96. STEROL METABOLISM OF MICRO-ORGANISMS

I. YEAST

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THE production of sterols, more particularly ergosterol, by micro-organisms is now a well-established fact. It is, especially, a metabolic accomplishment characteristic of the moulds and yeasts; whether bacteria produce sterols is a controversial point, with the weight of the evidence against this supposition. We became interested in elucidating the factors and metabolic intermediates concerned in the synthesis of sterols; and, consequently, in the function of sterols. We chose to use micro-organisms for this purpose, both on account of the uniformity of this biological material and its simple organization. In neither of these respects have micro-organisms entirely come up to our expectations. We give here the results with yeast; other results using other organisms, chiefly *Aspergillus niger*, will form the subject of a separate communication.

EXPERIMENTAL

Cultivation. We adopted a method similar in principle to that used by Smedley-MacLean [1922] in investigating the fat and carbohydrate metabolism of yeast, a method which has the combined advantages of speed and reproducibility. Four lots of moist baker's yeast, each of 20 g., were taken for each experiment. One lot was worked up without further treatment; this is the control. Each of the other three was suspended in 500 ml. of tap water, containing 50 g. of cane sugar and 0.5 g. KH_2PO_4 . One lot was aerated; another was allowed to ferment without aeration (i.e. it was essentially anaerobic); and to the last lot, which was aerated, was added the substrate under investigation. Incubation, at 28°, was usually for 3 days, although in some later experiments 1 day was deemed sufficient. Aeration was achieved by making use of the special type of flask (of 2 l. capacity) described by Kluyver *et al.* [1925], in which the incoming air-stream is broken up by means of a sintered glass filter.

Harvesting. The yeast from each flask was harvested by centrifuging, washed once with distilled water and re-centrifuged: the centrifugate was shaken up with 100 ml. of 75 % methyl alcohol and allowed to stand at 10° overnight. It was then filtered by suction and the filter cake broken up and air-dried at 32° for 24 hr. The yeast was finally dried over CaCl_2 in an evacuated desiccator at 32°, and then ground to a fine powder in a coffee mill.

2 g. of the powder were extracted with a boiling mixture of alcohol (two parts) and benzene (one part), as described by Bills *et al.* [1930]. The filtered extract was evaporated to dryness on the water bath, under reduced pressure, and the residue taken up in 100 ml. of absolute alcohol. Aliquots of this solution were then taken for the estimation of total alcohol-benzene-soluble material

(henceforward designated "total lipin"), the estimation of unsaponifiable matter and of sterol.

Estimations. Unsaponifiable matter was determined by adding 1 ml. of 20 % KOH to 20 ml. of the alcoholic solution and taking the solution to dryness on a boiling water bath. The residue, after drying over CaCl_2 in a desiccator, was extracted three times with ether. The ethereal solution was filtered into a tared beaker and allowed to evaporate spontaneously.

Sterol was estimated spectrographically, as a general rule, by measuring the extinction coefficient at 280 $\text{m}\mu$. The absorption of the alcoholic solution, suitably diluted, was measured with a small Hilger quartz spectrograph, used in conjunction with a rotating logarithmic sector and a Dobson microphotometer. The values obtained were frequently checked by gravimetric estimations on the same solution. This was considered desirable in case there should be any appreciable amount of sterol other than ergosterol, since the spectrographic method only estimates the latter. For instance α -dihydroergosterol would escape measurement by the spectrographic method. Further, the gravimetric method enables one to distinguish between ester and free sterol. Nevertheless, it has its drawbacks; the instability of ergosterol makes it susceptible to the rather drastic treatment involved in digitonide estimations. Further, the solubility of ergosterol digitonide has apparently never been investigated. Most workers have either assumed that it is the same as that of cholesterol digitonide or have ignored the question. In our hands the digitonide method gave excellent results with cholesterol, reasonably good results with solutions of pure ergosterol, and only approximate results with alcoholic extracts of yeast or *Aspergillus* "lipin".

The digitonide was prepared by treating a 20 ml. aliquot of the alcoholic solution with the requisite amount [Dam, 1928] of 1 % digitonin in 90 % alcohol. The mixture was brought to the boil and taken to dryness on the water-bath. The residue was washed with ether into a tared Gooch filter and there washed with water until frothing ceased. The filter was dried to constant weight in an evacuated desiccator (ergosterol digitonide decomposes when dried at high temperatures, e.g. 105°).

RESULTS

Table 1 brings out several points. First, it is clear, from a comparison of Exps. 1-4 with 5-8, that sterol production is a feature of aerobiosis. It is also clear that the increase in sterol under aerobic conditions is far in excess of the increased growth—a point emphasized later in the composite Table 9. On the other hand the apparent decrease in sterol in Exp. 5 is probably a dilution effect, the ergosterol of the total yeast (6.66 g. in this case) is the same, or slightly greater than, that of the control (4.44 g.). Nevertheless, we record later (see Exp. 15) a real case of decrease in sterol. The second point is concerned with the time of incubation. While increase in the incubation period leads to an increase in ergosterol production in the aerated suspensions, the ergosterol production after only 24 hr. is considerable. In most of our work on the effect of added substrates we have incubated for 72 hr., chiefly for reasons of our own convenience. In some later experiments, however, we cut the time down to 24 hr. The third point is that Table 1 brings out very clearly that most, if not all, of the ergosterol in the aerated yeast is esterified. The discrepancies between the amounts of ergosterol determined spectrographically and by the digitonide method are probably due to other digitonin-precipitable substances in yeast (e.g. α -dihydroergosterol).

Table 1.

1 Exp.	2 Duration of incubation hr.	3 Yield of dry yeast g.	2 g. of dry yeast contain				
			4 Total lipin mg.	5 Unsa- pon. matter mg.	6 Ergosterol (spectr.) mg.	Sterol by digitonide	
						7 Before sapon. mg.	8 After sapon. mg.
0	Control	4.443	30	4.0	5.3	—	—
Aerated							
1	24	8.83	123	35.5	18.9	2.6	23.2
2	48	8.92	97	34.5	16.7	2.1	23.0
3	96	9.9	136.5	43.0	25.7	8.3	33.6
4	120	9.47	142.5	44.0	25.0	2.4	29.4
Not aerated							
5	24	6.66	19.0	6.0	3.5	—	—
6	48	6.48	25.0	5.5	4.2	—	—
7	96	6.01	22.5	7.0	4.5	—	—
8	120	5.7	25.5	5.5	5.3	—	—

Column 4: total lipin represents the material soluble in alcohol-benzene and subsequently in absolute alcohol.

Column 6: ergosterol determined spectrographically.

Columns 7 and 8: sterol determined by digitonin precipitation. (Not attempted in Exps. 5-8.)

In Table 2 we record the results of investigating the effect of sodium acetate upon sterol production, and incidentally the effect of aeration in the absence of any carbon compound (Exp. 10). Smedley-MacLean [1922] has found sodium acetate effective in raising the fat content of yeast; and our own experiments with *Aspergillus niger* led us to believe that sodium acetate was worth investigation as one of the simpler precursors of ergosterol. The results justify this view.

Table 2

Exp.	Conditions of exp.	Yield of dry yeast g.	2 g. of dry yeast contain		
			Total lipin mg.	Unsa- pon. matter mg.	Ergosterol mg.
9	Control	4.516	27.5	4.0	4.3
10	No carbon	4.495	26.5	27.5	6.3
11	5 g. Na acetate	4.806	35.0	13.5	7.1
12	Cane sugar	8.411	111.5	37.5	20.1
13	Cane sugar + 5 g. Na acetate	8.569	127.0	38.5	24.1

In Exps. 10-13 the common medium was 500 ml. of tap water plus 0.5 g. KH_2PO_4 . In Exp. 10 nothing else was added and in Exps. 11, 12 and 13 the nutrients as indicated. The amount of cane sugar was 50 g. Incubation was for 24 hr. with aeration. Ergosterol was determined spectrographically.

We conclude that sodium acetate added to the basal medium leads to a slight increase of ergosterol production. The significance of this is referred to in the discussion.

Many of the commoner possible intermediates in carbohydrate metabolism can be ruled out after a consideration of the results obtained in studying the fat metabolism of yeast, as recorded by Smedley-MacLean [1922] and MacLeod & Smedley-MacLean [1938]. We refer here, of course, to the figures given by them

for unsaponifiable matter. We therefore turned our attention to certain substances as yet untried and, for various reasons, we elected to try in the first place the effect of ascorbic acid. The results are given below.

Table 3

Exp.	Conditions of exp.	2 g. of dry yeast contain					
		Yield of dry yeast g.	Total lipin mg.	Unsapon. matter mg.	Ergosterol (spectr.) mg.	Sterol by digitonide	
						Before sapon. mg.	After sapon. mg.
14	Control	4.671	28.0	18.0	6.8	6.3	7.3
15	Cane sugar anaerobic	6.146	30.0	16.0	3.1	3.2	4.4
16	Cane sugar aerated	8.217	113.0	45.0	21.8	7.3	37.1
17	Cane sugar aerated + ascorbic acid	9.723	138.0	55.0	28.7	8.5	72.0

Incubation was for 72 hr. Amounts of cane sugar and phosphate as in the preceding experiments. The amount of ascorbic acid added in Exp. 17 was 1 g., neutralized with *N* NaOH.

It will be seen that there is a marked increase in the ergosterol content of yeast incubated with aeration in the presence of ascorbic acid. Once again the importance of aeration is demonstrated; and also the fact that most of the ergosterol formed during aeration is esterified. Nevertheless this result is put forward with some reserve because up to the present we have not been able to repeat it. If, for instance, ascorbic acid were exercising any beneficial effect, it would be expected that increasing the amount of the acid would have correspondingly greater effects. Table 4 contains the results of our attempt to test this point.

Table 4

Exp.	Conditions of exp.	Yield of dry yeast g.	2 g. of dry yeast contain	
			Total lipin mg.	Ergosterol mg.
18	Control	4.712	19.0	3.8
19	Cane sugar	9.346	56.0	7.7
20	Cane sugar + 1 g. ascorbic acid	9.138	62.5	8.4
21	Cane sugar + 2 g. ascorbic acid	8.757	60.0	8.1
22	Cane sugar + 3 g. ascorbic acid	9.533	73.5	9.6
23	No cane sugar and no ascorbic acid	4.683	20.0	4.6
24	1 g. ascorbic acid	4.664	19.5	4.6

All incubated at 28° with aeration in the basal medium, tap water and phosphate (except 18).

The above series was, inadvertently, run for only 24 hr.; the original ascorbic acid experiment was run for 72 hr. This may be the reason for the failure to repeat. The matter is still under investigation. Another possibility is variation in the yeast, a point always to be borne in mind when comparing one series of experiments with another. In the work recorded in this paper 7 different batches of yeast were used. The synthetic capacities of 5 of them, as measured by the amounts of ergosterol formed from cane sugar alone, were remarkably similar. The other 2 (one of which was used in Exps. 18-24) were able to form only half the amount of ergosterol formed by the first 5. Table 5 illustrates this.

One of the reasons for trying ascorbic acid was its marked reactivity. The idea that the hexose unit undergoing metabolic change is in an especially "reactive" state is fairly widespread. This view is expressed by Harden [1932], and Massengale *et al.* [1931] resort to the same conception in discussing the conversion of carbohydrate into sterol; von Euler & Nilsson's [1930]

Table 5. *Ergosterol (in mg.) in 2 g. of dry yeast, after aeration in cane sugar phosphate medium*

Duration days	Different yeasts no.						
	1	2	3	4	5	6	7
1	18.9	20.1	—	—	—	7.7	9.4
2	16.7	—	—	—	—	—	—
3	—	—	21.8	22.1	19.4	—	—
4	25.7	—	—	—	—	—	—
5	25.0	—	—	—	—	—	—

“am-hexose” is another version of the same idea. For this reason we thought it worth while to test yeast glycogen as a “baustein” in the synthesis of sterol. The yeast glycogen which we used was hydrolysed immediately before use, with the aid of ptyalin; no attempt was made to separate the reducing sugar so formed; and as a control we used an equivalent amount of maltose. The results are given in Table 6 and provide no encouragement in the attempt to supply “active” carbohydrate. In arriving at this conclusion we are only considering Exps. 25, 26 and 27; these should not be compared with Exp. 28, in which the amount of cane sugar was the usual 50 g.; in the case of Exps. 26 and 27 the amount of sugar was only 2 g.

In Table 6 are two other results, representing two quite different points, but included here because performed on the same batch of yeast. Exps. 28 and 29 are controls. In Exp. 30 the air used for aeration was drawn through ethyl alcohol. Smedley-MacLean & Hoffert [1923] have shown that the addition of ethyl alcohol to the water in which yeast is oxygenated leads to a deposition of fat in the yeast; whilst Sobotka *et al.* [1935] have demonstrated an increase in the fat and sterol content of yeast left in contact with air saturated with alcohol vapour. There was a slight increase in sterol (see Table 6), which was a little more marked in a repetition experiment (not given in the table). But in neither case is there warrant for considering ethyl alcohol as a significant contributor to sterol synthesis. More interesting is the fact which emerges that ethyl alcohol is capable of sustaining growth of yeast aerobically, the actual yield being significantly greater than in the control sample.

Exp. 31 represents the effect of N_2 in place of air. The failure of yeast incubated without aeration to synthesize sterol might be attributed to inadequate nutrition of the yeast. In the non-aerated experiments the yeast settles to the bottom of the flask; consequently it must depend on diffusion for its supply of cane sugar and the lower layers of the settled yeast may be partially starved. In the aerated experiments, on the other hand, the yeast is kept uniformly suspended in the medium, whereby an adequate nourishment is ensured. The accumulation of CO_2 in the non-aerated experiments might also have an adverse effect on sterol synthesis. The substitution of N_2 for air enabled us to test these points; and it will be seen from Table 6 that sterol production in the presence of N_2 is no greater than that obtained in non-aerated experiments. There appears to be an actual decrease, but we are inclined to consider this a dilution effect. It will be noted, however, that the yield of yeast is greater than the yields from non-aerated experiments (cf. Exps. 5, 6, 7, 8 and 15). This probably means that the yeasts in the non-aerated experiments are indeed under-nourished. These experiments offer further proof that sterol synthesis is not dependent on growth; for a growth equal to that attained by the ergosterol-rich, aerated yeast is achieved here without the production of sterol. Exp. 31 has been repeated since with precisely similar results.

Table 6

Exp.	Conditions of exp.	Yield of dry yeast g.	2 g. of dry yeast contain	
			Total lipin mg.	Ergosterol (spectr.) mg.
25	Control	4.807	18.5	4.2
26	Maltose	5.457	26.0	6.6
27	Hydrolysed glycogen	4.686	24.0	6.9
28	Cane sugar	9.286	53.5	9.4
29	No carbon compound	4.985	26.5	6.2
30	Alcohol	5.682	26.0	5.7
31	Nitrogen	8.172	17.0	2.5

Incubated for 24 hr. with aeration (except 25 and 31).

The negative character of our findings so far led us to reconsider the possible intermediates. We had hitherto avoided introducing any N compounds, in order to keep as clear as possible of the metabolic processes accompanying growth. Yet the fact that the higher alcohols produced during fermentation are the result of decarboxylic deamination of amino-acids made it imperative that the effect of these should be tested, especially in conjunction with actively fermenting carbohydrate. In the first instance it was considered desirable to use a mixture of amino-acids. Table 7 contains the results of these experiments. Exp. 32 is the usual control; Exp. 33 represents cane sugar without any amino-acids; Exp. 34 cane sugar + a mixture of amino-acids (casein hydrolysed with trypsin) in an amount equivalent to 0.22 g. of amino-N. Exp. 35 is similar to 34 but anaerobic.

Table 7

Exp.	Conditions of exp.	Yield of dry yeast g.	2 g. of dry yeast contain		
			Total lipin mg.	Unsapn. matter mg.	Ergosterol (spectr.) mg.
32	Control	4.333	25.5	12.5	5.4
33	Cane sugar only	8.003	113.5	55.5	22.1
34	Cane sugar + amino-acids	10.984	58.5	26.5	12.6
35	Cane sugar + amino-acids: anaerobic	6.433	22.0	15.5	3.9

Incubation for 72 hr.; Exps. 33 and 34 aerated.

It would thus seem that the addition of amino-acids not only failed to increase sterol production but actually appeared to inhibit it. Despite this result we carried out further experiments, acting on a suggestion made to us by Prof. Robinson, that we should try specifically the effect of tyrosine. The results are contained in Table 8. Exps. 36 and 37 are the usual controls; Exp. 38 is the same as 37, but with the addition of 1 g. of *l*-tyrosine, whilst Exp. 39 is similar to 38 but anaerobic.

Table 8

Exp.	Conditions of exp.	Yield of dry yeast g.	2 g. of dry yeast contain		
			Total lipin mg.	Unsapn. matter mg.	Ergosterol (spectr.) mg.
36	Control	4.543	26.0	6.5	6.0
37	Cane sugar only	9.795	127.5	37.5	19.4
38	Cane sugar + tyrosine	9.480	95.5	34.0	14.6
39	Cane sugar + tyrosine: anaerobic	6.257	28.0	7.5	4.4

Incubated for 72 hr.; Exps. 37 and 38 aerated.

Once again it is quite clear that the addition of tyrosine leads to a decrease rather than an increase in sterol production. We may add here that we have also tried the effect of tyrosol in the system and found it to be the same as that of tyrosine.

In the foregoing tables we have expressed the results uniformly in terms of mg. per 2 g. of dry yeast—the amount actually taken for each estimation. In Table 9 we have brought together the data from the preceding tables in a different form. The first and second columns are self-explanatory. The third column contains the total ergosterol in the whole yeast, for any given experiment, less the amount of ergosterol in the control sample for that series. The fourth column represents a method of expressing the relationship of sterol production to growth. If

E_f = ergosterol in total yeast at conclusion of experiment,

E_0 = ergosterol in total yeast at beginning of experiment,

Y_f = total yeast at conclusion of experiment,

Y_0 = total yeast at beginning of experiment,

then the ratio expressed in the third column is $\frac{E_f/E_0}{Y_f/Y_0}$.

From this it follows that if sterol production ran parallel with growth, the value obtained should be 1.

Table 9

Conditions of exp.	Ergosterol % of dry yeast	Net increase in ergosterol mg.	Ratio sterol/growth
Control	0.26	—	—
Aerobic			
Phosphate	0.29	3.9	1.38
Phosphate + acetate	0.36	7.4	1.66
Phosphate + ascorbic acid	0.27	3.9	1.35
Phosphate + EtOH	0.30	7.9	1.47
Cane sugar (Cs.)	1.06	82.6	3.87
Cs. + ascorbic acid	1.44	123.6	4.22
Cs. + acetate	1.21	93.6	5.61
Cs. + amino-acids	0.63	57.5	2.34
Cs. + tyrosine	0.73	55.6	2.44
Cs. + tyrosol	0.70	48.7	3.25
Anaerobic			
Cs. + nitrogen	0.14	1.6	0.68
Cs.	0.21	0	0.75
Cs. + amino-acids	0.20	0.8	0.72
Cs. + tyrosine	0.22	0.2	0.73

It should be understood that phosphate was present in all experiments, in addition to the substances indicated. The figures for the ascorbic acid experiment given above are for the 3-day run; those of the 1-day run are not included here as the particular yeast used was abnormal in other respects (see Table 5). The relevant figures for that particular yeast (Table 4) showed that there was no effect due to ascorbic acid.

DISCUSSION

There are many different views concerning the mechanism of formation of sterols, a subject intimately bound up with the question of the metabolic function of sterols. Knowledge of the one is almost bound to illuminate the other. Perhaps the most attractive suggestion concerning sterol formation is that it arises by cyclization of a carotenoid or polyene chain. Thus Robinson [1934] considered squalene a possible precursor, whilst Bryant [1935] put

forward the merits of carotene for the role. Others [e.g. Artom, 1923] have postulated cyclization of oleic acid. Massengale *et al.* [1931] investigated the effectiveness of various sugars in the production of sterols by yeast and Bills [1935], reviewing the subject, infers that sterols are mainly the products of carbohydrate metabolism. Smedley-MacLean & Hoffert [1926] in a paper primarily concerned with fat metabolism of yeast, record the unsaponifiable matter found under the influence of a variety of substrates; this contributes valuable information concerning possible intermediates, but no positive suggestion arises from it. Nor can we, on the basis of the work reported here, point to any specific intermediate as the precursor of sterol. Our findings can be summarized under two headings. (1) We are able to exclude a certain number of substances as precursors, with a fair degree of confidence; and (2) we have extracted some information concerning the general metabolism of sterols by yeast.

Dealing with the first point, the evidence that amino-acids do not play any part in sterol formation seems conclusive. This is in agreement with the findings of previous workers [Massengale *et al.* 1931; Reindel *et al.* 1937; and Sobotka *et al.* 1935], who could demonstrate no connexion between sterol synthesis and N metabolism. (We can offer no explanation for the rather surprising discovery that the amino-acid mixture, tyrosine and tyrosol, all inhibited sterol production.) Nor can we find any support for the view that a "reactive hexose" is concerned in sterol formation; the results with hydrolysed yeast glycogen are negative. The effect of ascorbic acid is uncertain and that of ethyl alcohol has already been discussed.

We believe, on the basis of our experiments, that sodium acetate can provide the necessary carbon for sterol synthesis. It is known that acetate stimulates the formation of fat in yeast [Smedley-MacLean & Hoffert, 1923], and more recently Sonderhoff & Thomas [1937] have shown that the unsaponifiable material of yeast grown in a solution of trideutero-acetate contains 31.0 atoms % of deuterium. It is interesting to note in this connexion that Rittenberg & Schoenheimer [1937] concluded from a similar study, using deuterium as an indicator, that the synthesis of cholesterol in the animal body involves the coupling of a large number of small molecules. Further support for the view that acetate can serve as a sterol precursor may be found in the fact that *Aspergillus niger* can synthesize ergosterol when sodium acetate is its sole source of carbon [Maguigan, unpublished].

Under the second heading we have established five points.

(a) Sterol formation occurs at an early stage in any fermentation where it occurs at all. We may infer from this that it is a synthetic and not a degradative feature.

(b) Most of the sterol formed is present as ester. This has been claimed before [Smedley-MacLean, & Thomas 1920] and our experiments consolidate the point. If sterol formation be considered a consequence of the increased fat formation encountered under aerobic conditions, then an idea worth considering is that the sterol here is acting as a detoxicating agent against the excessive production of fatty acids; "neutralizing", as it were, by their esterification those fatty acids destined for fat storage in contradistinction to esterification with glycerol (or choline glycerophosphoric acid), a catabolic step.

As far as we are aware, the only sterol ester recorded as occurring in micro-organisms is ergosteryl palmitate, isolated from *Penicillium brevi-compactum* by Oxford & Raistrick [1933]. Our efforts to isolate and characterize the sterol ester of yeast fat have thus far been unsuccessful; but we are continuing with this point.

(c) The view, set forth above, that sterol formation may be a consequence of increased fat formation would lead one to expect a constant relationship between the sterol content and total lipin of yeast. Our results show that this relationship does tend to be constant, despite fairly wide variations in individual estimations. The average value (45 experiments) of the ergosterol content expressed as percentage of total lipin is 18.3%. In this conclusion we agree with Sobotka *et al.* [1935] and disagree with Bills [Massengale *et al.* 1931] who states that there is no relationship between the total lipin and sterol contents of yeast.

(d) Sterol formation is not directly related to growth. Not only is the rate of sterol formation much greater than the rate of growth, under the most favourable conditions investigated, but the former does not always vary in the same direction as the latter. Even in those experiments in which there was no growth, a small but definite increase in sterol was noted. It is true that a considerable growth occurred in the experiments yielding the largest amounts of ergosterol, but in these cases the greater sterol yields were often accompanied by lesser growths, and *vice versa*. In this connexion, it may be noted that the average value of the ratio: final weight of yeast/original weight of yeast for a group of 20 experiments in which the yeast was aerated in the presence of cane sugar with and without the addition of simple organic compounds was 2.00. The maximum variation in this ratio was $\pm 12\%$, showing that with respect to growth under the given conditions the yeast behaved as uniformly as can be expected of a biological population. The variations in sterol synthesis in the same experiments were, on the other hand, very great indeed.

The findings of Bills *et al.* [1930] and Reindel *et al.* [1937] that sterol synthesis tends to run parallel with growth can be explained by supposing that, in general, conditions favourable to growth are also favourable to sterol production. Under the conditions of our experiments we have been able to dissect the two processes.

(e) Sterol formation is a feature of aerobiosis. This is a conclusion which is capable of extension to organisms other than yeast. Without committing ourselves on the subject of sterol function in mammals (where it seems to be multiple), there must be some really fundamental function, involving sterol, common to the physiological processes of animals and plants (including fungi). To suppose that this process is intimately associated with aerobiosis is consonant with all the known relative facts. It is in accord also with the absence of sterols from bacteria where these are anaerobes; controversial cases may be due to the use of facultative anaerobes; aerobes should contain sterols. Evidence on this point is being sought.

SUMMARY

1. The production of sterols by yeast under a variety of conditions has been studied.

2. Of various substrates examined, sodium acetate led to a slight increase in sterol; ascorbic acid gave a definite increase in one case, and no effect in others; hydrolysed yeast glycogen was not effective; the role of ethyl alcohol as a sterol precursor is open to doubt; a mixture of amino-acids, tyrosine and tyrosol respectively, inhibited sterol formation.

3. It is established that sterol production (a) is a feature of aerobic metabolism, (b) arises early in the course of fermentation, (c) is accompanied by a corresponding increase in total lipin and (d) develops at a greater rate than growth, and is not directly correlated with it. (e) Most of the new sterol formed is esterified.

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