were given barbital sodium, 160 mg per kg, and half of them received Aresklene 400, 50 mg per kg, 2 minutes before the barbiturate. The onset of hypnosis averaged 14.8 minutes for the controls and the average time with Aresklene 400 was 2.4 minutes. Five matched pairs of dogs were given barbital sodium, 140 mg per kg with one of each pair receiving in addition Aresklene 400, 25 mg per kg, 1 minute before the barbiturate. The time from an injection until the dog would not stand up was an average of 12.8 minutes for the controls and 2.0 minutes for those receiving Aresklene 400. Ten other surface active agents in doses of one-half the LD_{50} were then tested for the effect on the onset of hypnosis in mice (Table V). Female mice were used for this experiment. Six of the agents showed a decrease in time of onset of hypnosis which was probably significant.

Summary. 1. The duration of hypnosis of pentobarbital sodium was increased for mice by all 11 surface active agents tested.

2. Aresklene 400 was selected for study on other animals. It increased the time of hypnosis due to pentobarbital in rats and rabbits but not in dogs.

3. Thiopental sodium showed an increase in effect with some of the surface active agents but not all.

4. Aresklene 400 increased the activity of thiopental apparently by decreasing the rate of destruction of thiopental in the body.

5. The onset of hypnosis but not the duration of hypnosis was influenced by Aresklene 400 with sodium barbital. Aresklene 400 decreased the time of onset of hypnosis when given with barbital sodium in mice, rabbits, and dogs but not in rats. Six of 10 other agents studied only in mice had the same effect on onset of hypnosis.

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Serological Tests on Soluble Antigens from Mice Infected with *Cryptococcus neoformans* and *Sporotrichum schenckii.** (17742)

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The purpose of the present paper is to show that soluble antigens of the fungus agents occur in various tissues and body fluids of mice infected with *Cryptococcus neoformans* and *Sporotrichum schenckii,* and that these antigens are readily detectable by direct serological tests analogous to those that have been used for the detection of soluble antigens in various bacterial, rickettsial and virus infections.

Methods. Thirteen strains of *Cryptococcus neoformans,* 4 strains of *Sporotrichum schenckii;* and, for control purposes, a strain of *Blastomyces dermatitidis* were used. The *Cryptococcus* included a representative strain of each of the types (A, B, C) recently reported by Evans(1), and also the Curtis strain which Benham(2) found to differ somewhat in agglutinating properties from other strains. The mice were infected by intraperitoneal injections, and the materials to be tested were obtained from animals that were obviously ill or that had recently died from the infection.

The antigens employed for serological precipitation tests were wash fluids from the peritoneal cavity and extracts made from the liver, spleen, testes and lungs. The peritoneal wash fluids were formalinized, and cleared by prolonged centrifugation. The organs were ground in mortars, suspended in 5 ml of salt solution, shaken for about 5 minutes in tightly stoppered test tubes, then heated 30 minutes

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2. Benham, R. W., *J. Infect. Dis.,* 1935, v57, 255.

at about 50°C and finally boiled for 10 minutes (preliminary experiments had proved that the reactive constituents were relatively heat stable). Centrifugation of the heated liver and spleen suspensions yielded clear supernatant fluids, but shaking treatments with chloroform were required for the lungs and testes.

All of the antigen solutions were tested, in a series of dilutions ranging from 1:5 to 1:1600, against at least one *Cryptococcus* and one *Sporotrichum* rabbit antiserum; in the case of the peritoneal washings and the liver extracts from the *Cryptococcus*-infected mice, 6 different *Cryptococcus* antiserums (produced by immunization with separate strains) were used. All the antiserums had relatively high homologous agglutinating titres and were used in 1:5 or 1:10 dilutions for the precipitation tests. For controls, antiserums of *Candida albicans* and of R pneumococci and normal rabbit serums were included in all the tests.

Results. The peritoneal wash fluids and the tissue extracts from all the mice infected with *Cryptococcus neoformans* and with *Sporotrichum schenckii* gave precipitation with the antiserum of the homologous fungus but not with any of the other serums included in the tests. Additional evidence of the specificity of the reactions was furnished by the fact that similar materials from mice infected with *Blastomyces dermatitidis* and from normal mice failed to react with the *Cryptococcus* and the *Sporotrichum* antiserums.

It is evident that the fungus antigens were present in considerable amount since positive reactions were obtained with 1:400 or higher dilutions of the solutions prepared from most of the mice infected with *Cryptococcus* and with 1:25 or 1:100 dilutions of the solutions from the mice infected with *Sporotrichum*. The precipitation was always apparent within 5 to 10 minutes at room temperature, although there was an increase in the intensity of the flocculation, and usually also in the maximum reacting dilution after incubation for 2 hours at 37°C plus icebox storage.

Soluble antigen was found also in the blood of *Cryptococcus*-infected mice. The animals were chloroformed and blood from the heart was citrated and centrifuged immediately. The plasma gave precipitation, in dilutions ranging from 1:5 to 1:25, against *Cryptococcus* antiserums and was non-reactive against control serums. Similar experiments were not made with *Sporotrichum*-infected mice.

In addition to the described precipitation tests, positive Quellung(3) reactions were obtained with the *Cryptococcus* and *Sporotrichum* cells contained in the sediments from the centrifuged peritoneal washings and in unheated samples of the tissue suspensions. The Quellung reactions made the capsules of both these fungi much more prominent and sharply defined, which facilitated the microscopic detection of the fungus cells and their differentiation from blood and tissue cells. With some of the *Cryptococcus* preparations which contained especially large amounts of free soluble antigen, washing of the fungus cells was important in order to get prominent Quellung reactions.

Since all strains of *Cryptococcus neoformans* are not serologically identical(1,2), our experiments are not comprehensive enough to prove that the antigens of all strains of that species could be detected with a single antiserum. However, the materials from mice infected with all the strains we tried gave both precipitation and Quellung reactions with all our antiserums, which had high homologous titres; the only difference observed was that lower dilutions of antiserum were required in the case of the materials from the mice infected with Evans'(1) type C strain.

Discussion. The present demonstration of soluble antigens in the tissues of mice infected with *Cryptococcus neoformans* and with *Sporotrichum schenckii,* is of academic interest because of the general lack of information on the presence and distribution of soluble antigens in hosts infected with mycotic agents.

Whether or not direct serological tests would be useful in the study of materials from human cases of cryptococcosis and of sporotrichosis can be determined only by trial in hospital laboratories. An application especially worthy of trial would be the test of spinal fluid from cases of suspected cryptococcal meningitis

3. Neill, J. M., Castillo, C. G., Smith, R. H., and Kapros, C. E., *J. Exp. Med.,* 1949, v89, 93.

(torulosis). With that material, one could try a combination of tests for serological precipitation upon the supernatant and of tests for Quelling reactions upon the sediment obtained by centrifugation of the spinal fluid, in a manner analogous to the procedures employed with spinal fluids from cases of pneumococcal, meningococcal, and *Hemophilus influenzae* infection.

It should be noted that antiserums of reasonably high potency† are essential for the optimum results both in precipitation of *Cryptococcus* soluble antigens and in Quellung of *Cryptococcus* capsules. Some cross-reactions occur between *Cryptococcus neoformans* and *Torulopsis rotundata,* various other encapsulated saprophytic yeasts, and type 2 pneumococci. These serological inter-relationships, which will be reported in a later paper, would have to be taken into account in the interpretation of precipitation reactions, but could readily be controlled.

Summary. The experiments showed that soluble antigens of the fungus agents occur in the tissues of mice infected with *Cryptococcus neoformans* and with *Sporotrichum schenckii,* and that these antigens are readily detectable by tests with appropriate antiserums. Soluble antigen was found also in the blood of *Cryptococcus*-infected mice.

† It has been our experience that adequate *Cryptococcus* antiserums can be produced with greater regularity and promptness than is indicated in the literature, provided closely spaced injections of relatively large doses of weakly encapsulated strains are employed for immunization(4). The antiserums could readily be produced in the usual hospital laboratory. However, for trials of practical applications of the antiserums, we could supply small samples to clinical workers who have access to materials from cases of human cryptococcosis.

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Variations in Proteolytic Activity of Serum of Animals Including Man.* (17743)

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Under normal conditions the blood plasma or serum of man shows little spontaneous proteolytic or fibrinolytic activity, although Dastré(1) early indicated that such enzymes were present. However, as shown by Delezenne and Pozerski(2) and others(3,4), treatment with chloroform produces definite proteolytic activity. Whether this be due to removal of inhibitor or true activation of the precursor has been a disputed subject. Yamakawa produced activity by use of other substances, including alcohols(5). The most consistent, definite and rapid activator has been streptokinase (6-8), but Gerheim *et al.* have also found an active staphylokinase(9). Grob(10) has

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