Tissue Cultures: Transplantation without Immune Suppression

An apparently immutable law of immunology is that transplantation of tissues to an antigenically dissimilar host inevitably leads to rejection of the transplant. Recent developments suggest, however, that this law may have a crucial loophole. According to William T. Summerlin of the Sloan-Kettering Institute for Cancer Research in New York City, certain types of animal and human tissues lose their ability to provoke an immune response in a new host if the tissues have been grown in a culture medium for a critical period of time prior to transplantation.

Summerlin's results have sparked a great deal of both enthusiasm and skepticism among immunologists: skepticism because his results contradict the conventional wisdom of the field, and enthusiasm because verification of his findings would greatly expand the potential applications of transplants. At the very least, burn victims and other patients requiring skin grafts might no longer have to serve as their own donors and simple organs such as corneas could be preserved for transplantation for much longer periods of time than is now possible. At best, an explanation of the phenomenon might lead to a much higher success rate for transplantation of large organs and to a greater fundamental knowledge of the immune system.

What Summerlin has observed, in essence, is that skin, corneas, and certain other types of tissue can be kept viable for as long as several months when they are grown in a conventional tissue culture medium. A substantial fraction of the cells in the cultured tissue die; but, after a critical period of time unique to each type of tissue and to each species, the surviving cells may be transplanted to the original donor or to an antigenically dissimilar host. In either case, the transplanted tissue thrives without rejection and apparently retains its original identity and function.

Human skin, for example, after 4 to 6 weeks in culture, may be transplanted onto individuals whose histocompatibility antigens are grossly different from those of the donor. (Histocompatibility antigens are sites on the surfaces of cells by which the body's immune system recognizes whether a cell is foreign or native. Generally, even a very close match of these antigens in the donor and recipient is insufficient to guarantee the acceptance of a transplant.) The transplanted cultured skin, Summerlin says, does not elicit the production of either thymus-derived lymphocytes or blocking antibody, responses that are normally associated with the rejection of a graft.

The cultured skin appears to be universally transplantable: Summerlin has successfully grafted human, pig, guinea pig, and rat skin onto mice. The transplanted skin and new tissue derived from it maintains its original identity, however. White skin or fur transplanted onto a black host remains white. Female skin grafted onto males retains the Barr bodies characteristic of female skin cells. And, in mice and humans, the histocompatibility antigens of the accepted graft are those of the donor rather than those of the recipient.

Summerlin has also found that corneas from several species, including humans, can be kept healthy for months in a culture medium and, after a critical period of time, can be transplanted into rabbits without rejection or apparent loss of function. Human corneas can usually be preserved no longer than 48 hours without degeneration, and the success rate for cornea transplants is widely variable.

Mouse adrenal glands maintain their function in culture, Summerlin says, and after 21 days, can be readily transplanted into antigenically dissimilar mice that have had their adrenal glands removed. The transplanted gland apparently functions quite normally in the recipient, as is indicated by measurement of adrenocorticotrophic hormone stimulation and corticosterone concentrations. (Summerlin uses as recipients a strain of mice that have no accessory adrenal tissue that could produce such hormones.) The adrenal gland is the most complex organ Summerlin has transplanted, although he has also found that some types of cultured malignant mouse tumors can be grafted onto mice that will not accept grafts of fresh tumors.

Summerlin's results have, not surprisingly, been greeted with a great deal of skepticism by members of the immunological community, primarily because similar experiments performed many years ago were largely unsuccessful. Although several laboratories have been unsuccessful in attempts to duplicate his results, Summerlin ascribes their failure to simple technical difficulties in the culturing procedure. Some investigators have, however, verified at least part of his results.

In work that preceded Summerlin's, Barbara B. Jacobs of the American Medical Center at Denver has demonstrated that cultured mouse tumors can be successfully transplanted into antigenically dissimilar mice. Her results differ from Summerlin's in several major respects, however.

Jacobs finds, for example, that the cultured tumor is rejected if a noncultured tumor is transplanted onto the host animal at the same time; with skin, Summerlin finds that only the noncultured tissue is rejected. She finds that the cultured tumor is also rejected if the host is sensitized to antigens from the donor before the transplant is made; he observes no effect from prior sensitization. And, she finds that mice which accept grafts of cultured tumors are subsequently more tolerant to grafts of noncultured tissue from the same source, indicating that the graft has somehow changed the host's immune response; he observes no change in the host's response. Jacobs has also transplanted cultured mouse ovaries, and her preliminary results suggest that the grafted ovaries may be permanently accepted and that they function in a near-normal fashion.

Marvin A. Karasek of the Stanford University Medical Center, Stanford, California, has successfully grafted cultured human skin to antigenically dissimilar patients with chronic leg ulcers. Because of certain well-known peculiarities in the way such ulcers heal, however, Karasek interprets his results very cautiously and is unwilling to assert that the grafts have been permanently accepted.

Steven Codish of Peter Bent Brigham Hospital, Boston, Massachusetts, has successfully transplanted cultured human and mouse skin onto mice, although his success rate has been somewhat lower than Summerlin's. It took Codish more than a year of experimentation with culturing tech-

niques, he says, before he obtained his first retained graft. He has also maintained viable mouse jejunum, colon, stomach, and aorta tissues and mouse tumors in culture for extended periods, but has not successfully transplanted these. At least one other investigator in the United States and three in Europe have also duplicated some of his results, Summerlin says.

The cause of the phenomenon observed by Summerlin and the others is still virtually a complete mystery, but a minimum of five possible explanations have been offered:

► Some immunologists have suggested that the cultured skin, in which large numbers of cells have died, serves only as an inert matrix to support intrusive growth of the host's own cells. The primary evidence against such a possibility is Summerlin's observation that a successful skin graft can be removed from the host several months or years later and, without further culturing, can then be transplanted to either the original donor or a second recipient without rejection. (Jacobs has found that an accepted tumor can be transplanted to another member of the host's strain or to the original donor, but not to a mouse of a different strain.)

► Karasek thinks the most likely hypothesis is that the phenomenon involves the blood supply of the grafted tissue. Blood vessel cells, he argues, may be particularly labile in culture, so that acceptance of the grafted tissue would require the formation of new blood vessels in the graft. Revascularization of the tissue by the host would have the effect of prolonging the period during which the graft was initially exposed to the host's immune system, thereby preventing or delaying rejection of the graft. Both Karasek and Summerlin are performing experiments to determine whether the blood vessels of the accepted transplant are derived from the host or the graft. Revascularization will, however, likely also be rejected as an explanation if Summerlin's observation of the retransplantability of the grafts is verified.

► The histocompatibility antigens of the cultured tissue might be blocked or masked by some unknown substance that either is already in the culture medium or is produced by the cell during culture. Masking of the antigens would prevent the host from becoming sensitized during acceptance of the graft, but removal of the masking agent at a later date could lead to delayed rejection.

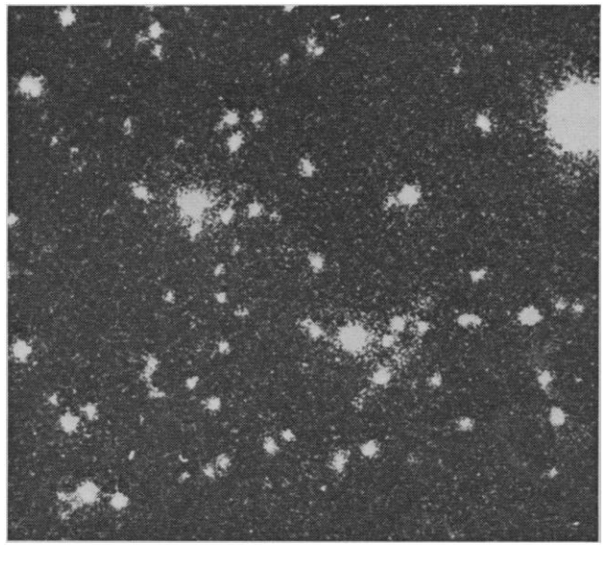
► Maintenance of the tissue in culture probably "washes out" the so-called passenger lymphocytes and other cellular components that might participate in sensitization of the host. This phenomenon, Summerlin says, might operate in conjunction with the revascularization of the graft.

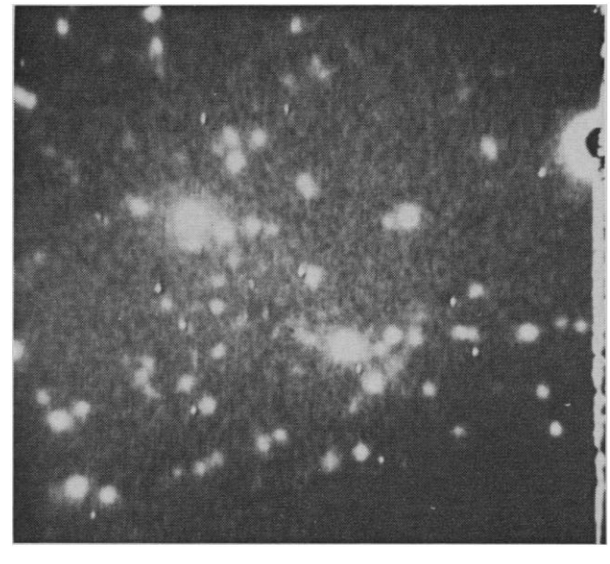
► Cultured skin cells, Summerlin says, superficially resemble the skin cells of embryos. It is thus possible that the cultured cells may be differentiating into a more primitive embryonic state in which they are unable to produce proteins, sugars, or other substances that might be recognized as foreign by the host. It is unclear how this type of differentiation would af-

Speaking of Science

Television-Type Sensors for Astronomy: New Pictures

At Mount Palomar, Mount Hamilton, Kitt Peak, and Mount Stromlo, indeed at almost every major observatory, astronomers are buying and testing television-type sensors. The small sensors alone cannot "see" very far, but the effect of attaching a sensor to a large telescope is to increase the telescope's effectiveness enormously. With this new bit of technology, the 200-inch telescope of the Hale Observatories could be made equivalent, for many purposes, to a 2000-inch telescope. In fact, because such great improvement can be achieved at relatively little cost with television sensors, the day of extremely large telescopes may be over. The most recently built U.S. telescope, dedicated on 20 June at the Kitt Peak National Observatory, has a mirror 158 inches (4 meters) in diameter and was built at about half the cost of a 200-inch instrument. With the exception of the





fect the functioning of larger organs.

There is, unfortunately, no biochemical evidence to support or reject any of these explanations. Summerlin reports that there are no other observable biological changes in the viable cells during the culturing, and that there are no gross changes in the culture medium. Investigators in his laboratory are now beginning a screening program to look for chemicals that might be shed by the cultured tissue, but this search is made difficult by the complexity of the culture medium—which makes identification of trace quantities of chemicals very laborious.

Confirmation and explanation of Summerlin's results could have great potential significance for the future of transplantation. Use of cultured tissues would, for example, provide a large new reservoir of skin for patients with burns and other superficial wounds, since skin from cadavers could be used routinely. Summerlin is now beginning studies with the Shriners Burn Institute in Cincinnati, Ohio, to explore this possibility. Culturing might also make it possible to store corneas and other simple organs for a much longer period of time than is now possible. An explanation of the phenomenon, moreover, might lead to a better understanding of the mechanism by which cancer cells are able to avoid stimulation of the body's immune system. But the potential for application of the technique to larger organs still remains questionable.

The most obvious problem is learning how to culture a large organ. Such organs are notoriously difficult to keep alive for any length of time. Isolated kidneys, for example, can be kept healthy by perfusion with blood or blood substitutes for no longer than 72 hours, by which time swelling of the endothelial lining of the blood vessels grossly restricts circulation through the organ. Total immersion of the organ in a culture medium might obviate this problem, but it would introduce the new problem of supplying internal tissues of the organ with sufficient oxygen. Summerlin's group is investigating techniques for culturing large organs, but their results, he says, while encouraging, are far too preliminary for comment.

Even if the organs can be cultured, other problems may arise. If the explanation of the phenomenon proves to be either intrusive growth by host cells or revascularization of the graft by the host, the culture technique would be patently unworkable for large organs. And if the explanation involves differentiation of the cultured cells to a more primitive state, it is quite possible that the transplanted organ would no longer be able to perform its function.

Transplantation of cultured tissue is obviously still at a germinal stage, and a great number of questions and problems remain. The most important problem, many immunologists argue, is confirmation that Summerlin's results are, indeed, valid. Beyond that lies explanation of the phenomenon and its application in clinical medicine. But the fruits of the continuing investigations promise to be very rewarding.

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Soviet Union, other countries constructing new telescopes are also building instruments with mirrors about 4 meters in diameter. (Five are planned.) An important element in the decision to build these telescopes on a smaller scale is the possibility of multiplying their light-collecting power many times with electronics.

The new sensors, which replace photographic plates in the operation of a telescope, are quite similar to the heart of a television camera in many cases, but are made of materials that are even more sensitive to low light intensities. The great advantage of television-type sensors is that they respond to as much as 90 percent of the light falling on them, whereas photographic plates typically respond to only 0.1 percent. The television-type sensors are also more effective at recording bright and faint objects side by side.

For about 2 years television-type sensors have been used to record the spectra of very faint objects, a process of recording data in essentially a one-dimensional form. But only recently have two-dimensional pictures been released for publication. The growth of research on the sensors has been rapid. A year ago there appeared to be only three systems actually ready to take data, but now there are many more. The excellent proceedings of a recent symposium at the University of British Columbia include 34 talks on different sensor systems (*1*).

Both pictures shown were made with the 200-inch telescope on Mount Palomar, as the telescope was pointed at a cluster of galaxies designated 0237 − 0138. To make the picture on the left, a photographic plate (103a-D) was placed at the prime focus of the telescope and exposed for 50 minutes. When the plate was replaced with an SIT (silicon intensified target) vidicon at the focus, only a 6-minute exposure was needed to make the image on the right. The vidicon picture appears worse on first viewing because it is fuzzier, but it actually contains more information than the photograph on the left. Because photographic plates have a threshold, the wispy outer parts of the galaxies do not show up in the photographic image. This deficiency for recording all the light that falls on the plate gives hard edges to the image and produces a pleasing picture, but important fine details are lost.

The image from the SIT vidicon is recorded directly onto magnetic tape in numerical form and simultaneously displayed on a small television so that the astronomer can see his picture immediately. All the electronics for the system, including a tape recorder that is compatible with a large computer, fit neatly into a compact 120-pound unit that rides aloft with the observer near the top of the telescope. The device was developed by James Westphal of the California Institute of Technology, Pasadena, California, and Hale Observatories.

Other types of vidicons are also being developed, and solid-state systems utilizing charge-coupled devices may soon be perfected to the point where an entire sensor, including a preamplifier and switching circuitry, can be contained on a thin piece of silicon. New sensors have already provided data on distant quasars that probably could not have been obtained any other way, and now that high-quality pictures can be made with electronics, the technology of television may revolutionize astronomy.

—WILLIAM D. METZ

References

1. Copies of the symposium, Astronomical Observations with Television-Type Sensors, 15–17 May 1973, are available for $10 from the Institute of Astronomy and Space Science, University of British Columbia, Vancouver 8, Canada.