OBSERVATIONS ON BONE TRANSPLANTS IN THE ANTERIOR CHAMBER OF THE EYE.

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Bone transplantation has been extensively performed for many years, and the results achieved have fully justified the procedure despite a lack of unanimity regarding many of the problems involved. It has been a matter of conjecture as to what exactly happens to the transplanted bone tissue. Does it continue to live, or does it become a dead framework? If new cells grow into a transplant, are these osteoblasts from existing bone or metaplastic cells from surrounding connective tissue? What is the comparative value of cortical and cancellous bone? Is periosteum a necessary factor? Does one large graft succeed as well as many small pieces and what is the comparative success of auto- and homo-transplants? These and many other problems have occupied the attention of a large field of workers and in order to study them further, it was decided to embark on an extensive series of animal experiments in which bone tissue was transplanted to various sites and its fate studied. The present contribution, which is only a small part of the larger work, describes a series of experiments in which bone transplants were introduced into the anterior chamber of the rabbit eye.

The technique of intra-ocular implantation was described in detail by Schochet in 1920 but writings, describing the use of the method appeared much earlier. As long ago as 1877 Cohnheim appears to have originated the method and it was subsequently described by Salomonsen (1879), Baumgarten (1880), and Klebs (1883).

The anterior chamber is for several reasons a suitable site for the growth of transplanted tissue. The implantation can be effected with ease, there is little or no disturbance to the animal and the tissue which is visible through the cornea, grows under optimum conditions of body temperature and nutrition. According to Greene (1940), the aqueous humour is not an isolated fluid, there is no blood aqueous barrier separating the anterior chamber from the rest of the body, and it participates in all the general body reactions. Bone transplanted to the anterior chamber is free from the influence of other bony tissue, and therefore living cells found in such a transplant are either survivors of the original transplanted bone, or tissue cells which have undergone metaplasia in response to some stimulus to bone formation. It is, in fact, an ideal and safe method of tissue culture.

METHOD.

The rabbits used were a mixed stock of animals obtained from various dealers. Considerable attention was paid to pre-operative preparation of the region of the iliac crest which was shaved and cleansed with soap and water and then swabbed with dettol. General anaesthesia consisting of nembutal pre-medication and open ether was used in all cases. Observing strict asepsis the iliac crest was exposed, a portion of bone removed by cutting forceps, sub-divided into small fragments of 1-2 mm. in diameter, and placed in sterile normal saline. The conjunctiva was grasped with forceps and the eye ball dislocated, a simple procedure in the rabbit. A small sclerotome was used to penetrate the cornea obliquely just in front of the limbus and fragments of bone were inserted through this small aperture into the anterior chamber. An iris respositor was then used to push the fragments well into the anterior chamber and if necessary to replace any herniation of the iris. Although the aqueous humour escaped freely from the anterior chamber, the normal tension was restored within 24 hours. Following the operation albucid drops were instilled into the conjunctival sac, but in no case was there any ocular infection and the animals seemed quite undisturbed by the operation.

Fifty such transplantations were carried out. Each animal had bone from its own ilium implanted into one eye (autotransplant) and bone from the ilium of a different animal into the other eye (homotransplant). The animals were killed and the specimens removed at intervals of 10 days, 21 days, 42 days, 90 days and 180 days, after operation. Each transplant was x-rayed on removal and sections were then prepared and stained for microscopic examination.

RESULTS.

10 days. In the 10-day autotransplant (Fig. 1) there is definite cytological evidence of living bone, with osteoblasts showing well stained nuclei. The normal rabbit ilium consists of bone containing islands of cartilage and these are apparent in the sections of the transplant. The host tissue reaction is considerable and consists of actively proliferating connective tissue cells. It is noticeable that these cells are in very close contact with the periphery of the bone fragments. In the homotransplants (Fig. 2) the bone is dead. The lacunae are empty and there are no nucleated osteoblasts. Where there are islets of cartilage these have, however, survived. The host tissue reaction is different from that of the autotransplant, it consists of round cells probably lymphocytes, and resembles bone marrow. It is not closely associated with the transplant and there is no attempt at the formation of a periosteal layer. On x-ray examination both autotransplants and homotransplants show normal bone shadow.

21 days. In the 21-day autotransplants (Fig. 3) most of the bone has survived. There is however necrosis in the central portion of the larger fragments. Living cartilage cells are evident but in all specimens examined there are empty lacunae. The host tissue reaction is similar to that seen at 10 days but in addition to massing at the periphery the cells appear to invade the matrix. The homotransplants (Fig. 4) present a picture similar to that seen at 10 days. A prominent feature is the appearance of cartilage tissue in the midst of dead bone. The cellular reaction is more pronounced and resembles marrow tissue with large alveolar spaces. On x-ray examination, there is rarefaction of the autotransplants but there is no change in the homotransplants compared with the 10-day specimens.

42 days. At 42 days the autotransplants (Fig. 5) consist of active living bone, the large nucleated bone cells resemble cartilage cells. Where the fragments are of considerable size central necrosis is present but at the periphery it is difficult in places to distinguish between bone cells occupying lacunae and those of the host cell reaction ; there is a multi-layer periosteal formation. The corresponding homotransplants (Fig. 6) present a very different picture. The bone is completely dead and the lacunae are empty even at the periphery, although odd living cartilage cells are still present. X-ray examination suggests marked decalcification of the autotransplants whereas the homotransplants give a dense shadow.

80 to 120 days. From 80 to 120 days the autotransplants (Fig. 7) consist of living bone with a well-stained matrix and large nucleated bone cells. They compare favourably with normal rabbit bone excepting the central portion of the larger fragments where necrosis is present. An interesting feature is the encirclement of the fragments by host tissue cells which form a line of demarcation between bone tissue and host tissue. The homotransplants (Fig. 8) consist of dead bone with a few surviving cartilage cells. On x-ray examination of the autotransplants it is possible to distinguish between cortical bone and cancellous bone, and compared with earlier specimens recalcification appears to have taken place. The homotransplants on the other hand show the dense shadow of a sequestrum.

120 to 180 days. From 120 to 180 days the autotransplants (Fig. 9) resemble normal rabbit bone, there is a well-marked periosteum and it appears in comparison with earlier stages that this is formed by the host tissue cells. The homotransplants (Fig. 10) are sequestra, there are no bone cells or periosteal formation. X-ray examination of the auto-transplants shows a normal bone shadow with central cancellous bone surrounded by a layer of cortical bone, comprising the periosteum which has been formed by the host cells. The dense shadow of the homo-transplant is that of a sequestrum.

DISCUSSION.

Although bone grafted to bone may react quite differently from transplants in the anterior chamber, the fate of these transplants does however give some answer to the problems mentioned at the beginning of the article.

Under the conditions of intra-ocular transplantation the auto-transplants survive while the homotransplants become sequestra. Of considerable interest are the changes which appear to occur in the auto-transplants. During the first 10 to 20 days the transplants lose vitality as evidenced by the areas devoid of osteoblasts. From about 40 to 80 days they have apparently revived and the picture is very much that of

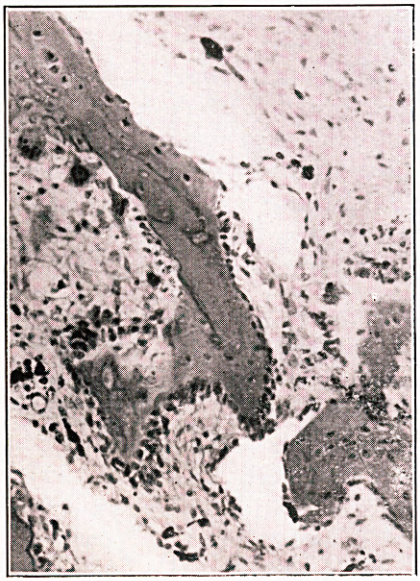

Fig. 1. 10 days autotransplant showing surviving bone cells, host tissue reaction, and commencing periosteal formation.

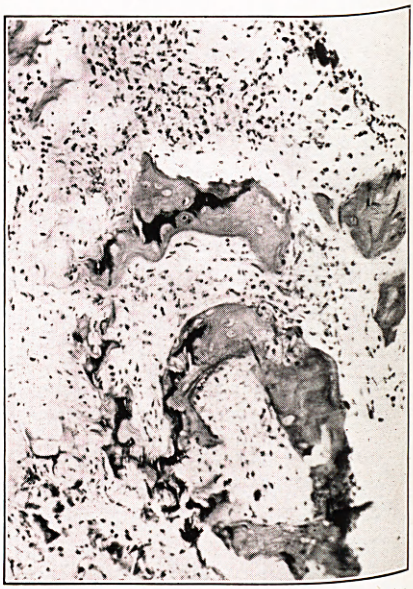

Fig. 2. 10 days homotransplant showing empty lacunae, persistence of cartilage cells and absence of periosteal formation.

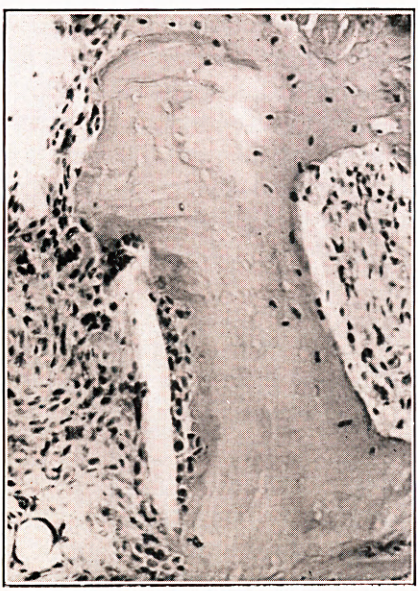

Fig. 3. 21 days autotransplant showing a proportion of living cells, active host tissue reaction and apparent invasion of matrix by large tissue cells.

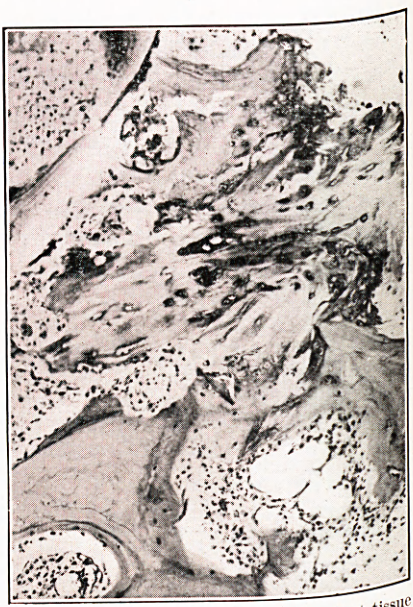

Fig. 4. 21 days homotransplant. The host tissue reaction has a ' marrow like ' appearance. Many cartilage cells are present, although most of the bone cells are dead.

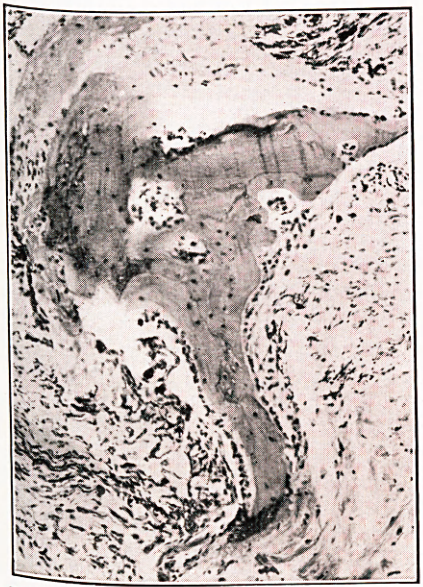

Fig. 5. 42 days autotransplant, bone cells are large and active looking. A distinct periosteal layer has formed.

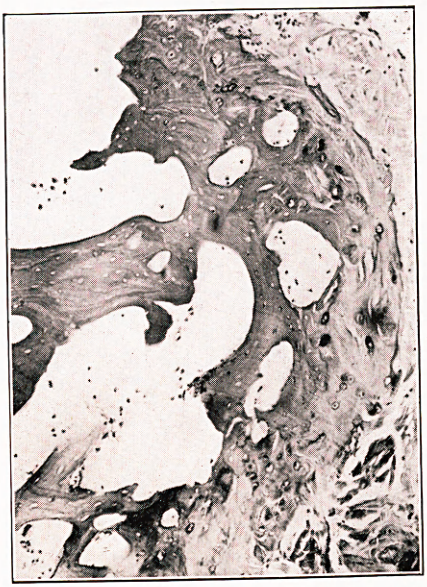

Fig. 6. 42 days homotransplant showing dead bone with empty lacunae, an odd cartilage cell persists.

Fig. 7. 100 days autotransplant showing active bone cells in normal bone tissue with a limiting periosteal layer.

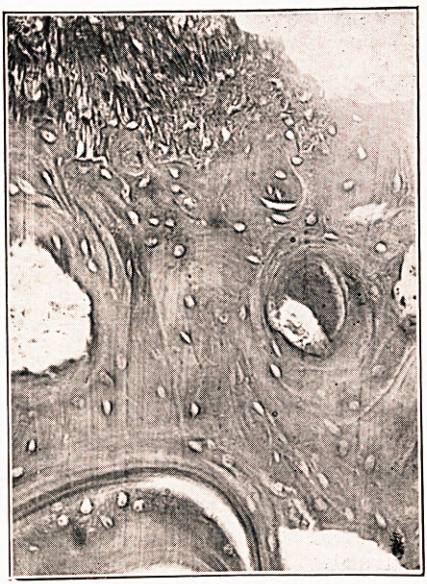

Fig. 8. 100 days homotransplant. The bone is quite dead and there is no periosteal formation.

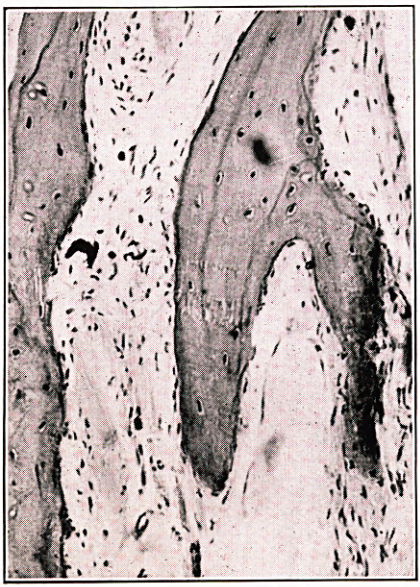

Fig. 9. 180 days autotransplant which has assumed the appearance of normal adult rabbit bone. The periphery of the bone is well demarcated by a layer of denser bone and the periosteal reaction has become a thin layer.

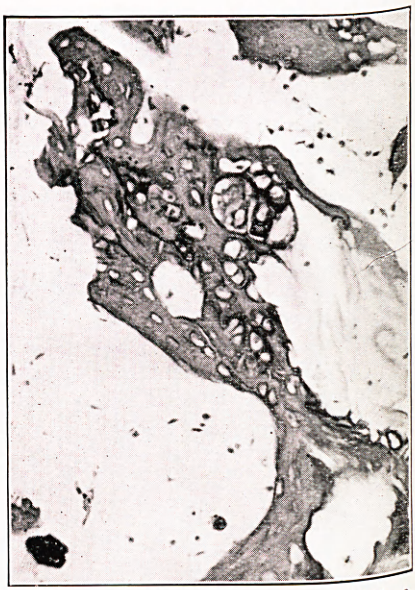

Fig. 10. 180 days homotransplant. It is a sequestrum like piece of bone with no live cells.

active living bone with an apparent increase in the number of cells each with a well-stained nucleus. These changes suggest that after an initial setback the bone revives and there are all the histological appearances of regenerating bone until about 120 to 180 days when the appearances are those of mature adult bone. Appearances at different stages certainly suggest that the cells of the host not only form a closely investing (periosteal) layer, but that they invade the bone and become bone-forming cells within the transplant. Examination of the oldest transplants in comparison with the earlier specimens suggests that the peripheral cortical layer is formed by the periosteum derived from these tissue cells.

There is considerable diversity of opinion amongst writers regarding the comparative value of cancellous or cortical bone transplants. This question has assumed greater importance because of the extensive use of cancellous bone in place of the older cortical grafts. Mowlem (1940) found that cancellous bone survived and developed a cortical layer around it but that cortical bone appeared dead at the end of five months. Brown (1946) described three cases of cortical graft used to replace excised tumours and concluded that the graft survived. Hellstadius (1944) experimented by implanting chips of cortical bone into one radius and chips of compact bone into the other and concluded after x-ray and histological study that cell survival was no better in the cancellous than

in the cortical grafts and that bony union occurred more rapidly on the side of the cortical grafts. The minute size of the fragments used in anterior chamber transplantation makes it difficult to answer this problem.

The small fragments of bone used for these experiments show initial central necrosis. It is therefore reasonable to assume that the much larger bone chips of the clinical graft will have correspondingly larger necrotic areas. It has been assumed here that the revival of the autotransplant is in part due to the invading host cells and this invasion is, of course, more rapidly and easily achieved with a large number of small fragments than with a few large fragments. From these experiments it would appear that autotransplants survive irrespective of the presence or absence of periosteum.

SUMMARY.

The important facts which emerge from this series of bone implantation experiments are :

The homotransplants do not survive but become sequestra. It is reasonable to assume that this is also their fate in clinical bone grafts. The autotransplants survive.

In the early stages there is some loss of vitality in the autotransplants, but they later revive. This revival appears to be due not only to the activity of the bone cells of the transplant but to invading host cells which become converted into and function as osteoblasts.

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