occasionally, minimal to moderate periglomerular fibrosis. Limited alterations were noted in the arteries and arterioles. For the most part these latter were sclerotic changes associated with aging processes and were similar in all groups studied. In a few scarred areas, perivascular fibrosis and mononuclear cells within the vessel wall were noted. These infrequent findings were thought to represent direct extension of the parenchymal inflammatory process to the wall of the arteriole.

Discussion. These results are consistent with most reports concerning the relationship between experimental pyelonephritis and hypertension(2,3,4). Exception to this was noted by Spitznagel and Schroeder(1) who produced pyelonephritis in the rat by intravenously injecting *E. coli* into animals with unilateral ureteral obstruction. Elevated blood pressures were recorded in many of these animals and the authors noted the occurrence of "vascular changes" in the kidneys. This finding may account for discrepancies with other published data. Other investigators have commented upon the absence of significant arteriolar nephrosclerosis and have postulated that this could explain the failure to note hypertension. This was noteworthy in the experiments described here.

Our observations and those recorded in the literature suggest that pyelonephritis does not cause hypertension in experimental animals despite the occurrence of progressive infection, marked tissue destruction and uremia. Whether this represents an animal specie variation is not known. It has suggested an alternative hypothesis to explain the clinically reported increased association between pyelonephritis and hypertension; that hypertension *per se* predisposes to renal infection. Experimental confirmation of this in rats has been reported(8).

Summary. Chronic, progressive, non-obstructed, enterococcal pyelonephritis in the rat is not associated with hypertension. It is suggested that this may be related to the absence of significant arteriolar nephrosclerosis.

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An Antigen for Detection of Hypersensitivity to *Cryptococcus neoformans.* (26977)

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Cryptococcus neoformans exists as a saprophyte in soils of various areas(1,2). The fungus has also been isolated from many specimens of pigeon droppings and nests(3-5), although the organism was not cultured from the internal organs of 20 pigeons(3). The possibility arises, therefore, that a reservoir for human infection can exist in pigeon excreta, for "pigeon" strains and "human" strains possess similar properties of virulence in mice(5).

Human cryptococcosis is world-wide in distribution(6). Most proven cases exhibit a severe meningitis, although pulmonary infec-

tion has been rarely found. It has been postulated that for every proven case of coccidioidomycosis there are approximately 2,000 undetected persons harboring the fungus. Perhaps cryptococcosis resembles coccidioidomycosis in this respect. If so, in New York City alone 5,000-15,000 persons could be infected with *C. neoformans*. As a means of proving or disproving this possibility, an epidemiologic survey with an effective skin-testing antigen would be effective(7-9).

Cryptococcal antigens thus far, however, have not been reported to be active in detecting skin hypersensitivity. Antigens, primarily polysaccharide in nature, have been isolated, but have been used essentially in precipitation-type serologic tests(10-12). The development of an effective skin-testing antigen may provide information on prior sensitization with *C. neoformans* and accordingly on the prevalence of undetected cryptococcosis. The present paper describes a method of extracting an antigen which elicits delayed reactions in guinea pigs previously infected with *C. neoformans*.

Materials and methods. Fungus. Two isolates (BET and MAG) of *C. neoformans* were used which originally had been obtained from patients with cryptococcosis. The fungus was maintained at room temperature on Sabouraud's glucose agar. For inoculation into animals or for preparation as antigen, the yeast cells were grown in an amino acid medium at 37°C for 24-48 hours(13).

Animals. White or albino guinea pigs of 300 to 500 g in weight were used. For induction of hypersensitivity, the animals were injected intraperitoneally approximately a week apart with 2 doses each of 5×10^5 living cells of *C. neoformans* in broth. Three weeks to 2 months later, guinea pigs were tested intradermally on the sides or back with 0.1 ml of antigenic solution, and diameters of areas of induration measured 4 hours and 18 to 24 hours after injection.

Method of preparation of antigen. The cells of *C. neoformans* were separated from the growth medium by centrifugation at 3,000 RPM for 1 hour. After having been washed in distilled water until the supernate was clear, the live cells were forced through a tiny aperture of a pressure cell at 60,000 lb/sq. in. (14,15). Subsequent washing and centrifugation served to eliminate much of the capsular material, according to analysis of the sediment under the electron microscope. Pressing the fungus cell suspension through the fine aperture for a second time at a pressure of 60,000 lb/sq. in. caused extensive disruption. The cell mixtures were washed again with several changes of distilled water, and finally centrifuged at 30,000 RPM for half an hour. The resulting sediment was examined under the electron microscope and consisted essentially of fragments of cell walls. This sediment was then resuspended in 50 ml of 0.1 M glycine buffer, pH 10.0, and held with occasional stirring for 2 days at 4°C. The suspension was centrifuged at 3,000 RPM for half an hour, the sediment resuspended in buffer, stirred, and immediately recentrifuged. The combined supernates were neutralized with 1 N HCl, dialyzed against physiologic saline, and filtered through ultra-fine sintered glass. There was no detectable loss of activity on filtration.

Analytical methods. The biuret test(16) was preferred for protein analysis because of its specificity for peptide bonds. When the amount of protein was near the lower limits of significance, analysis with the bromsulphalein method (BSP) was added(17). Egg albumin was used as the reference standard for both tests. Carbohydrate was determined by the modified Molisch test(18), and referred to glucose as a standard.

Results. Analysis. Analysis of typical lots of antigen yielded the following information:

	MAG I	BET IV	BET V
Biuret Protein (as egg albumin)	.06 mg/ml	.11	.38 (.64% of weight of crushed, washed cells)
BSP protein (as egg albumin)	.009 "	.030	.052
Kjeldahl nitrogen	.10 "	.05	.09
Carbohydrate (as glucose)	.090 "	.177	.247
Mean dilution yielding 10 × 10 skin reaction	1/6	1/31	1/96

TABLE I. Areas of Induration in Guinea Pigs Infected with *C. neoformans* and Subsequently Skin-Tested with Soluble Antigen.

Infected with	Diameters at 24 hr after intradermal injection of .1 ml of			
	10 μg/ml	1.0 μg/ml	.1 μg/ml	.01 μg/ml
BET	17 × 18	14 × 14	7 × 7	—
	17 × 17	14 × 13	13 × 12	—
	18 × 18	18 × 18	14 × 12	—
	16 × 17	14 × 17	9 × 11	4 × 4
	24 × 23	13 × 12	13 × 11	7 × 7
	19 × 17	14 × 13	9 × 11	5 × 6
	14 × 14	10 × 12	11 × 10	0
		11 × 15	11 × 10	7 × 9
		11 × 11	11 × 10	3 × 3
MAG	20 × 20	14 × 14	9 × 10	0
	15 × 15	10 × 10	8 × 9	6 × 6
	14 × 18	13 × 13	8 × 9	8 × 9
	18 × 18	12 × 12	8 × 9	6 × 6
	16 × 18	13 × 15	11 × 10	8 × 9
	28 × 28		17 × 17	
	17 × 17		14 × 14	

Intradermal tests. Guinea pigs were tested intradermally with varying dilutions of the soluble supernate; doses were measured according to the amount of protein (biuret) (Table I). Of 3 different lots of antigen from the BET isolate the mean value of dilutions which induced at least 10 × 10 mm induration in infected guinea pigs represented about 0.4 μg of protein (biuret) per animal. Fifteen guinea pigs hypersensitive to *Candida albicans,* 20 hypersensitive to *Histoplasma capsulatum,* and numerous normal ones did not develop reactions to intradermal injection of any of the *C. neoformans* antigen preparations.

A soluble antigen was prepared from a second isolate of *C. neoformans* (MAG).* Complete cross-reactivity occurred between animals infected with one strain and skin-tested with the other.

Two lots of *C. neoformans* were killed with ether (2 parts ether to 1 part broth) or 1:5000 merthiolate without treatment in the pressure cell. Extracts obtained from these with 0.1 M glycine at pH 10 had much higher carbohydrate content, but were low in protein and had no skin-testing activity.

Discussion. A soluble antigen was prepared from *C. neoformans* by crushing the yeast cell, discarding the supernatant and then extracting the insoluble portion. After the first exposure to high pressure, examination of the cells under the electron microscope revealed that most of the capsules had been removed from the cells. After these cells had been further washed, a second exposure to high pressure followed by extraction with 0.1 M glycine at pH 10.0 produced a solution which contained a skin-testing antigen. This technique of disrupting the live cells before extraction yielded an antigen relatively low in capsular polysaccharide. When the cells were killed with ether or merthiolate and then extracted, the carbohydrate content was much higher but skin-testing activity was absent.

The skin-testing antigens, prepared separately from 2 isolates, reacted similarly in animals infected with either homologous or heterologous isolate. Obviously, further experimentation is required before the importance of strain differences can be properly evaluated.

The aforementioned antigenic complex of *C. neoformans* appears to be effective in detecting hypersensitivity in guinea pigs due to prior infection. Further studies are now in progress to ascertain the activity of the antigen in man and the possible presence of benign cryptococcosis in human populations.

Summary. An antigen has been prepared from *Cryptococcus neoformans* which induces delayed skin reactions in guinea pigs previously infected with this fungus. Cross reactions did not occur in animals infected with *Candida albicans* and *Histoplasma capsulatum.*

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Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury. (26978)

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This study is concerned with the role of infection in the delayed deaths of mice following thermal burns; it does not deal with the problem of shock encountered during the first 2 days following injury. Extensive studies with experimental animals exposed to irradiation or to traumatic shock indicate that a reduction in natural resistance to infection follows this type of injury(1-5). In patients with extensive burn injury, the initial traumatic shock may be followed later by shock accompanying bacterial invasion and generalized "burn decompensation"(6-9); although the injured tissues may suffer localized infection within the first 4 days, invasion of the deeper tissues seldom occurs before the 5th post-burn day(10,11).

The delayed mortality in mice following extensive burn injury has long been investigated in our laboratories(12,13). This mortality occurs during the first 3 weeks after injury in animals surviving the initial period of traumatic shock. Therapy with 0.9% NaCl solution, given subcutaneously or intraperitoneally in amounts equivalent to 15% of body weight, produces over 85% survival at the end of the 2nd day. The mortality rate rises rapidly during the first 10 days but may not reach its maximum (approximately 75%) until the 3rd week of injury. The toes, tails and skin of the burned area become gangrenous and may slough off. Food consumption decreases and the animals become emaciated. No significant changes have been observed in body temperature, in the circulatory system (hematocrit, bleeding volume and plasma proteins), or in blood NPN levels. None of the histopathological changes observed in lungs, liver, spleen, adrenals, pancreas and kidneys of burned mice have been adequate to explain the deaths(14). Our studies indicate that infection is an important factor in the cause of delayed deaths of burned mice. Moreover, Rosenthal(14) and Millican and coworkers(15,16) have demonstrated that some chemotherapeutic agents are effective in promoting the survival of these burned mice.

Methods. Female albino mice (NIH or GP strains), weighing 16-22 g were employed. Burn trauma was produced by standardized procedures previously described(12,13). Anesthetized animals were dipped to the axilla in water at 70°C and held there for 8 seconds. This degree of injury is rapidly fatal to over