The control of growth in transplanted mammalian cartilage

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SUMMARY

Seven- to eight-day mouse tail vertebrae were transplanted under the renal capsules of syngeneic adult animals and allowed to grow for up to 6 months. Their curve of growth and histological pattern of ageing were similar to those of control vertebrae which had been left in their normal position on young animals.

Slices of the growth cartilage of seven- to eight-day rat tail vertebrae were similarly transplanted into adult syngeneic rats. Though some grafts failed to grow, a considerable number showed similar growth characteristics to the epiphyseal cartilage in intact control vertebrae.

These results have been used to support the view that in the mouse and rat the epiphyseal growth cartilage has a limited potential for growth. This intrinsic growth-limiting mechanism seems to be of relatively greater importance than age-dependent changes in blood hormone levels in determining the changes in the rate of growth of the vertebrae as the animals approach adulthood.

INTRODUCTION

This investigation represents the second part of a study of the factors controlling the rate of growth in length of mammalian vertebrae, using transplantation under the renal capsule as the principal experimental technique. The aim of the experiments was to try to distinguish between the effects of endocrinological factors and factors intrinsic to the vertebrae in the determination of the changes in the rate of growth as the animals approached the age of adulthood. It has been suggested (Siffert, 1966) that endocrinological factors are the major regulators of longitudinal bone growth in man. There is a considerable quantity of published data on the effects of the endocrine hormones on the rate of growth of the epiphyseal cartilage, e.g. Greenspan, Li, Simpson & Evans (1949), Silberberg & Silberberg (1956) and Tapp (1966). The experiments of Noel & Wright (1972) demonstrated that the growth rate of transplanted mouse vertebrae depended partly upon the age of the host animal and this variation was thought to be due mainly to age-dependent changes in blood hormone levels.

The experiments reported here were, however, directed towards finding out how far factors situated within the bones, or even within the epiphyseal cartilage itself, could be responsible for the changing growth rate with increasing age. The plan of the experiments was to transplant young vertebrae at an early stage

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of growth into fully adult syngeneic animals, where they would not be subjected to such great changes in hormonal stimulation as are thought to occur in young animals between the ages of youth and maturity. Any major changes in the rate of growth of the transplants, after an initial period of adjustment to their new environment, would be more likely to be due to changes taking place within the vertebrae than to changes in hormonal stimulation. Two experiments were carried out: in the first experiment, whole young mouse caudal vertebrae were transplanted under the renal capsules of syngeneic adult mice; in the second experiment, slices of the epiphyseal cartilage of young rat caudal vertebrae were similarly transplanted into syngeneic adult rats. In each case the growth and histological pattern of ageing were compared with control vertebrae which had been allowed to grow in their natural locations.

METHODS

Experiment 1: Transplantation of whole mouse vertebrae

(i) Animals. Inbred C₃H mice were used. Seven- to eight-day-old mice of both sexes were used as donors. Equal numbers of male and female 3- to 4-month-old mice were used as hosts. In each litter of 7- to 8-day-old mice, the animals were matched in experimental and control pairs with respect to tail length and body weight. In the experimental animal the distal vertebrae of the tail were transplanted into one of the host animals. In the control animal the tail was left intact.

(ii) Transplantation technique. The tail of each donor animal was amputated at about the position of the 17th caudal vertebra. The amputated vertebrae were then freed from the skin, placed in saline, and injected under the renal capsule of an anaesthetized host animal, using the technique described by Noel & Wright (1972). They were left to grow for varying periods ranging from 1 week to 6 months.

(iii) Technique of measurement and calculation of results. At the end of their allotted experimental period the host and corresponding control animals were killed. The vertebrae were dissected out, fixed, dehydrated, cleared and mounted on glass slides, and their length was measured using a low-power microscope. The results, presented in Fig. 1, are based on the length of the 19th caudal vertebra in each specimen. The mean length of the 19th caudal vertebra in each age group of the experiment was plotted against the age of the specimens at the time of dissection. Approximate curves of growth were then drawn in by eye. Each point on the graph represents the mean (plus or minus 1 s.e.) of six observations.

(iv) Histology. After the measurements had been completed, the vertebrae were freed from the mounting medium using xylene, decalcified, embedded in wax, and median longitudinal sections were cut at 5 μm and stained in haematoxylin and eosin (see Figs. 3A–D).
The pattern of ageing in the proliferative cartilage of the transplanted and control vertebrae was then compared. In each age group of the experiment, one section of the proximal epiphysis of the 19th caudal vertebrae of each of four transplanted and control specimens was examined. The proliferative cartilage was identified as the region where the cartilage cells were flattened in appearance and formed a series of irregular columns. The number of flattened cells in each of ten columns was counted on each section. The mean number of flattened cells per column in each age group of the experiment was then calculated (Fig. 4).

Experiment 2: Transplantation of slices of rat epiphyseal cartilage

(i) Animals. Inbred wistar rats were used. Seven- to eight-day animals of both sexes were used as cartilage donors. Three- to seven-month rats of both sexes were used as hosts. Since the supply of animals was extremely limited, control and experimental pairs could not be used. Therefore in each litter of 7- to 8-day rats, all but two of the animals were used to supply slices of cartilage for transplantation. Of the remaining two animals, one was used to supply similar slices of cartilage, which were not transplanted but fixed, cleared, mounted, measured and then sectioned to give an estimate of the size and histological composition of the slices at the age of transplantation. The last animal in each litter was left with its tail intact for use as a control and the growth of its vertebrae was followed throughout the experiment by repeated radiography.

(ii) Preparation of cartilage slices. The tail of each donor animal was cut off at about the position of the 17th caudal vertebra. The amputated vertebrae were then freed from the skin and examined microscopically. The epiphyses of the vertebrae appeared grey and translucent and could thus be distinguished from the diaphyses, which were hard and white and heavily calcified. A transverse slice about 0.3 mm thick was then cut through each epiphysis of the 19th, 20th and 21st caudal vertebrae. Six disc-like slices of cartilage were thus obtained from each animal. These slices usually contained the entire proliferative zone of the growth cartilage together with part of the articular cartilage, and surrounded by a ring of perichondrium and fibrous connective tissue (see Fig. 5 A).

(iii) Transplantation. The six slices of cartilage obtained from each animal were injected under the renal capsule of an adult rat, anaesthetized with chloral hydrate (300 mg per kg body weight). They were placed so that a circular transverse surface was parallel to the surface of the kidney and then left to grow for varying periods ranging from 2 weeks to 6 months.

(iv) Technique of measurement and calculation of results. At the end of their allotted experimental periods the host animals were killed and the grafts were dissected, fixed, cleared and mounted in the same way as the mouse vertebrae. The majority of the grafts differentiated into a zone of growth cartilage, bounded on one side by a ‘diaphysis’ of bone tissue, and on the other side first by enlarged cartilage cells and later by a centre of bone formation in the epiphysis (see Figs. 6 A, 7 A). The length from the tip of the diaphysis to the tip of the epiphysis was measured in each case. In the control animals the lengths of
the 19th, 20th and 21st caudal vertebrae were measured from radiographs taken at various ages.

Since the length of the cartilage slices at the start of the experiment was only a fraction of the length of the control vertebrae it was decided that it would be more informative to compare the amount of growth of the grafts and control vertebrae during the experiment, than simply to compare their final lengths. The results were therefore calculated as follows:

\[ \text{Growth of one graft} = b - a, \]

where \( b \) = observed final length of a single graft, \( a \) = average length of the 7- to 8-day cartilage slices which had not been transplanted.

\[ \text{Growth of one epiphysis of a control vertebra} = \frac{b_1 + b_2 + b_3 - a_1 + a_2 + a_3}{2} \]

where \( b_1, b_2, b_3 \) are the observed lengths of the 19th, 20th and 21st caudal vertebrae respectively in one animal at any given stage in the experiment; \( a_1, a_2, a_3 \) are the observed lengths of the same three vertebrae in the same animal at 7-8 days of age.

The mean growth of the grafts and control vertebrae was then plotted against their age at the time of dissection and an approximate curve of growth for the controls was drawn in by eye (see Fig. 2). Since the experimental variation was wide, the graph shows both individual and average results for the transplants.

(v) **Histology.** Longitudinal sections were later prepared using the method described above for mouse vertebrae.

## RESULTS

1. **Growth of transplanted mouse vertebrae**

Fig. 1 shows the mean length of the 19th caudal vertebra in the transplanted and control specimens of various ages in the first experiment. Although the length of the transplanted vertebrae was on average slightly lower than that of the controls at each stage, the general form of the curve of growth was very similar in the two groups. In both groups growth was rapid up to about 3 weeks of age. The growth rate then fell gradually so that by 12 weeks of age very little growth was taking place.

2. **Growth of transplanted slices of rat epiphyseal cartilage**

The results of this experiment were more varied. A small proportion of the grafts failed to grow and appeared at the end of the experiment as thin calcified discs. These have been discounted in calculating the results. The remaining 75% of the grafts became differentiated into a distinct epiphysis and diaphysis (see...
3. Histological pattern of ageing in the growth cartilage

In the epiphyses of the young mouse control vertebrae, the proliferative zone of the cartilage appeared as a broad band of flattened cells arranged in irregular columns (Fig. 3C). As the animal grew older, the number of cells present in each column decreased regularly (Fig. 4), so that by 6 months of age, when growth in length had virtually ceased, the columns of flattened cells had become very short, many columns contained hypertrophied cells only and between these columns there were occasional acellular spaces, filled with matrix (Fig. 3D).

Similar changes with increasing age took place in the transplanted vertebrae.

Fig. 1. A comparison of the mean (± s.e. mean) length at various ages of the 19th caudal vertebra in experimental specimens, grown as transplants under the renal capsules of adult mice, and control specimens left in their normal position on the tails of young mice. •—•, Transplanted vertebrae; ○—○, control vertebrae.

Figs. 6A, 7A) and showed an appreciable amount of growth, which has been plotted in Fig. 2. Their average growth was certainly considerably less than that of the controls and there was a tendency for them to reduce their growth rate at an earlier age than the controls. However, the growth of the most successful grafts followed approximately the general form of the curve of growth of the controls. Rapid growth continued up to about 8 weeks of age. After this age the growth rate declined and by 4 months growth had almost ceased.
Fig. 2. A comparison of the growth of transplanted slices of rat epiphyseal cartilage with the growth of control vertebrae left on the tail of the rat. ●, Mean growth of cartilage grafts; . . . . . . , growth of individual cartilage graft; ○—○, ½ x the mean growth of one control vertebra.

In the early stages of the experiment, the mean number of flattened cells per column was certainly slightly less than in the controls, but the general pattern of gradual shortening of the columns with age was similar in the two groups. The oldest specimens (Fig. 3B) showed occasional columns of a few flattened cells, several columns containing hypertrophied cells only and acellular spaces between the columns as in the oldest control vertebrae.

Figs. 5, 6 and 7 show parts of the growth cartilage of a series of rat cartilage grafts and control rat vertebrae at various stages in the second experiment. Again, in the control and transplanted specimens, increasing age has led to shortening of the columns of proliferative cells and the development of acellular spaces between the columns.
DISCUSSION

The results of the first experiment have shown that young mouse vertebrae could grow for long periods when transplanted into adult animals. It is likely that in the early stages their growth rate was depressed slightly by the adult hormonal environment, but this effect was partly masked by the growth-accelerating effect of the higher temperature in the kidney than the tail (Noel & Wright, 1972). Similarly, the decreased length of the proliferative cell columns in the transplanted vertebrae, during the early stages of the experiment, could be accounted for partly by the adult hormonal environment. It has previously been shown that the width of the epiphyseal cartilage can be related directly to the concentration of anterior pituitary growth hormone (Greenspan et al. 1949) and that the blood level of this hormone may be higher in children than adults (Greenwood, Hunter & Marrian, 1964).

The pattern of changing growth rate with increasing age followed a similar time sequence in the transplanted and control vertebrae, although the two groups of vertebrae must have been subjected to changes in hormonal stimulation at very different times. For the purpose of this discussion it has been assumed that the experimental vertebrae experienced a sharp change in
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hormonal stimulation at 7–8 days of age, when they were transplanted into adult animals, but rather less change later on. The vertebrae of the control animals are assumed to have experienced a more gradual change, between 1 and 8 weeks of age as these animals approached the age of adulthood. (These assumptions are based upon a study of the relationship between the rate of growth of young transplanted mouse vertebrae and the age of the supporting host animal; Noel & Wright, 1972.) The close similarity of the form of the curve of growth of the control and experimental vertebrae in the present experiments seems to suggest that the general pattern of a gradual reduction in the rate of growth, which took place particularly between 2 and 8 weeks of age, was due to a greater extent to factors situated within the vertebrae, than to changes in hormonal stimulation. This conclusion is further supported by the earlier experiments of Noel & Wright (1972) in which it was shown that senile adults, aged 17 months, which had themselves virtually ceased to grow, could

Fig. 5. Longitudinal section of (A) a slice of rat epiphyseal cartilage (× 120 approximately) and (B) a control rat vertebra at 7–8 days of age (× 105 approximately).
support a growth rate in young transplanted vertebrae which was 64% of that of similar vertebrae transplanted into young actively growing 1-week-old animals during the first three weeks after transplantation; i.e. the adult hormonal environment exerted only a minor effect on the growth rate of transplanted vertebrae.

The transplantation of slices of rat epiphyseal cartilage was carried out principally to see if there was any evidence of a growth regulating mechanism situated within the epiphyses of each vertebra. The results of this experiment were, however, difficult to interpret. Failure of some of the grafts to grow could have been due to various factors, including injury to the proliferative zone during transplantation and interaction between the tissues of the host and the graft. In some cases the graft became enclosed in a fibrous capsule, which may have impeded its growth. However, the most successful grafts showed similar patterns of growth and ageing to the controls and it seems likely that in these specimens the change in the growth rate with increasing age was due mainly to the same mechanism as in the controls.

It does therefore seem possible that the growth cartilage of each epiphysis contains some kind of growth limiting mechanism, although its nature is largely
unknown. It may be that as the animal becomes older, the proliferative cells gradually become 'senescent', and lose the ability for mitosis. In the sections of 4- to 6-month old cartilage plates shown in Figs. 3B and 3D and Figs. 7A and 7B, many columns in the proliferative zone contain hypertrophied cells only, while others are represented by acellular spaces, filled with matrix, where perhaps a column of cells has finally disappeared. However, on each section there remain at least a few columns of 4–7 flattened cells, which have a similar appearance, at least superficially, to the mitotically active cells of younger animals. Thus the columns of cells which make up one epiphyseal growth plate do not seem to be entirely synchronized in respect of ageing. Perhaps with increasing age it becomes increasingly likely that any one column, or its stem cell, will become 'senescent', and the overall effect is a general gradual decline in growth.

In the absence of malignant change, the growth cartilage of mouse and rat vertebrae can therefore be regarded as an organ with a limited potential for growth. Its rate of growth at any given time must depend upon a variety of interacting factors, some of which can be regarded as extrinsic in origin to the
vertebrae (e.g. endocrinological factors, temperature and blood supply), while others are intrinsic to the vertebrae (e.g. the number of cells present in the proliferative columns which are capable of mitosis and the degree of differentiation/ossification of the surrounding tissues in the vertebra). In the mouse the number of cells present in the proliferative columns of the epiphyses and the rate of mitosis in the cells remaining in these columns decrease at the same time as endochondrial ossification is proceeding in the diaphyses (Noel, 1968). Since endochondrial ossification also took place in the transplanted mouse vertebrae (possibly at a slightly accelerated rate) and in the transplanted slices of rat cartilage, the present experiments do not distinguish between the influences of ossification and factors situated solely within the epiphyseal cartilage in the control of cartilage growth.

RÉSUMÉ

Des vertèbres caudales de souris âgées de 7/8 jours ont été greffées sous les capsules surrenales d’animaux consanguins pour qu’elles se développent pendant au moins 6 mois. Leur courbe de croissance et comportement histologique de vieillissement ont été analogues à celles des vertèbres de contrôle laissées telles quelles chez de jeunes animaux.

Des prélèvements de cartilage de croissance des vertèbres caudales effectués sur des rats âgés de 7/8 jours ont été greffés de la même manière sur des rats adultes consanguins. En dépit du fait que certaines greffes n’ont pas prises, un nombre considérable a présenté les mêmes caractéristiques de croissance que celles du cartilage de l’épiphysse des vertèbres des contrôle laissées intactes.

Ces résultats ont été utilisés aux fins de prouver que chez le rat et la souris, le cartilage de croissance de l’épiphysse n’a qu’un potentiel de croissance limité. Ce mécanisme intrinsèque de limite dans la croissance semble être relativement plus important que les modifications dues à l’âge du taux d’hormones sanguines pour déterminer les modifications du rythme de croissance des vertèbres au fur et à mesure que l’animal approche de l’âge adulte.

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REFERENCES


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