# Lethality of Cell-Free Extract of *Candida albicans* for Chlortetracycline-Treated Mice.* (23002)

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Action of an endotoxin in pathogenesis of *Candida albicans* infection has been suggested (1,2). This communication reports the lethality for mice of a cell-free supersonic extract of *C. albicans* in combination with chlortetracycline (Aureomycin, Lederle) and the comparative toxicity of viable *C. albicans* singly and in combination with chlortetracycline for guinea pigs, rabbits and monkeys.

*Materials and methods. Candida albicans*, strain 780, was grown on glucose-peptone-yeast-extract agar at 27°C for 72 hours, harvested, washed 3 times in cold (4°C) 0.85% NaCl solution (saline), and resuspended in saline to a 1% concentration by volume (15 x $10^6$ cells/ml). *Heat-killed cells* were prepared by immersion of the 1% viable cell suspension in a 58°C water bath for 90 minutes. *Mechanical lysate* was obtained by shaking a 2% saline suspension of cells with glass beads at 4°C for 2 hours in a paint-shaking machine; cellular debris was separated from lysate by centrifugation. Microscopic observation showed that 60-70% of the cells had been ruptured. *Supersonic extract* was made by exposing a 10% saline suspension of viable cells to supersonic vibration at 4°C for 45 minutes in a Raytheon Sonic

TABLE I. Lethality for Antibiotic-Treated Mice of Preparations of *Candida albicans*.

| Antibiotic | *C. albicans* preparation | Deaths at 96 hr* | Hr to 50% death |
|---|---|---|---|
| Chlortetracycline† | Viable cells‡ | 50/50 | 31 |
| — | *Idem* | 4/25 | |
| Chlortetracycline | Heat-killed cells | 17/30 | 96 |
| — | *Idem* | 1/20 | |
| Chlortetracycline | Supersonic extract, 0.5 ml | 50/50 | 10 |
| — | *Idem* 1.0 | 0/10 | |
| — | " 2.0 | 0/10 | |
| Chlortetracycline | Mechanical lysate | 8/30 | |
| — | *Idem* | 1/20 | |
| Chlortetracycline/10 g body wt | | | |
| 1 mg | — | 0/20 | |
| 2 | — | 5/20 | |
| 3 | — | 18/20 | 48 |
| 4 | — | 20/20 | 24 |
| Oxytetracycline | Supersonic extract | 2/10 | |
| " | — | 0/10 | |
| Chloramphenicol | Supersonic extract | " | |
| " | — | " | |
| Streptomycin | Supersonic extract | " | |
| " | — | " | |

* Mice were observed for 96 hr after inj.; data are accumulative totals from replicate experiments.

† Mice were prepared for inoculation of viable cells or cell preparations by prior inj. of 1 mg of antibiotic/10 g body wt. Levels of chlortetracycline *alone* inoculated for control purposes are given in the table.

‡ Viable cells were inj. as 0.2 ml of 1% stock suspension; 0.5 ml of killed cells, extract or lysate were used except in high level controls of the most active preparation (supersonic extract).

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TABLE II. Toxicity of Viable *Candida albicans* Cells for Animals Pretreated with Chlortetracycline.

| Test animal | Cells/100 g body wt* | Chl./10 g† body wt, mg | Survival of animals, days 0 | 1 | 2 | 3 | 4 | 10 |
|---|---|---|---|---|---|---|---|---|
| Guinea pigs | $15 \times 10^6$ | 1 | 4 | 2 | 0 | 0 | 0 | 0 |
| | " | | 2 | 2 | 2 | 2 | 2 | 2 |
| | — | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| Rabbits | $15 \times 10^6$ | 1 | 4 | 0 | 0 | 0 | 0 | 0 |
| | " | | 6 | 6 | 5 | 4 | 3 | 3 |
| | — | 1 | 4 | 4 | 4 | 4 | 4 | 4 |
| | $12 \times 10^6$ | .75 | 4 | 3 | 3 | 3 | 3 | 3 |
| | " | | 4 | 4 | 4 | 4 | 4 | 3 |
| | — | .75 | 4 | 4 | 4 | 4 | 4 | 4 |
| | $10 \times 10^6$ | .67 | 2 | 2 | 2 | 2 | 2 | 2 |
| | " | | 2 | 2 | 2 | 2 | 2 | 2 |
| | — | .67 | 2 | 2 | 2 | 2 | 2 | 2 |
| | $5 \times 10^6$ | .34 | 2 | 2 | 2 | 2 | 2 | 2 |
| | " | | 2 | 2 | 2 | 2 | 2 | 2 |
| | — | .34 | 2 | 2 | 2 | 2 | 2 | 2 |
| Monkeys | $15 \times 10^6$ | 1 | 4 | 0 | 0 | 0 | 0 | 0 |
| | " | | 2 | 2 | 2 | 2 | 2 | 2 |
| | — | 1 | 2 | 2 | 2 | 2 | 2 | 2 |

* *C. albicans* was inoculated intraper. into test animals; each ml of inoculum contained from $15 \times 10^6$ to $5 \times 10^6$ cells.

† Chlortetracycline (chl.) was inoculated intraper. into test animals prior to inj. with *C. albicans*.

Oscillator, Model S-102A, 9 Kc/s. The supersonic extract was freed from intact cells by filtration through a 02 Selas filter to yield a water-clear sterile filtrate. Microscopic examination revealed that very few cells had been ruptured by supersonic vibration. *Supersonic lysate* was obtained from cells recovered from Selas filters: a 10% suspension of cells was autolyzed by storage at 4°C for 24 hours, and the opalescent lysate separated from cellular debris by passage through an 02 Selas filter. All cell-free preparations were stored at −70°C in sealed ampoules until used. *Crystalline antibiotics* reconstituted in saline were used within 2 hours after preparation. Antibiotics were injected intraperitoneally (IP) 30 minutes before IP inoculation of *C. albicans* cells or cell preparations. American Dutch Belt rabbits (1.5-2.0 kg), monkeys (*Macaca cynomolgus* 2.5-3.0 kg) and guinea pigs (0.5 kg) were used to test toxicity of viable *C. albicans*. Adult albino mice were employed to assay the toxicity of viable or heat-killed suspensions of *C. albicans* cells and cell-free preparations.

*Results.* It is evident from the findings summarized in Table I that: (a) lethality for mice of chlortetracycline and viable *Candida albicans* cells was equaled or exceeded by that of combinations of the antibiotic with supersonic extract or lysate, while combinations with heat-killed cells or mechanical lysate were less effective; (b) action of the antibiotic was associated with chlortetracycline; oxytetracycline was only slightly active and chloramphenicol and streptomycin were inactive; (c) antibiotic, extract or lysate singly was not lethal for mice. The supersonic extract was most potent of all *C. albicans* preparations tested. The data compiled in Tables I and II show that a) infectivity and toxicity of viable *C. albicans* for animals pretreated with chlortetracycline were separable properties, b) toxicity of viable *C. albicans* cells in combination with chlortetracycline was demonstrated in use of four diverse animal species; c) rabbits in response to graded doses of cells and antibiotic required for manifestations of toxicity a minimal threshold inoculum of yeast cells.

*Discussion.* Analysis of host-parasite relationships in experimental candidiasis is complicated by the potentiative effect of aureomycin(3,4,5). Possible stimulation by aureomycin of *C. albicans* growth has been reported (6,7,8) which ultimately may cause displacement of the normal flora of the gastrointestinal tract. Winter and Foley(5) showed that aureomycin could inhibit hematogenous spread of *C. albicans* in mice and alter the distribution of lesions. The findings reported here may aid clarification of this complex host-parasite relationship. The factor extractable from *C. albicans* cells may be regarded as an endotoxin(9) potentiated by chlortetracycline, or alternatively, as an agent capable of enhancing subliminal toxicity of the antibiotic. Either possibility is of clinical significance.

The origin of the extractable *C. albicans* factor is suggested by consideration of the nature and biological potency of the various cell-free preparations tested. Mechanical lysate (heavily opalescent), resulting from rupture of 60-70% of cells, was only slightly ac-

tive; supersonic lysate (slightly opalescent) was more active; and supersonic extract (water-clear), which resulted from lysis of very few cells, was most lethal to chlortetracycline-treated mice. This inverse relation between cell rupture and extract potency may indicate that the extractable factor is liberated from the surface of *C. albicans* cells. Surface origin is compatible also with the activity of heat-killed cells.

*Summary.* Supersonic vibration of viable *C. albicans* cells released a substance lethal to mice treated with chlortetracycline. In the absence of antibiotic the cell-free preparation was inactive. The combination of supersonic extract with chlortetracycline was highly lethal to mice, that with oxytetracycline was slightly active, while that with chloramphenicol or streptomycin was inactive. Evidence was presented for the combined toxicity of viable *C. albicans* cells and chlortetracycline to diverse animal species.

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## Continuous Cultivation of Epithelial Cell Strain (FL) from Human Amniotic Membrane.* (23003)

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Several investigators have succeeded in establishing epithelial-like cell lines from normal human tissues. Chang developed subcultures of cells from conjunctiva, liver, appendix and kidney(1), Perry *et al.* cultivated a cell line derived from normal human skin (2) and Jordan carried human nasal cells in continuous culture(3). Primary cultivation of cells from human amniotic membranes in tissue cultures has been reported(4). Further work in this and other laboratories has shown amnion cultures to be suitable for studies of several viruses(5-7) and as a source of normal human tissue for research on cancer problems. This stimulated attempts to develop amnion cells in subculture. We report here successful continuous cultivation of a strain (FL strain) of human epithelial-like cells derived from normal amniotic membrane. They grew as good confluent monolayer cultures of clear flat cells on the glass surface in medium without embryo extract. Their growth rate was high and they were cultivated in media containing human serum as well as several animal sera. For tissue culture work in which use of first generation material is not essential, the use of this strain of cells in serial cultivation offers several practical advantages, namely elimination of difficulties involved in collecting fresh material and a long trypsinization procedure. Susceptibility of this cell strain to polio and APC viruses will be reported later(8).

*Material and methods. Placental tissue*† was handled as aseptically as possible at the delivery room. Disinfectants of "surface active" type were completely avoided during de-

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