that protamine sulphate is in fact an anti-factor V but the author agrees with Ferguson (personal communication) that this interpretation is unjustified since such serum is an extremely complex reagent. The unrelated finding that the addition of extra platelets, $Al(OH)_3$ treated plasma or serum to a normal thromboplastin generation system preincubated with protamine sulphate tend to neutralize the effect of the latter should also be viewed in the same light and interpreted with extreme caution.

It is clear that protamine sulphate has more than one anticoagulant action and several workers have confirmed that very high concentrations cause precipitation of fibrinogen and that relatively lower concentrations have a fibrinoplastic action(3,7). The lowest concentrations of protamine required for the fibrinoplastic action are roughly equivalent to the lowest concentration necessary to interfere with the reaction between blood thromboplastin, plasma and calcium and this concentration is considerably higher than the minimal concentration affecting the yield of blood thromboplastin generation.

The inhibitory action of protamine in high concentrations on the reaction between fully formed blood thromboplastin, plasma and calcium appears an immediate one and is non-progressive. It is possible that this action of protamine is both antiprothrombic and antithromboplastic as suggested by Tocantins(6) but other interpretations are possible.

Summary. Protamine sulphate in low concentrations affects both rate and yield of blood thromboplastin but has no effect on formed thromboplastin. Relatively higher concentrations inhibit a reaction between blood thromboplastin, prothrombin and calcium.

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Effect of Chlorothiazide on Vascular Reactivity.* (23964)

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Chlorothiazide (Diuril) has been recently introduced as an orally effective diuretic agent (1). Clinical studies(2,3) have indicated a definite anti-hypertensive effect of this compound, and the suggestion has been made that it may have a direct hypotensive action(4). Thus far, however, there has been no indication of a mechanism whereby chlorothiazide might act to reduce blood pressure directly. Therefore, the present study was undertaken to determine the effect of this substance upon vascular response to certain pressor agents.

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Methods. 42 experiments were performed on adult mongrel dogs. They were anesthetized with sodium pentobarbital (30-35 mg/

TABLE I. Effect of Chlorothiazide on Vascular Reactivity.

Dose (μg/kg)	Control: No. of animals	Control: Before infusion (mm Hg)	Control: After infusion (mm Hg)	Control: Change	Chlorothiazide: No. of animals	Chlorothiazide: Before infusion (mm Hg)	Chlorothiazide: After infusion (mm Hg)	Chlorothiazide: Change	Level of significance*
A. Pressor responses to norepinephrine									
.05	9	17.3	16.7	− .6	8	20.4	12.6	−7.8	<.01
.10	9	24.1	22.7	− 1.4	7	26.7	17.8	−8.9	"
B. Pressor responses to epinephrine									
.05	7	9.8	9.7	− .1	14	11.1	7.8	−3.3	<.05
C. Depressor responses to isopropylnorepinephrine									
.05	9	16.0	17.6	+1.6	7	16.7	10.6	−6.1	"

* Considered significant if P <.05.

kg), vagotomized, and their arterial blood pressures recorded on a smoked drum by mercury manometer. In the first series, 2 responses to noradrenaline, 0.05 and 0.1 μg/kg were elicited, then 20 mg/kg chlorothiazide in 10 ml of 5% glucose was given intravenously followed by 20 mg/kg chlorothiazide in 60 ml 5% glucose infused over 30 minutes. The norepinephrine injections were then repeated. In a second series, 2 injections of .05 μg/kg epinephrine were given, chlorothiazide infused as in the first group, and epinephrine doses were repeated. A third group of dogs received .05 μg/kg isopropylnorepinephrine (Isuprel) before and after chlorothiazide infusion. Animals treated identically to the experimental groups with the exception that no chlorothiazide was present in the glucose solutions served as controls. The response to noradrenaline and adrenaline was considered to be the difference between mean arterial pressure before injection and highest mean pressure after injection. Similarly the response to Isuprel was determined by difference in mean pressure before administering Isuprel and lowest mean pressure following injection. Responses in all animals were determined from a base pressure which differed less than 10 mm Hg from control levels. Plasma sodium concentrations were measured by means of a Beckman flame photometer.

Results. Chlorothiazide infusion reduced the pressor response to norepinephrine and epinephrine (Table I, A & B). The decrease occurred in all dogs tested with norepinephrine, and failed to occur in only one animal challenged with epinephrine. Chlorothiazide also reduced the depressor response to isopropylnorepinephrine in a consistent manner (Table I, C).

The initial responses of experimental and control animals to norepinephrine, epinephrine, and isopropylnorepinephrine were not appreciably different, and the small increases or decreases in responsiveness after glucose infusion were not significant. On the other hand, the differences noted in reactivity before and after chlorothiazide were highly significant and comparisons of quantity changes in response to the 3 amines of the control and chlorothiazide groups were significant (Table I) when compared with each other.

This lowered responsiveness induced by chlorothiazide is somewhat slow in onset, being well developed after a 30 minute infusion, but is not evident 10-15 minutes following intravenous injection of 10 to 20 mg/kg of the material.

Plasma sodium concentrations after chlorothiazide infusions showed a small decrease from control values in 17 dogs averaging 2.4 meq/l, but this decrease did not approach significance when compared with the small plasma sodium variations in control groups. Elevation of plasma sodium by infusion of hypertonic sodium chloride into the chlorothiazide treated animal did not alter the lessened response to norepinephrine.

Discussion. Although a direct hypotensive action of chlorothiazide has been suggested from clinical evidence(4), a mechanism for this effect is lacking. Acute experiments involving infusion of chlorothiazide have shown no depression of blood pressures(2). The present study, illustrating that this diuretic agent decreases the pressor response to nore-

pinephrine and epinephrine, may shed light upon the clinically observed antihypertensive effect.

The possibility that chlorothiazide is a weak adrenergic blocking agent appears unlikely since its effect is not greater in diminishing the response to epinephrine than to norepinephrine and further since it also effectively decreases the depressor response to isopropylnorepinephrine.

Two other explanations of the decrease in responses are (1) chlorothiazide acts directly upon peripheral vascular smooth muscle inducing some refractoriness to agents tending to alter its state of contraction, or (2) the compound sensitizes the carotid sinus buffer mechanism and increases its effectiveness in opposing pressure changes. The reported reduction in dosage of ganglionic blocking drugs necessary for blood pressure reduction in hypertensive patients receiving chlorothiazide (5) would support the first possibility.

Summary. Chlorothiazide (Diuril), an orally effective diuretic, has been shown to decrease arterial pressure response to injections of norepinephrine, epinephrine and isopropylnorepinephrine in the anesthetized dog. The possible relationship of this finding to the reported hypotensive action of the compound is discussed.

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Indirect Staining Reaction with Fluorescent Antibody for Detection of Antibodies to Pathogenic Fungi. (23965)

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Methods for studying fluorescent antibody-antigen reactions have been widely used since their introduction by Coons *et al.*(1,2). They have made possible more definitive studies on the site of antibody formation in animals(3), on the distribution in tissues of polysaccharide (2), bacterial(4), viral(5) and Forssman antigen(6), and on specific localization of globulin in tissue from diseases believed to have hypersensitivity as an underlying cause(7). Recently Watson and Coons, as mentioned in Weller's and Coons' report(8), and Gitlin(9) have demonstrated that a rabbit antibody to human gamma globulin can be conjugated with fluorescein-isocyanate for the purpose of detecting any specific antibody of human origin after combination with its homologous antigen. Weller and Coons(8) were able to detect viral inclusion bodies using a 2-stage technic (indirect method) in which tissue suspected of containing varicella was treated with a convalescent serum and then fluorescein-labeled rabbit antihuman gamma globulin. This general technic has been used in the present work and describes the reaction of pathogenic fungi with human antisera and rabbit antihuman gamma globulin labeled with fluorescein. The possible usefulness of this technic is compared to standard serologic procedures. In at least one disease, cryptococcosis, preliminary evidence suggests that the indirect fluorescent-antibody technic used here may be superior to standard serologic procedures.

Materials and methods. Fungi studied. Stock cultures of *Histoplasma capsulatum, Candida albicans,* and *Blastomyces dermatitidis* were maintained on appropriate media in the yeast phase of growth. The strain of *Cryptococcus neoformans* was freshly isolated