testing possibilities for implanting homologous normal cells in anemic hosts are in progress.

In spite of these limitations the successful cases of permanent isologous implantation of normal blood-forming tissues in genetically anemic mice, including the lethal *WW* type, without prior radiation treatment, suggest that if methods for circumventing the homograft reaction can be found, it may be possible to use cell implantation generally for the therapy of genetic defects in erythropoiesis.

Summary. Successful implantation, without x-irradiation or other pretreatment of host, of isologous blood forming tissue from normal (*ww*) mouse fetal liver in viable severely anemic recipients (20 of 24 adult WW^v mice, 9 of 14 juvenile WW^v mice) and in lethally anemic recipients (4 of 15 juvenile *WW* mice) has been demonstrated. Criteria of success were permanent increase in rbc/mm^3 to near-normal levels, and decrease of mean cell volume from macrocytic to normocytic levels. These changes appeared in WW^v adult anemic hosts longer after cell injection than in similar transplant experiments with WW^v hosts subjected to 200-r whole-body irradiation. These findings fit well with the hypothesis of competition between slow-acting indigenous blood forming tissue of genetically anemic hosts and initially small implants of rapidly functioning blood forming tissue from genetically normal donors. If methods for circumventing homografts can be found, cell implantation may be generally useful for therapy of genetic defects in erythropoiesis.

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Effect of Degree of Encapsulation upon Virulence of *Cryptococcus Neoformans.** (25090)

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Capsule formation has been associated with virulence of many microbial species such as *Diplococcus pneumoniae, Bacillus anthracis, Hemophilus influenzae* and *Klebsiella pneumoniae.* However, the chemical nature of capsular substances formed by different microbial species is dissimilar, varying from complex polysaccharides composed of units of glucose, fructose, galactose, mannose, sugar acids and amino sugars, to polypeptides such as polymers of glutamic acid. The capsule of *Cryptococcus neoformans* contains 2 complex polysaccharides; an amylose of the starch group, and a serologically active pentosan which upon hydrolysis, yields units of xylose, mannose, galactose and glucuronic acid(1). To date, no single chemical constituent of cap-

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sular substance has been directly associated with virulence. Although literature contains many references re. encapsulation on virulence of bacterial species, studies of encapsulated yeasts are limited. Drouhet and Segretain(2) noted that an encapsulated, mucoid, dissociant strain of *C. neoformans*, isolated from a patient, was more virulent to mice than the thinly encapsulated, parent yeast strain. In contrast, Kao and Schwarz(3) failed to observe correlation between capsule thickness of *C. neoformans* and virulence in white Swiss mice with strains recovered from pigeon nests. Gadebusch(4) observed that variant strains of *C. neoformans* which possessed large capsules resisted phagocytosis by mouse polymorphonuclear leucocytes while thinly encapsulated yeast variants were readily ingested. The preceding studies were performed, however, before precise nutritional requirements of *C. neoformans* had been defined by Littman (1). In these studies the organism possessed an absolute requirement for thiamine and its moieties, a specific need for glutamic acid family of amino acids, and need for certain carbohydrates. A synthetic basal substrate, *Cryptococcus* Capsule Medium‡ (CCA), which satisfied complete nutritional need of *C. neoformans* and caused it to synthesize abundant capsular substance, has been described (1). It has also been observed that capsule synthesis diminishes rapidly when yeast is transferred from the medium to a dextrose-peptone agar such as Sabouraud dextrose agar§ (SAB). This provides a means for determining comparative virulence of the same yeast strain existing in different degrees of encapsulation.

Materials and methods. *C. neoformans* (D strain), isolated at our hospital from a fatal case of cryptococcal meningitis, was subcultured on both CCA and SAB agar media 4 days at 37°C. Culture growth in each medium was suspended in sterile, physiological saline. Measurements of yeast cell diameter, including the capsule, were made with ocular micrometer, using diluted fungicidal India ink mount.‖ Photometric measurements of saline suspensions of the organisms at 650 mμ revealed light transmission values of 19.3 and 19.5%, representing plate counts of 17.6 X 10^6 and 4 X 10^6 cells/ml, respectively. Tenfold, 1000-fold, and 10,000-fold saline dilutions of each cell suspension were prepared for injection. Ninety 6-week-old, female, white Swiss mice, each weighing approximately 15 g, were divided into 6 groups of 15 mice each. Three groups received intracerebral injections of 0.0625 ml of each of the 3 dilutions of thickly encapsulated CCA yeast cells, while the 3 remaining groups received thinly encapsulated SAB cells. Intracerebral injections were made midway between eye and ear, using 0.25 ml hypodermic syringe and 26 gauge 3 mm needle. Mice were weighed and examined each day for hydrocephalus. Upon death, gross pathological examination was made of all organs including brain. At autopsy, a platinum loop was inserted into the cerebral matter at site of injection and an India ink mount was prepared. Capsules of 30 yeast cells in each brain preparation were measured with ocular micrometer. Mouse survival time data were computed according to a modification of the nomograph method of Litchfield (5,6). Data were recorded as per cent cumulative fatality and plotted on 2-cycle logarithmic probability paper, with time expressed in days on logarithmic scale. Using least squares method, the best straight line was constructed. Standard deviation was computed by substitution of effective fatality times (ET_{80}, ET_{50} and ET_{20}) in the equation: Stand. dev. (S): $\frac{ET_{80}/ET_{50} - ET_{50}/ET_{20}}{2}$.

Results. Marked differences were observed in colonial and microscopic appearance of test strain of *C. neoformans* on SAB and CCA agar media (Fig. 1). Growth of the organism on

‡ Containing/liter of medium: KH_2PO_4, 2 g; $(NH_4)_2SO_4$, 2g; $MgCl_2.6H_2O$, 0.2 g; $CaCl_2.2H_2O$, 0.02; $FeCl_2.4H_2O$, 0.04 g; $MnCl_2.4H_2O$, 0.0015 g; $Na_2MoO_4.2H_2O$, 0.0015 g; thiamine, 0.001 g; maltose, 5 g; sucrose, 5 g; sodium glutamate, 2 g; washed agar, 20 g; double distilled water, 1000 ml; pH 7.0.

§ Containing/liter of medium: neopeptone (Difco), 10 g; dextrose 40 g; agar 15 g; pH 5.6.

‖ India ink, 15 ml; solution of Merthiolate (Lilly) 1:1000, 30 ml; Tween-80 1:100 aq. solution, 0.1 ml; filtered before use.

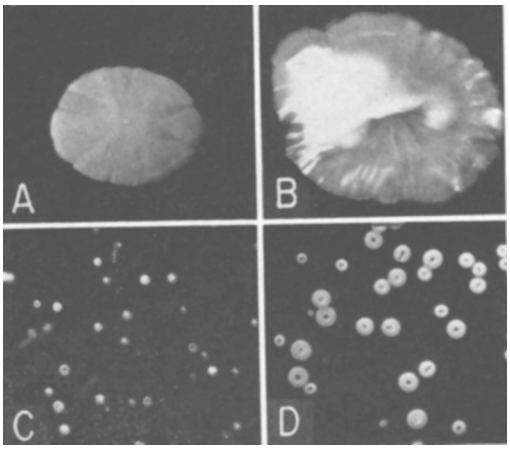

FIG. 1. Effect of culture substrate on *in vitro* encapsulation of *Cryptococcus neoformans*. (A) Giant colony on Sabouraud dextrose agar (SAB), 16 days at 37°C, ×.78. (B) Giant colony on *Cryptococcus* capsule agar (CCA), 16 days at 37°C, ×.78. (C) India ink mount of growth from SAB colony showing absent or sparse capsules, phase microscopy, unstained, × 117. (D) India ink mount of growth from CCA colony showing abundant capsules, × 117.

CCA medium was raised, mucoid, sectored, viscous, profuse and flowing; as a consequence a large colony was formed (Fig. 1B). Microscopic examination of this colony with India ink mount showed thickly encapsulated yeast cells (Fig. 1D). The colony on SAB medium, however, was considerably smaller (Fig. 1A). It was butyrous in consistency, showed sectoring, and appeared flat, dry and dull. Microscopic examination with India ink mount revealed, for the most part, thinly encapsulated cells (Fig. 1C). Of 100 cells grown on SAB, the average cell diameter, including capsule, was 9.12 μ, while CCA cells averaged 16.04 μ. While only 1% of the SAB cells were encapsulated, as many as 92% of the CCA cells demonstrated capsules. The majority of CCA cells possessed capsules that measured more than 14.4 μ in diameter.

Virulence of both CCA and SAB yeast cells in mice after intracerebral injection was approximately equal. This occurred over dose range of 25 to 1,100,000 cells (Fig. 2). Mouse mortality was directly proportional to intracerebral dose of yeast cells, without regard to their initial state of encapsulation. Thus, median effective fatality time (ET_{50}) for 110 CCA yeast cells was 14.8 days (Table I). Increasing the dose 10,000 times to 11 X 10^5 cells reduced ET_{50} value by only half, or 7.2 days. ET_{50} values for each dose of yeast cells were statistically similar for both types (Table I) and the curves of ET_{75}, ET_{50} and ET_{25} values were parallel.

Both thinly encapsulated SAB cells and thickly encapsulated CCA cells produced equally thick capsules after intracerebral injection. No appreciable differences in incidence of hydrocephalus were observed in mice that had received either type of yeast cell. Mice receiving the heaviest intracerebral dose of yeast cells died in 5 to 11 days (Fig. 2, Table II). Group II mice, given a smaller dose of yeast cells, died in 8 to 22 days, while Group III mice which had received the

TABLE I. Comparison of Median Effective Fatality Time (ET_{50}) in White Swiss Mice Produced by Test Strain of *Cryptococcus neoformans* Grown on *Cryptococcus* Capsule Agar (CCA) and Sabouraud Dextrose Agar (SAB).

Group	Intracer. dose, yeast cells	Culture medium	ET_{50} (days)	Stand. dev. (days)
I	11 × 10^5	CCA	7.2	1.22
	2.5 × 10^5	SAB	8.2	1.20
II	11 × 10^3	CCA	11.0	1.32
	2.5 × 10^3	SAB	13.2	1.33
III	110	CCA	14.8	1.39
	25	SAB	18.0	1.55

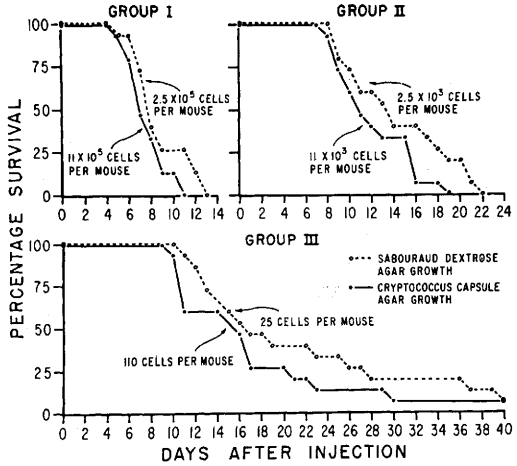

FIG. 2. Comparative virulence for white Swiss mice of thinly encapsulated SAB cells and thickly encapsulated CCA cells of *Cryptococcus neoformans*, following intracer. inj. of 0.0625 ml saline suspensions, 15 mice/group (arithmetic plot).

TABLE II. Comparison of Diameters of *Cryptococcus neoformans* Cells, Including Capsules, Grown on *Cryptococcus* Capsule Agar (CCA) and Sabouraud Dextrose Agar (SAB) (*In Vitro*) and following Intracerebral Injection (*In Vivo*).

Group	Intracer. dose of yeast cells	Culture medium	Avg capsule diameter (μ)* *In vitro*	Avg capsule diameter (μ)* *In vivo* mouse brain	% of mice with hydrocephalus on death	No. of days required to cause death
I	11×10^5	CCA	16.04	13.3	86	5-11
	2.5×10^5	SAB	9.12	14.1	80	5-14
II	11×10^3	CCA	16.04	13.1	93	8-19
	2.5×10^3	SAB	9.12	14.5	100	9-22
III	110	CCA	16.04	13.5	"	10-30
	25	SAB	9.12	14.3	"	11-40

* Avg of 100 cells.

smallest inocula, succumbed in 10 to 40 days. Mice that succumbed first (Group I) showed 80 to 86% hydrocephalus, while animals that survived for longer periods all developed hydrocephalus.

Discussion. It is known that strains of *C. neoformans,* cultivated on dextrose-peptone agar, vary considerably not only in thickness of their capsules but also in virulence for mice(7). Littman and Schneierson(7) noted that of 144 mice injected intracerebrally in pairs with 3 X 10^6 yeast cells, derived from 72 "pigeon" strains, 18% succumbed 10 days after injection, 40% within 3 weeks and 100% within 73 days. Thus it would seem that mouse virulence test for identification of *C. neoformans* is of limited diagnostic value where speed of diagnosis is desired. Cultural and biochemical procedures are preferred for identification of the organism(7).

In assaying the virulence of different strains that possess thin or thick capsules, the inherent virulence of the organism cannot be attributed solely to capsule size, since factors of strain virulence other than encapsulation may be involved. To eliminate the factor of strain differences, virulence studies herein reported were limited to a single strain of the organism. *In vitro* cultivation on CCA and SAB agar media permitted use of cells of the same strain since they differed widely in degree of encapsulation.

Assay of virulence of thinly and thickly encapsulated cells, derived from the same strain of *C. neoformans,* has not been previously reported. Our results show they possess the same degree of virulence for white Swiss mice. Thinly encapsulated SAB yeast cells also produced equally thick capsules *in vivo* as did thickly encapsulated CCA cells. The prompt increase in *in vivo* encapsulation of SAB cells is due probably to the fact that thiamine, glutamic acid, and certain carbohydrates and minerals, essential for growth of *C. neoformans,* exist in optimal concentrations in brain and CSF(1).

In computing mouse survival time according to the method of Litchfield(5,6), CCA and SAB yeast cells produced similar cumulative fatality curves and exhibited similar ET_{50} values. The results also show that, within certain limits, mouse virulence test for *C. neoformans* is dependent upon the intracerebral dose of yeast cells. For example, ET_{50} value of *C. neoformans* for white Swiss mice decreased from 18 to 8.2 days, with increased dosage of SAB yeast cells (Table I).

Summary. Thinly encapsulated *Cryptococcus neoformans* cells were equally as virulent for white Swiss mice as thickly encapsulated cells of the same strain. Yeast cells initially possessing thin capsules promptly developed thick ones upon intracerebral injection into mice, and upon subculture to synthetic capsule medium. Both types of cells produced hydrocephalus to an equal degree. Degree of encapsulation is not a factor in virulence of *Cryptococcus neoformans* for white Swiss mice.

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Electron Microscopic Study of Human Cholestasis.* (25091)

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Pathogenesis of jaundice associated with intrahepatic cholestasis is not established despite extensive research for many years. Jaundice with clinical and laboratory findings suggestive of biliary obstruction and without indications of injury of liver cells or hemolysis is present in the absence of a demonstrable obstacle in intrahepatic or extrahepatic bile ducts. On the basis of clinical, functional and light microscopic investigations, the alteration was localized into different sites, such as liver cells(1,2), Kupffer cells(3), cholangioles or ductules(4) as well as into lining of bile canaliculi(5,6). To throw light on this phenomenon, the ultrastructure of liver in human extrahepatic biliary obstruction, intrahepatic cholestasis and other types of hepatic jaundice were studied under the electron microscope. For comparison, liver of rats with experimental injuries from ethionine(7) and experimental extrahepatic biliary obstruction were studied. Previous electron microscopic observations have indicated abnormal communication between dilated bile canaliculae and perisinusoidal tissue spaces(8).

Material and methods. Biopsy specimens of liver of patients with hepatic injury and jaundice following intake of chlorpromazine, chlorpropamide, norethandrolone, and with primary biliary cirrhosis served as examples of intrahepatic cholestasis. Extrahepatic biliary obstruction was represented by carcinoma of the pancreas, jaundice associated with liver cell injury by viral hepatitis, and one case of chronic idiopathic jaundice was included. Small pieces of these as well as of livers of experimental animals mentioned above were fixed in 2% buffered ice-cold osmium tetroxide and thin sections examined with Philips model EM 100 B electron microscope. Other parts of tissue were routinely fixed in formalin and examined by standard light microscopic technics, which included particularly the periodic acid Schiff reaction after glycogen removal with diastase for demonstration of carbohydrate-bound protein.

Results. The bile canaliculus of normal human beings and rats appeared under the electron microscope as a round or elliptical lumen approximately one μ in diameter into which fine fingerlike projections extended (microvilli) (Fig. 1). It was separated from nearby extensions of the perisinusoidal tissue spaces by closely approximated straight cell borders. Liver cells projected as less regular microvilli into tissue spaces including their intercellular narrow extensions referred to above. Under the light microscope tissue spaces and some of the extensions gave a faint PAS reaction. Each liver cell usually had more than one bile canaliculus on its borders. The inner border of ductules was also lined by microvilli which were shorter and further apart than in liver cells.

In cases of intrahepatic biliary obstruction studied, the bile canaliculi appeared less numerous (Fig. 2). A few were the same size as normal canaliculi whereas others were dilated to a maximal diameter of 4 μ; in the latter, electron opaque debris was noted. In all canaliculi the microvilli were either entirely absent or further apart than normal and shortened, stunted or irregularly shaped. In a few instances a communication between dilated microvilli and extension of tissue spaces was

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