sis of fetal distress. In such cases, it appears that the fetus is being deprived of oxygen and is at risk of brain damage. With the advent and widespread use of the electronic fetal monitor in the past decade, diagnosis of fetal distress has become more common. But it is still difficult to decide whether fetuses are distressed. The task force felt it had insufficient data on mortality and morbidity to recommend decreasing the number of cesareans done for fetal distress.

With the increasing popularity of prepared childbirth classes, nonmedicated childbirth, the presence of husbands in delivery rooms, home births, and hospital birthing centers, many couples look forward to idealized vaginal deliveries and feel cheated and disappointed by cesarean deliveries. Recognizing this, the task force recommended that childbirth classes discuss the possibility of cesareans and that hospitals consider allowing fathers in operating rooms to observe cesarean deliveries. The task force also recommended that hospitals reconsider the practice of routinely separating healthy babies delivered by cesarean from their parents so that the babies can be observed in special nurseries.

In the final analysis, however, the task force could make specific recommendations only about repeat cesareans. "Somewhere along the line we have to look at the morbidity issue," Rosen says. Until then, the task force is somewhat shackled in its attempts to discourage cesarean deliveries.

—GINA BARI KOLATA

Transplants (II): Altering the Donor Organ

Culturing, use of fetal tissues make it less likely that donor organ will provoke an immune reaction

Most approaches to prevention of rejection of the donor organ in transplants have usually involved suppressing the recipient's immune system. Some of the newer techniques (*Science*, 3 October, p. 44) promise selectivity in immune suppression so that the transplanted organ will not be rejected, but the recipient will retain enough immune function to fight off virulent infections.

An alternative approach is to work with the donor organ in an attempt to reduce its immunogenicity (ability to stimulate an immune reaction). The two most common ways to do this have been to culture the organ before transplantation and to use fetal tissues as the donor. These methods, when used in conjunction with some of the techniques discussed previously, could make it possible to transplant organs that have been exceptionally difficult to work with in the past.

Culturing as a transplantation technique was very nearly discredited 6 years ago when William T. Summerlin, then of the Sloan-Kettering Institute in New York City and the foremost advocate of culturing, was accused of fabricating some research results and misinterpreting others. Summerlin had claimed that simple in vitro culture of skin, corneas, and certain other tissues for periods of 1 to 2 weeks changed the tissues somehow so that they could be transplanted across immunological barriers without rejection. Other investigators were not able to reproduce Summerlin's findings, but there were enough successful results with other kinds of culturing to keep a few investigators busy exploring the concept.

In 1975, Kevin J. Lafferty of the Australian National University in Canberra and David W. Talmage of the University of Colorado Medical Center in Denver reported that thyroid glands could be transplanted across immunological barriers in mice if the glands were first cultured for 12 days at 37°C in a controlled atmosphere consisting of 95 percent oxygen and 5 percent carbon dioxide. During the culture period, they observed, the thyroid tissue retained its structural integrity, but leukocytes (white blood cells) trapped in the tissue gradually disappeared. They concluded that it is these passenger leukocytes that initiate the rejection of uncultured thyroid tissue, and that the antigens on the thyroid itself are relatively weak immunogens. As support for this hypothesis, they note that injection of the organ recipient with leukocytes from the donor leads to rejection of a previously stable thyroid graft.

Subsequently, Lafferty and, independently, H. W. Sollinger and his colleagues at the University of Wisconsin at Madison have shown that thyroid tissue can be transplanted from rats to mice by culturing in this manner. Lafferty has also shown that the parathyroid gland can be transplanted across major immunological barriers in mice after it has been cultured, and Clyde F. Barker and his associates at the University of Pennsylvania School of Medicine in Philadelphia have achieved similar results with the parathyroid in rats.

These findings support the belief of some investigators that classical endocrine tissues (which produce hormones) are less immunogenic than exocrine tissues (all others). Talmage, meanwhile, has found that length of the culture period can be drastically reduced by increasing the partial pressure of oxygen in the incubator, since lymphocytes are very sensitive to oxygen. The culturing time can also be shortened by treating the donor, before the tissue is surrendered, with cyclophosphamide, a drug that kills rapidly proliferating cells, such as lymphocytes.

Lafferty and Talmage's technique has been adapted by Richard Hong of the University of Wisconsin and Erwin W. Gelfand of the University of Toronto to treat children with severe combined immunodeficiency disease (SCID), an inborn defect that leaves the children highly susceptible to infection. Gelfand's approach is to obtain thymic tissue from a donor, mince it, and grow it in an incubator for 14 to 21 days under conditions similar to those used by Lafferty. This allows growth of thymic epithelium with loss of T lymphocytes.

He then extracts segments of growing tissue from the culture and injects them in the SCID children. Within about 6 to 8 weeks after the transplant, immune function is established in some of the children, allowing them to live a normal life for the first time. Of special importance, Gelfand notes, is the fact that the cultured tissue rarely produces graft versus host disease—a condition where lymphocytes produced by the donor tissues attack the recipient's tissues—and which

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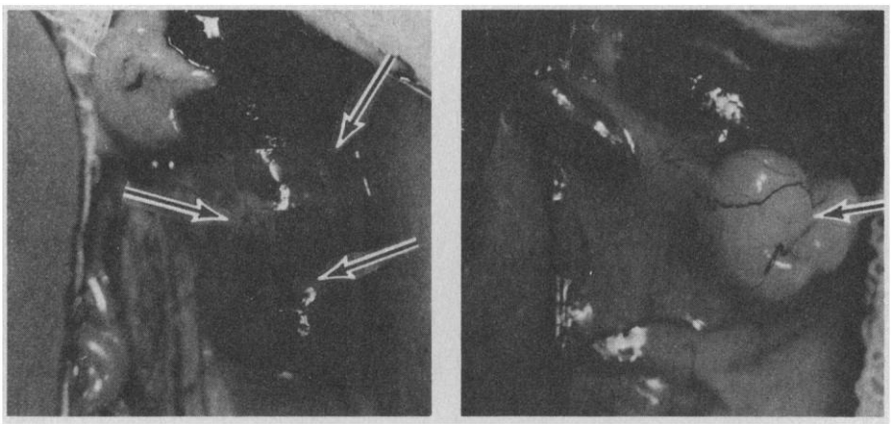

Pancreas transplants

Three fetal pancreases (left) indicated by the arrows have been transplanted under the kidney capsule of a rat. Six weeks later (right) the three have fused into a large fatty organ.

is a major source of problems if the graft is attempted without culturing.

Gelfand has transplanted thymic tissue in seven children, with a successful outcome in three cases. A similar technique has been used by Hong and by Raj Pahwa of Sloan-Kettering. The investigators are now at work solving several minor technical problems, including optimization of the culture medium and selection of the best candidates to receive grafts. They are also trying to learn more about the course of events during the culture period and whether culturing has any other effects on the tissues.

Much of the work on both culturing and fetal tissues has been conducted with the pancreas, the source of the hormone insulin, which is the primary regulator of sugar levels in the blood. The pancreas is a particularly difficult organ to transplant, says Peter J. Morris of Oxford University in England. For one thing, pancreatic tissue appears to be more immunogenic than either heart or kidney tissue. For another, diabetics are already very vulnerable to infection because of their disease, and immune suppression simply aggravates an already difficult situation.

Virtually all of the 101 patients who have received experimental pancreas transplants since 1968 have therefore been diabetics who were already immunosuppressed for a kidney transplant necessitated by the complications of diabetes. Most of the pancreas grafts have been rejected, according to David E. R. Sutherland of the University of Minnesota Health Sciences Center, and the longest surviving graft is only a little more than 4 years old. Many investigators thus hope that techniques to lower the immunogenicity of the pancreas might increase the success rate.

Because of the belief that endocrine tissues have a low intrinsic immunogenicity, some investigators have tried to separate islets of Langerhans, the clusters of cells that actually produce insulin, from the other pancreatic tissue. In addition to physical methods of separation, such as the use of enzymes that disrupt connective tissues, culturing seems to enhance separation. Several investigators have observed that islets seem to thrive in culture, while exocrine tissues wither away.

Paul E. Lacy and his colleagues at the Washington University School of Medicine in St. Louis reported in July that they were able to transplant rat islets into diabetic mice if the islets were first cultured for 7 days at 24°C under an atmosphere of air and 5 percent carbon dioxide (islets deteriorate rapidly under an atmosphere of 95 percent oxygen). The islets were injected into the portal veins of the mice, so that they could take up residence in the liver. Lacy also found it necessary to treat the recipients shortly before transplantation with a single injection of rabbit antilymphocyte serum prepared against rat lymphocytes.

Of ten mice treated this way, all retained functioning islets after 30 days; and seven retained the islets (and normal sugar metabolism) after 60 days. Several of these mice were still alive and required no exogenous insulin after more than 200 days. In contrast, all mice that received fresh rat islets rejected them within 12 days after the transplant. Lacy postulates that culturing removes most of the passenger lymphocytes that were initially present in the islets, and that the antilymphocyte serum scavenges any that are remaining.

Claes Hellerström and his colleagues at the University of Uppsala in Sweden and Orion D. Hegre and his associates at the University of Minnesota School of Medicine have, in a similar manner, transplanted islets across immunological barriers in mice. A group headed by Michelle Kedinger at INSERM in Strasbourg, France, has achieved the same feat in rats. Lafferty has recently shown that islets can be cultured in an atmosphere of 95 percent oxygen if 50 or so islets are aggregated into a large cluster. He has successfully transplanted the clusters across major immunological barriers in mice after culture periods of 7 to 12 days. None of these investigators has found it necessary to use antilymphocyte serum.

Morris, however, has observed no enhancement of graft survival with mouse islets cultured for 21 days. Why he alone has obtained negative results is not yet clear.

Lacy and his colleagues are now developing techniques to isolate islets from pig, hamster, and human pancreases; each of these is harder to isolate than rat or mouse islets, with human islets being the hardest of all. After working with each of these, they eventually hope to transplant islets in humans. They are also investigating other sites for injection of the islets since, in humans, they would like to inject them into the spleen. And finally, Lacy's group is investigating the potential of using antiserum against the Ia histocompatibility antigens found on T lymphocytes since this antigen does not appear in the islets themselves. If this should work, it might be possible to remove passenger lymphocytes without culturing the islets.

A partial foundation for use of islets in humans has been laid by John S. Najarian and his colleagues at the University of Minnesota Health Sciences Center. Najarian has removed the pancreas from eight patients with pancreatitis—a swelling of the pancreas accompanied by incapacitating, intractable pain—partially purified the islets, and injected them into the portal vein. At the present time, four of the patients treated in this manner require no exogenous insulin, two require only very small amounts, and two require slightly larger amounts. Using pancreases from cadavers, Najarian has also performed the same operation on seven diabetic kidney recipients. In none of these cases was the diabetes cured, but there was some transient evidence of islet function, including decreased insulin requirements and the presence in urine and blood serum of C peptide, a protein fragment produced during the synthesis of insulin.

Najarian's group was able to isolate from 24 to 55 percent of the islets from the pancreases they used, but most investigators who purify the islets to a greater extent obtain a much smaller per-

centage, too few to effect a cure of diabetes even if the graft were totally successful. Many transplant surgeons are therefore examining the use of fetal pancreases. Their hopes are based on observations by Barker and others that fetal skin is apparently less immunogenic than adult skin, since transplants of fetal skin survive signficantly longer than transplants of adult skin. There is little laboratory evidence indicating precisely when histocompatibility antigens appear in various tissues, and many investigators are trying to determine this now. There is also some evidence (*Science*, 26 December 1975, p. 1281) that endocrine tissues of the fetal rat pancreas develop before the exocrine tissues. It is thus possible to isolate the rat pancreas at a time, about 17 days after conception, when the islets are almost fully developed but when the exocrine tissue has only just begun to differentiate.

Among the first to adopt this approach were Josiah Brown and his colleagues at the University of California Medical School in Los Angeles. They began by transplanting fetal rat pancreases into diabetic rats of the same strain. They found that one fetal pancreas can, after a period of growth in the recipient, produce about 25 percent of the insulin produced by an adult pancreas, and that this amount is enough to restore normal sugar metabolism for the life of the rat. They also found that the animals' blood sugar must be carefully controlled with exogenous insulin for about 30 days after the transplant until the fetal organ becomes well established.

Brown and Yoko Mullen of UCLA, using a technique developed by Leslie Brent of the Medical Research Council in London, then began transplanting fetal pancreases across immunological barriers in rats. Transplanting skin in rodents, they found that rejection could be suppressed by injecting donor liver tissue into the recipient 2 weeks before the transplant—in effect, desensitizing the recipient to donor tissues in much the same way that a hay fever sufferer is desensitized to pollen and other allergens.

Early on, Brown and his group observed that fetal rat pancreas tissue is fully as immunogenic as adult tissues. Nonetheless, using Brent's technique as the primary tool for immune suppression, they found that fetal pancreases transplanted between strains with a minor degree of histoincompatibility were retained for life. Transplants across moderate barriers survived for 60 to 70 days, while those across severe barriers survived only 20 to 30 days. Similar results in which slightly different approaches were used have been achieved by Hellerström, Barker, Morris, and Klaus H. Usadel of the Johann Wolfgang Goethe University in Frankfurt, West Germany. Kedinger obtained similar results transplanting 15-day-old chicken pancreases into rats, but found that 18-day-old pancreases yielded little improvement in survival.

The goal of Brown and other investigators now is to further suppress the immune response in rats and other animals as a first step toward attempting transplants in humans. To do so, they will supplement fetal pancreases with the whole armamentarium of immunosuppressive regimens, including Brent's technique, antilymphocyte serum, total lymphoid irradiation, and culturing. Interestingly, Morris has found that cyclosporin A, a promising immunosuppressive agent for heart and kidney transplants, provides little benefit in transplanting islets.

Investigators are also starting to work with human tissues. Hellerström and John R. Turtle of the University of Syndey in Australia have each shown that fetal human pancreases can thrive in culture for as long as 3 weeks. Usadel has transplanted 26 human fetal pancreases into so-called nude rats and mice. The nude rodents do not have functional thymus glands, and thus do not reject transplants. Usadel found that 7- to 12-week-old pancreases grow and produce hormones after transplantation, while those that are older than 16 weeks do not grow. Brown and Hellerström are planning to do the same experiment with diabetic nude rodents to see whether the fetal human pancreases can cure diabetes.

Brown hopes to start human trials with fetal pancreases in about 3 years and will probably be the first in this country to do so; some investigators in Europe have already made preliminary attempts. Jean Meunier and his colleagues at the University of Bordeaux, France, report that they have eliminated diabetic symptoms in a young man by transplanting fragments from 21 fetal pancreases. The pancreases had been minced, frozen, and cultured for 5 days at body temperature. Menier plans to do more such transplants, but other investigators are skeptical about his results. Brown notes, for instance, that the patient had been diabetic for only 1 year before receiving the transplant, and he could therefore be passing through the well-known "honeymoon phase" of diabetes in which the disease temporarily undergoes a natural remission. It is also possible, he adds, that one of the fetal pancreases was fortuitously compatible with the patient.

Felix Largiader of Zurich has reported curing a diabetic man with the pancreas from a 2.5-month-old fetus. The recipient went through 6 months of recurrent rejection crises but is now stable and off insulin.

Other attempts have been less successful. Hellerström has attempted transplants in two patients with long-established diabetes. In the first case, six fetal pancreases were minced and cultured for periods ranging from 3 hours to 15 days (the pancreases were obtained at different times). The cultured tissues were then injected into the portal vein of the recipient. The patient's insulin requirement did not change, but after about 30 days C peptide appeared in his urine, suggesting that the grafted tissue was functioning. The concentration of C peptide was only about 5 percent of normal, however, and had completely disappeared after another 3 months. In the second case, Hellerström tried using more fetal pancreases, but no C peptide was ever observed.

Brown found that fetal pancreases transplanted between strains with a minor degree of histoincompatibility were retained for life.

Usadel dissected two fetal pancreases into about ten pieces and transplanted them into the brachioradialis muscle of the right arm of a woman diabetic who had recently undergone a kidney transplant. He also did not observe either C peptide or other evidence that the pancreases were functioning.

It is clear that both culturing and use of fetal tissues are very promising approaches to pancreas transplantation. It is equally clear, however, that neither technique is alone sufficient to provide success in humans. Although that success now begins to seem inevitable, it will most likely require a combination of immunosuppressive reagents and techniques in addition to manipulation of the donor organ before the cure of diabetes can be achieved in a significant number of patients.—THOMAS H. MAUGH II