Tissue Transplantation: Scope and Prospect

Partial solution of the problem of homograft rejection leaves other formidable problems still to be solved.

R. E. Billingham

The field encompassed by the term "transplantation biology" is extremely broad and has many facets, though it is unquestionably the clinical aspects of the subject which have been responsible for focusing so much professional and lay attention upon it during the past two decades. Transplantation of cells, tissues, and organs provides the basis of powerful analytical procedures applicable to a wide range of problems in genetics, embryology, physiology, oncology, and so forth. In embryology it is the classic method of studying the interactions of cells of different types, as, for example, in the process of induction. The manner in which genes determine skin and hair color has been elucidated in part by studies involving the incorporation, through grafting, of melanocytes with one genetic constitution into epidermal derivatives with a different one (*1*). There are striking, functionally important differences between the epidermis of different regions of human skin, as exemplified by the exquisite transparency of that of the cornea, the toughness of that of the sole of the foot, and the hardness of that of nails. To determine the manner in which these differences are conserved throughout life required grafting experiments in which epidermis from one region is artificially combined with dermis from another. These have shown that, with a few exceptions, maintenance of the specificity of most types of epidermis is the outcome of persistent inductive stimuli from the underlying dermis (*2*).

Transplantation has been crucial among the techniques which have led to the recent unraveling of the role of the thymus in the development and functioning of the machinery of immunological responses (*3*) and which have provided most of our knowledge of the life history of the small lymphocyte of the blood stream (*4*).

One of the most important approaches to the problem of senescence—apart from armchair contemplation—involves the application of grafting to produce heterochronic chimeras (5), that is, individuals in which a particular organ, or part of an organ, is derived from a donor very much older or younger than the host. Findings to date are consistent with the thesis that tissues and organs have a finite life span, however they are maintained.

The increasing repertoire of markers which can be employed to follow the fate of transplanted cells includes abnormal chromosomes, sex chromosomes and nuclear satellites or Barr bodies, isoantigens, enzymes, abnormal hemoglobins of red cells, and a variety of radioisotope labels. Their availability has greatly sharpened the discriminatory power of grafting techniques.

Not infrequently, transplantation affords a simpler and more convenient method than tissue culture for appraising the ability of some tissues and cells, and even organs, to withstand certain treatments in vitro, such as exposure to thermal and other stresses (as in the development of storage procedures) and exposure to drugs. As a means of evaluating tissue viability, transplantation is frequently superior to cultivation in vitro, since in vitro explants normally lose their characteristic histological organization. For example, a piece of skin grafted to an appropriate site will establish not only cell survival, but the survival of skin with its ancillary structures as an organized, functional tissue.

Types of Graft

Grafts may be single, discrete cells, such as those of tumors or fertilized ova (we mustn't forget that the implantation and development of the mammalian conceptus is a kind of transplantation). Grafts may be suspensions of cells in fluid media, such as blood and lymph; solid tissues, such as skin and cornea; or organs as complex as kidneys or livers. Mention must also be made of experimental parabiosis, the grafting of the body of one individual onto that of another. Even nonviable grafts of some tissues, including bone and cartilage, are now widely employed in replacement surgery, as indeed are blood-vessel segments, heart valves, corneas, and so forth, fashioned entirely of synthetic polymers. "Contour remodeling," both by insertion of solid prostheses and by injection of fluids such as dimethylpolysiloxane, is being practiced on an increasing scale by plastic surgeons (*6*). It would be foolish to omit such allegedly inert grafts from our deliberations since knowledge of the reactions of the host's tissues towards them is important. Will they prove oncogenic?

Technological and Immunological Problems

Whether one is concerned with transplantation of living tissues for experimental or therapeutic purposes, problems of two quite distinct categories are involved.

The first category includes problems concerned with the act of transplantation itself, which are essentially of a technological nature. They include the procurement, preparation, temporary storage, and, finally, the relocation of the graft in a living organism in such a manner that the normal healing processes, in conjunction with vessel and duct anastomoses where necessary, will procure its continued viability and

The author is professor and chairman of the Department of Medical Genetics, University of Pennsylvania Medical School, Philadelphia. This article is based on lectures given at Trinity College, Hartford, Connecticut, the National Institutes of Health, and the American Academy of Arts and Sciences.

conservation of normal structure and function. On the basis of extensive work on dogs and other animals, in most cases substantiated by trials in man, it can confidently be stated that the technical feasibility of replacement by grafting of bone marrow, lymphoid tissue, bone, cartilage, extensive areas of skin, corneas, some endocrine organs such as ovaries and parathyroids, kidneys, hearts, lungs, liver, the uterus, segments of the small intestine, and even entire limbs is firmly established (*7*). This impressive list indicates that adequate, though not necessarily completely satisfactory, solutions to these problems have now been evolved. Certainly there is still great scope for future investigation and improvement here, including the rationalization and better understanding of effective but empirically devised procedures. For example, it is well known that, provided it is not too thick, a sheet of skin grafted to a clean vascular bed and held in place for about 4 days will become united—that is, will "take" —and acquire a good blood supply of its own accord. But we are still uncertain whether the old blood vessels are utilized or whether they degenerate and are replaced by ingrowth and development of new vascular sprouts from vessels in the graft bed (*8*).

In surgery, skin grafting is necessary to make good a shortcoming of nature, for if the full thickness of the skin is lost or destroyed, it is never regenerated. The best that man can do is to produce a disfiguring and often function-impairing scar. However, some species of deer regenerate considerable areas of skin, each year, throughout life. The velvet that covers their growing antlers is *skin*, in every sense of the word. The application of transplantation to study this phenomenon of cutaneous neogenesis in antler formation may conceivably indicate ways of revealing and possibly exploiting a latent regenerative potential in human skin (*9*).

The second category of problems entailed in grafting is much more formidable and stems from the incompatibility of homografts—grafts exchanged between genetically dissimilar individuals of the same species. After a transient period of functional well-being that rarely exceeds a week or two, homografts normally fail where autografts of the same tissue or organ are permanently successful. This latent, unrelenting state of intolerance of our bodies towards grafts from someone else is a barrier to successful homotransplantation in man and practically all other vertebrates (*10*). Of course there are some special dispensations. Natural homografts, represented by mammalian fetuses, are not rejected for reasons that are as yet not fully understood (*11*). A large proportion of corneal homografts are also exempted.

Experimentalists can often circumvent the homograft barrier by employing animals of inbred, genetically uniform strains. Likewise, their genetic uniformity underlies the complete compatibility displayed by identical twins toward each other's grafts.

Little did the surgeons of the last century appreciate the formidable nature of the homograft problem when they predicted, as for example did George Lawson (*12*) in 1870, that " . . . The time will come when we shall beg portions of skin from a parent or friend who is willing to give of his abundance for the relief of a suffering child or neighbor."

Upwards of half a century of research has taught us that homograft destruction is an immunological phenomenon, the outcome of a highly specific attack on the part of the host against foreign genetically determined cellular isoantigens known as transplantation antigens.

By the early 1950's various means of prolonging the life of homografts in experimental animals had been discovered, including x-irradiation and administration of steroid hormones to the host (*10*). Most important of these was the discovery that if very young animals are inoculated with suspensions of living homologous cells, they may be rendered completely and specifically incapable of reacting against subsequent grafts from the donor of the perinatally inoculated cells, in later life, without impairment of their well-being. This induced specific state of unresponsiveness of antigenic material is known as immunological tolerance.

Once it was established that the homograft problem was, at least in principle, soluble, geneticists, immunologists, surgeons, biochemists, and others, in ever increasing numbers, began to focus their attention upon the clinical homotransplantation of organs. As a zoologist working in transplantation biology well before it had attained its current level of popularity, I was amused by a surgeon's recent pronouncement that " . . . workers in previously unpopular fields may be richly recognized when a meandering main stream of scientific investigation chances to flow their way. What atomic energy and satellites did for the physicist, transplantation and related problems in cancer and other fields, are doing for the geneticist, the immunologist and the histochemist" (*13*).

Improving Clinical Results

With this general outline of the scope and potentialities of transplantation biology as an essential background, I want to try and give some idea of what I believe might reasonably be accomplished in the next decade in the clinical application of homotransplantation. Rather than attempt a series of prophetic statements, I shall outline some current problems and try to indicate, where possible, the lines along which they may be solved.

We can no longer regard renal transplantation in man as an uncommon event, for since 1955 more than 1000 people have received kidney grafts. So far as kidney homografts are concerned, the procedure is still an experimental one because its results are unpredictable (*14*). By long-term administration of various immunosuppressive drugs, a host's resistance to a homograft can frequently be held in abeyance for a long time—for months, or even a year or two, or even more, in exceptional cases (*15*). Obviously, success depends upon the ability to find a dosage of the drug that will suppress the homograft reaction and yet will not impair the host's immunological defense machinery to an extent that renders him incapable of coping with the common pathogens of everyday life. High dosages of immunosuppressive agents are harmful to other cell systems of the body, too.

As one might have guessed from the genetic determination of homograft incompatibility, the results are very much better when donor and host are related, as between parent and offspring, or between full siblings, than when they are unrelated (*15*). The degree of "foreignness" of the graft—or its antigenicity—tends to be less in the former category. Weak histoincompatibilities are much easier to overcome with immunosuppressive drugs than

strong ones, requiring smaller dosages.

Two very important and unexpected facts have emerged from extensive studies carried out on renal homografts in dogs and man.

1) During the course of immunosuppressive therapy the host quite frequently does launch an immunological attack against the graft, of sufficient intensity to impair its function and threaten its life. If detected early enough, this reaction can often be reversed by treating the patient with corticosteroids, actinomycin C, and other agents such as x-rays (*14*). More important, once the tide of immunological attack has been turned, it may remain quiescent for a long time, despite resumption of the more conservative "pre-attack" drug schedule. Clearly, an understanding of the processes underlying such delayed attacks is of the utmost importance. Does this quiescent state following such an attack indicate the total destruction of a specific immunologically activated lymphoid-cell population?

2) For years, most experimentalists felt that study of the renal homograft problem was a waste of time until means of enforcing acceptance of a skin homograft had been devised. Skin is so much easier to transplant, they argued, and since there is no evidence of any tissue specificity in transplantation immunity, why go to all the trouble of grafting kidneys? Thanks to their enthusiasm and persistence, surgeons have finally disproved this premise, and we are now rid of what Medawar (*16*) has recently termed "the doctrinal tyranny of skin grafts."

Immunosuppressive drugs that will give renal homografts reasonable security of tenure are relatively ineffective with homografts of skin (*17*). Clearly, special dispensations apply to renal homografts, and it remains to be seen whether they apply to other organ homografts that require immediate vascular anastomoses. I believe they will be found to do so and that what is so peculiarly exacting about the skin graft is the site to which it is transplanted, rather than any antigenic peculiarity of this tissue.

There are three different lines of investigation which should lead to considerable improvement in the outcome of clinical homotransplantation:

1) *Development of more effective and more selective immunosuppressive drugs.* Immunological responses are a heterogeneous class of phenomena, put into effect in different ways. Furthermore, not all immunological responses are beneficial. Our reactivities to poison ivy, homografts, and possibly to certain ingredients of some of our own internal organs (in the so-called autoimmune diseases) do us a great disservice. It is generally believed that these reactivities are all put into effect by blood-borne cells of the lymphocytic series, in contradistinction to humoral antibodies which appear to mediate most of the beneficial immunological responses. There are some grounds for belief that there is a functional division of labor in the cellular components of our immunological response machinery (*18*), and consequently, at least some hope that it may be possible to produce drugs which act predominantly against the moiety responsible for the so-called cellular immunities.

Mention must also be made of the fact that we still do not know the proximate "cause of death" of a homograft. Most authorities believe that the agents of destruction are indeed the mononuclear cells of the lymphocyte series which leave the blood vessels and infiltrate the parenchyma of the graft in formidable numbers. Confirmatory evidence has been provided by recent studies on homograft reactivity at the cellular level in vitro (*19*). These have shown very clearly that lymphoid cells from specifically sensitized animals are able to destroy target cells in their vicinity; that is, that immunologically activated lymphocytes have cytocidal properties. Further work along these lines should indicate how this cytocidal effect is mediated. Is a "cell-bound" antibody involved, or the release of some kind of pharmacologically active substance analogous to histamine? If the latter proves to be the case, then another portal for possible pharmacologic attack will be available.

2) *Matching or typing donors and recipients.* The line of approach that seems to offer the most immediate promise involves devising means of donor selection, analogous to blood typing, which will enable surgeons to exclude donors whose grafts would be grossly incompatible for a given recipient.

Effective means of selecting the most compatible donor from a small available panel now seem to be almost within our grasp (*20*). However, the ultimate goal here is to be able to develop highly specific monovalent antibodies capable of identifying at least the more important transplantation antigens that contribute to an individual's antigenic uniqueness. We can confidently anticipate that within a few years individuals will be typed with respect to their transplantation antigens, as they can presently be typed with respect to their blood groups. As this work progresses we shall see the development of the genetics of tissue incompatibility in man, concerning which we know practically nothing at present. How many histocompatibility group systems are there in man, and how many of them are important—that is, determine strong antigens?

3) *Employment of the principle of immunological tolerance.* Although we may confidently anticipate that improved and more consistent results will occur from immunosuppressive drug therapy in conjunction with donor matching, application of this combination is likely to represent no more than an interim solution to the clinical homograft problem. The ideal solution requires a treatment that will render the host completely and specifically tolerant of the alien antigens of his homograft, obviating the requirement for continued drug therapy with its undesirable side effects. The principle of immunological tolerance, mentioned above, has been shown to apply to adult animals (*21*), as well as to infants, though when suspensions of living homologous cellular inocula are employed to induce tolerance, the repeated dosages required to overcome major incompatibilities are frighteningly large. In mice, they may amount to several donor equivalents of splenic cells. This approach in man would probably fail because of inability to obtain sufficient donor cells; it would certainly be very dangerous because of the risk of causing harmful graft-versus-host reactions, on the part of inoculated ingredients of the donor's lymphoid system, against the foreign antigens of their host (*10*). However, some of Medawar's (*22*) experiments conducted on mice indicate that partially degraded antigenic extracts of cells, appropriately administered, can lead to tolerance, and that they may do this more effectively if administered in conjunction with immunosuppressive agents. But this still leaves us with the dosage problem. Recent developments suggest that this may be far less formidable than anticipated. Certain rather crude evidence, based on studies of adult hosts exposed to tissue or cellular homografts under special

circumstances (*23*), in conjunction with Mitchison's sophisticated studies (*24*) on the responses of mice to a heterologous protein antigen, bovine serum albumin, indicate that there are two different dosage threshold requirements for tolerance-induction, rather than sensitization. The lower threshold for tolerance-induction may be very small indeed where chronic exposure is involved.

One of the most important problems in transplantation immunology, pertaining to the induction of tolerance discussed above, is concerned with the definition in biochemical terms of the nature and biological significance of transplantation antigens, concerning which our present knowledge is still very rudimentary (*10*). It has been suggested that antigenic material may one day be prepared in large amounts from different human cell lines propagated in vitro. Typed "antigen" then might be available to induce tolerance of appropriately matched organ homografts in patients.

A variety of possible means of improving the effectiveness of immunosuppressant agents, facilitating the induction of tolerance, or both, are currently under review. These include: thymectomy, splenectomy, reduction of the recirculating small-lymphocyte population of the body by placement of a cannula in the thoracic duct, by local irradiation of the blood stream by means of a cartridge containing radioactive yttrium or strontium suspended in a major vessel, or by extracorporeal irradiation of the blood as it passes through an arteriovenous shunt; or administration of an antiserum produced in an alien species, that is specific to the lymphocytes of the organism (*25*).

Although alteration of the antigenicity of a graft has long been discounted as a practical approach to the homograft problem, a recent report that skin homografts from cancer patients may long outlive similar grafts from normal donors (*26*) indicates the need for an open mind.

Procurement of Tissues and Organs

For years it was believed that the homograft barrier was the major obstacle to development of the surgery of organ replacement on a large scale, but now that effective means of overcoming this are practically within reach, we are becoming painfully aware of the existence of other formidable problems. It has been estimated that in the United States renal transplantation might be able to save 60,000 to 90,000 lives per year. With hearts and livers the figures might well be much higher, but these are not the only organs to be considered.

There are few organs a healthy, living person can donate. We can live perfectly normal lives on one kidney. Hence, if it becomes possible to ensure the long-term success of renal homografts, it may be possible to find enough live donors. But what about the other organs such as heart, liver, and lung? (Although one can live on one lung, to donate a healthy lung would probably involve too serious a surgical risk to be acceptable.) The only conceivable source of supply for these is the cadaver. However, few people die under circumstances in which donation of an organ is a practical possibility (*27*). With some notable exceptions, including skin, cornea, and possibly limbs and lungs, internal organs—certainly kidneys and livers—deteriorate very rapidly after death. Indeed, the process may have commenced in the terminal stages of life. To retard this process, steps must be taken immediately after death to cool the potential organ graft almost to the freezing point as rapidly as possible. An alternative is to keep the body alive with the aid of some kind of heart-lung machine. I need not emphasize the serious ethical and medicolegal problems associated with the employment of living or cadaver donors (*28*). Among other things they include having to make a prompt distinction between "the quick and the dead."

Even when a healthy, viable organ has been obtained, unless it can be transplanted immediately, there is the problem of its preservation. It is well established that when impregnated with certain protective agents, such as dilute solutions of glycerol or dimethyl sulfoxide, a wide range of cells, and even some tissues, can be chilled slowly and frozen to very low temperatures (preferably to that of liquid nitrogen, −196°C) and stored in what amounts to a state of suspended animation for long periods (*29*). Subsequent rapid warming will revitalize them. Unfortunately, attempts to extend these principles to the preservation of organs, particularly kidneys, have been unsuccessful. There is a tremendous gap between our knowledge of the conditions required to preserve isolated cells and small fragments of tissue and our knowledge of those required for an organ as large and complex as a kidney.

The current useful storage life of a human kidney, at a few degrees above freezing point, is well below 24 hours. If this could be extended only to 4 days it would have a considerable impact on the logistics of organ transplantation. Although several ingenious lines of research are being pursued, including the application of hyperbaric oxygen and attempts to inhibit metabolic activity by means of specific nontoxic chemical agents, I believe that this problem will take many years to solve. One fruitful line of study might be to investigate how a hibernating mammal, such as a woodchuck, is able to "store" itself for a week or two at temperatures near freezing.

Are there any alternatives to what Lederberg (*30*) has referred to as the "potential dehumanizing abuses of a market in human flesh"? Because of nonavailability of live human donors for human renal transplantation, a few intrepid surgeons have tried to use heterografts from baboons and chimpanzees. To everyone's great surprise, one patient was sustained for 9 months with a chimpanzee kidney (*31*). This finding is impressive in the light of the promptness with which heterografts are usually destroyed. However, chimpanzees are not abundant, nor do they breed rapidly. Furthermore, so far as man is concerned, it looks as though the problem of overcoming immunity to heterografts, even from near primate relatives such as chimpanzees, is much more exacting than overcoming resistance to homografts.

Other Therapeutic Applications

There are some serious hereditary and other diseases in man—the hemoglobinopathies—in which the red blood corpuscles are functionally defective. There is a sound experimental basis for the belief that solution of the donor-selection problem will make it possible to employ "grafts" of bone marrow to correct these conditions (*32*). Indeed, there are already several cases on record in which hemopoietic tissue grafts from identical twin donors have been successful in the treatment of severe hypoplastic anemia. Technically, marrow transplantation is a relatively simple procedure. This rather pulpy tissue can easily be aspirated from the mar-

row cavities of the donor (its loss is soon made good) and injected intravenously into the patient very early in life. The cells can find their own way to appropriate sites which may be "cleared" beforehand by x-irradiation or other means, though this may not be necessary.

Likewise, it may be possible to correct certain hereditary and acquired deficiencies on the part of the immunological defense machinery, such as hypogammaglobulinemia (*33*), by means of infusions of lymphocytic cells fractionated from the bloodstream of well-matched donors.

The possibility of being able to sustain the life of an individual during an acute self-limited illness, such as potentially reversible failure of the liver, by temporarily uniting his circulation with that of a healthy subject, has been voiced by clinicians for hundreds of years. The results of a few recent attempts to apply this principle (*34*) indicate that if its immunological hazards could be minimized by histocompatibility tests, it might have some usefulness.

Some partially successful attempts to treat patients suffering from acute liver disease by perfusing their blood through fresh, isolated pig livers for a few hours have been reported (*35*).

Epilogue

It is unfortunate that a not insignificant proportion of renal grafts, including some transplanted between identical twins where there is complete histocompatibility, ultimately succumb to the disease which destroyed the native organs (*15*). In such cases organ replacement represents the treatment of a symptom rather than of the basic disease process. Perhaps future studies will suggest means of early diagnosis of the disease and effective means of treatment will be found, obviating grafting in a significant proportion of cases. I believe that similar considerations will be found to apply to other organs for which replacement presently seems to be the only solution.

A few years ago surgeons were tremendously excited with the results obtainable with the aid of blood-vessel homografts, which, it was found, did not have to remain viable to fulfill a useful role. The fibrous matrix of the grafts became repopulated by cells of host origin and their lumens remained open. Then it was found that viability of the grafts, even at the time of transplantation, was not necessary; and finally, that perfectly satisfactory results could be obtained with vascular prostheses made of synthetic fibers. The viable graft was thus an important evolutionary stage in the surgical solution to a vascular problem.

So, I believe, it will ultimately prove to be with kidneys and hearts. Considerable progress has already been made in the development of mechanical prosthesis for both of these organs, especially the kidney (*36*).

So far as other organs are concerned, for which there are no reasonable grounds for belief that mechanical substitutes will be developed within the foreseeable future, the cadaver must be regarded as the only source of supply. It is ironic that the one tissue that can most easily be obtained from cadavers and which can easily be preserved at low temperatures for prolonged periods—skin—is the one against which the homograft barrier seems to be so unrelenting.

Although problems connected with its clinical application will dominate transplantation biology for many years to come, the field still has great potentialities in other directions, largely because of its versatility as a tool in fundamental biological research.

References and Notes

1. W. K. Silvers, *Science* **134**, 368 (1961).
2. R. E. Billingham and W. K. Silvers, in *Biology of the Skin and Hair Growth*, A. G. Lyne and B. F. Short, Eds. (Angus and Robertson, Sydney, 1965), p. 1.
3. R. A. Good and A. E. Gabrielsen, Eds., *The Thymus in Immunobiology* (Hoeber, New York, 1964).
4. J. L. Gowans and D. D. McGregor, *Progr. Allergy* **9**, 1 (1965).
5. P. L. Krohn, *Proc. Roy. Soc. London Ser. B* **157**, 128 (1962).
6. T. D. Rees, J. Platt, D. L. Ballantyne, *Plast. Reconstruc. Surg.* **35**, 131 (1965).
7. F. D. Moore, *Give and Take* (Saunders, Philadelphia, 1964); R. R. Lower, E. Dong, N. E. Shumway, *Surgery* **58**, 110 (1965); R. A. Malt and C. F. McKhann, *J. Amer. Med. Assoc.* **189**, 716 (1964).
8. J. A. Haller and R. E. Billingham, in *Wound Healing*, W. Montagna and R. E. Billingham, Eds. (Pergamon, New York, 1964), p. 165.
9. R. E. Billingham, R. Mangold, W. K. Silvers, *Ann. N.Y. Acad. Sci.* **83**, 491 (1959); R. J. Goss, in *Wound Healing*, W. Montagna and R. E. Billingham, Eds. (Pergamon, New York, 1964), p. 194.
10. P. S. Russell and A. P. Monaco, *The Biology of Tissue Transplantation* (Little, Brown, Boston, 1964).
11. R. E. Billingham, *New Engl. J. Med.* **270**, 667, 720 (1964).
12. G. Lawson, *Trans. Clin. Soc. Lond.* **4**, 49 (1870).
13. J. D. Hardy, *Surgery* **56**, 685 (1964).
14. T. E. Starzl, *Experiences in Renal Transplantation* (Saunders, Philadelphia, 1964).
15. J. E. Murray, R. Gleason, A. Bartholomay, *Transplantation* **3**, 294 (1965); B. A. Barnes, *ibid.*, p. 812.
16. P. B. Medawar, *Brit. Med. Bull.* **21**, 97 (1965).
17. J. E. Murray, A. G. Ross Shiel, R. Moseley, P. Knight, J. D. McGavic, G. J. Dammin, *Ann. Surg.* **160**, 449 (1964).
18. N. L. Warner, A. Szenberg, F. M. Burnet, *Austral. J. Exp. Biol. Med. Sci.* **40**, 373 (1962).
19. W. Rosenau and H. D. Moon, *J. Nat. Cancer Inst.* **27**, 471 (1961); D. B. Wilson, *J. Exp. Med.* **122**, 143 (1965).
20. J. W. Streilein, R. E. Billingham, W. K. Silvers, *J. Amer. Med. Assoc.* **195**, 351 (1966).
21. G. Gowland, *Brit. Med. Bull.* **21**, 123 (1965).
22. P. B. Medawar, *Transplantation* **1**, 21 (1963).
23. R. E. Billingham and W. K. Silvers, *Proc. Roy. Soc. London Ser. B* **161**, 168 (1964).
24. N. A. Mitchison, *ibid.* **161**, 275 (1965).
25. A. P. Monaco, M. L. Wood, P. S. Russell, *Science* **149**, 432 (1965); B. A. Barnes, G. L. Brownell, M. H. Flax, *ibid.* **145**, 1188 (1964).
26. D. B. Amos, B. G. Hattler, W. W. Shingleton, *Lancet* **1965-I**, 414 (1965).
27. N. P. Couch, W. J. Curran, F. D. Moore, *New Engl. J. Med.* **271**, 691 (1964).
28. M. F. A. Woodruff, *Brit. Med. J.* **1**, Pt. 2, 1457 (1964).
29. R. E. Billingham, *Surg. Gynecol. Obstet.* **121**, 275 (1965).
30. J. Lederberg, in *Man and His Future*, G. Wolstenholme, Ed. (Churchill, London, 1963), p. 263.
31. K. Reemtsma, B. H. McCracken, J. U. Schlegel, M. A. Pearl, C. W. Pearce, C. W. DeWitt, P. E. Smith, R. L. Hewitt, R. L. Flinner, O. Creech, *Ann. Surg.* **160**, 384 (1964).
32. E. S. Russell, *Fed. Proc.* **19**, 573 (1960).
33. R. A. Good, W. D. Kelly, J. Rötstein, R. L. Varco, *Progr. Allergy* **6**, 187 (1962).
34. J. M. Burnell, E. D. Thomas, J. S. Ansell, H. E. Cross, D. H. Dillard, R. B. Epstein, J. W. Eschbach, R. Hogan, R. H. Hutchings, A. Motulsky, J. W. Ormsby, P. Poffenbarger, B. H. Scribner, W. Volwiler, *Amer. J. Med.* **38**, 832 (1965).
35. B. Eiseman, D. S. Liem, F. Raffucci, *Ann. Surg.* **162**, 329 (1965).
36. *Proceedings of Conference on Hemodialysis* (U.S. Public Health Service Publication No. 1349, Government Printing Office, Washington, D.C., 1964).