RECENT ADVANCES IN ANTLER PHYSIOLOGY RESEARCH

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General introduction

In the last year significant advances in our understanding of the physiology of antler growth have been made at Invermay This manuscript describes two of these, namely the anatomy and physiology of nerves and the role of insulin like growth factor 1 (IGF1) *in vitro* in stimulating velvet growth

NERVES

Introduction

The pedicle/antler region of the deer receives sensory innervation from two branches of the trigeminal nerve, the supra-orbital and the zygomatico-temporal, and in a minority of animals the auriculo-palpebral branch of the facial nerve The pedicle receives sympathetic innervation from nerves originating in the superior cervical ganglion but sympathetic innervation does not normally reach the antler. Although this basic neuro-anatomy has been known for some time, the precise location of the nerves in the tissues of the pedicle and the antler have never been studied Apart from presumably conveying tactile and temperature sensory stimuli from the pedicle/antler to the brain, nerves appear to be responsible for partly controlling antler size but not the timing of the antler cycle Suttie and Fennessy (1985) who removed both branches of the trigeminal nerve from one side of young deer growing their first spike antlers found that denervation reduced antler size. Bubenik (1982) found that stimulating branches of the trigeminal nerve with a cardiac pacemaker increased antler size However the effects of sensory nerves on pedicle growth were not studied Also the anatomical distribution of these nerves in the pedicle and antler are not known

At the last meeting of the Deer Branch (Sydney 1991) much debate took place concerning analgesia, anaesthetic drug use and velvet antler removal One problem that was clearly identified was the lack of precise knowledge of the neuroanatomy of the pedicle and antler Although the current husbandry practice for velvet antler removal is to apply a ring block of local anaesthetic to the pedicle, it is not known with any degree of certainty that this in fact adequately desensitises the relevant area to be amputated This level of uncertainty is in part due to a lack of knowledge of the typical pattern of innervation

The aim of this study was to determine whether nerve tissue could be located in the pedicle/antler region using a specific monoclonal antibody directed against neuro-filaments If the technique could be used the aim was to determine the location of nerve bundles at the antler pedicle junction Available for study was antler tissue from antlers which had both branches of the trigeminal nerve surgically removed from both sides of the head prior to the start of pedicle growth

MATERIALS AND METHODS

Animals and management

Eight weaned red deer stag calves were allocated randomly to one of three treatments as follows·

1. Bilateral pedicle parasympathectomy (BP) (n=4)
2. Control, no manipulation (n=4)

The animals were kept indoors under natural conditions of illumination and fed to appetite a concentrate ration

Surgery

This basically followed Suttie and Fennessy (1985) Briefly under halothane/N_2O/O_2 anaesthesia the zygomaticotemporal branch of the trigeminal nerve was located in fat filled fossa posterior to the eye It was dissected free of vascular tissue and a one centimetre section removed The supraorbital branch was located in the subcutaneous tissue above the eye and one centimetre sections were removed from one or more branches of this nerve Successful ablation was confirmed by histology

Measurements

At two weekly intervals the deer were observed and pedicle/antler length and status were measured After the antler was almost completely grown in velvet, one antler was removed just below the antler pedicle junction under typical deer husbandry conditions (in a pneumatic crush 3 minutes after a ring block of Lignocaine, with a tourniquet applied) from each of the control and BP stags

Immunohistochemical localisation of nerves

Frozen sections of antler and pedicle were cut at 20 µm thickness with a cryostat and mounted on glass microscope slides treated with gelatin (subbed) The sections were dried at room temperature for 30 minutes The sections were incubated in a primary antibody (Monoclonal anti-200 KD neurofilament polypeptide) for 2 hr at room temperature The sections were washed in buffer and then incubated with a second antibody (biotinaylated sheep anti-mouse) for 1 5 hr at room temperature They were then incubated for 45 minutes at room temperature with biotin-streptavidin-Rhodamine All sections were examined under ultra-violet light using a Zeiss Axioplan microscope The numbers of neurofilaments in each whole cross section were counted and their positions noted

RESULTS

Parasympathectomy had no effect on the timing of pedicle or antler growth or pedicle length but reduced antler size.

The monoclonal antibody recognised nerve fibres in the vascular layer of the pedicle of control stags (Figure 1) Fibres were large and located in bundles typically close to blood vessels (Figure 2) The distribution of these fibres is indicative of parasympathetic innervation Fibres, probably sympathetic, were found innervating the smooth muscle of pedicle arteries (Figure 3) Parasympathetic nerves were located in the pedicle tissue of animals whose trigeminal nerves had been cut, but these were always lower in abundance compared with the control animals

In addition to the vascular layer overlying the periosteum large parasympathetic nerve bundles were located in the cancellous bone region of the antler-pedicle junction (Figure 4) and small fibres were located in the periosteum (Figure 5) in control stags but not in the BP stags

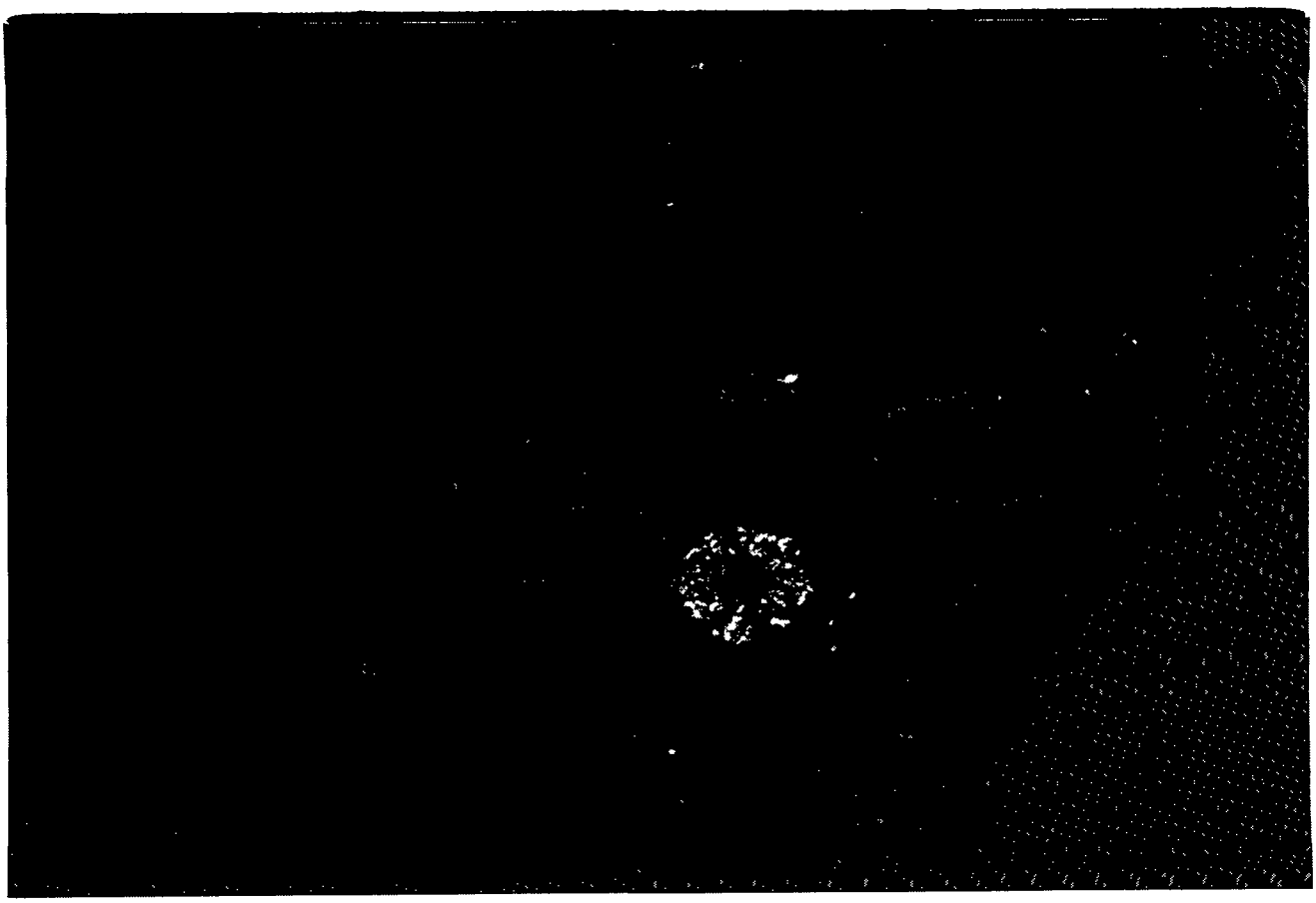

Figure 1 Nerve fibres in the vascular layer of the pedicle The nerves appear as a ring of white dots The ring shaped structures without labelling are blood vessels

Figure 2. Nerve fibre showing close proximity to an artery.

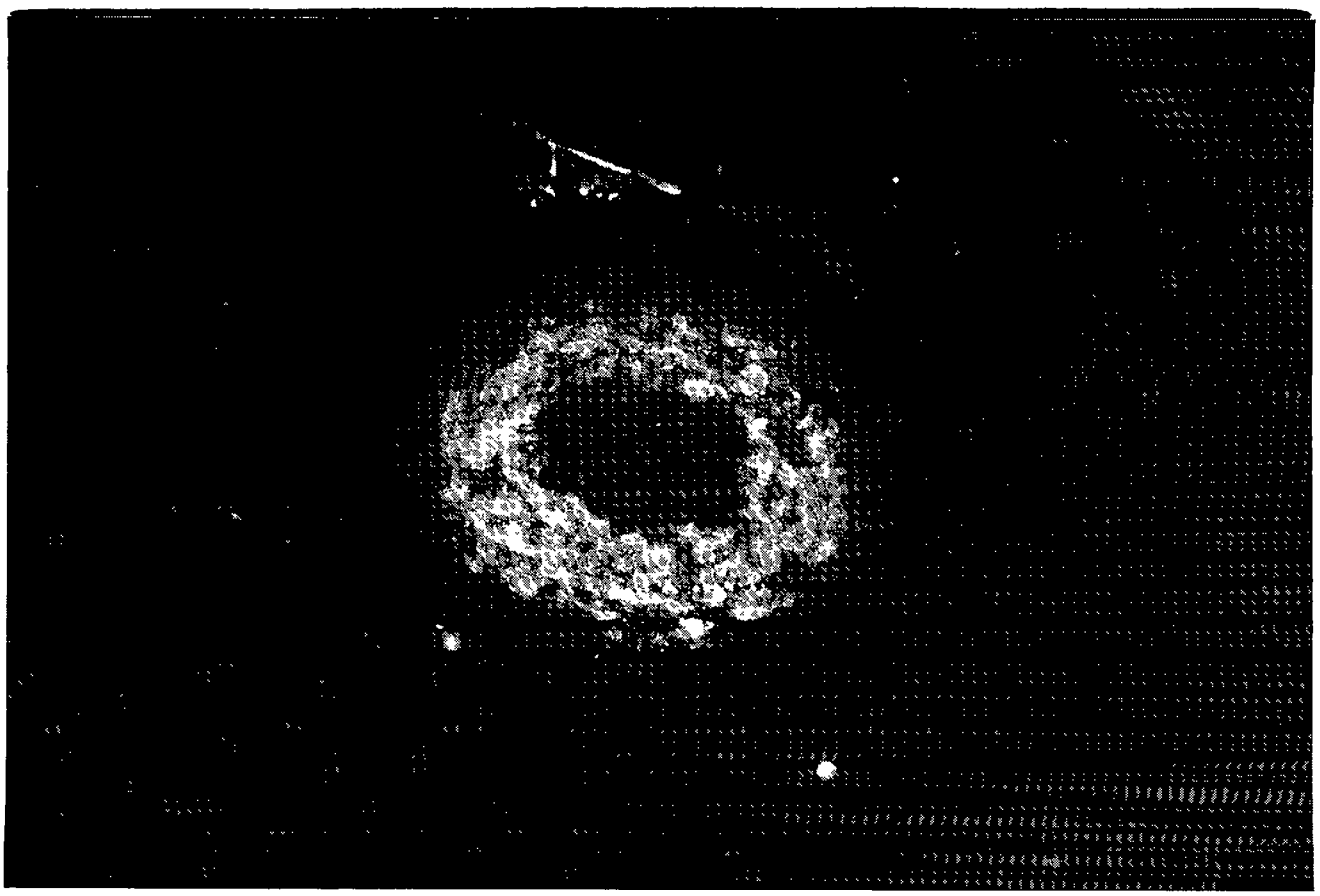

Figure 3 Sympathetic innervation of a blood vessel The nerve is the bright line The walls of the blood vessel are auto-fluorescing

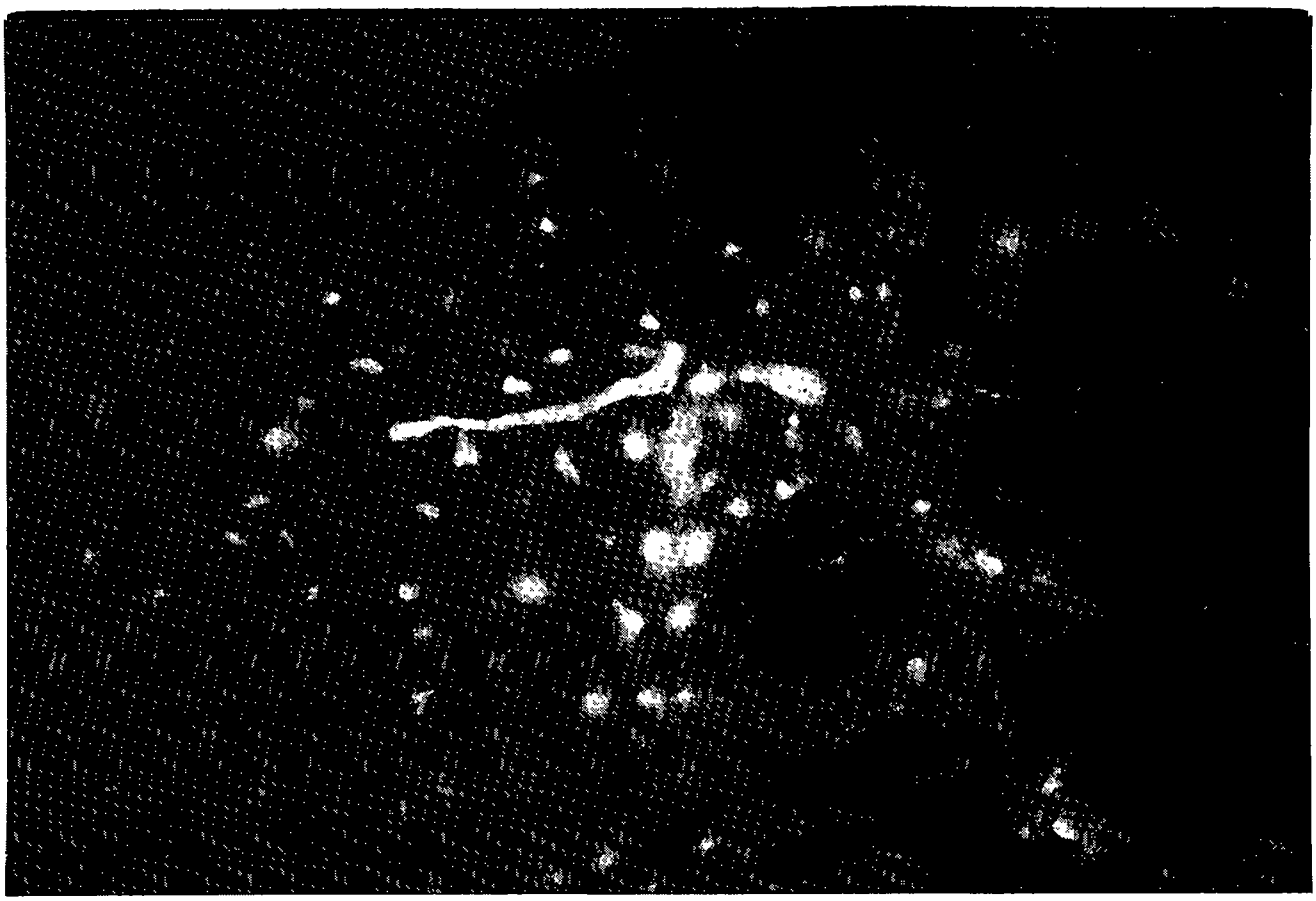

Figure 4 A small nerve fibre (line) in periosteum The dots are auto-fluorescence

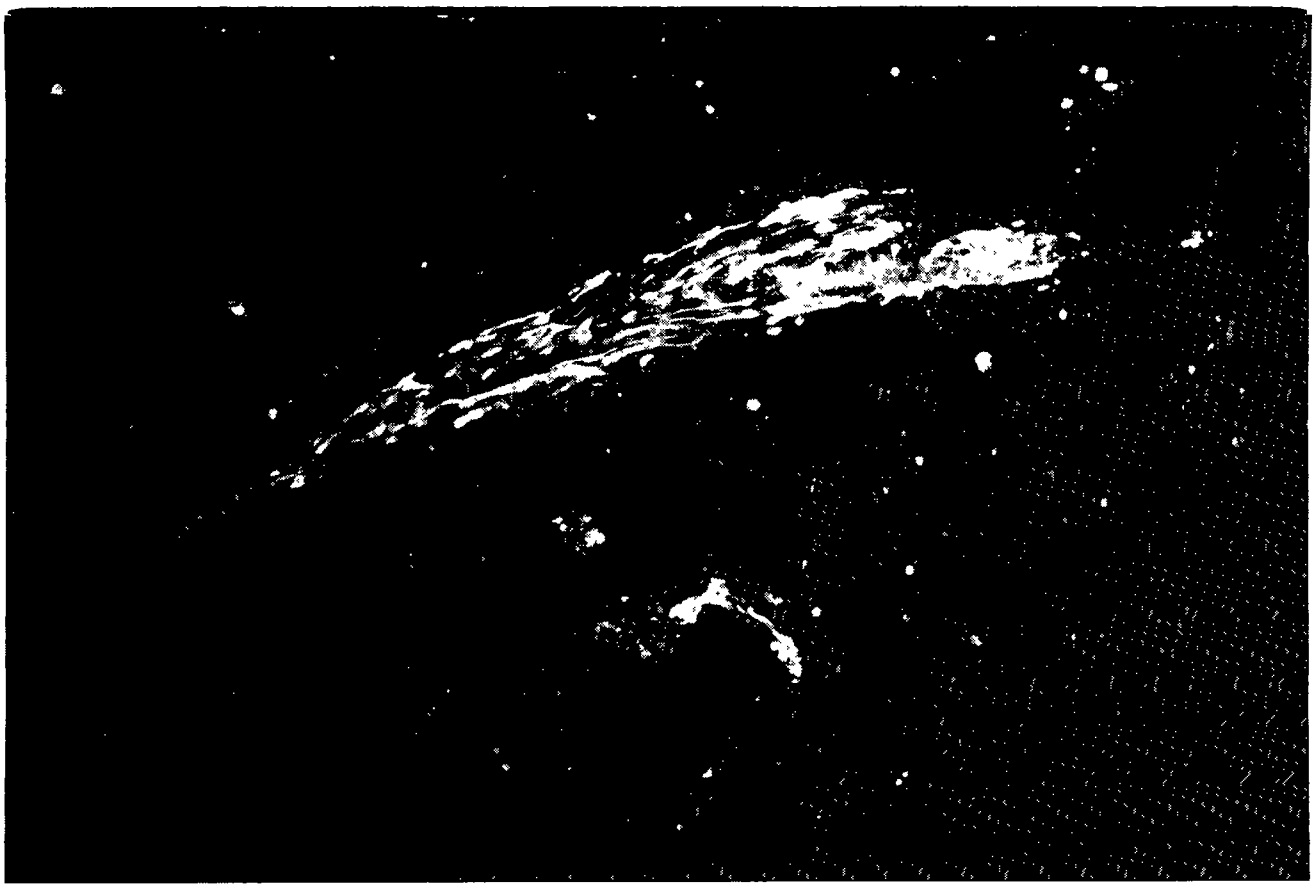

Figure 5 Large bundle of nerve fibres in cancellous bone Trabeculae in the bone are auto-fluorescing

In the antler (Figure 6) no sympathetic fibres were located in the smooth muscle walls of arteries but small bundles of nerves were found close to arteries in control stags No antler nerve fibres were located in any BP stag.

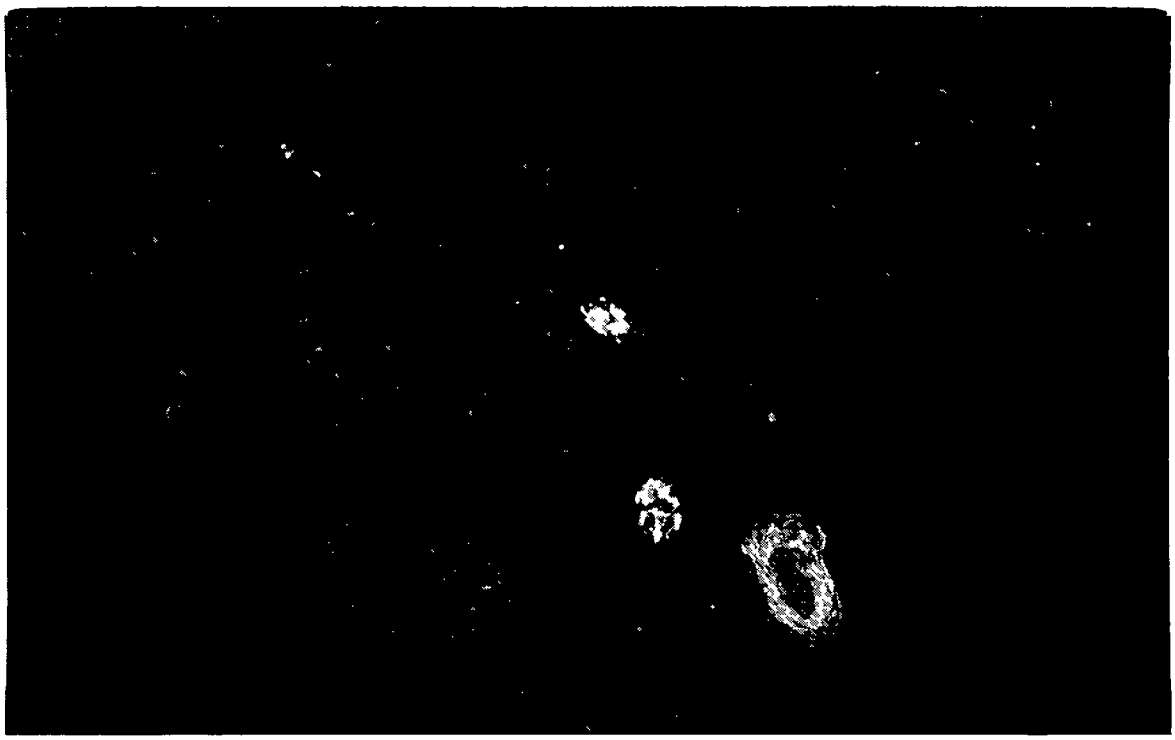

Figure 6 Small nerve fibres in antler The ring is auto-fluorescence around an artery

DISCUSSION

The effects of parasympathectomy on antler growth was as observed previously. The finding of nerves in the pedicles but not antlers of the BP stags was a surprise, however the monoclonal antibody technique cannot distinguish between parasympathetic and sympathetic nerves Therefore it is possible that either these nerves are sympathetic, or some nerve regeneration took place or the pedicle was re-innervated from another source

Anatomically it seems that the nerve distribution in the pedicle/antler falls into 2 categories peripheral and deep Peripheral nerves, both sympathetic and parasympathetic are located close to blood vessels in the pedicle Sympathetic nerves are located in the pedicle only However the finding of nerve fibre bundles in the bony core of the antler pedicle junction was unexpected. Whether these penetrate the antler itself must be a subject for further study but the serious question must be asked, does the current "ring block" technique of velvet antler removal abolish conductance of information in these deep nerves At this stage it would be most premature to refer to these nerves as sensory - although this would be a likely candidate role for them Were these nerves to be sensory and were they to penetrate the antler base then the possibility exists that the current analgesic policy does not adequately desensitise them

Further work on nerve supply to antler tissue is clearly required The fact that parasympathetic denervation of the pedicle destroys the deep nerve bundles indicates that branches of the trigeminal nerve must invade the bone of the pedicle core, these fibres are likely to be sensory Precisely where this occurs relative to administration of the ring block and whether these nerves penetrate the antler are topics requiring urgent attention This requires techniques of nerve tracing which are available in other systems and must be worked out for the antler pedicle.

IN VITRO ACTION OF IGF1 IN ANTLERS

INTRODUCTION

In the communication to the Deer Branch given in Sydney in 1991, we described our system for *in vitro* antler cell culture Briefly tissues from the reserve mesenchyme (fibroblast-like) and osteogenic (osteoblast-like) regions of the antler tip were dissected from antlers harvested 65 days after casting of the previous antler These cell types were separately cultured using standard techniques We report here that if these cells are treated with IGF1, dose dependent increases in growth are observed

MATERIALS AND METHODS

Tissues containing fibroblast and osteoblast-like cells were dispersed with collagenase and grown in 45% Fitton-Jackson modification media, 45% F12 nutrient 10% fetal bovine serum (FBS), penicillin (100 V/ml) and streptomycin (100 µg/mlo) Two x 10^4 cells/cm^2 were seeded in 24 well plates and incubated in a humidified 5% CO_2 atmosphere at 37°C After 48 h the media was changed to either 10% FBS or serum-free media (SFM) and incubated for a further 24 hr, followed by a 24 hr incubation in either 10% FBS, SFM or 10 nM IGF1. After 23 h 2 5 µl ^{3}H-thymidine was added to each well for one hour Reactions were terminated with 10% TCA ^{3}H-thymidine was counted in universal scintillant In a separate series of experiments conditions were the same except that graded doses of IGF1 were added to the SFM Results are the mean of triplicate experiments and were analysed by ANOVA.

RESULTS

10 nM IGF1 significantly increased DNA synthesis as measured by ^{3}H thymidine uptake in both fibroblast-like (Figure 7) and osteoblast-like (Figure 8) cells The growth of the cells in SFM was extensive and it appeared that the FBS might even inhibit this endogenous growth

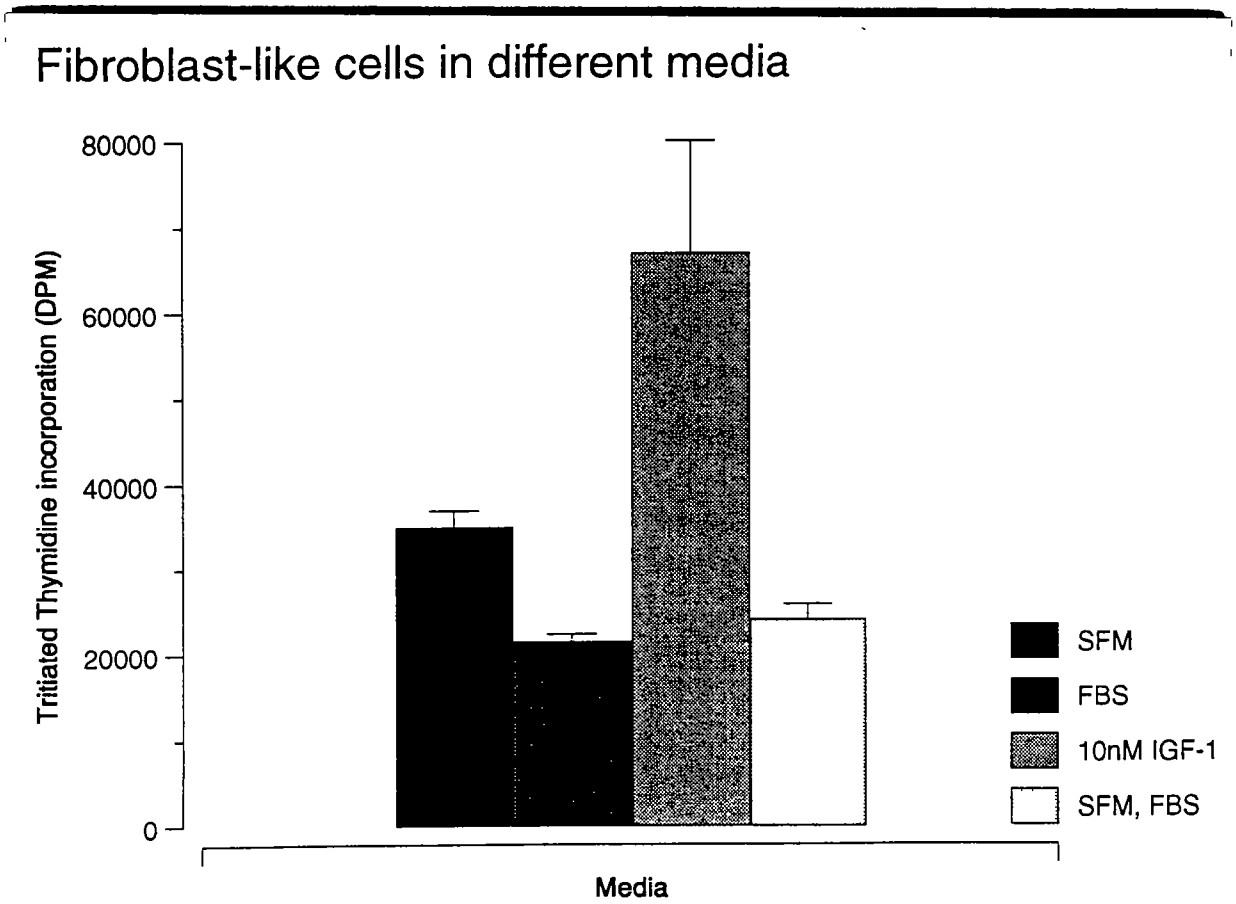

Figure 7 The effects of FBS, SFM and 10 nM IGF1 on antler fibroblast-like cells

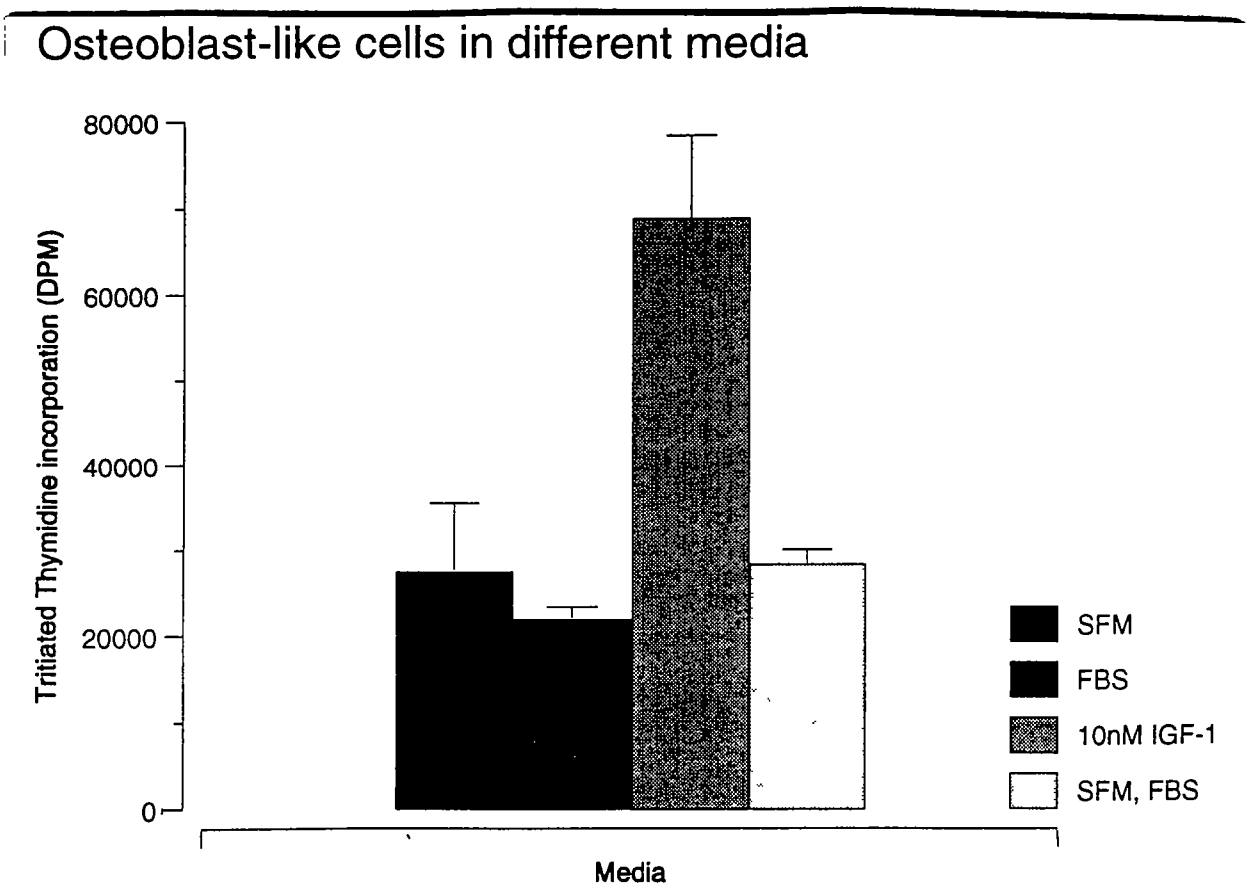

Figure 8. The effects of FBS, SFM and 10 nM IGF1 on antler osteoblast-like cells

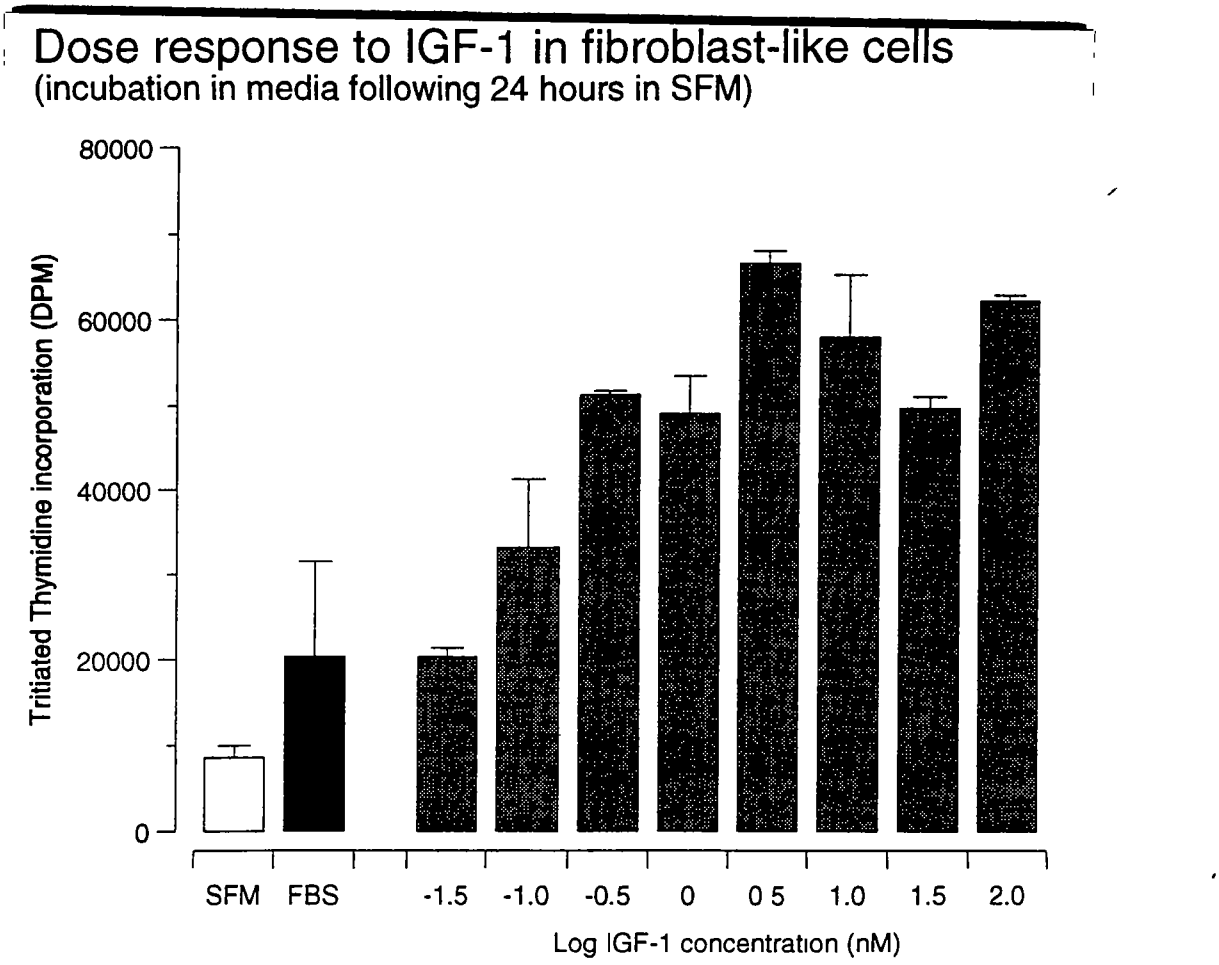

Figure 9 Dose dependant effects of IGF1 on fibroblast-like cells

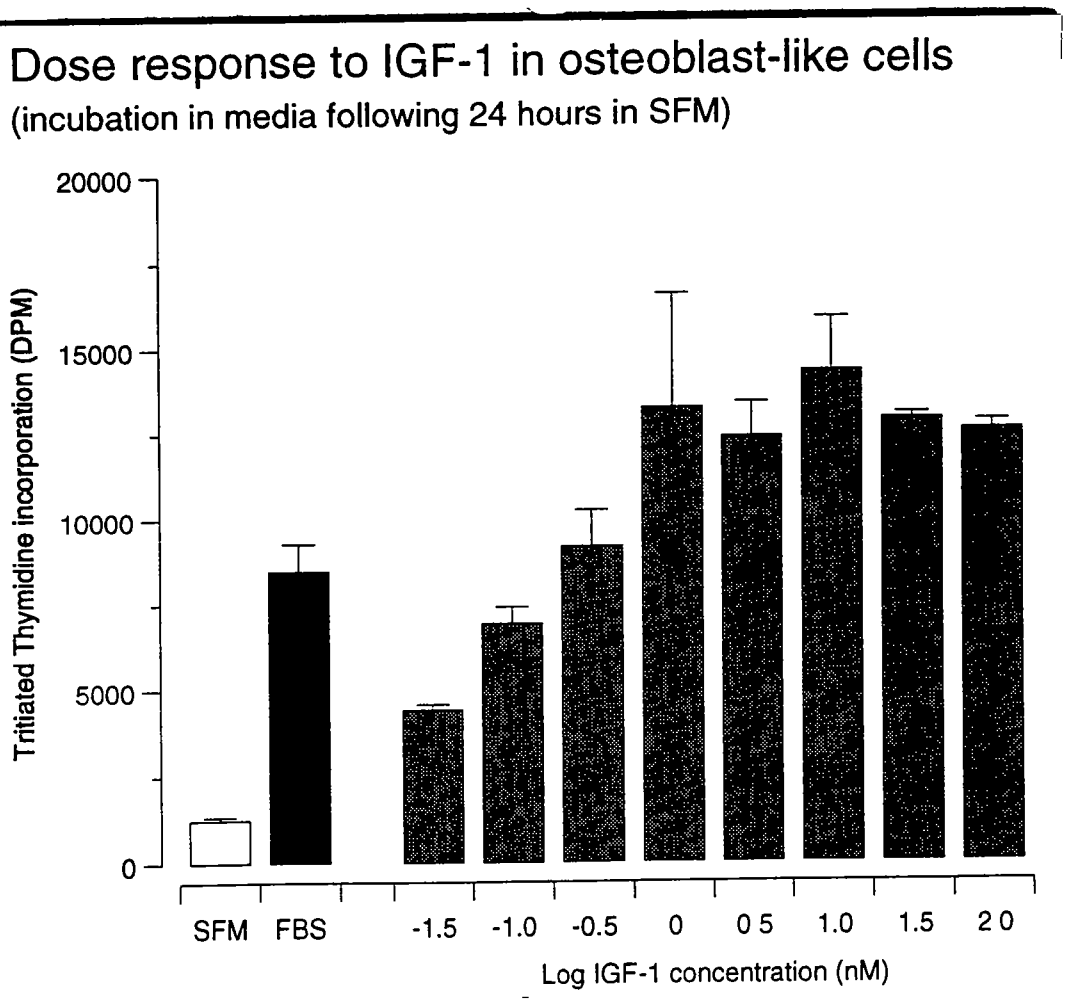

Figure 10 Dose dependant effects of IGF1 on osteoblast-like cells

There was a significant dose response to IGF1 in the fibroblast-like (Figure 9) and osteoblast-like (Figure 10) cells The fibroblast-like cells appeared slightly more responsive to IGF1 in that the maximum response was achieved with a lower dose of IGF1 than the osteoblast-like cells

DISCUSSION

The fact that IGF1 causes a dose dependent increase in cell multiplication (DNA synthesis) means that earlier reports which described correlations between antler growth and IGF1 and IGF1-receptors in antlers are almost certainly indicative of a normal control mechanism This is hardly surprising as growth of cartilage tissue in other organs is known to be under IGF1 control Whether the IGF1 normally reaches the antler via the blood stream or whether it is released by adjacent cells is not known As the antler lacks growth hormone (GH) receptors it seems that any local (adjacent cell) IGF1 is not released under GH control

Whether the knowledge that IGF1 can stimulate antler cells *in vitro* is useful in the production sense is not apparent At present we have no inkling whether increasing plasma IGF1 will increase or alter antler growth.

The next step is to explore the mode of action of IGF1 in increasing growth

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