# Tissue Transplantation

NEVILLE RUSSELL, '64

*Introduction*

The field of tissue transplantation is one of the most rapidly expanding frontiers of experimental and clinical medicine. It offers a wide range of new therapeutic advantages, not only to the surgeon, but to the internist, endocrinologist, cancer research worker and other scientists as well. Carefully controlled studies have already been made in tissue cultured embryonic adrenal and parathyroid glands have been transplanted to humans and animals. These homotransplanted embryonic organs have survived permanently, taking over the functions of the diseased or absent glands. One patient with Addison's disease now survives without hormone injections as a result of having received such transplanted adrenal tissue. Some patients have been relieved of hypoparathyroidism and tetany by the transplantation of embryonic parathyroid glands, but the cures and improvements have not always been consistent. At any rate the successful transplants demonstrate that the science of homotransplantation is no longer in the realm of hypothetical speculation.

*Background*

During the past decade, stimulated by the work of such men as Burnet and Medowar, interest in tissue transplantation has been renewed. Yet our era cannot lay claim to having pioneered in the field. The use of tissue transplants—more particularly homografts and heterografts—in reconstructive surgery and for the replacement of diseased and defective organs, has long stimulated the human imagination. Hardly an age has passed without some record of exploratory attempts in this type of surgery.

"Gambacurta, a female charlatan of the early 18th century, peddled a wound healing balsam in the public square of Florence. To dramatically advertize the concoction's remedical powers, it was her custom to cut a large piece of skin or "flesh" from her thigh and pass it around on a plate amongst the bystanders, for all to inspect. The fearless woman then replaced the bit of skin in the thigh defect and covered it with the miraculous herb." An eyewitness recalls that "the graft united so well that by the following evening it required no further medication." A fascinating account of free skin grafting.

Generally bibliographers and medical historians think of the early Hindus as the originators of skin grafting, although there is controversy as to the exact century and year the earliest authentic reports appeared. One historical account proceeds as follows:

"It was the custom in India to punish certain offences by cutting off the nose. As a necessary consequence, there approached at an early date, men skilled in plastic surgery, who belonged strangely enough to a low and despised class—the tilemaker's caste. Their work is said to have been excellent, even superior in some respects to that done at the present time. One of their most remarkable achievements was the replacement of the nose by a graft of thick skin from the gluteal region. The success obtained was marvellous, even with subcutaneous fat included, and one is tempted to ascribe some virtue to their "secret cement". Knowledge of the actual technique is so scarce that such speculation is of no value."

A personal description from an European who commanded the armies of an Indian prince tells of the restoration by gluteal graft of the nose of one of his soldiers.

The process was not begun until after cicatrization was occurring, but the result was "no disfiguration and only a hairline scar to show there had been a graft". Numerous accounts similar to the above appear throughout the literature of the ensuing centuries—many are fascinating, all are interesting, and some extremely amusing. An example of the latter is related by an 18th century Italian adventurer, and is supposedly authentic: He tells of witnessing a man having his nose cut off in a sword duel and goes on to say: "The poor gentleman remained without a nose, and I who picked it up, had it in my hand, all full of sand, and urinated on it. Having thus washed it, I attached it to him and sewed it on very firmly, and medicated it with balsam and bandaged it. And I had him remain thus eight days, believing it going to rot. However when I untied it, I found it was well attached once again, and I medicated it only once more and it was healthy and free and all Naples marvelled thereat".

Towards modern times reports begin to appear in the literature of verified cases of successful autographs and various techniques begin to be perfected—many of which form basic principles of the modern specialty of Plastic Surgery, e.g., Reverdin's Pinche grafts, split thickness grafts, etc.

Homografting (transfer of tissue between individuals of the same species) historically parallels the science of autografting, though was perhaps not so favourable a technique because of rather indifferent results. It too appears to have been initiated by man's attempts to restore to former presentability certain battered and deformed probosci. A 15th century poet writes in a letter to a friend of "a man of Sicily of wonderful talent, who has found out how to give a person a new nose, which he either builds from the arm or borrows from a slave". Reverdin, the 19th century Swiss of early plastic surgery fame, reported equally good results when skin homografts were employed, using on occasion skin from amputated limbs and even donating his own to patients who refused autografts. Subsequent medical history has demonstrated that most skin homografts are eventually rejected by the recipient patient or animal, but clinicians of Reverin's time were often firmly convinced that these grafts were permanently successful. Reports by early workers of the "sympathetic nose", i.e., when graft material was supplied by another person, a sympathetic union existed between it and the donor so that is sloughed on his death, indicate early experience with rejection phenomena.

Heterografting (transfer of tissue between different species) was experimented with by many, including the talented Reverdin. Without the threat of lawsuit or a vast body of preceding literature, workers of this age (before 1900) were free to conduct rather bizarre experiments. A paper read to the Academic des Sciences reporting three cases of leg ulcers, healed by grafts of rabbit skin, was so impressive that it was referred to the Committee for Prizes in Medicine. Davis, in his 1919 plastic surgery textbook, summed up these experimental results as follows:

"My own experience is that these grafts take readily and receive blood supply as promptly as ordinary grafts. Nevertheless, in every case observed by me, these grafts, after doing well, and often when the wound was entirely healed, suddenly and with no apparent cause have begun to melt away and have soon disappeared."

Now to consider tissue transplantation in the light of modern day knowledge of immunochemistry:

"Homograft Phenomenon"—so called is clinically described as follows:

1. Primary Response

(a) Lag period—Tissue is implanted and for five to six days no reaction is noted. The graft to all purposes is similiar to an autograph. During the first two to three days, vascularization and permeation of the graft by lymphatics occurs. The next two to three days are a latent period.

(b) Reactive phase—The fifth or sixth day histological changes are seen—infiltration by lymphocytes, eosinophils and immature plasma cells; next, dilatation of the graft's blood vessels, vascular congestion and eventually stoppage of the circulation. The cells die, collagen tissue is undermined and the graft sloughs or is overgrown.

2. Secondary or Accelerated Response

When a host reacts against a specific homograft, he becomes "immunologically deflowered" with respect to that donor. The sequence of events that occurs when a second graft is implanted after the first has sloughed differs markedly from the primary response. Rejection occurs immediately and so rapidly that there is no latent period. There is no opportunity for cellular infiltration or vascularization to any extent, the brunt of the reaction being directed against the graft margins.

A great deal of work has been done and is being done to try to identify the steps leading to the recognition of a homograft as foreign in a host animal. It is known that successful homografts occur under the following natural or physiological conditions:

(a) Close or identical (accidental) genetic similarity.

(b) Mother to offspring grafts—occasionally successful because of transfer of material cells into the developing fetus, with the production of acquired tolerance.

(c) Between identical twins or members of inbred strains.

(d) Fraternal cattle twins accept each other's grafts just as readily as the acceptance that occurs between identical cattle twins. This is because all bovine twins usually have common placental circulation and the mutual exchange of cells in fetal life results in acquired tolerance.

With the exception of the above mentioned conditions, transplants cannot be made to take permanently without pretreatment of recipient. Certain endocrine glands though have survived as homografts in defiance of the rules.

The vast weight of immological evidence recently accumulated points to antigen-antibody reactions as the basis of the homograft rejection phenomena. What is the source of the antigenic stimulus? How does the body "sense" the presence of foreign tissue? These are perhaps the vital questions which must be answered before graft rejection can be altered or combatted.

1. Histocompatability group of antigens: Genetically determined humeral antibodies of various types were first detected in studies on malignancy. A composite set of antigenic stimuli is responsible for these and by complicated laboratory methods an entire system of antigens (histocompatibility group) have been identified. These have been demonstrated to be present in all tissues of genetically identical animals, governed by proven sets of genes.

Using this information it has been calculated that there are approximately one million antigenic combinatons in the random human population and that random choice of donor and recipient should result in success in one of every three hundred transplants. All attempts to identify genetic transplantation mates have been unsuccessful.

It was noted that genetic loci determining transplantation antigenicity are the same as those determining the type of hemaglutinogens contained in normal RBC's. The obvious conclusion that RBC compatable patients would have greater chances of being graft compatable has not been experimentally borne out.

Attempts to transfer homograft immunity passively by means of serum, or to demonstrate it with serum in vitro, have been discouraging. This has lead to the feeling that the antigenic stimulus leading the appearance of the humeral response is different from that leading to homograft rejection.

2. The Cell as a source of antigen: It is now generally accepted that antigenicity of tissue with regards to transplantation immunity resides within the cell itself. Certain reasons—theoretical and experimental—for this assumption are as follows:

(a) Since transplantation immunity developed to one tissue imparts immunity to other tissues from the same donor, a common determination factor must be involved. DNA and RNA are involved in the cells' genetic make-up and certain evidence indicates that they may play a role in determining cellular antigenic specificity.

(b) A technically correct graft procedure survives for a short period indicating that it was not initially recognized as foreign. Information is imparted to the host while this tissue is viable and this may be in the form of a factor ordinarily present in an intact cell, eliciting immune response in foreign surroundings.

(c) Circulating tissue antigen is apparently absent in the homograft state despite violent immune responses. It may be that it is present in a form undetectable by present day methods.

(d) Cell damage may be necessary for antigenic information to be transferred from donor to host. This could explain the permanent nature of certain small grafts, i.e., antigen is carried to host at time of transplant, but is inadequate to initiate response. If it were coming from viable cells, one would expect outflow to be cumulative. This might also explain how using barriers, e.g., hamster's pouch lining, as a graft bed, facilitates survival or the mystery of preferred sites, e.g., anterior chamber of the eye, gonads, etc. These grafts are protected from the initial stimulus in cellular debris and when only viable cells remain, no further information is imparted.

To briefly summarize the foregoing: The homograft reaction is individually specific but has no tissue specificity. Every organ possesses the same antigenicity, i.e., any response to a graft may be obtained by previous sensitization with leucocytes, liver, spleen or kidney cell suspensions, etc. (RBC's do not have this power, adding to the theory that transplantation antigens reside in the cell nucleus.) It has been shown that a primary response to a homograft can be elicited between members of an inbred mouse strain by transferring into the recipient, lymphoid tissue from the draining nodes of an unrelated mouse undergoing a primary response. It may be said that regional lymphoid tissue becomes activated by homograft reactions leading to the production of immunologically potent cells or cell bound antibodies. These facts

along with other similarities, e.g., lag period, type of cellular infiltration, etc., would seem to justify our regarding the homograft response and hypersensitivity of the delayed hypersensitivity type as closely adjacent wave lengths on the immunological spectrum.

*Methods of Weakening the Homograft Reaction of Producing Graft Tolerance*

1. Non specific—a large number of substances have been found which decrease the violence of the reaction in a non specific way.

(a) Hormones—chiefly ACTH and Cortisone—systemically and locally have been found to prolong graft-survival time. Their effect is least in man.

(b) Irradiation—total body irradiation of recipient in large enough doses may actually produce total acceptance of the graft, by inducing a total failure of the central immunological mechanism. The hemopoietic tissue of mice so treated, has been replaced by that of normal mice with resultant take. However, it is found that while the host is completely tolerant of the donor tissue, immunologically competent cells of the latter eventually rebel against the host's tissues causing death.

(c) Dyes, e.g., Trypan blue—probably due to the selective interference with antibody producing cells, are known to prolong graft survival time.

(d) Agammaglobulinemia—in this condition non specific homografts are sometimes possible. This is due not to the lack of gammaglobulin and circulating antibodies, but to the general depletion of lymphoid tissue.

2. Specific

(a) Acquired tolerance—Burnet's self marker theory postulates that organisms during development learn to recognize their own tissues and that this process of learning occurs in a certain span of time and is not a spontaneous, inherent state of affairs. Thus if antigen is introduced into the young animal, during its immunologically formative years, it might become accepted as "self antigen", e.g., dizygotic cattle twins with their synchorial placenta are able to eventually accept each other's grafts.

The cells used for tolerance induction may actually repopulate the host's lymphoid tissue, i.e., taking over his immunological responsibilities, or they may act by some yet unknown mechanism. The result is a blockage of or deviation from the normal pathway of immunity production with respect to the specific antigen. This is inheritable and transferred to the entire immunologically active cell population, because otherwise any remaining immunologically potent cells would be able to destroy the graft.

*Clinical Application*

Much of the work done in the field of tissue transplantation is still in the realm of the experimental and theoretical. The vastly greater portion of the experimental work has been carried out in animals and animal experiments are not held in high regard for application to man.

Autoimplantation of various organs is highly developed and has been carried out with success in man. Homotransplantation is still not an effective reality despite the occasional successful transplant, e.g., kidney between identical twins. The widely pub-

licized bone and eye banks, where spare tissue is stored awaiting its use in other individuals do not mean routine successful homotransplantion. The cells of these tissues do not survive as such but merely serve as scaffolding along which the host develops his own healthy tissue.

Out of the attempts to overcome the homograft reaction has come an interesting practical breakthrough which utilizes the reaction itself. In choriocarcinoma the cells arise from the fertilized egg, i.e., they derive half their genetic complement from the male. This portion of the tumor would also contribute to its immunological expression and an attack against the malignancy might be induced by reinforcing the patient's immunological defences. In other words provide antibodies against male tissue. This has been done in cases with known metastases that were progressing despite conventional therapy. Immunization was carried out passively by subcutaneous injections of the husband's sperm and passively by serum from immunized rabbits.

Is it possible that some day the past futile struggles against malignant disease will be looked upon with the same incredularity which we now regard infectious disease in the pre-antibiotic days?

As regards the transplantation of organs, I have little doubt that the immune barrier will one day be crashed. The major work will, of course, come from the laboratory. The surgical techniques have in many cases already been mastered and the newer ones will be, with relative ease.

To the imaginative there are far reaching possibilities. Think of the young person with healthy tissues and organs who dies accidentally. His demise will not be so tragic, nor so wasteful, when we are able to transplant his healthy kidneys or liver into the dying uremic or cirrhotic. What of exchanging new hearts for old, or the wildest if imaginings—parts of the brain. We are on the threshold of a medical and surgical era, which is destined to affect clinical practise profoundly. During the years to come the surgeon will be called upon to do fewer resections of organs and portions of organs. The newborn science of homotransplantation with its close kin immunology will open the path to the surgery of the future—that of transplantation.

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