The growth of transplanted mouse vertebrae: effects of transplantation under the renal capsule, and the relationship between the rate of growth of the transplant and the age of the host

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SUMMARY

The tail vertebrae of 7- to 8-day C₃H mice were transplanted under the renal capsules of syngeneic animals of various ages. Morphological effects of transplantation included degeneration of the intervertebral joints, increase in the diaphyseal haemopoietic marrow and decreased formation of bone trabeculae. The growth rate of the transplanted vertebrae depended upon the age of the host. Those transplanted into animals of the same age as the donor grew faster than controls left on the tail. Those transplanted into young adults (aged 4 months) and senile adults (aged 17 months) grew at 71 % and 64 % respectively of the rate of those transplanted into 7- to 8-day hosts. These differences are interpreted as due primarily to age dependent changes in blood hormone levels.

INTRODUCTION

This paper represents the first part of a study of the factors controlling the rate of growth and development of cartilage bones, as revealed by growing the bones in a transplanted situation. Previous workers have shown that cartilage bones may continue to develop when transplanted into abnormal situations; for example, Fell (1956) showed that chick limb-buds could develop in vitro for up to 7 days. Lacroix (1943) showed that slices of rabbit epiphyseal cartilage could grow for up to 3 weeks under the renal capsules of litter-mate animals. Using strains of inbred animals transplants can now be maintained for longer periods. In the experiments reported in this paper, the caudal vertebrae of inbred C₃H mice have been transplanted under the renal capsules of syngeneic animals, and their growth has been followed for up to 4 months. The effects of transplantation on morphology and growth of the vertebrae have been studied, with particular reference to the relationship between the rate of growth in length of the transplants and the age and rate of growth of the vertebrae of the supporting host animal.

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Table 1. *External tail lengths in C₃H mice in our inbred colony*

<table>
<thead>
<tr>
<th>Age</th>
<th>Average length ± 1 s.e. mean cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>8 days</td>
<td>2.36 ± 0.11</td>
</tr>
<tr>
<td>13 days</td>
<td>3.71 ± 0.15</td>
</tr>
<tr>
<td>21 days</td>
<td>4.47 ± 0.22</td>
</tr>
<tr>
<td>6 weeks</td>
<td>5.54 ± 0.27</td>
</tr>
<tr>
<td>10-11 weeks</td>
<td>7.50 ± 0.10</td>
</tr>
<tr>
<td>15-20 weeks</td>
<td>7.57 ± 0.14</td>
</tr>
<tr>
<td>32-36 weeks</td>
<td>7.95 ± 0.26</td>
</tr>
<tr>
<td>50-55 weeks</td>
<td>7.99 ± 0.09</td>
</tr>
</tbody>
</table>

**METHODS**

(i) *Transplantation technique*

Seven- to eight-day-old C₃H mice were used as donors. Animals of varying ages from 7 days to 17 months were used as hosts. In all experiments the numbers of each sex of donor animals were approximately equal and in experiments which included host animals of varying ages the proportion of male to female host animals was balanced in the various groups. However, the difference in growth rate and final external length of tails of male and female animals in our colony was very small (Table 1). The external length of the tail of each 7- to 8-day animal was first measured using a graduated glass tube. The distal part of the tail was then amputated by a transverse cut about 8 mm from the tip. The exact position of the cut was determined later by radiographic examination of the stump. The vertebrae of the amputated part were then freed from the skin and placed in physiological saline. At this stage each specimen contained between eight and twelve vertebrae. The most distal four to six vertebrae were then usually cut off because the number of vertebrae that could be successfully grown in one kidney was limited to about five or six in an adult kidney and four or five in a juvenile kidney.

The recipient animal was then anaesthetized, using ether in the 7- to 8-day animals, and Nembutal (60 mg per kg body weight) injected via the intraperitoneal route in all older animals. The right or left kidney was drawn or squeezed through a small incision in the dorsal skin and lumbar muscles. The vertebrae were then placed in a thin-walled 18 s.w.g. hypodermic needle and 'injected' under the capsule of the kidney with the aid of a loose fitting metal plunger. The kidney was then returned to the abdominal cavity and the incision in the skin was closed.

After varying periods of time the host animals were killed and the implanted vertebrae were dissected free from the kidney. Fig. 1 shows a kidney with transplanted vertebrae that had been growing for 3 weeks.
Control animals of the same age as the donors were left with their tails intact. At the end of their allotted experimental periods they were killed and the vertebrae dissected free from the tail.

(ii) Measurement technique

The dissected vertebrae were fixed in formol-saline, dehydrated, cleared in beechwood creosote or chloroform and mounted in Dammarxylol on glass slides. They were then examined under a low-power (×32) microscope, fitted with an eye-piece micrometer. The length of each vertebra, from the end of the articular cartilage of one epiphysis to the end of the articular cartilage of the opposite epiphysis, was then measured (see Fig. 2).

(iii) Histological preparation

After the measurements had been completed, histological sections were prepared from some of the specimens. The mounting medium was dissolved in xylol and the specimens were decalcified in Kristensen’s Fluid and embedded in paraffin wax. Sections were cut at 5 μm and stained in haematoxylin and eosin.

Fig. 1. A kidney with transplanted vertebrae that had been growing for 3 weeks.
RESULTS

(i) Morphology of transplants

Fig. 3A shows a longitudinal section through part of a vertebra 3 weeks after transplantation into a host animal aged 3 months. Compared with the control (Fig. 3B) a number of minor abnormalities can be detected. The joint between two adjacent vertebrae was poorly developed. The intervertebral disc appeared compressed and the articular surfaces of the vertebrae were almost touching in the centre. The vertebrae were linked by a large amount of fibrous tissue which caused the chain of vertebrae in the transplant as a whole to become inflexible.

The area of cartilage between the articular zone and the proliferative zone was penetrated by a larger number of blood vessels in the transplant than the control. The proliferative zone retained its basic columnar organization although the diameter of the cartilage plate was slightly smaller. The zone of hypertrophied cartilage cells became narrower.

The marrow cavity was filled with haemopoietic marrow and extended further towards the epiphysis. In the diaphysis there was less development of bone trabeculae and the cortical bone was thinner.

(ii) Growth of autotransplanted vertebrae during the first 3 weeks after operation

To investigate the early effects of transplantation upon growth, ten 7- to 8-day-old animals were used. In five of the animals, four or five of the distal caudal vertebrae were autotransplanted under the renal capsule. In the remaining
five animals the tails were left intact to serve as controls. Three weeks later the animals were killed and the vertebrae were prepared for measurement. The mean lengths of the 6th–23rd caudal vertebrae inclusive were then calculated for each group (Fig. 4). It can be seen that at 28 days of age the lengths of the 6–17th caudal vertebrae were very similar in the control and experimental animals. These vertebrae had been growing in their natural positions in both groups. The fact that their average length was about 4% longer in the experimental animals than the controls reflected a slightly higher average tail length in the experimental group at the beginning of the experiment. The 18th caudal vertebra was usually crossed by the line of amputation in the experimental animals; it showed an extremely variable amount of growth which has not been presented in the results. Vertebrae 19–22 grown under the renal capsule showed an average length about 28% greater than that of the corresponding control vertebrae. Thus transplantation under the renal capsule has led to an overall increase in growth rate during the first three weeks after operation.

Fig. 3. Longitudinal section of the 19th caudal vertebra at 28 days. (A) An experimental vertebra transplanted at 7 days into a 3-month-old host. (B) A control vertebra left on the tail (×115).
Fig. 4. To compare the mean (±1 s.e. mean) lengths of the 6th–23rd caudal vertebrae at 28 days in five control animals and five experimental animals. The distal caudal vertebrae of the experimental animals had been growing as autografts under the renal capsules. ○—○, Vertebrae of control animals. ★—★, transplanted vertebrae of experimental animals. •—•, non-transplanted vertebrae of experimental animals. +, based on 4 observations only, due to line of amputation crossing 17th vertebra in one specimen.

(iii) Long-term growth of autotransplanted vertebrae

The next experiment was designed to see whether this accelerated growth rate of autotransplanted vertebrae was a short-term or long-term effect, and whether the final length of the vertebrae at the end of their period of active growth would be any greater than that of controls. A number of 7- to 8-day animals was therefore matched in control and experimental pairs, with reference to tail length and body weight. In experimental animals the tail was amputated at the level of the 18th–20th caudal vertebrae and the following four or five vertebrae were autotransplanted under the renal capsule. In the control animals the tail was left intact as before. The animals were killed after varying periods ranging from 1 to 16 weeks and the caudal vertebrae were prepared for measurement.

The results presented in Fig. 5 are based on the length of the 21st caudal vertebra in each specimen. The mean length of the 21st caudal vertebra in each age group of the experiment was calculated and plotted against the age of the vertebra at the time of dissection. Each point on the graph represents the mean (plus or minus 1 standard error of the mean) of four to seven observations. Approximate curves of growth for the control and experimental vertebrae were
Transplanted mouse vertebrae

Fig. 5. To compare the mean (± 1 s.e. mean) length of the 21st caudal vertebra at various ages in experimental specimens, autografted under the renal capsule at 7–8 days, and control specimens left to grow on the tail. •—•, Transplanted vertebrae; ○—○, control vertebrae.

then drawn in by eye. It can be seen that the accelerated growth rate of autotransplanted vertebrae was not a short-term effect but continued throughout the period of rapid growth. Only during the first week after operation did the transplants show a lower growth rate than the controls. This was presumably because they were ‘adjusting’ to their new environment and a blood supply was being established. In this group the difference in length between control and transplanted vertebrae was not statistically significant. In the remaining six experimental groups, the mean length of the transplants was considerably greater than that of the controls, and, with the exception of the group dissected at 12 weeks of age, the difference was statistically significant using a Wilcoxon matched pairs analysis ($P < 0.05$). The final mean length of the transplanted vertebrae at 17 weeks of age was about 20% greater than that of the controls.

(iv) Growth rate of vertebrae transplanted into host animals of varying ages

Since it appeared that vertebrae transplanted under the renal capsule of 7-day animals grew faster than controls, it was decided to investigate the growth rate of vertebrae transplanted into older animals. Seven-day vertebrae were therefore
grafted under the renal capsules of host animals of varying ages from 7 days to 17 months. They were left to grow for 3 weeks and then prepared for measurement. The results have been expressed as average growth rate per week during this 3-week period of the 19th plus 20th caudal vertebrae in each specimen. This was calculated as follows:

\[
growth \ rate \ per \ week = \frac{(a_2 + b_2) - (a_I + b_I)}{3},
\]

where \(a_2, b_2\) are observed lengths of the 19th and 20th caudal vertebrae in one specimen at the end of the experiment; \(a_I, b_I\) are estimated lengths of the 19th and 20th caudal vertebrae in the same specimen at the start of the experiment. The latter could not be measured accurately by a direct method, because the exact limit of each vertebra could not be clearly seen in living specimens at 7 days or in radiographs taken at that age. An indirect measure was therefore used. A graph had previously been constructed to correlate the external tail length of 30 7- to 8-day animals with cleared and mounted specimens of their 19th and 20th caudal vertebrae (Fig. 6). In the present experiment the external length of the tail of each 7- to 8-day animal had been measured before amputation and the length of the 19th plus 20th caudal vertebrae at this stage was estimated from the graph. In each experimental group the mean growth rate per week of the 19th plus 20th caudal vertebrae was finally calculated (see Table 2). The mean values were then plotted on a graph against the age of the host animals at a time half-way between receiving the graft and end of the experiment 3 weeks later. A negative exponential curve was fitted to these points (solid line,
Table 2. The mean growth rate per week (mm ± 1 S.E. mean) of the 19th plus 20th caudal vertebrae in transplants growing in hosts of varying ages

<table>
<thead>
<tr>
<th>Age of host at start of experiment (weeks)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1.14 ± 0.03 (4 M, 1 F)</td>
<td>1.12 ± 0.11 (2 M, 3 F)</td>
</tr>
<tr>
<td>4.5</td>
<td>0.98 ± 0.03 (6 M)</td>
<td>1.00 ± 0.04 (9 M)</td>
<td>1.01 ± 0.03 (4 M, 1 F)</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>0.78 ± 0.02 (6 M)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>0.83 ± 0.04 (5 M)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>0.80 ± 0.03 (4 M)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>39</td>
<td>0.74 ± 0.02 (6 M)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>51</td>
<td>0.72 ± 0.06 (7 M)</td>
<td>0.69 ± 0.02 (9 M)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>74</td>
<td>0.72 ± 0.03 (7 M)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Controls left on tails of 7-day mice</td>
<td>0.78 ± 0.07 (5)</td>
<td>—</td>
<td>—</td>
<td>0.72 ± 0.02 (5)</td>
</tr>
</tbody>
</table>

The figures in parentheses represent the number of specimens in each group and the sex of the host animals. Columns A, B, C and D represent tests carried out over different periods and employing differing methods of distribution of the donor animals.

(A) Tail vertebrae of donors of each litter distributed among hosts of as many different ages as possible or one animal kept as a control.

(B) Donors matched in pairs. Vertebrae of one member of each pair grafted into each group of hosts. Difference between two groups significant (P < 0.01).

(C) Donors matched in pairs. Vertebrae of one member of each pair grafted into each group of hosts. Seven-day hosts received grafts from donors derived from different litters. Difference between 2 groups significant (P < 0.05).

(D) Tail vertebrae of half the members of each litter transplanted as autografts. Difference between 2 groups significant (P < 0.05).

Fig. 7). It can be seen that the growth rate was highest in vertebrae transplanted into host animals aged 7–8 days at the time of operation. These vertebrae were in fact growing about 50% faster than controls left on the tails of 7- to 8-day animals (open hexagons in Fig. 7). The growth of the transplanted vertebrae decreased rapidly with increasing the age of the host up to 2 months, so that vertebrae growing in hosts aged 2–4 months were growing at a similar rate to those growing on the tails of 7- to 8-day animals. Increasing the age of the host animal beyond 3 months gave relatively little further change in the growth rate of the transplanted vertebrae.
The curve drawn as a dotted line in Fig. 7 represents the weekly growth rate of the 19th plus 20th caudal vertebrae in their natural locations at various ages. It was derived from a previous study in which the growth of the tail vertebrae of six normal animals from the same colony had been followed by measurements from radiographs taken at regular intervals (Noel, 1968). It shows that the growth rate of vertebrae in their natural location was highest at between 1 and 2 weeks of age, then fell rapidly with increasing age up to 2 months, after which it gradually approached zero. In the preceding transplantation experiment it may be assumed that the rate of growth of the host animals' own tail vertebrae was following approximately this curve. Thus it appears that at the time when the host animals were themselves growing rapidly they supported rapid growth in the transplanted vertebrae. As they approached 2 months of age, the growth rate of their own vertebrae dwindled and they also supported a lower rate of growth in the transplanted vertebrae. After 4 months of age, there was only a
Transplanted mouse vertebrae

very gradual rate of change in the growth of their own vertebrae, and similarly there was little change in the rate of growth of transplanted vertebrae with increasing the age of the host beyond four months.

However, it is important to note that while the growth rate of the 19th plus 20th caudal vertebrae in their natural locations at 4 months of age had fallen to about 0.04 mm per week, 7-day vertebrae transplanted into host animals aged 4 months maintained an average growth rate of 0.80 mm per week during the first 3 weeks after operation. Similarly, whereas the rate of growth in length of the 19th plus 20th caudal vertebrae in their natural locations at 12 months of age was near to zero, 7-day vertebrae transplanted into animals of this age still maintained an average growth rate of 0.72 mm per week during the first 3 weeks after operation.

DISCUSSION

(i) Morphological changes in transplanted vertebrae

The morphological changes in the transplanted vertebrae can possibly be explained partly by the differences in biophysical forces acting on the control and transplanted specimens. Fell (1956) recorded a similar malformation of the joint in foetal chick bones grown in organ culture. It was suggested that the development of a normal joint depended upon the normal pull of muscles in movement. In the present experiment a normal differentiated joint was present at the time of operation, but later degenerated in the absence of normal movement.

A second factor, which may be of considerable importance, is the difference in temperature between the kidney and the distal region of the tail. Although the temperature inside the tail vertebrae or inside the kidney could not easily be measured, the temperature of the skin overlying these organs was recorded, using an ‘Ellab TE 3’ electric thermometer, with a skin applicator. In mice reared at 22 °C the mean skin temperature was 36.8 °C under the fur in the pelvic region and 26 and 24.8 °C in the middle and distal regions of the tail respectively, which were only partly covered by hair. When a group of mice was reared in a hot environment (30 °C), the skin temperature in the distal tail was raised to 29.0 °C and histological examination of the distal vertebrae showed an increase in the haemopoietic marrow and reduction in the zone of hypertrophic cartilage cells similar to those observed in transplanted vertebrae (Noel, 1968). Similar findings have been noted in rats reared in a hot environment, and in rats whose distal tail vertebrae were transplanted into the peritoneal cavity (Huggins & Blocksom, 1936; Huggins, Blocksom & Noonan, 1936). In the present experiments the extension of haemopoietic marrow in vertebrae transplanted under the renal capsule and the associated reduction in the zone of hypertrophied cartilage cells, may thus have been due to the local rise in temperature.
(ii) Growth of autotransplanted vertebrae

The increased growth of vertebrae grown as autotransplants under the renal capsule may similarly be explained by the higher temperature in the kidney than the tail. It has previously been shown that when mice are reared in a hot environment there is an increase in the growth of the distal caudal vertebrae (Noel & Wright, 1970). Secondly, it is possible that the transplanted vertebrae received a greater supply of blood than the control vertebrae. Thirdly, it should be remembered that the diameter of the cartilage plate was narrower in the transplants than the control vertebrae, and it is possible that some cells which would normally have contributed to increasing the width of the vertebra, in some way contributed to increasing the length instead.

(iii) Relationship between growth rate of transplants and age of host

The observation that vertebrae transplanted into young host animals grew faster than those transplanted into older host animals can most easily be explained by assuming that there were differences in the endocrinological composition of the blood at various ages. The simplest interpretation would seem to be that there were growth promoting agents present in relatively high concentrations in the blood of the youngest host animals, which stimulated growth both in the animals' own vertebrae and in the transplanted vertebrae. This growth-promoting activity seems to have diminished by about 2 months of age. Sexual maturity in these mice was attained at 6–8 weeks of age.

Various products of the endocrine system are thought to have growth-promoting properties and the growth of epiphyseal cartilage is known to be particularly sensitive to anterior pituitary growth hormone (Greenspan, Li, Simpson & Evans, 1949). There is some evidence that, in man, the blood level of anterior pituitary growth hormone is much higher in children than adults (Greenwood, Hunter & Marrian, 1964). However, many other substances may have been involved in maintaining the high growth rate of vertebrae transplanted into young host animals.

It is also possible that the blood of the older host animals contained growth inhibiting substances. Silberberg & Silberberg (1956) showed that high concentrations of oestrogen and testosterone were inhibitory to cartilage growth although certain lower concentrations of testosterone accelerated growth. The result of the present experiment could therefore perhaps be explained partly by a high blood level of anterior pituitary growth hormone in young mice and partly by interaction of various other substances, whose concentration in the blood changes with increasing age. These might include the levels of the sex hormones, thyroid and parathyroid hormones, sugar and amino acids, and calcium and phosphates present in the blood.

Whatever may be the nature of the growth promoting or growth inhibiting agents circulating in the blood, these may not be considered the sole, or even
the major factor responsible for the changing growth rate of the epiphyseal cartilage as normal animals approach the age of adulthood. When 7- to 8-day vertebrae were transplanted into host animals aged 18 months and allowed to grow for 3 weeks, their average growth rate was 64% of that of vertebrae transplanted into host animals aged 7–8 days. Since these two groups of host animals were 17-3 months and 2-5 weeks of age respectively at a time half-way between operation and dissection, one may tentatively suggest that changes in the blood composition could account for a 36% fall in the growth rate of vertebrae between the ages of 2-5 weeks and 17-3 months. However, the growth rate of tail vertebrae in their natural locations at 17-3 months of age is only a negligible fraction of that at 2-5 weeks of age. The 17-month animals were, in fact, nearing the end of their life-span. Thus various factors other than endocrinological changes must be responsible for reducing the growth rate of the epiphyseal cartilage in adult mice.

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REFERENCES


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