It has been shown that the pressor effect of renin in rats depends upon the release of angiotensin I, which is converted to angiotensin II by circulating converting enzyme(9). This suggests that the effect of renin on adrenal steroidogenesis is mediated through angiotensin II. Thus, angiotensin II was taken in this experiment as representative of the renin-angiotensin system. Rapid inactivation of angiotensin II occurs in blood and tissues(10), and this might explain its failure to deplete adrenal ascorbic acid when given as a single injection. However, infusions of angiotensin II did not deplete ascorbic acid, so that rapid inactivation alone will not explain the lack of effect of angiotensin II. Transient bradycardia and apnea in all rats receiving injections of angiotensin served as evidence for its biologic activity. Thus, it is clear that ACTH and angiotensin II differ in their effect on rat adrenal ascorbic acid.

Although sensitivity of the assay has decreased somewhat by 48 hours after hypophysectomy, depletion of rat adrenal ascorbic acid by 0.37 mU of ACTH is still clearly demonstrable by this procedure(11). On the other hand, there is no appreciable increase in secretion of corticosterone into the adrenal vein of the dog with 0.5 mU of ACTH(12). Therefore, a dose of crude kidney extract which could stimulate corticosteroid secretion in the dog would, in our assay, deplete rat adrenal ascorbic acid if the active principle were ACTH alone. The assay can thus be used to distinguish renin from bound ACTH (5) in such extracts.

Summary. 1. ACTH and angiotensin II clearly differ in their effects on adrenal ascorbic acid in hypophysectomized rats. ACTH depletes adrenal ascorbic acid, whereas angiotensin II does not. 2. This difference can be used to determine the presence of ACTH in biological fluids or extracts which stimulate steroidogenesis by the adrenal cortex.

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Detection of Circumoval Precipitins by the Fluorescent Antibody Technic. (27748)

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Fluorescent antibody technics have been applied to the serological diagnosis of infections with *Schistosoma mansoni*(1). Cercariae preserved in 10% formalin were incubated with serum from known cases and then treated with fluorescent labeled anti-human globulin. Brilliant fluorescence was observed when cercariae were exposed to serum from infected cases and none with control sera.

The circumoval precipitin reaction observed when eggs of *Schistosome mansoni* are incubated in serum from individuals infected

with this parasite is highly sensitive and specific(2,3). The antigen-antibody reaction results in precipitate formation around the egg membrane. It was of interest to determine whether such circumoval precipitate could be detected by the fluorescent antibody technic (F.A.T.).

Materials and methods. Source of eggs of Schistosoma mansoni *and of immune serum.* Fresh eggs employed were isolated from the liver of previously infected mice according to the method of Ritchie(4). Immune sera were obtained from humans with known infections as confirmed by the presence of schistosome eggs in stools or rectal mucosa.

Fluorescent antibody technic. Immune sera with marked positive circumoval reaction were pooled and conjugated with fluorescein isothiocyanate. Globulins were prepared from pooled serum treated with an equal volume of cold saturated ammonium sulfate and allowed to precipitate at 10°C for 30 minutes. The precipitate and supernatant were thoroughly mixed and centrifuged for 1 hour at 3,000 $\times$ *g* in a refrigerated centrifuge. The precipitate was further treated with 40 ml of cold half-saturated ammonium sulfate and recentrifuged as above. Precipitation and centrifugation was performed several times. The globulins were dissolved in cold distilled water to bring the volume up to 10 ml and dialyzed against 0.85% sodium chloride with frequent changes of the saline until the dialysate was negative for the sulfate ion.

Total protein was determined by the Biuret method(5). The globulins in solution were labeled with fluorescein isothiocyanate as described by other investigators(6,7).

A globulin fraction was also obtained from a pool of normal sera from individuals not infected with *Schistosoma mansoni* and labeled with fluorescein isothiocyanate as done with the fraction from the infected persons.

Conjugates prepared from the immune and normal sera were tested for absence of denaturation by paper electrophoresis (rapid method)(8), and for antigenic reactivity by testing against human anti-globulin in agar-gel diffusion slides using the technic of Yakulis and Heller(9).

The direct fluorescent antibody technic was performed using a suspension of washed eggs as the antigenic substance and the labeled globulin as antibody. Drops of the labeled globulin were placed on a 3 $\times$ 1″ slide containing about 100 eggs; covered with a cover slip and sealed with vaseline, then incubated at 37°C overnight. The labeled globulin was also added to eggs in 13 $\times$ 100 mm test tubes and incubated at 37°C overnight. After incubation, the eggs from the test tubes were washed 3 times with phosphate-buffered saline at pH 7.5 to remove excess fluorescein; then mounted on slides with cover slips sealed with vaseline. The slide preparations were examined under a Reichert-Zetopan Research microscope using an Osram HBO-200 mercury vapor lamp with a combination of a primary (BG-12) with a heat absorbing filter; and a secondary (OG-1) filter to provide the optimum conditions to observe the fluorescence of fluorescein isothiocyanate. A darkfield condenser was employed to observe and photograph using an apochromatic 20 $\times$ objective and an 8 $\times$ eye-piece.

For the indirect fluorescent test(10), eggs were exposed overnight to sera from infected individuals and then washed 3 times with buffered-saline to remove excess antibody. Labeled rabbit antihuman-globulin was added, and the suspension incubated from 2 to 4 hours. The eggs were then examined microscopically for fluorescence.

Results. A total of 30 sera from individuals infected with *Schistosoma mansoni* were tested for fluorescent circumoval precipitins by the indirect method. The precipitate observed showed the characteristic greenish-yellow fluorescence. Best results were obtained in the wet slide preparations in which the fluorescence had not been washed (Fig. 1, 2). Apparently the autofluorescence of the eggs is quenched by excess fluorescent dye, thus making it easier to detect the specific fluorescence of the precipitate. No fluorescence was observed inside the eggs or in the empty shells.

Sensitivity of the test is enhanced by the ease with which the fluorescent circumoval bodies can be distinguished even when they are small and concealed behind the eggs.

The eggs from the egg-serum mixtures in test tubes showed some autofluorescence when washed with phosphate-buffered saline. However, the characteristic greenish-yellow fluorescence was distinctly observed in the precipitate around the eggs (Fig. 3).

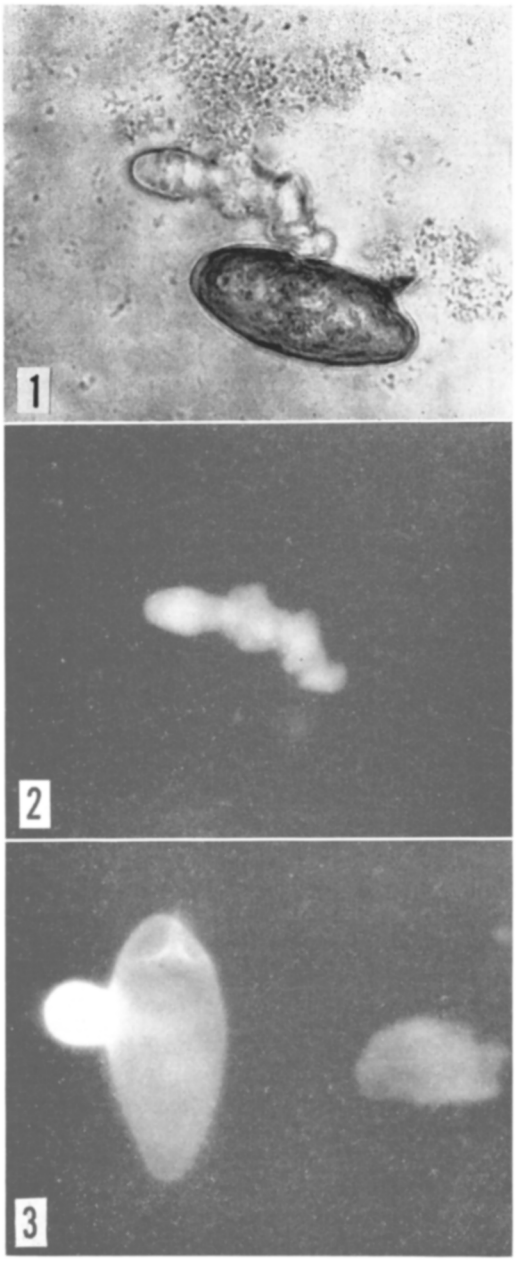

FIG. 1. Circumoval precipitin reaction by fluorescent antibody technic (4+). Excess fluorescein not washed off. Preparation observed under tungsten light 160×.

FIG. 2. Same preparation as Fig. 1. Observed under fluorescent light.

FIG. 3. Circumoval precipitin reaction by F.A.T. (2+). Excess fluorescein washed off. Preparation observed under fluorescent light 160×.

Twenty sera from individuals not infected with *Schistosoma mansoni* and 25 sera from children heavily parasitized with intestinal helminths, such as *Ascaris lumbricoides, Trichuris trichiura,* and hookworm, but not with *Schistosoma mansoni,* were tested using the indirect method. No fluorescent bodies were observed, and although some small blebs could be seen with some sera, these did not show the characteristic fluorescence of circumoval precipitate.

Discussion. Results indicate that the fluorescent antibody technic can be applied to the circumoval precipitin test with great ease and advantage. The test using fluorescent labeled anti-human globulin against eggs exposed to unknown serum (indirect test) has been shown to be one of the simpler and easier of the tests performed. Both the direct and indirect fluorescent antibody technics are equally reliable for this purpose. The indirect test seems the most practicable since it is not necessary to tag each unknown serum. Since anti-human globulin tagged with fluorescein isothiocyanate is available commercially and lyophilized schistosome eggs may be used(11) the test may conveniently be used as a routine diagnostic procedure. Since small volumes of sera are used (0.05 cc), sufficient blood can be obtained from finger punctures *via* capillary tubes, rendering an additional convenience of the test for field studies as also indicated for cercarial F.A.T. by use of finger blood taken on filter paper(12).

Since the precipitate is the only substance which fluoresces, the test also helps in distinguishing non-specific reactions and artifacts from true positive reactions.

Summary. 1. Whole sera and globulins from the blood of individuals infected with *Schistosoma mansoni* were labeled with fluorescein isothiocyanate. Eggs of *S. mansoni* were incubated in these labeled globulins with subsequent formation of circumoval precipitate. Examination of this precipitate by the direct and indirect FA technics revealed

a bright greenish-yellow fluorescence. 2. The fluorescent circumoval precipitin technic demonstrated excellent diagnostic performance. Positive reactions were obtained in 30 sera from individuals with known infections. Negative reactions were obtained in 20 sera from individuals not infected with *S. mansoni* and in the sera of 25 children, heavily parasitized with intestinal helminths, but not with *S. mansoni*. 3. The sensitivity and specificity of the circumoval reaction, in addition to the advantages introduced by the fluorescent antibody technic, might make the fluorescent circumoval precipitin technic highly reliable for serological diagnosis of schistosomiasis.

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Propagation in Tissue Culture of Cytopathic Agents from Patients with Rubella-Like Illness.* (27749)

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Recognition of the association between maternal rubella and congenital anomalies of the human fetus has stimulated increasing interest in a clinical entity previously considered benign. Although a viral etiology of rubella was suggested by the demonstration of the infectiousness for human volunteers of filtered inocula(1,2), reports of transmission to monkeys(3,4) and of propagation of the agent in the embryonated hen's egg(4) or tissue culture(5) have not been confirmed. Thus, the nature of the etiologic agent remains uncertain. The lack of specific diagnostic procedures has made evaluation of rubella-like illness in the pregnant female a perplexing problem. Similarly, interpretation of reports such as the isolation of adenoviruses from individuals with rubella(6) has, of necessity, been speculative.

In October, 1960, attempts to isolate an agent from the urine of a boy with an exanthematous illness resulted in recovery of a virus characterized by unique cytopathic activity in primary human amnion cell cultures. The attendant circumstances led to investigation of an institutional outbreak of rubella at Phillips Exeter Academy, Exeter, N. H., in February, 1961. Similar viruses were recovered from the urine or blood of 2 patients; this was made possible by Dr. James T. Heyl, Medical Director, and our indebtedness to him for clinical data and for collection of sera is gratefully acknowledged. During the

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