THE GROWTH OF TRANSPLANTED FOETAL BONES IN DIFFERENT IMMUNOLOGICAL ENVIRONMENTS*

J. CHALMERS†, EDINBURGH, SCOTLAND, and R. D. RAY, CHICAGO, UNITED STATES OF AMERICA

*From the Department of Orthopaedics, Presbyterian-St Luke's Hospital, and
the University of Illinois Research and Education Hospitals, Chicago, Illinois*

Substantial growth of immature bone homografts in various sites has been observed by many workers including Leopold (1881), Fischer (1882), Helferich (1899), Willis (1936, 1939), Huggins and Blocksom (1936), Hancox (1947); Ray, Degge, Gloyd and Mooney (1952); Felts (1955, 1957*a* and *b*). Such growth is contrary to the behaviour of mature bone homografts which are capable of only very limited growth, restricted to the first few days after implantation (Chalmers 1959). If immaturity allows a greater chance of bone homograft survival such bone might have greater value in clinical use than the homograft preparations currently in use.

A closer examination of the work of the above authors suggests that the difference may be more apparent than real, and might in some instances be explained by the use of particular host sites, or by the use of partly inbred laboratory animals. For example, Willis (1936) used brain as the host site and it is now known that homografts of adult tissues transplanted to the brain may enjoy prolonged survival (Medawar 1948). Willis (1939) had less success with the use of a subcutaneous site. Leopold (1881) and Ray *et al.* (1952) also made use of a privileged site, the anterior chamber of the eye, in which prolonged homograft survival may take place (Woodruff and Woodruff 1950). Felts (1955) reported the growth of bone homografts exchanged between five-day-old litter mate rats, but in further studies in which he made use of pure strains of mice, he found that homografts of bones from two-day-old mice grew only to a limited extent. Huggins and Blocksom (1936) had a variable experience with homografts of tail bones from newborn rats transplanted intraperitoneally, some growing well whereas others showed limited cartilage growth only.

In view of the general uncertainty in this field a further investigation appeared desirable, and the following experiments were planned to show the influence of a range of different immunological environments on a foetal bone.

METHOD AND MATERIALS

To overcome the technical difficulties of a foetal autograft, pure strains of mice were used. Grafts exchanged between individuals of a pure strain behave as autografts and are known as isografts.

The grafts used in all experiments were femurs taken from eighteen-day-old foetal Strong A mice. These were transplanted into the spleens of hosts of both sexes aged six to seven weeks at the time of grafting as detailed in Table I.

Each series of experiments was arranged so that the progress of a pair of femora from one foetus could be observed in differing hosts:

- Series I Compared isograft and homograft.
- Series II Compared first and second set homograft.
- Series III Compared homograft and heterograft.

A total of 180 transplants was done.

In Series I a pair of animals was killed at intervals from two days to three months, but in Series II and III the greatest duration was eight weeks because it was found that few changes

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† Holder of British Postgraduate Medical Federation Travelling Fellowship.

occurred in the grafts after that time. The transplants were dissected free from soft tissues and were weighed, photographed and radiographed under standard conditions so that measurements of total length and length of diaphysis could be made. Each graft was examined histologically.

TABLE I
DETAILS OF HOSTS AND GRAFTS

Host	Status of graft
Strong A mice	Isograft
C ₃ H mice	Homograft
C ₃ H mice previously immunised against Strong A tissues	Second set homograft
Long Evans rats	Heterograft

TECHNICAL DETAILS

The graft—The femora were dissected from eighteen-day-old foetal Strong A mice with the aid of a dissecting microscope. They were kept moist with sterile saline during the dissection and were stored in saline at 37 degrees Fahrenheit until all the femora from the litter had been obtained, a process usually taking about two hours. The transplantations were carried out immediately thereafter.

The eighteen-day-old foetal femur is largely cartilaginous with a small diaphysis of true bone (Fig. 1). The total length and the length of bony diaphysis were measured by means of an ocular micrometer, and the weight of the femur was recorded. The values obtained from 180 femora were: total length 4.1 (± 0.2) millimetres; weight 2.8 (± 0.7) milligrams.

Operative procedure—Under intraperitoneal Nembutal anaesthesia a small incision was made in the left flank of the host mouse and the spleen exteriorised. A stab wound was made in the spleen and the foetal femur inserted with its long axis parallel to the long axis of the spleen. The abdominal wall and skin were closed with a single suture. Excessive bleeding was not encountered.

Method of immunisation—The technique used for the initial immunisation in Series II was based on that described by Billingham, Brent and Mitchison (1957). Spleens, removed from two adult Strong A mice, were cut into small pieces and placed in a thimble of 50 mesh tantalum gauze. Gentle pressure with a glass rod was used to work the tissue through the mesh into 10 millilitres of saline. Clumps of cells were allowed to settle and a cell count was made of the supernatant suspension (usually about 30×10^6 nucleated cells per millilitre). Half of the C₃H host mice in the series were then given an intraperitoneal injection of about six million cells. The foetal bone homograft was inserted three weeks later.

Histology—The grafts were fixed and decalcified in Bouin's solution, embedded in celloidin, sectioned on their long axes and stained with haematoxylin and eosin.

The data for the growth of the normal femur were obtained by killing a pair of mice, usually one male and one female, at each of the time intervals indicated in Table I. Measurements of total length, diaphysial bone length and weight of the femur were made by the techniques described previously.

RESULTS

Series I. Comparison of eighteen-day foetal femur isograft and homograft—There was very little interruption in growth in length and weight of both isograft and homograft after transplantation (Table II and Figs. 23 and 24). There was, however, a delay of two days before the diaphysis began to increase in size. Thereafter growth proceeded in an orderly manner and both isograft and homograft developed identically until the sixth day (Fig. 2). Histological preparations showed growth of bone and cartilage in both groups, with normal endochondral ossification (Figs. 9 and 10).

After the sixth day the progress of the two grafts differed (Figs. 3 to 19). The isograft continued to grow uninterruptedly, secondary centres of ossification appeared in the correct sequence, although a day or two earlier than in the normal femur (Figs. 3 to 6). The greatest growth in length of the isograft at about eight weeks was 80 per cent of that of the normal

TABLE II
SUMMARY OF BONE MEASUREMENTS

	Days																Weeks											
	Age of normal femur -2 0 2 4 6 8 10 12 14 16 18 20																4 5 6 7 8 9 10 11 12											
Equivalent graft age	 0 2 4 6 8 10 12 14 16 18 20 —																4 5 6 7 8 9 10 11 12											
	Normal 4.1 4.8 5.3 6.0 6.7 7.3 8.1 8.7 9.1 9.5 9.9 10.1 11.3 11.9 12.1 13.2 14.1 14.0 14.2 14.4																11.3 11.9 12.1 13.2 14.1 14.0 14.2 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4											
Total length in millimetres	Isograft — 4.4 5.3 5.5 5.6 6.7 7.5 7.5 8.0 8.8 8.9 — 9.3 9.7 10.7 9.2 10.6 11.5 9.5 12.0 11.3																11.3 11.9 12.1 13.2 14.1 14.0 14.2 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4 14.4											
	Homograft. — 4.4 5.2 5.3 5.7 6.6 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0 7.0																Too distorted to measure											
	Homograft in pre-immunised host — 4.5 5.0 5.4 5.9 6.5 6.5 5.9 6.5 6.5 6.5 6.5 6.5 6.5 6.5 6.5 6.5 6.5 6.5 6.5 6.5																Too distorted to measure											
	Heterograft — 4.2 4.5 5.5 — 4.9 5.1 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0																5.5 5.1 5.5 — 5.6 —											

Mean values only are recorded whenever two or more pairs of hosts of the same series were killed at the same time interval.

femur (Table I and Figs. 21 and 22). This limitation of growth was not related to the age of the host for several pairs of femora were implanted isogenously into six-weeks- and eight-months-old mice, and no difference was observed in the length of the grafts. The articular cartilage of the isograft was thinner and more irregular than that of the normal femur.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

Figure 1—Femur of an eighteen-day-old mouse foetus. ($\times 8$.) Figure 2—Isograft (*on the left*) and homograft (*on the right*) at six days. ($\times 8$.) Up to six days there is a general similarity between isograft and homograft both in total length and length of the diaphysis. Figure 3—Isograft (*on the left*) and homograft (*on the right*) at eight days. ($\times 8$.) Figure 4—Isograft (*on the left*) and homograft (*on the right*) at twelve days. ($\times 8$.) While ossification has proceeded normally in the isograft, the diaphysis of the homograft has ceased to grow and has become paler in colour. Cartilage growth has continued in the homograft to the same extent as in the isograft, although fractures and distortions may cause a difference in length. The increasing opacity in the cartilage in the homograft is due to calcification.

FIG. 5

FIG. 6

Figure 5—Isograft (*on the left*) and homograft (*on the right*) at fourteen days. ($\times 6.5$.) Figure 6—Isograft (*on the left*) and homograft (*on the right*) at twenty days. ($\times 6.5$.) In these radiographs the trabecular arrangement of the bone distinguishes it from the calcified cartilage. In the homograft the length of the column of bone in the diaphysis remains approximately the same whereas in the isograft it increases steadily. Note the progressive reduction in the width of the epiphysal cartilage as the rate of growth diminishes.

After six days the homograft showed no further growth of bone, and the bone which had developed up to that time largely died, with only a few osteocytes persisting (Figs. 12 and 19). The epiphysal cartilage plate differentiated normally, but the hypertrophic, calcified cartilage, which accumulated in large amounts both in the metaphysal region and in the epiphyses, was unreplaced by bone. This hypertrophic cartilage was so fragile that

fractures frequently occurred, resulting in distortions which made measurements of length impossible.

The duration of growth of homograft cartilage corresponded closely to that of the isograft as judged by weight measurement and by the microscopic appearance of the epiphyseal cartilage plate. In both groups the plate became much narrower after the eighth week, corresponding to the reduction in the rate of growth (Figs. 15 and 16).

The hypertrophic cartilage of the homograft was invaded increasingly by blood vessels from the periphery after the third week, causing irregular areas of resorption in which an accumulation of inflammatory cells occurred. These areas were examined carefully for evidence of new bone formation, but this was found in only one graft at twelve weeks in which some new bone had developed in the distal epiphysis (Fig. 20).

FIG. 7

FIG. 8

Figure 7—Posterior view of homograft (*on the left*) and isograft (*on the right*) at eleven weeks. ($\times 8$.)
Figure 8—Radiograph of the homograft and isograft pair shown in Figure 7. ($\times 8$.) Note that the homograft has retained a well marked lesser trochanter and intertrochanteric ridge, but that these features are missing in the isograft.

The medullary space of the homograft became occupied with fibrous tissue and infiltrated with inflammatory cells, chiefly plasma cells and lymphocytes, contrasting with the haemopoietic marrow of the isograft (Figs. 18 and 19).

A most interesting difference was observed in the macroscopic appearance of the isograft and homograft. In both, the growing cartilage began to lay down in some detail the secondary characteristics of a femur such as the lesser trochanter and intertrochanteric ridge, but in the isograft these secondary characteristics became submerged and disappeared as the cartilage was replaced by bone. In the homograft, on the other hand, these secondary characteristics persisted throughout the period of growth. Tubulation or remodelling of the "metaphysis" did not occur in the homograft (Figs. 7 and 8).

Series II. Comparison of homografts in normal and pre-immunised hosts—In the hosts which had been immunised against Strong A tissues three weeks before the foetal bone homograft the grafts showed one clear and consistent difference from similar homografts in the untreated

FIG. 9

FIG. 10

Figure 9—Isograft at six days, showing normal endochondral ossification proceeding at the junction of cartilage and bone. (Haematoxylin and eosin, $\times 105$.) Figure 10—Homograft at six days, showing a corresponding field to Figure 9. Active endochondral ossification is proceeding, and there is a close resemblance to the isograft. (Haematoxylin and eosin, $\times 105$.)

FIG. 11

FIG. 12

Figure 11—Isograft at ten days, showing the normal appearance of endochondral ossification at the junction of cartilage and bone. (Haematoxylin and eosin, $\times 105$.) Figure 12—Homograft at ten days, showing a similar field to Figure 11, but no osteogenesis has occurred and the metaphyseal zone appears avascular. (Haematoxylin and eosin, $\times 105$.)

FIG. 13

FIG. 14

Figure 13—Isograft at five weeks showing the normal appearance of the epiphyseal cartilage plate. The epiphyseal centre of ossification is now fully developed. (Haematoxylin and eosin, $\times 105$.) Figure 14—Homograft at four weeks. This is a similar field to Figure 13, and there is a general correspondence in the appearance of the growing cartilage, but no replacement of the hypertrophic cartilage by bone. (Haematoxylin and eosin, $\times 105$.)

FIG. 15

FIG. 16

Figure 15—Isograft at nine weeks, showing that the growth plate persists but is becoming narrower, and endochondral replacement by bone is less obvious, indicating a slowing down of growth. (Haematoxylin and eosin, $\times 105$.) Figure 16—Homograft at ten weeks. This field of the growth plate likewise shows less activity and less clear differentiation than hitherto. (Haematoxylin and eosin, $\times 105$.)

FIG. 17

Homograft at four weeks. This is a low power view of the upper end of the femur. The general shape is well maintained and the epiphysal cartilage plates of the head and greater trochanter are normally sited. There is a vast accumulation of mature cartilage and complete absence of bone. (Haematoxylin and eosin, $\times 25$.)

FIG. 18

Isograft at ten days, showing living bone and haemopoietic marrow in part of the diaphysis. (Haematoxylin and eosin, $\times 135$.)

FIG. 19

Homograft at ten days, also showing part of the diaphysis in which the bone is mostly acellular with no new bone formation. The marrow space is infiltrated with inflammatory cells. (Haematoxylin and eosin, $\times 135$.)

FIG. 20

Homograft at twelve weeks, showing some living bone within the epiphysis of a homograft. It is surrounded by a highly vascular connective tissue but no haemopoiesis is in evidence. This is the only example of bone formation seen after the sixth day in the homograft femur. (Haematoxylin and eosin, $\times 135$.)

hosts. The diaphysial bone did not grow at all after implantation whereas growth during the first six days was seen in the normal homograft (Table I and Figs. 25 to 27, 32 and 33). Growth and organisation of the cartilage was similar to that of a normal homograft.

Unfortunately, the histological preparations from this group were unsatisfactory, but the material that was available failed to show any evidence of new bone formation in the grafts to pre-immunised hosts—even during the initial six days.

FIG. 21

Increase in length of normal and isograft femora.

FIG. 22

Increase in weight of normal and isograft femora.

Series III. Comparison of homograft and heterograft—The heterograft differed from the homograft in the following respects. The diaphysial bone did not increase in size after implantation (Figs. 32 and 33). The total length of the graft increased during the first six days by 1.5 millimetres at the most and thereafter grew no more; the weight increased during the

same period by an average of 2 milligrams and subsequently showed no change (Table 1). Calcification of the epiphyses occurred at inconstant and variable times and in irregular patterns. After six days no changes took place in the macroscopic appearance of the heterografts, so that at two months they remained miniature but well proportioned femora (Figs. 28 to 30). A dense fibrous capsule developed around the heterograft, which although not adherent to the graft, was so tough that it was extremely difficult to dissect without damaging the bone.

Histologically no evidence of surviving bone cells was found in the heterografts after transplantation. Much of the cartilage, particularly towards the centre of the mass, lost its cellular character and took on a confluent amorphous appearance which failed to stain with haematoxylin and eosin. However, some of the cells, particularly at the periphery, retained their normal morphology for at least six weeks, and in the early period after transplantation an attempt at differentiation of an epiphyseal cartilage plate occurred (Fig. 31). The marrow space became the site of an intense inflammatory reaction after fourteen days—up to that time it appeared avascular and largely acellular.

Figures 32 and 33 summarise some of the characteristic features of the eighteen-day-old foetal femoral grafts in the four different series.

FIG. 23
Increase in length of isograft and homograft femora.

DISCUSSION

In each of the experiments described above the foetal femur was observed to grow to a greater or lesser degree. However, the foetal femur is comprised of two principal tissues—cartilage and bone—which individually showed great differences in behaviour.

FIG. 24
Increase in weight of isograft and homograft femora.

If each of these tissues is considered separately a clearer understanding of the different behaviour of the grafts is possible.

The bone—In the isografts the diaphysial bone growth continued almost without interruption and replaced the maturing cartilage by the normal process of endochondral ossification until

FIG. 25

FIG. 26

FIG. 27

Figure 25—Homografts to normal (*on the left*) and pre-immunised hosts (*on the right*) at four days. ($\times 8$.) Figure 26—Homografts to normal (*on the left*) and pre-immunised hosts (*on the right*) at five weeks. ($\times 8$.) These photographs show the similarity in total growth of the femur homografts in normal and pre-immunised hosts (Series 2). The only difference between these grafts lay in the bony diaphysis, which in the pre-immunised hosts did not increase in size after transplantation, while in the unprepared hosts the diaphysis increased in size during the first six days. The disparity between the diaphysial total lengths and thicknesses is well shown in these photographs. Figure 27—A radiograph of homografts to normal (*on the left*) and pre-immunised hosts (*on the right*) at two weeks ($\times 6.5$) which shows very clearly the difference in diaphysial size which was the striking feature of Series 2. The length of the diaphysis in the homograft to the pre-immunised host has not increased following transplantation, while the cartilage growth in the two grafts is comparable.

FIG. 28

FIG. 29

FIG. 30

Figure 28—Homograft (*on the left*) and heterograft (*on the right*) at two days. ($\times 8$.) Figure 29—Homograft (*on the left*) and heterograft (*on the right*) at eight weeks. ($\times 8$.) In these homograft and heterograft pairs from Series 3 the homograft is shown to increase steadily in size, whereas the heterograft remains almost unaltered in size following transplantation. Figure 30—Homograft (*on the left*) and heterograft (*on the right*) at eight weeks. ($\times 6.5$.) This radiograph shows the unchanged appearance of the heterograft. The diaphysis has not altered in size since transplantation.

growth of the transplant ceased. In the homografts to normal hosts the bone grew for a period of only six days and thereafter most of it died, while in the homografts to pre-immunised hosts no bone growth was observed.

These findings agree so closely with the behaviour of the first and second set homografts of mature tissues in general and of mature bone in particular (Chalmers 1959) that little doubt is left that, from an immunological standpoint, there is no difference in behaviour between eighteen-day-old foetal bone and adult bone homografts. There is no reason, therefore, to suppose that bone from an immature source will have any advantages over mature bone homografts in clinical use.

The failure of bone survival in the foetal heterografts is entirely in accordance with the fate of heterografts in general.

The cartilage—The isograft cartilage grew and differentiated in a manner which differed from that of normal bone only in that the articular cartilage was thinner and more irregular.

The homograft cartilage also grew and differentiated in a normal fashion and was responsible for the increase in size of the femoral homograft. This growth appeared uninfluenced by the development of immunity, except in so far as the death of the true bone and reduction of circulation prevented the replacement of hypertrophic cartilage by bone, so that large masses of hypertrophic and calcified cartilage accumulated. This continued growth of the foetal homograft cartilage does not, however, indicate an immunological difference between foetal and adult cartilage because cartilage homografts in experimental animals and in man have been shown to survive and even grow when taken from more mature donors (Nunnemacher 1939, Lacroix 1951, Peer 1954, Bacsich and Wyburn 1947, 1955). This property of cartilage stems from the fact that the cells of cartilage are surrounded by a continuous matrix which prevents their contact with host tissue cells. Physical contact between host and donor cells is necessary for the fulfilment of the homo-immune response.

The heterograft cartilage grew to a very limited extent and survived only in part. This agrees with the experience of others. Cartilage heterografts appear to be unique amongst tissues in showing even this limited capacity for survival (Loeb and Harter 1926).

The organisation of bone growth—The different behaviour of cartilage and bone growth in these experiments has afforded an excellent opportunity of observing the relative importance of these two tissues in influencing the form and size of the developing femur. It has been known since the classic studies of Fell and her colleagues (Fell and Robison 1929, Fell and Canti 1934, Fell 1939) that the cartilage precursor of bone has the ability to develop recognisable characteristics of a particular bone even in the tissue culture chamber.

In the homograft transplants described above in which the cartilage growth continued but not that of bone, it was apparent that not only was there an inherent growth pattern in immature cartilage, which could produce the characteristic features of a particular bone in great detail, but that the duration of growth and ultimate size of the bone were also governed to a great extent by intrinsic factors within the young cartilage.

FIG. 31
Heterograft at seven days. There has been a partial differentiation of the epiphysal cartilage plate at the periphery of the femur, while the central part of the cartilage mass has lost its staining character and the chondrocytes in this area are shrunken. (Haematoxylin and eosin, $\times 105$.)

FIG. 32

Increase in diaphysal length of the foetal femur in the different circumstances described in the text.

FIG. 33

Drawing to scale illustrating some of the differences which were observed in growth and development of the foetal femur in the various experimental situations. The shaded areas represent bone.

In the isograft in which the normal sequence of endochondral ossification took place, the cartilage continued to lay down the characteristics of a femur and to regulate its final size but, as bone replaced the cartilage, many of the secondary characteristics of the femur became submerged in the course of the remodelling which took place at the metaphysis.

These results indicate the importance of the relative roles of bone and cartilage in shaping the skeleton. Immature cartilage is endowed with a potential for growing into a specific shape of finite size independent of its normal anatomical relationships and even of its normal endocrinological environment. Bone on the other hand appears to be more dependent on extrinsic influences for the maintenance of form. If guided by a cartilage model to assume a specific form, it quickly loses many of its secondary prominences to become a slender tubular structure in the absence of muscular attachments.

Induction of bone by cartilage—Transplants of growth cartilage have been found by other workers to be potent inducers of bone formation in surrounding soft tissues (Lacroix 1956, Bridges and Pritchard 1958).

It was expected that evidence of such induced bone might be found in or around the cartilage of the homograft transplants, but, although a particular search was made for this, in only one graft at twelve weeks was living bone found which might be accounted for in this way. Huggins, McCarroll and Blocksom (1936) found the spleen to be a site in which bone induction by bladder mucosa transplants did not occur unless fascia was transplanted in conjunction. Perhaps the use of a site other than the spleen might have favoured induction in these experiments.

SUMMARY

1. Homografts of eighteen-day-old foetal femora in pure strains of mice showed no fundamental difference in behaviour from grafts of more mature bone and cartilage.
2. Growth of bone was limited to a short period after transplantation and was abolished by previous immunisation. Cartilage growth alone was responsible for the increase in size of these transplants and did not appear to be influenced by the presence of immunity.
3. There is no reason to suppose that bone from an immature source is likely to behave more favourably than more mature bone homografts in clinical use.
4. The limited growth of cartilage and the total failure of bone survival in the heterografts indicate an immune reaction of a different order from that which develops against the homograft.
5. The different effect of the homograft immune reaction on cartilage and bone enabled certain conclusions to be drawn concerning the part played by these two tissues in determining the form of a bone. Cartilage growth and development is shown to be regulated in large part by intrinsic factors. Bone growth and form on the other hand is shown to be dependent largely upon extrinsic influences.

The work reported in this paper formed part of a thesis by one of the authors (J. C.), which was accepted by the University of Edinburgh for the degree of M.D.

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