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THE EFFECT OF BIOTIN DEFICIENCY ON THE SYNTHESIS OF FATTY ACIDS BY YEAST*

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SUMMARY

Biotin was replaceable by the unsaturated long-chain fatty acids, oleic and palmitoleic, when added with aspartic acid to biotin-deficient baker's yeast under aerobic conditions. Baker's yeast grew normally with ethyl stearate and palmitate under similar conditions. This is explained by the ability of the esters to penetrate into the yeast cells, where the saturated acids were converted into unsaturated ones.

When baker's yeast grew in the absence of biotin under aerobic conditions, the content of C_{18} fatty acids, including oleic acid, decreased, and the content of C_{16} fatty acids, particularly palmitoleic acid, and of acids with less than 16 carbon atoms, increased. Thus the view is supported that biotin plays a role in fatty acid synthesis, the absence of biotin being reflected as reduced formation of C_{18} fatty acids *in vivo*.

During industrial propagation, the biotin content of baker's yeast fell from about 2 μg to 0.5 μg/g dry wt. The amount of C_{18} acids simultaneously decreased from about 50% to 30%, oleic acid from about 37% to 27%, while palmitoleic acid rose from 35% to 53% of the total fatty acid. Addition of biotin prevented the decrease of C_{18} acids, especially of oleic acid, as well as the increase of palmitoleic acid. Corresponding results were obtained with biotin-deficient baker's yeast under aerobic growth conditions on a laboratory scale.

INTRODUCTION

Biotin is essential for the growth of many microorganisms. The importance of biotin has also been proved in the technical production of baker's yeast[1-4]. Biotin participates in yeast anabolism in many ways: in the carboxylation of pyruvic acid, in the synthesis of pyridine nucleotides[5], in nucleic acid synthesis[6], in the formation of both purine and pyrimidine bases[7], in protein synthesis[6,8], in the synthesis of polysaccharides[9] and in the synthesis of fatty acids[10-13]. Aspartic acid or asparagine, added to baker's yeast, to some extent compensate for biotin deficiency[14]. In some bacteria unsaturated, long-chain fatty acids, primarily oleic acid, also have an effect on growth similar to that of biotin. These observations suggested that lack of biotin might affect the synthesis of fatty acids. We have therefore investigated the influence of biotin

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deficiency on fat composition, and tested whether long-chain fatty acids can replace biotin in baker's yeast.

MATERIALS AND METHODS

In laboratory experiments the anaerobically cultured A_2 stage of baker's yeast, produced by the Rajamäki Factories of the Finnish State Alcohol Monopoly, was used as seed yeast. The influence of different compounds on growth was examined using the culture medium and apparatus for biotin determination suggested by WHITE[15]. 1.1 l of culture medium was inoculated with 300 mg (fresh wt.) of baker's yeast of stage A_2 and kept for 24 h at 30°; the aeration rate was 3.5 l/min. In the experiments with aspartic acid, an amount of ammonium sulphate corresponding to the aspartic acid nitrogen was omitted to keep the total nitrogen of the culture medium at the same level in all experiments. The growth of the yeast was followed by dry-matter determinations: a sample was filtered on a glass sinter G4 and dried overnight at 105°. Several laboratory experiments were performed on a larger scale, 11-l lots being used in a thermostatic fermentometer constructed by Messrs O. DICH (Denmark) and equipped with an automatic feeding and aeration apparatus. The culture medium was inoculated with 9 g of A_2 stage baker's yeast. The aeration rate was 9 l/min and the air was suspended in small bubbles. The fatty acids were always determined from the whole yeast culture. The biotin content and the fatty acid composition at the various technical stages of yeast production were determined directly on 100 g fresh-yeast samples from the Rajamäki Factories. The industrial propagation and the treatment of the yeast samples have been described previously[16].

Fatty acids were extracted by boiling for 3 h in a methanol–chloroform mixture, 1:2 (see ref. 17). The yeast remaining in the funnel after filtration was boiled again in a further lot of the same mixture for 30 min and filtered. The combined filtrates were evaporated in vacuum at 30–40° and the fats extracted from the remainder with light petroleum (boiling range 40–60°)[18]. The light petroleum was evaporated under vacuum, and the fats were saponified by boiling for 3 h with 0.5 N KOH in absolute methanol. The unsaponified residue was removed by extraction with light petroleum, the solution was made acid with 5 N sulphuric acid, and the acids liberated were extracted with light petroleum[19]. The extract was dried with anhydrous sodium sulphate and evaporated to 5-10 ml. Diazomethane prepared from "Diazald" (*N*-methyl-*N*-nitroso-*p*-toluene-sulphonamide, Aldrich Chemical Co., Inc., Wisc. (U.S.A.)) was added in excess according to the method of DE BOER AND BACKER[20]. Esterification was allowed to proceed for 30 min at room temperature and the ether solution of diazomethane was then evaporated. The methyl esters of the fatty acids were identified by gas chromatography, with a ^{90}Sr detector[21] and argon as carrier gas. The column used was a glass tube 120 cm long (diameter 4 mm), filled with acid-washed Celite (120–140 mesh), saturated with 25 % Silicone or Abiezon M. The unsaturated fatty acids were separated by addition of 25–35 % Rheoplex-400. The fatty acid composition of the yeast samples was planimetrically determined from the peaks of the chromatogram.

The biotin content of the yeast samples was determined according to WHITE[15] by autolysis of the yeast in the presence of toluene for 7 days at 30° followed by microbiological determination of the liberated biotin. The A_2 stage of baker's yeast

was used as test organism. The nucleic acid content of the yeast was determined by the method of DICARLO, SCHULTZ AND FISHER[22]. The disappearance of aspartic acid from the culture medium was followed by the method of POPE AND STEVENS[23], and ammonia consumption was determined with a Parnas–Wagner apparatus.

RESULTS

The effect of addition of fatty acids on the growth of biotin-deficient baker's yeast

It has been suggested[14] that the yeast's requirement of biotin could be satisfied by replacing the ammonia nitrogen of the culture medium with aspartic acid or asparagine. This is possible, but only to a certain extent (Fig. 1). On adding long-chain fatty acids, saturated or unsaturated, to a culture medium containing only ammonia nitrogen, no noticeable increase in the yeast could be observed. When, however, the fatty acids were added with aspartic acid, all the acids with an even number of

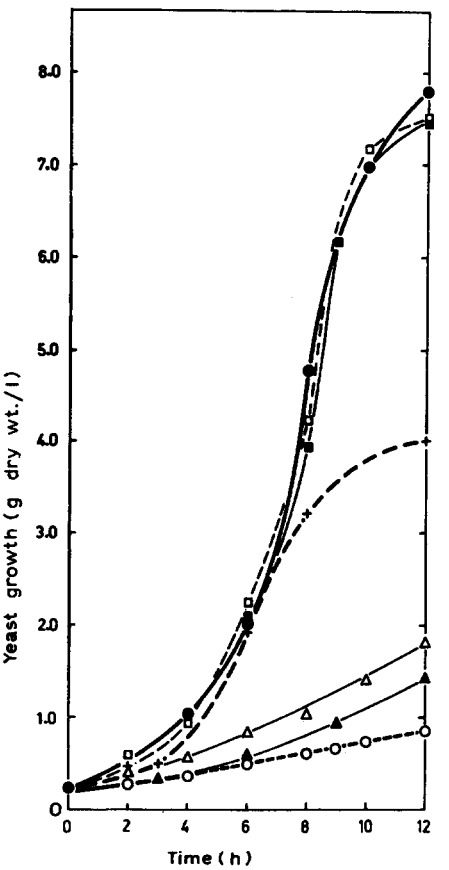

Fig. 1. The growth of baker's yeast with (●—●) and without (○---○) biotin, and without biotin but with aspartic acid (+----+), oleic acid (△—△) palmitoleic acid (▲—▲), aspartic acid + oleic acid (□----□), or aspartic acid + palmitoleic acid (■—■).

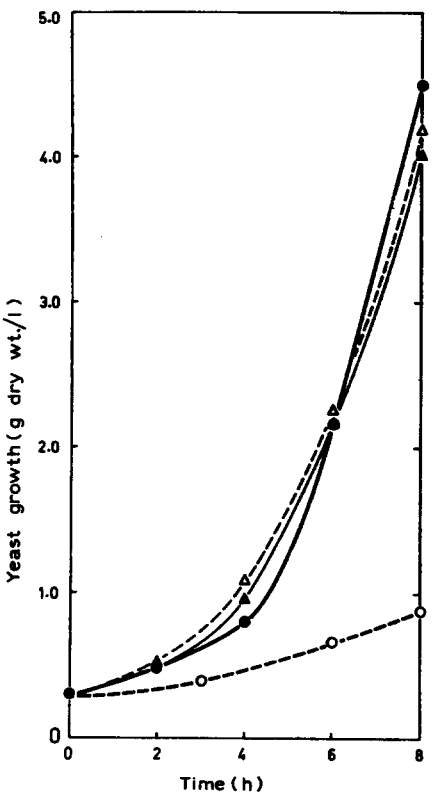

Fig. 2. The growth of baker's yeast with (●—●) and without (○---○) biotin, and without biotin but with addition of aspartic acid + ethyl stearate (▲—▲), or aspartic acid + ethyl palmitate (△----△).

TABLE I

THE EFFECT OF DIFFERENT FATTY ACIDS ON THE GROWTH OF BAKER'S YEAST IN THE ABSENCE OF BIOTIN, WITH OR WITHOUT ADDITION OF ASPARTIC ACID

Growth in 1.1 l during 24 h at 30°. Control experiments were run with ammonium sulphate, without aspartic acid, fatty acids or biotin and the values subtracted in each case from the results obtained.

Addition			*Increase of yeast growth (g dry wt./l)*
Aspartic acid N (per cent of total N)	*Fatty acid (g)*		
	Oleic acid	1.0	0.72
	Linolenic acid	0.5	0.35
30			0.73
30	Oleic acid	0.5	4.57
30	Linolenic acid	0.5	4.04
50	Lauric acid	1.0	1.73
50	Tridecanoic acid	1.0	2.48
50	Myristic acid	1.0	1.67
50	Pentadecanoic acid	1.0	2.49
50	Palmitic acid	1.0	1.37
50	Stearic acid	1.0	2.05
50	Oleic acid	0.5	5.99
50	Oleic acid	1.0	5.23
50	Linolenic acid	0.1	2.96
50	Linolenic acid	0.5	4.31
50	Linolenic acid	1.0	4.09
	Biotin	10.0 μg	7.68

carbon atoms, from lauric to stearic acid, promoted the growth of the yeast (Table I). A stronger growth-promoting effect was obtained when some long-chain unsaturated fatty acid, palmitoleic, oleic or linolenic acid, was added with the aspartic acid (Tables I and II). With palmitoleic or oleic acid the growth even caught up with that produced by addition of biotin (Fig. 1). Aspartic acid supplied about 50% of the nitrogen of the culture medium, and Table III shows that no further increase was obtained with larger amounts of this acid.

Linolenic acid and succinic acid hardly affected the growth of the yeast. On the other hand, caprylic, pelargonic, capric and enanthic acids inhibited the growth.

When yeast was grown in the absence of biotin, its nucleic acid content was about 13–15% of the dry matter, but increased to 18–21% when the yeast was cultured in the presence of biotin, or without biotin but with aspartic acid (Table II). Similar results have been reported by AHMAD AND ROSE[24].

When the effect of the addition of oleic acid was studied (Table IV) the consumption of aspartic acid nitrogen from the medium was found to rise only slightly during 9 h, while the consumption of ammonia nitrogen increased by 50%.

The effect of biotin deficiency on the fat composition of baker's yeast

Lack of biotin is known to reduce the yield of baker's yeast in industrial propagation when sugar beet molasses is used as carbohydrate source[1–4]. We have previously observed that the biotin content of yeast in the industrial process falls from more than 2 μg to less than 0.5 μg per g dry weight[8]. The full data are given in Table V.

TABLE II

THE EFFECT OF PALMITOLEIC AND OLEIC ACIDS ON THE GROWTH OF BAKER'S YEAST IN THE ABSENCE OF BIOTIN, WITH OR WITHOUT ASPARTIC ACID

Growth in 11 l at 30°.

Addition	*Time (h)*	*Yeast growth* (g dry wt./l)*	*Nucleic acid content* (per cent of dry wt.)*
	0	0.27 (10)	12.2 (10)
Without biotin	6	0.50 (5)	14.7 (4)
	10	0.73	12.0
	12	0.85	10.5
Without biotin + 5 ml palmitoleic acid	6	0.59 (1)	12.6 (1)
	9	0.94	13.5
	12	1.44	—
Without biotin + 5 ml oleic acid	6	0.85 (1)	13.8 (1)
	10	1.40	14.1
	12	1.82	—
Without biotin + 50% aspartic acid N	6	1.90 (4)	21.3 (3)
	9	3.22	18.0
	12	3.98	—
Without biotin + 50% aspartic acid N + 5 ml palmitoleic acid	6	2.10 (2)	20.8 (1)
	9	6.17	18.4
	10	6.95	—
	12	7.49	—
Without biotin + 50% aspartic acid N + 5 ml oleic acid	6	2.25 (4)	19.6 (3)
	10	7.16	17.1
	12	7.54	—
Biotin 100 μg	6	2.02 (5)	18.5 (5)
	10	6.96	15.7
	12	7.82	15.0

* The figures in parentheses indicate the number of determinations from which the mean values are calculated.

TABLE III

THE EFFECT OF ASPARTIC ACID ON THE GROWTH OF BAKER'S YEAST, WITH AND WITHOUT ADDITION OF LINOLENIC ACID

Growth in 1.1 l during 24 h at 30°. Control experiments were run with ammonium sulphate, without aspartic acid, linolenic acid or biotin and the values in each case subtracted from the results obtained.

Addition		*Increase of yeast growth (g dry wt./l)*
Aspartic acid N (per cent of total N)	*Linolenic acid (g)*	
5		0.70
5	0.5	0.98
30		0.73
30	0.5	4.04
50		0.75
50	0.5	4 31
75	0.5	4.19
100	0.5	3.39

TABLE IV

THE UTILIZATION OF ASPARTIC ACID AND AMMONIUM SULPHATE NITROGEN DURING THE GROWTH OF BAKER'S YEAST WITHOUT BIOTIN, WITH AND WITHOUT OLEIC ACID

Growth in 11 l at 30°. Aspartic acid nitrogen 30 %, and ammonium sulphate nitrogen 70 %, of total.

Addition	*Time (h)*	*Yeast growth (g dry wt./l)*	*Consumption of*	
			aspartic acid N (g)	*ammonium sulphate N (g)*
Aspartic acid, 25 g	0	0.28	—	—
	6	2.00	1.45	0.64
	9	3.10	2.13	1.16
Aspartic acid, 25 g + oleic acid, 5 ml	6	2.48	1.48	1.06
	9	4.54	2.45	2.39

TABLE V

THE BIOTIN CONTENT OF BAKER'S YEAST AT DIFFERENT STAGES OF INDUSTRIAL PRODUCTION

The results are expressed as mean values of two series of propagation.

Yeast stage	*Biotin (µg/g dry wt.)*
R_3	2.14
A_2	2.29
A_3	1.83
A_4	0.82
A_5	0.46

TABLE VI

FATTY ACID COMPOSITION OF BAKER'S YEAST AT DIFFERENT STAGES OF INDUSTRIAL PRODUCTION

Yeast stage	R_3	A_1	A_2*	A_3	A_4	A_5*
Fatty acid	*Per cent of total fatty acids*					
C_{18}	5.1	2.2	4.1	6.2	3.9	3.3
C_{18}:1	28.5	47.5	46.4	43.1	31.7	27.2
C_{17}	1.1	0.1	0.6	0.6	1.7	0.6
C_{16}	23.7	12.5	12.7	12.5	9.9	11.8
C_{16}:1	39.0	36.9	34.5	35.8	46.5	52.8
C_{15}	0.4	0.2	0.7	0.5	1.0	1.2
C_{14}	0.4	0.2	0.3	0.6	traces	4.0
C_{13}	0.2	0.1	0.3	0.4	0.2	0.1
C_{12}	0.9	0.1	0.2	0.4	1.1	0.8
C_{11}	0.6	traces	0.1	—	—	0.1
C_{10}	—	—	0.1	—	—	0.4
$C_{18} + C_{18}$:1	33.6	49.7	50.5	49.3	35.6	30.5
$C_{16} + C_{16}$:1	62.7	49.4	47.2	48.3	56.4	64.6
C_{10}–C_{15}	2.5	0.5	1.7	1.9	2.3	6.6

* Mean of two experiments.

If biotin deficiency affects the synthesis of fatty acids, this fact ought to be reflected in the composition of the fat at different stages of yeast growth. Samples were therefore taken from different phases of technical production and the yeast fat separated and analysed. Table VI shows a sharp decrease in the amount of oleic acid, as well as in the total amount of fatty acids with 18 carbon atoms, during transfer from stages A_1–A_3 to stages A_4–A_5. On the other hand, the amount of acids with 16 carbon atoms or less, particularly palmitoleic acid, increased.

In the technical scale it was shown that the decrease in C_{18} fatty acids and particularly of oleic acid, as well as the increase in the amounts of palmitoleic acid and of acids with less than 16 carbon atoms, was due to biotin deficiency. During the propagation of the commercial yeast stage with A_4 stage as seed yeast, 300-mg and 600-mg lots of biotin were added respectively to two parallel batches of 125 m^3. Table VII shows the results obtained in the analysis of the fatty acid composition of the commercial A_5 yeast stage.

TABLE VII

THE EFFECT OF BIOTIN ADDITION ON THE FATTY ACID COMPOSITION OF THE COMMERCIAL A_5 STAGE OF BAKER'S YEAST

Biotin added 300 or 600 mg per batch of 125 m^3. The fatty acid composition of A_4-yeast, used as seed yeast, appears in Table VI.

	Biotin added	
	300 mg	*600 mg*
Fatty acid	*Per cent of total fatty acids*	
C_{18}	0.8	2.2
C_{18}:1	22.3	29.4
C_{16}	10.3	11.7
C_{16}:1	64.2	55.9
C_{14}	2.1	0.7
C_{12}	0.3	0.1
$C_{18} + C_{18}$:1	23.1	31.6
$C_{16} + C_{16}$:1	74.5	67.6
Less than C_{16}	2.4	0.8

In the industrial process, the yeast is transferred to vigorously aerated, poorer culture medium. The changes of the culture conditions might be expected to influence the composition of the fatty acids: therefore, several laboratory experiments in absence and presence of biotin were performed under standard conditions. When biotin was absent, addition of aspartic acid proved to be essential for the growth of the yeast. The clearest results were obtained in the experiment described in Table VIII. In all essentials this table confirms the results presented in Table VI, *i.e.* in yeast grown without biotin the amount of C_{18} fatty acids and oleic acid decreased while the amount of C_{16} acids, particularly palmitoleic acid, and the total amount of acids with less than 16 carbon atoms increased.

The effect of saturated long-chain fatty acids on the growth of biotin-deficient baker's yeast

It has been shown that unsaturated long-chain fatty acids added to the growth medium with aspartic acid, were able to compensate for biotin deficiency in the

TABLE VIII

FATTY ACID COMPOSITION OF BAKER'S YEAST GROWN ON A LABORATORY SCALE WITH OR WITHOUT BIOTIN, AND WITHOUT BIOTIN BUT WITH ASPARTIC ACID

Growth in 11 l for 6 h at 30°. A_2-yeast (9 g) used as seed yeast. The fatty acid composition of A_2-yeast appears in Table VI.

	With biotin	*Without biotin*	*Without biotin but with 50% aspartic acid N*
Yield of yeast (g dry wt./11 l)	*23.13*	*6.79*	*21.88*
Fatty acid	*Per cent of total fatty acids*		
C_{18}	2.2	1.8	3.0
$C_{18}:1$	35.0	24.3	24.7
C_{16}	16.8	11.4	8.8
$C_{16}:1$	45.3	57.7	53.1
C_{15}	0.1	1.0	0.6
C_{14}	0.9	1.0	3.7
C_{13}	0.1	0.8	0.3
C_{12}	0.5	0.9	1.7
C_{11}	traces	0.3	0.2
C_{10}	0.3	0.5	1.3
$C_{18} + C_{18}:1$	37.2	26.1	27.7
$C_{16} + C_{16}:1$	62.1	69.1	61.9
C_{16}–C_{15}	1.8	4.5	10.4

culture medium of baker's yeast. Saturated fatty acids, such as palmitic or stearic, had a weaker effect (Table I). Tables VI and VIII show that biotin-deficient yeast does not suffer from lack of palmitoleic acid. Hence it is hard to comprehend how the compensating effect of the long-chain fatty acids could be due to the absence of unsaturated fatty acids. This is even more difficult to understand because—according to recent investigations[25,26]—the unsaturated fatty acids appear to be formed from the corresponding saturated acids, palmitoleic from palmitic and oleic from stearic acid.

TABLE IX

YIELD OF FATTY ACIDS, EXPRESSED AS METHYL ESTERS, FROM BAKER'S YEAST GROWN WITH OR WITHOUT BIOTIN, WITH ASPARTIC ACID, AND WITH ASPARTIC ACID + OLEIC ACID

Growth in 11 l for 6 h at 30°.

Addition	*Yield of yeast (g dry wt./11l)**	*Yield of fatty acid methyl esters (per cent of yeast dry wt.)**
With biotin, 100 μg	23.49 (4)	3.4 (4)
Without biotin	8.78 (4)	3.4 (4)
Without biotin but with 50% aspartic acid N	19.76 (2)	3.2 (2)
Without biotin but with 50% aspartic acid N + 5 ml oleic acid	23.03 (2)	1.5 (2)

* The figures in parentheses indicate the number of determinations from which the mean values are calculated.

In the discussion as to why baker's yeast was differently influenced by saturated and unsaturated fatty acids, it was suggested that the growth effect might be correlated with the different rates of penetration into the cells. The unsaturated acids, having an oily consistency at the growth temperature, remain suspended in the culture medium and might penetrate into the yeast cells more easily than the solid saturated long-chain acids. This assumption was supported by the observation that even oleic acid, when added to the growth solution was only taken up by the yeast in limited amounts (Tables IX and X). The content of fatty acids was markedly lower in yeast which had been given oleic and aspartic acids together than in yeast grown in the presence of biotin under similar conditions (Table IX). Even when oleic acid was added to the culture medium, the yeast fat did not contain disproportionate amounts of oleic acid (Table X).

TABLE X

FATTY ACID COMPOSITION OF BAKER'S YEAST GROWN WITH ASPARTIC ACID + OLEIC ACID

Growth in 11 l for 6 h at 30°. A_2-yeast (9 g) used as seed yeast. The fatty acid composition of A_2-yeast appears in Table VI. The yield of yeast was 23.03 g and the results with each acid are expressed as means of two determinations.

Fatty acid	*Per cent of total fatty acids*
C_{18}	0.7
$C_{18}:1$	53.1
C_{17}	0.3
C_{16}	13.0
$C_{16}:1$	28.6
C_{10}–C_{15}	4.5

Esters of organic acids penetrate more easily than free acids into yeast cells[27]. Esterification of palmitic and stearic acids lowers their melting points, and makes them more readily emulsified. These acids were therefore added to the medium as ethyl esters together with aspartic acid. The esters are presumed to be hydrolysed within the cells and the results (Fig. 2) show that both of these saturated long-chain fatty acids added as ethyl esters together with aspartic acid helped to induce normal growth in biotin-deficient baker's yeast. In the first phases of propagation they even increased the rate of growth.

DISCUSSION

The composition of fatty acids in yeast fat has hitherto been little investigated. TÄUFEL, THALER AND SCHREYEGG[28] have reported that the fat of brewer's yeast contains palmitic, stearic and linoleic acids, and, in addition, oleic acid up to two thirds of the total fatty acids. According to NEWMAN AND ANDERSON[29], about 25% of the total content of unsaturated acids in baker's yeast consists of some unsaturated fatty acid with a chain of 16 carbon atoms. The fatty acid composition has been determined in *Torulopsis utilis*[30], *T. lipofera*[31] and *Rhodotorula gracilis*[32]. In the first-mentioned yeast, oleic and linoleic acids accounted for up to 70% and in the

two latter yeasts up to 50–60 % of the total. The palmitic acid content in the latter yeasts was also comparatively high, 25–30 %. BARAUD AND GENEVOIS[33] have analysed the fatty acids of baker's yeast by gas chromatography and report palmitic and oleic acids as the main components, which comprise 50.3 % and 36.8 % of the total. These results are broadly in agreement with ours, although the authors do not mention palmitoleic acid which seems to be included with the palmitic acid.

As mentioned above, it has been observed that aspartic acid is able to compensate for the absence of biotin in the culture medium of baker's yeast[14,34]. POTTER AND ELVEHJEM[35] found that aspartic and oleic acids when given together almost completely replace biotin in promoting the growth of *Lactobacillus plantarum*. In yeast, however, the biotin-like effect of the unsaturated long-chain fatty acids has only recently been discovered, obviously because their effect is not manifested unless aspartic acid is also present. AHMAD AND ROSE[24,24a] have reported that oleic acid does indeed influence the growth of biotin-deficient baker's yeast but that the amount of growth in a biotin-free medium containing aspartic acid + oleic acid was still only a small fraction of that occurring in the stationary phase of biotin-optimal cultures. In our experiments, palmitoleic or oleic acid added with aspartic acid almost completely replaced the biotin. This effect was discovered under aerobic culture conditions in biotin deficiency. Thus our observation differs from the finding reported by ANDREASEN AND STIER[36] that unsaturated fatty acids are indispensable for the growth of yeast under strictly anaerobic conditions[37].

In our opinion it is evident that biotin participates in the fatty acid synthesis of yeast *in vivo*. The results also seem to be compatible with the opinion expressed by BLOOMFIELD AND BLOCH[25] as well as by MARSH AND JAMES[26] that the unsaturated fatty acids, palmitoleic and oleic, are formed from the corresponding saturated acids, palmitic and stearic acids.

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