rises with anaphylaxis and uniform falls with the pyrogenic reaction. (3) The mechanism of these hematologic changes is not touched upon in the present study. (4) It is concluded that the leukocyte changes after anaphylaxis are not due to pyrogen contamination.

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Serologically Reactive Material in Spinal Fluid, Blood, and Urine from a Human Case of Cryptococcosis (Torulosis).* (18924)

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The purpose of this paper is to report the occurrence and the serological detection of reactive substances ("soluble antigens") in the spinal fluid, blood, and urine of a patient with cryptococcal meningitis; and also to describe the serological reaction of the capsules of the *Cryptococcus* cells contained in the sediment of the spinal fluid.

Materials and methods. The materials were from a patient with cryptococcal meningitis at Okmulgee, Okla.; a clinical report of the case will be published elsewhere by Dr. McCauley(1). The samples were obtained about 5 weeks after the patient's admission to the hospital and about a week before the fatal outcome; the spinal fluid was taken 2½ days earlier than the blood and the urine.

The spinal fluid was clear in gross appearance and the urine (a first morning sample of about 200 ml) proved normal in routine chemical analysis. *Cryptococcus* cells were present in considerable number in the spinal fluid, but none were demonstrable in either the blood or the urine by culture or by microscopic examination. The samples were transported to New York City for the serological tests.†

The serological tests on the spinal fluid, blood serum, and urine were made with supernatants cleared by centrifugation. In the case of the urine, tests were also made with solutions of alcohol precipitable material, prepared by 3 precipitations with 2.5 volumes of alcohol and extractions of the precipitate with water; the final solution, made in one-fourth the original volume, was water clear and more suitable for serological tests than the original urine. The precipitation test mixtures consisted of equal volumes (0.2 ml) of antigen and of serum; observations were made after 1½ to 2 hours at room temperature, and again after 3 days storage in the icebox. The complement fixation tests were made with similar mixtures of antigen and of serum plus 2 units of complement (0.2 ml); after overnight storage at 5°C followed by a 10 minute period in a 37°C water bath, 0.2 ml of sensitized sheep r.b.c. were added to the mixtures which were then put at 37°C for a one-hour hemolysis test period.

Experimental. A series of dilutions of the patient's fluids were tested for both precipitat-

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1. McCauley, D. W., *Okla. State Med. J.* in press.

† Merthiolate (0.01%) was added to all of the samples immediately after they were taken from the patient, and an equal volume of 95% alcohol was added to the major portion of the urine. The blood and the urine samples were maintained below 5°C throughout the entire time of transport, but the spinal fluid which was shipped separately by air mail was not packed in ice. Although the merthiolate (0.01%) did not kill all of the cryptococci in the spinal fluid, it was apparently sufficient to have prevented any growth during the transport of the sample, since cultures of the strain isolated failed to grow when large inoculums were tried in broth and in Berkefeld filtered spinal fluid (from other patients) to which either 0.01 or 0.001% merthiolate had been added.

TABLE I. Serological Reactions of Spinal Fluid, Blood Serum and Urine of a Cryptococcosis Patient and of Solutions of Purified *Cryptococcus* Polysaccharides.

Materials	Titres* of the materials in tests against *Cryptococcus* antiserum			
	Precipitation		Complement fixation	
	1:5 serum	1:20 serum	1:40 serum	1:160 serum
Patient's spinal fluid	100	100	400	400
" blood serum	200	200	800	800
" urine	U	U	20	10
Control spinal fluids, serums, urines	0	0	0	0
Solutions of *Cryptococcus* polysaccharide, containing 0.01 mg/ml	50	50	400	200

* The titres represent the maximum dilution of the materials which gave positive reactions when mixed with an equal volume of the antiserum. U represents a positive reaction obtained only with undiluted material.

ing and complement-fixing capacities against a potent *Cryptococcus* antiserum and also against a pool of normal rabbit serums and the pre-immunization (normal) bleeding of the rabbit from which the test antiserum had come. Two samples of normal human serum, 10 samples of spinal fluid, and 6 morning samples of urine obtained from patients in the New York Hospital with illnesses other than cryptococcosis, and a solution of alcohol precipitable material prepared from one normal urine were included as controls. For comparison, solutions of 2 different preparations of purified *Cryptococcus* polysaccharides,‡ each containing 0.01 mg per ml, were tested in the same series of dilutions as those employed with the patient's fluids; one of these polysaccharides had been prepared from the strain which had been employed to produce the antiserum used in the experiment.

The dilutions of the rabbit serums were 1:5 and 1:20 for the precipitation and 1:40 and 1:160 for the complement fixation tests; these dilutions represented, respectively, about 1/64 and 1/16 of the maximum titre of the antiserum in preliminary standardization experiments against solutions of purified *Cryptococcus* polysaccharides. An antiserum sufficiently potent to permit the employment of relatively high serum dilutions for the complement fixation tests was utilized in order to avoid the possibility that normal rabbit serum in low dilution might have some complement-fixing capacity with the patient's serum.

Results. All the fluids from the patient reacted with the *Cryptococcus* antiserum (Table I). In the case of the spinal fluid and blood serum, 1:5 dilutions gave strong flocculation immediately, 1:100 dilutions gave definite clouding within a few minutes, and 1:400 to 1:800 dilutions gave positive complement fixation; in contrast, the control spinal fluids and blood serums from other persons proved entirely negative even when tested undiluted. Further evidence that the amount of reactive material contained in the patient's spinal fluid and blood was relatively great is that both of them precipitated in slightly but definitely higher dilutions than did solutions that contained 0.01 mg per ml of purified *Cryptococcus* polysaccharides. All of the tests with the control rabbit serums were negative and are omitted from the table.

The large amount found in the blood is of particular interest because no *Cryptococcus* cells were demonstrable in the sample either by culture or by microscopic examination. This finding is analogous to the occurrence, in some cases of pneumonia, of pneumococcal

‡ The polysaccharides were kindly supplied by Dr. Edward J. Hehre and coworkers. One was prepared from a culture grown in a synthetic fluid medium(2) and the other from washed *Cryptococcus* cells that had been grown on agar(3).

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3. Hehre, E. J., and Abrahams, I. Unpublished manuscript.

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5. Neill, J. M., Abrahams, I., and Kapros, C. E., *J. Bact.*, 1950, v59, 263.

reactive substances in blood samples that contain no demonstrable pneumococci(6), and indicates that *Cryptococcus* reactive substances (probably capsular polysaccharide material) may be liberated into the blood from the site or sites of the infection without being accompanied by the fungus cells. The concentration of reactive material in the morning sample of urine was considerably less than that in the blood sample obtained at approximately the same time, which suggests that the major portion of the reactive *Cryptococcus* material contained in the blood of this patient consisted of substances that do not readily pass through the kidney. It is worthy of note that the lower concentration in the urine in comparison to the blood is different from the relationship that occurs in at least some cases of pneumococcal lobar pneumonia(6-8).

The reactive substances in all the fluids of the present patient were relatively heat stable, since boiling for 10 minutes caused no loss in reactivity. Moreover, absorption experiments were made which showed that treatment with solutions of purified *Cryptococcus* capsular polysaccharide‡ removed the capacity of the antiserum to react with the patient's spinal fluid and blood serum. These results furnish evidence of the immunological specificity of the reactions obtained with the patient's fluids and also strongly indicate that the reactive substances represent capsular polysaccharides derived from the fungus with which the patient was infected.

Capsular reactions. The *Cryptococcus* cells from the sediment of the centrifuged spinal fluid gave Quellung-like reactions§ in moist mounts containing antiserum. These reactions of the capsules, (Fig. 1 and 2), are of academic importance because there is no previous evidence in the literature that capsules of *Cryptococcus* from human infection contain serologically reactive constituents. The capsular reactions seem also of some practical importance because the greater visual prominence of the fungus cells in preparations containing a reactive antiserum (Fig. 1) should aid in the microscopic search for *Cryptococcus* cells in materials from patients and in the distinction of the fungus cells from lymphocytes. The capsules also become evident in ordinary India preparations(9) but the serological preparations in our opinion are less likely to be complicated by artefacts. In practice, of course, the Quellung test would be useful only with materials containing sufficient numbers of the fungus cells to be detected microscopically.

The *Cryptococcus* cells in Fig. 1 and 2 had been washed once with salt solution to remove the excess of free soluble antigen. In similar preparations of unwashed cells, long strands and amorphous masses (presumably soluble antigen precipitated by the antiserum) occurred, especially around the clumps of agglutinated cells, which simulated the "reticulated" appearance described by Frisch(10) for the Quellung reaction of some type 3 pneumococcal sputums. The same sort of phenomena was observed also in Quellung tests upon the peritoneal fluids of *Cryptococcus*-infected mice(11).

Comment. The data are of academic importance because they present the first demonstration of the occurrence of serologically reactive *Cryptococcus* substances in body fluids of a human infected with that fungus. The samples tested were obtained at a late and grave stage of the patient's infection and, *a priori*, might be expected to contain larger

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§ The term Quellung-like is used for convenience and also because of the similarity in immunological principle between the reactions of the capsules of *Cryptococcus* and the Quellung reactions of pneumococci and other bacteria. However, as pointed out previously(4), the major part of the visible phenomenon in the case of *Cryptococcus* seems to be due to a change which makes the capsule more readily seen rather than to any great increase in the size of the capsule.

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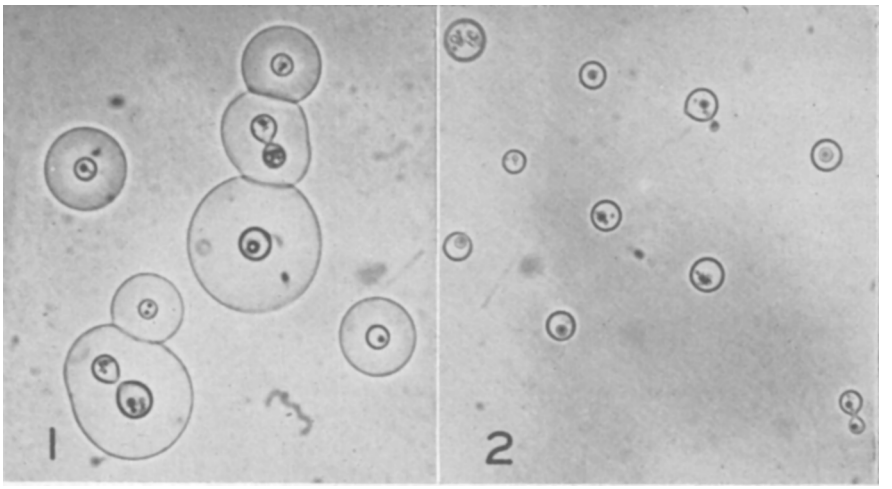

FIG. 1. *Cryptococcus* cells from the sediment of the patient's spinal fluid plus *Cryptococcus* rabbit antiserum. FIG. 2. *Cryptococcus* cells from the same suspension plus normal rabbit serum. Note the prominence and the sharply outlined borders of the capsules§ in Fig. 1 whereas only faint and equivocal halos are evident in the control mount illustrated in Fig. 2. The magnification of the illustrations is × 560. The cells in Fig. 1, including the capsules which comprised from ⅔ to ¾ of the total measurement, range from about 160 to 300 μ in diameter, but larger cells, some of which were as much as 475 μ in diameter were not uncommon in the sediment from the spinal fluid.

amounts of reactive material than would samples from earlier stages or milder forms of cryptococcal infection. However, the relatively large amounts of the reactive material that occurred in this case and the ease and speed of the serological detection suggest that tests upon body fluids of patients may have some practical importance in the study of human cryptococcosis. That question, however, can be determined only by trials with materials from a reasonably large number of patients. We are now attempting to collect material for that purpose, and would appreciate receiving samples from clinical workers who encounter cases of cryptococcal infection.

Summary. Serologically reactive substances ("soluble antigens") which were readily detectable by precipitation and by complement fixation with *Cryptococcus* antiserum were found in the spinal fluid, blood serum, and urine of a patient with cryptococcal meningitis. Absorption of the antiserum with solutions of purified *Cryptococcus* polysaccharide removed its capacity to react with the fluids of the patient. The reactive substances in the patient's fluids were relatively heat stable and it is probable that they represent capsular polysaccharides of the fungus with which the patient was infected.

Serological reactions of the Quellung type were obtained with the capsules of *Cryptococcus* cells from the centrifuged sediment of the spinal fluid.

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Influence of Female Hormones on Motility of Cat's Uterus and Its Responses to Oxytocics. (18925)

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The uteri of cats chosen at random are frequently quite insensitive to α-hypophamine (Pitocin) and synthetic oxytocics. To overcome this limitation an extended study was