Disorders of the Skeletal System: New Perspectives in Bone Grafting

Uptodate

Presented by Mr H B S Kemp, Mr C W Manning, Dr M W Elves, Institute of Orthopaedics (University of London), Royal National Orthopaedic Hospital.

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Directed by Trevor A Scott.

Black-and-white
Duration: 00:51:33:16

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<Opening titles>

<Mr Kemp to camera>

What is a bone graft? How does it function? There are many reasons why in orthopaedic surgery and traumatology, we need to use bone grafts and, as surgical techniques have advanced, so they play a more significant role in the management of patients. Their major applications can be described in broader terms. First, and most commonly, they are used in the management of delayed union or in non-union of fractures. Secondly, they may be used to arthrodese these joints affected by disease.

<Kemp narrates over x-rays>

And thirdly, they are used to replace bony deficiencies that can be consequent upon trauma: infection or bone cysts, and furthermore, in certain circumstances, they can

be used to replace bone that has been removed in the management of certain tumours.

<Kemp to camera and then over slides illustrating different types of bone graft>

There are three types of bone grafts: the autograft, this consists of bone which is removed from one part of the patient's skeleton and subsequently transplanted to the site under treatment. When the patient's own skeletal depot is inadequate for the provision of donor bone, then a homograft or allograft is employed. Such grafts introduce the problem of immunological response on the part of the recipient. The most successful homografts are probably those taken from siblings for, in these instances, there is likely to be a relatively close antigenic relationship. However, such donor grafts are not necessarily available and in most instances the surgeon is then dependent on the availability of a bone bank to provide adequate grafting material.

<Kemp narrates over illustration of Adam and Eve>

Incidentally, it is interesting to reflect that the earliest use of a homograft is described in the Book of Genesis, Chapter 2, Verse 21: 'And the Lord God caused a deep sleep to fall upon Adam and he slept and he took one of his ribs and closed up the flesh thereof'.

<Kemp to camera and then over further slides, interspersed with talk to camera>

Unfortunately, in the majority of centres, bone banks are not available and it is for this reason that bone from animals has been used in an attempt to provide alternative sources of bone for grafting. These grafts are called heterografts or xenografts. When prepared on a commercial basis, they are usually derived from calf bone. Various techniques are used in grafting; the details of the methods employed are fully documented in most orthopaedic surgical textbooks. In general terms,

however, grafts are employed as inlay or as onlay grafts and only very rarely are they used as strut grafts.

The physical nature of the graft is of importance. It may consist of either the compact outer layer of the bone shown here in white, that is, the cortical bone, or it may be taken from a central medullary, or spongy bone, which is shown here in black.

<Kemp narrates over photomicrographs>

Cortical bone is most suitable for bridging defects and for providing support. It is, however, dense and, in consequence, if it is to be incorporated, osteoclasts must actively erode into it by forming channels which can then be invaded by blood vessels and osteoblasts which lay down new bone in the interstices which have been created. Medullary bone is quite incapable of providing tensile strength. On the other hand, it stimulates osteogenesis and, further, the numerous inter-trabecular spaces are freely invaded by blood vessels so that not only woven bone is laid down but, in addition, bone is deposited on the abundant trabecular scaffold.

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<Kemp to camera>

In consequence, when using autografts, providing that the supportive techniques are adequate, it is reasonable to anticipate that the graft will be successfully incorporated. In children such grafts usually take about 3 months to unite with the recipient bone and a further 3 months to incorporate, while in adults the process can take twice as long.

<Kemp narrates over x-rays of fractured femur>

An example of an autograft in an adult patient is seen in these radiographs. This patient had the fracture of his femur treated by internal fixation. It became infected and failed to unite. And so the internal fixation was removed and the infection was

treated with antibiotics. An external compression plate was applied in conjunction with Phemister onlay type of autografts. Twelve months later, the graft was incorporated and the bone was soundly united.

<Kemp to camera>

Where support is required, it is usually achieved by a compromise which combines the strength of the cortical bone with the osteogenic potential of the medullary bone. It is possible to remove the equivalent of a sandwich of cortical bone, containing medullary bone, from the wing of the ilium. And this is used for grafting defects in adult spines. Such grafts usually incorporate in 3 to 6 months and consolidate in about 12 months. However, difficulties are encountered when bone bank bone is used.

<Kemp narrates over X-rays of spine, interspersed with talk to camera>

These difficulties are exemplified in this patient in whom previous grafting had been unsuccessful. On the second occasion, homogeneous bone was also used in conjunction with a patient's own autogenous bone. The autogenous bone incorporated successfully, but the homogeneous bank bone responded indolently. It showed evidence of stress fractures, and it was 9 years later that it eventually incorporated.

<*To camera*> In similar situations, heterogeneous bone has been used. Aware of the rejection phenomena that apply to such bone, attempts have been made to remove the fat content and in particular the protein fraction, for it is well recognised that it is the presence of this foreign protein that stimulates the immune response on the part of the host. Unfortunately, because of the peculiar structure of bone, it is impossible to remove completely this foreign protein without damaging the matrix of the bone. In consequence, using heterogeneous bone, certain paradoxical changes occur. In general, the response of the body is an attempt to isolate the foreign graft and to remove it progressively by mobilising foreign body giant cells and osteoclasts. <*Further x-rays*> Occasionally such an implant, particularly when inlaid into bone, will

not only stimulate such a response but it will also induce an osteogenic response on the part of the host so that the heterogeneous graft is in a state of almost total isolation, walled off by the host in a tomb of bone from which it can be slowly removed and replaced. Now, this process, while not the original intent of the surgeon, serves a useful role even though its ultimate completion is inevitably slow.

<Kemp to camera>

What then does the surgeon require for the achievement of successful grafting? Ideally, he requires an adequate source of autogenous bone from the patient. Failing this, he must find a source of homogeneous bone from the bone bank, but if he is to achieve this, how can he be certain that an adequate match can be obtained between the homogeneous graft and the host? Further, if such a match cannot be achieved, is it feasible or even justifiable to invoke the use of drugs that are cytotoxic to minimise the immuno-rejection response from the part of the recipient?

Now, my colleague Mr Charles Manning has a particular problem in the field of bone grafting.

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<Intertitle>

Bone grafting in treatment of patients with scoliosis

<Manning narrates over intertitle and then over illustration of woman with scoliosis>

Spinal deformity and instability may lead to serious respiratory insufficiency as well as mechanical failure in the spine itself. A proportion of patients with scoliosis may therefore require stabilisation of the spine after correction of the deformity as far as possible.

<Manning to camera and then narrates over series of photographs, x-rays and intertitles, interspersed with talk to camera>

To secure a firm arthrodesis of up to 12 or 15 spinal segments, bone may have to be added to reinforce the local arthrodesis. Here is a photograph of a girl of 12 with a scoliosis and here is the radiograph of her spine. After operation, there still appears to be quite a severe deformity, but the spine is stable. And here is the girl's appearance 2 years after operation.

<Intertitle>

Technique of spinal fusion

Operation consists of exposure of the posterior aspect of the spine by division of the muscles in the midline. Separation to either side to lay bare bone. Individual joints are dissected free. The articular surfaces excised and bone punched into the rawed areas as indicated in this diagram from a paper by John Moe. This is a photograph of a joint exposed and here the articular surfaces are being removed and bone punched into the excised joint.

<Intertitle>

Stabilising the cancellous bone grafts

After thorough decortication of the posterior arches of the spine, bone is added, homogeneous or autogenous, and internal fixation is secured, here by a homogeneous bone bank strut.

<Intertitle>

Devitalised bone is incorporated relatively easily in children

<Intertitle>

How is homogenous[1] bone obtained?

Homogeneous bone may be obtained at operation and may be prepared under strict asepsis for storage for use as required.

<Intertitle>

Bone preparation

A preparation bench covered by sterile material is required with all tools and instruments similarly prepared. The bone source, in this case an amputated leg, is prepared and the bone exposed and removed.

<Intertitle>

Storage

The bone is then cut into convenient sizes and stored in screw-cap jars, half-filled with autoclave-distilled water and containing a solution of antibiotics. And this bone is stored frozen solid in the solution in a refrigerator at minus 10 degrees centigrade. Bacteriological checks are made before the bone is used, and careful records are kept of the source of bone and its subsequent use.

The possible presence of jaundice, syphilis or septicaemia is checked in the donors before the bone is accepted. Another type of bone for the bank is that collected without sterile precautions, usually cadaveric bone, shaped as required, freeze-dried and sealed into these evacuated glass containers, which are sterilised by exposure to 4 megarads of radioactive cobalt. The containers can then be stored indefinitely at room temperature.

The sealed container is opened by scoring the thick glass with a diamond cutter, applying heat which cracks the glass along the score so that the bone, which is then very dry and brittle, can be removed and shaped with a high-speed circular saw. And then fixed firmly in the spine to support the cancellous graft.

[1] Spelling as on film. In these transcriptions the word has been spelt as: homogeneous.

Internal fixation with metal rods, as described by Harrington, is now preferred to bone bank struts as the fixation is more efficient. Similarly, autogenous iliac bone has superseded the homogeneous graft since it was found possible to reduce the high postoperative infection rate, at one time almost 30%, to negligible proportions by the use of much smaller quantities of autogenous bone, removed from the patient's own iliac crest, without any reduction in the rate of successful fusion. This shows a graft exposed I year after operation, demonstrating the conversion of the rough crumbs of bone to a smooth hard surface resembling a long bone.

Although bank bone is no longer used for scoliosis in most centres, fresh homogeneous bone is still preserved for use in small children who need massive implants, for instance, in stabilising dislocated hips. The common source now is ribs, usually from young women who've had resection of the prominent posterior projection for scoliosis after they've reached skeletal maturity.

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<Intertitle>

Can we improve the value of the bank for use in patients by modification of the bone – or by influencing the response of the host?

<Manning to camera>

Well now, Dr Michael Elves will try to explain some of the ways in which this can be done.

<Elves, standing, to camera>

In the first part of this programme, my colleagues have outlined some of the clinical problems which they encounter in bone grafting. And it's a surprising fact that apart from skin grafting, bone grafting is the most widely practised form of transplantation. And yet, so many of the biological factors involved are barely known to us at all.

We've been attempting to investigate some of these factors using an animal model, and the animal we have chosen is the inbred rat.

<Elves refers to illustrations on display wall and narrates over them>

By using inbred animals, we are able to achieve a large degree of genetic uniformity. The graft tissue that we use is from the pelvis of the donor animal. This gives us a cortico-cancellous type of bone graft. By using animals of the same genetic strain as the recipient, and transferring some of his pelvis to a subcutaneous site in our recipient, we can achieve an isograft situation. There are no problems of the immune response in these animals. By choosing a second genetically unrelated strain of rat and transferring his pelvis also into a subcutaneous site, we can examine what goes on within a homograft.

Now, one of the major difficulties in bone transplantation research is, in fact, how one can quantify or assess the state of well being of the graft. The most widely used methods have been based on the histology of the graft. Now, if we look at the next slide, […]

<Elvis narrates over series of photomicrographs>

[…] we will see here a graft that is comprising mainly dead grafted bone. That's the dark material. And smaller light-staining areas of bone can be seen attached to the dark bone and this is the living bone. And here one sees the situation where the dead bone is by far the most preponderant type. Now, in contrast to this, the next slide shows us the reverse situation. In this case, the largest part of the bone is newly formed with only small spicules of graft remaining. Here we would see two extremes. Unfortunately, however, these two slides were both taken from different areas of the same graft and this pinpoints the problem of histology. In order to quantify new bone formation in a graft by the histological methods, serial sectioning of the graft from one end to the other must be carried out. If it isn't, this sort of situation will exist and one can get completely spurious results.

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<Elves to camera and then refers to further diagrams and narrates over them>

And so, we've been attempting to devise a more quantitative method of estimating new bone formation in the whole of the graft. And to do this, we have used the radioactive tracer compound strontium-85. We are interested in our animal in two compartments. Firstly, the bloodstream into which the strontium is first injected. And from the bloodstream the strontium can enter the bone, and it does this by one of two methods: firstly, by a purely physicochemical process of exchange. Strontium from the blood is exchanged against calcium ions in the bone mass and this is a reversible process. And the strontium can be removed from the system by excretion via the kidney. And the second method by which the strontium can find itself in the bone is by actual incorporation. The strontium is incorporated into new bone as it is formed and is irreversibly bound there for some time. And in any method of quantitation employing this principle, we must measure that mode of uptake *<indicates incorporation>* and remove that mode *<indicates exchange>* of uptake from our consideration.

The way that this can be achieved is shown in the next diagram, which shows the three compartments of the body in which we are interested: the blood plasma, the calcium mass of the skeleton, and the newly formed bone. When strontium is injected into the body, all three compartments will take up the isotope but this will be removed by excretion via the kidney, first from the plasma compartment. This in turn will create a gradient between the calcium mass of the skeleton and the plasma, and cause strontium to be removed by exchange from the calcium mass. And we are finally left with the state of affairs where the only strontium remaining is in the new bone compartment which is the compartment that we are interested in measuring. In point of fact, this state is never achieved, but in rat experiments, we have found that if we leave the animal for 4 days after injection of the strontium, 95% or more of the isotope which can be counted in the graft will be bound into the new bone, and, hence, this is a valid method of measuring the amount of new bone formed.

Here then is our experimental system. The recipient animal has already received two grafts which we want to assess. 4 days before he is killed, he is injected with strontium-85, and 4 days later he is killed. The grafts are then removed into convenient-sized glass tubes, together with the pelvis of the recipient animal which will also be labelled, of course, with the strontium-85. These pieces of material are then weighed and fixed in a histological fixative. After this, they're transferred to a scintillation counter and the strontium-85 content is assessed. And following that, the grafts can be sent for histology, which we always use as a check on the radiochemical method. To quantify new bone formation in the graft, we relate it to counts per minute in the graft per milligram to counts per minute per milligram of the host's cancellous skeleton. And this gives us a figure which we refer to as the osteogenic index. This then is the technique that we have used to explore some aspects of bone transplantation.

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<Elves narrates over series of photomicrographs>

Now, before we look at the bone homograft, the foreign bone that is, it is worth examining what happens in an autograft, and this picture shows a 7-day-old autograft in which there is luxuriant but randomly organised new bone being laid down in between the scenes of the old bone. The old bone looks obviously dead, its osteocytes have disappeared. By 3 weeks, which we can see in the next picture, the new bone is becoming reorganised and has a clean-cut endosteum evident. The cavity between the trabeculae is, however, filled at this stage still with a fibroblastic type of tissue.

Next slide. 8 weeks after grafting, the bone has become very small, indeed, and is organised to form a small ossicle in which lamellar bone is most prominent. And in cavities within the ossicle can be seen haemopoietic marrow. And the next picture shows a higher power of the marrow in which all haemopoietic elements, from megakaryocytes, granulocytes and red cell series, can be seen.

<Elves narrates over slide showing graph>

Now, examining the osteogenic activity of these grafts by means of the strontium technique, this is the type of pattern of osteogenesis that we find. 1 week after grafting, the osteogenic activity in the graft is below the level of osteogenesis in the host's skeleton, that is, the osteogenic index is below 1. By the second week, however, the graft is exceeding the skeleton and we will see that a peak in osteogenesis is reached at about 3 weeks. The new bone formation rate then declines and forms a plateau between 4 and 6 weeks. And then a second wave of new bone formation is clearly detectable from 8 weeks onwards. So, then we have a situation in the autograft where there are clearly two phases of new bone formation: an early phase, lasting for about 2 to 3 weeks, and here we have evidence that the graft itself is largely responsible for providing the osteogenic progenitor cells for new bone formation. This subsides and its place is taken by a second phase of osteogenesis, and again we have good evidence here that the cells responsible are derived from the host itself.

It's clear from our experiments that the second phase of osteogenesis is intimately related to the success of the first phase and is dependent on it. Because in situations, as we will see, where the first phase is absent or curtailed, so too will the second phase be.

<Elves narrates over photomicrograph>

Now, let us turn our attention and examine the osteogenic potential in conventional bone bank materials. This slide shows what happens in a graft of frozen autologous bone. The graft itself is totally dead and inert. There is no evidence at all of an endosteal layer of cells anymore. And the graft itself after 3 weeks shows very little new bone formation at all. And the inter-trabecular spaces become filled with the granulation tissue that tends to become fibrotic with time.

<Elves to camera>

Occasionally, in the 8-week graft, one does find small amounts of new bone formation on the surface of the dead bone, but it is very sparse indeed and nothing like that compared with the fresh autograft. We can use the strontium technique to assess the osteogenesis in these grafts as well. We've examined three types of bone bank material. Firstly, the frozen bone. This has been taken from the animal fresh and then kept at minus 20 degrees centigrade for 2 to 3 weeks.

<Elves narrates over slides showing graphs>

This type of graft shows very little new bone formation in the early phase of osteogenesis. They are all, in fact, very much below the host's level. But by 8 weeks, there is a slight but not really significant increase in the amount of new bone formed. Essentially, the same type of picture is seen in the case of freeze-dried bone grafts. Again, the first phase is curtailed and the second phase is very feeble indeed. In the case of decalcified freeze-dried grafts, we start off with a very low level of strontium uptake because, of course, we have removed any possible exchange element as there is no calcium in these grafts. And it will be seen that the first phase is very poor, but there is now some clear evidence of a second phase in some of the grafts, but again they rarely reach the host's normal skeletal osteogenic level.

And so to summarise then, in the case of the preserved, or bone bank type of material, the first phase of osteogenesis is absent or virtually absent. And the second phase is present but is extremely poor.

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<Elves to camera>

And now we come to consider the homograft situation and the most important question that we must try to answer is: what sort of an immune response do bone homografts elicit? When considering the immune response to any type of transplanted tissue, there are two mechanisms which we must consider. There is the

humoral immune mechanism which results in the production of antibodies which are shed into the serum. And secondly, and probably more important in the transplantation context, there is the cell-mediated immune response, and in this case the effector system are killer lymphocytes which are generated during the response.

Now firstly, as far as humoral antibody production is concerned, we have examined inbred animals that are carrying homografts from donors who are compatible with the recipient for the major transplantation antigen locus. And in this situation, we find no evidence at all of any humoral immune response.

<Elves narrates over slides showing graphs>

Where an incompatibility for the major transplantation antigen system does exist, then recipients of cancellous marrow-containing bone grafts soon show antibodies. And by 3 weeks following grafting, antibody is evident in the serum of 80% of the recipients, and this level is maintained for at least 8 to 10 weeks. The pattern of antibody production in these animals is almost identical to the antibody pattern that we find in recipients of skin grafts. One of the controversies, that has raged for the last 15 years or so, concerns the origin of the antigenicity of these grafts. And for long it has been assumed that the red marrow is the most important antigenic component of these grafts. And so, we have examined the immune response in recipients of cancellous bone from which the marrow has been removed. And this is shown in the next diagram, where we will see that the immune response is a little bit delayed in its onset, reaching a peak at about 6 weeks following grafting instead of 3 weeks. And in this case too, 40 to 50% of the recipients only develop antibodies. And so although marrow is an important antigenic component of these grafts, it is by no means the only antigenic component, and bone cells, per se, can act as antigens.

The third type of graft we've examined is the cortical bone graft and, in this case, there is no antibody production until 6 weeks after grafting, but from then onwards, we get a rapidly increasing number of animals showing antibody and they eventually reach a maximum at about 80%, in other words, the same sort of level of antibody

production is eventually found in these animals as was found in the marrow containing cancellous bone grafts.

In the case of the preserved bone bank material, we find no evidence at all of any antibody production.

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<Elves to camera>

In order to investigate the cell-mediated immune response to bone grafts, we've employed the leukocyte migration inhibition assay. The basis of this assay is the observation that if lymphocytes from a sensitised individual are cultured for a short time in the presence of the antigen to which they are sensitised, they will produce a lymphokine into the medium. This is a factor which will act on other leukocytes and prevent their migration. It inhibits their mobility. Thus, in this test, we take lymphocytes from the animal under test, pack them into small capillary tubes and place them in culture dishes containing culture medium, and leave them at 37 degrees centigrade for 24 hours. After this time, the migration of the cells from out of the capillary tube is measured. And we can see this in the next picture.

<Elves narrates over slides of photomicrographs and then graphs>

This, in fact, is a test with lymphocytes from an individual who is sensitised against the bone graft, but the culture medium contains no graft antigen. In contrast, the next slide shows what happens if the graft antigen is present in the culture medium; and there is a severe impairment in the migration fan from the capillary tube. If the area of migration, in the presence of antigen, is less than 80% of that in the absence of the antigen, we regard the animal as sensitised.

Now, we can look at some of the results we've obtained using bone grafts. First of all, for comparison purposes, we've examined recipients of skin grafts, and 100% of these animals show sensitivity to graft antigen in this test system. And a very similar

sort of response was found in recipients of fresh cancellous marrow-containing bone grafts. Removal of the marrow from the cancellous bone has very little effect on the immune response. One animal only gave no evidence of sensitisation in this test.

In the case of recipients of cortical bone grafts, all animals were very clearly sensitised. And surprisingly, in view of the observations on humoral activity, recipients of frozen bone also clearly were sensitised. The only different type of response was found with freeze-dried bone grafts, and here the majority of recipients showed no evidence of sensitivity at all.

<Elves to camera>

So, summarising then, we can say then that fresh bone grafts, whether containing marrow or not, certainly have an immunogenicity as far as cell-mediated immunity is concerned. Frozen bone, on the other hand, although it is inert as far as the humoral immune response is concerned, is powerfully immunogenic in the cell-mediated immune system. Cortical bone is also clearly immunogenic. The only type of material we found which is not immunogenic, either for humoral immunity or cell-mediated immunity, is the freeze-dried bone graft. But, I ought to stress that this type of material is inert also as far as osteogenesis is concerned.

How, then, does the immune response affect the bone graft itself?

<Elves narrates over photomicrographs>

This picture shows the histological appearance within a homograft of bone 2 weeks after grafting. And it can clearly be seen that there is a small amount of old bone still present, in the top of the picture and at the very bottom of it. But the old bone that is present is also clearly dead. Its lacunae are usually empty and there's also quite a significant amount of new bone present as well in the inter-trabecular space. This isn't true, however, of all 2-week grafts, and at the other extreme, one finds grafts more like this one in appearance. With very small amount of new bone evident at all, the bulk of the bone in the graft is the old grafted material and this is clearly dead.

And the centre of the graft becomes filled with a fibrous granulation type of tissue. By 3 weeks, the picture is becoming more clear and there is now very little new bone evident that is still viable. If we can look at this new bone at higher power, we can see that it is extremely ragged in appearance and only occasional osteocytes are still seen to be occupying their lacunae. The majority of the lacunae are empty and there is no evidence at all of an endosteum. This appearance suggests that the early phase of new bone formation in these grafts is graft derived and, therefore, will be sensitive to the immune response. The other very striking feature of these grafts is, of course, the presence in the spaces of numerous small mononuclear cells, the lymphocytes.

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<Elves narrates over slides showing graphs>

We've also quantified osteogenesis in the homograft using the strontium technique. And here to remind you is the pattern of osteogenesis in the autograft. The first types of homograft that we examined were those that were incompatible with the host for the major H antigen system. And it can be seen that although the grafts start off a little better in the first week than autografts, the first phase is soon cut down and is very poor. The second phase too is very poor but is evident in about one third of recipients.

A second type of graft, we've examined, is compatible for the major histocompatibility antigen system with the donor but has up to 14 minor antigen disparities and these grafts also showed very poor first phase osteogenesis, and surprisingly in these recipients, the second phase of osteogenesis was non-existent.

To summarise, then, as far as the homograft or allograft is concerned, the first phase begins but then is curtailed very rapidly and a second phase is rarely present. This is equally true for grafts which are compatible for the major H antigen system as for those that have a major disparity.

<Elves to camera>

In the unmodified recipient, then, tissue typing would appear to have no significant effect on the behaviour of bone allografts. We've also examined the state of affairs that exists in the recipient that has been immunosuppressed before receiving bone grafts. And for convenience we have used whole body x-irradiation as the means of immunosuppression to bring this state of affairs about.

<Elves narrates over series of photomicrographs>

This picture shows an 8-week-old graft of bone, which has a major H antigen disparity with the host. And there is a small amount of ragged new bone still present, but this is now clearly dead and the space is filled with an almost acellular fibrous tissue. The same type of graft, but this time in an irradiated animal, gives essentially the same appearance. The small amount of new bone, which is still evident, is clearly dead and, again, this fibrous tissue, rather poorly cellular, occupies the spaces. Now, we see a similar type of bone but taken this time from an animal which is compatible with the host for the major H antigen system, but it has got multiple minor antigen disparities. One can again see the essentially same dismal picture. The new bone that has been formed is now clearly dead and unhealthy and, once more, the intervening spaces are filled with the rather acellular tissue. However, the same type of graft put into an animal that has been treated with irradiation is completely different. It is smaller in size and there has been considerable reorganisation of the new bone which is now lamellar in structure, as you can see from the bottom of the picture. And there is a tendency for the graft to become an ossicle as in the case of the autograft at 8 weeks. In the centre there is a healthy haemopoietic marrow present, and in the high power microscope, we can see that most elements of haemopoiesis are present.

<Elves narrates over slide showing graph>

This graph summarises our results with the strontium assay at 8 weeks. In the normal recipient, the AS, we find the expected impairment of osteogenesis in the two

types of homograft compared with the autografts; the impairment being greatest again in the major antigen compatible but multiple minor incompatible type of combination, that is, the HS. However, in the irradiated animal, we find no significant improvement in performance, osteogenesis-wise, in the major incompatible combination of the AS2. But the major antigen compatible combination shows definite and significant improvement in its osteogenic level, achieving almost the autograft level in the unmodified host. It is interesting also to observe from this data that the irradiation seems not to have had any harmful effect on the autografts in this system, quite the contrary, in fact, as the autografts now show almost half as much osteogenesis again as compared with the same type of graft but is in the normal animal.

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<Elves narrates over slides summarising conclusions>

And so we can come to some conclusions. Firstly, it is clear from these studies that the graft is important as a source of osteogenic cells during its early life. Secondly, the host cells come to assume osteogenic roles in order to maintain the graft. Further, we can say that the second phase of osteogenesis is dependent upon a successful first phase.

Traditional bone bank material lacks any intrinsic osteogenic potential.

As far as the immunology of bone is concerned, it is clear that fresh bone allografts are immunogenic and do stimulate both humoral and cell-mediated immunity.

The immune response that is elicited does act to curtail the first phase of osteogenesis.

Immunogenicity with regard to antibody production is clearly abolished in our experiments by both freezing or freeze-drying the bone, whereas freeze-drying the bone only reduces its ability to stimulate cell-mediated immunity altogether.

Immunosuppression has little effect on major antigen compatible grafts. But immunosuppression does significantly improve the survival of major antigen compatible grafts.

<Elves to camera>

And so we've got evidence from our studies that we can expect a much better performance from a bone graft if it is tissue typed and compatible with the recipient, provided that immunosuppression is used as well. The hopeful feature of this work is that in the case of bone grafting, unlike that of kidney and heart grafting, one would not expect to have to use lifelong immunosuppression of the patient. One would require only immunosuppressive measures to be taken during the first phase of osteogenesis in order to protect the graft's contribution: a period in the rat of about 3 weeks, and in the human this hasn't been determined but is likely to be of the order of 2 months.

<Elves narrates over intertitle and slide>

<Intertitle>

Possible future developments in bone grafting

And so, what about future developments in the field of bone transplantation? Firstly, it's important that we have a viable bone bank in the future. Secondly, allografts should be tissue matched to the recipient. And thirdly, immunosuppressive cover could be used in order to protect the first phase of osteogenesis.

<Elves to camera>

It has long been taught that the bone graft acts simply as a scaffold on which the host's cells can lay down new bone. It is now becoming increasingly clear that this view needs to be considerably modified. The success of the bone graft lies in its

viability. And efforts should be made, therefore, to preserve this both before the graft is put into the patient and also after the graft is in its host.

<End credits>