STUDIES ON THE GROWTH AND COMPOSITIONAL DEVELOPMENT OF ANTLERS IN RED DEER (*CERVUS ELAPHUS*)

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Paul David Muir

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P.D. Muir

The experiments described in this thesis investigated nutritional and physiological aspects of antler growth in red deer stags. The initial experiment (Section 3) examined the effects of winter nutrition on subsequent antler casting date and velvet antler weight. Mature stags on two different farm types (hill country, Farm H and irrigated lowland, Farm L) were offered three levels of winter nutrition, two levels of a concentrate supplement (ad libitum pellets and 1/2 ad libitum pellets) and a basal hay ration. On both properties liveweight gains occurred in supplemented groups and liveweight losses in unsupplemented groups. At antler casting there were significant differences in liveweight of approximately 10 kg between fully supplemented and unsupplemented groups. Realimentation of winter liveweight losses subsequently occurred so that by the following rut the effects of winter undernutrition had been eliminated. On Farm H poor winter nutrition (hay only) resulted in a significant delay in casting date (13 days) and lower velvet antler yields (0.24 kg), than in stags offered the ad libitum concentrate ration. Stags on Farm H were 13 kg lighter at commencement of the trial than at Farm L and the differences in treatment effects obtained between farms may have been due to differences in body condition at commencement of the trial.

An association was demonstrated between liveweight and date of antler casting, with heavier stags casting earlier than lighter stags. There was no effect of age of stag on casting date. Of the liveweights recorded, liveweight prior to the rut showed the best relationship with casting date, possibly because the seasonal nature of liveweight change meant that a weight recorded at this time gave the best indicator of the true frame size of a stag. Both age and liveweight significantly

affected velvet antler weight, with increases of velvet antler weight of 0.26 kg between 3 and 4-year-old stags and of 0.30 kg between 4 and 5-year-old stags at the same liveweight. Within an age group velvet weight increased by 0.12 kg for each 10 kg increase in pre-rut liveweight.

The experiments described in Section 4 comprised studies on antler growth and composition. In order to obtain data on antler growth and composition individual antlers were removed sequentially from mature red deer stags between 28 and 112 days after casting of hard antlers. Contralateral antlers were removed after stripping of velvet. Wide variation occurred in antler casting date (53 days) compared to date of velvet stripping (24 days). The duration of the period of antler growth may therefore be governed more by date of casting than by date of velvet stripping. Mean duration of the antler growth period was 164 days. Growth in length of the antler appeared to follow a sigmoid curve. However, between 28 and 112 days after casting, rates of elongation were close to linear. Mean length of hard stripped antlers was 0.71 m and between 28 to 112 days after casting mean rate of antler elongation was 0.62 cm/day. Over this period indivdual antlers increased in fresh weight at a rate of 13.7 g/d, with heaviest weight recorded 112 days after antler casting, at approximately 130% of final hard antler weight. Between 28 and 91 days of growth, volume of blood in the antler increased linearly at a rate of 194 ml/kg. Three phases of mineralization were demonstrated in developing antlers. Tips of growing antlers were cartilaginous and poorly mineralized. A zone of mineralization occurred 5.0 to 7.5 cm behind the antler tip which corresponded histologically to the transition from mineralized cartilage to trabecular bone. The second phase of mineralization occurred through continued accretion of trabecular bone in the antler shaft. The third phase, described as "terminal mineralization" in this study, appeared to be associated with a rapid increase in density of cortical bone in the periphery of the antler shaft. Terminal mineralization (between 91 and 112 days after casting of hard antlers) coincided with the slowing of growth in length, a decrease in relative blood volume in the antler and an increase in levels of plasma testosterone. These events occurred close to the summer solstice.

At velvet stripping individual antlers had a mean weight of 1.12 kg and contained 81.1% dry matter (DM). Fat free organic matter (FFOM) and ash concentration in DM were 36.6 and 60.0%, respectively. Peak daily rates of FFOM and ash deposition occurred between 91 days and 112 days after casting, at rates of 1.4% of hard antler FFOM and 1.6% of hard antler ash. For a stag producing 2.24 kg of hard antler mean rates of FFOM and ash deposition over this period were 9.3 and 18.3 g/d, respectively. On a whole antler basis calcium concentration in antler ash remained constant, at around 35%. Therefore peak rate of antler calcium deposition would be 6.4 g/d.

In the final experiment (Section 5) mature stags were offered a maintenance ration of greenfeed oats during the period of peak calcium requirement for antler growth and the kinetics of calcium metabolism were examined using a radio-isotope (^{45}Ca). Rates of faecal endogenous loss were low and at approximately 6.4 mg/kg BW per were half the estimated requirements of ARC (1980) for sheep and cattle. Availability of calcium from greenfeed oats was low (mean, 37%) and less than 30% of total calcium requirements were derived from the diet. Poorly mineralized skeletal bones indicated that the shortfall in antler calcium was derived from the skeleton. In spite of a severely negative calcium balance stags were capable of maintaining high and apparently normal rates of antler calcium deposition (mean, 44 mg/kg BW per day). Antlers appear to be acting as a sink with calcium being irreversibly deposited in the antler and lost to the animal's body. On the assumption therefore that antler calcium behaves like calcium lost during lactation a kinetic model of calcium metabolism in the stag was developed.

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PART B

SECTION 1

Introduction

Red deer (Cervus elaphus) have been farmed in New Zealand since 1969 (Coop and Lamming, 1976), producing high per hectare returns from antlers, venison and sale of live animals. Antlers are frequently removed during the annual growth phase for use in traditional Asian medicine (Luick, 1983). Developing antlers are covered with sparse and soft epidermal hairs and because of this appearance are referred to as "velvet antlers". Removal, grading for sale and subsequent marketing of velvet antlers is subjective, with highest returns obtained for antlers supposedly with maximum blood content and minimum calcification, at the heaviest possible weight. Profitability of velvet antler production is therefore influenced by both antler quality, and the weight of velvet antler harvested per stag.

Until recently deer have been available for study in limited numbers only. Consequently our knowledge of the factors which affect antler growth, and of the compositional development of antler tissue, is fragmentory. One of the initial objectives of this thesis was to investigate the effects of winter nutrition on antler growth. Groups of stags in two different farm environments were offered three levels of nutrition during the winter prior to antler growth. The effects of winter nutrition on date of antler casting and velvet antler yield are described in Section 3. The study which followed (Section 4) examined nutrition during the period of antler growth. A control diet was compared with both low calcium and high protein rations. However a large part of this study was concerned with a quantitative description of the pattern of antler growth, blood content and mineralization which was achieved by sequential antler removal. The results of Section 4 allowed calculation of the calcium requirements for antler growth. This was taken a step further and Section 5 describes calcium metabolism in stags offered low calcium diets during the period of peak calcium requirement for antler growth. The data from this experiment have provided estimates of the requirement for calcium in red deer.

SECTION 2

Literature review

Introduction

Antlers are bony structures carried by most members of the deer family (Cervidae) and are distinctly different from horns. Of the 41 species of deer in 17 genera, all bear antlers except musk deer (Moschus moschiferus) and Chinese water deer (Hydropotes inerma), (Whitehead, 1972). Antlers are borne only by the males with the exception of the reindeer (Rangifer tarandus) and in rare instances other species (Donaldson and Doutt, 1965). Antler size between species ranges from the 2 to 3 cm spike antlers of the South American pudu (Pudu pudu) to the palmate antlers of the extinct Irish elk (Megaceros giganteus), which had a span of up to 3.3 m (Savage, 1966).

The annual cycle of antler growth consists of rapid growth and differentiation of vascular tissue which is subsequently mineralized to hard bone within a few months. Antlers are carried until separation of the antler-pedicle junction results in casting and growth of new antlers.

Antler growth and development

1) Pedicles

Two bony pedicles are the sites of antler development and are extensions of the frontal bone of the skull. Pedicle tissue is influenced by androgens and is essential for antler growth (Goss, Severinghaus and Free, 1964). Although pedicles first become apparent as conspicuous swellings following sexual differentiation of the male foetus between 75 and 100 days of gestational age (Lincoln, 1973), they remain dormant until the onset of puberty. Castration of the pre-pubertal stag inhibits pedicle development (Wislocki, Aub and Waldo, 1947) but subsequent pedicle development may be induced in castrated stags by testosterone administration. Traumatisation of pedicle rudiments may result in antler growth (Jaczewski and Kryzywinska, 1974). In females ovariectomy followed by testosterone administration can also induce the development of pedicles and, as in castrates, traumatisation of pedicles may result in antler growth (Jaczewski, 1983). These studies suggest

that wound healing is an important feature of antler initiation (Goss, 1983).

The first antler produced after puberty is normally unbranched and referred to as a "spike antler". In this antler, growth of the pedicle and antler is continuous with no clear definition between the pedicle and antler tissue. With subsequent antlers the zone between antler tissue and pedicle is marked by the antler burr or coronet (Fig. 2.1a).

2) Antler Casting

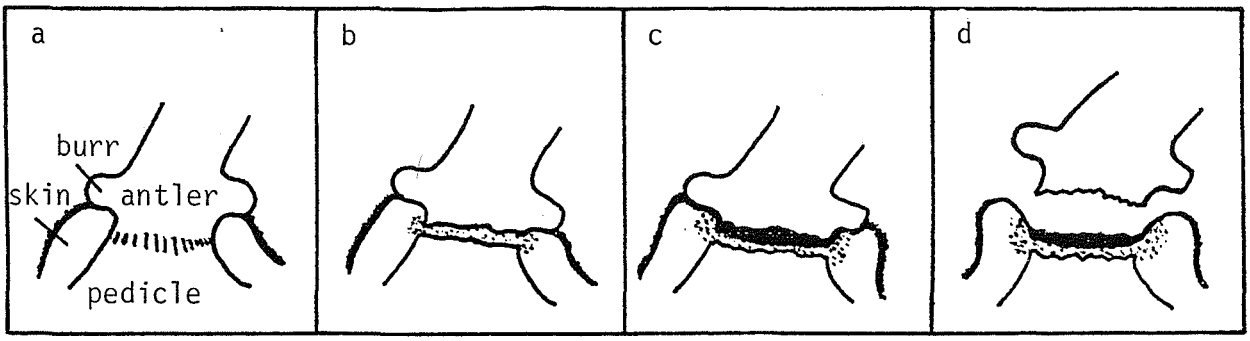
Antler casting occurs along the interface between the dead woven bone of the antler and the living trabecular bone of the pedicle (Fig. 2.1a). Resorption of bony connections between antler and pedicle was considered by Waldo and Wislocki (1951) to commence two weeks prior to casting (Fig. 2.1b). Wislocki and Waldo (1953) subsequently proposed that as bone is resorbed it is replaced by vascular fibrocellular connective tissue. Invagination of pedicle skin along the antler-pedicle junction may assist the casting process (Fig. 2.1c and d).

3) Antler morphology

a) Early development

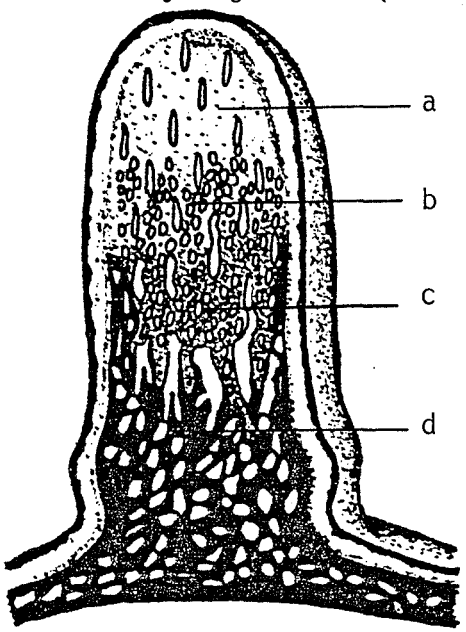
Antler growth commences with proliferation of fibrocellular tissue and invagination of pedicle skin into the concavity of the casting site. Pedicle skin subsequently forms the integument covering the antler (Wislocki, 1942) and reserve mesenchyme beneath the dermis of the developing antler tip differentiates to form chondroblasts (Banks, 1974). Goss (1983) has shown that in sika deer (Cervus nippon) cartilage in the antler tip is arranged in columns, interspersed vertically with vascular channels. In this respect antler cartilage differs from normal cartilage which is avascular (Ham, 1969). However Frasier, Banks and Newbrey (1975) have demonstrated that in white-tailed deer (Odocoileus virginianus) antler cartilage is histochemically similar to cartilage in the cartilaginous plates of long bones. Antler growth continues through differentiation in the distal regions while chondrocytes in the proximal region of the antler hypertrophy as the cartilaginous matrix is calcified (Sayegh, Solomon and Davis, 1974; Newbrey and Banks, 1975). Alkaline phosphatase is an enzyme associated with calcification and may be synthesized by osteoblasts and hypertrophied cartilage (Bourne, 1972).

Figure 2.1
Stages in the process of antler casting.
(from Wislocki and Waldo, 1953)



a) dissolution of the bony connections between antler and pedicle
b) replacement of the bony connections between antler and pedicle by connective tissue
c) growth of pedicle skin, contributing to antler loosening
d) casting of the antler from the pedicle

Figure 2.2
Morphological development of a young antler (from Banks, 1974)



a) Proliferative zone of reserve mesenchyme
b) Cartilaginous zone
c) Primary spongiosa
d) Secondary spongiosa

This enzyme also appears to be produced in the antler and as a result plasma levels of the enzyme are high during antler growth (Graham et al., 1962). Kuhlman, Rainey and O'Neill (1963) further demonstrated that alkaline phosphatase was present in high concentrations in the preosseus zone. Bourne (1972) has suggested that a role of alkaline phosphatase may be in assisting the precipitation of bone salts.

b) Ossification

Antler ossification has been described histologically in white-tailed deer by Banks (1974) and in a number of deer species by Banks and Newbrey (1983) and is illustrated diagrammatically in Fig. 2.2. Calcified cartilage is replaced by woven bone (primary spongiosa), through the action of cartilage eroding cells (chondroclasts) and bone forming cells (osteoblasts) on the surface of the cartilaginous trabeculae. Woven and lamellar bone is deposited peripherally as a sleeve of periosteal bone at the interface between calcified cartilage and the dermis. These authors considered that secondary spongiosa is formed by total replacement of the primary spongiosa (calcified cartilage and woven bone) by lamellar bone.

The pattern of antler development is therefore of growth at the antler tip, and at the tips of the tines. Antler mineralization initially occurs through endochondral bone formation. Periosteal sleeve bone in the periphery of the antler shaft is formed through intramembranous bone formation (Banks, 1974; Banks and Newbrey, 1983).

c) Velvet

The developing antler is enclosed within a sheath, referred to as velvet because of its covering of short, soft, pigmented hairs. These are formed de novo in the antler tip and are not associated with erector muscles (Billingham, Mangold and Silvers, 1959). Sebaceous glands are associated with hair follicles (Goss, 1983) and although sweat glands have been demonstrated in the antlers of red deer, fallow (Dama dama) and roe deer (Capreolus capreolus) they have not been found in the antler velvet of white-tailed deer (Chapman, 1975). Pigmentation of antler velvet results from melanocytes in its basal layer of dermal tissue. Beneath the epidermis and dermis of the velvet is a vascular collagenous tunic (Wislocki, 1942). Underlying the vascular layer is a layer of periosteal fibrocellular tissue which binds velvet tissue to developing

antler bone. Death of velvet antler tissue is associated with antler ossification and the velvet tissue strips off to expose hard mineralized antlers.

d) Blood supply

Antlers are supplied with blood from two branches of the superficial temporal artery (Waldo, Wislocki and Fawcett, 1944). These branch and lesser arteries ascend the antler in the subdermal connective tissue of the velvet. Blood supply to the developing antler is via arterioles through the antler shaft (Rhumbler, 1929,1931; cited Goss, 1983) and from arterial blood passing from the antler tip down to medullary sinuses in the cartilage. Initially venous drainage is internal via the antler core to the pedicle but Waldo, Wislocki and Fawcett (1944) considered that following ossification of the antler base venous blood passes out radially to large veins in the vascular layer of the velvet. These authors also suggested that velvet stripping occurs as a consequence of ossification of the peripheral zone of the antler shaft, which restricts venous return from the antler.

e) Innervation

Nerve supply to the antlers is via the infratrochlear and zygomaticotemporal branches of the trigeminal nerve (Adams, 1979). These branch and ascend the antler along with the major arteries in the vascular layer of the velvet and innervate the antlers to the tips (Wislocki and Singer, 1946). No specialised nerve endings have been demonstrated in velvet antlers. Nerve supply is not essential to antler growth since antlers, albeit malformed through damage, occurred despite antler denervation (Wislocki and Singer, 1946).

4) Sequence of antler development

Antler growth is initiated in most species with casting of the dead, fully mineralized antlers in late winter. In deer of the genus Odocoileus, antlers may be cast in early winter, but antler growth is not initiated until spring (Goss, 1983). Antler growth in length, as a function of time, follows a sigmoid curve (Fennessy, 1982; Van Ballenberghe, 1983) with growth being slow initially. During the phase of rapid growth, antler elongation rate is close to linear (Jacobsen and Griffin, 1983), and Goss (1970) cites growth rates of up to 27 mm per day

in antlers from large wapiti (Cervus canadensis). In the multi-branched antler in adult red deer (Fig. 2.3), brow tine development occurs approximately 16 days after hard antler casting, and bey and trey tine development after approximately 30 and 44 days of growth, respectively (Corson and Fennessy, 1985). There are few accurate accounts of the duration of the antler growth period. Bergerud (1976) recorded 20.9 weeks and 23.9 weeks as the period from casting to velvet stripping for the third and fourth set of antlers of a captive reindeer. There appear to be no such data on red deer.

In red deer in New zealand amputation of actively growing antlers is carried out commercially between 60 and 70 days of growth, at the stage of bulbing of the antler shaft, prior to formation of royal tines (Fig. 2.4). These velvet antlers are processed and sold for use in traditional Asian medicine (Luick, 1983).

5) Antler composition

Kay et al. (1982) described ash deposition in the antler shaft of velvet antlers of unknown age. According to that study a zone of mineralization occurred in a discrete band 2 to 4 cm behind the antler tip. Mineralization increased down the antler shaft. Brown, Cowan and Griel, (1978) used radiograph densitometry of whole antlers to describe the increase in relative bone mass during antler growth. However as yet no quantitative study of the composition of developing antlers has been reported.

Ash of fully mineralized, cast antlers is similar in mineral composition to that of skeletal bone. Antlers from 2-year-old to 4-year-old red deer had specific gravities ranging from 0.98 to 1.38 (Hyvarinen, Kay and Hamilton, 1977), and these authors suggested that antler ash concentration increased with increasing age of stags and improved nutrition. Bernard (1963) demonstrated a similar range of specific gravities for hard antlers from eight other deer species, and suggested that differences in specific gravity were due to differences in relative area of compact and spongy bone, since spongy bone was found to contain 10% less ash than compact bone. Calcium and phosphorus concentrations in antler ash had mean values of 38.4% and 17.9% respectively, which represents a calcium to phosphorus ratio of 2.1:1 (Hyvarinen, Kay and Hamilton, 1977). These workers calculated that total

Figure 2.3
Antler structure in red deer.

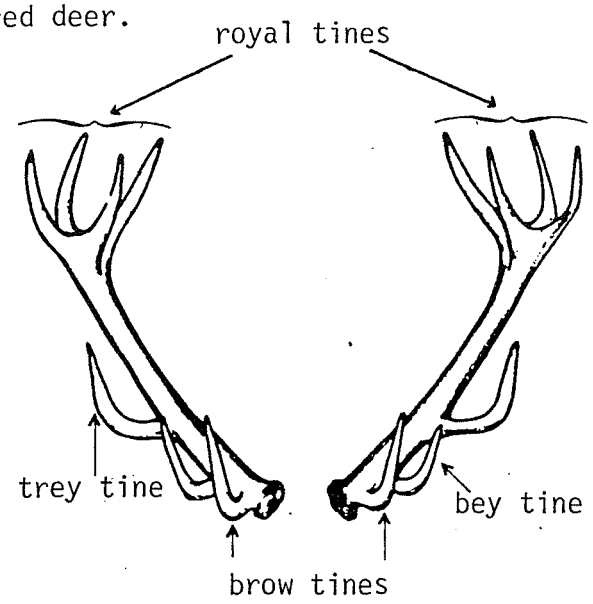


Figure 2.4
Red stag in "velvet antler" suitable for removal and commercial sale

calcium and phosphorus requirements for the production of 2 kg of hard antler would be similar to the total lactation requirements of a red deer hind. However, because no quantitative data have been available on the pattern of antler mineralisation it has not been possible to derive daily mineral requirements for antler growth.

a) Sources of minerals for growth of antler bone

Despite high mineral requirements for antler bone formation there is evidence to suggest that plasma calcium and phosphorus concentrations remain stable during antler growth (Graham et al., 1962; Morris and Bubenik, 1983). Histological studies by Meister (1956) demonstrated osteoporosis of bone mineral in skeletal long bones during antler growth, suggesting a net loss of skeletal bone during this period. Rerabek and Bubenik (1963) however, were sceptical about these findings and from studies with phosphorus, considered that phosphorus could not be stored within the body, and that minerals for antler growth were derived from the diet. Subsequent histological work (Banks et al., 1968a; Banks and Davis, 1966) together with chemical analyses (Banks et al., 1968b; Ullrey et al., 1973) has confirmed that skeletal osteoporosis may occur during antler growth, particularly on low calcium diets (Ullrey et al., 1973). Skeletal resorption during antler growth was observed to be proportionally greater in ribs than in long bones (Hillman, Davis and Abeldaki, 1973). Cowan, Hartsook and Whelan (1968) labelled skeletal calcium by injection of radioactive calcium into white-tailed bucks prior to antler growth. Antlers between 10 to 12 cm in length were removed and found to contain the injected radio-isotope. These authors concluded that some of the skeletal calcium was mobilised and used for antler mineralization. However it has not been clearly established whether skeletal mobilisation of calcium is due to the process of antler growth, or whether it is a seasonal phenomenon which occurs regardless of calcium supply.

Factors affecting antler growth

1) Age

Pedicle diameter increases annually through deposition of concentric rings of bone (Banfield, 1960) and date of hard antler casting advances with increasing age of the stag (Behrend and McDowell,

1967; Jacobson and Griffin, 1983). There is also evidence to suggest earlier velvet stripping in older stags (Bergerud, 1976). Huxley (1926) demonstrated that cast antlers of red deer stags increased in size and weight with increasing age of stags, reaching peak size at the tenth set (i.e. in 10-year-old stags). Wolfe (1983) produced similar data from a population of wapiti deer, with heaviest and longest antlers also being obtained from 10-year-old bulls.

2) Nutrition

a) Pedicles

Pedicle development may be influenced by plane of nutrition with restricted feeding in yearling stags delaying development of pedicles and the first set of antlers (Long et al., 1959; Suttie and Kay, 1983). In some stags (hummels) pedicles remain rudimentary indefinitely, possibly as a result of extremely impoverished nutrition (Lincoln and Fletcher, 1976). Such stags are often fertile as adults (Lincoln, Guinness and Fletcher, 1973), suggesting that pedicle tissue may only be sensitive to the androgen environment during the stag's first year of life (Lincoln and Fletcher, 1976). The work of Suttie, Lincoln and Kay (1984) suggests that undernutrition may delay the testosterone surge which is necessary for pedicle development. If puberty is delayed for too long primordial pedicle tissue may no longer be responsive to androgens, and, as a consequence, pedicle and antler development may not occur.

b) Casting

In red deer antler casting may be delayed in stags of poor body condition following a severe winter (Watson, 1971). In contrast white-tailed deer have a different pattern of antler casting, with antlers frequently being cast in early winter (Goss, 1983), but antler growth remains dormant until spring. Date of antler casting in white-tailed bucks was advanced by winter feed restrictions (Long et al., 1959) suggesting that nutrition may have a different effect on antler casting in this species than in red deer. However this conflicts with field observations of white-tailed deer where bucks in poor condition have had delayed antler casting (Einarsen, 1956; French et al., 1956).

c) Antler growth

Numerous authors refer to the role of nutrition in antler development but much of the literature is anecdotal. Large, multi-branched antlers have been noted in yearling red stags (Huxley, 1926; J. Henshaw, pers. comm., 1981) and attributed to improved nutrition. Whitehead (1950) cited Vogt (1936) as having obtained spectacular increases in cast antler weight from 1.6 kg to 6.0 kg from the best of his 3-year-old stags over a 6 year period. This was attributed to the feeding of sesame cake, a feed with high protein and calcium content. It is not clear whether the overall level of nutrition was constant between animals or between years. Therefore between animal or between year feeding effects, or some other environmental effect may have confounded these results. Baihle-Grohman (1896) recorded larger antlers on properties with soils of limestone parent material and Huxley (1926) described a decrease in antler size after lime application to pastures was discontinued. It is not clear whether lime was affecting antler growth directly or whether pasture growth and therefore general level of nutrition decreased with cessation of liming.

Further evidence suggesting that improved nutrition may increase antler weight was produced by Huxley (1931) who found that a positive relationship existed between liveweight and antler weight. However effects of age, sire and body condition could have confounded this result. Hyvarinen, Kay and Hamilton (1977) obtained a linear relationship between peak summer liveweight and hard antler weight within an age group. Subsequently Fennessy (1982) also demonstrated a relationship between liveweight and velvet antler weight, with a 10 kg increase in late summer liveweight associated with an increase of 92 g in velvet antlers removed after 60 days of growth.

Variation in level of nutrition during the year may affect antler production. Severinghaus et al. (1950) and Bergerud (1976) suggested that poor winter nutrition may impair antler growth. Long et al. (1959) concluded there was no adverse effect of winter feed restriction provided stags were well fed during the period of antler growth. However Ullrey (1983) found that protein and energy supplementation prior to casting led to increased antler weights. During this period, antler growth may be adversely affected by severe dietary restrictions of energy and protein (French et al., 1956; Ullrey, 1983), Unfortunately these authors based

their conclusions on treatment groups consisting of only one or two animals. There is also evidence which suggests that composition of the diet may affect antler composition. Low dietary calcium resulted in antlers of lower specific gravity and ash content without apparent alteration in the calcium or phosphorus concentration of antler ash (Ullrey, 1983).

3) Photoperiod

In temperate zones antler growth in most deer species is seasonal, with hard antler casting occuring in late winter or early spring and full mineralization and velvet stripping in late summer. Antler cycles are asynchronous in equatorial regions, where seasonal changes in daily photoperiod are small. Goss (1983) suggested that the transition from asynchrony in the tropics to synchronous antler cycles in temperate regions occurs between 10° and 17°N. Transportation of stags from the Nothern hemisphere to New Zealand resulted in a complete shift in the timing of antler growth to match the Southern hemisphere seasons (Donne, 1924). Jaczewski (1954) demonstrated the first clear link between changes in daily photoperiod and the antler cycle, with antler casting and subsequent growth occurring with increasing daily photoperiod, and mineralization and stripping with decreasing daily photoperiod. Goss (1969a) subsequently demonstrated that acceleration of antler cycles can be induced by increasing the freqency of annual daylight cycles with up to four complete antler growth cycles being achieved in 12 months. Changes in food intake, behaviour and plasma hormone concentrations (testosterone and prolactin) of red deer stags were also fully entrained to a 6-monthly photoperiod cycle (Brown et al., 1979).

On constant short (8h) or long (16h) days, antler growth underwent normal cycles, but at irregular intervals (Goss, 1969b). In later studies Goss (1976) showed that antler cycles could be triggered by either increasing or decreasing daily photoperiod and further suggested that it is the seasonal alteration in daily photoperiod which primarily controls antler cycles, rather than the direction of photoperiodic change.

4) Hormones

Antlers are secondary sexual characteristics and as such it is probably not surprising that thay are primarily affected by fluctuations in the secretion of gonadal hormones.

a) Androgens

Testosterone is required for pedicle development prior to initiation of first antlers since castration of pre-pubertal stags will inhibit pedicle development (Wislocki, Aub and Waldo, 1947). However, in post-pubertal stags antler casting and initiation of antler growth occurs at a time when the testes are quiescent (Wislocki, 1949). Casting of hard antlers follows castration, and is prevented by testosterone administration (Wislocki, Aub and Waldo, 1947). This indicates that testicular hormones are instrumental in preventing antler casting and the subsequent initiation of antler growth. Antler casting occurred in normal male black-tailed deer (Odocoileus hemionus columbianus) when serum testosterone concentrations decreased to less than 1 ng/ml (West and Nordan, 1976a). Testicular testosterone is not essential for antler growth since castrate stags will develop antlers. However, Lincoln (1971) and Bubenik (1983a) have suggested that low levels of testosterone are necessary for antler development. Adrenal androgens may be responsible for antler development in castrated animals (Bubenik, Tachezy and Bubenik, 1976) although the antlers appear not to fully mineralize (Belanger, Choquette and Cousineau, 1967; Bubenik et al., 1975a). Mineralization of the antlers in castrated white-tailed bucks, however, can be induced by administration of androgens, particularly 19-OH-testosterone (Morris and Bubenik, 1983). The association of hard antler development and velvet stripping with increasing serum levels of androgens as the breeding season approaches has been demonstrated in entire stags by a number of authors (e.g. McMillan et al., 1974; West and Nordan, 1976a; Brown, Cowan and Griel, 1978). Following the rut (mating period), circulating testosterone levels decrease rapidly (McMillan et al., 1974; and Barrell, Muir and Sykes, 1985). Hard antlers are cast in early winter in some species (e.g. Odocoileus, moose and reindeer) although antler growth may not ensue until spring. In others (e.g. wapiti, red, sika and fallow deer) hard antlers are retained until casting in late winter/early spring (Goss, 1983).

b) Oestrogens

Oestrogens in males originate from aromatization of androgens, and by synthesis in the testes and adrenal cortex (Kelch et al., 1972; cited by Bubenik, Bubenik and Zamecnik, 1979). Oestrogens and androgens appear to have similar effects on the antler cycle. In intact stags antler casting is prevented, and premature antler mineralization and velvet stripping are induced by oestrogens (Goss, 1968). Oestradiol-17β in particular is considered more potent than testosterone and in comparatively small doses can induce antler mineralization and rutting behaviour in castrated stags (Fletcher and Short, 1974). Although circannual rhythms of oestradiol-17β are evident in the plasma of intact white-tailed stags (Bubenik, Bubenik and Zamecnik, 1979) its role in antler bone formation is not clear.

c) Other hormones

Photoperiodic change governs the antler cycle (Goss, 1983), the pineal gland presumably being important in mediating these changes. However pinealectomy of white-tailed bucks only caused deviations of a few months from the twelve month antler cycle (Brown, Cowan and Kavanaugh, 1978; Mazur, 1974; and Plotka et al., 1978). Lincoln (1984) has suggested that in longer-lived species, such as the red deer, other seasonal cues from the environment may influence the timing of the breeding cycle. In intact animals melatonin is released from the pineal gland in response to changes in lighting and in some way inhibits gonadotrophin release. The pattern of melatonin release may be important and if this pattern is modified, photoperiodic control of the seasonal cycle can be disrupted (Lincoln, 1984). In support of this theory, the work of Bubenik (1983b) has demonstrated that antler mineralization and early rutting behaviour can be induced by melatonin administration to white-tailed deer.

Of the gonadotrophins released by the pituitary gland, luteinizing hormone (LH) stimulates testicular androgen secretion and has been linked with plasma testosterone levels and the antler growth cycle (Lincoln and Kay, 1979; Suttie, Lincoln and Kay, 1984). Immunisation against gonadotrophin releasing hormone (Lincoln, Fraser and Fletcher, 1982) or use of a synthetic progestagen (Muir, Barrell and Sykes, 1982) to block LH release can therefore induce early antler casting and prevent full

antler mineralization.

Prolactin, another pituitary hormone, reaches high levels in blood during the period of antler growth (Suttie, 1980; Barrell, Muir and Sykes, 1985). Wislocki, Aub and Waldo (1947) considered it as a possible "antler stimulating hormone". However West and Nordan (1976b) were unable to elicit an antler growth response to exogenous prolactin. Recently Suttie, Lincoln and Kay (1984) speculated that since the increase in plasma prolactin levels coincided with antler casting, this hormone may be acting as an anti-gonadotrophin.

The possibility of an "antler stimulating hormone" has again been raised by the work of Suttie, Fennessy and Gluckman (1983). These authors found that plasma levels of insulin like-growth factor-one (IGF-1), a hormone known to be associated with cartilage synthesis, were increased during the period of active antler growth.

Secretory patterns of a number of other hormones have been described in male deer; those of: cortisol (Bubenik et al., 1975b), growth hormone (Bubenik et al., 1975b; Ryg and Jacobsen, 1982; Barrell, Muir and Sykes, 1985) and thyroid hormones (Bubenik and Bubenik, 1978; Ryg and Jacobsen, 1982). However no link between these hormones and antler growth has been demonstrated.

SECTION 3

Effects of winter nutrition on antler development

Introduction

There is evidence to suggest that the weight of antler produced by a stag is related to its age, liveweight, nutrition and date of hard antler casting. However the precise nature of these relationships is not clear. Older stags (which are also heavier), tend to cast earlier (Behrend and McDowell, 1967). However data on the role of nutrition, and liveweight, on casting date is conflicting between species. Long *et al.* (1959) have indicated that in white-tailed deer antler casting may be advanced by poor nutrition during the 10 weeks preceding casting. In red deer stags however, antler casting occurred earliest in stags in good body condition (Watson, 1971).

Huxley (1926, 1931) indicated that antler weight was related to age and liveweight of the stag, although in these studies the effect of liveweight was confounded by age, genetic and environmental effects. Hyvarinen, Kay and Hamilton (1977), provided the first evidence that within stags of the same age, weight of hard antlers increased with increasing liveweight. Subsequently, Fennessy (1982) demonstrated that within an age group velvet antler weight also increased with increasing liveweight.

P.F. Fennessy (*pers. comm.*, 1979) also suggested that within an age group hard antlers might be cast earlier by heavier stags and that these stags would subsequently produce heavier velvet antlers. This study was therefore undertaken to test the hypothesis that an improvement in winter nutrition could advance the date of hard antler casting and increase the yield of velvet antler.

Experimental methods

A winter feeding trial was carried out in 1979 on two Canterbury properties in association with Ministry of Agriculture and Fisheries staff at Invermay Agricultural Research Station. Farm L is an irrigated lowland property situated at 130 m above sea level, and at latitude 43° 56'S and longitude 171° 36'E. Farm H is a North Canterbury high country station which farms deer at 600 m above sea level at latitude 42° 36'S and longitude 172° 30'E. Deer on both properties were likely to be genetically diverse since both farms were originally stocked with feral deer. Animals on Farm L came from the Rakaia area, whereas deer on Farm H were descended from animals originally liberated in the Nelson area.

On both properties three nutritional levels were used, hereafter referred to as groups 1, 2 and 3.

(1) Ad libitum feeding of high quality pellets (Timaru Milling Limited) based on barley, lucerne and linseed meal (Table 3.1) with an estimated energy content of 11.5 megajoules of metabolizable energy (MJME) per kg of dry matter (DM).

(2) Offered half the quantity of pellets consumed by group 1 in the previous week.

(3) No pellets were offered.

All groups had unrestricted access to lucerne hay on Farm L and meadow hay on Farm H. Stags were confined in the smallest fenced areas available to minimise pasture intake. At Farm L groups of stags were confined to 1.0 ha of improved pasture under border dyked irrigation. On Farm H groups 1, 2 and 3 were restricted to paddocks of 4.5, 2.0 and 2.0 ha respectively. These sloping areas were dominated by matagouri (Discaria toumatou), silver tussock (Poa laevis) and semi-improved hill country pasture species. The terrain on Farms L and H is illustrated in Plate 3.1 (a and b).

Plate 3.1

Plates a and b contrast treatment groups 2 (½ ad libitum pellets) on irrigated farmland (Farm L) with the harsher terrain of Farm H.

a) Farm L - treatment group 2

b) Farm H - treatment group 2

Table 3.1

Ration formulation and chemical analysis of a pelleted ration offered to groups of red deer stags during a winter feeding trial.

Ration formulation

Component	Proportion of ration (%)
Barley	46.0
Lucerne	35.0
Linseed	15.0
Dicalciphate	3.5
NaCl	0.25
Vitamins, trace elements	0.25

Analysis

Crude protein (g/kg DM)	170.0
Calcium (g/kg DM)	16.0
Phosphorus (g/kg DM)	9.0

Prior to the trial stags on each property were managed as a single mob. Fifty three stags in three age classes 23 (3), 10 (4) and 20 (5 years of age) were available at Farm L and 48 in 5 age classes 16 (3), 15 (4), 5 (5), 5 (6) and 8 (9 years of age) at Farm H. Stags were allocated to treatments in a stratified random manner so that groups were balanced for age and liveweight. Feeding commenced in mid winter; on 27 June at Farm L and on 5 July at Farm H. Winter feeding continued until antler casting was complete. This occurred on 15 and 28 October at Farm L and Farm H, respectively. During the course of the trial a 5-year-old stag in trial group 2 at Farm L was injured and at Farm H a 5-year-old stag in group 3 died as a result of an accident. Records from both stags were excluded.

Feed consumption was monitored throughout the period of winter feeding. Pellets were offered to group 1 in a covered hopper which was refilled weekly. Subsamples of pellets offered and refused were taken weekly for DM determination. Group 2 were fed one seventh of the weekly

allocation each day and this was always rapidly consumed. Numerous feeders were used to prevent high intakes by dominant stags. Hay was available from racks which were always well filled. Hay offered and refused (collected fortnightly) was weighed and subsampled for dry matter determination. Pasture intakes were determined monthly by the Australian difference technique (Lynch, 1966). Six randomly selected quadrats (0.5 m^2), were taken within each treatment group, three from inside exclusion cages and three outside. Herbage was cut to ground level and washed prior to drying. All pellet, hay and pasture samples were oven dried at 70°C, to constant weight.

Rainfall, and daily maximum and minimum temperature, were recorded (by farm staff) throughout the period of winter feeding. Normal winters were experienced on both properties, with total rainfall during the period of winter feeding of 254 mm and 273 mm at Farms L and H, respectively. Mean daily temperatures were slightly higher on Farm L (8.4°C) than on Farm H (6.4°C). A lower incidence of frosts was recorded on Farm L, with 24 frosts out of the 106 days over which recordings were made, compared with 38 frosts out of 110 days on Farm H.

Liveweights were recorded fortnightly until casting using a enclosed weighing crate on a set of Avery clockface scales mounted on a small trailer. On Farm L liveweights also were recorded after velveting and again on 5 March prior to the rut.

Antlers are normally cast in close succession (Wislocki and Waldo, 1953) but in some instances one antler has been observed to remain in place in spite of new growth having commenced on the antler site. Consequently casting date was recorded as the date when the first antler button was cast. Dates of antler casting were recorded daily at Farm L. On Farm H, stags that had cast were drafted weekly, and estimates made as to when in that week casting had occurred. After casting, all stags on both properties were run together in a single mob in large paddocks until velvet harvest. As single deer cannot be safely left alone a stag which cast 17 days earlier than its contemporaries at Farm L had to be maintained in its treatment group. Antler casting began at Farm H before spring pasture growth commenced and barley grain was provided ad libitum until spring feed was readily available.

Velvet antlers were judged to be ready for commercial harvesting by farm staff. Stags were sedated with 3 ml of 2% xylazine hydrochloride (Rompun, Bayer) and each pedicle nerve blocked with 2 ml Xylocaine (2%, Astra) injected subcutaneously. Antlers were sawn immediately above the antler-pedicle junction. Cut antlers were inverted immediately to prevent blood loss and frozen in polyethylene bags to prevent weight loss. Prior to sale frozen antlers were weighed, and antler length recorded from tip to base around the external curvature. At Farm H velvet antler records were available from only 40 of the original 48 stags, from 14, 15, and 11 stags in groups 1, 2 and 3, respectively.

Data were analysed statistically using the general statistical programme, Genstat, 4.03 (Rothamstead Experimental Station) and Minitab, version 81.1 (Pennsylvania State University). The significance of differences between means were determined by Duncan's multiple range test using a program written by J. Baird (Agricultural Engineering Institute, Lincoln College).

Results

1) Liveweight changes

At the commencement of the trial there was a marked difference in mean stag liveweight between the two properties (123.4 ± 1.42) at Farm L and (110.2 ± 1.75) at Farm H (Fig. 3.1). Supplemented groups (1 and 2) at Farm L had lower winter liveweight gains (41 and 33 g/d, respectively) compared with 68 and 54 g/d, respectively, at Farm H. On the other hand the unsupplemented group at Farm L tended to have a greater winter liveweight loss (-71 g/d) than that at Farm H (-49 g/d). On both properties there were significant differences in daily winter liveweight change ($P < 0.01$) between the group fed hay only and both supplemented groups. These differences were reflected in liveweight at casting, with both supplemented groups on both farms being significantly heavier ($P < 0.05$) than the corresponding group 3 (Table 3.2).

Liveweight realimentation occurred on Farm L during both spring and summer (Fig. 3.1). Stags offered hay only (group 3) and which lost weight during the winter gained weight at 264 g/d, significantly faster than did stags in both group 1 ($P < 0.05$; 204 g/d) and group 2 ($P < 0.01$; 189 g/d). There were no significant differences in liveweight at velvet harvest, values being 140.9 ± 3.93, 139.0 ± 3.42 and 136.5 ± 2.36 kg, respectively. Realimentation occurred after velveting and at the pre-rut weighing (5 March) mean stag liveweight had increased to 163.7 ± 2.21 kg. There were no differences between groups.

It was impossible to obtain liveweight data from Farm H after antler casting.

2) Feed Intake

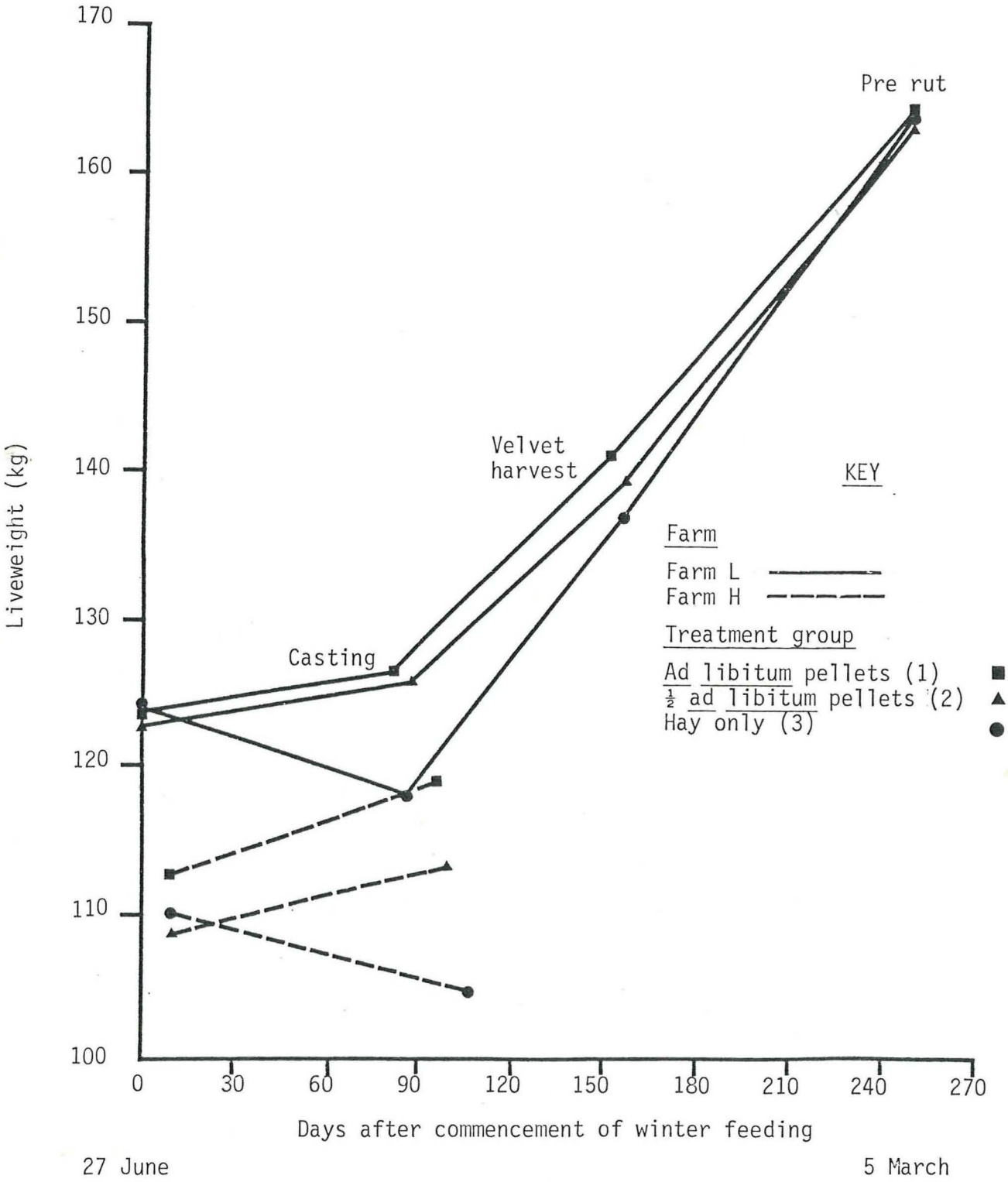
Mean daily dry matter intakes of the groups are presented in Table 3.3. Supplemented groups consumed similar quantities of pellets on both properties. Hay intake decreased as level of supplementation increased but was consistently higher at Farm L than in comparable groups on Farm H. Pasture comsumption at Farm L accounted for between 11 and 17% of total dry matter intake. On the other property, the nature of the terrain (see Plate 3.1b) made sampling difficult, as a consequence replication was inadequate to provide reliable estimates of pasture intake and the data were excluded. Data from Farm L have previously been

Table 3.2

Liveweight, antler casting date and velvet antler data recorded from red deer stags offered three levels of winter nutrition on two Canterbury farms.

Property	Treatment group	No. of stags	Mean liveweight at commencement of study (kg)	Mean liveweight at casting (kg)	Winter liveweight change (kg)	Mean casting date	Velvet antler weight (kg)	Days of antler growth	Antler growth rate (g/d)
Farm L	1 Ad libitum pellets	18	123.7	126.4	+2.7 a	15 Sept a	2.17 a	70 a	31.0 a
	2 ½ Ad libitum pellets	16	122.6	125.5	+2.9 a	21 Sept a	2.30 a	70 a	32.9 a
	3 Hay only	18	123.9	118.0	-6.1 b	20 Sept a	2.28 a	69 a	33.0 a
	S.E.D.		±3.59	±3.86	±1.55	±4.32	±0.192	±2.9	±2.20
Farm H	1 Ad libitum pellets	16	112.8	118.9	+6.1 a	25 Sept a	1.46 a	51 a	28.6 a
	2 ½ Ad libitum pellets	16	108.7	113.2	+4.5 a	29 Sept ab	1.38 ab	54 a	25.6 a
	3 Hay only	15	110.1	104.7	-4.6 b	8 Oct b	1.22 b	54 a	22.6 a
	S.E.D.		±4.34	±3.70	±1.55	±5.57	±0.100	±2.0	±1.80

Figure 3.1
Liveweight changes in red deer stags offered three levels of winter nutrition on two Canterbury farms.



used to derive the energy requirements for red deer (Fennessy, Moore and Corson, 1981).

Table 3.3

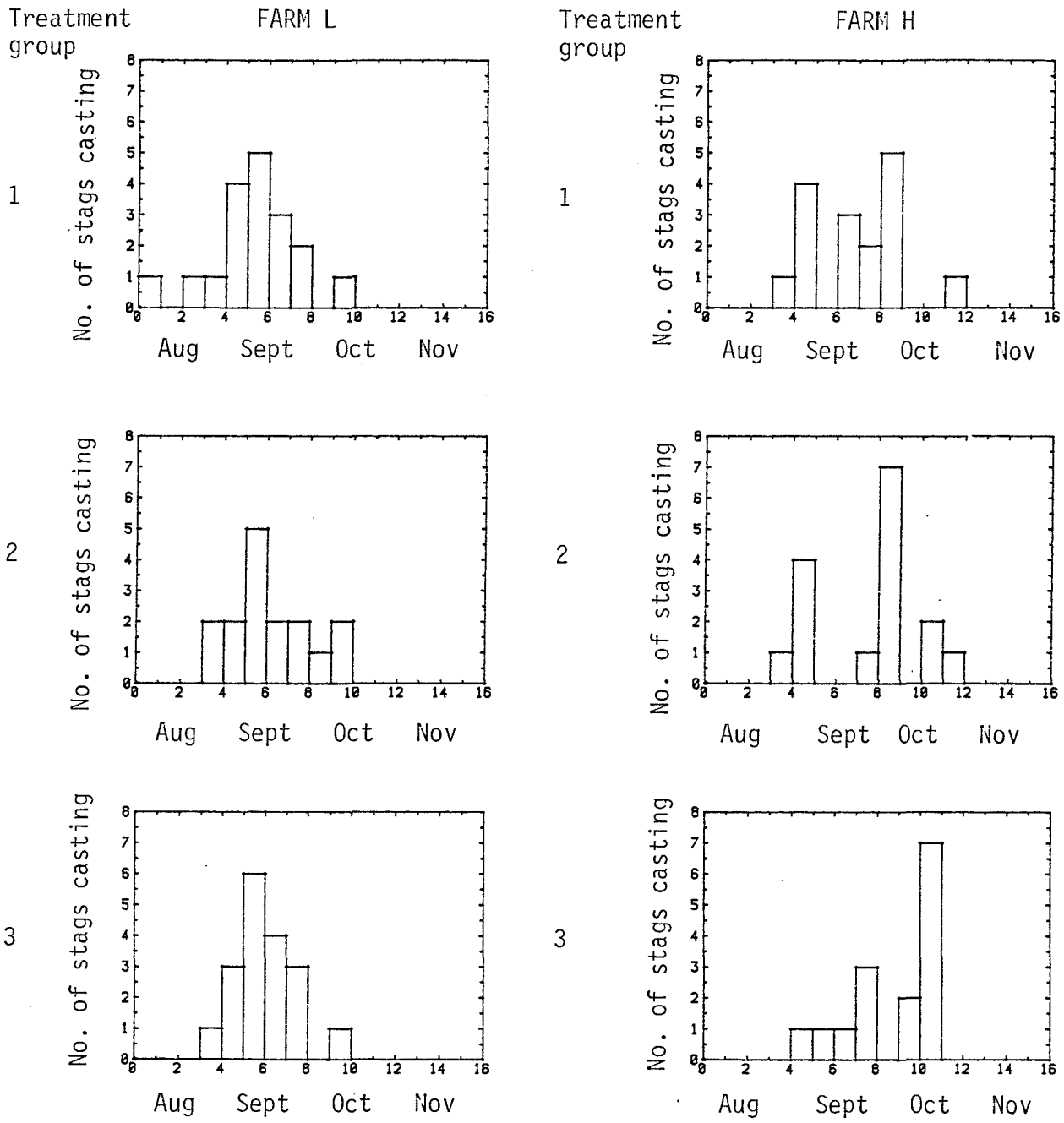
Mean daily winter feed intake of hay, pasture and high quality pellets by red deer stags on two Canterbury properties.

Property	Treatment group	Mean daily intake (kg DM/day) Pasture	Hay	Pellets
Farm L	1) Ad libitum pellets	0.62	0.80	2.27
	2) 1/2 Ad libitum pellets	0.36	1.58	1.20
	3) Hay only	0.35	2.19	-
Farm H	1) Ad libitum pellets	-	0.26	2.23
	2) 1/2 Ad libitum pellets	-	0.69	1.15
	3) Hay only	-	1.77	-

3) Casting Date

There was no effect of nutritional treatment on casting date at Farm L. Mean casting date was 19 September, with a range of 68 days. In each treatment group the pattern of antler casting was normally distributed with similar modal casting dates. The wide spread of casting date on Farm L was caused by a single stag casting 17 days earlier than the first of its herd-mates. Mean casting date on Farm H was 30 September with a range of 59 days. Winter nutrition significantly ($P < 0.05$) affected mean casting date, there being a difference of 13 days between groups 1 and 3 (Table 3.2). Moreover, nutritional treatment appeared to change the pattern of antler casting on this property

Figure 3.2
Number of stags casting hard antlers each week on two Canterbury properties subsequent to three levels of winter feeding on each farm.



(Fig. 3.2). Casting was normally distributed in group 1 stags and to a lesser degree within stags of group 2. In group 3 however, which was offered only hay, the pattern of antler casting appeared to be skewed and the modal casting date delayed by two weeks.

On both properties the left and right antler buttons from each stag were shed in rapid sequence, within a mean of 1.7 days at Farm L (range, 0-6). There were no differences between treatment groups. Weekly recordings only were made at Farm H therefore it was not possible to accurately ascertain the sequence of casting.

At Farm L mean casting date for 5-year-old stags was 11 September, significantly earlier ($P < 0.05$) than for either 4-year-old stags (19 September), or 3-year-old stags (25 September). A relationship was detected between the pre-rut liveweight recorded six months after casting, and date of antler casting. Heavier stags tended to have earlier casting dates and analysis of covariance indicated that this relationship was not due to an age effect. Consequently a common regression was fitted through all age groups to describe the relationship between pre-rut liveweight, and date of casting (Fig. 3.3). Pre-rut liveweight tended to give a better relationship with date of antler casting than did liveweight recorded at either the start of the trial (mid winter), the date of antler casting or the time of velvet antler removal. This is illustrated in the regression equations below where X refers to liveweight (kg) and Y the date of antler casting (days from the winter solstice).

Mid winter	$Y = 132.2 - 0.338\ X$ ($r = 0.32^{*}$, RSD = 11.0)
Casting	$Y = 113.2 - 0.184\ X$ ($r = 0.19$, RSD = 11.4)
Velvet removal	$Y = 126.1 - 0.257\ X$ ($r = 0.31^{*}$, RSD = 11.0)
Subsequent pre-rut	$Y = 146.5 - 0.344\ X$ ($r = 0.48^{**}$, RSD = 10.2)

4) Antler Growth

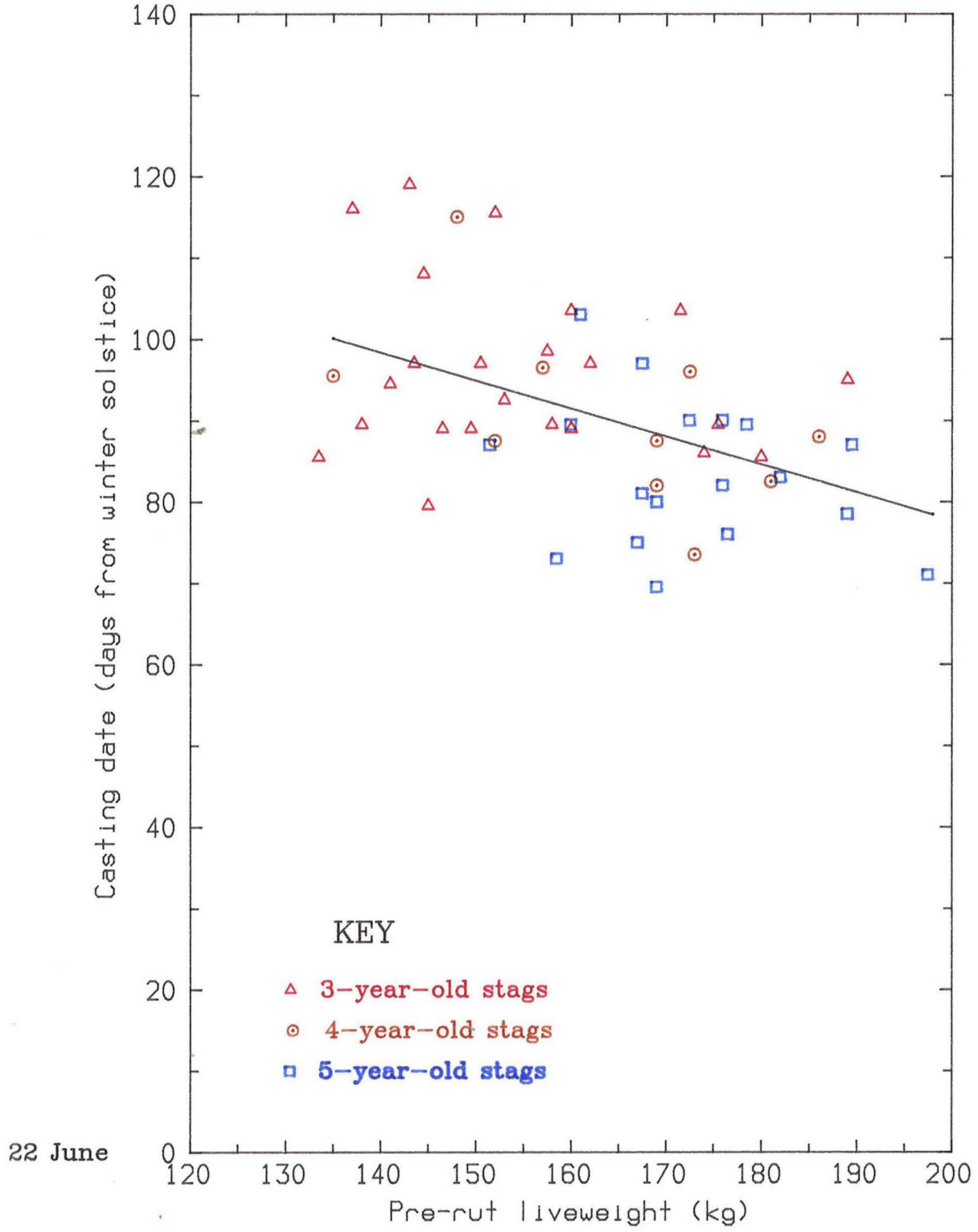
Antler growth rates were lower, and dates of antler removal earlier at Farm H (Table 3.2). Consequently antlers were smaller, weighing on average 1.36 ± 0.045 kg, and measuring 0.33 ± 0.092 m in length, compared with 2.25 ± 0.076 kg and 0.52 ± 0.140 m in antlers from Farm L.

Figure 3.3
Relationship between liveweight (kg) prior to the subsequent rut and date of hard antler casting (days from winter solstice).

$Y = 146.5 - 0.344\ X$ ($r = 0.48^{**}$, RSD = 10.2)

where r = correlation coefficient and RSD = residual standard deviation

Figure 3.3
Relationship between liveweight and date of antler casting in 3, 4 and 5–year–old stags on Farm L. Date of casting is the number of days from the winter solstice (22 June). Liveweights were recorded prior to the following rut (5 March). See facing page for equation.



Poor winter nutrition was associated with decreased velvet antler weight at Farm H. Mean velvet weight was significantly lower ($P < 0.05$) in group 3 than in group 1 (Table 3.2).

Antler weight was not, however, affected by winter nutrition at Farm L, nor were there significant differences in mean date of antler removal between age groups of stags, these being 69, 68 and 72 days in 3, 4 and 5-year-olds, respectively. These data have therefore been used to study the effects of casting date and liveweight within an age group on velvet antler yield. A 5-year-old stag which cast exceptionally early (51 days after the winter solstice) and which had to be maintained within its treatment group for three weeks after antler casting gave a very poor fit in these analyses, and was excluded. Significant relationships ($P < 0.01$) existed between date of antler casting and weight of velvet antler produced within 3-year-old ($r = 0.54$) and 4-year-old ($r = 0.74$) stags (Fig. 3.4), greater weights of velvet antler being removed from stags which cast earliest. No such relationship could be detected within 5-year-old stags. The fitted regression coefficients were not significantly different between age groups and have been adjusted to a common slope (Fig. 3.4). The model describing the relationship between age, casting date and velvet antler weight, as demonstrated in Figure 3.4 has a correlation coefficient of 0.76.

A relationship also existed between liveweight and velvet antler weight. Of the liveweights obtained during the experiment, (mid winter, casting, velvet removal, pre-rut) subsequent pre-rut liveweight provided the best relationship with velvet antler weight (Fig. 3.5). For example the regression equations relating liveweight (X) and velvet antler weight (Y), within 3-year-old stags, are given below.

Mid winter	$Y = 0.881 + 0.0850\ X$ ($r = 0.23$, RSD = 0.39)
Casting	$Y = 1.194 + 0.00591\ X$ ($r = 0.18$, RSD = 0.40)
Velvet removal	$Y = 0.633 + 0.00950X$ ($r = 0.33^{*}$, RSD = 0.38)
Subsequent pre-rut	$Y = 0.0996 + 0.0116\ X$ ($r = 0.44^{**}$, RSD = 0.36)

Significant effects ($P < 0.05$) of liveweight on velvet antler weight were obtained within 3-year-old ($r = 0.44^{**}$, RSD = 0.36) and 4-year-old ($r = 0.68^{*}$, RSD = 0.25) stags, but not for 5-year-olds. No significant differences were found in slope of the fitted regressions within each age

Figure 3.4
Relationship between antler casting date (days from winter solstice) and subsequent weight of velvet antler (kg). Regression equations have been adjusted to a common slope.

3-year-old stags

$Y = 3.91 - 0.026\ X$

(Prior to adjustment; r = 0.54**, RSD = 0.34)

4-year-old stags

$Y = 4.16 - 0.026\ X$

(Prior to adjustment; r = 0.74**, RSD = 0.23)

5-year-old stags

$Y = 4.37 - 0.026\ X$

(Prior to adjustment; r = 0.39, RSD = 0.46)

Figure 3.4
Relationship between casting date and velvet antler weight in 3, 4 and 5-year-old stags on Farm L. Date of antler casting is the number of days after the winter solstice (22 June) and velvet antlers were removed approximately 69 days after casting of hard antlers. See facing page for equations.

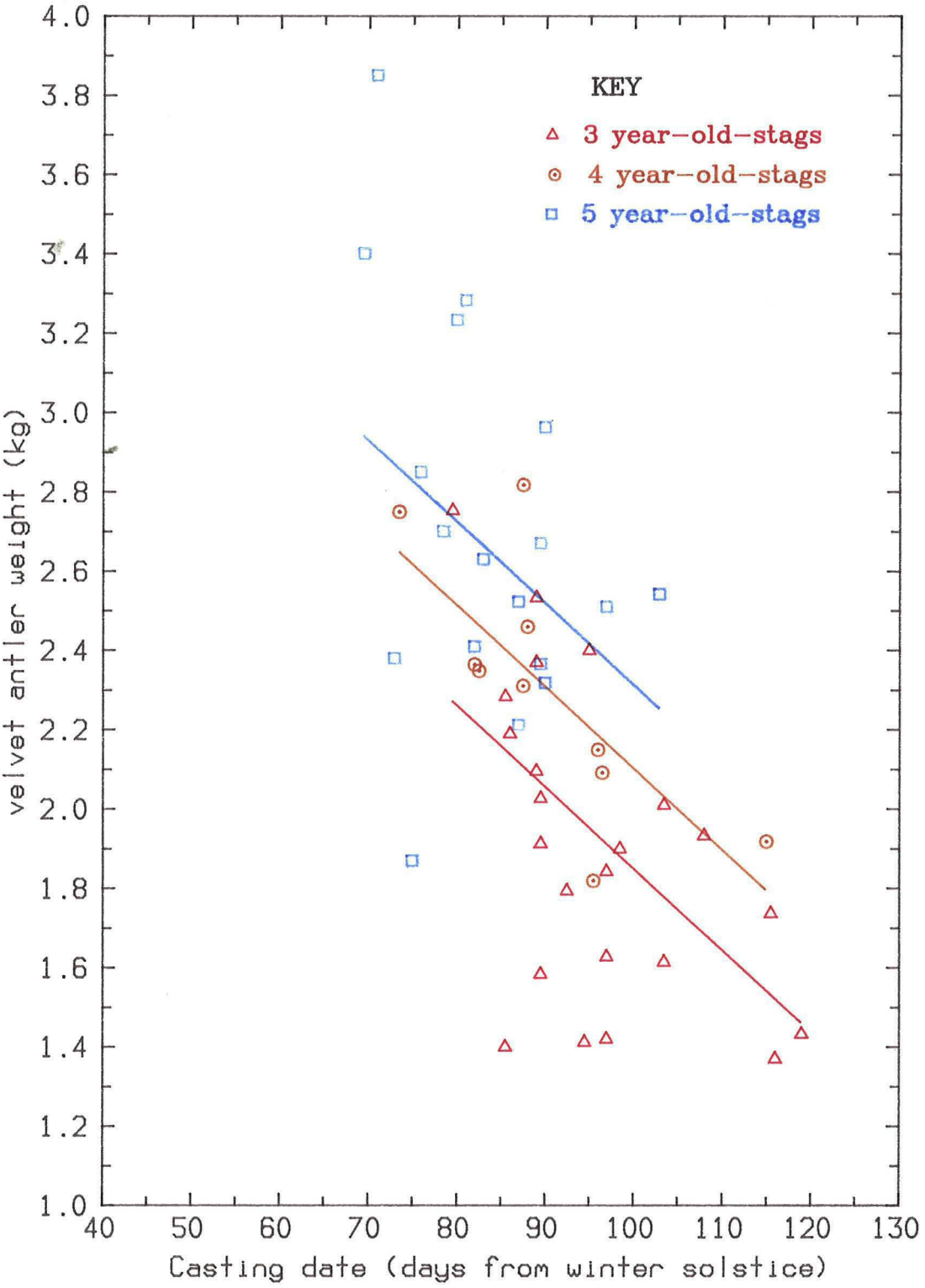


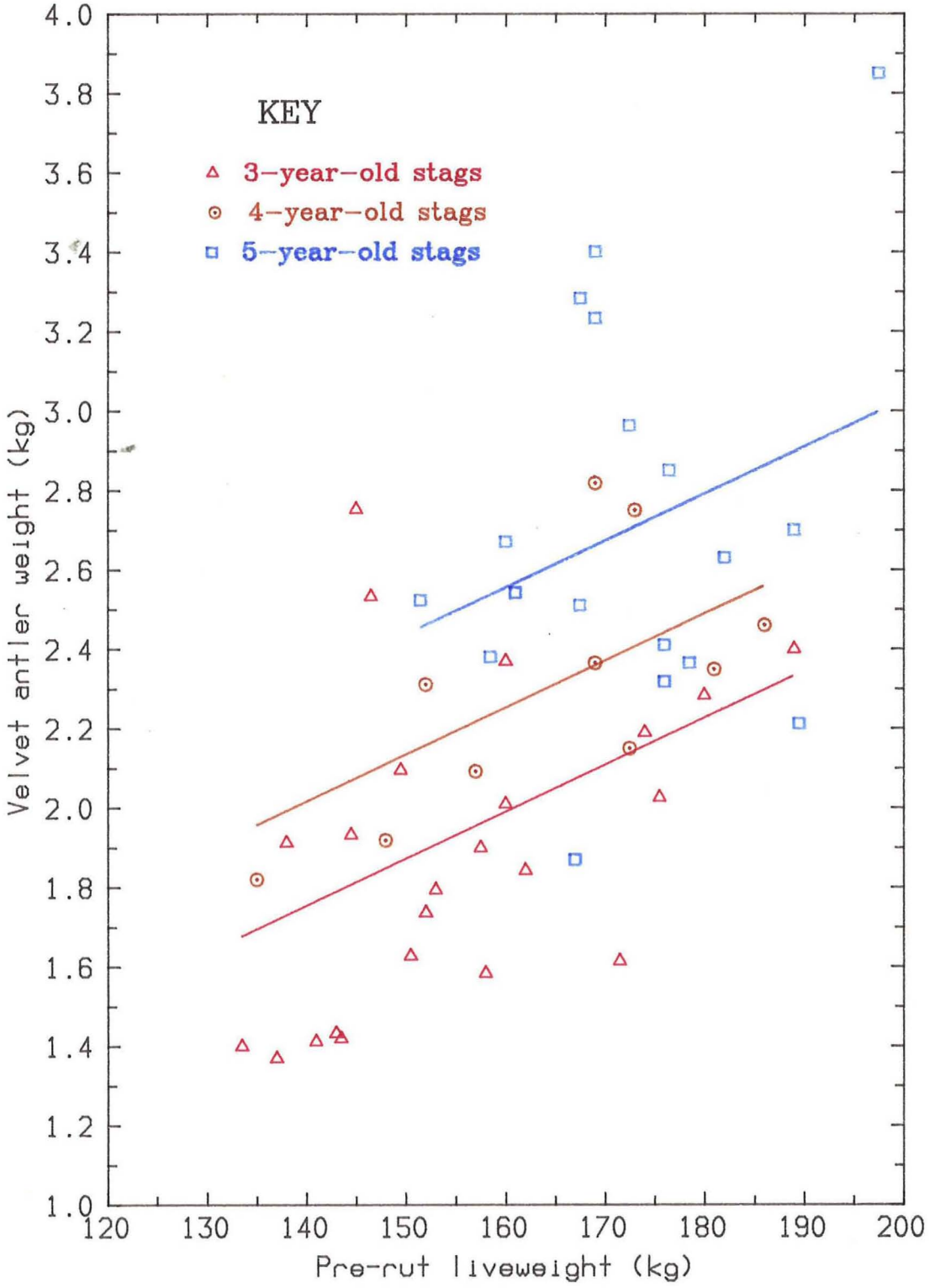
Figure 3.5
Relationship between liveweight (kg) and velvet antler weight (kg). Regression equations have been adjusted to a common slope.

3-year-old stags
$Y = 0.099 + 0.0118\ X$
(Prior to adjustment; $r = 0.44^*$, RSD = 0.36)

4-year-old stags
$Y = 0.361 + 0.0118\ X$
(Prior to adjustment; $r = 0.68^*$, RSD = 0.25)

5-year-old stags
$Y = 0.664 + 0.0118\ X$
(Prior to adjustment; $r = 0.25$, RSD = 0.48)

Figure 3.5
Relationship between liveweight and velvet antler weight in 3, 4 and 5–year–old stags on Farm L. Antlers were removed approximately 69 days after casting of hard antlers. Liveweights were recorded prior to the following rut (5 March). See facing page for equations.



group and a common slope was fitted, and adjusted for age (Fig. 3.5). Velvet antler weight also increased with age and at the same liveweight velvet weight increased by 0.27 kg between 3 and 4-year-old stags and by 0.30 kg between 4 and 5-year-old stags. The overall correlation coefficient for this model was 0.73.

Multiple regression analysis on antler weight was conducted within age groups using casting date, pre-rut liveweight and spring and summer liveweight gain. Neither liveweight or liveweight gain explained significantly more of the variation in antler weight than did casting date alone.

No regrowth velvet of any consequence was obtained from stags at either property with the exception of 0.68 kg harvested from the stag at Farm A which cast its antler buttons exceptionally early, on the 13 August.

Discussion

Differences in winter nutrition resulted in delayed antler casting and impaired antler production on one of the farms in this study. The levels of winter feed offered caused significant liveweight differences between treatment groups at casting on both properties. However these differences resulted from liveweight losses in the groups fed hay only rather than from liveweight increases in groups which were offered pellets. Stags in group 1 were offered a high quality supplement during winter, yet intake and liveweight gain was low on both properties over this period. Following winter feeding at Farm L, rapid bodyweight gain occurred between the time of antler casting and the pre-rut weighing (5 March). Other workers have also demonstrated seasonal rhythms of voluntary feed intake in red deer stags (Kay, 1980) and in reindeer stags (Ryg and Jacobsen, 1982) with a reduced feed intake during winter followed by an increased intake during spring and summer. The results obtained at Farm L indicate that liveweight realimentation occurred following differential winter feeding, the effects of liveweight loss in group 3 having disappeared by the time of the pre-rut weighing. On the other hand Suttie et al. (1983) demonstrated persistent effects of winter feed restrictions in red deer fawns and yearlings. Their results were obtained by restricting the intake of a group of red deer fawns to 70% of the intake of a control group fed a high quality ration ad libitum. Compensatory growth occurred during the period of unrestricted feeding (summer) although there were still significant differences in liveweight (approximately 10 kg) at the end of the summer period. The experiment was repeated on the same animals during the following year, resulting in a difference of approximately 15 kg in peak summer liveweight. Although rates of summer liveweight gain were 300 g/d the period of unrestricted feeding was insufficient to allow full realimentation. These authors concluded that poor winter nutrition, particularly during the first year of life, was an explanation for the reduced mature size of wild Scottish stags. It is likely that undernutrition during early life could affect bone growth and therefore ultimate frame size of the animal. However the stags in the present study were older and although undernutrition may influence muscle and fat content of the carcass it would be less likely to affect bone growth. Under these circumstances (i.e. where frame size is not jeopardised) full compensatory growth may be easier to achieve.

Stags on the hill country property (Farm H) appeared in poorer condition and had faster liveweight gains in group 1, and slower liveweight losses in group 3 at Farm H than at Farm L. In addition mean stag liveweights differed markedly between properties at commencement of the trial, with stags from Farm H being, on average, 13 kg lighter than than those at Farm L. Body condition of the stags was not assessed, so it is not possible to attribute this difference to body size, or to body condition. There is evidence in the literature to suggest that the faster liveweight gains in the lighter stags at Farm H are due to poor body condition. The appetite of animals is increased following undernutrition (Allden, 1970) and in non-lactating cows higher feed intakes have been obtained in thin compared with fat animals (Bines, Suzuki and Balch, 1969). Moreover, even at the same allowance faster growth rates and slower liveweight losses have been observed in sheep in poor condition compared with sheep in good condition (Thompson, McEwen, Kelly and Crosbie,1983). Bines and Morant (1983) suggested that at restricted intakes thin cows may have a better utilization of feed, through an increased rate of utilization of acetate. Differences in winter liveweight gain between properties may therefore be attributable to differences in body condition. Such differences in body condition between properties might arise from variation in liveweight losses over the rut, or as a result of differences in early winter nutrition. Stags at Farm L were farmed on ryegrass/white clover irrigated swards and those at Farm H on semi-improved tussock country. Poor agronomic species (Suckling, 1960), together with lower winter temperatures (Langer, 1973) would probably make high levels of nutrition more difficult to attain on the hill country property.

Poor winter nutrition significantly altered antler casting date in the group offered hay alone at Farm H, and changed the pattern of casting by delaying for two weeks the modal casting date. This lends further support to the findings of Watson (1971) that antler casting in Scottish red deer stags is delayed in stags in poor condition. The precise mechanism by which antler casting occurs has not been established. It is difficult, therefore, to provide an explanation for the effects of undernutrition on casting date. There may be a link with the pituitary hormone, prolactin. Prolactin secretion has been shown to be lower in animals fed a restricted ration than in those offered food *ad libitum* (Forbes *et al.*, 1979). Suttie *et al.* (1984) speculated that prolactin

may be acting as an anti-gonadotrophin. If this is the case it is possible that in poorly nourished animals, such as those at Farm H, there may be a delay in the late winter decline in plasma testosterone levels which precedes antler casting. However, a different mechanism may operate in white-tailed deer since Long et al. (1959) found antler casting in this species may be advanced following poor nutrition.

In the present study weight of velvet antler subsequently removed was also lower in the group of stags in which antler casting was delayed. Animals in this group produced 0.24 kg less than those offered pellets ad libitum. Similar liveweight losses occurred at Farm L without significantly affecting either casting date or velvet antler production. This may imply a critical level of body condition below which both casting date and velvet antler production is affected. Stags in poorer condition may have impaired antler growth as a consequence of priority partitioning of nutrients to more essential body processes in early spring. Identification of stags in poor condition, possibly those which have sustained greatest rut liveweight losses, for preferential winter feeding may achieve increased velvet antler weights. An on-farm condition score, as used in both the dairy (Grainger and McGowan, 1982) and sheep industries (Jeffries, 1961), may be more useful than liveweight in predicting and improving antler production. Riney (1955) demonstrated the usefulness of backfat thickness in determining condition in wild red deer during summer and was able to detect significant environmental differences in condition. During the winter months however back fat thickness did not provide a good index of body condition whereas the depth of kidney fat did. Riney's explanation was that during periods of inanition back fat is mobilised first and abdominal fats, the main fat storage reserves, are mobilised last. Therefore it seems unlikely that the method used to condition score sheep and cattle (i.e. assessing back fat thickness) would be useful in stags during winter. Further information is needed establish a method of measuring condition and to define a critical liveweight/condition score above which improved winter nutrition may prevent a reduction in antler weight.

Antler casting occurred earlier in older stags, as noted by Behrend and McDowell (1967) and Jacobsen and Griffin (1983). However older stags are usually heavier and, as shown by the present study, liveweight rather than age may effect casting date. A relationship was

demonstrated between casting date and liveweight recorded prior to the rut, but not with liveweight recorded at other times during the year. Hyvarinen, Kay and Hamilton (1977) considered that because of the seasonal nature of liveweight change, liveweight recorded in late summer best represents net growth (i.e. frame size) over the lifetime of the stag and that frame size may explain why this relationship could be obtained only at this time of year. Seasonal liveweight losses of the stag, are almost entirely comprised of body fat losses (Drew, 1985) and tend to be greater in older animals (Fennessy, Moore and Corson, 1981). Consequently there is likely to be a trend for narrowing of the range of liveweights at antler casting. This is borne out by the greater range in mean liveweight observed at the pre-rut liveweight recording (163.7 ± 2.21 kg) than in the liveweight recorded at the start of the trial (123.4 ± 1.42 kg) or at date of hard antler casting (123.3 ± 1.62 kg), at Farm L. Ranking of stags on pre-rut liveweight is highly repeatable between years (r = 0.89; Muir, unpublished data from Lincoln College stags). The most critical liveweight may therefore be the liveweight attained in the previous summer. Since this weight probably reflects the true size of the animal, casting date may be responding to frame size of the stag.

Within an age group heavier stags cast earlier and produced heavier velvet antlers. If mature liveweight affects antler weight the mature size penalty associated with feed restrictions in early life (Suttie et al., 1983) may affect antler production in the stag. The relationship between pre-rut liveweight and velvet antler yield in the present study, of 0.12 kg for each 10 kg increase in liveweight, was comparable to that derived for 2-year-old stags by Fennessy (1982) who found velvet antler yield increased by 0.09 kg for each 10 kg increase in liveweight. The present study however, also indicated a definite increase in velvet antler weight between age groups, irrespective of bodyweight. For stags at the same liveweight, antler production increased by 0.26 kg between 3 and 4-year-old stags, and by 0.30 kg between 4 and 5-year-old stags. Changes in pedicle diameter may be contributing to the age effect on velvet antler weight since Banfield (1960) described the annual deposition of concentric bone rings within caribou pedicles which implies an annual increase in pedicle size.

Between farm effects were apparent in casting date and weight of velvet antler harvested. Casting date was 11 days later on Farm H, the higher altitude property, and velvet antler weights were also 0.90 kg lower on this property. These are large effects compared with the within property effects attributed to or associated with age, liveweight and nutrition. Banwell (1970) has suggested that stags descended from those initially liberated in the Nelson area (Farm H) produce lighter antlers than those descended from stags liberated in the Rakaia area (Farm L). Therefore genetic effects may have contributed to the differences in mean antler growth rates, of 25.6 and 32.3 g/d observed at Farm H and Farm L, respectively. However the single factor contributing most to differences in velvet antler weight between farms was date of antler removal, with antlers from Farm L stags being removed, on average, 70 days, and Farm H, 53 days after hard antler casting. It is not clear whether this reflects differences in antler growth between properties, or differences between operators in assessment of the best stage for antler removal for commercial sale.

SECTION 4

Antler growth and composition

Introduction

Antler weight was not markedly affected by major differences in general winter nutrition (Section 3). However there is evidence to suggest that specific nutrients, namely protein and calcium, may influence antler growth. Whitehead (1950) cited work by Vogt which indicated that hard antler weight increased from 1.6 kg in the best of 3-year-old stags to 6.0 kg in the best of 3-year-old stags six years later. This was attributed to the feeding of sesame cake. Sesame cake not only has a high protein content (412 to 491 g/kg; MAFF, 1975) but is prepared by a heat treatment process and as a consequence the protein may be protected from degradation in the rumen. This bypass protein may have elicited a response in a similar manner to that which has been demonstrated in other ruminants (Kempton, Nolan and Leng, 1977; Orskov, 1982).

During antler growth there is likely to be a high requirement for bone mineral since antler growth occurs rapidly over a few months and, when fully mineralized, antlers contain concentrations of calcium and phosphorus in ash similar to those in skeletal bone (Chapman, 1975). There is anecdotal evidence to suggest that antler growth may be impaired if stags are "deficient" in dietary calcium during this period. Baihle-Grohman (1896) compared deer from the same source on two neighbouring parks. Superior antler weights were reported on the park where soils were derived from limestone parent material compared to those on the park with limestone overlaid by sandstone. Huxley (1926) reported decreases in antler weights when liming was discontinued on an English red deer park. Neither author described stocking rate or feed intake, or considered that lime may have improved pasture growth and feed supply available to the stags.

Financial returns from velvet antler in New Zealand also are influenced by composition. Velvet antler of the highest economic value is said to be that which has both maximum blood content and minimal calcification, at the heaviest possible weight. The optimal association

is claimed to occur at the time of bulbing in the main antler beam prior to branching to form the royal tines. However, morphological variation in antlers, together with our lack of knowledge of antler growth and composition often results in wide differences in date of antler removal. There is therefore considerable morphological variation in velvet antlers offered for sale and, as a consequence, a lower return for some of the product.

The experiments described in this section were designed to investigate the sequential pattern of antler growth and the pattern of change in blood content and mineralization. Determination of values for rates of mineral deposition would then enable estimates of mineral requirements for antler growth to be made.

Part A was conducted to establish methods for antler sectioning and for the measurement of antler blood content using radio-isotopes. In addition, techniques for chemical and histological analysis of antler tissue were evaluated. Part B was a sequential study on antler composition using techniques developed in Part A, together with an investigation of the effects of protected dietary protein and low dietary calcium during the period of antler growth on antler growth rate and composition.

PART A

Experimental methods

1) Experimental outline

A preliminary trial was undertaken at Invermay Agricultural Research Centre, Mosgiel to evaluate techniques for assessing antler blood volume, mineralization and histology. Four stags in velvet antler were available, comprising two mature and two 2-year-old stags. Antlers from both mature stags had been removed for commercial sale during spring and regrowth antler had occurred from the antler stump. On the other hand the 2-year-old stags had first growth antler. All antlers were assessed to be at a stage suitable for commercial harvest and marketing.

Calculation of blood volume from the measured plasma volume of an organ multiplied by the venous haematocrit may be invalid due to innaccuracies caused by plasma streaming in capillaries (Hobbs, 1967). To determine if antler blood volume could be obtained reliably by this method antler blood volume was measured in this pilot experiment using a double isotope technique (Luick, 1963). Red blood cells labelled with sodium chromate (^{51}Cr-RBC) and radio-iodinated human serum albumin (^{125}I-HSA) were used. Both radio-isotopes were supplied by The Radiochemical Centre, Amersham, England. Initial estimates indicated that 800 µCi of ^{51}Cr-RBC and 100 µCi of ^{125}I-HSA would be necessary to produce acceptable counts in antler tissue. Only 200 µCi of ^{125}I-HSA were available. Consequently only one adult stag and one 2-year-old stag were used for measurement of antler plasma content. ^{51}Cr-RBC was used in the determination of antler red blood cell volume in all 4 stags.

2) Radio-isotope preparation

a) ^{51}Chromium labelled red blood cells (^{51}Cr-RBC)

Whole blood (400 ml) was collected from a 2-year-old stag into a flask containing 80 ml sterile acid citrate dextrose solution. After centrifugation at 3000 rpm for 10 min the supernatant plasma and buffy coat were discarded. Sodium chromate containing 4 mCi of ^{51}Cr was added to the red blood cells, mixed gently and incubated at 37°C. A labelling efficiency of 81% was achieved by this procedure. Labelled red blood cells were washed in cold sterile isotonic saline, centrifuged at 3000

rpm for 10 min and the supernatant discarded. This washing procedure was repeated once. Approximately 120 ml of labelled red blood cells were produced and four aliquots of 30 ml prepared and made up to 50 ml with isotonic saline prior to injection into the stag.

b) Iodinated human serum albumin (^{125}I-HSA)

No preparation was required with ^{125}I-HSA, and aliquots of approximately 1 ml (i.e. 100 μCi) were used for each stag.

c) Injection and blood sampling procedure

Once stags were sedated (see below) a blood sample was obtained by jugular venepuncture and a 1.7 x 51 mm teflon catheter (angiocath, Deseret) inserted into the right external jugular vein. Isotope solutions were introduced slowly into the jugular vein and the catheter flushed 2 to 3 times with sterile saline, and removed. Blood samples were obtained by venepuncture from the left external jugular vein at 5, 10, 15 and 20 minutes after isotope injection, in order to check equilibration of the isotope. Immediately after the final blood sampling (20 minutes after injection of isotope) a tourniquet was applied to the right antler pedicle and the antler removed as described below.

3) Antler removal, measurement and sectioning

a) Antler removal

Stags were sedated with 3 ml 2% xylazine hydrochloride (Rompun, Bayer) and nervous conduction in both infratrochlear and zygomaticotemporal branches of the trigeminal nerve supplying each pedicle blocked with 2 ml lignocaine hydrochloride solution (2% Xylocaine, Astra) injected intradermally. A thin rubber strip was applied as a tourniquet to the left pedicle and the antler removed by sawing immediately above the coronet.

b) Antler measurements

Antlers were inverted, immediately after removal, to prevent blood loss. They were then weighed and length recorded around the external curvature from tip (distal) to base (proximal). The number of tines was noted and tine length recorded around the external curvature from tip to point of intersection with the main beam.

c) Antler sectioning

Sectioning of antlers did not commence until 30 minutes after removal so that blood coagulation could occur. Antlers were sectioned at approximately 5.0 cm intervals from tip to base (Fig. 4.1). Length of base sections therefore varied, depending on initial length of the antler. Antler tines were sectioned in a similar manner. Cylinders from the antler tips were bisected longitudinally and a thin slice, approximately 2 to 3 mm thick, removed for histology (Fig. 4.2a). Histological sections were also obtained by removing a transverse sliver of tissue from the base of each antler cylinder (Fig. 4.2a and b). These sections were fixed in 10% buffered formalin (Humason, 1962). Poorly calcified sections were pinned to slices of cork to prevent twisting due to cell shrinkage, prior to being immersed in 10% buffered formalin. The remaining antler cylinders were placed in polyethylene bags and frozen for later analysis. These were weighed and bisected longitudinally whilst frozen. One half cylinder was refrozen for later chemical analysis; the other was transversely sectioned at 2.5 cm intervals (Fig. 4.2a and b). These two half cylinders were used for radio-isotope counting to determine blood content, as described below.

4) Blood volume

The blood sample taken prior to isotope injection was mixed thoroughly and aliquots transferred to four micro-haematocrit tubes which were then sealed. Tubes were centrifuged at 7000 rpm for 20 min and the haematocrit was measured on a micro-haematocrit reader (Hawksley, England). Radioactive blood samples were centrifuged at 3000 rpm for 20 min and plasma aspirated into polypropylene tubes and weighed.

Frozen antler half cylinders were split longitudinally into sections 0.5 to 1.0 cm in diameter. Maceration did not improve counts obtained in a gamma scintillation counter (Packard) and tubes were therefore weighed, packed with antler sample and reweighed. Sample height in the counting tube did not exceed 2.5 cm. Counts per minute exceeded 10 times background for all samples counted and which were subsequently corrected for background activity.

Figure 4.1
Method of sectioning antlers prior to analysis.

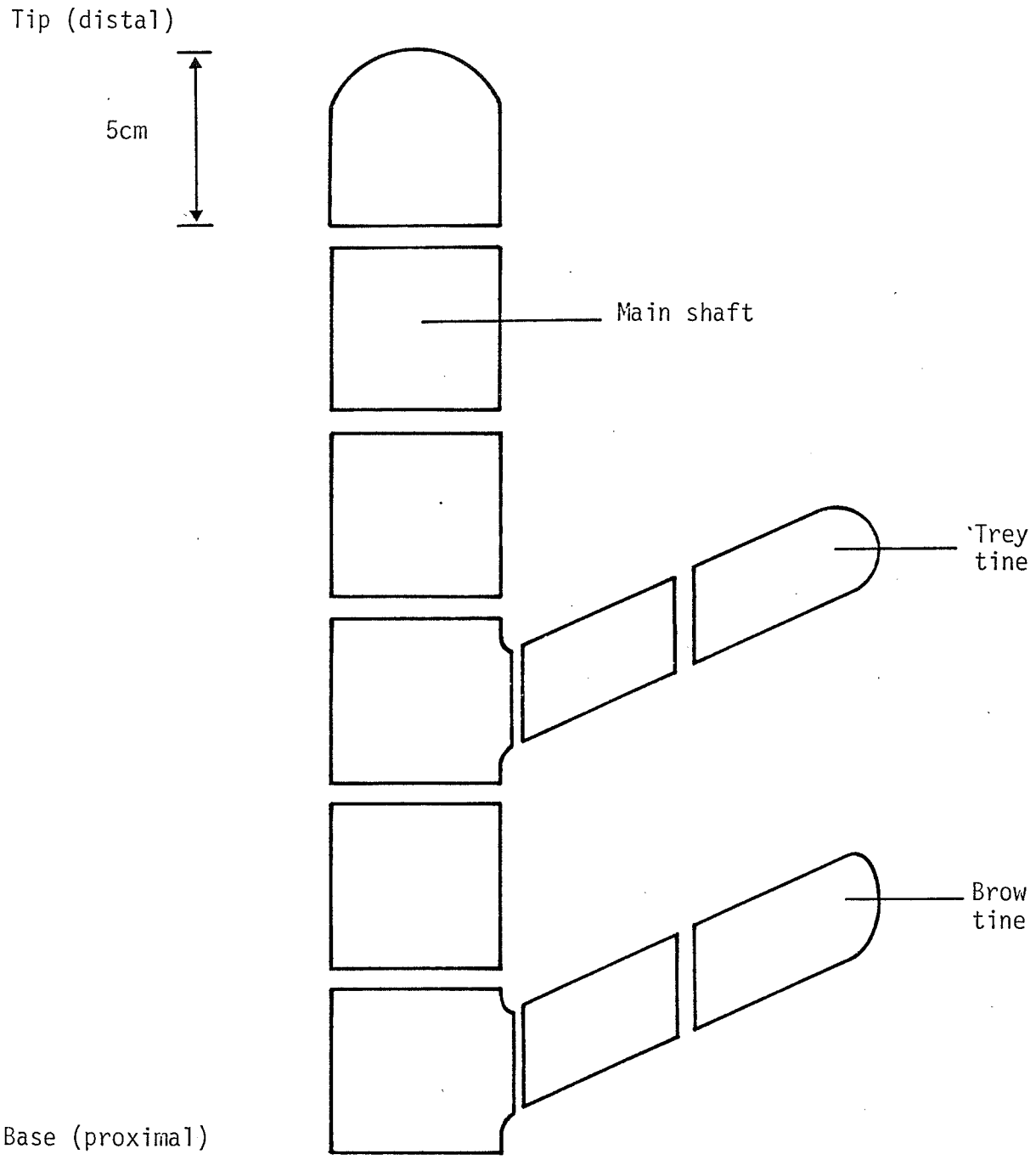
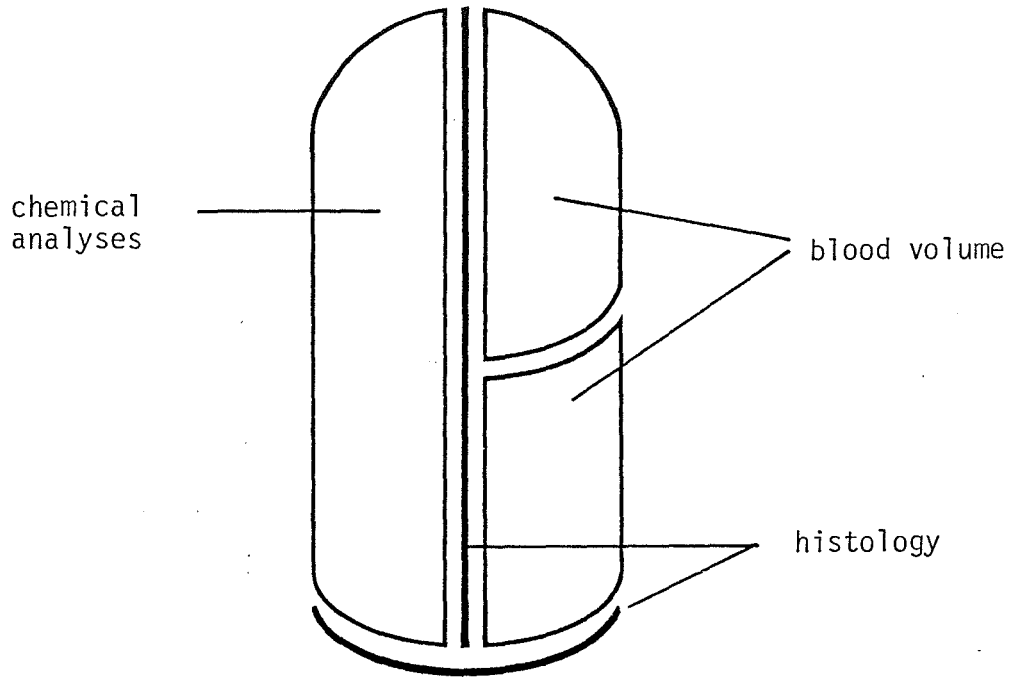
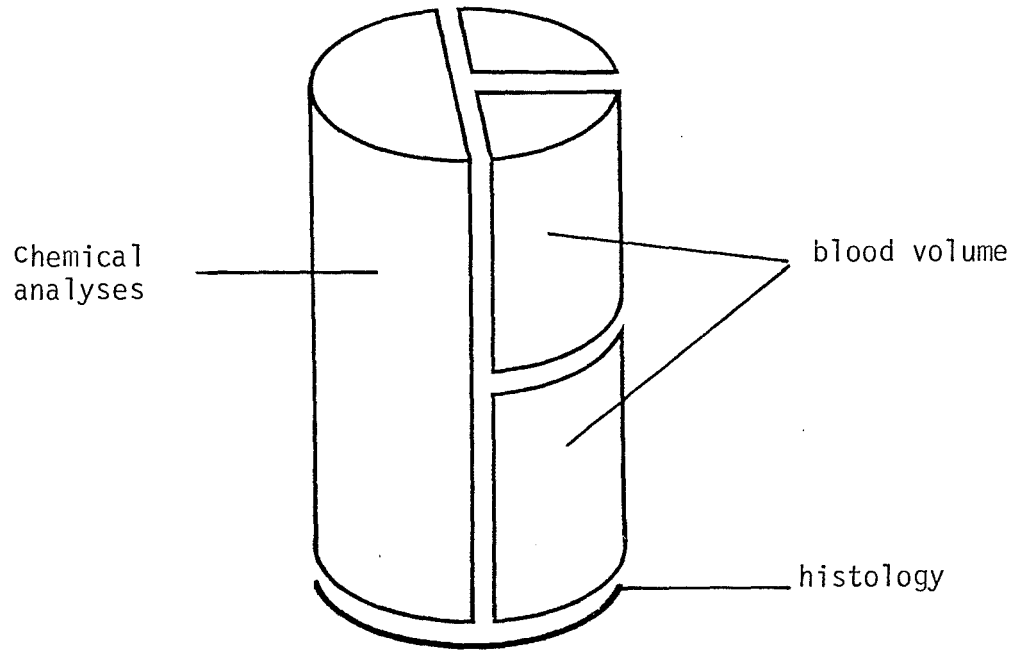


Figure 4.2
Antler sectioning of a) tip sections and b) shaft sections for blood volume, chemical and histological analysis.

a) antler tip sections



b) antler shaft sections



For each antler section, ^{125}I and ^{51}Cr radioactivity was expressed as mean counts per minute per gram (cpm/g) of antler tissue. From the activity of plasma and red blood cells at jugular equilibration, weight of plasma and red blood cells in the antler were calculated. Plasma and red blood cell volume for each antler section were calculated using the mean values of 1.027 and 1.084 for specific gravity of plasma and red blood cells, respectively, reported for sheep and cattle (Swenson, 1977). Plasma and red blood cell volumes were calculated relative to antler section fresh weight and have been expressed as relative plasma volume (RPV) and relative red blood cell volume (RRBCV).

5) Chemical analysis

Half cylinders from each antler section were analysed chemically. Antler tissue was weighed and half cylinders immersed in water for 8 h prior to volume measurement by water displacement. Subsequently each half cylinder was further split longitudinally into slices 0.5 to 1.0 cm in diameter and oven dried at 100°C to constant weight (48 h) for dry matter determination. Fat was extracted by boiling twice for 6 h in petroleum ether and fat-free dry weight determined by re-drying to constant weight. Samples were then dry ashed at 550°C to constant weight (16 h) in a muffle furnace.

The ratio of ash to organic matter (A:R ratio) was used to indicate the degree of mineralization of bone matrix in the antler (Stewart, 1965).

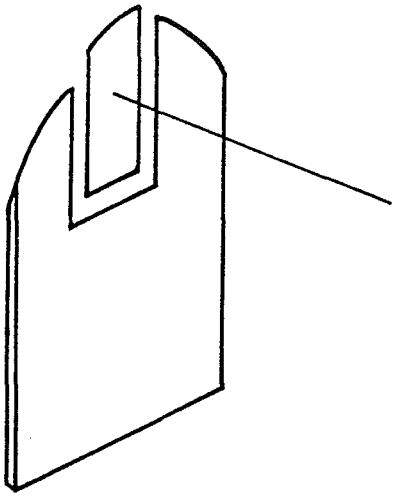
5) Histology

Isotopes were successfully injected into only two of the stags. Lack of success with the other two stags was caused by difficulties associated with catheterisation. Tissue from non-radioactive antlers was used to determine the best methods for fixing, decalcifying and staining. The following procedure was an effective method for preparation of antler tissue.

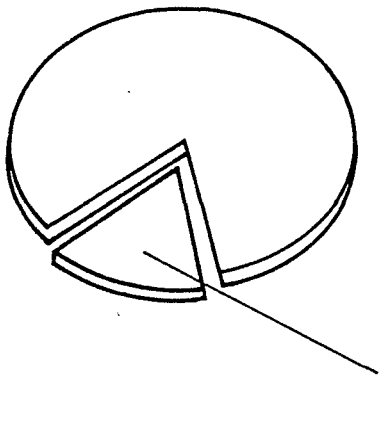
Representative sections were taken from antler shaft sections and from the antler tip (Fig. 4.3), and rinsed in flowing water for three days to remove 10% buffered formalin prior to decalcification in 10% formic acid. Tip sections required no treatment but heavily mineralized sections required up to 2 weeks of decalcification. Two pieces from each

Figure 4.3
Subsampling of antler tissue for histological preparation.

a) Longitudinal section from the antler tip



b) Transverse section from the antler shaft



section were decalcified and degree of demineralization was determined by ease of passage of a needle through one piece of tissue. Decalcification was arrested by soaking in 5% sodium metabisulphite for 24 h. Sections were again rinsed for 3 days in water, which was subsequently removed using a series of ethyl alcohol baths (containing 50%, 70%, 70%, 96%, 96%, 100%, 100%). Alcohol was removed using 2 toluol baths prior to rinsing in 3 paraffin baths. Sections were embedded in paraffin wax prior to sectioning with a microtome (Spencer "820").

Staining was conducted using haemotoxylin and eosin (Culling, 1963) and Ralis tetrachrome (Ralis and Ralis, 1975).

Results and discussion

When stags were fully recumbent difficulties arose in maintaining the short catheters within the jugular vein. Consequently only two stags, a mature stag with regrowth antler and a 2-year-old stag with first growth antler received complete doses of ^{125}I-HSA into the jugular vein. The stag with regrowth antler was also successfully injected with ^{51}Cr-RBC. Antler blood content and mineralization data obtained from these two stags only is discussed below.

1) Antler measurements and sectioning

Both of these antlers were of similar age at date of removal. The regrowth antler was removed 65 days after the date of first (commercial) velvet harvest. The first growth antler was removed 62 days after casting and was assessed to be at the optimum stage for commercial sale. The regrowth antler weighed 0.76 kg and was 27 cm in length, compared with values of 0.81 kg and 41 cm for the first growth antler.

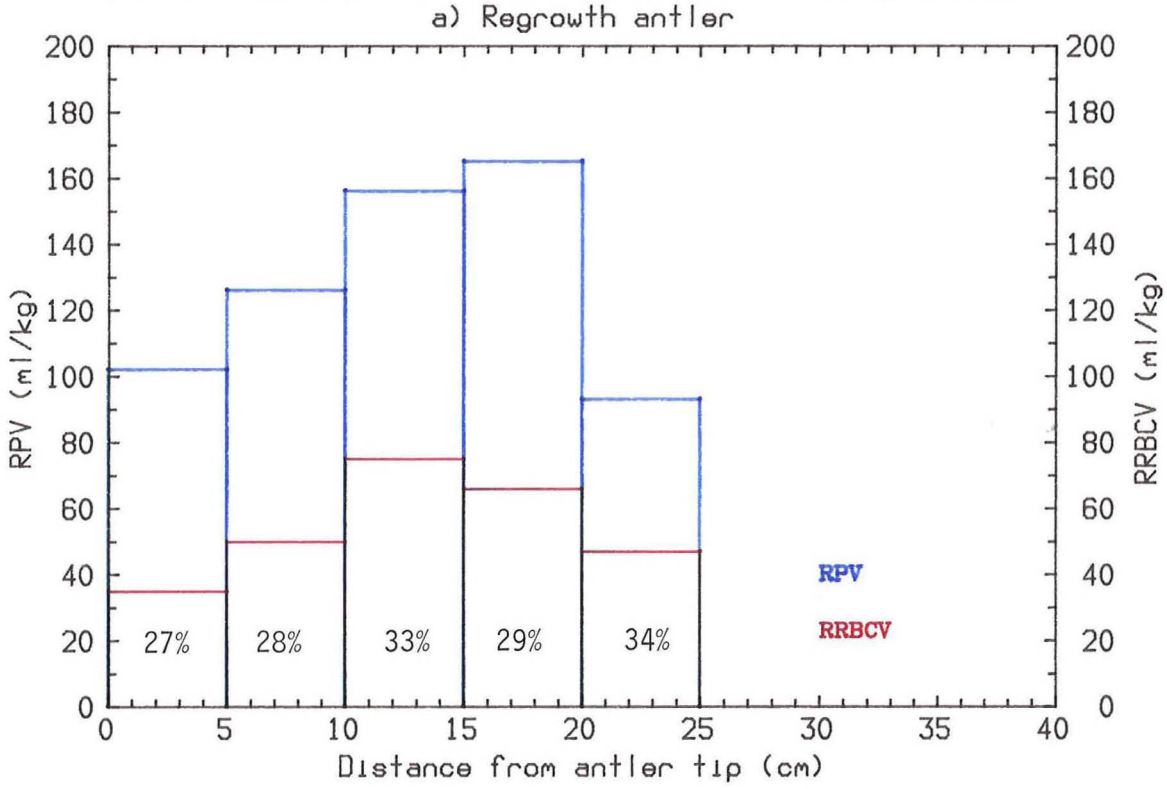
At the time of antler sectioning (30 minutes after removal) blood had not coagulated in the antlers and losses occurred during sectioning. Sectioning antlers whilst fresh was therefore considered impractical. Some method of freezing prior to sectioning and counting was necessary to allow determination of accurate antler blood volume.

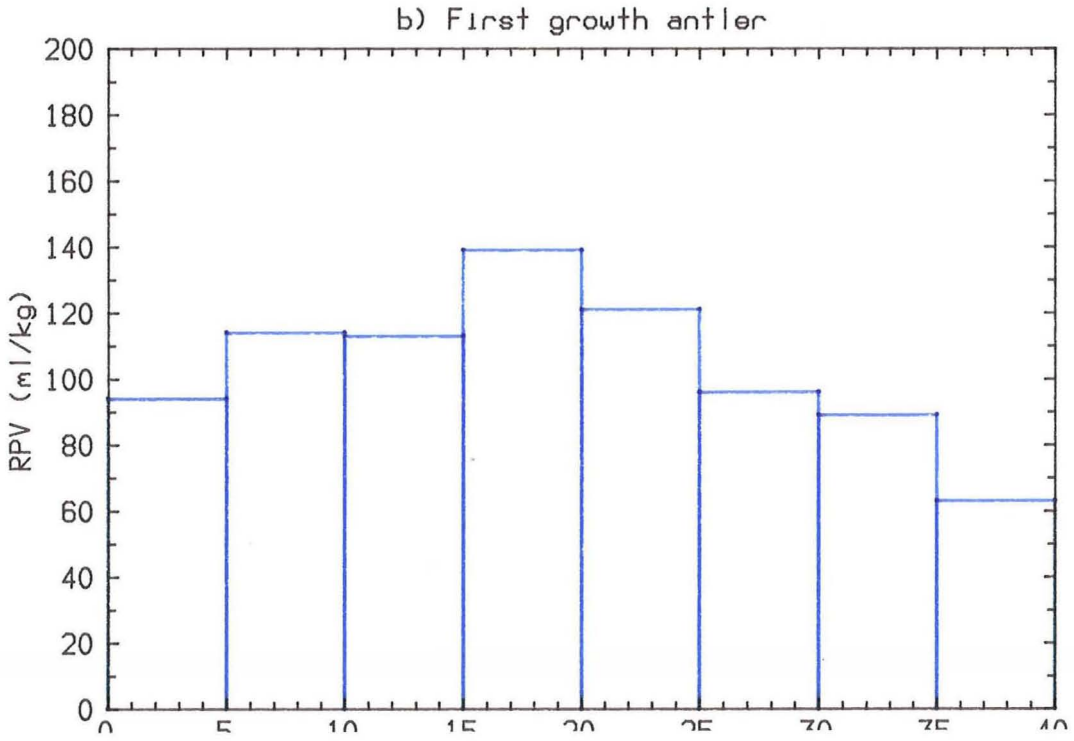
2) Blood volume

Jugular haematocrits were found to be 30.0% in the stag with regrowth antler and 30.5% in the stag with first growth antler.

The patterns of distribution of blood in the two antler shafts are given in Fig. 4.4 (a and b). Relative plasma volume (RPV) only is given for the antler shaft of the first growth antler (Fig. 4.4b). In both antlers, RPV tended to reach a peak 15 to 20 cm below the tip, and then to decrease towards the antler base. Relative red blood cell volume (RRBCV) in the regrowth antler followed a similar pattern. In this antler (Fig. 4.4a) there was little variation in haematocrit within the antler shaft, and therefore no consistent evidence of plasma streaming.

Figure 4.4
Distribution of blood in the shaft of velvet antlers. Haematocrits, together with relative plasma volumes (RPV's) and relative red blood cell volumes (RRBCV's) are presented for a regrowth antler. Relative plasma volumes (RPV's) only are presented for a first growth antler.





Average RRBCV and RPV in the regrowth antler were 52.5 ml/kg and 112.8 ml/kg respectively, with an average relative blood volume (RBV) of 165 ml/kg. The calculated antler haematocrit of the stag was therefore 31.7%, similar to the jugular haematocrit value of 30.0%.

Average RPV in the first growth antler was calculated to be 108.9 ml/kg. If the jugular haematocrit value of 30.5% is applied the average relative antler blood volume (RBV) in the whole antler could be calculated to be 157 ml/kg, similar to the 165 ml/kg measured in the regrowth antler.

3) Mineralization

The pattern of distribution of mineralization in the antler shaft and tines of the two antlers is given in Table 4.1. Tip sections were poorly mineralized, particularly in the regrowth antler, but considerable mineralization had occurred in the subjacent section. In the regrowth antler, degree of mineralization in the shaft below the tip was relatively constant whereas in the first growth antler mineralization increased down the shaft towards the antler base.

Average mineralization of the whole antler was almost identical, with an A:R ratio of 0.75 in the regrowth antler and 0.79 in the first growth antler.

Table 4.1

Pattern of mineralization (A:R ratio) within the shaft and tines of a) a regrowth antler from a mature red stag and b) a first growth antler from a two-year-old stag

	A:R ratio			
Distance from tip (cm)	Shaft	Trey	Bey	Brow
a) Regrowth antler				
0 - 5	0.06	-	0.48	0.41
5 - 10	0.98	-	-	0.75
10 - 15	0.86	-	-	-
15 - 20	0.81	-	-	-
20 - 25	0.96	-	-	-
b) First growth antler				
0 - 5	0.20	0.23	-	0.28
5 - 10	0.70	-	-	-
10 - 15	0.61	-	-	-
15 - 20	0.72	-	-	-
20 - 25	0.76	-	-	-
25 - 30	0.85	-	-	-
30 - 35	0.91	-	-	-
35 - 40	1.10	-	-	-

Conclusions

In spite of the differences in type of antler (first growth versus regrowth antler) both had similar composition in terms of relative blood volume and degree of mineralization. Although this observation was based on only two single antlers it is noteworthy in that it was contrary to the currently held belief in the deer industry, namely that regrowth antlers are excessively mineralized and contain a lower blood "content" than first growth antlers removed for commercial sale, thereby making regrowth antler an "inferior" product.

The regrowth antler from the stag which was injected with both ^{51}Cr-RBC and ^{125}I-HSA showed no evidence of plasma streaming down the antler shaft. On a whole antler basis the proportion of whole blood which was red blood cells (31.7%) agreed closely with the haematocrit of 30.0% obtained in jugular blood. Determination of antler plasma volume and then application of this figure to the jugular haematocrit should therefore give an accurate yet simple method for estimating the distribution and total relative blood volume in the antler. This would eliminate the need for measurement of both plasma and red blood cell volume of antlers using the double isotope technique.

It was concluded that it would be desirable to freeze antlers prior to sectioning to prevent blood loss. Preparation of histological sections from radioactive and frozen tissue is, however, undesirable in terms of both isotope contamination and tissue damage. It was decided, therefore, that a group of stags not used for antler blood volume work would be needed to provide histology data. The techniques described previously proved satisfactory for the fixing, decalcification and staining of antler tissue. The ashing technique, together with use of A:R ratio, appeared to provide a satisfactory description of antler mineralization.

PART B

Experimental methods

1) Experimental outline

a) Nutritional treatments

Eighteen mature red deer stags were provided from a variety of sources by members of the New Zealand Game Industry Association. The exact age of most of the stags was not known but all were older than 3 years. Stags were individually penned (1.6 x 2.4 m pens) in the Lincoln College deer shed and bedded on sawdust. Animals were exposed to natural lighting and adjusted to pelleted rations over periods ranging from 2 to 3 weeks as they became available from farms in late July. Stags were allocated, using a stratified random design on the basis of previous velvet antler weight (where known) and current liveweight, to three nutritional treatment groups to equalise mean velvet antler weight and liveweight. The trial commenced in August. One group (A) was offered a control diet; a second group (B) was offered a similar diet with a high protected-protein content (formaldehyde treated linseed meal) and group C a low calcium diet. Unfortunately it was not possible to obtain a diet with very low calcium content (i.e. 1.0 g/kg DM) and still retain a diet with high protein and energy content. Consequently the diet offered to stags in group C contained a level of calcium of 3.0 g/kg DM. The composition of the diets is given in Table 4.2. Diets were isocaloric (12.5 megajoules of metabolisable energy (MJME) per kg DM) and were based on wheat, peas and linseed meal. Rations were offered daily at the rate of 0.95 MJME per kg of metabolic bodyweight ($W^{0.75}$). It was anticipated that this would allow a liveweight gain over the trial of at least 300 g/day (P.F. Fennessy, pers. comm., 1980). Stags were weighed weekly and individual dry matter intake recorded daily.

Table 4.2

Ration formulation and protein, calcium and phosphorus contents of three pelleted rations offered to groups of pen fed red deer stags.

Ration formulations	A Control	B High protein	C Low calcium
Barley	-	10.0	-
Wheat	66.8	40.0	74.0
Wheat pollard	20.0	-	15.0
Peas	10.0	10.0	10.0
"Protected" linseed	-	36.0	-
Limestone	2.2	2.4	-
Salt	1.0	1.0	1.0
Vitamin and trace element pre-mix was added to all rations			
Analysis			
Crude protein (g/kg DM)	130	199	132
Calcium (g/kg DM)	7.6	6.7	3.0
Phosphorus (g/kg DM)	5.0	5.5	5.7

b) Antler sampling

Within each treatment group the six stags were allocated at random to six dates for removal of the developing antler. The left antler was removed from one stag in each treatment group at 28, 42, 56, 70, 91, and 112 days after casting of hard antler remnants. These harvesting times were designed so as to provide maximum information about antler composition at the stage of removal for commercial sale, which normally occurs between 60 and 70 days after casting of hard antler buttons. The contralateral antler was allowed to harden and was removed at commencement of stripping of velvet. Any regrowth of the left antler was also removed at this time.

The date at which each stag attained the optimal stage for commercial antler harvest (bulbing to form royal tines) was recorded. This observation was made on the right antler which was destined to remain intact until velvet stripping.

2) Antler removal, measurements and sectioning

All antlers were analysed chemically. In addition, velvet antlers from stags in group A were used to provide tissue for a histological study of antler growth and were therefore processed while fresh. Blood content was measured in the velvet antlers from stags in groups B and C. The method of sedation, ^{125}I-HSA administration and antler removal were as described in Part A although only one velvet antler was removed from each stag. Hard and regrowth antler was removed from all stags once velvet fraying had commenced. For this operation stags were less tractable than previously and approximately 5 ml of 2% Rompun was required for sedation.

Antler weight, tine and beam lengths were recorded as in Part A. In addition antler beam circumference was measured immediately above the brow and bey tine. Brow tine circumference was measured immediately above the point of intersection with the main beam.

All antlers were sectioned at approximately 5.0 cm intervals from tip to base as in Part A (Fig. 4.1). Control group antlers (group A) were sectioned within 30 minutes of antler removal and histological sections obtained as previously outlined (Fig. 4.2). Radioactive antlers from group B and C stags were frozen, weighed and sectioned within one week. One half cylinder was refrozen for later chemical analysis, while the other was sectioned transversely at 2.5 cm intervals (Fig. 4.2). These two half cylinders were then bisected longitudinally and an upper and lower quarter prepared for ^{125}I counting. The remaining tissue was refrozen and retained for later analysis.

No histological or blood volume studies were undertaken with hard or regrowth antlers and all were sectioned as above, with one half cylinder being analysed chemically and the other half being retained.

3) Blood volume

Blood and antler sample preparation for the counting of ^{125}I radioactivity was identical to that described in Section 4, Part A. Stags from groups B and C were injected intravenously with approximately 20 μCi of radio-iodinated human serum albumin (^{125}I-HSA) to determine antler plasma volume. A longer catheter (600 mm, Bard, England) inserted through a 14 g needle was used instead of the shorter catheters used previously. These antlers were subsequently sectioned while frozen to minimise loss of blood, and blood volume calculated using plasma volume and haematocrit (Hobbs, 1967). For each antler section, antler whole blood volume was calculated from plasma volume and haematocrit.

4) Chemical analysis

a) Antler samples

Initially half cylinders from each antler section were analysed. However, in order to provide more data on a zone of mineralization in the antler tip recourse was made to stored tissue for further analysis. The two sections from the antler tip (0 to 5 cm and 5 to 10 cm) were further subdivided and analysed at 2.5 cm intervals. Methods for measurement of volume, fat content and dry matter and ash were described previously (Part A). Ashed samples were ground through a 1mm sieve and digested for 15 min in 2N HCl at 60°C. Inorganic phosphorus (in ashed samples) was measured using an automated technique, based on the method of Hurst (1964) and adapted to the Technicon AutoAnalyzer system by Kraml (1966). Calcium content of samples was measured following the method of Willis (1960). Samples were diluted at a rate of 1 to 49 with strontium chloride and calcium concentrations determined using a single beam atomic absorption spectrophotometer (Instrumentation Laboratory 151). Calcium and phosphorus concentrations were measured in all sections of all velvet antlers and in all sections in three hard antlers randomly selected from each nutritional treatment group. A:R ratios were calculated for each antler section, and for each complete antler.

b) Feed samples

Samples were oven dried at 60°C for 48 h and DM% determined. After grinding to 1 mm sieved size, nitrogen concentration was determined using standard Kjeldahl digestion techniques used in a Tecator digestion and distillation system. For this analysis 0.5 g of dried ground sample and 1 g of catalyst (19:1; K_2SO_4:$CuSO_4.{}_5HSO$) was added to a constant boiling mixture of 20 ml H_2SO_4 and 5 ml H_2O_2. Samples were also dry ashed and calcium and phosphorus levels measured as described in the above section. The degree of protection of dietary protein was to be assessed from its degradability, using the dacron bag technique described by Mehrez and Orskov (1977). Unfortunately stored feed samples were destroyed by rodents before this could be undertaken.

5) Histology

Sectioning, tissue subsampling, decalcification and staining was carried out using the method described in Part A.

Colour photographic slides to record macroscopic details were obtained directly from microscope slides. Photomicrographs were taken on slide transparency film (Kodak Ektachrome 64) using a light microscope (Olympus BH-2). Both colour and black-and-white prints were prepared from colour slides.

After analysis it became apparent that further sections would be valuable to demonstrate a particular aspect of antler morphology. Further sections were therefore subsequently prepared from the frozen quarter sections remaining of (previously radioactive) antlers from groups B and C stags. Sections were taken 15, 30 and 45 cm from the antler tip of 70, 91 and 112 day antlers in group B and from 91 and 112 day antlers in group C.

6) Blood sampling and testosterone assay

Blood samples (10 ml) were obtained weekly by jugular venepuncture into heparinised (14 i.u. per ml) evacuated glass tubes. Initially all stags were bled, but due to problems of temperament with some stags and the associated possibility of antler damage, only five were bled throughout antler growth. Blood samples were immediately chilled and later centrifuged at 3000 rpm for 15 min. Plasma was aspirated and stored at -20°C. Testosterone concentrations in plasma were measured by

direct radioimmunoassay (Garnier, Cotta and Terqui; 1978) using non-extracted techniques described by Schanbacher and D'Occhio (1982).

7) Data analysis

Two deaths, caused by Malignant Catarrhal Fever, occurred in Group B after sampling at 4 and 8 weeks. No suitable replacement stags were available. Since both stags were healthy at time of antler harvest, blood content and mineralization data from velvet antlers have been included.

The ratio of ash to organic matter (A:R ratio) was used to indicate the degree of mineralization of bone in the antler (Stewart, 1965). To provide estimates for growth and ash deposition and to allow for individual variation in antler size the length, weight and ash content at the various stages of growth have been expressed as a proportion of that of the contralateral hard antler at velvet stripping. Antlers were removed approximately 1 cm above the coronet, and these portions of antler were therefore accounted for in determining rates of antler growth in length.

Data were analysed using the general statistical program Genstat, 4:04 (Rothamstead Experimental Station) and Minitab, version 81.1 (Pennsylvania State University, 1982).

Results

1) Effects of treatment

Feed intake, (Fig. 4.5a) and liveweight gain, (Fig. 4.5b) were similar for the three treatment groups and results for these parameters have been pooled. Mean level of feed intake rose from 1.75 ± 0.048 kg DM/day (0.58 MJME/kg$W0.75$/day) in mid August, to 2.75 ± 0.055 kg DM/day (0.95 MJME/kg $W0.75$/day) in early November, with full consumption of the ration offered occurring in the latter stages of the trial (Fig. 4.5a). Mean liveweight at the start of the trial was 121.0 ± 2.67 kg, had risen to 125.0 ± 2.93 kg at the time of antler casting and rose further to 179.1 ± 5.08 kg at velvet stripping (Fig. 4.5b). Mean liveweight gain for the duration of the trial and over the period of antler growth was 304 ± 18.7 g/d and 327 ± 16.9 g/d, respectively.

Heavier mean hard antler weights were attained by stags in group B (1.32 ± 0.220) than by stags in groups A (1.12 ± 0.162) or C (0.99 ± 0.117) but these differences, although large, were not statistically significant even when liveweight was used as a covariate. Similarly, no differences in hard antler length, period of antler growth, or degree of mineralization could be attributed to dietary treatment (Table 4.3). Treatment results were therefore combined in order to describe the pattern of antler growth and composition.

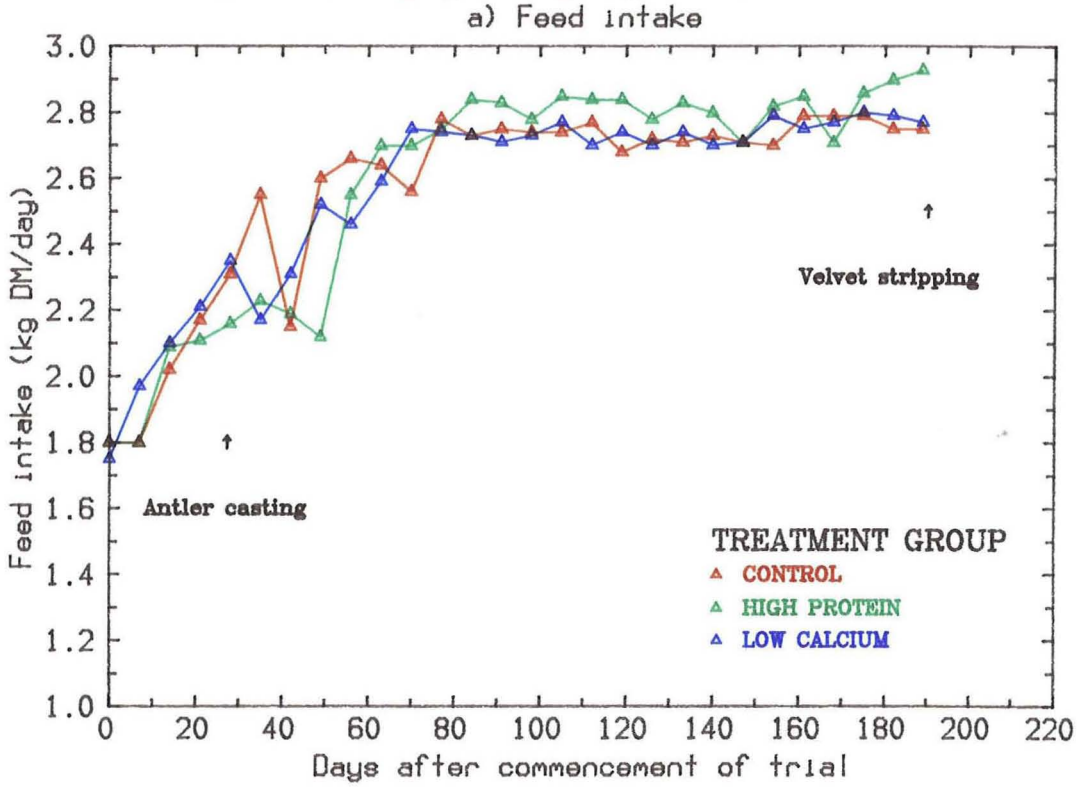
A possible consequence of early velvet antler removal was that some form of compensatory growth might have occurred in the contralateral hard antler. The data in Table 4.4 do, in fact, suggest that there may be a non-significant trend towards heavier hard antlers in those stags from which velvet antler was removed during early growth.

2) Growth of the antler

a) Antler casting to velvet stripping

The period of antler growth, from casting to velvet stripping, ranged from 142 to 187 days with a mean of 164 days. Antlers reached a stage suitable for commercial removal and sale (subjectively assessed) at an average of 64 days (range, 57-79 days).

Figure 4.5
Pattern of a) feed intake and b) liveweight change in three groups of red deer stags offered three treatment rations throughout the period of antler growth. For the control and low calcium treatment groups n = 6 and for the high protein group, n = 4.



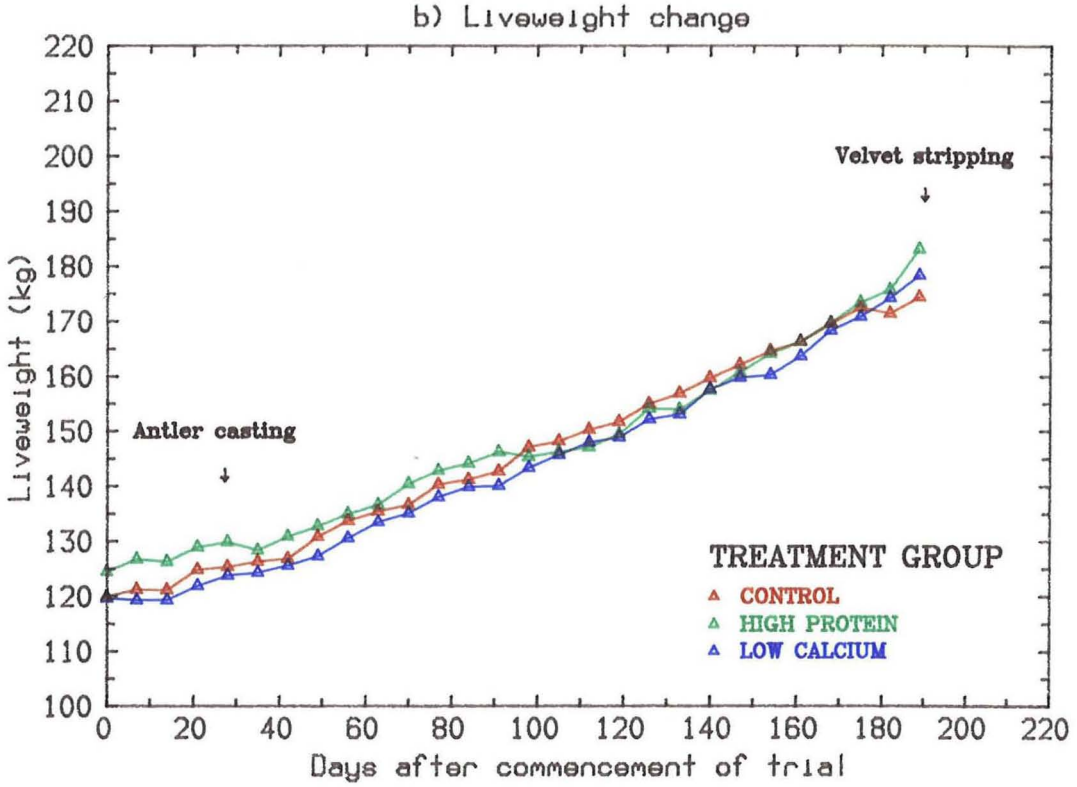


Table 4.3

Mean (± S.E.D.) hard antler weight, length, degree of mineralization, casting date, velvet stripping date and duration of the antler growth period in mature red deer stags on three nutritional treatments.

	Treatment Group			
	Control	High protein	Low calcium	S.E.D
No of stags	6	4	6	
Weight (kg)	1.12	1.32	0.99	± 0.214
Length (m)	0.71	0.71	0.72	± 0.326
Mineralization (A:R)	1.76	1.72	1.72	± 0.068
Mean casting date	8 Sept	19 Sept	15 Sept	± 8.6
Mean velvet stripping date	20 Feb	27 Feb	26 Feb	± 4.1
Duration of antler growth (days)	166	161	164	± 8.4

Table 4.4

Mean (± S.E.D.) hard antler weight, length, degree of mineralization and liveweight (at hard antler removal) following early removal of the contralateral antler at two and three weekly intervals during the period of velvet antler growth.

	Date when contralateral antler removed (Days after casting)						
Hard Antler	28	42	56	70	91	112	S.E.D.
No of stags	2	3	2	3	3	3	
Weight (kg)	1.38	1.25	1.32	0.99	1.11	0.85	± 0.317
Length (m)	0.73	0.72	0.76	0.68	0.72	0.69	± 0.046
Mineralization (A:R)	1.72	1.65	1.85	1.69	1.79	1.72	± 0.091
Liveweight (kg)	162.3	176.7	201.0	178.7	190.2	167.5	± 19.69

The pattern of distribution of antler casting was normally distributed (Fig. 4.6a), about a mean of 13 September, and with a range of 53 days from 18 August to 11 November. Left and right antler buttons from each stag were usually cast in close succession (mean difference, 1.7 ± 0.5 days). Stripping of velvet on the single hard antler and on any regrowth from the contralateral antler stump occurred simultaneously and as a result both were removed on the same day. Overall, the date of velvet stripping was much more tightly distributed than date of antler casting (Fig. 4.6b) with a mean date of 24 February and a range of 24 days from 9 February to 5 March. A significant negative correlation was obtained between date of antler casting (number of days after commencement of the trial) and length of the period of antler growth ($r = -0.88$, $P < 0.01$).

b) Tine development

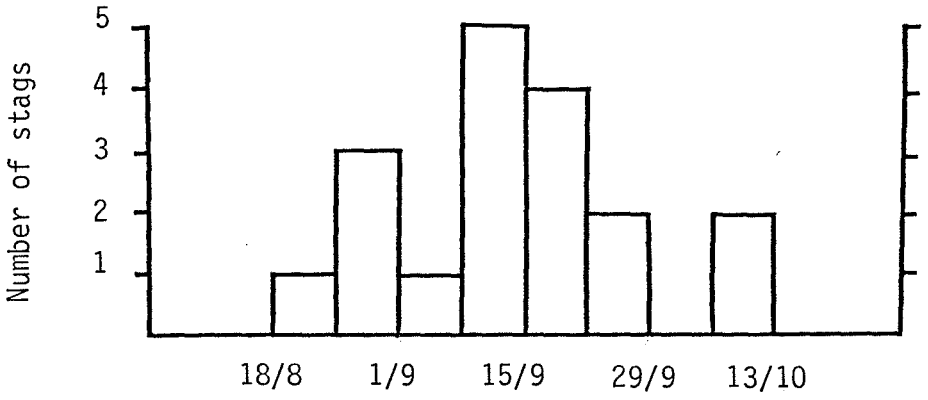
Total number of tines on individual hard antlers varied considerably between stags, with a mean of 4.1 ± 0.22 (range, 3-6). Brow tines were present on all velvet and hard antlers. However, bey tines were present on only 9 of the 18 harvested velvet antlers and 7 of the 16 stripped hard antlers. This may reflect variation in genetic background of these animals. Banwell (1970) claimed that stags descende from Rakaia liberations usually lack a bey tine whereas those from Otago and Nelson herds frequently have a full complement of tines. Trey tines were present on all 16 hard antlers but because they do not form until approximately 44 days after casting (Corson and Fennessy, 1985) none wer present on antlers removed during the early stages of development. Roya tines varied in number from 1 to 3 with a mean of 1.6 ± 0.15. Six stags had an unbranched main beam which was counted as a single royal tine.

c) Increase in length

Hard antlers attained a mean stripped length of 0.71 ± 0.013 m (range, 0.65-0.79 m). Growth in length of the main shaft has been expressed as a percentage of final stripped antler length (Fig. 4.7). Twenty eight days after casting antlers had attained 21.2% of final length and after 112 days 94.7% of growth in length had been completed. Insufficient data points were obtained in the early and latter stages of the antler growth period to fit a growth curve to the data and between 2 and 112 days after casting orthogonal polynomials were used to test for

Figure 4.6
Distribution pattern of date of hard antler button casting and velvet stripping in pen fed red deer stags

a) Hard antler button casting (n = 18)



b) Velvet stripping (n = 16)

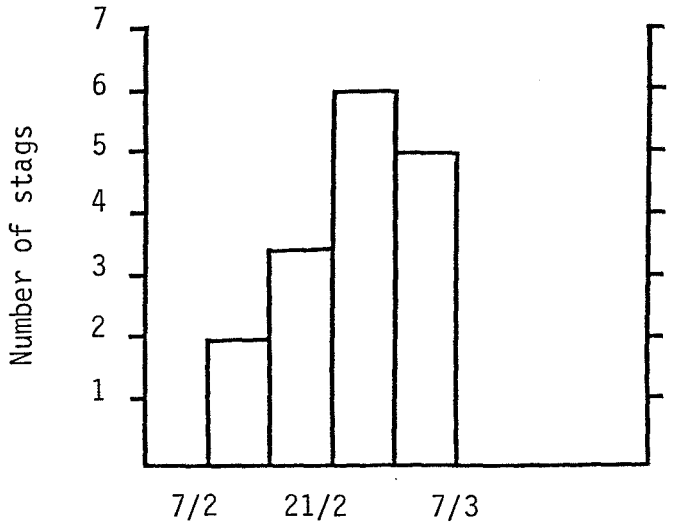


Figure 4.7

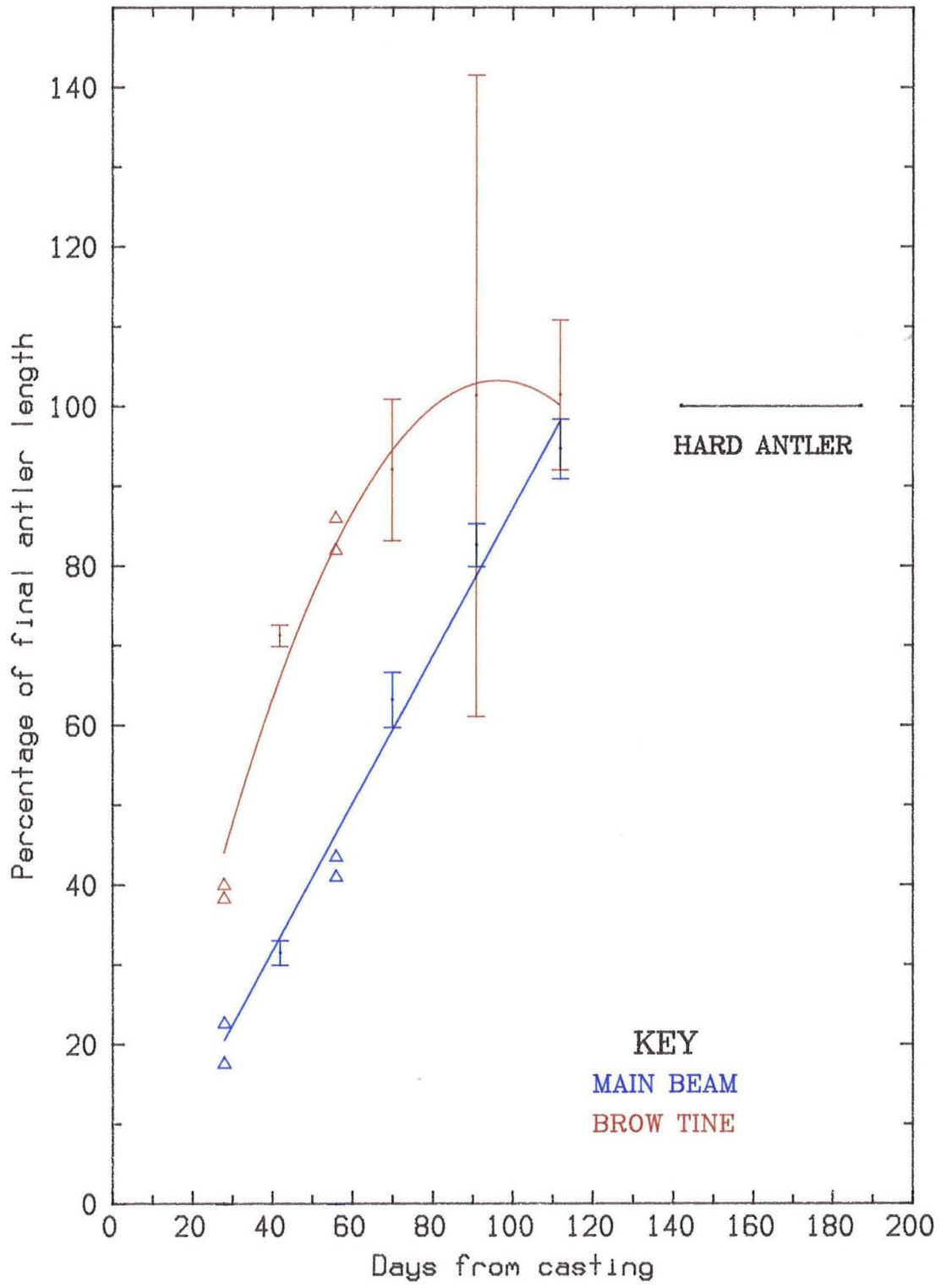
Elongation of the antler shaft and brow tine, where Y = the percentage of final antler length and X = days from antler casting.

Antler shaft; $Y = -5.31 + 0.925\ X$ ($r = 0.98^{**}$, RSD = 5.3)

Brow tine; $Y = 14.34 + 2.44\ X - 0.0133\ X^2$

Note: The brow tine equation was fitted through the means

Figure 4.7
Elongation of the antler shaft and brow tine (mean ± S.E.M.). Length of harvested antler shaft and brow tine has been expressed as a percentage of that of the contralateral antler at velvet stripping. The range of hard antler stripping dates is indicated by the horizontal line. See facing page for equations.



non-linearity. There was no significant departure from linearity of antler elongation velocity and a mean rate of antler elongation of 0.62 cm/day was calculated. However, the data (Figure 4.7) do also suggest that between casting and the 28th day, growth was slower and that by 112 days increase in length had almost ceased.

Considerable variation in brow tine length existed between stags (mean, 0.23 m; range, 0.12-0.31 m), and between contralateral brow tines from the same stag. No significant relationship could be fitted to describe rate of elongation of the brow tine. However a quadratic equation was fitted through the means and suggests a different pattern of growth to that of the antler shaft (Fig. 4.7). At 28 days 39.2% of total length had been reached and at 56 days, when 84.0% of growth had occurred, growth appeared to slow. Although not linear, growth between 28 to 56 days after casting has been estimated at 0.53 cm/day. At 70 days growth was almost completed at 92.1% of final brow tine length (Fig. 4.7).

The mean diameter of the main shaft was significantly greater than that of the brow tine (3.61 ± 0.146 versus 2.89 ± 0.111 cm, respectively; $t = 4.95$, $P < 0.001$, $df = 30$).

d) Increase in weight

The mean weight of stripped hard antler was 1.12 ± 0.092 kg (range, 0.55-1.90 kg). Antler growth in weight, expressed as a percentage of final stripped antler weight (Fig. 4.8) was close to linear ($P < 0.01$) over the sampling period from 28 to 112 days. As with growth in length, increase in weight was slow in the initial stages increasing from 25.2% of hard antler weight at 28 days post casting to 127.4% at 112 days. This represents a mean antler growth rate for individual antlers of 13.7 g fresh weight/day. Between 112 days and date of hard antler harvest (164 days) antler weight decreased.

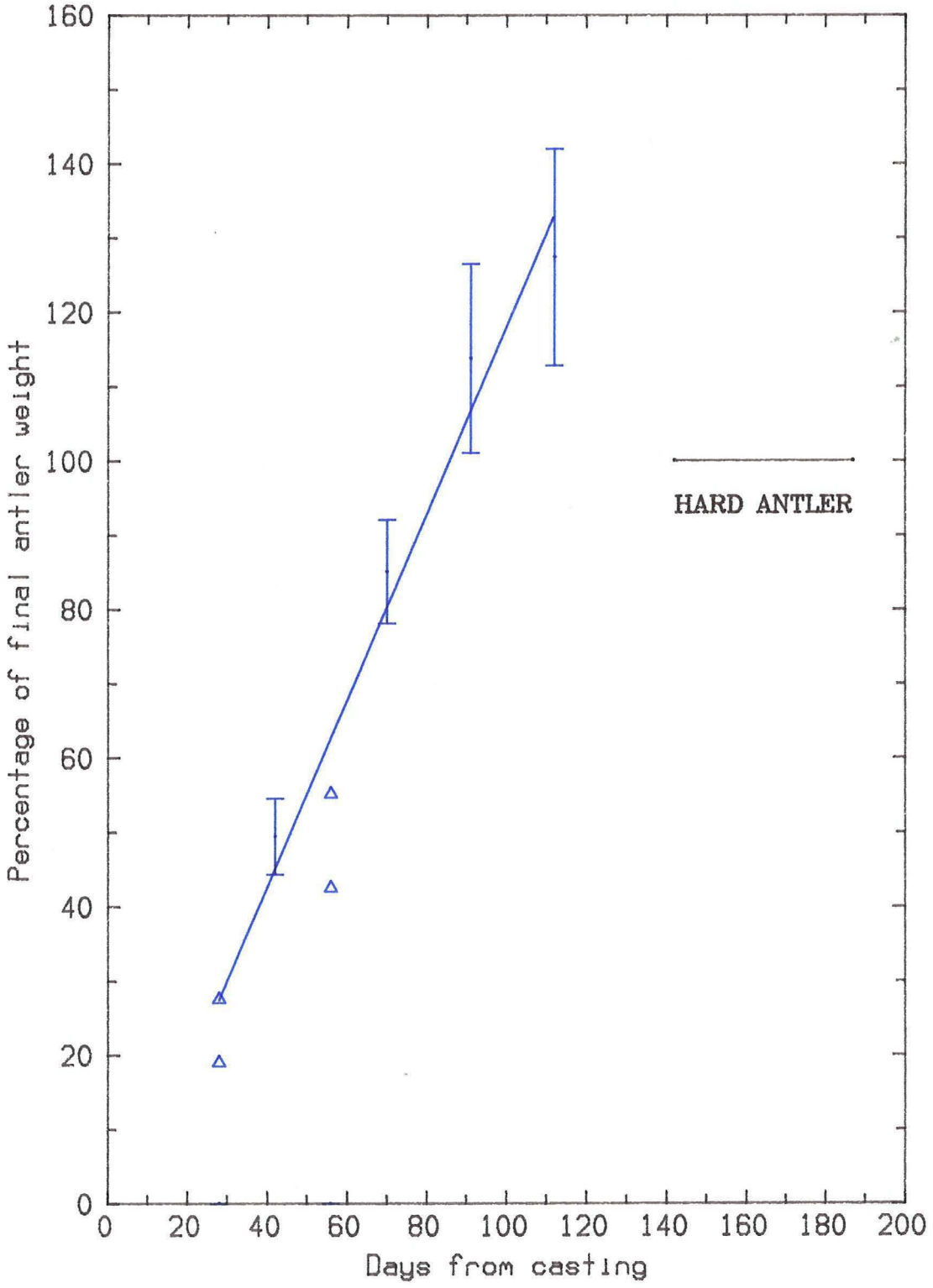
e) Antler regrowth

Antler tissue regrew from the velvet antler stump in 11 of the 16 stags and was removed at velvet stripping. The extent of regrowth was related to date of antler harvest (Table 4.5); the earlier the date of harvesting, the greater the amount of regrowth. No regrowth of antler tissue occurred when velvet antlers were removed later than 11 December,

Figure 4.8
Antler growth in weight, where Y = the percentage of hard antler weight and X = days from antler casting.

$Y = -7.70 + 1.26\ X$ ($r = 0.92^{**}$, RSD = 15.9)

Figure 4.8
Antler growth in weight (mean ± S.E.M.). Weight of harvested antlers has been expressed as a percentage of the contralateral antler weight at velvet stripping. The range of hard antler stripping dates is indicated by the horizontal line. See facing page for equation.



or from the stump of antlers removed 112 days after antler casting.

Table 4.5

Regrowth antler weight following early removal of one antler at varying stages of growth. Regrowth antler weight has been expressed as a percentage of the hard stripped weight of the contralateral antler.

	Date of antler removal (Days from hard antler casting)					
	28	42	56	70	91	112
No of stags with regrowth antler	2	3	2	2	2	-
Mean regrowth antler weight (proportion of hard antler)	48.0	42.8	31.0	19.9	19.3	-

Antler tissue which regrew to a height greater than 1 cm above the abscission line was defined as "regrowth antler", and removed.

3) Antler blood volume

a) Distribution in the antler shaft and brow tine

The amount of blood contained in the antler has been expressed as relative blood volume (RBV; ml/kg fresh weight). The distribution of blood in the antler shaft and the brow tine, in antlers removed at six stages of growth, is given in Tables 4.6 and 4.7, respectively. In antlers at all stages of growth RBV's were low in distal sections 0 to 5 cm from the antler tip (Table 4.6). RBV's varied considerably in the shaft of antlers between 28 and 70 days after casting. RBV's were low in the basal 10 cm of the shaft of antlers removed at 91 and 112 days of growth. The oldest antlers sampled (112 days) tended to have a lower RBV than those aged between 28 and 91 days.

There was no change in RBV with location in the brow tine (Table 4.7). There was however a time-related trend, with RBV's generally being higher in the brow tines of 28 to 56 day old antlers (range, 37-138 ml/kg), but declining to low levels in 91 to 112 day old antlers (range, 7-31 ml/kg)

Table 4.6

Blood distribution in the antler shaft of antlers harvested at 6 stages of growth. Blood volumes have been expressed as relative blood volume (RBV). Antlers were sampled at 5cm intervals from the antler tip.

Distance from antler tip (cm)	Relative blood volume (RBV)											
Age of antler (days)	28		42		56		70		91		112	
Stag ID	184	53	451	405	101	627	10	231	887	622	60	87
0- 5	78	71	84	49	115	92	132	121	277	106	59	19
5-10	175	185	142	184	273	144	239	226	267	260	96	33
10-15	133	188	209	185	204	148	223	139	206	250	64	33
15-20		158	189	227	330	196	215	73	380	202	81	27
20-25				153	188	165	220	149	279	127	101	33
25-30					153	242	118	157	349	183	171	41
30-35					186		227	46	175	210	120	136
35-40							225		277	298	217	83
40-45							144		201	180	176	130
45-50							161		43	136	117	33
50-55										143	27	19

Table 4.7

Blood distribution in the brow tines of antlers harvested at 6 stages of growth. Blood volumes have been expressed as relative blood volume (RBV). Brow tines were sampled at 5cm intervals from the tip.

Distance from brow tine tip (cm)	Relative blood volume (RBV)											
Age of antler (days)	28		42		56		70		91		112	
Stag ID	184	53	451	405	101	627	10	231	887	622	60	87
0-5	63	81	60	54	50	52	26	20	15	10	16	7
5-10	74	54	138	116	54	37	60	26	18	18	18	7
10-15				79	93	99	49	31	21	17	13	20
15-20					127		28	58	31	22	19	

b) Relative blood volume in the antler shaft and brow tine

In the antler shaft total RBV appeared to increase between 28 and 91 days after commencement of antler growth and subsequently to decline (Fig. 4.9a). This relationship was best described by a significant quadratic equation ($P < 0.05$).

In the brow tine (Fig. 4.9b) the pattern of change in total RBV was best described by an equation with cubic components ($P < 0.01$). RBV was highest between 28 and 56 days after casting, but declined rapidly to low levels after 91 days of growth.

A relationship between blood volume and antler weight was examined (Fig. 4.10a). During the period when antler growth in length was occurring (between 28 and 91 days) blood volume increased linearly ($P < 0.01$) with increasing antler weight, at a rate of 225 ml/kg. This relationship did not hold for antlers removed after 112 days of growth and these were not included when deriving the equation. A relationship was also demonstrated within the brow tine where blood volume increased linearly ($P < 0.01$) with increasing brow tine weight (28 to 56 days) but at a lower rate of 110 ml/kg (Fig. 4.10b).

c) Relative blood volume in the whole antler

The relationship between total blood volume and antler weight was similar to that described for the antler shaft in Figure 4.10a. Between 28 and 91 days of growth total blood volume increased linearly with increasing antler weight and was described by the following equation.

$$Y = -29.3 + 194 \quad X \quad (r = 0.97^{**}, \text{RSD} = 17.9)$$

where Y = total blood volume and X = weight of antler tissue
Over this period the rate of increase in blood volume of the whole antler was 194 ml/kg.

4) Antler mineralization

a) Antler shaft

The density of organic matrix (ratio of fat free organic matter to volume, R:V, Fig. 4.11a) and of ash (ratio of ash to volume, A:V, Fig. 4.11b) was used to describe changes in composition within the antler shaft. Antler tips had high R:V (Fig. 4.11a) but low A:V ratio

Figure 4.9

Relationship between relative blood volume (RBV) and age of antler, in the antler shaft and brow tine. Y = RBV (ml/kg) and X = age of antler (days from casting).

Antler shaft;

$$Y = 42.28 + 4.21\ X - 0.0323\ X^2 \quad (r = 0.59^*,\ RSD = 38.8)$$

Brow tine;

$$Y = -120.25 + 11.08\ X - 0.182\ X^2 - 0.000839\ X^3$$

$$(r = 0.95^{**},\ RSD = 11.3)$$

Figure 4.9
Relationship between relative blood volume (RBV) and age of antler in a) the antler shaft and b) the brow tine of antlers removed at six stages of growth. See facing page for equations.

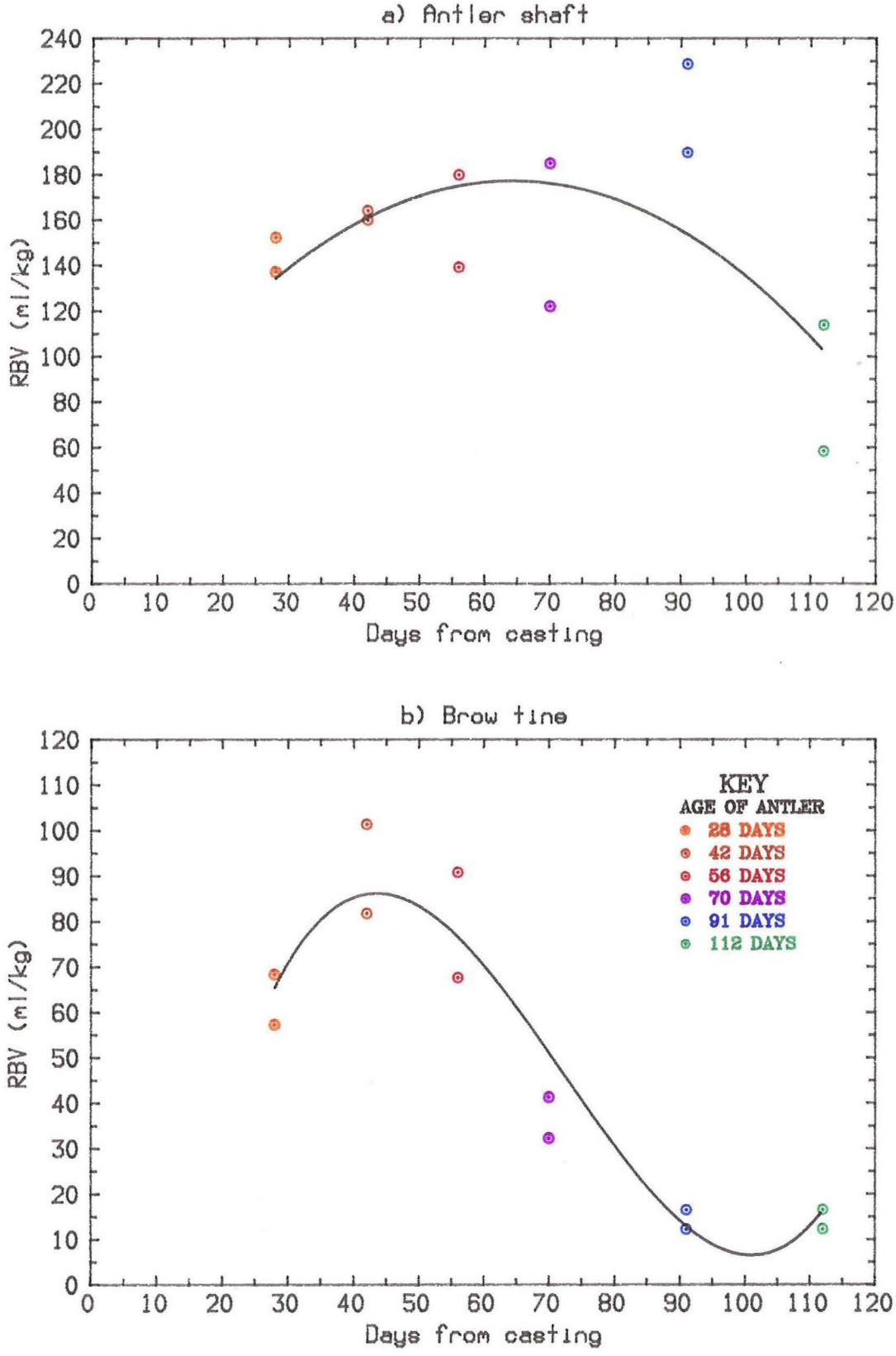


Figure 4.10

Relationship between total blood volume and antler weight in the antler shaft and brow tine. Y = total blood volume (ml) and X = antler weight (kg).

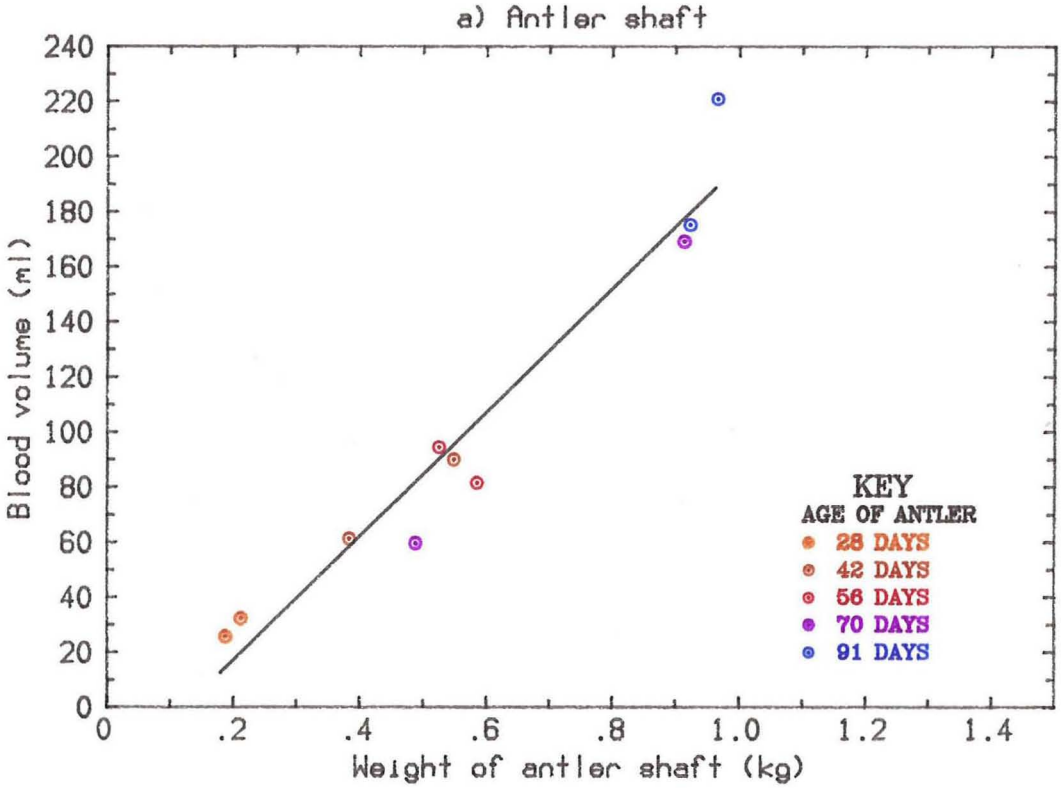
Antler shaft;

$Y = -27.94 + 225x$ ($r = 0.97^{**}$, RSD = 17.4)

Brow tine;

$Y = -1.68 + 110\ X$ ($r = 0.96^{**}$, RSD = 1.2)

Figure 4.10
Relationship between blood volume and antler weight during the period of elongation in a) the antler shaft and b) the brow tine. See facing page for equations.



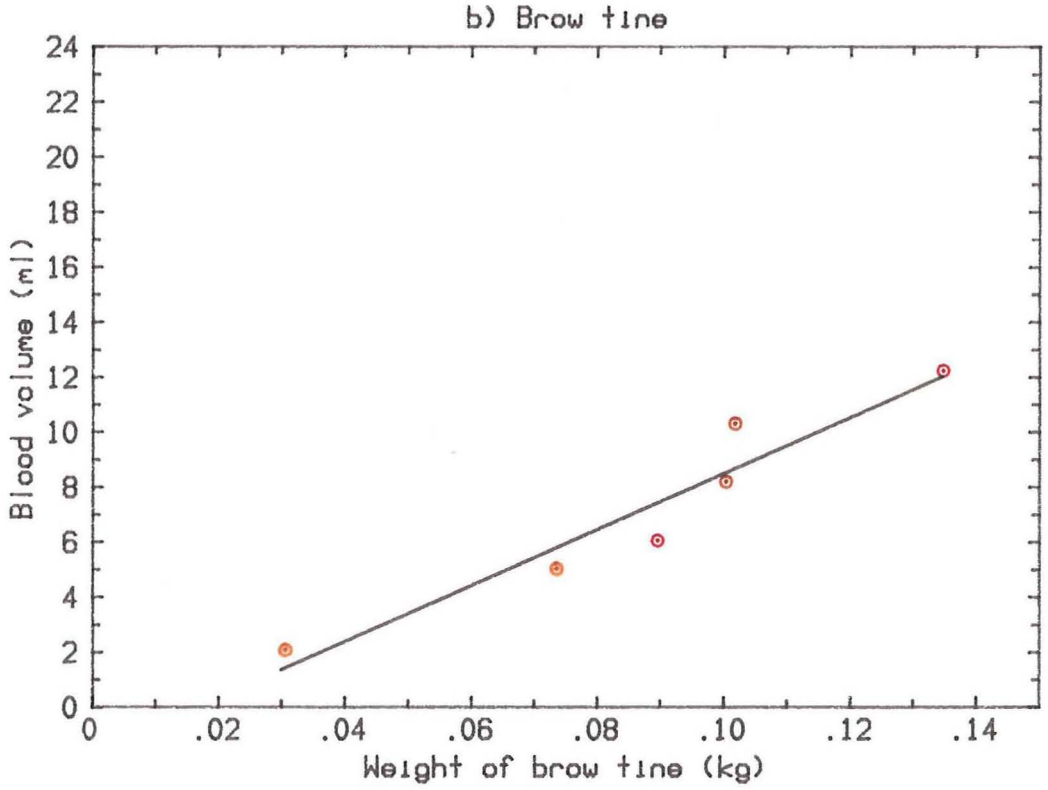


Figure 4.11

a) Ratio of fat free organic matter to volume (R:V) in the antler shaft, where Y = R:V ratio and X = distance from the antler tip (cm).

In antlers removed between 28 and 91 days after casting (blue line) $Y = 0.127 + 0.00265\ X$ (Prior to adjustment to a common slope; $r = 0.89^{**}$, RSD = 0.02)

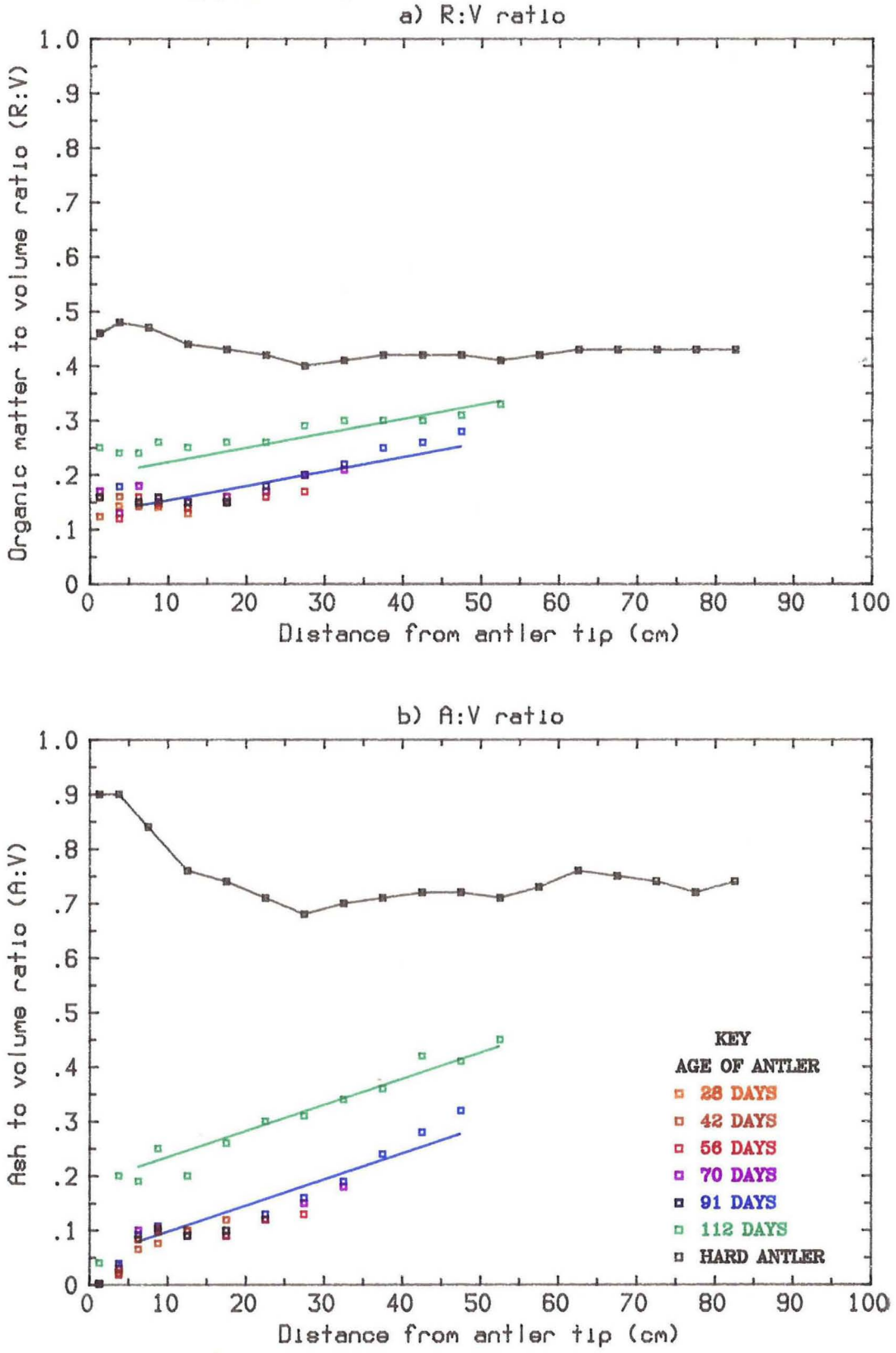
In antlers removed 112 days after casting (green line) $Y = 0.197 + 0.00265\ X$ (Prior to adjustment to a common slope; $r = 0.72^{**}$, RSD = 0.04)

b) Ratio of ash to volume (A:V) in the antler shaft, where Y = A:V ratio and X = distance from the antler tip (cm).

In antlers removed between 28 and 91 days after casting (blue line) $Y = 0.0499 + 0.00479\ X$ (Prior to adjustment to a common slope; $r = 0.50^{**}$, RSD = 0.09)

In antlers removed 112 days after casting (green line) $Y = 0.187 + 0.00479\ X$ (Prior to adjustment to a common slope; $r = 0.87^{**}$, RSD = 0.05)

Figure 4.11
Ratio of a) fat free organic matter to volume (R:V) and b) ash to volume ratio (A:V) in the shaft of antlers removed at six stages of growth. Points represent means of three antler sections. See facing page for equations.



(Fig. 4.11b). A marked increase in A:V ratio occurred in the section 5.0 to 7.5 cm below the tip. Accordingly tip sections (those in the distal 5.0 cm of the antler) have been excluded from the regression equations in Figures 4.11a and 4.11b. There were no significant differences in the R:V and A:V ratio in antlers between 28 and 91 days after casting and common regression equations have been fitted to describe the pattern of increase from the section 5.0 to 7.5 cm from the antler tip, to the antler base (Fig. 4.11a, 4.11b). R:V and A:V ratio increased with distance down the antler shaft, but increase in ash deposition occurred at a greater rate. However, between 91 and 112 days of antler growth there was a significant increase ($P < 0.01$) in both the amount of organic matrix and amount of ash in the antler shaft with the increase in density of ash being almost twice that of the increase in density of organic matrix. There were, however, no changes in the relative rate of organic matrix and ash deposition with distance from the antler tip in this latter phase and regression equations have been adjusted to a common slope. Overall correlation coefficients of the adjusted regression equations for the models describing R:V ratio (Fig. 4.11a) and A:V ratio (Fig. 4.11b) in the antler shaft were 0.94 and 0.82, respectively.

Ash to organic matter ratio (A:R) has been used to describe the degree of mineralization of organic matrix (Fig. 4.12a). Tip sections were poorly mineralized with no detectable change in A:R ratio occurring between 28 days and 91 days. However after 112 days of growth, A:R ratio of the tip section had increased to 0.19 (Fig. 4.12a). In the adjacent proximal section (2.5 to 5.0 cm from the tip) A:R ratio had increased from 0.12 in 28 day antlers to 0.22 after 91 days. In antlers removed 112 days after casting A:R ratios in this particular section had increased to 0.41. A:R ratios were higher in the section 5.0 to 7.5 cm below the antler tip, but did not change with increasing antler age (e.g. at 28 days A:R ratio was 0.61 and at 112 days was 0.64). No significant differences in mineralization in the antler tip could be detected and a common regression line (a) has been fitted to describe mineralization in the distal 7.5 cm of velvet antlers between 28 and 112 days of growth (Fig. 4.12a).

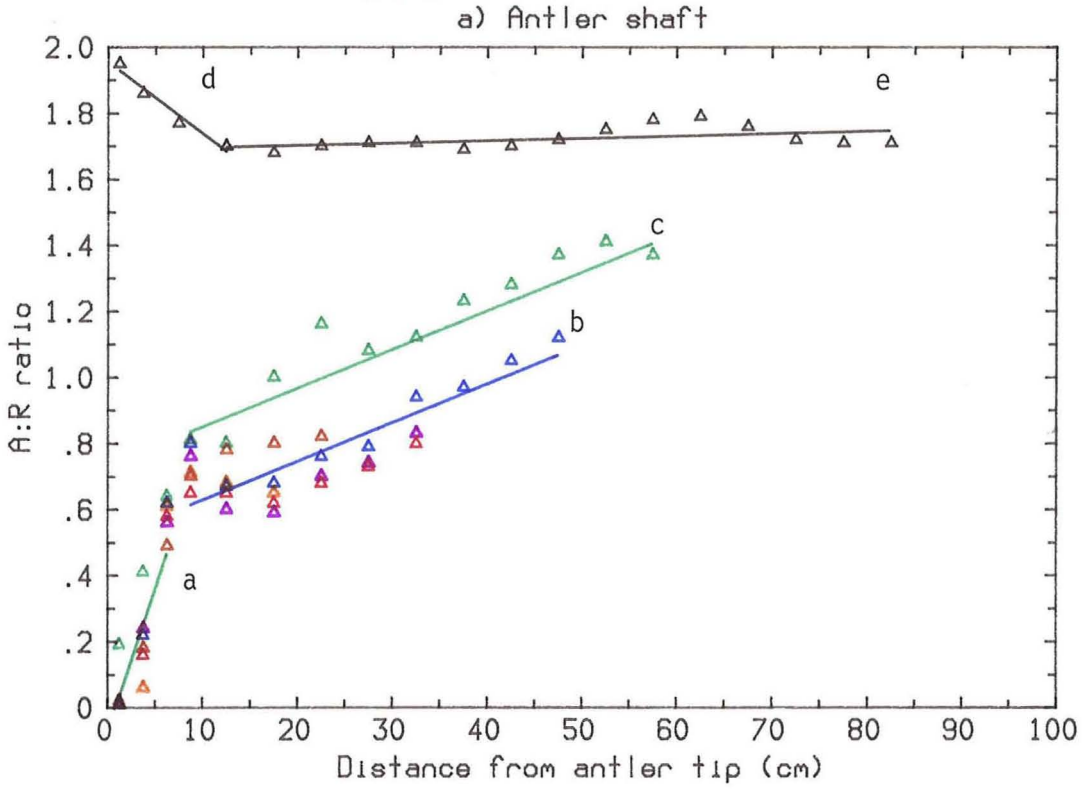
Mineralization of the antler shaft continued progressively towards the antler base and at a constant rate between 28 and 91 days of growth (Fig. 4.12a). A single regression line (b) has therefore been fitted to

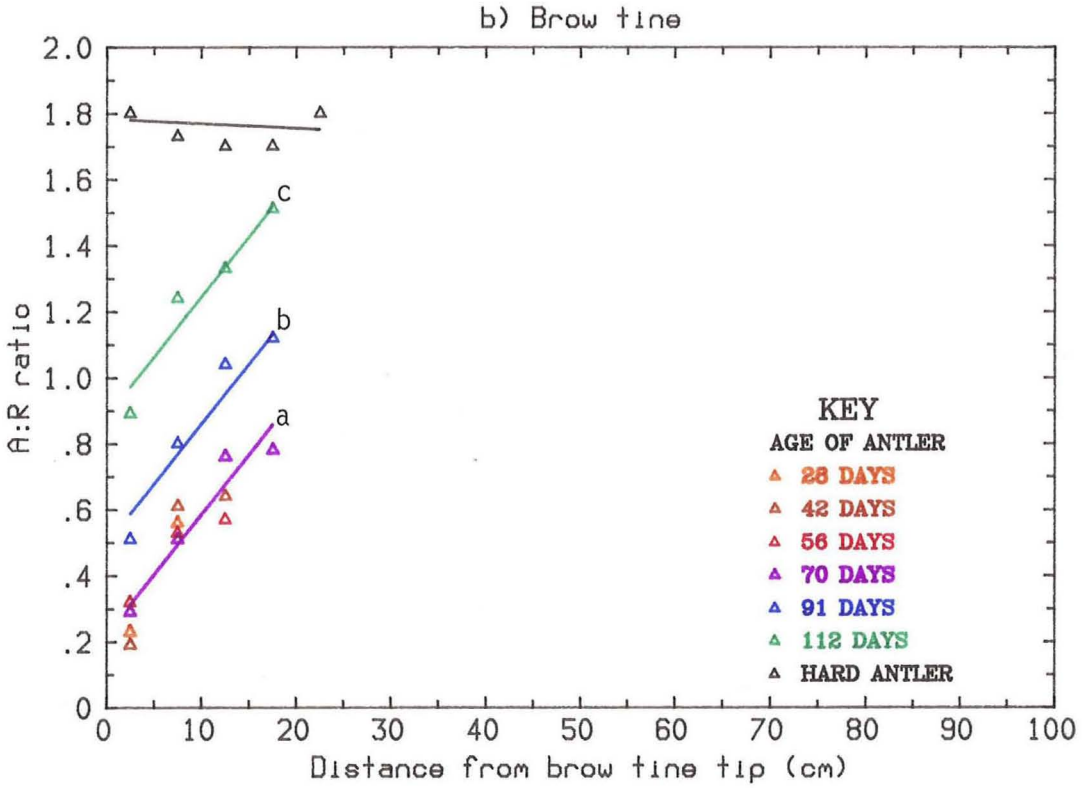
Figure 4.12

a) Mineralization in the antler shaft, where Y = ash to organic matter ratio (A:R) and X = distance from the antler tip (cm) for regression equations a, b, c, d and e
   a) $Y = -0.0805 + 0.0875\,X$ (Prior to adjustment to a common slope; $r = 0.87^{**}$, RSD = 0.14)
   b) $Y = 0.512 + 0.0117\,X$ (Prior to adjustment to a common slope; $r = 0.79^{**}$, RSD = 0.17)
   c) $Y = 0.732 + 0.0117\,X$ (Prior to adjustment to a common slope; $r = 0.68^{**}$, RSD = 0.11)
   d) $Y = 1.96 - 0.0217\,X$ (Equation fitted through the means)
   e) $Y = 1.69 - 0.000729\,X$ (Equation fitted through the means)

b) Mineralization in the brow tine, where Y = ash to organic matter ratio (A:R) and X = distance from the brow tine tip (cm) for regression equations a, b, c and d
   a) $Y = 0.881 + 0.0364\,X$ (Prior to adjustment to a common slope; $r = 0.85^{**}$, RSD = 0.15)
   b) $Y = 0.497 + 0.0364\,X$ (Prior to adjustment to a common slope; $r = 0.85^{**}$, RSD = 0.16)
   c) $Y = 0.222 + 0.0364\,X$ (Prior to adjustment to a common slope; $r = 0.73^{**}$, RSD = 0.16)
   d) $Y = 1.78 - 0.00143\,X$ (Equation fitted through means)

Figure 4.12
Pattern of mineralization in a) the antler shaft and b) the brow tines of antlers removed at six stages of growth and at velvet stripping (hard antler). Points represent means of three antler sections. See facing page for equations.





describe antler mineralization in the antler shaft between the section 5.0 to 7.5 cm below the tip, and the antler base. Antlers removed at 112 days showed a significantly greater increase in mineralization throughout the antler shaft, indicated by a significant increase ($P < 0.01$) in elevation of the fitted regression line (c). No differences were detected in slope of the two fitted regression lines (b and c) and both have been adjusted to a common slope (Fig. 4.12a). The overall correlation coefficient (r) for this model incorporating lines b and c was 0.87.

Antler base sections were invariably more heavily mineralized than distal sections (Fig. 4.12a). At 112 days of growth A:R values in base sections were 1.41 ± 0.094, which represented 82% of the degree of mineralization of hard stripped antlers. In the period from 112 days of growth to velvet stripping rapid mineralization of the antler shaft continued, particularly in the distal 20 cm.

In the hard stripped antler degree of mineralization was greatest in the distal 10 cm, mean A:R values falling from 1.95 ± 0.039 in the tip to 1.70 ± 0.047 in the section 10 to 15 cm from the tip. Degree of mineralization was relatively constant in the remainder of the antler shaft. During sectioning of hard stripped antlers high ratios of compact to spongy bone were observed in the distal 10 cm, with spongy bone absent from the most distal sections. Consequently in Figure 4.12a separate regression lines have been fitted (through the mean values) to describe mineralization in the distal 10 cm (d) and in the remainder of the hard antler shaft (e).

The composition of antler ash was not constant within antlers (Table 4.8). Linear contrasts were used to demonstrate that calcium concentrations in extreme tip sections were not significantly different between 28 and 91 days after casting (mean; 21.1 g/100g ash), but that these were significantly different ($P < 0.01$) to calcium concentrations in the tip of the 112 day antler and in hard antlers (mean; 35.7 g/100g ash). Changes in phosphorus concentration followed a similar but less marked pattern, with a significant increase ($P < 0.01$) from a mean of 14.0 g/100g ash in the tip sections of antlers removed between 28 and 91 days, to a mean of 19.0 g/100g ash in the tip sections of antlers removed either at 112 days after casting, or at hard antler stripping. In hard stripped antlers no change in calcium or phosphorus concentrations

Table 4.8

Calcium and phosphorus concentrations and Ca:P ratios in the distal sections and base of hard stripped antlers and of velvet antlers harvested at 6 stages of growth. For velvet antlers n = 3 and for hard antlers n = 9. Calcium and phosphorus concentrations are expressed as g/100g ash.

Age of antler (days)	Distance from antler tip (cm)											
	0 - 2.5			2.5 - 5.0			5.0 - 7.5			Base		
	Ca	P	Ca:P	Ca	P	Ca:P	Ca	P	Ca:P	Ca	P	Ca:P
28	19.7	14.4	1.4	33.6	17.5	1.9	34.7	19.7	1.8	38.3	20.7	1.9
42	22.3	13.9	1.6	30.1	15.4	2.0	33.7	19.3	1.8	37.6	20.6	1.8
56	20.9	12.8	1.6	31.1	16.3	1.9	33.8	18.6	1.8	37.0	20.2	1.8
70	19.9	13.7	1.4	33.3	17.7	1.9	35.3	20.0	1.8	36.0	20.0	1.8
91	21.5	13.5	1.6	35.2	19.6	1.8	35.8	21.0	1.7	36.2	20.0	1.8
112	35.8	17.3	2.1	36.4	17.5	2.0	35.3	19.3	1.9	34.3	18.3	1.9
160 (Hard)	35.5	19.6	1.8	36.1	19.9	1.8	35.7	19.7	1.8	35.2	19.0	1.9
S.E.D.	±2.13	±0.82	±0.11	±1.65	±0.83	±0.06	±1.07	±0.69	±0.04	±1.09	±0.63	±0.05

occurred within the antler shaft.

Calcium to phosphorus ratio in antler tip sections also increased as antlers aged. There was no significant difference between 28 and 91 day antlers but in antlers removed after 112 days and at velvet stripping Ca:P ratio in tip sections had increased significantly (Table 4.8). In antlers at all sampling ages calcium and phosphorus concentrations, and calcium to phosphorus ratios in the section immediately behind the antler tip (2.5 to 5.0 cm) had increased to levels similar to those in the remainder of the antler shaft (Table 4.8).

b) Brow tine

A:R ratio increased from tip to base of the brow tine (Fig. 4.12b). A poorly mineralized tip and a zone of mineralization was not observed within the brow tine. Three regression equations were fitted to the data which describes mineralization in the brow tines of growing antlers (Fig. 4.12b). The common regression line (a) fitted to the brow tines of antlers between 28 and 70 days of age had a significantly different intercept ($P < 0.05$) to that of the 91 day antler (b). In the 112 day old antlers the regression line (c) had a significantly different intercept ($P < 0.01$) to that of the 91 day antler. Slopes were not significantly different and all were adjusted to a common slope. The overall correlation coefficient (r) for this model was 0.92. From the regression coefficients of 0.36 and 0.12 in the brow tine and antler shaft, respectively, it appears that mineralization per unit distance from the tip had occurred at a greater rate in the brow tine than in the antler shaft.

Although compact bone appeared to form a higher proportion of bone in the tips of brow tines, this observation was not associated with correspondingly high A:R ratios as in the antler shaft (Fig. 4.12a). A single regression line was therefore fitted through the means to describe mineralization in the brow tine of the hard stripped antler (Fig. 4.12b).

c) Total mineralization in the antler shaft, brow tine and whole antler

The pattern of mineralization of the whole antler shaft has been contrasted with that of the brow tine (Fig. 4.13) and of the whole antler (Fig. 4.13 overlay). Time of brow tine initiation was not recorded, consequently brow tine A:R ratio has been expressed in terms of days from casting rather than as time from initiation. All trends were non-linear containing significant quadratic components ($P < 0.05$). With the small sample numbers being compared ($n = 3$), no significant differences could be demonstrated in degree of mineralization between the antler shaft and brow tine at any stage of growth. However degree of total mineralization tended to be greater in the antler shaft at earlier stages of growth; at 28 days A:R ratio in the antler shaft was 0.51 ± 0.035 compared with 0.39 ± 0.038 in the brow tine ($t = 2.15$, $P < 0.10$, $df = 4$). From the fitted curves (Fig. 4.13) A:R ratios in the brow tine and antler shaft at date of commercial harvest (64 days) were estimated at 0.56 and 0.68, respectively. Total mineralization in the brow tine accelerated after 70 days and by 91 days after casting the degree of mineralization in the brow tine (0.82 ± 0.166) had approached that of the antler shaft ($0\ 86 \pm 0.035$; $t = 0.24$, $P < 0.83$, $df = 4$). By 112 days the brow tine was mineralized to a greater degree (1.28 ± 0.090) than the antler shaft (1.20 ± 0.117). However, these differences were not significant ($t = 0.54$, $P < 0.62$, $df = 4$). Mean A:R ratios were similar in both the shaft and brow tine at velvet stripping.

Since the antler shaft comprised a large proportion of antler weight, the pattern of total antler mineralization (antler shaft and all tines) followed closely that of the antler shaft (Fig. 4.13, overlay). By the time of hard antler removal (mean, 164 days), mean A:R ratio had increased to 1.73 ± 0.028.

There was no change in total calcium (31-36 g/100g ash) or phosphorus concentration (17-20 g/100g ash) in ash of antlers aged between 28 days and 112 days, or at hard antler stripping (Table 4.9). Consequently calcium to phosphorus ratio remained relatively constant, at between 1.8 and 1.9 to 1.

Figure 4.13
Pattern of mineralization in the antler shaft, brow tine and whole antler; Y = A:R ratio and X = age of antler (days from casting). Quadratic equations have been fitted through the means.

Mineralization in the antler shaft;
$Y = 0.6064 - 0.00399\,X + 0.0000807\,X^2$

Mineralization in the brow tine;
$Y = 0.4836 - 0.00579\,X + 0.000113\,X^2$

Mineralization in the whole antler;
$Y = 0.5578 - 0.00371\,X + 0.0000810\,X^2$

Figure 4.13

Pattern of mineralization (A:R ratio, mean ± S.E.M.) during antler development in the antler shaft and brow tines of antlers removed at six stages of growth. The range in A:R ratio, and in date of velvet stripping of hard antlers is indicated in the upper right hand corner. Overlay : Pattern of whole antler mineralization. See facing page for equations.

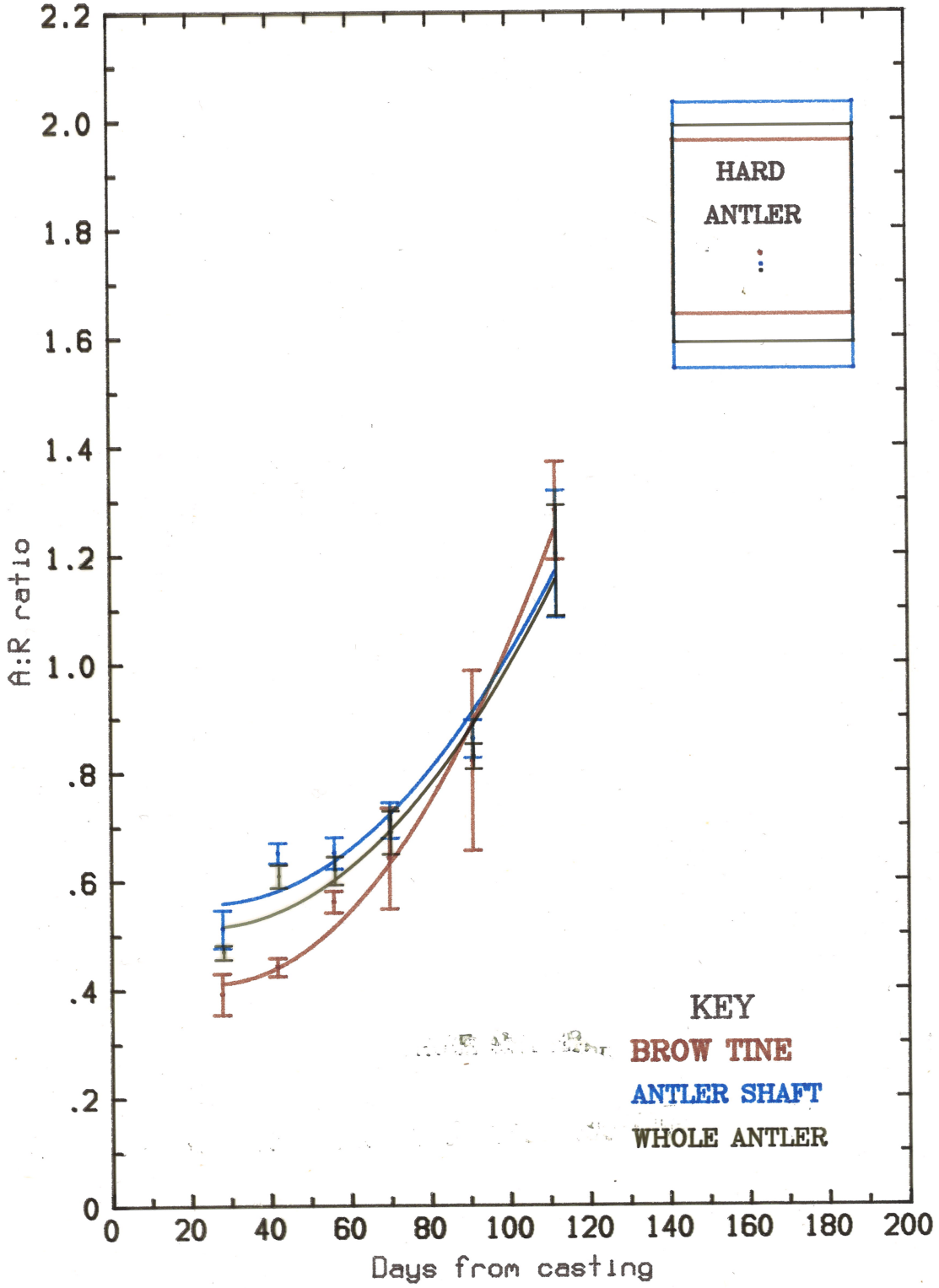


Figure 4.13

Pattern of mineralization (A:R ratio, mean ± S.E.M.) during antler development in the antler shaft and brow tines of antlers removed at six stages of growth. The range in A:R ratio, and in date of velvet stripping of hard antlers is indicated in the upper right hand corner. Overlay : Pattern of whole antler mineralization. See facing page for equations.

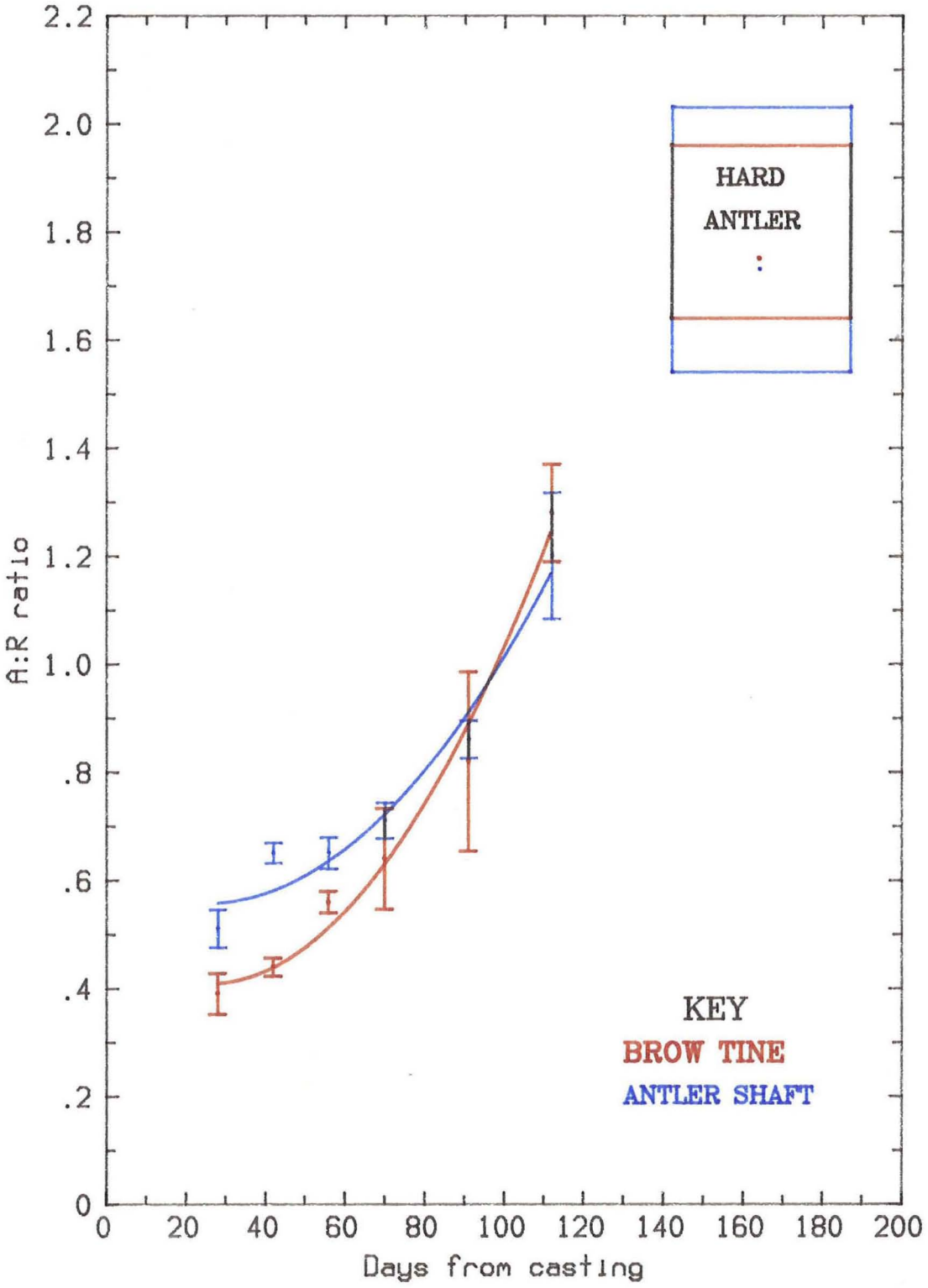


Table 4.9

Total calcium (Ca) and phosphorus (P) concentrations, and calcium to phosphorus ratios (Ca:P) in hard antlers (n = 9), and in velvet antlers (n = 3) removed at 6 stages of growth.

Age of antler (days)	Ca (g/100g Ash)	P (g/100g Ash)	Ca:P
28	35.3	19.7	1.8
42	35.5	19.8	1.8
56	36.6	20.0	1.8
70	35.8	19.7	1.8
91	36.6	20.4	1.8
112	35.0	18.9	1.9
164 (Hard)	35.1	18.9	1.9
S.E.D.	0.84	0.54	0.03

Regrowth antlers removed at velvet stripping were less well mineralized (A:R 1.62 ± 0.046; range 1.37-1.73) than their hard antler counterparts (A:R 1.71 ± 0.045; range, 1.57-1.99) although the difference was not significant ($t = 1.36$, $P < 0.19$, $df = 16$).

d) Rate of organic matrix deposition

In order to calculate rates of organic matrix deposition in antlers the fat free organic matter content (FFOM) of each harvested antler has been expressed as a proportion of the total FFOM in the contralateral antler allowed to grow to completion (Fig. 4.14). The proportion of FFOM deposited increased exponentially with time and a good fit was obtained to the data with an allometric exponential ($y = ax^b$; Fig. 4.14). FFOM accumulation was slow initially but increased rapidly throughout the period of rapid antler elongation (i.e. between 28 and 91 days after casting) and by 112 days after casting approximately 98% of final FFOM was present. The most rapid rate of increase in FFOM occurred between 91 and 112 days after casting, at a rate of 1.4 g/100g hard antler FFOM per day.

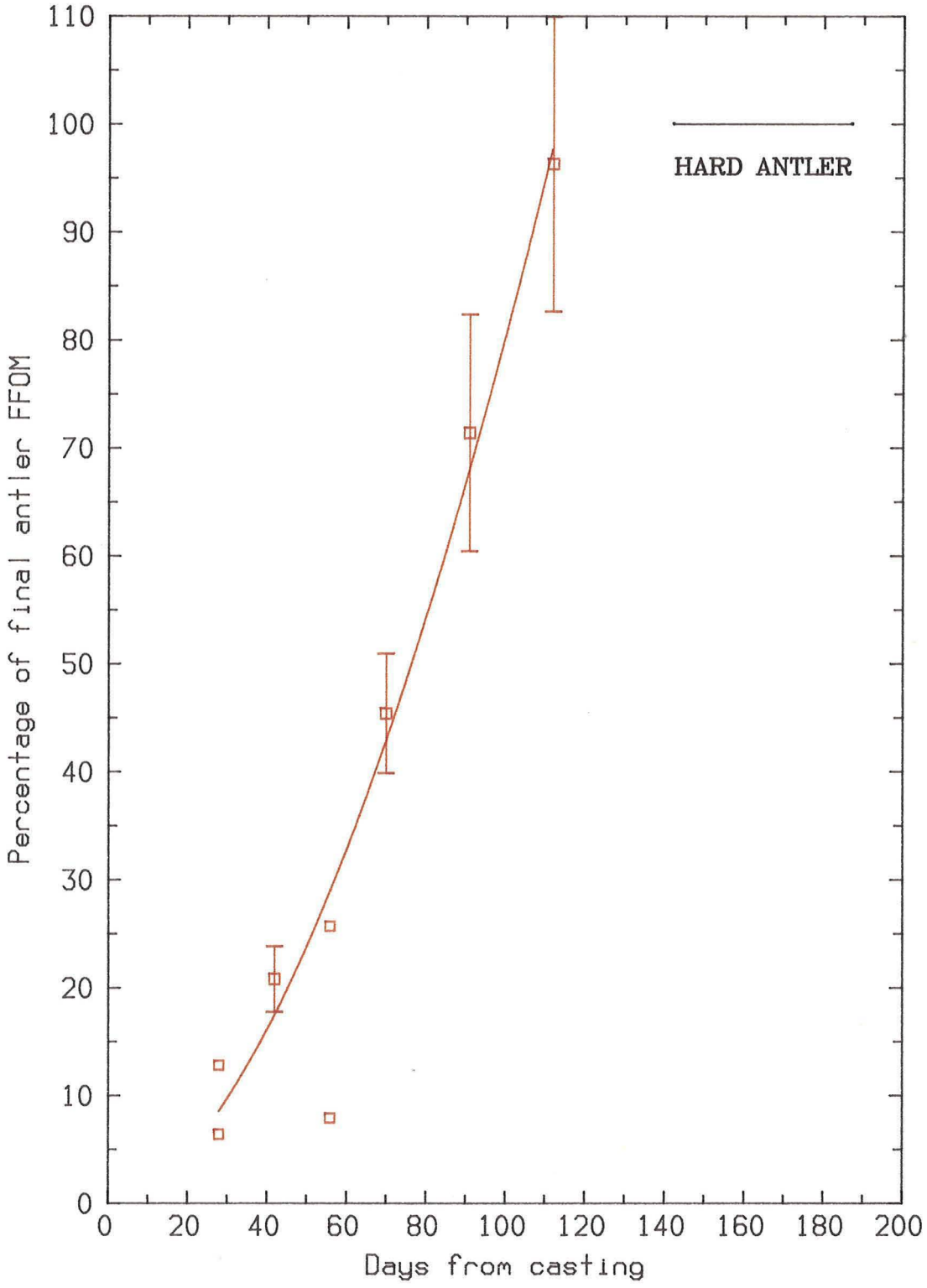
Figure 4.14

Rate of fat free organic matter (FFOM) deposition during antler growth, where Y = percentage of final antler FFOM and X = age of antler (days from casting).

$Y = 0.0244X^{1.76}$ ($r = 0.92^{**}$, RSD = 13.8)

Figure 4.14

Pattern of fat free organic matter (FFOM) deposition during growth of the antler. FFOM content of individual velvet antlers removed at six stages of growth has been expressed as a percentage of the FFOM content of the contralateral antler removed at velvet stripping. See facing page for equati The range in date of hard antler stripping is indicated by the horizontal lir



Mean weight of hard stripped antlers was 1.12 kg, which on a dry matter basis (DM 81.1 ± 0.77%) contained 36.6 ± 0.37% FFOM. Therefore a typical mature red deer stag (such as one in this study, but producing two antlers) would have a total antler FFOM content of 0.66 kg. Between 91 and 112 days after casting maximum rate of deposition of FFOM in the antlers would be 9.3 g/d.

e) Rate of ash deposition

Rate of antler ash deposition has been derived in the same manner as rate of FFOM deposition described in the previous paragraph. An allometric exponential has also been fitted to the data (Fig. 4.15). Ash deposition was slow in the initial stages (by 70 days after casting only 19% of final antler ash had been deposited compared with 43% of final antler FFOM) but accelerated rapidly in the latter stages of growth. By the time of final antler sampling at 112 days 68% of final antler ash had been deposited. Peak rate of ash deposition occurred between 91 and 112 days (33%) representing maximum daily rate of 1.6 g/100g hard antler ash.

On a dry matter basis hard stripped antlers contained 63.0 ± 0.34% ash. A typical stag producing 2.24 kg of hard antler would therefore have a total antler ash content of 1.14 kg and a maximum rate of ash deposition of 18.3 g/d.

Total antler calcium content remained constant at around 35% of antler ash (Table 4.9) therefore rate of calcium accumulation in antlers must have been equivalent to 35% of the rate of ash deposition (as illustrated in Figure 4.15).

5) Antler histology

a) Growth of the antler tip

Longitudinal sections (Plate 4.1) indicated a marked decrease in size of the cartilaginous growth plate between antlers aged 70 days and 112 days. Both photomicrographs were taken at the distal end of the chondrocytic zone such that the upper edge of the photograph depicts tissue in close proximity to the dermis of the velvet. Plate 4.1(a) from an antler tip at 70 days has an extensive area of cartilage. There was no distinct columnation or zoning of the cartilage with proliferating cartilage beneath the periosteum and dermis of the velvet merging with

Figure 4.15

Rate of ash deposition during antler growth, where Y = percentage of final antler ash and X = age of the antler (days from casting).

$Y = 0.000120\, X^{2.81}$ ($r = 0.96^{**}$, RSD = 6.7)

Figure 4.15
Pattern of ash deposition during growth of the antler. Ash content of individual velvet antlers removed at six stages of growth has been expressed as a percentage of the ash content of the contralateral antler removed at velvet stripping. See facing page for equation. The range in date of hard antler stripping is indicated by the horizontal line.

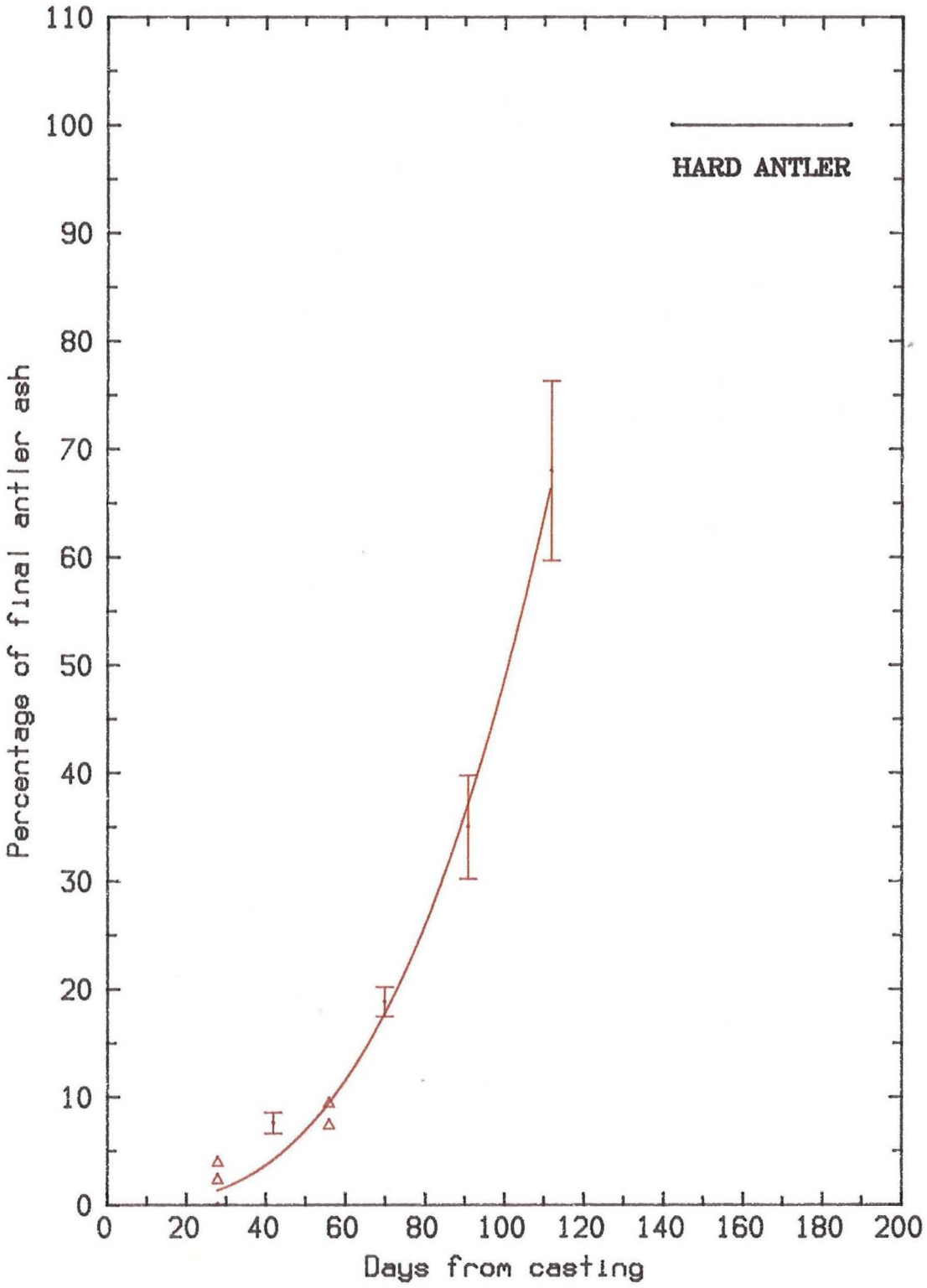


Plate 4.1

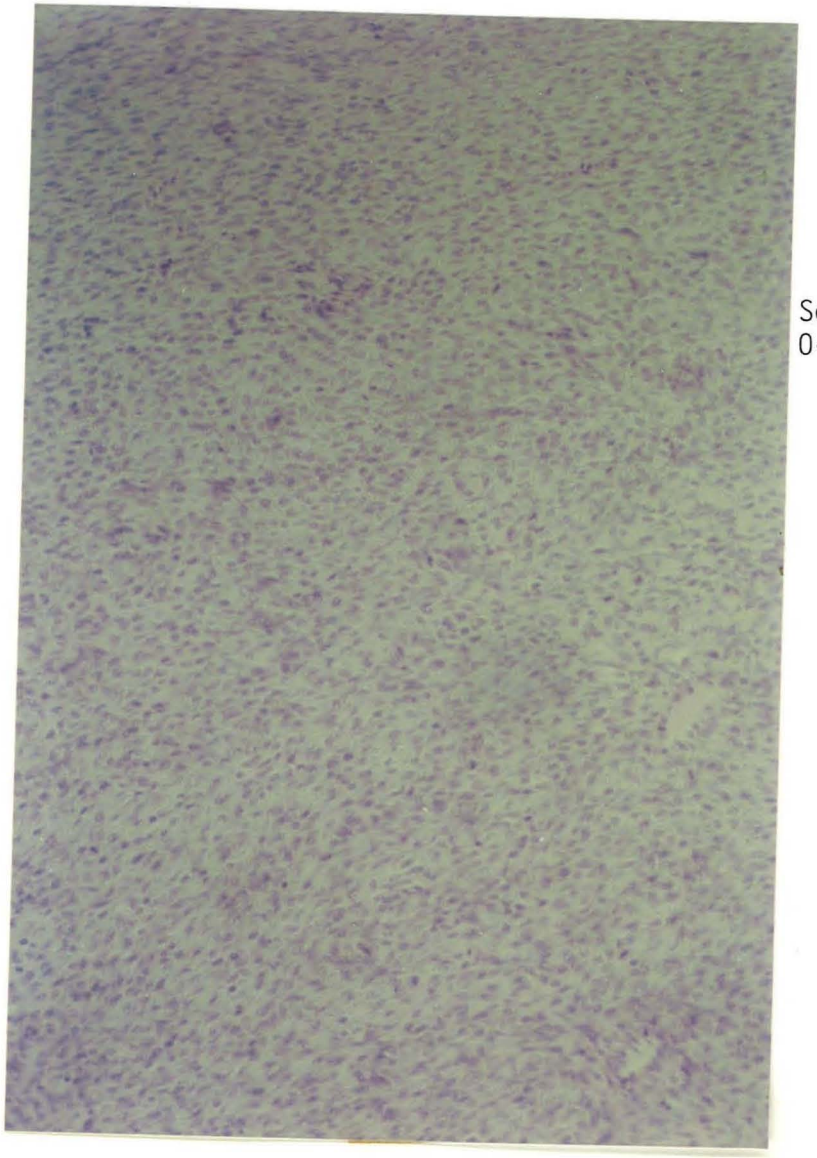
Growth of the antler tip. Both photomicrographs are of histological sections taken from the distal end of the cartilaginous zone, such that the upper border of each section is in close proximity to the periosteum and dermis of the velvet.

a) Longitudinal tip section from an antler after 70 days of growth.
b) Longitudinal tip section from an antler after 112 days of growth. Both mineralized cartilage (n) and trabecular bone (o) are apparent near the bottom of the photograph.

Magnification: 100x. Haematoxylin and eosin.

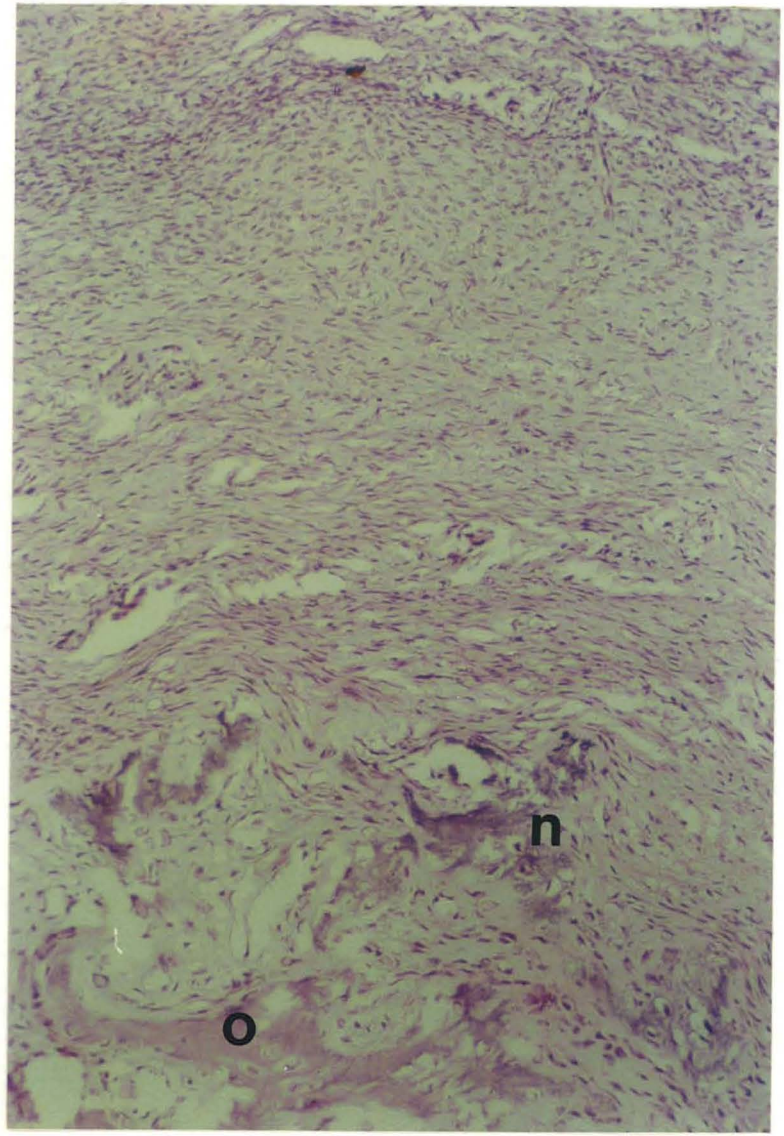
Plate 4.1

a) Antler tip - 70 day antler (L.S.)



Scale
0-10 mm

b) Antler tip - 112 day antler (L.S.)



hypertrophying chondrocytes. No distinct blood vessels were apparent in the area of proliferating cartilage (Plate 4.1a). Deeper in the chondrogenic zone numerous longitudinally orientated vascular channels were observed. By 112 days of growth only a narrow band of cartilage (5 mm) was apparent in the antler tip. Both mineralized cartilage (n) and trabecular bone (o) were present immediately beneath the chondrocytic zone and in close proximity to the antler tip (Plate 4.1b).

b) Zone of mineralization

Plates 4.2a and b show transverse sections taken 5.0 and 10.0 cm, respectively, beneath the tip of an antler aged 42 days. Both sections are representative of the interior of the antler shaft, which ultimately becomes spongy bone in the stripped hard antler. In the section 5.0 cm from the antler tip (Plate 4.2a) chondrocytes (p) are clumped in trabeculae. Numerous cells are hypertrophied and mineralized. This area of cartilage is highly vascular (q) and there is evidence of osteogenic cells (r) proliferating under the endothelium of the blood vessels. However in the section 10 cm below the tip (Plate 4.2b) mineralized cartilage has been replaced by trabecular bone (s). Rows of osteoblasts (t) are visible on the bone surface. Some calcified cartilage cells (u) are still present, surrounded by trabecular bone (Plate 4.2b).

c) Mineralization of the antler shaft

Plate 4.3 illustrates the general pattern of mineralization in the antler shaft of antlers removed 91 days after casting (Plates 4.3a, b and c) and 112 days after casting (Plates 4.3d, e and f). In the antler removed 91 days after antler casting mineralization of antler bone occurred through slow mineral accretion and thickening of bony trabeculae on progression from the growth point to the base (Plate 4.3a, b and c). A change in the pattern of mineralization appeared to have occurred, however, in the shaft of the antlers removed at 112 days, with a large increase in density of peripheral trabecular bone. As a result well developed osteones had formed in the periphery of the antler shaft. The formation of dense compact cortical bone apparently occurred simultaneously down the antler shaft, rather than gradually towards the antler base. It was this finding that prompted the preparation of sections from frozen tissue of group B and C antlers which subsequently supported these findings.

Plate 4.2

Zone of mineralization.

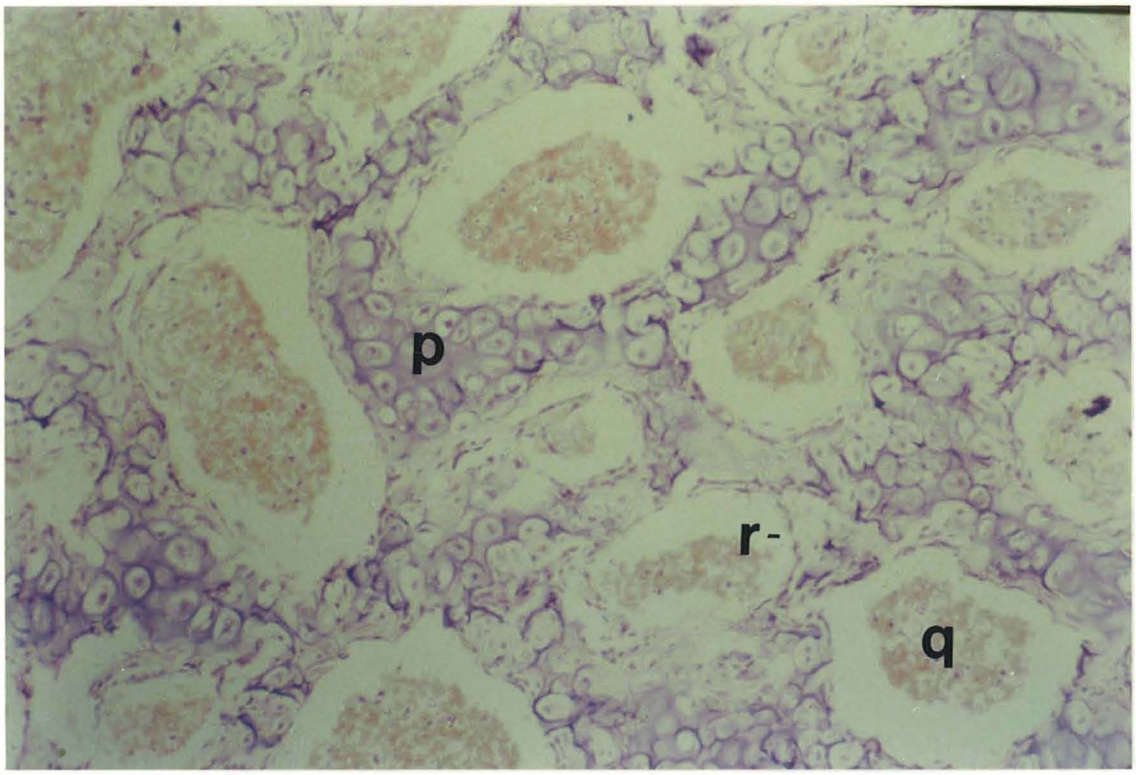
a) Transverse section taken 5 cm below the tip of an antler after 42 days of growth. Chondrocytes are clumped in cartilaginous trabeculae (p) and surrounded by numerous vascular channels (q). Osteogenic cells (r) are present on the surface of the cartilaginous trabeculae.

b) Transverse section taken 10 cm below the tip of the same antler. Cartilaginous trabeculae have been replaced by trabecular bone (s) and osteoblasts are evident on bone surfaces (t). Some calcified cartilage cells (u) remain trapped within trabecular bone.

Magnification: 100x, Haematoxylin and eosin.

Plate 4.2

a) 42 day antler - 5 cm from tip (T.S.)



b) 42 day antler - 10 cm from tip (T.S.)

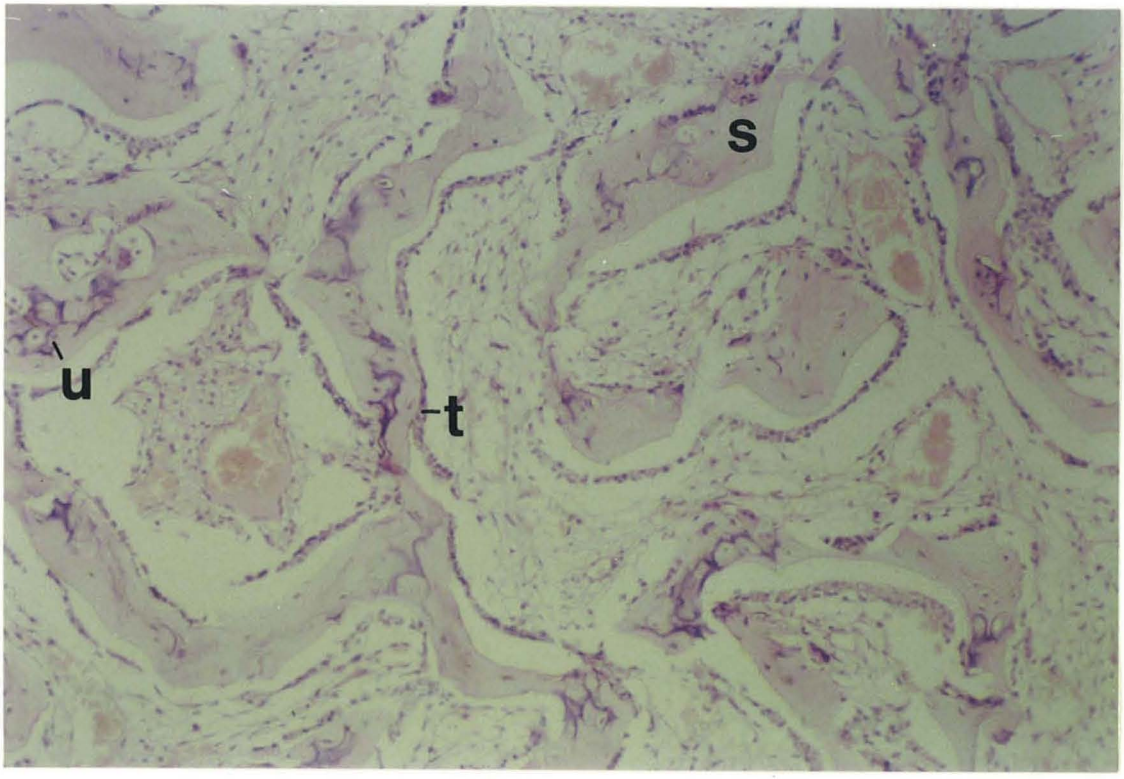


Plate 4.3

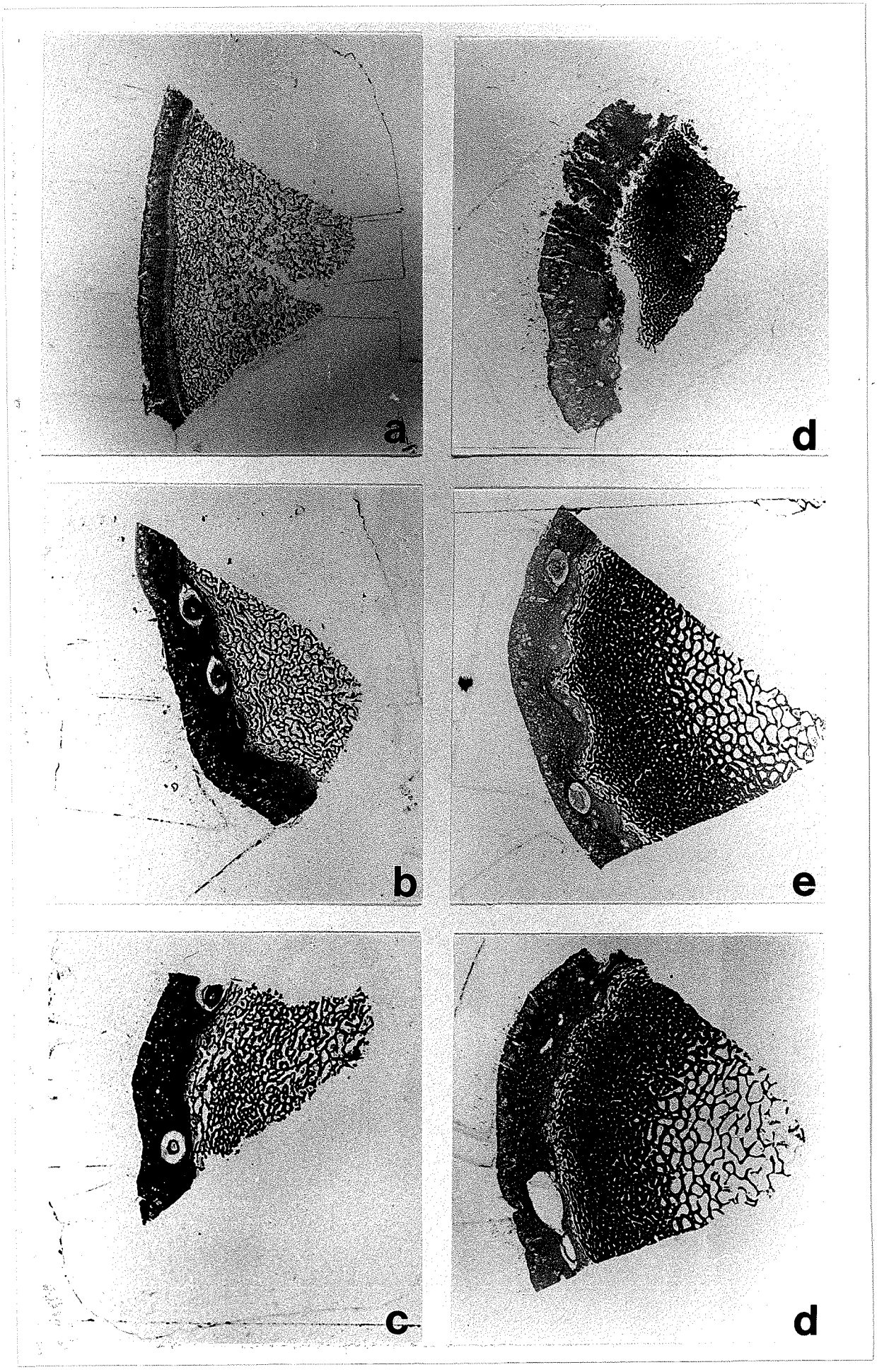
Mineralization of the antler shaft.

Bone development in the periphery of the antler shaft in antlers removed 91 days after casting (a, b and c) and 112 days after casting (d, e and f).

Transverse sections were taken; a and d - 15, b and e - 30, and c and f - 45 cm respectively, from the antler tip.

Magnification: approximately 4x, Ralis tetrachrome

Plate 4.3



The timing of appearance of compact bone in the shaft coincided with the formation of compact bone in the brow tine. Plates 4.4 (a, b and c) indicate the degree of mineralization in transverse sections taken 30 cm from the antler tip from antlers harvested at 70, 91 days and 112 days of growth, respectively. A gradual increase in bone density at days 70 and 91 (Plates 4.4a and b) contrasted with the marked increase in the 112 day antler (Plate 4.4c). A similar phenomenon was observed within the brow tine. Transverse sections taken 10 cm from the tip of the brow tine of the same antlers are illustrated in Plates 4.4 (d, e and f). These indicate large amounts of cortical bone thickening at 112 days (Plate 4.4f) which is not evident in the brow tines of antlers removed at 70 or 91 days after casting (Plates 4.4d and e respectively).

6) Plasma testosterone

In the 5 stags sampled, mean plasma testosterone levels declined from 1.4 ± 0.51 ng/ml on 13 August (5 days prior to trial commencement) to 0.4 ± 0.13 ng/ml at date of casting (22 September), (Fig. 4.16). Mean casting date for these stags was 9 days later than for the group as a whole. Plasma testosterone levels remained low during early antler growth (< 0.2 ng/ml), but increased rapidly from 0.24 ± 0.040 ng/ml 89 days after casting (20 December) to 0.9 ± 0.15 ng/ml 105 days after casting (5 January). Testosterone levels in plasma continued to rise and were elevated to 3.3 ± 1.93 ng/ml at velvet stripping on 26 February. Mean stripping date for these stags was 2 days later than for the whole group.

Plate 4.4

Bone development in antlers removed 70, 91 and 112 days after casting of hard antlers. Transverse sections a, b and c were taken 30 cm from tip of their respective antlers. Transverse sections taken 10 cm from the tip of the brow tine of the same antlers have been included for comparison (d, e and f, respectively).

Magnification: approximately 4x, Ralis tetrachrome.

Plate 4.4

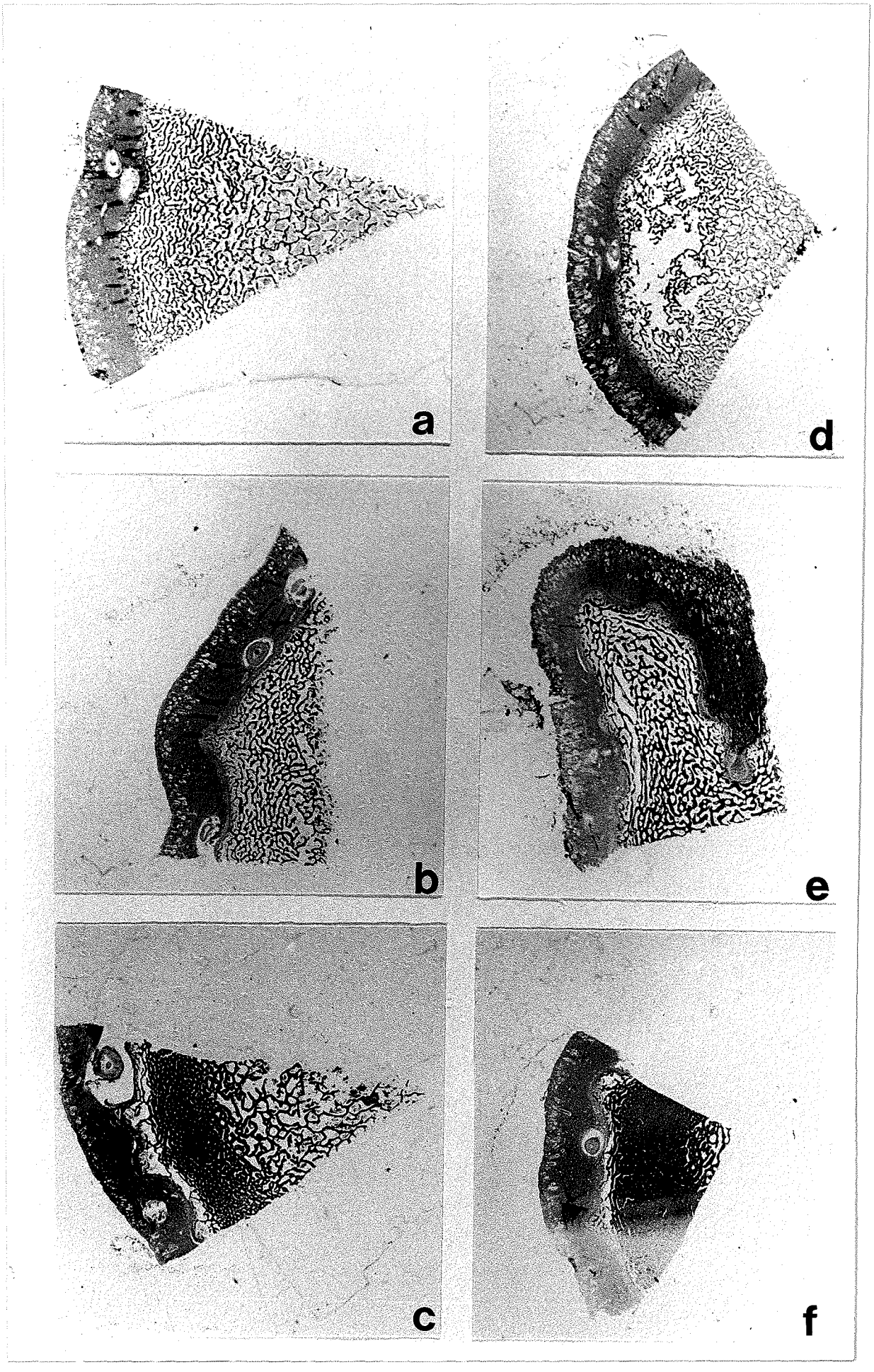
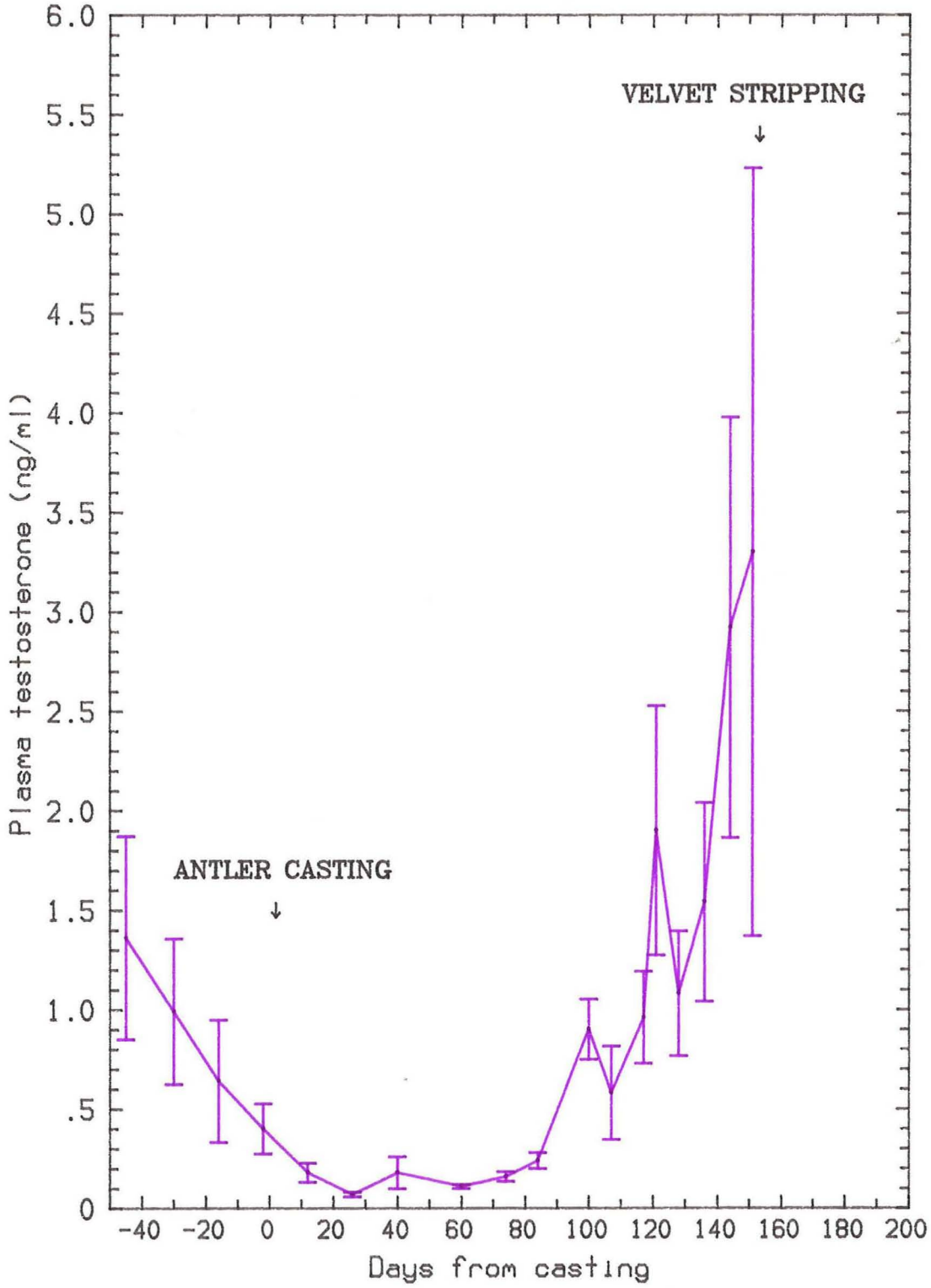


Figure 4.16
Mean plasma testosterone concentrations (± S.E.M.) recorded in five red deer stags at approximately two weekly intervals throughout the period of antler growth.



Discussion

1) Treatment effects

Stags in the high protein group (group B) had slightly higher mean antler weights (1.32 kg) than those in either the control group (1.12 kg), or those in group C offered the low calcium ration (0.99 kg). These higher antler weights in the high protein group may simply be a result of the large variation in hard antler weight (0.55-1.90) which probably reflects the wide range in liveweight, age and genetic background of the stags available for this trial. As a result of the variation in hard antler weight between stags at least 24 animals per treatment group would have been necessary to demonstrate that the differences in hard antler weight, between treatment groups, were significant at the 5% level.

An effect of nutritional treatment on hard antler weight cannot be ruled out although it seems unlikely that stags in this study would have been limited for dietary protein. The organic matrix of bone is comprised almost entirely of protein, of which 89% is collagen (Herring, 1972), therefore rate of FFOM deposition in the antler should be equivalent to the rate of protein synthesis. Therefore for the average stag in the present study (if both antlers had been allowed to grow to completion) maximum rate of antler protein deposition would have been approximately 9 g/day (see page 82). This is a relatively low net protein requirement, corresponding to the protein loss in milk in a ewe producing approximately 1.4 litres of milk (Perrin, 1958). Stags in this study were increasing in bodyweight at 327 g/d during the period of antler growth. A small proportion of this gain would be antler growth and actual liveweight gain would have been approximately 300g/d. A mean value for the protein concentration of empty bodyweight gain of 145 g/kg has been calculated from the data of the ARC (1980). Therefore the protein requirement for liveweight gain of the stags in this study would have been approximately 44 g/d, or five times the requirement for antler growth. Since mean daily DM intake was approximately 2.75 kg, and the protein content of feed was 130 and 199 g/kg for the control and high protein diet respectively, dietary protein intake would have been in the order of 350 and 550 g/d for stags in the respective groups. Unless rates of dietary protein N absorbability were extremely low it seems

unlikely that stags in either group were "protein deficient".

It is possible, however, that the heavier antler weights observed in group B stags were due to greater availability of an essential amino acid. Linseed meal was used as the high protein source for this ration and was formaldehyde treated, hence amino acid supply to the duodenum should have been enhanced.

There appeared to be no effect of the low calcium diet on antler growth, since the degree of mineralization of hard antler was not significantly different between treatment groups. This may be due to a number of factors. Initially it was hoped to have a very low a calcium concent in the ration formulated for group C (close to 10 g/kg), but it proved impossible to achieve this. Secondly, with the removal of one antler and the subsequent impaired development of regrowth, stags had lower calcium requirements than for two normal antlers. Calcium levels in the low calcium ration (C) may therefore have been sufficient to allow full antler mineralization from dietary calcium. The degree of adequacy of calcium in the ration is discussed further on pages 100 and 101.

2) Growth of the antler

Antler casting and subsequent growth is considered to be dependent on the absence of testosterone. This has been demonstrated both by surgical castration experiments (Goss, 1963) and hormonal manipulation (Muir, Barrell and Sykes, 1982). In the present study casting of hard antler buttons in September was associated with a slight decrease in plasma testosterone, which is consistent with the hypothesis of G.A. Lincoln (pers. comm., 1983) that a decline in plasma testosterone levels from greater than 1 ng/ml to levels which are barely detectable permits casting of hard antlers to occur. Rapid antler growth occurred while mean plasma testosterone levels were low (< 1.0 ng/ml). Antlers were sampled between 28 and 112 days after casting to provide maximum information on antler composition at the time of commercial harvest. Between these dates antler growth was linear. The data suggest, however, that if additional antlers had been sampled prior to 28 days and subsequent to 112 days, the overall pattern of growth of the antler would have been that of an sigmoid growth curve as observed in spike antlers (Fennessy, 1982) and in antlers from a single mature moose (Von Ballenberghe, 1983). Stags in this study produced antlers 77.3 cm in

length, and assuming a linear rate of antler elongation between 28 and 112 days growth, mean daily rates of antler growth were 0.62 cm.

Slowing of growth in length and an increase in mineralization in the tip of the 112 day antler coincided with increasing plasma testosterone levels. Similarly the initiation of regrowth antler tissue was inhibited (Table 4.5) when antlers were removed after 11 December, at or around the time when testosterone levels rose. The marked decrease in size of the cartilaginous growth plate, together with the appearance of ossified tissue at the antler tip coinciding with this increase, suggests that this hormone was involved in maturation of the cartilaginous antler tip. This is perhaps consistent with the view of Silberberg and Silberberg (1971) that although low levels of testosterone in humans have a skeletal growth promoting effect, high levels of testosterone prematurely close growth zones and stimulate early development of ossification centres. Increasing plasma testosterone levels in the present study may have accelerated antler mineralization, particularly the formation of compact bone in the periphery of the antler shaft (see later in this dicussion).

Elongation of the brow tine followed a different pattern to that of the main shaft. Brow tines were almost fully formed 56 days after casting (84% of final antler length), at which stage the antler shaft was still growing actively, and whilst plasma testosterone levels were still low. This suggests that control of elongation in the brow tine may operate through a different mechanism, or that there are differences in effectiveness of the same mechanism. Other evidence in the literature indicates that within an individual animal there are differences in the time of epiphyseal closure of bones and Smith (1956) found that in sheep the proximal epiphysis of the radius fused at around 4 months but the distal epiphysis of the same bone was not fused until 20 months after birth. In the present study longitudinal histological sections were not taken from the tines but it must be concluded that cessation of growth was also due to "epiphyseal closure".

Growth in total weight of the antler followed a similar pattern to growth in length and was linear, at a rate of 13.7 g antler fresh weight/day, over the period between 28 and 112 days after casting. Heaviest antler weights were obtained at the 112 day sample date and were approximately 130% of hard antler weight. It is not clear if this

represents peak antler weight, or whether antlers would have continued to increase in weight as mineralization progressed. The observed decrease in fresh weight between time of final sampling and hard antler stripping would be due in part to the decrease in relative blood volume, together with the loss of soft tissues at velvet stripping.

The narrow time span over which velvet stripping occurred, viz. 24 days, compared with the wide range of casting dates, viz. 53 days, indicates that variation in the period of antler growth (142-187 days) was due largely to differences in casting date. Velvet stripping occurred simultaneously from both the regrowth antler and the contralateral fully developed antler. Therefore greater weights of regrowth antler tissue were obtained from the stumps of antlers removed at an early stage of growth. These data indicate that the period of antler growth was terminated by mineralization and velvet stripping. If the rise in testosterone was responsible for the second phase of mineralization, it occurred when mean plasma testosterone levels (0.9 ± 0.15 ng/ml) were low and suggests extreme sensitivity of the tissue to this hormone. Velvet stripping in late February occurred when mean levels of plasma testosterone (3.3 ± 1.93 ng/ml) were considerably below the peak levels (up to 48 ng/ml) recorded in red stags during the rut (Barrell, Muir and Sykes, 1985).

3) Antler blood volume

The histological appearance of the antler tip indicates that the chondrocytic zone of the growing tip is vascular and this supports the findings of Goss (1983) that vascularized cartilage is present in the antler tips of Sika deer. These findings disagree with recognized texts such as Ham (1969) which states that cartilage is a non vascular tissue. Since the antler is a rapidly growing tissue simple diffusion of nutrients may be insufficient to maintain cells at the base of the chondrocytic zone, hence the necessity for vascularized cartilage in this tissue. RBV's were found, on analysis, to be lowest in the upper 5 cm of the antler shaft. It is not clear, however, whether RBV's in cartilage of the growing antler are actually lower than in ossifying regions of the antler or whether there is a drainage from soft cartilaginous tissue after antler removal. Considerable variation in RBV occurred within the antler shaft (Table 4.6) compared to results obtained in the preliminary experiment described in Part A of this section (Fig. 4.4). This may be a

result of the different sizes of tissue used (half cylinders versus quarter cylinders in Parts A and B respectively) and ideally half cylinders should be used for this type of analysis. It was possible to discern a trend of increasing RBV down the antler shaft. There was no apparent gradation in RBV from tip to base in the brow tine and this may be due to the relatively larger section sizes taken from the brow tines than from the antler shaft. Although the same size sections (5 cm) were taken in the brow tine and antler shaft that taken from the brow tine tip represents a much larger proportion of the brow tine. If the cartilage in the brow tine tip was proportionately the same size as that of the antler shaft a low blood volume would have been masked by higher blood volumes further down the tine.

A time trend was demonstrated for relative blood volume in the antler shaft and brow tine. However over the period of active growth in length total blood volume increased linearly with increasing weight, at a rate of 224 ml/kg and 110 ml/kg in the antler shaft and brow tine (Fig. 4.10), respectively, and at 194 ml/kg in the whole antler. This suggests that over the period of active growth, up to 91 days in the shaft, and to 56 days in the the brow tine antler, much of the variation in antler blood volume is attributable to variation in antler weight. Thus heavier antlers will invariably have a higher blood volume.

Irvine (1974) has described plasma volumes in a number of body tissues. Assuming negligible plasma streaming and a packed cell volume of 40% (Swenson, 1977) relative blood volumes in the kidney (255 ml/kg), liver (192 ml/kg), heart (185 ml/kg) and rib (57 ml/kg) have been calculated. Relative blood volumes in growing antlers (194 ml/kg) are therefore comparable to liver and heart muscle but substantially higher than the RBV of rib bone. Although RBV only measures the amount of trapped blood, the high values obtained here are suggestive of a high rate of blood flow through the antler.

The decrease in RBV between 91 and 112 days after casting may be a result of the rapid increase in cortical bone formation since blood volume of cortical bone is considerably lower than that of cancellous bone (Ray, 1976). RBV in bone may decrease as mineralization accelerates simply because with increased bone density there may be less blood space available. However total RBV in the brow tine declined 56 days after casting. At this time growth in length of the brow tine was almost

complete yet cortical bone was still comprised of relatively open trabeculae (Plate 4.4). Whether the decrease in RBV (and presumably blood supply), is the cause or effect of the slowing in growth, is not clear.

RBV in the antler shaft was twice that of the brow tine (224 and 110 ml/kg respectively) during the period of active growth in length. The reason for this difference is not clear although the multiple growth zones (i.e. other tines) associated with the main antler shaft could demand a higher blood supply.

4) Antler mineralization

Antler tip sections remained poorly mineralized while active growth of the antler continued. Kay et al., (1982) analysed short velvet antlers (10 to 15 cm; antler age unknown) from 2-year-old stags and also described poorly mineralized tips with a low calcium to phosphorus ratio in antler ash. However the present study indicates that low concentrations of calcium and phosphorus in ash only occurred in antler tip sections (0 to 2.5 cm) during the period of active growth in length (28 to 91 days after casting). The low values for calcium to phosphorus ratios in the tips of growing antlers were similar to those of the initial mineral deposits in epiphyseal cartilage (1.48; Urist, 1976). Calcium and phosphorus concentration in antler ash and calcium to phosphorus ratio increased in the tip of the 112 day antler. This, together with the appearance of mineralized cartilage close to the tip and slowing or cessation of growth in length at that stage, is evidence for a form of "epiphyseal closure" in antler cartilage. There were no differences in calcium and phosphorus concentrations or calcium to phosphorus ratios in other regions of the antler shaft. On a whole antler basis tip sections made up a very small proportion of antler ash. Consequently total antler ash concentrations of calcium (35.8%), phosphorus (19.8%), and calcium to phosphorus ratios (1.8:1) remained constant throughout antler growth.

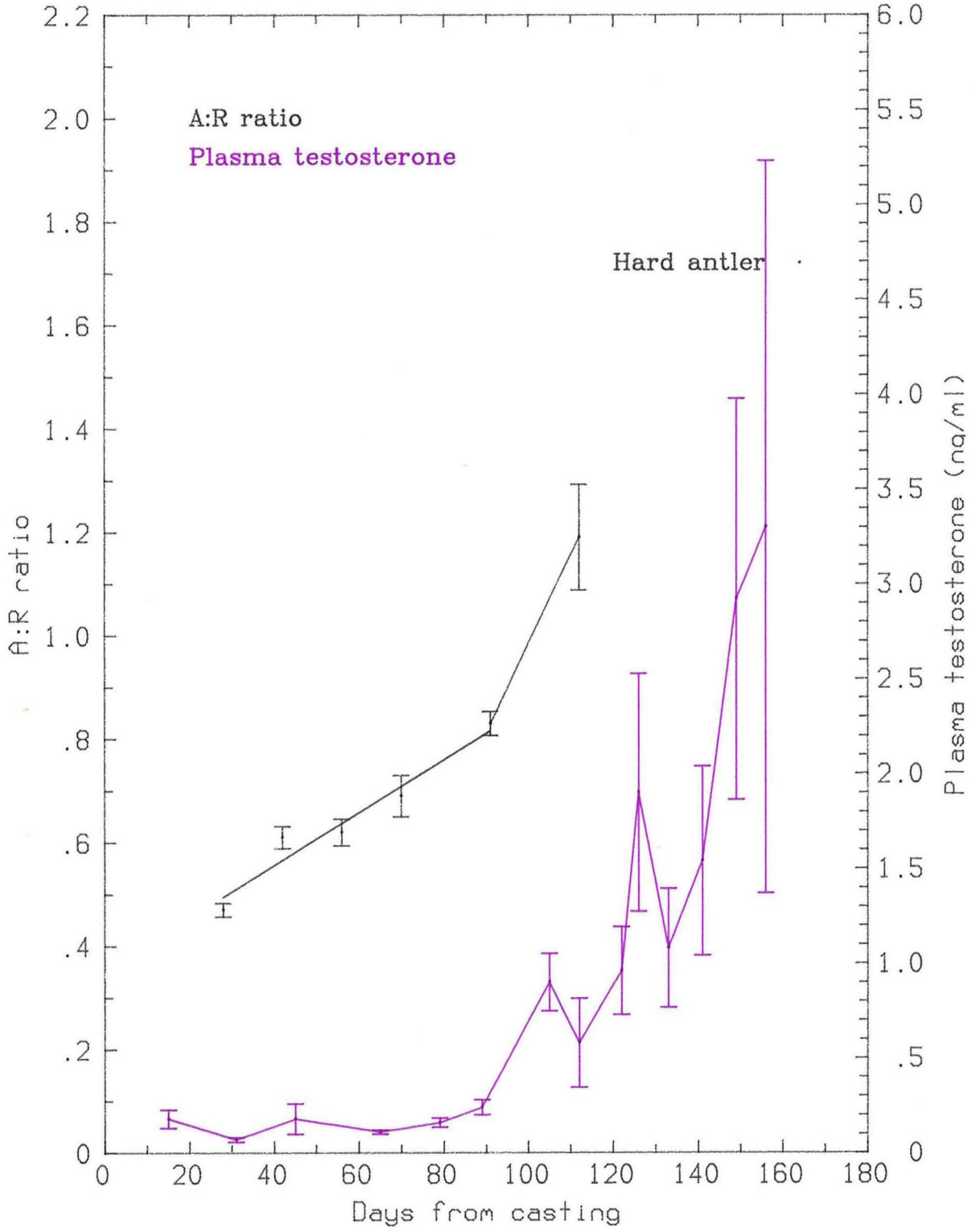
Three distinct phases of mineralization were apparent within the growing antler. In the first phase rapid mineralization occurred in a discrete band 5.0 to 7.5 cm below the antler tip and histologically this corresponded with the transition from mineralized cartilage 5 cm from the tip to trabecular bone 10 cm down the shaft. The zone of mineralization

occurred consistently in the section 5.0 to 7.5 cm below the antler tip, and therefore presumably the amount of poorly mineralized tissue (cartilage) at the antler tip remained constant during the period of active growth. A poorly mineralized tip and zone of mineralization was not demonstrated in the brow tine but may have been due to the sampling method used, i.e. 5 cm sections in the brow tine, compared with 2.5 cm sections in the tip of the antler shaft. Kay et al., (1982) described a zone of mineralization 2 to 4 cm below the antler tip of 10 to 15 cm antlers (age unknown) in 2-year-old stags. This difference may be due to differences in sampling technique, or to size of the antlers. Antlers from young stags are smaller (Huxley, 1926), and may have smaller growing points which would contribute to the discrepancy in location of the band of mineralization.

In the second phase there appears to be a gradual increase in density of trabecular bone in the antler shaft. Thus the extent of mineralization of a particular piece of bone is dependent on distance from the antler tip.

The third phase could be described as "terminal mineralization" and appears to reflect a significant change in the pattern of mineralization of the antler shaft. This occurred between 91 and 112 days after casting in association with a rapid increase in density of trabeculae in the cortex of the antler shaft and the formation of well developed osteones. Banks (1974) and Banks and Newbrey (1983) were unable to demonstrate any marked histological changes in the pattern of bone deposition within the secondary spongiosa but this may have been due to the timing of their biopsy samples. In the present study the phase of increased mineralization appeared to coincide with the most rapid rate of whole antler FFOM and ash deposition, cessation of growth at the antler tip, and an increase in plasma testosterone (Fig. 4.17). The data indicate that this phase may be the "terminal mineralization" phase of antler growth. In antlers removed at 112 days there was a general trend of increasing mineralization down the antler shaft with base sections almost fully mineralized. However in the hard antler, tip sections were the most heavily mineralized, possibly as a result of the higher ratio of compact to cancellous bone which was observed. In order to attain this status, the pattern of mineralization must have changed, with the direction of increased matrix deposition and mineralization of matrix

Figure 4.17
Change in A:R ratio (mean ± S.E.M.) in antlers between removed between 28 and 112 days after casting of hard antlers. Plasma testosterone levels (mean ± S.E.M.) were recorded in five red deer stags during the same period.



being reversed and occurring from the antler base to the antler tip. The increase in A:R ratio between antlers removed at 91 and 112 days of growth may be due to the appearance of cortical bone. Cortical bone contains more ash per unit of matrix than trabecular bone (Arnold, 1960) and skeletal bones with high proportions of cortical bone (long bones) have significantly higher A:R ratios than those such as vertebrae and ribs which have higher proportions of trabecular bone (Sykes, Nisbet and Field; 1973). It could be argued that the "terminal mineralization" phase occurs to provide support for the almost fully formed antler, and is not dependent on testosterone. However Morris and Bubenik (1983b) recently provided histological evidence that cortical bone in antlers could be modified by androgens. Testosterone (19-OH) was injected into a castrate white-tailed buck, carrying 5-month-old antlers. Prior to androgen injection the antler tip consisted of slender bony trabeculae. Subsequent to injection of 19-OH-testosterone antlers became fully mineralized and cortical bone was demonstrated in the antler tip. Since the antlers of castrate stags normally consist of trabecular bone the induction of cortical bone with 19-OH-testosterone provides further support for a "terminal mineralization" phase of antler mineralization which is under androgenic control.

Brow tine mineralization proceeded three times as rapidly as mineralization within the antler shaft. Mean brow tine lengths were only 0.23 m (in the stripped hard antler) compared to mean lengths of 0.72 m in the shaft. The pattern of mineralization occurring in the brow tine appears, therefore, to be simply an accelerated version of the mineralization which is occurring in the shaft. As a consequence therefore there was little difference in overall brow tine and antler shaft mineralization in antlers of a similar age. The data suggest that 70 days after casting less total mineralization had occurred in the brow tine than in the antler shaft. However if time of brow tine initiation had been recorded and degree of mineralization expressed as days from initiation, degree of mineralization would have been close to or greater than that of the antler shaft. In spite of a slowing in rate of elongation after 70 days of growth and a decrease in relative blood volume there was no evidence of substantial cortical bone formation at this stage (Plate 4.4).

In summary therefore in both the antler shaft and brow tine initial mineralization proceeded in spite of low testosterone levels. However plasma testosterone levels increased rapidly between 90 and 100 days after casting and coincided with the appearance of cortical bone and a rapid increase in mineralization. Chronologically these events occurred in mid to late December, close to the summer solstice. A biphasic mineralization process, under an androgenic influence, is proposed (Fig. 4.17). In a mature stag antlers increase in length and weight, blood volume increases and mineralization progresses slowly up to approximately 90 days after casting. A phase of "terminal mineralization" coincides with the slowing of linear growth, a decrease in relative blood volume and may be attributable to increasing levels of plasma testosterone.

A relationship may exist between liveweight of the stag and degree of antler mineralization. Hard antlers from stags in this study were well mineralized (ash comprising 63.0% of DM) in comparison with other antlers reported in the literature (51.7%, Kay et al., 1982; 55.3-59.0%, Hyvarinen, Kay and Hamilton, 1977). Hyvarinen, Kay and Hamilton (1977) reported that hard antlers of pen fed deer were larger and better mineralized than those of poorly nourished stags grazing hill pasture, and that antler quality decreased with increasing stocking rate. Since pen fed stags in the present studies were also heavier this effect may operate through liveweight, with heavier stags casting earlier (Section 3), and having a longer period of antler growth during which mineralization can occur. That regrowth antlers in this study had both a shorter growth period and tended to be less well mineralized than their normal counterparts would further support the relationship between length of antler growth period and degree of mineralization.

5) Mineral requirements for antler growth

Requirements for antler calcium can be derived from the rate of ash and calcium deposition. For example, using the stag in this study with the heaviest antler (1.89 kg) it is possible to calculate the total ash and therefore calcium content if both antlers had been left to mineralize fully. Assuming a hard antler composition of 81.1% DM, 63.0% ash in DM and a calcium content of antler ash of 35.0%, a total of 676 g calcium would be present. Calcium deposition follows the same pattern as total ash deposition (Fig. 4.15) therefore a peak rate of calcium

deposition (33% of total antler calcium) would occur between 91 and 112 days after casting. Peak antler calcium requirements would be 10.7 g/day.

Similarly it is possible to calculate whether the low calcium diet in the present study would have provided adequate levels of dietary calcium for normal antler growth. Mean weight of single antlers removed from stags offered the low calcium diet (group C) was 0.99 kg. Using the same calculation as above (for one antler only, ignoring regrowth antler) peak requirement for antler calcium would have been 2.8 g/d between 91 and 112 days after casting. An estimate of the calcium required to maintain other body processes (faecal endogenous loss) can be made using estimates made for sheep and cattle of 16 mg/kg liveweight per day (ARC, 1980). During the latter stages of antler growth mean stag liveweight was approximately 170 kg, therefore faecal endogenous loss would have been in the order of 2.7 g/d. Total daily calcium requirement of the stag over this period would have been approximately 5.5 g. Total calcium available to the stag also can be estimated. Mean feed intake was 2.75 kg DM, and calcium content of the ration 3.0 g/kg DM therefore total calcium intake would have been 8.3 g/d. However not all dietary calcium may be available to the stag and using a value of 68%, as recommended for sheep and cattle by the ARC (1980), a value of 5.6 g/d was calculated for available dietary calcium. This calculation suggests that these stags were not deficient in dietary calcium.

In other ruminants (ARC, 1980) mobilisation of skeletal calcium occurs during lactation to meet a dietary deficiency of calcium and a similar phenomenon may occur during antler growth in stags. Meister (1956) and Hillman, Davis and Abeldakhi (1973) provide histological evidence of skeletal bone resorption, and Brown, Cowan and Griel (1978) demonstrated radiographically a decrease in metacarpal density during antler growth. A further kinetic study of calcium metabolism in stags, employing a radio-isotope was therefore undertaken to provide quantitative data on the degree to which skeletal calcium is mobilised to provide calcium for antler development, and to provide data on endogenous loss and availability of dietary calcium in red deer stags.

SECTION 5

Calcium metabolism in stags during peak antler mineralization

Introduction

Calcium is the most predominant mineral in the body and is essential for a variety of functions, such as blood coagulation, membrane permeability, neuromuscular excitability, transmission of nerve impulses and activation of certain enzyme systems. Most calcium in the body (99%) is stored in the skeleton where it functions as support for body tissues and as a reservoir of calcium, being continually removed (resorption) and added to (accretion). The remaining 1% is present in body fluids and other tissues of the body. Much of our knowledge regarding calcium metabolism in ruminants has come from detailed studies with sheep and the following review deals largely with this species.

Calcium is continually excreted from the body in urine and faeces (faecal endogenous loss), with additional losses during lactation. Calcium must be absorbed from the diet to replenish these losses. The amount of dietary calcium available to the animal is therefore determined by the rate of absorption of calcium from the small intestine. Absorption of dietary calcium is a two phase process (Wasserman et al., 1971), involving passive diffusion down a concentration gradient and an active process requiring vitamin D. Vitamin D supplementation has been shown to improve calcium availability in dairy cows (Ward, Dobson and Dunham,1971) although the active forms of vitamin D are actually the metabolites of vitamin D produced in the body. Vitamin D is hydroxylated to 25-hydroxycholecalciferol in the liver and further hydroxylated in the renal cortex. Of the vitamin D metabolites, $1,25(OH)_2D_3$ has the most marked effects, that of increasing bone resorption and absorption of calcium from the intestine (Care, Barlet and Abdel-Hafeez, 1980). Parathyroid hormone and calcitonin are also intimately involved in calcium homeostasis, with parathyroid hormone raising plasma calcium concentration through elevating the production of $1,25(OH)_2D_3$ and increasing bone resorption. Calcitonin on the other hand has a role in the reduction of plasma calcium concentration through the reduction of bone resorption. One theory concerning calcium absorption, proposed by

Nicolaysen, Eeg-Larsen and Malm (1953) is that the active phase of calcium absorption may be dependent upon degree of demineralization of the skeleton. This argument was also advanced by Sykes and Dingwall (1975) and Braithwaite (1978) as a possible explanation for high rates of calcium absorption obtained during lactation in ewes which had demineralized skeletons.

Requirement of the animal for calcium is an important determinant of the rate of absorption. For example, Braithwaite and Riazuddin (1971) found that calcium absorption in growing lambs declined with age, presumably as the requirements for skeletal growth declined. Similarly higher rates of calcium availability have been demonstrated in lactating than non lactating ewes (Braithwaite, 1983). Calcium content of the diet (Luick, Boda and Kleiber, 1967) and level of feed intake (Braithwaite, 1975) may also affect the rate of calcium absorption.

A number of other factors may affect the rate of calcium absorption, for example a 2:1 ratio of calcium to phosphorus in the diet is optimal, and extreme ratios below 1:1 and above 7:1 may result in decreased availability (Payne, 1977). Level of dietary protein may also affect availability of dietary calcium. Where animals are protein deficient skeletal protein may be mobilised and the resulting liberation of calcium may decrease the requirement for dietary calcium absorption (Sykes and Field, 1972). These authors considered that in sheep on low calcium diets but fed adequate protein, availability of dietary calcium might approach 100%.

When insufficient dietary calcium is absorbed rate of skeletal bone calcium resorption is elevated to maintain homeostasis. For example, Braithwaite, Glascock and Riazuddin (1969) compared lactating and non-lactating sheep and found rates of skeletal bone resorption of 64.4 and 14.1 mg/kg bodyweight (BW)/day, respectively. Rates of skeletal bone accretion were also increased in the lactating ewes compared to the non-lactating ewes (30.2 and 20.3 mg/kg BW/day, respectively). Therefore net bone resorption was increased to meet the demands of lactation. Subsequently remineralization and net accretion occurred during periods of low calcium requirement (Braithwaite, Glascock and Riazuddin, 1969).

Few data are available on calcium metabolism in the stag and no studies have yet clearly examined the extent to which skeletal calcium is utilised for antler growth. From studies with phosphorus Rerabek and Bubenik (1963) considered that all the minerals necessary for antler growth were derived from the diet. However Meister (1956) provided histological evidence of an increase in skeletal porosity during antler growth. Banks et al. (1968a) further demonstrated that the number of resorption spaces in ribs increased during antler growth and this was associated with a decrease in rib density and ash to volume ratio (Banks et al., 1968b). Recent work by Stephenson and Brown (1984) examined calcium metabolism in white-tailed deer and provided estimates of whole body calcium flux in these animals. Unfortunately in this study antlers were not sampled and these authors could not differentiate between calcium deposited in the skeleton or in the antler. Therefore although skeletal bone minerals are undoubtedly mobilised during antler growth the amount of antler calcium derived from the skeleton has, as yet, not been quantified.

This study was therefore conducted to examine calcium metabolism in the stag over the period of peak antler mineralization (91 to 112 days after antler casting, Section 4) and to determine the possible source of antler calcium. Stags were offered maintenance levels of a forage with a low calcium content to allow determination of calcium availability under conditions where requirement for calcium is high, yet intake of calcium low. Rates of skeletal turnover and deposition of calcium into antlers over this period were quantified using radio-isotopes. The results of this study also have enabled measurement of urinary and faecal endogenous loss of calcium in red deer.

Experimental methods

1) General

Two mature red deer stags (589 and 103) were used during the period of peak antler mineralization between 91 and 112 days after casting. A low calcium ration was offered during two calcium balance periods, the second of which employed kinetic analysis using a radio-isotope (^{45}Ca). The stags were individually penned in the Lincoln College deer shed prior to casting of hard antler stubs, which occurred on the 5 and 13 September for stags 103 and 589 respectively.

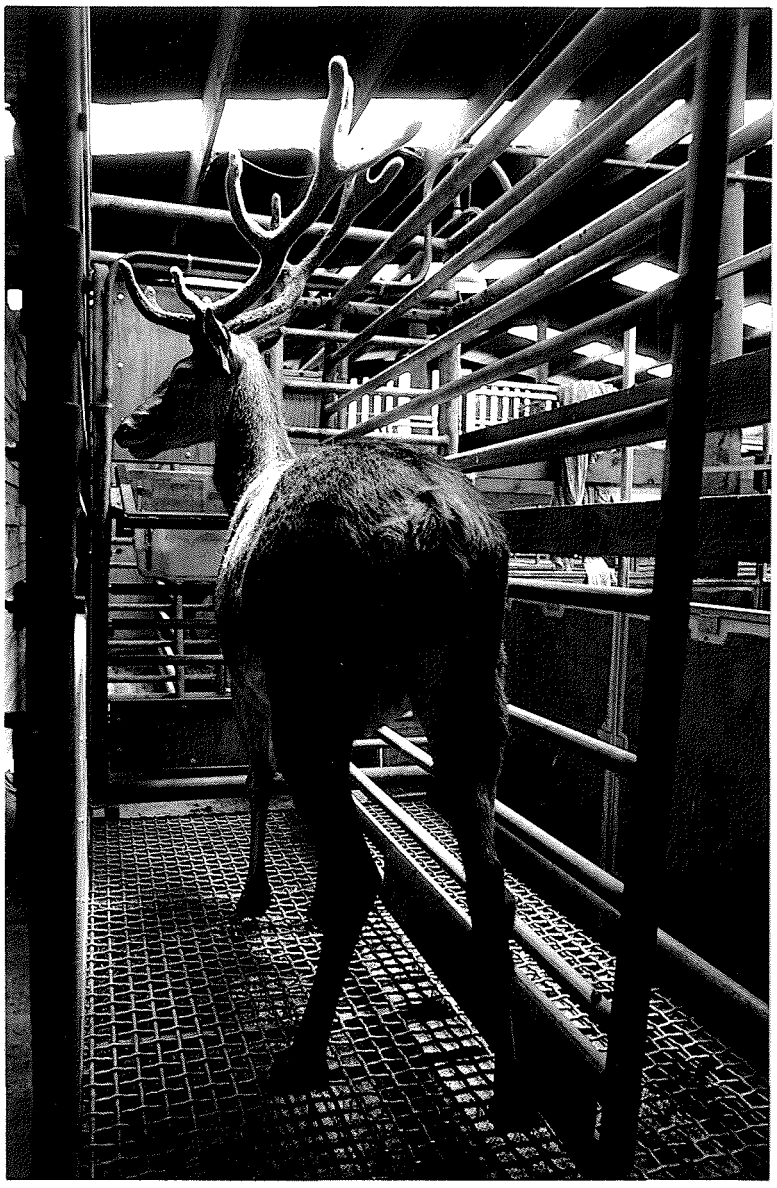
Throughout the pre-trial period the stags were offered proprietary deer nuts (NRM, Christchurch) ad libitum and weighed weekly. Final liveweights were recorded 27 days prior to commencement of the first balance period, further weighings being precluded by the possibility of damage to antlers. Stags were then adjusted to the low calcium ration (fresh greenfeed oats) and were not placed under any feed restriction. They were placed in metabolism crates on the 24 November. The metabolism crates (Plate 5.1) were constructed from 35 mm galvanised steel pipe with a floor composed of 8 mm galvanised steel mesh (grid size 30 mm). The front and one side of the crate were movable so that width and length of the crate could be adjusted to prevent the stags from turning around. A perforated galvanised iron screen overlaying a sloping galvanised iron tray allowed separate collection of faeces and urine.

Balance Trial 1 commenced on 30 November (77 and 85 days after casting for stags 589 and 103, respectively) and samples were collected over a 12 day period. Final faecal and urine collections were completed immediately prior to the start of Balance Trial 2. At commencement of Balance Trial 2 (11 December) antlers of stags 589 and 103 were aged 89 and 97 days respectively.

2) Low calcium diets

A progressive deterioration in feed quality normally occurs over summer so the greenfeed oats were cut on two occasions in late November to avoid any changes in feed quality. Daily rations were weighed into cotton bags and stored at -15°C. Rations from the first cut (Cut 1, 27 November) were used in the first calcium balance trial and those from the second cut (Cut 2, 30 November) in the subsequent radio-isotope

Plate 5.1
The type of metabolism crate used during the present experiment.



Note: The photograph was taken post-experimentally

balance trial. Feed was offered at a level expected to be approximately 25% above maintenance. This was calculated using an estimated M/D value for greenfeed oats at the stage immediately prior to ear emergence ("boot" stage) of 11.5 MJME/kg DM (Ulyatt et al., 1980) and a spring maintenance requirement for pen fed red deer stags of 0.57 MJME/kg $W^{0.75}$ (Fennessy, Moore and Corson, 1981).

3) Balance Trials

Faeces were collected daily throughout both balance periods, weighed, and sub-samples frozen. Urine volume was recorded daily and sufficient 2N HCl added to adjust pH to 3.0 to prevent precipitation of calcium. Sub-samples of urine were stored frozen at -20°C. Blood samples were obtained on Day 1 (11 December) and at three day intervals thereafter by jugular venepuncture using heparinised vacutainer tubes. During Balance Trial 1 (8 December) indwelling polyvinyl catheters were placed in each of the jugular veins. Both catheters were sewn to the skin of the animal's back such that blood sampling could be conducted from a site external to the crate and without manual restraint. Patency was maintained with heparinised saline (50 i.u. sodium heparin per ml).

At the commencement of the second balance period both stags were injected with a single dose of ^{45}Ca (800 μCi $CaCl_2$, Amersham) in the left jugular vein, and blood samples were obtained through the contralateral catheter in the right jugular vein at 30 second intervals for the first 2 min and thereafter at 3, 4, 5, 8 and 10 min. Between 10 and 30 min after injection samples were taken at 5 min intervals and then at 10 min intervals between 30 and 60 min. Sampling was conducted at 20 min intervals over the next hour, 2 hourly intervals between 2 and 12 hours and at 4 hourly intervals over the next 12 hours. Blood samples were then taken at 6 hourly intervals for the next six days and thereafter at 12 hourly intervals until the end of the balance period, 13.5 days after isotope injection. Prior to each sampling, saline and blood were drawn from the catheter into a 50 ml syringe and discarded. The sample (approximately 10 ml) was then drawn into a 20 ml syringe and the time recorded when half the sample had been collected. Blood samples were placed in heparinised 10 ml glass vacutainer tubes and plasma separated by centrifugation within 60 min of sampling.

Faeces and urine were collected daily, sub-sampled and stored as in Balance Trial 1. Upon completion of the experiment (24 December) the stags were killed by intravenous injection of a lethal dose of sodium pentobarbitone (pentobarb 500, South Island Chemicals, N.Z.). Antlers (aged 102 days and 110 days for stags 589 and 103, respectively) were removed by sawing immediately below the coronet. Antlers were weighed, had their length and tine measurements recorded (as described in Section 4), and were frozen at -20°C for later analysis. The 3rd rib and tibia (both from the left side) and the 3rd lumbar vertebra were removed from each stag and stored at -20°C.

4) Sample preparation and analysis

Blood samples were centrifuged at 3000 rpm for 20 min. Duplicate 1 ml samples were placed in 20 ml scintillation vials. The remaining plasma (2 to 3 ml) was stored at -20°C.

Faeces samples were freeze dried, their dry matter content calculated and then ground to less than 1 mm sieved size. Ground faeces (5 g sample size) were wet ashed in 60 ml nitric acid and 10 ml perchloric acid (72%) at 150-200°C and made up to 20 ml with distilled water.

The left antler from each stag was sectioned longitudinally in a similar manner to that described in Section 4, though complete antler cylinders rather than half cylinders were used. Skeletal bone samples were scraped clean of muscle and connective tissue prior to determining their fresh weight, before analysis. Bone volume was not measured, but antlers and bone samples were dried, extracted in petroleum ether, ashed and A:R ratios calculated. Ground ash samples (1 g) of skeletal bones and antler sections were digested using the technique described above.

Calcium concentration in faeces, antler and skeletal ash digesta, urine and plasma, was then determined by atomic absorption spectrophotometry as outlined in Section 4.

5) Radio-isotope counting

Samples were counted in a Phillips PW4700 liquid scintillation counter. Nine ml of scintillation cocktail were added to 1 ml of acid digests of faecal, antler and skeletal ash, and of plasma and urine. For the scintillation cocktail, one litre of triton X-100 was added to each

litre of filtered toluene. Five g of primary scintillant (PPO, 2,5-diphenyl- oxazole) and 0.3 g of secondary scintillant (POPOP, 1,4-di-2-(-5-phenyl- oxazolyl) was added, completely dissolved, and the mixture stored in the dark at 3°C. This cocktail gave the following efficiencies of counting : plasma 81.5%, antler and skeletal ash 81.3%, faeces 67.8% and urine 76.8%. Sample counts were corrected for quenching using an external quench correction curve derived from a series of coloured samples, using haemolysed blood for plasma samples, and ashed faecal material for the ash, faeces and urine quench series.

6) Data analyses

Counts per minute were corrected for counting efficiency to calculate disintegrations per minute (dpm), and all samples were corrected for decay to a standard time. A time curve of plasma specific activity (dpm/g of calcium) was plotted and the integral of the curve, which gives the mean plasma specific activity over the duration of the experiment (Braithwaite and Glascock, 1975), was calculated using a program written by J. Bird (Biochemistry Department, Lincoln College).

Faecal endogenous loss was calculated from the total radioactivity (dpm) collected in faeces over the duration of Balance Trial 2, divided by mean calcium specific activity (SA) of plasma (Braithwaite and Glascock, 1975).

Net calcium accumulation in antlers was calculated by the same method, using total radioactivity in the antlers at time of removal.

Rate of urinary calcium excretion was determined both metabolically, and from the isotope data, as with faeces and antlers.

Rate of absorption of calcium (V_a) was calculated from the difference between total calcium intake (V_i) and total faecal output (V_F), corrected for faecal endogenous loss (V_f), (Braithwaite and Glascock, 1975).

$$V_a = V_i - V_F + V_f$$

7) Model of calcium metabolism

The model of calcium metabolism in the stag described on pages 123 and 124 and in Table 5.3 was derived using the SAAM (Simulation, Analysis and Modelling) computer program (Berman and Weiss, 1978). The model was similar to that used for lactating dairy cattle (Ramberg et al, 1970) and calcium deposited in antlers was considered to be a loss from the system, as in lactation (Fig. 5.1). The justification behind this assumption is discussed on page 120. Four exponentials, representing four compartments or exchangeable pools were obtained by manual curve peeling from a semi-log plot of plasma specific activity versus time. An auxillary program (Mapper) compiled by L.P.R. Danielson and C.F. Ramberg of Ruakura Agricultural Research Centre and described by Wastney (1980) was used to convert these observed exponential equations to fractional flow rates between compartments. These initial estimates of fractional flow rates, together with rate of loss of ^{45}Ca from the body (expressed as a percentage of initial dose accumulated in faeces, urine and antlers) were submitted to the program. Plasma isotope data was also included in the form of percentage of initial dose. The SAAM program used an iterative process to give a least squares fit to the experimental data and produce a steady state solution. As well as rate transfers between compartments the model produced data for compartment masses, urinary excretion, faecal endogenous loss and accretion of calcium in the antlers and skeleton, together with an output of expected and observed data for urine, faeces and antlers.

Since a steady state is assumed (i.e. the total mass of calcium remains constant over the experiment) the amount of calcium resorbed from bone (V_{o-}) must equal the difference between total animal requirement for calcium (faecal endogenous loss (V_f), urinary excretion (V_u), antler accretion (V_{antler}) and skeletal accretion (V_{o+})) and calcium absorbed from the gut.

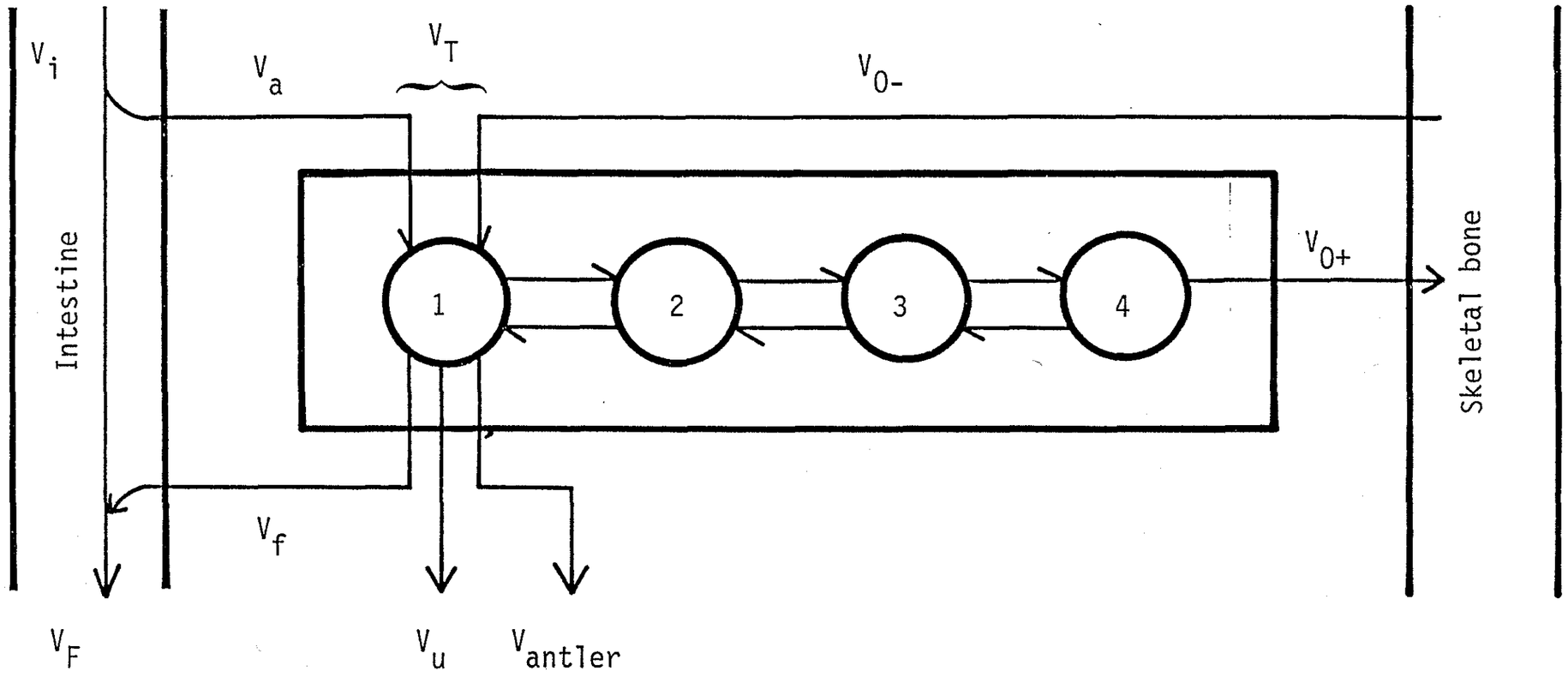
$$V_{o-} = (V_f + V_u + V_{antler} + V_{o+}) - V_a$$

Calcium balance $\triangle$ (i.e. the difference between calcium accretion in skeletal bone V_{o+} and resorption from skeletal bone V_{o-}) can also be calculated.

$$\triangle = V_{o+} - V_{o-}$$

Figure 5.1

Scheme of calcium metabolism in the stag (adapted from Ramberg et al., 1970). Compartments 1 to 4 represent exchangeable calcium pools. Inputs of calcium to the system (V_T) via compartment 1 are absorption from the alimentary tract (V_a) and resorption from the skeleton (V_{0-}). Calcium is lost from compartment 1 to the intestine (V_f), urine (V_u) and antlers (V_{antler}). Calcium also enters skeletal bone from compartment 4 (V_{0+}). V_i represents total dietary calcium intake and V_F the total amount of calcium lost in faeces.



Results

Twenty seven days prior to commencement of Balance Trial 1 stags 589 and 103 weighed 167.5 and 148.0 kg, respectively. Over the period prior to commencement of Balance Trial 1 the animals had been adjusted from a pelleted concentrate ration to fresh greenfeed oats and introduced into and become accustomed to metabolism crates. Liveweight gains would probably have been low and liveweights during the balance periods have therefore been estimated at 170 and 150 kg, respectively.

Data obtained from Balance Trials 1 and 2 have been summarised in Table 5.1. Only parameters measured metabolically were available for Balance Trial 1. Data presented for Balance Trial 2 includes faecal endogenous loss and antler calcium accretion (both measured from the total cumulative output of isotope divided by the mean plasma specific activity) and calculated rate of calcium absorption. No data derived from the SAAM program has been included, but is discussed later in the model of calcium metabolism in the stag (pages 123 and 124).

1) Nutrient intake

Digestibility of the oat ration over the two balance periods was found to be 71%, and M/D values for the ration would therefore have been closer to 10.5 MJME/kg DM than the previously estimated 11.5 MJME/kg DM. This meant that the stags were offered ME at approximately 10% above maintenance. During Balance Trial 1 stags 589 and 103 consumed 2.89 and 2.55 kg of DM, respectively. During Balance Trial 2 greater feed refusals, particularly in the case of stag 589, resulted in lower daily feed intakes, 2.40 and 2.37 kg DM for stags 589 and 103, respectively. These intakes represented energy intakes of approximately 90% and 100% of maintenance for stags 589 and 103 respectively.

Calcium content of the feed was higher in the oats cut for Balance Trial 1 (3.79 g/kg DM) than in those cut for Balance Trial 2 (2.78 g/kg DM). As a result of lower calcium content and lower intake total calcium intake declined between Balance Trials 1 and 2 (Table 5.1). Phosphorus levels in feed offered also declined, from 2.89 g/kg DM (Balance Trial 1) to 2.43 g/kg DM in Balance Trial 2. This resulted in a slight increase in calcium to phosphorus ratio in the feed consumed, from 1:1.31 to 1:1.14 between the two balance studies.

Table 5.1

Calcium metabolism in two red deer stags offered low calcium diets over two balance periods, during late antler growth. Values have been calculated both metabolically (mean ± S.E.M. have been given) and from the radio-isotope data (mean values only are given).

	Stag 589	Stag 103
Balance Trial 1		
Liveweight (kg)	170	150
Age of antlers at commencement of balance period (days after casting)	77	85
Dry matter intake (kg)	2.89 ± 0.078	2.58 ± 0.076
Rate (mg/kg BW/day) of :		
Ingestion of calcium	64.5 ± 1.75	64.4 ± 1.91
Total calcium in faeces	41.8 ± 4.82	50.2 ± 2.49
Excretion of calcium in urine	6.3 ± 0.94	5.9 ± 0.67
Apparent availability of calcium (%)	35	22
Mean plasma calcium (n = 3) (mg/l)	86 ± 1.2	83 ± 2.1
Balance Trial 2		
Liveweight (kg)	170	150
Age of antlers at commencement of balance period (days from casting)	89	97
Dry matter intake (kg)	2.40 ± 0.032	2.37 ± 0.042
Rate (mg/kg BW/day) of :		
Ingestion of calcium	39.2 ± 0.52	43.8 ± 0.78
Total calcium in faeces	27.2 ± 2.99	39.3 ± 1.65
Excretion of calcium in urine	2.0 ± 0.46	2.7 ± 0.24
Faecal endogenous loss †	6.6	6.1
Absorption of calcium	18.5	10.6
Net calcium accretion in antlers†	48.8	38.8
Apparent availability of calcium (%)	47	24
Mean plasma calcium (n = 60) (mg/l)	85 ± 0.13	82 ± 0.12

† Calculated from radio-isotope data

2) Antler composition

Total antler tissue removed from stags 589 and 103 at the end of Balance Trial 2 weighed 4514 and 3004 g, respectively. Differences in antler length (0.710 m, 589; versus 0.705, 103) were less marked. The antlers removed from stag 589 were also younger (102 days) than those from stag 103 (110 days). The antler from each stag sectioned for analysis comprised 4 tines. Both contained brow and bey tines, but whereas the antler from stag 589 had two royal tines, that from stag 103 had a trey tine and a single unbranched main beam.

Distal tip sections (0 to 2.5 cm from the antler tip) were less well mineralized than other sections in the antler shaft. In the antler removed 102 days after casting from stag 589 A:R ratio increased from 0.16 in the section 0 to 2.5 cm below the tip, to 0.45 in the subjacent section. The values in comparable sections in the 110 day antler removed from stag 103 were 0.32 and 0.56, respectively. Mineralization was greatest nearer the antler base. In the antler from stag 589 A:R ratio increased from 0.52, in the section 5.0 to 7.5 cm below the tip, to 1.30 in the antler base. For the antler from stag 103 values were 0.66 and 1.10 respectively. For the same reasons as those discussed in Section 4, namely the differences in pattern of mineralization in the antler tip, regression equations were fitted to describe the pattern of mineralization in the antler shaft between the section 5.0 and 7.5 cm from the antler tip and the antler base. The respective regression equations are given below; where Y = A:R ratio and X = distance from the antler tip (cm).

a) Stag 589; $Y = 0.0180 X + 0.365$ ($r = 0.97^{**}$; RSD = 0.31)

b) Stag 103; $Y = 0.00835X + 0.692$ ($r = 0.96^{**}$; RSD = 0.14)

Calcium concentration in antler ash was low (283 mg/g) in the distal tip section of the younger antler (589), but increased to 368 mg/g in the subjacent section. Thereafter calcium concentration remained constant down the antler shaft of both antlers, with mean calcium concentrations of 375 mg/g and 388 mg/g antler ash in stags 589 and 103, respectively.

Total antler A:R ratios were similar (0.97 and 0.95 in stags 589 and 103, respectively). Antlers removed from stag 589 were considerably heavier than those from stag 103. Consequently the total amount of antler ash (both antlers) was correspondingly greater (1038 g and 699 g, respectively). Of the radio-isotope administered, 73.4% of the initial dose was recovered in the antlers of stag 589, and 61.8% in the antlers of stag 103. Net daily flow of calcium into the antlers was obtained from total antler specific activity divided by the mean plasma specific activity. This calculation was based on the assumption that radioactive calcium deposited in the antler matrix was unavailable and could not re-enter the body pool. Over the 13 days of Balance Trial 2, calculated rates of net calcium deposition in the antler were 8.29 and 5.82 g per day for stags 589 and 103, respectively. On a bodyweight basis this represents a net rate of calcium deposition in the antler matrix of 48.8 and 38.8 mg/kg BW per day.

3) Calcium concentration in plasma and rates of excretion of calcium in urine and faeces

No changes in plasma calcium concentration were observed in either stag over the course of the study. During Balance Trial 1 mean plasma calcium concentrations were 86 and 84 mg/l and during Balance Trial 2 were 85 and 81 mg/l for stags 589 and 103, respectively. In both stags rate of loss of radioactivity from plasma was extremely rapid during the first few hours after isotope injection but slow over the last 12 days of the experiment (Fig. 5.2a). Mean plasma specific activity, as measured by the integral of the curve (Braithwaite, 1975) was 62,493,000 and 74,385,000 dpm/g calcium/day for stags 589 and 103 respectively.

Calcium was excreted in urine at rates of 6.3 and 5.9 mg/kg BW/day for stags 589 and 103 respectively, during Balance Trial 1. Rates of excretion during Balance Trial 2 (also calculated metabolically) were 2.0 and 2.7 mg/kg BW respectively (Table 5.1). These latter results differed from the results obtained from the radio-isotope data. Over the 13 day balance period 2.0 and 8.4% of total injected ^{45}Ca was excreted in urine of stags 589 and 103 respectively (Fig. 5.2b). These represented rates of excretion of calcium in urine of 1.3 and 5.3 mg/kg BW per day for stags 589 and 103, respectively.

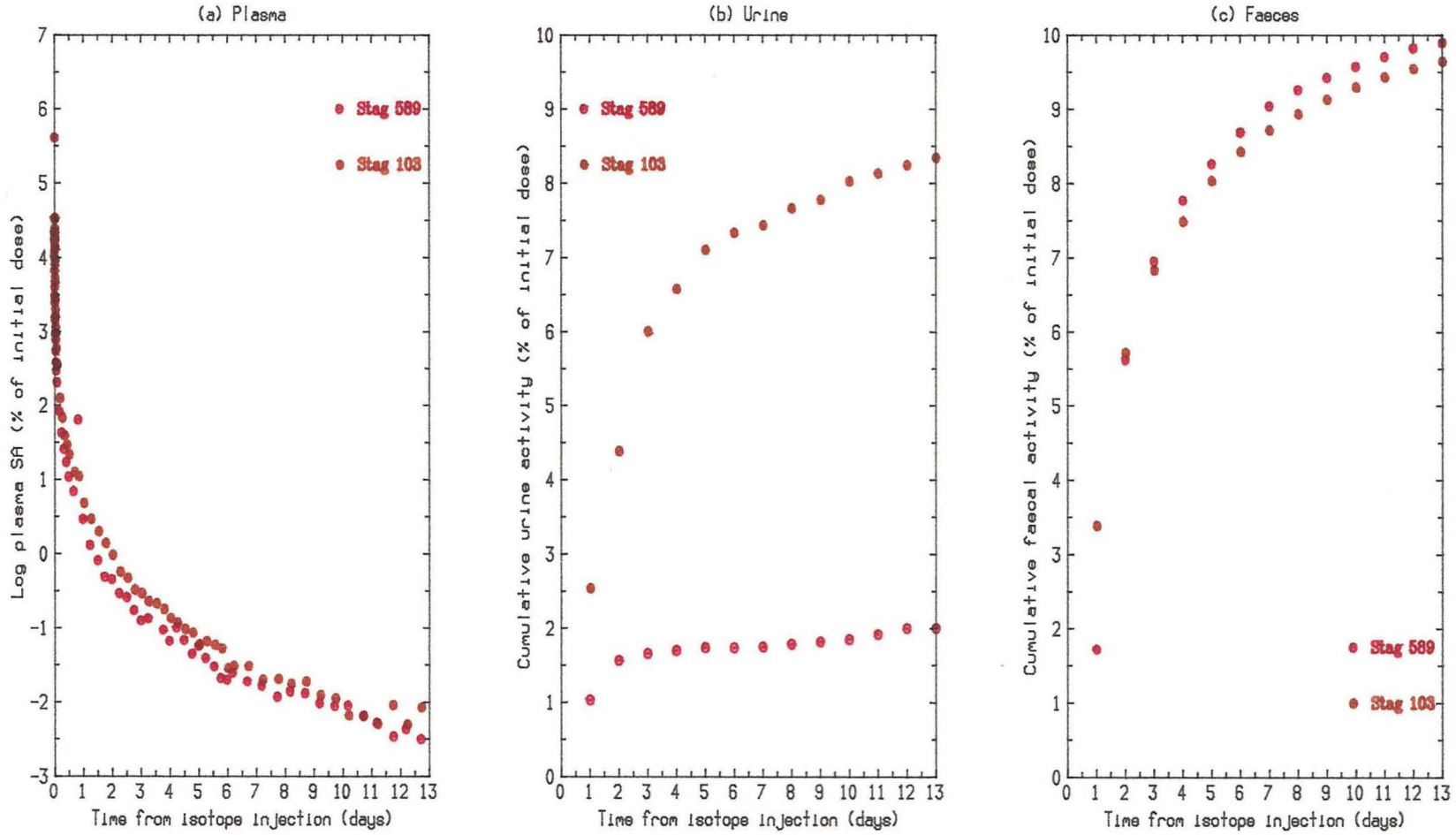
Figure 5.2

Radio-isotope (^{45}Ca) data recorded in two red deer stags during late antler growth:

a) Rate of loss of specific activity (SA) from plasma (log SA versus time)

b) Cumulative excretion of ^{45}Ca in urine

c) Cumulative excretion of ^{45}Ca in faeces



Calcium was excreted in faeces at rates of 41.8 and 50.2 mg/kg BW per day for stags 589 and 103 respectively, during Balance Trial 1. Total faecal calcium excretion declined during Balance Trial 2 to 27.2 and 39.3 mg/kg BW per day (Table 5.1).

4) Faecal endogenous loss and rate of calcium absorption

Total radioactivity collected in faeces represented 9.9 and 9.7% of total injected radioactivity in stags 589 and 103, respectively (Fig. 5.2c). Rates of net faecal endogenous loss (calculated from the total radioactivity collected in faeces, divided by mean plasma specific activity) were 6.6 and 6.1 mg/kg BW per day in stags 589 and 103 respectively during Balance Trial 2.

The calculated true rate of absorption of calcium was 18.5 mg/kg BW/day for stag 589, almost twice the daily rate of 10.6 mg/kg BW/day calculated for stag 103 over the same period.

5) Skeletal composition

Degree of mineralization (A:R ratio) and specific activity per gram of bone calcium in the tibia, rib and vertebra of the two stags are given in Table 5.2. In both stags the tibia had the greatest degree of mineralization of matrix, and vertebra the poorest. There was an inverse relationship between degree of mineralization and calcium specific activity, with the poorly mineralized vertebra having the highest specific activity. Mean specific activities of antler calcium in the stags 589 and 103 at 1,325,400 and 1,605,300 dpm/g, respectively were 10 to 40 times that in bone.

Table 5.2

Degree of mineralization (A:R ratio) and specific activity of ash (SA; dpm/g calcium) in the tibia, rib and vertebra of two red deer stags slaughtered during late antler growth and 13 days after the intravenous infusion of ^{45}Ca (800 µCi $CaCl_2$).

	Stag	
	589	103
Tibia		
A:R	1.60	1.49
SA	34,100	47,600
Rib		
A:R	1.25	1.28
SA	84,300	123,600
Vertebra		
A:R	0.99	0.87
SA	100,500	150,800

Discussion

1) Antler mineralization

Within the antler shaft mineralization followed a similar pattern to that described in Section 4. Mineralization increased from tip to base down the antler shaft, although at a greater rate in the antler from stag 589 than that from stag 103. A poorly mineralized tip section in the antler from stag 589 suggests that active elongation may still have been occurring. On the other hand a greater degree of mineralization in the tip section of the antler from stag 103 suggests that growth in length may have been completed by the time of antler removal. However in spite of the difference in age between antlers (8 days) there was little difference between the two antlers in terms of total A:R ratio. This may simply reflect normal between-animal antler variation.

Calculation of net rate of antler calcium accretion from the radio-isotope data gave values of 8.29 and 5.82 g/d for stags 589 and 103, respectively. However if resorption of antler calcium had occurred over the isotope balance period these values would underestimate the net rate of daily increase in antler calcium. Using the equations derived in Section 4 it was possible to calculate the expected rate of net antler calcium deposition for the stags in the present study. Expected hard antler weight was calculated from the linear relationship (Fig. 4.8) describing growth in weight of the antler between 28 and 112 days after casting, where Y is the proportion of hard antler weight and X is the age of the antler in days.

$$Y = -7.70 + 1.26\ X \quad \text{(Fig. 4.8)}$$

Antler age and weight at removal were 102 and 110 days and 4514 and 3004 g for stags 589 and 103, respectively. Calculated stripped hard antler weight (for the respective stags) was 3731 and 2295 g, respectively. Hard antler composition has been described in Section 4 (81.1% DM, 63.0% ash in DM, 35.0% calcium in ash). From these data and using total hard antler weight, total hard antler calcium contents of 667 and 410 g were calculated for stags 589, and 103, respectively. The percentage of final antler calcium deposited at the beginning and end of Balance Trial 2 was then derived from the equation describing rate of calcium deposition in the antler, Y being the percentage of hard antler calcium, and X the age of the antler (see page 83).

Note: These calculations have been based on the assumption that calcium deposition in antler matrix was linear over the duration of the experiment (see Fig. 4.15). If calcium deposition was greater in the latter stages then proportionally more calcium with a low specific activity would be deposited. In these circumstances antler calcium would have a lower specific activity than if calcium deposition was linear. This may explain why net calcium deposition appeared to be slightly lower than the expected rate.

$$Y = 0.00012\ X^{2.81}$$ (Fig. 4.15)

On this basis it can be calculated that 16.8% and 19.5% of total antler calcium should have been deposited, respectively, during Balance Trial 2. Therefore estimated daily rates of calcium deposition were 8.62 g and 6.15 g for stags 589 and 103, respectively. Actual rates of net calcium deposition in the antlers (calculated from radio-isotope data) were 8.29 and 5.82 g/d and were therefore 95% of the expected rate. The assumption that the antler is, in fact, acting as a sink for calcium seems justified. The present data also suggest that calcium deposited in antler matrix is unavailable for further turnover (i.e. antlers are acting as a sink for calcium). This is a new and unexpected finding. Payne (1977) states that rapidly forming bone mineral has a high surface area and therefore is rapidly available for resorption. The current findings also conflict with the histological observations of Belanger, Choquette and Cousineau (1967) who described osteocytic osteolysis in the trabecular bone of actively growing antlers of male and female reindeer. These authors did not quantify the amount of osteolysis and it is possible that such resorption was negligible with respect to the total amount of calcium accretion during the period of rapid antler growth. These authors also suggested that osteolysis also continued after the period of active growth. Moreover Care et al., (1984) reported that antlers from a normal red deer stag had a lower specific gravity and contained more resorption lacunae than did hard antlers from a parathyroidectomised/ thyroidectomised stag, suggesting that lack of parathyroid hormone may have led to a reduced capacity for resorption of antler bone. In the latter stages of antler growth rate of calcium accretion could be expected to slow and under these circumstances the effect of any resorption would be more pronounced.

These results indicate that stags in this study were capable of maintaining high and probably normal rates of antler calcium deposition despite being offered a low calcium ration. These findings conflict with the results obtained by Ullrey (1983) who found that white-tailed deer produced antlers with a low specific gravity on a ration as low as 1.8 g calcium/kg DM. These latter stags were offered this ration over an extended period (twelve months) and following severe skeletal demineralization antler mineralization may have been reduced. In the short term at least, the stag appears to be able to maintain antler

mineralization at the expense of considerable skeletal demineralization.

2) Plasma calcium

Plasma calcium levels of 85 and 82 mg/l for stags 589 and 103 respectively, were within the normal range reported for sheep (8 to 12 mg/100ml, Field and Suttle, 1969) but lower than the mean value of 10.9 mg/100ml obtained in white-tailed deer by Graham et al. (1962). The latter authors and Morris and Bubenik (1983a) found no evidence for seasonal changes in plasma calcium levels in white-tailed deer. Although significant decreases in plasma calcium levels have been demonstrated in sheep maintained on low calcium rations during gestation and lactation (Benzie et al. 1956) it is generally accepted that plasma calcium levels are not a good indicator of the true mineral status of the animal. In the present study plasma calcium concentrations did not alter in spite of a high calcium demand and suggest a well-regulated control of calcium metabolism during the period of antler mineralization and, as occurred in this experiment, a period of severe skeletal bone demineralization.

3) Faecal endogenous loss and urinary excretion of calcium

Calcium requirements for maintenance of red deer appeared to be low in this study. Rates of faecal endogenous loss were 6.1 and 6.6 mg/kg BW per day, for stags 589 and 103 respectively, which contrast markedly with estimated requirements for sheep and cattle of 16 mg/kg BW per day (ARC, 1980). More recent estimates have suggested faecal endogenous loss of calcium may be affected by DM intake. Braithwaite (1982) derived a formula relating faecal endogenous loss (V_f) to feed intake (F_I) in sheep:

$$V_f \text{ (mg/kg BW per day)} = 3.38 + 0.572\ F_I \text{ (g/kg BW per day)}.$$

If this relationship is applied to the deer in the present study faecal endogenous losses should have been 11.5 and 12.4 mg/kg BW per day for 589 and 103, respectively, twice that obtained in this study. The recent data of Stephenson and Brown (1984) can be recalculated to yield further estimates of endogenous loss in white-tailed deer. The mean endogenous loss of four stags over three balance periods was 6.4 mg/kg BW per day, in very close agreement with the results of the present study. A possible explanation for the low endogenous losses is that deer may be better adapted to the demands for calcium during antler growth

(and possibly lactation) than domestic ruminants.

Rates of urinary calcium excretion were within the wide though low range of values reported for sheep during lactation (e.g. Braithwaite, Glascock and Riazuddin, 1969; range 0.4-2.1 mg/kg BW/day and Braithwaite, 1983; range, 4.7-11.1 mg/kg BW/day). The decrease in urinary calcium excretion (observed in the data calculated chemically) during Balance Trial 2 indicates a possible conservation mechanism in operation during the period of increasing calcium requirement. However the reason for the discrepancy between these results and those calculated from the isotope data, is not clear.

4) Calcium absorption

Although there was a high demand for calcium, both stags in this study had very low rates of absorption, i.e. at 18.5 and 10.5 mg/kg BW per day. These were similar to the 17.6 mg/kg BW per day reported for mature sheep at maintenance (Braithwaite and Riazuddin, 1971) but well below the 115 mg/kg BW/day reported for lactating ewes by Sykes and Dingwall (1975). Rates of calcium absorption can be estimated for the first balance period using the rates of faecal endogenous loss calculated for Balance Trial 2. These yielded rates of 16.2 and 8.1 mg/kg BW per day for stags 589 and 103, respectively. These results suggest that the decrease in total faecal excretion of calcium observed during Balance Trial 2 resulted from the decrease in calcium intake rather than any change in rate of calcium absorption. The high calcium demand of the stags in this study apparently did not lead to a high rate of absorption of dietary calcium, as occurs in growing lambs (Braithwaite and Riazuddin, 1971; Braithwaite, 1975) and lactating ewes (Braithwaite, Glascock and Riazuddin, 1969, Braithwaite, 1983). In addition calcium availability is generally higher when animals are offered low calcium diets (Luick, Boda and Kleiber, 1967, Braithwaite, 1983) as were the stags in the present study. For example the latter author offered lactating ewes a plentiful (calcium intake, 285 mg/kg BW/day) versus a low calcium ration (calcium intake, 91mg/kg BW/day) and obtained mean dietary calcium availabilities of 22 and 66%, respectively. Therefore the high calcium demand coupled with a low calcium intake should have resulted in a high calcium availability. That calcium availabilities remained low, at 47 and 24% for stags 589 and 103 respectively, suggests that some factor may have been influencing the uptake of dietary calcium.

There are little data available on the calcium availability of fresh herbage, since most work on calcium metabolism has been conducted with dried rations. Thompson and Gelman (1984) obtained calcium availabilities of 35% when growing lambs were offered fresh herbage and it is not inconcievable that calcium availability from fresh herbage differs from that of dried feeds.

It is possible that a component of the feed may have interfered with calcium absorption resulting in the low availabilities in this study. Ewer and Bartrum (1948) described a high incidence of rickets when lambs were wintered on green cereal crops, particularly oats. From experiments with mice Grant and O'Hara (1957) demonstrated a rachitogenic factor which they concluded was carotene or vitamin A. To what extent a component of the diet interfered with the action of vitamin D in the active phase of calcium transport in the current study is not clear.

A further possibility is that stags in the present study may have been energy and/or protein deficient since they were restricted to a maintenance ration of a low protein feed. The possibility of skeletal demineralization in protein deficient lactating ewes, to provide protein for lactation, has been raised by Sykes and Field (1972). In these circumstances calcium in the skeleton would be liberated along with proteins from the organic matrix and the requirement for dietary calcium would decrease. Greenfeed oats contain relatively low levels of crude protein (94 g/kg; MAFF, 1975) and the present stags were restricted to a low level of feeding, therefore intakes of crude protein should have been low, at approximately 226 and 222 g/d for stags 589 and 103 respectively during Balance Trial 2. However in the absence of data on protein status of these animals any relationship between dietary protein and calcium availability in the current study must remain speculative.

5) Model of calcium metabolism in the stag

Comparison of rate of accumulation of ^{45}Ca in antler ash and the earlier model of antler mineralization has indicated that little or no calcium deposited in the antler is resorbed and returned to the body. It seemed reasonable therefore to simulate a model of calcium kinetics in the stag in which the antler calcium was treated in a similar way to the calcium loss during lactation (i.e. an irreversible loss). The SAAM program could therefore be used to separate antler calcium accretion from

the accretion of calcium in the skeleton and allow calculation of skeletal calcium resorption. Output from the program also included masses of the four compartments and rate of calcium transfer between compartments (Table 5.3).

On a per kg BW basis the sizes of the exchangeable calcium pool (M_T) of 0.21 and 0.24 g/kg were in close agreement with the mean value (0.21 g/kg) obtained for lactating dairy cattle by Ramberg et al., (1970) but considerably lower than the mean value of 0.50 g/kg obtained during late antler growth by Stephenson and Brown (1984). This discrepancy may be due to the failure of the latter authors to separate an antler component. Consequently this model may have been unable to separate the mass of the calcium entering the antler from that of the exchangeable pool.

In both stags accretion of calcium in the skeleton occurred only slowly (Table 5.3) particularly in the stag with the highest calcium requirement (589). It is possible that accretion of calcium in the skeleton of this stag was decreased in response to a high calcium demand. However the expected rate of accretion of calcium in the antler of this stag was not in close agreement with the observed values (9.93 versus 8.29 g/d) whereas a very good fit to the antler data was obtained in stag 103 (5.79 versus 5.82 g/d). This may therefore reflect a poorer fit of the data to the model, for this stag.

Both stags were in severe negative calcium balance (Table 5.3) with high rates of bone calcium resorption occurring to meet the high calcium demands of antler growth. On a bodyweight basis these rates were 48 and 50mg/kg BW/day for stag 589 and 103, respectively, a total of 71.8 and 81.3% of total calcium requirement being met from the skeletal reserves. The degree of skeletal demineralization over Balance Trial 2 can be estimated using the data of Sykes, Field and Gunn (1974) where total body calcium of ewes in early pregnancy was calculated to be 0.95% of total bodyweight. Using this assumption stags 589 and 103 would have had total body calcium contents of 1620 and 1430 g, respectively. Since negative calcium balances of -8.2 and -5.9 g/d were demonstrated for stags 589 and 103, respectively, approximately 6 to 7% of total body calcium was lost from the skeleton of both stags. Presumably a similar rate of loss would have occurred during Balance Trial 1 and therefore total body calcium loss may have exceeded 10%. Nicolaysen, Eeg-Larsen

Table 5.3

Calcium balance in two red deer stags offered low calcium diets during late antler growth. Compartmental masses (g) and flow rates of calcium between compartments (g/d) and into antlers, faeces, urine and the skeleton are those estimated by the SAAM program. From these data estimated rates of calcium absorption from the diet, total calcium requirement and therefore amount of skeletal calcium resorption have been estimated. Values in parentheses have been calculated from the metabolic data (Table 5.1).

	Stag 589	Stag 103
M_1 (g)	0.81	0.92
M_2 (g)	2.61	3.12
M_3 (g)	9.09	10.08
M_4 (g)	23.41	22.31
M_T (g)	35.92	36.43
R_{12} (g/d)	197.80	103.29
R_{21} (g/d)	197.82	104.78
R_{23} (g/d)	38.15	17.83
R_{32} (g/d)	38.16	19.32
R_{34} (g/d)	6.47	3.95
R_{43} (g/d)	6.49	5.44
Calcium intake (g/d)	(6.66)	(6.57)
Calcium output (g/d)		
- faeces	(4.71)	(5.90)
- antlers	9.93	5.79
- urine	0.24	0.76
- faecal endogenous loss	1.28	1.03
Skeletal bone accretion	0.02	1.49
Total calcium requirement (g/d)	11.47	9.07
Calcium absorption (g/d)	3.23	1.70
Calcium availability (%)	48.4	25.9
Net skeletal resorption (g/d)	8.24	7.37
Skeletal calcium balance (g/d)	-8.22	-5.88
Percentage of total calcium requirements derived from diet	28.2	18.7

Notation : Compartment masses are signified by $M_{(1-4)}$ and flow of calcium from compartment 1 to compartment 2 by R_{21}.

and Malm (1953) postulated that skeletal demineralization of 5% may result in the release of a bone factor which triggers an increase in dietary calcium absorption. It seems unlikely that stags in this study did not reach this "critical" level of skeletal demineralization necessary for this proposed phenomen to occur.

The proposed model of calcium metabolism in the red deer stag is supported by the skeletal bone data (Table 5.4) which indicates that a net loss of skeletal bone reserves may have occurred. Degree of skeletal mineralization was poor in comparison with that of ewes during late pregnancy (Sykes, Nisbet and Field, 1973; Table 5.4). No information is available on skeletal mineralization in deer. However a mature stag which died suddenly of an intestinal torsion late in the winter of 1982 provided tibia, 3rd rib and 3rd lumbar vertebra which were analysed in an identical manner to those in the present study (Table 5.4). This stag had been carrying hard antler remnants for 6 months and since no active antler growth had been occurring over this period the bones would be expected to be well mineralized. The two stags slaughtered during late antler growth did indeed have poorly mineralized bones. Greatest differences in A:R ratio were evident in the vertebra, being 1.38 for the ewes, and 1.46 in the stag in late winter compared with a mean value of 0.93 for the two stags in the present study. Smaller differences were apparent in the tibia, 1.95 (ewe), 1.87 (dead stag, 1982) and 1.55 (mean of 2 stags). This perhaps suggests that non load bearing bones were selectively demineralized during antler growth and this suggestion is supported by the higher specific activities of bone ash in the rib and vertebra indicating an greater rate of calcium turnover in these bones than in the tibia. These findings are in agreement with those of Benzie et al., (1956) who found that cancellous bone is drawn upon before compact bone during skeletal resorption in sheep. Vertebrae, which contain a high proportion of cancellous bone, also were found by these authors to be the most severely affected of the skeletal bones examined.

Table 5.4

Degree of mineralization (A:R ratio) of selected skeletal bones removed from 2 red deer stags during late antler growth, a stag which died during late winter and data collected from ewes during late pregnancy.

		Skeletal bone		
		tibia	rib	vertebra
A:R ratios of skeletal bones collected during late antler growth (present study)	589	1.60	1.25	0.99
	103	1.49	1.28	0.87
A:R ratios of bones from a stag which died in late winter (present study)		1.87	1.76	1.46
Mean A:R ratios of bones from ewes slaughtered in late pregnancy (Sykes, Nisbet and Field, 1973)		1.95	1.41	1.38

In summary therefore the close agreement (95%) between the rate of calcium deposition obtained in this study and the calculated expected rate has validated the model of antler mineralization proposed in Section 4. Furthermore it appears that during growth of the antler little or no bone turnover occurs within the antler, and that calcium entering the antler may be lost to the animal. In this respect antler calcium may be regarded in much the same way as calcium secreted in milk during lactation.

Maintenance requirements (endogenous loss) for calcium in deer also have been calculated and appear to be considerably lower than those estimated for sheep. Stags in this study derived no more than 30% of their total calcium requirements from the diet, yet were capable of maintaining high rates of antler mineralization, presumably through

mobilization of skeletal reserves. In spite of a high calcium demand, rates of calcium absorption remained very low. Further study is necessary to establish whether these low availabilities are attributable to some component of the greenfeed ration used, or a low physiological stimulus for calcium absorption.

SECTION 6

General discussion and summary

The nutritional study described in Section 3 demonstrated that winter nutrition may affect both antler casting date and velvet antler weight. On a property where mature stags were of low liveweight (110 kg) and in poor condition in early to mid-winter, restricted winter nutrition delayed antler casting date by 13 days, and velvet antler yield was 0.24 kg lighter compared with another group on the same property offered a high quality ration ad libitum (Section 3). This relationship between poor winter nutrition and decreased antler weight only occurred on one property and within stags of poor body condition. Therefore it is clearly important to establish a method of condition scoring deer, and define a critical liveweight or condition score above which a response to improved winter feeding might be obtained.

The same treatments were conducted on another property with stags of heavier mean liveweight (123 kg) and different genotype. Those fed a high quality ration through the latter stages of the winter gained 41 g/d. By comparison the group fed a poor quality ration lost 71 g/d over the same period. No effect on antler weight was noted and full liveweight realimentation had occurred in these adult stags by the following rut. The same level of compensatory growth however may not occur following winter feed restrictions in young stags. Suttie et al. (1983) offered male red deer calves a restricted feed intake during winter (70% of ad libitum), and repeated this treatment the following winter with the same animals as yearlings. This feeding regime prevented the attainment of mature body size. In the present study (Section 3) a positive relationship was demonstrated between velvet antler weight and pre-rut liveweight, within an age group. The implication is that poor winter nutrition during the first or second year of a stag may adversely affect the liveweight of a stag, relative to unrestricted animals and that the resulting lower liveweight may decrease the animal's antler growth potential. The relationship between antler weight and liveweight, of 0.12 kg for each 10 kg of liveweight gain, as obtained here for 3 and 4-year-old-stags agrees closely with that obtained by Fennessy (1982) for 2-year-old stags. The increase in velvet antler weight between age groups (Section 3) indicates that the

relationship of increasing hard antler weight with age of stags, described for red deer stags by Huxley (1926), holds true for antlers in velvet. Velvet antler yields appeared, in the present work, to be best predicted from the pre-rut or late summer liveweight and it has been suggested by Hyvarinen, Kay and Hamilton (1977), from their work with stags in hard antler, that this liveweight provides the best indicator of the animal's lifetime potential for liveweight gain. The findings of the present study, that differences in liveweight between age groups were greatest prior to the rut than at other times of the year would also suggest that pre-rut liveweight is the best indicator of the stags mature size and therefore of velvet antler production. This may be a result of the seasonal liveweight changes in the stag: older and heavier stags having greater weight losses during the rut than younger and lighter stags (Fennessy, Moore and Corson, 1981).

Antler growth is terminated by mineralization and velvet stripping (Section 4) and the period of antler growth appears to be governed more by date of hard antler casting than by date of velvet stripping. The data in Section 3 suggests that date of hard antler casting is affected more by liveweight than age and it is argued that older stags may cast earlier because they are heavier rather than because they are older. Behrend and McDowell (1967) and Jacobsen and Griffin (1983) also noted a relationship between age and casting date but were unable to examine the effect of liveweight and casting date.

Antler elongation has the characteristics of a sigmoid growth curve as shown by Fennessy (1982) and Von Ballenberghe (1983), with slow growth in the early and latter stages and an intermediate period of rapid growth. In this study (Section 4) growth rate was almost linear over the period from 28 to 112 days after casting of hard antler remnants, with 95% of antler growth in length completed by 112 days of growth, at which stage final antler sampling occurred. Beyond the sampling points of the present study (i.e. prior to 28 days and after 112 days) the data also appeared to follow a sigmoid growth curve. Over the period of linear growth in length total antler relative blood volume increased linearly at a rate of 194 ml/kg. Slowing of antler elongation coincided with an increase in plasma testosterone levels, mineralization of the antler tip and a decrease in relative blood content in both the antler shaft and whole antler. Growth in length of the brow tine slowed at 70 days and

was also associated with a decrease in relative blood volume and suggests a different mechanism, or effectiveness of the same mechanism, in control of growth of the brow tine, compared with that of the antler shaft.

Three phases of antler mineralization have been demonstrated in these studies. The first phase of antler mineralization occurs in a zone 5.0 to 7.5 cm behind the growing tip which is the region where transition from cartilage to trabecular bone is occurring. Antler tips are poorly mineralized during active growth of the antler. The second phase of antler mineralization appears to constitute continued trabecular bone accretion with older regions near the antler base having a greater degree of mineralization. The third phase is that of compact bone formation in the periphery of the antler shaft, which in this study was followed closely by mineralization of the antler tip, decrease in antler relative blood volume and slowing of growth in length. The third phase of antler mineralization has been termed "terminal mineralization" and appears to be associated with an increase in circulating levels of plasma testosterone.

These findings of increasing mineralization occurring at the same time as increasing plasma testosterone provide quantitative data to support the work of Brown, Cowan and Griel (1978). These authors described a positive correlation between degree of antler mineralization (as measured by X-ray densitometry) and the increase in level of androgens in plasma. However a certain degree of antler mineralization occurs independently of high levels of circulating androgens since trabecular bone mineralization occurs in the antlers of castrate reindeer (Belanger, Choquette and Cousineau, 1967). Moreover, in the present study mineralization of trabeculae occurred whilst plasma testosterone levels were low (< 0.2 ng/ml). Further histological work by Morris and Bubenik (1983b) indicated that complete antler mineralization (but not velvet stripping) could be achieved by the administration 19-OH-testosterone to castrated white-tailed bucks. Terminal mineralization appears to be under androgenic control and a biphasic pattern of antler mineralization has been proposed (Fig. 4.17). In the stags in this study these events occurred between 91 and 112 days after hard antler casting and close to the summer solstice.

From the model of antler growth proposed in Section 4 it is now possible to calculate the rate of antler calcium deposition and therefore the calcium requirements for antler growth, when the weight and age of an antler are known. Deer are now regarded as a domesticated animal species (Short, 1985) and therefore these data are essential to enable derivation of total mineral requirements. Between 28 and 112 days after casting the proportion of total ash and calcium in the antler increased exponentially, with 33% of final antler ash being deposited between 91 and 112 days after antler casting. A hypothetical stag producing 3.8 kg of hard antler would have a requirement for antler calcium of approximately 10.7 g/d over this period. A further study employing radio-isotopes (Section 5) has allowed calculation of rates of faecal endogenous loss in deer so that it is possible to calculate the total animal requirement for calcium. Rates of faecal endogenous loss in deer (6.1 and 6.6 mg/kg BW/day) appear to be much lower than the daily requirement of 16 mg/kg BW cited by the ARC (1980) for sheep and cattle. The study described in Section 5 also indicates that antlers may behave quite differently to normal skeletal bone in that calcium deposition in antler tissue appears to be essentially a one-way process (i.e. calcium accretion). This contrasts with normal skeletal bone where calcium is in a state of flux with both calcium accretion and resorption occurring. It was found that antler mineralization does not appear to be dependent on dietary calcium and skeletal demineralization may occur to provide minerals for antler growth. Over a 13 day period during peak antler mineralization stags on a low calcium ration were capable of meeting up to 70% of their antler calcium requirements from skeletal reserves whilst rates of gut absorption of calcium remained extremely low (Section 5). Other studies concerned with calcium absorption in ruminants (e.g. Braithwaite, 1983) suggest that during lactation when requirements are high the availability of dietary calcium can be increased by the animal. In addition calcium availability is greater on low calcium diets (Luick, Boda and Kleiber, 1967), as were the rations used in the present study. Therefore the reasons for the low rates of calcium absorption, and relatively low calcium availabilities in the present stags are not clear. Possible explanations are either that red deer normally mobilise large amounts of skeletal calcium during antler growth or that some component of the feed contributed to the low rate of calcium absorption from the diet. For example, the role of dietary protein on calcium

availability has been discussed by Sykes and Field (1973) who suggested that where a low calcium diet included adequate dietary protein, calcium availability might approach 100%. It is possible that in the present study an insufficiency of dietary protein resulted in the mobilisation of skeletal protein leading to liberation of skeletal calcium which would in turn decrease the requirement for absorption of dietary calcium. Another possibility is that the greenfeed oats offered contained a rachitogenic factor (Grant and O'Hara, 1957) which may have impaired the action of vitamin D and impaired calcium absorption from the gut.

In conclusion, the present study has examined the effects of winter nutrition on antler growth, described the pattern of growth and composition of antlers, derived the calcium requirements for antler growth and examined calcium metabolism in the red deer stag. However the mechanisms which regulate calcium metabolism are not clear. In particular the present study has demonstrated the need to establish whether the low rates of absorption obtained are simply a result to the normal metabolism of the red deer, or are in fact attributable to some component of the feed offered.

Practical aspects and conclusions

Velvet antler weights may be decreased by a low level of winter nutrition if stags are in poor condition in early winter. Amongst stags used for mating, dominant stags suffer greater weight losses than subordinate stags (Kelly and Moore, 1977). Therefore it may be profitable to feed preferentially the stags used for mating after the rut and through the winter to prevent a possible reduction in antler growth.

Suttie et al. (1983) demonstrated that calves and yearlings fed at a restricted level of winter nutrition lost weight, and although compensation occurred, these stags failed to attain the same mature size as a control group fed ad libitum. There may be some carry-over effect of poor nutrition of young animals on future antler weight since a relationship has been demonstrated between liveweight and velvet antler weight, with heavier stags generally producing heavier velvet antler weights. This perhaps highlights the importance of achieving rapid growth rates in early life.

The field study described in Section 3 illustrated considerable differences in date of removal of velvet antler between properties (mean values were 53 and 70 days after casting). This greatly affects the amount of velvet antler harvested. That differences in time of antler removal might be due to difficulties in assessing stage of ideal commercial harvest was supported by the observations made during the subsequent experiment. The most appropriate stage for antler removal (prior to branching of the main shaft to form the royal tines) was subjectively assessed (Section 4) and found to have a mean date of 64 days after casting (range; 57 to 79 days). In a number of stags failure of the antler shaft to branch to form royal tines made assessment of commercial harvest date more difficult.

Degree of mineralization of antler bone can be determined from the age of the antler. Although antler mineralization increases throughout antler development, the most rapid phase of mineralization did not occur until after 91 days of growth, when compact bone began to form in the antler shaft. Antlers remained highly vascular during the period of antler elongation, with relative blood volume increasing linearly at a rate of 194 ml/kg. Therefore total blood volume can be predicted from antler weight and degree of mineralization from the antler age. It is

therefore difficult to standardise the blood and mineral composition of velvet antlers by removing antlers at a fixed morphological stage (i.e. at the stage of royal tine branching). Removal of velvet antlers at a given age (e.g. 64 days after casting) should result in a commercial product with a fairly consistent composition, at least in terms of degree of mineralization. The wide range in hard antler casting dates observed in Section 4 might make this difficult in a commercial situation. However the technique described by Muir, Barrell and Sykes (1982) to advance antler casting using a progestagen could be applied to synchronize antler casting and velvet antler removal.

Composition also varies within an individual antler. Low relative blood volumes found in the cartilaginous antler tip conflict with the belief held in the deer industry that the antler tip is a high quality product because of its reputed high blood volume and low degree of calcification. A price differential also exists between the brow tine and the remainder of the antler as brow tines are claimed to be excessively mineralized. However if antlers were removed 70 days after casting there would be little difference in total mineralization of the brow tine and antler shaft. Brow tines may contain a lower relative blood volume, which may result in a more calcified appearance but it is difficult to justify down-grading all of the brow tine on the basis of excessive mineralization. In older antlers, (e.g. 70 to 90 days after casting) the base may have an unacceptable degree of mineralization but the upper and middle sections of the antler will still be poorly mineralized with high relative blood volumes. Trimming of the base and lower tines from these older antlers should still produce a marketable antler product with the desired composition.

As a result of this study it is now possible to assess the weight of velvet antler a stag would have produced if hard antler weight is known. By using the equation describing antler growth in weight (weight as a percentage of final hard antler weight; Fig. 4.8) it can be calculated that 73% of hard antler weight could have been removed commercially as velvet antler 64 days after hard antler casting. This will provide a rule-of-thumb estimate of the velvet production potential of a captured stag, or of a breeding stag whose antlers have been left to fully mineralize.

Another useful outcome of this work is the ability to estimate the calcium requirements for a stag producing velvet antlers. An average stag (as in Section 4 of this study) would produce 2.24 kg of hard antler with DM of 81.1%, an ash content 63.0% of DM and a calcium content 35% of ash . This would contain 399 g of calcium. Over the first 64 days of antler growth 13.8% (or 55 g) of calcium would be deposited at a maximum rate of 2.4 g/d when branching of royal tines approaches. However stags generally have a high feed intake during spring and fresh pasture contains a high calcium content (4 to 10 g/kg DM, McDonald, Edwards and Greenhalgh, 1973) so it is unlikely that a deficiency of dietary calcium will occur unless dietary calcium availability is extremely low. If such a deficiency should occur red deer stags appear to be able to mobilise skeletal calcium in order to maintain antler mineralization.

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