lated and inoculated cultures. Other constituents were found in infected and not in uninfected tissue cultures, but their occurrence was not unique to any one of the four virus-infected cultures—for example, metabolites having retention times of 80, 125, 165, 200, 265, 290, 510, 565, and 1130 seconds. However, as shown in Table 1, two compounds observed in the cell cultures of dog kidney had the same retention times as compounds found in the serums of animals inoculated with the hepatitis virus—R_t values of 420 and 1380 seconds; these two metabolites were not present in cultures receiving any of the other viruses investigated. Four distinctive peaks were noted in cultures inoculated with the herpes virus, and three different products were observed in distemper-infected cultures, while two differentiating peaks were found in cultures receiving the parainfluenza virus.

The degree of specificity of the products of these particular virus infections remains unknown; more viruses must be investigated. However, correlation between the appearance of products having R_t values of 420 and 1380 seconds and infection with infectious hepatitis is suggested by the observations that the same two compounds were present in all 11 animals infected with the virus, metabolites having identical chromatographic characteristics were observed in tissue cultures receiving this virus but in none of the others, and the disappearance of these metabolites coincided with recovery of the animals.

If a specific or possibly unique metabolite is produced in minute amounts in animals as a consequence of or prelude to a particular infection, ultrasensitive gas-chromatographic methods may be able to detect these distinguishing substances. By means of such highly sensitive procedures, it may then be possible to perform an early or rapid diagnosis of microbial infections of animals and even humans.

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8 March 1968

Electrically Supported Column of Liquid

Abstract. *Application of an electric field normal to the interface of certain liquids causes a liquid column of uniform diameter to be formed parallel to the electric field. A column of amyl alcohol supported in air by a high voltage was investigated experimentally; the diameter of the column varied as the voltage raised to the 3.5 power.*

Recently the various phenomena (such as pumping, stirring, electroconvection, production of a spray) associated with the interaction of an electric field with a dielectric fluid have attracted much attention (*1*). During investigation of the stability of a fluid interface in the presence of an electrostatic field (*1*) it was observed that certain liquids form columns, of uniform diameters, extending from the surfaces of the liquids to the upper electrode (Fig. 1). To the best of my knowledge, electrical formation and support of a liquid column had never been mentioned (*2*, *3*).

A strong electrostatic field was applied (Fig. 2) normal to various liquids in order to determine which liquids would form a column surrounded by air and extending from the liquid surface to the upper electrode. For initiation of the column, the upper electrode was first lowered until distance h was quite small. A liquid having moderate electrical conductivity, such as tap water, underwent much surface activity (formation of surface waves) but formed no column because the air between the liquid surface and the upper electrode broke down. A liquid having lower conductivity, such as ethylene glycol (10^{-5} mho/m), formed a peak extending from the liquid surface to the upper electrode. A liquid low in conductivity, such as fuel oil, experienced strong pumping in that a fountain of liquid was continuously circulated between the liquid surface and the upper electrode.

Of the various liquids tested, amyl alcohol formed easily the longest (up to 3 cm) uniform-diameter column in air. At a relatively low field strength (preceding collapse of the column) the top of the column narrowed down; at a relatively high field strength (when h was quite small) the top of the column widened and resembled in shape the base of the column. Thus the experimental investigation with amyl alcohol excluded the cases of high and low field strengths.

The height of the column was measured by displacement of the string from its reference point (Fig. 2); this height was used also to calculate the average electric field: $E = \psi_A/h$. The relative diameter of the column (the diameter

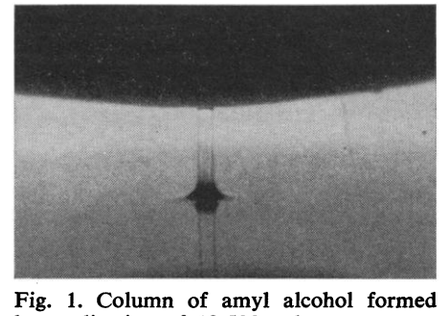

Fig. 1. Column of amyl alcohol formed by application of 12,500 volts.

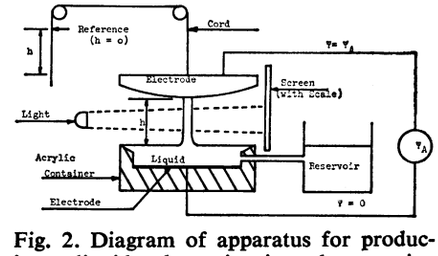

Fig. 2. Diagram of apparatus for producing a liquid column in air and measuring its diameter and height.

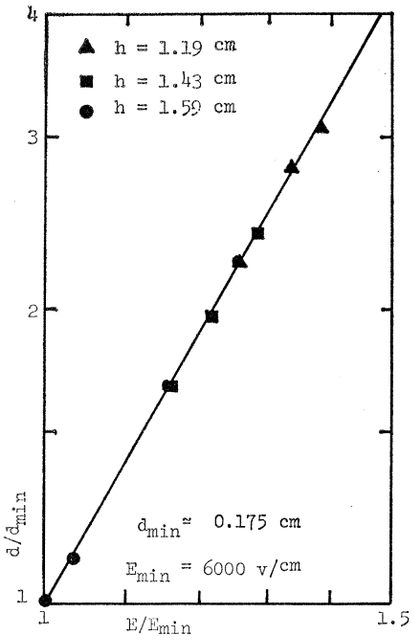

Fig. 3. Diameter of column of amyl alcohol versus average electric field.

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.

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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