Archiv für Mikrobiologie, Bd. 28, S. 145—152 (1957)

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Biological Transformation of Thiourea

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(Eingegangen am 7. August 1957)

Comparatively little is known about the biological transformation of thiourea which exerts a general toxic effect in the animal organism and inhibits certain oxidizing enzymes in plants (see CROCKER 1948), probably through its chelating effect on copper. To higher plants thiourea seems unavailable as a source of nitrogen and also toxic (NICOLAS and NICOLAS 1925), and it is strongly inhibitory to *Nitrosomonas* in soil (QUASTEL and SCHOLEFIELD 1951) as well as *in vitro* (LEES 1952, JENSEN and SÖRENSEN 1952). Its effect on heterotrophic organisms is less pronounced; KLÖPPING (1951) found the growth of a few species of fungi checked by 200 to 10,000 p.p.m. (0.0026 to 0.132 molar) thiourea.

Occurrence of thiourea in higher plants has sometimes been reported; KJAER (personal communication) was unable to confirm this, but substituted thioureas may arise through reaction between certain amides and naturally occurring isothiocyanates (KJAER and GMELIN 1956). OVCHAROV (1938) stated that certain fungi synthesize thiourea in synthetic media with ammonia, asparagine or protein (but not nitrate) as nitrogen source, but the specificity of the qualitative reactions employed seems open to doubt.

Thiourea is apparently not readily decomposed by biological agencies. According to NICOLAS and NICOLAS (1925) it is not hydrolyzed by soy bean urease. MUNRO (1886), KASTLE and ELVOLVE (1904) and BEESLEY (1914) found no nitrification of thiourea by impure cultures of nitrifying bacteria even after very long incubation, and its inhibition of soil nitrification is quite protracted (QUASTEL and SCHOLEFIELD 1951). It was further found unserviceable as source of nitrogen to penicillia, yeasts and mixed cultures of fungi and bacteria by KASTLE and ELVOLVE (1904), as source of sulphur to *Aspergillus niger* by STEINBERG (1941), and as source of energy to the autotrophic *Thiobacillus thiocyanoxidans* by YOUATT (1954).

Positive findings of thiourea metabolization have rarely been reported. TANNER (1917—1918) stated that several bacteria, yeasts and related

fungi produce sulphide from thiourea. RIPPEL (1925) found *Aspergillus niger* unable to use thiourea as sole nitrogen source, but a small fraction of its sulphur was oxidized to sulphate in the presence of nitrate. DEN DOOREN DE JONG (1926) observed a moderate to sparse growth of a few bacteria, e.g., *Aerobacter aerogenes*, on glucose-thiourea-agar, and non-exacting strains of *Corynebacterium diphtheriae* can according to BRAUN (1938) use thiourea as an inferior substitute for cystine. FULLER et al. (1950) report that 100 p.p.m. thiourea-nitrogen was slowly nitrified and 200 p.p.m. slowly converted into ammonia during 10—14 weeks in alkaline soil. FREDERICK et al. (1957) found only 9% of the sulphur in thiourea (added in the rather large dose of 1%) oxidized to sulphate after 21 weeks in soil of pH 6.1, where the numbers of microorganisms were strongly depressed for 42 weeks; no significant sulphate formation was observed on perfusion of the same soil for six weeks with a 0.5% thiourea solution.

The present contribution deals with the metabolization of thiourea by fungi encountered in experiments on the behaviour of this compound in soil—a problem to which its suggested use as a fly larvicide in dungheaps lends some actuality.

I. Isolation and methods

Microorganisms utilizing thiourea as nitrogen source were isolated by plating on a medium of basal glucose solution (see below) + 0.05% thiourea and 2% agar, either directly from soil or else after enrichment in soil-infected glucose-thiourea-solution. The following fungi showed evidence of activity: 1—2: two non-identified green aspergilli of the *A. glaucus* group. 3: a green penicillium of the *P. citrinum* group. 4: *Trichoderma viride*. 5—6: two unidentified *Dematiaceae*, here called Fungus *Th. 5* and Fungus *Th. 6*. None of these fungi produced more than a trace of growth in sugar-free thiourea solution.

A few strains of bacteria, particulary a fluorescent and rather weakly proteolytic *Pseudomonas*, showed in the primary culture a definitely better growth on glucose-thiourea agar than on corresponding nitrogen-free medium, but this property was lost after a few transfers. These organisms were not studied any further.

The following basal medium was used: Glucose 20.0 gm.; K_2HPO_4 1.0 gm.; $MgSO_4 \cdot 7H_2O$ 0.2 gm.; $CaSO_4$ 0.2 gm.; $FeSO_4 \cdot 5H_2O$ 0.05 gm.; $ZnSO_4 \cdot 7H_2O$ 5 mgm.; $MnSO_4 \cdot 4H_2O$ 0.75 mgm.; $CuSO_4 \cdot 5H_2O$ 1.5 mgm.; in 1000 ml. distilled water. The medium was dispensed in 300-ml.-Erlenmeyer flasks, mostly in portions of 40 or 50 ml., and autoclaved, whereupon thiourea was added from stock solutions sterilized by filtration. Cultures of *Aspergillus* and *Penicillium* were incubated at 28—30° C, the others at 25° C. After growth the mycelium was carefully collected, washed, dried, and weighed, or else nitrogen was determined directly in the moist mycelium after washing.

Nitrogen determinations in mycelium and culture filtrates were made by the Kjeldahl method; the micro-modification (Parnas-Wagner) was used when nitrogen contents less than 0.5 mgm. were expected. Sulphate was determined gravimetrically after precipitation as barium salt. Unless specified, the data in the subsequent tables represent averages of duplicate cultures.

II. Experimental results

1. Utilization of thiourea as nitrogen source

A tentative experiment with *Aspergillus A* (Table 1) shows that thiourea is a rather inferior source of nitrogen which allows only a slow rate of growth and a production of mycelium less than one-half of that obtained with ammonia-nitrogen offered in nearly the same quantity (7.28 mgm., of which 78% was assimilated) as thiourea-nitrogen (7.37 mgm. of which 38% was assimilated).

Table 1. *Growth of Aspergillus A with thiuorea as source of nitrogen.* (40 ml. medium, 2.0% glucose)

Source of nitrogen	Dry weight of mycelium mgm			N-content of mycelium mgm after 21 days
	7 days	14 days	21 days	
N-free medium	1.1	1.3	1.4	0.013
Thiourea 0.05%	9.6	40	88	2.52
NH_4-acetate 0.10% . .	190	219	208	5.67

Varying concentration of thiourea was tested in another tentative experiment (Table 2) which shows that 0.05 and 0.10% thiourea have almost the same effect; 0.20% thiourea causes a delayed growth which after 4 weeks reaches much the same extent as with the lower concentrations. Distillation of the combined filtrates and washings from the mycelia with magnesium oxide showed only insignificant amounts of ammonia-nitrogen (0—0.04 mgm.) in the cultures as well as in sterile control media.

Table 2. *Growth of Aspergillus A with varying concentration of thiourea.* (50 ml. medium, 2.0% glucose)

Thiourea %	Dry weight of mycelium mgm				N-content of mycelium mgm after 28 days
	7 days	14 days	21 days	28 days	
0.05	9	46	66	68	1.58
0.10	12	40	66	69	1.69
0.20	6	17	33	63	1.60

The slow increase in mycelial growth and the incomplete uptake of thiourea-nitrogen might suggest that the decomposition of thiourea is accompanied by loss of nitrogen in gaseous form. A more elaborate experiment was therefore performed with the two *Aspergilli* and the *Penicillium* at a lower thiourea concentration. As seen in Table 3, the uptake of nitrogen within approx. six weeks is again less than one-half in *Aspergillus A*, and less than one-third in the two others. The sum of nitrogen in the mycelia and culture fluids agrees, within the expected

limits of error, with the amount of thiourea-nitrogen originally added. There is thus no indication that nitrogen was lost or converted into a form not detectable by the Kjeldahl method.

An additional experiment was made with *Aspergillus A* in medium with still lower thiourea concentrations. Table 4 shows that the amount of mycelium nitrogen is roughly proportional to that of added thiourea, and the uptake is not greater than from the higher thiourea concentrations.

Table 3. *Utilization of thiourea by Aspergillus and Penicillium.* (40 ml. medium, 2.0% glucose, 0.025% thiourea, theoretical N-content of thiourea, 3.68 mgm.)

Organism and initial N mgm found	Inc. days	Dry weight of mycelium mgm	N per culture mgm in		
			Mycelium	Filtrate	Total
Aspergillus A	14	14	0.54	3.02	3.56
N: 3.45 mgm.	21	52	1.30	2.20	3.50
	28	58	1.39	2.15	3.54
	40	75	1.70	1.92	3.62
Aspergillus B	14	13	0.48	3.27	3.75
N: 3.66 mgm.	30	34	0.72	3.07	3.79
	45	48	1.01	2.67	3.68
Penicillium sp.	14	18	1.00	2.77	3.77
N: 3.82 mgm.	28	31	1.03	2.77	3.80
	45	40	1.14	2.82	3.96

Table 4. *Utilization of thiourea in low concentration by Aspergillus A* (40 ml. medium, 2.0% glucose)

Thiourea %	N in mycelium mgm			Uptake of thiourea-N[1] % after 35 days
	10 days	20 days	35 days	
0	—	—	0.018	—
0.0025	0.058	—	0.074	15
0.005	0.096	0.141	0.154	19
0.010	0.207	0.285	0.53	36

[1] N-content of mycelium from N-free medium subtracted.

The three remaining fungi, which grew more sparsely, were tested in medium with 0.02—0.025% thiourea. *Trichoderma viride* and Fungus *Th. 5* assimilated only 4—5% of the added nitrogen, while 16% was assimilated by Fungus *Th. 6*, within 45 days. Addition of soil extract to the medium resulted in a somewhat better mycelial growth but no greater uptake of thiourea-nitrogen.

All six fungi were tested in an experiment with very low thiourea-concentration (Table 5). Even under these conditions the thiourea is far from quantitatively utilized. The nitrogen uptake by *T. viride* and Fungus *Th. 5* is indeed better but that by the Aspergilli and the Peni-

cillium lower than at higher thiourea concentration. Tests of the culture filtrates with GROTES reagent (GROTE 1931) showed that the thiourea had almost completely disappeared and must have undergone some transformation other than assimilation by the fungi.

Table 5. *Utilization of thiourea in very low concentration*
(100 ml. medium, 1.0% glucose, 0.001% thiourea = 0.368 mgm. N)

Organism	Incubation days	N in mycelium mgm		Uptake of thiourea-N %
		+ thiourea	—N	
Aspergillus A	14	0.073	0.012	17
	40	0.072	0.013	16
Aspergillus B	14	0.060	0.014	12
	40	0.068	0.015	14
Penicillium sp.	14	0.099	0.007	25
	40	0.083	0.011	20
Trichoderma viride	18	0.054	0.005	13
	42	0.047	0.010	10
Fungus *Th. 5*	15	0.048	0.004	12
	45	0.057	0.008	13
Fungus *Th. 6*	15	0.061	0.010	14
	45	0.085	0.008	21

Very long periods of incubation were tried in a final experiment. *Aspergillus A*, *Penicillium* sp. and Fungus *Th. 6* were grown for about 15 weeks, during which time the evaporation losses were approximately restored by periodical addition of sterile distilled water. As seen in Table 6, again little more than one-fourth to one-third of the thiourea-nitrogen is assimilated after 46 days, and rather less than one-half after 102 days. At the final stage, however, the sums of nitrogen in mycelia and filtrates tend to be higher than the initial, probably owing to uptake of some combined nitrogen from the air during the prolonged incubation. Tests with GROTES reagent showed that very little thiourea remained in the medium even after 46 days, when comparisons with thiourea solutions of known concentration suggested a content not exceeding 0.001—0.002% thiourea. The greater part of the nitrogen in the filtrates was apparently present as unknown transformation products of the thiourea (Table 6).

2. Oxidation of thiourea-sulphur

The metabolization of thiourea would be expected to involve a transformation of its sulphur. Several heterotrophic microorganisms are known to oxidize reduced sulphur compounds to sulphate (see RIPPEL 1925, STARKEY 1956, FREDERICK et al. 1957). Tentative experiments with *Aspergillus A* suggested the same effect on thiourea, and this was confirmed by other experiments in glucose-thiourea medium where

calcium was omitted and magnesium and iron supplied as chlorides; thiourea thus served as simultaneous source of nitrogen and sulphur (beside the traces of sulphate added with the heavy metals). The mycelia were harvested as usual, and sulphate was determined in the combined filtrates and washings. The results in Table 7 show that *Aspergillus A* and *Penicillium* sp. form small but significant amounts of sulphate,

Table 6. *Utilization of thiourea by long incubation.* (50 ml. medium, 2.0% glucose, 0.01% thiourea. Initial N-content in thiourea 1.84 mgm., in basal medium 0.04 mgm., total 1.88 mgm.)

Organism	*Aspergillus A*		*Penicillium* sp.		Fungus *Th.* 6	
	a	b	a	b	a	b
After 46 days						
Dry mycelium mgm.	42.3	40.9	24.9	31.3	15.6	15.5
N in mycelium mgm.	0.58	0.55	0.68	0.66	0.46	0.47
N in filtrate mgm.	1.30	1.31	1.26	1.23	1.59	1.48
Total N mgm.	1.88	1.86	1.92	1.89	2.05	1.94
After 102 days						
Dry mycelium mgm.	52.1	49.9	45.9	52.6	20.0	34.4
N in mycelium mgm.	0.91	0.79	0.84	0.86	0.58	0.79
N in filtrate mgm.	1.07	1.53	1.19	1.27	1.43	1.28
Total N mgm.	1.98	2.32	2.03	2.13	2.01	2.07
Uptake of thiourea-N						
% after 46 days	32	30	37	36	25	26
% after 102 days	49	43	46	47	32	43

while the effect of the more weakly growing strain *Aspergillus B* is scarcely significant. The amount of sulphur added with the heavy metals corresponds to only 0.09 mgm. per 100 ml.; the bulk of the sulphate in the sterile control media presumably consists of impurities, or may have arisen by slight chemical oxidation of the thiourea. The two active strains convert in 50 days roughly 15—17% of the thiourea-sulphur into sulphate; this is considerably more than found by RIPPEL (1925) in *Aspergillus niger* which did not utilize thiourea-nitrogen. It is also noteworthy that nitrogen is assimilated and sulphur oxidized in approximate stoichiometric proportion (ratio 0.62—1.14, theoretically 0.875).

An additional experiment on the general sulphur-oxidizing power of the fungi was made in sulphate-free medium (zinc, manganese and copper sulphates omitted) with cystine as simultaneous source of nitrogen and sulphur. A strain of *Aspergillus niger* was included for comparison. Table 8 shows the results after 14 days' incubation. *A. niger* grows vigorously and oxidizes most of the sulphur. The growth of the other fungi is only moderate to scant, but sulphate is formed in all cases, and when the results are expressed on the basis of weight of synthesized mycelium, at least the two green Aspergilli *A* and *B* appear little less active than *A. niger*.

Table 7. *Oxidation of thiourea-sulphur by Aspergillus and Penicillium.* (100 ml. medium, 2.0% glucose, 0.05% thiourea = 18.4 mgm. N and 23.8 mgm. S)

Organism	*Asp. A*	*Asp. B*	*Penicillium*
After 20 days			
(Number of replicates)	(2)	(2)	(3)
Dry mycelium mgm.	110	16	28
N in mycelium mgm.	2.55	0.248	1.49
SO_4-S in filtrate mgm.	2.54	0.33	2.73
SO_4-S excess over sterile control (oxidized S)[1]	2.23	(0)	2.42
Ratio $\frac{\text{assimilated N}}{\text{oxidized S}}$	1.14	—	0.62
After 50 days			
(Number of replicates)	(2)	(3)	(3)
Dry mycelium mgm.	116	48	72
N in mycelium mgm.	2.57	0.58	3.54
SO_4-S in filtrate mgm.	4.06	0.69	4.41
SO_4-S excess over sterile control (oxidized S)[1]	3.67	(0.30)	4.02
Ratio $\frac{\text{assimilated N}}{\text{oxidized S}}$	0.70	—	0.88

[1] SO_4-S in sterile medium 20 d. (5 repl.): 0.14—0.41, mean 0.31 mgm.
SO_4-S in sterile medium 50 d. (6 repl.): 0.26—0.63, mean 0.39 mgm.

Table 8. *Oxidation of cystine-sulphur.* (100 ml. medium, 2.0% glucose, 0.10% cystine)

Organism		Dry mycelium mgm	SO_4-S in filtrate mgm	Cystine-S oxidized to SO_4	
				%	mgm per gm mycelium
Aspergillus A	a)	150	5.5	21	37
	b)	151	4.5	17	30
Aspergillus B	a)	119	4.5	17	38
	b)	128	5.9	22	46
Penicillium	a)	80	2.2	8	26
	b)	55	1.1	4	20
Asp. niger	a)	406	22.0	83	54
	b)	343	19.7	74	57
Sterile medium		—	<0.02	—	—

III. Conclusions

Thiourea can according to the present experiments obviously be used by certain fungi as a source of nitrogen, although an inferior one which allows only a slow growth and never appears to be quantitatively assimilated. The reason why no more than one-half and usually much less thiourea-nitrogen was recovered in the mycelium is somewhat obscure. It might be imagined that the thiourea-molecule becomes available through reversion of the process $NH_4SCN \rightarrow CS(NH_2)_2$, and that only the ammonium radical of the ammonium thiocyanate is utilized. This hypothesis seems

supported by the fact that a separate experiment showed all six strains of fungi unable to use potassium thiocyanate as a source of nitrogen, although some fungi do possess this property (KOSSOWICZ 1914). Nevertheless the hypothesis appears untenable, firstly because no thiocyanate reaction with ferric chloride was detected in the culture fluids, and secondly in view of the fact that sulphur is oxidized in amounts roughly proportional to those of assimilated nitrogen. It seems possible that the unknown nitrogen compounds in the culture filtrates are a mixture of miscellaneous products of excretion and cell autolysis during the long continued growth, and that some thiourea-nitrogen in addition to that recovered in the mycelium has gradually passed through the mycelial stage.

Summary

Six strains of fungi: two *Aspergilli* of the *A. glaucus* group, *Penicillium* sp., *Trichoderma viride*, and two unidentified dark fungi, were found able to use thiourea as a source of nitrogen. One of the Aspergilli and the Penicillium showed the best utilization, but the growth was always slow, and no more than 50% (usually much less) of the thiourea-nitrogen was recovered in the mycelium.

Assimilation of thiourea-nitrogen by *Aspergillus* and *Penicillium* was accompanied by oxidation of the sulphur to sulphate in roughly approximate stoichiometric proportion. The same fungi were able to produce sulphate from cystine as combined source of nitrogen and sulphur.

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