(*Manduca sexta*) [R. T. Yamamoto and G. S. Fraenkel, *Ann. Entomol. Soc. Amer.* **53**, 499 (1960); L. M. Schoonhoven and V. G. Dethier, *Arch. Neer. Zool.* **16**, 497 (1966)], both of which have been found to have specific gustatory receptors for this compound.
10. *Trans*-2-hexenal was added since it is an attractant for *B. mori* larvae (*11*) and is also the substance in oak leaves which initiates the release of sex pheromone by female polyphemus [L. M. Riddiford, *Science* **158**, 139 (1967)] and stimulates oviposition in this species (J. W. Truman, unpublished observations in this laboratory).
11. T. Watanabe, *Nature* **182**, 325 (1958); T. Ito and Y. Horie, *J. Insect Physiol.* **8**, 569 (1962).
12. Such wing defects are presumably a result of fatty acid deficiency; see G. Fraenkel and M. Blewett, *J. Exp. Biol.* **22**, 172 (1946).
13. Nutritional Biochemical Corporation now markets a mixture of the first five ingredients under group 1 as the "Vanderzant Adkisson Wheat Germ Diet" and a mixture of all the compounds in group 2 except the aureomycin, kanamycin, and linseed oil as "Vanderzant's Vitamin Mixture for Insect Diets."
14. C. M. Ignoffo, *Ann. Entomol. Soc. Amer.* **56**, 178 (1963); T. M. O'Dell and W. D. Rollinson, *J. Econ. Entomol.* **59**, 741 (1966).
15. The aureomycin was provided by the Lederle Laboratories; the kanamycin by the Bristol Laboratories. The work was supported by research funds of Harvard University and NSF grant GB 6730. I thank Mrs. Su-Mei Wang for technical assistance and Prof. C. M. Williams for reading the manuscript.

8 April 1968

Lectins in Extracts of Certain Polygonaceae Seed Precipitate Animal and Human Serums

Abstract. *Seeds of four species of Polygonaceae were tested for lectins that precipitate human and animal serums.* Rumex crispus, Polygonum convolvulus, *and* Polygonum pennsylvanicum *developed specific precipitate bands on double diffusion on agar gel plates. These bands were enhanced and increased in number when extracts were tested against serums from patients with certain diseases. When tested against lyophilized serum, no precipitate bands developed. The active substance cannot be dialyzed through cellulose membrane against running tap water for 16 hours, and it is heat stable. Extracts from* Fagopyrum esculentum *developed no precipitate bands.*

Agglutinating lectins from extracts of various plant seeds are specific for blood group A and its subgroups (*1*). Specific lectins against M, N, and H groups have also been discovered (*2*). These lectins have aided in elucidating the ABO and MNSs blood groups, and they are of practical value in legal medicine and anthropology. If a satisfactory lectin against group B (*1–3*) were discovered, the use of lectins in blood grouping for transfusion would become routine, since the present methods are more expensive.

The precipitating lectins in plant extracts have not been as extensively studied as the agglutinating lectins (*4*). One probable reason is that agglutination of red cells is very sensitive and the tests are easy to perform; another is that agglutinating lectins are used in blood grouping for transfusion. The precipitating tests present more difficulties. Some preciptating lectins are specific for blood-group substances in various body fluids (*5*). Because some leguminous plant lectins give specific precipitates with serum proteins (*6*), there is the possibility that they may be used in the diagnosis of certain diseases.

Rumex crispus (Curly dock), *Polygonum convolvulus* (Wild buckwheat), *Polygonum pennsylvanicum* (Smartweed), and *Fagopyrum esculentum* (Domestic buckwheat) (*7*) were examined. The active precipitating substances could be extracted with water or ethanol. For extraction in ethanol, 6 g of seed was ground in a hand coffee mill, then pulverized to an average fineness of 500 μm in a water-cooled micropulverizer. Five grams of the pulverized seed were then transferred to a flask with 100 ml of absolute ethanol and extracted overnight (16 hours) in a refrigerator at 7°C.

The flask was occasionally shaken, when convenient. The ethanol was filtered off, and the residue was washed four times, each time with 50 ml of absolute ethanol. The fourth washing was free of color. The ethanol filtrate was evaporated to dryness at 37°C. At this stage, depending upon the intended tests, the dry residue was suspended either in 50 ml of distilled water or in 0.85 percent unbuffered saline and the *p*H was adjusted to 7.0 with 0.4*N* NaOH. For routine gel-diffusion tests, the dry residue was suspended in saline containing 0.01 percent Merthiolate as a preservative. The suspension was centrifuged, filtered, distributed approximately equally into six test tubes, and stored at −15°C. As needed the extract was thawed at 37°C in a water bath, adjusted to *p*H 7.0 with 0.1*N* NaOH, and centrifuged at 2000 rev/min for 10 minutes. The supernatant was used for gel-diffusion tests or was filtered and used in ring-precipitin tests. The extract was stored at 7°C.

Serums were obtained from the hospital blood bank and from patients admitted to the hospital. Animal serums, obtained from local sources, were first used fresh, and then after 0.01 percent Merthiolate was added as a preservative. All serums were stored at 7°C. Initially, the ring-precipitin test was done with these serums; if significant precipitate developed, then the Ouchterlony gel-diffusion procedure was employed (*8*). We prepared our own plates using Noble agar in phosphate buffer at *p*H 7.4. Merthiolate (0.01 percent) was added as a preservative. The test plates were allowed to develop at room temperature. Ethanol extracts in saline were used in red-cell agglutination and complement fixation tests.

Extracts of the four species were tested against serum from a patient afflicted with lupus erythematosus (Fig. 1). Both extract of *R. crispus* dialyzed through a cellulose membrane against running tap water for 16 hours and undialyzed extract developed three precipitate bands. The extracts from *P.*

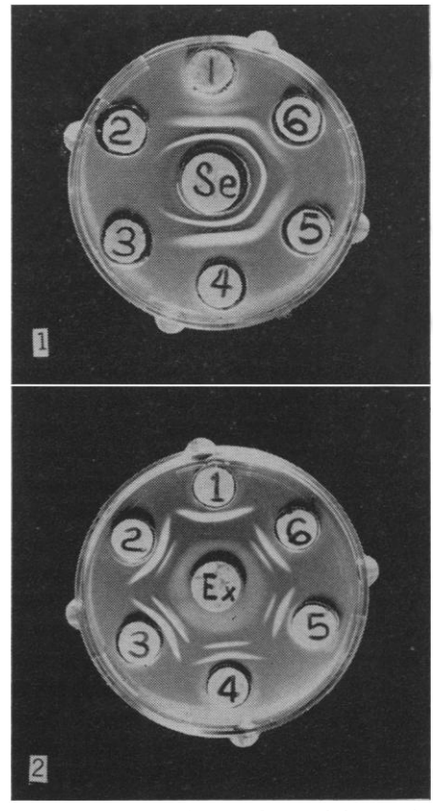

Fig. 1. Comparison of precipitates in Noble agar gel diffusion of four species of Polygonacae against lupus erythematosus serum. Well 1, dialyzed *R. crispus* extract; wells 2 and 3, *F. esculentum*, silver hull and black varieties, respectively; well 4, undialyzed *R. crispus*; well 5. *P. convolvulus;* and well 6, *P. pennsylvanicum; Se,* lupus erythematosus serum. Note the identity of precipitate bands and that *F. esculentum* varieties develop no precipitates. Photographed at 24 hours. Fig. 2. Precipitate bands formed by *R. crispus* extract against different animal serums including human donor serum in Noble agar gel with phosphate buffer at *p*H 7.4. Well 1, human donor serum; well 2, Rhesus monkey serum; well 3, turkey serum; well 4, horse serum; well 5, cow serum; and well 6, hog serum; *Ex, R. crispus* extract. Note only one precipitate band with donor serum. Photographed at 48 hours.

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.

ERNEST B. HANAN
Laboratory, Burge-Protestant Hospital, Springfield, Missouri
JAMES W. SPINDLER
2949 Laclede Avenue, St. Louis, Missouri 63103

References and Notes

1. W. C. Boyd and R. M. Reguera, *J. Immunol.* **62**, 333 (1949); W. C. Boyd, *Introduction to Immunochemical Specificity* (Interscience, New York, 1962); W. C. Boyd, E. Werzcaeuko-Zackarczenko, S. M. Goldwasser, *Transfusion* **1**, 374 (1961); G. W. G. Bird, *J. Immunol.* **69**, 319 (1952).
2. W. C. Boyd, D. L. Everhart, M. M. McMaster, *J. Immunol.* **81**, 414 (1958); F. Ottensooser and K. Selberschmidt, *Nature* **172**, 914 (1935); K. O. Renkonen, *Ann. Med. Exp. Biol. Fenn.* **26**, 66 (1948); O. Makela, *ibid.* **35** suppl. 11, 1 (1957).
3. F. Ottensooser, R. Sato, M. Sato, *Transfusion* **8**, 44 (1968).
4. E. C. Schneider, *J. Biol. Chem.* **11**, 47 (1912).
5. W. C. Boyd and E. Shapleigh, *Science* **119**, 419 (1954).
6. M. A. Leon, *ibid.* **158**, 1325 (1967); S. Nakamura, K. Tanaka, S. Murakawa, *Nature* **188**, 144 (1960).
7. J. A. Steyermark, *Flora of Missouri* (Iowa State Univ. Press, Ames, 1963).
8. P. L. Carpenter, *Immunology and Serology* Saunders, Philadelphia, ed. 2, 1965), p. 425.
9. J. Munter, *Ann. Med. Exp. Biol.* **27**, 12 (1949).
10. We thank Drs. N. R. Lewis and P. S. Quinn for their encouragement in these studies; the laboratory personnel for technical assistance; and P. Glynn, K. Janes, and Miss C. Hanan for help in securing material and recording data.

10 April 1968

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