17283. An Immunologic Comparison of Twelve Strains of *Cryptococcus neoformans* (*Torula histolytica*).

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The results of various authors concerning the immunogenic properties of *Cryptococcus neoformans* have not been in agreement.[1-7] Some investigators have been unable to demonstrate antibody formation in animals[4,7] although others have reported agglutinin titers ranging from 1:9[5] to 1:280.[3] Benham[1] obtained serum with an agglutinin titer of 1:160 against pathogenic strains by injecting capsule free cells into rabbits. Working with pathogenic and non-pathogenic strains she has divided the genus Cryptococcus into 4 groups on the basis of serologic and morphologic characteristics. The present investigation is concerned only with strains isolated from human infections (Benham Group III).

Methods. Rabbits were immunized with encapsulated, formalin-killed cells of *C. neoformans*. Intravenous injections were made on three consecutive days of each week. Nine strains of *C. neoformans* were employed for immunization: strains BT, RE, DU, 1523, RO, L2, and LE from the Los Angeles County General Hospital and strains 732 and 2526 from the American Type Culture Collection. Three additional strains were included in the agglutination tests: HA,† BE,‡ and ATCC 4189.

TABLE I.
Agglutination* in Serum Absorbed with Cells of Heterologous Type.

		Serum		
Type	Cells	Type A (RE)†	Type B (1523)‡	Type C (LE)§
A	732	++++	—	—
	2526	++	—	—
	4189	++	—	—
	BT	++++	—	—
	RE	++++	—	—
	DU	++++	—	—
	BE	++++	—	—
	HA	++++	—	—
B	1523	—	+++	—
	L2	—	+++	—
	RO	—	++	—
C	LE	—	—	++++

* Microscopic slide technic; serum dilution 1:20.

† Absorbed with strain L2. Similar results were obtained when other Type B strains were used for absorption. (See Table II).

‡ Absorbed with strain RE. Similar results were obtained when other Type A strains were used for absorption.

§ Absorbed with Type B cells.

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† Obtained from Dr. M. Marples, Otago University Medical School, New Zealand.

‡ From the Los Angeles County General Hospital.

TABLE II.

Agglutination* in Type A (RE) Serum after Absorption with Homologous and Heterologous Cells.

Cells	Unabsorbed serum	Serum absorbed with: 732	2526	4189	BT	RE	DU	BE	HA	1523	L2	RO	LE
732	+	|	|	|	|	|	|	|	|	+	+	+	+
2526	++	|	|	|	|	|	|	|	|	++	++	++	++
4189	++	|	|	|	|	|	|	|	|	++	++	++	++
BT	+	|	|	|	|	|	|	|	|	+	+	+	+
RE	+++	|	|	|	|	|	|	|	|	+++	+++	+++	+++
DU	++	|	|	|	|	|	|	|	|	++	++	++	++
BE	++	|	|	|	|	|	|	|	|	++	++	++	++
HA	+	|	|	|	|	|	|	|	|	+	+	+	+
1523	++	|	|	|	|	|	|	|	|	++	++	++	++
L2	++	|	|	|	|	|	|	|	|	++	++	++	++
RO	±	|	|	|	|	|	|	|	|	±	±	±	±
LE	|	|	|	|	|	|	|	|	|	|	|	|	|	|

* Microscopic slide technic; serum dilution 1:20.

Agglutinations were performed by the serial dilution tube test and by a microscopic slide technic using serum dilutions of 1:20.

After twelve weekly series of injections agglutinating titers of 1:320 were obtained against strains DU, RE, and 1523. Strain LE produced a titer of 1:40.* The other strains with the exception of RO and ATCC 732 had developed low titers (1:10 to 1:40) at the end of 12 weeks.

Agglutinin absorptions were conducted by the method of Krumwiede, Cooper and Provost.[8]

Results. From the data presented in Table I, it is evident that serologic differences exist among the strains employed. For convenience, the 3 varieties have been designated as Types A, B, and C. With the exception of anti-DU serum, it was necessary to absorb all serum with heterologous cells to prevent cross-reactions. The anti-DU serum agglutinated all Type A strains to a titer of 1:320, but failed to agglutinate Types B and C. Table II presents the results of slide agglutinations in anti-RE (Type A) serum following absorption with homologous and heterologous cells. These data were confirmed by similar absorptions of Type B (1523) and Type C (LE) sera.

Discussion. There appears to be a difference in the immunogenic properties of different strains of *C. neoformans*. Although the rabbits used in the present investigation received equal numbers of killed cells, high titers were produced against only 3 strains. This variation may account for the failure of some authors to elicit antibody formation.

Although it is obvious that there are differences in the antigenic structure of the strains used here, no conclusions can be drawn regarding the location of the antigens responsible. It seems possible that the capsular carbohydrate may be important since Neil and his associates[5] have reported a polysaccharide

* Although this serum is of considerably lower titer than the others, it was included in the reciprocal agglutination tests because strain LE appeared to be heterologous to all other strains used.

[8] Krumwiede, C., Cooper, G., and Provost, C. J., *J. Immunol.*, 1925, **10**, 55.

from Cryptococcus which was serologically active up to a dilution of 1:2,000,000.

Summary. 1. Rabbits were immunized with 9 strains of *Cryptococcus neoformans*. After 12 weekly series of injections, 3 strains produced agglutinin titers of 1:320, 4 strains gave titers ranging from 1:10 to 1:40 and 2 strains failed to produce antibodies.

2. On the basis of reciprocal agglutinin absorptions, 3 serologic types of the organism are described. These have been designated as Types A, B, and C.

Received June 8, 1949. P.S.E.B.M., 1949, **71**.

17284. Electron Microscopy Study of Chick Embryo Erythrocytes.

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A few electron micrographs of erythrocytes are found in the literature.[1-7] Erythrocytes with cytoplasm made transparent to electrons apparently by the hypotonic solutions which were used in specimen preparation were discovered accidentally in preparations of isolated liver cell nuclei which were being examined in an electron microscope.

Materials and methods. Nuclei of liver cells from healthy, 16-day-old chick embryos were isolated by the differential centrifugation technic of Dounce[8] as modified by Hoerr,[9] and a portion of the final sediment was resuspended in distilled water for immediate deposition upon electron microscope specimen screens. Three other portions were resuspended for ten minutes in 10% formalin, in 2% osmic acid and in 1% silver nitrate, respectively, and specimens of each were prepared as before. The proportion of reagent volume to specimen volume was about 10:1 and seemed excessive, but smaller proportions of reagent and less time for reagent action gave bad results also. Some of the prepared screens which were formalin-fixed only were stained with Harris hematoxylin for 5 minutes; others with 1% safranin for one minute and still others with 1% methyl green for one minute and were washed with distilled water to remove the excess stain. Wet preparations were examined with the ordinary light microscope for control purposes.

Micrographs were taken employing a biased electron gun and an objective aperture with relatively low plate magnifications between 3,000 and 4,000 times. Crystalline residues, which are recognizable in electron micrographs were often observed, and examples of some are indicated at the arrows in Fig. 4, 5 and 12. No effects of electron bombardment were noted upon the morphology of the cells, except that they shrunk when focussed beams were directed upon them, but none of the fields reproduced here have been subject to focussed beams.

Observations and discussion. Unfixed, unstained erythrocytes are identified in this work by their large size, almost filling the whole 2 inches square field at 3,600 times; by their characteristic oval shape; by the centric position and the diffuse, elliptical outline of the nucleus within them; by the proportion of their cytoplasmic to nuclear area; by the homogeneity of their cytoplasm and

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