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

from a case of cryptococcal meningitis; the patient's serum was used for serologic studies. Other human antisera were obtained from patients with systemic manifestations of mycotic infection. The complement fixation titers of the blastomyces and histoplasma human antisera were 1:256 and 1:128 respectively. The yeast cell agglutination titer of the candida serum was 1:256. *Preparation and fluorescein-labeling of rabbit antihuman gamma globulin serum.* White rabbits weighing approximately 5 lb. were immunized with alum precipitated(10) human gamma globulin. The rabbit sera were pooled and fractionated by Cohn's ethanol method as described by Deutsch(11) to obtain the globulin fraction. The globulin was conjugated with fluorescein isocyanate using Goldman and Carver's modification(12) of Coons' technic (1). The fluor was stored at –20°C. *Preparation and staining of slides.* The fungi, with the exception of *B. dermatitidis,* were killed with 1% phenolized-saline, washed several times with normal saline and adjusted to a No. 2 MacFarland nephelometer tube. *B. dermatitidis* was killed with pyridine according to the method of Fischer and Labzoffsky (13). A loopful of organisms was spread on a clean slide ranging in thickness from 1.2 to 1.28 mm and allowed to air dry. A drop of undiluted or diluted human sera was then placed over the dried smear. After standing for 30 minutes under a petri dish cover with moistened filter paper liner, the slide was washed gently with 10 ml of isotonic phosphate-buffered saline (pH 7.4). A drop of fluorescein-labeled rabbit antihuman globulin was then placed on the slide and allowed to act for 30 minutes at room temperature. The slide was then washed in the above buffer for 10 minutes. A drop of glycerol-buffer (9 parts glycerol to 1 part isotonic phosphate-buffered saline, pH 7.4) was placed over the stained smear and a cover slip applied. *Light source and microscope used.* A carbon arc with automatic electromagnetic feed operated at 12 amperes D.C. was used with a Leitz monocular microscope fitted with a standard darkfield condensor and Wratten 2A filter in the eyepiece. Details are given in articles by Coons(1) and Coffin(14). *Controls for specific staining.* The autofluorescence of organisms was studied in unstained preparations and with the fluorescein-labelled rabbit antihuman globulin (Rabbit-fluor). To test the specificity of the positive reactions obtained when human antiserum and Rabbit-fluor were used together various absorptions of human sera with homologous and heterologous organisms were performed.

Results. C. albicans. Unstained *C. albicans* yeast cells killed with phenol, gave a dull bluish-green autofluorescence under the fluorescent microscope; with the Rabbit-fluor alone, they were somewhat greener. With human antiserum plus the Rabbit-fluor, a bright greenish-yellow fluorescent "ringing" of the individual cells was noted (Fig. 1). The term ringing best describes the bright staining reaction which appears to involve all or part of the cell wall structure. Ringing was present at serum dilutions comparable to those causing agglutination of yeast cells in the standard serologic test. Absorption of the human antiserum with *C. albicans* yeast cells removed the ringing while absorption with *H. capsulatum* cells did not. Several normal sera caused ringing, in keeping with the known fact that antibodies to *C. albicans* can be demonstrated in a large percentage of the population.

C. neoformans. Unstained *C. neoformans* cells appeared bluish-gray under the fluorescent microscope and essentially the same when the Rabbit-fluor was added. Capsules were not visible. When serum from the patient with cryptococcosis was added plus the Rabbit-fluor, the capsules were visible and appeared as opaque fluorescent halos about the cells. The capsular reaction was noted with diminished intensity at a 1:2 dilution and was negative at a 1:4 dilution of serum. Fig. 2 and 3 show the positive and negative capsular reaction obtained with serum from the patient with cryptococcosis and a normal serum respectively. A serum from this patient 4 months later, when he was in remission as a result of treatment, gave only a slight capsular reaction. Several normal sera gave a slight capsular reaction, although far less than the specific human sera. It was found subsequently that absorption of these sera with *C.*

albicans removed the slight capsular reaction from normal serum but not from the patient's serum.

H. capsulatum. Unstained *H. capsulatum*

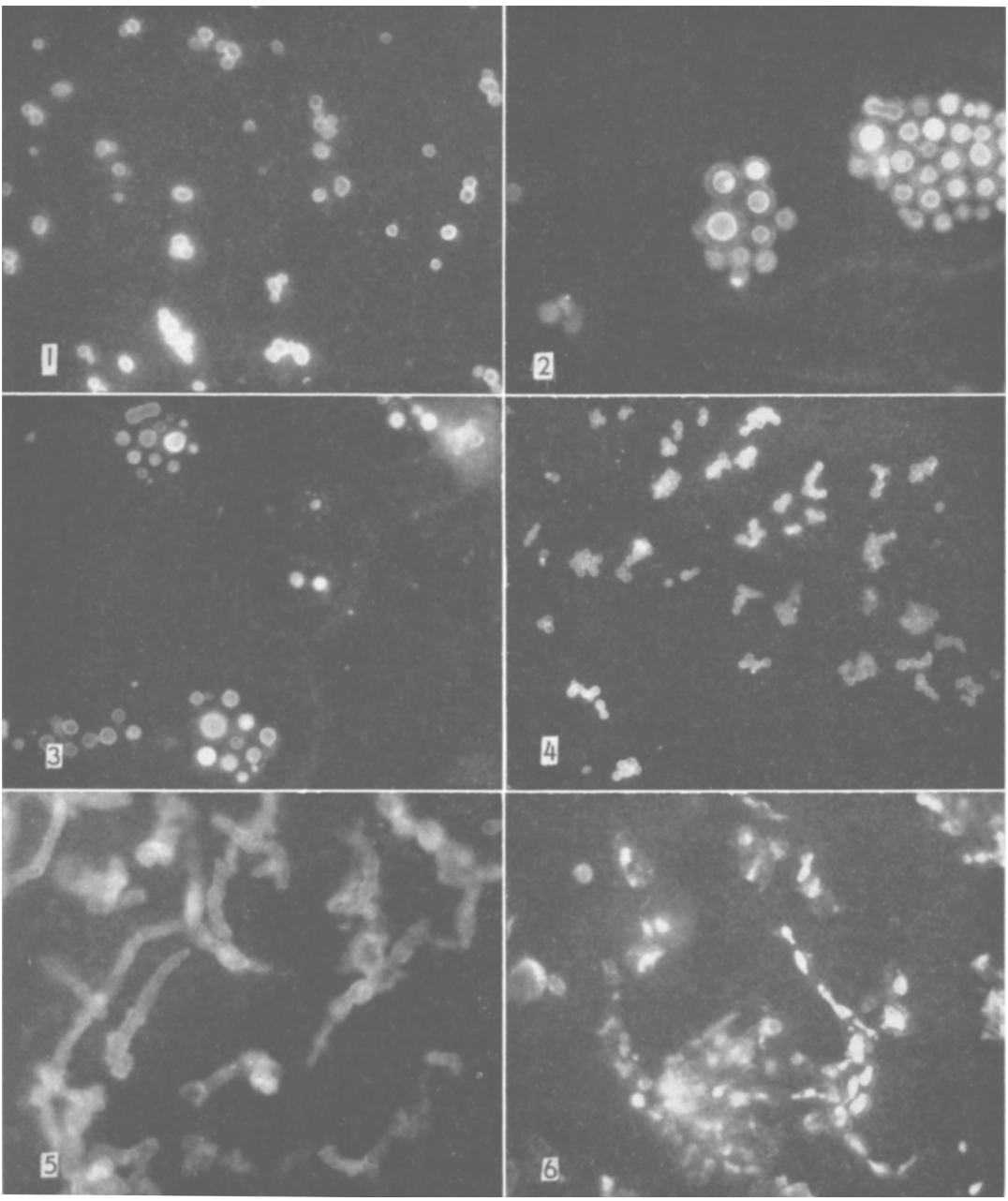

FIG. 1. *C. albicans* cells plus human antiserum and rabbit anti-human gamma globulin fluor. All figures 550 ×.

FIG. 2. *C. neoformans* cells plus human antiserum and rabbit anti-human γ globulin fluor.

FIG. 3. *C. neoformans* cells plus normal human serum and rabbit anti-human γ globulin fluor.

FIG. 4. *H. capsulatum* cells plus human antiserum and rabbit anti-human γ globulin fluor.

FIG. 5. *B. dermatitidis* cells plus human antiserum and rabbit anti-human γ globulin fluor.

FIG. 6. *B. dermatitidis* cells plus normal human serum and rabbit anti-human γ globulin fluor.

cells appeared dull green under the fluorescent microscope, and somewhat brighter when the Rabbit-fluor was used. With human antiserum and Rabbit-fluor, the cells took on a ringed, greenish fluorescent appearance (Fig. 4) much like the Candida cells mentioned above. Several normal sera were noted to give this effect also, despite negative complement fixation tests to *H. capsulatum* antigens. Absorption of the antiserum with Histoplasma cells abolished the ringing effect from all sera, however absorption with *C. albicans* or sheep red blood cells appeared to intensify the ringing effect in both normal and specific sera. The ringing effect, with specific human antiserum dilutions, persisted at approximately the same titer (1:128) as the complement fixation test while some normal human sera ringed cells in a dilution as high as 1:16. The reason for the activity of Histoplasma cells in normal sera is obscure at the present time.

B. dermatitidis. Unstained *B. dermatitidis* cells appeared bluish-green when observed under fluorescent illumination and somewhat greener when stained with the Rabbit-fluor alone. In Fig. 5 and 6 are shown *B. dermatitidis* cells in the yeast phase with some mycelial transformation, not infrequently observed in such cultures when a temperature of 37°C is not rigidly maintained. Fig. 5 shows the appearance of cells when a positive human Blastomyces serum and Rabbit-fluor are added. A bright greenish-yellow fluorescence which stains the cytoplasm diffusely is seen: this tends to make the cells appear larger than normal. The striking appearance of the positive reaction was seen through dilutions comparable to the complement fixation titer of the serum (1:256). Normal human serum and Rabbit-fluor failed to give this reaction; the fluorescence in this case was concentrated in the cytoplasmic inclusions of the cells (Fig. 6).

Reactions with fluorescein-labeled human gamma globulin. A sample of the immune human gamma globulin used to immunize rabbits was conjugated with fluorescein-isocyanate and used as an additional control. It failed to cause specific fluorescence with *H. capsulatum* or *B. dermatitidis* cells but did ring *C. albicans* cells brightly in what is considered a positive reaction. This unusual result would indicate that gamma globulin (American Red Cross) contains a substantial amount of antibody reacting with *C. albicans*. A positive agglutination obtained with human gamma globulin and *C. albicans* yeast cells confirms the presence of such antibody in this material.

Discussion. In the present report all fungi examined in unstained preparations fluoresced with either a bluish-gray or light-greenish hue. It was impossible therefore to have a perfectly negative control composed of non-fluorescing organisms. It subsequently was found however, that when specific human serum and a fluorescein-labeled rabbit antibody to human gamma globulin were applied to the organisms, a characteristic fluorescing reaction took place with each fungus which clearly allowed the identification of specific antibody. These phenomena were fluorescent ringing of cells in the case of *C. albicans* and *H. capsulatum*, fluorescing capsular opacity with *C. neoformans*, and a diffusely even staining, bright fluorescence of hyphal elements in *B. dermatitidis*. The technic used here was primarily an attempt at the detection of antibody in sera from patients with suspected mycotic infections. In the case of blastomycosis, histoplasmosis, and candidiasis it would not seem to have any superiority over recognized serologic tests such as complement fixation or agglutination tests since the fluorescent titer in each case equalled the known serologic titer of the serum; however, in one disease, cryptococcosis, this technic may prove of value. Serum from a patient with cryptococcosis was tested unsuccessfully for presence of antibodies using the polysaccharide-coated resin particle test described by Evans(15), the capsular reaction test of Neill *et al.*(16) and the complement fixation test of Fischer and Labzoffsky(13). A positive test was obtained, however, with the fluorescent antibody technic suggesting that it is a more sensitive test.

Normal human sera were found to react with *H. capsulatum* cells in the fluorescent antibody technic described here; this activity could not be characterized as a cross reaction with either *C. albicans* or sheep red blood cells. It is possible that another antibody in

normal serum cross reacts with *H. capsulatum* cells or that a low titer of histoplasma antibodies is present and not detectable in the complement fixation test.

Summary. Antibodies in sera from patients with systemic mycoses were demonstrated by the indirect method of staining with fluorescent antibody. In spite of a general autofluorescence of pathogenic fungi, addition of human antiserum and a fluorescein-labeled rabbit antihuman gamma globulin resulted in characteristic fluorescing reactions. In the case of cryptococcosis antibodies were demonstrated by the fluorescent technic but not by other serologic methods.

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Comparison of Opacimetric and Hematocrit Methods in Measurement of Mitochondrial Swelling.* (23966)

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Study of the osmotic properties of isolated mitochondria is undeveloped in comparison with the maturity of similar studies of the red blood cell. The opacimetric method has been most widely used in mitochondrial studies for it is simple and lends itself readily to kinetic studies of volume changes. For the erythrocyte, the results obtained with the opacimetric method are generally in good agreement with the results of a variety of other methods which depend on different properties of the red cell system(1). For mitochondria, comparisons of the opacimetric method with others have been reported for only 2 alternative technics. Tedeschi and Harris(2) have shown that the optical density of mitochondrial suspensions is inversely proportional to the mitochondrial volume measured by photomicrography. The per cent dry weight of mitochondrial pellets

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