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 Guillon, 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 B6, 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 monoleate (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 distilled water follows: glucose, 15 g; NH4CL, 5 g; anhydrous Na-acetate, 3 g; adenine sulfate, guanine hydrochloride, and thymine, 5 mg each; K2HPO4, 0.5 g; KH2PO4, 0.5 g; MgSO4·7H2O, 0.2 g; KI, 0.05 mg; boric acid, 0.5 mg; NaCl, 10 mg; FeSO4·7H2O, 10 mg; MnCl2·4H2O, 10 mg; CuSO4·5H2O, 0.05 mg; ZnSO4·7H2O, 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-oxobiotin 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 \mu g to 0.01 \text{mg} 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 mg 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. (1945b) and Winnick et al. (1945).

The activity of dethiobiotin compared favorably with that of biotin in amounts up to about 0.1 \text{mg} 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., 1945a), 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.

Of the two analogues of biotin with different length side chains, norbiotin, which has three CH₂ groups, displayed appreciably more activity for C. albicans than homobiotin which has five CH₂ 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. Bioeytin

<table>
<thead>
<tr>
<th>Analogue or Other Compound</th>
<th>Relative Activity (Biotin = 100%)</th>
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<tbody>
<tr>
<td>Oxybiotin</td>
<td>20(%)</td>
</tr>
<tr>
<td>Dethiobiotin</td>
<td>10 to 100(%)</td>
</tr>
<tr>
<td>Norbiotin</td>
<td>0.1</td>
</tr>
<tr>
<td>Homobiotin</td>
<td>&lt;0.001(%)</td>
</tr>
<tr>
<td>Biotinol</td>
<td>0.01</td>
</tr>
<tr>
<td>Bioeytin</td>
<td>75 to 100</td>
</tr>
<tr>
<td>Aspartic acid, 100 \mu g/ml</td>
<td>40(%)</td>
</tr>
<tr>
<td>Tween 80, 100 \mu g/ml</td>
<td>0(%)</td>
</tr>
<tr>
<td>Aspartic acid plus Tween 80, each 100 \mu g/ml</td>
<td>60(%)</td>
</tr>
</tbody>
</table>

* When compared at two suboptimal levels. With larger amounts the activity of oxybiotin approached that of biotin.
* One hundred per cent only for suboptimal amounts of dethiobiotin up to about 0.1 \text{mg} per ml. Larger amounts caused some inhibition of growth.
* Virtually inactive at 1 \text{mg} per ml.
* Based upon 100 \mu g of the test compound and an amount of biotin just sufficient for optimal growth.
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. Dethiobiocytin activity equalled that of biotin in amounts up to about 0.1 mg 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 sorbital monooleate (Tween 80) was virtually inactive but produced some stimulation of growth in the presence of aspartic acid.

### REFERENCES


