Virulence in Mice of Colonial Variants of *Candida albicans*. (20086)

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A previous note(1) on the chemotherapeutic activity of 6-sulfanilamido-2,4-dimethylpyrimidine (Elkosin), reported that this sulfonamide exerted a high degree of effectiveness against infections in mice induced by a number of micro-organisms, including *Candida albicans*. Based on 2 separate tests made with the latter organism, Elkosin was found to be active whereas sulfadiazine and sulfisoxazole exhibited no activity. The results of the earlier tests have since been confirmed and extended in many subsequent experiments with the exception that we have been unable to reproduce the activity of Elkosin in *C. albicans* infections. This discrepancy between the earlier and later trials could have been caused by a number of factors. Several workers(2-4) have clearly shown that cultures of *C. albicans* undergo spontaneous variation particularly when maintained for long periods under laboratory conditions. George and Plunkett(4) found that of 10 cultures maintained for several years on Sabouraud's glucose agar, 6 strains exhibited varying degrees of colonial dissociation proceeding from the smooth, moist type to the rough, dry form with the development of a much-branched "bushy" mycelium in contrast to the compound verticils of blastospores observed in the original, freshly isolated cultures.

In order to determine whether such colonial variations result in an alteration in virulence or could have influenced the results of our chemotherapeutic assays with Elkosin, we have investigated, in addition to the strain (culture No. 300) used for the earlier chemotherapy tests, 2 other *C. albicans* strains maintained in our culture collection.

1. *Cultural characteristics.* Two of these strains were composed of colonial variants consisting primarily of either yeast forms (smooth, colony type) or pseudomycelial elements (rough, colony type). The smooth type was present in 2 variations with respect to the gross aspect of the colonial growth, one variant producing cream-colored, flat colonies and the other yielding similar colonies but with deeper, yellow centers. *Table I* describes the colonial and cell morphologies of these variants. In Sabouraud's dextrose broth after 24 hours of incubation at 37°C, all strains grew compactly at the bottom except for Strain No. 238 which produced a cottony-like type of growth in the lower third of the broth.

2. *Virulence studies.* The different variants were grown and prepared according to a modification of Strauss and Kligman's method (5). The yeast phase of a culture less than 10 days old was transferred to Sabouraud's broth and incubated with constant agitation at 37°C for 24 hours. After centrifuging and noting the volume of packed cells, the organ-

TABLE I. Morphological Characteristics of *C. albicans* Colonial Variants.

Culture	Colony characteristics on Sabouraud's agar (24 hr at 37°C)	Wet-mount microscopic appearance of cells
#300 S*	Flat, cream-colored smooth colonies	Many budding blastospores in small clusters and chains. Very few branching pseudomycelial cells.
#238 R	White, round, rough colonies with domed raspberry-like surface	Many long-branching pseudomycelial cells with attached clusters of blastospores and chlamydospores. Few free blastospores.
#285 S	Flat, cream-colored smooth colonies with yellowish centers	Many budding blastospores in pairs, chains and clusters. Very few branching pseudomycelial cells.
#285 R	Small, white, rough colonies with domed raspberry-like surface	Many elongated and oval budding blastospores in pairs, chains and rosettes.

* S = Smooth type; R = Rough type.

TABLE II. Mouse Virulence of Colonial Variants of *Candida albicans*.

Culture	Conc. of packed organisms	No. of mice	Survival time (days) Avg	Range
#300 S*	1:10	16	5.1	<1 – >18
	1:50	10	2.8	<1 – 11
	1:100	12	4.4	<1 – >18
	1:500	11	7.8	2 – >18
	1:1000	6	8.2	2 – 12
#238 R	1:10	11	6.6	<1 – >18
	1:50	5	7.8	2 – >18
	1:100	11	16.5	2 – >18
	1:500	11	18.8	17 – >18
	1:1000	11	19.0	>18
#285 S	1:10	11	1.1	< 1
	1:50	10	4.6	<1 – >18
	1:100	8	2.1	<1 – 3
	1:500	8	6.5	2 – >18
	1:1000	8	16.2	4 – >18
#285 R	1:10	13	4.0	<1 – >18
	1:50	10	1.5	< 1
	1:100	8	9.6	<1 – >18
	1:500	8	9.1	2 – >18
	1:1000	8	12.6	2 – >18

* S = Smooth type; R = Rough type.

isms were resuspended at varying concentrations in Sabouraud's broth containing 4% gastric mucin. Male CFW mice were infected intraperitoneally with 0.5 ml amounts of the different concentrations of culture and observed for a total of 18 days. As seen from *Table II* the results of these experiments suggest that the different colonial types possess varying degrees of virulence for mice. This is indicated by a comparison of the rough and smooth types with regard to the average survival times for the different culture dose levels. In this connection, it is of interest to note that George and Plunkett(4) found no differences in fermentative properties among the dissociated strains.

3. *Chemotherapeutic studies.* All variants were used for chemotherapeutic assays. Elkosin did not influence the infections induced by any of our strains of *C. albicans*.

Summary. 1. Several variants isolated from 3 cultures of *C. albicans* exhibited differences with regard to colonial morphology, microscopic appearance as well as virulence. The smooth colonial type of strain No. 300 proved to be more virulent than the rough colonial form of Strain No. 238. 2. The results of the previous chemotherapeutic tests with Elkosin in *C. albicans* infections could not be reproduced in tests performed with the newly isolated variants.

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An Activator System in Blood Indispensable for Formation of Plasmin by Streptokinase.*† (20087)

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The fibrinolytic agent produced by certain streptococci (streptokinase) was originally thought to be a fibrinolytic enzyme(1). Milstone(2) showed that in addition to the streptococcal factor, a "lytic factor" in serum was necessary for the production of fibrinolysis. Later, Christensen(3) found that plasminogen, a precursor in blood, was transformed to the enzyme, plasmin, by streptokinase. Streptokinase is considered a direct activator of plasminogen. Discrepancies have been ob-

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