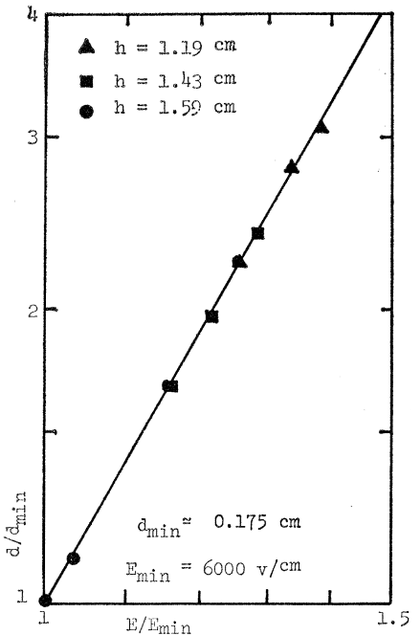

Fig. 3. Diameter of column of amyl alcohol versus average electric field.

of its shadow) was read directly from the scale on the screen; since the column always formed in the same location, only one absolute diameter size, determined from a photograph, was needed.

The column diameter as a function of the average electric field (Fig. 3) suggests a relation of the form

$$d/d_{min} = (E/E_{min})^m$$

with m approximating 3.5. So far no analytical work has attempted to explain this result because other parameters such as density, viscosity, conductivity, surface tension, and dielectric constant also are important (*1*). Furthermore the mechanism is not purely electrostatic, because the open-circuit voltage is higher than the voltage when a column is present, so that a small flow of current is indicated.

ROLAND J. RACO
Newark College of Engineering, 323 High Street, Newark, New Jersey

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Concanavalin A in vivo: Induction of Hemorrhagic Skin Lesions (Arthus-Like Reactions) in Mice

Abstract. *Concanavalin A injected into the skin of mice induced the formation of hemorrhagic Arthus-like lesions. No lesions resulted if concanavalin A was adsorbed with insoluble α_2-macroglobulin or if mice were first treated with nitrogen mustard. The ability of concanavalin A, phytohemagglutinin, or pokeweed mitogen to induce skin lesions seemed to parallel their ability to precipitate serum proteins.*

Various plant lectins, as well as immunoglobulins, possess the properties of hemagglutination, leukagglutination, and mitogenicity (*1*). The lectins most studied with regard to these qualities are pokeweed mitogen (PWM) (*2*) derived from the plant *Phytolacca americana* and phytohemagglutinin (PHA) (*1*) obtained from the kidney bean *Phaseolus vulgaris*. The ability of these materials to transform resting peripheral blood lymphocytes to "blast-like" cells in vitro has been of particular interest since such transformed cells are similar to cell types engaged in antibody synthesis (*3*). Because of its mitogenic property, the effects of PHA on antibody formation in vivo (in animals) have been tested. It has been injected into patients with aplastic anemia to stimulate the production of marrow stem cells (*4*). The accidental ingestion of PWM (in pokeweed berries) by children has resulted in plasmacytosis (*5*). Because of the effects of lectins in vivo and the marked ability of concanavalin A to precipitate serum proteins (a property which suggested it would be biologically active) we have studied the effects of concanavalin A in vivo.

Concanavalin A (Con A), the lectin of the jack bean *Canavalia ensiformis*, is a protein which interacts with α-D-glucopyranosyl, α-D-mannopyranosyl, or β-D-fructofuranosyl residues of polysaccharide chain ends (*6*). Various polysaccharides and serum glycoproteins will precipitate with Con A (*7–10*) and precipitation can be inhibited by low-molecular-weight haptens such as α-methyl glucoside. Con A, like PHA and PWM, has mitogenic effects on lymphocytes in vitro (*11*) and will agglutinate the red blood cells of many species (*7*). Chemically, however, Con A (*12*) appears to be quite distinct from PHA (*2*) and PWM (*2*).

Con A was extracted from jack bean meal (General Biochemicals, Chagrin Falls, Ohio) by the method of Cifonelli and Smith (*13*). It was purified by adsorption and elution from Sephadex G 50 (*14*), and preparations have been obtained which produced a single band on disc-gel electrophoresis. Female mice (CFW, 18 to 25 g) were shaved (dorsal surface) and on the following day were injected intracutaneously with 0.05 ml of Con A (23 μg) in physiologic saline. Sites of skin tests were examined 4 and 24 hours later, and some were removed for sectioning and staining.

The injected sites responded with the edema and confluent petechial hemorrhages characteristic of the Arthus reaction. Hemorrhagic areas 9 to 15 mm in diameter were visible 4 hours after challenge and became more intense after 24 hours (Fig. 1). Histologic examination of the test sites at 24 hours revealed an accumulation of leukocytes on both sides of the panniculas and extending into the subcutaneous fat. Congestion of capillaries and free red cells in the dermis were also observed.

To prove that the observed skin reactions were due to the activity of Con A rather than to some toxic trace contaminant in the injected material, we adsorbed a solution of Con A with insoluble α_2-macroglobulin (α_2M). α_2-Macroglobulin (human fraction IV, Hyland Lab., Los Angeles) was made insoluble by cross linkage with formaldehyde (*15*) and was used in excess to ensure complete adsorption. Approximately 1 g of α_2M was incubated with 2.5 ml of Con A (475 μg/ml) at 37°C for 1 hour, and the mixture was kept at refrigerator temperature for several days, with occasional stirring. The mixture of α_2M and Con A was then centrifuged, and 0.05 ml of the supernatant was injected intracutaneously into mice. To test the specificity of the reaction between Con A and α_2M, we then added 2 ml of physiologic saline containing 0.1*M* D-arabinose to the adsorbent; the mixture was stirred at room temperature for 30 minutes, and the supernatant was removed after centrifugation. The complex of α_2M and Con A was washed several times with saline and was then mixed with 2 ml of 0.1*M* methyl α-D-glucopyranoside. The complex was centrifuged 30 minutes later, and 0.05 ml of this supernatant as well as 0.05 ml of the supernatant obtained after the addition of D-arabinose were tested for their ability to induce hemorrhages in the skin of mice. The eluates were not dialyzed to remove D-arabinose or methyl

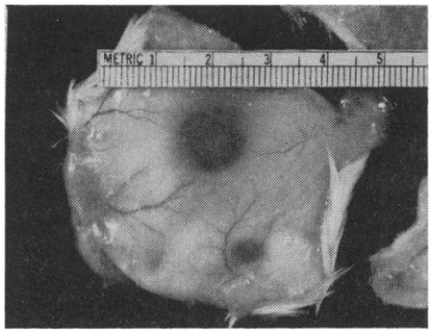

Fig. 1. Hemorrhagic skin lesion (upper dark circular area) 24 hours after intracutaneous injection of 23 μg of concanavalin A.

α-D-glucopyranoside since previous experiments had indicated that these sugars did not inhibit the skin reactions produced by solutions of Con A. Concanavalin A adsorbed with α_2M lost its ability to induce hemorrhagic skin reactions in mice (even if volumes larger than 0.05 ml were injected). The active material could not be eluted from the α_2M by D-arabinose but could be eluted with 0.1*M* methyl α-D-glucopyranoside. Goldstein *et al.* (*6*) have shown that methyl α-D-glucopyranoside is a potent inhibitor of the precipitin reaction between dextran and Con A, whereas D-arabinose does not inhibit this system. The ability of solution of Con A to cause skin reactions could also be removed by the addition of mouse serum to Con A and removal of the resultant precipitate by centrifugation.

Arthus reactions can be markedly suppressed in rabbits whose polymorphonuclear leukocytes have been reduced to a very low number by the administration of nitrogen mustard (*16*). Mice were injected intravenously with 5 mg of Mustargen (Merck) per kilogram of weight. Four days later, when their average polymorph count was six per cubic millimeter compared with 1000 per cubic millimeter for the control animals, the mice were injected intracutaneously with 60 μg of Con A. Ten hours later, the animals were killed, and the inner surface of the skin was examined macroscopically for hemorrhagic skin lesions. Of the 16 mice previously treated with nitrogen mustard, 15 mice failed to produce hemorrhagic skin lesions after the administration of Con A, whereas all 16 of the control animals produced lesions.

We then compared the abilities of Con A, PHA (PHA-P, Difco), and PWM (Grand Island Biological Co.) to induce skin lesions in mice and to precipitate with mouse serum. Con A precipitated with undiluted mouse serum at a concentration of 1 mg/ml, whereas PHA and PWM at concentrations of 10 mg/ml failed to form precipitates in agar. Five micrograms of Con A produced 5- to 10-mm skin lesions in 50 percent of mice, whereas with PHA 50 μg was required; 500 μg of PWM produced no visible reaction. Three groups of mice containing seven animals per group were used to calculate the ED_{50} (effective dose in 50 percent of the animals). Others (*9*) have demonstrated by the technique of crossed electrophoresis that PHA will combine with α_2M, and a noncommercial preparation of PHA precipitates in agar with undiluted human serum (*17*).

It thus appears that the ability of Con A, PHA, and PWM to induce Arthus-like reactions parallels their ability to precipitate with serum proteins (Con A > PHA > PWM). Although the formation of a precipitate of Con A and serum glycoprotein in the wall of blood vessels is likely the initial step in the development of the observed hemorrhagic skin lesions, it is conceivable that Con A and other lectins may combine with glycoproteins or polysaccharides on cell surfaces to trigger subsequent complement fixation, polymorph accumulations, and vascular damage. It has been demonstrated that heterophile antiserums which likely react with Forssman antigen on endothelial cells will produce severe hemorrhagic reactions in guinea pig skin (*18*).

We have some evidence that complement may play a role in the skin lesions under discussion. Depletion of mouse complement by the polysaccharide carrageenin prevented Con A from inducing Arthus-like reactions. Ward and Cochrane (*19*) had previously inhibited Arthus reactions in rats and guinea pigs with this material. We have also observed that the addition of Con A to fresh mouse serum deprived that serum of its ability to lyse sensitized sheep cells. It has been stated that Con A will precipitate with each of the components of complement as well as with other serum glycoproteins (*10*). It is thus conceivable that Con A may generate the polymorphonuclear chemotactic factor required for production of Arthus reactions by direct combination with individual complement components rather than by interaction with immunoglobulin G or immunoglobulin M followed by sequential binding and activation of complement components. Evidence exists that human complement components 3 or 5 can be acted upon directly by various materials (independent of prior acting components of complement) to yield biologically active fragments (*20*).

Phytohemagglutinin reportedly produces erythema and induration in guinea pig (*21, 22*) and human (*21, 23*) skin, and these reactions have been interpreted to be examples of delayed hypersensitivity (*21, 23*). Airo *et al.* (*23*) on the basis of such an interpretation found it difficult to explain the strong reactivity of patients with chronic lymphocytic leukemia to intradermal injections of PHA at a time when their lymphocytes in cell culture were hyporeactive to PHA. Our data on the likely interaction of Con A and PHA with glycoproteins in vivo would seem to resolve this dilemma.

LEON S. KIND
WARREN A. PETERSEN
Department of Asthma-Allergy,
National Jewish Hospital,
Denver, Colorado

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