convolvulus and *P. pennsylvanicum* each developed two precipitate bands. The two precipitate bands which all three species form are identical. Extracts of all varieties of *F. esculentum* caused no precipitates to form in gel-diffusion plates. A similar reaction has been observed when these extracts are tested against serums of certain patients with multiple myeloma.

Because the *R. crispus* extract frequently develops three distinct precipitate bands, we used it in gel diffusion experiments with one donor serum and five different animal serums (Fig. 2). The donor serum used in this experiment developed only one precipitate band. However, in a series of 50 donor serums tested against a similar *R. crispus* extract, 19 developed two precipitate bands. All of the different animal serums developed two precipitate bands except turkey serum, which developed three bands.

In human and animal serums tested by gel diffusion, the first precipitate band develops near the serum well, and it is present for all serums tested. The second and third bands (Figs. 1 and 2) may or may not develop.

For comparative studies it is desirable to test the different serums in groups (Fig. 2). However, this posed a problem since some serums deteriorated before all for the group could be collected. The customary addition of Merthiolate and refrigeration at 7°C satisfactorily preserved the serums. We tried to preserve serum by lyophilization; however, no precipitate bands developed when lyophilized serum was tested. This is interesting since lyophilization is the usually accepted procedure of preserving serum antigens and antibodies in the dried state. This finding indicates that lyophilization has made the active serum component inactive against these plant lectins.

Extracts of species of the same genus may differ widely in their strength and specificity reactions (*1–6, 8, 9*). That *F. esculentum* developed no precipitate bands is of interest since this species (*5*) does not agglutinate human red cells when tested for hemagglutinins. *Rumex crispus* gives a weak reaction (*8*).

There is evidence that the agglutinating behavior of lectins extends to specific precipitating power for related antibody-like substances in body fluids (*6*). The *R. crispus* precipitating lectin agglutinates A, B, and O red cells (titer not determined), but we have observed no relation between blood groups and the precipitate bands developed in gel-diffusion tests. However, almost all human red cells contain H substance, which is also present in body fluids. Some blood group substances, identical or similar, are present in animal red cells and body fluids. We suggest that these substances may cause some of the precipitate bands.

Rumex crispus extract fixes complement in 1:40 dilution. However, the absence of complement in serums inactivated by heat (56°C for 30 minutes) had no influence upon the development of the precipitate bands when they were tested with *R. crispus* extract. Heating the extract for 40 minutes in a boiling water bath does not appreciably destroy the active substance.

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Rejection of Renal Allografts: Specific Immunologic Suppression

Abstract. *Kidneys were transplanted across a major genetic barrier (Ag-B locus), from Lewis×BN F_1 hybrid rats into bilaterally nephrectomized Lewis rats. Survival of grafts is prolonged (indefinite?) in rats treated with a combination of (i) intravenous injection of donor spleen cells 1 day before the graft, and (ii) passive immunization with antiserum prepared in rats of the recipient strain against donor spleen and lymph-node cells. The recipient's immune response to other antigens is not impaired.*

Delayed-type hypersensitivity, the primary if not exclusive mechanism of allograft rejection (*1*), was suppressed in rats by treatment with both antigen and antibody before sensitization (*2*). We now report that antigen and antibody used in combination suppress specifically and almost completely the rejection of renal allografts in the rat.

Transplantation was performed between two isohistogenic strains, Lewis and BN, and the Lewis×BN F_1 hybrid (*3*), which are histoincompatible at the major Ag-B locus (*4*). The left kidney from a male Lewis×BN F_1 donor was transplanted into the abdomen of a male Lewis recipient by a microvascular surgical technique (*5*); the recipient's own kidneys were then removed immediately.

The antigen injected into each recipient before the transplantation was 10^8 viable donor spleen cells obtained by gentle dispersion of splenic fragments in a loose-fitting, hand-operated, glass, tissue-grinding vessel, and three washings in saline. Viability of the cells was 90 percent on the basis of ability to exclude trypan blue dye.

Lewis antiserum to BN, used to immunize passively graft recipients, was prepared by injection of Lewis rats with BN spleen and lymph-node cells; each rat received a total of 10^8 cells, suspended in 0.85 ml of 0.9 percent NaCl and 0.15 ml of *Bordetella pertussis* vaccine as an adjuvant, with injections in the pads of all four feet. Booster injections without adjuvant followed after 5 weeks. Serial weekly bleeding started with the 6th week when the hemagglutinin titer was at least 1 : 2048 (*6*); multiple pools of antiserums were collected.

Function of the grafted kidneys was evaluated primarily by measurement of the concentration of urea nitrogen in the blood (BUN); normal concentration in the rat is less than 20 mg/100 ml. It was measured daily during the 1st week after the transplantation, on alternate days during the 2nd week, and weekly thereafter. The concentration rose to 50 to 100 mg/100

ml on the 1st day after the transplantation; usually it was lower than 35 mg/100 ml by the 4th day and remained low until the onset of rejection.

Fourteen recipients of transplants were given no treatment. The mean onset of rejection, based on an abrupt rise of BUN, was 6.1 ± 0.2 days after the transplantation; the mean survival was 17.3 ± 2.7 days. Nine rats showed progressive increase in BUN until the time of death 8 to 17 days after the graft; in five the concentration fell somewhat between the 10th and 15th days but remained abnormally high until the time of death 18 to 45 days after the graft (Table 1).

In a group of ten recipients treated with antigen and antiserum, survival ranged from 80 to more than 230 days, with no deaths due to rejection; they received both antigen (10^8 donor spleen cells) intravenously 18 to 24 hours before the transplantation and 1 ml of antiserum intravenously (Lewis antiserum to BN, with a hemagglutinin titer of 1 : 2048) 2 hours before and 1 hour after the transplantation. Intravenous injections of antiserum were continued on alternate days between 6 and 62 days (Table 1) without observed untoward effects. Seven of the ten animals had minor elevations of BUN during the first 15 days after the transplantation; the remaining three showed an early transient rise in BUN to 100 to 140 mg/100 ml by the 5th day after the graft, yet recession was such that since the 15th day the mean BUN in treated rats has been 30 mg/100 ml or less. Rats receiving antiserum for only 6 days have fared as well as those injected as long as 62 days. Of the ten treated rats, five remain alive 164 to 231 days after the graft; the other five died between 80 and 177 days, during an epidemic of pneumonia in our rat colony, without functional or microscopic evidence of rejection.

This observed suppression of allograft rejection was immunologically specific. Treated rats developed the usual delayed-type hypersensitivity response to *B. pertussis* vaccine or to rabbit γ-globulin (*7*), and a normal hemagglutinin response to injected sheep erythrocytes.

Suppression of rejection was not as pronounced in two smaller groups of rats that received either antigen or antiserum alone. Four received antiserum alone; the mean onset of rejection was delayed to 9.0 ± 0.7 days after the graft, and BUN did not exceed 150 mg/100 ml during the initial rejection episode. In two of the four the concentration returned to less than 35 mg/100 ml by the 60th day and remained there; they remain alive after 195 and 210 days. The other two had persistently elevated concentrations after the 10th day and died of rejection 120 and 132 days after the graft.

Seven rats received antigen alone. The mean onset of rejection was 6.6 ± 0.6 days after the transplantation, BUN reaching a peak of 150 to 200 mg/100 ml by the 30th day; five have died of rejection after 20, 49, 53, 75, and 83 days; two remain alive after 126 and 141 days, with BUN's of 187 and 94 mg/100 ml, respectively.

Although either antigen or antiserum alone has prolonged the survival of grafts and enhanced the growth of tumors (*8*), the effect of combined treatment is greater than that of either alone (*9*). Our treatment of the recipient with both resulted in maximum suppression that was specific and apparently nearly complete.

Obviously, passively administered antiserum must not cause significant injury to the graft. Although isoantibodies are cytotoxic for some tumor cells and dissociated normal cells (*10*), harmful effects on tissue or organ grafts have not been consistently observed (*11*). Passive immunization, with isoantiserums prepared against kidney, reportedly causes mild transient renal injury (*12*), but in our study antiserum prepared against lymphoid tissue caused

Table 1. Concentrations of blood urea nitrogen (mg/100 ml) and survival after renal transplantation.

Days after transplantation											Survival (days)
5	10	15	20	30	60	90	120	150	180		
After no treatment											
20	165	45	100	75							45
90	210	80	90	120							31
40	65	50	105								22
60	330	75									21
45	145	100									18
75	115	185									17
30	230	270									15
40	280										14
85	235										12
15	150										11
85											10
110											9
50											9
190											8
Means ± S.E.											
67 ± 12	192 ± 25	115 ± 31	98 ± 4								17.2 ± 2.1
After treatment with antigen and antiserum											
25	20	25	20	35	20*	20	10	10	15	20	231
140*	55	25	30	25	20	30	20	15	30	15	212
35	40	30	20	35	30*	20	20	25	20	15	210
30	60	20	40	35	20*	20	20	25	20		209
60	45	30	30	35	30*	15	20	20			177†
35*	25	30	30	15	20	25	20	15			164
100	40	30	25	25	25*	20	10				161†
100	50	35	35	25*	15	20					103†
25*	25	30	30	20	20						84†
80	25	25	35	20*	25						80†
Means ± S.E.											
53 ± 13	38 ± 4	28 ± 1	30 ± 2	27 ± 2	22 ± 2	21 ± 2	17 ± 2	18 ± 2	21 ± 3	17 ± 2	

* Last day on which antiserum was administered. † Death.

no apparent injury to the transplanted kidneys.

Perhaps in our study the failure of passive immunization alone, to prevent completely rejection of the graft, may be due to the absence of antibody directed against strain-specific renal antigens. It has been shown that maximum suppression of rejection of tumor allografts requires antibody against all antigens present in the donor and absent from the host (*13*).

Antibodies against donor antigens were prepared in animals syngeneic to the recipients. Therefore, in this model system, the antiserum used for passive immunization is thought to contain antibodies against all histocompatibility antigens present in the donor and absent from the recipient. In genetically diverse populations difficulty is apparent in obtaining a single antiserum reacting specifically with all allograft histocompatibility antigens that the recipient lacks. Use of antiserum from multiple donors may provide a sufficiently wide spectrum of antibody activity. Alternatively it may be possible to immunize the recipient with donor antigen so that antibody is produced but delayed hypersensitivity does not develop; in this way the recipient could acquire antibody capable of reacting against all graft antigens.

The broadly nonspecific immunosuppressive agents in current clinical use depress the host's ability to react to all antigens, and impair his defenses against ubiquitous pathogens; consequently infection, not rejection, is the most frequent cause of death among recipients of transplants. The treatment with antigen and antiserum combined suggests a means for specific suppression of graft rejection after transplantation of organs.

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Hypersensitivity: Specific Immunologic Suppression of the Delayed Type

Abstract. *Delayed-type cutaneous hypersensitivity to sheep erythrocytes was induced in rats by intradermal injection of the antigen mixed with Freund's adjuvant; hypersensitivity was sustained by weekly injections. Either passive immunization with rat antiserum to sheep erythrocytes or intravenous injection of sheep erythrocytes partially suppressed induction of hypersensitivity; these procedures used together specifically and completely suppressed induction of hypersensitivity. Complete suppression was sustained by antigen given intravenously before each weekly injection of the mixture of antigen and adjuvant. These findings provide the rational basis of a simple method for prolonging survival of allografts with only the biological agents, antigen and antibody, of the immunological response.*

Immunological reactions to an antigen divide into two broad categories: (i) antibody and (ii) cell-mediated (delayed-type hypersensitivity) responses. Either or both responses may be induced, depending on immunization procedures. Selective, specific suppression of the antibody or the cell-mediated response may be desirable for special purposes. For example, the antibody response of animals not previously exposed to an antigen can be specifically and profoundly suppressed by passive immunization with antibody to the antigen (*1*). Advantage is taken of this phenomenon to prevent immunization of Rh-negative mothers by fetal Rh-positive erythrocytes (*2*). On the other hand, a cell-mediated rather than an antibody response probably mediates rejection of allografts (*3*). We now report use of antigen and antibody together to suppress specifically induction of delayed-type hypersensitivity in the rat.

Adult female Sprague-Dawley rats were sensitized to sheep erythrocytes (SRBC). Emulsion (0.5 ml) consisting of equal parts of 5 percent SRBC in saline and of Freund's complete adjuvant (FCA) was injected intradermally in three depots in abdominal skin. For demonstration of hypersensitivity, 0.1 ml of a 7.5-percent suspension of SRBC in saline was injected intradermally on the dorsum of a hind paw; the extent of swelling provided a measure of hypersensitivity; measurements could be made rapidly, accurately, and repeatedly by weighing of the volume of mercury displaced by the paw (*4*). Hypersensitivity was most severe 9 to 11 days after sensitization; swelling of the paw began after about 12 hours and became most severe 18 to 24 hours after challenge. In the following experiments rats were challenged 9 days after sensitization, and responses were measured 21 hours later. By multiple criteria the challenge reaction was completely analogous to a cell-mediated, delayed-type hypersensitive reaction previously described for the rat (*4*, *5*). A high degree of cutaneous hypersensitivity to SRBC could be maintained by weekly intradermal injections of SRBC with FCA; weekly injections of either antigen or adjuvant alone did not maintain hypersensitivity.

Development of hypersensitivity was reduced by either of two procedures: (i) intravenous injection of antigen, or (ii) passive immunization. Rats were injected intravenously with 1.0 ml of 5 percent SRBC in saline 1 day before sensitization by SRBC with FCA. Challenge responses were reduced in severity by about 60 percent. Other rats were passively immunized with a total of 4.0 ml of hyperimmune-rat antiserum to SRBC given intravenously in 1.0-ml amounts 12 hours before and 12, 36,