ample iodide 85-90% of plasma iodine is inorganic, but the PBI level also rises many-fold with increasing iodine supply. Apparently this is due to loose binding of I^- by plasma protein since N-butanol extractable iodine is lower than PBI(4,5).

Both *in vitro* uptake studies(8) and the present *in vivo* data indicate that the only significant iodine concentration in the internal tissues and organs of the trout occurs in the lower jaw, containing the thyroid follicles. Although ovarian tissue was not sampled in this study, it is also known to concentrate iodine(3,9).

Summary. Iodine content of trout tissues is directly related to dietary and/or habitat water levels of iodine. With ample iodide in the water (10 μg/l), trout can extract iodide directly from water and maintain tissue iodine levels comparable to those of sea-run trout. High PBI levels were correlated with high plasma inorganic iodide. An isotopic equilibrium method was applied to estimate the iodine content of trout tissues.

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Behavior of Candida Cells Within Leukocytes.* (28840)

DONALD B. LOURIA AND ROBERT G. BRAYTON (Introduced by E. Alture-Werber)
Infectious Disease Laboratory, Second (Cornell) Medical Division, Bellevue Hospital, New York and Department of Medicine, Cornell University Medical College, New York

We have previously reported that *C. albicans* intravenously inoculated into mice multiply progressively only within the kidneys, and that Candida cells can be visualized within polymorphonuclear leukocytes(1). Therefore, we undertook to analyze the ability of polymorphonuclear leukocytes to phagocytose Candida cells *in vitro* and to study the behavior of the fungus after phagocytosis.

Materials and methods. Six strains of *Candida albicans,* 5 of *Candida tropicalis,* and one of *Candida guilliermondi* were used. Each was maintained by weekly subculture on Sabouraud's glucose agar slants incubated at 30°C. Each maintained a consistent virulence for mice during the experimental period. In virulence studies, groups of 10 Carworth Farms white, male, 17-20 g Swiss mice were inoculated *via* a lateral tail vein with 10^6 to 5×10^6 cells of an overnight culture in a 0.5 ml volume. Mortality was studied for a 30-day observation period. A minimum of 3 experiments was performed on each strain.

Candida cells were harvested in physiologic saline from 24-hour slant cultures, counted directly in Neubauer-Levy hemocytometer, and diluted appropriately in saline. Normal human blood was drawn from an antecubital vein into siliconized syringes and was immediately transferred into siliconized tubes containing 100 units of heparin per 10 ml of blood. Two ml samples were transferred into smaller tubes and Candida cells were added in a 0.1 ml volume so that there was approximately a 2 to 1 Candida to polymorphonuclear leukocyte ratio. The size of the inoculum was determined by standard pour plate enumeration techniques. The blood-Candida

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mixture was rotated at 22 rotations per minute in a 37°C incubator. At 30 minutes and at 4 hours, single drops of the mixture from each tube were placed on 2 glass slides, dried, and stained with Wright's stain. A drop was also mixed with 1% trypan blue and studied immediately. The number of fungus cells within the phagocytes and the number growing out of leukocytes were analyzed in a sampling of 100-200 dried white blood cells and in an additional 100-200 supravitally stained polymorphonuclear cells which did not take the trypan blue stain and were therefore considered viable. Survival within leukocytes was evaluated by grinding the 4-hour specimens in a teflon tissue homogenizer and determining the number of viable fungi by making pour plates of serial 100-fold dilutions. In the studies on intra-leukocytic survival, only *Candida tropicalis* and *C. guilliermondi* strains were used because normal human plasma is fungicidal for *C. albicans* but not for *C. tropicalis* or *C. guilliermondi*(2). Homogenization of saline suspensions of yeast phase Candida cells did not alter the number of cultivatable organisms when compared to non-homogenized control suspensions.

The functional capacity of the leukocytes which had ingested Candida and did not take the trypan blue stain was analyzed by adding coagulase-positive, hemolytic *Staphylococcus aureus* in a 2:1 staphylococcus to leukocyte ratio after 4 hours of incubation of the Candida-leukocyte suspension. The Candida-leukocyte staphylococcus mixture was rotated for an additional 30-60 minutes and phagocytosis was then determined by Wright-stained smears of dried samples and by performing differential centrifugation studies according to the technique of Maaløe(3). Phagocytosed bacteria were found in the sediment after centrifugation whereas non-phagocytosed bacteria remained in the supernatant. Blood rotated for 4 hours without Candida served as the control for staphylococcus phagocytosis studies.

Results. 1. *Comparative virulence.* Thirty-day mortality after infection with 10^6 to 5×10^6 cells of the 12 strains is indicated in Fig. 1. Ninety-five to 100% of mice inoculated

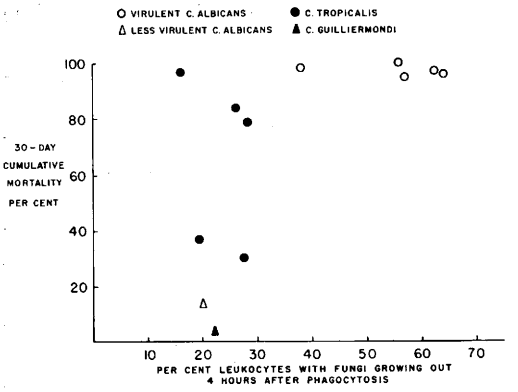

FIG. 1. Relationship between capacity of Candida strains to grow out of viable polymorphonuclear leukocytes and lethality.

with 5 of the *C. albicans* strains died during the experimental period. Cumulative mortality was less with the 5 *Candida tropicalis* strains, 30 to 97% of the animals dying in a similar time interval. Strain 851, a less virulent strain of *C. albicans,* produced only 13.7% deaths, and 3.0% of animals died after infection with the one strain of *C. guilliermondi* tested.

2. *Phagocytosis.* After incubation at 37°C for 30 minutes, there was extensive phagocytosis by polymorphonuclear leukocytes. Individual polymorphonuclear leukocytes ingested from 1 to 7 Candida cells (Fig. 2),

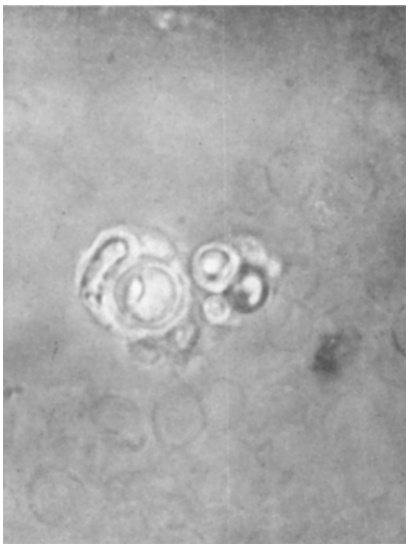

FIG. 2. Phagocytosis of Candida cells by human polymorphonuclear leukocytes after 30 min incubation. Trypan blue, × 1200.

phagocytes with larger numbers of fungi frequently showing marked displacement of nuclei. When the number of fungi ingested by 100 leukocytes was totaled, and appropriate calculations made, virtually the whole inoculum could be accounted for within cells. At the 30-minute period, less than 2% of polymorphonuclear leukocytes took the trypan blue stain. Strains or species of Candida did not differ, all appearing to be readily phagocytosed in normal human blood.

3. *Behavior within phagocytes.* Four hours after incubation at 37°C, 2 to 10% of phagocytes (and infrequently up to 20%) took the trypan blue stain and were deemed not viable. Many of the viable polymorphonuclear leukocytes contained fungus cells, but in addition, fungi of each of the 12 strains could be seen apparently penetrating the cell wall of viable leukocytes (Fig. 3A, 3B). Although the leukocyte was markedly distorted by the pseudomycelia which often appeared far away from the body of the cell, it seems possible that a thin cell membrane continued to surround the elongated pseudomycelia although documentation of this will have to await electron microscopy studies. The different Candida species and strains showed striking differences in ability to "grow out" of apparently viable leukocytes within 4 hours. Each of the fungus strains was studied on 3 to 20 separate occasions, and in each instance, vital stains were confirmed by study of Wright stains. The results of these studies are summarized in Fig. 1. The 5 more virulent strains of *C. albicans* showed the greatest capacity to "grow out" of viable leukocytes, 38 to 64% of phagocytosing leukocytes having one or more pseudomycelia penetrating the cell wall after 4 hours' incubation. Of the other 7 strains, the percentage of living leukocytes which had performed phagocytosis and had fungi "growing out" of the cell ranged from 16 to 28.2%. In 6 of the 7, there was rough correlation between virulence for mice and ability to "grow out" of leukocytes after phagocytosis (Fig. 1), whereas with the 7th strain, *C. tropicalis* strain NH, only 16% of viable leukocytes which had participated in phagocytosis had pseudomycelia penetrating the cell wall, but

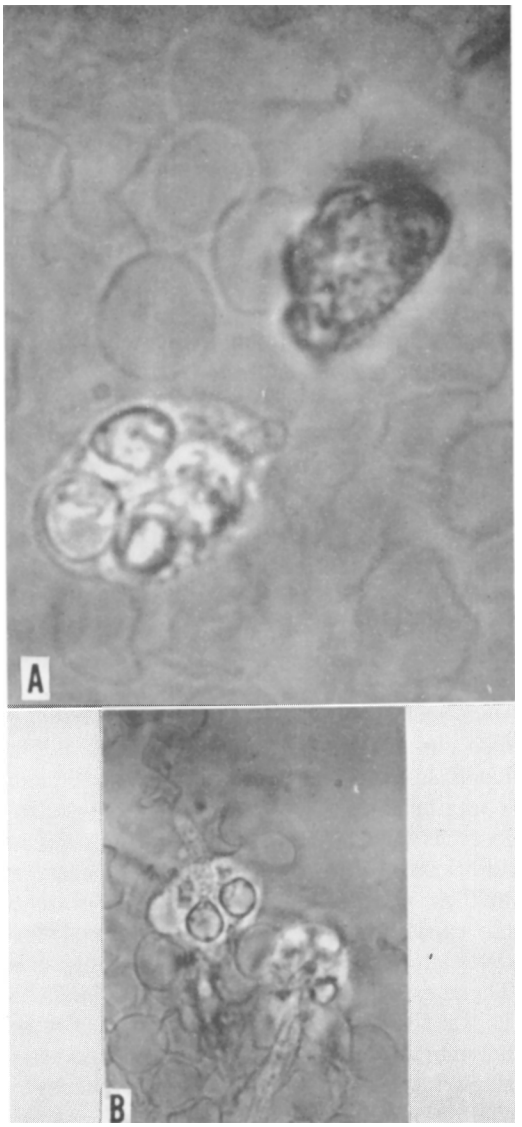

FIG. 3A, 3B. Candida cells penetrating viable human leukocyte wall 4 hr after *in vitro* phagocytosis. Trypan blue, × 1200-2500.

the cumulative mortality was 97% in 30 days.

4. *Survival within phagocytes.* Normal human plasma inhibited multiplication but did not kill the 5 strains of *C. tropicalis* or the one strain of *C. guilliermondi* during the 4-hour test period. There was no evidence that leukocytes killed any of the 6 Candida strains. In each instance, the total number of viable fungi recovered from whole blood and cell-free plasma was identical 4 hours

TABLE I. Phagocytosis of Staphylococci by Leukocytes Which Have Ingested Candida.

Exp	Control leukocytes	Leukocytes with Candida in cell	Leukocytes with Candida "growing out"
	Phagocytosing *S. aureus*/Total (%)		
1-2	240/400 (60%)	527/854 (61.7%)	437/939 (46.5%)
3-7	184/500 (36.8%)	105/250 (42.0%)	142/539 (26.3%)

after incubation, and in each case, approximated the original inoculum.

5. *Further studies on viability and functional capacity of leukocytes at the time fungal mycelia apparently penetrate the cell wall.* Although the overwhelming majority of polymorphonuclear leukocytes which had performed phagocytosis and had pseudomycelia growing through the cell wall 4 hours later did not take the trypan blue stain, the cells appeared sluggish and granular activity seemed to be reduced. Seven experiments were performed, therefore, in which *Staphylococcus aureus* was added to the Candida-blood suspension after 4-hour incubation on a rotator at 37°C. The blood-fungus-staphylococcus mixture was allowed to rotate for an additional 30 minutes and stained smears as well as vital stains were made to evaluate the capacity of the fungus-containing leukocytes to perform bacterial phagocytosis. These experiments are summarized in Table I. In the first 2 studies, 60% of control polymorphonuclear leukocytes phagocytosed staphylococci as did 61.7% of leukocytes containing Candida. In the other 5 experiments, 36.8% of control and polymorphonuclear cells participated in phagocytosis as did 42% of those containing candida. In contrast to the control and Candida-containing leukocytes, those polymorphonuclear cells which had pseudomycelia apparently penetrating the cell wall were less able to perform phogocytosis, 46.5% participating in phagocytosis in experiments 1 and 2 as contrasted to 60% of controls, and 26.3% participating in phagocytosis in Exp. 3-7 as contrasted to 36.8% of controls. These results are statisically highly significant. Phagocytosis of staphylococci by Candida-containing leukocytes is depicted in Fig. 4A and 4B.

These studies indicating that phagocytosis was impaired in cells with Candida pseudomycelia growing through the cell wall were confirmed in differential centrifugation studies. Five experiments were performed in which 2 control specimens were studied immediately after adding staphylococci and 2 control and 2 Candida-containing mixtures were studied 1 hour after adding staphylococci. In 4 of the studies, an average reduction of 64-fold was found in supernatant staphylococcal census in controls as compared to a 14-fold reduction in Candida-containing specimens. In the 5th experiment, phagocytosis (reduction in supernatant census) was identical in controls and Candida-containing specimens. Reduction in the sediment count indicates intracellular destruction of phagocytosed bacteria. This was found to be unaltered by prior phagocytosis of Candida.

These studies show clearly that the leukocytes which have phagocytosed Candida, even those which have pseudomycelia apparently penetrating the cell are functionally viable although it is also clear that at least those leukocytes distorted by pseudomycelia have diminished phagocytic capacity.

Discussion. Studies in experimental animals and in man indicate both that the kidney is the target organ in systemic moniliasis and that the polymorphonuclear leukocyte is of paramount importance in limiting the infection(1,4,5). In normal mice, *Candida albicans* appears to be incapable of proliferating outside the kidneys(1,6), and we have suggested that renal parasitism depends on the ability of the fungus to penetrate the tubular lumen and thus escape host cellular defenses (1). These studies further suggest that more virulent strains of Candida (especially *Candida albicans*) possess an additional adaptive mechanism which enhances their capacity to establish residence within the renal tubular lumen, namely the ability to evade host cellular defenses by growing out of viable or non-viable leukocytes following phagocytosis. It is apparent that virulence and the ability to grow out of leukocytes are not always

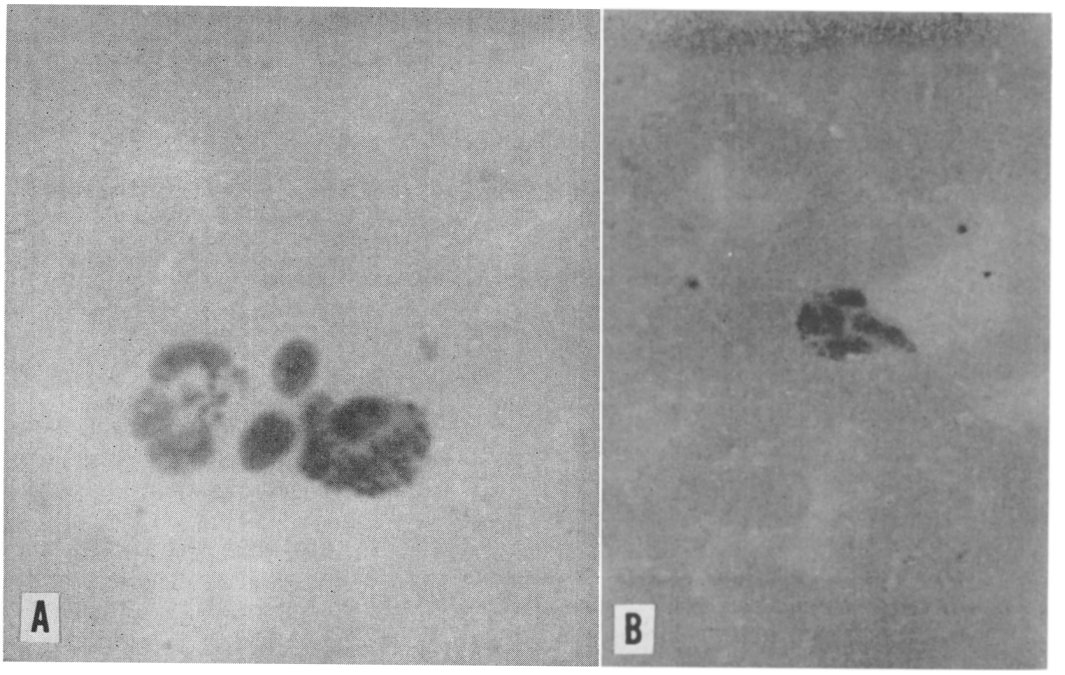

FIG. 4. (A) Phagocytosis of staphylococci *in vitro* by human polymorphonuclear leukocyte 4 hr after ingestion of Candida cell. Leukocyte-fungus-staphylococcus suspension rotated at 37°C for an additional 30 min. Wrights stain, × 2000. (B) Phagocytosis of staphylococci by human polymorphonuclear leukocyte which has Candida penetrating cell wall. Wrights stain, × 1200.

closely correlated, as evidenced by *C. tropicalis* strain NH. It will be interesting to see whether the pseudomycelia have actually penetrated the wall of viable leukocytes. It seems more likely that distorted cells which do not take the trypan blue stain have a thin cell membrane surrounding the pseudohyphae and when the pseudomycelia actually rupture through the limiting membrane of the leukocyte, the cell dies.

The fate of phagocytosed fungi which do not grow out of the leukocyte is not clear. In the present studies, there was no evidence (even after 4 hours of intracellular residence) that the leukocyte could kill the fungus. Killing may be far more effective *in vivo* than in this *in vitro* model. It is also possible that killing would have been observed if the study had been carried out beyond the 4-hour time limit set. Furthermore, the result of phagocytosis may not be death of the fungus but rather reduced growth of at least some strains of Candida into the renal tubular lumen. Subsequent killing of the fungus may then be related to release of fungicidal substances from living or dead cells following formation of interstitial abscesses.

Summary. Candida cells are rapidly phagocytosed by human polymorphonuclear leukocytes. After 4 hours' incubation at 37°C, the fungus survives within the leukocyte and may form hyphae which appear to penetrate the wall of viable leukocytes. More virulent Candida strains usually, but not always, exhibit a greater capacity to grow out of viable leukocytes after phagocytosis. This ability to circumvent host cellular defenses may permit the fungus to gain readier access to the renal tubular lumen, a site where Candida can proliferate unhampered by ordinarily effective defense mechanisms.

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Cardiac Dynamics of Intact, Splenectomized and Hepatic Artery Ligated Dogs During Acute Oligemic Shock.* (28841)

STEVEN M. HORVATH AND A. DOUGLAS BENDER†
Environmental Stress Laboratory, University of California, Santa Barbara

Shock has been said to be a problem of combined failure of the circulatory system (1). Myocardial deterioration, as a result of reduction in coronary flow(2-6) and thus a decrease in oxygen availability, is a major contributor to circulatory failure in irreversible hemorrhagic shock(7,8). In oligemic shock cardiac output is markedly reduced (2-5) as a result of a decrease in venous return. This situation and other conditions requiring increased cardiac output are attended by splenic contraction(9). In the intact dog, hemorrhage invariably produces a reduction in size of the spleen(9,10), while the response of the liver is extremely variable(9). Thus the splenectomized dog is more vulnerable than the intact animal to the consequences of oligemic shock when equal volumes of blood (on the basis of body weight), are removed from each animal(11,12). When both are bled to the same hypotensive level, however, this hemodynamic disadvantage should be nullified, for the contribution of the spleen in the intact animal would only necessitate removal of a greater blood volume in order to achieve the desired blood pressure level. Under this condition a finding of a greater depression in cardiac performance in the splenectomized animal could not be completely explained by the lack of reservoir function, and may imply a humoral contribution by the spleen in the intact animal. In the present study myocardial responses were observed in intact, splenectomized, and hepatic artery ligated dogs bled to a mean arterial blood pressure of 40 mm Hg.

Methods. A total of 41 mongrel dogs, comprising 3 groups, were used in 41 experiments. Group I consisted of 11 intact (control) animals, 15.1 to 26.6 kg in weight; Group II, 14 animals, 14.0 to 24.2 kg, which had undergone removal of their arterial supply to the liver(13) more than 300 days prior to the experiment; Group III, 16 animals, 10.5 to 21.4 kg, splenectomized 3-13 months prior to the study. Each animal was anesthetized with sodium pentobarbital (30 mg/kg) and heparinized (5 mg/kg). The procedure for positioning of the catheters at various sites, and the methods employed for determination of oxygen consumption, blood oxygen content, cardiac output (Fick), coronary blood flow (nitrous oxide), and other parameters were the same as previously outlined(4). Calculations of various metabolic and hemodynamic functions of the myocardium were made using the equations of Bing *et al*(14).

Following control observations, the animals were bled freely from a femoral artery cannula until the mean arterial blood pressure reached 40 mm Hg. The time required to reach this level and the volume of blood removed were recorded. Arterial pressure was maintained at 40 mm Hg for one hour by withdrawing or infusing blood. At the end of this time the additional volume of blood removed or infused was recorded. Cardiovascular and metabolic determinations were made when the pressure first reached 40 mm Hg and one hour later.

Most of the animals employed were sacrificed after the experiment, thus no attempt

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† Present address: Research Development Division, Smith Kline and French Laboratories, Philadelphia.