1959

LIVER TRANSPLANTATION: FROM LABORATORY TO CLINIC

Edited by

FRANCO FILIPPONI and **FRANCO MOSCA**

AZIENDA OSPEDALIERA
PISANA

CONSIGLIO NAZIONALE
DELLE RICERCHE

1. Liver transplantation: from the laboratory to the clinic and beyond

I.R. Marino, T.E. Starzl

INTRODUCTION

During the past 33 years, orthotopic liver transplantation has become a highly successful form of therapy, and as of this writing it is being performed at more than 100 institutions in the U.S., with a similar number in Europe. This is testimony to the great advances achieved in this field since the 1960s, when there were essentially only two teams actively engaged in liver transplantation, one at the University of Colorado and the other at Cambridge University (England). Essential to its success has been the work in the laboratory, where the surgical technique and all the refinements of this operation, as well as a panoply of relevant physiologic concepts, were developed before being introduced in the clinical arena.

The laboratory work also allowed many surgeons around the world to practice, in order to be able to do liver transplantation safely. This is still perhaps the most complex operation in routine use today, and good technique is crucial to a satisfactory outcome. A fall-back system such as that for kidney, heart, pancreas, and intestinal transplantation (i.e. dialysis, ventricular assist device, insulin, and parenteral hyperalimentation) does not exist for the liver. Thus, the smallest mistake in the surgical management of the patient may prove fatal.

In this chapter we will describe how liver transplantation came to be a clinical discipline. We also will consider its future developments, including drug-free graft acceptance (based on the "2-way paradigm") and the prospects of clinical xenotransplantation.

THE LABORATORY-CLINIC INTERFACE

Experimental work in the laboratory has been critical to each major step in the evolution of liver transplantation. Evidence obtained in the animal models has been transferred to the clinics, and conversely, problems encountered in the patients have been brought back to the animal laboratory for clarification (1), as had been done earlier for kidney transplantation (2). This flux has been continuous for almost 40 years. It resulted in the development of operative techniques, the improvement of immunosuppression, and clarification of previously enigmatic physiologic principles. The objective was to avoid human experimentation, rather than depending on it, when the time came to apply this potentially life-saving procedure in the clinic. The first attempt to replace a human liver was preceded by more than 7 years of animal experiments involving hundreds of animals (3).

A co-product of these efforts was the concept of team construction in the laboratory. The more experience gained in the laboratory, the better the team will perform in the human operating room. In addition, a clinical liver transplant demands the strict cooperation among specialists in different fields including health care administration. The recipient candidate is evaluated by surgeons, hepatologists, anesthesiologists, radiologists, psychiatrists/psychologists, nurses, social workers, and many other care providers. The actual operation requires separate donor and recipient teams, the activities of which must be closely knit. During the transplant operation, cooperation among surgeons, anesthesiologists, perfusionists, and nurses is essential. From the very beginning, one of the objectives of laboratory work was to create harmony amongst the specialists and also those with whom the team would react.

The core roles of the surgeons and the steps involved in the operation are identical to those of everyday clinical practice. However, the operation and postoperative care are more complex. We have stated earlier: "It is unlikely that anyone would attempt clinical liver transplantation without first personally recapitulating in the laboratory at least some of the earlier experiments in dogs or alternatively in pigs" (4). Someone inevitably will emerge from this experience as the team leader, most commonly but not necessarily a surgeon. Rigidity, impatience, selfishness, dishonesty, inhumanity, ignorance, and poor organizational skills are disqualifying characteristics. In addition to possessing these graces, as well as professional competence,

the leader must have those scientific instincts which allow advances.

There are two different approaches to transplantation of the liver. With one method an auxiliary graft is inserted heterotopically at a non-anatomical site, without removal of the native liver. The other is to transplant the graft orthotopically (in its natural location), after removal of the recipient liver (total hepatectomy).

Auxiliary Liver Transplantation

The first report of liver transplantation appeared in the scientific literature in 1955 when C. Stuart Welch of Albany (New York), described the insertion of an auxiliary canine liver into the right paravertebral gutter or pelvis of non-immunosuppressed dogs (Figure 1.1) (5). The liver arterial supply was derived either from the aorta or the iliac artery. The allograft portal vein was revascularized by rerouting high volume systemic venous flow into the transplanted liver's portal system. This was done by end-to-end anastomosis of the host iliac vein or inferior vena cava to the portal vein of the allograft (Figure 1.1). The graft outflow was directed into the host systemic venous system by an end-to-end anastomosis between the suprahepatic inferior vena cava of the graft and the inferior vena cava (or common iliac vein) of the host. The "blind" distal stump of the graft inferior vena cava was ligated, and the biliary tract reconstruction was performed by a cholecysto-duodenostomy (Figure 1.1).

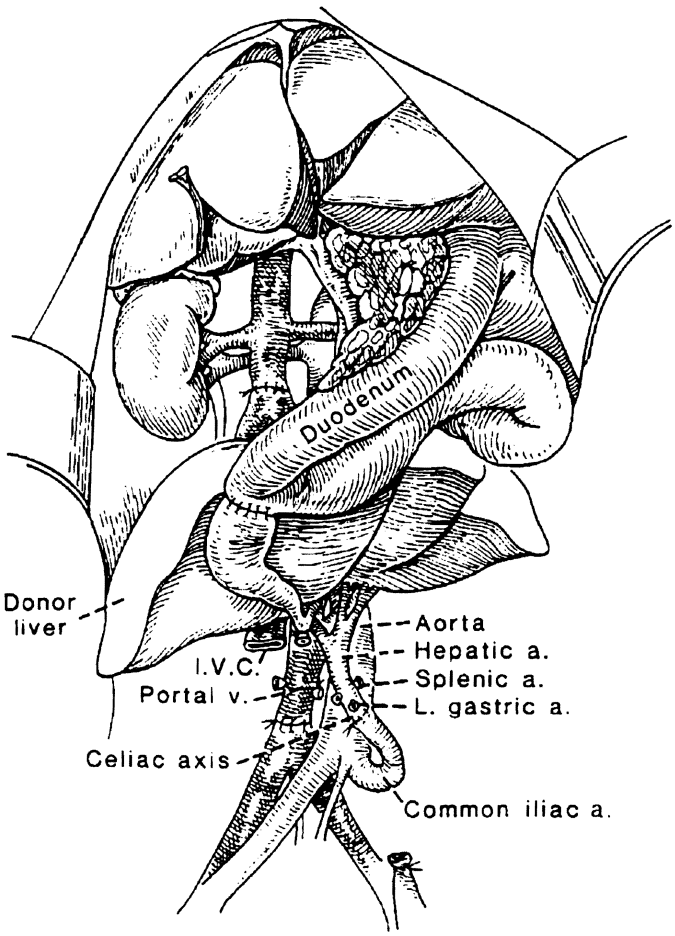

Figure 1.1 – Auxiliary liver transplantation in a dog by a modification of Welch's original technique. Note that the reconstituted portal blood supply is from the distal inferior vena cava. (From: Starzl TE, et al. Immunosuppression after experimental and clinical homotransplantation of the liver. Ann Surg 1964;160:411-439. Used by permission).

The livers transplanted with this technique into non-immunosuppressed dogs produced bile for several days and then ceased to function. When grafts underwent astonishingly rapid atrophy, the shrinkage was considered at first to be one of the manifestations of unmodified rejection (5, 6). The misconception was not corrected until the early 1960s, when Welch's experiments were repeated in mongrel dogs immunosuppressed with azathioprine, the drug whose laboratory testing in a canine renal transplant model had been spearheaded by Calne (7, 8). Although protection of the auxiliary hepatic allografts was unreliable, it was good enough in many experiments to completely prevent cellular rejection for 7 to 30 days. Yet, the livers had profound atrophy by the end of the first week (9) (Figure 1.2). The conclusion from these observations was some constituent(s) of the splanchnic venous blood (either a hormone [suspected to be insulin] or nutrients) that was present in high concentration in normal portal, but not systemic, blood had liver supporting qualities (9). Efficient removal of this "hepatotrophic" substance(s) by the first pass through the native liver apparently made it unavailable to the graft. This hypothesis was strengthened by the demonstration that the auxiliary livers had a normal or supernormal volume of flow (10). The shrunken porta-prival liver had characteristics that were indistinguishable from an Eck fistula liver.

The Ripple Effect – The importance of this discovery was recognized immediately. Until these experiments were performed under immunosuppression, an entrenched dogma of hepatic physiology had been that the volume, not the source, of portal venous blood flow was the critical factor in hepatic homeostasis (the flow hypothesis [11, 12]). Now, overthrow of the flow hypothesis had begun (13, 14). Fifteen more years were required, however, for its completion, culminating in the demonstration that insulin was the principal splanchnic hepatotrophic factor (15-19). Eventually, the identification of a family of factors with insulin-like hepatotrophic properties that controlled liver structure, function, and the capacity for regeneration defined the new field of hepatotrophic physiology (20, 21).

These developments were made possible by recognition of the double liver principle whereby one liver dominates the other by its avid clearance of hepatotrophic factors (9) (Figure 1.3A). The consequent pervasive impact on the study of liver physiology in general and upon the *in vivo*

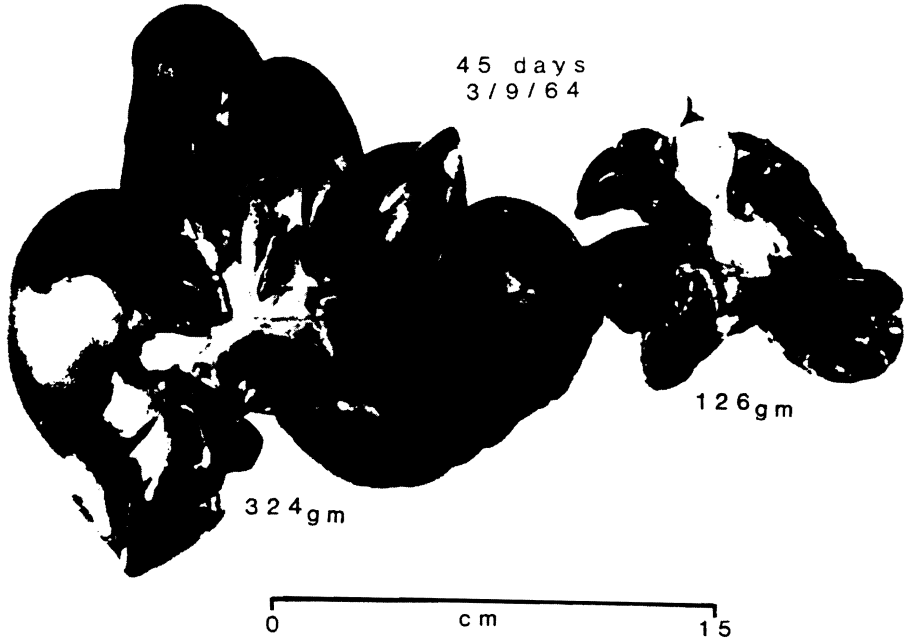

Figure 1.2 – The auxiliary allograft (right) and the recipient dog's own liver (left) 45 days after the experimental transplantation was performed in 1964. Immunosuppression was with azathioprine, and there was no histopathological evidence of rejection. Note the well-preserved but dimensionally reduced general structure of the allograft. The gallbladder did not shrink proportionately. (From: Starzl TE, et al. Immunosuppression after experimental and clinical homotransplantation of the liver. Ann Surg 1964;160:411-439. Used by permission).

study of hepatic growth factors in particular was slow to evolve. After the primary discovery, a series of non-transplant models was developed (13-19), in which the animal's own liver was divided into competing fragments (Figure 1.3B-D). With these models and particularly the final one shown in Figure 1.3D, 8 growth factors with potent hepatotrophic effects (18-30) as well as 2 with the opposite action (29, 30) were identified (Table 1).

The first clearly delineated hepatotrophic factor was insulin, (18, 19) followed 4 years later (22) by the cytosol of hyperplastic liver cells containing the "augmenter of liver regeneration" (ALR), a molecule originally called hepatic stimulatory substance (HSS). When infused by itself into the defunctionalized hilar portal vein after portacaval shunt, each of the 8 substances could prevent the hepatocyte and whole organ shrinkage caused by the Eck fistula. In addition, they augmented the hyperplasia characteristic of the Eck fistula liver with a response similar to but more sustained than the regeneration response following a moderate partial hepatectomy. The development of the *in vivo* Eck fistula assay (Figure 1.3D) was critical for the study of liver growth control. Because only 2 of the 8 hepatotrophic factors (transforming growth factor-alpha [TGF-α] and hepatocyte growth factor [HGF]) stimulate mitoses in tissue culture, the other 6 could not be identified as growth factors with *in vitro* techniques.

The most elusive of the 6 non-mitogenic "occult" growth factors was the "intrinsic hepatic growth factor", ALR, which is present in the hyperplastic livers of weanling rats or in the residual liver fragment of adult animals of several

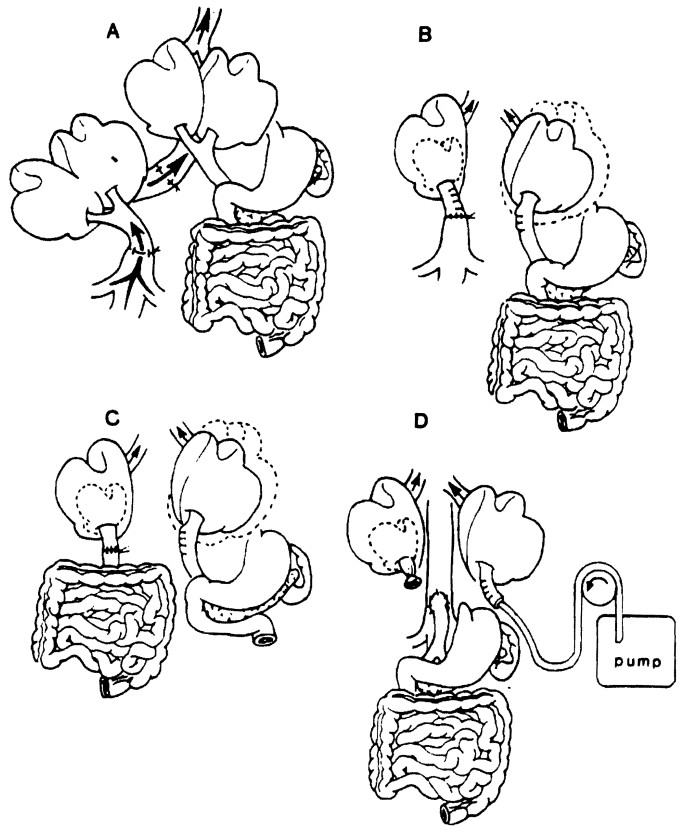

Figure 1.3 – Growth factor detection with coexisting liver and Eck fistula models alone or in combination: (A) auxiliary liver transplantation; (B) partial portacaval transposition; (C) splanchnic venous division; (D) Eck fistula plus selective infusion of tested substance. (From: Francavilla A, et al. Augmenter of liver regeneration: its place in the universe of hepatic growth factors. Hepatology 1994;20(3):747-757. Used by permission).

TABLE 1
GROWTH FACTORS REVEALED BY STUDIES IN ECK FISTULA MODELS (1994)

	Reference No.
Stimulatory	
Hormones:	
Insulin	18, 19
Growth Factors:	
Cytosol substrate and ALR	21, 22, 23, 24
IGF II	25
TGF-α[a]	25
HGF[a]	25
Immunosuppressants:	
Cyclosporine	26
FK506	27
Immunophilins:	
$FKBP_{12}$	28
Inhibitory	
Growth factors:	
TGFβ[b]	29
Immunosuppressant:	
Rapamycin[b]	30

[a]Mitogenic in tissue culture
[b]Inhibitory in tissue culture

species following partial hepatectomy. Its purification to >800, 000 (23, 31, 32) and cloning (24) after 16 years of effort in our laboratories added another important component to the complex network of modulators, both stimulatory and suppressive, which regulate hepatocyte proliferation and hepatic regeneration at the organ level. In these days of cellular and molecular biology, it is striking that the 2 operations of portacaval shunt (Eck's fistula) (33) and auxiliary liver transplantation (5) described in dogs 78 years apart played an indispensable role in this development.

The new growth factor ALR is a unique heat stable peptide whose gene exhibits a 50% homology with the dual function nuclear yeast gene ERV1 (34). The ERV1 gene is required for oxidative phosphorylation (respiratory chain) and also is essential for mitosis, which ceases in 3-4 days following gene deletion in the yeast, *Saccharomyces cerevisiae*. If, as we suspect, ALR is the mammalian homologue of ERV1, it is apt to be a major growth regulatory gene. Subsequent to the discovery of the rat ALR gene, we have identified the mouse and human ALR genes (and the protein products) (35). These were all found in all species to be highly conserved and preferentially expressed in the testis and the liver (24, 35). The ALR gene maps to the mouse chromosome 17, in a region syntenic with human chromosome 16, where the allele-rich T/t region involved in spermatogenesis is located. The ALR protein appears to be a protein with important potential physiologic properties, not exclusively limited to liver regeneration, but rather with roles that are involved in the synthesis or stability of the nuclear and mitochondrial transcripts that are present in actively regenerating cells, and particularly the germ cells of the testis.

The Practical Applications – Although auxiliary liver transplantation was envisioned by Welch as a potential treatment for liver cirrhosis or other non-neoplastic diseases, the laboratory experiments showed at the beginning that the procedure had a fundamental design defect. The immediate impact of its laboratory investigation (36) was prevention of a tragedy in which unwise clinical trials of the Welch operation might have been undertaken, as appeared originally to be logical. The liver is an unpaired organ. Instead of removing and replacing it, why not transplant a second (auxiliary) liver in some convenient location in the abdomen? Implantation of an auxiliary liver had a special appeal for teams already involved in kidney transplantation. Kidney grafts are essentially never placed in a normal anatomic location and frequently they are implanted without removing the 2 native kidneys. It was easy to envision how an extra liver could go in the same general location. This was, in fact, Welch's original intention.

The first discoveries of the hepatotrophic effect of splanchnic venous blood pushed the pendulum of the early 1960s toward the seemingly more difficult operation of liver replacement. Here also, the necessity was apparent for assuring portal venous inflow from the effluent blood of the other visceral organs. The same principle applies today. If orthotopic liver transplantation is to be performed in patients who have portal-systemic shunts, the shunts should be disconnected. In patients who have thrombosis or damage to their own portal veins, extension or jump grafts should be used to ensure delivery to the liver of the hormone and nutrient-rich splanchnic venous blood (37-40).

Finally, it was possible at long last to understand what caused the manifestations of Eck's fistula in animals and in humans subjected to portacaval shunt. The reason was that portal systemic shunt procedures placed the liver in a variably insulinoprival state (18, 19, 41). In 1961, Bollman, the world's leading authority on the subject, wrote: "In the 83 years since it was first reported, the Eck fistula has been reasonably successful in hiding its secrets as well as giving rise to many additional questions fundamental to an understanding of the functions of the intestine, liver, and brain." (42). The hepatotrophic field that grew from the earliest efforts to evaluate the potential clinical value of auxiliary liver transplantation blew away the fog.

Canine Orthotopic Liver Transplantation

The concept of liver replacement first appeared in the literature in 1956 in a one page notation Jack Cannon of the University of California, Los Angeles, entitled "Brief Report" (43). In it, he indicated that he had attempted the operation in dogs, apparently after reading Welch's article. Cannon suggested that the liver, by virtue of its possible role in the still poorly understood immune system, might not contribute to its own rejection. No technical information or results were given except for the statement that "several successful operations" had been performed ... "without any survival" (43). Frustration in accomplishing operative survival also was encountered at the first 2 research programs that made this a formal objective.

These were begun independently in the summer of 1958 in Boston at the Peter Bent Brigham Hospital (Harvard) (44-46), and in Chicago at Northwestern University (47, 48). Because there was no way to prevent or control rejection, maximum survival was 12 and 20.5 days in the 2 laboratories, respectively. By the time of the April 1960 meeting of the American Surgical Association (49), 31 of these procedures had been done in Boston and 80 in Chicago. Rejection was always present after 5 or 6 days in both series, and generally it was the principal cause of death. The principles that emerged from this collective experience were: 1) the need for splanchnic venous blood for optimal portal revascularization (see preceding section), 2) core cooling of the allograft by infusion of chilled solutions into the portal vein as is practiced clinically today, and 3) decompression of the occluded splanchnic and systemic venous pools into the upper vena caval system through external venous bypasses during the anhepatic stage.

In addition to liver transplantation alone, modifications had been added by the end of 1959, including the multivisceral engraftment procedures (50, 51) that would be used clinically with essentially no change 3 decades later (52). The interrelationship of all abdominal viscera transplant operations (53) was already apparent by the early 1960s (Figure 1.4).

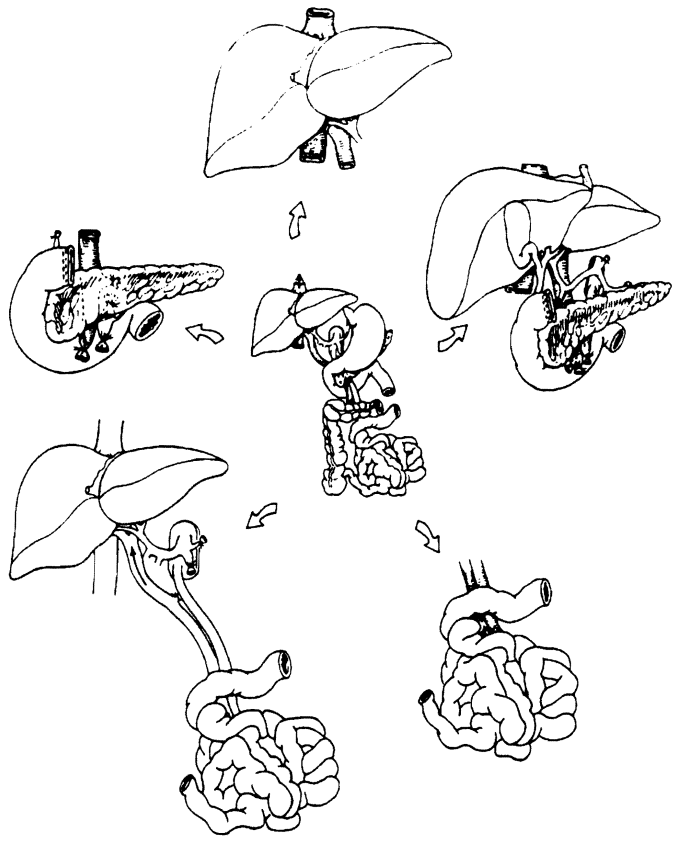

Figure 1.4 – The intra-abdominal viscera (center) that constitute a multivisceral graft (50, 51). In the periphery as shown, the liver, intestinal, and pancreas grafts that are part of this complex and can be transplanted alone or in various combinations (53). (From: Starzl TE. The contribution of transplantation to gastroenterologic knowledge. In: The Growth of Gastroenterologic Knowledge during the 20th Century, JB Kirsner [Ed.], Lea and Febiger, Philadelphia, Pennsylvania, 1994, pp. 348-369. Used by permission).

LIVER PRESERVATION

Hypothermia

The efforts in 1958-1959 to preserve dog livers came only a short time after the preservative value of cooling *per se* was described. While studying total body hypothermia for cardiac surgery in the 1950s, Owens, Prevedal, and Swan (54) observed that lower temperatures protected the kidneys and other abdominal organs from the injury of cross-clamping of the thoracic aorta. This effect of renal cooling was promptly confirmed in simpler ischemia models (55). When Lillehei and his associates began attempts at bowel autotransplantation and allotransplantation in 1958, they cooled the intestine by immersing it in cold electrolyte solution (56). Refrigeration through the thin-walled bowel was rapid, an advantage that did not pertain to solid organs.

Core Cooling

It was recognized during the canine liver transplant experiments of 1958-1959 that core cooling of the organ to be removed was an essential component of any procurement procedure. After this was applied to experimental liver transplantation (47), the concept of core cooling was promptly extrapolated to human kidney transplantation (57). It remains today the single most important step in any organ procurement technique. The easiest way to avoid warm ischemia is by *in situ* infusion of

the preservation solution, chilled to 4°C, at the time of the circulatory arrest. The remaining technical aspects of organ retrieval, and the refinements added in the following years, are secondary to this critical maneuver (58).

The Slush Techniques – The first solution used was chilled lactate Ringer's solution, replaced in the late 1960s by the so-called Collins solution, which has an electrolyte composition close to the intracellular one (59). This solution was successfully used for about 20 years, until the introduction of the University of Wisconsin solution (60, 61), which significantly extended the duration of organ viability. In 1975 and 1976, Benichou et al (62) in Denver and Wall et al (63) in Cambridge showed that with Collin's solution and with a plasma-like solution, respectively, dog livers could be cold-stored for 12 hours or more. Now, it became possible to ship livers from city to city, but within a safe time limit of only about 6 hours. After Jamieson et al (64) demonstrated that canine livers could be preserved reliably for 24 hours with solution at the University of Wisconsin, the promise of expanded organ-sharing between regions and countries, or even trans-Atlantically, became practical and commonplace.

Multiple Organ Procurement – In the early days of multiple organ procurement, the individual organs were skeletonized. After all of the dissection was completed, the kidneys were removed and cold perfused on the back table. The liver and heart then were removed simultaneously. However, the removal of all four organs was a rare event. The first time the kidneys, liver, and heart were removed from a single donor was on 17 April, 1978 during a visit by the University of Colorado team to the University of Minnesota. It quickly became obvious that *in situ* cooling of organs was going to be necessary if extrarenal organ transplantation were to flourish. During the times when the numbers of liver or heart transplants were small, the annoyance caused for renal transplant surgeons by multiple-organ procurement was relatively minor. As demand for extrarenal organs increased, a "civil war" loomed between those solely interested in the kidney, and the gastrointestinal and thoracic surgeons.

Consequently, a major educational effort was required to unite these diverse groups with a common plan. The procedures developed in Denver and Pittsburgh were demonstrated throughout the eastern two-thirds of the United States and Canada, and at the request of the Surgeon General of the United States (Dr. C.E. Koop), a description of the new operation of multiple-organ procurement was published (58). This was followed by modifications for unstable donors or even for donors whose hearts have ceased to beat (65). In the space of less than five years, these standardized multiple-organ procurement techniques were being used interchangeably, not only from city to city but from country to country throughout the world.

Continuous Perfusion

The alternative to infusion and cold storage is continuous perfusion. In a prototype of many later efforts, whole body or regional cadaveric perfusion was developed in dogs (66) and in humans (67, 68) using a pump oxygenator into which a heat exchanger was incorporated for cooling. Subsequently, *ex vivo* perfusion of dog livers for 24 hours was shown to be feasible by Brettschneider et al (69) using whole blood and a simple oxgenerator that was housed along with the grafts within a hyperbaric oxygen chamber as described earlier for kidneys by Ackerman and Barnard (70). Although several human livers were preserved with this method, the complexity of the approach and the potential dangers of the high compression oxygen chamber caused the technique to be abandoned. Nevertheless, the concept of continuous *ex vivo* organ preservation has never lost its appeal, because it is widely thought that only this technology will permit true organ banking.

VENOVENOUS BYPASS

In the dog, survival after liver replacement is not possible without shunting the venous return from the occluded splanchnic and inferior vena cava vascular beds to the superior vena cava during the anhepatic phase (45, 47). This early observation provided the foundation for the later development of the venovenous bypass used nowadays in clinical practice. However, venovenous bypasses were not used systematically for orthotopic liver transplantation in humans until 1983 (71). The result was that liver transplantation repetitively was performed in patients under conditions that limited its usefulness, increased its perioperative risk and made training of the next generation of hepatic surgeons difficult. The mistake was made of believing that the fundamental bypass principle delineated in animals was not relevant in humans.

A Recycling of History

How this error was rectified cannot be traced easily from the articles describing the steps. The stimulus for reassessment was a persistent 5-10% intraoperative mortality that was due almost entirely to poor patient tolerance during the venous occlusions of the anhepatic phase. However, nothing decisive was done to remedy the situation until a tragedy occurred in Pittsburgh on 13 May, 1982 that utterly demoralized the transplant team. A teenage hemophiliac male with chronic active hepatitis died on the operating table from the combination of bleeding, third space fluid sequestration and cardiovascular instability that was then common during hepatectomy and the sewing-in of the new liver.

The emotional burden caused by the death of this

popular patient was not lightened by the thought that we might have been hardened by our own repeated failures to the point of no longer making improvements. The program was closed until 15 June, 1982 when one of us (TES) requested Dr. Henry T. Bahnson, Chairman of the Department of Surgery at the University of Pittsburgh, to devise a pump-driven bypass for a liver replacement scheduled for that evening. Bahnson grasped the essence of the problem instinctively, and agreed immediately. That night, liver transplantation was carried out under venovenous bypass in a 6-year-old child with biliary atresia. The bypass was performed under 3 mg/kg heparin with a roller pump and other conventional equipment used for open-heart surgery. This technique of a pump-driven bypass had been described in dogs 10 years earlier by Cutropia et al (72), but their article was unknown to us at the time. There was little trouble in reversing the heparin effect afterwards. All those who were there that night (including Luigi Fassati of Milan) were ecstatic about the ease and non-stressful nature of the transplantation under bypass conditions.

By 1 July, 1982 abstracts describing the technique were submitted to the Southern Surgical Association and to the American Association for the Study of Liver Diseases. Both were rejected. In the meanwhile, problems had been encountered with reversal of the heparin effect in several of the adult recipients. Venovenous bypass under systemic heparinization had worked marvelously in those patients with relatively "simple" diseases such as primary biliary cirrhosis and in recipients who had never had any previous operations. The same was not true in patients with difficult pathology or exceptionally advanced disease and especially in those who had undergone multiple procedures previously. Here, the bleeding from the raw surfaces was so great and the heparin effect was reversed with such difficulty that the value of bypass technique was vitiated. Two patients died of hemorrhage when clotting could not be restored.

The Heparin-Free Bypass

From a lifetime of experience with cardiopulmonary bypass techniques, Bahnson was convinced that eliminating the systemic heparinization would not be safe. However, two of Bahnson's young associates, Dr. Bartley Griffith and Dr. Robert Hardesty, had avoided systemic heparinization in patients with pulmonary insufficiency who had been supported with extracorporeal membrane oxygenators perfused with an atraumatic centrifugal pump. The rest of their equipment, including the cannulas and tubing, was not much different than in the first trials under heparin. In September, 1982, they proposed the use of venovenous bypass without heparin.

On 30 September, 1982, work began on dogs in the laboratory. The project was assigned to Dr. Scot Denmark, the resident on cardiac surgical research rotation. Griffith and Denmark provided the bypass equipment. The liver transplantations were performed by members of the transplantation service including the senior fellow, Dr. Byers Shaw, Jr. By the end of 1982, the preclinical preparation reported by Denmark at the *Surgical Forum* of the American College of Surgeons in October, 1983 already had been completed (73). However, clinical trials of the non-heparin bypass were not started, in part because it was difficult to predict which patients really needed it. In addition, there still was uneasiness about the possibility of clot formation in bypass tubing and consequent pulmonary emboli. Finally, Shaw (by now a junior faculty member), who later became an enthusiastic proponent of the technique (74), was initially opposed to its use.

During the Christmas season of 1982 and in January of 1983, three more deaths occurred on the operating table in much the same way as with the earlier hemophiliac patient. As a consequence, a command decision was made that venovenous bypasses must be used henceforth for all adult recipients of liver transplants. In view of Bahnson's previous trepidation about using non-heparin bypass, Griffith, as an additional precaution, added the heparin-bonded (Gott) cannulas for both the outflow and inflow limbs of the system (75); even though this was not an integral part of the animal technique. The problems with bleeding that had been aggravated by systemic heparinization were greatly ameliorated. It was obvious that from that moment onward liver transplantation could be a far more reasonable procedure that would be within the capability of many general and vascular surgeons and which henceforth could be taught to surgeons in training in a systematic way.

The sequence of articles from this laboratory-generated advance was orderly, but the actual publication schedule was not. The rejection of Bahnson's two abstracts expunged the beginning of these events from the record with the exception of a brief notation in a 1982 review article (71). The animal work on non-heparin bypasses by Denmark (73) appeared promptly in the literature. However, the descriptions by Griffith et al (75) of the first clinical application of the new method (Figure 1.5) as well as details of technique were delayed. These were sent to *Surgery, Gynecology & Obstetrics* in July, 1983, but the article was not published until more than 1-1/2 years later (75). In the meantime, an account of the advantages of venovenous bypasses in liver transplant recipients was given by Shaw at the American Surgical Association in the spring of 1984 and published the following October in *Annals of Surgery* (74).

Such priority questions were unimportant. However, noteworthy was Bahnson's encouragement of Griffith to pursue an idea in the laboratory that he, Griffith's Chief, initially did not believe to be sound. After this, movement to the clinic was inexorable.

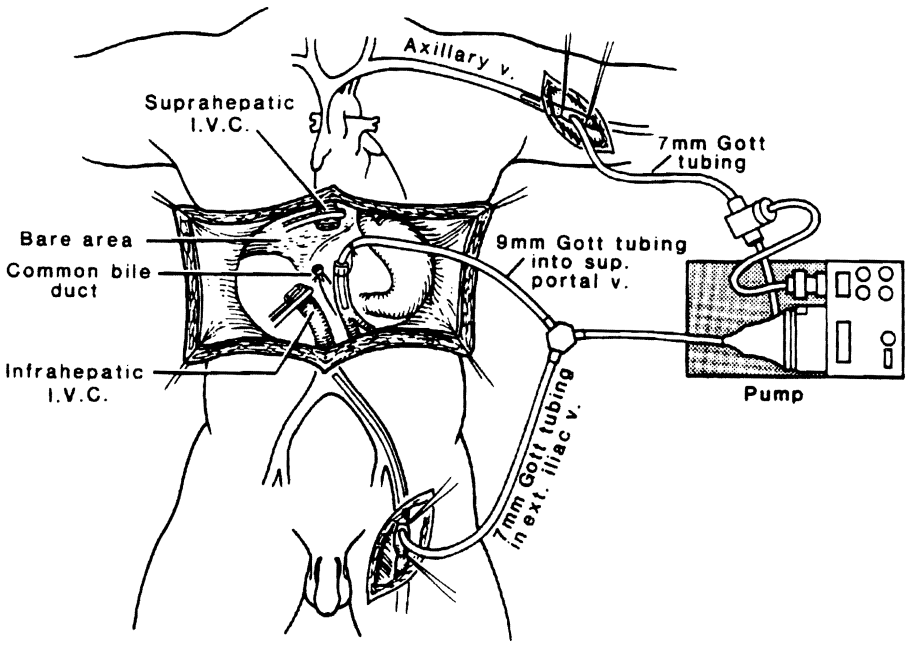

Figure 1.5 – Veno venous by-pass used during the anhepatic stage of liver transplantation. (From: Starzl TE. History of the liver and other splanchnic organ transplantation. In: Transplantation of the Liver, Busuttil R and Klintmalm G [Eds.], WB Saunders, Philadelphia, Pennsylvania, 1996. Used by permission).

Are Venovenous Bypasses Necessary?

Although not all liver transplant surgeons use venovenous bypasses routinely, the practicality of liver transplantation with venovenous bypass is so much greater compared to that in previous times that the principle of venovenous bypass, at least in selected cases, is widely accepted.

Some surgeons use a test period of portal and venacaval cross-clamping before making a decision about the use of bypass. The development of hypotension and/or low cardiac output is a warning that the bypass is needed. In infants or very small children, the difficulty of achieving an effective bypass may preclude its use. For these cases, the piggy-back operation has a special appeal (see later).

Ironically, severe liver disease may reduce the need for venovenous bypass. Picache et al (76) showed that even canine liver replacement, which cannot be done reliably in normal animals without bypass, is relatively safe when venous collateralization is stimulated by ligation several weeks in advance of the recipient's common bile duct.

REFINEMENTS OF SURGICAL TECHNIQUE

In principle, liver transplantation is exceptionally straightforward, involving the removal of the diseased native liver and its replacement with the liver of a cadaveric donor in as anatomically a normal way as possible (Figure 1.6). All of the essential steps were described in the first clinical report (77), including a new technique for intraluminal suturing of vessels that had to be reconstructed without redundancy in close quarters. More complete details of the operation were summarized in a 1969 text (78). The operation used by Calne was essentially the same (79). However, technical difficulties continued for years.

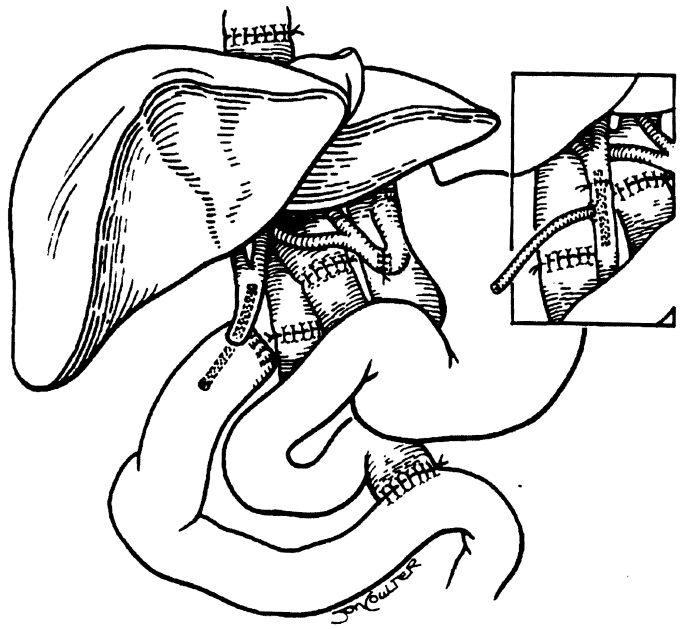

Figure 1.6 – Completed orthotopic liver transplantation (liver replacement). Biliary tract reconstruction is usually with choledochojejunostomy (to a Roux limb) or (inset) with a choledochocholedochostomy, which is stented with a T tube. (From: Starzl TE, et al. Liver transplantation: a 31-year perspective. Part I. Curr Probl Surg 1990; XXVII(2):49-116. Used by permission).

Recipient Hepatectomy

Performing a total hepatectomy in a patient with cirrhosis and severe portal hypertension is a stressful experience. In the early trials of liver transplantation, both donor and recipient procedures usually were done by the same team. This required a marathon effort. Today, recipient operations usually have a seasoned surgeon plus two or three assistants who are familiar with the procedure. Sometimes the presence of adhesions from previous surgery makes the standard technique of total hepatectomy unsuitable. If the hilum cannot be directly approached the suprahepatic vena cava should be isolated first, and the liver mobilized from cephalad to caudad approaching the hilum posteriorly, where it is usually relatively free of adhesions.

Another useful technique is hepatectomy with preservation of the inferior vena cava. This method, called the piggyback operation, which was first reported by Calne (79) and popularized by Tzakis (80), is based on the total one-stage canine hepatectomy that was described in 1959 (81) (Figure 1.7). This technique cannot be used in liver malignancies, where the resection margins should be as wide as possible. However, it has special value if a liver from a substantially smaller donor is to be used, because it is easier to adjust disparities in length and size of the donor and recipient vessels as was necessary in our two recent baboon-to-human liver xenotransplantations (82, 83) (Figure 1.8). Also, smaller raw surfaces are created with the piggyback dissection, thus making subsequent hemostasis easier.

In small children in whom venovenous bypass may not be feasible, the combination of the piggyback technique and a temporary portacaval shunt (84) minimizes the physiologic disturbances of the anhepatic period. Finally, the fact that there are only 3 vascular anastomoses to do will always allow the donor liver to be reperfused simultaneously with portal and arterial blood. Otherwise, the piggyback technique of liver implantation is similar to the standard one, except for the outflow anastomosis which is made to the anterior surface of the recipient vena cava, on a large common funnel fashioned by opening and interconnecting two or all three of the main suprahepatic veins. The lower end of the donor inferior vena cava is either ligated or sutured (Figure 1.8).

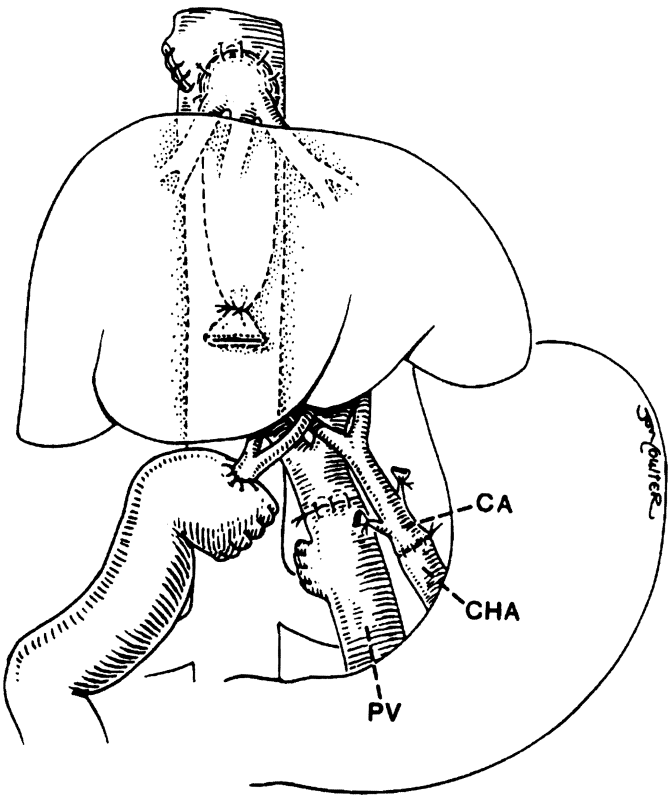

Figure 1.8 – Piggyback baboon to human liver xenotransplantation performed in June, 1992 (82), and January, 1993 (83). The animal livers were approximately one quarter the ideal size for the adult male recipients. (From: Starzl TE, et al. Prospects of clinical xenotransplantation. Transplant Proc 1994;26(3):1082-1088. Used by permission).

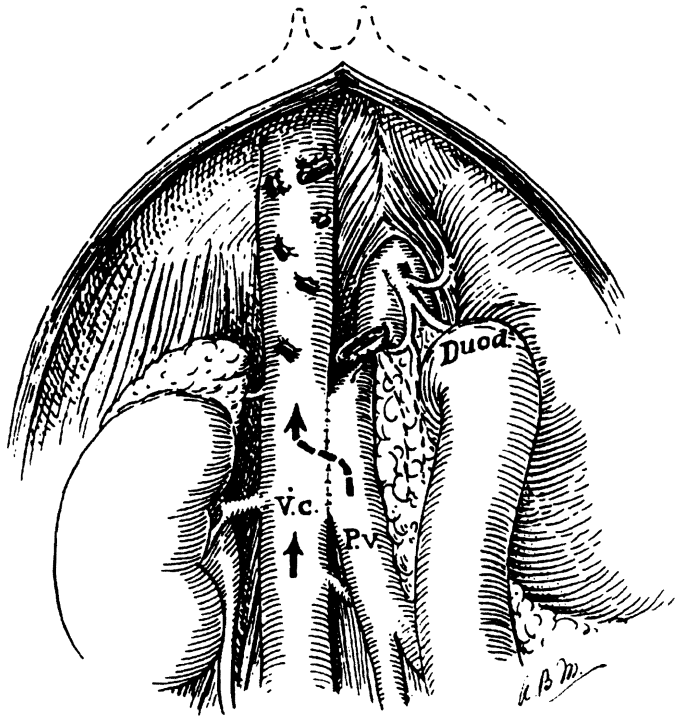

Figure 1.7 – Operative field after completion of total canine hepatectomy with preservation of the inferior vena cava. This experimental procedure, published 38 years ago (81), is the basis of the "piggyback" variation of orthotopic liver transplantation used clinically today. (From: Starzl TE, et al. A new method for one-stage hepatectomy in dogs. Surgery 1959;46:880-886. Used by permission).

Revascularization

A number of anatomical problems that precluded liver transplantation up to the last decade are no longer absolute contraindications. When the recipient portal vein is thrombosed, or significantly smaller in diameter than the donor portal vein, the venous anastomosis can be performed at the confluence of the recipient splenic and superior mesenteric veins. If the donor portal vein is not long enough to reach this low, a free interposition graft, from donor iliac vein, can be used to bridge the gap (37, 38). In cases where the portal thrombosis extends inferiorly, a venous graft can be "jumped" from the graft hilum to the host superior mesenteric vein (39, 40, 85).

If a direct arterial reconstruction is not feasible, or when the arterial inflow is unsatisfactory, a so-called arterial conduit may be used (4, 47, 86-88). Many routes have been used in the past to bring a donor iliac graft from the recipient infrarenal aorta into the subhepatic area.

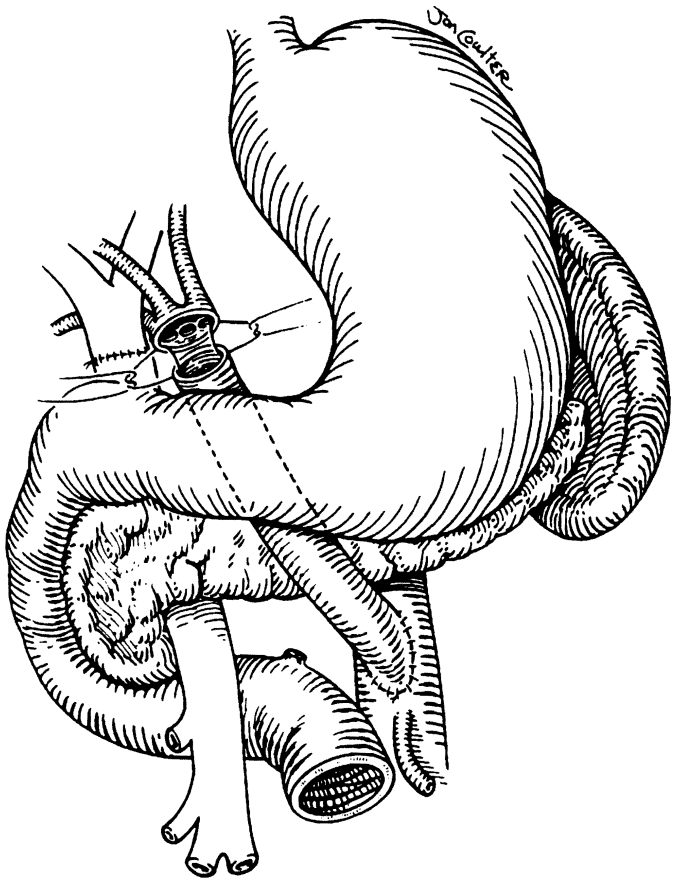

Figure 1.9 – The route of free graft of donor iliac artery from the recipient aorta to the hilar area of an orthotopic liver graft. Note the donor arterial anomaly in which the right, middle, and left hepatic arteries came separately from the aorta. The origin of these vessels was converted to a Carrel patch which was anastomosed to the end of the "conduit" graft. (From: Tzakis A, et al. The anterior route for arterial graft conduits in liver transplantation. Transplant Int 1989;2:121. Used by permission).

Currently, the preferred route is to pass the graft from the recipient aorta through an opening in the transverse mesocolon, behind the pylorus (Figure 1.9). The arterial graft can also be placed in parallel with a venous jump graft from the superior mesenteric vein, if both are required. The various vascular graft techniques described in this section enable today's liver transplant surgeons to accomplish a liver implantation with minimal hilar dissection.

Biliary Tract Reconstruction

In most of the first human cases, the homograft common bile duct was anastomosed to the recipient common duct over a T-tube stent (Figure 1.6, inset). Unfortunately, this approach was temporarily abandoned in favor of cholecystoduodenostomy, a technically easier operation that had worked well in canine experiments. The first inkling that unrecognized biliary obstruction was common came in a report by Martineau et al (89). Subsequently, the incidence of bile duct complications including obstruction, fistula and cholangitis was shown to be more than 30% (90-92). Eventually, these problems were minimized by a return to choledochocholedochostomy with a T-tube stent or, if this was not feasible, choledochojejunostomy to a Roux limb (71, 91, 92) (Figure 1.6).

In England, Calne had encountered the same problems with biliary reconstruction (93), and he recommended a modification of the procedure developed by Waddell et al (94) at the University of Colorado. With the so-called Waddell-Calne operation, the common duct of the homograft is anastomosed to the homograft gallbladder that then serves as an extension conduit to the recipient common duct or bowel. The Waddell-Calne operation is not used very often in most centers because of a high risk of stone formation (95), but it can be useful or even lifesaving in some complicated cases in which an extra length of the homograft common duct is needed.

IMMUNOSUPPRESSION

Earliest Efforts

Total body irradiation (TBI) (96), adrenal cortical steroids (97, 98), and the myelotoxic drug 6-mercaptopurine (6-MP) (99, 100) were shown between 1953-1959 to modestly prolong skin allograft survival in several animal species. Using TBI, successful kidney transplantation from fraternal (dizygotic) twin donors was accomplished in patients at the Peter Bent Brigham Hospital (Boston) in January, 1959 and again 5 months later at the Hopital Necker (Paris). Although the genetic barrier to transplantation of the kidney finally had been breached in humans, liver transplant operations still had no conceivable application. Preoperative conditioning of hepatic canine recipients with

TBI appeared to preclude even perioperative, much less extended, survival (101).

An historic turn in the road came in 1958 and 1959, when Schwartz and Dameshek described the immunosuppressive effect of 6-MP in non-transplant models (102, 103). Schwartz's intention from the outset was to use 6-MP in a different transplant strategy than previously. In September 1959, he presented a paper at an international oncology meeting in London describing a dose-related prolongation of rat skin grafts, using non-myelotoxic doses of the new drug. His manuscript was submitted to the *Journal of Clinical Investigation*, but was delayed in publication until June 1960 (99). In the meanwhile, Robert Good's team at the University of Minnesota had done similar skin allograft studies which were published rapidly in *Proc Soc Exp Biol Med* in December 1959 (100) with acknowledgment of the Schwartz-Dameshek priority. Also aware of the Schwartz/Dameshek breakthrough, Roy Calne did the first of his 6 kidney transplants in dogs under 6-MP in late 1959 and early 1960. Two of his animals survived for 21 and 47 days (7). Independent similar observations also were recorded by Charles Zukoski, a young surgeon working with Dave Hume at the Medical College of Virginia (104).

Zukoski, Lee, and Hume submitted their abstract to the *Surgical Forum* of the American College of Surgeons before the deadline of February 15, 1960, at the same time as Calne's results were reported in *The Lancet*. However, their manuscript was not published (*Surgical Forum* of the American College of Surgeons) until the first week of October, 1960 (104). The work of Schwartz and Dameshek, Meeker, Calne and Zukoski was widely known in the United States and England by the spring and summer of 1960. In fact, Rene Kuss in Paris already was using 6-mercaptopurine for delayed therapy of his irradiated human kidney recipients as early as April, 1960 (105); in the same month, the drug was given to a human kidney transplant recipient at the Brigham (106).

In July 1960, Calne moved to Boston, determined to press forward with preclinical trials of the purine analogues in collaboration with Joseph Murray. Because of the poor therapeutic margin of 6-mercaptopurine (and azathioprine), Calne and Murray attempted to find drug combinations (8, 107, 108). Inexplicably, their experiments failed to show that the value of azathioprine was increased by combining it with adrenal cortical steroids (8, 106-108). Consequently, Calne and Murray focused their intention instead on adjunct cytotoxic agents such as actinomycin C and a drug called azaserine.

The preclinical kidney transplant studies in Boston and Richmond with 6-MP and its analogue, azathioprine, were viewed by us in a different light than anything previously done. Whereas kidney transplantation with long survival had never previously been possible in mongrel dogs, about 5% of animals given one or the other of the new drugs lived >100 days after renal transplantation. Consequently, the objective of exploiting hepatic replacement to treat human liver disease was settled upon as a high priority during discussions in June, 1961, between one of us (TES) and William R. Waddell, who left the Massachusetts General Hospital to assume the chairmanship of surgery at the University of Colorado 5 months before TES moved from Chicago to Denver. The plan called for first establishing a track record in renal transplantation, a procedure under formal clinical development in the United States only in Boston and Richmond.

Our clinical plans for both the kidney and liver were shelved in January, 1962. Until this time, we had been following the tracks laid by the American kidney transplant pioneers (Murray [with Calne] and David Hume) and those in Paris (Rene Kuss and Jean Hamburger), only to eventually realize that we had joined them in a therapeutic *cul de sac*. As late as March 1, 1963, the date of our first liver transplantation, only 6 recipients of kidney allografts in the world had survived ≥ one year (one in Boston and 5 in Paris), all treated with TBI. The clinical results with azathioprine-based immunosuppression were little better (106, 109). Although the longest surviving kidney recipient treated by Murray solely with azathioprine or 6-MP-based therapy between April 1960 and April 1962 was now 11 months postoperative, we knew from contact with Murray that the patient had deteriorating renal allograft function.

The Colorado Drug Cocktail

The experimental results with 6-MP and azathioprine in the Denver VA canine laboratory were no better than those in Boston and Richmond, with one notable exception (110). During the summer of 1962, high doses of prednisone were shown to reliably reverse kidney (and in pilot studies liver) rejection that usually developed under primary azathioprine therapy (111). Although most of the dogs died from complications of steroid-induced peptic ulceration, several lived for years after discontinuance of prednisone and even when azathioprine also was stopped. Using the "double drug cocktail", the Colorado clinical kidney transplant program finally was opened in November, 1962.

The first 10 cases under azathioprine/prednisone immune suppression were compiled rapidly and reported in the October, 1963, issue of *Surgery, Gynecology, and Obstetrics* (112), preceding by 2 months the article in the same journal describing the first trials of liver transplantation (77). Four of the 10 renal recipients survived ≥ 25 years including 2 who still bear the longest continuously functioning kidney allografts in the world after a third of a century (113). It already was obvious that these patients could return to an unrestricted environment on reduced maintenance immunosuppression, suggesting that a state of relative host/graft non-reactivity had been accidentally but regularly induced by the renal allografts.

The controversial, but as it turned out apposite, term "tolerance" (see later) was used to describe the change (112).

THE FIRST CLINICAL LIVER TRIALS

The breakthrough with the "Colorado cocktail", and its successful application for kidney recipients was the signal that triggered the liver trials. The first 3 patients entered were: a moribund child with biliary atresia, a 48-year-old man with Laennec's cirrhosis and an unresectable hepatoma, and a 62-year-old man with a completely obstructing bile duct carcinoma who had previously undergone bilateral above-knee amputations for peripheral vascular disease. Their high risk factors would preclude candidacy today. Although 2 survived the operation, neither left the hospital alive. The redacted summary of the report of these trials (77) is given below.

"A number of problems are described which must be surmounted for the clinical use of liver homotransplantation, based upon experience with 3 patients. The first patient died of hemorrhage during conclusion of the operation. The second and third patients lived for 22 and 7-1/2 days, respectively, both ultimately dying from multiple pulmonary emboli . . . Procurement of a viable and relatively undamaged donor organ [was] . . . accomplished with the use of an extracorporeal circuit which perfuses and cools the [donor] liver immediately after death . . . It has been found necessary to decompress only the inferior vena cava during [the anhepatic] time with an external bypass from the inferior to the superior vena caval systems . . . During operation, a bleeding diathesis was regularly detectable . . . The use of the external bypass and [delayed] hypercoagulability may have contributed to the formation of the [pulmonary] emboli . . . Hepatic functions were immediately deranged [postoperatively], probably as the result of injury incurred during the transplantation, with progressive improvement thereafter . . . Biochemical evidence of [irreversible] homograft rejection was not observed, and at autopsy in the last 2 patients there was surprisingly good gross and histologic preservation of graft structure . . . Therapy with azathioprine, prednisone, and actinomycin C had forestalled the rejection process."

Most failed trials are doomed to be footnotes, if that much, in the pages of history. The 1963 liver transplant article escaped obscurity because it was based on principles that were enduring. Aside from the manifold details of the difficult operation, including the role of and complications from veno-venous bypass, there already was accurate insight into the importance of hepatotrophic physiology, and into the cause and treatment of metabolic acidosis. The only non-surgeon author, Kurt von Kaulla, anticipated the intraoperative coagulation disorders, monitored them with serial thromboelastograms, and provided treatment with blood components and epsilon amino caproic acid (an analogue of the currently used aprotinine). Lessons from the research preceding the clinical trial already had cross-fertilized to the kidney and eventually were exploited for all kinds of allografts: core cooling by infusion of chilled intravascular fluids, *in situ* procurement procedures that presaged the standard flexible procedures of today (58, 65), and the intravascular techniques required for close-quarter anastomoses.

The Liver Moratorium

The Colorado kidney center mushroomed overnight while the spark that had ignited it, liver transplantation, was consigned by the end of 1963 to a self-imposed 3-1/2 year worldwide moratorium following 4 more failures: 2 in Denver (9) and one each in Boston (114) and Paris (115). Three advances applicable to all organs were made during this period: 1) the purification and clinical introduction in 1966 of antilymphocyte globulin (ALG) for use with azathioprine and prednisone in a triple drug regimen (116); 2) preservation techniques that allowed livers to be stored *ex vivo* for one to 2 days (69); and 3) the demonstration (with Paul Terasaki of UCLA) that the quality of donor/recipient HLA matching had little association with kidney transplant outcome (117). It was assumed (correctly) that the same non-discrimination of tissue matching would apply with other organs.

Clinical Success (1967)

When the liver program was reopened in July, 1967, during the 2-year fellowship of Carl Groth (Stockholm), multiple examples of prolonged liver recipient survival were produced (4, 118). A second liver transplant program was founded in 1968 by Roy Calne of Cambridge University (79) and fostered by a long lasting inter-university collaboration with the hepatologist Roger Williams at King's College Hospital, London. The American and English teams sustained each other for the next dozen years, joined in the early 1970s by Rudolph Pichlmayr in Hannover and by Henri Bismuth in Paris, always tantalizingly close to making liver transplantation a service. In Denver, 170 patients underwent the operation between 1963 and 1979. Although only 56 survived for 1 year, 25 were alive after 13 to 22 years at the end of 1992 (119), and 19 remain today with follow-ups of 17 to 27 years.

Cyclosporine and Tacrolimus

Although the feasibility of liver transplantation was established, the results remained unacceptable until Sir Roy Calne, who had presided over the preclinical development

of the thiopurine drugs in Boston nearly 2 decades before, repeated the feat with cyclosporine and then reported the first clinical series which included 2 liver recipients (120). In another visit to the past, the full potential of the new drug was realized for transplantation of the kidney (121), liver (122), and eventually all organs when it was combined with prednisone or used in triple drug cocktails. The stampede to develop extrarenal transplant centers began. Nine years later, expectations moved up another notch with the advent of tacrolimus (123, 124) (Figure 1.10).

It is noteworthy that intensive efforts over a span of 35 years to develop better immune suppression have resulted in only a handful of widely used regimens (Table 2). These were based on azathioprine (or cyclophosphamide), cyclosporine, and tacrolimus with or without antilymphoid agents (polyclonal ALG and monoclonal refinements) or myelosuppressive adjuncts. The one indispensable second drug was prednisone.

TABLE 2
IMMUNOSUPPRESSIVE DRUGS IN ORGAN TRANSPLANTATION

Agents	Year Reported (Ref)	Institution
Azathioprine	1962 (106)	Peter Bent Brigham Hospital
Azathioprine-steroids	1963 (112)	University of Colorado
Cyclosporine	1979 (120)	Cambridge University
Cyclosporine-steroids	1980 (121)	University of Colorado
Tacrolimus-steroids	1989 (123)	University of Pittsburgh

CHIMERISM AND THE TWO-WAY PARADIGM

As it turned out, none of the individual drugs, or all together, remotely approached in importance the realization in the summer of 1962 that rejection could be engineered into prolonged allograft and recipient survival by the strategic use of multiple agents, always including adrenal cortical steroids (112). The cyclic pattern of convalescence and the consequent achievement of allograft acceptance remained enigmatic until it was discovered in 1992 that long-surviving organ recipients had donor leukocyte chimerism (119, 125).

The Phenomenology

This link between bone marrow and organ transplantation was provided when microchimerism was detected with sensitive immunocytochemical and polymerase chain reaction (PCR) techniques in the tissues or blood of all 30 human kidney or liver recipients studied from 2.5 to 30 years postoperatively (119, 125). The donor cells were multilineage, but paradoxically many appeared to be dendritic cells (DC), a potent antigen presenting cell (APC) (126). Individual samples from patients often do not contain the donor leukocytes, which wax and wane (127). However, disseminated donor cells, including DC, and/or donor DNA are consistently demonstrable if rodents bearing long-term grafts are completely studied (128-131).

Along with peripheral migration of the donor cells from a successfully transplanted graft, there is an influx of host leukocytes which do not cause graft damage (Figure 1.11A) (119). Thus, both the allograft and recipient become genetic composites. A mirror image condition exists after

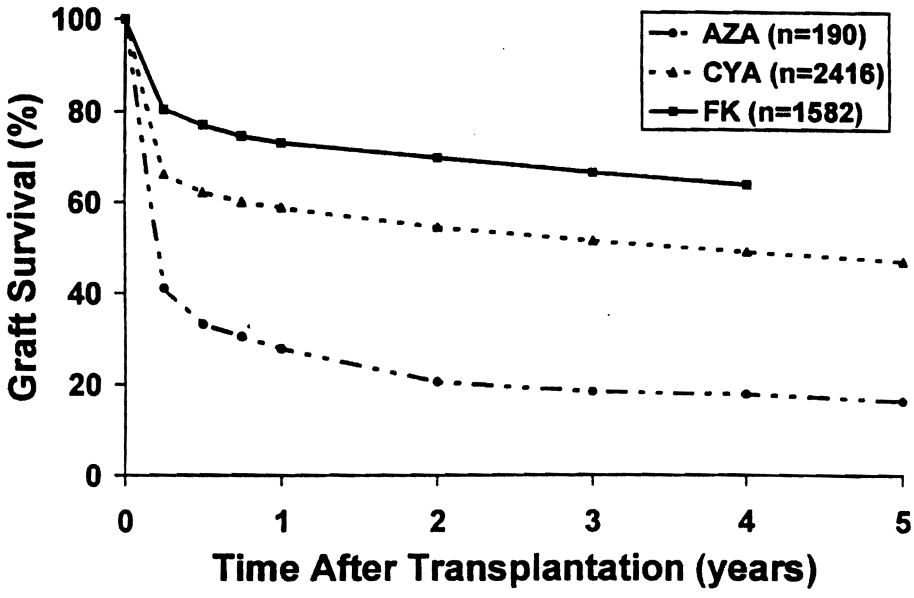

Figure 1.10 – The 3 eras of orthotopic liver transplantation at the Universities of Colorado (1963-80) and Pittsburgh (1981-1993), defined by immune suppression based on azathioprine, cyclosporine, and FK 506 (tacrolimus). Graft survival (shown here) was about 10% lower than patient survival in the cyclosporine (1980-89) and tacrolimus eras (1989-93) because of retransplantation, an option that did not exist previously (data from 124).

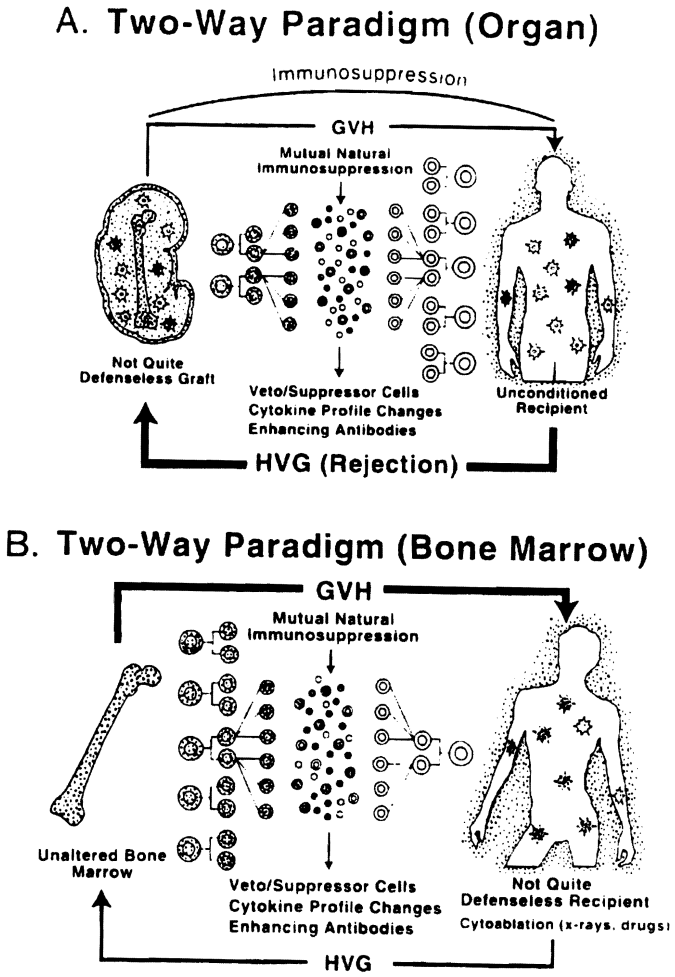

Figure 1.11 – Two-way paradigm with which transplantation is seen as a bidirectional and mutually cancelling immune reaction that is **(A)** predominantly HVG with whole organ grafts, and **(B)** predominantly GVH with bone marrow grafts.

bone marrow transplantation (132) (Figure 1.11B), proved by demonstrating a trace residual population of host leukocytes in essentially all stable human bone marrow recipients who previously were thought to have complete donor-cell chimerism (133).

Previous Enigmas

Graft Acceptance – These discoveries have provided an important framework for a better understanding of allograft "acceptance", for analysis of management problems, and for therapeutically oriented transplantation research (134). In the new context (the 2-way paradigm), the donor leukocytes in organ recipients constitute the small member of antagonistic but reciprocally attenuated or abrogated host-versus-graft (HVG) and graft-versus-host (GVH) arms, each of which can induce specific non-reactivity (tolerance) in the other (119, 125, 132, 134) (Figure 1.12). Deletion of the host arm by cytoablation prior to bone marrow but not organ transplant alters the balance in this mutual interaction and is thus responsible for the disparities in the two different kinds of transplantation (Table 3).

The 2-way paradigm defines success and failure after transplantation in a different way than before. Success implies that chimerism has been introduced which may or may not be immunosuppression-dependent. Failure connotes the therapeutically uncontrollable ascendancy of HVG or GVH. Pathologic evidence of both processes frequently is found in failed liver or intestinal transplant cases, but the ultimate result is predominantly rejection or GVHD. In kidney recipients who are exposed to a small load of passenger leukocytes, pathologic findings in the recipient and allograft are usually those of rejection only.

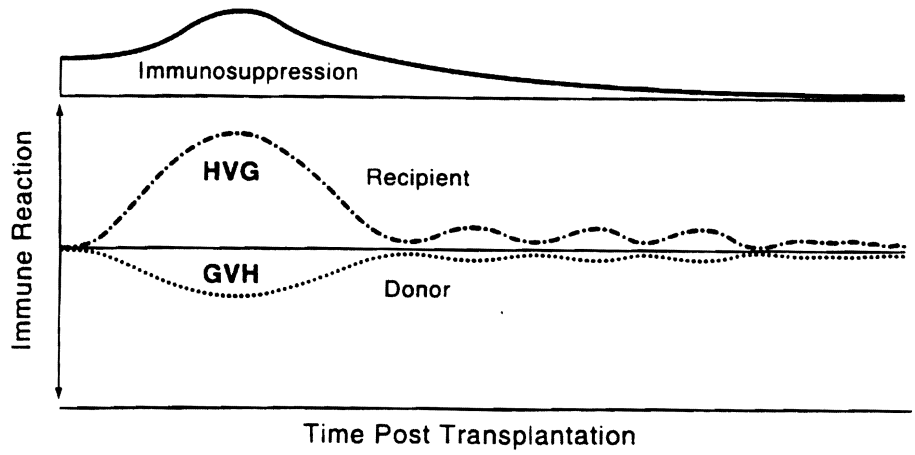

Figure 1.12 – Contemporaneous host versus graft (HVG) and graft versus host (GVH) reactions in the two-way paradigm of transplantation immunology. Following the initial interaction, the evolution of non-reactivity of each leukocyte population to the other is seen as a predominantly low-grade stimulatory state that may wax and wane, rather than a deletional one.

TABLE 3
DIFFERENCES BETWEEN CONVENTIONAL BONE MARROW AND ORGAN TRANSPLANTATION

Bone Marrow				*Organ*
Yes	←	Recipient Cytoablation*	→	No
Critical	←	MHC Compatibility	→	Not Critical
GVHD	←	Principal Complication	→	Rejection
Common	←	Drug Free State	→	Rare
Tolerance	←	Term for Success	→	"Acceptance"**

* Note: All differences derive from this therapeutic step which in effect establishes an unopposed GVH reaction in the bone marrow recipient whose countervailing immune reaction is eliminated.
** Or "operational tolerance"

After liver transplantation, however, stigmata of GVHD are not rare (119).

Other Enigmas – The dynamic 'nullification' effect of the two arms makes it obvious why organ recipients can sometimes stop immunosuppression without losing their allografts (134). It also explains the poor prognostic value of HLA matching for organ transplantation; the rarity of GVHD following the engraftment of immunologically active organs such as the intestine and liver.

The Counter-Argument

In the previous conception of organ allograft acceptance, which excluded a role for lymphoid cell microchimerism, it was axiomatic that antigens of the parenchymal (or vascular endothelial) cells of transplanted organs permitted or induced allograft acceptance (135) in various ways e.g. via veto/suppressor cells, cytokine profile changes or enhancing antibodies. In an extension of such reasoning, it has been contended that the microchimerism associated with successful organ transplantation, and conversely its disappearance with or just after irreversible rejection in experimental models (128, 130), is epiphenomenal (136) and inconsistent.

Such arguments have been skillfully summarized in a debate format by Wood and Sachs (137). However, there is no credible evidence to support their contention that the microchimerism found in organ recipients is the effect, rather than the cause of allograft acceptance. Failure with limited tissue or blood sampling to find peripheral microchimerism in patients after successful organ transplantation (127) connotes an incomplete search. In our clinical studies in which sampling was from multiple sites, the yields from individual locations were comparable to those of other investigators. However, when the results were pooled from the different sites in individual cases, all 30 of our originally tested patients had microchimerism (119). In rat experiments where tissues can be retrieved without limit, the association of chimerism with avoidance of chronic rejection has been absolute in our hands (131).

Post-Transplant Lymphoproliferative Disorders (PTLD)

The 2-way paradigm has shed light on the pathogenesis of PTLDs (138). Except for their frequent Epstein-Barr virus (EBV) association, these human B-cell lymphomas are indistinguishable from those induced by Robert S. Schwartz in a mouse chimerism model (139) 3 years before the PTLD complication was first recognized clinically (140) and explained by simple loss of surveillance (141). Rather than simple loss of surveillance, Schwartz (the same man who introduced 6-MP and azathioprine, see earlier) ascribed the tumors to an active lymphoproliferative response by the dominant immune apparatus to the persistent subclinical GVH counterattack of the minority donor leukocyte population. The relevance of his observations to clinical PTLD could only be appreciated 30 years later with the discoveries of microchimerism.

Then, it could be seen that PTLD is a complication of the joint activation of the coexisting immune populations, to which powerful co-factors are added that act upon both (138). Because host leukocytes in most organ recipients vastly outnumber the chimeric donor cells, with a similar obverse disproportion in successfully engrafted bone marrow recipients, clinical PTLD are usually of recipient origin in the first instance (142-144), and of donor phenotype in the second (143, 145, 146). There are exceptions to this generalization, but not to the principle.

For example, if the minority immunocyte population of an organ recipient unexpectedly gains ascendancy, the recipient is at primary risk of GVHD, but there is a corollary hazard of donor type PTLD. Conversely, the presence of a large recipient cell population in a bone marrow recipient with mixed chimerism implies a proportionate risk of a host PTLD. The exceptional PTLD of donor phenotype in organ recipients usually are seen during the early post-transplant period of mutual co-option of the 2 immune systems.

In the Schwartz model, heightened leukocyte cell renewal was ascribed solely to smoldering GVH. In human PTLD, however, this and all other B cell-stimulating influences would seemingly be dwarfed in importance by the presence of EBV with its uniquely potent B cell-transforming quality (147). However, the pathogenetic factors in the Schwartz model and the EBV infected transplant recipient are not mutually exclusive. It requires no imagination to postulate that their combination would send already heightened B cell proliferation into overdrive.

Also different from the Schwartz model, heavy immunosuppression typically precedes the appearance of the tumors in the human organ recipient. Conversely, reduction or discontinuance of the anti-rejection drugs (148) can allow restoration of immune surveillance, manifested by PTLD regression. Tumor involution frequently is coincident with organ rejection, but in most cases a level of immune suppression can be reached by trial and error that

permits salvage of the allograft without precipitating regrowth of the lymphoma (148-150).

The way in which this fresh insight can be used to map strategies of PTLD treatment is discussed elsewhere (138). The point here is that PTLD syndromes are aberrations, heavily influenced by co-factors, of the same interactions between donor and recipient cell populations by which allograft acceptance is achieved (119, 125, 132, 134). Immune suppression is a dominant co-factor, particularly when it is T cell-directed. Consequently, it was not surprising to note an incremental increase in PTLD with successively more potent immunosuppressants. However, the risk of PTLD can be reduced at the outset by avoiding the joint use of the biologic antilymphoid agents (i.e. ALG and OKT3) with cyclosporine and tacrolimus except as a last resort, and then with extreme caution. When PTLD is diagnosed early in development, it usually is a trivial problem requiring only drug dose reduction.

THE FUTURE OF TRANSPLANTATION

Tolerance Induction

In the context of the 2-way paradigm, historic efforts to improve transplantation results with donor-specific blood transfusion (151) and the donor bone marrow augmentation of organ recipients (152, 153) were based on sound therapeutic principles involving the unrecognized augmentation of chimerism. It is also obvious why whole organs are inherently tolerogenic. Their engraftment involves a small coincidental extramedullary bone marrow transplantation, including pluripotent stem cells (154).

Understanding the concept of the subsequent donor/recipient leukocyte dialogue helps predetermine what can (and cannot) be accomplished with various tolerance-inducing strategies, all of which are attempts to influence this interaction. Our first clinical premise was that the spontaneous microchimerism of organ transplantation could be greatly augmented by the co-administration of unmodified donor bone marrow cells without a significant risk of GVHD, providing the two immunocyte populations were initially competent and that immunosuppression was delivered to both equally. It also was predicted that the timing, severity, and frequency of acute rejection would be approximately the same as in non-marrow augmented control patients (125, 155, 156).

These expectations have been fulfilled in 200 human organ recipients treated at the University of Pittsburgh (156-158), including 86 who were given kidneys. The presence of donor DNA in the myeloid and erythroid colonies generated from recipients PBMC as measured in standard (157) or innovative clonal hematopoietic progenitor cell assays (159) has provided unequivocal evidence of augmented stem cell chimerism. There were no examples of significant GVHD.

The hypotheses of therapeutic efficacy being tested are that the threat of delayed (acute or chronic) rejection can be reduced and that the frequency of ultimate drug independence can be increased by the higher persistent level of chimerism. An efficacy evaluation is expected to take 5 to 10 years (134, 155), roughly the same time frame for tolerance induction learned from clinical experience with MHC-incompatible liver and bone marrow transplantation.

Other chimerism-enhancing strategies (e.g. G-CSF, GM-CSF or the new hematolymphopoietic growth factor, glycosulated Flt-3 ligand [160]) should follow the same safety/efficacy rules. In contrast, procedures that alter only one of the interacting arms must be approached with caution, as exemplified by the historical experience with GVHD following cytoablation and bone marrow transplantation. When the converse tactic of leukocyte or T-cell specific depletion of intestinal allografts was attempted as GVHD-prophylaxis in the 1980s, virtually every bowel recipient who survived the perioperative period developed lethal Epstein-Barr virus-associated B-cell lymphomas (53).

Unbalance also can be caused by delayed provision of donor leukocytes (e.g. repeat infusion of adjunct donor bone marrow to an organ recipient). To the extent that the first exposure (whether to infused leukocytes or to passenger leukocytes in a transplanted organ) induces tolerance, the result of the second stage delivery can resemble the effect of a parent to defenseless offspring F_1 hybrid experiment (128). Investigators signing on for multicenter trials of serial bone marrow augmentation should be made aware of this serious and predictable risk of GVHD.

Xenotransplantation

Further growth or transplantation will depend on the use of animal organs. This inevitably must conform to the guidelines painfully acquired over 35 years with allo-transplantation. The creation of transgenic animals is in essence an attempt to improve the cross species tissue match, and is specifically designed to eliminate the barrier of hyperacute humoral rejection by transfecting human complement regulatory genes (161-163). This has been accomplished in pigs, but it will not remove the necessity of maintaining cohabitation of the animal and human immune systems, as defined by the 2-way paradigm.

The possibility of achieving the latter objective has been demonstrated by Zanjani et al (164, 165), who inoculated sheep embryos intraperitoneally at the 40 to 50 day stage of the 120 day gestational period with leukocytes from human fetal livers, or with human stem cells purified from adult bone marrow. A handful of the sheep fetuses

completed their intrauterine life in a healthy state and have stable with > 5% human leukocyte chimerism 6 to 7 years later (165).

We have shown that stable chimerism also can be accomplished in piglets given unaltered adult bone marrow intravenously a few hours after birth, without any immunosuppression. One year after birth, there was evidence of donor-specific as well as species hyporeactivity in the healthy chimeric animals (166). At 14 months, the chimeric cells could be dramatically upregulated with injections of human GM-CSF (unpublished observations, University of Pittsburgh).

Because the primary source of complement is the liver, not the hematolymphopoietic system, it is unlikely that humoral rejection caused by the interspecies complement activation will be abrogated, no matter what the duration of chimerism (166). However, by inducing chimerism in pigs who already have human complement regulatory proteins in their organs at birth, the barrier of complement activation and cellular tolerance may be jointly approached. Such experiments are underway at the University of Pittsburgh.

CONCLUSION

The specialty of organ transplantation was an anomaly which acquired shape and substance by trial and error rather than springing from a well formulated scientific base or from a theoretical model. Its supporting struts are prevention of rejection, tissue matching, organ preservation, and surgical technique. However, only the last of these conformed to expectations. Consequently, the steps leading to practical clinical application of transplantation were empirical, each higher level of success appearing to violate more egregiously biologic (including genetic) rules that had been viewed as immutable. The consequence was a succession of unexpected small and great discoveries in the laboratory and in the clinic about how the immune system actually worked in the whole animal or human. Because of its complex structure and function, the liver, above all organs, was the supreme instrument of scientific exploration because it also served as the ultimate metabolic and physiologic probe.

The inability to explain why transplantation succeeded at all with any organ, much less routinely, made the evolution of this field the most enigmatic in the history of medicine. The assumption that stem cell-driven hematolymphopoietic chimerism was irrelevant to successful whole organ transplantation as currently practiced led to alternative inadequate explanations of organ allograft acceptance, clouded the meaning of successful bone marrow transplantation, and precluded for more than three decades the elucidation of a cardinal principle of transplantation. The recent recognition of this error and the incorporation of the chimerism factor into a two-way paradigm has allowed previous mysteries of organ as well as bone marrow engraftment to be explained and should allow the genius of basic science to be more meaningfully exploited in transplantation, including across species barriers. Thus, far from nearing its end, the history of transplantation and its role in medicine has only just begun.

REFERENCES

1. Waddell WR. Foreword. In: Starzl TE: *Experience in Hepatic Transplantation*. p. ix, 1969. W.B. Saunders Co., Philadelphia, PA.
2. Waddell WR. Foreword. In: Starzl TE: *Experience in Renal Transplantation*. p. ix, 1964. W.B. Saunders Co., Philadelphia, PA.
3. Starzl TE. The contribution of transplantation to gastroenterologic knowledge. In: *The Growth of Gastroenterologic Knowledge During the 20th Century* (JB Kirsner, ed) Lea and Febiger, Philadelphia, Pennsylvania, 1994, pp. 348-369.
4. Starzl TE. *Experience in Hepatic Transplantation*. WB Saunders Company, Philadelphia, PA, 1969, pp.3-553.
5. Welch CS. A note on transplantation of the whole liver in dogs. Transplant Bull 1955; 2:54.
6. Goodrich EO, Jr, Welch HF, Nelson JA, Beecher TS, Welch CS. Homotransplantation of the canine liver. Surgery 1956; 39:244.
7. Calne RY. The rejection of renal homografts: Inhibition in dogs by 6-mercaptopurine. Lancet 1960; 1:417-418.
8. Calne RY. Inhibition of the rejection of renal homografts in dogs with purine analogues. Transplant Bull 1961; 28:445.
9. Starzl TE, Marchioro TL, Rowlands DT Jr, Kirkpatrick CH, Wilson WEC, Rifkind D, Waddell WR. Immunosuppression after experimental and clinical homotransplantation of the liver. Ann Surg 1964; 160:411-439.
10. Daloze PM, Huguet C, Groth CG, Stoll F. Blood flow in auxiliary canine liver homografts. J Surg Research 1969; 9:10-12.
11. Mann FC. The William Henry Welch Lectures: II. Restoration and pathologic reactions of the liver. J Mt Sinai Hosp 1944; 11:65-74.
12. Fisher ER, Fisher B. Ultrastructural hepatic changes following partial hepatectomy and portacaval shunt in the rat. Lab Invest 1963; 12:929-942.
13. Marchioro TL, Porter KA, Brown BI, Otte J-B, Starzl TE. The effect of partial portacaval transposition on the canine liver. Surgery 1967; 61:723-732.
14. Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, Putnam CW. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973; 137:179-199.
15. Starzl TE, Porter KA, Kashiwagi N, Lee IY, Russell WJI, Putnam CW. The effect of diabetes mellitus on portal blood hepatotrophic factors in dogs. Surg Gynecol Obstet 1975; 140:549-562.
16. Starzl TE, Porter KA, Kashiwagi N, Putnam CW. Portal hepatotrophic factors, diabetes mellitus and acute liver atrophy, hypertrophy and regeneration. Surg Gynecol Obstet 1975; 141:843-858.
17. Starzl TE, Lee IY, Porter KA, Putnam CW. The influence of portal blood upon lipid metabolism in normal and diabetic dogs and baboons. Surg Gynecol Obstet 1975; 140:381-396.
18. Starzl TE, Porter KA, Putnam CW. Intraportal insulin protects from the liver injury of portacaval shunt in dogs. Lancet 1975; 2:1241-1246.
19. Starzl TE, Watanabe K, Porter KA, Putnam CW. Effects of insulin, glucagon, and insulin/glucagon infusions on liver morphology and cell division after complete portacaval shunt in dogs. Lancet 1976; 1:821-825.

20. Starzl TE, Porter KA, Terblanche J. Inter-organ communications: With particular reference to hepatotrophic factors and intrinsic liver growth factors. In: *Communications of Liver Cells* Proceedings of Falk-Symposium No. 27, (H Popper, F Gudat, L Bianchi, W Reutter, eds) PTP Press LTD, Lancaster, UK, 1980, pp. 93-97.
21. Francavilla A, Hagiya M, Porter KA, Polimeno L, Ihara I, Starzl TE. Augmenter of liver regeneration (ALR): its place in the universe of hepatic growth factors. Hepatology 1994; 20:747-757.
22. Starzl TE, Jones AF, Terblanche J, Usui S, Porter KA, Mazzoni G. Growth-stimulating factor in regenerating canine liver. Lancet 1979; 1:127-130.
23. Francavilla A, Ove P, Polimeno L, Coetzee M, Makowka L, Rose J, Van Thiel DH, Starzl TE. Extraction and partial purification of a hepatic stimulatory substance in rats, mice and dogs. Cancer Res 1987; 47:5600-5605.
24. Hagiya M, Francavilla A, Polimeno L, Ihara I, Sakai H, Seki T, Shimonishi M, Porter KA, Starzl TE. Cloning and sequence analysis of the rat augmenter of liver regeneration (ALR) gene: Expression of biology active recombinant ALR and demonstration of tissue distribution. Proc Natl Acad Sci 1994; 91:8142-8146.
25. Francavilla A, Starzl TE, Porter K, Scotti-Foglieni C, Michalopoulos GK, Carrieri G, Trejo J, Azzarone A, Barone M, Zeng QH. Screening for candidate hepatic growth factors by selective portal infusion after canine Eck's fistula. Hepatology 1991; 14:665-670.
26. Mazzaferro V, Porter KA, Scotti-Foglieni CL, Venkataramanan R, Makowka L, Rossaro L, Francavilla A, Todo S, Van Thiel DH, Starzl TE. The hepatotrophic influence of cyclosporine. Surgery 1990; 107:533-539.
27. Starzl TE, Porter KA, Mazzaferro V, Todo S, Fung J, Francavilla A. Hepatotrophic effects of FK 506 in dogs. Transplantation 1991; 51:67-70.
28. Starzl TE, Schrieber SL, Albers MW, Porter KA, Foglieni CS, Francavilla A. Hepatotrophic properties in dogs of human FKBP, the binding protein for FK506 and rapamycin. Transplantation 1991; 52:751-753.
29. Francavilla A, Azzarone A, Carrieri G, Scotti-Foglieni C, Zeng QH, Cillo U, Porter K, Starzl TE. Effect on the canine eck fistula liver of intraportal TGF-ß alone or with hepatic growth factors. Hepatology 1992; 16:1267-1270.
30. Francavilla A, Starzl TE, Scotti C, Carrieri C, Azzarone S, Zeng QH, Porter KA, Schrieber SL. Inhibition of liver, kidney, and intestine regeneration by rapamycin. Transplantation 1992; 53:496-498.
31. Francavilla A, Polimeno L, Barone M, Azzarone A, Starzl TE. Hepatic regeneration and growth factors. J Surg Oncol Suppl 1993; 3:1-7.
32. Francavilla A, Barone M, Van Thiel DH, Mazzaferro V, Prelich JG, Starzl TE. Further steps of hepatic stimulatory substance purification. Dig Dis Sci 1991; 36:674-680.
33. Eck NV. K voprosu o perevyazkie vorotnois veni: Predvaritelnoye soobschjenye. Voen Med J 1877; 130:1-2. (Translation: Ligature of the portal vein). Child C. G. III: Surg Gynecol Obstet 1953; 96:375-376.
34. Lisowsky T. Dual function of a new nuclear gene for oxidative phosphorylation and vegetative growth in yeast. Mol Gen Genet 1992; 232:58-64.
35. Giorda R, Hagiya M, Seki T, Shimonishi M, Sakai H, Michaelson J, Francavilla A, Starzl TE, Trucco M. Analysis of the structure and expression of the ALR gene. Molecular Med 1996; 2:97-108.
36. Marchioro TL, Porter KA, Dickinson TC, Faris TD, Starzl TE. Physiologic requirements for auxiliary liver homotransplantation. Surg Gynecol Obstet 1965; 121:17-31.
37. Starzl TE, Halgrimson CG, Koep LJ, Weil R III, Taylor PD. Vascular homografts from cadaveric organ donors. Surg Gynecol Obstet 1979; 149:737.
38. Shaw BW Jr, Iwatsuki S, Bron K, Starzl TE. Portal vein grafts in hepatic transplantation. Surg Gynecol Obstet 1985; 161:66-68.
39. Sheil AGR, Thompson JF, Stevens MS, Eyers AA, Graham JC, Bookallil MJ. Mesoportal graft for thrombosed portal vein in liver transplantation. Clin Transplant 1987; 1:18.
40. Tzakis A, Todo S, Stieber A, Starzl TE. Venous jump grafts for liver transplantation in patients with portal vein thrombosis. Transplantation 1989; 48:530-531.
41. Starzl TE, Porter KA, Francavilla A. The Eck fistula in animals and humans. Curr Probl Surg 1983; 20:687-752.
42. Bollman JL. The animal with an Eck fistula. Physiol Rev 1961; 41:607-621.
43. Cannon JA. Brief report. Transplant Bull 1956; 3:7.
44. Moore FD, Smith LL, Burnap TK, Dallenbach FD, Dammin GJ, Gruber UF, Shoemaker WC, Steenburg RW, Ball MR, Belko JS. One-stage homotransplantation of the liver following total hepatectomy in dogs. Transplant Bull 1959; 6:103-110.
45. Moore FD, Wheeler HB, Dimissianos HV, Smith LL, Balankura O, Abel K, Greenberg JB, Dammin GJ. Experimental whole organ transplantation of the liver and of the spleen. Ann Surg 1960; 152:374-387.
46. McBride RA, Wheeler HB, Smith LL, Moore FD, Dammin GJ. Homotransplantation of the canine liver as an orthotopic vascularized graft. Histologic and functional correlations during residence in the new host. Am J Pathol 1962; 41:501-520.
47. Starzl TE, Kaupp HA Jr, Brock DR, Lazarus RE, Johnson RV. Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet 1960; 111:733-743.
48. Starzl TE, Kaupp HA Jr, Brock DR, Linman JW. Studies on the rejection of the transplanted homologous dog liver. Surg Gynecol Obstet 1961; 112:135-144.
49. Starzl TE. In: Moore et al. (Reference n.45). Discussion. Ann Surg 1960; 152:386-387.
50. Starzl TE, Kaupp HA Jr. Mass homotransplantation of abdominal organs in dogs. Surg Forum 1960; 11:28-30.
51. Starzl TE, Kaupp HA Jr, Brock DR, Butz GW Jr, Linman JW. Homotransplantation of multiple visceral organs. Am J Surg 1962; 103:219-229.
52. Starzl TE, Rowe MI, Todo S, Jaffe R, Tzakis A, Hoffman AL, Esquivel C, Porter KA, Venkataramanan R, Makowka L, Duquesnoy R. Transplantation of multiple abdominal viscera. JAMA 1989; 261:1449-1457.
53. Starzl TE, Todo S, Tzakis A, Alessiani M, Casavilla A, Abu-Elmagd K, Fung JJ. The many faces of multivisceral transplantation. Surg Gynecol Obstet 1991; 172:335-344.
54. Owens TC, Prevedal AE and Swan H. Prolonged experimental occlusion of thoracic aorta during hypothermia. Arch Surg 1955; 70:95-97.
55. Bogardus GM and Schlosser RJ. The influence of temperature upon ischemic renal damage. Surgery 1956; 39:970-974.
56. Lillehei RC, Goott B, Miller FB. The physiologic response of the small bowel of the dog to ischemia including prolonged in vitro preservation of the bowel with successful replacement and survival. Ann Surg 1959; 150:543-560.
57. Starzl TE. Donor Nephrectomy. In: *Experience In Renal Transplantation*. WB Saunders Company, Philadelphia, PA, 1964, pp. 68-83.
58. Starzl TE, Hakala TR, Shaw BW Jr, Hardesty RL, Rosenthal TJ, Griffith BP, Iwatsuki S, Bahnson HT. A flexible procedure for multiple cadaveric organ procurement. Surg Gynecol Obstet 1984; 158:223-230.
59. Collins GM, Bravo-Shugarman M, Terasaki PI. Kidney preservation for transportation. Lancet 1969; 2:1219-1222.
60. Belzer FO, Southard JH. Principles of solid organ preservation by cold storage. Transplantation 1988; 45:673-676.
61. Todo S, Tzakis A, Starzl TE. Letter to the Editor. Preservation of

livers with UW or Euro Collin's solution. Transplantation 1988; 46:925-926.
62. Benichou J, Halgrimson CG, Weil R III, Koep LJ, Starzl TE. Canine and human liver preservation for 6 to 18 hr by cold infusion. Transplantation 1977; 24:407-411.
63. Wall WJ, Calne RY, Herbertson BM, Baker PG, Smith DP, Underwood J, Kostakis A, Williams R. Simple hypothermic preservation for transporting human livers long distances for transplantation. Transplantation 1977; 23:210-216.
64. Jamieson VN, Sundberg R, Lindell S, Laravuso R, Kalayoglu M, Soutyhard JH, Belzer FO. Successful 24 to 30 hour preservation of the canine liver: A preliminary report. Transplant Proc 1988; 20(Suppl 1):945-947.
65. Starzl TE, Miller C, Broznick B, Makowka L. An improved technique for multiple organ harvesting. Surg Gynecol Obstet 1987; 165:343-348.
66. Marchioro TL, Huntley RT, Waddell WR, Starzl TE. Extracorporeal perfusion for obtaining postmortem homografts. Surgery 1963; 54:900-911.
67. Starzl TE. Role of Cadaveric Donors. In: *Experience In Renal Transplantation*. WB Saunders Company, Philadelphia, PA, 1964, pp. 54-62.
68. Starzl TE. Donor Hepatectomy and Liver Preservation. In: *Experience in Hepatic Transplantation*. WB Saunders Company, Philadelphia, PA, 1969, pp. 41-64.
69. Brettschneider L, Daloze PM, Huguet C, Porter KA, Groth CG, Kashiwagi N, Hutchinson DE, Starzl TE. The use of combined preservation techniques for extended storage of orthotopic liver homografts. Surg Gynecol Obstet 1968; 126:263-274.
70. Ackerman JRW and Barnard CN: A report on the successful storage of kidneys. Brit J Surg 1966; 53:525-532.
71. Starzl TE, Iwatsuki S, Van Thiel DH, Gartner JC, Zitelli BJ, Malatack JJ, Schade RR, Shaw BW Jr, Hakala TR, Rosenthal JT, Porter KA. Evolution of liver transplantation. Hepatology 1982; 2:614-636.
72. Cutropia JC, Coratolo F, Spinetta A, Kei J, Ribas A, Assini L, Delle Donne G, Bianchi M, Guinazu A, Verdaguer JA. Trasplante hepatico ortotopico experimental (Experimental orthotopic liver transplantation). Rev Esp Enfermed Aparato Dig 1972; 38:553-567.
73. Denmark SW, Shaw BW Jr, Starzl TE, Griffith BP. Venovenous bypass without systemic anticoagulation in canine and human liver transplantation. Surg Forum 1983; 34:380-382.
74. Shaw BW Jr, Martin DJ, Marquez JM, Kang YG, Bugbee AC, Iwatsuki S, Griffith BP, Hardesty RL, Bahnson HT, Starzl TE. Venous bypass in clinical liver transplantation. Ann Surg 1984; 200:524-534.
75. Griffith BP, Shaw BW Jr, Hardesty FL, Iwatsuki S, Bahnson HT, Starzl TE. Venovenous bypass without systemic anticoagulation for transplantation of the human liver. Surg Gynecol Obstet 1985; 160:270-272.
76. Picache RS, Kapur BML, Starzl TE. The effect of liver disease on the need for venous decompression during the anhepatic phase of canine orthotopic liver transplantation. Surgery 1970; 67:319-321.
77. Starzl TE, Marchioro TL, Von Kaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation of the liver in humans. Surg Gynecol Obstet 1963; 117:659-676.
78. Starzl TE. The Recipient Operation in Man. In: *Experience in Hepatic Transplantation* WB Saunders Company, Philadelphia, PA, 1969, pp:112-143.
79. Calne RY, Williams R. Liver transplantation in man: I. Observations on technique and organization in five cases. Br Med J 1968; 4:535-540.
80. Tzakis A, Todo S, Starzl TE. Piggyback orthotopic liver transplantation with preservation of the inferior vena cava. Ann Surg 1989; 210(5):649-652.
81. Starzl TE, Bernhard VM, Benvenuto R, Cortes N. A new method for one-stage hepatectomy in dogs. Surgery 1959; 46:880-886.
82. Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle HR, Zeevi A, Warty V, Michaels M, Kusne S, Rudert WA, Trucco M. Baboon to human liver transplantation. Lancet 1993;341:65-71.
83. Starzl TE, Valdivia L, Murase N, Demetris AJ, Fontes P, Rao A, Manez R, Marino IR, Todo S, Thomson AW, Fung JJ. The biological basis of and strategies for clinical xenotransplantation. Immunol Rev 1994;141:212-244.
84. Tzakis AG, Reyes J, Nour B, Marino IR, Todo S, Starzl TE. Temporary end-to-side portocaval shunt in orthotopic liver transplantation. Surg Gynecol Obstet 1993; 76:180-182.
85. Stieber AC, Zetti G, Todo S, Tzakis AG, Fung J, Marino IR, Casavilla A, Selby R, Starzl TE. The spectrum of portal vein thrombosis. Ann Surg 1991; 213:199-206.
86. Todo S, Makowka L, Tzakis AG, Marsh JW Jr, Karrer FM, Armany M, Miller C, Tallent MB, Esquivel CO, Gordon RD, Iwatsuki S, Starzl TE. Hepatic artery in liver transplantation. Transplant Proc 1987; 19:2406-2411.
87. Shaw BW Jr, Iwatsuki S, Starzl TE. Alternative methods of arterialization of the hepatic graft. Surg Gynecol Obstet 1984; 159:490-493.
88. Tzakis A, Todo S, Starzl TE. The anterior route for arterial graft conduits in liver transplantation. Transplant International 1989; 2:121.
89. Martineau G, Porter KA, Corman J, Launois B, Schroter GT, Palmer W, Putnam CW, Groth CG, Halgrimson CG, Penn I, Starzl TE. Delayed biliary duct obstruction after orthotopic liver transplantation. Surgery 1972; 72:604-610.
90. Starzl TE, Ishikawa M, Putnam CW, Porter KA, Picache R, Husberg BS, Halgrimson CG, Schroter G. Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction. Transplant Proc 1974; 6:129-139.
91. Starzl TE, Porter KA, Putnam CW, Schroter GPJ, Halgrimson CG, Weil R III, Hoelscher M, Reid HAS. Orthotopic liver transplantation in ninety-three patients. Surg, Gynecol, Obstet 1976; 142:487-505.
92. Starzl TE, Putnam CW, Hansbrough JF, Porter KA, Reid HAS. Biliary complications after liver transplantation: With special reference to the biliary cast syndrome and techniques of secondary duct repair. Surgery 1977; 81:212-221.
93. Calne RY, McMaster P, Portmann B, Wall WJ, Williams R. Observations on preservation, bile drainage and rejection in 64 human orthotopic liver allografts. Ann Surg 1977; 186:282-290.
94. Waddell WR and Grover FL. The gallbladder as a conduit between the liver and intestine. Surgery 1973; 74:524-529.
95. Halff G, Todo S, Hall R, Starzl TE. Late complications with the gallbladder conduit biliary reconstruction after transplantation. Transplantation 1989; 48:537-539.
96. Dempster WJ, Lennox B, Boag JW. Prolongation of survival of skin homotransplants in the rabbit by irradiation of the host. Br J Exp Pathol 1950; 31:670-679.
97. Billingham RE, Krohn PL, Medawar PB. Effect of cortisone on survival of skin homografts in rabbits. Br Med J 1951; 1:1157-1163.
98. Morgan JA. The influence of cortisone on the survival of homografts of skin in the rabbit. Surgery 1951; 30:506-515.
99. Schwartz R, Dameshek W. The effects of 6-mercaptopurine on homograft reactions. J Clin Invest 1960; 39:952-958.
100. Meeker WR, Condie R, Weiner D, Varco RL, Good RA. Prolongation of skin homograft survival in rabbits by 6-mercaptopurine. Proc Soc Exp Biol 1959; 102:459-461.
101. Starzl TE, Butz GW Jr, Brock DR, Linman JT, Moss WT. Canine liver homotransplants: The effect of host and graft irradiation. Arch Surg 1962; 85:460-464.

102. Schwartz R, Stack J, Dameshek W. Effect of 6-mercaptopurine on antibody production. Proc Soc Exp Biol Med 1958; 99:164-167.
103. Schwartz R, Dameshek W. Drug-induced immunological tolerance. Nature 1959; 183:1682-1683.
104. Zukoski CF, Lee HM, Hume DM. The prolongation of functional survival of canine renal homografts by 6-mercaptopurine. Surg Forum 1960; 11:470-472.
105. Kuss R, Legrain M, Mathe G, Nedey R, Camey M. Homologous human kidney transplantation. Experience with six patients. Postgrad Med 1962; 38:528-531.
106. Murray JE, Merrill JP, Dammin GJ, Dealy JB Jr, Alexandre GW, Harrison JH. Kidney transplantation in modified recipients. Ann Surg 1962; 156:337-355.
107. Calne RY, Murray JE. Inhibition of the rejection of renal homografts in dogs by Burroughs Wellcome 57-222. Surg Forum 1961; 12:118.
108. Calne RY, Alexandre GPJ, Murray JE. A study of the effects of drugs in prolonging survival of homologous renal transplants in dogs. Ann NY Acad Sci 1962; 99:743.
109. Murray JE, Merrill JP, Harrison JH, Wilson RE, Dammin GJ. Prolonged survival of human kidney homografts by immunosuppressive drug therapy. N Engl J Med 1963; 268:1315-1323.
110. Starzl TE. The Rocky Mountains. In: *The Puzzle People: Memoirs of a Transplant Surgeon*. University of Pittsburgh Press, Pittsburgh, Pennsylvania, 1992, pp. 83-95.
111. Marchioro TL, Axtell HK, LaVia MF, Waddell WR, Starzl TE. The role of adrenocortical steroids in reversing established homograft rejection. Surgery 1964; 55:412-417.
112. Starzl TE, Marchioro TL, Waddell WR. The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet 1963; 117:385-395.
113. Braun WE. World Transplant Records 1995. Patients who currently have functioning transplants. Kidney (living-related). In: *Clinical Transplants 1995* (J.M. Cecka and P.I. Terasaki, Eds.) UCLA Tissue Typing Laboratory, Los Angeles, CA, 1996, pp. 503.
114. Moore FD, Birtch AG, Dagher F, Veith F, Krisher JA, Order SE, Shucart WA, Sammin GJ, Couch NP. Immunosuppression and vascular insufficiency in liver transplantation. Ann NY Acad Sci 1964; 102:729-738.
115. Demirleau, Noureddine, Vigne, Prawerman, Reziciner, Larraud, Louviers. Tentative d'homogreffe hepatique (Attempted hepatic homograft). Mem Acad Chir (Paris) 1964; 90:177-179.
116. Starzl TE, Marchioro TL, Porter KA, Iwasaki Y, Cerilli GJ. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal homotransplantation. Surg Gynecol Obstet 1967; 124:301-318.
117. Terasaki PI, Vredevoe DL, Mickey MR, Porter KA, Marchioro TL, Faris TD, Starzl TE. Serotyping for homotransplantation. VI. Selection of kidney donors for thirty-two recipients. Ann NY Acad Sci 1966; 129:500-520.
118. Starzl TE, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ Jr, Porter KA. Orthotopic homotransplantation of the human liver. Ann Surg 1968; 168:392-415.
119. Starzl TE, Demetris AJ, Trucco M, Murase N, Ricordi C, Ildstad S, Ramos H, Todo S, Tzakis A, Fung JJ, Nalesnik M, Rudert WA, Kocova M. Cell migration and chimerism after whole organ transplantation: The basis of graft acceptance. Hepatology 1993; 17:1127-1152.
120. Calne RY, Rolles K, White DJG, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Henderson RG, Aziz S, Lewis P. Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers. Lancet 1979; 2:1033-1036.
121. Starzl TE, Weil R III, Iwatsuki S, Klintmalm G, Schroter GPJ, Koep LJ, Iwaki Y, Terasaki PI, Porter KA. The use of cyclosporin A and prednisone in cadaver kidney transplantation. Surg Gynecol Obstet 1980; 151:17-26.
122. Starzl TE, Klintmalm GBG, Porter KA, Iwatsuki S, Schroter GPJ. Liver transplantation with use of cyclosporin A and prednisone. N Engl J Med 1981; 305:266-269.
123. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramanan R, Jain A. FK 506 for human liver, kidney and pancreas transplantation. Lancet 1989; 2:1000-1004.
124. Todo S, Fung JJ, Starzl TE, Tzakis A, Doyle H, Abu-Elmagd K, Jain A, Selby R, Bronsther O, Marsh W, Ramos H, Reyes J, Gayowski T, Casavilla A, Dodson F, Furukawa H, Marino I, Pinna A, Nour B, Jabbour N, Mazariegos G, McMichael J, Kusne S, Venkataramanan R, Warty V, Murase N, Demetris AJ, Iwatsuki S. Single center experience with primary orthotopic liver transplantation under FK506 immunosuppression. Ann Surg 1994; 220:297-309.
125. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. Lancet 1992; 339:1579-1582.
126. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 1973; 137:1142-1162.
127. Schlitt HJ, Hundrieser J, Hisanaga M, Uthoff K, Karck M, Wahlers T, Wonigeit K, Pichlmayr R. Patterns of donor-type microchimerism after heart transplantation. Lancet 1994; 343:1469-1471.
128. Demetris AJ, Murase N, Fujisaki S, Fung JJ, Rao AS, Starzl TE. Hematolymphoid cell trafficking, microchimerism, and GVHD reactions after liver, bone marrow, and heart transplantation. Transplantation Proc 1993; 25:3337-3344.
129. Qian S, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE. Murine liver allograft transplantation: Tolerance and donor cell chimerism. Hepatology 1994; 19:916-924.
130. Murase N, Starzl TE, Tanabe M, Fujisaki S, Miyazawa H, Ye Q, Delaney CP, Fung JJ, Demetris AJ. Variable chimerism, graft versus host disease, and tolerance after different kinds of cell and whole organ transplantation from Lewis to Brown-Norway rats. Transplantation 1995; 60:158-171.
131. Murase N, Demetris AJ, Tsamandas AC, Ye Q, Starzl TE. The heterogenous distribution of chimerism produced by rat organ and bone marrow allotransplantation. Transplantation 1996; 61:1126-1131.
132. Starzl TE, Demetris AJ. Transplantation milestones: Viewed with one- and two-way paradigms of tolerance. JAMA 1995; 273:876-879.
133. Przepiorka D, Thomas ED, Durham DM, Fisher L. Use of a probe to repeat sequence of the Y chromosome for detection of host cells in peripheral blood of bone marrow transplant recipients. Am J Clin Pathol 1991; 95:201-206.
134. Starzl TE, Demetris AJ, Murase N, Trucco M, Thomson AW, Rao AS. The lost chord: Microchimerism. Immunology Today, December, 1996.
135. Morecki S, Leshem B, Eid A, Slavin S. Alloantigen persistence in induction and maintenance of transplantation tolerance. J Exp Med 1987; 165:1468-1480.
136. Bushell A, Pearson TC, Morris PJ, Wood KJ. Donor-recipient microchimerism is not required for tolerance induction following recipient pretreatment with donor-specific transfusion and anti-CD4 antibody. Transplantation 1995; 59:1367-1371.
137. Wood K, Sachs DH. Chimerism and transplantation tolerance: cause and effect. Immunology Today, December, 1996.
138. Starzl TE, Nalesnik MA, Rao AS, Klein G, Demetris AJ, Fung JJ. Pathogenesis and treatment of post-transplant lymphoproliferative disease. Lancet, in press.
139. Schwartz R, Andre-Schwartz J, Armstrong MYK, Beldotti L. Neoplastic sequelae of allogenic disease. I. Theoretical considerations and experimental design. Ann New York Acad of Sciences 1966; 129:804-821.

140. Penn I, Hammond W, Brettschneider L, Starzl TE. Malignant lymphomas in transplantation patients. Transplant Proc 1969; 1:106-112.
141. Starzl TE, Penn I, Putnam CW, Groth CG, Halgrimson CG. Iatrogenic alterations of immunologic surveillance in man and their influence on malignancy. Transplant Rev 1971; 7:112-145.
142. Jaffe R, Trager JDK, Zeevi A, Sonmez-Alpan E, Duquesnoy R, Todo S, Rowe M, Starzl TE. Multivisceral intestinal transplantation: Surgical pathology. Pediatr Pathol 1989; 9:633-654.
143. Weissmann DJ, Ferry JA, Harris NL, Louis DN, Delmonico F, Spiro I. Post-transplantation lymphoproliferative disorders in solid organ recipients are predominantly aggressive tumors of host origin. Am J Clin Pathol 1995; 103:748-755.
144. Chadburn A, Suciu-Foca N, Cesarman E, Reed E, Michler RE, Knowles DM. Post-transplantation lymphoproliferative disorders arising in solid organ transplant recipients are usually of recipient origin. Am J Pathol 1995; 147:1862-1870.
145. Shapiro RS, McClain K, Frizzera G, Gajl-Peczalska KJ, Kersey JH, Blazar BR, Arthur DC, Patton DF, Greenberg JS, Burke B, Ramsay NKC, McGlove P, Filipovich AH. Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation. Blood 1988; 71:1234-1243.
146. Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, Castro-Malaspina H, Childs BH, Gillio AP, Small TN, Young JW, Kernan NA, O'Reilly RJ. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. New Engl J Med 1994; 330:1185-1191.
147. Klein G, Klein E. Evolution of tumours and the impact of molecular oncology. Nature 1985; 315:190-195.
148. Starzl TE, Nalesnik MA, Porter KA, Ho M, Iwatsuki S, Griffith BP, Rosenthal JT, Hakala TR, Shaw BW Jr, Hardesty RL, Atchison RW, Jaffe R, Bahnson HT. Reversibility of lymphomas and lymphoproliferative lesions developing under Cyclosporine-steroid therapy. Lancet 1984; 1:583-587.
149. Nalesnik MA, Makowka L, Starzl TE. The diagnosis and treatment of post transplant lymphoproliferative disorders. In: *Current Problems In Surgery* (M Ravitch, ed) Year Book Medical Publishers, Inc, 1988; 25:367-472.
150. Reyes J, Zeevi A, Ramos H, Todo S, Demetris AJ, Nour B, Nalesnik M, Trucco M, Abu-Elmagd K, Fung JJ, Starzl TE. Frequent achievement of a drug-free state after orthotopic liver transplantation. Transplant Proc 1993; 25:3315-3319.
151. Salvatierra O Jr, Vincenti F, Amend WJ, Potter D, Iwaki Y, Opelz G, Terasaki P, Duca R, Cochrum K, Hanes D, Stoney RJ, Feduska NJ. Deliberate donor-specific blood transfusions prior to living related renal transplantation. A new approach. Ann Surg 1980; 192:543-552.
152. Monaco AP, Clark AW, Wood ML, Sahyoun AI, Codish SD, Brown RW. Possible active enhancement of human cadaver renal allograft with antilymphocyte serum (ALS) and donor bone marrow: Case report of an initial attempt. Surgery 1976; 79:384-392.
153. Barber WH, Mankin JA, Laskow DA, Dierhoi MH, Julian BA, Curtis JJ, Diethelm AG. Long term results of a controlled prospective study with transfusion of donor-specific bone marrow in 57 cadaveric renal allograft recipients. Transplantation 1991; 51:70-75.
154. Murase N, Starzl TE, Ye Q, Tsamandas A, Thomson AW, Rao AS, Demetris AJ. Multilineage hematopoietic reconstitution of supralethally irradiated rats by syngeneic whole organ transplantation: with particular reference to the liver. Transplantation 1996; 61:1-4.
155. Starzl TE, Demetris AJ, Murase N, Thomson AW, Trucco M, Ricordi C. Donor cell chimerism permitted by immunosuppressive drugs: A new view of organ transplantation. Immunology Today 1993; 14:326-332.
156. Fontes P, Rao A, Demetris AJ, Zeevi A, Trucco M, Carroll P, Rybka W, Ricordi C, Dodson F, Shapiro R, Tzakis A, Todo S, Abu-Elmagd K, Jordan M, Fung JJ, Starzl TE. Augmentation with bone marrow of donor leukocyte migration for kidney, liver, heart, and pancreas islet transplantation. Lancet 1994; 344:151-155.
157. Rao AS, Fontes P, Dodson F, Zeevi A, Rugeles MT, Abu-Elmagd K, Aitouche A, Rosner G, Trucco M, Demetris AJ, Rybka W, Todo S, Fung JJ, Starzl TE. Augmentation of natural chimerism with donor bone marrow in orthotopic liver recipients. Transplant Proc 1996; 28:2959-2965.
158. Shapiro R, Rao AS, Fontes P, Zeevi A, Jordan M, Scantlebury VP, Vivas C, Gritsch A, Corry RJ, Egidi F, Rugeles MT, Rilo H, Aitouche A, Demetris AJ, Rosner G, Trucco M, Rybka W, Irish W, Fung JJ, Starzl TE. Combined simultaneous kidney/bone marrow transplantation. Transplantation 1995; 60:1421-1425.
159. Garcia-Morales R, Esquenazi V, Zucker K, Gomez CI, Fuller L, Carreno M, Cirocco R, Alamo A, Karatzas T, Burke GW III, Cianico G, Temple D, Fernandez H, Ricordi C, Tzakis A, Miller J. Assessment of the effects of cadaver donor bone marrow on kidney allograft recipient blood cell chimerism by a novel techinique combining PCR and flow cytometry (PCR-FLOW). Transplantation, In Press.
160. Iyengar A, Bonham CA, Antonysamy M, Rao AS, Murase N, Demetris AJ, Starzl TE, Thomson AW. Striking augmentation of haematopoietic cell chimerism and donor-derived dendritic cells in lymphoid tissue of non-cytoablated allogeneic bone marrow recipients treated with Flt_3 ligand and tacrolimus. Transplantation, In Press.
161. Bach FH, Turman MA, Vercellotte GM, Platt JL, Dalmasso AP. Accommodation: A working paradigm for progressing toward clinical discordant xenografting. Transplant Proc 1991; 23:205-207.
162. Parker W, Saadi S, Lin SS, Holzknecht ZE, Bustos M, Platt JL. Transplantation of discordant xenografts: a challenge revisited. Immunology Today 1996; 17:373-378.
163. Bach FH, Winkler H, Ferran C, Hancock WW, Robson SC. Delayed xenograft rejection. Immunology Today 1996; 17:379-384.
164. Zanjani ED, Pallavicini MG, Ascensao JL, Flake AW, Langious RG, Reitsma M, MacKintosh FR, Stutes D, Harrison MR, Tavassoli M. Engraftment of long-term expression of human fetal hemotopoietic stem cells in sheep following transplantation in utero. J Clin Invest 1992; 89:1178-1188.
165. Zanjani ED, Almeida-Porada G, Flake AW. Retention and multilineage expression of human hematopoietic stem cells in human-sheep chimeras. Stem Cells 1995; 13:101-111.
166. Starzl TE, Demetris AJ, Murase N, Valdivia L, Thomson AW, Fung J, Rao AS. The future of transplantation: with particular reference to chimerism and xenotransplantation. Transplant Proc, In Press.