

133
all pages 104-115

Sonderdruck

Transplantation von Organen und Geweben

*Internationales Symposium in Bad Homburg v. d. H.
vom 6.-8. Oktober 1966*

Herausgegeben von

Priv.-Doz. Dr. K. E. Seiffert

Oberarzt der Chirurgischen Universitätsklinik
Frankfurt a. M.

und

Prof. Dr. R. Geißendörfer

Direktor der Chirurgischen Universitätsklinik
Frankfurt a. M.

103 Abbildungen, 49 Tabellen

1967



Georg Thieme Verlag
Stuttgart

two strong precipitates in the betazone. And diffusing against serum of the same individual they yield only one precipitate. Fibrinogen is the difference between plasma and serum, thus the comparison of the two last immunoelectrophoreses demonstrates that the antileukocyte sera are strongly fibrinogen precipitating.

On this statement we inverted the immunological reasoning and started to look for an antileukocyte effect of an antifibrinogen serum. It was obtained by immunising rabbits tolerant to serum with plasma. It was not absorbed, thus a rather specific and pure antifibrinogen serum. We have applied it on the same leukocyte mixture aforementioned and observed a strong leukoagglutination. The very few cells non-agglutinated were mostly polymorphonuclear, that is to say, the antifibrinogen serum was principally antilymphocyte. Of course, we have checked that leukoagglutination is neither a general feature for a normal rabbit serum nor for an antiserum even a strong one.

In summary antileukocyte serum is antifibrinogen and antifibrinogen serum seems to be antileukocyte. We wish to provoke other remarks on the problem of the specificity of antileukocyte and antilymphocyte sera. Their beneficial action on graft tolerance may not be so simple as it was firstly hypothesised.

The Prospects of Successful Liver Transplantation in Man

With Special Emphasis on the Possible Use of Antilymphoid Globulin*

By Th. E. STARZL, Denver/Col., Th. L. MARCHIORO, Denver/Col., L. BRETTSCHEIDER, Denver/Col., Y. IWASAKI, Chiba (Japan)

That homotransplantation of the liver is a feasible procedure is already known. In the spring and summer of 1964, a large series of orthotopic hepatic transplantations were carried out in dogs, using homografts obtained from nonrelated mongrel donors. The only measure taken to prevent rejection was the administration of azathioprine, which is still the single best immunosuppressive agent known, but which is a drug that is markedly hepatotoxic in dogs. In spite of the latter handicap, survival was at least as consistent in these experiments (16) as can be obtained with comparable treatment after canine renal homotransplantation. Four of the dogs are still alive, a convincing demonstration, inasmuch as their own livers were totally excised at the time of the original operation. All 4 of these animals received azathioprine for only 4 months. Since its discontinuation, they have received no therapy whatsoever.

Important information has also accrued on the alternative method of auxiliary liver transplantation, an operation which does not involve recipient hepatectomy. The physiologic prerequisites for the successful use of this procedure have been defined as the result of studies in dogs which demonstrated a metabolic competition between the homograft and the autologous liver (9). The capacity for function which either liver possesses determines the extent to which the other organ is „starved“. Presumably, the more severe the damage to the host liver, the less hostile the environment would be for the homograft. The relative advantages which the coexistent organs enjoy can be manipulated by the method selected for revascularization of the homograft or by the performance or omission of host portacaval shunt (4, 9, 20).

* Aided by United States Public Health Service grants AM 06283, AM 06344, HE 07735, AM 07772, AI 04152, Fr 00051 and FR 00069

In spite of this progress, all efforts to apply hepatic homotransplantation for the treatment of human liver disease have failed. The reasons have been multiple. The technical procedures of either orthotopic or auxiliary transplantation were particularly difficult in patients with hepatic disease who had poor liver function, portal hypertension, and coagulation disorders. The problem of obtaining well preserved cadaveric organs was a serious one. Even after satisfactory operations, a high incidence of hypercoagulability was encountered which led to pulmonary embolization from 1 to 3 weeks later. Frequent gastrointestinal ulceration and hemorrhage were noted. All of these problems as well as others have been extensively discussed in recent reviews (17, 18) and will not be treated further here except to say that none seems to constitute an insurmountable obstacle.

Instead, the single most unsatisfactory element of clinical care has concerned the immunosuppressive regimen. The combination of azathioprine and high doses of prednisone, which is usually required to control rejection after homotransplantation of either renal or hepatic homografts obtained from nonrelated donors, has been poorly tolerated by liver recipients. All patients, who survived for more than a few days after both auxiliary or orthotopic procedures, developed infectious complications. Pneumonitis was almost invariable. There was difficulty with dose control of azathioprine, probably because this agent has an important hepatic pathway of metabolism. With the reduced liver function at the time of rejection, several recipients developed bone marrow depression including a patient whose survival of 34 days has been the longest yet attained (4). It has become increasingly clear that there will not be a reasonable hope of long-term success until means are found to reduce the need for such dangerous immunosuppression.

There are 2 possible ways in which this objective can be achieved. The first is to perfect methods of histocompatibility matching between recipients and prospective donors. One such antigen matching method, developed by Dr. PAUL TERASAKI of Los Angeles, has been tested clinically in cases of renal homotransplantation. By selecting nonrelated donors on the basis of the best possible antigen match, it was possible to appreciably increase survival (18). Nevertheless, the mortality of 37% in the first year as well as the morbidity in those who survived were unacceptable.

The alternative solution would be to develop better methods to prevent or mitigate rejection. The most promising new agents amongst the many recently evaluated have been antilymphoid serum or its derivatives. Although the potential immunosuppressive value of such substances has been known for a number of years, WOODRUFF (22) and WAKSMAN (21) were the first to suggest their use in transplantation. Since then, a number of valuable confirmatory reports have been published (2, 6—8, 11—13, 23, 24) dealing with skin graft test systems in inbred rodents. For the past year and a half, much of the effort in our laboratory has been devoted to the development and testing of various antilymphoid products for protection of whole organs in the outbred canine and human populations. Similar efforts have been made by ABAZA (1) and MITCHELL (10) and their associates.

Preparation of Antilymphoid Agents

Twelve horses were immunized subcutaneously with lymphoid tissue, obtained from dog lymphnodes or spleens in 10 cases, and from cadaveric human lymphnodes, thymuses or spleens in the other 2. Each horse received tissues from 10 to 40 donors. The

equine antibody response was monitored by the ability of the horse serum to agglutinate or to lyse dog or human white cells. There was considerable variability in the effect of the serum products upon the leukocytes of different tested members of the species which had been used for immunization, suggesting some individual specificity of the horse antibodies (15).

Although the details of immunization and of the subsequent absorption and purification of the horse plasma or serum have been fully described (5), a few comments are pertinent. First, the most important factor in obtaining a potent horse antibody response is the cell dose as shown in Figure 1. In this case, 0.18 to 1.4 billion subcutaneous human lymphocytes were given for several months. The leukoagglutinin titer rose to 1:32. Subsequently, when 10 to 50 times more splenic lymphocytes were administered per injection, the titer quickly rose to 1:16,000. When large doses were used from the beginning, high titers could be reached in 20 to 75 days.

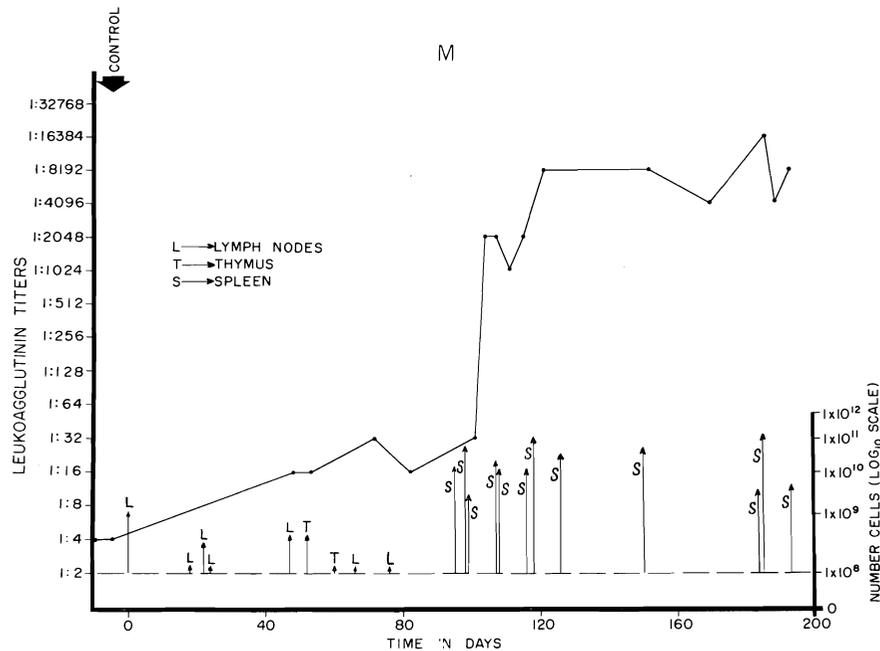


Fig. 1 Effect of immunizing dose upon the leukoagglutinin titer of a horse inoculated with cadaveric human lymphoid tissue. Note that the rise in titer was very modest during the first 3 months, during which time small doses of cells were used. When the quantity of antigen was increased by the use of spleen cells, abrupt increases in titer were observed within a few days. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 1)

The plasma prepared from the horse blood was toxic when given to dogs intraperitoneally, causing the death of 11 of 36 animals within 15 days. Acute anemia was invariable, which was apparently due at least in part to hemagglutinins which had developed against canine red cells. Subsequently, it was found that both the anemia and the mortality could be eliminated by absorbing the dog plasma (or serum) with dog red cell pack until all hemagglutinins were removed, and by further absorption with pooled dog plasma (5). Neither procedure affected the leukoagglutinin titer.

A number of studies were performed to identify the anti-leukocyte antibody employing chromatography with the DEAE cellulose column, electrophoresis and immunoelectrophoresis (5). An analysis of horse serum containing strong antihuman activity is shown in Figure 2. The leukoagglutinating antibody was located in the gamma G and T-equine globulin fractions probably with an additional small component in the beta globulin. It is probable that chromatographic separation will be a useful commercial method to separate such globulin for therapeutic use. With a pH of 6, and a phosphate buffer of 0.05, 60% of the antibody activity can be retained.

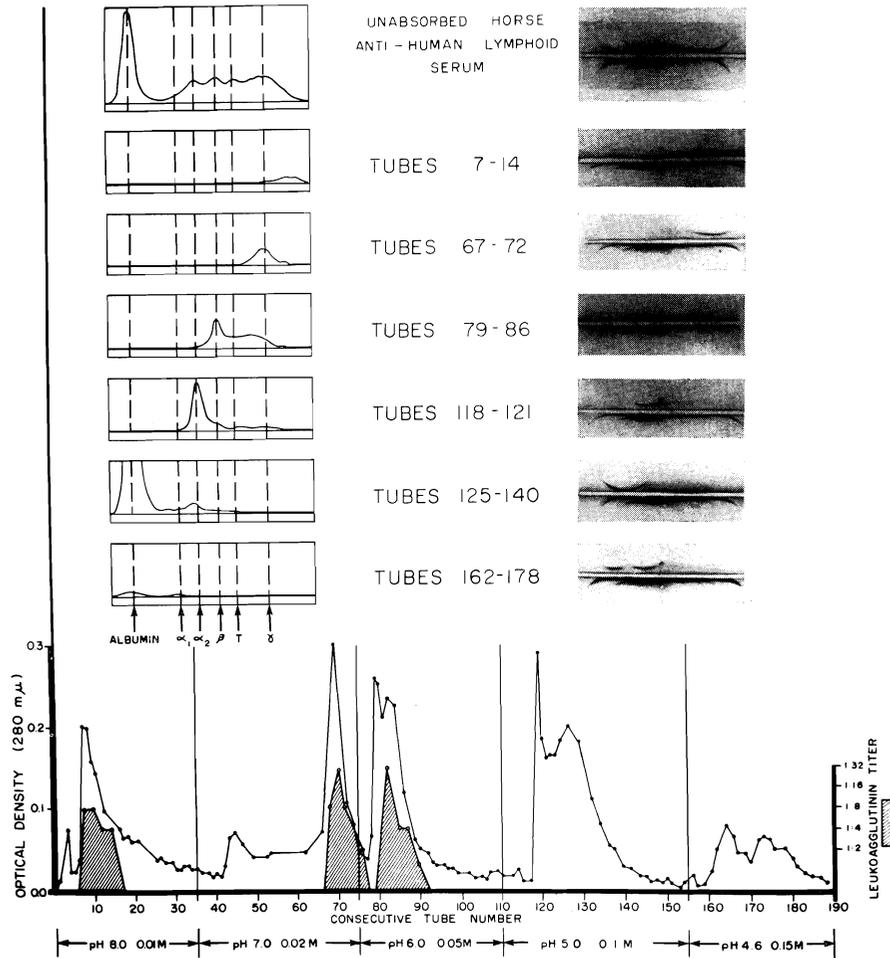


Fig. 2 Studies of the leukoagglutinin-containing fractions in antihuman lymphoid serum employing column chromatography, electrophoresis, and immunoelectrophoresis. The various eluates from the DEAE cellulose column were analyzed spectrophotometrically for protein content (expressed as optical density), and the presence or absence of leukoagglutinins determined for each collection tube. The electrophoresis and immunoelectrophoresis permitted relatively complete classification of the active immunoglobulins. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 1)

However, partly for practical purposes of bulk preparation, the alternative technic of double or quadruple ammonium sulphate precipitation was used to extract raw globulin for testing. The quality of the product obtained by this method can be seen in Figure 3.

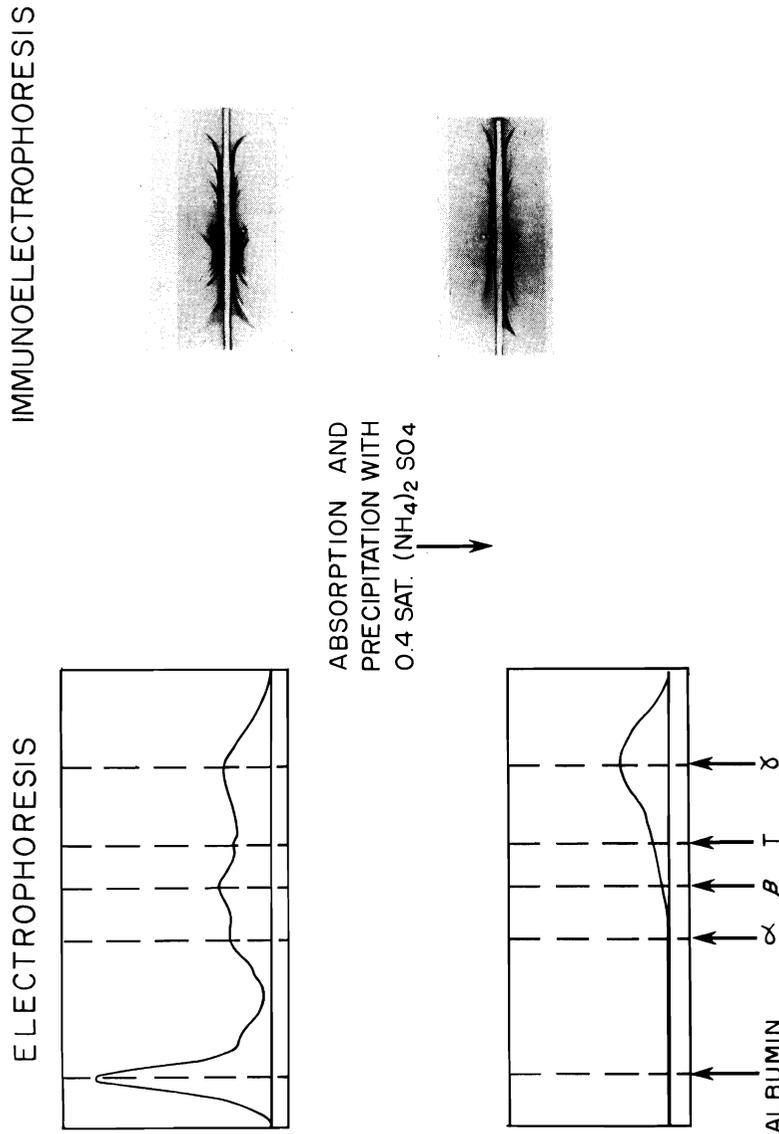


Fig. 3 Electrophoresis and immunoelectrophoresis of absorbed antihumanlymphoid serum and the protein obtained from it by 2 precipitations with 0.4 saturated ammonium sulphate, 2 dialyses and lyophilization. The final product, which was used clinically, consists almost entirely of gamma G Globulin. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 1)

Unabsorbed immune plasma or serum, or globulin removed after absorption of this serum all produced lymphopenia in dogs when injected intraperitoneally, intravenously or subcutaneously (Fig. 4), compared to an absence of this effect when normal horse serum was employed. There is little reason, however, to infer that the responsible equine antibodies have an immunologically specific action against these target cells.

If the antilymphoid serum was absorbed repeatedly with canine kidney or liver cell pack, most of the anti-leukocyte titer was removed (5). LEVEY and MEDAWAR (8) have shown that an effective antilymphoid serum can be produced by immunizing the heterologous serum donor with epithelial or L-cells. These findings suggest, first, that the lymphoid system may be a particularly sensitive target to these agents and, secondly, that the hazard of injury to the kidney or other organs is a real possibility. The demonstration of electron dense bodies in the glomeruli of a number of dogs treated with „antilymphoid“ products (5) suggests that a more proper term might be „antidog“ or „antihuman“.

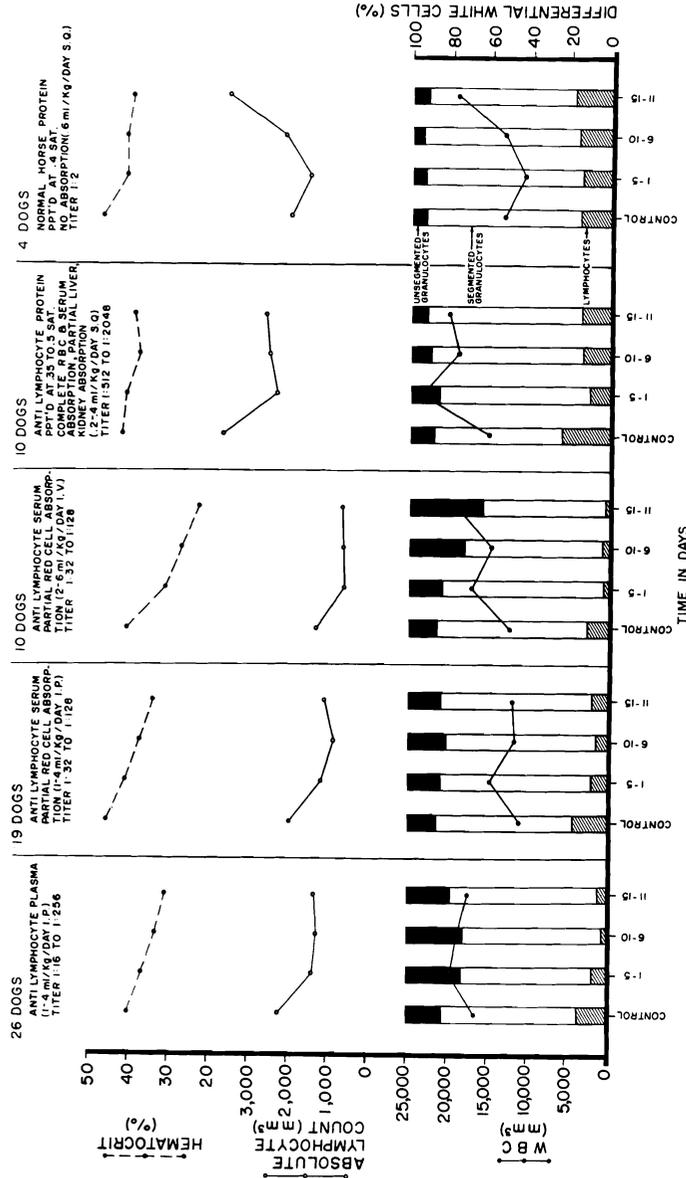


Fig. 4 The effect of horse plasma or serum and crude horse globulin upon the hematocrit, lymphocyte count, total white count, and white count differential during 15 days of daily administration. In all but the control experiments on the right, the agents were prepared from horses immunized against dog lymphoid tissue. Note that acute anemia was largely prevented only when complete absorption had been carried out with canine red cell pack. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 1)

In spite of this danger, antilymphoid globulin has been well tolerated in animals. Even in nontransplanted dogs which were shown to have renal lesions, there was no change in kidney function. The reaction of the treated animals was different from that of dogs given serum from normal horses (Fig. 5) in that the canine precipitin response to horse protein was greatly attenuated. Apparently there is some self-antidotal quality of the antilymphoid products as suggested by earlier workers (3, 8) which reduces its hazard.

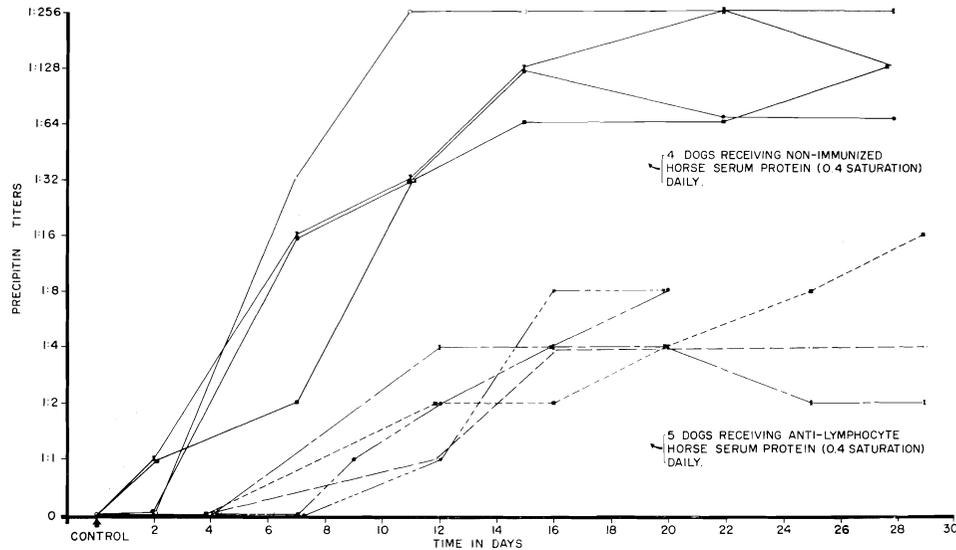


Fig. 5 The response of canine precipitin titers to horse protein during daily injection of globulin prepared from the serum of non-immunized and immunized horses. Note the striking difference in the 2 groups of dogs. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 1)

Canine Homotransplantation

Eighteen dogs have received orthotopic liver transplantation while under treatment with either intraperitoneal antilymphoid serum (9 animals) or subcutaneous globulin (9 animals). No other therapy was used (19).

Of the 9 animals in the first group, 5, 4, 3, and 2 lived for more than 15, 20, 30, and 50 days respectively. With limitation of survival credit to 70 days for any dog, the mean postoperative survival was 26.8 ± 26 (SD) days compared to 6.8 ± 3 (SD) days for 32 untreated controls ($p < 0.05$). Two of these animals are still alive after 5 and 6 months. One had therapy stopped 3 weeks after transplantation (Fig. 6) and the other was treated only prior to operation (Fig. 7). The success or failure of an experiment was not correlated with the presence or absence of lymphopenia.

Also limiting individual survival credit to 70 days, the results with subcutaneous globulin therapy were similar (19), with a mean survival of 36 ± 30 (SD) days. Of these 9 dogs, 5, 5, 4, and 4 lived for more than 15, 20, 30, and 50 days. The prolongation of life was significant ($p < 0.01$). Maximum survival in a dog still living is 4 months.

A definite protective effect upon renal homografts was also demonstrated in a much larger series of renal homotransplantations (19), but not to the same degree as in the livers. Not only was the mean survival lower, but the degree of histologic damage was more severe and acute even long after operation. These findings suggested, as did an earlier study on azathioprine (16), that the liver may actually be a more favorable organ than the kidney in term of the inherent ease with which it can be shielded from immunologic repudiation. The experiments with kidneys (19) also clearly showed that treatment for some days before as well as after operation was distinctly superior to that started on the day of transplantation.

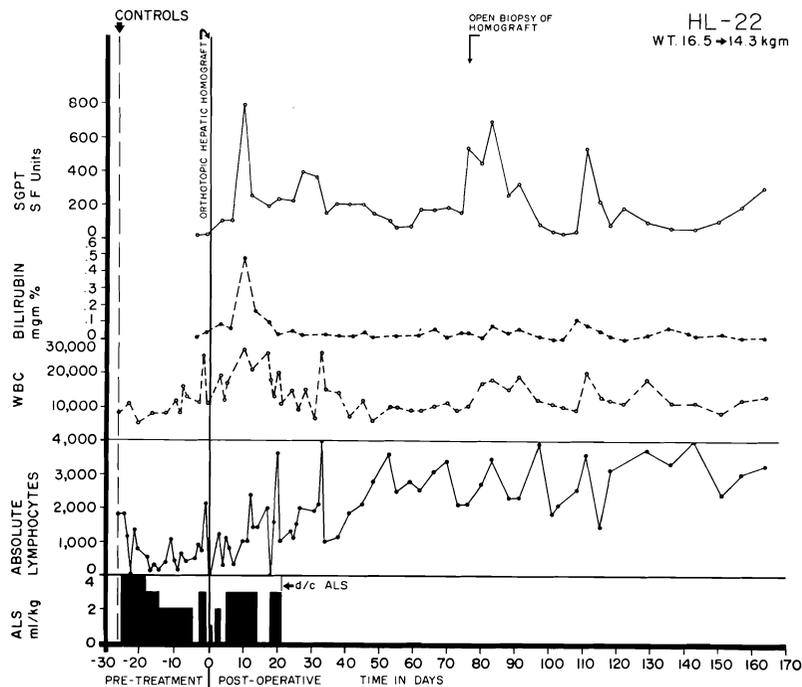


Fig. 6 A chronically surviving dog which was treated before and for 20 days after orthotopic liver transplantation with intraperitoneal antilymphoid serum (ALS). Note the pronounced lymphocytosis late in the postoperative period. The animal is in excellent health after almost 6 months. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 38)

Clinical Use of Antilymphoid Globulin

The foregoing canine studies provided firm guidelines for clinical application. First, although the immunosuppressive action of antilymphoid globulin was unequivocal, its effect in individual experiments was unpredictable and somewhat weaker than that known to be attainable with azathioprine. Secondly, the genuine threat of serum sickness might limit its use to relatively short periods. Consequently, the globulin was added as an adjuvant to the basic regimen of azathioprine and prednisone, after first obtaining evidence that it would not cancel the efficacy of the standard drugs.

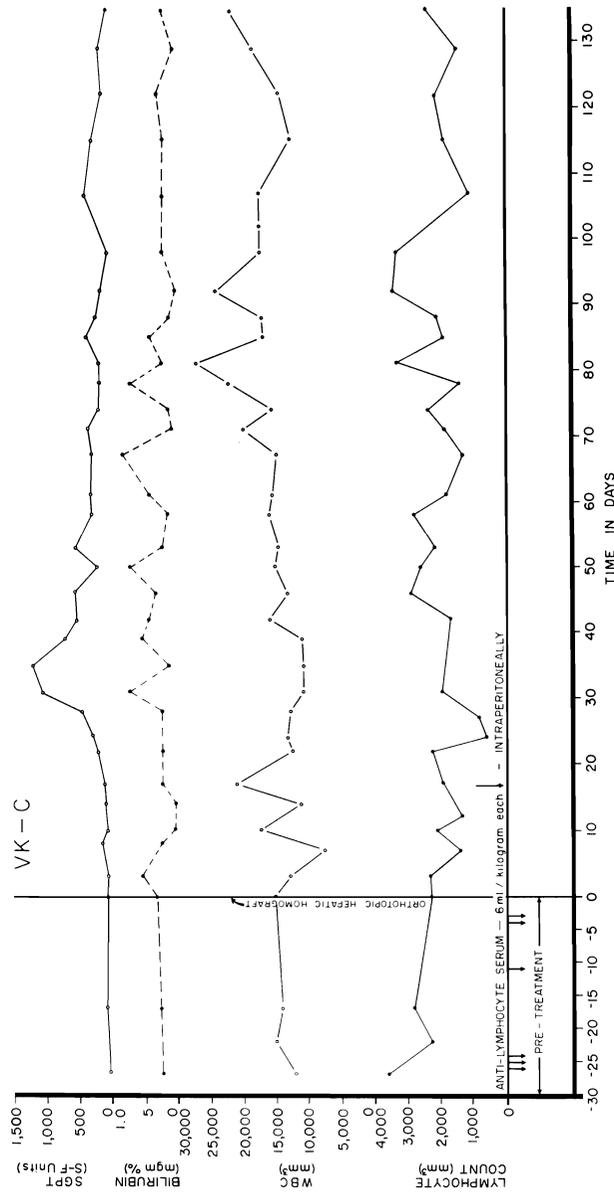


Fig. 7 A dog which received an orthotopic liver homograft after 6 intraperitoneal injections of antilymphoid serum (ALS). No postoperative therapy was ever given. Note that the lymphocyte count was little changed. The dog is still alive. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 38)

A detailed analysis of these results has been presented elsewhere (19), but some summary comments follow. There were 8 newly transplanted patients who received intramuscular antilymphoid globulin beginning from 5 to 35 days before renal homotransplantation and continuing thereafter as shown in Figure 8. All 8 patients are well after 9½ to 14 weeks. Their average daily doses of azathioprine and prednisone as well as their BUN's and creatinine clearances for the first 63 postoperative days are summarized in Figure 9 and compared to the data of 3 previous series of comparable patients. The patients in the antilymphoid globulin series required considerably less than half the

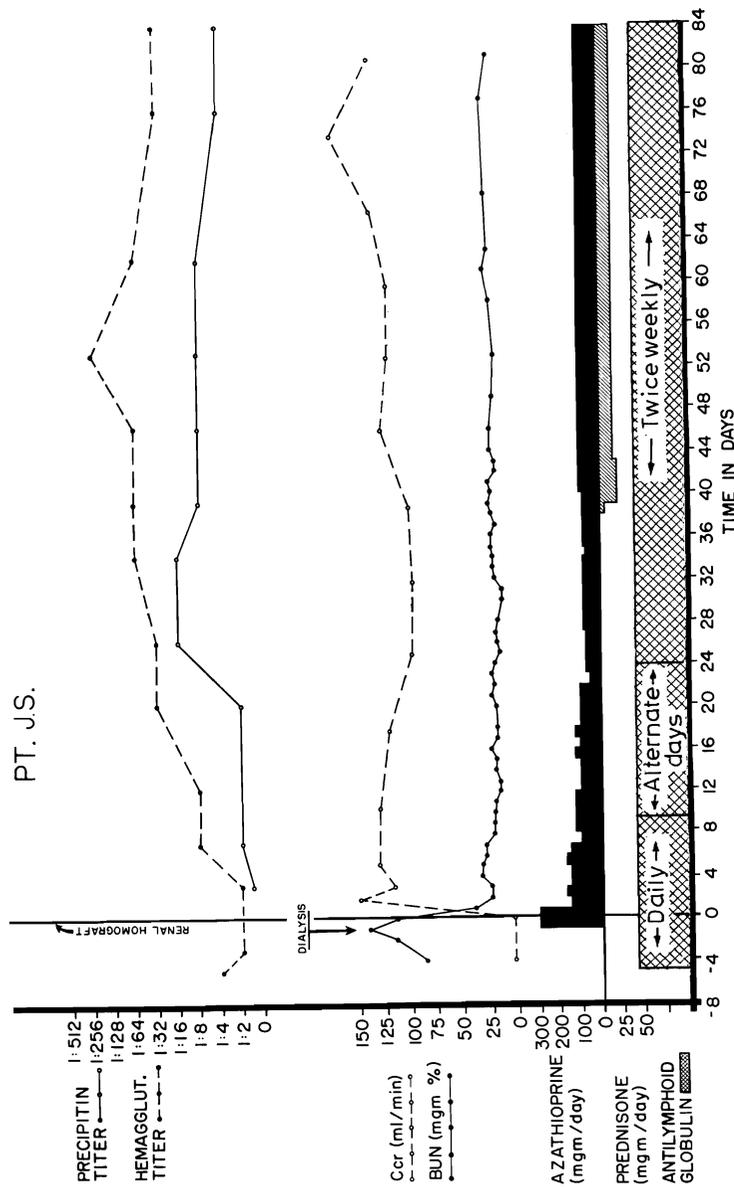


Fig. 8 Course of a patient treated before and after renal homotransplantation with antilymphoid globulin. No rejection occurred. Note the rises in precipitin and hemagglutinin titers, findings which prompted institution of prednisone therapy. These titers subsequently fell. This patient had a good antigen match with his sibling donor. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 38)

steroid therapy than any of the 3 preceding groups. They were also given smaller quantities of azathioprine. This relaxation of standard immunosuppressive therapy was not paid for with a loss of renal function. The pooled BUN's and creatinine clearances in the first 3 series were not significantly different from those in the test group.

Three other patients with progressively failing late renal homografts were placed on antilymphoid globulin therapy, after which prednisone dosage was rapidly reduced to $\frac{1}{2}$ to $\frac{1}{5}$ of the original level. Two of these 3 patients had stabilization or improvement of renal function in the ensuing 10 and 13 weeks. The third patient, whose BUN and

creatinine clearance were 115 and 5 ml/minute respectively had a slight further deterioration. She died rather suddenly 13 weeks later of a cardiac arrest which followed a bout of acute pulmonary edema.

Serious systemic toxicity has not yet been observed, although moderate fever has been common. Hemagglutinin titers against sheep red cells and precipitin titers rose slightly in most patients but with a later tendency to decline (Fig. 8).

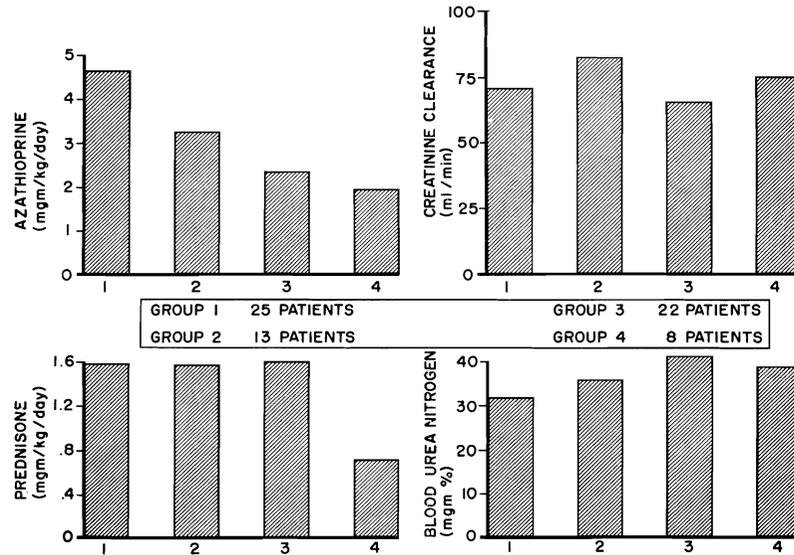


Fig. 9 Variations in immunosuppression and renal function during the first 63 postoperative days in 4 successive groups of patients who received kidneys from blood relatives. Since the blood urea nitrogen and creatinine clearance were not determined each day, these were compiled on a weekly basis. Those in Series 4 received adjuvant therapy with antilymphoid globulin. Note the drastic reduction in average prednisone dose which was achieved in these patients without significant loss of renal function. The progressive diminution of azathioprine dosage in the succeeding series is evident. (By permission of *Surgery, Gynecology & Obstetrics*, 124 [1967], 38)

Discussion

Early experience with antilymphoid globulin has awakened hopes that it can be used in a safer and more effective program of immunosuppression, which could be applied to the problem of homotransplantation of the liver. Tested as the sole therapy in canine hepatic homotransplantation, it allows a definite prolongation of life. In a clinical trial with renal homotransplantation, it has seemed to permit effective therapy with smaller doses of prednisone and azathioprine than had been previously possible.

It has been pointed out to us by Dr. K. A. PORTER of London, who examined all the tissues from our studies, that this hope may be fleeting in view of the report by PARO NETTO and POPPER (14). These authors showed a synergistic effect of horse serum upon the minor hepatotoxicity produced with the appropriate dose of halogenated hydrocarbons. If azathioprine damages the human liver, a question which has not yet been decisively resolved, the same risk could be expected in man.

In any case, it is important to stress the experimental nature of antilymphoid globulin therapy in view of the unknown risk from serum sickness. The magnitude of this hazard and the related question of nephrotoxicity can be completely assessed only with further observation and by study of renal homograft biopsies in those patients now under treatment. Wider clinical trial is not recommended until this information has been obtained.

References

- 1 Abaza, H., B. Nolan, J. Watt, M.F.A. Woodruff: The effect of antilymphocytic serum on the survival of renal homografts in dogs. *Transplantation* 4 (1966), 618
- 2 Gray, J. G., A. P. Monaco, P. S. Russell: Heterologous mouse anti-lymphocyte serum to prolong skin homografts. *Surg. Forum* 15 (1964), 142
- 3 Gray, J. G., A. P. Monaco, M. L. Wood, P. S. Russell: Studies on heterologous anti-lymphocyte serum in mice. I. In vitro and in vivo properties. *J. Immunol.* 96 (1966), 217
- 4 Halgrimson, C. G., T. L. Marchioro, T. D. Faris, K. A. Porter, G. N. Peters, T. E. Starzl: Clinical and experimental auxiliary hepatic homotransplantation with emphasis on host portacaval shunt. *AMA Arch. Surg.* 93 (1966), 107
- 5 Iwasaki, Y., K. A. Porter, J. Amend, T. L. Marchioro, V. Zühlke, T. E. Starzl: The preparation and testing of horse antidog and antihuman antilymphoid plasma or serum and its protein fractions. *Surg., Gynec. & Obst.* 124 (1967), 1
- 6 Jeejeebhoy, H. F.: Immunological studies on the rat thymectomized in adult life. *Immunology* 9 (1965), 417
- 7 Levey, R. H., P. B. Medawar: Some experiments on the action of anti-lymphoid antisera. *Ann. N. Y. Acad. Sci.* 129 (1966), 164
- 8 Levey, R. H., P. B. Medawar: Nature and mode of action of antilymphocyte antiserum. *Proc. National Acad. Sci.* (1966), 1130
- 9 Marchioro, T. L., K. A. Porter, T. C. Dickinson, T. D. Faris, T. E. Starzl: Physiologic requirements for auxiliary liver homotransplantation. *Surg., Gynec. & Obst.* 121 (1965), 17
- 10 Mitchell, R. M., A. G. R. Sheil, S. F. Slafsky, J. E. Murray: The effect of heterologous immune serum on canine renal homografts. *Transplantation* 4 (1966), 323
- 11 Monaco, A. P., M. L. Wood, P. S. Russell: Effect of adult thymectomy on the recovery from immunologic depression induced by heterologous antilymphocyte serum. *Science* 149 (1965), 423
- 12 Monaco, A. P., M. L. Wood, J. G. Gray, P. S. Russell: Studies on heterologous antilymphocyte serum in mice. II. Effect on the immune response. *J. Immunol.* 96 (1966), 229
- 13 Nagaya, H., H. O. Sieker: Allograft survival: Effect of antiserum to thymus glands and lymphocytes. *Science* 150 (1965), 1181
- 14 Paronetto, F., H. Popper: Aggravation of hepatic lesions in mice by in vivo localization of immune complexes (Auer hepatitis). *Am. J. Path.* 47 (1965), 519
- 15 Putnam, C. W., N. Kashiwagi, Y. Iwasaki, P. I. Terasaki, T. L. Marchioro, T. E. Starzl: The demonstration of interspecies reactivity and intraspecies "specificity" of antilymphoid globulin. *Surgery*, in press
- 16 Starzl, T. E., T. L. Marchioro, K. A. Porter, P. D. Taylor, T. D. Faris, T. J. Herrmann, C. J. Hlad, W. R. Waddell: Factors determining short- and long-term survival after orthotopic liver homotransplantation in the dog. *Surgery* 58 (1965), 131
- 17 Starzl, T. E., T. L. Marchioro, K. A. Porter: Progress in homotransplantation of the liver (Chapter). In *Advances in Surgery* (Ed. Claude Welch). Year Book Medical Publishers, Inc., Chicago, Illinois, 1966, pp. 295-370
- 18 Starzl, T. E., T. L. Marchioro, T. D. Faris, R. J. McCardle, Y. Iwasaki: Avenues of future research in homotransplantation of the liver: With particular reference to hepatic supportive procedures, antilymphocyte serum and tissue typing. *Am. J. Surg.* 112 (1966), 391
- 19 Starzl, T. E., T. L. Marchioro, K. A. Porter, Y. Iwasaki, G. J. Cerilli: The use of heterologous antilymphoid agents in canine renal and liver homotransplantation, and in human renal homotransplantation. *Surg., Gynec. & Obst.* 124 (1967), 381
- 20 Tretbar, L. L., E. G. Beven, R. E. Herrmann: Homotransplantation of an auxiliary dog liver into the pelvis-Effect of portacaval shunt in the prevention of liver atrophy. *Surg. Forum* 16 (1965), 219
- 21 Waksman, B. H., S. Arbouys, B. G. Arnason: The use of specific "lymphocyte antisera" to inhibit hypersensitive reactions of the delayed type. *J. Exp. Med.* 114 (1961), 997
- 22 Woodruff, M. F. A.: *The Transplantation of Tissues and Organs*. Charles C. Thomas, Springfield, Ill., 1960
- 23 Woodruff, M. F. A., N. F. Anderson: The effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocyte serum on the survival of skin homografts in rats. *Nature (Lond.)*, 200 (1963), 702
- 24 Woodruff, M. F. A., N. F. Anderson: The effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocyte serum on the survival of skin homografts in rats. *Ann. N. Y. Acad. Sci.* 120 (1964), 119