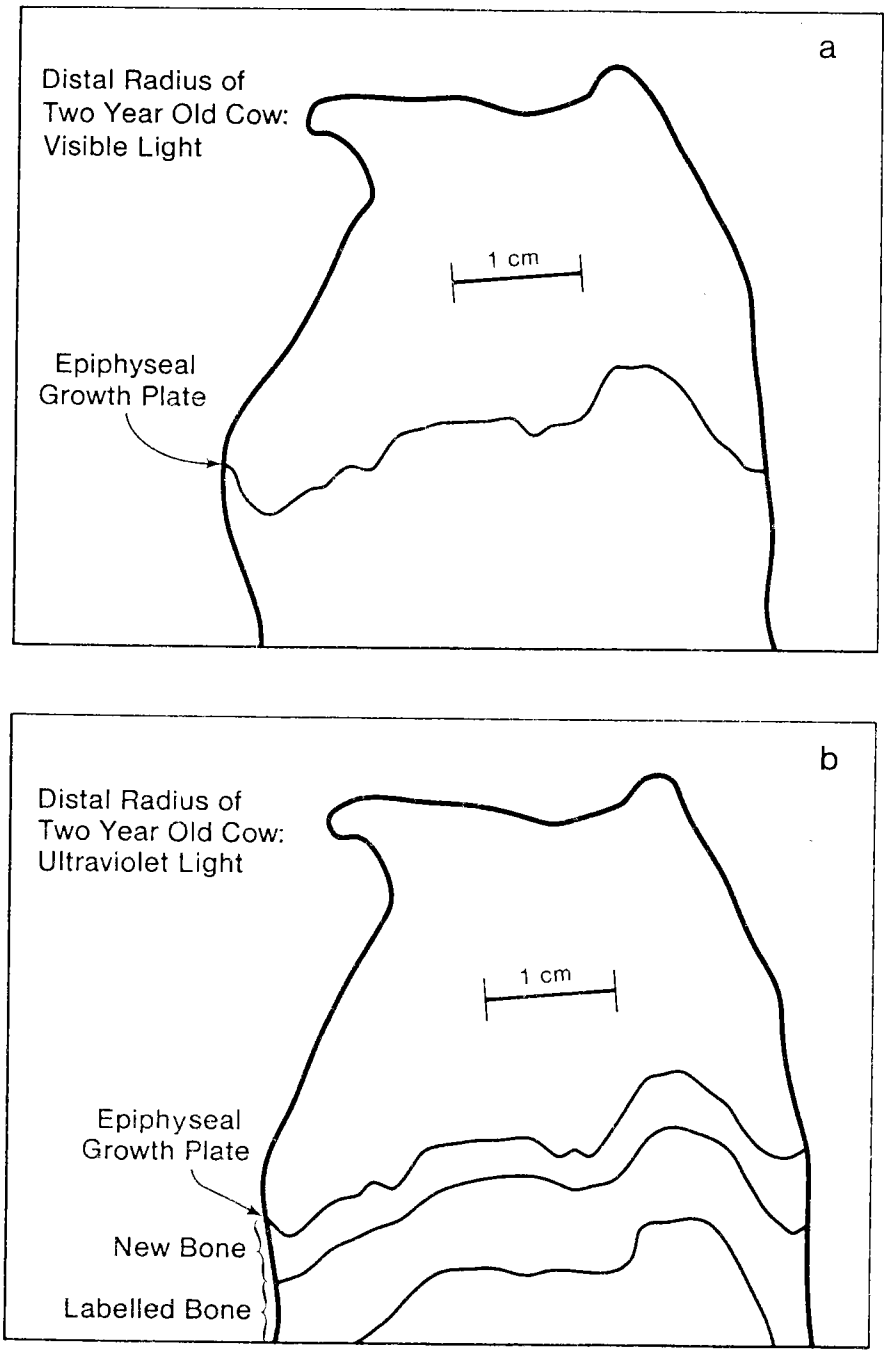


Fig. 1. The appearance of the longitudinally split distal radius (a) under visual light (b) under ultraviolet light.



a further 5 wk. The cows were fed a high grain finishing diet throughout the experimental period.

Following slaughter and overnight chilling of each carcass, the distal radius was removed and longitudinally split. One of the resulting halves was clamped with the cut surface facing upward. A camera and two ultraviolet lamps (366 mm, 115 volts, .14 amps) were mounted above the bone. Each bone was photographed twice: the first exposure, made under visual light, recorded the position of the growth plate within the bone. The second exposure, made under ultraviolet light, recorded the position of the yellow-green fluorescent lines demarcating the zones of active bone formation at the times of labelling. Under ultraviolet light, an ultraviolet blocking filter (to block reflected ultraviolet light) and a medium yellow filter (to block visible blue light emitted by the lamps) were fitted to the camera. All photographs were taken with black and white film (A.S.A. 125), and printed on 20 cm × 25 cm paper to give a linear magnification of about three times actual size.

Examples of these photographs are shown in Fig. 1.

The use of tetracyclines for in vivo labelling of actively ossifying bone has been discussed by Frost (1968). The method has been used to quantify bone growth in length and circumference, particularly in rats (Tapp 1966). In cattle and horses the boundaries between labelled and subsequently formed bone are quite distinct due to the rapid removal of tetracycline from the bloodstream (MacCallum et al. 1972).

Figure 1 shows the appearance of the distal radius of a 2-yr-old cow under normal (1a) and ultraviolet (1b) light. The labelled bone can be seen as a white band in Fig. 1b, having a distinct distal boundary, but no distinct proximal boundary. The distinct distal boundary represents the position of the epiphysial growth plate on the day of labelling. The proximal boundary represents the depth to which the tetracycline penetrated the recently formed bone at that time. Although not apparent from the figure, the fluorescent zone represents two bands of label, separated by 5 wk growth. Only where bone growth was rapid enough, or when the time interval between labels was long enough, would it be possible to obtain distinct separation of the two labels.

Measurements of growth were made by ruler directly on the photographs and standardized with the 1-cm calibration mark. The mean of about three measurements of the thickness of the band was used. The bands measured were: from the proximal edge of the growth plate to the distal edge of the fluorescent area (Fig. 1b), which represented growth in the 5 wk before slaughter and, where possible (see below), from the distal edge of the first fluorescent band to the distal edge of the second fluorescent band, representing the previous 5 wk growth.

No increase in length occurred in the distal radius of the 4-yr-old cows. The absence of a tetracycline band next to the growth plate indicated that closure of the epiphysial growth plate (fusion of epiphysis to diaphysis) was already complete when the tetracycline infusions were made. In the case of the 3-yr-old cows, there was a visible band of new bone between the cartilage growth plate and the second label. The amount of growth in 5 wk, however, was not sufficient to clearly separate the two labels. In the 2-yr-old cows, a more rapid rate of bone growth led to a greater distance between the growth plate and the second label. Furthermore, in three of the five cows in this age group there was sufficient separation of the fluorescent lines to allow a measurement of growth in each 5-wk period.

A measurement between the label and the epiphyseal growth plate may be less accurate than a measurement between two labels because at the growth plate the zone of resorption and ossification is permanently labelled while the zone of calcification (formation of calcified cartilage) is not (MacCallum et al. 1972). With the naked eye it was impossible to identify the different zones within the epiphysial growth plate although the error

introduced here is probably very small.

The growth in length of the distal radius in the 14 cows during the 5-wk period before slaughter was found to be: 2-yr-olds ($n = 5$) 0.0732 ± 0.02680 (SD) mm/day; 3-yr-olds ($n = 5$) 0.0205 ± 0.00979 (SD) mm/day; 4-yr-olds ($n = 4$) 0.0000. In the three cases where both tetracycline labels could be identified, the growth rate in the first 5 wk was not different to the growth rate in the second 5 wk. The results for the three age groups show the expected decline in growth rate with age. According to Sisson and Grossman (1953) closure of the epiphysial cartilage at the distal radius occurs between 3½ and 4 yr of age. This is supported in the present work since closure had occurred in all 4-yr-old cows, but not in the 3-yr-olds.

Labelling bone in vivo with tetracycline is a relatively simple procedure which is capable of recording the increase in length that occurs at each individual growth plate over quite short time intervals, in a single animal. It is similar to the classical staining technique using madder (*Rubia tinctorum*) root (Payton 1932) except that the labelling substance is given in a single infusion rather than including it in the feed. Bone growth can also be measured radiographically after the insertion of metal markers into the shaft (Sissons 1953; Bisgard and Bisgard 1935). This method, however, requires a surgical operation for each bone that is to be measured. Tetracycline, being relatively non-toxic, gives a minimum of interference with the growth of the experimental animal. Under ultraviolet light a tetracycline label was also seen at the periosteum and, in a similar fashion, bone growth at this site could also have been measured. In vivo labelling of bone can provide an alternative approach to the study of relative bone growth which can be used in place of, or in conjunction with, the comparative slaughter techniques commonly used at present.

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BERG, R. T., ANDERSON, B. B. and LIBORIUSSEN, T. 1978. Growth of bovine tissues. 4. Genetic influences on patterns of bone growth and distribution in young bulls. Anim. Prod. **27:** 71-77.

BERG, R. T. and BUTTERFIELD, R. M. 1976. New concepts of cattle growth. Sydney University Press, Sydney.

BISGARD, J. D. and BISGARD, M. E. 1935. Longitudinal growth of long bones. Arch. Surg. (Chicago). **31:** 568-578.

JONES, S. D. M., PRICE, M. A. and BERG, R. T. 1978a. Effects of breed and sex on the relative growth and distribution of bone in cattle. Can. J. Anim. Sci. **58:** 157-165.

JONES, S. D. M., PRICE, M. A. and BERG, R. T. 1978b. The density of bovine limb bones. Can. J. Anim. Sci. **58:** 105-106.

MacCALLUM, F. J., KRAMER, L.L., LEINER, D. J. and HUSKA, G. R. 1972. Prenatal and postnatal tetracycline labelling in equine and bovine ossification. Amer. J. Vet. Res. **33:** 1277-1284.

PAYTON, C. G. 1932. The growth in length of the long bones in the madder-fed pig. J. Anat. **66:** 414-425.

SISSON, S. and GROSSMAN, J. D. 1953. The anatomy of the domestic animals. W. B. Saunders Company, Philadelphia, Pa.

SISSONS, H. A. 1953. Experimental determination of rate of longitudinal bone growth, J. Anat. **87:** 228-236.

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