GROWTH PROMOTING EFFECT OF SOME BIOTIN ANALOGUES FOR *CANDIDA ALBICANS*

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Previous reports have indicated that many strains of *Candida albicans* and related species require biotin for growth and that thiamine is stimulatory or essential for some strains (Schopfer and Guilloud, 1944; Hijner, 1946; McVeigh and Bell, 1951; Bona and Hedrick, 1952). Later, Drouhet and Couteau (1954) and Drouhet and Vieu (1957) studied the detailed requirements of over 100 strains in the genus *Candida*. Practically all of them required biotin; a few needed also thiamine, vitamin B_6, or nicotinic acid, either singly or in different combinations. This was particularly true of the *Candida krusei* subgroup. Pantothenic acid stimulated growth of some strains of *Candida pseudotropicalis*.

It is evident that biotin is the principal vitamin requirement of *C. albicans*. Since there is no information, to the best of our knowledge, on the activity of biotin analogues for *C. albicans* the present study was undertaken. The effect of aspartic acid and polyoxyethylene sorbitan monooleate (Tween 80) as substitutes for biotin was also determined.

MATERIALS AND METHODS

Cultures. Four strains of *C. albicans* which appeared to be representative on the basis of conventional morphological and physiological characteristics were selected from a larger collection of candida cultures maintained in this laboratory. They were isolated from saliva samples, mostly from patients in the dental clinic or from laboratory personnel who showed no active candida infection at the time. All cultures were kept in stock in a tomato juice yeast extract liquid medium as prepared by Orland (1946) and transferred about once a month with interim storage in the refrigerator. The four cultures used for these tests all required biotin for satisfactory growth unless aspartic acid was supplied. For inoculation, cultures were grown for 48 hr in the experimental basal medium with 0.001 μg biotin per ml. A 1 to 1000 dilution of the culture in sterile saline was used to inoculate each tube in a series of tests.

Basal medium. A simplified amino acid-free medium which supported growth of *C. albicans* when supplemented with biotin was used for the experiments. This medium was chosen in preference to a casein digest medium because aspartic acid supports growth of candida in the absence of biotin. Growth in the simpler medium is somewhat slower than in a medium with a variety of preformed amino acids, but is nevertheless satisfactory for tests. The composition of the basal medium without biotin, in amounts per 1000 ml of redistilled water follows: glucose, 15 g; NH_4CL, 5 g; anhydrous Na-acetate, 3 g; adenine sulfate, guanine hydrochloride, and thymine, 5 mg each; K_2HPO_4, 0.5 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; KI, 0.05 mg; boric acid, 0.5 mg; NaCl, 10 mg; $FeSO_4 \cdot 7H_2O$, 10 mg; $MnCl_2 \cdot 4H_2O$, 10 mg; $CuSO_4 \cdot 5H_2O$, 0.05 mg; $ZnSO_4 \cdot 7H_2O$, 0.5 mg.

The medium was adjusted to pH 6.8 with sodium hydroxide, tubed in 8 ml amounts in tubes standardized for turbidity determinations, and autoclaved for 15 min at 121 C (15 lb).

Biotin and analogues. Some of the biotin analogues were not available commercially but were kindly provided for experimental purposes. For biotinol, *d*-homobiotin and *d*-norbiotin we are indebted to Hoffmann-LaRoche Inc., for *d,l*-oxybiotin to Dr. K. Hofmann of the University of Pittsburgh, and for biocytin to Merck Sharp and Dohme. Commercial preparations of (+)-biotin and *d,l*-dethiobiotin were obtained from Nutritional Biochemicals Corporation.

Experimental procedures. Solutions of each compound were prepared separately in redistilled water and sterilized by filtration through glass filters. All test substances were added singly to the basal medium in place of biotin at the time tests were started and after the medium had been tubed and autoclaved. The over-all concentra-

tions of the analogues subjected to test varied from 10 μg to 0.01 mμg per ml; for each analogue several amounts tested were based upon expected activity as determined in preliminary tests. Final results were based on at least 3 and often 4 or 5 different concentrations. At least two different series of tests were made at different times with each compound and with each test several concentrations of biotin were used for comparison. Controls of the basal medium without biotin or analogue were also inoculated.

Incubation was at 35 C. The growth of candida was followed by measurements of turbidity using a Lumetron colorimeter with a 580 mμ filter. Readings were made at 2, 3, 4, and in some cases at 6 days or later. Thus, any delayed growth could be taken into account in determining the result.

RESULTS

The growth response of *C. albicans* to the biotin analogues and to aspartic acid and Tween 80 is summarized in table 1 in terms of per cent of biotin activity. Since similar results were secured with each strain, the data can be presented briefly in the form of an average for the four candida strains. The per cent activity is stated in round numbers since it seemed unnecessary to attempt to determine the relationships more exactly.

Oxybiotin supported good growth of candida, but at suboptimal levels about 10 times as much was required for comparable growth. Assuming that only the *d*-form of *d,l*-oxybiotin is active, then the activity of oxybiotin for *C. albicans* is about one-fifth that of biotin. These results are within the same range as the 20 to 25 per cent activity reported for *Saccharomyces cerevisiae* by Rubin *et al.* (1945*b*) and Winnick *et al.* (1945).

The activity of dethiobiotin compared favorably with that of biotin in amounts up to about 0.1 mμg per ml. Larger amounts of dethiobiotin were inhibitive. Previous reports by others have shown that the effect of dethiobiotin on microorganisms differs greatly with different species. For *S. cerevisiae* this sulfur-free analogue of biotin was found to be quite active (Melville *et al.*, 1943; Dittmer *et al.*, 1944; Lilly and Leonian, 1944; Rubin *et al.*, 1945*a*), but for *Lactobacillus casei* it was inactive as a growth promoting agent and also inhibited the response to biotin. The work of Perlman (1948) with several species of clostridia well illustrates the differences in the effect of both dethiobiotin and oxybiotin on different species, even those within the same genus.

TABLE 1

Comparative growth promoting activity of some biotin analogues and compounds of related interest for Candida albicans

Analogue or Other Compound	Relative Activity (Biotin = 100%)
	%
Oxybiotin	20[a]
Dethiobiotin	10 to 100[b]
Norbiotin	0.1
Homobiotin	<0.001[c]
Biotinol	0.01
Biocytin	75 to 100
Aspartic acid, 100 μg/ml	40[d]
Tween 80, 100 μg/ml	0[d]
Aspartic acid plus Tween 80, each 100 μg/ml	60[d]

[a] When compared at two suboptimal levels. With larger amounts the activity of oxybiotin approached that of biotin.

[b] One hundred per cent only for suboptimal amounts of dethiobiotin up to about 0.1 mμg per ml. Larger amounts caused some inhibition of growth.

[c] Virtually inactive at 1 μg per ml.

[d] Based upon 100 μg of the test compound and an amount of biotin just sufficient for optimal growth.

Of the two analogues of biotin with different length side chains, norbiotin, which has three CH_2 groups, displayed appreciably more activity for *C. albicans* than homobiotin which has five CH_2 groups; even so, the activity of norbiotin was but a small fraction of that of biotin. Yeasts have shown a variable response to these two compounds. Belcher and Lichstein (1949) found that the effect differed markedly for different strains of *S. cerevisiae* as well as for other *Saccharomyces*. With *S. cerevisiae* strain T and *S. globosus* both analogues supported good growth in a biotin-free medium, though greater concentrations were needed to produce this effect. With *S. cerevisiae* (139) and two other yeasts growth was inhibited by these two analogues. They advanced reasons for believing that the growth promoting effect, where evident, was not due to biotin contamination of the analogues.

Biotinol, the alcohol derivative of biotin, showed very low activity for *C. albicans*. Biocytin

is the conjugate of biotin with lysine obtained and identified by Wright *et al.* (1950, 1951). Its activity for *C. albicans* was found comparable to that of biotin. Wright and associates reported that biocytin was active for a number of other microorganisms, including *Saccharomyces carlsbergensis*, although a few lactic acid bacteria were unable to utilize it.

The lower part of table 1 gives the results obtained with aspartic acid and Tween 80 as substitutes for biotin. Aspartic acid partially replaces biotin but the growth promoting effect is not equal to that of optimal amounts of biotin. The result with *C. albicans* is similar to that obtained originally with *Torula cremoris* when the aspartic acid replacement of biotin was first reported (Koser *et al.*, 1942). Tween 80 supported little or no growth of *C. albicans*, but when added with aspartic acid growth was stimulated somewhat over that secured with aspartic acid alone.

SUMMARY

Tests of growth promoting activity of some biotin analogues and compounds of related interest were made with 4 strains of *Candida albicans*. Biocytin and oxybiotin were both readily utilized in place of biotin and exhibited activities, respectively, of 75 to 100 per cent, and 20 per cent that of biotin. Dethiobiotin activity equaled that of biotin in amounts up to about 0.1 mμg per ml but larger amounts partially inhibited growth. Norbiotin and biotinol exhibited little activity and homobiotin was virtually inactive. Aspartic acid partially replaced biotin. Polyoxyethylene sorbitan monooleate (Tween 80) was virtually inactive but produced some stimulation of growth in the presence of aspartic acid.

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