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# Long-Term Survival after Renal Transplantation in Humans:

## (With Special Reference to Histocompatibility Matching, Thymectomy, Homograft Glomerulonephritis, Heterologous ALG, and Recipient Malignancy)

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AT the University of Colorado Medical Center, 189 patients have been given kidney homografts at a remote enough time to permit relatively long follow-up in the event of continued survival. On the basis of this experience, a reassessment will be attempted of the practical value of renal transplantation. In addition, the effect of various factors upon short and long-term survival will be examined including the organ source, the quality of HL-A antigen matching, thymectomy before or after transplantation, the addition of heterologous antilymphocyte globulin (ALG) to the immunosuppressive regimen, the development of glomerulonephritis in the transplants, and the occurrence of a significantly increased incidence of *de novo* malignancies in the recipients.

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## Case Material

### Temporal Subdivision of Experience

The cases were divided on the basis of potentially important changes in either immunosuppressive management or donor selection.

**Series 1.** Between the autumn of 1962 and March 1964, immunosuppressive therapy was provided with azathioprine and prednisone.[64] Except for the usual (though not invariable) precaution of assuring compatibility of red cell groups, donor selection was not guided by any pre-transplant immunologic evaluation. Actinomycin C was given to a few patients during episodes of rejection, and/or the homografts were locally irradiated.

**Series 2.** Immunosuppression continued to be provided with azathioprine and prednisone between October 1964 and April 1966. However, an attempt was made to type lymphocyte antigens[83, 86] which became known later as the HL-A system.[46] On the basis of these serologic analyses, the best matched donor was selected from among those available. For the intrafamil-

ial transplantations of Series 2, the number of potential donors for any given recipient was so small that there was no statistically significant improvement of the quality of matching over that which had been achieved in Series 1.[83] In the non-related cases of Series 2, it was thought that the use of a donor pool of as many as 100 volunteers would permit an appreciable upgrading of matching.[83] In retrospect, the improvement was not particularly great, since the antigen measurements were so incomplete by present-day standards.

**Series 3.** In these patients, horse antilymphocyte globulin (ALG) was added to the immunosuppressive agents azathioprine and prednisone.[66, 71] The ALG was given intramuscularly before or at the time of transplantation and in the average instance was continued for 4 months.[70] Tissue typing was almost always performed prospectively but failure to obtain a good tissue match was not considered to be a contraindication to operation. As a consequence, the quality of matching in Series 3 was not significantly different than in Series 1 and 2.

### Subdivision According to Donor

**Consanguineous Homotransplantation.** Excluding identical twins, there were 131 consecutive intrafamilial transplantations. The primary renal homografts were donated by 66 siblings, 55 parents, four uncles, four cousins, an aunt and a niece. The 131 recipients underwent operations between November 1962 and February 1968. Consequently, potential follow-ups of 2 to 7½ years are now available.

**Non-related Homotransplantation.** The first homografts for the other 58 recipients were obtained from non-related donors. The operations were performed between February 1963 and March 1969, assuring potential follow-ups of one to more than 7 years. The minimum observation period for patients still alive was shorter for the non-related transplantations (one year) than for the consanguineous cases (2 years). The shorter follow-up was accepted since the sample of non-related cases acquired late in our experience would otherwise have been too small to permit meaningful statistical comparisons with the earlier cases. The donors were living volunteers in 35 instances and in the other 23 they were recently deceased cadavers. Only cadaveric donors have been used in non-related cases since November 1965.

### Pathologic Material

Kidney homograft samples have been obtained from 163 of the 189 recipients as a result of open biopsy, transplant nephrectomy or autopsy. In some cases more than one homograft was examined from a given recipient. All the tissues were examined by light microscopy. Sections were cut at both 5 $\mu$ and 0.5 $\mu$. The former were stained with hematoxylin and eosin, periodic acid Schiff (PAS), Weigert's for elastic counterstained with hematoxylin and van Gieson, and methyl green pyronin. The 0.5 $\mu$ thick sections were stained with Azure 2.

Electron microscopy was carried out on 126 of the specimens. The tissue was fixed in Palade's buffered osmium tetroxide and embedded in Epon 812. Ultra-thin sections were stained with lead citrate and examined in a Philips 300 electron microscope.

From 96 of the homografts, samples of tissue were frozen in liquid nitrogen and stored at −70° centrigrade until examined. Sections 4 $\mu$ thick were cut in a cryostat and stained with fluorescein conjugated antisera.[78] The fluorescent reagents used were antisera made in rabbits and goats to human IgG, IgM, fibrinogen, C'1q and $\beta$1C/$\beta$1A globulin. Specificity of the antisera was confirmed before application to the tissue by immuno-electrophoresis and double diffusion in agarose. Specificity of the fluorescence was established by block-

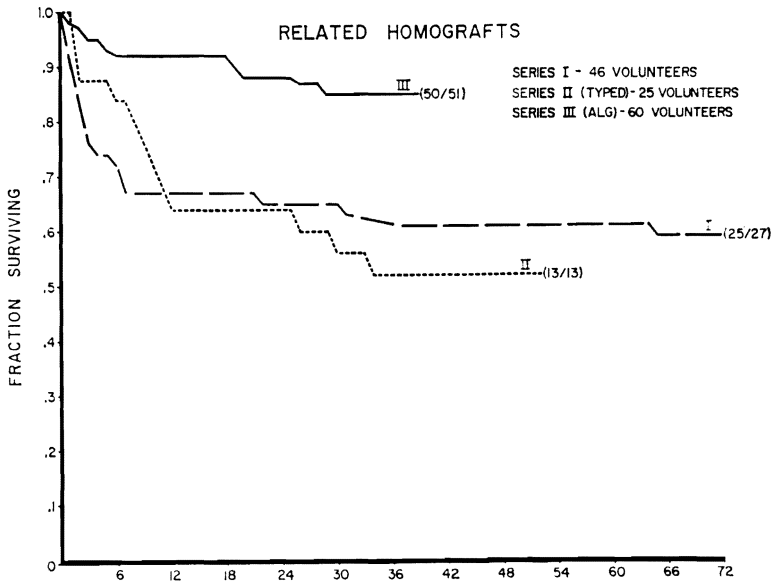


FIG. 1. Life survival curves in the three series of intrafamilial renal homotransplantations described in the text. In Series 3, the patients have been studied for at least 26 months. The follow-ups in Series 2 and 1 are for a minimum of 4 and 6 years, respectively. At the end of each survival curve a numerator and denominator are given. The denominator tells the number of living patients, and the numerator denotes the number of original homografts that are still functioning.

ing positive reactions with unconjugated antisera, by absorption of the labelled antisera with specific antigens, and by the use of an anti-human serum albumin control.

In so far as the method of tissue collection permitted, the presence or absence of the following features was determined in the homografts: (1) subendothelial glomerular capillary basement membrane thickening; (2) subepithelial glomerular capillary basement membrane thickening; (3) increased cellularity of the glomerular tufts; (4) increased amount of mesangial matrix; (5) tubular atrophy; (6) interstitial fibrosis; (7) mononuclear cell infiltration of the interstitium; (8) "hyaline" in the arteriolar walls; (9) thickening of the intima of the interlobular arteries; (10) deposits of IgG, IgM, complement or fibrinogen in the glomeruli. The lesions and deposits were graded in severity from 0 to 4.

In 159 cases, the patients' own diseased kidneys were examined and the findings were ultimately compared to those in the homografts. These specimens were all studied by light microscopy and in a few instances by immunofluorescence.

## Overall Mortality

### After Intrafamilial Transplantation

**According to Series.** In Series 1, there was a heavy early mortality in that a third of the recipients (15/46) had died by the end of 7 months (Fig. 1). From this point on, the losses were encouragingly slight and only one patient each died in the second, third, fourth and sixth postoperative years. Today, 27 (58.7%) of the 46 recipients are still alive after 6 to 7½ years (Fig. 1). Twenty-five of these 27 patients are still living on the function of their original homografts. The two others received second transplants after 5½ and 6 years. None of the 27 remaining patients is on dialysis.

The acute mortality of Series 2 was reduced to the extent that 84% of the recipients were alive at 7 months. However, in spite of these first prospective attempts at histocompatibility typing, the results were slightly worse after 2, 3, and 4 years than in Series 1 (Fig. 1). Of the 25 recipients, 13 (52%) still survive after 4 to 5½ years, in each case with function of the original homograft.

TABLE 1. *Results with Sibling Transplantation in Series 1–3 and in Combined Series*

| | Time in Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | 18 | 24 | Current |
| Series 1 | 23 | 16 | 15 | 14 (61%) | 14 | 13 (57%) | *10 (43%) |
| Series 2 | 9 | 9 | 9 | 7 (78%) | 7 | 7 (78%) | **5 (56%) |
| Series 3 | 34 | 33 | 32 | 32 (94%) | 32 | 31 (91%) | ***29 (85%) |
| Total experience | 66 | 58 | 56 | 53 (80%) | 53 | 51 (77%) | 44 (67%) |

* The 3 late deaths occurred after 30, 37, and 64 months. The 10 current survivors have continuing function of the original homografts after 6 1/6–7 1/3 years.

** The 2 late deaths occurred after 25 and 33 months. The original homografts in the 5 survivors have functioned for 4–5 1/2 years.

*** The 2 late deaths were after 25 and 28 months. Twenty-eight of the 29 current survivors have function of the original homografts after 2 1/6–3 5/6 years.

Follow-ups of more than 2 to almost 4 years are available for the patients of Series 3 who received a 4-month course of ALG as well as indefinite azathioprine and prednisone. At 1 and 2 years, the patients (and kidneys) survived at rates of 92 and 88%. There are 51 (85%) of the 60 recipients still alive and in all but one, the original homografts are still providing life-supporting function. The exceptional patient had a successful retransplantation using a cadaver kidney after 3⅞ years.

**According to Donor Relation.** The largest experience with intrafamilial transplantation was with sibling cases of which there were 66 (Table 1). Through Series 1–3, there was a steady improvement in the one year, 2 year, and ultimate survival rate. Exactly two thirds (44/66) of the sibling recipients treated from 27 to 87 months ago are still alive and all but one of these patients have continuing function of their original homografts.

The pooled survival in Series 1–3 after parent to offspring transplantation (Table 2) was similar to that with siblings in that 73% (40/55) of all recipients of parental kidneys are still alive from 26 to 90 months after their initial operation. Furthermore, 38 of these 40 patients still have adequate function of the first grafts. In the two exceptional instances, retransplantation was carried out between 5 and 6 years. As with sibling transplantation, the best results were obtained in the ALG treated patients (Series 3).

TABLE 2. *Results with Parent to Offspring Transplantation in Series 1–3 and in Combined Series*

| | Time in Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | 18 | 24 | Current |
| Series 1 | 20 | 16 | 15 | 14 (70%) | 14 | 14 (70%) | *14 (70%) |
| Series 2 | 15 | 12 | 12 | 9 (60%) | 9 | 9 (60%) | **8 (53%) |
| Series 3 | 20 | 20 | 19 | 19 (95%) | 19 | 18 (90%) | ***18 (90%) |
| Total experience | 55 | 48 | 46 | 42 (76%) | 42 | 41 (75%) | 40 (73%) |

* The 14 survivors have follow-ups of 6 1/6–7 1/2 years. Twelve of these 14 patients have continuing function of their original kidneys. The other 2 had retransplantation at 5 1/2 and 5 5/6 years, respectively.

** The 8 surviving patients are 4–5 1/2 years. All 8 have continuing function of their original homografts.

*** Eleven of the 18 surviving patients are 2 1/2–3 5/6 years. The other 6 are 2–2 1/2 years. All 18 survivors have continuing function of their original homografts.

TABLE 3. *Results with Transplantation from Uncles, Cousins, an Aunt and a Niece**

| | Time in Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | 18 | 24 | Current |
| Series 1 | 3 | 3 | 3 | 3 (100%) | 3 | 3 (100%) | 3 (100%) |
| Series 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Series 3 | 6 | 4 | 4 | 4 (67%) | 4 | 4 (67%) | 4 (67%) |
| Total experience | 10 | 8 | 7 | 7 | 7 | 7 | 7 (70%) |

* All 7 surviving patients have had function of their original definitive homografts after 2 1/3–6 1/6 years.

Ten patients received homografts from relatives more distant than siblings or parents. The pooled patient and kidney survival to date (Table 3) remains at 70% (7/10) with follow-ups of 2⅓ to 6½ years.

### After Non-Related Transplantation

In the unrelated Series 1, 2, and 3, respectively, there were 18, 23, and 17 patients. At first (Series 1), all the donors were living volunteers. In Series 2, 6 cadaveric donors (26% of the 23 cases) were used and this source of organs was utilized exclusively for Series 3.

At the end of 1 year, the survival in Series 1, 2, and 3 was 33%, 52%, and 82%. Subsequent to 1 year, the loss of lives and of homografts continued at a steady rate (Fig. 2). For example, the 41 patients in the pooled Series 1 and 2 now have potential follow-up intervals of 4½ to more than 7 years. Only eight (19.5%) of these 41 early recipients are still alive and only five of them are being supported by their original transplants; the other three required retransplantation after 1, 2½, and 3 years. In Series 3, three deaths after 17½, 25, and 27½ months have reduced the survival from 82.3% at one year to the present level of 65%.

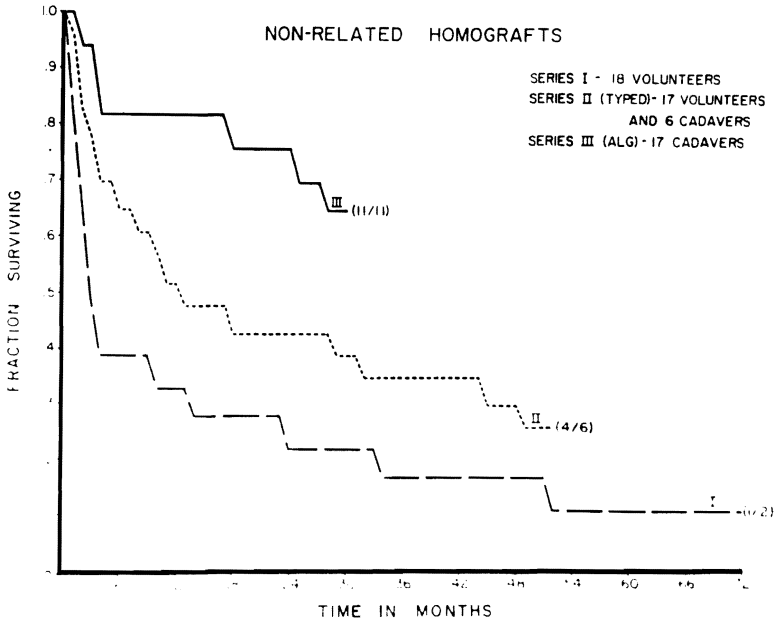


FIG. 2. Life survival curves in the three series of non-related renal homotransplantations described in the text. The minimum follow-ups in Series 1, 2, and 3 are 6, 4½, and 1 years. The significance of the numerators and denominators at the end of each curve is the same as in Figure 1.

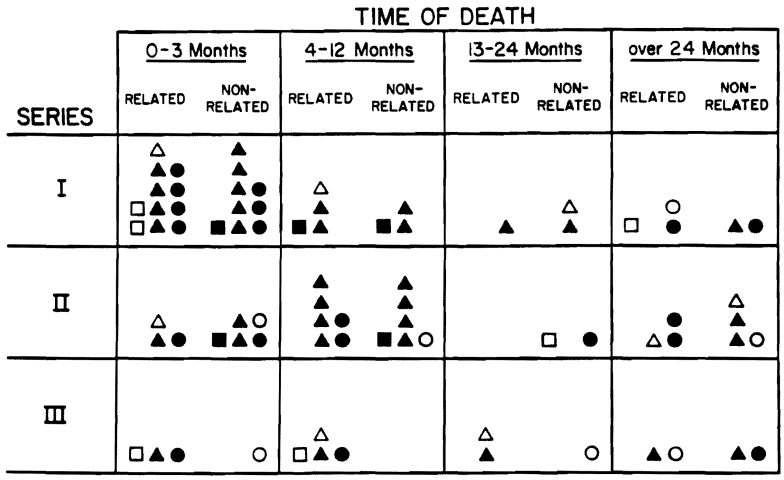


FIG. 3. The contribution of imperfect or failed renal function and of infections to the deaths of 79 patients. The shading of symbols indicates that infection either caused or was a major contributor to death. The squares indicate excellent premortem renal function. The triangles indicate subnormal but life-supporting premortem renal function. The circles indicate that the homografts had failed.

## Causes of Death

Seventy-nine of the 189 patients have died, 40 after receipt of related kidneys and 39 after receiving non-related organs. Autopsies were performed in all but two. In reviewing the causes of the 79 deaths, a specific notation was made in every case about the roles of renal insufficiency and infection in the fatal outcome. Two associations were obvious. First, 68 of the 79 patients had less than normal (42 examples) or failed renal function (26 examples) in the interval before death (Fig. 3). Secondly, a major infectious complication was present in 58 (73%) of the cases (Fig. 3). The most difficult therapeutic dilemma was posed by the co-existence of variable degrees of renal impairment in conjunction with sepsis. In this situation, life was threatened by reduced or failing function of the homograft and by measures taken to prevent further deterioration or complete loss of the transplant. In 53 of the deaths (70%) the combination of imperfect (or failed) homograft function and infection was present.

The timing of death differed in the three periods of our experience. In Series 1, the mortality was heavily concentrated in the first 3 months (Fig. 3) due in large part to a tendency to administer doses of azathioprine that caused bone marrow depression in the event of renal impairment. In Series 2, this error was avoided. However, the mortality from infection was not prevented but only forestalled to the 4 to 12 month post-transplantation period (Fig. 3). With the more balanced form of immunosuppression used in Series 3, the deaths became rather evenly distributed throughout the first 2 years. Moreover, only half of the patients in the last series had significant infection at the time of death, compared to 80% and 76% in Series 1 and 2.

The locations of the infections were highly variable but the most common were the lung (31 examples), the central nervous system (five brain abscesses and three meningitides), the transplant wound (eight examples) and the peritoneal cavity (four examples). The infecting micro-organisms were frequently multiple but it was usually possible to determine the dominant agent. Deaths in the first three months were due mainly to well known bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* (Table 4). After the early post-transplantation months, nonbacterial (fungal, protozoan, viral) infection played a major role (Table 4). Between the 4th and 12th month after transplantation, Pneumocystis carini accounted for five deaths and fungal

infections killed an additional five patients. After 4 months, four patients died from viral causes alone, three with viral hepatitis and the other with pan-intestinal varicella. Multi-organ cytomegalic inclusion disease was found in many patients, often in association with pneumocystis carini.

There were two deaths in the early postoperative period from acute pancreatitis. Pulmonary emboli killed three patients (after 3, 18½ and 28½ months) and contributed to the death of at least 10 others. Two recipients died of myocardial infarction 2½ and 4⅓ years post-transplantation. As will be mentioned later, reticulum cell sarcoma accounted for two late deaths. Isolated causes of mortality included suicide, inanition, stroke, and jejunal necrosis.

TABLE 4. *Pathogenic Organisms Involved in the Deaths* of 79 Patients*

| | Number of Patients Death with Infection at: | | |
|---|---|---|---|
| | 0–3 mo. | 4–12 mo. | over 12 mo. |
| Bacterial: | | | |
| *Staphylococcus aureus* | 3 | 4 | 2 |
| *Hemolytic Streptococcus* | 4 | 0 | 0 |
| *Listeria monocytogenes* | 0 | 1 | 0 |
| *Diplococcus pneumoniae* | 1 | 0 | 0 |
| *Escherichia coli* | 8 | 1 | 2 |
| *Pseudomonas aeruginosa* | 12 | 8 | 5 |
| *Klebsiella aerobacter* | 4 | 1 | 2 |
| *Proteus mirabilis* | 3 | 0 | 0 |
| *Paracolon species* | 1 | 0 | 0 |
| Viral: | | | |
| Hepatitis | 0 | 1 | 2 |
| Cytomegalic inclusion dis. | 3 | 1 | 2 |
| Varicella | 0 | 0 | 1 |
| Protozoan: | | | |
| Pneumocystis carini. | 1 | 8 | 1 |
| Fungal: | | | |
| *Aspergillus fumigatus* | 2 | 3 | 2 |
| *Candida albicans and stelloidea* | 4 | 2 | 1 |
| Nocardia | 0 | 2 | 0 |
| Histoplasmosis | 0 | 1 | 0 |
| Cryptococcus | 0 | 0 | 1 |

* Some patients died with 2–3 types of microorganisms. For each patient the full spectrum of clinically significant microbiologic data has been entered into the table.

## Histocompatibility Determinations

Tissue typing in these cases [73] was begun in the spring of 1964 with the serologic technic of lymphocyte antigen detection [80] that has become widely used.[84] Patients from our previous experience who had died by 1964 could not be studied but matching was carried out retrospectively upon the recipients still surviving and upon their donors. Subsequently, tissue typing was almost always carried out in advance of operation. At all times, the reagents used for typing were lymphocytotoxin-rich human isoimmune antisera obtained from persons who had been sensitized accidentally or deliberately to white cell antigens.

The cytolysis of test lymphocytes by such antisera indicated the presence of the same or a similar antigen as that which originally sensitized the serum donor. Failure of such a reaction implied the absence of the antigen. When the lymphocytes of both the donor and recipient reacted the same to a given antiserum, *identity* of that antigen was said to be present. The absence of an antigen in a donor which was present in a recipient was defined as *compatibility*. When an antigen was found in the donor lymphocytes but not on those of the recipient, a *mismatch* existed. Identity of antigens was preferable, compatibility was the next most satisfactory condition, and the least desirable was an overt mismatch.

The number of antisera used for typing has been as large as 200. Even when human typing was first performed it was appreciated that many of the antisera in the total panel measured the same or similar lymphocyte antigens. Between 1963 and 1968 [2, 7, 9, 10, 29, 42, 49, 82, 91, 93] those antisera were classified by direct testing and by computer technics according to their specificity of action. In this way, it eventually became possible to define human lymphocyte HL-A antigens against which groups of antisera reacted.[22, 23]

Since tissue typing was performed on our

TABLE 5. *57 Sibling Renal Homografts*

| Match | Survival Time in Months | | | | | Current |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | 24 | |
| A | 17 | 17 | 16 | 16 | 16 (94%) | 14 (82%)* |
| B | 18 | 17 | 17 | 17 | 17 (94%) | 14 (78%)** |
| C | 16 | 16 | 16 | 14 | 12 (75%) | 10 (63%)*** |
| D | 6 | 6 | 6 | 6 | 6 (100%) | 5 (83%)**** |

The fate of 57 sibling renal homografts in relation to the histocompatibility matches. Loss of the homograft is considered the equivalent of death. The sibling cases of 1962–1964 have been included even though they were studied retrospectively since 14 of the 23 recipients of that era lived long enough to be typed. With $\chi^2$ analysis, the groups were not significantly different.

* The late deaths occurred at 28 1/2 and 33 1/2 months.

** Late kidney loss or death after 25 1/2, 42 and 66 months.

*** Late deaths after 25 1/2 and 37 1/2 months.

**** Late death is after 37 1/2 months.

patients before as well as after the definition of HL-A groups, it was necessary to convert the year to year data into uniform terminology. This was done in December 1969 and January 1970 by re-analyzing the results obtained with the original antisera in the early cases and by converting the findings into HL-A designations. At the least, it was possible to define eight antigenic groups and at the time of the most completely studied later cases, 11 groups [23] could be identified. These groups will be referred to below as phenotypes.

## HL-A Phenotype Correlations

With the definition of antigen groups, a histocompatibility grade (A–D) was given. An A match indicated identity of the measured donor and recipient HL-A antigen groups. With a B match, no incompatibilities were present, but there were one or more examples of non-identity. C and D matches were progressively less satisfactory with frank mismatches of one or more antigen groups. Because new HL-A groups were discovered and characterized through-

TABLE 6. *49 Parental Renal Homografts*

| Match | Survival Time in Months | | | | | Current |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | 24 | |
| A | 5 | 5 | 5 | 3 | 3 (60%) | 2 (40%)* |
| B | 19 | 16 | 16 | 16 | 16 (84%) | 15 (79%)** |
| C | 22 | 22 | 21 | 20 | 20 (91%) | 18 (82%)*** |
| D | 3 | 3 | 3 | 3 | 2 (67%) | 2 (67%)**** |

The fate of 49 parental renal homografts in relation to the histocompatibility matches. Loss of the homograft is considered the equivalent of death. The parental cases of 1962–1964 have been included even though they were studied retrospectively since 14 of the 20 recipients of that era lived long enough to be typed.

* In the late case, the maternal kidney has deteriorated so much that the patient began to require occasional dialysis after 6 1/2 years.

** The late death was caused by reticulum cell sarcoma after 29 1/2 months; renal function was subnormal with a creatinine clearance of 20–30 ml./min.

*** The late homograft losses occurred at 5 1/2 and 5 5/6 years; the patients were both successfully retransplanted.

**** The 2 surviving patients have completely normal renal function after 6 3/4 and 4 2/3 years.

out the 6-year period of analysis, the basis for grading was more complete and consequently more accurate at the end of the study than at the beginning.

**Survival.** After intrafamilial transplantation, survival was not associated to a statistically significant degree with either compatibility or incompatibility. This conclusion applied with sibling to sibling (Table 5), and with parent to offspring combinations (Table 6) as well as with transplantation from more distant relatives.

Histocompatibility typing was carried out prospectively in only 34 of the 58 unrelated cases and retrospectively in another five. The five retrospectively typed recipients were not included in this analysis since by the time they were studied they represented such a small number (28%) of patients remaining alive from the original 18 of Series 1 that an unacceptable degree of selection had already occurred. In the 34 prospectively typed cases there was not significant correlation of the match with survival (Table 7).

TABLE 7. *34 Unrelated Renal Homografts*

| Match | Survival Time in Months 0 | 3 | 6 | 12 | Current |
|---|---|---|---|---|---|
| B | 8 | 5 | 5 | 5 (62.5%) | 3 (37.5%)* |
| C | 17 | 15 | 12 | 12 (71%) | 8 (47%)** |
| D | 9 | 9 | 8 | 6 (67%) | 4 (44%)*** |

The fate of 34 unrelated renal homografts in relation to the prospective histocompatibility matches as determined by serologic analysis. For this table, loss of the homograft is considered the equivalent of death. Since prospective matching was not carried out from 1962 to March 1964, cases from that era have not been included.

* The three current survivors are 14, 53, and 59 months. The late deaths occurred at 17 1/2 and 44 1/2 months.

** The eight current survivors are 15, 15, 18, 24, 26, 28, 57, and 59 months. The late deaths occurred at 12 1/2, 13, 27 1/2, and 31 1/2 months.

*** The four current survivors are 12, 21, 23, and 33 months. The late deaths occurred at 28 1/2 and 48 1/2 months.

**Function and Immunosuppression.** For

TABLE 8. *Antigen Match Versus Kidney Function and Steroid Doses*

| | Donor | Time Post-Op (Years) | Antigen Match A | B | C | D |
|---|---|---|---|---|---|---|
| BUN mg./100 ml. | Sibling | 1 | 23.8 | 24.5 | 29.7 | 26.5 |
| | | 2 | 23.2 | 21.4 | 30.6 | 29.6 |
| | Parental | 1 | 22.7 | 24.3 | 28.5 | 23.7 |
| | | 2 | 23.6 | 24.6 | 33.4 | 20.5 |
| Creatinine Clearance ml./min. | Sibling | 1 | 86.7 | 87.0 | 73.3 | 88.1 |
| | | 2 | 93.4 | 87.9 | 71.1 | 77.1 |
| | Parental | 1 | 72.6 | 69.1 | 69.5 | 65.2 |
| | | 2 | 73.3 | 74.1 | 63.8 | 83.0 |
| Daily Prednisone Dose (mg./Kg.) | Sibling | 1 | 0.21 | 0.26 | 0.24 | 0.22 |
| | | 2 | 0.16 | 0.21 | 0.25 | 0.23 |
| | Parental | 1 | 0.24 | 0.24 | 0.30 | 0.30 |
| | | 2 | 0.20 | 0.21 | 0.25 | 0.16 |
| No. of Patients | Sibling | 1 | 16 | 17 | 14 | 6 |
| | | 2 | 16 | 16 | 12 | 6 |
| | Parental | 1 | 3 | 16 | 20 | 3 |
| | | 2 | 3 | 15 | 20 | 2 |

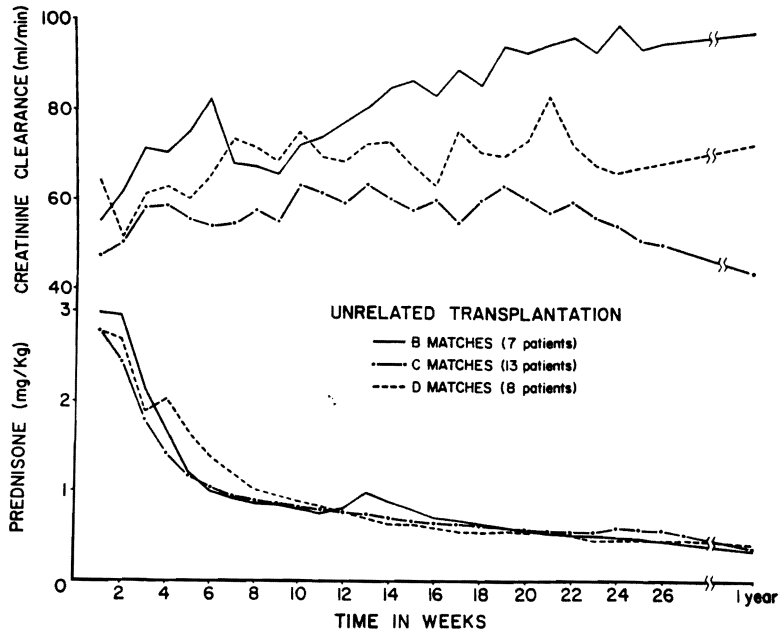


FIG. 4. Average daily Prednisone doses and creatinine clearances for the first 26 weeks post-transplantation and at one year in 28 recipients of non-related renal homografts, grouped according to histocompatibility grades. Inclusion in the analysis was contingent upon one year survival after transplantation.

these comparisons, A and B matches were pooled and compared with the combined C and D matches. In both the sibling and parental recipients the patients with A and B matched kidneys had slightly better function in that the average BUN's were lower and the creatinine clearances were higher at the end of the first and second postoperative years (Table 8). However, the differences were significant ($p < 0.02$) only in the sibling cases at 2 years. There was no correlation between the typing results and the average daily immunosuppression with prednisone and azathioprine at the first two annual follow-up times. Subtle late deterioration of function of the badly as opposed to the well matched kidneys was not evident.

There were 28 non-related cases, five from Series 1, 9 from Series 2, and 14 from Series 3 in which good enough histocompatibility typing was available to permit correlation of the match with the immunosuppressive treatment and homograft function. During the first postoperative year, the seven patients who lived this long after receipt of B matched kidneys had higher average creatinine clearances (Fig. 4) and lower BUN's than patients with C and D matched kidneys. The prednisone (Fig. 4) and azathioprine doses were much the same in all three groups. Although suggestive, the advantage of better renal function enjoyed in this small series by non-related patients with B matched kidneys was not statistically significant. The credibility of the observation was further weakened by the fact that the function of the C matched homografts was inferior to that of the organs from donors with D matches (Fig. 4).

**Pathology.** There were 95 intrafamilial cases in which both phenotype data and tissue specimens were available; 50 were in the sibling, 39 in the parental, and six in the more distantly related categories. Pathologic changes were assessed in all the kidneys by light and electron microscopy. In many instances, immunofluorescent data was also available and was taken into account.

In the sibling kidneys examined at least 1 year after transplantation, most of the 13 kinds of immunopathologic lesions were

less frequent and less severe in the A matches than in the combined others (Table 9). The differences were significant ($p < 0.05$) in the amount of mesangial matrix in the glomeruli, tubular atrophy, interstitial fibrosis, mononuclear infiltration of the interstitium, "hyaline" in the arterial walls, thickening of the intima of the interlobular arteries and in IgM deposition (Categories 4, 5, 6, 7, 8, 9, and immunofluorescence, Table 9). The differences between the B, C, and D matched kidneys were not significant.

In the parental kidneys there was no clear relationship between phenotype grades and the frequency and severity of the immunopathologic lesions (Table 10). The same was true in homografts obtained from more distant relatives but these were too few in number to permit meaningful correlation of the pathologic changes with the antigen match.

In addition to the foregoing intrafamilial cases, there were 29 nonrelated transplantations in which homograft samples were obtained from a few days to more than 4 years after operation. In eight instances the homografts were B matched, in 12 others there was a C match and in the other nine the match was designated D. Pretransplantation thymectomy had been carried out in three, five, and one of the B, C, and D-matched recipients, respectively. There was some inequality of immunosuppression in the groups being compared in that more of the patients with C and D matches received treatment with ALG since they fell into the Series 3. For the purposes of the analysis the influence of both thymectomy and ALG therapy were not given weight. The average damage to the B, C, and D grafts was approximately the same (Table 11).

## HL-A Genotype Correlations

From family studies, it became evident that the determinants of the HL-A antigens were on one chromosome[3, 4, 7, 10, 11, 62, 93] and were probably localized to two subloci.[8, 23, 29, 62] These antigens were shown to be transmitted in combinations from generation to generation in accordance with classical Mendelian law.[7, 11, 29] As a consequence, it was possible within families to use the pattern of the HL-A phenotypes as a means of establishing geno-

TABLE 9. *Sibling Donors*

Pathologic Lesions vs. Tissue Match

| Tissue Match | No. of Cases | Mean Score* for Each of 9 Types of Histopathologic Abnormality† and for Four Kinds of Glomerular Deposit | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | IgG | IgM | C′ | Fib. |
| A | 14 | 0.88 | 0.58 | 0.22 | 0.36 | 0.45 | 0.64 | 0.50 | 0.86 | 0.29 | 0.67 | 0.58 | 0.67 | 0.17 |
| B | 16 | 1.38 | 0.63 | 0.50 | 1.13 | 1.50 | 1.56 | 0.88 | 1.63 | 1.31 | 0.31 | 1.08 | 0.54 | 0.31 |
| C | 12 | 1.58 | 0.50 | 0.92 | 0.67 | 1.42 | 1.92 | 1.58 | 1.25 | 2.08 | 1.78 | 1.55 | 1.22 | 1.11 |
| D | 5 | 1.20 | 0.40 | 0.40 | 1.60 | 2.00 | 2.20 | 1.40 | 1.80 | 1.60 | 0.25 | 1.25 | 1.00 | 0.50 |
| ** P values | | 0.1 | 0.5 | 0.1 | 0.03 | 0.01 | 0.001 | 0.01 | 0.05 | 0.001 | 0.6 | 0.05 | 0.6 | 0.1 |

*See text for details of both histopathologic grading systems and for discussion of the timing of tissue sample procurement.

† Type 1 = Subendothelial glomerular capillary basement membrane thickening. 2 = Subepithelial glomerular capillary basement membrane thickening. 3 = Increased cellularity of glomerular tufts. 4 = Increased amount of mesangial matrix. 5 = Tubular atrophy. 6 = Interstitial fibrosis. 7 = Mononuclear cell infiltration of the interstitium. 8 = "Hyaline" in arteriolar walls. 9 = Thickening of intima of interlobular arteries. IgG = immunoglobulin G; IgM = immunoglobulin M; C′ = complement; Fib. = fibrinogen.

** Comparison is between A and combined B-D kidneys.

TABLE 10. *Parental Donors*

Pathologic Lesions vs. Tissue Match

| Tissue Match | No. of Cases | Mean Score for Each of 9 Types of Histopathologic Abnormality and for Four Kinds of Glomerular Deposit* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | IgG | IgM | C′ | Fib. |
| A | 4 | 0.75 | 0 | 0.75 | 0.75 | 1.75 | 1.75 | 1.25 | 1.00 | 2.50 | 1.00 | 1.50 | 1.00 | 0 |
| B | 13 | 1.23 | 0.15 | 0.62 | 0.54 | 1.23 | 1.31 | 0.92 | 0.85 | 1.09 | 0.31 | 0.85 | 0.31 | 0.31 |
| C | 16 | 1.13 | 0.13 | 0.50 | 0.50 | 1.44 | 1.56 | 1.50 | 0.88 | 1.50 | 0.72 | 1.44 | 0.64 | 0.43 |
| D | 3 | 1.00 | 0 | 1.33 | 0.67 | 1.33 | 1.67 | 1.67 | 1.00 | 1.00 | 0 | 0 | 0 | 0 |

* Footnotes same as in Table 9.

types,[7, 10, 29] and to then correlate the genotype designations with the fate of skin or renal homografts.[3, 5, 6, 92, 93]

In parent to offspring transplantations, half of the recipient HL-A alleles (one haplotype) are by definition from the donor whereas the other haplotype is in essence unrelated in respect to the donor. With transplantation between siblings, it is theoretically possible[3, 6, 11] for brothers and/or sisters to inherit two identical HL-A haplotypes from their parents at about a 25% incidence, to have a single haplotype in common in about half the cases, and to share neither haplotype in the other fourth.

In 51 of the 66 sibling transplantations of the present study, an attempt was made to convert the HL-A phenotypes into genotypes. The information about antigens was programmed for computer evaluation by Dr. M. R. Mickey to determine in which cases the donor and recipient had double haplotype identity at a probability of 0.7 or greater. The probability figures depended upon several factors including the results and completeness of the antigen analysis as well as the frequency distribution of the different measured antigens in the non-related population. The outcome in 16 HL-A identical sibling cases was compared to that observed with the other 35 sibling transplantations for which double haplotype identity had not been established.

**Survival.** At 2 years, the patient (and kidney) survival in the double haplotype identical cases was 94% as compared to 89% in the less favorably matched group (Table 12). The advantage of double haplotype identity was accentuated after 2 years until at the present time the survival is still 88% versus 74% for the pooled remaining cases (Table 12). However, the better results in the double haplotype identical cases were not statistically significant.

**Function and Immunosuppression.** At 1 and 2 years, 15 recipients with double

TABLE 11. *Unrelated Donors*

Pathologic Lesions vs. Tissue Match

| Tissue Match | No. of Cases | Mean Score for Each of 9 Types of Histopathologic Abnormality and for Four Kinds of Glomerular Deposits* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | IgG | IgM | C′ | Fib. |
| B | 6 | 1.81 | 0.33 | 0.67 | 1.00 | 1.33 | 1.50 | 0.83 | 1.33 | 1.50 | 0.60 | 1.60 | 0.80 | 0.60 |
| C | 8 | 1.88 | 0.25 | 1.50 | 1.38 | 2.13 | 2.13 | 1.75 | 1.63 | 2.25 | 0.60 | 1.20 | 1.00 | 0.60 |
| D | 8 | 2.13 | 0.38 | 1.00 | 1.38 | 1.75 | 1.63 | 2.0 | 1.50 | 1.63 | 1.0 | 1.67 | 1.33 | 0.17 |

* Footnotes same as in Table 9.

TABLE 12. *51 Sibling Renal Homografts*

| | Survival Time in Months | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | 24 | Current |
| Two haplotypes identical ($P \geqslant 0.7$) | 16 | 16 | 15 | 15 | 15 (94%) | 14 (88%)* |
| All others | 35 | 35 | 35 | 33 | 31 (89%) | 26 (74%)** |

The fate of 51 sibling renal homografts in relation to haplotype. Loss of the homograft is considered the equivalent of death.

* The late death occurred at 33 1/2 months from Torula meningitis.

** Late kidney loss or death after 25 1/2, 31, 37, 42, and 66 months.

haplotype-identical donors had somewhat better average renal function and were receiving smaller maintenance doses of prednisone than 30 other sibling recipients without double haplotype identity who also survived for at least 2 years (Table 13). However, the differences between the two groups were not statistically significant except for the 1 year BUN ($p < 0.05$).

**Pathology.** Homograft tissue became available 1 year or longer after operation from 13 of the 16 patients with double haplotype identity. The abnormalities in the transplanted organs were distinctly less than in 28 other sibling cases in which the double haplotype identity could not be established. The advantage enjoyed was statistically significant for 10 of the 13 kinds of tabulated abnormalities (Table 14). In particular, the amount of subendothelial thickening of the glomerular capillary basement membranes, the amount of mesangial matrix, the degree of interstitial fibrosis, the number of mononuclear cells infiltrating the interstitium, and the severity of the arteriolar and arterial obliterative changes were much less in the double haplotype siblings.

## Homograft Glomerulonephritis

Homografts were studied after residence for from a few days to more than 5 years in 163 of the 189 recipients. All of the specimens were submitted to light microscopy, 96 were studied with immunofluorescence, and 126 were examined by electron microscopy.

### Definition of Terms

**Definite Glomerulonephritis.** A diagnosis of glomerulonephritis was made if, by light microscopy, over 50 per cent of the glomeruli showed one or more of the following features: (1) focal or diffuse hypercellularity of the tufts; and (2) focal or

TABLE 13. *Sibling Transplantation*

Haplotype Versus Kidney Function and Steroid Dose

| | Time Post-tx (Years) | No. Patients | BUN (mg./100 ml.) | Ccr (ml./min.) | Pred (mg./Kg.) |
|---|---|---|---|---|---|
| Two haplotype identity ($p \geqslant .07$) | 1 | 15 | 20.8 | 84.9 | .23 |
| | 2 | 15 | 20.4 | 93.4 | .17 |
| Other siblings | 1 | 30 | 26.1 | 84.9 | .23 |
| | 2 | 30 | 24.5 | 83.9 | .23 |

TABLE 14. *Double Haplotype Identity**

| | No. of Cases | Mean Score for Each of 9 Types of Histopathologic Abnormality and for Four Kinds of Glomerular Deposits** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | IgG | IgM | C′ | Fib. |
| Double haplotype identity | 13 | 0.57 | 0.23 | 0 | 0.23 | 0.62 | 0.77 | 0.46 | 0.62 | 0.38 | 0.44 | 0.56 | 0.22 | 0.22 |
| Other siblings | 30 | 1.50 | 0.70 | 0.70 | 1.33 | 1.53 | 1.77 | 1.27 | 1.53 | 1.50 | 0.88 | 1.12 | 0.88 | 0.40 |
| P values*** | | =0.01 | >0.2 | <0.01 | <0.05 | <0.01 | <0.005 | <0.001 | <0.01 | <0.01 | >0.1 | =0.01 | <0.05 | >0.22 |

* Probability ⩾ 0.70.
** Footnotes same as in Table 9.
*** Comparison of kidneys with double haplotype identity with all other sibling transplants.

generalized thickening of the capillary basement membranes. These two basic pathologic changes might, or might not, be accompanied by epithelial cell crescents, by a focal or generalized increase in the amount of mesangial matrix, and by the presence of deposits of immunoglobulins and complement on the glomerular capillary wall.

**Possible Glomerulonephritis.** Glomerulonephritis was considered a possibility if the glomeruli appeared normal on light microscopy but: (1) electron microscopy revealed an excess of predominantly noncellular material on either aspect of the glomerular capillary basement membranes: the material could be of any degree of electron density; and/or (2) immunofluorescence demonstrated deposits of immunoglobulins and complement on the glomerular capillary walls. The deposits could be in a granular, linear or mixed pattern.

## Incidence of Glomerulonephritis

**Definite Glomerulonephritis.** By light microscopy, 56 of the 163 homografts were diagnosed as having glomerulonephritis for an uncorrected incidence of 34.4%. In the kidneys examined at least 1 year after transplantation this diagnosis was made in 50 of 105 instances giving a corrected incidence of 47.6% (Table 15).

**Possible Glomerulonephritis.** An additional 25 of the 163 kidneys (15.3%) had ultrastructural or immunopathologic glomerular lesions that were compatible with glomerulonephritis. Twenty-three of these specimens were among the 105 kidneys examined a year or more after transplantation, for a corrected incidence of 21.9% (Table 15).

**Non-related Versus Related Cases.** Only corrected incidences will be given since early deaths occurred so much more frequently in the non-related recipients. Amongst the 105 grafts examined a year or more after transplantation 21 were from

TABLE 15. *Glomerulonephritis* in 105 Renal Homografts One Year or More After Transplantation, and Correlations with Original Disease*

| | Original Disease | No. of Cases | No Evidence Glomerulonephritis | Definite Glomerulonephritis | Possible Glomerulonephritis |
|---|---|---|---|---|---|
| Related donors | Glomerulonephritis | 64 | 18 | 30 | 16 |
| | Pyelonephritis | 9 | 3 | 2 | 4 |
| | Other Renal Disease | 3 | 2 | 0 | 1 |
| | Unknown | 8 | 4 | 2 | 2 |
| | Total | 84 | 27 (32.1%) | 34 (40.5%) | 23 (27.4%) |
| Unrelated donors | Glomerulonephritis | 11 | 2 | 9 | 0 |
| | Pyelonephritis | 3 | 1 | 2 | 0 |
| | Other Renal Disease | 2 | 2 | 0 | 0 |
| | Unknown | 5 | 0 | 5 | 0 |
| | Total | 21 | 5 (23.8%) | 16 (76.2%) | 0 |
| Total cases | Glomerulonephritis | 75 | 20 (26.7%) | 39 (52.0%) | 16 (21.3%) |
| | Pyelonephritis | 12 | 4 (33.3%) | 4 (33.3%) | 4 (33.3%) |
| | Other Renal Disease** | 5 | 4 (80%) | 0 | 1 (20%) |
| | Unknown | 13 | 4 (30.8%) | 7 (53.8%) | 2 (15.4%) |
| | Total | 105 | 32 (30.5%) | 50 (47.6%) | 23 (21.9%) |

* See text for criteria of diagnosis.
** Three polycystic, one medullary cystic disease, one surgical accident and loss of single kidney.

non-related and 84 were from intrafamilial donors.

Sixteen of the 21 non-related kidneys (76.2%) had stigmata of glomerulonephritis that were evident on light microscopy (Table 15). In contrast, this definite diagnosis could be made in only 34 of the 84 related kidneys (40.5%). The difference was found to be significant ($2 \times 2$ contingency table: $chi^2 = 8.50$; $p < 0.01$).

However, more subtle glomerular lesions detectable only by electron microscopy or immunofluorescence were found in a further 23 related kidneys but not in any additional non-related organs (Table 15). If the definite and possible diagnoses of glomerulonephritis were pooled, the incidence for the related group became 67.9%, a figure that was not significantly different than the 76.2% in the non-related grafts.

### Relationship to Original Disease

The patients' own diseased kidneys were examined histopathologically in 159 of the 189 cases of the present report and the findings were eventually compared to those in the homografts. In 105 of these cases the homograft tissue was obtained a year or more after transplantation; the results in this special collection are summarized in Table 15.

When the original disease had been clearly glomerulonephritis (75 patients), a diagnosis of glomerulonephritis in the homograft was either definitely made (39 grafts) or else considered a possibility (16 grafts) for an overall incidence of 73.3% (Tables 15 and 16). The native kidneys in these cases were found to have various morphologic varieties (membranous, proliferative, lobular, etc.) of glomerulonephritis. Features of the glomerulonephritis in 23 of these 55 organs were either identical or similar to those found in the host's own kidneys. However, the majority of the affected grafts (32 of 55) had glomerulonephritis changes that were not a faithful or nearly faithful anatomic recapitulation of the original disease.

When pyelonephritis or other non-glo-

TABLE 16. *Incidence of "Transmission Glomerulonephritis" in Renal Homografts Examined One Year or Longer after Transplantation to Patients Whose Original Disease was Glomerulonephritis*

| | No. of Cases | Definite or Possible Glomerulonephritis in Homograft | Glomerulonephritic Changes in Homograft | | |
|---|---|---|---|---|---|
| | | | Identical to Host's Own Kidneys | Similar to Host's Own Kidneys | Different than Host's Own Kidneys |
| Related | 64 | 46/64 (71.9%) | 8/64 (12.5%) | 10/64 (15.6%) | 28/64 (43.8%) |
| Unrelated | 11 | 9/11 (81.8%) | 3/11 (27.3%) | 2/11 (18.2%) | 4/11 (36.4%) |
| Total | 75 | 55/75 (73.3%) | 11/75 (14.7%) | 12/75 (16.0%) | 32/75 (42.7%) |

merulonephritic disorders were thought to have been the cause of the patients' renal failure (17 recipients), nine of the transplanted kidneys (53%) examined 1 year or more later had findings either diagnostic of (four grafts) or compatible with (five grafts) glomerulonephritis (Table 15).

The pathologic differentiation of glomerulonephritis from pyelonephritis is sometimes very difficult in end-stage kidney disease. Consequently, a small group of 13 recipients was of special interest because the original renal disease was neither glomerulonephritis nor pyelonephritis and it was almost certainly not of immunologic etiology. Six of the patients had suffered from polycystic disease and two from obstructive uropathy. In two more, an only kidney had been accidentally removed. The other three diagnoses were congenital medullary cystic disease, cystinosis and oxalosis. Five of these recipients died within the first 2 postoperative months. At autopsy, one of the five transplants had definite signs of glomerulonephritis. In this case, the recipient's original renal failure was due to polycystic disease. The eight other homografts were studied more than 2 months after operation. There was glomerulonephritis after 7½ months in the kidney transplanted for the treatment of oxalosis. Ultrastructural lesions compatible with glomerulonephritis were present after 2 years in the graft of another patient whose original diagnosis was polycystic disease.

## Relation of Glomerulonephritis to Histocompatibility

Except for the sibling cases there was not a relationship between the quality of histocompatibility match and freedom from glomerulonephritis in the kidneys examined at least 1 year after transplantation. In the siblings the glomerular lesions tended to be somewhat less frequent and less severe in the A-matched kidneys. An advantage of good sibling matching was evident to a significant degree in the double haplotype identity cases. This fact can be appreciated by reviewing those immunopathologic features in Table 14 that define the presence or absence of glomerulonephritis, namely the lesion categories 1–3 as well as the immunofluorescent studies.

## Chronic Adverse Effects of Homograft Glomerulonephritis

There were 34 kidneys from related donors that were afflicted with definite histopathologic signs of glomerulonephritis but which supported life for at least 1 postoperative year. Between 12 and 24 months, three of these kidneys became totally nonfunctional; two of the three patients died and the third required dialysis. The function of six more of the organs (20%) deteriorated in terms of BUN and creatinine clearance. In contrast, only one of the 28 intrafamilial recipients with nonglomerulonephritic transplants died during the same interval, and functional deterioration occurred in only one other instance (3.7%).

In the non-related cases, the rate of death or homograft deterioration was also higher (63%) with glomerulonephritis than without (40%).

At 2 years, the diagnosis of definite homograft glomerulonephritis in 44 recipients was associated with significantly poorer renal function than in 51 patients whose kidneys were without such distinct histopathologic changes (Table 17); the correlation applied in both related and unrelated cases. The incidence of proteinuria in the patients with definite glomerulonephritis was 50% (22/44) compared to 2% (2/51) in the other cases (Table 17) in which glomerular lesions were either absent (no glomerulonephritis) or detectable only with ultrastructural or immunofluorescent study (possible glomerulonephritis) (Table 17).

### Immunosuppression Versus Histopathology

For this analysis, the biopsy, autopsy and homograft nephrectomy specimens obtained 1 year or more after transplantation in Series 1 and 2 were pooled. These patients were treated with azathioprine and prednisone. Comparisons were made with the specimens of Series 3 taken from patients who were also given ALG. Only the related cases were considered because the number of unrelated cases in Series 3 was too small to permit useful statistical analysis. Even in the related cases, valid statistical evaluations were difficult because the pooled Series 1 and 2 represented a more selective collection. In Series 1 and 2, the mortality before the end of the first year was in excess of 30%, whereas in the consecutive ALG-treated patients more than 90% potentially qualified for inclusion in the study by virtue of their survival for the requisite interval.

In the homografts examined, there was not a striking difference in the kidneys of Series 1 and 2 as compared to those in Series 3 (Table 18). There may have been more IgM in the kidneys of Series 1 and 2, but several other kinds of findings were somewhat prominent in Series 3, including IgG and fibrinogen (Table 18).

### Hypertension and Vascular Lesions

Acute arterial hypertension is a very common but highly reversible manifestation of acute homograft rejection.[74] Chronic hypertension is a less well recognized complication. The incidence of this late complication was evaluated in 105 patients who lived for at least 1 year and whose homografts became available for histopathologic study. Related and unrelated cases were considered together. A blood pressure score was assigned on a 0–4 scale in which 0 represented a normotensive state without any necessity for medication, four indicated uncontrolled hypertension in spite of

TABLE 17. *Renal Function and Proteinura After Two Years Vs. Glomerular Pathology*

| Pathology | No. of Patients | BUN (Mg./100 ml.)* | Ccr (Ml./Min.)* | Proteinuria** |
|---|---|---|---|---|
| Definite glomerulonephritis*** | 44 | 34.5 ± 2.5 | 61.5 ± 4.8 | 22/44 (50%) |
| Possible glomerulonephritis | 23 | 22.3 ± 2.4 | 78.7 ± 5.2 | 1/23 (4.4%) |
| No glomerulonephritis | 28 | 19.6 ± 1.1 | 90.2 ± 5.7 | 1/28 (3.6%) |

* Deviations are standard errors of the mean.
** Fraction of patients having more than 300 mg./24 hrs.
*** Differences in the renal functions and in proteinuria were all significant ($P < 0.05$) when compared to either of the other groups at each time period.

TABLE 18. *Histopathology of ALG Cases Vs. Others*

| Series | No. of Cases | Mean Score for Each of 9 Types of Histopathologic Abnormality and for Four Kinds of Glomerular Deposit* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | IgG | IgM | C′ | Fib |
| 1 and 2 | 48 | 1.31 | 0.29 | 0.56 | 0.88 | 1.13 | 1.40 | 1.02 | 0.88 | 1.25 | 0.51 | 1.28 | 0.69 | 0.29 |
| 3 | 36 | 1.22 | 0.44 | 0.58 | 0.97 | 1.39 | 1.53 | 1.25 | 1.61 | 1.31 | 0.97 | 0.94 | 0.68 | 0.53 |

* Footnotes same as in Table 9.

multiple drugs in large doses, and 1–3 represented intermediate permutations.

Only a third of the patients had completely normal blood pressures (Table 19), although in most of the others satisfactory control was obtained with drug administration. When the histopathologic findings in the homografts of these 105 recipients were plotted against the hypertension scores, good correlations were found between raised blood pressure and almost all of the kinds of lesions, especially the presence of thickening of the intima of the interlobular arteries, tubular atrophy, and interstitial fibrosis in the homograft (Table 19).

## Thymectomy

### Before Transplantation

In eight of the first renal homograft recipients at the University of Colorado, thymectomy was carried out prior to transplantation.[72] The unusually untroubled early and late courses of some of these patients was alluded to in past reports,[64, 73] and attention drawn to the possible contributing role of the thymectomies. In order to clarify this issue, a prospective study of the effect of thymectomy was carried out in an additional group of patients treated between October 1964 and June 1966. These cases belonged to Series 2 except for the first four cases of Series 3. Transthoracic thymectomy was performed in 24 of the recipients; the other 22 served as controls. A similar spectrum of donor-recipient lymphocyte antigen compatibility was present in both the test and control series.

The results of the random thymectomy investigation have been fully documented in a recent report[75] and will only be summarized here. In both the related and nonrelated cases and with follow-up intervals of 4 to 5½ years, there has not been a significant advantage of thymectomy in terms of homograft survival (Fig. 5), reduced drug dosages or quality of renal function. However, pathologic studies with light and electron microscopy and with immunofluorescence revealed that the homografts in the 24 thymectomized patients had not suf-

TABLE 19. *Pathologic Lesions vs. Hypertension*

| Grade of Hypertension 1 Year after Transplantation* | No. of Cases | Mean Score for Each of 9 Types of Histopathologic Abnormality* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 0 | 35 | 1.00 | 0.20 | 0.46 | 0.60 | 0.94 | 1.09 | 0.94 | 0.74 | 0.86 |
| 1 | 28 | 1.39 | 0.32 | 0.61 | 0.89 | 1.32 | 1.54 | 1.07 | 1.18 | 1.43 |
| 2 | 23 | 1.70 | 0.30 | 0.74 | 1.35 | 1.83 | 1.87 | 1.48 | 1.83 | 1.43 |
| 3 | 15 | 2.07 | 0.80 | 1.13 | 1.73 | 2.00 | 2.00 | 1.40 | 1.87 | 2.40 |
| 4 | 4 | 1.75 | 0 | 0.75 | 1.00 | 2.50 | 2.50 | 2.25 | 1.75 | 3.25 |
| Correlation coefficient r | | 0.85 | 0.40 | 0.70 | 0.59 | 0.99 | 0.99 | 0.91 | 0.86 | 0.95 |

* See text for details of hypertension grading system. Other footnotes same as Table 9.

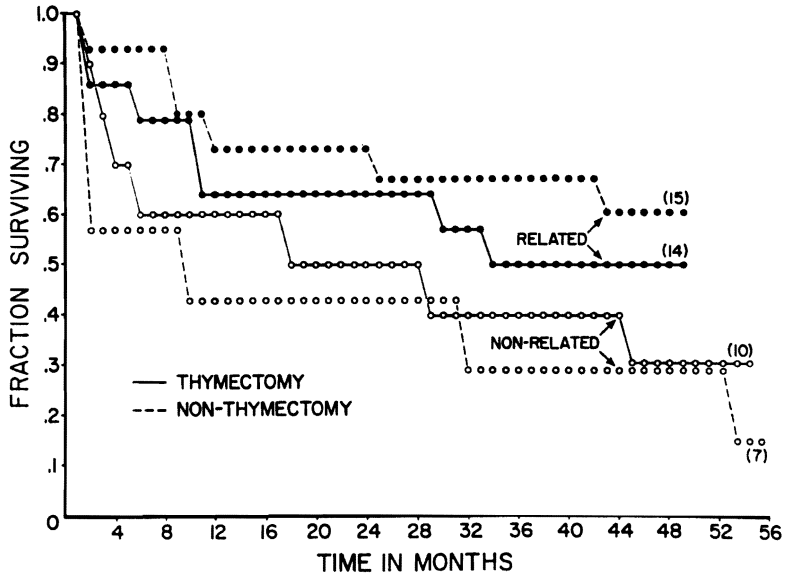


FIG. 5. Life survival curves in a series of 46 patients of whom 24 were submitted to thymectomy prior to transplantation. Note that thymectomy had no statistically significant influence upon survival after transplantation from either related or non-related donors. The figures at the end of each survival curve indicate the number of recipients at the outset of the study.

fered as much long-term damage as in the 22 non-thymectomized controls.[75] The kidneys in the thymectomy series had less severe cellular infiltration and fewer lesions of the vascular, glomerular and fibrotic varieties described elsewhere in this report.

### Late Thymectomy

Five years ago a separate group of 9 patients was described in which thymectomy was carried out from 8½ to 17 months after renal homotransplantation in the hope that the requisite level of maintenance immunosuppression could thereby be reduced.[73] There had been complications in the postoperative courses of these recipients. Five had developed late rejection, two had steroid induced cataracts, one had a ureterovesical stricture requiring an extensive reparative operation and one had become jaundiced.

At the time these cases were reported, there was no evidence that the delayed thymectomy had had any influence upon the subsequent course. Five years later the same is still true. Within the group of nine patients, there were seven recipients of familial kidneys (five maternal, two sibling); two of these seven homografts failed after two and six years. The other two patients had received kidneys from non-related living volunteers. The transplants became unable to support life after 2½ and 4⅓ years, respectively. The first patient was successfully retransplanted and is still alive, but the second one died of a perforated sigmoid diverticulitis about two months after retransplantation.

### The Risk of Malignancy

It was predicted by several authors [18, 59, 64] that chronically surviving organ transplant recipient would have an increased risk from *de novo* malignancy but the first formal reports of this complication were not made until 1968.[65, 67] Since then, it has become obvious [15, 50-52, 66] that a significant risk of neoplasia must be accepted as part of the price for success after renal transplantation. The point can be illustrated from our personal experience as well as by summarizing obervations made in other centers and compiled in Denver through an informal tumor registry.

### Colorado Experience

Among the 189 recipients of the present report, there have been 10 (5.3%)

who have developed primary malignancies (Table 20), either of epithelial (seven examples) or mesenchymal origin (three examples). In no instance was there any objective evidence of a neoplasm prior to transplantation. Afterwards, the diagnosis of tumor was made following intervals of 5¼ to 75 months. The donors of the 10 homografts were living volunteers who are still alive and in good health from 2½ to 7 years later. None has ever had a malignancy, either before or after nephrectomy.

The true incidence of neoplasia was actually higher than 5.3%. Forty of the 189 patients died from a variety of non-neoplastic complications before the end of the fourth postoperative month. At autopsy, the only tumor in these 40 recipients was a papillary adenocarcinoma in a patient who died 2 days post-transplantation of a splenic arterial hemorrhage. It was in the remaining 149 recipients who lived beyond the fourth postoperative month in whom the 10 neoplasms developed, for a partially corrected incidence of almost 7%. The rate of tumor development in these young patients (5 to 49 years, average 32) compares with the yearly risk of about 0.06% in the general population at a comparable age range.[16]

There were several conditions that were common to all 10 recipients; the continuous presence of a renal homograft, postoperative immunosuppressive treatment with azathioprine and prednisone, and splenectomy. Factors which were present in some but not all patients included thymectomy before transplantation (four) or afterwards (one), treatment with actinomycin C (four), local homograft irradiation (five), or intramuscular ALG (three). It was of particular interest to note the incidence of malignancy in patients given azathioprine and prednisone (Series 1 and 2) as opposed to the incidence when ALG was used as well (Series 3). The number of patients at risk for 1 and 2 years in Series 3 was slightly greater than in the combined Series 1 and 2. During this time and even between 2 and 3 post-transplantation years the incidence of malignancy was not proportionately higher in patients given triple agent therapy.

The behavior of the malignancies differed according to their origin (Table 20). Those rising from epithelium (skin and cervix) appeared from 32 to 75 months after transplantation and in patients whose average age was 38 years. The tumors were relatively slow growing and in every case they were apparently controlled with excision by conventional surgical technics.

In contrast, the three lymphomas appeared earlier (after 5¼, 7 and 30 months) and in patients who averaged only 19 years of age. In two instances, the neoplasms were fatal within a short time. The third patient was saved[51] by applying radiotherapy to the space occupying lesion in the brain and by drastically reducing the maintenance doses of immunosuppression.

### Experience from Other Centers

The malignant tumors known to have been observed in renal homograft recipients in other transplantation centers are listed in Table 21. There were 14 carcinomas and 13 neoplasms of mesenchymal origin. As in our cases, the prognostic implications of the mesenchymal tumors were grave and only two of these patients remain alive. In contrast, nine of 14 patients with carcinomas have been successfully treated.

## Discussion

At the April, 1965, meeting of the American Surgical Association, an account was given of the 36 patients of Series 1 who were still alive after follow-ups of one to 2½ years.[73] The chronic survival of so many (56%) recipients from an original group of 64 was an encouraging notation, particularly since it seemed likely that the majority of the homografts were going to continue functioning for a long time. Nevertheless, the opinion was offered that . . .

TABLE 20. *Malignant Tumors in Renal Homograft Recipients (Colorado)*

| No. | Age at Time of Tx | Sex | Donor | Splenectomy | Thymectomy | Imuran | Prednisone | ALG | Type of Tumor | Diagnosis. Time after Transplantation | Organs Involved | Outcome* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 40 | M | Unrelated living donor | Yes | Yes (Late) | Yes | Yes | No | Squamous cell carcinoma | 32 months | Skin of ear | No recurrence after excision. Died 4 1/2 years of other causes |
| 2 | 43 | M | Uncle | Yes | No | Yes | Yes | Yes | Basal cell carcinoma | 33 months | Nasolabial fold | Alive 3 1/2 years. No recurrence after excision. |
| 3 | 39 | M | Brother | Yes | Yes | Yes | Yes | No | Superficial squamous cell carcinoma | 36 months | Lower lip | Alive 4 years. No recurrence after excision. |
| 4 | 37 | F | Unrelated living donor | Yes | Yes | Yes | Yes | No | Squamous cell carcinoma *in situ* | 50 months | Cervix of uterus | Alive 5 years. No recurrence after hysterectomy |
| 5 | 40 | M | Unrelated living donor | Yes | No | Yes | Yes | No | Superficial squamous cell carcinoma | 66 months | Lower lip | Alive 6 years. No recurrence after excision |
| 6 | 34 | M | Sister | Yes | No | Yes | Yes | No | Squamous cell carcinomas | 74 months | Left forearm; right forearm | Alive 7 years. No recurrence after excision |
| 7 | 30 | M | Brother | Yes | Yes | Yes | Yes | No | Basal cell carcinoma | 75 months | Nasolabial fold | Alive 7 years. No recurrence after excision |
| 8 | 14 | M | Mother | Yes | No | Yes | Yes | Yes | Reticulum cell sarcoma | 5 1/4 months | Brain | Fatal 6 1/4 months. |
| 9 | 20 | F | Father | Yes | No | Yes | Yes | Yes | Unclassified lymphoma | 7 months | Brain | Alive 2 1/2 years. Treated with radiotherapy |
| 10 | 23 | M | Father | Yes | Yes | Yes | Yes | No | Reticulum cell sarcoma | 30 months | Thyroid, liver, lung, stomach, prostate, pituitary, skin, psoas muscle | Fatal 30 months. |

* Survival times are dated from transplantation.

TABLE 21. *Malignant Tumors in Renal Homograft*

| No. | Transplant Center | Age at Time of Transplant | Sex | Donor | Splenectomy | Thymectomy | Imuran | Prednisone | ALG |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Minneapolis | 16 | F | Cadaver | No | No | Yes | Yes | No |
| 2 | Ghent, Belgium | 53 | F | Cadaver | No | No | Yes | Yes | No |
| 3 | Richmond | 33 | F | Sister | No | No | Yes | Yes | No |
| 4 | San Francisco | 46 | M | a) Son<br>b) Cadaver | Yes | No | Yes | Yes | No |
| 5 | Nashville | 35 | M | Cadaver | No | No | Yes | Yes | No |
| 6 | Louisville | 36 | M | Brother | No | No | Yes | Yes | No |
| 7 | Louisville | 32 | M | Brother | Yes | No | Yes | Yes | No |
| 8 | Minneapolis | 27 | M | Brother | Yes | No | Yes | Yes | No |
| 9 | Nashville | 48 | M | Cadaver | No | No | Yes | Yes | No |
| 10 | Minneapolis | 28 | F | Brother | No | No | Yes | Yes | No |
| 11 | Montreal | 38 | F | Cadaver | No | No | Yes | Yes | Yes |
| 12 | Los Angeles | 27 | M | Mother | No | No | Yes | Yes | No |
| 13 | Los Angeles | 25 | F | Brother | No | No | Yes | Yes | No |
| 14 | Los Angeles | 38 | F | Mother | No | No | Yes | Yes | No |
| 15 | Edinburgh, Scotland | 26 | F | Mother | No | No | Yes | Yes | Yes |
| 16 | Cleveland | 32 | M | Cadaver | No | No | Yes | Yes | Yes |

*Recipients (Other Transplant Centers)*

| Type of Tumor | Time after Transplantation | Organs Involved | Outcome | Ref.* |
|---|---|---|---|---|
| Dysgerminoma | 32 months | Peritoneum; ovary; mediastinal & axillary lymph nodes | Fatal | Dr. W. Kelly |
| Adenocarcinoma | 35 months | Sigmoid colon | Alive following extensive left hemicolectomy | Dr. F. Derom |
| Squamous cell carcinoma *in situ* | 36 months | Cervix of uterus | Cone biopsy. Patient being observed at regular intervals. | Dr. H. Lee |
| Alveolar cell carcinoma of lung | 9 months | Lungs | Died | Dr. S. Kountz |
| Squamous cell carcinoma | 63 months | Metastases in lymph nodes of neck. Primary site of tumor unknown | Alive following radical neck dissection | Dr. C. Zukoski |
| Squamous cell carcinoma | 8 months | Lower lip | Alive. No recurrence after excision. | Dr. D. Leb |
| Embryonal cell carcinoma | 2 months | Testis; abdominal organs; ureter of transplanted kidney; lung. | Died | Dr. D. Leb |
| Undifferentiated carcinoma | 10 months | Liver; brain; bone marrow | Died | Dr. C. Hitchcock |
| Undifferentiated carcinoma | 17 months | Lung; mediastinal lymph nodes; brain; liver | Died | Dr. C. Zukoski[99] |
| Squamous cell carcinoma *in situ* | 30 months | Cervix of uterus | Alive. No recurrence after simple hysterectomy | Dr. R. L. Simmons |
| Squamous cell carcinoma *in situ* | 6 months | Cervix of uterus | Alive. No recurrence following cryosurgery | Dr. K. Pritzker |
| Squamous cell carcinoma | 25 months | Lower lip | Alive. No recurrence after excision. | Dr. R. Goldman |
| Squamous cell carcinoma | 35 months | Lower lip | Alive. No recurrence after excision. | Dr. R. Goldman |
| Squamous cell carcinoma *in situ* | 35 months | Cervix of uterus; anterior wall of vagina | Alive. No recurrence after excision. | Dr. A. Gordon |
| Reticulum cell sarcoma | 25 months | Lymph nodes; pleura; spleen; liver; ovary; adrenal; bone marrow; & transplanted kidney | Fatal | Prof. M. Woodruff[96] |
| Reticulum cell sarcoma | 22 months | Buttock; lungs, aortic lymph nodes | Fatal | Dr. S. D. Deodhar[13] |

TABLE 21.

| No. | Transplant Center | Age at Time of Transplant | Sex | Donor | Splenectomy | Thymectomy | Imuran | Prednisone | ALG |
|---|---|---|---|---|---|---|---|---|---|
| 17 | New York | 35 | F | Cadaver | No | No | Yes | Yes | No |
| 18 | Richmond | 35 | M | Cadaver | No | No | Yes | Yes | No |
| 19 | Auckland, New Zealand | 34 | M | Cadaver | No | No | Yes | Yes | No |
| 20 | Auckland, New Zealand | 46 | F | Cadaver | No | No | Yes | Yes | No |
| 21 | New York | 18 | M | Uncle | No | No | Yes | Yes | No |
| 22 | New York | 36 | M | Cadaver | No | No | Yes | Yes | No |
| 23 | Richmond | 29 | M | Brother | Yes | No | Yes | Yes | No |
| 24 | Little Rock, Arkansas | 21 | M | Father | Yes | No | Yes | Yes | No |
| 25 | Boston | 34 | M | a) Cadaver b) Half-sister | No | No | Yes | Yes | No |
| 26 | Montreal | 36 | M | Cadaver | No | No | Yes | Yes | No |
| 27 | San Francisco | 39 | F | Sister | Yes | No | Yes | Yes | No |

* Reported cases annotated; all other cases by personal communication from the indicated physician or surgeon

"for the present it would seem most reasonable to regard homotransplantation as an effective, but incompletely characterized, form of palliative therapy."

Although 29 (45.3%) of the original 64 patients in Series 1 remain alive after an additional interval of 5 years, there is no reason now to change the foregoing point of view since late failures have continued to be observed. Admittedly, the attrition has been slow in the familial cases but the delayed loss rate of non-related kidneys or their recipients has been excessive. The same conclusions are evident in the late reports of other workers in transplantation who were active in the 1962–1963 era and who now have follow-up data of comparable duration.[20, 24, 33, 38, 41, 44, 63, 97]

In Series 1 as well as in Series 2 and 3 of the present report, it is of interest to examine the causes of the 24 deaths that occurred after 1 year. Since the transplants were still providing life supporting function in the majority of these cases (14 of 24), it could be tempting to erroneously ascribe such failures exclusively to extrarenal factors. Examples, even years after transplantation, might be fatal myocardial infarction, pulmonary embolization, lymphoma, or infection. It is of the utmost importance to appreciate that most of these patients who died late had less than nor-

(*Continued*)

| Type of Tumor | Time after Trans-plantation | Organs Involved | Outcome | Ref.* |
|---|---|---|---|---|
| Visceral Kaposi's sarcoma | 10 months | Lungs; esophagus; stomach; urinary bladder; mediastinal and abdominal lymph nodes. | Fatal | Dr. J. H. Siegal[61] |
| Reticulum cell sarcoma | 31 months | Lung | Fatal | Dr. J. Pierce |
| Reticulum cell sarcoma | 7 months | Tongue; esophagus; liver | Fatal | Dr. P. Doak[15] |
| Reticulum cell sarcoma | 9 months | Brain | Fatal | Dr. P. Doak[15] |
| Reticulum cell sarcoma | 9 months | Brain | Fatal | Dr. R. Porro |
| Reticulum cell sarcoma | 10 months | Brain | Fatal | Dr. F. Veith |
| Reticulum cell sarcoma | 67 months | Lymph nodes; liver; vertebrae | Fatal | Dr. H. Lee |
| Reticulum cell sarcoma | 24 months | Brain | Alive, following radiotherapy | Dr. C. Araoz |
| Leiomyosarcoma | 47 months | Stomach | Alive, receiving local radiotherapy | Dr. R. E. Wilson |
| Leiomyosarcoma | 51 months | Small bowel; liver; pancreas | Fatal | Dr. L. D. Maclean |
| Reticulum cell sarcoma | 14 months | Brain; lungs | Fatal | Dr. F. O. Belzer |

mal renal function, that many had hypertension or other complications as a consequence, and that greater than average maintenance immunosuppression was usually being given in order to prevent further functional losses. Thus the typical late death, whatever its immediate cause, tended to occur against a background of subnormal renal function and relatively excessive immunosuppression, especially with steroids. Only two of the patients who died after 12 postoperative months had excellent homograft function prior to their terminal illness (Fig. 3).

If the above described delayed deterioration in renal function were due mainly to incomplete long-term control of the rejection process, they should have been predictable with any system that identified major donor-recipient histoincompatibilities. In the present study, the clinical observations were compared with the widely used[34] A-D antigen match classification. With this system, the letters were a statement in code about the compatibility, incompatibility or identity of the HL-A antigens.

Following inter-sibling transplantation, it was encouraging that histocompatibility matching by this method correlated at least partially with the outcome. Specifically, the designation of an A match endowed a slight advantage in terms of survival and quality of homograft function as well as a

highly significant advantage in terms of the histopathologic appearance of the kidneys at varying times postoperatively. In practical fact, the designation of an A match in sibling cases very often was an indication that the donor and the recipient both had the same two histocompatibility haplotypes, one from each parent, and had therefore achieved identity of the HL-A antigens; for the HL-A chromosome it could be said that there was genotype as well as phenotype identity. These observations have supported the conclusion from other skin or renal transplantation studies within families about the relevance of HL-A antigens to histocompatibility.[3, 5, 6, 92]

In contrast, significant clinical or histopathologic correlations could not be demonstrated between the phenotype grades and the results with the parent to offspring transplantations. With this familial combination, half of the HL-A antigens of the donor (one haplotype) are by definition identical to those in the recipient. The remaining 50% of HL-A antigens are in essence unrelated. It is the unrelated half of the donor-recipient genetic mass which finds expression in the A-D score.

In the parental cases, there could be several explanations for the failure to find significant relationships between the alphabetical grades and survival, function, or histopathology. The possibility that the HL-A antigens are irrelevant to histocompatibility cannot be seriously entertained in view of the evidence to the contrary cited above in connection with sibling cases and in view of the evidence from other kinds of studies.[12, 17, 57] A second possibility could be that "immunologic artefact" caused by the transmission of preexisting host glomerulonephritis to the transplants was responsible. However, it will be emphasized farther on that the latter factor alone could not explain all or probably even the majority of the poor correlations. It could also reasonably be speculated that: (1) the completeness and/or accuracy with which HL-A phenotypes currently can be measured is substantially poorer than is generally realized[31]; (2) significant and presently undetected histocompatibility loci existed on other than HL-A chromosomes; (3) variable host immunologic reactivity in different patients[27] was comparable in importance to the antigen match in determining the outcome; (4) or, host presensitization to antigens present in the homografts[26, 28, 70, 79, 85, 94] jeopardized the outcome in a number of instances but was not always recognized as a factor.

In addition, there remains the possibility that the A-D system is not an optimal one with which to express the real meaning of the data obtained by determining individual donor and recipient antigens. The point of view was emphasized at the Histocompatibility Conference held in Los Angeles in January, 1970.[23] Based upon recent progress in understanding the genetics of the HL-A system, Rapaport and Dausset[56] have proposed that the A-D scale be replaced by a numerical index of donor-recipient histocompatibility called the Net Histocompatibility Ratio (NHR).*

Whatever the explanation, the experience with the parental cases reaffirms a finding first noted several years ago. In many of these same patients as well as in mismatched siblings, it was learned[48, 73, 87] that immuno-suppressive treatment could very often override significant or apparently ominous incompatibilities with subsequent renal graft survival for many years. The durability of related but mismatched kidneys has become even more evident with the longer follow-ups of 2 to 7½ years in the present study. As a consequence, our policy continues to be to accept parental donors despite the fact that they may be badly matched. Moreover, siblings and

* In preliminary studies of the cases in the present study, Dr. Rapaport believes he has found correlations between the NHR and the survival of recipients of parental and unrelated kidneys as well as after inter-sibling transplantation.

other relatives are not arbitrarily excluded from giving kidneys simply because of antigen incompatibilities, although an effort is usually made in this circumstance to determine if the donor and recipient share one HL-A haplotype and are therefore genetically similar to a parent-offspring combination (see earlier section "HL-A Genotype Correlations" on page 447). If only one donor is available, the only absolute immunologic contraindication to intrafamilial transplantation in our center is the demonstration of performed antidonor humoral antibodies.

Since the A-D grades failed to conform to any identifiable spectrum of outcome after the parent to offspring transplantations, it was hardly surprising to find the same lack of correlation within the much less fortunate group of patients who received non-related kidneys and who tended to die or lose their transplants at a higher rate both early and late after operation. Reducing the risk in this kind of case has become one of the great challenges of applied immunology. It remains to be seen if more assiduous application of serologic tissue typing technics will be instrumental in improving the outlook of the non-related recipient. If so, it now seems certain, in view of the negative results thus far obtained, that improved methods of interpretation must be evolved or else that nearly perfect matching of a truly complete set of antigens will be required.

Of course, the foregoing antigenic, clinical, and histopathologic correlations were an oversimplification since a significant number of early deaths were not immunologic in etiology. Even delayed deterioration of a renal homograft was not necessarily due to inadequate control of the rejection process. Three alternative possibilities that were always considered in our patients were obstructive uropathy, pyelonephritis, and glomerulonephritis. In the cases of the present report, there were no unrelieved high-grade chronic urinary obstructions as has been recently documented in a separate publication.[68] Moreover, pyelonephritis was diagnosed on histopathologic grounds in only two of the 105 homografts examined 1 or more years after transplantation.

In contrast, 73 of the 105 specimens (70%) examined pathologically 1 or more years post-transplantation contained either obvious (50 examples) or subtle (23 examples) glomerular changes of the kind caused in experimental glomerulonephritis models by antiglomerular basement membrane (anti-GBM) antibodies (Masugi-like nephritis) or by the lodgment of soluble antigen-antibody complexes in the renal microvasculature (complex nephritis). Even before a year, these lesions were commonly noted, at the earliest after only 2 weeks' residence of a transplant in its new host. Glomerulonephritis-like lesions have also been noted in renal homografts in a number of past reports.[19, 21, 32, 35, 36, 39, 40, 41, 47, 53, 55]

Tabulation of such a high incidence of glomerular abnormalities has made it desirable to try to determine whether they are a manifestation of humoral antibody rejection or a continuation of pre-existing glomerulonephritis. The criteria of the differentiation have been necessarily vague since, as McPhaul and Dixon have pointed out,[39] analogous nephritogenic mechanisms could be responsible under both circumstances, differing primarily in the nature of the antigen.

The circumstantial evidence from the present study has suggested that a common and possibly the predominant cause of glomerulonephritis in transplanted kidneys was indolent rejection. For one thing, glomerulonephritis developed in the homografts of several patients whose original renal disease did not have an auto-immune etiology. Another observation in support of the rejection pathogenesis was that the incidence of obvious glomerulonephritis was less with good histocompatibility as in

double haplotype identical siblings. Conversely, the incidence was exceedingly high in the generally less well matched nonrelated kidneys.

However, there were also strong indications that many renal homografts were adversely affected by "transmission" of the disorder which had made transplantation necessary in the first place. If the original host disease was glomerulonephritis, the chance of having such a diagnosis made in the transplant was greatly increased. Moreover, 23 (22%) of the 105 homografts examined a year or more after operation had glomerulonephritic lesions that were strikingly similar or absolutely indistinguishable from those in the native kidneys. These observations of "transmission glomerulonephritis" support the opinions expressed by McPhaul and Dixon and their associates [39] but are at variance with the interpretations of Merrill,[40] Najarian and Foker,[45] and Williams, Lee and Hume.[95] Although the exact incidence of "rejection" as opposed to "transmission" glomerulonephritis is not known, it is obvious that the immunosuppressive technics used thus far do not consistently prevent this complication.

Only two programs of immunosuppressive therapy have been thoroughly tested in humans. The first, which was introduced in 1962 and 1963,[25, 43, 74, 98] consisted of double drug therapy with azathioprine and prednisone. With the other regimen, heterologous antilymphocyte globulin (ALG) was added to these two agents in a triple drug combination, usually confining the globulin treatment to the first 4 postoperative months. In our center, the death and kidney loss rates fell sharply after June 1966 when ALG was introduced.[69, 71, 76] Similar clinical trials have been reported with ALG in other institutions. With one exception,[58] the experience with the triple drug treatment has been reported as favorable [1, 14, 30, 54, 60, 77, 88-90] with a reduced mortality and/or better homograft function.

In the present study, the magnitude of tissue injury at about 2 years has been added to survival and function as a third measure of the effectiveness of treatment. Although the patients reached this level of convalescence more consistently than in the past, the grafts under treatment with triple drug therapy were in no better condition than kidneys sampled at comparable times in the double agent era. The findings are not different from those reported from our earlier experience with the triple drug treatment.[76]

In addition to the effects of various immunosuppressive agents, there may be a slowly evolving attenuation of host immunologic responsiveness as a consequence of recipient thymectomy. In a recent full report [75] of the human thymectomy experience that was briefly summarized in the present communication, the evidence was reviewed from animal experimentation that predicted an effect in adult humans. Despite this expectation, there did not seem to be any influence at all upon either survival or homograft function in our patients who were submitted to this adjuvant procedure. However, the homografts transplanted to the recipients with absent thymus glands developed significantly fewer and less severe abnormalities.[75] In no sense can this latter observation be construed as a recommendation that thymectomy be included as part of the standard preparation for transplantation. Yet it has reopened the question of conducting further well controlled studies to clarify the issue of thymectomy in humans.

It is probable that a final judgment about the value of organ transplantation will depend upon more knowledge of the liabilities associated with chronic immunosuppression. It has been proved that patients can live in good health for many years providing they can maintain function of their transplants without the need for excessive drug doses. In such recipients, there has not been an alarming tendency for the de-

velopment of life threatening late infection. A more serious long-term risk may be that of malignancy. The findings in the present report reinforce previous warnings [15, 50-52, 65, 67] that an increased incidence of neoplasia may be one of the more serious ultimate limitations in the application of transplantation procedures for the treatment of human disease.

## Summary

Kidney transplantation was performed for 189 victims of renal failure during 3 periods: 1962–1964 (Series 1), 1964–1966 (Series 2), and after June 1966 (Series 3). The patients of Series 1 and 2 were treated with azathioprine and prednisone; despite the acquisition of a large experience during this 4-year interval, the mortality remained relatively fixed. In Series 3, horse ALG was also given, usually for the first 4 postoperative months. This change was associated with an improvement in survival but not with any definite quantitative or qualitative changes in the histopathology of the chronically functioning homografts. Seventy nine of the 189 patients have died. The causes of early as well as late mortality in all three series were usually associated with toxicity from immunosuppressive therapy, with failure of the immunosuppression to completely control rejection, or most commonly to a combination of both factors.

In all three eras of our experience, the majority of the recipients of related kidneys derived long lasting benefits with one-year survival rates in Series 1, 2, and 3 of 67%, 68, and 92%, respectively. With follow-ups of 2 to 7½ years in these familial cases, 91 of the 131 consecutive patients (69.5%) are living and 88 (67.2%) of the originally transplanted homografts are still functioning. The type of consanguinity was not a critical factor in determining the outcome since the 1-year, 2-year and current survival was approximately the same with the use of organs from siblings, parents and more distant relatives.

With non-related donors (both volunteer and cadaveric) the salvage of life and the degree of social and vocational rehabilitation were less gratifying. When Series 1 was compiled in 1962 and 1963, two thirds of the recipients of non-related kidneys (12/18) died within the first postoperative year. In Series 2, the one year mortality dropped to 48% (11/23) whereas in Series 3 only 17.7% (3/17) failed to live for at least 12 months with life-supporting function of the transplant. In all three series, late failure of unrelated homografts (after a year) was far more common than with the familial kidneys. Consequently, only 19 (33%) of the 58 patients treated 1 to 7 years ago are still surviving, and in three of these 19 cases by virtue of retransplantation.

In most of the cases, typing of the HL-A antigens of both the donors and recipients was carried out with a lymphocytotoxicity test. From these measures, matches of the antigen phenotypes were designated on a scale of A to D. After inter-sibling transplantation, the recipients of A-matched kidneys had better average renal function and an increased long-term survival. In addition the incidence and severity of histopathologic abnormalities in the matched transplants were minimal. It was shown by genetic analysis that these favored grafts tended to have been given by donors who had double haplotype identity of the HL-A chromosome with the recipients.

With parent to offspring transplantation or with transplantation between more distant relatives or non-related people, no correlation existed between the A-D phenotype matching grades versus survival, function and histopathology. Several explanations for these negative findings were discussed, including the possibility that the A-D scoring was not an optimal way of converting the raw typing data to an expression of the quality of HL-A compatibility.

The possible role of glomerulonephritis in obscuring histocompatibility correlations was evaluated and found to be a factor of potential, though probably not overriding significance. There were 105 renal homografts examined one year or more after operation. By light microscopic examination 50 (47.6%) had one variety or other of glomerulonephritis. Another 23 transplanted kidneys (21.9%), without obvious glomerular abnormalities by light microscopy, had more subtle findings detectable by ultrastructural or immuno-fluorescence examination. Evidence was presented that the most common cause of homograft glomerulonephritis was slow rejection mediated primarily by humoral antibodies. In support of this contention were the facts that: (1) the diagnosis of homograft glomerulonephritis was made in some cases in which the original host disease had not been of autoimmune etiology; (2) good histocompatibility matching (as in the double haplotype identical siblings) was associated with a decreased rate of glomerulonephritis; (3) histoincompatibility predisposed to glomerulonephritis as shown by the higher incidence in unrelated as opposed to related cases. On the other hand, there were many patients in whom the transplanted organs apparently were adversely affected by continuation of the same disease that had destroyed the native kidneys. The evidence favoring this sequence of events was that: (1) The incidence of glomerulonephritis in the transplants was higher if this was the original diagnosis; (2) glomerular changes in the grafts were very often (23 well documented examples) a faithful or nearly faithful anatomic recapitulation of the disease that had necessitated transplantation in the first place.

Thymectomy as an adjuvant immunosuppressive measure was evaluated in a group of 46 patients treated from 4 to 5½ years ago. Before transplantation the thymus gland was removed transthoracically in 24 randomly selected recipients whose subsequent fate was compared to that of the 22 others who did not have thymectomy. The patients with thymic excision did not have better survival or superior renal function, but their homografts tended to have fewer and less severe histopathologic abnormalities when examined long after transplantation.

Ten (5.3%) of the 189 patients in the present report have developed carcinomas (seven examples) or mesenchymal malignancies (three examples). In addition, 27 cases of *de novo* malignancy have been collected from other institutions and summarized. From the data, it was concluded that an increased risk of neoplasia is yet another penalty for chronic iatrogenic immunosuppression and that the complication is not related to the use of any individual agent. In our recipients, eight of the 10 malignancies were cured by conventional forms of treatment; the other two died of widespread reticulum cell sarcoma.

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## Discussion

Dr. Thomas C. Moore (Los Angeles): Two years ago Dr. David Hume and I carried out an indepth review of the Richmond experience with first kidney transplants. The results of this study have been published as three articles in the July 1969 issue of *Annals of Surgery*. All but a handful of these patients had been followed from 1 to 6½ years following transplantation.

At the time of our review 49 of 75 related living donor (RLD) recipients were living with functioning transplants. The initial and continuing level of function as mirrored in BUN and creatinine levels was considered to be of considerable prognostic importance.

In the two years since our review, eight of these 49 functioning RLD transplants have been lost; four due to patient death and four as a result of transplant failure lead to nephrectomy. Three of the deaths involved patients with excellent transplant function 6½ to 7 years after transplantation (all had creatinines in the 1.0 range). Two died of malignancy (one of leukemia and one of lymphoma) and one was suspected suicide. The other death resulted from liver failure 4 years post-transplant. One additional patient is living in a tenuous way with severe liver damage which recently required portocaval shunting for portal hypertension and bleeding varices. Three of the four transplants lost due to transplant failure and nephrectomy were in trouble at the time of the last report. The other transplant which failed had functioned at an intermediate level and failed during a period of serious pulmonary infection.

Twenty-four living RLD patients continue, three to 7½ years post-transplant, to have the excellent level of function of 20 BUN-1.2 creatinine or better. None of the patients who had this level of function 2 years ago have suffered any deterioration of function in the interval since our last report. Seven additional patients have creatinines (Cr) in the 1.3–1.5 range. The patient whose function deteriorated moderately during pregnancy but recovered in the initial 8 months post-partum, currently has a BUN of 18 and a Cr of 1.0 5½ years after transplantation, and the surviving C-match Wilm's tumor patient has a BUN of 19 and a Cr of 1.0 six years after transplantation.

With a recent BUN of 18 and a Cr of 0.9, the patient who was given Isoniazid (isonicotinic acid hydrazide) during first and second transplants for tuberculosis continues to survive with a functioning transplant 53 months after receiving a CD second transplant.

At the time of our study, 2 years ago, 15 first transplant cadaver donor (CD) recipients had functioning transplants. One of these kidneys still are functioning. Four kidneys have been lost due to patient death, all at comparatively short-term risk, and one from failure and transplant nephrectomy. Of the ten functioning CD kidneys currently at risk from 2½ to 6 years, nine have creatinines of 1.6 or better. Of the nine CD kidneys at risk more than 2 years at the time of our study 2 years ago, only one kidney and no patients have been lost and seven of the eight surviving kidneys are functioning at a surprisingly satisfactory level. None of these seven have shown any significant deterioration in function in the past 2 years and all are in the 4- to 6-year range post-transplant. One patient 5 years and 9 months post-transplant has a BUN of 23 and a Cr of 1.3 and one 5 years and 1 month has a BUN of 17 and a Cr of 1.1. This is a very encouraging finding for CD recipients at risk 2 and more years and with reasonably adequate function. From a group of 20 consecutive CD first transplants at risk from 4 to 6 years, eight (40%) continue to function.

In the 2 years since our study, two new hazards in long-term survivors have risen; the threat of reticuloendothelial and bone marrow types of malignancy and the hazard of liver failure from chronic hepatic injury. Both of these late hazards probably are related to long-term azathioprine administration. Because of these findings, we must raise the question of the feasability and desirability of substantial, gradual reduction in azathioprine administration in long-term survivals. Other agents including drugs such as the antihistamines and isonicotinic acid hydrazide we are using at UCLA might be utilized to facilitate reduction or elimination of azathioprine administration in the period after 2 or 3 years.

Dr. Starzl has mentioned their experience with spontaneous malignancy. I would like to ask him about their problems with hepatic damage in long-term survivors.

DR. RICHARD E. WILSON (Boston): I would like to try and supplement Dr. Starzl's data with findings from our transplant program at Peter Bent Brigham Hospital. I think this will enable the members and guests to arrive at a consensus which is important at this stage in the management of patients with renal and other transplants.

I wish to discuss three aspects of his presentation. One of the typing, the second is the use of antileukocyte globulin and the third is the problem of the late rejecting kidney, or glomerulonephritis.

We have cooperated with Dr. Teraski as has he, since the onset of his typing work, and have had all of our patients typed in Los Angeles, as well as their being typed locally in Boston. We have never rejected a patient because of a poor match except when they were presensitized, Teraski-"F" match.

Our living donors with an A match have done very well, but our living donors with B matches have not done any better than C or D matches; in fact many were worse. Some of them have had very early rejections and I am sure, as Dr. Starzl mentioned, this probably represents a fault in the sophistication of typing more than anything else. Our cadaver donor kidneys have done equally well, B, C and D matches and, again, if anything the C and D matches have done better than the B's.

Our philosophy about this typing program is much as that which Dr. Starzl summarized: that is, we believe we must continue to type, that we must become more sophisticated but we certainly do not believe that at the moment we have all of the antigens properly matched, nor can we for any given patient predict the exact outcome of a given kidney.

In regard to antileukocyte globulin, about 2½ years ago we began using antileukocyte globulin in a controlled study with alternate living donor and cadaver donor patients receiving ALG and the other half not receiving ALG. To this date, we have about 80 patients with about 20 in each of the four groups. We cannot find any difference in survival at 3 months and at 1 year in these patients and the only thing that appears to be better in the ALG treated patients is less severe and probably fewer rejections. This is much more subjective difference. We are continuing to work at this with hopes that better ALG and better ways of using it may make it more effective for immunosuppression in patients. All of this has been subcutaneously administered.

Finally, we would agree completely with Dr. Starzl's points about late rejection and failure to be able to really differentiate this from chronic glomerulonephritis. The changes in these late kidneys are certainly severe, vascular in nature and are the end result of immunologic damage, be it glomerulonephritis or transplant rejection.

DR. DAVID M. HUME (Richmond): I would certainly be willing to accept that Dr. Starzl's figures seem to indicate that ALG has improved his results, particularly in the unrelated donor groups. However, I would like to sound a word of caution about the ready acceptance of this conclusion. It is unwise to judge the results of organ transplantation, particularly from cadaver donors, until at least 2 years have gone by. It is gratifying that this is now the case for Dr. Starzl's series, but the results *without* ALS in our own series at this time interval are comparable to those he just presented. The most recent group related living donor transplants had a functional survival at 1 year of 92%, and a survival to 3½ years of 80%. This is survival of functioning grafts, not just patient survival, which is, of course, better.

In the cadaver donor group, the 1 year survival of 19 consecutive recent cadaver donor transplants was 95%, while the 2- to 4-year survival was 53%. These figures do not greatly differ from Dr. Starzl's with ALG, and illustrate the difficulty in comparing recent results with past results as a consequence of the over-all improvement in the handling of the patient, apart from the addition of ALG.

All ALG's are certainly not the same, and it may well be that the difference between Dr. Wilson's results and Dr. Starzl's relate to the ALG itself. ALG is assuredly a tremendously important tool in transplantation, but the details of its production, assay, and utilization still remain to be clarified and standardized.

The second comment I would like to make relates to the incidence of recurrent nephritis. In our own series, the incidence has been very much less than the 22% reported by Dr. Starzl. We have had only two instances of definite recurrent nephritis in which the disease in the transplant was identical in every respect to the disease in the original host kidneys. In two additional instances the host developed nephritis in the transplanted kidney of a type different from that in his own kidneys. A fifth patient developed anti GBM nephritis in the transplant, but had no known disease in his own kidneys prior to the development of cortical necrosis after an accident.

We have noted many instances in which the transplanted kidney has developed chronic rejection nephritis, and there appear to be at least three circumstances which predispose to its appearance. The first is in children who had nephritis in their own kidneys, who are much more apt to develop proteinuria in the transplant than adults. The second are those A-matched related living donors who had glomerulonephritis, of whom 32% have had the nephrotic syndrome in the transplant. The third group are the poorly matched patients, whether or not they originally had glomerulonephritis. The poorly matched related living donors had a 65% incidence of proteinuria, while the poorly matched cadaver donors had a 90% incidence. I should like to ask Dr. Starzl if he has noted similar findings?

DR. THOMAS E. STARZL (Closing): I will try to respond to one or two of the issues that were raised by each of the three gentlemen.

Dr. Moore, Dr. Israel Penn did a formal study on the incidence of liver disease in our renal transplant patients (*Current Topics in Surgical Research*, 1: 67, 1969) and found a significant incidence of liver disorders at all times postoperatively. Over-all, more than half the patients had abnormal liver function tests at some time. The evidence of hepatic injury ranged from minor rises in SGOT all the way to death from liver failure. The time of greatest risk was early postoperatively. However, we also had two late deaths from liver failure of exactly the kind that you mentioned. These fatalities were after 18 and 25 months.

I agree in principle with everything that Dr. Wilson said, including the need for improved tissue typing. However, it does not seem likely that the mere discovery of new antigens will really help us to improve practical typing. Rather, longer lists of antigens could make the whole problem more unmanageable than it already is if the major objective is to match up these increasingly numerous phenotypes. At the 1970 Histocompatibility Conference in Los Angeles, one of the main items of discussion was the means by which the phenotypes could be used to establish more meaningful genotypes even when matching between unrelated people. This approach forms the basis for the formulae of Rapaport and Dausset that are discussed in the text. The outcome of such studies may ultimately lead to simplification of matching of fewer rather than more antigenic determinants.

I would also agree with much of what Dr. Hume said including the desirability of controlled clinical studies in which some patients receive ALG while other comparable controls do not. Because we are convinced of the value of ALG, we cannot in good conscience perform such studies which will have to be left to those who are more skeptical. We feel approximately the same way about ALG for hepatic and cardiac transplantation.

As to the factors that underlie transplant glomerulonephritis, Dr. Hume's main point was one that we would very definitely agree with, namely that bad histocompatibility predisposes to this complication. The best way to support the contention is to point out that recipients of nonrelated kidneys have an incidence of graft glomerulonephritis that is greater than that in recipients of related kidneys. By definition, the nonrelated cases should have a higher average rate of histo*in*compatibility whether you can accurately measure it or not. Moreover, familial kidneys that are known to be exceptionally well matched (because they come from double haplotype identical sibling donors) tend to have the lowest incidence of glomerulonephritis of any subgroup of cases. Of course, these observations are circumstantial evidence that glomerulonephritis can be a manifestation of graft rejection. In the text, evidence will also be considered of the alternative possibility that transplant glomerulonephritis may be a continuation of the original host disease that necessitated transplantation in the first place. In our opinion, both mechanisms play a role.

The question about the incidence of glomerulonephritis in children is one that we are looking at at the present time. I do not have the data organized with which to answer the question accurately at this time. In a general way I can say that since we have seen "transmission glomerulonephritis" in all age groups we do not consider it a unique risk of pediatric recipients.