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Cyclophosphamide and human renal transplantation

C.W. PUTNAM, M.D., B. LAUNOIS, M.D., J. CORMAN, M.D.,
G. SCHROTER, M.D.
A. S. BOOTH, Jr., M.D. I. PENN, M.D. C. G. HALGRIMSON, M.D.
C. G. GROTH, M.D. T. E. STARZL, M.D., Ph. D.

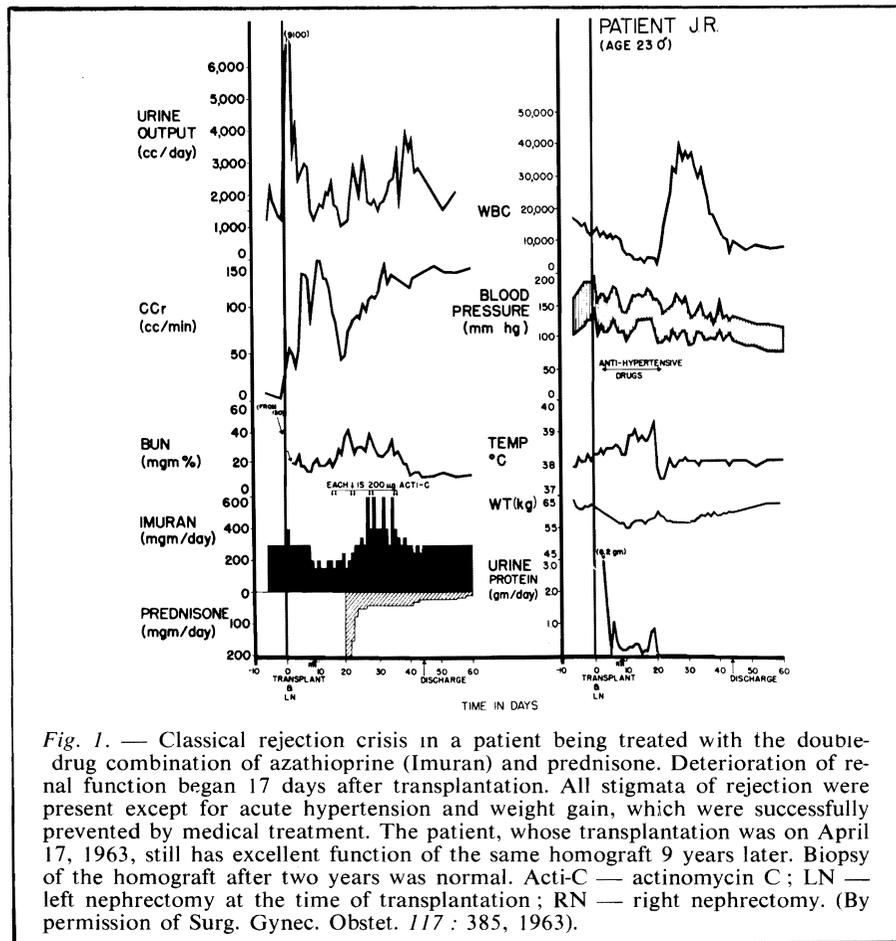
From the Departments of Surgery and Pediatrics,
University of Colorado School of Medicine and the Veterans Administration Hospital
Denver, Colorado, U.S.A.

In this discussion we propose to describe the use of cyclophosphamide in immunosuppressive regimens that recently have been employed for humans after whole organ transplantation. Before doing this it might be well to briefly go back over some major principles of immunosuppressive therapy that were worked out almost a decade ago with drugs other than cyclophosphamide. Almost all of this basic information came from the accurate observation of patients subjected to the relatively simple operation of renal homotransplantation.

REVERSAL OF REJECTION AND RELATIVE GRAFT « ACCEPTANCE »

Some typical events subsequent to renal homotransplantation are illustrated in Figure 1. This 23-year-old patient was given a renal homograft by his younger brother in early 1963. After transplantation, the drug azathioprine (Imuran[®]) was given in an attempt to prevent rejection. There was prompt and excellent function of the new kidney with large volumes of urine, a spectacular rise in creatinine clearance, and a fall in BUN. However, after a little more than 2 post-operative weeks the new kidney began to fail. The creatinine clearance dropped sharply, the BUN rose secondarily, the urine volume diminished and the patient gained weight. In addition, there was an increase in the white cell count. He developed hypertension which required control by antihypertensive medications,

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became acutely febrile with temperatures of almost 40 °C, and developed proteinuria. In short, all the manifestations of acute rejection had developed in spite of immunosuppressive therapy, namely signs of acute homograft failure plus an acute systemic febrile illness.

Before experience with this patient and others of the same era in the early 1960's, it had been assumed that rejection was one of biology's most vigorous and persevering reactions and that, once begun, it would continue to the death of the transplant. It was now proved that this assumption was false and that rejection was a highly reversible process. In this case, immunosuppressive treatment was intensified by the addition of prednisone to the azathioprine. The findings of rejection promptly disappeared. The BUN fell, the creatinine clearance rose, and the proteinuria disappeared as did the high blood pressure. The fever was gone within a few hours of the institution of the prednisone therapy. Rejection had thus been shown to be a reversible process. This was a fundamental disclosure by clinicians and one

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which had not been at all anticipated by previous workers in basic research laboratories.

The second equally important thing learned from these early patients can also be demonstrated by the same case. In the weeks after rejection had been reversed in this patient, the prednisone dose was very rapidly reduced, and eventually it was discontinued altogether after only about 5 months. Then the azathioprine dose was also lowered until within a year the patient was receiving only about half the daily quantities that at the outset had failed to prevent the onset of a moderately severe rejection. It is quite possible that all immunosuppressive therapy could be stopped in this patient who is still alive with perfect renal function after almost 9 years. Although we have never felt justified in taking the final drastic step of stopping treatment in any human recipient, we have thoroughly tested the hypothesis in dogs. We have animals in our laboratory surviving for as long as 9 years and 8 years, respectively, after renal and hepatic homotransplantation from non-related mongrel donors. These remarkable dogs who, it is now clear, are apt to die of old age, were treated with azathioprine for only a few months post-operatively and have not now received any treatment for approximately 2/3 of a canine lifetime.

Even in this introduction it is worth summarizing the two concepts which have just been stated. First, rejection is a highly reversible process. Second, a favorable change often occurs after transplantation by virtue of which the host comes to better tolerate the presence of the homograft, thereby allowing immunosuppression to be reduced. If either of these statements were not true, organ transplantation would not be feasible. The fact that they *are* true is the inside story of clinical transplantation. The observations supporting these two conclusions indicate that whole organ homotransplantation in conjunction with non-specific immunosuppressive therapy can and often does lead to selective abrogation of the host rejection response. From a practical point of view, this objective is most easily achieved by the use of pharmacologic agents in combination. In fact, the modern era of clinical transplantation was ushered in by the realization (illustrated in Figure 1) that azathioprine and prednisone can advantageously be used together with an effect exceeding the simple sum of the individual drugs. This combination of agents has been referred to as the « double drug regimen. »

THE TRIPLE DRUG REGIMEN OF AZATHIOPRINE, PREDNISONE, AND ALG

A third potent immunosuppressive agent that has been widely employed is heterologous antilymphocyte globulin (ALG). When given subcutaneously, intramuscularly, or intravenously, it has been shown as the sole treatment to mitigate or prevent rejection. Moreover, it has an additive effect when administered with either azathioprine or prednisone.

In our clinics, ALG has been administered as a third agent, added to azathioprine and prednisone, in almost all organ recipients treated from May, 1966 to the end of 1970. In most cases the course of ALG therapy was limited to the first 4 post-operative months, during that critical time

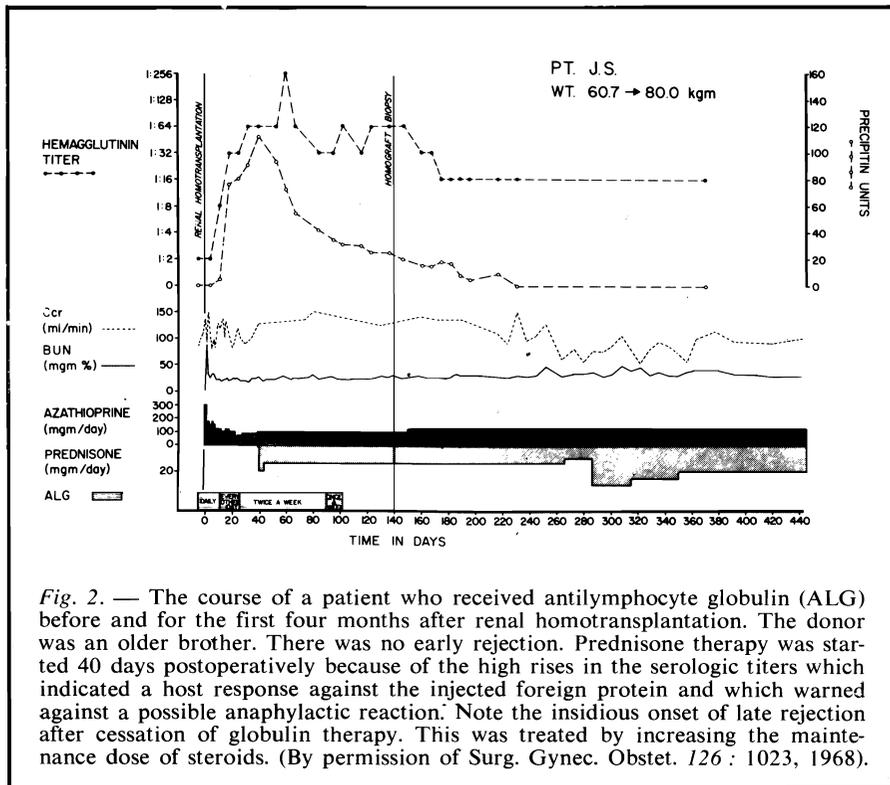


Fig. 2. — The course of a patient who received antilymphocyte globulin (ALG) before and for the first four months after renal homotransplantation. The donor was an older brother. There was no early rejection. Prednisone therapy was started 40 days postoperatively because of the high rises in the serologic titers which indicated a host response against the injected foreign protein and which warned against a possible anaphylactic reaction. Note the insidious onset of late rejection after cessation of globulin therapy. This was treated by increasing the maintenance dose of steroids. (By permission of Surg. Gynec. Obstet. 126 : 1023, 1968).

when « graft acceptance » is hoped for (Figure 2). We have referred to this treatment program as a triple drug regimen as opposed to a double drug regimen consisting of azathioprine and prednisone only that was used before 1966.

With the institution of the foregoing triple drug program there has been an improvement in the results after renal transplantation at our center. Other workers including those in Lyon, Minneapolis, Salt Lake City, Sydney and Boston have shared this same opinion of the benefits of adding ALG, although it must be conceded that acceptance of ALG as an important component of treatment has not yet been universal. This question is an interesting and unresolved one but we will not be able to dwell on it since it is outside the main focus of the present communication.

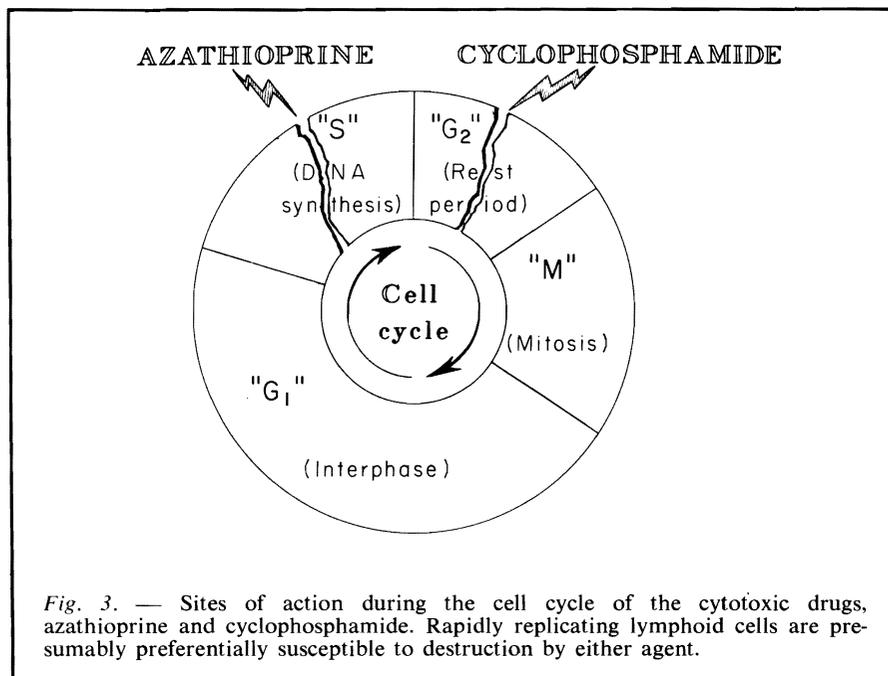
CYCLOPHOSPHAMIDE

With both the double drug and triple drug programs just described, azathioprine has been considered the cornerstone agent. As such, azathioprine has acquired the mystique that goes with presumed indispensibility. The present report will show that another well-known

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cytotoxic drug, namely cyclophosphamide (or Cytoxan) is approximately as potent and safe as azathioprine. At the least, it can be administered interchangeably with azathioprine, but hopefully its use may lead to something more than just the advantage of having a major « back-up » drug.

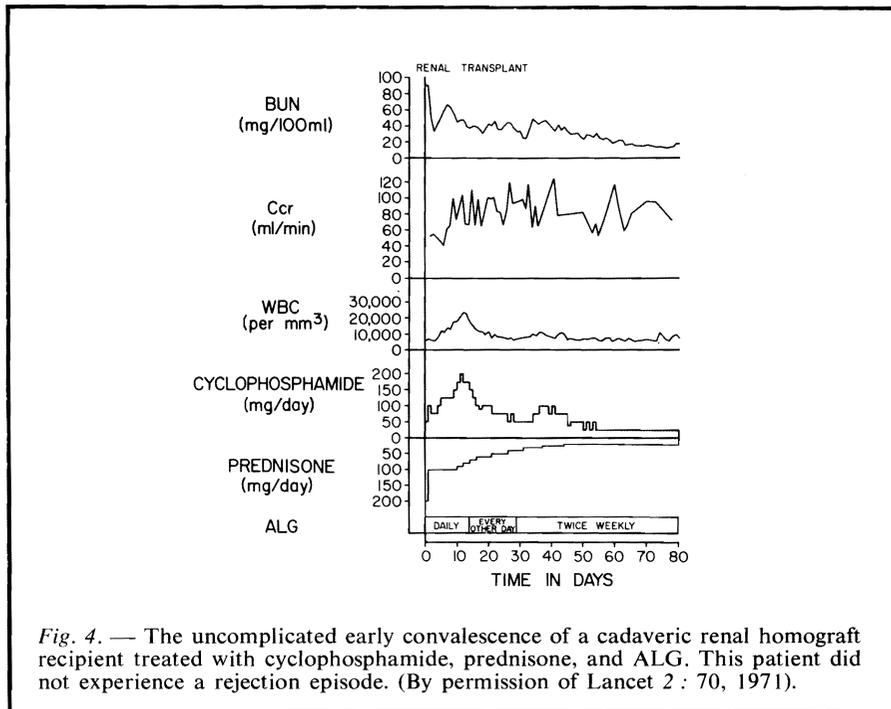
As many of you know, azathioprine is a purine analogue. Its site of action has been described by Elion and Hitchings to be principally at the DNA synthesis (or S) phase of the cell cycle (Figure 3). The presumption is that



the metabolically active lymphoid tissue which subserves cell-mediated rejection is thus injured and thereby prevented from mounting an effective rejection. In contrast, cyclophosphamide is classified as an alkylating agent, chemically similar to nitrogen mustard. Its site of action, according to Wheeler, is at the G₂(or resting)phase of the cell cycle, just preceding mitosis (Figure 3).

Cyclophosphamide in the Triple Drug Regimen

Patient and Kidney Mortality — Although cyclophosphamide is fundamentally different from azathioprine it has proved capable of performing precisely the same role as exemplified in Figure 4. The patient whose early course is depicted received a kidney more than seven months ago from a mismatched cadaveric donor. Cyclophosphamide was given instead of azathioprine but in doses that were less than half of what would



have been anticipated with azathioprine. Rejection in this case was never diagnosed despite the rapid withdrawal of prednisone therapy. ALG was also given initially and later stopped.

Figure 5 illustrates the course of another recipient, this time of a maternal kidney. A week after operation there was a severe rejection crisis of the kind first observed under azathioprine therapy almost a decade ago. The findings included recurrent azotemia, a fall in creatinine clearance, oliguria, proteinuria, and hypertension. The crisis was controlled and reversed by temporarily increasing the prednisone dosage. The patient was never given enough cyclophosphamide to depress her white count. This we believe to be the secret of successful cyclophosphamide therapy. In this case, the eventual daily doses were 25 mg or less, or smaller than a half a milligram per kilogram per day.

Six to 11 months ago (February to July, 1971) we treated 18 consecutive intrafamilial kidney recipients with the cyclophosphamide-containing triple drug regimen. Two of the kidneys underwent hyperacute rejection and were removed immediately or within a few hours; these patients subsequently died after retransplantation. Two other recipients died of perforated diverticulitis and pneumonitis, respectively, for a total patient mortality of 22%. The other 14 homografts are all functioning satisfactorily (Table 1).

In the 3 months from December, 1970 to March, 1971, a comparable group of 20 intrafamilial recipients were treated with the standard triple

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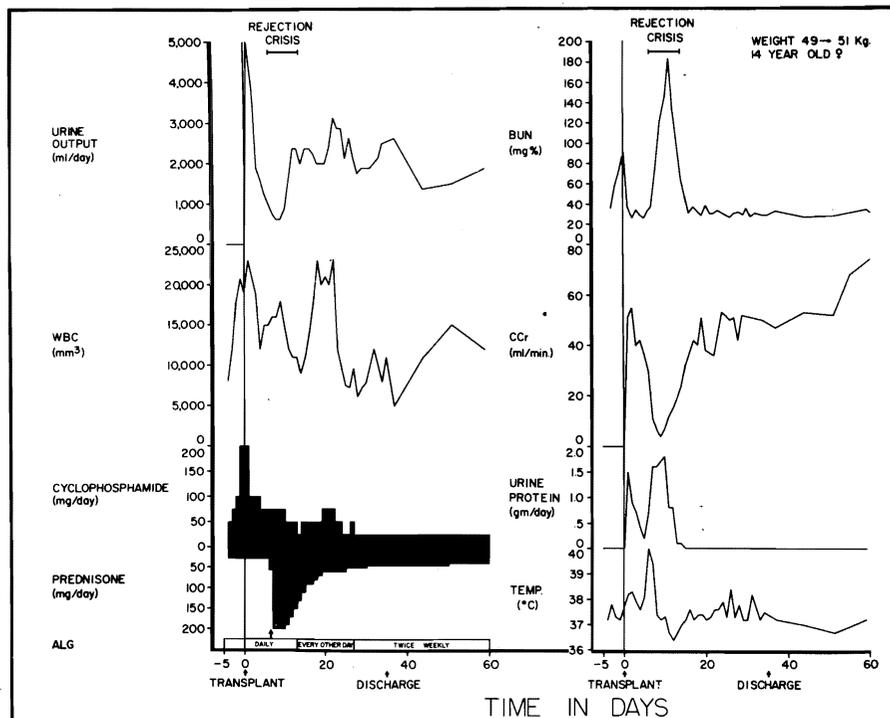


Fig. 5. — The first 60 days after the transplantation of a kidney from a mother to her daughter. Although the rejection crisis after a week was a severe one, it was easily and completely reversed. Note that leukopenia was never produced by the daily doses of cyclophosphamide that were usually between 0.5 to 1.0 mg per kg/day. ALG — horse antilymphocyte globulin; BUN — blood urea nitrogen; CCr — creatinine clearance; WBC — white blood cell count; ARROW — 625 mg methyl prednisolone intravenously. (By permission of Surg. Gynec. Obstet. 133 : 981, 1971).

Table 1. — Intrafamilial transplantation.

	18 Cyclophosphamide	20 Azathioprine
Functioning Grafts*	14 (78 %)	16 (80 %)
Hyperacute Rejection	2 (11 %)**	1 (5 %)
Patient Dead*	4 (22 %)	3 (15 %)

* Follow-ups in the cyclophosphamide cases are 6 to 11 months; the follow-ups in the azathioprine cases are 11 to 15 months.

** Both these patients died.

drug program containing azathioprine. The results were essentially the same (Table 1).

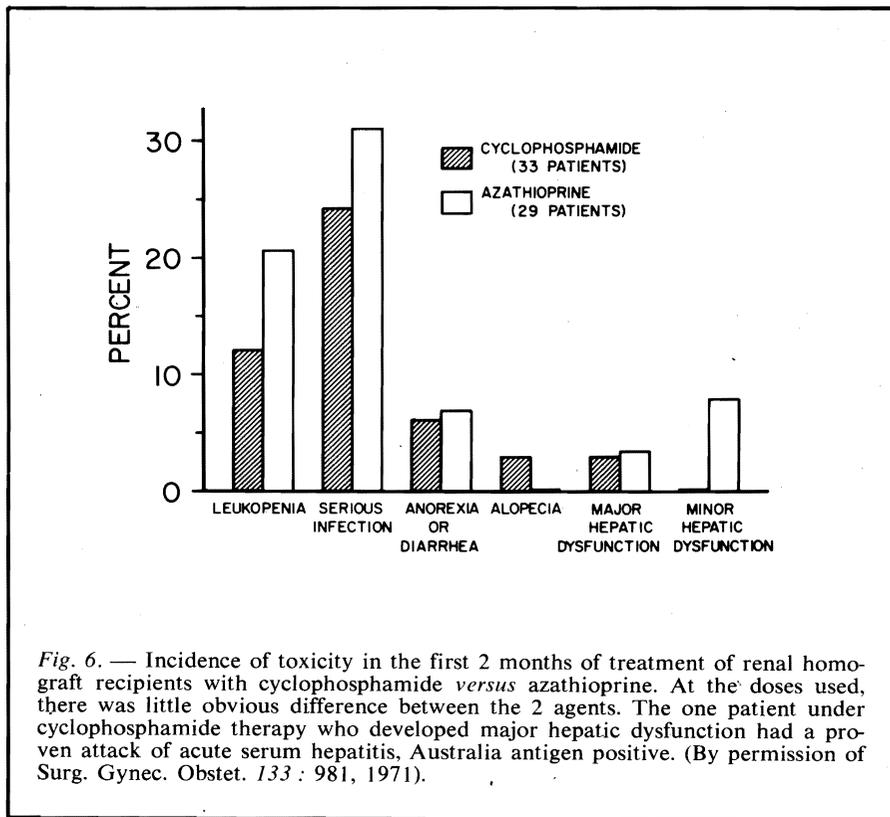
In addition to these intrafamilial cases, 17 cadaveric renal transplantations were also performed under cyclophosphamide. Comparisons with azathioprine were difficult here since the cases were more

complicated and included numerous retransplantations. In fact, 46 % of the recipients (12 of 26) were undergoing retransplantation for the second to the fifth time. In the cyclophosphamide group the kidney survival to date has been about two-thirds, somewhat better than in the azathioprine controls (Table 2).

Table 2. — Cadaveric transplantation*

	17 Cyclophosphamide**	9 Azathioprine**
Functioning Grafts	11 (65 %)	5 (56 %)
Hyperacute Rejection	1	1
Graft Rejected	2	2
Patient Dead	3 (18 %)	2 (22 %)

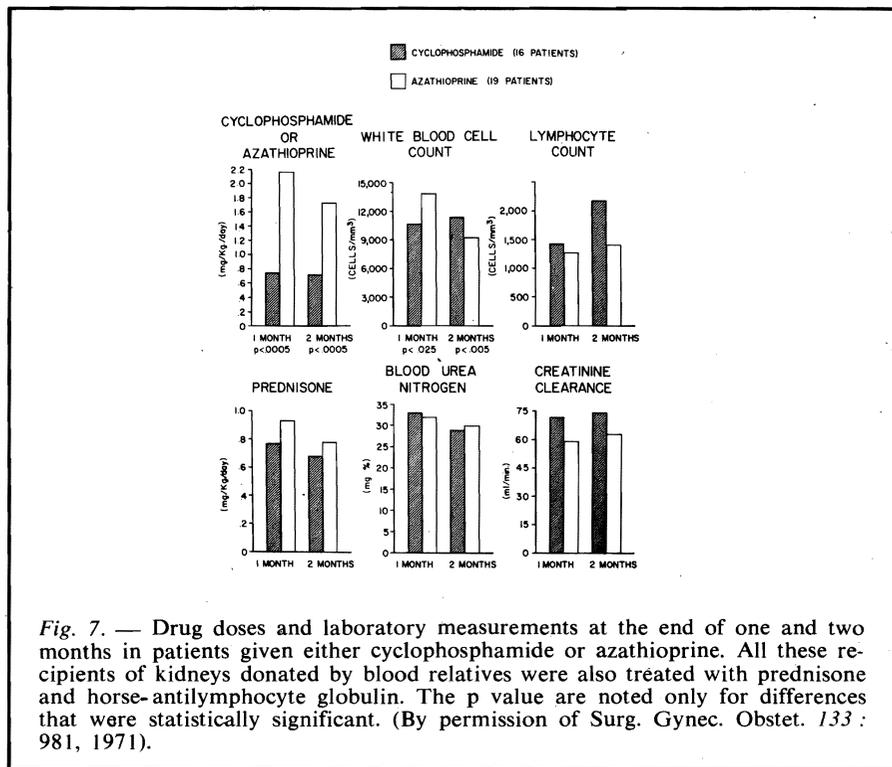
* 12 of these 26 recipients were undergoing retransplantation from the second to the fifth times, helping to explain the relatively poor results in both groups.
 ** Follow-ups in the cyclophosphamide cases are 6 to 11 months; the follow-ups in the azathioprine cases are 11 to 15 months.



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Relative Toxicity — As with so many other really important things in organ transplantation, Dr. Will Goodwin of Los Angeles pioneered the use of cyclophosphamide almost a decade ago. Unfortunately, in these early days of its use in transplantation and for cancer chemotherapy, cyclophosphamide acquired the reputation of being terribly toxic. We now believe that this bad name was the product of overdosage. In the doses used for our patients, the incidence of serious infections and bone marrow depression were not different than in control cases using azathioprine as shown in Figure 6. The relatively specific complications of cyclophosphamide, such as gastrointestinal complaints, alopecia and hemorrhagic cystitis were either not common or were not observed at all.

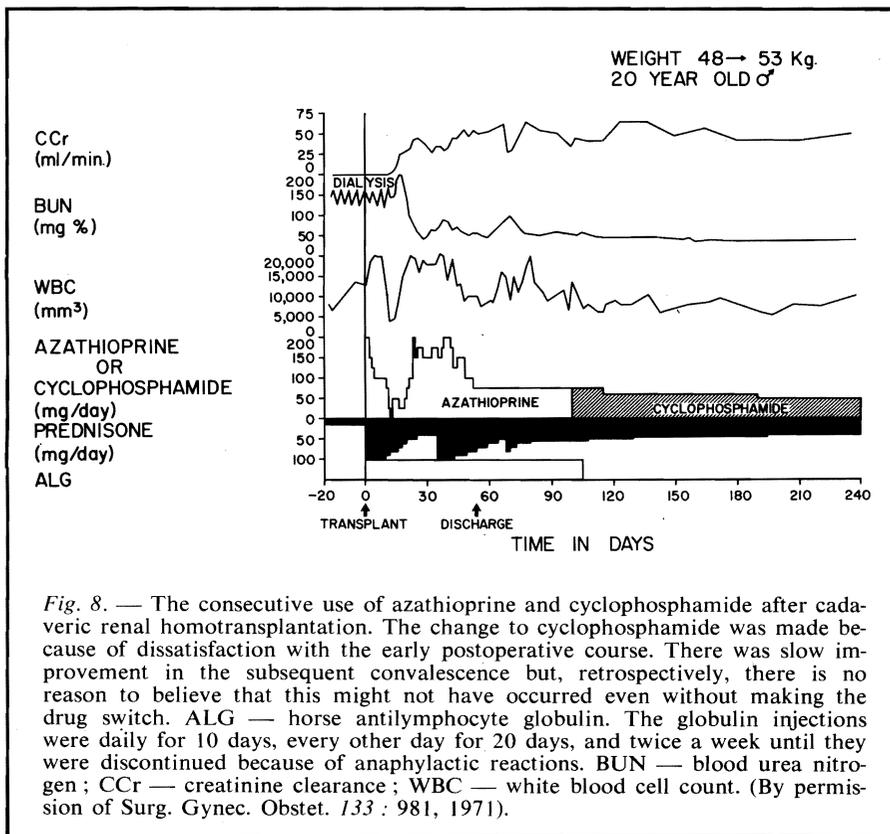
To give some idea of relatively safe doses of cyclophosphamide in relation to those of azathioprine, the statistical study depicted in Figure 7 was



carried out in the patients described in the preceding section who received consanguineous kidneys and who lived for at least two postoperative months. The doses of cyclophosphamide per body weight were about 40 % of those with azathioprine. With these 2 different cytotoxic drugs the creatinine clearances, the BUN, the daily quantities of prednisone, and the average white cell counts were not significantly different.

Cyclophosphamide Substitution

From the foregoing observations, it seemed that cyclophosphamide and azathioprine played a very similar role as the cytotoxic agent in triple drug therapy. This conclusion was supported from the study of an additional 49 patients in whom pre-existing therapy with azathioprine was stopped from 6 weeks to 8 years after renal transplantation and replaced with cyclophosphamide as in the recipient of a cadaveric kidney graft whose course is shown in Figure 8. After the change in this patient, renal function

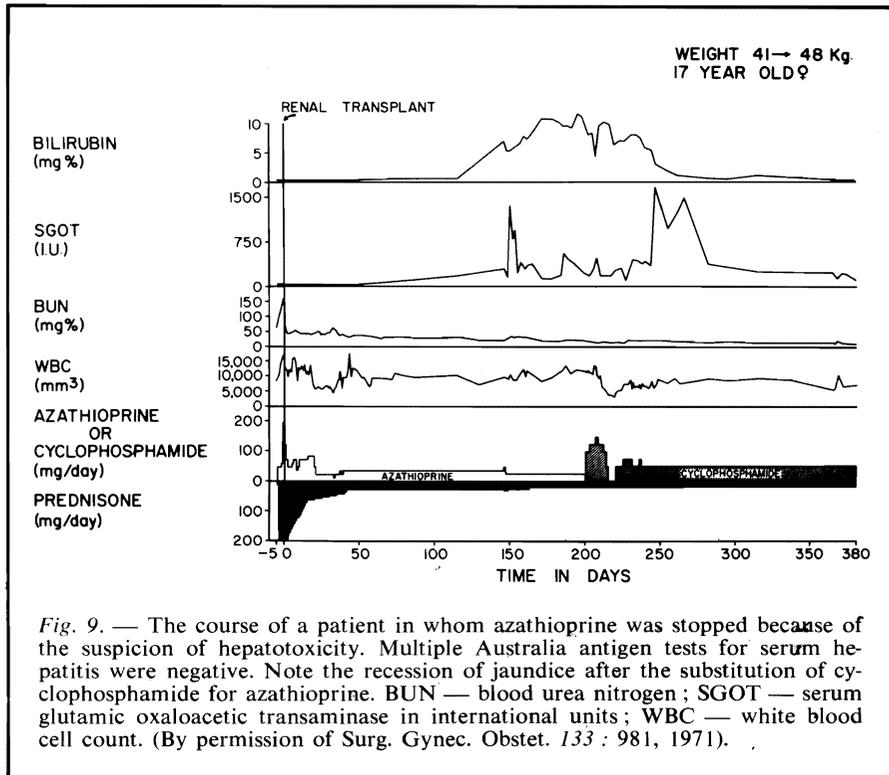


was maintained and, in fact, it became possible to reduce the doses of prednisone. With almost no exceptions, the preexisting condition of the 49 patients was not altered by the drug change.

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Specific Present Indications For Cyclophosphamide Substitution

For the moment, we would like from this experience to leave with you one small but practical point. There will be specific indications for cyclophosphamide as exemplified by a patient (Figure 9) who was dying of

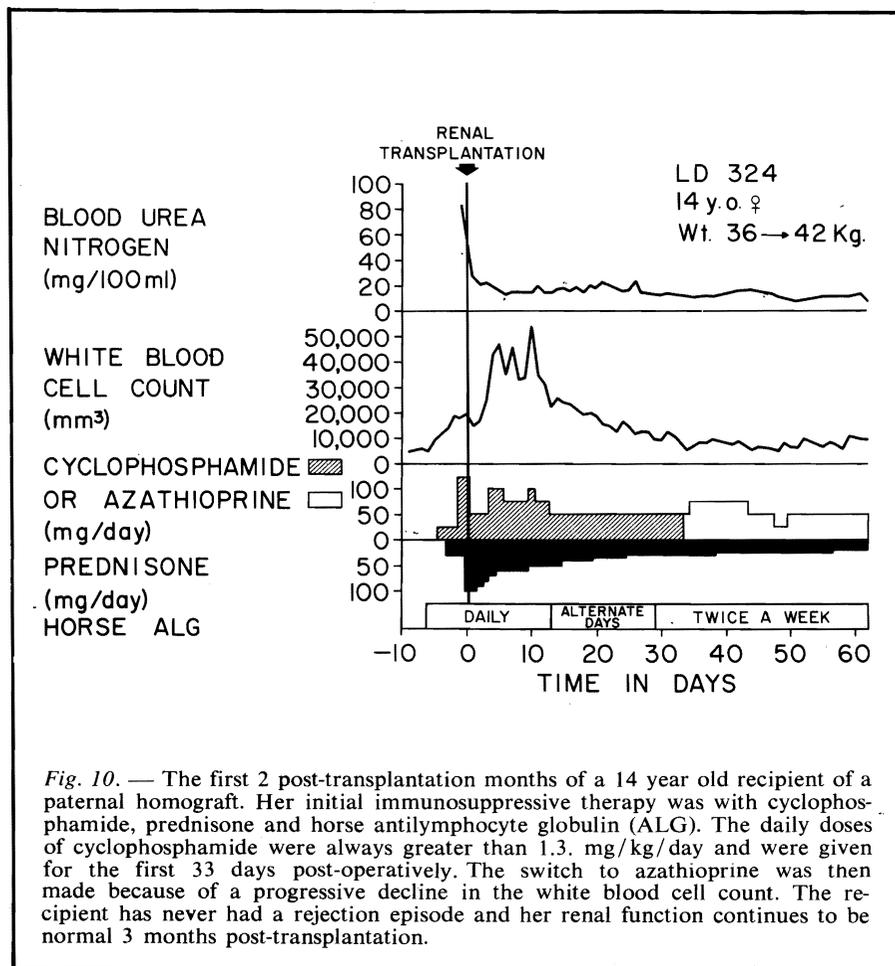


liver disease after an otherwise successful renal transplantation. The hepatic injury was suspected to be due to azathioprine for which reason this agent was stopped and replaced with cyclophosphamide almost one year ago. The renal function was perfectly maintained and eventually there was complete recovery from the liver injury. We have encountered other examples of relief of hepatotoxicity after making the drug switch.

Future Prospects

It is of considerable theoretical interest that azathioprine and cyclophosphamide can be used essentially interchangeably since they are of

different drug classes and are thought to have different actions. But does this fact imply that sweeping improvements in clinical immunosuppression will be made possible simply by the wider routine use of cyclophosphamide? If so, we believe that cyclophosphamide and azathioprine will have to be used by techniques of sequential rather than cotemporaneous administration. Since last summer, we have treated about 35 kidney recipients with this principle, which is an extremely simple one.- For the first three to eight post-operative weeks the triple drug treatment is given with cyclophosphamide, prednisone, and horse ALG. The doses of cyclophosphamide are kept at or above 1mg/kg/day. As soon as these doses cause significant drops in the white count, or arbitrarily at the end of 8 weeks, the cyclophosphamide is stopped and replaced with azathioprine for chronic maintenance therapy (Figure 10).



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It is too early to judge the efficacy of this most recently evolved therapeutic protocol but at the moment the results are encouraging. Only one patient has died. One additional kidney has been lost, but this was the consequence of a technical error. While we do not know if this therapeutic program is superior to the standard triple drug program used in the past, we are quite certain it is at least as good.

Summary

In one regimen or other, cyclophosphamide has been given to almost all renal homograft recipients treated in Denver in the last year, to a total of nearly 100. In addition, cyclophosphamide was substituted for azathioprine in 49 more recipients at varying times post-transplantation. The immunosuppressive potency and the safety of cyclophosphamide have been equivalent to that of azathioprine, indicating that there are now *two* first-line and apparently interchangeable agents for use in whole organ transplantation. The present practice is to use cyclophosphamide for all fresh cases for 3 weeks to 2 months and then to switch to azathioprine for chronic therapy. Since cyclophosphamide and azathioprine have different actions on their target cells, it is hoped that the transition from one to the other agent might promote a more effective kill of immunologically competent cells which are replicating in response to the antigenic stimulus. Whether or not this is true will have to be determined by further clinical observations and controlled laboratory experiments.

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