

# An autoradiographic study of equine hoof growth

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## Introduction

GROWTH in length of the equine hoof wall is usually estimated by marking the hoof wall and measuring the increase in distance of the mark, from the coronary band hair line, over a period of time. It has been estimated that the new horn grows at the rate of about 8 to 10 mm per month, so that nine to twelve months are required for the wall at the toe to grow from the proximal to the distal border (Stump 1967; Schummer, Wilkens, Vollmerhaus and Habermehl 1981). Toe growth rates of the hooves of unshod mature horses, measured weekly for 126 days, ranged from 0.19 to 0.28 mm/day (Glade and Salzman 1985).

The sole and frog are replaced every two months (Schummer *et al* 1981). It has also been stated that the lamellar epidermis of normal hoof does not produce any significant amount of horn (Stump 1967; Sack and Habel 1977). Each dermal lamella terminates in a series of papillae which merge and are continuous with the papillae of the dermis of the sole. The epidermis on and between the terminal papillae continuously produces tubular and intertubular horn thus filling the area between the distal ends of the primary epidermal lamellae (Sack and Habel 1977) and contributes to the formation of the white zone.

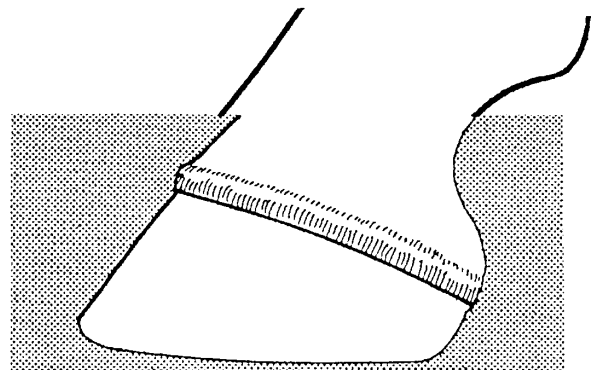
The use of radioisotopes in the measurement of wool growth (Downes, Clarke and Dagg 1967) suggested the possibility of developing an accurate way of measuring growth of the entire equine hoof and of investigating the nature of hoof growth.

## Materials and methods

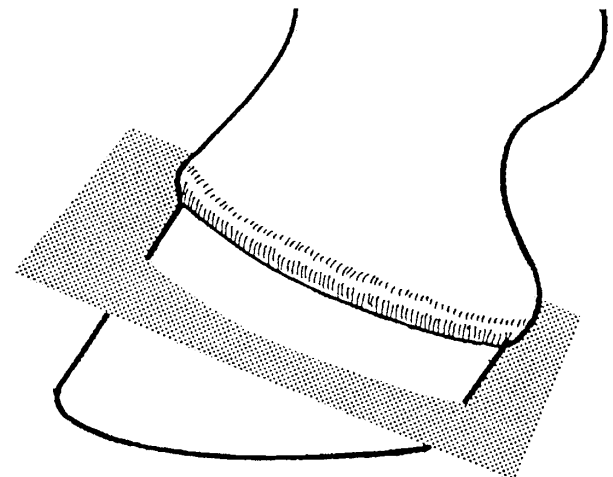
Three 1 ml doses, each containing 0.33 mCi of radioactive L-[<sup>35</sup>S] methionine (Amersham Australia, Sydney), were injected at 14 day intervals into the jugular vein of a 350 kg, clinically normal, five-year-old Australian pony. The half-life of sulphur 35 is 87.2 days and the third injection was given when the total activity had decayed to 0.751. The pony was killed, using intravenous (iv) pentobarbitone sodium, 24 h after the final injection. The digits were amputated through the proximal interphalangeal joint and immediately deep frozen at -70°C, freeze dried for seven days and sectioned with a band saw through the planes shown in Figure 1. Each 10 mm thick section was embedded in polyester resin, (Mawhinney and Ellis 1983) mounted on plate glass using double sided adhesive tape (Cellotape, SM) and disc sanded to a thickness of 1 mm. X-ray film (Kodak Safety Film AR) was placed in close contact with each specimen and after 90 days of exposure the film was developed in an automatic x-ray film processor. Exposure commenced when the total activity of the <sup>35</sup>S methionine had decayed to 0.656 and ended approximately 80 per cent through the total life of the isotope.

## Results

The developed autoradiographs showed good contrast. The keratinised horn which had incorporated the <sup>35</sup>S methionine or its metabolites had turned the film black whereas the old, non-radioactive horn left the film clear. The polyester resin had chemically fogged the film but fortunately in section A (Fig 1) this served to outline the hoof, the hoof corium and the phalangeal and navicular bones.



(a) Plane of sagittal section



(b) Plane of transverse section

Fig 1. Diagrams showing the planes of section used in the autoradiographic study of hoof growth

There were three intensely labelled zones of  $^{35}\text{S}$  uptake, corresponding to the three iv injections. The space between the first and last zone represented 28 days of growth. The first zone was furthest from the coronary band. The three major zones appeared to represent the hard alpha keratin of intertubular horn.

Below the coronary band the growth zones of the hoof wall at the toe were not parallel; they were widest at the internal wall and about 50 per cent narrower at the external wall. Thus autoradiographic predictions of hoof wall growth made on the external surface of the hoof differ from those made on deeper areas of the growth zone. In 28 days the external wall had grown 7 mm (0.25 mm/day) and the internal wall had grown 10 mm (0.36 mm/day). Only the former figure is within the range (0.19 - 0.28 mm/day) of Glade and Salzman (1985). Near the external surface it would take about 280 days for the wall to grow from the proximal to the distal border, in agreement with Stump (1967) and Schummer *et al* (1981). Measurements made on the internal wall, however, suggest a faster rate of hoof wall growth (about 220 days). Also, the internal wall would reach the ground about 28 days earlier than the external wall if the radioactive bands remained in the same configuration during their proximo-distal journey. Alternatively it is possible that the radioactive zones could reach the ground surface in a changed, more compressed 'form' in response to ageing and the forces of weight-bearing and locomotion.

There were five dark bands of radioactivity in the lamellar region (arrowed) of the sagittal section (Fig 2). Because new primary epidermal lamellae are formed from epidermal basal cell proliferation, on the internal part of the coronary groove, the four most proximal lamellar bands probably resulted from obliquely sectioned primary epidermal lamellae. The presence of a similar band, arising from an obliquely sectioned primary epidermal lamella in the mid-wall region (the fifth lamellar band), is surprising. Perhaps the keratinised cells of the primary epidermal lamellae move distally at a far greater rate than hitherto appreciated.

The three curved zones of radioactivity at the distal lamellae, white zone region (Fig 2), merged with the corresponding three zones of the sole and frog. The distance between the first and third of the white zone bands (representing 28 days of growth) was approximately equal to the growth rate of the inner proximal hoof wall. In 28 days the

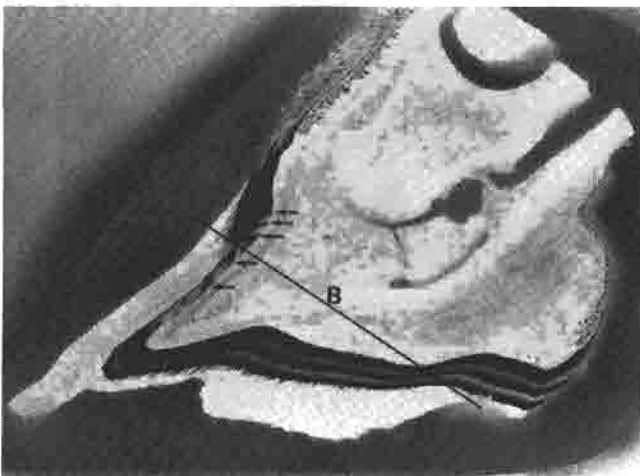


Fig 2. Sagittal autoradiograph: three intensely labelled zones of  $^{35}\text{S}$  uptake correspond to three iv injections of radioactive L- $^{35}\text{S}$  methionine. Five dark bands of radioactivity in the lamellar region are arrowed. The line B shows the plane of the transverse section in Figure 3

sole and frog had grown over half their total thickness (0.19 mm/day and 0.32 mm/day respectively), thus confirming the statement of Schummer *et al* (1981) that the sole and frog are replaced every two months. The pattern of growth of the sole, frog and heel was not uniform (Fig 2). The bands of radioactivity diverged at the apex of the frog and at the heel suggesting that these areas either grow more rapidly or less obliquely than the convergent narrow zones.

Three layers of radioactive horn tubules penetrated the hoof of the wall, sole and frog (Fig 2) ahead of the corresponding major zones of  $^{35}\text{S}$  uptake. They were oriented parallel to the dorsal surface of the distal phalanx. The horn tubules were longest at the hoof wall and sole and shortest in the terminal papillae region and frog. Tubules were very short or non-existent in the heel region. Gaps in the third zone of radioactivity represent the spaces occupied by dermal papillae. They were less conspicuous in the first and second zones.

The skin of the pastern was represented as a single fine line which merged with the third, most recent zone of radioactivity at the periople of the wall and heel. There was no autoradiographic evidence of the first two injections of  $^{35}\text{S}$  in the skin, probably because of constant epidermal desquamation. Autoradiographs of hair follicles and hair shafts were present on either side of the skin epidermis.

The autoradiograph of the transverse section (Figs 1b and 3) showed the three major radioactive zones only where the sole and frog are sectioned obliquely. Small clear spots within the dark major zones represent the areas occupied by dermal papillae (Fig 3). The small dark dots adjoining the dark major zones are autoradiographs of the distal tips of horn tubules in cross section (Fig 3).

The dark zone in the hoof wall of the toe is the autoradiograph made by the  $^{35}\text{S}$  methionine of the first injection (Figs 1, 3 and 4). The intertubular horn is the darkest region. The dark circles and dots in the outer hoof wall are the autoradiographs of horn tubules in cross section (Figs 3 and 4). Small clear spots within the dark zone and the larger dark circles represent the areas occupied by dermal papillae.

All of the primary epidermal lamellae had uniformly taken

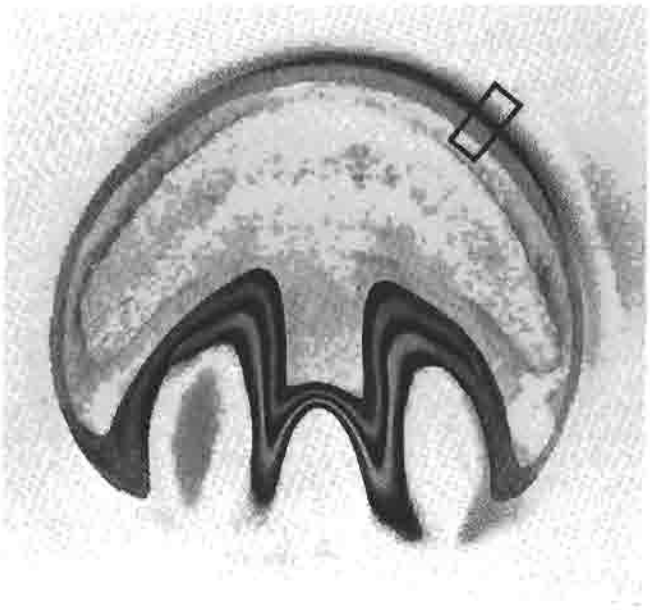


Fig 3. Transverse autoradiograph: only the obliquely sectioned sole and frog show the three intensely labelled zones of radioactivity. The dark zone in the hoof wall of the toe was made by the  $^{35}\text{S}$  methionine of the first injection. All of the primary epidermal lamellae are shown autoradiographically. The area shown in Figure 4 is boxed

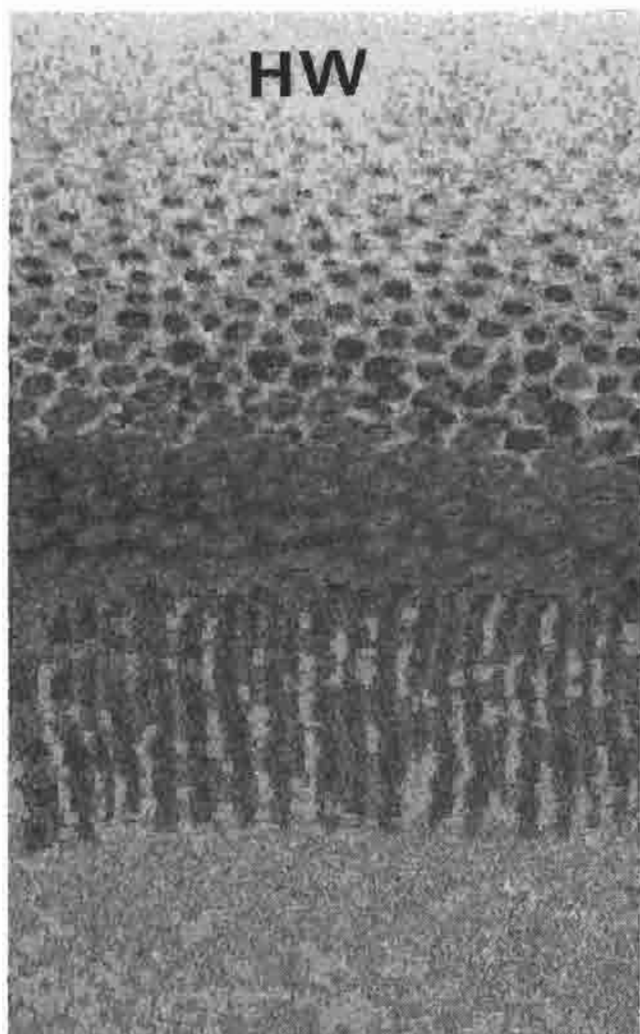


Fig 4: Enlargement of the area boxed in Figure 3. Small clear spots within the darkest radioactive zone represent the areas occupied by dermal papillae. The dark circles and dots in the hoof wall (HW) are autoradiographs of the tips of horn tubules in cross section

up  $^{35}\text{S}$  and were shown autoradiographically (Figs 3 and 4). Even the lamellae adjacent to hoof wall showing no  $^{35}\text{S}$  uptake were radioactive suggesting that the lamellar epidermis either had a continuous requirement for methionine or had grown distally at a greater rate than the labelled inner hoof wall. The primary epidermal lamellae at the toe were longer than the lamellae at the heels (2.8 mm and 1.5 mm respectively); a similar finding to that of Linford (1987) who used histomorphometry to compare the lengths of primary lamellae at toe and heels.

## Discussion

If the incorporation of  $^{35}\text{S}$  into epidermal cells is a marker for cell division and growth, then finding autoradiographs of the lamellar epidermis contradicts the statement that the lamellar epidermis does not produce any significant amount of horn (Sack and Habel 1977). Germinal cell activity of the secondary epidermal laminae may add cornifying cells to the sides and edges of primary epidermal laminae at a rate sufficient to keep up with the downgrowth of the hoof wall (Trautman and Fiebiger 1949). Perhaps the epidermal lamellar cells metabolise sulphur for reasons other than growth and cell division. Continuous detachment and reattachment between the cells of the primary and secondary epidermal lamellae as they move past one another could be one such metabolic process (Leach and Oliphant 1983).

This report introduces an autoradiographic method of studying the growth of entire equine hoof sections. The advantage is that accurate measurements can be made of changes in the rate of growth before and after treatment such as dietary supplementation with biotin or the development of laminitis.

The differential rates of growth of the regions within the hoof can be compared because each dose of radioactivity establishes a simultaneous time mark accurate to within  $\pm 2$  h (Downes *et al* 1967). The method is non-invasive and does not require serial biopsies. The amount of radioactivity needed is so small that radiation effects are unlikely. The great sensitivity of the method is gauged from the fact that, in addition to the hooves, each hair of the skin was individually labelled (Fig 2).

For longer term experiments such as labelling the entire growth cycle of the hoof wall,  $^{14}\text{C}$  methionine may be used. Because carbon 14 has a half-life of more than 5,000 years a 'permanent' time mark could be placed on the hooves and the autoradiography performed any time thereafter.

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