be direct toxicity, but, rather, an effect of diuresis. Hypoglycemia was the most disturbing. We saw this in two patients.

DR. METCOFF: How many patients had received the drug?—Two instances of hypoglycemia out of approximately how many trials with the drug?

DR. MAHER: We gave the drug to 23 patients on 81 distinct occasions, most often for 24-hour exposures. These two, however, had severe renal failure, and one of them was the man who was given 16 mg/Kg, which turned out to be something like a hundred pills in a day. Weeks after that, he presented with convulsions. His blood sugar was 60 mg% and he responded to glucose. The other patient had a blood sugar of 20 mg%, after otherwise unexplained convulsions, which occurred three weeks after ethacrynic acid was administered. But both of these patients were severely ill with advanced uremia when we studied them.

CHAIRMAN BARNETT: Dr. Maher, did you have any impression of any effect on blood pressures?

DR. MAHER: No. There are several other groups studying this drug, one paying particular attention to hypertension, and they have the feeling that it is less effective therapy for the hypertension than thiazides.

DR. CLARK: Dr. Maher, I wonder if either you or Dr. Goldberg could comment on any changes in phosphate excretion, following ethacrynic acid?

DR. GOLDBERG: We have had acutely untitratable acid and ammonia excretion. In a few studies on water diuresis, there was a moderate increase in titratable acid and ammonia.

CHAIRMAN BARNETT: Thank you. The next talk, authored by Dr. Thomas Marchioro, will be presented by Dr. Conrad Riley, who has been working with the group at Colorado University, since Dr. Marchioro was unable to be present.

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Results in 40 Cases of Human Renal Homotransplantation

THOMAS MARCHIORO, THOMAS STARZL, WILLIAM WADDELL, AND CONRAD M. RILEY

I regret that Dr. Marchioro could not present this talk in person, because he, together with Dr. Thomas Starzl and Dr. William Waddell, has been on the project from the beginning. I am glad to say, however, that since these men are performing a transplant on a ten-year-old patient of mine, I am happy to substitute for them.

I am going to show you, first, the over-all score box and then I'll go into some of the details: things move so fast in Denver that I am not sure Table 39 is up to date.

TABLE 39

Human Kidney Homotransplants by Colorado University Group
(Nov. 20, 1962–Oct. 7, 1963)

36 Living donors in 33 patients	Deaths	11
2 from cadavers in 2 patients	Have left hospital	17
Causes of Death:		
Rejection		0
"Toxicity" without rejection		7
Progressive uremia (cadaver kidneys)		2
? Suicide		1
Electrolyte imbalance		1

There is an error in the title of this talk in that, although we have performed transplants from a total of 40 donors, 2 were between identical twins, so that there have been only 38 donors of homotransplants to 35 patients. The breakdown of these over-all figures is explained in Table 39. Three patients, in whom the kidney failed to

function at all, had the initial kidney removed and a second one from another donor implanted at a later date.

It is interesting that, among the causes of death, as far as we could tell from the anatomic appearance of the kidneys, rejection was not a factor. The toxicity is very disturbing. The individuals seem to waste away, no matter how much food you give them. They continue to be in negative nitrogen balance, and succumb. One of those with "toxicity" suffered a fatal pulmonary embolus.

As you can see, the two cadaver kidney recipients died because the kidneys never really worked. There was one patient who came in from the outside, dead on arrival, who the team judges was probably a suicide.

The patient with electrolyte imbalance was a tragic case, and I think we all feel that this really should have been preventable. In any event, the post-transplant diuresis was so rapid that we were not able to keep up with the fluid needs and she died within 12 hours after the operation.

Figure 87 shows the time when the operations were done. The survivors are shown above. Those who died, and the length of their survival, are indicated by the heavy bordered boxes, and the living patients are shown as blank columns. You see, we have something like 7 who have passed the three-month period, at least.

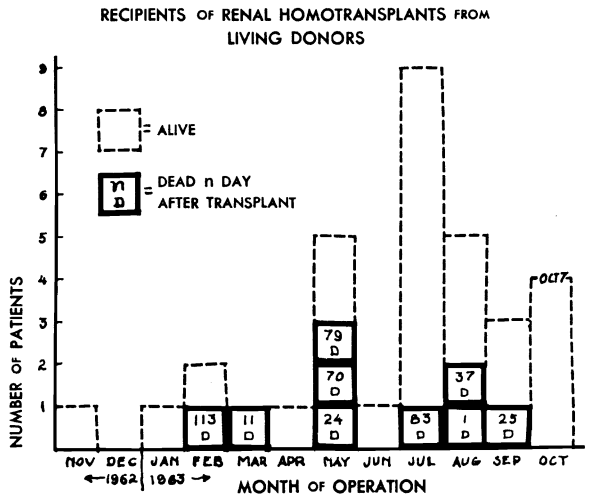

FIGURE 87. Chart to show time relationships in experience at Colorado University.

For patients with transplantations of less than three months, as Dr. Merrill said, we cannot be too sure of the eventual outcome, but we can be hopeful. The one patient who died nearly four months after transplant had multiple complications, but had satisfactory renal function.

TABLE 40

Donor-Recipient Relationships in Homographs

Relation	No.	Alive
Father-offspring	1	0
Mother-offspring	6	6
Sibling	14	10
Unrelated	12	8
	33	24

To give you an idea of the sources of the organs (Table 40), we have used parent-offspring where we could; we used siblings where we could. Others have come from unrelated donors. It is a wonderful thing that so many strangers are willing to donate kidneys when a suitable sibling or mother is unavailable. People volunteer literally by the hundreds to donate kidneys to these patients.

TABLE 41

*Direction of Acceptable Mismatched Tissue Transfer**

O to Non-O	Safe
Rh− to Rh+	Safe
Rh+ to Rh−	Relatively safe
A to Non-A	Dangerous
B to Non-B	Dangerous
AB to Non-AB	Dangerous

* O is universal donor; AB is universal recipient.

Table 41 shows our conclusions about the problem of blood-matching. It was our first thought that if one washed out the kidney well, prior to transplant, one could use major blood group mismatches with impunity. Subsequent experience has taught us that this is wrong. Where we have gone from a major group to a patient who has naturally occurring antibodies against that group, we have run into very obvious trouble on at least two occasions, and probable trouble on a third

occasion. We now feel that we should abide by the same rules as apply for transfusions: that O to A is probably safe but that A to O is not safe.

In two instances where this kind of mismatch was attempted, the implanted kidney failed to "pink-up," and after several hours' observation it was removed. In a third, the kidney immediately functioned well, but after 24 hours renal function diminished markedly. Now, over two months later, the kidney is functioning fairly well, but definitely less well than other experience would lead us to expect. Whether this doubtful result is related to the blood-group problem or to the underlying disease I shall discuss later.

Examination of the kidneys which failed to gain adequate circulation under observation showed grossly a very pale cortex. After attempted perfusion with radiopaque material, X-ray study in one showed filling of only the larger vessels. Microscopic examination showed hemagglutination in the small vessels. Presumably the small vessels never did open up.

To discuss the technical aspects a little: when we were using mismatched blood, we perfused the kidney with buffer at 15°C and procaine, in an effort to prevent vascular spasm. When we were using donors in whom the matching was satisfactory, we cooled the donor so that we started with a cooled organ. At the time of operation, one expects urine flow to begin immediately; within 5 to 90 minutes, I think, has been the time when the urine flow does begin. It is very rapid—up to 500 ml per hour for 12 to 18 hours.

The time of ischemia in the transplants has varied from as short as 17 minutes to as high as 81 minutes, with a mean of 33 minutes. The longer the ischemia, the less likely the transplant seems to take. The third instance in which a second implant had to be used was one in which technical difficulties prolonged the ischemia to the maximum noted above.

As far as the methods of trying to control rejection are concerned, at first, as you probably know, we were doing thymectomies, but this proved to be so difficult from the surgical point of view that we have given that up now; there were several cases where uncontrollable oozing and infection at the site of the wound took place.

Splenectomy is being done routinely as a means of removing excess lymphocytes; nephrectomy is being done routinely. Usually, the splenectomies are done at the same time as the transplant, so only one operative procedure is involved.

The type of immuno-suppressive therapy that we have been using has, in general, been limited to drugs. In the first case, we did use total body irradiation, but since that time we have been using drugs only.

These drugs consist of azothioprine, or Imuran®, which is started 10 to 12 days before the operation, and then steroids as well as actinomycin C are given in large doses when signs of rejection occur.

Figure 88 presents a chart of the first patient, who had everything: a thymectomy, indicated by T; a bilateral nephrectomy and splenectomy (BLNS); he was being dialyzed all this time (D) preoperatively, and he was given X-irradiation before the operation and again after the operation. Finally, he was given azothioprine, or Imuran, subsequent to operation, and steroids in large doses.

One interesting point, that does not show here, is the diuresis that occurs immediately after operation. At the time of his rejection crisis, he had a marked drop in clearance of creatinine, in spite of the fact that he had an extremely low blood count, presumably due to total body irradiation.

The average diuresis in 25 cases has been of the order of 450 ml per hour in the first 12 hours, so that you can see that keeping up with the fluid balance can be a difficult thing.

The signs of rejection occurred with the rising BUN, the rising temperature, and the drop in his creatinine clearance. He was immediately treated with steroids and actinomycin C. The rejection apparently reversed itself, his creatinine clearance improved, and he

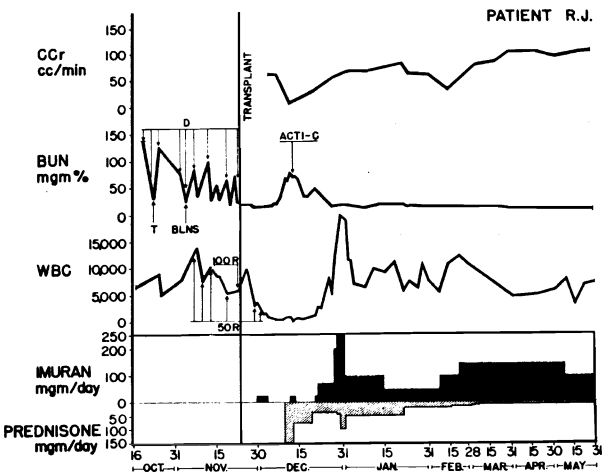

FIGURE 88. Graphic representation of early course on one patient, a 12-year-old boy whose mother was the donor. (Permission received from A.M.A.)

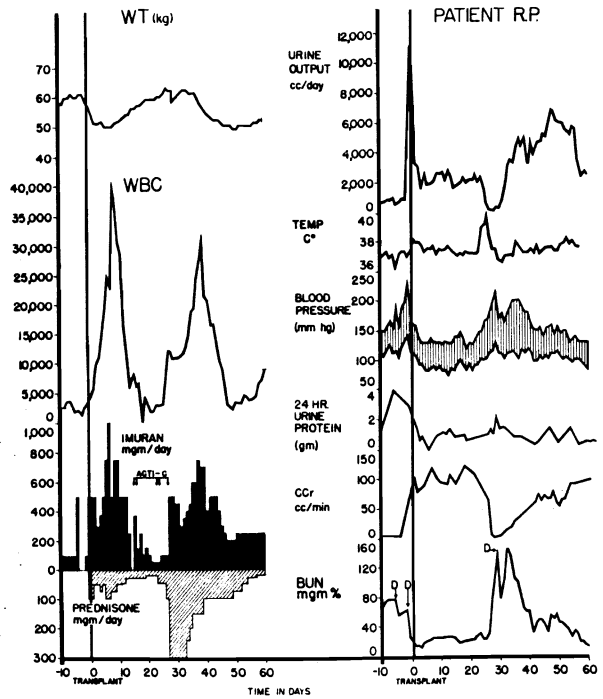

FIGURE 89. Charts to show features in early course of an older patient who is still doing well.

recovered—in fact, he is now working as a technician in Dr. Marchioro's laboratory.

Figure 89 represents the course of another patient. It shows the enormous diuresis which occurs immediately postoperative, the rejection coming about 1 to 2 weeks afterward, with a rise in blood pressure, a fall in creatinine clearance, and a drop in urine volume, again responding, presumably, to the treatment with steroids and actinomycin C.

Thus, we have quite a group of patients who have been treated, and at the moment they are looking pretty well. The youngest, a six-year-old boy, the only one who was nephrotic originally, is an interesting case. The others had chronic glomerulonephritis, pyelonephritis, or polycystic kidneys. This boy was one of our mismatches. His mother

was a blood group A and he was an O. Whether this had anything to do with it, we are not entirely sure, but in his postoperative period he had massive proteinuria, his blood serum took on a very typical nephrotic pattern, and his cholesterol rose to the 500 to 600 mg range. The question is, was this a recurrence of his original disease in his mother's kidney, or was it because of the mismatching that he got into this trouble? At this moment, his blood pattern looks normal, about two months after operation, but his renal function is not what we would like it to be.

DISCUSSION

CHAIRMAN BARNETT: Thank you, Dr. Riley. Are there any questions or comments?

DR. LEWIS: We have studied 20 transplants. The duration of our work is more than four years. Our survival rate is very different, because we have observed that most deaths occur between one hour after operation up to the first month. Once the patients survive the first month, it seems to us that they have a very good hope of living a long time. Nine patients lived longer than one month, 2 are now dead, and 7 are alive. Of the two who died after the first month, one lived less than 6 months and another one for 22 months. The others are alive. The oldest one has lived more than four and a half years; another for 19 months, and two others for 14 months. Three have lived between six months and one year, and the others are between three and six months.

In the beginning, during the first six months, we have observed what we think to be a kind of early rejection. After the sixth month, there is another late disease, which is probably the original one, often with very great enlargement of the spleen and, also, in some cases, of the liver. Dr. Riley, has your group observed the same late accidents after the sixth month? What do you mean by "toxicity" without rejection?

DR. RILEY: I would say that the deaths we have had have mostly been in the first three months. Those who survived three months are alive with the exception noted.

This "toxicity" without rejection, I put in quotes because I cannot define it very well. It is a wasting disease, where the patients seem to be in negative nitrogen balance, and nothing you do seems to reverse it. They just gradually go down and fade away completely. Have you seen this at all?

DR. LEWIS: No.

DR. LANGE: Could you tell us whether all the patients are still on the immuno-suppressive therapy and how long you continue them on such therapy and with what dose? Do you think this so-called "toxicity" may be something similar to homologous or runt disease?

DR. RILEY: That is what it looks like, but I do not know the answer to that question, as far as the runt disease goes. Our longest patient, who is now rounding out eleven months, has been taken off steroids, but is still getting relatively small doses of Imuran. Imuran, as you know, is very similar to 6-mercaptopurine, and our dose, as a starter, has usually been around 2.5 to 3 mg/Kg. It is being tapered at the moment.

DR. CLARK: I would be interested in knowing about three things. First, in regard to infection, while these patients are on immuno-suppressive therapy, one can have a transplant, as we have had, at 68 days, that died of a spirillosis. We have been concerned about this, giving antibiotic therapy or germ-free living and so on.

Secondly, have you been paying a great deal of attention to the urine sediment in, perhaps, early rejection that is—whether it may be a clue?

Lastly, could you comment on the histologic findings in the transplanted kidney of those who have died?

DR. RILEY: First, about the infection: we have put the recipients into a "germ-free environment" which is fairly complete, with gloves and masks, etc. We have not routinely put them on antibiotics. We treat when signs of infection occur. In those who did not have signs of infection prior to operation, we have had pretty good luck. Some of those in the early stages had infected wounds or some other cause beforehand, and those did not take to this treatment very well. It is quite alarming to see the white count go down to 300 or even less.

We have not looked at the urinary sediment, and I am not well enough informed to discuss the histology in detail. In general, I am told that the kidneys have looked essentially normal, and that the deaths have been from causes apparently unrelated to the kidney.

DR. ELKINTON: Have you given up all consideration of the cadavar as the source of kidneys?

DR. RILEY: There has been practically no morbidity among our donor group. One had a minor wound infection and one had a spontaneous pneumothorax, unexplained. But, other than those, there have been no complications among the donors. The two kidneys that came from cadavers were so long in getting from the donor when alive into the recipient that they never did work, and there is a very strong feeling among our surgeons that the time of ischemia is extremely important. If you could get a prospective cadaver to agree to a living removal, I think that this could be possible. But, what with getting family permission and removing the kidney, the time interval is so great that our men are not doing it.

CHAIRMAN BARNETT: Didn't Dr. Merrill say that they have some survivals with cadaver kidneys?

DR. RILEY: Yes; one, a long-time survivor, too.

DR. ELKINTON: The time of ischemia did not exceed 71 to 81 minutes in your cadaver kidney transplants. Ours was two hours and did quite well.

DR. RILEY: The two that we have done, taking 71 and 81 minutes, were from living donors, and never functioned. I do not know the exact time of the ischemia of the cadaver transplants. It was, however, in the 2-hour range.

DR. BERNSTEIN: Dr. Riley, was there any relation between the pathologic disease and the kidneys of the recipient?

DR. RILEY: I do not think there has been. For the most part, we do not try to be too accurate in the diagnosis—we call it "terminal renal failure."

DR. LANGE: Did you have any incidence of lupus in your surviving cases? After giving massive therapy to lupus cases for long periods of time, I have seen many flareups. I wonder whether this is just specific to lupus erythematosus, or whether it represents the result of prolonged steroid therapy.

DR. RILEY: We have not seen any. It may be the short duration of time.

DR. MANN: We have a rather perplexing problem that just came up. We performed a transplant in apparently identical twins. About four months later, the recipient developed serum hepatitis. About two weeks later, he developed apparent rejection of the kidney, and succumbed. The pathology demonstrated the typical rejection reaction. We are wondering whether anyone has experienced any similar problem?

DR. RILEY: No, I have not.

CHAIRMAN BARNETT: Thank you, Dr. Riley. The last talk is by Dr. Paul Kimmelstiel.

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"Sterile Pyelonephritis" and "PPLO"

PAUL KIMMELSTIEL, GUY PANDOLA, SILAS FARMER, AND LIEN-HWA HO

Last year I reported to you a few pilot experiments in which we found PPLO in tissues of rat kidneys with "sterile pyelonephritis." It took me all year to come to the conclusion that my statement then was probably correct. During the past year we worked with rat kidneys, which, one month after ligation of the ureter, showed hydronephrosis and chronic, partially purulent pyelonephritis. At no time during the three months' duration of the experiments could organisms be cultured with conventional methods.

Sterile tissue emulsion was streaked on one-half of a commercial PPLO agar plate. The controlled side and streaks of normal rat kidney from the contralateral side always showed occasional colonies of similar type. The diseased kidney emulsions, however, showed between 10 and 50 times more colonies. This may indicate (1) that horse serum normally contains PPLO and that there is a factor (enzyme) in the kidney that accelerates its growth; or (2) that it merely forms artifactual structures from substances in the agar; or (3) that there really is an increased number of such organisms in the diseased kidney in which they normally occur in small numbers.

The type of colony we obtained on appropriate media has long been regarded as an artifact. It was identified as a soap crystal and later, in 1940, by Brown as a "pseudocolony." It has not been heard from since.

Because we found these "pseudocolonies" in such large numbers in diseased kidneys, in contrast to the contralateral normal kidney, we studied these colonies in detail and found:

1. A battery of stains revealed that the colonies are much like classic PPLO and most probably contain RNA since acridine orange fluorescence was inhibited by specific ribonuclease. Characteristically, these colonies grow in a swirl-like pattern.