Studies on Biological Methylation. Part XV. The Formation of Dimethyl Selenide in Mould Cultures in Presence of* D- *and* L-*Methionine, or of Thetins, All containing the* $^{14}CH_3$ *Group.*

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D- and L-Methionine (as also DL-methionine; Part XIV *) labelled with $^{14}CH_3$, in cultures of *Aspergillus niger* containing selenate, give radioactive dimethyl selenide, the methylation percentage being 90—100. This figure is reduced to 53—61 when labelled D-methionine competes with an equal weight of unlabelled L-isomer in the same culture, and *vice versa*. The two stereoisomers appear to be equally available as methyl donors. Dimethylacetothetin and dimethyl-β-propiothetin chloride are not direct methyl donors and are very poor methyl sources. The availability of *S*-methyl-DL-methioninesulphonium iodide, labelled in one methyl group, as a methyl source has been examined.

In *A. niger* cultures containing labelled methionine about one-third of the radioactivity appears as an acid which contains sulphur but no keto- or amino-group.

CHALLENGER, LISLE, and DRANSFIELD (Part XIV *) found that methionine is the most important source of methyl groups in the production of dimethyl selenide or trimethylarsine by *Scopulariopsis brevicaulis* and of dimethyl selenide by *Aspergillus niger*. Betaine, choline, and formate are of secondary importance and probably act mainly as methyl sources in the synthesis of methionine. Dimethylacetothetin chloride $Cl^-\{Me_2S^+\cdot CH_2\cdot CO_2H$

* Part XIV, *J.*, 1954, 1760.

and dimethyl-β-propiothetin chloride $Cl^-\{Me_2S^+\cdot CH_2\cdot CH_2\cdot CO_2H$ are not direct methyl donors in *A. niger* cultures, but their significance as methyl sources was not assessed. A similar experiment showed that *S*-methyl DL-methioninesulphonium iodide, $Cl^-\{Me_2S^+\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$, though probably not a direct methyl donor to selenium, was probably an effective source of methionine, (*a*) by loss of one methyl group partly as carbon dioxide (see Table 2) and (*b*) by transfer of this methyl to homocysteine, possibly as an oxidised fragment. These results and those of workers with higher plants and animals (see Part XIV) suggest that methionine occupies a key position as a methyl donor, and in animals is probably first converted into *S*-adenosinylmethionine (Cantoni, *J. Biol. Chem.*, 1953, **204**, 403).

Cantoni (*ibid.*, 1951, **189**, 208, 211) found that L-methionine is twice as active as the DL-isomer in the methylation of nicotinamide to *N'*-methylnicotinamide by the enzyme nicotinamide methyl kinase. This system involves the formation of *S*-adenosinylmethionine (see Part XIV for references to Cantoni's work, and also Baddiley and Jamieson, *Chem. and Ind.*, 1954, 375). Cantoni concludes that D-methionine is inactive as a methyl donor in this system. Betaine and dimethylacetothetin were also inactive but, in presence of DL-homocysteine, methylation of nicotinamide occurred readily. Betaine is 2—5 times more effective as a methyl donor to selenium in *A. niger* cultures in presence of homocysteine (Part XIV). Cantoni's result with dimethylacetothetin agrees with our own (see pp. 1156, 1160), but we have not studied the behaviour of the thetins in presence of added homocysteine.

Handler and Bernheim (*J. Biol. Chem.*, 1943, **150**, 335) found that L-methionine was twice as effective as the D-isomer in the methylation of guanidinoacetic acid to creatine by rat liver slices. However, the α-keto-acid, $MeS\cdot CH_2\cdot CH_2\cdot CO\cdot CO_2H$ was as effective as L-methionine itself. Possibly D-methionine is converted through the keto-acid into the L-amino-acid before transmethylation occurs : this is supported by the observation that transmethylation from D-methionine did not take place in the presence of benzoic acid, a D-amino-acid oxidase inhibitor.

We have now studied the relative utilisation of D- and L-methionine in the mycological methylation of sodium selenate, giving dimethyl selenide. The results (see Expts. 1—4, Table 1) show that D-methionine is quite as effective as L- or DL-methionine.

In the liquid medium used in these experiments the total amount of sulphate present per culture is 0·67 mmole. Hence the maximum amount of " natural methionine " which the mould could synthesise is only 0·67 mmole per culture, which is small compared with 2 mmole of methionine added to each culture. The actual amount of natural methionine formed is probably much less. A rough estimate of this can be made from our experimental figures. If we assume methionine to be the only direct methyl donor present and a maximum theoretical methylation of 100%, then, from the average figure of 95% methylation for the earlier stages of the experiment obtained by adding 2 mmoles of radioactive L-methionine to the culture, we can calculate that $2(100—95)/95 = \sim 0{\cdot}1$ mmole of " natural methionine " is actually formed from sulphate in the medium.

If this small amount of " natural methionine " is always present in the culture at the time of addition of the 2 mmoles of radioactive methionine, there would be present twenty times as much radioactive as non-radioactive " natural " methionine. If the amount of radioactive methionine added was halved, this ratio would be 10 : 1 and there would be approximately 90% methylation. Clearly, under these experimental conditions such a large excess of radioactive methionine is being added that if D-methionine were, say, only half as effective as the L-enantiomorph, the amount of methylation would be only 5% less, *i.e.*, 90 instead of 95%. Consequently experiments (p. 1157) were set up in which equal amounts of D-[*Me*-^{14}C]methionine and non-radioactive L-methionine were added to the selenate cultures. Since absolute values for amount of methylation were not known, owing to uncertainties such as the magnitude of the " back-scatter " correction for mercuri-chloride precipitates (see p. 1156), a similar set of experiments (Table 1, Expts. 5—7) employing radioactive L- and non-radioactive D-methionine was carried out simultaneously. The results showed that the L- and the D-enantiomorph of methionine are utilised with equal facility in mycological methylations. This agrees with the results of work on the

utilisation of D- and L-methionine for the growth of rats (Jackson and Block *J. Biol. Chem.*, 1937, **122**, 425; Rose, *Physiol. Rev.*, 1938, **18**, 109; Bach, "The Metabolism of Protein Constituents in the Mammalian Body," Clarendon Press, Oxford, 1952, p. 161). It seems that (*a*) the methyl group of D-methionine *as such* is labile in mould cultures, or (*b*) if D-methionine cannot be utilised then it must be converted in the mould cell into the L-form as rapidly as the competing L-methionine can be utilised, or (*c*) that both processes (*a*) or (*b*) occur simultaneously. Information from other sources can be found in support of each of these possibilities. Horowitz (*J. Biol. Chem.*, 1944, **154**, 141) found by the Warburg technique that a D-amino-acid oxidase obtained from the mould *Neurospora crassa* (wild, *i.e.*, normal type) deaminates many DL-amino-acids, and DL-methionine most rapidly of all. In this case γ-methylthio-α-oxobutyric acid was isolated from the medium as its 2 : 4-dinitrophenylhydrazone. When L-methionine was used no oxidation occurred. A mutant strain of *N. crassa* which cannot synthesis methionine (methionine-deficient) also utilises D-methionine. Horowitz concludes that the requirements of this mutant strain for L-methionine are met by oxidative deamination of the D-isomer to the keto-acid, followed by re-amination to the L-form (alternative *b*). Unlike the D-amino-acid oxidase presumably present in Handler and Bernheim's enzyme system (see p. 1154), Horowitz's D-amino-acid oxidase is not inhibited by benzoic acid. It resembles a D-amino-acid oxidase from kidney and liver which also oxidises DL-methionine more readily than any other DL-amino-acid (Krebs, *Biochem. J.*, 1935, **29**, 1620). Rydon (*Biochem. Soc. Symposia*, 1948, **1**, 40) states that the D-forms of some amino-acids can probably be used directly in certain enzyme reactions, depending upon the points of attack and attachment. Consequently possibility (*a*) (see above) must not be overlooked since, in the interaction of adenosine triphosphate with methionine to form "active methionine" (*S*-adenosinylmethionine), only the sulphur atom of the amino-acid is involved and this is separated by two carbon atoms from the centre of asymmetry. Cantoni, however, regards D-methionine as inactive in the methylation of nicotinamide by the liver enzymes. Whether D-methionine can form an *S*-adenosinyl derivative under biological conditions is not known, nor has it yet been shown that "active methionine" is concerned in plant methylation. Nevertheless, the isolation of 5′-deoxy-5′-methylthioadenosine ("adenine thiomethyl pentoside" $C_5H_4N_5 \cdot C_4H_5O_3 \cdot CH_2 \cdot SMe$, which is a hydrolysis product of Cantoni's *S*-adenosinylmethionine) from yeast (Mandel and Dunham, *J. Biol. Chem.*, 1912, **11**, 85; Suzuki, Odake, and Mori, *Biochem. Z.*, 1924, **154**, 278), suggests that the significance of *S*-adenosinylmethionine is not confined to animals.

Horowitz remarks that (in 1944) the occurrence of D-amino-acid oxidase in fungi had not previously been recorded. It appears from our experiments that it may be present in *A. niger*.

Though it is clear that D-methionine is utilised as readily as L-methionine in the mycological methylation of selenium, the mechanism of the process has not been determined.

In experiments with liquid cultures containing radioactive L-, D-, or DL-methionine 30—50% of the added radioactivity was always found in what appeared to be a single acid. By the use of ion-exchange resins a very small quantity of this acid was isolated as an oil. It appeared from its behaviour on a paper chromatogram to be almost pure. It contained sulphur but no keto-group, and the ninhydrin reaction was negative. It was presumably a degradation product of methionine.

Obvious possibilities, such as β-methylthiopropionic acid $MeS \cdot CH_2 \cdot CH_2 \cdot CO_2H$, and methylthioacetic acid $MeS \cdot CH_2 \cdot CO_2H$ and its sulphone, were excluded by chromatographic comparisons with authentic compounds. The acid was formed early in the experiments, and accompanied by a large quantity of other acids (produced by *A. niger* from the sucrose of the medium) which disappeared later. No further degradation of this acid took place till most other food sources were exhausted. The remainder of the radioactivity then appeared as $^{14}CO_2$. Figures for methylation percentage were low even before this unknown acid was attacked, so probably it does not possess a labile methyl group. Its investigation is being continued.

It was concluded (Part XIV) that dimethylacetothetin chloride (I), dimethyl-β-propiothetin chloride (II), and probably *S*-methyl-DL-methioninesulphonium iodide (III) are not

direct methyl donors in mycological methylation. These compounds, labelled with ^{14}C in one methyl group were prepared (see Ferger and du Vigneaud, *J. Biol. Chem.*, 1950, **185**, 54, for the first two) and their significance as methyl sources studied. Methylmethioninesulphonium iodide proved to be a good source of methyl groups in the formation of dimethyl

$Cl^-\{Me_2S^+\cdot CH_2\cdot CO_2H$ (I) $\quad Cl^-\{Me_2S^+\cdot CH_2\cdot CH_2\cdot CO_2H$ (II) $\quad I^-\{Me_2S^+\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$ (III)

selenide by *A. niger* (see Table 2). This agrees with the observation that it can replace methionine in the diet of white rats (Bennett, *ibid.*, 1941, **141**, 573; Handler and Benheim, *ibid.*, 1943, **150**, 335). The aceto- and propio-thetin (I and II) were very ineffective under the same conditions. Since choline also is a poor methyl source for selenium in *A. niger* cultures under certain conditions (Part XIV), it was decided to study the behaviour of thetins as methyl sources in bread–selenate cultures of *S. brevicaulis*. The results (Table 3) show that under these conditions thetins cannot be utilised as methyl sources, even to the same extent as betaine, choline, or formate. Hence the " thetin transmethylase " found in rat liver preparations by Borsook and Dubnoff (*J. Biol. Chem.*, 1948, **176**, 789) is presumably not present in the moulds *A. niger* or *S. brevicaulis* under our conditions. Du Vigneaud (Harvey Lectures, 1942—43, **38**, 39) and Maw and du Vigneaud (*J. Biol. Chem.*, 1948, **174**, 381) found that (I) and (II) are as effective as choline as sources of methyl groups for the growth of rats. Borsook and Dubnoff (*loc. cit.*) and du Vigneaud (" A Trail of Research," Cornell Univ. Press, Ithaca, 1952, pp. 102—104) found thetins effective methyl sources for the methylation of homocysteine to methionine in rat tissue prepartions or in intact rats respectively.

Du Vigneaud showed that 27% of the methyl groups of dimethylacetothetin and dimethyl-β-propiothetin was oxidised to carbon dioxide in the rat. We find that oxidation of the methyl groups of these three sulphonium compounds is much less than that of the methyl groups in betaine under comparable conditions.

The culture media at the end of our experiments with (I) and (III) (see p. 1159) contained no radioactive compounds other than the chloride and iodide initially added, showing that in *A. niger* cultures the two dimethylthetins are relatively stable except with respect to the elimination of dimethyl sulphide by S–C fission, observed with the methylmethioninesulphonium iodide (cf. Challenger and Liu, *Rec. Trav. chim.*, 1950, **69**, 334).

The magnitude of the " back-scatter " correction for mercurichlorides (see Part XIV) has now been determined. [*Me*-^{14}C]Dimethyl-β-propiothetin chloride was decomposed with alkali, and the dimethyl sulphide aspirated into 3% aqueous mercuric chloride. The resulting precipitate ($2Me_2S,3HgCl_2$; Blackburn and Challenger, *J.*, 1938, 1878) was filtered on to a standard " plate " and its composition checked by analysis. Its activity per mole was 6—10% higher than that of the original propiothetin chloride. This correction should be applied to all figures for methylation percentages given in Part XIV. Since, however, all conclusions drawn from these figures are relative, this is unnecessary. The existence of such a correction, however, does partly explain why in Table 1 when using D-[*Me*-^{14}C]methionine + L-methionine (Expts. 5 and 6) and L-[*Me*-^{14}C]methionine + D-methionine (Expt. 7), the methylation percentages were greater than the theoretical value of 50%.

Experimental

Radioactive Assay.—The material was plated on " Perspex " discs and counted at infinite thickness, as described in Part XIV (*J.*, 1954, 1760).

The methylation percentage was calculated from the expression

$$\text{Methylation (\%)} = \frac{100}{nf} \times \frac{\text{Radioactivity of methylated product per mole}}{\text{Radioactivity of methyl source per mole}}$$

where n is the number of methyl groups produced by methylation per molecule of the product, and f is the fraction of the total of labelled methyl groups or carbon atoms per molecule which are theoretically labile. Thus, $f = 1$ for the methylmethioninesulphonium ion since both methyl groups are theoretically labile, methionine being formed by the loss of one methyl group. In

the case of the thetins however, *f* was taken as 0·5, since by the loss of one methyl group from the acetothetin methylthioacetic acid is formed which has no labile methyl group (Maw and Du Vigneaud, *J. Biol. Chem.*, 1948, **176**, 103; Dubnoff and Borsook, *ibid.*, p. 789).

Culture Technique.—The moulds, culture media, and experimental procedure are described in Part XIV.

Preparation of Starting Materials.—The preparation of the radioactive D- and L-methionine was described in Part XIV. DL-[*Me*-^{14}C]methylmethioninesulphonium iodide was prepared from DL-[*Me*-^{14}C]methionine and non-radioactive methyl iodide by Toennies and Kolb's method (*J. Amer. Chem. Soc.*, 1945, **67**, 849). A paper chromatogram, in butanol–acetic acid, developed with ninhydrin demonstrated the absence of other amino-acids.

Dimethylacetothetin and dimethyl-β-propiothetin chlorides, labelled in one methyl group with ^{14}C, were prepared from radioactive methyl iodide and unlabelled methylthioacetic acid and β-methylthiopropionic acid (Ferger and Du Vigneaud, *J. Biol. Chem.*, 1950, **185**, 54).

Stable substrates were sterilised under pressure but the two thetins and methylmethioninesulphonium iodide were sterilised by Seitz filtration. The specific rotations of D- and L-methionine were unchanged after sterilisation at 120° for 20—30 min.

Activities (μc *per mmole*) *of Starting Material.*—DL-[*Me*-^{14}C]Methionine, 0·356; [*Me*-^{14}C]-betaine hydrochloride, 0·102; D-, 0·725, and L-[*Me*-^{14}C]methionine, 1·440 (for the preparation of these specimens see Part XIV); DL-[*Me*-^{14}C]methylmethioninesulphonium iodide, 0·202; [*Me*-^{14}C]dimethylacetothetin chloride, 0·494; [*Me*-^{14}C]dimethyl-β-propiothetin chloride, 0·601.

These activities were found by comparison with a plated sample of choline picrate (see Part XIV).

The hygroscopic thetin chlorides were kept over phosphoric oxide before counting, which was performed rapidly. Exposure to air for 2—3 min. did not appreciably affect the results.

Determination of Probable "Back-scatter" Correction for Mercurichlorides.—Radioactive dimethyl sulphide mercurichloride was prepared by boiling [*Me*-^{14}C]dimethyl-β-propiothetin chloride with sodium hydroxide and aspirating the sulphide (Challenger and Simpson, *J.*, 1948, 1591) into aqueous 3% mercuric chloride. Specimens of the thetin chloride and the sulphide mercurichloride were plated and left for one night over phosphoric oxide and counted with an accuracy of ±2%.

The observed activity for the mercurichloride was 8% higher than that calculated from the activity of the thetin chloride (see above) and the known composition of dimethylsulphide mercurichloride. Hence, the back-scatter correction for a mercurichloride can be roughly estimated as 6—10% (see Part XIV).

The mercurichloride had m. p. 154—155° (decomp.; uncorr.), the usual value obtained by one of us (P. B. D.) for pure specimens. Challenger and Simpson (*J.*, 1948, 1593) record m. p. 156—158°. A weighed amount was decomposed with hot alkali, the radioactive dimethyl sulphide recovered, the alkaline suspension of mercuric oxide acidified and treated with hydrogen sulphide, and the mercuric sulphide weighed (Found: Hg, 63·8. Calc. for $C_4H_{12}S_2Cl_6Hg_3$: Hg, 64·1%).

Results.—(*a*) Table 1 shows experiments with *Aspergillus niger* grown on medium G of

TABLE 1.

Expt. no.	Methionine used and its concn. (mmole per flask)	(1) Aspiration time (days)	(2) Methylation (%)	(3) CO_2 (mmole)	(4) Activity (%) recovered as CO_2	(5) Activity (%) recovered from medium in "acid and neutral" fractions
1	DL-[*Me*-^{14}C], 2·0	10	95·2			
		26	86·6	1420	16	35
		41	13·3			
		50	3·2	170	13·9	
2	L-[*Me*-^{14}C], 2·0	19	95	490	3·5	30
		31	99			
3	D-[*Me*-^{14}C], 2·0	18	88	560	4·2	40
		30	100			
4	D-[*Me*-^{14}C], 2·0	11	74	1010	6·5	15
		24	93			
		35	15	770	27·5	
		50	3			
5	D-[*Me*-^{14}C], 1·0 + L-, 1·0	10	61·0	1280	7·0	30
		22	48·0			
6	D-[*Me*-^{14}C], 1·0 + L-, 1·0	13	53·0	Not measured	—	40
7	L-[*Me*-^{14}C], 1·0 + D-, 1·0	10	63·0	1200	9·4	35
		22	37·0			

Part XIV, with sodium selenate (2·5 mmole per flask) as substrate. Dimethyl selenide evolved was collected, and counted, as the mercurichloride, m. p. and mixed m. p. 152—153° (decomp.) in all cases.

In experiments (2) and (3) the cultures developed and spored much better than the others. The yield of dimethyl selenide during the aspiration was only 2·5% compared with the usual 10%, and evolution of selenide ceased completely after 30 days although the food sources of the medium were not exhausted (as shown by the relatively small evolution of carbon dioxide).

Cultures 5 and 7 were made up, grown, and aspirated simultaneously under identical conditions.

Investigation of culture media after aspiration. The cultures were killed by addition of 5—10 c.c. of chloroform, and the mycelium was filtered off, pressed, well washed with water, again pressed, and dried overnight at 30°, and for 2 hr. at 80°. The dry solid was weighed, powdered, plated, and counted.

The radioactivity in the medium was found by counting the viscid solid obtained by evaporating 10% of the medium and washings at 50°/30 mm.

In experiment 1, 40% of the activity originally added as DL-[*Me*-^{14}C]methionine was found in the medium and 10% in the mycelium, the remainder in the carbon dioxide (30%) and dimethyl selenide (20%).

The radioactive material in the medium was probably not methionine since the amount of methylation at the end of the experiment was only 5%. After removal of chloroform at 50—60° the medium was passed slowly through sulphonated cross-linked polystyrene resin to remove bases. These were eluted with 0·2N-ammonia and concentrated by evaporation *in vacuo* over potassium hydroxide.

Only 5% of the original radioactivity was found in the basic fractions, from which methionine was absent (Sullivan test; *J. Biol. Chem.*, 1941, **141**, 871; 1943, **151**, 635; Borsook and Dubnoff, *ibid.*, 1948, **176**, 789).

A chromatogram of the concentrated bases was run on Whatman No. 1 filter paper in butanol–acetic acid. With ninhydrin several spots appeared corresponding to various amino-acids, but not to methionine. No localisation of the radioactivity in any particular spot could be detected.

The effluent from the column, containing acidic and neutral substances, was evaporated. The radioactivity was 35% of the original. Acidic and neutral substances were separated by " Deacidite FF " resin. The " neutral " eluate was almost inactive. The acids (AX) were eluted with 2N-hydrochloric acid and the radioactivity found in the fraction emerging just before the chloride ions. A chromatogram of this fraction, on Whatman No. 54 paper with ethanol–ammonia, was developed with alkaline thymol-blue in ethanol. Four prominent spots at R_F approx. 0, 0·2, 0·45, and 0·6 were obtained but only the third was radioactive. The acid was separated by running a large paper " strip " falling-front chromatogram with a portion of the concentrated fractions. The radioactive band at R_F 0·5 was washed out with water, and the solution evaporated in a vacuum over potassium hydroxide, giving 20 mg. of a viscous brown material of high activity. Several rising-front chromatograms run with this material in ethanol–ammonia showed a radioactive acid spot at R_F 0·45 and a fainter inactive spot at R_F 0·2. The aqueous extract of the main acid spot contained nitrate but no sulphate, sulphite, or chloride. Fusion of the viscous radioactive acid with sodium gave a strong odour of an unidentified sulphur compound. The nitroprusside test was strongly positive; the nitrogen test very faintly positive [(?) nitrate]; halogen was absent. The acids (AX) gave an inactive 2 : 4-dinitrophenylhydrazone but the active material gave no such derivative. The unknown compound is therefore not a keto-acid but contains sulphur and, possibly, nitrogen, and is probably a derivative of the original methionine with radioactive carbon still attached to sulphur. That so much of the original radioactivity is present in a single compound supports this view.

The Sullivan test however was negative, showing that methylthio-acids such as 2-methylthiopropionic or methylthioacetic acid were absent, and the R_F's of these acids when run simultaneously in ethanol–ammonia were 0·75 and 0·65 respectively, compared with 0·45 for the unknown acid. Oxidation of methylthioacetic acid to the sulphone lowered the R_F only to 0·55.

Experiment 4 continued much longer than the others. The bases, after separation as before, contained no methionine and were only slightly radioactive. The small amount of the " acid and neutral " fraction indicated almost complete exhaustion of food sources in the medium, and 90% of the sucrose was converted into carbon dioxide. Very little " acid and neutral " radioactive material was obtained. The activity was contained in one acid spot of R_F 0·45 in

alcohol–ammonia and 0·15 in acetone–ammonia. The radioactive acids in experiments 1 and 4 are therefore identical. Much of this acid was probably oxidised as the activity of the carbon dioxide during the last 14 days was very high. In experiments 2—7 the basic fractions containing amino-acids from the younger cultures were highly radioactive. Paper chromatograms in butanol–acetic acid, developed with ninhydrin, showed several spots including a prominent methionine spot which contained all the radioactivity, showing that methionine did not all disappear in four weeks.

TABLE 2.

Expt. no.	Thetin	Concn. (mmole per flask)	Aspiration time (days)	Methylation (%)	CO_2 (mmole)	Activity (%) recovered as CO_2
8	(I) $Cl^-\{^{14}CH_3\cdot\overset{+}{S}(^{12}CH_3)\cdot CH_2\cdot CO_2H$	2	11	1·4	520	5·2
			22	2·3		
9	,, ,, ...	2	14	1·4	360	2·0
10	(II) $Cl^-\{^{14}CH_3\cdot\overset{+}{S}(^{12}CH_3)\cdot CH_2\cdot CH_2\cdot CO_2H$	2	11	1·8	500	4·4
			22	2·8		
11	(III) $I^-\{^{14}CH_3\cdot\overset{+}{S}(^{12}CH_3)\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$	2	6	35·5	610	2·4
			11	36·5		
			14	28·7		
—	$Cl^-\{^{14}CH_3\cdot\overset{+}{N}(^{12}CH_3)_2\cdot CO_2H$	2	11	11·1	948	22·1
			22	19·6		
			40	22·6		

(*b*) Table 2 shows experiments with thetins and *A. niger* 17 grown on medium G (300 c.c. per flask) with sodium selenate (2·5 mmole per flask) as substrate. Dimethyl selenide was collected and counted as mercurichloride (mixed m. p. 152—153°).

Experiments 10 and 11 each yielded approx. 1% of radioactive dimethyl sulphide. This was separated from dimethyl selenide as described in Part XIV. The yield of selenide in experiments 8—10 was approx. 8% in 22 days and in experiment 11 approx. 12% in 14 days.

In experiment 8, where labelled dimethylacetothetin chloride was used a balance was worked out in the same way as in experiment 1; the results were: basic fractions of medium 75%, neutral and acidic fractions 0, carbon dioxide 5%, dimethyl selenide 0·5%, and mycelium 15% (total 95·5%).

Two chromatograms in butanol–acetic acid on Whatman No. 54 paper developed with ninhydrin and bromophenol-blue, respectively, revealed a basic strongly radioactive spot, giving no colour with ninhydrin and identical in R_F with the dimethylacetothetin spot; so much thetin was unchanged. Challenger and Liu (*Rec. Trav. chim.*, 1950, **69**, 338) found that dimethylacetothetin bromide yields no dimethyl sulphide with *S. brevicaulis*. The absence of radioactivity in the acid fraction is important and shows that no methylthioacetic acid had accumulated. This could arise by demethylation of the thetin and would have been easily detected as the thetin salt was strongly radioactive. Possibly both methyl groups are oxidised. [The behaviour of methylthioacetic acid with *A. niger* is unknown but in bread cultures of *S. brevicaulis* methyl- and ethyl-thioacetic acids give neither alkanethiol nor alkyl methyl sulphide (Lowther, Thesis, Leeds, 1949; Challenger and Charlton, *J.*, 1947, 425).]

The " acid and neutral " fractions of the medium from experiment 11 which had originally contained DL-[*Me*-^{14}C]methylmethioninesulphonium iodide were non-radioactive, and methionine was not detected in the basic fractions, the radioactivity being located in one spot on a paper chromatogram run in butanol–acetic acid and developed with ninhydrin or bromophenol-blue. This corresponded exactly in R_F and colour to that given by the methylmethioninesulphonium ion. This fraction gave with phosphotungstic acid a radioactive precipitate and an almost non-radioactive filtrate. Apparently the methylmethioninesulphonium ion is not degraded in the same way as methionine since neither the amino-acid itself nor the acid of R_F 0·45 was found in the medium.

(*c*) Table 3 shows results with *S. brevicaulis* grown on bread with sodium selenate (2·5 mmole per flask). Dimethyl selenide was collected and counted as mercurichloride. Aspiration was through mercuric cyanide and (*a*) acid and (*b*) neutral mercuric chloride. The thetins were labelled in one methyl group.

The experiments were simultaneous and m. p.s and mixed m. p.s for the purified mercurichloride were 152—153°. Results are based on radioactivities of precipitates collected in acid mercuric chloride only. These, even when crude, were almost inactive and

free from sulphides (by m. p.). The slight radioactivity present was unchanged on the usual purification (Part XIV). The crude precipitates from experiments 12 and 13 (from material collected in *neutral* mercuric chloride) were highly radioactive. This activity was removed on purification and was due to dimethyl sulphide mercurichloride formed by S–C fission of the

TABLE 3.

Expt. no.	Thetin	Concn. (mmole per flask)	Aspiration time (days)	Methylation (%)	CO_2 (mmole)	Activity (%) recovered as CO_2
12	(I)	2·0	7	0·9	1830	9
			14	1·6		
13	(II)	2·0	7	0·8	2050	8
			14	1·5		
14	$Cl^-\{^{14}CH_3{\cdot}NMe_2{\cdot}CH_2{\cdot}CO_2H$	2·0	7	4·1	1510	19
			14	2·0		

thetins. This fission was only 0·5% in experiment 12 and 2·0% in experiment 13. The activity of the neutral mercuric chloride precipitate from experiment 14, however, was of the order of that of the acid mercuric chloride precipitate and was unchanged by purification.

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