

# EFFECTS OF CERTAIN LIMITING CONDITIONS ON THE SYNTHESIS OF B VITAMINS BY YEAST

By MARGARET A. EPPRIGHT AND ROGER J. WILLIAMS

(From the Clayton Biochemical Institute and the Department of Chemistry,  
University of Texas, Austin)

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## I

### INTRODUCTION

The function of the B vitamins in metabolism is a challenging problem and to a large extent an unsolved one. In microorganisms which possess the ability to synthesize all or most of the B vitamins and to retain relatively large amounts of them within their cells, it appears reasonable to suppose that fabrication of the vitamins is not merely an expression of functionless creative ability but rather is the response of the organisms to a requirement for these substances. When the medium used for culturing such organisms is modified, either by qualitative alterations or by the addition of certain metabolic inhibitors, it seems likely that as a result the synthetic activities of the cells will reflect this modification. Any correlation between changes in extent of synthesis of any two or several vitamins might serve as indirect evidence as to how these vitamins function.

In this investigation the effects of certain qualitative modifications in the culture medium on the extent of growth and on the total synthesis of B vitamins by a microorganism have been studied. Further, the vitamin contents of cells produced in qualitatively altered media have been determined, and changes in the content of cells cultured in the presence of various inhibitors have been measured.

The organism chosen for this study was a strain of *Saccharomyces cerevisiae* which was isolated from a cake of Fleischmann's yeast (1). This strain, designated as F. B. yeast and carried in pure culture since the time of its isolation, has the ability to synthesize many of the B vitamins. In a study of the effects of the five vitamins which are growth factors for yeast, Williams *et al.* (1) found that an exogenous supply of thiamine, pyridoxine, and inositol was not required by F. B. yeast and made the tentative assumption that synthesis of these substances by the organism is the basis for their dispensability. Leonian and Lilly (2), who also used a strain of yeast isolated from a Fleischmann's cake, reported that by successively subculturing this yeast in a medium from which first pantothenic acid and then pantothenic acid and biotin were omitted a variant was obtained which grew well in a medium containing none of the B

vitamins except biotin. In the absence of biotin reduced growth was observed, regardless of whether or not other vitamins were present.

Qualitative modifications in the culture medium were obtained by (1) substitution of other carbohydrates for sucrose and/or (2) omission of various vitamins. Casein hydrolysate was added to several of the media in which growth was scant in order to determine whether or not its presence would increase the amount of growth in the same manner that it does in a medium which supports relatively heavy growth of yeast (3). Urea was also added to several of the media to serve as an additional source of nitrogen.

In the two modified media in which growth was sufficiently heavy to warrant separation of the yeast cells from the culture medium, content of the various vitamins in the cells rather than total synthesis was determined.

Similarly, yeast crops grown in the presence of the following inhibitors were assayed: potassium cyanide, hydroxylamine, sodium sulfite, semicarbazide, 4,4'-diamidino-1,3-diphenoxypropane (propamidine), sulfaguanidine, urethane, chloral hydrate, sodium fluoride, and camphor. These reagents, for the most part, have been shown to suppress fermentation, respiration, or growth of microorganisms. In some cases they are known to act on certain enzymic transformations, but it is highly probable that in no case are all of the sites of inhibition known.

## II

### EXPERIMENTAL

The medium described by Williams (4) was used in this study and is referred to as the supplemented medium; the basal medium contained no added vitamins.

All cultures were incubated at 30°C., and amounts of growth were determined by measurement with a thermocouple turbidimeter or by weighing the dried yeast crops.

In making the vitamin assays, microbiological methods were used exclusively. Thiamine, niacin, pantothenic acid, inositol, riboflavin, folic acid, and biotin assays were made using the methods summarized by Williams (4). Thiamine determinations on most of the samples were also made using the method of Niven and Smiley (5). *p*-Aminobenzoic acid values were obtained using the test developed by Lewis (6), and vitamin B<sub>6</sub> assays were made according to the method of Atkin *et al.* (7).

#### *Determination of Extent of Growth of Yeast Cultured in Qualitatively Modified Media*

In order to study the effects of certain qualitative modifications in the nutrient medium on the growth of F. B. yeast, the following media were employed:

- (1) Basal medium.
- (1a) Basal medium + 200 mg. per liter of casein hydrolysate.
- (1b) Basal medium + 40 mg. per liter of urea.
- (2) Basal medium + vitamin supplement with biotin omitted.

- (2a) Basal medium + vitamin supplement with biotin omitted + 200 mg. per liter of casein hydrolysate.  
 (2b) Basal medium + vitamin supplement with biotin omitted + 40 mg. per liter of urea.  
 (3) Basal medium + 1  $\gamma$  per liter of biotin ("biotin medium").  
 (4) Basal medium with sucrose replaced by xylose (20 gm. per liter).  
 (5) Supplemented medium with sucrose replaced by xylose (20 gm. per liter).  
 (5a) Supplemented medium with sucrose replaced by xylose (20 gm. per liter) + 200 mg. per liter of casein hydrolysate.

Duplicate sterile portions (50 ml. in 125 ml. Erlenmeyer flasks) of the various media were seeded with 0.3 mg. of moist F. B. yeast from a 24 hour inoculum and incubated for 124 hours. Growth measurements were made, and the yeast

TABLE I  
*Extent of Growth of F. B. Yeast Successively Subcultured in Various Qualitatively Modified Media*

Culture sequence	Seeding (moist yeast)	Incubation period	Extent of growth* in various media					Supplemented medium (control)
			Medium (1)	Medium (2)	Medium (3)	Medium (4)	Medium (5)	
	<i>mg. per culture</i>	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1	0.3	124	7	40	58		20	200
2	0.3	136	1	1.2	110	0.3	17	220
3	0.3	120	1.8	1.8	110		19	200
4	0.3	72	4.8	25	170		13	180
5	0.2	72	13	29	210		12	190
6	0.2	72	11	11	170		12	180
7	0.2	90	15	11	220		17	
8	0.2	72	9.5	5			11	
12	0.2	168	25	10			20	
13	0.2	192	28	16			14	

\* The extent of growth is expressed as milligrams of moist yeast per 50 ml. of culture medium.

was then successively subcultured over an extended period of time in the respective media. Amounts of growth are listed in Table I together with the respective incubation intervals and inoculums used. Values are not included for media (1a), (1b), (2a), and (2b) since it was found that neither casein hydrolysate nor urea improved the amount of growth in these deficient media. In medium (5a) growth differed by only 20 to 30 per cent from that in medium (5), so these values are also omitted.

*Determination of Extent of Vitamin Synthesis by Yeast Cultured in Qualitatively Modified Media*

In the case of cultures obtained on the twelfth transfer in media (1), (1*b*), (2), (2*b*), (5), and (5*a*), the yeast cells together with the medium in which they had grown for 8 days were assayed for the B vitamins. For those cultures containing added amounts of one or several of the vitamins, assay values were corrected for the amount of each vitamin added.

TABLE II  
*Amounts of B Vitamins Synthesized by F. B. Yeast in Various Qualitatively Modified Media Compared with the Vitamin Content of Cells Grown in the Control Medium*

Material assayed	Yeast crop*	Thiamine†	Niacin	Pantothenic acid	Inositol (acid-extracted)	Inositol (enzyme-extracted)	Riboflavin	Folic acid‡	Vitamin B <sub>6</sub>	<i>p</i> -Aminobenzoic acid	Biotin
	mg. per liter	γ per liter	γ per liter	γ per liter	γ per liter	γ per liter	γ per liter	γ per liter	γ per liter	γ per liter	γ per liter
Cells + culture medium from											
Medium (1).....	560	40	520	160	5,100	100	60	25	240	42	0.071
Medium (1 <i>b</i> ).....	490	40	480	120	3,900	0	80	27	230	49	0.088
Medium (2).....	320	45	530	110	9,800	1,100	40	12	50	8.1	0.062
Medium (2 <i>b</i> ).....	450	64	630	80	11,000	2,200	40	14	60	9.2	0.065
Medium (5).....	280	58	270	60	1,300	2,400	20	3	Toxic	0.32	0
Medium (5 <i>a</i> ).....	420	79	340	50	2,400	2,100	40	4	Toxic	0.83	0
Yeast separated from 1 liter of supplemented medium.....	3,800	100	600	46	1,400		37	24	19	24	0.7

\* The amount of yeast produced is expressed on a moist basis.

† Thiamine values were determined by the yeast growth assay method.

‡ Folic acid values represent micrograms of material of "potency 40,000."

Samples prepared according to the procedure of Cheldelin *et al.* (8) were assayed for thiamine, niacin, pantothenic acid, inositol, riboflavin, and folic acid. Inositol assays were also run on samples made 2 N in HCl and autoclaved for 1 hour. The acid-treated samples were assayed for vitamin B<sub>6</sub>, *p*-aminobenzoic acid, and biotin. Results of the assays are presented in Table II; for comparative purposes the vitamin content of yeast cells grown in the supplemented medium is included.

*Determination of Vitamin Content of Yeast Cultured in Qualitatively Modified Media*

Medium (3) was found to support heavy growth of yeast, and for a month, the yeast was transferred in this medium at 3 day intervals; then a crop of the

resulting "biotin yeast" was prepared in the following way. Each of two 2,000 ml. Erlenmeyer flasks containing 500 ml. of sterile "biotin medium" was seeded with 2 mg. of moist "biotin yeast" and incubated for 72 hours. The yeast was centrifuged out, washed, and dried.

A portion of the dried yeast was treated with enzymes according to the method of Cheldelin *et al.* (8), and the extract was assayed for thiamine, niacin, pantothenic acid, riboflavin, and folic acid. Preliminary studies were made

TABLE III  
Vitamin Content of Yeast Cultured in Various Kinds of Media

Yeast crop from	Dry weight of crop	Thiamine ( <i>Streptococcus</i> <i>sakazarius</i> test)	Thiamine (yeast growth test)	Niacin	Pantothenic acid	Inositol	Riboflavin	Folic acid*	Vitamin B <sub>6</sub>	<i>p</i> -Aminobenzoic acid	Biotin
	gm. per liter	$\gamma$ per gm. †	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.	$\gamma$ per gm.
Supplemented medium (control)...	1.2	30	84	500	39	1,100	30	20	16	20	0.57
Cyanide medium (shaken culture).....	1.2	14	62	400	81	980	38	4.0	16	17	0.58
Cyanide medium (unshaken culture).....	1.2	17	72	360	100	810	45	4.3	11	36	1.4
Hydroxylamine medium.....	1.1	15	40	330	46	820	30	10	17	9.8	1.6
Sulfite medium.....	1.2	18	64	270	9.3	770	35	46	19	35	0.83
Semicarbazide medium.....	0.70	11	27	54	4.1	730	14	9.2	3.0	9.7	1.2
Propamidine medium.....	0.90	30	78	520	32	570	25	20	9.9	24	1.2
Sulfaguanidine medium.....	0.81	34	90	750	18	1,600	45	5.6	40	13	2.4
Urethane medium.....	0.94	28	100	520	4.8	980	25	23	5.9	20	0.69
Chloral hydrate medium.....	1.1	28	73	400	32	850	22	2.5	47	19	0.78
Fluoride medium.....	0.48	12	27	38	12	720	4.4	0.72	16	2.4	1.6
Camphor medium (0.001 M).....	1.2	30	78	610	31	980	42	13	13	17	0.84
Camphor medium (saturated).....	0.54	21	82	730	24	810	25	2.5	3.0	8.5	1.6
"Biotin medium".....	1.2	27	100	760	31	770	31	23	31	41	0.59
Galactose medium (crop 2).....		60	250	440	79	1,100	38	4.2	24	9.2	4.8
Galactose medium (crop 6).....		17	65	540	61	1,600	38	3.1	28	34	0.56
Galactose medium (crop 8).....		29	80	440	37	1,500	36	6.3	47	18	0.49

\* Folic acid values represent micrograms of material of "potency 40,000."

† All vitamin contents are expressed as micrograms per gm. of dry yeast.

to determine the most satisfactory extraction procedures for the remaining vitamins. For biotin and inositol, the yeast was autoclaved for 1 hour in 20 volumes of 3 N HCl; and for *p*-aminobenzoic acid and vitamin B<sub>6</sub>, it was autoclaved for 30 minutes with 20 volumes of 2 N H<sub>2</sub>SO<sub>4</sub>.

Galac yeast was produced by successive subculture in a medium containing galactose (25 gm. per liter) and Difco yeast extract (9). Three crops of the dried yeast were assayed. The crops selected for assay were those obtained on the second, sixth, and eighth transfer in the galactose medium, so that any trend in changing content of the respective vitamins might be discerned.

Assay values for the "biotin yeast" and crops 2, 6, and 8 of the galac yeast are shown in Table III.

*Determination of Vitamin Content of Yeast Cultured in the Presence of Various Inhibitors*

By means of preliminary experiments it was found that each of the following inhibitors at the indicated concentration would restrict the growth of F. B. yeast in a 24 hour period but permit heavy growth in 72 hours; potassium cyanide (0.001 M), hydroxylamine (0.001 M), sodium sulfite (0.001 M), semicarbazide (0.01 M), propamidine (0.001 M), sulfaguanidine (0.005 M), urethane (0.2 M), chloral hydrate (0.01 M), sodium fluoride (0.001 M), camphor (0.001 M), and camphor (saturated). These concentrations were obtained by adding the calculated weight of inhibitor to the sterile supplemented medium just prior to inoculation with yeast. Two levels of camphor were used because it was observed that in the saturated medium, involutinal cells developed; no such effect was noted in the 0.001 M medium.

A crop of F. B. yeast was grown in the presence of each inhibitor and harvested using the procedure described for obtaining the "biotin yeast" crop. In the cyanide medium it was necessary to extend the incubation period since no discernible growth had occurred in 72 hours. After 13 days a slight increase in turbidity was noted, and 3 days later the crop was harvested.

Extracts of these crops were assayed, and for control purposes a crop of F. B. yeast grown in the supplemented medium was assayed. The results are presented in Table III.

### III

#### DISCUSSION

The data in Table I show that F. B. yeast requires an exogenous source of biotin in order for extensive proliferation to occur. Neither casein hydrolysate nor urea improved the restricted growth of yeast in a biotin-free medium, so evidently a limited ability to synthesize necessary amino acids is not the factor responsible for reduced growth.

Xylose will not support the growth of F. B. yeast in a vitamin-free medium, but in a supplemented medium slight growth does occur. It has been reported that most yeasts multiply a little in a xylose medium but that there is only a small consumption of sugar (10).

Cells cultured in the basal medium were found to synthesize significant amounts of all of the B vitamins except biotin. When the basal medium contained addenda of thiamine, pyridoxine,  $\beta$ -alanine, and inositol, the amounts of the respective vitamins synthesized were lower than those produced in the vitamin-free medium except in the case of thiamine, niacin, and inositol. The most marked decreases were observed in *p*-aminobenzoic acid and vitamin B<sub>6</sub> formation. Apparently one or several of the added nutrilites suppresses the formation of these substances or is able to replace them in function.

Since the yeast crops in the media discussed above were only one-twelfth to one-seventh as large as that in the supplemented medium, while the total amounts of the respective vitamins synthesized were as great or greater than the amounts present in the yeast crop from the supplemented medium, it appears probable that in no case is the entire amount of a synthesized vitamin retained by the cells.

In the supplemented medium in which xylose was substituted for sucrose, the amounts of the B vitamins found were sufficient to indicate that some synthesis of every one except biotin and vitamin B<sub>6</sub> had occurred. No values were obtained for the latter because the extracts were toxic to the test organism.

The inositol values listed in Table II were determined on samples extracted with acid and with enzymes. Inspection of the values suggests that most of the inositol which is synthesized by the yeast is bound in such a fashion that acid hydrolysis is required for its liberation.

Inasmuch as F. B. yeast is able to synthesize considerable amounts of all of the B vitamins except biotin it is not surprising that the vitamin content of the "biotin yeast" is very like that of the control yeast. In "biotin yeast" the amounts of vitamin B<sub>6</sub> and *p*-aminobenzoic acid present are approximately twice the values for the control. These were the two vitamins synthesized in larger amounts in a vitamin-free medium than in a medium containing addenda of thiamine, pyridoxine,  $\beta$ -alanine, and inositol. Biotin appears not to be involved in this effect, but further investigation is necessary in order to determine which of the other four nutrilites are responsible for these reduced contents and what is the significance of the changes.

Although no conclusions can be drawn concerning the synthetic activity of galac yeast, cells of this derived yeast appear to require a significantly larger amount of vitamin B<sub>6</sub>, a slightly larger amount of riboflavin, and a considerably smaller amount of folic acid than do those of the parent yeast. The acclimatization of F. B. yeast in the galactose medium was accompanied by no significant change in niacin content, but for the remaining four vitamins certain eccentricities were observed which cannot be satisfactorily explained.

In making a critical study of the changes in the vitamin content of yeast cultured in the presence of inhibitors, it is necessary to recognize that an observed lowering in content may be interpreted as arising from (1) a diminished need for, (2) an interference in the synthesis of, (3) a lessened retention of, or (4) in some cases, a reduced absorption from the medium of that vitamin. An observed increase may be regarded as evidence for (1) an enhanced requirement, (2) a stimulation of synthesis, (3) an increased retention, or (4) in some cases, an increased absorption from the medium. The extent of variation in content of the respective vitamins and the significance of these changes will be considered next.

*Thiamine and Niacin.*—Since there was a fair degree of parallelism between

thiamine and niacin values, they will be considered together. It has been reported that the thiamine content of *Saccharomyces cerevisiae* increases in anaerobic culture and that the fermentative energy increases with increased thiamine content (11). It seems reasonable to assume that thiamine content may be used to determine the extent to which various inhibitors interfere with or enhance fermentation, as may the correlative niacin content.

On this basis, propamidine, urethane, camphor (0.001 M), and chloral hydrate had no effect on the course of fermentation. The reagents for the carbonyl group, *viz.* potassium cyanide, sodium sulfite, semicarbazide, and hydroxylamine, caused considerably lowered contents of these two vitamins as was to be expected. Sodium fluoride also caused a sharp reduction which was entirely expected inasmuch as it is known to inhibit two of the enzymes, enolase and carboxylase (12), active in the chain of fermentation reactions, and to prevent the formation of cozymase (13) as well. Yeasts from the sulfaguanidine and saturated camphor media had niacin contents considerably higher than the corresponding thiamine contents. This may mean that niacin functions in processes other than the oxidation-reduction changes in fermentation, or it may be that these inhibitors alter cell retention of these vitamins differently.

The thiamine values referred to in the foregoing discussion are those obtained with *Streptococcus salivarius* as the test organism, because the yeast method has been shown not to be completely specific for thiamine (14). Although values obtained in this study by the yeast growth method were consistently higher (2 to 4 times) than corresponding values from the *S. salivarius* test (Table III), two sets of ratios obtained by dividing the thiamine content of each derived yeast by that of the control yeast agreed within 10 per cent, respectively, for seven of the yeast crops produced in the presence of inhibitors. This agreement indicates that changes in thiamine content are attended by corresponding changes in content of a non-thiamine material measured in the yeast growth method. This stimulative material may be regarded as a precursor of the vitamin or as a degradation product.

*Pantothenic Acid.*—In only one case, *viz.* in the "cyanide yeast," was an increase of more than 20 per cent in pantothenic acid content observed. The absence of any correlation between changes in pantothenic acid content and thiamine and niacin values makes it appear probable that the chief metabolic rôle of pantothenic acid does not reside in the reactions involved in fermentation. Since a sharp decrease in content was observed in "urethane yeast" and since urethane is a recognized dehydrogenase inhibitor (15), it is conceivable that pantothenic acid acts in a dehydrogenase system which is put out of operation by urethane and is called into increased activity in the presence of cyanide, a reagent which poisons oxidases but not dehydrogenases (16). An alternate interpretation is that a dehydrogenase system is involved in the formation of pantothenic acid.



*Inositol*.—Of the inhibitors employed, only propamidine caused the inositol content to fall as low as 50 per cent of the value for the control yeast, and only sulfaguanidine caused an increased content of this nutrilitite.

To a certain extent, changes in inositol values parallel those for thiamine and niacin which suggests that inositol may also be involved in fermentation reactions. Previously, in a study on rat tissues, Williams (17) showed that inositol and biotin exhibit a positive correlation with both the aerobic respiratory rate and the anaerobic glycolysis rate. If inositol functions in both aerobic and anaerobic processes in yeast, it is not surprising that the cells maintain a relatively constant amount of the substance even though they are grown under adverse conditions.

*Riboflavin*.—Two of the carbonyl reagents, hydroxylamine and sulfite, caused no appreciable changes in riboflavin content, while semicarbazide caused a reduced content and cyanide an increased one. The apparent lack of consistency in effect of these reagents indicates that riboflavin probably is not directly involved in the reactions occurring in fermentation. An increased content of riboflavin in baker's yeast cultured in the presence of cyanide has been observed previously by Pett (18). He suggested that respiration in yeast may take place along two paths, one of which involves the cytochromes and one in which a flavoprotein is active. Some supporting evidence for this hypothesis has been obtained by Stier and Castor (19).

If riboflavin actually participates in such an auxiliary system in yeast, changes in the amount of the substance contained in the cells should serve as a measure of the extent of activity of this secondary system. From this standpoint, sulfaguanidine and camphor (0.001 M), as well as cyanide, must increase the activity of the flavin system. Of the inhibitors which caused reductions in riboflavin content, fluoride had the most drastic effect. This action of fluoride probably relates to its property of phosphatase inhibition.

*Folic Acid*.—No correlation was observed between changes in folic acid content and changes in the other nutrilitites. Only in one instance was the content of the vitamin increased, and that was in the presence of sulfite.

In the yeast crops grown in the two camphor media, the difference in folic acid content was greater than that in any other vitamin under investigation. This, however, does not explain the fact that the more concentrated camphor medium caused the development of involution in the yeast since other yeasts had similar folic acid contents but did not exhibit pleomorphism.

Unfortunately the folic acid values obtained are not an actual measure of the total combined folic acid in yeast. Since this investigation was started, Totter *et al.* (20) showed that extraction with clarase liberated only a fraction of the folic acid from brewers' yeast.

*Vitamin B<sub>6</sub>*.—It seems probable that vitamin B<sub>6</sub> is not involved in the fermentation processes of yeast since changes in this vitamin show no significant correlation with thiamine and niacin values.

Yeast cultured in the presence of fluoride was quite low in every vitamin except vitamin B<sub>6</sub>, biotin, and inositol. Since this reagent inhibits fermentation at two sites and is also an inhibitor of yeast phosphatases, it appears likely that the three vitamins named function in processes which are not part of the normal chain of fermentative reactions and which are not linked with the action of phosphatases.

*p-Aminobenzoic Acid.*—Changes in *p*-aminobenzoic acid content were not found to correlate with those for any other vitamin.

The "fluoride yeast" contained only 12 per cent as much of this vitamin as was in the control yeast, which may mean that the formation of *p*-aminobenzoic acid or its action in yeast depends on reactions involving fluoride-sensitive phosphatases.

The yeast crop from the sulfaguanidine medium contained 65 per cent as much *p*-aminobenzoic acid as the control, indicating that this inhibitor did not markedly block synthesis of the vitamin even though its interference with function was to be expected because of structural similarity.

*Biotin.*—In no case did the biotin content of any yeast fall below that of the control; this serves as further evidence that F. B. yeast must contain within its cells a limiting level of biotin in order to carry on those reactions essential for extensive growth.

In a number of cases the biotin content of yeast was increased by culturing in the presence of inhibitors. In some instances, this increase can be accounted for without assuming that any synthesis of the vitamin occurs, since the biotin available in the medium is sufficient to account for the increase. But apparent synthesis occurred in the presence of cyanide, fluoride, hydroxylamine, camphor (saturated medium), and sulfaguanidine. The most striking increase was observed in yeast from the sulfaguanidine medium. This yeast had a content more than 4 times that of the control yeast and more than twice the amount of biotin added to the medium. An attempt to obtain a yeast capable of extensive growth in a sulfaguanidine medium from which biotin was omitted was unsuccessful.

#### IV

#### SUMMARY

In yeast crops which were grown in the presence of various inhibitors, there was considerable variation in content of the various B vitamins. A higher degree of parallelism in variation in content was found to exist between thiamine and niacin than between any other pair of vitamins; this has been interpreted as indicating that the predominant functions of these two vitamins are their established rôles in fermentation. Values for inositol indicate that it may be involved in fermentation processes, but this is not the case for other members

of the B complex. Biotin appears to be unique since in no case did the biotin content of yeast grown in the presence of an inhibitor fall below that of the control yeast. There was some evidence of synthesis of biotin, or a material with biotin activity, in the presence of certain inhibitors, the most striking instance being with sulfaguanidine.

An exogenous supply of biotin was essential for extensive proliferation of F. B. yeast, and yeast grown in a medium to which biotin was the only added vitamin contained the B vitamins in amounts very similar to those found in the control yeast, the most marked differences being in increased vitamin B<sub>6</sub> and *p*-aminobenzoic acid contents.

In the absence of biotin, significant amounts of all of the B vitamins except biotin were synthesized, both in the presence and absence of certain other members of the B complex. The addition of thiamine, pyridoxine, inositol, and  $\beta$ -alanine to the culture medium caused a reduction in the amounts of vitamin B<sub>6</sub> and *p*-aminobenzoic acid synthesized.

F. B. yeast was able to grow in a xylose medium only when certain of the B vitamins were present, and even then growth was limited. Evidence was obtained for some synthesis of all of the vitamins investigated except biotin and vitamin B<sub>6</sub>.

The most significant differences in vitamin content between galac yeast and the parent F. B. strain were in folic acid and vitamin B<sub>6</sub>, the former being considerably reduced in amount, the latter being increased.

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