SULPHUR METABOLISM OF YEAST[1]

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Introduction

Two general conclusions are warranted as to the sulphur metabolism and nutrition in animals. The first is that although most of the sulphur taken into the system is in the reduced form, that is, mainly as cystine or possibly also as cysteine, nevertheless the main end product of normal sulphur metabolism is the completely oxidized sulphate form. This is well established by numerous investigations. The other important conclusion is that cystine is a very important amino acid in growth and maintenance.

Recently the importance of sulphur in nutrition of plants has also been so well shown that the use of sulphates in fertilizers is emphasized. ARMSTRONG (1) also studied the effect of various sulphur compounds in the development of *Aspergillus niger, Penicillium glaucum,* and *Botrytis cinerea.* He concluded that the best form of food sulphur is sulphate, and that even here the end product of sulphur metabolism is the sulphate form.

Recent studies (7) in this laboratory indicate that cystine also may be of some importance in yeast growth and that studies on yeast, when properly controlled, may throw more light on certain phases of metabolism. The object of this investigation was to determine more definitely the forms of sulphur available for yeast growth, and to investigate further the changes cystine undergoes in yeast growth and metabolism.

In all the studies here reported the concentration of the yeast growth stimulant was carefully controlled just as in the work by MILLER (5) and SWOBODA (7). In the first part of the work various forms and amounts of sulphur, as found in biological material, were added to an artificial medium containing a known, but very small amount of sulphur, and the rate of yeast growth determined by the weighing method. In the second part of the study the yeast was separated and analyzed for sulphur, and the filtrate analyzed for total sulphur and for "inorganic sulphate."

Experimental

1. MEDIA AND SULPHUR COMPOUNDS.—The following stock media were used from time to time with or without other additions as indicated under the observations made. The materials used in these media and the various

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sulphur compounds added were for the most part prepared or purified in these laboratories.

The saccharose first employed was the commercial rock candy, but we soon found this to contain sufficient amounts of sulphur to influence the results. It was then purified by solution in water and precipitation by redistilled absolute alcohol. By this procedure the sulphur content was reduced from the equivalent of 0.7 mg. to 0.1 mg. barium sulphate per gram of saccharose.

The asparagine was of the C.P. grade but was again recrystallized from water. The inorganic salts used were the "analyzed chemically pure" grade. The cystine was obtained from horn in the usual way and then reprecipitated five times from an aqueous solution of the hydrochloride by ammonium hydroxide. Cysteinic acid was prepared from cystine by FRIEDMANN'S method (**2**). It was recrystallized from hot water and washed with alcohol. Taurocholic acid was prepared from ox bile by HAMMARSTEN'S method (**3**), and taurine therefrom by the HAMMARSTEN (**4**) and TAUBER methods (**8**). The purity of these substances as measured by the sulphur content is indicated in table I.

TABLE I

SULPHUR CONTENT OF MATERIALS USED

SUBSTANCE	$BaSO_4$ OBTAINED PER GRAM	PER CENT. OF SULPHUR	THEORETICAL PER CENT. OF SULPHUR
	mg.		
Purified saccharose	0.1	.....	.....
Asparagine	0.2	.....	.....
Vitamine solution 5 per cent.	0.085	.....	.....
KH_2PO_4	1.4		
Cystine	.....	26.59	26.69
Cysteine	.....	26.59	26.47
Taurocholic acid	.....	5.33*	6.22
Taurine	.....	25.0	25.63
Cysteinic acid	.....	18.88	18.87
Dried yeast	.....	0.35	.....

* The low value is probably due to an admixture of moisture and metallic impurities. An ash estimation confirmed the latter interpretation.

The vitamine solution was prepared by drying Fleischmann's best grade of stock yeast in a current of air at room temperature. This material was next thoroughly ground in a mill and extracted six times with hot absolute

ether. The ether extract was discharged and the yeast powder next extracted six times with boiling 75 per cent. alcohol. After cooling, the combined alcoholic extracts were filtered and then concentrated to a paste under diminished pressure. The material was next redissolved in hot 90 per cent. alcohol and again cooled in the refrigerator and filtered. Thus 500 cc. of stock solution were obtained from five pounds of fresh yeast. The vitamine solution as added to the media in most cases was a 5 per cent. solution of this alcoholic stock solution. The media used are shown in table II.

TABLE II

COMPOSITION OF MEDIA EMPLOYED

SUBSTANCE	MEDIUM NUMBER						
	1	2	3	4	5	6	7
	grams	grams	grams	grams	grams	grams	grams
Saccharose	20.0	20.0	20.0	20.0	20.0	100.0	100.0
Asparagine	1.5	1.5	1.5	.....	1.5	7.5	7.5
$NH_4H_2PO_4$	.....	3.0	.....	.....	.....	.....	.....
KH_2PO_4	2.0	2.0	2.0	2.0	2.0	10.0	10.0
$MgSO_4$	.....	.....	.....	.....	.....	.....	1.20
$MgCl_2$	.....	0.25	0.25	0.25	.....	1.01	.....
$CaCl_2$	.....	0.25	0.25	0.25	0.25	1.25	1.25
Diluted to	1000	1000	1000	1000	1000	500	500

2. GROWTH METHOD.—The methods of WILLIAMS (**9**), MILLER (**5**), and SWOBODA (**7**) were used with slight modifications. A 125 cc. volume of the medium in a 500 cc. Erlenmeyer flask was sterilized in an autoclave at ten pounds for ten minutes and after cooling 1 cc. of the usual yeast suspension was added. After growth at 30° C. for 20 hours a few drops of formaldehyde were added so as to stop the growth of yeast. The yeast cells were at once filtered off into the weighed Gooch crucible, dried in the oven for 30 minutes at 103° C. and weighed after cooling.

3. ANALYTICAL METHOD FOR SULPHUR.—The method of alternate oxidation by hydrogen peroxide and by nitric acid as devised by STOCKHOLM and KOCH (**6**) was employed. It gave excellent checks and theoretical values for the well purified sulphur compounds and is very satisfactory for small amounts of sulphur.

Experimental observations

A. THE IMPORTANCE OF CALCIUM, MAGNESIUM AND SULPHATE IN YEAST GROWTH.—To 100 cc. of medium no. 1 varying amounts of M/10 $CaCl_2$, M/10 $MgCl_2$, M/10 $MgSO_4$, M/10 H_2SO_4 and vitamine solution were added

in various combinations and then diluted to 125 cc. Yeast was then grown in these special media, filtered off, dried and weighed. The results given in table III are with the unpurified saccharose and 5 cc. of the 5 per cent. vitamine solution per 100 cc. medium no. 1. In table IV we have a similar procedure, only here we have one-fifth of the amount of vitamine solution. In table V we have similar observations on medium no. 1 with the purified saccharose. Similar results were obtained repeatedly in connection with many other growth tests.

TABLE III

GROWTH OF YEAST IN UNPURIFIED SACCHAROSE, WITH 5 CC. VITAMINE IN MEDIUM NO. 1 WITH VARYING SALTS

NUMBER	$MgCl_2$ M/10	$CaCl_2$ M/10	$MgSO_4$ M/10	$(NH_4)_2SO_4$ M/10	H_2SO_4 N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	cc.	mg.
1	2	2	...	...	...	17.7
2	...	2	2	...	...	49.1
3	...	...	2	...	1	44.2
4	...	...	...	2	1	1.6
5	2	2	...	...	1	48.5
6	...	2	2	2	...	51.0
7	2	...	2	...	1	47.4
8	2	2	...	2	...	42.1
9	2	2	2	...	...	52.2
10	...	2	2	...	1	48.4

TABLE IV

GROWTH OF YEAST IN UNPURIFIED SACCHAROSE, WITH 1 CC. VITAMINE IN MEDIUM NO. 1 WITH VARYING SALTS

NUMBER	$CaCl_2$ 1%	$MgCl_2$ 1.5%	$MgSO_4$ 1.5%	$(NH_4)_2SO_4$ 2%	H_2SO_4 N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	cc.	mg.
1	2	2	...	...	...	9.6
2	2	...	2	...	...	12.1
3	2	...	1	1	...	12.8
4	2	...	2	1	...	14.4
5	2	...	2	...	1	12.3
6	2	...	4	...	...	11.8
7	2	...	...	4	...	1.2
8	2	...	8	...	...	11.8
9	2	...	...	8	...	1.4
10	2	...	4	4	...	14.6

TABLE V

GROWTH OF YEAST IN PURIFIED SACCHAROSE, WITH VITAMINE IN MEDIUM NO. 1 PLUS SALTS

NUMBER	$CaCl_2$ M/10	$MgCl_2$ M/10	$MgSO_4$ M/10	H_2SO_4 N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	mg.
1	2	...	...	...	7.0
2	2	2	...	...	7.6
3	2	...	1	...	8.6
4	2	...	2	...	9.9
5	2	2	2	...	11.2
6	2	...	4	...	11.4
7	2	...	8	...	9.7
8	2	2	...	1	9.7
9	2	2	...	2	10.2

B. THE SUBSTITUTION OF REDUCED SULPHUR FOR SULPHATE SULPHUR IN THE MEDIUM.—In the first part of these studies the cystine was dissolved in a KH_2PO_4 solution and this was then added to a modified medium no. 3 together with a standard KH_2PO_4 solution so that the total amount of KH_2PO_4 always remained 2.0 grams per liter. Many series of growth tests were made and in all cases the results were essentially as indicated in table VI. However, if asparagine is not present in the medium the results in

TABLE VI

GROWTH OF YEAST IN MEDIUM NO. 3, PLUS VITAMINE AND CYSTINE AS INDICATED WITH AND WITHOUT ASPARAGINE

NUMBER	VITAMINE SOL. 5 PER CENT.	CYSTINE	WEIGHT OF DRIED YEAST WITH ASPARAGINE	WEIGHT OF YEAST WITHOUT ASPARAGINE IN THE MEDIUM
	cc.	mg.	mg.	mg.
1	...	...	2.9	0.5
2	1	...	8.9	2.5
3	1	1	10.0	.....
4	1	2	10.5	2.2
5	1	3	12.4	.....
6	1	4	14.3	2.5
7	...	1	3.0	.....
8	...	2	3.6	1.3
9	...	3	4.2	.....
10	...	4	5.1	1.3

the last column of table VI are obtained. By adding various mixtures of the salts and cystine to medium no. 6, together with vitamine in each case,

the values in table VII were obtained. Practically the same observations are given in table VIII, where medium no. 6 also was used as the stock medium with 1 cc. vitamine solution in each test.

TABLE VII

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS VITAMINE WITH SALTS AND CYSTINE ADDED, AS INDICATED

NUMBER	$MgCl_2$ M/10	$MgSO_4$ M/10	NH_4Cl M/10	CYSTEINE	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	mg.	mg.
1	2	...	...	...	9.2
2	2	...	...	1	10.7
3	2	...	...	3	11.4
4	...	2	...	...	14.4
5	...	2	...	1	14.9
6	...	2	...	3	14.5
7	2	...	2	...	11.2
8	2	...	2	1	11.7
9	2	...	2	3	12.0
10	2	...	20	1	10.4
11	2	...	20	3	11.2

Cysteine, used in amounts ranging from two to twenty milligrams, gave results very similar to cystine.

TABLE VIII

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS 1 CC. VITAMINE, AND WITH SALTS AND CYSTINE ADDED

NUMBER	$MgCl_2$ M/10	$MgSO_4$ M/10	NH_4Cl M/10	CYSTINE	WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	mg.
1	2	...	...	...	8.6
2	2	...	...	1	10.2
3	2	...	...	3	13.9
4	2	...	...	10(?)	10.4
5	...	2	...	...	14.3
6	...	2	...	1	16.5
7	...	2	...	3	13.3
8	...	2	...	10(?)	12.7
9	2	...	20	...	8.2
10	2	...	20	1	10.9
11	2	...	20	3	12.8
12	2	...	20	10(?)	12.4

Hydrogen sulphide seems to be able to supply sulphur for yeast growth to a limited extent. The results, using medium no. 6 as the stock medium, are given in table IX. In each case 1 cc. of the vitamine solution was added.

TABLE IX

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS 1 CC. VITAMINE, WITH SALTS, AND H_2S ADDED AS INDICATED

NUMBER	$MgSO_4$ M/10	$MgCl_2$ M/10	H_2S 1 cc. = 0.00216 mg.	WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	mg.
1	2	...	...	13.1
2	2	...	1	10.8
3	2	...	2	10.9
4	2	...	4	10.2
5	...	2	...	8.4
6	...	2	1	10.7
7	...	2	2	11.0
8	...	2	4	9.9
9	...	2	6	10.2
10	...	2	10	7.3
11	...	2	20	6.9

C. THE VALUE OF SULPHONATE SULPHUR.—In tables X and XI are given the results obtained with taurocholic acid, cysteinic acid and taurine. In table X are given the results, using medium no. 3 and no. 4 as stock media. In table XI the stock medium was no. 5.

TABLE X

GROWTH OF YEAST WITH TAUROCHOLIC ACID AS SULPHUR SOURCE

NUMBER	5 PER CENT. VITAMINE SOLUTION	TAUROCHOLIC ACID ADDED	MEDIUM NO. 4 WEIGHT OF DRIED YEAST	MEDIUM NO. 3 WEIGHT OF DRIED YEAST
	cc.	mg.	mg.	mg.
1	1	0	8.5	12.3
2	1	1	8.6	11.5
3	1	4	6.3	10.2
4	1	10	5.0	7.1
5	0	0	2.6	3.0
6	0	1	2.5	1.8
7	0	4	1.6	1.5
8	0	10	1.1	1.3

TABLE XI

GROWTH OF YEAST IN MEDIUM NO. 5, CYSTEINIC ACID AND TAURINE TESTS

NUMBER	5 PER CENT. VITAMINE SOLUTION	$MgCl_2$ M/10	$MgSO_4$ M/10	CYSTEINIC ACID	TAURINE	AVERAGE WEIGHT OF YEAST
	cc.	cc.	cc.	mg.	mg.	mg.
1	1	2	...	...	...	9.0
2	1	2	...	2	...	10.6
3	1	2	...	6	...	10.0
4	1	2	...	10	...	9.9
5	1	2	...	20	...	9.7
6	1	2	...	...	2	9.3
7	1	2	...	...	6	9.5
8	1	2	...	...	10	9.7
9	1	2	...	...	20	10.2
10	1	...	2	...	...	14.9
11	1	...	2	2	...	13.4
12	1	...	2	6	...	11.0
13	1	...	2	10	...	10.4
14	1	...	2	20	...	9.9
15	1	...	2	...	2	14.6
16	1	...	2	...	6	14.6
17	1	...	2	...	10	13.9
18	1	...	2	...	20	14.3

D. UTILIZATION OF INORGANIC SULPHATE SULPHUR.—Yeast was grown in 500 cc. of medium no. 7 with the addition of 5 cc. vitamine solution. After the usual growth period the yeast was separated by centrifuging and washed twice by centrifuging and decanting. After drying and weighing, the total sulphur was estimated by the method referred to previously. The filtrate was carefully recovered and the "total sulphate" estimated by the usual method. The original medium contained 0.0244 grams sulphur as sulphate; the filtrate from the yeast contained 0.0225 gram or a difference of 0.0019

TABLE XII

UTILIZATION OF CYSTINE SULPHUR BY YEAST, MEDIUM NO. 6

SUBSTANCE	TOTAL SULPHATE	TOTAL SULPHUR	CYSTINE SULPHUR	SUM
	gm. S	gm. S	gm. S	gm. S
Original medium	0.00137	.....	0.0127	0.01407
Yeast filtered off (0.6752 gram)	.....	0.00462	.....	0.00462
Filtrate from yeast	0.00541	.....	0.00322	0.00863

gram sulphur. This amount apparently was removed from the medium during growth. The yeast filtered off contained 0.0016 gram sulphur.

E. UTILIZATION OF CYSTINE SULPHUR.—In this case 0.05 gram cystine was added to 500 cc. of medium no. 6 and the sulphate, cystine and total sulphur determined in the original medium and in the filtrate after growth. The cystine was determined by the phosphotungstic acid precipitation method as employed in the VAN SLYKE procedure for protein analysis. The results are given in table XII.

Discussion of results

A comparison of tables III and IV first of all shows the stimulating action of increased amounts of vitamine under otherwise constant conditions. Both of the tables very clearly show the value of both magnesium and sulphate in yeast growth; and it is particularly noteworthy to observe that in the presence of a liberal supply of the growth-stimulating vitamine, the effect of the same addition of magnesium and sulphate is eight to ten times that observed with a low vitamine content. The results also show that the optimum concentration of magnesium is very soon attained even where growth is extensive as shown in table III. An addition of magnesium beyond that amount neither improves nor injures the medium, no matter whether the vitamine concentration is low or high. The same general conclusions hold for sulphate. Comparison of no. 4 and no. 3 in table III very strikingly shows how much more important magnesium is in this medium than the ammonium. Calcium does not appear to be nearly so important as magnesium.

The observations on the effect of sulphate clearly show that growing yeast is able to utilize sulphate and presumably convert it into protein (probably cystine) sulphur. The quantitative studies on the loss of sulphate from a sulphate medium confirm the same general conclusions as to the utilization of sulphate in yeast growth.

The studies with cystine again confirm the slight stimulating effect thereof when in the lower concentrations, but that one can soon observe an inhibiting effect when the concentration is increased beyond say five to ten milligrams per 125 cc. of medium. This inhibiting effect is observed much more readily when sulphate also is present in the medium. In a sulphate-free medium an otherwise minimal inhibiting concentration of cystine is still stimulating. So also the stimulating effect of cystine is much more marked in a medium containing asparagine than in one free therefrom. In no case, however, could cystine sulphur be satisfactorily substituted for sulphate sulphur. An amount of cystine, equivalent in sulphur content to a good concentration of sulphate as magnesium sulphate or ammonium

sulphate, is toxic in any case. Thus ten milligrams of cystine contain 3.6 milligrams of sulphur whereas 6.4 milligrams of sulphur as sulphate is a very favorable concentration. The observations on cysteine lead to the same general conclusions.

Hydrogen sulphide can also be drawn upon for yeast growth in the absence of sulphate, but one soon reaches a toxic concentration which is far below the sulphur content of a desirable sulphate concentration. Whether the sulphide itself is exceedingly toxic and only that part of it which has become oxidized in the medium is utilized by the yeast, or whether a trace of the sulphide is the real intermediate form between sulphate and yeast tissue sulphur is not determined. Here again an extremely low concentration of hydrogen sulphide (0.00216 milligram H_2S per 125 cc.) is toxic to yeast growth when sulphate is added to the medium, but when the sulphate has not been added it requires ten times the concentration of hydrogen sulphide before a toxic effect is observed. However, even then the stimulating effect is very slight indeed. The action of hydrogen sulphide is very similar to that of cystine, but it is very much more toxic. Possibly cystine itself is harmless, but hydrogen sulphide liberated from it in traces may be the real reason for the apparent toxicity of cystine.

Apparently the yeast is able to protect itself against cystine to some extent by oxidizing the sulphur in cystine to the sulphate form. This is shown in table XII. This observation is very interesting because the yeast metabolism of excess cystine appears to be very similar to that in man where the main excretory form is sulphate. The results also indicate that of the cystine sulphur which was changed, approximately equal amounts were found in the yeast as yeast protein and in the filtrate as sulphate respectively.

The results with taurocholic acid, cysteinic acid and taurine indicate that taurocholic acid is toxic in any case no matter whether other more available forms of sulphur are present or not. Cysteinic acid aids only slightly in an otherwise sulphur-free medium; and in the other media, containing more available forms of sulphur, it is toxic. Cysteinic acid thus stands between cystine and taurocholic acid. Taurine is without appreciable action in the concentrations employed. These results suggest that the cholic acid part in taurocholic acid introduces the toxic effect, but also that the sulphonate form of sulphur is the least available for yeast growth.

Summary and conclusions

1. Magnesium and sulphate appear equally important in yeast growth.

2. Of the forms of sulphur studied, the inorganic sulphate form is the most available.

3. Sulphate sulphur is a true nutrient in that it is actually converted into yeast protein and probably at least in part into cystine.

4. Cystine, cysteine, and hydrogen sulphide stimulate yeast growth in a sulphate-free medium up to certain, but low concentrations. Above those concentrations they retard growth. Cystine also stimulates slightly in a sulphate-containing medium, but hydrogen sulphide retards growth in such a medium even in extremely low concentrations.

5. When yeast grows in a sulphate-free medium containing cystine as the source of sulphur it converts part of the cystine into new yeast protoplasm and about an equal amount into sulphate left in the medium.

6. Taurocholic acid in very low concentrations retards yeast growth. Cysteinic acid acts intermediate between taurocholic acid and cystine.

7. Taurine is without action on yeast growth in the concentrations here employed.

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