84. *Studies on Biological Methylation. Part X. The Fission of the Mono- and Di-sulphide Links by Moulds.*

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The fungus *Schizophyllum commune* converts inorganic sulphate into methylthiol (Birkinshaw *et al.*, see below). The authors have detected dimethyl sulphide and dimethyl disulphide among the products. *S. commune* forms dimethyl selenide in presence of sodium selenate. Methylthiol and dimethyl sulphide are produced from dimethyl disulphide by the fungus. Diethyl and di-*n*-propyl disulphides yield thiols. *Scopulariopsis brevicaulis* with methionine and the *S*-alkylcysteines (Me, Et, *n*-Pr) gives alkylthiol and alkyl methyl sulphides. This fission of the Alkyl-S–C link appears to be a new type of microbiological action which presents analogy with the fission of cystathionine in animal tissues.

It was shown by one of us with Rawlings (*J.*, 1937, 868) and Blackburn (*J.*, 1938, 1872) that bread cultures of *Scopulariopsis brevicaulis* cause fission of the –S–S– link of disulphides, R·S·S·R, (R = methyl to *n*-amyl) giving the thiol, R·SH, and the alkyl methyl sulphide, R·SMe. Inorganic sulphates are unaffected by *S. brevicaulis*, but Birkinshaw, Findlay, and Webb (*Biochem. J.*, 1942, **36**, 526) showed that the wood-destroying fungus *Schizophyllum commune* when grown on a medium containing glucose and inorganic sulphate evolves methylthiol and traces of hydrogen sulphide. This is the first recorded instance of the methylation of inorganic sulphur by a microbiological process. Mr. Findlay kindly sent us a culture of this organism. Cultures on wort, and especially on bread, without added substrate evolved methylthiol, due to combined sulphur in the medium. When the cultures were almost odourless other substrates were added. Sodium selenate gave dimethyl selenide in small quantity. Potassium tellurite gave black tellurium, and the odour of dimethyl telluride could hardly be detected. Arsenious oxide, and methylarsonic, cacodylic, and *n*-propylarsonic acids gave no more than a very faint garlic odour. *S. commune* therefore appears to have a weaker methylating action than *S. brevicaulis*.

Birkinshaw *et al.* do not mention the production of dimethyl sulphide by *S. commune*, but we find that this is evolved in small quantities with some dimethyl disulphide, probably arising from oxidation of methylthiol. Absorption in mercuric chloride, after removal of methylthiol by mercuric cyanide, gives a mixture of dimethyl sulphide mercurichloride, $2Me_2S,3HgCl_2$, and methylthiomercuric chloride, MeSHgCl, or its mercurichloride $MeSHgCl,xHgCl_2$. The first compound yields dimethyl sulphide with sodium hydroxide. The other two, which arise from the disulphide by fission (*J.*, 1938, 1872, 1878), evolve no sulphur compound under these conditions.

The concentrated culture medium contained traces of pyruvic acid.

The dimethyl sulphide might be produced by a mechanism analogous to that suggested by one of us (*Chem. and Ind.*, 1942, **61**, 414; *Chem. Reviews*, 1945, **36**, 343) to explain the formation of dimethyl selenide from sodium selenate, thus: $Na_2SO_4 \longrightarrow Na_2SO_3 \longrightarrow MeSO_2{\cdot}ONa \longrightarrow Me{\cdot}SO_2Na \longrightarrow Me_2SO_2 \longrightarrow Me_2SO \longrightarrow Me_2S$.

Sodium ethanesulphonate and ethanesulphinate, dimethyl sulphoxide nitrate, and diethyl sulphone in well-grown cultures of *S. commune* gave no dialkyl sulphide. Probably therefore dimethyl sulphide arises by further methylation of the methylthiol.

It is possible that dimethyl disulphide is a primary product of *S. commune* and yields methylthiol on reduction, but this is uncertain. *S. commune* causes fission of dimethyl, diethyl, and di-*n*-butyl disulphides giving the corresponding thiols, and, in the first case, some dimethyl sulphide.

The traces of hydrogen sulphide evolved from *S. commune* cultures (see p. 424 and below) may arise by reduction of sulphate or thiosulphate to sulphide, a reaction effected by certain bacteria (see Stephenson, "Bacterial Metabolism", Longmans, 1930, 76; Ellis, "The Sulphur Bacteria", Longmans, 1931, 22), or possibly by fission of a disulphide, *e.g.*, MeS·SMe giving MeSH and MeSOH, the sulphenic acid giving formaldehyde and hydrogen sulphide by Schöberl's reaction (*Annalen*, 1933, **507**, 111; 1936, **522**, 97).

At one time it appeared possible that the methylthiol produced by *S. commune* might arise by fission of the C–SMe group of methionine, $SMe{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$, synthesised by the fungus. Addition of *dl*-methionine to well-grown cultures of *S. commune*, however, gave only traces of methylthiol or dimethyl sulphide. When *dl*-methionine was present in a suitable medium from the outset as sole source of sulphur, some methylthiol and traces of hydrogen sulphide were evolved, but the amount was very slight in comparison with that obtained using the Birkinshaw medium (*loc. cit.*)

It seemed worth while to determine whether a similar stability would be exhibited by methionine in bread cultures of *S. brevicaulis*. Somewhat to our surprise the amino-acid was readily converted into methylthiol and dimethyl sulphide. With *S*-methyl-, *S*-ethyl- and S-*n*-propyl-cysteine under identical conditions, the corresponding alkylthiol and alkyl methyl sulphide were produced in each case. This fission of the C–S link may be reductive giving homoalanine as the other primary product, or hydrolytic giving homoserine,

$$CH_2(OH){\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H.$$

Methionine is converted by kidney or liver slices into the keto-acid, $SMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CO_2H$ (Borek and Waelsch, *J. Biol. Chem.*, 1941, **141**, 99) and on feeding to rats (Waelsch, *ibid.*, 1941, **140**, 313); see also Handler and Bernheim, *ibid.*, 1943, **150**, 335). The methionol, $SMe{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}OH$, isolated from soya sauce by Akabori and Kaneko (*Proc. Imp. Acad. Japan*, 1936, **12**, 131) doubtless arises from methionine. Akobe (*Z. physiol Chem.*, 1936, **244**, 14) states that with *O. lactis* and *l*-methionine small amounts of methylthiol and diethyl sulphide are formed, but gives no details of their detection or characterisation. No other record of the formation of methylthiol from methionine occurs in the literature, although the keto-acid yields methylthiol with hot acid or alkali (Waelsch and Borek, *J. Amer. Chem. Soc.*, 1939, **61**, 2252; see also Behagel and Ratz, *Ber.*, 1939, **72**, 1257; Nicolet, *J. Amer. Chem. Soc.*, 1931, **53**, 3066).

The thiols obtained in *S. brevicaulis* cultures from methionine and the *S*-alkylcysteines may therefore be formed from keto-acids rather than directly from the amino-acids themselves. The behaviour of other methylthiol derivatives of carboxylic acids is under investigation. Ethylthioacetic acid, $SEt{\cdot}CH_2{\cdot}CO_2H$, yields neither ethylthiol nor methyl ethyl sulphide in bread cultures of *S. brevicaulis*.

Borek and Waelsch (*loc. cit.*) state that homocystine and homocysteine are not deaminised by tissue slices, and refer to "the formation of hydrogen sulphide from homocysteine as from cysteine (compare Smythe, *J. Biol. Chem.*, 1942, **142**, 387) by the action of tissue slices". This may possibly have a botanical significance. Allyl *iso*thiocyanate, a product of the hydrolysis of the glycoside sinigrin occurring in black mustard seeds, may be formed by elimination of hydrogen sulphide from homocysteine (arising from methionine) followed by decarboxylation and interaction of the resulting amine with hydrogen sulphide and carbon dioxide:

$$SH{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H \xrightarrow{-H_2S} CH_2{:}CH{\cdot}CH(NH_2){\cdot}CO_2H \xrightarrow{-CO_2}$$

$$CH_2{:}CH{\cdot}CH_2{\cdot}NH_2 \underset{+H_2S}{\overset{+CO_2}{\longrightarrow}} CH_2{:}CH{\cdot}CH_2{\cdot}NCS + 2H_2O$$

The relation existing between the *iso*thiocyanates of the *Cruciferæ* (see Armstrong and

Armstrong, " The Glycosides ", Longmans, 1931, p. 66) and the corresponding amino-acids has been pointed out by Barger and Coyne (*Biochem. J.*, 1928, **22**, 1417) with reference to methionine and cheirolin, $SO_2Me{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NCS$, which occurs as the glycoside glucocheirolin in wall-flower seeds, and also for phenylethyl *iso*thiocyanate (from the glycoside gluconasturtiin, in the garden nasturtium) and phenylalanine.

The existence of erysolin, $SO_2Me{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NCS$ (found as glucoerysolin in the seeds of the bright orange wall-flower), suggests the advisability of a search for homomethionine among the products of protein hydrolysis.

Diallyl disulphide, which occurs in oil of garlic (Semmler, *Arch. Pharm.*, 1892, **230**, 434) and was isolated as an oxygenated derivative, probably $C_3H_5{\cdot}S{\cdot}S(\rightarrow O){\cdot}C_3H_5$, from the same source by Cavallito *et al.* (*J. Amer. Chem. Soc.*, 1944, **66**, 1950, 1952; 1945, **67**, 1032), may also be formed from homocystine by loss of ammonia and carbon dioxide, a suggestion supported by the well-known enzymic equilibrium existing between fumaric and aspartic acids,

$$[CO_2H{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}S]_2 = 2CO_2 + 2NH_3 + (CH_2{:}CH{\cdot}CH_2{\cdot}S)_2.$$

The fission of the C–SMe link in methionine and the *S*-alkylcysteines has, we believe, only one biological counterpart, namely the fission of cystathionine,

$$CO_2H{\cdot}CH(NH_2){\cdot}CH_2{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

(Brown and du Vigneaud, *J. Biol. Chem.*, 1941, **137**, 611; du Vigneaud, Brown, and Chandler, *ibid.*, 1942, **143**, 59). In presence of rat liver or its saline extracts or kidney slices, this gives cysteine and probably homoserine, $CH_2(OH){\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$, or its phosphoric ester (Binkley, Anslow, and du Vigneaud, *ibid.*, p. 559; Binkley, *ibid.*, 1944, **155**, 39). This fission is probably reversible (Binkley and du Vigneaud, *ibid.*, 1942, **144**, 506), and cystathionine appears to play an important part in the biological conversion of methionine into cystine (Stetten, *ibid.*, p. 501; du Vigneaud *et al.*, *ibid.*, 1944, **155**, 650).

We find that methionine methiodide (Toennies and Kolb, *J. Amer. Chem. Soc.*, 1945, **67**, 849) gives dimethyl sulphide but no methylthiol in bread cultures of *S. brevicaulis*. Homoserine or α-aminovinylacetic acid may be the other product.

$$CO_2H{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}SMe_2\}X + H{\cdot}OH = Me_2S + HX + CO_2H{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}OH$$

This suggests that formation of a sulphonium derivative is not involved in the C–S fission of methionine discussed above. Trimethylsulphonium iodide yields no volatile sulphur compound under similar conditions.

Methyl β-methylthiopropionate, $SMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Me$ (*A*), occurs in pine-apple juice (Haagen-Smit *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 1651), and the chloride of *S*-dimethyl-β-propiothetine, $Me_2\overset{+}{S}(\overset{-}{Cl}){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$, has been isolated from the red alga *Polysiphonia fastigiata* by Miss M. I. Simpson and one of us (forthcoming publication). This sulphonium chloride (*B*) (or other salt) is the precursor of the dimethyl sulphide shown by Haas (*Biochem. J.*, 1935, **29**, 1258) to be evolved by the alga. *A* and *B* may originate from either methionine or cystine, and the free base of *B* and the ester *A* may be biologically connected by a change analogous to the conversion of betaine into methyl dimethylaminoacetate (Willstätter, *Ber.*, 1902, **35**, 584). This comparison of a β-thetine with an α-betaine is put forward with reserve, because *B* with alkali gives dimethyl sulphide and acrylic acid (Holmberg, *Arkiv Kemi, Min. Geol.*, 1946, **21**, *B*, 1). (The liberation of dimethyl sulphide by the alga is probably a very similar reaction.) On the other hand, β-propiobetaine readily gives acrylic acid and trimethylamine, and does not undergo the isomeric change (Willstätter, *loc. cit.*, p. 611).

Experimental.

Growth of S. commune *on a Medium containing Sulphate. Formation of Dimethyl Sulphide and Methylthiol.*—Twenty 1-l. flasks each containing 300 c.c. of the medium used by Birkinshaw *et al.* [glucose (3%) in tap water, with $(NH_4)_2SO_4$ (0·3%), KH_2PO_4 (0·25%), $MgSO_4{,}7H_2O$ (0·1%), " Marmite " (0·01%), and traces of $FeCl_3$, $CuSO_4$, $MnSO_4$, $ZnSO_4$, and borax] were sterilised, inoculated with mycelium from a wort agar culture of *S. commune*, and incubated for 6 days at 32° and 3 days at room temperature. The flasks were arranged in four parallel sets of 5 in series, and volatile products aspirated through 4% aqueous mercuric cyanide and 3% aqueous mercuric chloride for 53 days. The deposits in the cyanide (3·81 g.) melted between 130° and 140°, and yielded mercury dithiomethoxide, m. p. 174°, on recrystallisation, not depressing the m. p. of an authentic specimen, m. p. 175°. The first and third contained traces of the yellow double compound $(MeS)_2Hg{,}2HgS$, but the second was white.

The deposits in the mercuric chloride (2·74 g.) melted between 143° and 147° (decomp.) but some from the bottles nearest the cultures were not completely fused at 260°. The deposits were treated with sodium hydroxide and volatile products aspirated through mercuric cyanide and chloride. No deposit formed in the cyanide but a white solid (0·02 g.) separated in the chloride during 24 hours. It had m. p. 157° and mixed m. p. 156—157° with authentic dimethyl sulphide mercurichloride of m. p. 157—158°. There was much less dimethyl sulphide than methylthiol.

In a second experiment the dimethyl sulphide was identified as before and also absorbed in alcoholic benzyl chloride giving benzyldimethylsulphonium picrate, m. p. 134°, and m. p. 133·5—134° in admixture with a specimen, m. p. 133·5—134° (see Baker and Moffitt, *J.*, 1930, 1722).

After removal of dimethyl sulphide from the crude mercurichloride the alkaline residue was acidified. Aspiration into mercuric cyanide gave mercury dithiomethoxide, m. p. and mixed m. p. 173—174°. The original deposits in the mercuric chloride therefore contained a derivative of methylthiol (MeSHgCl or $MeSHgCl,xHgCl_2$ or both). As no methylthiol escaped from the original mercuric cyanide tubes this must have arisen from dimethyl disulphide formed from methylthiol during aeration of the cultures. MeSHgCl is unmelted at 260° and preponderates if dimethyl disulphide in excess reacts with mercuric chloride. This probably explains the high m. p. of the deposits in the first two mercuric chloride tubes.

Evolution of Methylthiol from S. commune *Cultures on Breadcrumbs containing no Added Substrate.*—Five 1-l. flasks each containing 150 g. of bread and 25 c.c. of water were sterilised, inoculated with *S. commune*, and incubated at 32°. An odour developed in 3—6 days, so the flasks were connected in series and sterile air drawn through into mercuric cyanide and chloride. In the cyanide a yellow deposit formed which, after recrystallisation from ethyl acetate (to remove $Hg(SMe)_2,2HgS$), gave mercury dithiomethoxide, m. p. 175°. The slight deposit in the chloride melted at 150—153° and at 140—145° in admixture with dimethyl sulphide mercurichloride (m. p. 157°). It gave an odour of dimethyl sulphide with sodium hydroxide but consisted mainly of $MeSHgCl,xHgCl_2$. With hydrochloric acid it gave an odour of methylthiol. A second experiment gave a similar result.

Formation of Pyruvic Acid by S. commune *on a Synthetic Medium containing Glucose.*—The fungus was grown on Birkinshaw's medium (see p. 426) for 3 months. 600 C.c. were then concentrated and treated with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The yellow precipitate (0·2 g.), m. p. 210—212° (decomp.), was completely soluble in aqueous sodium carbonate and was reprecipitated by acid, unchanged in m. p. Recrystallisation from alcohol gave orange-yellow crystals, m. p. 216—217°, not depressing the m. p. of pyruvic acid 2 : 4-dinitrophenylhydrazone (m. p. 218°).

With *p*-nitrophenylhydrazine a similar procedure (800 c.c.) gave an orange solid (0·2 g.) which on crystallisation from alcohol–ether had m. p. 219—220° (decomp.) alone and in admixture with pyruvic acid *p*-nitrophenylhydrazone [m. p. 220—222° (decomp.)].

No inorganic sulphide, cystine, or cysteine could be detected in the medium. Extraction with chloroform yielded no dialkyl sulphone.

S. commune *and Diethyl Disulphide.*—Four 1-l. flasks each containing 200 c.c. of wort were inoculated with *S. commune*. After 10 days evolution of methylthiol (see above) had ceased. Each flask then received 25 c.c. of a 2% aqueous suspension of carefully purified diethyl disulphide. After 24 hours, aspiration into mercuric cyanide and chloride was begun. Deposits in the cyanide of m. p. range 70° to 76°, sintering at 60°, were obtained. Recrystallisation from alcohol gave mercury dithioethoxide, m. p. 73° and mixed m. p. 74—75° with a specimen of m. p. 74—75°. Later, traces of mercuric sulphide formed in the cyanide solution. The first two mercuric chloride tubes contained deposits which were unmelted at 260° owing to the presence of EtSHgCl formed from the disulphide (see p. 424).

The deposit from the fourth mercuric chloride tube did not depress the m. p. (151°) of an authentic specimen of $EtSHgCl,HgCl_2$, but was not quite pure. It gave ethylthiol with hydrochloric acid, but with sodium hydroxide gave a distinct odour of an alkyl sulphide (cf. Challenger and Rawlings, *J.*, 1937, 872). Extraction with benzene yielded no methyl ethyl sulphide mercurichloride, however, and $EtSHgCl,HgCl_2$ remained insoluble.

In a second experiment with 6 cultures, deposits in the mercuric chloride were treated with sodium hydroxide. Aspiration through mercuric chloride (see p. 426) gave negative results.

S. commune and *Di-n-butyl Disulphide.*—Four 1-l. flasks (200 c.c. of sterile wort) were inoculated and incubated for 12 days at 32° and six days at room temperature. Each then received 25 c.c. of a 2% suspension of di-*n*-butyl disulphide. Aspiration as usual after 36 hours yielded a deposit (0·9 g.) in the mercuric cyanide which, after two crystallisations from alcohol to remove traces of mercuric sulphide, had m. p. 82—83° alone and 83—84° in admixture with mercury di-*n*-thiobutoxide (m. p. 84—85°).

Deposits in the mercuric chloride after 30 days had m. p. 177° unchanged by admixture with chloromercuri-*n*-thiobutoxide, BuSHgCl, and doubtless arose by fission of di-*n*-butyl disulphide. On examination it was clear that only traces of the mercurichloride of methyl *n*-butyl sulphide were present.

S. commune *and Dimethyl Disulphide.*—The disulphide, prepared by Stutz and Schriner's method (*J. Amer. Chem. Soc.*, 1933, **55**, 1242), was free from methylthiol and hydrogen sulphide.

Five wort cultures of *S. commune*, grown for 6 days at 32° and 3 days at room temperature, received 25 c.c. of a sterile 2% aqueous suspension of the disulphide. Aspiration through mercuric cyanide and chloride yielded, after 5 days, 3·7 g. of mercury dithiomethoxide, m. p. 174—175°, containing traces of yellow $(MeS)_2Hg,2HgS$. The deposits in the chloride were treated with sodium hydroxide. Aeration through mercuric chloride gave dimethyl sulphide mercurichloride, m. p. and mixed m. p. 157—158°.

Blank experiment. About 6·5 g. of the dimethyl disulphide were mixed with excess of aqueous mercuric chloride, and the $MeSHgCl,xHgCl_2$ was separated and treated with sodium hydroxide. Aspiration through aqueous mercuric chloride gave no precipitate, indicating the complete absence of dimethyl sulphide.

S. commune *and Sodium Selenate.*—Four 1-l. flasks each containing 200 c.c. of sterile wort were inoculated and incubated for 10 days at 32° and 17 days at room temperature. Addition to each of 25 c.c. of a sterile 0·7% sodium selenate solution and aspiration through Biginelli's solution (*J.*, 1933, 98) gave, after 22 days, 0·01 g. of solid, m. p. 154° and mixed m. p. 152° with dimethyl selenide mercurichloride of m. p. 153° (all with decomp.). Using 25 c.c. of a 2·4% solution of sodium selenate, growth was partly inhibited.

Behaviour of Sodium Sulphite and Sulphate in developing Cultures of S. commune *on Wort.*—In an attempt to compare the ease of methylation of sulphite and sulphate by *S. commune* the fungus was grown on sterile wort. Suitable quantities (about 0·25%) of sodium sulphate and sulphite were added on cessation of the evolution of methylthiol (10 days). Aspiration into mercuric cyanide and chloride gave only a faint yellow turbidity during 30 days.

Possibly, therefore, the methylation of inorganic sulphate may be associated with the early stages of the growth of the fungus. In view of the very slight formation of methylthiol (see p. 425) on addition of *dl*-methionine to established cultures of the organism, the experiment was repeated with methionine present from the outset.

Growth of S. commune *on a Medium containing Glucose with* dl-*Methionine as Sole Source of Sulphur.*—An aqueous medium was made up containing glucose (3%), NH_4NO_3 (0·3%), KH_2PO_4 (0·25%), $MgCl_2$ (0·1%), and *dl*-methionine (0·2%). This corresponded to the medium used on p. 426 with addition of NO_3', Cl' and methionine, and omission of SO_4'', the " trace elements ", and " marmite ".

Four 1-l. flasks each containing 200 c.c. of sterile medium were inoculated with *S. commune*. The flasks were placed in an incubator at 32° and immediately connected in series, and a stream of sterile air was passed through them and into mercuric cyanide and chloride. After about 5 days at 32° a yellow deposit formed in the first cyanide tube but only increased slowly. It was removed after 28 days, weighed 0·10 g., and consisted of mercury dithiomethoxide and traces of its double compound with mercuric sulphide.

Even after 28 days at 32° the mould growth was scanty. No deposit formed in the mercuric chloride.

S. brevicaulis *and* dl-*Methionine.*—Five 250-c.c. flasks each containing 50 g. of sterile bread were inoculated with *S. brevicaulis* (Strain Washington 2, see *J.*, 1933, 98) and incubated for 4 days at 32° and one day at room temperature. 10 C.c. of a 2% solution of *dl*-methionine in sterile water were added to each flask (concn. in the bread = 0·4%), and the whole was connected in series and sterile air passed through into aqueous mercuric cyanide and chloride. After 7 days the white deposit in the cyanide when recrystallised from ethyl acetate had m. p. and mixed m. p. 174—175° with mercury dithiomethoxide of m. p. 175° (all with decomp.).

The deposits in the mercuric chloride on recrystallisation from benzene had m. p. and mixed m. p. 158° with dimethyl sulphide mercurichloride of m. p. 158°, and gave a strong odour of the sulphide with sodium hydroxide.

Control experiments. (*a*) Four 1-l. flasks each containing 250 g. of sterile bread were inoculated with the same strain of *S. brevicaulis* and incubated for 4 days at 32° and 2 days at room temperature. On aspiration through mercuric cyanide and chloride, except for a very faint trace of deposit in the first cyanide tube on the 4th day (disappearing later) there was no deposit in any tube during 23 days, and no odour.

(*b*) 10 C.c. of a 1% solution of methionine in sterile water were added to each of 4 flasks each containing 50 g. of sterile bread. Aspiration as before gave no deposit during 26 days.

S. brevicaulis *and* S-*Methylcysteine.*—This was prepared by du Vigneaud's method (*J. Biol. Chem.*, 1933, **101**, 719) and purified by repeated solution in water and precipitation with excess of alcohol, giving a specimen of constant m. p. 233° (uncorr.) on 3 successive precipitations [du Vigneaud *et al.* (*loc. cit.*) give m. p. 248° (decomp.) after sintering at 240°] (Found: C, 35·6; H, 6·7; S, 23·3, 23·5. Calc.: C, 35·5; H, 6·7; S, 23·7%).

20 C.c. of a solution of *S*-methylcysteine (0·71 g.) in sterile water (80 c.c.) were added to each of 4 bread cultures (250 g. of bread in 1-l. flasks; concn. in bread = 0·07%) which had been grown for 4 days at 32° and 4 at room temperature. On aspiration, deposits formed in the mercuric cyanide and chloride within a few hours and steadily increased during 25 days. Two deposits, about 0·35 g. in all, from the cyanide after recrystallisation from ethyl acetate had m. p. 175° and 173—174° and mixed m. p. 174—175° and 173—174° with mercury dithiomethoxide of m. p. 174° (all decomp.). Precipitates were obtained from the mercuric chloride of m. p. 147—148°, 151—152°, 135—140°. On crystallisation from benzene the m. p. and mixed m. p. was 157—158° with dimethyl sulphide mercurichloride of m. p. 157—158°. Traces of $MeSHgCl,xHgCl_2$ were present as evidenced by the small amount of residue insoluble in benzene.

S. brevicaulis *and* S-*Ethylcysteine.*—A specimen of *S*-ethylcysteine of m. p. 245—247° (Clark and Inouye, *J. Biol. Chem.*, 1931, **94**, 548) was recrystallised thrice from hot water by addition of alcohol. The four specimens when immersed in a bath at 150° all melted simultaneously at 245—246°, after sintering at 243—244°. That used for the experiment had the same m. p. (Found: C, 40·6; H, 7·4; S, 21·8. Calc.: C, 40·2; H, 7·4; S, 21·5%).

The cultures were as before and were incubated for 4 days at 32° and 3 days at room temperature. To each were added 25 c.c. of a 1% solution of *S*-ethylcysteine in sterile water (concn. in bread = 0·1%). Aspiration during 14 days yielded deposits in the cyanide (0·15 g.) which after two recrystallisations from alcohol had m. p. 76—77° and mixed m. p. 76—77° with mercury dithioethoxide of m. p. 75—76°. Deposits in the mercuric chloride of m. p. 127—128° and 125—127°, after crystallisation from acetone–light petroleum and benzene respectively had m. p. 127—128° and 126—127° not depressing the m. p. (127—128°) of methyl ethyl sulphide dimercurichloride.

Control experiment. 10 C.c. of a 1% solution of *S*-ethylcysteine in sterile water were added to each of four flasks each containing 250 g. of sterile bread. Aspiration into mercuric cyanide and chloride gave no deposits during 14 days.

S. brevicaulis *and* S-n-*Propylcysteine.*—*S-n*-Propylcysteine seems to be new. It was prepared from *l*-cystine as described by du Vigneaud (*loc. cit.*) for the *S*-methyl derivative. The crude product was repeatedly recrystallised from hot water, by addition of alcohol. The main *product* was then twice recrystallised and had a constant m. p. 244—245° (decomp.) (Found: C, 43·8; H, 7·6; S, 19·8. $C_6H_{13}O_2NS$ requires C, 44·1; H, 8·0; S, 19·6%).

Four bread cultures of the Washington strain of the mould were grown for 2 days at 32° and 12 days at room temperature. 25 C.c. of a solution of 0·53 g. of *S-n*-propylcysteine in sterile distilled water (100 c.c.) were added to each flask (concn. = 0·05%). During 4 weeks five deposits (0·28 g. in all) from the mercuric chloride all melted between 163° and 165° and did not depress the m. p. of methyl *n*-propyl sulphide mercurichloride (m. p. 163—164·5°). A grey deposit (0·05 g.) from the cyanide tubes, after recrystallisation from alcohol, had m. p. and mixed m. p. 67—68° with mercury di-*n*-thiopropoxide of m. p. 66—67°.

Other Moulds and dl-*Methionine.*—Bread cultures of *Penicillium notatum* and *P. chrysogenum*

(National Collection of Type Cultures Nos. 4222 and 589), of *P. notatum* var. (Bird and Challenger *J.*, 1939, 163) and of *Aspergillus niger* (strains " W 17 " and " 17 " kindly supplied by Dr. T. K. Walker, College of Technology, Manchester) containing 0·1% of *dl*-methionine gave no methylthiol or sulphide on aeration through mercuric cyanide and chloride for 3—4 weeks. With 0·5% of *dl*-methionine the first two moulds and *A. niger* " W 17 " gave faint odours resembling a thiol.

S. brevicaulis *and Homocystine, Cystine, and Cysteine.*—25 C.c. of a 1·2% solution of (*a*) homocystine and (*b*) cystine in sterile sodium carbonate (1%) and 25 c.c. of a 2% solution of cysteine in sterile water were added to 3 bread cultures in each case. No odour was produced and no deposit formed on aspiration for 30 days through mercuric cyanide and chloride.

S. brevicaulis *and* dl-*Methionine Methiodide.*—The iodide was prepared by the method of Toennies and Kolb (*loc. cit.*) (see p. 426) who give m. p. about 155° (decomp.). Our specimen melted at 165° (decomp.) and was unaffected by a further crystallisation (Found : C, 25·1; H, 5·0; I, 43·7. Calc. for $C_6H_{14}O_2NIS$: C, 24·7; H, 4·8; I, 43·6%).

Six cultures (50 g. of bread) were grown for 6 days at 32° and 2 days at room temperature. 10 C.c. of a solution of the methiodide (1·0 g.) in sterile water (60 c.c.) were added to each flask, and volatile products aspirated through mercuric cyanide and chloride. During 30 days deposits (0·51 g. in all) were removed from the chloride tubes. On crystallisation from benzene the m. p. and mixed m. p. with dimethyl sulphide mercurichloride of identical m. p. was 157—158°. No deposit formed in the cyanide during this time.

Control experiment. 10 C.c. of a solution of the methiodide (0·6 g.) in sterile water (40 c.c.) were added to each of four flasks containing 50 g. of sterile bread. Aspiration through mercuric cyanide and chloride for 32 days produced no deposit.

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