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Remodelling of bone and bones: growth of normal and transplanted caudal vertebrae

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INTRODUCTION

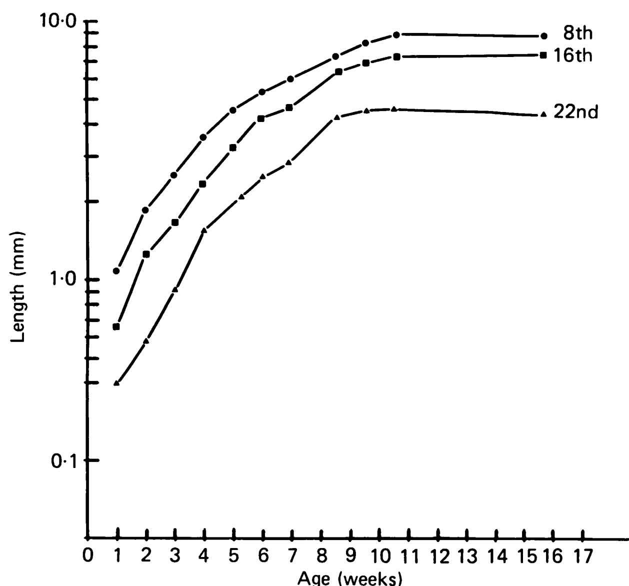
Although many investigators have studied the transplantation of whole bones into non-functional sites the results obtained appear conflicting, regarding both the rate of growth and the final form of the transplant. Some bones grew at a normal rate, at least initially (Chalmers, 1965), some more slowly (Harkness & Trotter, 1980) but with wider ends relative to length (Felts, 1955), while others grew at a faster rate but were narrower than normal (Noel & Wright, 1972). From both *in vitro* (Fell, 1956) and *in vivo* (Felts, 1959) transplantation experiments it is generally accepted that the cartilage model will develop its basic form due to the operation of intrinsic cellular factors. However, the modifying effect on transplants of such factors as, for example, the age of the donor and/or the host and the transplant site with attendant differences in temperature, vascularity and function has not been clarified. One reason for this has been the lack of a suitable experimental model. The rat tail may provide such a model for it consists of a series of bones of progressively decreasing size and, during growth, of maturity. These bones grow at predictable rates and remodel symmetrically (Hammond & Storey, 1970) or, when the forces applied to them are altered, asymmetrically (Storey, 1972). Furthermore, they are easily accessible for experimental work involving surgery, application of mechanical or electrical forces and homologous and autologous transplantation.

Little use has been made of the tail in experimental studies of bone growth and development. Bert (1863) and Huggins & Anderson (1976) found that transplants of immature caudal vertebrae grew well. Noel & Wright (1972) observed that transplants grew faster the younger the host. This study explores the alterations in the rate of growth and form of transplants of different age, size and maturity. Three relatively non-functional sites were used to examine the effects of environmental factors, other than stress, on bones of different maturity. The influence of tissue constraints on a transplant was also studied.

MATERIALS AND METHODS

Sprague–Dawley rats fed a diet of GR 2+ pellets (Clark King & Co., Melbourne, Vic.) and tap water *ad libitum* were housed at a relatively constant temperature of 22 °C. Several groups of animals were used, the experimental procedures being outlined below.

In each of two separate experiments (one commencing at birth and the other at 50 g body weight) the tails of two male and two female rats were serially radio-



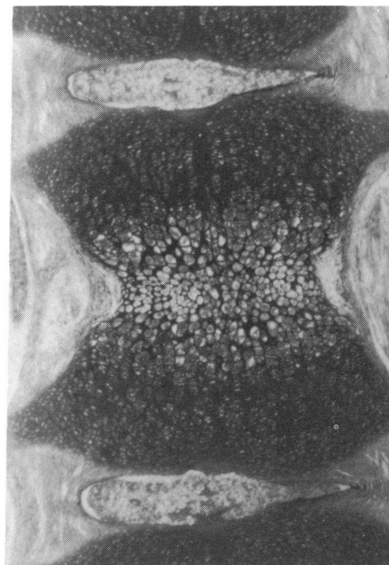


Fig. 1. Change with age in the diaphyseal lengths of the 8th (●), 16th (■) and 22nd (▲) caudal vertebrae, using combined data from both control groups.

Fig. 2. Cartilage anlage of the 8th caudal vertebra from a 4 days old animal undergoing normal development, showing central hypertrophied cells surrounded by a calcifying matrix and the early formation of a primary bone collar. × 80.

graphed, at weekly intervals at 1–8 weeks, and at 12 weeks after the start of each experiment. From these radiographs measurements were made of the diaphyseal lengths of the 8th, 16th and 22nd caudal vertebrae.

Transplants

(1) *Young donors*

The tails of 4 days old male and female rats were cut off, skinned, either left whole or divided into two segments and, after radiography, transplanted into 50 g (24–27 days old) hosts. Three groups of hosts were used with transplants:

(*a*) into subcutaneous pockets in the anterior abdominal wall (36 specimens);
(*b*) with the distal ends of the segments under the kidney capsule (36 specimens);
(*c*) free in the abdominal cavity (36 specimens).

(2) *Older donors*

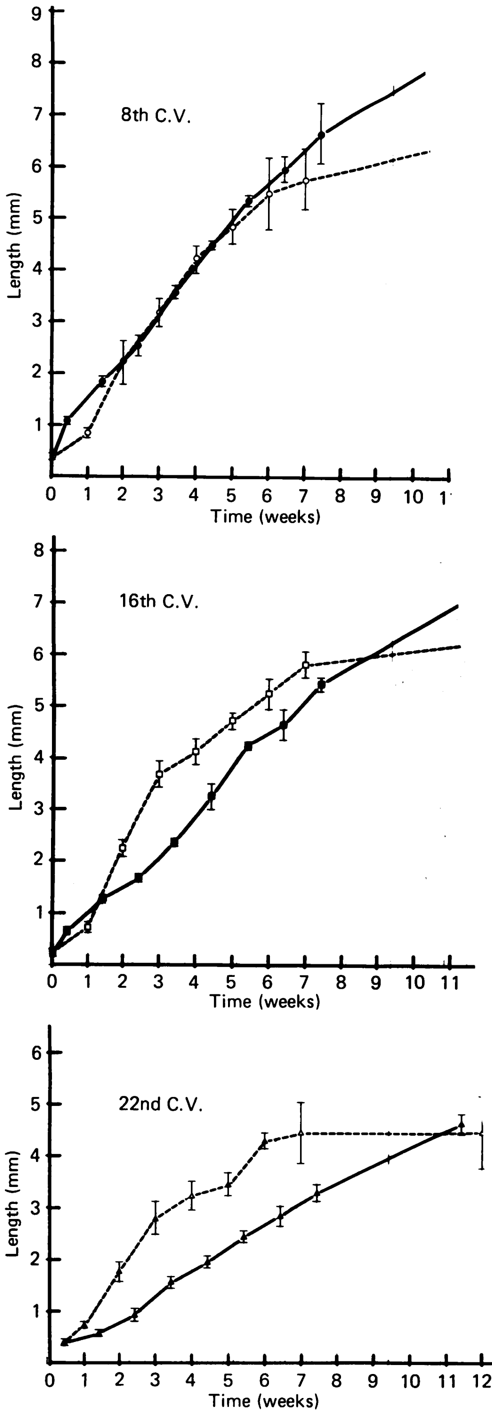
Equal numbers of male and female 50 g (24–27 days old) rats were radiographed, the tails skinned and treated as follows:

(*a*) segments containing the 7th, 8th and 9th caudal vertebrae were transplanted subcutaneously into litter mates (24 specimens);

(*b*) segments containing the 15th, 16th and 17th caudal vertebrae were transplanted autologously into subcutaneous pockets (4 specimens);

(*c*) segments containing the caudal vertebrae from the 20th to the tail tip were transplanted subcutaneously both autologously and into litter mates (36 specimens in each category).

Four hosts of each series with transplants from the young and older donors were killed at weekly intervals from 1–7 weeks and at 12 weeks, when the transplants were dissected out and radiographed. Group 2(*b*) animals were radiographed at



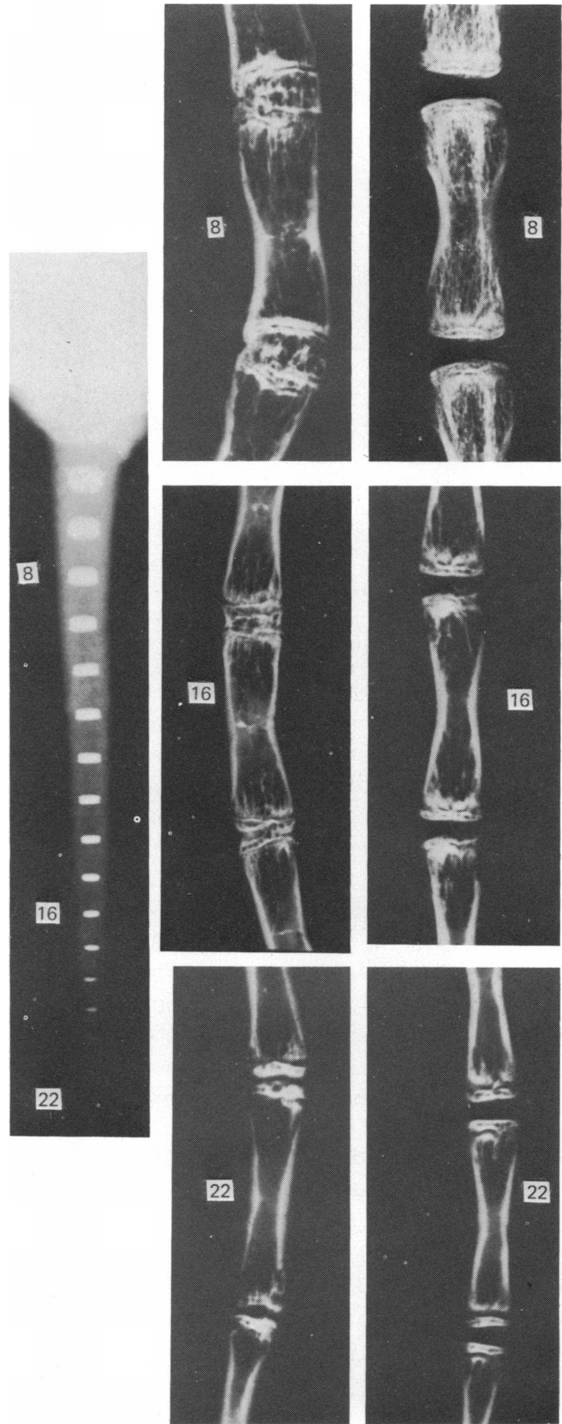


Fig. 3. Change in the diaphyseal lengths with time after transplantation of the 8th, 16th and 22nd caudal vertebrae (C.V.) from 4 days old donors (- - -) with the corresponding controls (—).

Fig. 4. Radiographs showing the 8th, 16th and 22nd caudal vertebrae from 4 days old donors at the time of transplantation (left), 12 weeks after transplantation (centre), and the corresponding controls after 12 weeks (right). × 5.

Table 1. *Diaphyseal length (mm) of caudal vertebrae 12 weeks after transplantation, with corresponding controls**

Vertebra	Young donors	Controls	Older donors	Controls
8	6·63 ± 1·20	8·57 ± 0·37†	4·42 ± 0·48	8·98 ± 0·50‡
16	6·29 ± 0·65	7·23 ± 0·32†	4·71 ± 0·93§	7·62 ± 0·23†
22	4·47 ± 0·69	4·74 ± 0·21 NS	4·39 ± 0·64	4·35 ± 0·25 NS

* Values are means ± S.D.; $n = 4$ in each group; NS = not significant;
† $P < 0.05$;
‡ $P < 0.001$;
§ $n = 3$.

weekly intervals up to 10 weeks with the transplants *in situ*. From the radiographs, measurements were made of the 8th, 16th and 22nd caudal vertebrae, and in addition the 15th and 17th caudal vertebrae in Group 2(*b*) were measured. Additional animals were used as necessary to obtain specimens for histological examination and microradiography at the same time intervals as for the controls.

Transplantation procedures

Animals were weighed prior to being anaesthetised with an intraperitoneal injection of chloral hydrate at a dose level of 30 mg/100 g body weight and the tail being radiographed to identify the caudal vertebra required. To prevent haemorrhage, a black silk suture was tied around the tail proximal to the segment required; then the tail was severed, stripped of skin and immersed in normal saline solution till transplantation.

In some host animals, a subcutaneous transplant pocket was created between the skin and the underlying muscle of the anterior abdominal wall. In others, the distal end of the segment was inserted under the perforated kidney capsule. For intra-abdominal transplants, the tail segment was slipped into the cavity through a perforation in the abdominal wall. Wounds were closed with interrupted absorbable Dexon sutures size 000 (Dexon polyglycolic acid, American Cyanamid Co., Pearl River, N.Y.).

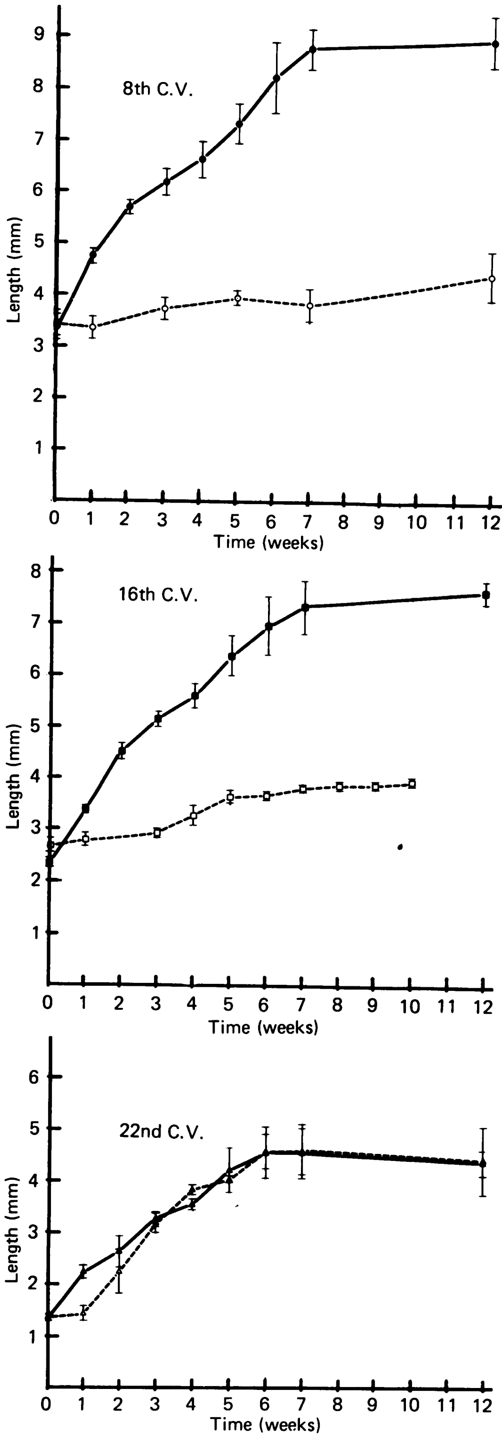
Radiography

A Faxitron 43805 N X-ray System Unit with a current of 2·5 mA and a film-to-source distance of 61 cm was used for all radiographs. Radiographs of live, anaesthetised animals were made using Kodak X-Omat S Medical X-ray film with an exposure time of 3/10 minutes. The voltage was varied for bones of different sizes and densities. Serial radiographs of segments of tail transplanted into the anterior abdominal wall were taken by placing anaesthetised animals on their sides and using tape to attach the area of skin containing the transplant onto the film.

When transplants, rather than whole animals, were radiographed, Kodak Industrex MX 54 film with an exposure time of 2 minutes was used. All films were developed in Kodak Liquid Developer Type 2 for 5 minutes at 20 °C.

Histology

Tissues for histological examination were fixed in 10% buffered formol saline. Those to be embedded in paraffin were decalcified in a solution of sodium formate 3·4%, formic acid 17·5% and distilled water 79·1%. Sections 3–4 μm thick were



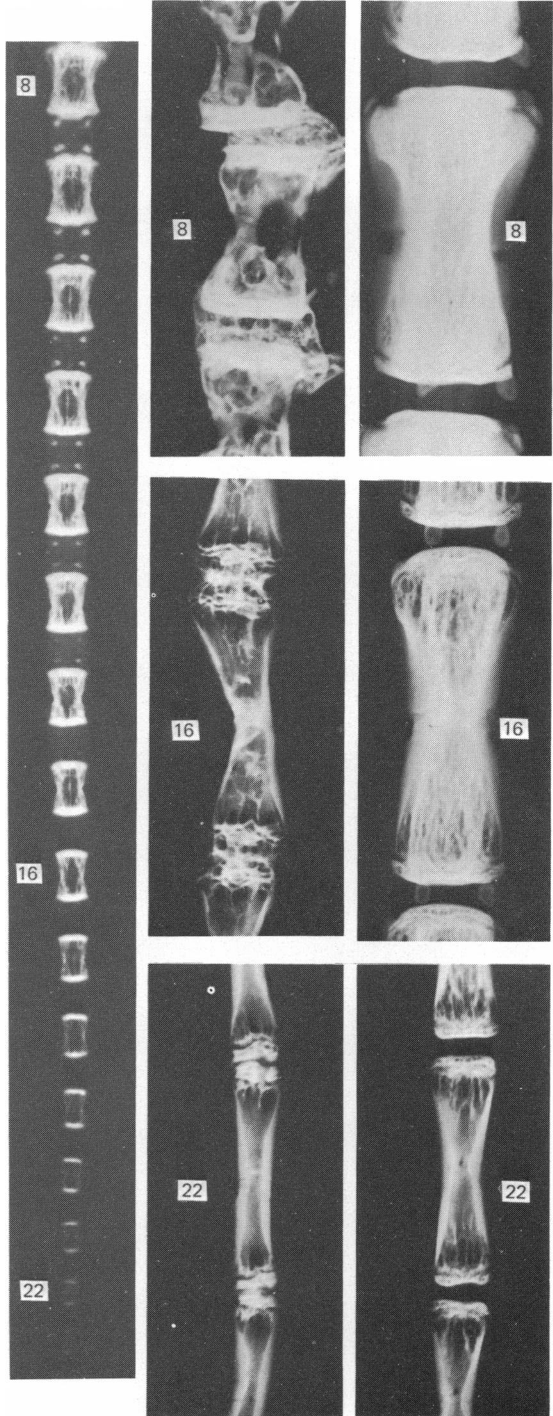
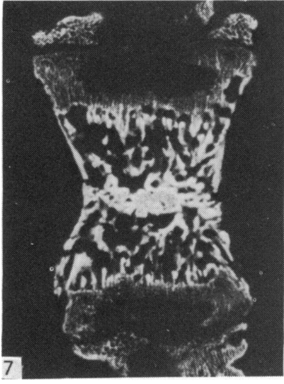


Fig. 5. Change in the diaphyseal lengths with time after transplantation of the 8th, 16th and 22nd caudal vertebrae (C.V.) from 50 g donors (- - -) with the corresponding controls (—).

Fig. 6. Radiographs showing the 8th, 16th and 22nd caudal vertebrae from 50 g donors at the time of transplantation (left), 12 weeks after transplantation (centre) and the corresponding controls after 12 weeks (right). ×5.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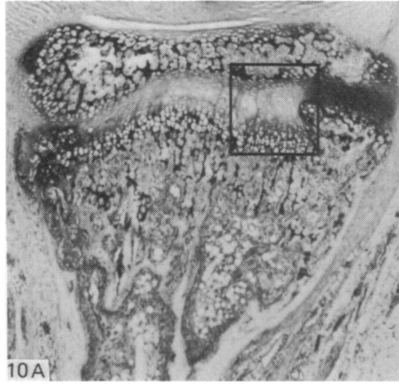
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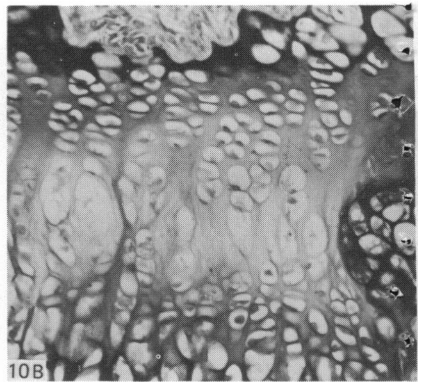
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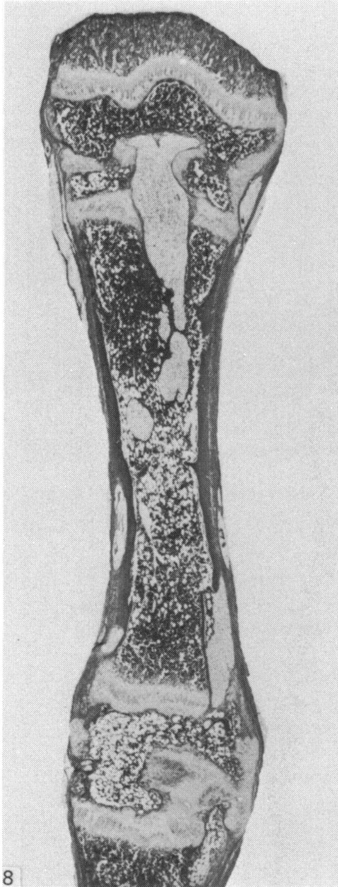
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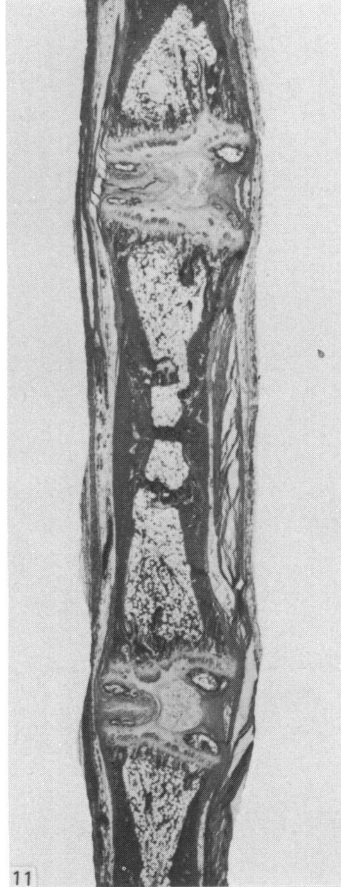
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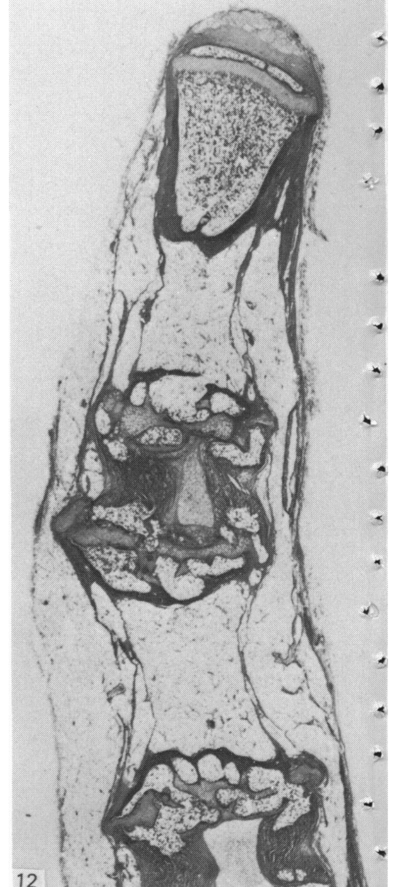
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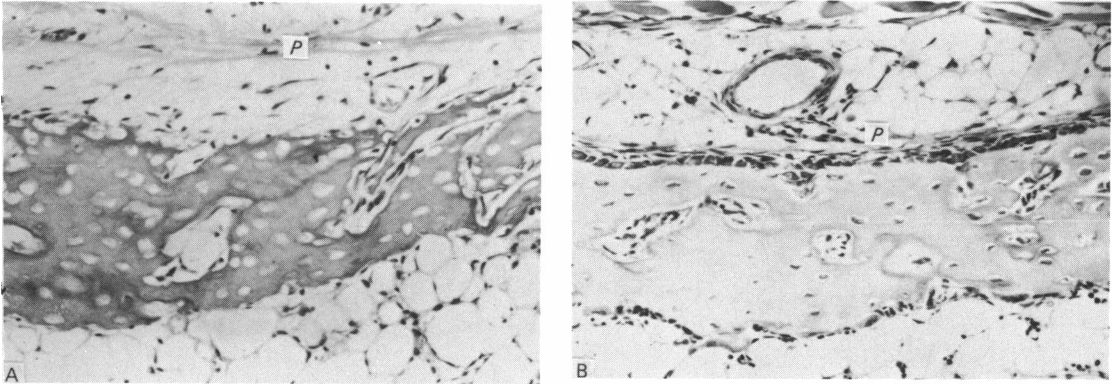

Fig. 9(A-B). Diaphyseal bone. (A) 8th caudal vertebra from a 50 g donor 7 days after transplantation. The periosteum (*P*) has separated from the bone in which osteocytes stain faintly or not at all. (B) Control. ×290.

cut and stained routinely with Ehrlich's haematoxylin and Putt's eosin or with 5% toluidine blue. Selected sections were stained with Van Gieson's stain.

For undecalcified sections, tissues were dehydrated through graded alcohols, cleared by extraction in acetone for 8 hours using a Soxhelt apparatus and embedded in clear embedding resin (R.F. Services, Fitzroy). After 2–3 weeks curing, sections were cut on a Microslice – 2 to approximately 50–60 μm. Some 30 μm sections were obtained by lapping on a Sectilap Mk-2 (Malcolm, 1975). The undecalcified sections were stained with 0·15% basic fuchsin or 5% toluidine blue.

Microradiography

Microradiographs were taken on Kodak Spectroscopic plate type 649-0 on an MSH apparatus (Malcolm, 1972). A routine exposure time of 12 minutes at 15 Kv was used for the 50–60 μm sections and 10 minutes for the 30 μm sections. Better definition was obtained with thinner sections using a 60 minutes exposure time at 10 Kv.

Measurements of length of bones

The distance between the diaphyseal borders of the growth cartilages was measured directly with vernier calipers (Tesa, Switzerland) from radiographs placed on

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Fig. 7. Microradiograph of the 8th caudal vertebra from a 4 days old donor 3 weeks after transplantation. A transverse calcified bar is located centrally and uncalcified areas are present in the cartilage. ×10.

Fig. 8. 16th caudal vertebra from a 4 days old donor 7 weeks after transplantation. The joint has ankylosed and the nucleus pulposus is extruding through the defect in the cartilage into the marrow cavity at one end and under the periosteum of the shaft at the other end of the bone. ×15.

Fig. 10(A-B). (A) 16th caudal vertebra from a 50 g donor 14 days after transplantation. An extensive central area of poorly stained cartilage is flanked by peripheral proliferating cartilage and some new endochondral growth. ×25. (B) Detail of cartilage seen in (A); non-vital cartilage beginning to be torn apart. ×290.

Fig. 11. 22nd caudal vertebra from a 50 g donor 5 weeks after transplantation. The remnants of dead cartilage and bone, encased by new bone, in the central area of the shaft indicate the size and location of the vertebra at the time of transplantation. ×15.

Fig. 12. A transplanted segment, consisting of the 16th and 17th caudal vertebrae from a 50 g donor at 7 weeks. There is complete resorption of the original diaphyseal bone, leaving behind the fibrous component of the periosteum. The free end of the 17th vertebra has developed rapidly, whereas little longitudinal growth has occurred at the enclosed ends of the bones. ×11.

a viewing screen. Four animals at each time interval were used to obtain the mean diaphyseal lengths (± 1 S.D.). In the case of the immature caudal vertebrae, measurements were begun as soon as a calcified bar appeared in the anlage. Confirmation of the changes in length of the bones was made, first, by measuring the distance between the epiphyses when they appeared in the radiographs and, secondly, by measuring the changes in length of very immature bones from histological sections.

RESULTS

Control tail growth and structure

Radiographs showed that calcification in only the first few proximal caudal vertebrae had started at birth and by 7 days it had progressed down to the 23rd caudal vertebra. By 21 days of age, 26 primary calcification sites, as well as two secondary sites, were present and a typical mature caudal vertebral structure was apparent – rectangular bones with biconcave sides and slightly narrower epiphyses. Measurements of the metaphyseal lengths of the 8th, 16th and 22nd caudal vertebrae showed that the three increased in length at a similar rate, the rate decreasing with increasing age of the animal (Fig. 1).

Histological examination showed that the development of the caudal vertebrae was basically similar to that of other long bones formed in cartilage. The central cells of the cartilaginous anlage hypertrophied and the adjoining extracellular matrix calcified. As this process extended, the perichondrium became osteogenic and formed the primary bone collar (Fig. 2). Removal of the central endochondral trabeculae resulted in the formation of a marrow cavity. Ossification within the epiphysis commenced later as two peripheral zones which eventually fused centrally.

As growth proceeded the original form of the bone was maintained by symmetrical periosteal and endosteal remodelling. The haemopoietic marrow became fatty, except at the metaphyseal ends where bone growth continued. The main blood supply to the bone was via the nutrient artery which penetrated the shaft in the middle and ended in arborizations at the metaphyses. This vessel supplied the marrow, cortex and a large part of the metaphyses. The peripheral parts of the metaphyses and growth plates were supplied by circumferential arteries and the epiphyses and the remainder of the growth plates by epiphyseal networks. Small intervertebral muscles ran from one vertebra to the next. Outside these were dense bands of longitudinally orientated collagen fibres which comprised the four main vertebral ligaments running all the way down the tail.

Transplants: radiographic changes

Young donors

While the whole tails grew well, they curved around within the subcutaneous transplantation pocket. Bones in straight segments retained a relatively normal shape whereas, if the tail bent, the bone form changed. As transplants matured some joints ankylosed. This latter aspect is discussed in detail elsewhere (Feik & Storey, 1982). Most transplants of smaller segments of tails grew relatively straight, enabling the measurements of length and changes in form reported here.

After an initial lag phase the growth of the 8th caudal vertebra kept pace with that of the control for approximately 7 weeks and then slowed, so that by 12 weeks the transplants were shorter than the control bones. Secondary centres of epiphyseal ossification appeared by 2 weeks in transplants but not until later in normal bones.

Central calcified cartilage remnants were visible in the radiographs of the transplants in the early stages. They remained for up to 7 weeks after transplantation but were not present in control bones.

The transplanted 16th caudal vertebra showed an initial retardation in growth but then grew faster and matured earlier than normal. The growth of transplants had almost ceased by 7 weeks and by 12 weeks most were shorter than normal but there was a wide variation in growth rate.

Calcification in the 22nd caudal vertebra had not begun at the time of transplantation. When it could be detected radiographically the length of the vertebra and its maturation greatly exceeded that of the controls. The difference in length was still obvious at 7 weeks but bones were thinner with less cortical inwaisting than normal. Growth of transplants then slowed so that by 12 weeks they were approximately the same length as the control bones and with a similar cortical thickness and radiodensity (Figs. 3, 4).

Comparison of the lengths of the subcutaneously transplanted 16th and 22nd caudal vertebrae with those transplanted under the kidney capsule showed essentially no difference (Table 1). Most transplants into the abdominal cavity failed to grow unless they were vascularized through attachment to the abdominal wall or to a vascular organ such as the liver or kidney; in such cases the growth was similar to that seen in the subcutaneous transplants.

Older donors

The caudal vertebrae from older donors grew more slowly than those from younger ones. The 8th caudal vertebra showed very little increase in length, but some lateral growth and thickening of the metaphyses and epiphyses occurred while the bone shaft became progressively rarefied. The transplanted 16th caudal vertebra grew faster than the 8th but still much more slowly than the control. In this group, the same four transplants were measured at weekly intervals and because of the longitudinal nature of this series the standard deviations were smaller. By 12 weeks the vertebrae were shorter and more inwaisted due to the relative lack of lateral expansion of the diaphyses with continued lateral growth of the epiphyses. A radiopaque image of the original bone was present in the central area of the shaft. The 22nd caudal vertebra showed an initial growth lag followed by a period of rapid elongation so that by 7 weeks it was similar in length to the control bone and then both bones continued to grow at the same rate until the 12th week (Table 1). Two radiopaque transverse bars still remained in the central area of the shaft, marking the location of the metaphyses of the original transplant at the time of transplantation (Figs. 5, 6).

In transplants of segments of tail (from 50 g donors) consisting of only the 15th, 16th and 17th caudal vertebrae, the 16th grew more slowly than the other two. This difference was largely due to the rapid enlargement of the free ends of the other two vertebrae, particularly the distal end of the 17th. A radiopaque metaphyseal bar separated the newly formed bone from the original shaft.

Histological and microradiographic changes

Young donors

Transplantation of the 22nd caudal vertebra of the 4 days old rat was associated with a slight inflammatory reaction in the host tissues which subsided within a few days. A few scattered centrally located cells stained poorly but most appeared

normal and by 7 days growth had resumed and was qualitatively normal. The inflammatory reaction following transplantation of the 16th caudal vertebra was more prolonged and more central chondrocytes failed to stain normally. The periosteum survived and continued to elaborate a bony collar around the pre-existing bone in which osteocytes stained only faintly. The dead zone of central cartilage remained for up to 3 or more weeks, being gradually surrounded by bone and then remodelled to form a transverse calcified bar. The 8th caudal vertebra differed in that, in addition to the changes seen in the 16th, a wedge-shaped area of uncalcified poorly stained cartilage appeared in the epiphyseal ends of the bone. By 3 weeks the only remaining signs of interruption to growth were the centrally located calcified transverse bar across the bone shaft and some small areas of pre-existing bone with absent or very faintly staining eosinophilic osteocytes and, in some cases, a wedge of poorly stained uncalcified cartilage in the epiphyses (Fig. 7). The joints between the larger vertebrae ankylosed and the nucleus pulposus, which was occasionally extruded from the intervertebral space through defects in the non-vital cartilage, appeared in the marrow cavity of the shaft or under the diaphyseal periosteum (Fig. 8).

Older donors

The bones from older donors already had a differentiated bone shaft, marrow and endochondral cartilages. A few days after transplantation the periosteum had separated from the diaphyseal bone in which osteocytes stained faintly (Fig. 9). The marrow contained engorged blood vessels and some polymorphonuclear leucocytes. A poorly stained area of cartilage appeared towards the ends of the bones or, if epiphyseal ossification had already taken place, in the middle of the endochondral cartilage (Fig. 10A). In the 22nd caudal vertebra, even though the periosteum also appeared initially to be stretched away from the dead shaft, by one week a layer of new bone was being deposited over the original bone. Revascularization of the metaphyses occurred through lateral ingrowth of vessels from the surrounding host tissue so that by 10 days after transplantation new endochondral growth and bone remodelling were in progress. The marrow had revascularized and osteoclastic resorption of the dead bone shaft was occurring.

In some bones by 14 days the poorly stained area in the centre of the cartilage had disappeared or had been left behind in the marrow and a new metaphysis had formed. Enlarged spaces appeared in the centre of more extensive areas of poorly stained cartilage (Fig. 10B) which were invaded by connective tissue cells and vessels from the metaphysis. Subsequently, the dead cartilage remnant separated from the living part and eventually was removed or remained as part of a calcified transverse bony bar in the shaft (Fig. 11).

Resorption of the original transplanted bone shaft continued periosteally, end-osteally and within vascular canals, so that by 7 weeks little remained. In some specimens, all the bone was resorbed leaving behind a periosteal fibrous layer enclosing fatty marrow. Where cartilage growth resumed, a new metaphysis formed which subsequently remodelled to form a thin bone cortex. This was particularly well shown in the transplanted segments from 50 g donors at the exposed ends of the 15th and 17th caudal vertebrae. These resumed growing in a normal manner and at a faster rate than the cartilages at the other end of the bones enclosed by inter-vertebral joint structures. Concomitantly, new bone was progressively laid down by

the periosteum extending from the epiphysis to the original metaphysis. As growth proceeded this underwent remodelling, with resorption on the periosteal and formation on the endosteal surfaces (Fig. 12).

DISCUSSION

Increasing size, age and maturity of transplanted caudal vertebrae are associated with decreasing longitudinal growth. While such vertebrae may, after an initial lag period, grow faster, at normal rates or more slowly than normal, most reach a plateau at similar times to control rats. This supports the suggestion that a limited intrinsically determined potential for growth exists (Chalmers, 1965; Harkness & Trotter, 1980) but in most cases this is not realized because of temporary or permanent growth aberrations resulting from transplantation. This may affect one or more aspects of growth with resultant changes in the rate, shape and structure of developing bones.

In the early stages of transplantation, diffusion of nutrients into cartilage must be the most important growth rate limiting factor. The presence of any diffusion impediment, such as a calcified bone collar, encasing joint tissues or even a large volume of cartilage, may be sufficient to cause death or disorganization of deeply located chondrocytes and chondroblasts and inhibition of longitudinal growth. This is clearly shown in the case of older transplants where the free ends of epiphyses directly exposed to the host tissues grow rapidly whereas the other ends encased in joint structures do not.

Felts (1955) and Chalmers (1965) found that transplants grew more slowly than normal. This is consistent with the findings presented here for older transplants. The delay in growth was associated not only with central necrosis of endochondral cartilage but also with its sequestration to allow continued elongation of the bone. Yabsley & Harris (1965) produced a similar effect in rabbit tibiae after stripping the periosteum and destroying the nutrient artery. However, prolonged growth inhibition does not always follow central necrosis of the endochondral cartilage because, in young caudal vertebrae, the surviving peripheral cartilage may grow sufficiently to maintain near normal growth rates even though a permanent perforation exists in the cartilage.

Even when conditions are optimal for diffusion of nutrients into cartilage, the process of vascularization is necessary for the continued growth and development of cartilage anlages. This is shown by the failure of intra-abdominal transplants to develop unless attachment and vascularization occur.

In contrast to older or larger caudal vertebrae, those towards the tail tip from the younger donors grow faster than normal for some weeks before slowing down. This is consistent with the findings of Noel & Wright (1972) in the mouse but the growth acceleration was much less in their experiments. The use of caudal vertebrae from somewhat older mice, which are also more mature at a comparable age than those of the rat, probably accounts for the difference.

Stimulation of endochondral bone growth is said to occur in conditions which produce hyperaemia, such as inflammation (Phemister, 1933) and venous stasis (Janes & Musgrove, 1950). The inflammatory process associated with the transplants from young donors is very transient, lasting only a matter of days, and does not coincide with the period of accelerated growth. Thus, it is unlikely to be an important factor in this instance. Increased vascularization has also been cited as

the reason for the overgrowth of bone after periosteal stripping (Khoury, Silberman & Cabrini, 1963) and fracture of bone (Trueta, 1953). However, the transplants on the well vascularized kidney grew no better than those in the subcutaneous pockets so other factors must also be important.

Noel & Wright (1970) found that the caudal vertebrae grew faster in mice maintained in a hot environment or transplanted under the kidney capsule (Noel & Wright, 1972). Such a rise in local temperature may have induced rapid growth and earlier maturation of anlages in the present experiment but it had no demonstrable effect on older transplants.

Functional factors must also modify the growth of cartilage, for the forces exerted on the caudal vertebrae *in situ* are considerable (Storey, 1972; Levy, 1978). The importance of the restraining effect of the periosteum on growth has also been demonstrated recently by Crilly (1972), Warrell & Taylor (1976) and Harkness & Trotter (1978). Crilly interpreted the stimulation of growth after circumferential division of the periosteum as being due to release of periosteal tension leading to decompression of the endochondral cartilage. Consequently, it is not surprising that longitudinal growth, producing long, thin caudal vertebrae, is accelerated when the external restraints imposed by muscles and ligaments are removed.

The final shape of transplants differed greatly in the present experiments. This ranged from normal, to longer and thinner or shorter and excessively dumb-bell shaped bones. Longer and thinner transplants were associated with excessive rates of longitudinal growth, and shorter and more inwaisted ones with retarded longitudinal growth. The variation in form appeared to be, primarily, the result of a change in the direction of growth of cartilage, and, secondarily, the result of a similar change in periosteal bone formation or resorption. Where longitudinal cartilage growth was impeded in older transplants, lateral growth occurred instead, so that the epiphyses widened. Felts (1955) also noted that implants tended to become wider at the growing ends as compared to controls. This is not surprising, for if the central necrotic cartilage still connects the shaft to the epiphysis then, while longitudinal growth of surviving cartilage is not likely to occur, as internal stresses increase, cartilage will expand laterally. This is consistent with the *in vitro* experiments of McMaster & Weinert (1970) who found that when compressive forces on cartilage are increased it expands laterally.

Felts (1955) and Chalmers (1965) found an increased inwaisting of older transplants which is consistent with the present findings. This occurs not only because of increased widening of the epiphyses but also because of lack of lateral periosteal growth together with increased resorption of the dead shaft. Death of calcified bone cannot be primarily responsible for resorption because, in some cases, the transplanted, dead shaft became encased in new diaphyseal bone. Neither can resorption be attributed simply to lack of function, because transplanted bones grown from anlages have relatively normal bone shafts. The stimulus for resorption is more likely to be associated with the absence of a living periosteum.

The maintenance of the general form of a growing bone, despite its transplantation into a non-functional site, can be explained in terms of the elasticity of the periosteum. This contains longitudinally orientated elastic fibres (Murakami & Emery, 1967) and seems to function as an elastic membrane (Harkness & Trotter, 1978) which, through its attachment to bone, influences the orientation of subperiosteal bone deposition. If this is so, then it must also exert pressure on the outer surface of the widening metaphysis and account for the remodelling resorption

which occurs here in non-functioning transplanted as well as in functioning, normal, growing bones.

Structural changes may occur in transplanted caudal vertebrae and these are associated with cell death and disorganization of the endochondral cartilage, periosteum and calcified bone shaft. The resultant spectrum of pathological structural changes embraces some, but not all, of the changes described in different experiments by other authors (Felts, 1955, 1959; Chalmers, 1965; Harkness & Trotter, 1978; Noel & Wright, 1972). The changes may range from apparently normal shaped bones with most of the dead remnants of the original bony transplant encased within the bone shaft, to perforations of the epiphysis and endochondral plate and sequestration of the nucleus pulposus into the diaphyseal marrow or between the periosteum and the shaft. The rapid growth of the free ends of the older transplants, which has already been mentioned, illustrates that while bones may possess an inherent potential for growth this requires ideal conditions for its expression.

The shape of the bone is also influenced by whether or not periosteal osteoblastic growth continues after transplantation. The results of transplantation differ depending on the experimental conditions (Felts, 1955, 1959; Harkness & Trotter, 1978). Although osteocytes largely die after transplantation, new periosteal bone develops only in small and young caudal vertebrae. In older ones, where the osteoblastic layer is absent, resorption occurs so that the bone shaft may become narrower than normal. Thus, the use of transplants as models for studying growth requires careful selection of donor bones to minimize the pathological changes associated with transplantation.

SUMMARY

Changes in the rate of growth, shape and structure of the 8th, 16th and 22nd caudal vertebrae of 4 and 24–27 days old Sprague–Dawley rats were studied *in situ* and in three different non-functional transplantation sites for 12 weeks. With increasing size, maturity and age the three vertebrae showed progressively decreasing growth, changes in shape and structural abnormalities. The smallest anlages grew faster and matured sooner than normal, so that their length equalled that of controls. Central endochondral necrosis in older bones was associated with decreased longitudinal growth but in some younger ones, despite a perforation of the cartilage and herniation of the nucleus pulposus into the marrow cavity of the shaft, growth proceeded at near normal rates. The free ends of older, larger transplants grew faster than the abutting ends joined by joint connective tissue, indicating that central necrosis of cartilage resulted from impaired nutrient diffusion.

The results suggest that the cartilage model may possess an inherent capacity to produce a certain limited amount of bone tissue which may be distributed either in the form of long and thin or short and inwaisted bones, depending on the balance of forces between interstitial cartilage expansion and the restraining ensheathing periosteal-perichondrial tissues. This basic form may be modified further by functional forces.

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