SULFUR AND TRACE-ELEMENT NUTRITION OF ASPERGILLUS NIGER [1]

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INTRODUCTION

Though it is known that sulfate sulfur disappears in the green plant to reappear as disulfide (cystine) and sulfhydryl (cysteine), little is known concerning this process of reduction, or of the intermediate compounds involved. Nightingale et al. (*12*) [3] found that sulfate is reduced to sulfite and apparently to sulfhydryl in the comparatively alkaline phloem region of roots and tops. Neither Heiserich (*5*) nor Mothes (*10*) was able to bring about reduction of sulfate with macerated green tissue. However, reduction of sulfate to disulfide was reported by Hammett and Reynolds (*4*) to take place with extracts of *Phaseolus vulgaris* root tips. They considered the process to be enzymatic, since the heated extracts did not cause a diminution in sulfate.

According to summaries by Pfeffer (*13*), Jost (*6*), and Miller (*9*), green plants require sulfate and are unable to assimilate any of its reduction products. Recently, however, increased yields have been found to occur with atmospheric sulfur dioxide (0.1 to 0.2 p.p.m.) by Setterstrom (*14*). Though the possibility exists that oxidation of sulfur dioxide occurred prior to use, the experimental data would indicate the desirability of a restudy of sulfur utilization in green plants. There can be little doubt of the presence in plants of sulfur compounds intermediate in oxidation between sulfhydryl and sulfate (*10, 12*). It appears improbable that sulfur is introduced into organic compounds as sulfate by the green plant without previous reduction.

The situation is quite different with respect to fungi. These have been grown successfully with a large number of sulfur compounds (*2, 7, 18, 19*). Here, again, insufficient evidence is available to determine the course of sulfur assimilation except in a general way. Mothes (*10*) was able to determine, however, that the sulfur metabolism of *Aspergillus niger* parallels that of green plants, whereas it diverges from that of the yeasts. Fuller information, in his opinion, may reveal that specific differences in sulfur metabolism also exist between green plants and fungi.

A general survey of the relation between constitution and assimilability of sulfur compounds was therefore undertaken with this fungus.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 126.

A fuller knowledge of sulfur metabolism in *Aspergillus niger* should prove of value in working out that of the green plant. The absence of rapid and sensitive methods for the chemical identification of suspected intermediary forms of sulfur made necessary the adoption of comparisons upon an exactly quantitative basis. This was accomplished by measuring assimilability of sulfur by the yield obtained with 25 mg. of sulfur per liter at 35° C., after 4 days of growth in an optimum solution. The maximum dry weight (1,100 to 1,300 mg.) obtained with sulfate sulfur served as a standard of comparison.

The sulfur compounds studied fall into five groups of which the known reduction products of sulfuric acid are the most important. Other sources of inorganic sulfur are the thionic acids. The naturally occurring sulfur compounds, such as cystine and methionine and their derivatives, fall into a special category. Lastly, there are the miscellaneous organic sulfur compounds, some of natural occurrence, including the oxidation-reduction series of mercaptan sulfonate and of sulfide-sulfone. These are treated separately. These substances are, on the whole, the simple sulfur-containing compounds into which the more complex forms would be transformed before assimilation under ordinary conditions.

The general assumption underlying the experimental procedures and interpretation of data is that the initial stages of sulfur assimilation are essentially a process of chemical reduction (digestion). Armstrong (*2*) and others have found *Aspergillus niger* to be capable of carrying this reduction to the production of elemental sulfur. Inability of the fungus to grow when supplied with sulfur in a reduced form was assumed to imply, in addition, that oxidation of nutritive sulfur is a matter of difficulty for the fungus.

Paralleling the studies on sulfur assimilation were others with the trace elements iron, zinc, copper, manganese, molybdenum, and gallium. The effects of a deficiency of each of these elements were studied with a variety of sulfur compounds in an attempt to determine whether a specific role was played by one of these elements in the (enzymatic) reduction of sulfur compounds. A relationship of this sort has been found between nitrate reduction and molybdenum (*17*).

MATERIAL AND METHODS

Aspergillus niger Van Tiegh. (No. 215–4247 in the collection of Dr. Charles Thom) was grown on 50-cc. portions of a nutrient solution in 200-cc. Erlenmeyer flasks at 35° C. for 4 days. The nutrient solution consisted of redistilled water 1,000 cc., sucrose 50 gm., reagent ammonium nitrate 2.06 gm., reagent dipotassium phosphate 0.35 gm., and reagent magnesium sulfate ($7H_2O$) 0.25 gm. This solution contained 25 mg. of sulfur per liter, or 1.25 mg. of sulfur per culture. Practically spectroscopically pure iron, zinc, copper, manganese, molybdenum, and gallium were supplied as chlorides in concentrations of 0.30, 0.30, 0.075, 0.075, 0.02, and 0.02 mg. per liter, respectively. The sucrose contained 0.00087 percent of ash. All cultural vessels and accessory apparatus were of transparent quartz, except only the boiling flask of the still and the storage bottle for redistilled water, which were of silica ware. Inoculation was by means of a spore suspension.

When nutrient-solution purification (*15*) was employed, the ammonium nitrate was increased to 2.60 gm. per liter, the dipotassium

phosphate to 0.70 gm., and the magnesium sulfate to 1.10 gm. in the above formula. After the addition of 1 gm. of calcium carbonate and heating to 100° C. for 20 minutes (steamer), the solution was filtered through a fritted quartz crucible of No. 4 porosity. Trace elements were added subsequently.

In solutions provided with other than sulfate-sulfur, magnesium was supplied as $MgCl_2.6H_2O$, 0.21 gm. per liter, or 0.91 gm. per liter when the solution was purified with calcium carbonate. Sulfur compounds other than sulfate were also supplied in concentrations sufficient to provide 25 mg. of sulfur per liter, or, if subjected to nutrient-solution purification, 50 mg. of sulfur per liter

In performing the starch tests a drop of N/20 iodine solution was placed on the reverse of the mycelial felts during filtration, washed several times with water, and examined for starch at ×10 magnification after the lapse of an hour or more. A quinhydrone electrode was used to determine acidity. Yields are given per culture of 50 cc., the mycelial felts having been dried at 103° C. overnight after filtration with fritted glass crucibles of No. 3 porosity.

The sulfur compounds used were reagent chemicals or the purest available.

ASSIMILABILITY OF INORGANIC SULFUR

The maximum yields obtained with sulfate and its products of reduction are tabulated in table 1. It can be seen that as sulfate was successively reduced to sulfite, hyposulfite, sulfoxylate, and sulfide, no diminution in assimilation occurred until the sulfide was formed. Sulfoxylate, though unobtainable, is included here because sodium hyposulfite hydrolyzes in slightly acid solution to form an equimolecular mixture of sulfoxylic and sulfurous acids, which is assimilated as readily as sulfurous acid alone. Utilization of sodium hyposulfite is therefore considered to depend wholly on the formation of sulfoxylate. The bisulfite residue is presumably reduced again to sodium hyposulfite by the fungus. It does not seem probable from the chemical data as summarized by Mellor (*8*) that sulfoxylate has been produced directly by reduction of hyposulfite, though sulfide can be so formed. It is clear, at any rate, that sodium sulfoxylate should also be capable of giving maximum yields, and that it is the lowest state of oxidation in which inorganic sulfur can be utilized efficiently.

TABLE 1.—*Growth data for Aspergillus niger after 4 days at 35° C. with inorganic compounds providing 25 mg. of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. sucrose	Sporulation [1]
		Mg.	
Potassium persulfate	$K_2S_2O_8$	716.2+	2
Sodium sulfate	Na_2SO_4	1,173.0	8
Sodium bisulfite	$NaHSO_3$	1,099.3	8
Sodium hyposulfite	$Na_2S_2O_4.2H_2O$	1,104.3	9
Sodium sulfoxylate	Na_2SO_2	[2]	
(Hydrogen sulfide)	H_2S	(912.5)	6
Sodium disulfide (pH=6.62)	Na_2S_2	602.3	8
Ferrous sulfide	FeS	166.1	9
Sodium sulfide (pH=6.70)	$Na_2S.9H_2O$	256.4	10
Sodium thiosulfate	$Na_2S_2O_3.5H_2O$	1,029.8	4
Sodium dithionate	$Na_2S_2O_6$	2.4	0
Sulfamic acid	NH_2SO_3H	949.3+	4

[1] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.
[2] Maximum.

Results with sulfide and disulfide require further explanation (see also tables 2 and 5). An old sample of sodium sulfide gave maximum yields, as did also the sodium disulfide formed by solution of the calculated amount of sulfur in a 9-percent solution of the sulfide. Inasmuch as this same sample has been used in a previous study (*16*) and found unserviceable as a source of sulfur, additional tests were made to clear up the discrepancy. The data obtained indicated that the sulfur of old and presumably altered (oxidized) samples of sodium sulfide (or disulfide prepared with it) was fully assimilable. Fresh samples gave poor growth, showing symptoms of sulfur deficiency. The transformation of nonassimilable sulfur of sodium sulfide and disulfide to assimilable sulfur was found to be accelerated even by slight alkalinity. Yields with disulfide were uniformly greater than with sulfide. This difference will be discussed in connection with tables 2 and 5.

Growth with sulfur as hydrogen sulfide was almost optimum, but little weight is placed on these data, inasmuch as passage of gas through the nutrient solution (5 minutes) caused precipitation of much sulfur. As will be noticed, ferrous sulfide served poorly as a source of sulfur, though decomposed by the organism, unless a large excess was present. Turbidity of the substrate in the latter case would indicate that a similar decomposition of hydrogen sulfide presumably occurred as with passage of the gas.

An oxidation product of sulfuric acid, namely, persulfate, proved freely available as a source of sulfur, and it is believed that it would have given maximum yields with adjustment of other components of the nutrient solution. A like interpretation of the results with sulfamic acid is also probable. Only two of the six known thionic acids could be tried as sources of sulfur, namely, thiosulfate and dithionate. Sodium thiosulfate gave maximum yields when used as a source of sulfur. Practically no growth occurred with sodium dithionate.

TABLE 2.—*Effect of acidity and age of sample on the utilization of sulfide and disulfide by Aspergillus niger (4 days' growth at 35° C.)*

Source of sulfur	Initial acidity of nutrient solution	Yield per 2.5 gm. of sucrose	Starch [1] in mycelium	Sporulation [2]
	pH	*Mg.*		
Sodium sulfide (old sample)	6.62	1,088.6	1	6
	7.83	1,140.4	0	9
Sodium sulfide (new sample)	6.62	254.6	0	10
	7.69	446.3	0	10
Sodium disulfide (old sample)	6.70	1,086.9	2	5
	7.46	1,111.9	0	9
Sodium disulfide (new sample)	6.70	602.3	4	8
	7.61	986.1	1	6
Sodium sulfate (control)	5.78	1,188.9	0	6
	7.08	1,173.0	0	8

[1] Starch is rated from 0 (none) to 5 (very profuse).
[2] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.

Further insight into the mechanism of sulfide and disulfide utilization is afforded by the data of table 2. Attention is called to the increased yields accompanying increased age of the sample of sulfide. A slight initial alkalinity of the nutrient solution led in all cases to an increase in yield, particularly with the fresh and presumably unoxi-

dized sample. This is interpreted to imply that sulfide and disulfide are more rapidly altered in alkaline solution to forms assimilable by *Aspergillus niger*. It is a matter of general knowledge to mycologists that even higher acidities than are here dealt with promote increased growth of this fungus.

Insufficient evidence is available to form a final opinion on the cause of the higher yields with disulfide as compared with sulfide. The use of sulfur washed with alcohol and water for the preparation of disulfide has little effect on yield. Sulfur is quite difficult to obtain pure and free from oxygen except by recrystallization in carbon disulfide and subsequent distillation in nitrogen (*1*). Washing the sulfur as aforesaid, prior to the preparation of disulfide, would appear of little value in this connection (*8*).

The possibility exists that chemical transformations that result in the formation of assimilable sulfur are more readily undergone by disulfide than by sulfide. Stock solutions of the former alter more rapidly on standing than do those of the sulfide (yield of fungus).

ASSIMILABILITY OF SULFUR IN CYSTINE, METHIONINE, AND THIAMIN [4]

Cysteine and oxidized derivatives (table 3) gave maximum growth when supplied as sources of sulfur. The same is true for taurine, which might be considered as formed from cysteic acid by decarboxylation, and for taurine disulfoxide. Deaminization of taurine to form ethane sulfonic acid does not diminish the assimilability of the contained sulfur, as is shown by the yields obtained with potassium ethane sulfonate. Benzylation of the sulfhydryl group of cysteine was only moderately effective in decreasing the availability of cysteine sulfur. Methionine, cystine, cysteine, and homocystine were equally effective as sources of sulfur supply. A test with thiamin chloride revealed that this essential metabolite in the nutrition of the fungi was unable to serve as a general source of sulfur supply.

TABLE 3.—*Growth data for Aspergillus niger after 4 days at 35° C. with metabolites or their derivatives providing 25 mg. of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. of sucrose	Sporulation [1]
		Mg.	
No sulfur (control)		19.4	2
Cysteic acid	$CH_2(SO_3H).CH(NH_2)COOH$	1,140.7	8
Cysteine sulfinic acid	$CH_2(SO_2H).CH(NH_2)COOH$	1,239.0	8
Cysteine disulfoxide	$(C_3H_6O_2N)SO_2.S(C_3H_6O_2N)$	1,161.5	8
l-Cystine	$[-S-CH_2CH(NH_2)COOH]_2$	1,218.9	8
Cysteine hydrochloride	$HSCH_2CH(NH_2.HCl).COOH$	1,087.3	8
Taurine	$NH_2CH_2CH_2SO_3H$	1,039.5	8
Taurine disulfoxide	$NH_2CH_2CH_2SO_2.SCH_2CH_2NH_2$	1,126.9	8
Potassium ethane sulfonate	$C_2H_5SO_3K$	1,176.9	8
dl-Methionine	$CH_3SCH_2CH_2CH(NH_2)COOH$	1,183.2	8
Homocystine	$[-SCH_2CH_2CH(NH_2)COOH]_2$	1,103.8	4
S-benzylcysteine	$C_6H_5CH_2.SCH_2CH(NH_2)COOH$	623.3	8
Thiamin chloride [2]	$C_{12}H_{17}N_4SOCl$	80.5	4

[1] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.
[2] Only 22.2 mg. S/L.

[4] Lanthionine $[(HO_2C.CH(NH_2)CH_2)_2S]$ and "optically inactive cystine" were obtained, subsequent to completion of this manuscript, from Dr. D. B. Jones and Dr. M. J. Horn, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. The yield with this new amino acid was only 159.3 mg., while that with "optically inactive cystine" was 1,099.2 mg., or about that obtained with *l*-cystine.

The state of oxidation of sulfur in anabolites is immaterial, therefore, in their use by the organism as a general source of sulfur supply. This is in marked contrast to the results previously discussed on the assimilation of inorganic sulfur. It is evident, therefore, that a clear distinction must be made in the nutrition of this fungus between compounds requiring digestion and those utilized without a preliminary alteration, because they are identical with substances (anabolites) necessarily formed during development.

ASSIMILABILITY OF SULFUR IN MISCELLANEOUS ORGANIC COMPOUNDS

Growth data for *Aspergillus niger* with sulfur compounds of the mercaptan-sulfonate series, the sulfide-sulfone series, and miscellaneous compounds are given in table 4. The slight increases in yield with *n*-propyl sulfone, *n*-amyl disulfide, benzyl isothiourea, and benzoyl persulfide are perhaps fortuitous and due to impurities of other sulfur compounds. An interesting feature of these responses is the failure of ethyl, propyl, and heptyl mercaptans to serve as sources of sulfur. It can probably be assumed that all alkyl mercaptans are useless as sulfur supply. Nevertheless, ethane sulfonic acid, an oxidation product of ethyl mercaptan, furnishes sulfur in readily available form, as does isoamyl sulfonic acid (table 5).

TABLE 4.—*Growth data for Aspergillus niger after 4 days at 35° C. with miscellaneous organic compounds providing 25 mg. or more of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. of sucrose	Sporulation [1]
		Mg.	
No sulfur (control)		4.7	0
Dimethyl sulfone	$(CH_3)_2SO_2$	6.7	1
Ethyl mercaptan	C_2H_5SH	3.1	0
n-Propyl mercaptan	C_3H_7SH	6.3	0
n-Propyl sulfide	$(C_3H_7)_2S$	6.8	0
n-Propyl sulfone	$(C_3H_7)_2SO_2$	243.8	8
n-Propyl disulfide	$C_3H_7S.SC_3H_7$	3.7	0
n-Heptyl mercaptan	$C_7H_{15}SH$	5.5	0
n-Heptyl sulfide	$(C_7H_{15})_2S$	6.4	0
n-Amyl disulfide	$(C_5H_{11}S)_2$	241.2	1
Sodium hydroxymethane sulfinate	$HOCH_2SO_2Na$	1,193.8	9
Thioglycolic acid	$HSCH_2COOH$	0	0
Potassium ethyl xanthate	$C_2H_5OCS.SK$	0	0
Benzyl isothiourea hydrochloride	$C_6H_5CH_2SC(NH_2)NH$	152.4	8
Di(β-phenylpropiophenone)-β-sulfide	$[C_6H_5CH(CH_2COC_6H_5)]_2S$	13.1	1
Thiourea	$(NH_2)_2CS$	7.6	0
Sulfonal	$(CH_3)_2C(SO_2.C_2H_5)_2$	6.2	0
Diphenyl thiocarbazone	$C_6H_5N.NCSNHNHC_6H_5$	23.7	2
Sodium benzene sulfinate	$C_6H_5SO_2Na$	29.5	2
Diphenyl sulfone	$(C_6H_5)_2SO_2$	15.3	2
Diphenyl disulfide	$C_6H_5S.SC_6H_5$	0	0
Diphenyl sulfoxide	$(C_6H_5)_2SO$	16.6	2
Benzoyl persulfide	$(C_6H_5CO.S)_2$	188.5	1
Benzyl disulfide	$(C_6H_5CH_2S)_2$	2.1	0
Benzyl sulfide	$(C_6H_5CH_2)_2S$	0	0
Trithiomethylene	$(HCHS)_3$	2.6	0
Thioacetic acid	$CH_3CO.SH$	0	0
Thioacetamide	$CH_3CS.NH_2$	600.5	2
β,β-Dihydroxyethyl sulfide	$(HOCH_2CH_2)_2S$	460.8	3
Potassium dithiooxalate	$(COSK)_2$	750.5	1

[1] Sporulation is rated from 0 (sterile) to 10 (maximum). All spores were black.

TABLE 5.—*Effect of barium ion on growth of Aspergillus niger (4 days at 35° C.) with various sulfur compounds*

Source of sulfur (25 mg. of sulfur per liter)	Yield per 2.5 gm. of sucrose	
	No barium added	Barium added (120 mg. per liter)
	Mg.	*Mg.*
Sodium sulfate	1,209.7	414.8
Taurine	1,190.4	1,024.1
Cysteic acid	1,147.1	1,131.9
Potassium ethane sulfonate	1,216.4	1,204.3
Sodium iodomethane sulfonate	116.6	83.7
Sodium isoamyl sulfonate	1,183.0	885.7
Sodium 2-bromethane sulfonate	40.0	35.5
Thioacetamide	481.0	433.1
β, β-Dihydroxyethyl sulfide	568.2	475.5
Potassium dithiooxalate	463.2	454.0

Trial of alkyl sulfinates could not be made, but growth with sodium hydroxymethane sulfinate (sodium formaldehyde sulfoxylate) was fully equal to that of any source of sulfur. Its sulfur is in the lowest stage of oxidation compatible with maximum efficiency of assimilation of all organic compounds investigated except methionine, cysteine, homocystine, and cystine. It is important to note that, like all sulfinates, it may be considered a derivative of sulfoxylic acid, whose sulfur is in the lowest stage of oxidation for maximum efficiency of assimilation of all inorganic compounds investigated. That is to say, sulfoxylate and sulfinate are synonymous terms, the one used for inorganic compounds, the other for organic. The chemistry of sodium hydroxymethane sulfinate has been discussed by Bazlen (*3*) and Whitmore (*20*).

The difference in nutritive value of sulfur in sulfhydryl and disulfide anabolites (cysteine, cystine, and methionine) and of catabolites (alkyl mercaptans, alkyl disulfides) is sharp and clean-cut. It would indicate that the important factor, at least in metabolism, is molecular configuration as a whole, and not the configuration of specific groups in the molecule.

Other interesting features are nonassimilability of benzene sulfur derivatives, the availability of sulfur in dithiooxalic acid in contrast to the unavailability of that in thioacetic acid, the effect of an amino group attached to the same carbon to which sulfur is attached by a double bond (thioacetamide), and the effect of introduction of oxygen into the alkyl groups of ethyl sulfide (β, β-dihydroxyethyl sulfide). These citations furnish examples of the influence of adjacent groups in the organic molecule upon the rate and ease of assimilation of its contained sulfur.

Availability of a number of alkyl sulfonates permitted a closer experimental study of the method whereby they are utilized as a source of sulfur by the fungus. Several possibilities exist. Sulfur may be used only after hydrolytic removal from the molecule as free sulfonic, sulfinic, or sulfenic acid, depending on concomitant reduction. The nature of the alkyl group would be of little influence in this event. If the barium ion is without effect upon sulfur utilization, it can be assumed that its hydrolytic removal does not occur as the sulfate (*16*).

If the composition of the alkyl residue does determine the extent of sulfur assimilation, the possibility exists that sulfur is assimilated only in combination with a specific carbon group, or that the presence of substituents may prevent nonhydrolytic removal of sulfur.

Examination of table 5 discloses that a considerable difference in composition of the alkyl group [CH_3CH_2— as compared with $(CH_3)_2CH_2CH_2CH_2$—] had no influence on the efficiency of sulfur utilization. However, the presence of halogen substituents in sodium iodomethane sulfonate and sodium 2-bromoethane sulfonate definitely prevented the assimilation of sulfur. It is improbable that toxicity plays a part in this response, inasmuch as the former compound is claimed to be inert in the animal after ingestion and suitable for X-ray diagnoses. The possibility is slight that the presence of halogen prevents the hydrolytic removal of sulfur, since in compounds such as trichloracetic acid and trichloracetaldehyde its effect is to weaken the carbon-to-carbon bond. It seems likely, therefore, that cleavage of sulfur from these compounds by the organism is nonhydrolytic, a residue with a double bond being formed.

Several reasons render probable the interpretation that the sulfur is split off in the form of free sulfoxylic acid (HSO_2H). The ineffectiveness of the presence of barium ion would indicate that sulfur is not removed hydrolytically or otherwise as sulfuric acid. Its removal as free sulfurous acid (HSO_3H) is possible, of course, but improbable from the chemical point of view. Lastly, alkyl sulfinate is equivalent to sulfate-sulfur in assimilability and may undergo this type of cleavage with production of free sulfinic acid (HSO_2H). Whether, however, sulfur is reduced still further before cleavage to free sulfenic acid (HSOH) must await further investigation. As far as the evidence goes, the indications are that sulfur is removed from organic compounds as free sulfinic acid, and that the process is probably enzymatic and due to the presence of "desulfinase."

Since the splitting off of the sulfonic acid group is doubtful under these conditions from what is known of its chemistry, the evidence would indicate that reaction occurs only after reduction at least to sulfinate has occurred. This conclusion is identical with that arrived at on the basis of the data on assimilation of inorganic sulfur and on that of sodium hydroxymethane sulfinate.

EFFECTS OF TRACE-ELEMENT DEFICIENCIES WITH VARIOUS SULFUR COMPOUNDS

Further data on the relation of inorganic sulfide and disulfide to growth of *Aspergillus* are brought out in table 6. The yields would indicate that age of sample and alkalinity of the nutrient solution favor the rapid transformation of sulfide and disulfide into forms readily assimilable by the fungus. The addition of sodium disulfide to the nutrient solution invariably causes the formation of a profuse precipitate, presumably of finely divided sulfur. This precipitate largely disappeared during the course of growth of the fungus. A precipitate is also formed with sodium thiosulfate in acid solution and disappears similarly.

TABLE 6.—*Effect of age of sulfide and disulfide sulfur on growth of Aspergillus niger for 4 days at 35° C. and on the responses of the fungus to deficiencies in trace elements*

Element omitted	Sodium sulfide (old sample)					Sodium disulfide (old sample)					Sodium sulfide (fresh sample)					Sodium disulfide (fresh sample)					Sodium sulfide (fresh sample in non-alkaline solution)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,140.4	100.00	1.83	0	9	1,111.9	100.00	1.86	0	9	446.3	100.00	2.01	0	10	986.1	100.00	1.88	1	6	359.0	100.00	2.14	0	10
Fe	161.2	14.14	2.00	0	4	172.4	15.51	1.99	0	4	102.1	22.88	2.23	0	4	88.5	8.98	2.26	0	2	232.7	64.82	1.99	0	10
Zn	416.7	36.54	1.79	0	10	414.6	37.29	1.81	0	10	310.1	69.46	2.02	0	10	242.5	24.59	1.96	0	10	290.2	80.82	2.14	0	10
Cu	1,154.9	101.27	1.93	0	6, br	1,144.9	102.97	2.05	0	6, t	464.4	104.05	1.85	0	8	892.2	90.48	2.07	1	2	293.4	81.71	2.04	0	10
Mn	651.7	57.15	1.49	3*	0	639.3	57.50	1.50	3	0	369.5	82.79	1.64	3*	2	456.3	46.27	1.64	3*	1	236.8	65.96	1.67	2	0
Mo	1,126.4	98.77	1.78	0	9	1,065.3	95.81	1.83	0	4	532.6	114.34	1.84	1	10	1,042.7	105.74	1.95	0	6	323.9	90.21	2.02	0	10
Ga	1,128.6	98.96	1.85	0	9	1,111.8	99.99	1.84	0	8	501.2	112.19	1.91	1	10	979.2	99.30	1.97	0	6	315.6	87.89	2.11	0	10
Maximum [3]	1,146.1					1,159.8					539.4					1,102.8					388.1				
C. U. [4]		45.84					46.39					21.58					44.11					15.52			
pH [5]			7.83					7.46					7.69					7.61					4.18		

[1] Starch is rated from 0 (none) to 5 (very profuse), an asterisk denoting the immediate formation of a blue color on addition of iodine. Starch in substrate indicated in parentheses. Erythrodextrin indicated by E.

[2] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores are black unless otherwise stated; br=brown, t=tan, y=yellow, w=white.

[3] Maximum individual yield.

[4] Coefficient of utilization or yield per 100 gm. of sucrose.

[5] Initial acidity of nutrient solution.

The acidities employed in the acid nutrient solution in table 6 are not considered too high, since Mellor (*8*) states that a solution of 0.5 N-HCl (pH=0.36±) has been found to dissolve 2.54 times its volume of hydrogen sulfide. This is more than ample to supply the quantity of sulfur required for maximum growth. The data for sodium disulfide in acid solution have been omitted, since yields were maximum owing to complete aging of disulfide.

The sample of sulfur used in the preparation of the disulfide gave appreciable increases in growth when used as a sole source of sulfur. It is possible, therefore, that elemental sulfur in a very finely divided form is capable of utilization by the fungus in the presence of reduced inorganic sulfur compounds because it is more readily converted to assimilable sulfur chemically. Trials with equal quantities of elemental sulfur and of sulfur as sulfide, hyposulfite, sulfite, or thiosulfate gave increases over and above those attributable to the individual constituents with sulfur and thiosulfate and with sulfur and hyposulfite.

Results with trace elements proved disappointing in view of the insolubility of sulfides (except gallium). The marked acidification of the substrate during the course of development was probably responsible for the negative results obtained. As previously mentioned, ferrous sulfide readily dissolves under these conditions. Nevertheless, cupric sulfide is insoluble at much higher acidities than were here encountered. The data would indicate, however, that the usual trace elements were required for growth. Deficiency of individual trace elements was without marked effect on acidity of the cultures at harvest, or on starch in the mycelial felts, except in those cases in which addition of manganese was omitted.

The effects of a trace-element deficiency with various sulfur compounds serving as sources of sulfur in the nutrition of *Aspergillus niger* are tabulated in table 7. It is evident that the trace elements required with sulfate sulfur are also needed for growth with other inorganic sources of sulfur, no matter in what stage of reduction. A similar condition was found to exist with cystine, taurine, methionine, and their derivatives. No evidence was obtained that reduction of inorganic sulfur to a form suitable for conversion into organic sulfur is specifically associated with the presence of one of the essential trace elements. It should be realized, on the other hand, that further refinement of experimental technique may lead to quite different results in the future. Results on sporulation, formation of starch, and acidity of the substrate at harvest were also much the same with all sources of sulfur.

Interpretation of the data with propyl sulfone requires further study, though growth with this compound has been previously assumed to be due to other sulfur impurities. Dimethyl sulfone cannot supply assimilable sulfur. Nor were alkyl sulfoxides available for trial. The fact that propyl sulfide cannot be utilized should not be considered as confirmatory evidence, since ethyl mercaptan cannot be assimilated, whereas its oxidation product, ethyl sulfonate, can be used.

TABLE 7.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C., with various sources of sulfur supply* [1]

Element omitted	Magnesium sulfate					Sodium thiosulfate					Sodium bisulfite					Taurine					Potassium ethane sulfonate					Cysteine hydrochloride				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,041.7	100.00	3.14	3	8	917.5	100.00	2.53	4	4	1,020.8	100.00	2.76	2	8	1,006.5	100.00	2.24	4	8	1,023.6	100.00	2.41	3	8	1,006.1	100.00	2.41	4	8
Fe	71.3	6.61	2.39	0	1	78.0	8.51	2.39	0	2	134.6	13.18	2.08	0	1	193.0	19.17	2.11	1	4	100.2	9.79	2.19	0	1	141.2	14.03	2.24	1	4
Zn	134.9	12.95	2.33	0	1	30.8	3.36	2.99	0	1	47.9	4.69	2.59	(?)	1	22.3	2.22	2.96	0	1	52.8	5.16	2.57	(?)	1	41.1	4.08	2.75	1	1
Cu	930.9	89.46	3.14	4	2	650.7	70.93	2.30	5*[1]	1	742.0	72.69	2.57	3	1	873.2	86.76	2.25	4	3	817.7	79.89	2.47	3"E"	1	954.8	84.94	2.47	2	2
Mn	976.5	93.94	1.98	4	2	812.5	88.56	2.07	2	1	1,099.3	107.69	1.98	4	1	994.1	98.77	2.02	4	1	1,100.4	107.50	2.05	4	2	1,087.3	108.07	2.08	5	2
Mo	763.0	73.24	2.33	4	3	825.7	90.00	2.39	3	4	813.5	79.69	2.12	5*	2	876.5	87.09	2.09	4	4	981.7	95.90	2.36	4	6	950.8	94.57	2.19	4	6
Ga	972.3	93.33	3.09	3	6	823.3	89.74	2.39	5*	2	979.1	95.91	2.82	3	6	926.4	92.04	2.13	4	4	984.1	96.14	2.51	3	6	973.9	96.87	2.31	4	7
Maximum	1,080.0					1,029.8					1,099.3					1,039.5					1,100.4					1,087.3				
C. U		43.20					41.19					43.97					41.58					44.02					43.49			
pH			7.01					7.28					7.12					6.94					7.00					6.40		

[1] See footnotes to table 6.

TABLE 7.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C., with various sources of sulfur supply*—Continued

Element omitted	*dl*-Methionine					*n*-Propyl sulfone					Cysteine disulfoxide				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	932.7	100.00	2.16	3	8	243.8	100.00	2.19	0	10	806.2	100.00	2.25	5	2
Fe	124.5	13.34	2.10	0	1	156.4	64.17	2.26	0	10	53.4	6.62	2.48	0	1
Zn	41.3	4.43	2.72	4	1	103.4	42.42	2.77	0	6	189.0	23.45	2.09	4	1
Cu	840.8	90.15	2.21	5 "E"	2,y	217.6	89.26	2.14	0 (1)	8	681.8	84.57	2.39	4 (1)	0
Mn	912.1	97.79	1.96	4	4	243.1	99.73	2.11	2 (1)	10	1,082.3	134.24	2.13	5 (2)	0
Mo	702.0	75.26	1.90	5*	4	271.5	111.37	1.95	2 (1)	10	579.9	71.93	1.85	5*(5)	0
Ga	876.6	93.98	2.11	4	6	186.3	76.44	2.25	1	6	772.3	95.79	2.22	5	1
Maximum	963.0					297.3					1,082.3				
C. U		38.52					11.89					43.27			
pH			6.89					7.09					6.84		

Element omitted	Cysteic acid					Sodium hyposulfite					Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,020.8	100.00	1.86	0	8	1,082.6	100.00	3.10	2	9	1,183.3	100.00	1.83	1	8
Fe	267.3	26.19	1.94	0	6	116.0	10.71	2.13	0	0	343.8	29.06	1.74	2	8
Zn	26.6	2.61	2.79	0	2	129.1	11.92	2.52	0	1	486.7	41.14	1.74	0	10
Cu	1,069.6	104.78	2.21	0	4,t	813.1	75.15	2.71	3	2	1,176.1	99.42	1.87	1	4
Mn	576.5	56.48	1.62	0	4	1,059.1	97.82	1.85	3	0	552.8	46.98	1.55	5	0
Mo	1,012.5	99.19	1.86	0	6	699.3	64.60	1.91	5*(5)	3	1,044.2	88.26	1.62	1	4
Ga	1,107.7	108.52	1.94	0	8	1,029.2	95.06	3.01	2	8	1,198.0	101.26	1.84	0	6
Maximum	1,140.7					1,104.3					1,200.7				
C. U		45.63					44.17					48.03			
pH			6.62					6.85					7.39		

EFFECTS OF NUTRIENT-SOLUTION PURIFICATION WITH VARIOUS SULFUR SOURCES

Results with trace-element deficiencies after nutrient-solution purification with calcium carbonate (table 8) emphasize the results obtained with unpurified solutions. There would seem little doubt that all the essential trace elements are required irrespective of the source of sulfur. Results with iron and zinc deficiencies were good from the experimental point of view with every sulfur compound tested. Omission of copper led to the greatest decrease in growth with sodium hydroxymethane sulfinate. Best results on manganese were obtained with methionine. Cysteic acid gave maximum decreases in yield on omission of molybdenum and of gallium, but the results were poor. Little weight is placed on the data for *n*-propyl sulfone. Interpretation of these results, however, must await further evidence that they are not fortuitous.

Increased mycelial deposition of starch occurred most frequently with a deficiency in manganese, sometimes with a deficiency in molybdenum, and more rarely with a deficiency in copper or iron. This phenomenon was usually accompanied by specially high increases in acidity of the substrate and a sharp diminution in sporulation. In no instance did similar responses accompany the omission of zinc. Since yields on omission of zinc were far less than on omission of copper, manganese, or molybdenum, these differences may be only concentration effects and not specific.

DISCUSSION OF RESULTS

The growth data on sulfur utilization with compounds of the sulfide-sulfate series and the mercaptan-sulfonate series would appear to justify the assumption that reduction is the normal preliminary process in the utilization of sulfur compounds. This has been assumed in the case of sulfate assimilation (*2*, *4*, *12*). These data would indicate, however, that reduction may be carried too far. When carried beyond a certain stage (sulfoxylate or sulfinate) the nutritive value of sulfur compounds disappears. Evidently the reverse process of oxidation of sulfur is performed by the fungus with great difficulty, if at all.

A distinction should be made in this connection as concerns metabolites since the organism was able to assimilate all cystine derivatives regardless of the state of oxidation of the contained sulfur. Metabolites, or their derivatives, would appear to follow a definite metabolic channel whether synthesized by the fungus or added to the solution as a nutrient. It is possible that all assimilated sulfur passes through the amino acid stage, since cystine and methionine afford excellent sources of sulfur for general use in metabolism, equal to that of sulfate-sulfur or any other. However, it is not definitely known whether cystine and methionine are assimilated as a whole when fed to the fungus, though this is assumed to be the case. Thiamin chloride was practically unavailable as a general source of sulfur. The fungus does not suffer from the inability of mammalian forms to convert cystine into methionine.

TABLE 8.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C. with various sources of sulfur supply after purification of the nutrient solution with calcium carbonate* [1]

Element omitted	Magnesium sulfate					Sodium thiosulfate					Sodium bisulfite				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,212.6	100.00	2.54	0	10	1,355.5	100.00	2.26	0	9	1,272.5	100.00	1.97	0	8
Fe	3.2	.26	3.77	0	0	6.0	.44	3.27	0	1	3.2	.25	3.28	0	0
Zn	87.8	7.24	2.67	0	6	15.6	1.15	3.00	0	1	21.0	1.65	2.88	0	2
Cu	922.5	76.08	2.34	2	2,y	1,169.3	83.99	2.21	0	0	1,120.3	88.04	2.19	0	4,y
Mn	573.0	47.25	1.58	5* (4)	0	430.1	31.71	1.59	4* (2)	0	482.9	37.95	1.56	5* (5)	0
Mo	1,174.3	96.84	2.29	0	4	1,222.6	90.13	1.93	0	6	1,136.8	89.33	1.69	0 (2)	6
Ga	1,266.0	104.41	2.95	0	10	1,332.3	98.20	2.40	0	9	1,245.2	97.85	2.08	0	8
Maximum	1,266.0	---	---	---	---	1,362.0	---	---	---	---	1,300.2	---	---	---	---
C. U.	---	50.64	---	---	---	---	54.48	---	---	---	---	52.00	---	---	---
pH	---	---	7.19	---	---	---	---	7.69	---	---	---	---	7.06	---	---

Element omitted	Taurine					Potassium ethane sulfonate					Cysteine hydrochloride				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	832.5	100.00	1.91	2	8	1,252.9	100.00	2.09	0	8	1,305.3	100.00	2.88	0	10
Fe	0	0	6.25	0	0	6.1	.48	3.57	0	0	18.0	1.38	2.85	0	1
Zn	24.5	2.94	3.01	0	1	17.5	1.40	2.89	0	1	33.8	2.59	2.83	0	2
Cu	362.3	43.52	1.77	1	4,t	595.4	47.52	1.72	2 (2)	1,t	858.4	65.76	2.28	0	0
Mn	386.2	46.39	1.55	5* (2)	0	441.3	35.22	1.57	5* (3)	0	539.1	41.30	1.58	5* (5)	0
Mo	675.6	81.15	1.61	5 (5)	6	1,180.4	94.21	1.98	0	4	1,172.2	89.80	1.91	4	4
Ga	746.6	89.68	1.85	2	7	1,271.6	101.49	2.25	0	6	1,336.6	102.40	2.74	0	8
Maximum	887.9	---	---	---	---	1,327.7	---	---	---	---	1,338.2	---	---	---	---
C. U.	---	35.52	---	---	---	---	53.11	---	---	---	---	53.53	---	---	---
pH	---	---	7.24	---	---	---	---	7.41	---	---	---	---	7.07	---	---

[1] See footnotes to table 6.

TABLE 8.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C. with various sources of sulfur supply after purification of the nutrient solution with calcium carbonate*—Continued

Element omitted	*dl*-Methionine					*n*-Propyl sulfone					Cysteine disulfoxide					Cysteic acid					Sodium hyposulfite					Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,009.0	100.00	1.89	0	6	54.9	100.00	3.01	0	4	1,229.9	100.00	2.09	0	6	672.9	100.00	1.79	0	8	1,303.5	100.00	2.08	0	8	1,281.7	100.00	2.25	1	6
Fe	0	0	6.37	0	0	0	0	6.61	0	0	3.1	.25	3.48	0	0	3.6	.53	3.32	0	1	5.6	.43	3.09	0	2	18.3	1.42	2.97	0	1
Zn	28.3	2.81	2.87	0	4	15.0	27.25	3.18	0	4	30.4	2.47	2.90	0	3	17.3	2.58	3.02	0	2	16.3	1.25	2.93	0	2	5.7	.45	3.00	0	0
Cu	1,146.0	113.58	2.06	0	4,y	28.9	52.66	3.03	0	4	1,217.5	99.03	3.32	0	3,br	500.9	74.45	1.74	0	8	750.5	57.58	1.78	2	4,br	466.5	36.40	1.67	5* (3)	0
Mn	225.8	22.38	1.78	5*	0	28.3	51.43	2.67	0	4	335.6	27.37	1.62	5* (2)	0	261.1	38.81	1.68	5*(0)	0	373.7	28.66	1.55	5*(3)	0	523.9	40.88	1.53	5* (3)	0
Mo	826.7	81.94	1.60	5* (5)	4	44.1	80.33	2.91	0	4	977.2	87.24	1.76	0 "E"	4	544.8	80.99	1.61	0 (2)	8	1,267.0	97.20	1.96	0	6	1,189.7	92.82	1.99	1	4
Ga	1,041.0	103,17	1.95	0	6	38.3	69.67	2.99	0	4	1,259.2	102,38	2.19	0	6	555.2	82.53	1.71	0	8	1,305.5	100.15	2.16	0	8	1,264.6	99.67	2.26	1	6
Maximum	1,146.0					74.2					1,259.2					834.8					1,361.5					1,326.2				
C. U		45.84					2.97					50.37					33.39					54.46					53.05			
pH			7.00					7.23					7.12					7.01					7.08					7.86		

If metabolites serving as nutrients may be pictured as passing into the normal channels present in the organism for their use, the case of nonmetabolites falls into a different category. Nutrients must first be altered to a form suitable for assimilation (digestion) and so brought into normal metabolic channels.

While mercaptans are formed by various organisms, they are probably products of catabolism (waste products) and therefore without a normal channel for anabolism. In this case also, sulfur as alkyl mercaptan was unassimilable, whereas it became readily assimilable after oxidation to alkyl sulfonate. Possibly alkyl sulfinates [5] (RSO_2H) or alkyl sulfinates (RSOH) will be found equally effective. It appears probable, therefore, that with organic compounds also a basic distinction exists in the absence of oxygen and its presence in combination with sulfur, or in an adjacent group. The partial utilization of substances like β, β-dihydroxyethyl sulfide, whereas ethyl sulfide presumably cannot supply sulfur, illustrates the point under discussion.

Though sulfur in alkyl sulfones is combined with oxygen, these proved to be poor sources of sulfur supply. The reason for this may be the inability of the fungus to effect their reduction. It is known that even nascent hydrogen cannot bring about their reduction. Investigation of the assimilability of sulfur in alkyl sulfoxides should help to decide whether this is the correct or sole interpretation.

Other factors of molecular configuration may prevent the utilization of organic sulfur. Sulfur attached to the benzene ring was unsuitable for use in assimilation irrespective of its state of oxidation. However, further tests may reveal that the presence of adjacent groups may considerably modify this response. Sulfur in the thiazole ring (thiamin chloride), or other rings presumably, will probably prove of little value as a nutrient, unless special conditions exist that permit of its ready release in combination with oxygen. The fungus seems unable to avail itself of sulfur attached to carbon, as in thiourea, though again exceptions may exist. The number of sulfur derivatives available for test was far too small to arrive at final conclusions. There is some indication that the presence of adjacent groups (HO—, CO=, NH_2—) may increase the availability of sulfur.

Nevertheless, the different lines of approach employed in the study of sulfur assimilation by *Aspergillus niger* have yielded an identical conclusion. Sulfur, it was found, was reduced and transformed to sulfoxylic acid when supplied as sulfate or alkyl sulfonate. Hydroxymethane sulfinate, a derivative of sulfoxylic acid, was as effective as sulfate in supplying sulfur. Miscellaneous compounds effective as sources of sulfur supply seem to require the presence of oxygen in the molecule. A further reduction to sulfenate before assimilation is a theoretical possibility, though no alkyl sulfenates are known. Whether other available sulfur compounds (β, β-dihydroxyethyl sulfide, etc.) also decompose to form sulfinate is unknown and must await further investigation. It should not be overlooked, however, that anabolites need not conform to this behavior.

Nicolet (*11*) has suggested the possibility of methylene pyruvic acid being a precursor in the utilization by the plant of sulfur in the

[5] A supply of sodium *n*-butane sulfinate ($CH_3CH_2CH_2CH_2SO_2Na$) became available through the kindness of Dr. William H. Ziegler and Dr. Ralph Connor, of the John Harrison Laboratory, University of Pennsylvania, Philadelphia, Pa., after completion of this manuscript. Yield with 25 mg. per liter of sulfur supplied as this compound was 1,173.2 mg. In the presence of barium ion (see table 5) the yield was 768.0 mg.

form of inorganic sulfide or disulfide or as mercaptan, to account for the formation of methionine. Experimental results with *Aspergillus niger* would indicate, however, that the forms of sulfur postulated by Nicolet are unassimilable by this fungus. Other organisms (bacteria) may be capable of assimilating sulfide, disulfide, and mercaptan and may possibly follow the Nicolet course of assimilation. A clear distinction should be made by investigators of this question between utilization for energy and as units for essential metabolites. Experimental proof with aerobic forms would be difficult or impossible because of atmospheric oxidation. Conditions with anaerobes would probably be such as to afford adequate proof.

Nonassimilation of sulfur in sulfide, disulfide, and alkyl mercaptan by *Aspergillus* does not necessarily imply that methylene pyruvic acid does not serve with this organism as a precursor of methionine. The available information might readily be interpreted on the basis of side reactions that lead to the locking of sulfur in unavailable form. Addition of sulfoxylic acid at the double bond of methylene pyruvic acid is quite probable chemically. Other biological data would indicate that forms of sulfur (sulfide, thiourea, etc.) unavailable to *Aspergillus niger* are readily assimilated by certain of the bacteria.

SUMMARY

Alterations in the source of sulfur supply were practically without effect on the trace-element requirements of *Aspergillus niger* Van Tiegh. Iron, zinc, copper, manganese, molybdenum, and gallium were apparently required in approximately equal degree, whatever the state of oxidation of sulfur supplied as a nutrient. The slightly better results obtained through omission of copper with sodium hydroxymethane sulfinate, of manganese with methionine, and of molybdenum and gallium with cysteic acid may prove to have been due to chance.

A survey of the assimilability of inorganic sulfur compounds indicated that sulfur is reduced to sulfoxylate prior to its conversion to organic sulfur. Sulfide and disulfide were not assimilated.

Assimilability of organic sulfur varied with molecular configuration and was also correlated with the presence of attached or adjacent oxygen in the molecule. Alkyl mercaptans, sulfides, and disulfides could not be used as a source of sulfur, whereas alkyl sulfonate and alkyl sulfinate were readily available. Utilization of sulfur in alkyl sulfonates and alkyl sulfinates was considered to depend on their decomposition into free sulfinic acid and an unsaturated residue.

Anabolites, particularly cystine and its derivatives, homocystine, and methionine, were readily available as sole sources of sulfur supply, irrespective of the state of oxidation of their contained sulfur, and were assumed to follow the normal channel for their metabolism. Catabolites and miscellaneous synthetic organic sulfur compounds were assumed to require a process of digestion before assimilation.

ADDENDUM

The data in table 9 were obtained after completion of the manuscript of this paper and are included as a demonstration of the assimilability of alkyl sulfinate by *Aspergillus*. The difficulty with which

compounds of this type can be obtained also made it desirable to report on its utilization as fully as possible.

TABLE 9.—*Growth of Aspergillus niger at 35° C. for 4 days with sulfur supplied as sodium n-butane sulfinate* [1]

Element omitted	Unpurified (25 mg. sulfur per liter)					Purified with $CaCO_3$ (50 mg. sulfur per liter)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	1,041.0	100.00	1.71	0	8	1,262.5	100.00	2.54	0	10
Fe	221.6	21.28	1.96	0	6	6.8	.54	2.94	0	0
Zn	379.6	36.47	1.75	0	10	8.4	.66	2.94	0	0
Cu	1,150.6	110.53	1.84	2	8	1,016.7	80.55	2.05	2	4
Mn	812.0	78.01	1.57	3	4	728.2	57.60	1.53	4	4
Mo	1,025.4	98.50	1.62	1	6	1,096.5	86.85	1.89	2	6
Ga	1,108.7	106.51	1.66	1	8	1,269.0	100.51	2.64	2	10
Maximum	1,150.6					1,295.7				
C. U		46.02					51.83			
pH			7.35					7.47		

[1] See footnotes to table 6.

LITERATURE CITED

(1) ARCHIBALD, E. H.
1932. THE PREPARATION OF PURE INORGANIC SUBSTANCES. 383 pp., illus. New York.

(2) ARMSTRONG, GEORGE M.
1921. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XIV. SULPHUR NUTRITION: THE USE OF THIOSULPHATE AS INFLUENCED BY HYDROGEN-ION CONCENTRATION. Mo. Bot. Gard. Ann. 8: 237–281, illus.

(3) BAZLEN, MAX.
1927. ZUR KENNTNIS DER HYDRO-SCHWEFLIGEN SÄURE. Deut. Chem. Gesell. Ber. 60: 1470–1479.

(4) HAMMETT, FREDERICK S., and REYNOLDS, ARTHUR.
1937. DISULFIDE FROM AMMONIUM SULFATE IN THE PRESENCE OF MASHED ROOT-TIPS OF PHASEOLUS VULGARIS. Science 86: 498–499.

(5) HEISERICH, ERNST.
1935. UNTERSUCHUNGEN ÜBER DEN SCHWEFELSTOFFWECHSEL AN MAIS UND TABAK. Ztschr. f. Pflanzenernähr., Düngung u. Bodenk. 37: 55–72.

(6) JOST, LUDWIG.
1907. LECTURES ON PLANT PHYSIOLOGY. 564 pp., illus. Oxford.

(7) KOSSOWICZ, ALEXANDER, and LOEW, WALTER.
1912. ÜBER DAS VERHALTEN VON HEFEN UND SCHIMMELPILZEN ZU NATRIUMTHIOSULFAT. Ztschr. f. Gärungsphysiol. 2: [87]–103.

(8) MELLOR, J. W.
1930. A COMPREHENSIVE TREATISE ON INORGANIC AND THEORETICAL CHEMISTRY. v. 10, 958 pp., illus.

(9) MILLER, EDWIN C.
1931. PLANT PHYSIOLOGY, WITH REFERENCE TO THE GREEN PLANT. 900 pp., illus. New York and London.

(10) MOTHES, K.
1938. ÜBER DEN SCHWEFELSTOFFWECHSEL DER PFLANZEN II. Planta 29: [67]–109.

(11) NICOLET, BEN H.
1938. BIOCHEMISTRY BY ANALOGY: THE SULFUR OF CYSTINE. Wash. Acad. Sci. Jour. 28: 84–93.

(12) NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R.
1932. EFFECTS OF SULPHUR DEFICIENCY ON METABOLISM IN TOMATO. Plant Physiol. 7: 565–595, illus.

(13) PFEFFER, W.
1900. THE PHYSIOLOGY OF PLANTS; A TREATISE UPON THE METABOLISM AND SOURCES OF ENERGY IN PLANTS. Ed. 2, rev., 3 v., illus. Translated and edited by Alfred J. Ewart. Oxford.
(14) SETTERSTROM, CARL.
1940. EFFECTS OF SULFUR DIOXIDE ON PLANTS AND ANIMALS. Indus. and Eng. Chem., Indus. Ed. 32: 473–479.
(15) STEINBERG, ROBERT A.
1935. NUTRIENT-SOLUTION PURIFICATION FOR REMOVAL OF HEAVY METALS IN DEFICIENCY INVESTIGATIONS WITH ASPERGILLUS NIGER. Jour. Agr. Res. 51: 413–424.
(16) ———
1936. EFFECTS OF BARIUM SALTS UPON ASPERGILLUS NIGER AND THEIR BEARING UPON THE SULPHUR AND ZINC METABOLISM OF THE FUNGUS IN AN OPTIMUM SOLUTION. Bot. Gaz. 97: 666–671.
(17) ———
1937. ROLE OF MOLYBDENUM IN THE UTILIZATION OF AMMONIUM AND NITRATE NITROGEN BY ASPERGILLUS NIGER. Jour. Agr. Res. 55: 891–902.
(18) VOLKONSKY, MICHEL.
1933. SUR LES CONDITIONS DE CULTURE ET LE POUVOIR DE SYNTHÈSE DE SAPROLEGNIA SP. ÉTUDE QUALITATIVE DE L'ALIMENTATION CARBONÉE, AZOTÉE, ET SULFURÉE. Inst. Pasteur [Paris] Ann. 50: [703]–730, illus.
(19) ———
1934. SUR LA NUTRITION DE QUELQUES CHAMPIGNONS SAPROPHYTES ET PARASITES. Inst. Pasteur [Paris] Ann. 52: [76]–101, illus.
(20) WHITMORE, FRANK C.
1937. ORGANIC CHEMISTRY. 1,080 pp., illus. New York.

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