TABLE 2. Comparison of graft function in CS and MP kidneys

	CS (n=13)	MP (n=13)
Immediate function	7.6%	38.5%[a]
Primary nonfunction	7.6%	0%
Postop. dialysis (ATN)	84.6%	61.5%[a]
Preservation-related	69.2%	61.5%
Early rejection	15.4%	0%
Mean period of postop. hemodialysis	12.4 days	8.0 days[a]
Best serum creatinine (mean)	1.70 mg/dl	1.69 mg/dl
One-month graft survival	76.9%	100%

[a] $P<0.05$.

rum creatinine level, and the one-month graft survival, the MP group gave a better result.

With waiting lists growing progressively larger, new efforts to increase the number of kidney transplants must be made if we are to cope with the present demand and expected future need. For this reason, all potential donors must be identified. Non–heart-beating donors should be considered. Historically, in an attempt to make the warm ischemic time as short as possible, the insertion of a specially designed double balloon catheter into the aorta via the femoral artery followed by in situ perfusion has been performed (*5*). Several approaches have been developed to improve the quality of organs from non–heart-beating donors (*5–7*). However, postoperative renal failure or nonfunction often occurs (*6*). Certainly, immediate function prevents rejection, permits early use of cyclosporine, simplifies fluid and electrolyte management, and obviates the cost and hazards of dialysis in the postoperative patient. It has been shown that, if delayed function occurs, there is a 17% reduction in graft survival (*8*).

For cadaver kidney transplantation, two preservation methods are widely used—CS and MP. Recent studies have reported that neither patient nor graft survival is significantly affected by the preservation method (*9–11*). As a result, a decided trend toward CS has emerged because it is simpler and more economical. However, there is some disagreement about the effect of the preservation method on graft function (*2*). To evaluate the relative merits of CS and MP, particularly with regard to early function, we initiated a prospective, randomized trial of CS versus MP in 13 Japanese non–heart-beating donors. This study indicates that MP may be superior to CS as a method for achieving optimal early graft function. MP was significantly better than CS in reducing the frequency of ATN and its severity in terms of length of dysfunction. Organ harvesting from non–heart-beating donors would greatly contribute to the donor pool. On the basis of the present study, MP can alleviate the severe early graft dysfunction that results in increased graft loss. We conclude that MP is a useful modality for improving the function of compromised renal grafts from marginal donors.

NAOTO MATSUNO
ETSUO SAKURAI
ISAO TAMAKI
MASAMI UCHIYAMA
KOICHI KOZAKI
MSAMI KOZAKI
Department of Organ Transplantation
Hachioji Medical Center
Tokyo Medical College
Hachioji, Tokyo, Japan

REFERENCES

1. Japanese Transplantation Society. The annual report of kidney transplantation in Japan. Jap J Transplant 1991; 26: 504.
2. Halloran P, Appile M. A randomized prospective trial of cold storage versus pulsatile perfusion for cadaver kidney preservation. Transplantation 1987; 43: 827.
3. Hoffman RM, Stratta RJ, D'Allesandro AM, et al. Combined cold storage perfusion preservation with a new synthetic perfusate. Transplantation 1989; 47: 32.
4. Canafax DM, Torres A, Fryd DS, et al. The effects of delayed function on recipients of cadaver renal allografts: a study of 158 patients randomized to cyclosporine or ALG-azathioprine. Transplantation 1986; 41: 177.
5. Anaise D, Yland MJ, Ishimaru M, et al. Organ procurement from non-heartbeating cadaver donors. Transplant Proc 1989; 21: 1211.
6. Anaise D, Smith R, Ishimaru M, et al. An approach to organ salvage from non-heartbeating cadaver donors under existing legal and ethical requirements for transplantation. Transplantation 1990; 49: 290.
7. Koyama I, Hoshino T, Nagashima N, Aadachi H, Ueda K, Omoto R. A new approach to kidney procurement from non–heart beating donors: core cooling on cardiopulmonary bypass. Transplant Proc 1989; 21: 1203.
8. Terasaki PI. Clinical kidney transplants 1985. Los Angeles, CA: UCLA Tissue Typing Laboratory, 1985: 170.
9. Vaughn W, Mendez-Picon G, Humphries A, Spees EK. Method of preservation is not a determinant of graft outcome in kidneys transplanted by Southeastern Organ Procurement Foundation institutions. Transplantation 1981; 32: 490.
10. Spees E, Vaugh W, Medez-Picon G, Humphries AL. Preservation methods do not affect cadaver renal allograft outcome: the SEOPF prospective study 1977–1982. Transplant Proc 1984; 16.
11. Rothental J, Herman J, Taylor R, Broznick B, Halaka T. Comparison of pulsatile machine perfusion with cold storage for cadaver kidney preservation. Transplantation 1984; 37: 425.

Received 14 July 1992.
Accepted 15 June 1993.

THE RECTUM AS A NOVEL SITE FOR ISLET CELL TRANSPLANTATION

Islet cell grafting has been proposed as an alternative to pancreas transplantation in the treatment of type I diabetic patients (*1*). The potential advantages of transplanting free islets as opposed to an organ (segmental or whole) are in the technical simplicity of the procedure, lack of complications related to the exocrine component of the pancreas, and avoidance of major surgery. Several sites for islet cell grafting have been explored, both experimentally and clinically. These can be considered in two groups: sites with systemic venous drainage such as the kidney capsule (*2*), subcutaneous and intramuscular planes (*3*, *4*), thymus (*5*), testis (*6*), and cisterna magna (*7*), and sites with portal venous drainage such

s the peritoneal cavity (*8*), omental pouch (*9*), spleen (*10*), nd liver (*11*).

Of all these sites, the one that would appear most acces- ible for implantation procedures and subsequent biopsy of mplanted islets is the subcutaneous or intramuscular pocket *3, 4*). However, studies have indicated that islet function in his milieu is insufficient to materially affect the course of the liabetic state, possibly due to poor perfusion of the grafted issue or systemic route of drainage. It has been argued that ites with portal venous drainage may afford better meta- olic control of the diabetic state (*12*). Studies in rats suggest hat less islet tissue is needed to reverse diabetes and im- prove metabolic control if the tissue is placed within the portal rather than the systemic venous system. Therefore, ites with portal venous drainage are probably the more ap- propriate choice for clinical transplantation, since it seems ikely that any clinical islet transplants performed at present will result in only marginal quantities of functional islet issue.

In this report, we describe the lower rectum as a novel site or transplantation of free islets. The reasons for selection of he rectum as a potential site are first, that there is a rich plexus of veins draining into the portal venous system, and second, that it is safe and convenient for access, both for grafting procedures and biopsies.

The superior hemorrhoidal venous plexus lies in the sub- mucosa of the upper part of the anal canal and the lower 2 cm of the rectum. Thence five or six collecting veins pass up- ward in the wall of the rectum; at first they run in the sub- mucosa but gradually they penetrate the muscle coat to be in the perirectal fat where they unite to form two main veins and eventually the single superior hemorrhoidal trunk, which is an important tributary of the portal vein. In addition, the inferior hemorrhoidal vein, which drains into the subcutaneous or external hemorrhoidal plexus and lower part of the anal canal, probably has communications also with the submucous or internal hemorrhoidal plexus and normally drains partly upward along the superior hem- orrhoidal veins unless there is some obstruction of the por- tal system (*13*).

Arising from a knowledge of the venous drainage system of the anorectum, it appears that the optimal site for transplan- tation of islets is the submucosal plane of the lower rectum and upper anal canal around the region of the dentate line. This study reports our preliminary experience in islet cell grafting using this site.

Syngeneic islet cell grafts were performed in PVG (RT1A) rats weighing 200–250 g of either sex. Pancreases were re- moved surgically under aseptic conditions, and islets were obtained by a modification of the method of Ballinger (*14*). Pancreases were digested with a prewarmed mixture of 2 mg/ml of collagenase (Sigma type XI), 0.3 mg/ml DNase I (Sigma Chemicals), and 1 mg/ml $CaCl_2$ for 12 min at 37°C at a shaking speed of 300 rpm. The digestion was stopped by adding 4 volumes of cold Hanks' balanced salt solution (4°C), and the crude islets were washed 3 times with cold HBSS. The crude islets were suspended in 5 ml of 25% Ficoll (Sigma Chemicals) and then layered with 3 ml each of 23%, 20%, and 11% Ficoll, and centrifuged initially at 400 rpm for 4 min and subsequently at 2000 rpm for 16 min at 4°C. The concen- trated islets present at the two interfaces between 11% and 20% and 20% and 23% Ficoll were aspirated and washed three times with cold HBSS. Intact islets were hand-picked with the aid of a dissecting microscope and then cultured for three days in a humidified incubator (10% CO_2, 37°C), in petri dishes containing 15 ml of RPMI 1640 supplemented with 5% fetal calf serum, 100 U/ml of penicillin, and 100 µg/ml of streptomycin.

Each rat pancreas yielded between 400 and 600 islets. The combined islets from 3 donor animals (approximately 1500 intact islets) were used for each isograft. Islet cells suspended in 1 ml of RPMI 1640 were implanted into the rectum of hypnorm-anesthetized recipient PVG rats (n=31) by an in- jection technique using a size 27 gauge hypodermic needle. The needle was introduced into the submucosal plane of the lower rectum above the dentate line. There were no compli- cations related to this simple procedure.

Initial difficulty in retrieving implanted islets and frequent contamination of grafted tissue obtained in the first 15 cases done were thought to be due to the fecal loading and difficulty in achieving good bowel preparation in rats. This problem was overcome using a diverting sigmoid colostomy for subsequent cases (n=16). This was performed by division of the mobile sigmoid colon. The proximal end was brought up to the ab- dominal wall as an end colostomy. The distal end was sutured and returned to the abdominal cavity. In these animals, dia- betes was induced by intravenous injection of 35 mg/kg of streptozotocin (Zanosar, Upjohn) (*15*). After 2 weeks, the rats became diabetic with repeated fasting glucose levels of greater than 400 mg/dl. This procedure was well tolerated and excel- lent engraftment of islets in the anorectum was subsequently achieved in 10/16 rats studied over one month. This was dem- onstrated by histology using routine hematoxylin and eosin and indirect immunoperoxidase techniques. Free islets were seen in the submucosal plane of the anorectum, with an abun- dance of insulin-positive cells demonstrated (Fig. 1, A and B). The precise location of the grafts was in the submucosa of the rectum (n=6), the submucosa of the anal canal (n=2), and subserosa of the rectum (n=2).

Initial functional studies performed using streptozotocin- induced diabetic rats showed that rectal islets were able to reverse diabetes in 6/10 rats studied. Fasting glucose levels fell from more than 400 mg/dl preimplantation to less than 150 mg/dl 1 week after transplantation and continued to remain normoglycemic for more than 60 days.

The lower rectum as a site for clinical islet cell transplan- tation is attractive, since the technique can be simply per- formed under direct vision via proctoscopy as an outpatient procedure, similar to the injection of hemorrhoids (*16*). This technique also facilitates later islet retrieval for biopsy, or even repeat grafting procedures. Initial difficulty was en- countered in localizing islet cells accurately in the submuco- sal planes. We think that this difficulty is unique to the small animal model that we have used for our project and should not be encountered in the larger animal models or humans. However, further refinement of the technique is currently underway in rodents to achieve consistent local- ization of islet cells in the submucosa of the lower rectum, where venous drainage is into the portal system. This in- cludes the use of smaller hypodermic needles. Functional studies are also in progress to determine if the implantation of islets cells in the rectum is comparable to other sites of portal venous drainage in the ability to reverse diabetes in streptozotocin-treated rats. Our initial results are encourag-

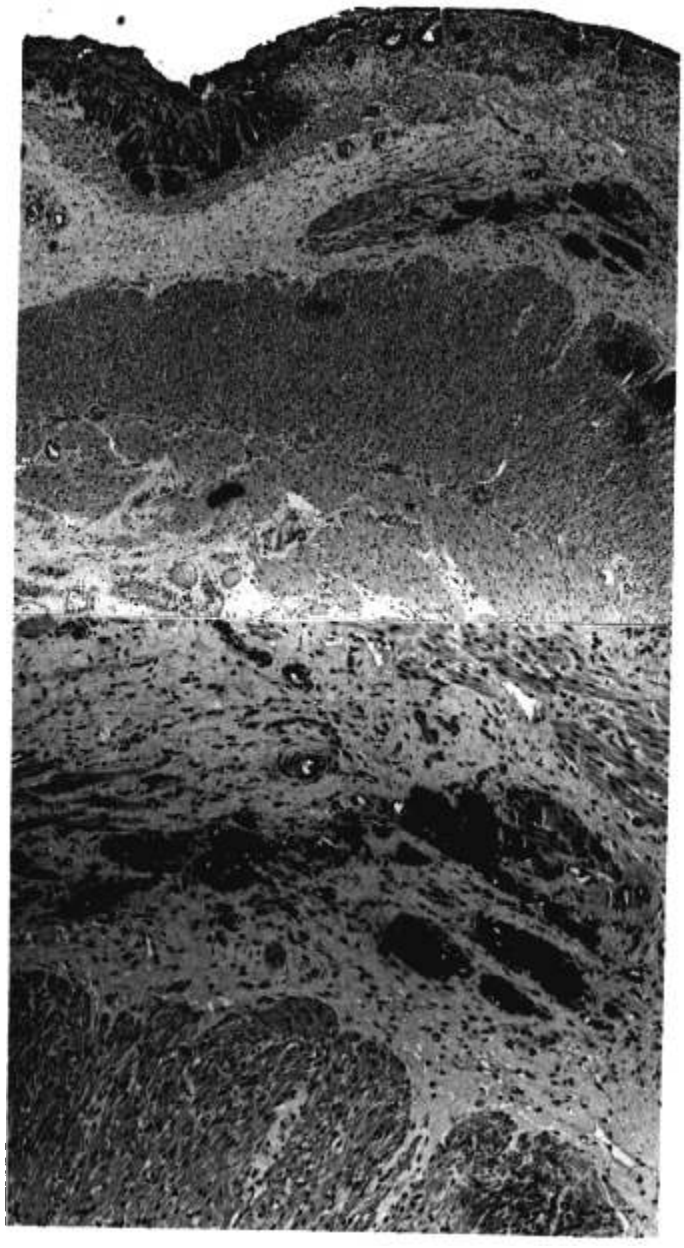

FIGURE 1. Photomicrograph of an islet isograft in the submucosa of the lower rectum and upper anal canal one month after transplantation (Immunoperoxidase stain: [A] ×40, [B] ×100, demonstrating numerous insulin-positive cells).

ing as we have been able to reverse diabetes in 6 of our streptozotocin-induced diabetic rats from fasting glucose levels of more than 400 mg/dl for 2 weeks to less than 150 mg/dl one week posttransplant. They continued to remain normoglycemic for more than 60 days.

Acknowledgments. We gratefully acknowledge the National Science and Technology Board and the National University of Singapore for funding this project. We would also like to thank Miss Margaret Lee, Miss Chng Yi-Mei, and Mr. Subhas Balan for technical assistance.

SUSAN M. L. LIM[1]
S. Q. LI
L. H. POH
N. K. LIM
M. L. SEAH
K. K. HENG
Department of Surgery
National University of Singapore
Singapore

[1] Address correspondence to: A/Prof. Susan M. L. Lim, Department of Surgery, National University Hospital, 5 Lower Kent Ridge Road, Singapore 0511.

REFERENCES

1. Gray DWR, Morris PJ. Developments in isolated pancreatic islet transplantation. Transplantation 1987; 43: 321.
2. Recce-Smith H, DuToit DF, McShane P, Morris PJ. Prolonged survival of pancreatic islet allografts transplanted beneath the renal capsule. Transplantation 1981; 31: 305.
3. Gray BN, Watkins E. Isolated islet transplantation in experimental diabetes. Aust J Exp Biol Med Sci 1976; 54: 57.
4. Tuch BE, Grigoriou S, Turtle JR. Growth and hormonal content of human fetal pancreas passaged in athymic mice. Diabetes 1986; 35: 464.
5. Posselt AM, Barker CF, Tomaszewski JE, et al. Induction of donor-specific unresponsiveness by intrathymic islet transplantation. Science 1990; 249: 1293.
6. Ferguson J, Scothorne RJ. Extended survival of pancreatic islet allografts in the testis of guinea-pigs. J Anat 1977; 124: 1.
7. Lee HC, Ahn KJ, Lim SK, et al. Allotransplantation of rat islets into the cisterna magna of streptozotocin-induced diabetic rats. Transplantation 1992; 53: 513.
8. Ziegler MM, Reckard CR, Barker CF. Long term metabolic and immunological considerations in transplantation of pancreatic islets. J Surg Res 1974; 16: 575.
9. Yasunami Y, Lacy PE, Finke EH. A new site for islet transplantation—a peritoneal-omental pouch. Transplantation 1983; 36: 181.
10. Feldman SD, Hirshberg GE, Dodi G, et al. Intrasplenic islet isograft. Surgery 1977; 82: 386.
11. Trimble ER, Karakash C, Malaisse-Lagae F, et al. Effects of intraportal islet transplantation on the transplanted tissue and the recipient pancreas. Diabetes 1980; 29: 341.
12. Kemp CB, Knight MJ, Scharp WF, et al. Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia 1973; 9: 486.
13. Goligher JC, Duthie HL. Surgical anatomy and physiology of the colon, rectum and anus. In: Goligher JC, ed. Surgery of the anus, rectum and colon. London: Bailliere Tindall, 1980: 25.
14. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery 1972; 72: 175.
15. Jiao L, Gray DWR, Gohde W, et al. In vitro staining of islets of Langerhans for fluorescence-activated cell sorting. Transplantation 1991; 52: 450.
16. Graham-Steward CW. Injection treatment of haemorrhoids. Br Med J 1962; 1: 213.

Received 17 March 1993.
Accepted 14 June 1993.