

method of studying the distribution of organisms, in which whole organs were cultured, agreed in general with those using plate counts of weighed tissue homogenates though it must be kept in mind that a larger quantity of tissue was cultured by the former than by the latter method in which less than 10 organisms could not have been counted. Organisms were cleared with greater rapidity from the tissues of vaccinated than unvaccinated mice. When whole organs were cultured, the difference in clearance rate between vaccinated and control mice was not conspicuous until the third day for *Ps. aeruginosa* and the eighth day for *P. mirabilis*. By the quantitative procedure, the difference in rate of clearance between vaccinated and control mice was observed as early as the first day after iv infection for *Pseudomonas* and the third day for *Proteus*.

Summary. Mice were vaccinated with 1 dose of 0.25 ml of *P. mirabilis* or *Ps. aeruginosa* antigen by various routes 1 week before iv challenge with homologous culture. The incidence of gross damage and positive kidney cultures compared with controls was greatly reduced whichever the route of immunization. Vaccination after pyelonephritis had become established was ineffective. Cross-vaccination studies showed considerable protection among 4 strains of *Pseudomonas* and 3

species of *Proteus*. LPS administered before *P. mirabilis* pyelonephritis was induced did not alter the outcome of the infection; when given after the disease was established, tolerance was noted. Qualitative and quantitative cultures of tissues of normal and vaccinated mice at various intervals following iv infection revealed that *P. mirabilis* and *Ps. aeruginosa* were cleared with greater rapidity from the blood, spleen, liver, and kidneys of vaccinated than of unvaccinated mice.

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Toxic Effects of Intraperitoneal Juglone in Rats* (32747)

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Juglone, 5-hydroxy 1:4 naphthoquinone, originally extracted from the Black Walnut tree, *Juglans nigra*, was found to be depressant when administered to several species of animals (1), and toxic in larger doses. Preliminary investigations of toxicity indicated that juglone caused accumulation of fluid in the abdominal cavity of rats when administered intraperitoneally (ip), and this study was done to determine the possible mechanisms involved.

Materials and Methods. Adult Sprague-Dawley rats of both sexes and approximately 350–400 gm in weight were used. They were divided into 3 groups of which 1 group served as controls and the remaining 2 as experimentals. Juglone, obtained commercially and repurified prior to use by sublimation, was dissolved in corn oil in concentrations of 2 or 3 mg/ml. Experimental Group A received 2 mg/kg of juglone and Group B, 3 mg/kg. Controls received corresponding amounts of corn oil. All animals were maintained on food and water *ad libitum*. Parameters measured

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TABLE I. Effect of Juglone on Hematocrit, Lung Weight Ratio, and Quantity of Abdominal Fluid Aspirated.

Group	No. of animals	Dose (mg/kg)	Hematocrit (% \pm SD)	Abdominal fluid (ml/kg \pm SD)	Wet/dry lung (wt. ratio \pm SD)
Control	19	—	42 \pm 2.32	Trace	4.98 \pm 0.12
A	24	2	52 ^a \pm 5.85	37.8 ^a \pm 10.7	4.63 ^a \pm 0.17
B	15	3	58 ^a \pm 9.0	39.8 ^a \pm 8.11	4.47 ^a \pm 0.08

^a $p < 0.05$ using Student *t* test.

TABLE II. Effect of Juglone on Plasma Proteins, Sodium, and Potassium Levels.

Group	Plasma proteins (gm/100 ml \pm SD)	Plasma sodium (meq/liter \pm SD)	Plasma potassium (meq/liter \pm SD)
Control	6.86 \pm 1.31 (8) ^b	143 \pm 4.30 (8)	4.18 \pm 0.55 (8)
A	6.67 \pm 1.05 (6)	137 \pm 5.64 (7)	7.04 ^a \pm 2.36 (7)
B	5.50 ^a \pm 1.51 (6)	140 \pm 6.54 (5)	6.28 ^a \pm 1.56 (5)

^a $p < 0.05$ using Student *t* test.

^b Figures in parentheses represent number of animals.

were microhematocrit, plasma sodium and potassium levels using a Beckman flame photometer model 105, and plasma proteins by the biuret method of Gornall *et al.* (2) adapted for use with the Beckman ultramicro spectrophotometer model 150. Twenty-four hours following administration of a single dose of juglone or corn oil, animals were anesthetized with sodium pentobarbital, 32 mg/kg of body weight, blood was drawn by cardiac puncture, and then the animals were sacrificed. Abdominal fluid, when in any quantity, was aspirated and measured. Lungs were removed, stripped of extraneous tissues, weighed, and dried to constant weight to obtain the wet/dry weight ratio.

Results. Tables I and II show the results obtained for the various parameters measured. As shown there was a significant increase in the hematocrit of both experimental groups over that of the control. Also noted was a significant amount of abdominal fluid accumulation in both experimental groups, whereas there was no measurable amount in the control. This fluid readily formed soft clots, indicating the presence of coagulating proteins.

Also of significance was the decrease in wet/dry lung weight ratios in both experimental groups compared to controls. This suggests that fluid was lost from lung tissues of

animals receiving juglone. Plasma proteins, when measured, indicated a significant decrease in experimental Group B but not Group A. However, the trend appeared to be downward in Group A, suggesting a loss of plasma proteins in both experimental groups. Another finding of interest was the significant increase of plasma potassium of both experimental groups over that of the control, but no significant change in plasma sodium. These findings suggest that there may be cell permeability changes permitting potassium to leak out of the cell into the plasma.

Discussion. Results obtained indicate that juglone caused accumulation of peritoneal fluid that apparently was due to increased capillary permeability. Other factors that support the assumption of increased permeability of capillary membranes were the rise in hematocrit, the decrease in lung fluids, and loss of plasma proteins. The loss of fluid may lead to depression since Nungesser *et al.* (3) reported that when hypertonic mannitol solution was given ip to dogs, approximately 80 ml/kg of body weight of peritoneal fluid accumulated and led to fatal shock. However, the fluid loss in our rats was not as great as that reported by these investigators, nor did it cause death.

The increased permeability may be related to irritation of the peritoneal lining by

juglone and subsequent injury to capillaries. In conscious rats, massive abdominal contractions were illicited along with movements suggestive of discomfort. This continued for approximately 10 min and was followed by a period of depression. However, these depressed animals were readily aroused when stimulated. There were few reactions from animals that received corn oil only.

Summary. Juglone, administered ip, was found to produce an accumulation of fluid in the abdominal cavity of rats. That the fluid from the circulatory system was accompanied by proteins was suggested by soft clots that formed in the fluid. Changes in hematocrit,

plasma proteins, lung weights, and electrolytes suggested that increased capillary permeability resulted from the irritative properties of juglone.

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Antigenic Enhancement of Ether-Extracted Influenza Virus Vaccines by $AlPO_4$ * (32748)

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It has been shown that the antibody response of humans vaccinated with hemagglutinins prepared by extraction with ether (HA) is equal to or greater than that found after administration of the same dose of intact virus vaccine (1-3). Furthermore, HA vaccines proved to be as potent as virus vaccines when tested in rabbits (4). However, one preparation of HA vaccine that yielded a superior antibody response in man failed to meet minimum potency requirements when tested in mice (3).

The present investigations were carried out to obtain further information on the relative potency of HA vaccines in mice. It was found that dilutions of HA vaccines yielded lower hemagglutination inhibition (HI) antibody titers and lower degrees of protection than equivalent dilutions of virus vaccines, but that addition of a mineral carrier largely restored

the antigenic potency of the HA vaccines.

Materials and Methods. Viruses. The strains utilized were selected from the collection of the Virus Laboratory, Department of Epidemiology, School of Public Health, the University of Michigan and are described by currently accepted nomenclature.

Vaccines. Monovalent vaccines containing virus plus $AlPO_4$, HA plus $AlPO_4$, or HA without mineral carrier were prepared with prototype strains of influenza A and B. For each set, a single pool of infected allantoic fluid was employed. Virus was concentrated tenfold by adsorption and elution from chicken RBC. The concentrates were further purified by adsorption and elution from $BaSO_4$ (5). To aliquots of virus, Tween 80 was added to yield a final concentration of 1.25 mg/ml. After the addition of an equal volume of fresh anesthetic ether, the mixture was shaken for 10 min at 4°C. The aqueous phase was reextracted with ether until gelatinous material no longer was visible at the interphase. Five or six treatments generally sufficed. Excess ether was removed by bubbling with nitrogen at room temperature. Virus

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