terval between administration of the drug and donor nephrectomy, hydration of the donor, the interval and severity of hypotension, the length of warm ischemia, and the preservation time.

In summary, pretreatment with MPSS provided protection against ischemic injury, improving the percentage of survival as well as renal function when given at the appropriate interval prior to ischemia (2 hr) and in the appropriate dose (30 mg/kg). The interval between administration and the onset of ischemia was especially critical.

H. Charles Miller
J. Wesley Alexander
Department of Surgery
University of Cincinnati
College of Medicine and
Shriners Burns Institute
Cincinnati, Ohio 45229

LITERATURE CITED

1. Ferguson, W. W.; Glenn, T. M.; Lefer, A. M. 1972. Amer. J. Physiol. *222:* 450.
2. Johnson, R. W. G.; Anderson, M.; Morley, A. R.; Taylor, R. M. R.; Swinney, J. 1972. Transplantation *13:* 174.
3. Lotke, P. A.; Schwartz, S. L. 1970. Surgery *67:* 488.
4. McCabe, R. E.; Fitzpatrick, H. F. 1972. J. Amer. Med. Assoc. *219:* 1056.
5. Miller, H. C.; Alexander, J. W. 1972. Transpl. Proc. *4:* 595.
6. Miller, H. C.; Alexander, J. W.; Nathan, P. 1972. Surgery *72:* 193.
7. Moberg, A. W.; Mozes, M. F.; Campos, R. A.; Santiago, E. A.; Najarian, J. S. 1972. Transpl. Proc. *4:* 200.
8. Pinkerton, J. A.; Walkup, R. H.; Hattori, H.; Killen, D. A. 1972. Amer. Surg. *38:* 333.
9. Starling, J. E.; Ferguson, W. W.; Rudolf, L. E.; Wangensteen, S. L. 1972. Surg. Forum *23:* 259.
10. Weissman, G. 1965. New Eng. J. Med. *273:* 1084.
11. Woods, J. E.; Brown, J. S.; Donovan, J. L.; Mathews, L. E. 1972. Amer. J. Surg. *123:* 289.

Received 5 January 1973.
Accepted 20 February 1973.

UNSUCCESSFUL ATTEMPTS TO CONTROL HYPERACUTE REJECTION OF HUMAN RENAL HOMOGRAFTS WITH $F(ab')_2$ AND CITRATE ORGAN PRETREATMENT[1]

The presence of preformed cytotoxic antidonor antibodies in the serum of potential allograft recipients leads to the rapid destruction of the graft by the now well known events of hyperacute rejection (*10, 21, 24–27*). Experimental work in the past several years at our center and in other laboratories has been oriented to the solution of this difficult immunological problem, not only in the presensitized homotransplantation model but also in strongly incompatible xenograft combinations. Antibody and complement depletion (*3–5, 7, 8, 13, 16, 19, 21, 23*), or treatment by the chelating agents, sodium citrate (*12, 14*) and ethylenediaminetetraacetate (EDTA) (*1*), has been shown to delay hyperacute kidney rejection in both experimental models, whereas anticoagulation with heparin (*15*) or cobra venom (*6*) has yielded equivocal results. Even the most effective of these therapeutic procedures only delayed the destruction of the graft.

More recently, encouraging results were obtained by several workers (*11, 20, 22*) with pretreatment of the organ with antidonor IgG fragments ($F(ab')_2$). It was suggested that $F(ab')_2$ fragments were protective by occupying the donor antigen receptor sites.

Unsuccessful attempts to control hyperacute rejection in one of our patients who had preformed circulating cytotoxic antibodies are reported here, using homografts pretreated with sodium citrate or digested IgG.

CASE REPORT

A 42-year-old multiparous female with chronic glomerulonephritis had been on chronic hemodialysis since 1969 and had received more than 80 blood transfusions. A bilateral nephrec-

[1] This work was supported by research grants from the Veterans Administration, by Grants RR-00051 and RR-00069 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health, and by Grants AI-10176-01, AI-AM-08898, AM-07772, and HE-09110 from the United States Public Health Service.

tomy and splenectomy were performed in June 1970 and in July she underwent a thymectomy. She received her first renal homograft in August 1970 from a sibling with a C match (one HL-A incompatibility). Although no cytotoxic antibodies had been detected prior to transplantation, the graft function deteriorated rapidly and the organ was removed 5 days after surgery. It showed histopathological evidence of hyperacute rejection.

A second transplant from a cadaveric donor was performed in January 1971. At this time, the crossmatch for detection of preformed anti-donor cytotoxic antibodies was weakly positive. The homograft was hyperacutely rejected. On several occasions thereafter, the patient's serum was tested against a panel of lymphocytes both in our and in Dr. Paul Terasaki's laboratory in Los Angeles and was found positive for preformed cytotoxic antibodies against 90% of the 94 panel members. She also possessed cytotoxic antibodies against the lymphocytes of her third, fourth, and fifth renal donors to be described below.

$F(ab')_2$ pretreatment. Recipient plasma was obtained by plasmaphoresis. A $F(ab')_2$ preparation of the immunoglobulins was made by the method of Nisonoff and Wissler (*18*), obtaining 50 ml with a $F(ab')_2$ concentration of 6.2 g/100 ml that had the protective effects shown in Table 1. A panel of lymphocytes was pretreated with recipient $F(ab')_2$, washed with Hanks' balanced solution, and then submitted to Terasaki's microcytotoxicity test (*17*), using unaltered recipient serum as the reagent. Nondiluted $F(ab')_2$ completely inhibited the cytotoxicity to all of the test lymphocytes (Table 1). Dilution of the $F(ab')_2$, however, decreased the inhibitory activity. The low temperature (4 C) did not affect the results.

TABLE 1[a]

Lymphocyte panel	HL-A profile	Cytotoxicity titer using unaltered recipient serum	Cytotoxicity titer after exposure of target cells to $F(ab')_2$ dilutions			
			1:1		1:10	1:100
			37 C	4 C		
1. B.W	1,2 12, 4A	1:128	0	0	1:16	1:64
2. Y.A	2,10 4B, 4C	1:64	0		1:4	1:64
3. B.B.	2,9 W10, 4C	1:32	0			
4. F.B.	2,5 4A, 4B	1:64	0			
5. J.A.	1 7,8	1:64	0			

[a] Recipient HL-A profile, 3,11 W15.

In November 1971 a kidney from a 15-year-old cadaver donor was pretreated with the $F(ab')_2$ fragments by perfusion for 2 hr at 7 C, pH 7.15 (corrected to 37 C), and 40 mm Hg systolic pressure. The perfusate consisted of 450 ml of deflocculated homologous plasma to which 2.6 g of recipient $F(ab')_2$ were added. Before starting the perfusion, the perfusate was tested for its ability to protect the cells of one of the panels of lymphocyte donors (Y.A., Table 1) against the cytotoxic action of unaltered recipient serum. After exposure to the perfusate for 45 min, the cells were destroyed at a cytotoxicity titer of 1:4, compared to the previous titer of 1:64. At the end of the perfusion, however, this titer was 1:16, indicating that the perfusate became less protective with time and that $F(ab')_2$ fragments had probably been partly absorbed by the kidney.

Following perfusion the homograft was transplanted into the right iliac fossa of the recipient. No biopsies were taken. After revascularization, the color of the graft was pale but the organ did not show gross evidence of hyperacute rejection. However, the kidney never produced urine, and a renal scan at 24 hr failed to show any radioisotope uptake. The kidney was removed on the 3rd postoperative day. Histopathological examination confirmed the diagnosis of hyperacute rejection, with complete cortical necrosis rimmed by polymorphonuclear leukocytes, thrombosis of many of the arteries, and fibrin plugs and platelet aggregates in glomerular capillaries. IgG and C3 were detected in the vascular endothelium by immunofluorescence.

Sodium citrate pretreatment. In February 1972 a kidney from a cadaver donor, 1½ years old, was pretreated with 15 g of sodium citrate which had been added to a perfusate consisting of deflocculated crossmatch-positive recipient plasma collected by plasmaphoresis and prepared by the Belzer technique (*2*). The organ was perfused for 2 hr at 7 C (perfusion unit, Waters Instrument Co., Inc., Rochester, Minnesota), being thus exposed to recipient cytotoxic antibodies, but under conditions of citration that prevented complement binding and completion of the immune reaction.

Under regional heparinization the kidney was then connected to the recipient's circulation through a peripheral arteriovenous fistula, which had been previously established for hemodialysis

treatment. A roller pump was used to return venous blood to the patient. Perfusion of the organ lasted 2½ hr and was discontinued because the patient became hypotensive. During perfusion the kidney was initially pink but became slightly bluish between 20 and 50 min when the color again became normal. The consistency of the organ felt normal throughout the perfusion. Transient hematuria occurred, and the kidney produced 105 ml of urine. Initially, blood flow was poor and vascular resistance was high, but these improved considerably after 35 min of perfusion.

Cytotoxic titers of the perfusate were studied before and after the addition of citrate to the recipient plasma and several times during graft perfusion. During the procedure the concentration of IgG in the perfusate decreased by one-half, but IgM and β_{1c} values remained stable, and the total complement level was unchanged. Biopsies of the kidney were taken at regular intervals for routine histopathological and immunofluorescence studies. The biopsy sites bled normally until the end of the perfusion.

Light microscopy showed a progressive increase of polymorphonuclear leukocytes, with a mean number of 9.8 polymorphs/tuft at 150 min in the glomeruli. Platelet aggregates, practically absent at 30 min, were occupying the capillary loops at the subsequent biopsies. However, no fibrin was detected. The tubules showed some damage with eosinophilic casts and flattening of the lining of proximal convoluted tubules near the end of the perfusion. Finally, occasional fibrin thrombi appeared at 90 min in the interlobular arteries and at 150 min in the arcuate arteries. In spite of this evidence of mild but progressive hyperacute rejection through the perfusion period, immunofluorescence failed to detect IgG or complement deposit in any of the specimens.

Seven weeks later a cadaver kidney from a donor, 4 years old, was studied in a similar fashion. Results were much the same except that the urinary output while the graft was under perfusion was 72 ml. The histopathological studies of the sequential biopsies were entirely comparable to the previous experiment. However, linear binding of IgG and C3 was observed mainly in the glomerular endothelium, indicating with an unusual precision the exact localization of the antigen-antibody reaction. In human cases of hyperacute rejection, this had never been so clearly delineated as in this homograft (G. Andres, personal communication).

The contralateral kidney of the second cadaver donor was submitted to the same citrate pretreatment except that perfusion was for 5 instead of 2 hr. The kidney was then transplanted to the right iliac fossa of the recipient who had been systemically heparinized with 1.25 mg/kg. After revascularization, the homograft was observed for 4 hr. After ½ hr, it turned slightly bluish, but within another 30 min it regained its normal pink color. No urine was ever produced. Twenty-four hours later an arteriogram failed to show any cortical blood flow and the graft was removed. The histopathological diagnosis was hyperacute rejection.

CONCLUSIONS

Both experiments were designed to expose the homograft to preformed antidonor recipient antibodies which were prevented from reacting normally, in one instance by digestion of the antibodies to the noncomplement-binding $F(ab')_2$ fragment and in the other by citrate inhibition of complement. These efforts to prevent hyperacute rejection may have had some transient effect, but since the organs were eventually hyperacutely rejected, no practical benefit was thereby achieved. The negative results with $F(ab')_2$ are similar to the recently published experience of Habal et al. (*9*) in the difficult pig-dog xenotransplantation model.

J. L. CORMAN
N. KASHIWAGI
K. A. PORTER
G. ANDRES
S. IWATSUKI
C. W. PUTNAM
M. POPOVTZER
I. PENN
T. E. STARZL
Departments of Surgery
University of Colorado School of Medicine
and
Veterans Administration Hospital
Denver, Colorado 80220
Department of Pathology
St. Mary's Hospital Medical School
London, England
Department of Pathology

State University of New York
Buffalo, New York 14214

LITERATURE CITED

1. Belitsky, P.; Popovtzer, M. M.; Corman, J.; Launois, B. A.; Porter, K. A. 1973. Transplantation *15:* 248.
2. Belzer, F. O.; Ashby, B. S.; Dunphy, J. E. 1967. Lancet *2:* 536.
3. Bier, M.; Beavers, C. D.; Merriman, W. G.; Merkel, F. K.; Eisman, B.; Starzl, T. E. 1970. Trans. Amer. Soc. Artif. Intern. Organs *16:* 325.
4. Clark, D. S.; Foker, J. E.; Pickering, R.; Good, R. A.; Varco, R. L. 1966. Surg. Forum *17:* 264.
5. Clark, D. S.; Gewurz, H. G.; Varco, R. L. 1965. Fed. Proc. *24:* 621.
6. Gewurz, H.; Clark, D. S.; Cooper, M. D.; Varco, R. L.; Good, R. A. 1967. Transplantation *5:* 1296.
7. Gewurz, H.; Clark, D. S.; Finstad, J.; Kelley, W. D.; Varco, R. L.; Good, R. A.; Gabrielson, A. G. 1966. Ann. N.Y. Acad. Sci. *129:* 673.
8. Giles, G. R.; Boehmig, H. J.; Lilly, J.; Amemiya, H.; Takagi, H.; Coburg, A. J.; Hathaway, W. E.; Wilson, C. B.; Dixon, F. J.; Starzl, T. E. 1970. Transpl. Proc. *2:* 522.
9. Habal, M. B.; Misra, M. K.; Busch, G. J.; Carpenter, C. B.; Birtch, A. G. 1972. Transplantation *14:* 796.
10. Kissmeyer-Nielsen, F.; Olsen, S.; Peterson, V. P.; Fjeldborg, O. 1966. Lancet *2:* 662.
11. Kobayashi, K.; Hricko, G.; Lukl, P.; Hunsicker, L.; Patel, R.; Reisner, G. S.; Birtch, A. G. 1971. Surg. Forum *22:* 246.
12. Kux, M.; Boehmig, H. J.; Amemiya, H.; Torisu, M.; Yokoyama, T.; Launois, B.; Popovtzer, M.; Wilson, C. B.; Dixon, F. J.; Starzl, T. E. 1971. Surgery *70:* 103.
13. Linn, B. S.; Jensen, J. A.; Portal, P.; Snyder, G. B. 1968. J. Surg. Res. *8:* 211.
14. Linn, B. S.; Jensen, J.; Pardo, V.; Levi, D. F.; Hudson, D. G. 1970. J. Amer. Med. Assoc. *212:* 864.
15. Macdonald, A.; Busch, G. J.; Alexander, J. L.; Pheteplace, E. A.; Menzoian, J.; Murray, J. E. 1970. Transplantation *9:* 1.
16. Merkel, F.; Bier, M.; Beavers, C. D.; Merriman, W. G.; Starzl, T. E. 1970. Surg. Forum *21:* 261.
17. Morris, P. J.; Williams, G. M.; Hume, D. M.; Mickey, M. R.; Terasaki, P. I. 1968. Transplantation *6:* 392.
18. Nisonoff, A.; Wissler, F. D. 1960. Arch. Biochem. *89:* 230.
19. Perper, R. J.; Najarian, J. S. 1966. Transplantation *4:* 377.
20. Shaipanich, T.; Vanwijck, R. R.; Kim, J. P.; Lukl, P.; Busch, G. J.; Wilson, R. E. 1971. Surgery *70:* 113.
21. Simpson, K.; Bunch, D. L.; Amemiya, H.; Boehmig, H. J.; Wilson, C. B.; Dixon, F. J.; Coburg, A. J.; Hathaway, W. E.; Giles, G. R.; Starzl, T. E. 1970. Surgery *68:* 77.
22. Smith, G. V.; Kenyon, A. J.; Lovett, E. J., III; Owens, G. 1971. J. Surg. Res. *11:* 57.
23. Snyder, G. B.; Ballesteros, E.; Zarco, R. M.; Linn, B. S. 1966. Surg. Forum *17:* 478.
24. Starzl, T. E.; Boehmig, H. J.; Amemiya, H.; Wilson, C. B.; Dixon, F. J.; Giles, G. R.; Simpson, K. M.; Halgrimson, C. G. 1970. New Eng. J. Med. *283:* 383.
25. Starzl, T. E.; Lerner, R. A.; Dixon, F. J.; Groth, C. G.; Brettschneider, L.; Terasaki, P. I. 1968. New Eng. J. Med. *278:* 642.
26. Terasaki, P. I.; Marchioro, T. L.; Starzl, T. E. 1965. p. 83. *In* J. J. van Rood and D. B. Amos (eds.). *Histocompatibility testing 1965.* National Academy of Sciences—National Research Council, Washington, D.C.
27. Williams, G. M.; Lee, H. M.; Weymouth, R. F.; Harlan, W. R., Jr.; Holden, K. R.; Stanley, C. M.; Millington, G. A.; Hume, D. M. 1967. Surgery *62:* 204.

Received 29 January 1973.
Accepted 13 April 1973.

PULSATILE FLOW AND VIABILITY OF ISOLATED PERFUSED KIDNEYS[1]

At present, the easiest and most commonly used criterion in kidney evaluation before clinical transplantation is its pulsatile flow, which, associated with pressure, pulsation, and other subjective criteria aids in determining kidney viability. However, some kidneys which showed an excellent pretransplant flow had severe renal insufficiency after transplantation (7). On the other hand, canine kidneys with poor flow during preservation have had excellent renal function following autotransplantation. It is our pur-

[1] Supported by Grant 5-PO2-AML-3083-04 from the National Institutes of Health.