FIG. 3. Nucleus largely filled by glycogen. Magnification = 6,000×.
FIG. 4. Nucleus containing 5 droplets of glycogen, one of which lies within a possible nucleolus. Magnification = 5,200×.
FIG. 5. Cytoplasm containing irregular, sharply circumscribed, moderately dense masses resembling glycogen in nuclei. Magnification = 2,933×.
FIG. 6. Typical nucleus in duplicate section after diastase digestion. Magnification = 4,800×.

observed (Fig. 5).

To determine whether diastase affects this material 5 μ, 1 μ and ultrathin sections were incubated at 37°C in 0.1% USP IX malt diastase in Lillie's pH 6.0 saline phosphate buffer for 1 hour(6,7). Examination of stained sections in the light microscope as well as ultrathin sections in the electron microscope reveal complete removal of the presumed nuclear and cytoplasmic glycogen. Often in nuclei a faint ring is left on the formvar film, suggesting the outline of a pre-existing deposit of glycogen (Fig. 6). Occurring in the cytoplasm are masses of material which are poorly demarcated, larger and uniformly less dense than the presumed glycogen particles (best seen in Fig. 5 and 6). These are not removed by diastase. Other cytologic features appear unaltered. Sections incubated in the buffer solvent without diastase show no effect upon the glycogen. Because of the ribonuclease-like activity demonstrated after prolonged incubation of sections in malt diastase, and apparently due to contaminating traces of ribonuclease, duplicate sections were incubated in a solution of crystalline ribonuclease (Armour's, bovine pancreas) with control sections of pancreas (8). No removal of material regarded as nuclear or cytoplasmic glycogen is observed in the electron microscope.

Summary. In sections of human liver examined by the electron microscope, material is visible in the nuclei and cytoplasm, which, because of its resemblance in morphology and location to glycogen seen in stained sections examined in the light microscope and because of its removal by diastase, is presumed to be glycogen.

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Hemolysin from the Yeastlike Phases of Some Pathogenic Fungi. (18653)

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The only hemolysin previously described in a pathogenic fungus was isolated from the ground mycelium of *Aspergillus fumigatus*(1). The present investigation is concerned with an hemolysin or hemolysins from the yeast-like phases of *Histoplasma capsulatum, Blastomyces dermatitidis, Candida albicans,* and *Cryptococcus neoformans.*

The fungi used were from the collection maintained in this laboratory. *H. capsulatum* (strain 6515) and *B. dermatitidis* (strain 6046) were grown for 5 days in flasks containing an amino-acid broth at 37°C, and kept in constant rotation during this period of growth (2). *C. albicans* (strain 3148) and *C. neoformans* (strain 3715) were grown for 7-10 days at 25°C in a similar amino-acid broth in 1-liter Blake bottles(3). The cells, after exposure to 0.5% formalin for 72 hours, were thoroughly washed in saline at 3°-5°C, and stored in 1:10,000 merthiolate until ready to

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TABLE I. Properties of Soluble Supernates from Four Pathogenic Fungi.

	Dry wt (after dialysis), mg/ml	Kjeldahl N, mg/ml	Total carbohydrate, mg/ml (Molisch)
(6046) *B. dermatitidis*	3	.22	.32
(6515) *H. capsulatum*	10	.46	.30
(3148) *C. albicans*	1.8	0	.66
(3715) *C. neoformans*	1.8	0	1.02

be dissociated. Eight ml aliquots of concentrated cell suspensions were vibrated with 10 g of glass beads* in a container of a Mickle tissue disintegrator† for 5-6 minutes at 4°C, and the resulting suspension centrifuged at 4°C for one-half hour at 40,000 r.p.m. The resulting supernates from typical lots of the four fungi studied had the characteristics shown in Table I after prolonged dialysis in redistilled water at 4°C. In attempts at hemagglutination, 0.5 ml of each supernate was added to an equal amount of a 0.25% suspension of erythrocytes of hen, guinea pig, rabbit, human (types O and AB), goat, mouse, rat, sheep, and horse. No distinct hemagglutination was observed; subsequently the concentration of erythrocytes was varied from 0.1% to 1%, and the time of incubation from 0.5 to 3 hours, both at room temperature and at 37°C. No marked hemagglutination was obtained.

In the hemolysin tests, 0.5 ml of a 1% cell suspension was exposed to the supernate for 3 hours at 37°C, then allowed to remain overnight at 5°C before readings were made. Experiments on several types of erythrocytes showed the best hemolysis characteristically on cells from hens and guinea pigs (Table II). Accordingly, in the studies on the effects of environmental factors on the action of the fungal hemolysin, guinea pig or hen cells were employed.

To determine the influence of pH on hemolysis, the supernates from *B. dermatitidis* and *H. capsulatum* were dialyzed for 48 hours at 5°C against a Na_2HPO_4-NaH_2PO_4 buffer of 0.1 ionic strength and the action of the resulting solutions studied on the hemolysis of guinea pig erythrocytes (Table III). At pH's of 6.0 and below, the controls in buffer produced hemolysis. The highest significant hemolytic activity occurred at pH 6.5 and the optimum point for differentiating *B. dermatitidis* and *H. capsulatum* at pH 7.0.

The ionic strength of this Na_2HPO_4-

TABLE II. Species Differences in Erythrocytes on Hemolysis by Supernates from the Yeast-like Phases of *B. dermatitidis* and *H. capsulatum*.§

		Dilutions of antigens					
		2	4	8	16	32	64
Guinea pig	Bd	*	*	†	‡		
	Hc	*	*	*	†	†	‡
Hen	Bd	‡					
	Hc	*	*	*	†	‡	
Horse	Bd	‡					
	Hc	*	*	†	‡		
Mouse	Bd	†	†	‡			
	Hc	†	†	†	‡		
Rat	Bd	‡					
	Hc	*	†	‡			
Sheep	Bd	‡					
	Hc	‡					
Human "O"	Bd	‡					
and "AB"	Hc	‡					

Bd = Supernate from *B. dermatitidis*.
Hc = Supernate from *H. capsulatum*.
* Complete hemolysis.
† Partial hemolysis.
‡ No hemolysis.
§ Goat not included because cells in diluent (minus supernate) hemolyzed.
Note: Controls with broth, broth treated with formalin (as in preparation of antigen), saline, and PO_4 buffer (of 0.1 ionic strength and at pH 7) produced no hemolysis with any of the erythrocytes.

TABLE III. Effect of pH on Hemolysis of Guinea Pig Erythrocytes.

	pH*				
	6.0	6.5	7.0	8.3	Saline
B. dermatitidis supernate	>128†	64	8	4	4
H. capsulatum supernate	>128	64	32	4	32

* All buffers were at 0.1 ionic strength.
† Titer denotes dilution of antigen producing partial or complete hemolysis.

* Type 511—Pavement Marking Beads, Minn. Mining and Mfg. Co., St. Paul, Minn.

† Mickle tissue disintegrator. H. Mickle Co., Hampton, England.

TABLE IV. Influence of Ionic Strength (μ) on Hemolysis of Guinea Pig Erythrocytes.

Supernates from	μ .05	.1	.15	.2	.4
B. dermatitidis	32	32	16	8	4
H. capsulatum	64	64	64	32	16

NaH_2PO_4 buffer was varied at pH 6.7 from 0.05 to 0.4, and the effect on hemolysis of guinea pig cells examined (Table IV).

An ionic strength of 0.1 was therefore used for future experiments.

The hemolysins of *B. dermatitidis* and *H. capsulatum* lost no activity after exposure to 60°C for 30 minutes, but were destroyed after autoclaving for 15 minutes at 15 lb. pressure.

C. albicans and *C. neoformans* hemolyzed guinea pig erythrocytes in supernate dilutions of 1:4 and 1:2 respectively, when buffered at pH 6.7. This action seemed especially interesting since these two fungus supernates had no detectable nitrogen (Table I), but had the highest carbohydrate concentration. In addition, since *H. capsulatum* supernate had the highest nitrogen content and the highest hemolysin activity, the probability presents itself that the hemolysin is a protein or protein-like substance.

Attempts were made to detect specific antibodies (both human and rabbit) by absorption of the hemolysin with specific immune serum. Thus far, however, the test has not proved of value because of the strong hemolysin-absorption properties of "normal" rabbit and human sera.

Summary. One or more hemolysins were demonstrated in soluble extracts prepared from the yeastlike phases of *Histoplasma capsulatum, Blastomyces dermatitidis, Candida albicans,* and *Cryptococcus neoformans.* The hemolysin was most active against hen and guinea pig erythrocytes. Some properties of the hemolysin were investigated.

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Local Effects of Cortisone on Granulation Tissue and the Role of Denervation and Ischemia.* (18654)

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The effect of cortisone upon connective tissue structures has been well established. All the elements of such tissues seem to be influenced by this steroid(1-10). The mechanisms, by which cortisone acts upon connective tissue structures, are unknown. In the light of our present knowledge, a number of possible mechanisms warranted investigation. It has been well established that C_{11} oxysteroids have a catabolic effect on tissue proteins(11). It therefore seemed possible that the inhibition of granulation

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