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METHUEN'S
MONOGRAPHS ON
BIOCHEMICAL SUBJECTS

General Editors: SIR RUDOLPH PETERS, F.R.S.
and F. G. YOUNG, F.R.S.

THE METABOLISM OF
SULPHUR COMPOUNDS

The Metabolism of Sulphur Compounds

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LONDON: METHUEN & CO LTD
NEW YORK: JOHN WILEY & SONS, INC.

First published 1958
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CATALOGUE NO. 4147/U (METHUEN)
PRINTED IN GREAT BRITAIN BY
BUTLER AND TANNER LTD
FROME AND LONDON

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Preface

It has long been recognized that compounds containing sulphur participate in a variety of biochemical processes, and much progress has been made towards an understanding of the chemical changes which these compounds undergo in living systems. This book is our attempt to provide an introduction to the main features of the metabolism of sulphur compounds in higher animals and in micro-organisms. Recent years have been marked by mounting activity in this field of research, some indication of which can be gained from the fact that nearly half of the eight hundred papers cited in this book have been published since 1950. Although reviews dealing with the metabolism of sulphur compounds have appeared from time to time, these have been concerned mainly with the sulphur-containing amino acids and vitamins. Under these circumstances it seemed to us that there would be a place for a monograph giving a more general account of sulphur metabolism, and we hope that our book will do something to meet this need.

We have been fortunate in that one of our colleagues, Dr M. B. Thorn, has read the manuscript most carefully and has made valuable comments on it. We are greatly indebted to him for this help and to Dr Mary Barber for her kindness in reading and criticizing the microbiological section. We are also grateful to Miss Lesley Gosney and Miss Diane Burridge for the secretarial assistance they have given in the preparation of the book.

CHAPTER I

Introduction

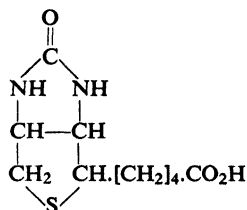
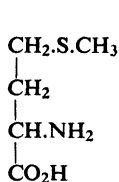
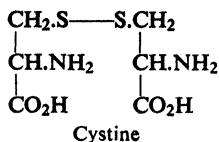
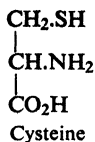
THE NATURALLY-OCCURRING COMPOUNDS OF SULPHUR

All living organisms require sulphur in some suitable form, and in higher animals this need is met by the amino acids L-cysteine, L-cystine and L-methionine, together with the heterocyclic compounds biotin and thiamine. Most plants and micro-organisms are less exacting than animals in their sulphur requirements, for these can often be satisfied by the provision of inorganic sulphate. In most living organisms the amount of energy contributed by sulphur compounds is small by comparison with that derived from other sources. Certain autotrophic bacteria, however, are able to use reactions involving inorganic forms of sulphur as their primary source of energy [1].

Representatives of most of the main types of inorganic and organic sulphur compound have been identified in biological material. Although the function of many of these compounds has yet to be determined, the importance of the sulphur-containing amino acids in biochemical processes is well established. For example, the biological activity of proteins is not infrequently related to the presence of these amino acids in their molecules [2]. Cysteine is a component of the tripeptide glutathione and is a precursor of coenzyme A and taurine. It undergoes oxidation in the animal body and is a major source of sulphate. Methionine is one of the chief biological sources of methyl groups, and is thereby concerned in the synthesis of such compounds as choline, acetylcholine, creatine and adrenaline [3].

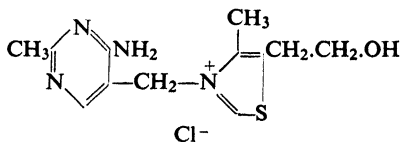
The vitamins biotin and thiamine take part in a number of enzymic processes. Biotin occurs in several forms, including biotin sulphoxide and ϵ -N-biotinyl-L-lysine [4], and it appears to be concerned in carbon dioxide fixation [5]. Thiamine takes part in the decarboxylation of α -keto acids, such as pyruvic acid.

INTRODUCTION



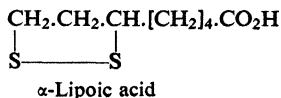
Methionine

Biotin



Thiamine chloride

It occurs in the free form and also as its pyrophosphate, and is believed to act in conjunction with another sulphur compound, α -lipoic acid [6].

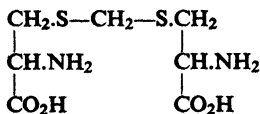


Inorganic sulphate is present in the diet of animals in small amounts. It is poorly absorbed from the intestinal tract and is readily excreted by the kidneys. It is required for the synthesis of sulphomucopolysaccharides and other sulphuric acid esters, and its formation in the body for these purposes is an important aspect of the oxidative metabolism of the sulphur-containing amino acids. Most plants obtain their supplies of sulphur from the sulphate present in the soil, and it is their ability to reduce this form of sulphur and incorporate it into organic compounds

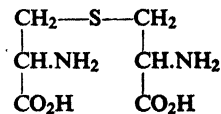
which ensures the supply of sulphur-containing amino acids needed by higher animals. Among other naturally-occurring inorganic forms of sulphur is sulphite, which plays an intermediary role in sulphate metabolism and has been detected in seminal plasma [7]. Mammalian urine generally contains thiosulphate and thiocyanate, and the latter is known to occur in secretions such as saliva.

In addition to cysteine and cystine, other naturally-occurring thiols and disulphides are known. One of the most important of these is coenzyme A (for structural formula, see p. 45), a component of which is cysteamine, a thiol related to cysteine. Some of the simple thiols, for example, methanethiol, *n*-propanethiol [8] and dithioisobutyric acid [9], have been separated from plant sources. Diallyl disulphide occurs in onions and garlic and is a breakdown product of the more complex substance alliin. Methanethiol, together with dimethyl disulphide and dimethyl sulphide, is a metabolic product of the wood-rotting fungus, *Schizophyllum commune* [10], and there are indications that it may be a catabolite of methionine in animals. Two other thiols, *n*-butanethiol and isopentanethiol are found together with their corresponding disulphides in the secretion of the anal gland of the skunk. α -Lipoic acid, to which reference has already been made, appears to be widely distributed and has been isolated from yeast, in which it occurs in several bound forms. Ergothioneine, the betaine of 2-thiolhistidine, is another thiol formed by micro-organisms [11].

Several of the naturally-occurring thioethers are *S*-substituted derivatives of cysteine. Among these are *S*-methylcysteine, $\text{CH}_3\text{.S.CH}_2\text{.CH(NH}_2\text{).CO}_2\text{H}$, found in legumes [12], djenkolic acid, a constituent of the Java djenkol bean [13], and lanthionine, a component of the antibiotics nisin and subtilin [14, 15]. The urine of the cat has been reported to contain another cysteine derivative which has been named felinine [16].



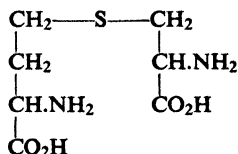
Djenkolic acid



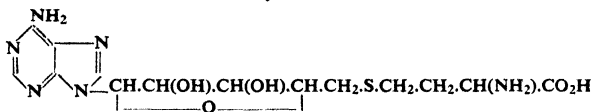
Lanthionine

Various derivatives of methionine are also found in nature.

Cystathionine is excreted in urine under certain conditions, and is also a metabolic product of a mutant of *Neurospora crassa* [17]. *S*-Adenosylhomocysteine has been identified as a product of the demethylation of methionine in enzyme experiments, although it has not yet been isolated from tissues.

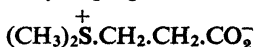


Cystathionine

*S*-Adenosylhomocysteine

Another thioether of importance is the nucleoside 5'-methylthioadenosine. This compound has been known for some considerable time as a constituent of yeasts [18], and it is now thought to be concerned in the synthesis and metabolism of methionine in these organisms. Other thioethers present in plants include dimethyl sulphide [19], methionol, $\text{CH}_3\text{S} \cdot [\text{CH}_2]_3\text{OH}$ [20], and methyl *S*-methylthiopropionate, $\text{CH}_3\text{S} \cdot \text{CH}_2\text{CH}_2\text{COO} \cdot \text{CH}_3$. The last-named ester has been obtained from pineapple juice [21].

Three naturally-occurring sulphonium compounds have been identified with certainty, but there is good evidence for the existence of others. Dimethyl- β -propiothetin,



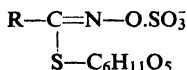
has been isolated in the form of its salts from various marine algae [22], and methionine methylsulphonium chloride,

$(\text{CH}_3)_2\text{S}^+\text{CH}_2\text{CH}_2\text{CH(NH}_2\text{)}\text{CO}_2\text{H Cl}^-$, has been obtained from several plant sources [23, 24]. The occurrence of these compounds in animal tissues has not been established, but a related sulphonium salt, *S*-adenosylmethionine, is an intermediary metabolite of methionine in mammalian liver.

A number of sulfoxides occur in plants. Alliin, or *S*-allyl-

cysteine sulphoxide, has been isolated from garlic [25] and is regarded as a precursor of the diallyl disulphide obtained from this plant. A similar compound, which has been identified as *S*-methylcysteine sulphoxide, is now known to be a constituent of cabbage and other plants [26, 27]. Radish seeds contain the sulphoxide sulphoraphene, $\text{CH}_3\text{.SO.CH=CH.CH}_2\text{.CH}_2\text{.NCS}$, and the corresponding nitrile [28]. It has been reported that after the administration of methionine to animals, the sulphoxide of the amino acid is present in the urine, but there are grounds for believing that under these conditions it is no more than an artifact [29]. $\beta\beta'$ -Dihydroxydiethyl sulphoxide, reputed to be a constituent of ox adrenal glands, may also be an artifact, for it is possibly formed during the process of extraction.

Two sulphones, cheirolin, $\text{CH}_3\text{.SO}_2\text{.[CH}_2\text{]}_3\text{.NCS}$, and erysolin, $\text{CH}_3\text{.SO}_2\text{.[CH}_2\text{]}_4\text{.NCS}$, are present as glucosides in wallflower seeds. These and other *isothiocyanates* found in plants (30, 31) occur as combined sulphuric acid esters and glucosides of the following general structure:



There have been several reports of the existence of dimethyl sulphone in ferns and also in the blood and adrenal glands of cattle.

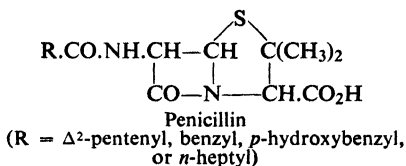
The most important naturally-occurring sulphonic acid is taurine, $\text{H}_2\text{N.CH}_2\text{.CH}_2\text{.SO}_3\text{H}$, which is found in the free state in mammalian tissues and urine, as well as in the muscles and organs of many invertebrates. It is also a component of conjugated bile acids. Isethionic acid, $\text{HO.CH}_2\text{.CH}_2\text{.SO}_3\text{H}$, the hydroxy analogue of taurine, is believed to be a constituent of squid axoplasm [32]. Among the sulphonic acids found in plants are methanesulphonic acid, $\text{CH}_3\text{.SO}_3\text{H}$, and sulphoacetic acid, $\text{HO}_2\text{C.CH}_2\text{.SO}_3\text{H}$, both of which occur in combination with alkaloids [33]. A sulphinic acid, $\text{HO}_2\text{S.CH}_2\text{.CH(NH}_2\text{).CO}_2\text{H}$, derived from cysteine, has recently been isolated from rat brain [34], and is an important intermediate in the metabolic oxidation of this amino acid.

The sulphomucopolysaccharides, in which sulphuric acid is linked to various aminosugars, are the commonest sulphuric acid

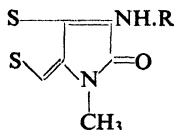
esters of natural origin. They occur, for example, in mucous secretions and as components of cartilaginous tissues. Sulphuric acid esters are normal constituents of urine. Certain steroids are excreted in this form, as are a number of phenols formed by bacterial action in the intestine. The presence of tyrosine-*O*-sulphate in urine has recently been established, and this compound has also been identified as a component of fibrinogen [35]. Another compound of this class is the sulphate ester of choline,

$(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{O}\cdot\text{SO}_2\text{O}^-$, which has been isolated from the mycelium of the mould *Aspergillus sydovi* [36].

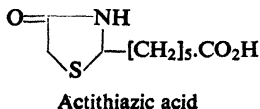
Several sulphur-containing heterocyclic compounds have been shown to occur naturally, and reference has already been made to two of these, namely biotin and thiamine. Some of the antibiotics are compounds of this type, for example, the penicillins.



Other examples are provided by the antibiotics thiolutin and aureothricin. These have the following structure, in which R denotes an acetyl and a propionyl grouping, respectively [37]:



Gliotoxin and actithiazic acid are also sulphur-containing heterocyclic compounds. The latter substance, which is toxic to mycobacteria *in vitro*, is of interest by virtue of its structural similarity to biotin, and its ability to act as an antimetabolite of this growth factor in bacteria [38].



METHODS USED IN STUDYING THE METABOLISM OF
SULPHUR COMPOUNDS

Most of the methods which have been developed for the investigation of metabolic problems have found application in the study of sulphur metabolism. One type of approach which has led to important findings in this field has been based on the use of nutritional experiments. The first significant experiments of this kind were those of Osborne and Mendel [39] on the growth-promoting properties of cystine in the diet of the rat. Attempts by later workers to replace dietary cystine by related sulphur compounds led to the recognition of the fact that methionine rather than cystine is an indispensable constituent of the diet.

Feeding experiments have generally been carried out with rats maintained on basal diets low in their content of the sulphur-containing amino acids. Such diets may be prepared using proteins known to be poor in these compounds, such as casein (which is low in cystine) or arachin (which is low in methionine). Use may be made of proteins treated chemically so as to render their sulphur-containing amino acids unavailable to the animal, as occurs when casein is treated with hydrogen peroxide and the methionine present is thereby oxidized to the sulphone. Alternatively, the protein component of the diet may be replaced by a mixture of purified amino acids from which methionine and cystine have been excluded. When animals are fed on the basal diet alone, their growth is arrested and they develop various deficiency symptoms. Growth is restored when the diet is suitably supplemented, as, for example, with methionine and cystine together, or with methionine alone. Growth experiments have also been used in the study of compounds which compete with normal, sulphur-containing metabolites, or which deprive an animal of a particular sulphur compound by combining with it. Ethionine and oxythiamine are examples of the former type of compound, and they bring about growth inhibition as a result of being metabolic antagonists of methionine and thiamine respectively. Bromobenzene is an example of the latter type of compound, for by being excreted in conjugation with cysteine it induces a deficiency of sulphur-containing amino acids.

Studies have also been made of the effect of sulphur compounds on the growth of micro-organisms, and an interesting outcome

of this has been the development of methods for the microbiological assay of methionine, cystine, biotin and thiamine. The substitution of normal sulphur-containing nutrients by compounds believed to be metabolic intermediates has been investigated, and an example of this is provided by the substitution of sulphite for sulphate in the media of bacteria which reduce sulphate [40]. Another approach has made use of artificially-induced mutants which have been rendered more exacting in their nutritive requirements as a result of their inability to perform certain metabolic processes (see Chapter IX).

Inorganic sulphate is the chief end-product of sulphur metabolism in animals, and numerous quantitative studies of its excretion in urine have been made. As a result of the work of Baumann [41, 42, 43] it became recognized that sulphuric acid is excreted in combination with organic compounds as well as in the free form. These two forms do not account for all the sulphur of the urine, however, and it is now customary to refer to the occurrence of three sulphur fractions in urine, inorganic sulphate, ethereal (or ester) sulphate, and neutral sulphur. In order to establish the magnitude of these fractions it is necessary to determine the total sulphur content of the urine, the total sulphate (i.e. inorganic sulphate together with all sulphate liberated by acid hydrolysis) and the inorganic sulphate content. The individual fractions are calculated from these values as follows:

$$\text{Neutral-S} = \text{Total-S} - \text{Total sulphate-S}$$

$$\text{Ethereal sulphate-S} = \text{Total sulphate-S} - \text{Inorganic sulphate-S}$$

There is still some uncertainty concerning the identity of the compounds which comprise the neutral sulphur fraction of the urine. It is generally accepted that the sulphur-containing amino acids, thiols, taurine, thiocyanate and thiosulphate are present. The ethereal sulphate fraction includes sulphate excreted in conjugation with hydroxy compounds such as steroids and phenols, including tyrosine. Inorganic sulphate makes up the major sulphur fraction of normal urine, and as a normal diet usually contains little inorganic sulphur, most of the inorganic sulphate of the urine is derived from the oxidation of the sulphur-containing amino acids.

Much of the early work on the metabolism of sulphur compounds was carried out by determining the amount of sulphur

excreted in urine under various conditions and its distribution between the three sulphur fractions. By this means it has been possible to gain some indication of whether an inorganic or organic sulphur compound is oxidized to sulphate or whether it is excreted unchanged. In a number of such investigations, however, insufficient attention was given to certain important experimental details. The food intake and sulphur content of the diet of experimental animals were not always carefully controlled, and the possibility that a compound administered by mouth may be acted upon by intestinal micro-organisms before absorption was sometimes overlooked. Furthermore, it was generally accepted that any 'extra' sulphur which appeared in the urine was derived entirely from the sulphur compound administered, an assumption which was not necessarily justified. Fortunately, some of these difficulties can now be avoided by the use of ^{35}S -labelled compounds.

One of the end-products of sulphur metabolism, taurine, is excreted in the bile in conjugation with bile acids, and use has been made of this fact in the study of taurine formation. Bergmann [44], using dogs with biliary fistulae, found that the taurocholic acid content of the bile was increased after administering sodium cholate. By continuing this treatment, however, the amount of taurine available was depleted to the point where taurocholic acid formation began to diminish and the compound eventually almost ceased to be present in the bile. When cystine was administered to the animals the amount of taurocholic acid in the bile was restored, a finding which suggested that cystine is a precursor of taurine. This technique was subsequently developed and used by Virtue and Doster-Virtue [45].

Many of the processes of sulphur metabolism have been studied with the aid of tissue slices, minces and suspensions, and a number of the enzymes concerned have been separated and their properties investigated. In some instances perfused organs have been employed. The reactions brought about by various tissues and enzymes have been followed by chemical analysis of the reaction products or by manometric techniques. The first worker to employ tissue slices successfully in the investigation of the metabolism of sulphur compounds was Pirie [46], who used them in his work on the oxidation of cystine and methionine to sulphate. In these experiments Pirie separated inorganic sulphate

as the insoluble benzidine salt and determined the benzidine in the precipitate colorimetrically. Essentially the same procedure has since been used to study the oxidation of other sulphur compounds by tissues *in vitro* [47, 48]. Measurements have also been made of the uptake of oxygen by tissues in the presence of sulphur-containing substrates [47, 49]. The conclusions reached have not always been convincing, however, because of uncertainty concerning the identity of the products formed. Reactions in which there is acid formation, such as those brought about by sulphatases [50], transmethylases [51] and penicillinase [52], have been carried out in bicarbonate media and the carbon dioxide evolved has been measured by manometric methods. Other enzymic reactions have been followed by the chemical determination of the products, such as hydrogen sulphide liberated from cysteine by desulphydrases, and phenols set free from arylsulphuric acids by arylsulphatases.

Of the isotopes of sulphur, only ^{35}S has been used extensively in tracer studies. This isotope is an emitter of β -particles and has a half-life of 87.1 days. Its radiation, like that of ^{14}C , has a low energy (0.167 Mev), and for the accurate measurement of ^{35}S in biological material [53, 54, 55] it is usually necessary to separate the radioactive sulphur in the form of a solid compound. The procedure commonly followed is to oxidize the sulphur to sulphuric acid, and then to separate this as an insoluble salt, the radioactivity of which is measured by a suitable means, such as the use of a counter with a thin-window Geiger-Müller tube. Although the sulphur has been oxidized in various ways [53, 54, 56, 57, 58, 59] the methods which have been used for precipitating the sulphuric acid have been limited to its conversion to the barium salt or the benzidine salt. Barium sulphate offers the advantage that the conditions for its quantitative precipitation are less critical than those for the benzidine salt. On the other hand, the physical character of the barium sulphate precipitate renders it less satisfactory than benzidine sulphate for collection and for the measurement of its ^{35}S content. It is essential that the precipitation of benzidine sulphate be carried out at about pH 3 [60], and in situations where ^{35}S -labelled compounds are present in addition to radioactive sulphate, care needs to be taken to ensure that they also are not precipitated by benzidine [61].

Methods have been developed for labelling with ^{35}S various compounds of biological importance, and the chemical procedures employed have been reviewed by Tarver [62]. For the preparation of some ^{35}S -labelled compounds biosynthetic methods have proved especially useful. For example, both L- ^{35}S]cystine and L- ^{35}S]methionine have been obtained by the hydrolysis of the proteins of yeast grown on a medium containing sodium ^{35}S]sulphate [63]. Penicillin labelled with ^{35}S has also been prepared by biological methods [64, 65]. The simultaneous administration of ^{35}S]cystine and a suitable aromatic compound (e.g. bromobenzene) to animals permits the isolation from the urine of a mercapturic acid labelled with ^{35}S [66], and radioactive mercapturic acids prepared in this way have themselves been used for metabolic studies [67]. The development of autoradiographic techniques suitable for use with ^{35}S has made possible the study of the distribution of this isotope in tissue sections and other biological materials [68, 69, 70].

In the study of sulphur metabolism, as in so many other fields, ion exchange methods and paper chromatography have proved to be of great value. Methods have been developed for the separation of sulphur-containing amino acids by means of paper chromatography [71, 72]. Paper chromatographic procedures have also been used in conjunction with ^{35}S -labelled compounds, and the chromatograms have been scanned for ^{35}S by means of suitable counting devices [73, 74, 75]. Autoradiographic methods have also been employed to establish the positions of ^{35}S -labelled compounds on chromatograms [69, 76].

This brief account, though by no means complete, serves to indicate the diversity of the methods used in the study of the metabolism of sulphur compounds.

REFERENCES

1. BUNKER, H. J. (1936). *A Review of the Physiology and Biochemistry of the Sulphur Bacteria*. London: H.M.S.O.
2. BARRON, E. S. G. (1951). *Advanc. Enzymol.* **11**, 201.
3. DU VIGNEAUD, V. (1942-43). *Harvey Lect.* **38**, 39.
4. WRIGHT, L. D., CRESSON, E. L., SKEGGS, H. R., WOOD, T. R., PECK, R. L., WOLF, D. E. and FOLKERS, K. (1952). *J. Amer. chem. Soc.* **74**, 1996.

5. LARDY, H. A. and PEANASKY, R. (1953). *Physiol. Rev.* 33, 560.
6. REED, L. J. (1953). *Physiol. Rev.* 33, 544.
7. LARSON, B. L. and SALISBURY, G. W. (1953). *J. biol. Chem.* 201, 601.
8. CHALLENGER, F. and GREENWOOD, D. (1949). *Biochem. J.* 44, 87.
9. JANSEN, E. F. (1948). *J. biol. Chem.* 176, 657.
10. BIRKINSHAW, J. H., FINDLAY, W. P. K. and WEBB, R. A. (1942). *Biochem. J.* 36, 526.
11. MELVILLE, D. B., EICH, S. and LUDWIG, M. L. (1956). *Fed. Proc.* 15, 314.
12. THOMPSON, J. F., MORRIS, C. J. and ZACHARIUS, R. M. (1956). *Nature, Lond.* 178, 593.
13. VAN VEEN, A. G. and HYMAN, A. J. (1935). *Rec. Trav. chim. Pays-Bas*, 54, 493.
14. ALDERTON, G. (1953). *J. Amer. chem. Soc.* 75, 2391.
15. NEWTON, G. G. F., ABRAHAM, E. P. and BERRIDGE, N. J. (1953). *Nature, Lond.* 171, 606.
16. WESTALL, R. G. (1953). *Biochem. J.* 55, 244.
17. HOROWITZ, N. H. (1947). *J. biol. Chem.* 171, 255.
18. SUZUKI, U., ODAKE, S. and MORI, T. (1924). *Biochem. Z.* 154, 288.
19. HAAS, P. (1935). *Biochem. J.* 29, 1297.
20. AKABORI, S. and KANEKO, T. (1936). *Proc. imp. Acad. Japan*, 12, 131.
21. HAAGEN-SMIT, A. J., KIRCHNER, J. G., DEASY, C. L. and PRATER, A. N. (1945). *J. Amer. chem. Soc.* 67, 1651.
22. CHALLENGER, F. and SIMPSON, M. I. (1948). *J. chem. Soc.* p. 1591.
23. McRORIE, R. A., SUTHERLAND, G. L., LEWIS, M. S., BARTON, A. D., GLAZENER, M. R. and SHIVE, W. (1954). *J. Amer. chem. Soc.* 76, 115.
24. CHALLENGER, F. and HAYWARD, B. J. (1954). *Chem. & Ind.* p. 729.
25. STOLL, A. and SEEBECK, E. (1948). *Helv. chim. acta*, 31, 189.
26. SYNGE, R. L. M. and WOOD, J. C. (1955). *Biochem. J.* 60, xv.
27. MORRIS, C. J. and THOMPSON, J. F. (1956). *J. Amer. chem. Soc.* 78, 1605.

28. SCHMID, H. and KARRER, P. (1948). *Helv. chim. acta*, **31**, 1017, 1087, 1497.
29. DENT, C. E. (1947). *Science*, **105**, 335.
30. ETTLINGER, M. G. and LUNDEEN, A. J. (1956). *J. Amer. chem. Soc.* **78**, 4172.
31. KJAER, A., GMELIN, R. and JENSEN, R. B. (1956). *Acta chem. scand.* **10**, 26.
32. KOECHLIN, B. A. (1954). *Proc. nat. Acad. Sci., Wash.* **40**, 60.
33. BERSIN, T. (1950). *Advanc. Enzymol.* **10**, 223.
34. BERGERET, B. and CHATAGNER, F. (1954). *Biochim. biophys. Acta*, **14**, 297.
35. BETTELHEIM, F. R. (1954). *J. Amer. chem. Soc.* **76**, 2838.
36. WOOLLEY, D. W. and PETERSON, W. H. (1937-38). *J. biol. Chem.* **122**, 213.
37. BINKLEY, S. B. (1955). *Annu. Rev. Biochem.* **24**, 597.
38. UMEZAWA, H., OIKAWA, K., OKAMI, Y. and MAEDA, K. (1953). *J. Bact.* **66**, 118.
39. OSBORNE, T. B. and MENDEL, L. B. (1915). *J. biol. Chem.* **20**, 351.
40. POSTGATE, J. R. (1951). *J. gen. Microbiol.* **5**, 725.
41. BAUMANN, E. (1876). *Pflüg. Arch. ges. Physiol.* **12**, 63, 69.
42. BAUMANN, E. (1876). *Pflüg. Arch. ges. Physiol.* **13**, 285.
43. BAUMANN, E. (1878-79). *Hoppe-Seyl. Z.* **2**, 335.
44. VON BERGMANN, G. (1904). *Beitr. chem. Physiol. Path.* **4**, 192.
45. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1937). *J. biol. Chem.* **119**, 697.
46. PIRIE, N. W. (1934). *Biochem. J.* **28**, 305.
47. MEDES, G. and FLOYD, N. (1942). *Biochem. J.* **36**, 259.
48. MAW, G. A. (1954). *Biochem. J.* **58**, 665.
49. BERNHEIM, F. and BERNHEIM, M. L. C. (1939). *J. biol. Chem.* **127**, 695.
50. LOWENSTEIN, J. M. and YOUNG, L. (1952). *Biochem. J.* **52**, xxv.
51. MAW, G. A. (1955). *Biochem. J.* **60**, xxxii.
52. HENRY, R. J. and HOUSEWRIGHT, R. D. (1947). *J. biol. Chem.* **167**, 559.
53. HENRIQUES, F. C. jun., KISTIAKOWSKY, G. B., MARGNETTI, C. and SCHNEIDER, W. G. (1946). *Industr. Engng Chem. (Anal.)*, **18**, 349.

54. YOUNG, L., EDSON, M. and McCARTER, J. A. (1949). *Biochem. J.* **44**, 179.
55. ELDJARN, L. and NYGAARD, O. (1954). *Scand. J. clin. Lab. Invest.* **6**, 160.
56. TARVER, H. and SCHMIDT, C. L. A. (1939). *J. biol. Chem.* **130**, 67.
57. SELIGMAN, A. M. and FINE, J. (1943). *J. clin. Invest.* **22**, 265.
58. DZIEWIATKOWSKI, D. D. (1945). *J. biol. Chem.* **161**, 723.
59. BOURSNELL, J. C., FRANCIS, G. E. and WORMALL, A. (1946). *Biochem. J.* **40**, 743.
60. OWEN, E. C. (1936). *Biochem. J.* **30**, 352.
61. HAWKINS, J. B. and YOUNG, L. (1954). *Biochem. J.* **56**, 166.
62. TARVER, H. (1951). *Advanc. biol. med. Phys.* **2**, 281.
63. WILLIAMS, R. B. and DAWSON, R. M. C. (1952). *Biochem. J.* **52**, 314.
64. HOWELL, S. F., THAYER, J. D. and LABAW, L. W. (1948). *Science*, **107**, 299.
65. ROWLEY, D., MILLER, J., ROWLANDS, S. and LESTER-SMITH, E. (1948). *Nature, Lond.* **161**, 1009.
66. GUTMANN, H. R. and WOOD, J. L. (1950). *Cancer Res.* **10**, 8.
67. MARSDEN, C. M. and YOUNG, L. Unpublished work.
68. HOWARD, A. and PELC, S. R. (1951). *Nature, Lond.* **167**, 599.
69. DZIEWIATKOWSKI, D. D. (1952). *Trans. Fourth Conference, Josiah Macy Jr. Foundation*, p. 74. Oklahoma City: The Foundation.
70. DAVIES, D. V. and YOUNG, L. (1954). *J. Anat., Lond.* **88**, 174.
71. TOENNIES, G. and KOLB, J. J. (1951). *Analyt. Chem.* **23**, 823.
72. PASIEKA, A. E. and MORGAN, J. F. (1955). *Biochim. biophys. Acta*, **18**, 236.
73. LOWENSTEIN, J. M. (1953). Ph.D. Thesis, University of London.
74. EDWARDS, L. J. (1954). *Biochem. J.* **57**, 542.
75. CORNER, E. D. S. and YOUNG, L. (1955). *Biochem. J.* **61**, 132.
76. AWAPARA, J. and WINGO, W. J. (1953). *J. biol. Chem.* **203**, 189.

CHAPTER II

Thiols and Disulphides

CYSTEINE AND CYSTINE

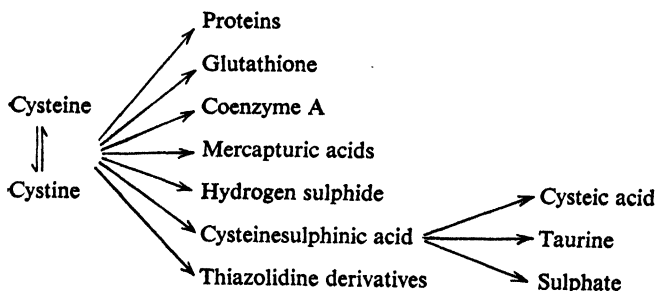
Cystine was the first amino acid to be discovered. Although it was isolated from a urinary calculus by Wollaston in 1810 [1], almost a century elapsed before its structure was established by Friedmann [2, 3] and its occurrence in proteins was demonstrated by Mörner [4]. Methionine was not discovered until 1922 [5, 6] and it was only after this event that there began to emerge a real understanding of the nutritional significance of cysteine and cystine and their place in the metabolism of sulphur compounds.

It is generally accepted that cysteine and cystine are interconvertible in the living organism and that in a nutritional sense they can replace each other. In higher animals, the dietary need for these amino acids is bound up with the intake of methionine, for methionine will maintain the growth of young rats in the absence of dietary cystine [7, 8] whereas cystine cannot promote growth in the absence of methionine. As a result of these and other findings it has become apparent that in higher animals methionine-sulphur can become the sulphur of cysteine and cystine, but that the reverse process does not occur (see Chapter III). Some micro-organisms, however, can use cystine-sulphur for the synthesis of methionine (see Chapter IX).

Cysteine and cystine occur as components of proteins and therefore of enzymes and certain hormones. The activity of a number of enzymes is dependent on the integrity of the sulphydryl groups of their constituent cysteine residues, and the inhibitory action of agents such as arsenic compounds and iodoacetic acid is an outcome of their ability to combine with the sulphydryl groups of the enzymes. On the other hand, for the full activity of certain biologically important compounds such as insulin, thrombin and oxytocin, the disulphide linkages within their

molecules must be intact. The formation of disulphide linkages between cysteine residues in separate polypeptide chains makes an important contribution to the maintenance of the configuration of certain proteins. This is especially so in fibrous proteins, such as the keratins, which may contain over 12% of cysteine.

In addition to being incorporated into proteins, cysteine and cystine undergo a number of other metabolic reactions. These are summarized below and are treated in a detailed manner later in the present chapter.



Various types of organism have been shown to use cysteine in the synthesis of glutathione, a tripeptide which has an important effect on the redox conditions prevailing in cellular systems, as well as having other functions. Cysteine is used in the synthesis of coenzyme A and it would appear that in higher animals no other compound can substitute for cysteine in this process. Coenzyme A plays a key role in intermediary metabolism and its activity is related to the ability of its sulphydryl group to react with acyl compounds to form thiol esters. A further metabolic process in which cysteine participates is that whereby certain foreign cyclic compounds give rise to mercapturic acids, i.e. S-substituted derivatives of *N*-acetylcysteine.

Cysteine undergoes desulphuration, and this leads to the formation of hydrogen sulphide and other products which may include pyruvic acid and ammonia [9, 10]. It is now generally agreed that cysteinesulphinic acid is an important intermediary in the oxidative metabolism of cysteine. Cysteinesulphinic acid is converted *in vivo* to β -aminoethanesulphinic acid [11] and to cysteic acid [12], both of which serve as precursors of taurine. The chief

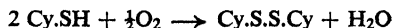
end-product of cysteine oxidation is sulphate, and evidence has been obtained that cysteinesulphinic acid is converted to alanine and sulphite [13] and that the latter compound is oxidized to sulphate. The formation of sulphate is an important aspect of sulphur metabolism inasmuch as sulphate is required for the production of sulphomucopolysaccharides as well as sulphuric acid esters of phenolic compounds and certain steroids.

When cystine reacts with cyanide, one of the products formed is 2-iminothiazolidine-4-carboxylic acid [14]. This compound has been isolated from the urine of rats dosed subcutaneously with sodium cyanide [15], a finding which may explain the protective effect of cystine against cyanide poisoning in animals [16]. Recently a mitochondrial system has been shown to bring about the condensation of cysteine and formaldehyde to yield thiazolidine-4-carboxylic acid, and further enzymic action leads to the formation of *N*-formylcystine. An interesting feature of these reactions is that the formaldehyde-carbon is transferred from the sulphur to the amino group of cysteine [17].

Interconversion of cysteine and cystine

The ready interconversion of cysteine and cystine which can be brought about by purely chemical means is believed to have its parallel in living systems. In spite of the importance of the thiol-disulphide relationship in biochemical processes, however, surprisingly little work has been carried out on the enzymic aspects of the interchange.

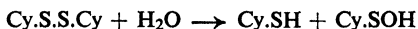
There seems no doubt that oxidation of cysteine (Cy.SH) to cystine (Cy.S.S.Cy) takes place *in vivo*.



This reaction can be catalysed by traces of metal ions such as Fe^{3+} , Cu^{2+} and Mn^{2+} , and Keilin [18] has shown that it can be accelerated by cytochrome *c* and cytochrome oxidase acting together but not separately. The action of this system is inhibited by agents such as cyanide which are inhibitors of cytochrome oxidase. The oxidation of cysteine to cystine by rat-liver slices and by liver suspensions has been demonstrated by Medes [19]. Cysteine reacts with elementary sulphur to form cystine and hydrogen sulphide, and from the observations of Meister and his

coworkers [20] it would appear that this reaction has a metabolic significance.

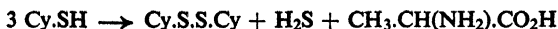
There is good evidence [21] to show that enzymic reduction of cystine to cysteine takes place in certain yeasts (see Chapter IX). It is generally accepted that this reduction also occurs in higher animals, and some evidence in support of this belief was obtained by Lewis, Updegraff and McGinty [22], who observed that when phenyluraminocystine or dibenzoylcystine was administered to rabbits orally or by injection, about half of the compound was excreted in the urine as the cysteine derivative. Furthermore, in his experiments on sulphur metabolism in tissue slices, Pirie [23] found that sulphate formation from cystine ceased when the system no longer gave a positive nitroprusside reaction, and he expressed the opinion that any oxidation of cystine to sulphate which occurred in his experiments took place after the cystine had been reduced to cysteine. One suggestion put forward by Pirie [24] was that formation of cysteine occurs as a result of a hydrolytic dismutation of cystine:



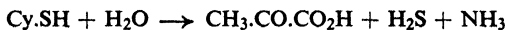
Support for this possibility has come from chemical experiments by other investigators [25, 26, 27].

Desulphuration of cysteine and cystine

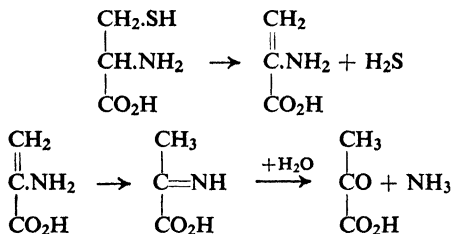
The enzymic nature of the process whereby micro-organisms liberate hydrogen sulphide from cysteine and cystine was first demonstrated by Tarr [28, 29], who obtained active cell-free preparations from *Proteus vulgaris*. The occurrence, in mammalian tissues, of a system which brings about the production of hydrogen sulphide from cysteine was described by Fromageot, Wookey and Chaix [9] and by Smythe [10]. This reaction was attributed to an enzyme which was described as a desulphurase by Fromageot [9] and as a desulphydrase by Smythe [30]. The enzyme system appears to be of general occurrence among higher animals, where it is localized mainly in the liver, although it is also present in lesser amounts in kidney and pancreas [31]. Fromageot and his coworkers [9] concluded that the overall reaction which they observed was as follows:



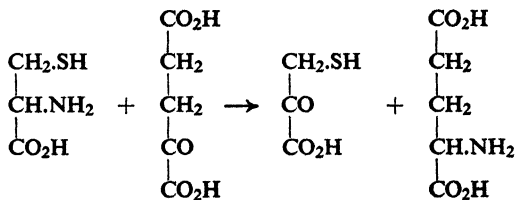
Smythe [10], however, obtained results which indicated the occurrence of the following reaction:



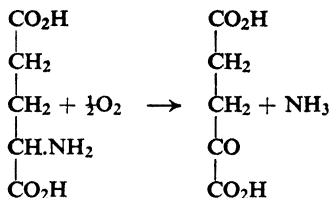
This led to the suggestion that a desulphydrating enzyme catalyses the conversion of cysteine to α -aminoacrylic acid and hydrogen sulphide, and that the α -aminoacrylic acid is converted to pyruvic acid and ammonia with the intermediate formation of an imino acid:



This presupposes that liberation of hydrogen sulphide is the first stage in the process. There is now evidence, however, that this might not be so, for it has been reported that the addition of arsenate prevents the production of hydrogen sulphide, but not the formation of ammonia [32]. As an alternative mechanism, ammonia might first be removed with the formation of β -mercaptopyruvic acid which subsequently gives rise to pyruvic acid and hydrogen sulphide [10]. Although oxidative deamination of cysteine does not occur readily in higher animals, it is known that transamination can take place between cysteine and α -ketoglutaric acid [33]. It is probable that the occurrence of this transaminative reaction in liver gives rise to β -mercaptopyruvic acid and glutamic acid, and that the latter compound then undergoes oxidative deamination with the liberation of ammonia:



THIOLS AND DISULPHIDES



Support for the occurrence of a transaminative step in the desulphuration of cysteine can be derived from the fact that pyridoxal phosphate acts as a coenzyme in the desulphurating system of liver. Compared with preparations obtained from normal animals, those from the livers of rats deficient in vitamin B₆ have a reduced ability to produce hydrogen sulphide from cysteine [34]. Furthermore, the desulphurating activity of purified enzyme preparations is lost when they are dialysed, and is restored by the addition of pyridoxal phosphate and inorganic phosphate [32]. Pyridoxal phosphate appears to be without effect on the desulphuration of β -mercaptopyruvic acid [20], and it would appear, therefore, that its effect is exercised in the transaminative phase of the process.

A new insight into the breakdown of cysteine has been provided by the work of Meister, Fraser and Tice [20]. In experiments in which β -mercaptopyruvic acid and glutamine were incubated with a liver glutaminase preparation, these workers observed the formation of alanine rather than cysteine or cystine. As the system they were using converted neither cysteine nor cystine to alanine, it appeared that β -mercaptopyruvic acid was giving rise to pyruvic acid which was then converted to alanine by transamination. Pyruvic acid was, in fact, shown to be formed when β -mercaptopyruvic acid was incubated with preparations from rat tissues and from certain micro-organisms. In a large-scale experiment with a liver preparation the important observation was made that elementary sulphur separated in amounts stoichiometrically equivalent to the pyruvic acid formed. When the reaction was carried out in the presence of a suitable reducing agent, such as β -mercaptoethanol, there was formation of stoichiometric amounts of pyruvic acid and hydrogen sulphide. The preparations used in these experiments did not act on cysteine or cystine, but this could have been due to the absence of enzymes which convert cysteine to β -mercaptopyruvic acid [35].

As a result of these various findings it becomes possible to envisage the occurrence of the following reactions during the desulphuration of cysteine in higher animals:

- (1) Cysteine + α -ketoglutaric acid $\rightarrow$
 β -mercaptopyruvic acid + glutamic acid
- (2) β -Mercaptopyruvic acid $\rightarrow$ sulphur + pyruvic acid
- (3) Sulphur + hydrogen donor $\rightarrow$
hydrogen sulphide + oxidized donor

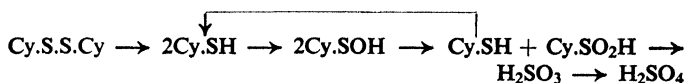
This scheme explains how different desulphurating preparations might yield the various products which have been reported. Reaction (1) requires pyridoxal phosphate, and in the presence of the appropriate deaminating system the glutamic acid formed would give rise to ammonia. The pyruvic acid formed in reaction (2) might undergo transamination with the formation of alanine, and if cysteine were to act as the reducing agent in reaction (3), formation of cystine would take place, as has been observed by Fromageot and his coworkers [36]. It would now appear that there are two mechanisms for the desulphuration of cysteine. In one of these there is direct formation of hydrogen sulphide, whereas in the other elementary sulphur is first formed. The relation between these two processes has yet to be clarified.

Evidence has been obtained for the occurrence of desulphurating systems which act on substrates other than cysteine. Fromageot and Desnuelle [37] demonstrated the presence, in the tissues of higher animals, of a system, distinct from cysteine desulphhydrase, which acts on homocysteine with the liberation of hydrogen sulphide. Another enzyme system, exocystine desulphhydrase, which brings about hydrogen sulphide formation during the breakdown of peptides and other derivatives of cystine, has been shown by Greenstein and Leuthardt [38] to occur in rat liver.

Oxidation of cysteine and cystine to sulphate

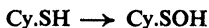
Cysteine, cystine [39, 40] and methionine [41, 42] have all been shown to give rise to sulphate in intact animals. The oxidation of these amino acids to sulphate by tissue preparations was not demonstrated until 1934 when Pirie [23] showed that they can be oxidized by slices of rat liver and kidney. Pirie's findings led

him to propose a scheme for the oxidation of cystine and cysteine which may be represented as follows:

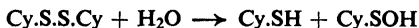


An important feature of this scheme was the introduction of the possibility that cysteinesulphinic acid is an intermediary compound, and as will be shown later, this has been borne out by subsequent work.

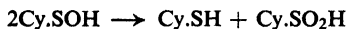
It has been suggested that formation of cysteinesulphinic acid, Cy.SOH , is the first step in the oxidation [23, 24, 43]:



and that this sulphinic acid is also formed by the hydrolytic cleavage of cystine [23, 44]:



Cysteinesulphinic acid is unstable and under physiological conditions it would be expected to break down to cysteine and cysteinesulphonic acid [23, 24, 45]:

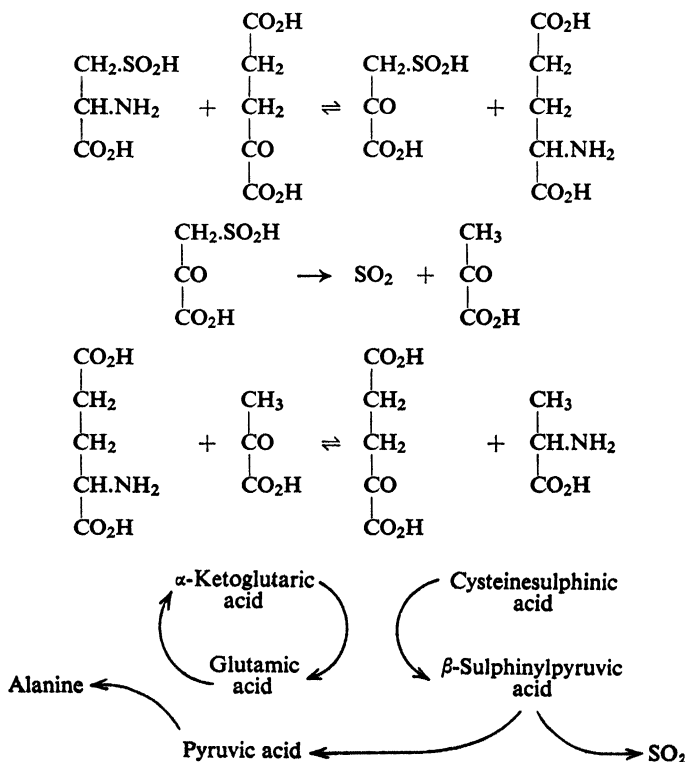


The role of cysteinesulphinic acid as an intermediate in the oxidation of cysteine is supported by the readiness with which it is oxidized by various types of tissue preparation [13, 19, 43]. Furthermore, it has been shown to be oxidized to sulphate in normal human subjects [40] and in animals [46]. Its presence in the animal body was demonstrated in 1954, when Bergeret and Chatagner [47] showed it to be present in the brain of the rat.

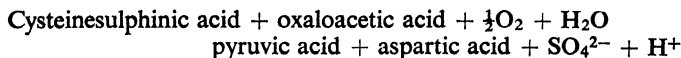
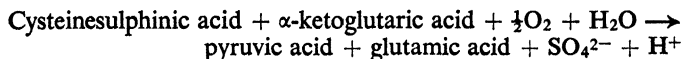
The findings of various workers in recent years have provided abundant evidence in favour of Pirie's [23] suggestion that cysteinesulphinic acid is metabolized to sulphite which then undergoes oxidation to sulphate, and this has come to be regarded as a main pathway of sulphate formation in the organism [48]. It is also well established that sulphite is oxidized to sulphate *in vivo* [49], although only recently has evidence been put forward [50] that this is an enzymic process (see Chapter VIII).

Evidence that cysteinesulphinic acid breaks down to sulphite was obtained by Fromageot, Chatagner and Bergeret [13] from

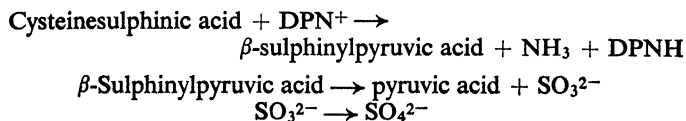
experiments in which they incubated the sulphinic acid with liver preparations under anaerobic conditions. They gave the name 'desulphinicase' to the enzyme system responsible for the production of the sulphite and they showed that formation of alanine also took place. Purified preparations of the enzyme system were found to require a dialysable factor which was subsequently identified as α -ketoglutaric acid [51], and this pointed to the occurrence of a transaminative step in the process. The various findings can be represented by the following set of reactions, and evidence has been obtained that these take place in the intact rat [52].



Further information about the metabolism of cysteinesulphinic acid has been obtained by Singer and Kearney [53]. Using acetone-dried powders of mitochondria from animal tissues they have shown that the oxidation is initiated by a transamination with α -ketoglutaric acid or oxaloacetic acid, and that the following overall reactions occur:



Singer and Kearney also showed that the oxidation of cysteinesulphinic acid by these mitochondrial preparations took place in the presence of a suitable dye and that diphosphopyridine nucleotide (DPN) participated in the process.



β -Sulphinylpyruvic acid is known to undergo enzymic oxidation to β -sulphonylpyruvic acid, but this latter compound does not appear to be an intermediate in sulphate formation from cysteinesulphinic acid.

It is interesting to note that Chapeville and P. Fromageot [54] have been able to bring about the synthesis of [^{35}S]cysteinesulphinic acid by incubating [^{35}S]sulphite, pyruvic acid and glutamic acid under anaerobic conditions with an acetone-dried powder prepared from rabbit kidney. It is reasonable to suppose that the first step in this process is the formation of β -sulphinylpyruvic acid from pyruvic acid and sulphur dioxide, and as β -sulphinylpyruvic acid is a sulphinic acid analogue of oxaloacetic acid, this reaction can be considered as a counterpart of the Wood-Werkman reaction.

Mention should also be made of thiosulphate as a possible end-product of the oxidation of cysteine and cystine. Its occurrence in small amounts in urine has been discussed by Fromageot and Royer [55]. Whether it is formed enzymically from other inor-

ganic forms of sulphur or whether it is a normal intermediate in organic sulphur metabolism has yet to be established.

Conversion of cysteine and cystine to taurine

The metabolism of taurine is considered in detail in Chapter V, and is therefore reviewed only briefly here.

It has long been accepted that cysteine and cystine undergo conversion to taurine in the animal body, and it has been shown that the intravenous administration of [^{35}S]cysteine to a rat is followed by the appearance of [^{35}S]taurine in the liver [56]. Studies of the steps in this process have led to the formulation of a number of hypotheses, the chief of which are as follows:

- (1) Cysteine $\rightarrow$ cysteinesulphinic acid $\rightarrow$ cysteic acid $\rightarrow$ taurine
- (2) Cystine $\rightarrow$ cystine disulphoxide $\rightarrow$
cystamine disulphoxide $\rightarrow$ taurine
- (3) Cysteine $\rightarrow$ cysteinesulphinic acid $\rightarrow$
 β -aminoethanesulphinic acid $\rightarrow$ taurine

As is shown in Chapter V, the current view is in favour of (3), with (1) as an alternative but quantitatively less important pathway.

Taurine occurs in the free form in various animal species, and as there is no indication that it undergoes any metabolic change other than conjugation with bile acids, it is generally regarded as an end-product of sulphur metabolism.

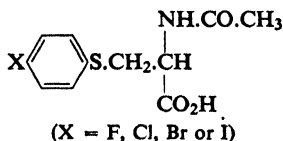
Nitrogen metabolism of cysteine and cystine

The little information which is available concerning the oxidative deamination of cysteine and cystine suggests that this does not occur readily in the animal body. Green and his coworkers [57, 58] observed that the L-amino acid oxidase of rat kidney acts only slowly on cystine and it is doubtful whether this enzyme has any action on cysteine. In the presence of α -ketoglutarate, however, cysteine serves as a substrate for a transaminase present in heart muscle and in liver [59]. β -Mercaptopyruvic acid, the α -keto acid derived from cysteine, has not been detected in body fluids, but as already mentioned (see p. 28), many tissues of the rat contain an active system which converts this compound to pyruvic acid. Experiments by Arnstein and Crawhall [60] on the metabolism of DL-[β - ^{14}C]cystine and DL-[^{35}S]cystine by the rat

do in fact point to pyruvic acid as an important intermediate in cystine metabolism. If cysteine and cystine are metabolized in this way, they might be expected to have a gluconeogenic action. The results obtained have been conflicting, however, for whereas some workers have observed no glucogenic effect with these compounds [61, 62], others have reported the opposite result [63, 64].

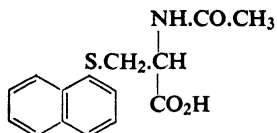
Mercapturic acids

The first mercapturic acid to be discovered was *p*-bromophenylmercapturic acid, i.e. *N*-acetyl-*S*-(*p*-bromophenyl)-*L*-cysteine. In 1879 Baumann and Preusse [65] described the separation of this compound from the urine of dogs to which they had administered bromobenzene, and very shortly afterwards Jaffé [66] reported the isolation of *p*-chlorophenylmercapturic acid from the urine of dogs dosed with chlorobenzene. These workers established that the urine of the dosed animals contained acid-labile compounds which broke down to give the mercapturic acids, and they considered that these precursors were glucuronic acid derivatives. The isolation of *p*-iodophenylmercapturic acid from the urine of a dog which had been given iodobenzene was described by Baumann and Schmitz [67] in 1895, and more recently *p*-fluorophenylmercapturic acid was isolated by Young and Zbarsky [68] from the urine of rats dosed with fluorobenzene. The mercapturic acids derived from the monohalogenobenzenes have the following structure:



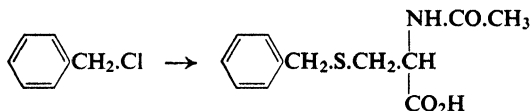
Evidence has also been obtained for the presence of mercapturic acids in the urine of animals dosed with certain di- and trichlorobenzenes [69, 70, 71].

Mercapturic acids have been separated from urine excreted by animals following the administration of unsubstituted cyclic hydrocarbons. The first such compound to be isolated was 1-naphthylmercapturic acid:

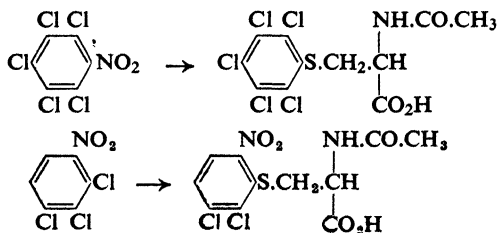


This was obtained in 1934 by Bourne and Young [72] from the urine of rabbits which had been dosed with naphthalene. Subsequently Boyland and Levi [73] separated 1-anthrylmercapturic acid from the urine of rabbits and rats to which anthracene had been administered, and Zbarsky and Young [74] isolated phenylmercapturic acid from the urine of rats dosed with benzene. There has been some speculation concerning the possibility that carcinogenic hydrocarbons give rise *in vivo* to mercapturic acids. This has not been demonstrated, however, and there is chemical evidence which indicates that cysteine derivatives of the higher polycyclic hydrocarbons are unstable [75].

As was first shown by Stekol, benzyl chloride [76] and *p*-bromobenzyl bromide [77] give rise to mercapturic acids in the animal body. This type of reaction is of interest, inasmuch as conjugation occurs with the displacement of halogen:

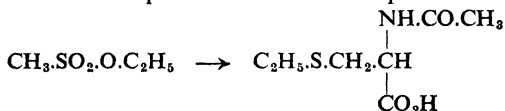


Thorpe and his co-workers have studied the metabolism of all 19 members of the chloromononitrobenzene series in the rabbit. They have found that mercapturic acids are formed by most members of the series and that this occurs either by replacement of the nitro group or chlorine by an acetylcysteyl group, for example:



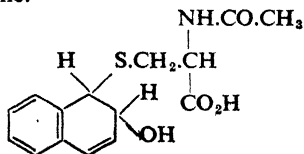
The results of these extensive investigations have been summarized and discussed by Bray, James and Thorpe [78].

Among the compounds reported to give rise to mercapturic acids in the animal body, one of the most interesting is the cytotoxic alkylating agent, ethyl methanesulphonate. Roberts and Warwick [79] were able to show the presence of ethylmercapturic acid in the urine of rats to which they had administered this ester, and thereby provided the first demonstration of the metabolic formation of a mercapturic acid from an aliphatic compound:



Various *S*-arylcysteines, for example *S*-phenylcysteine [80] and *S*-(*p*-bromophenyl)-cysteine [81], have been found to undergo acetylation *in vivo* to give mercapturic acids, and evidence has been obtained that this type of reaction occurs in liver and kidney [82]. Benzylmercapturic acid is formed in the rat from *S*-benzylcysteine [76], *S*-benzylglutathione [83] and *S*-benzyl- β -mercaptopyruvic acid [84], whereas the α -hydroxy analogue of the last of these compounds appears to be excreted unchanged [85].

A number of the observations of Baumann and Preusse and of Jaffé on the excretion of acid-labile precursors of mercapturic acids have recently been confirmed and extended by Boyland, Sims and Solomon [86] and by Knight and Young [87]. The name 'premercapturic acids' has been applied to these compounds by Knight and Young [87], who have obtained evidence of their formation in the rat from benzene, naphthalene, anthracene and the four monohalogenobenzenes. The premercapturic acid which yields 1-naphthylmercapturic acid when decomposed by acid has been isolated by Boyland, Sims and Solomon [86] from the urine of rabbits dosed with naphthalene, and it has been identified provisionally as *N*-acetyl-*S*-(2-hydroxy-1:2-dihydro-1-naphthyl)-L-cysteine:



It would seem that not all the mercapturic acids isolated from urine are derived from the breakdown of premercapturic acids, for studies of the metabolism of benzyl chloride have given no evidence of the formation of a premercapturic acid from this compound [87].

Sherwin and his coworkers [88, 89, 90] were of the opinion that mercapturic acids serve as metabolic intermediates, and in support of this belief they described experiments in which they found that these compounds were oxidized to a considerable extent to ethereal sulphate when administered to the pig and the rabbit. No support for these findings was obtained by Coombs and Hele [91] and by Lawrie [92], and in recent experiments in which ^{35}S -labelled mercapturic acids were administered to rats, less than 1% of the ^{35}S was excreted in the ethereal sulphate fraction of the urine [93].

When rabbits are dosed repeatedly with naphthalene they develop cataract [94] and the cysteine content of the lens is decreased [95], as also is the glutathione content of the eye [96]. The demand for sulphur-containing amino acids which attends the metabolism of naphthalene may be a contributory factor in cataract production [72], but it is insufficient to explain the role of naphthalene in this process, for other compounds which form premercapturic acids or mercapturic acids in the rabbit do not give rise to cataract.

When bromobenzene [97] or naphthalene [98] is administered to young rats on a diet which normally provides just sufficient sulphur-containing amino acids for good growth, it is found that the growth of the animals is inhibited. Supplementation of the diet with suitable amounts of cystine or methionine leads to restoration of growth. These findings may be explained on the grounds that conjugation of bromobenzene or naphthalene with cysteine can produce a deficiency of sulphur-containing amino acids in the organism. On the other hand, retardation of growth has been produced by the administration of certain polycyclic hydrocarbons such as pyrene, methylcholanthrene and 3:4-benzopyrene [99], for which no evidence of premercapturic acid or mercapturic acid formation has been forthcoming.

Cystinuria

Cystine is present in normal human urine in amounts of the order of 30 mg. per day [100]. Certain individuals excrete much larger quantities, however, and amounts exceeding 1 g. daily have been reported. This condition, usually described as 'cystinuria', is said to occur in about one individual in 600 [101]. In a mild form it is unlikely to give rise to symptoms, but in more severe form it is liable to be accompanied by the formation of cystine calculi in the renal tract. Cysteine, homocysteine and methionine were reported by Brand, Cahill and Harris [102] to have increased the excretion of cystine when they were administered to a cystinuric subject, whereas when cystine was administered to this subject, it was metabolized normally and none was excreted in the urine. These workers concluded that cystine can be metabolized without being reduced to cysteine. They were of the opinion that individuals with cystinuria suffer from an error in the metabolism of cysteine, and that dietary methionine, by undergoing conversion to cysteine, is the chief source of the urinary cystine. Recent studies of the pathogenesis of cystinuria, however, have revealed that the disorder does not arise from a defect in the intermediary metabolism of one or other of the sulphur-containing amino acids, but is of renal origin. In cystinuric subjects, according to Dent and Rose [103], there is a low renal threshold for cystine which appears to be due to a failure by the tubules to reabsorb cystine. The increased cystine excretion which occurs is associated with an increased excretion of lysine and sometimes of arginine and ornithine [103, 104, 105]. Dent and his coworkers [106, 107] have shown by chromatographic, microbiological and polarographic methods that the plasma cystine levels are no higher in cystinuric subjects than in normal individuals. When cysteine, cystine and methionine were fed separately to normal and also to cystinuric subjects it was found that only cysteine markedly increased the amount of cystine in the plasma and in the urine [106, 107]. These findings have been explained on the ground that cysteine is rapidly absorbed and converted into cystine, whereas cystine itself is absorbed slowly, and methionine, although it is absorbed readily, is converted only slowly and in part to cystine [107]. On the basis of these and other findings Dent and his coworkers [106] have

suggested that there is probably no clear-cut difference in the metabolism of cysteine between normal and cystinuric subjects, and that in cystinuria there is no 'error of metabolism' of the kind envisaged by Garrod [108].

OTHER THIOLS AND DISULPHIDES

Homocysteine and homocystine

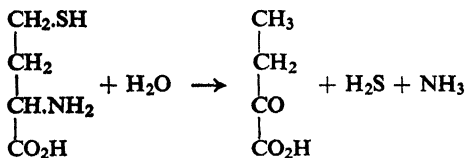
Homocysteine is thought to play an important role in the metabolic processes linking methionine and cysteine, and this aspect of its metabolism is described in the two succeeding chapters. In Chapter III an account is given of the formation of homocysteine from methionine and its participation in reactions leading to the production of cysteine. The synthesis of methionine from homocysteine is considered in Chapters III and IV. Although there is evidence that homocystine is excreted in the urine of animals dosed with methionine, neither homocysteine nor homocystine has so far been detected in the free state in tissues. Formation of *S*-adenosylhomocysteine, however, has been demonstrated during the enzymic synthesis of creatine from glycocyamine and *S*-adenosylmethionine [109], and this compound can be broken down by rat liver preparations to homocysteine [110].

It has been claimed that the metabolism of homocysteine may differ from that of homocystine, and that although both compounds undergo oxidation to sulphate [111, 112], this is accompanied by a significant formation of taurine from homocysteine, but not from homocystine [46, 113]. It is now generally assumed, however, that homocysteine and homocystine are interconvertible *in vivo*, and that the relationship between them has much in common with that between cysteine and cystine. The nature of the system which brings about the oxidation of homocysteine has not been established, but Racker [114] has described a transhydrogenase in liver which catalyses the reduction of homocystine in the presence of glutathione. The administration of either D- or L-homocystine to rats on a diet deficient in cystine leads to growth [115, 116], and Borsook and Dubnoff [117] have described a system in rat liver which brings about the conversion of homocystine to methionine. These observations may be considered to indicate that conversion of homocystine to homocysteine can

take place. Some evidence suggests that there is an enzyme system in rat liver which can bring about the oxidation of homocysteine to homocysteic acid [43]. The metabolic oxidation of the sulphur of homocysteine appears to be prevented by *S*-ethylation or *S*-benzylation [112] and when *S*-benzylhomocysteine is administered to dogs it is converted to the *N*-acetyl derivative [118], a homologue of benzylmercapturic acid.

Although Borek and Waelsch [119] were unable to obtain any evidence of the oxidative deamination of either homocysteine or homocystine by slices of rat liver and kidney, reference has already been made to the fact that both D- and L-homocystine can promote the growth of rats on cystine-deficient diets. This raises the possibility that D-homocystine is reduced to D-homocysteine which then undergoes inversion to L-homocysteine as a result of deamination followed by reamination. The L-homocysteine thus formed could then give rise to L-methionine and L-cysteine.

When homocysteine is incubated with preparations of liver, pancreas or kidney, there is a liberation of hydrogen sulphide, and according to Fromageot and Desnuelle [37, 120], this is brought about by homocysteine desulphydrase. This enzyme appears to be quite distinct from cysteine desulphydrase, for the ratio of the amounts of hydrogen sulphide derived from homocysteine and from cysteine varies with different enzyme preparations. Furthermore, homocysteine desulphydrase is more unstable than cysteine desulphydrase, and enzyme preparations can be obtained which act on cysteine but not on homocysteine. The identity of all the products formed by desulphydration of homocysteine in animals has not been established, but it is noteworthy that Kallio [121] has prepared a cell-free extract of *Proteus morganii* which brings about a stoichiometric conversion of homocysteine to hydrogen sulphide, ammonia and α -keto-butyric acid.



Some of the possible roles of homocysteine in the metabolism of sulphur-containing amino acids have been discussed by Cantoni [122].

Glutathione

Glutathione, γ -glutamylcysteinylglycine, is a tripeptide which is widely distributed in living systems, and it accounts for a large proportion of the thiol groups of cells. Many types of cell are able to synthesize the compound and it has become apparent that it participates in a variety of metabolic processes. It undergoes a rapid turnover in animal tissues, for when Waelsch and Rittenberg [123, 124] administered [^{15}N]glycine and [^{15}N]glutamic acid to rats and rabbits they found that these amino acids were introduced into the glutathione of the liver much faster than they were incorporated into the liver protein. They calculated that the half-life of rat-liver glutathione is about 2–4 hours, whereas the corresponding value for the total protein of the liver is 5–7 days. Evidence of the rapid turnover of glutathione was also obtained by Anderson and Mosher [125] in their experiments with [^{35}S]cystine.

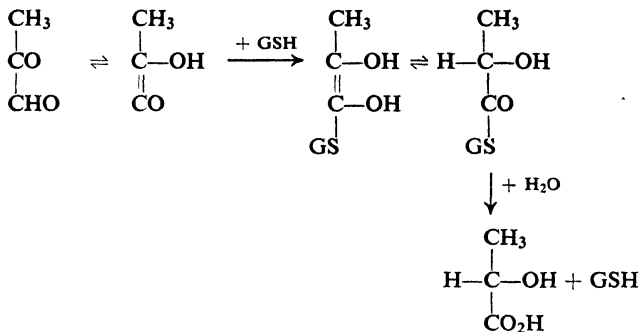
The synthesis of glutathione by tissue preparations has been investigated in detail by Bloch and his coworkers. It is a subject which has a special interest in that it may throw light on the general process of peptide bond formation from amino acids. Extracts of acetone-dried pigeon-liver preparations were shown to form glutathione from glutamic acid, glycine and cystine when Mg^{2+} and adenosine triphosphoric acid (ATP) were present [126], and the livers of various mammals were found to contain the enzymes which catalyse this process [127]. At least two consecutive steps occur under these conditions [128]. The first step is the combination of glutamic acid and cysteine to form γ -glutamylcysteine, and the dipeptide then reacts with glycine to give glutathione. Considerable progress has been made in the purification of the enzymes which bring about these two reactions [129, 130] and evidence has been obtained which suggests that the second of them may be reversible [131].

Olson and Binkley [132] separated from pig kidney an enzyme which hydrolysed the γ -glutamyl linkage of glutathione and another which hydrolysed cysteinylglycine. They found that glutamine activated the first of these reactions [133], but it has

since become apparent that this property is shown by a number of amino acids and that some are more active than glutamine in this respect [134]. Meanwhile, Hanes, Hird and Isherwood [135] observed that in the presence of a kidney preparation, glutathione reacted with various added amino acids to form new peptides containing glutamic acid and that these were probably γ -glutamyl peptides. It was demonstrated that with glutathione, kidney preparations gave rise to γ -glutamyl dipeptides from phenylalanine, glycine [135], arginine [136] and other amino acids [134]. It was further shown that glutathione is not unique as a γ -glutamyl donor, for in the presence of the transferring enzyme other γ -glutamyl peptides act in the same manner [135, 137]. One example of this is the formation of glutathione from cysteinyl-glycine and γ -glutamyl peptides [137]. The rapid metabolic turnover of glutathione and the observation that an enzyme-catalysed transfer of the glutamyl radical of glutathione occurs suggested that glutathione may have a role in protein synthesis [136, 138]. The recognition of the widespread occurrence of transpeptidation reactions, however, has led to less emphasis being placed on this possibility.

The enzyme glyoxalase, which catalyses the conversion of methylglyoxal to lactic acid [139, 140, 141], is widely distributed in animals, plants and micro-organisms, and it requires the presence of reduced glutathione for its action [142]. It has become apparent that there are two enzymic steps in the conversion of methylglyoxal to lactic acid [143, 144, 145, 146], and the enzymes responsible have been separated by Racker [146] and named glyoxalase I and II. Racker has shown that the first step in the process is catalysed by glyoxalase I, and is the condensation of the enol form of methylglyoxal with glutathione (GSH) to give an intermediate which rearranges to a thiol ester which is hydrolysed to lactic acid and glutathione as a result of the action of glyoxalase II. These reactions can be represented as shown on the following page.

Krimsky and Racker [147] have shown that glyceraldehyde-3-phosphate dehydrogenase contains a firmly-bound prosthetic group which they have identified as glutathione. This finding, when taken in conjunction with Racker's observations on the glyoxalase system, led them to suggest that the sulphhydryl group takes an active part in triose phosphate oxidation [148]. They



have presented evidence to show that glyceraldehyde-3-phosphate reacts with the sulphydryl group of the dehydrogenase and is oxidized to a thiol ester which is then split phosphorolytically with the formation of 1:3-diphosphoglyceric acid. Both steps require DPN and are catalysed by glyceraldehyde-3-phosphate dehydrogenase [148].

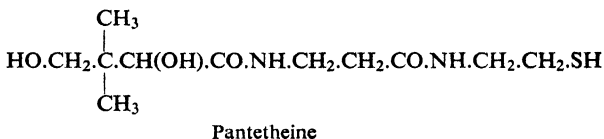
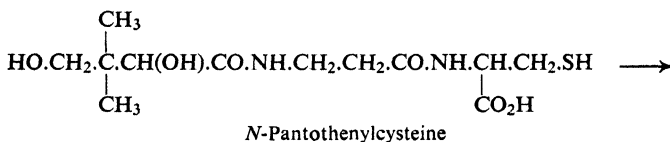
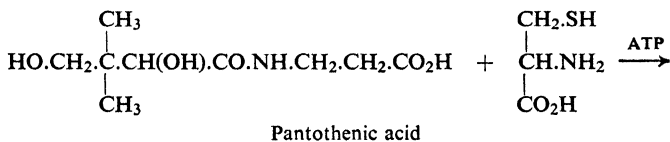
It is clear that in recent years the study of glutathione has been fruitful. A valuable account of work in this field has appeared in the form of the proceedings of a symposium on glutathione [149].

Coenzyme A

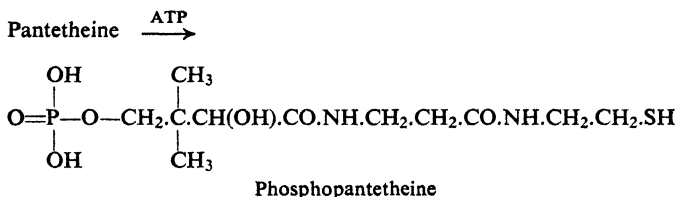
Coenzyme A contains a β -mercaptoethylamine grouping, the sulphydryl group of which is the biochemically functional part of the coenzyme molecule [150]. For this reason coenzyme A is often represented as HS.CoA. The discovery of coenzyme A by Lipmann [151] has opened the way to an understanding of a variety of metabolic processes involving acyl compounds, such as the formation and breakdown of fatty acids and ketone bodies, and the formation of citric acid, acetylcholine and *N*-acetylaryl-amines.

The steps whereby coenzyme A is synthesized in mammals have been elucidated by Hoagland and Novelli [152]. There is a requirement for cysteine in the biosynthesis of coenzyme A which apparently cannot be met by any other sulphur compound. In the presence of ATP, cysteine combines with pantothenic acid to form *N*-pantothenylcysteine which is then decarboxylated to give pantetheine. These reactions have been shown to be catalysed

by enzymes present in the supernatant fraction from rat-liver homogenates:



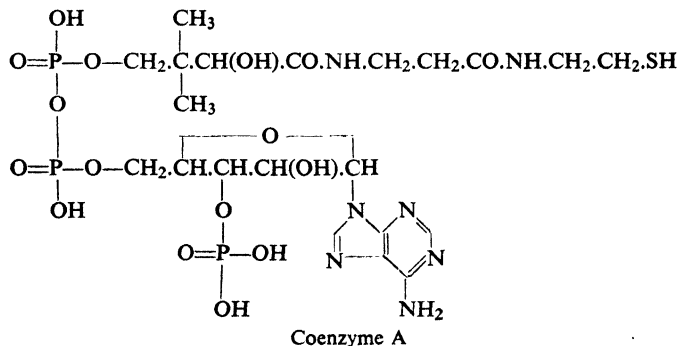
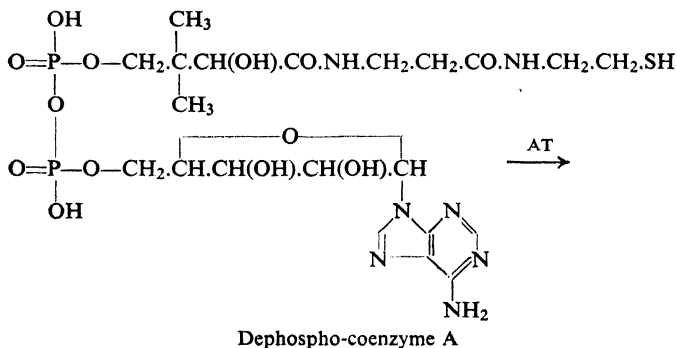
The next step is the phosphorylation of pantetheine to give phosphopantetheine. This requires ATP, and a preparation containing an enzyme, pantetheinekinase, which catalyses the reaction, has been obtained from the supernatant fractions of pigeon-liver preparations by Levintow and Novelli [153]:



The last two steps in the biosynthesis of coenzyme A also require ATP and have been shown to be brought about by enzymes present in both the mitochondrial and the supernatant fractions of liver. In the first of these reactions phosphopantetheine and ATP react to give inorganic pyrophosphate and dephospho-coenzyme

A. This last compound is converted to coenzyme A by the phosphorylation in the 3-position of the ribose component:

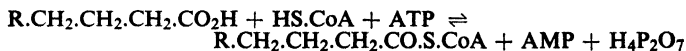
Phosphopantetheine $\xrightarrow{\text{ATP}}$



Hoagland and Novelli [152] have shown that Mg^{2+} must be present for these various reactions to occur, and that the substrates must be present in their reduced forms. It is probable that each organ synthesizes its own coenzyme A, for the compound does not appear to circulate in the plasma [154], and Govier and Gibbons [155] have reported that when it is injected into dogs it is mostly excreted as phosphopantetheine.

Coenzyme A acts as a carrier for acyl groups by virtue of its

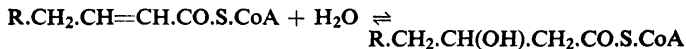
ability to form thiol esters with the general formula $R.CO.S.CoA$. One of the important functions of coenzyme A is concerned with the synthesis and degradation of fatty acids, and the following brief account of its role in the oxidation of fatty acids is given to illustrate the type of process in which it participates. The first step in the series of reactions is the formation of the coenzyme A derivative of the fatty acid. This requires ATP and the presence of Mg^{2+} , and an overall reaction occurs [156] which can be represented as follows:



The fatty acid derivative of coenzyme A then undergoes dehydrogenation and in the process flavin adenine dinucleotide (FAD) is reduced:



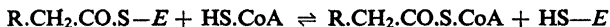
The unsaturated derivative takes up a molecule of water to give a derivative which is dehydrogenated in the presence of DPN:



The β -ketoacyl derivative of coenzyme A formed in this last reaction undergoes thiolytic cleavage catalysed by the enzyme β -ketothiolase as follows:

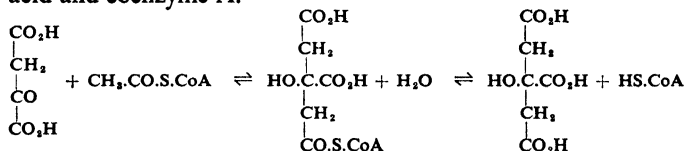


It would appear that β -ketothiolase has a functional sulphhydryl group, for it is inhibited by iodoacetic acid and arsenite, and Lynen [157] has obtained evidence that the enzyme ($HS-E$) takes part in the thiolytic reaction in the following manner:



As a result of these various reactions the original fatty acid derivative of coenzyme A, $R.CH_2.CH_2.CH_2.CO.S.CoA$, is con-

verted to a derivative with two carbon atoms less. This degradative process occurs repeatedly until all the fatty acid has been broken down. The acetyl-coenzyme A, $\text{CH}_3\text{CO.S.CoA}$, formed in these reactions can condense with oxaloacetic acid to form citryl-CoA, which is acted upon by a deacylase and yields citric acid and coenzyme A:



The citric acid then undergoes the series of reactions which constitute the tricarboxylic acid cycle.

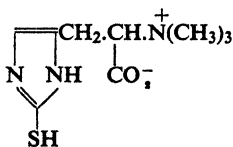
Detailed accounts of the various roles of coenzyme A in intermediary metabolism are to be found in reviews by Lipmann, Lynen, Green and others [151, 158, 159, 160].

Lipoic acid

Lipoic acid has been studied primarily in micro-organisms (see Chapter IX), where it has been shown to take part in the oxidative decarboxylation of α -keto acids [161, 162]. In higher animals a dietary need for lipoic acid has not been demonstrated, and its formation and breakdown do not appear to have been investigated. It has been reported [163, 164, 165] to be present in preparations of the pyruvic acid oxidase and α -ketoglutaric acid oxidase of animal tissues, however, and it is probable that it has a role in animal metabolism.

Ergothioneine

Ergothioneine was originally isolated from the ergot of rye by Tanret [166] and was shown by Barger and Ewins [167] to be a betaine of 2-thiolhistidine.



Ergothioneine

This compound has since been found to be present in many tissues of animals and man [168, 169, 170, 171], in particular the liver, but it appears to be absent from plasma and brain. More recently Leone and Mann [172, 173] have reported its occurrence in boar semen, where it is confined to the seminal plasma. It also occurs in plants, notably in cereal grains.

The possible synthesis of ergothioneine by animals has been studied with the aid of labelled compounds considered to be likely precursors. Heath and his coworkers [174] found that neither sulphate nor 2-thiolhistidine labelled with ^{35}S gave rise to labelled ergothioneine in the boar, but that the sulphur of methionine appeared to be incorporated into seminal ergothioneine. Melville [175, 176] showed that 2-thiolhistidine was unable to serve as a precursor in the rat. In careful experiments with ^{35}S - and ^{14}C -labelled methionine he found, however, that the synthesis of ergothioneine from this amino acid in the pig and certain other animals did not take place. Histidine, cystine and glycine were also shown not to give rise to the compound.

Melville and his associates have reached the conclusion that the ergothioneine present in animal tissues originates entirely from the diet. Feeding experiments with animals have shown that as little as 1 part of ergothioneine in 100,000 of diet leads to an accumulation of the compound in the erythrocytes [176]. When rats are maintained on purified diets free of the compound, however, its level in blood and tissues falls to negligible amounts [177]. The isolation of ergothioneine from oats by Melville and Eich [178] explains the earlier observation of Baldrige and Lewis [179] that the addition of oats to the diet of rabbits is particularly effective in raising the blood ergothioneine level.

It has recently become apparent that ergothioneine can be formed by some micro-organisms. For example, *Neurospora crassa* synthesizes this compound using inorganic sulphate, cystine or methionine as a source of sulphur, and appears to derive the carbon and nitrogen skeleton from histidine [180]. It is possible that some of the ergothioneine in cereals originates from the synthetic activities of micro-organisms. On the other hand, it has been established that animals do not derive significant amounts of ergothioneine from their intestinal flora [181].

Little is known of the metabolism of ergothioneine. Heath [182] has shown that when the ^{35}S -labelled compound is adminis-

tered orally to rats, about one-third of the dose is oxidized to sulphate. The related compound, 2-thiohistidine, undergoes little oxidation *in vivo*.

Miscellaneous thiols and disulphides

Metabolic studies have been made of the formation of sulphate from various thiols and disulphides in addition to those already considered. Many of these experiments were carried out before ^{35}S became available and in consequence the results lack the precision which characterizes the study of this type of problem by the use of labelled compounds. It is clear, nevertheless, that among thiols and disulphides marked differences are encountered in the extent to which oxidation to sulphate takes place *in vivo*. For example, isocysteine, $\text{H}_2\text{N}.\text{CH}_2.\text{CH}(\text{SH}).\text{CO}_2\text{H}$, unlike cysteine, gave no significant increase in the urinary excretion of sulphate when it was administered orally or subcutaneously to rabbits. Instead, most of the extra urinary sulphur was present as a disulphide which was probably isocystine [183]. Thioglycollic acid, $\text{HS}.\text{CH}_2.\text{CO}_2\text{H}$, thiolactic acid, $\text{CH}_3.\text{CH}(\text{SH}).\text{CO}_2\text{H}$ [184], dithiodiglycollic acid, $(-\text{S}.\text{CH}_2.\text{CO}_2\text{H})_2$, $\beta\beta'$ -dithiodipropionic acid, $(-\text{S}.\text{CH}_2.\text{CH}_2.\text{CO}_2\text{H})_2$, and the hydroxy analogue of cystine, $\alpha\alpha'$ -dihydroxy- $\beta\beta'$ -dithiodipropionic acid, $(-\text{S}.\text{CH}_2.\text{CH}(\text{OH}).\text{CO}_2\text{H})_2$ [185], all undergo considerable oxidation to sulphate in the animal body. It is noteworthy that these three disulphides are all unable to replace cystine in the diet of the rat [186, 187].

In recent years methanethiol has acquired a biochemical significance as a result of the finding that it is formed from methionine by various micro-organisms and also by rat-liver mitochondrial preparations. Administration of ^{14}C -labelled methanethiol to rats has shown that the compound serves as a source of methyl groups, for in these experiments ^{14}C was found to be present in the methyl groups of choline and methionine. In similar experiments with methanethiol labelled with ^{35}S , the compound became bound to liver proteins by disulphide bonds, and the ^{35}S was not transferred either to methionine or to cystine [188], but appeared in the urine mostly as sulphate [189]. It has been shown that yeast contains an enzyme which catalyses the formation of a methylthiol ester from 3-phosphoglyceric acid and methanethiol in the presence of ATP [190]. Ethanethiol, when given to dogs

by mouth, was found by Smith [191] to be oxidized to sulphate. Snow [192] recently studied the metabolism of compounds related to ethanethiol, and showed that when diethyl disulphide and ethyl thiolbenzoate labelled with ^{35}S were administered to mice and guinea pigs, most of the ^{35}S excreted in the urine was present as sulphate. In the urine of guinea pigs dosed with diethyl disulphide, however, it was also possible to show the presence of ethyl methyl sulphone. Unlike the aliphatic thiols, neither thiophenol nor thiocresol gives rise to sulphate in the rabbit [184].

Considerable interest attaches to the metabolism of 2:3-dimercaptopropanol (BAL, British Anti-Lewisite), because of the importance of this compound as an antidote to arsenic and certain other heavy metal poisons [193, 194, 195, 196]. Three groups of workers [197, 198, 199] have carried out metabolic studies with ^{35}S -labelled 2:3-dimercaptopropanol, and there is general agreement that the ^{35}S of much of the radioactive BAL which enters the body is excreted in the urine, and that most of this ^{35}S is present as neutral sulphur and very little as sulphate. When they applied isotope dilution techniques to the urine of rats dosed with ^{35}S -labelled BAL, Simpson and Young [198] found no evidence of the presence of unchanged BAL, a result which has also been obtained by Tamboline, Matheson and Zbarsky [199] by the use of radiochromatographic methods. Although the chief metabolites of BAL have yet to be identified, some light was thrown on this matter by Stocken and Thompson [200], who observed that the administration of relatively large doses of BAL to rats or rabbits led to the excretion of a thiol, and obtained indications that this was a dithiol not identical with BAL, but closely related to it [201].

REFERENCES

1. WOLLASTON, W. H. (1810). *Phil. Trans.* p. 223.
2. FRIEDMANN, E. (1902). *Beitr. chem. Physiol. Path.* **2**, 433.
3. FRIEDMANN, E. (1903). *Beitr. chem. Physiol. Path.* **3**, 1.
4. MÖRNER, K. A. H. (1899). *Hoppe-Seyl. Z.* **28**, 595.
5. MUELLER, J. H. (1922). *Proc. Soc. exp. Biol., N.Y.* **19**, 161.
6. MUELLER, J. H. (1923). *J. biol. Chem.* **56**, 157.
7. JACKSON, R. W. and BLOCK, R. J. (1932). *J. biol. Chem.* **98**, 465.

8. BEACH, E. F. and WHITE, A. (1939). *J. biol. Chem.* **127**, 87.
9. FROMAGEOT, C., WOOKEY, E. and CHAIX, P. (1940). *Enzymologia*, **9**, 198.
10. SMYTHE, C. V. (1942). *J. biol. Chem.* **142**, 387.
11. CHATAGNER, F. and BERGERET, B. (1951). *C.R. Acad. Sci., Paris*, **232**, 448.
12. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1939). *J. biol. Chem.* **127**, 431.
13. FROMAGEOT, C., CHATAGNER, F. and BERGERET, B. (1948). *Biochim. biophys. Acta*, **2**, 294.
14. SCHÖBERL, A., KAWOHL, M. and HAMM, R. (1951). *Ber. dtsh. chem. Ges.* **84**, 571.
15. WOOD, J. L. and COOLEY, S. L. (1956). *J. biol. Chem.* **218**, 449.
16. VOEGTLIN, C., JOHNSON, J. M. and DYER, H. A. (1926). *J. Pharmacol.* **27**, 467.
17. HARRIS, J. and MACKENZIE, C. G. (1955). *Fed. Proc.* **14**, 223.
18. KEILIN, D. (1930). *Proc. Roy. Soc. B.* **106**, 418.
19. MEDES, G. (1939). *Biochem. J.* **33**, 1559.
20. MEISTER, A., FRASER, P. E. and TICE, S. V. (1954). *J. biol. Chem.* **206**, 561.
21. NICKERSON, W. J. and ROMANO, A. H. (1952). *Science*, **115**, 676.
22. LEWIS, H. B., UPDEGRAFF, H. and MCGINTY, D. A. (1924). *J. biol. Chem.* **59**, 59.
23. PIRIE, N. W. (1934). *Biochem. J.* **28**, 305.
24. PIRIE, N. W. (1933). *Biochem. J.* **27**, 1181.
25. LAVINE, T. F. (1937). *J. biol. Chem.* **117**, 309.
26. SCHÖBERL, A. and HORNING, T. (1938). *Liebigs Ann.* **534**, 210.
27. SCHÖBERL, A. and RAMBACHER, P. (1939). *Liebigs Ann.* **538**, 84.
28. TARR, H. L. A. (1933). *Biochem. J.* **27**, 1869.
29. TARR, H. L. A. (1934). *Biochem. J.* **28**, 192.
30. SMYTHE, C. V. (1945). *Advanc. Enzymol.* **5**, 237.
31. FROMAGEOT, C. (1951). *The Enzymes*, Vol. I, p. 1237. Ed. by J. B. Sumner and K. Myrbäck. New York: Academic Press.
32. SUDA, M., KISU, Y., SAIGO, T. and ICHIHARA, K. (1953). *Med. J. Osaka Univ.* **3**, 469.

33. CAMMARATA, P. S. and COHEN, P. P. (1950). *J. biol. Chem.* **187**, 439.
34. BRAUNSTEIN, A. E. and ASARKH, R. M. (1950). *C.R. Acad. Sci. U.R.S.S.* **71**, 93.
35. CHATAGNER, F. and BERGERET, B. (1955). *Ann. Nutr., Paris*, **9**, 93.
36. FROMAGEOT, C., CHAIX, P. and THIBAUT, Y. (1946). *Bull. Soc. chim., Paris*, **13**, 202.
37. FROMAGEOT, C. and DESNUELLE, P. (1942). *Bull. Soc. Chim. biol., Paris*, **24**, 1269.
38. GREENSTEIN, J. P. and LEUTHARDT, F. M. (1944). *J. nat. Cancer Inst.* **5**, 209.
39. HELE, T. S. and PIRIE, N. W. (1931). *Biochem. J.* **25**, 1095.
40. MEDES, G. (1937). *Biochem. J.* **31**, 1330.
41. MUELLER, J. H. (1923). *J. biol. Chem.* **58**, 373.
42. PIRIE, N. W. (1932). *Biochem. J.* **26**, 2041.
43. MEDES, G. and FLOYD, N. (1942). *Biochem. J.* **36**, 259.
44. LAVINE, T. F. (1937). *J. biol. Chem.* **117**, 309.
45. LAVINE, T. F. (1936). *J. biol. Chem.* **113**, 583.
46. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1939). *J. biol. Chem.* **128**, 665.
47. BERGERET, B. and CHATAGNER, F. (1954). *Biochim. biophys. Acta*, **14**, 297.
48. FROMAGEOT, C. (1953-54). *Harvey Lect.* **49**, 1.
49. SCHMIDT, C. L. A. and CLARK, G. W. (1922). *J. biol. Chem.* **53**, 193.
50. HEIMBERG, M., FRIDOVICH, I. and HANDLER, P. (1953). *J. biol. Chem.* **204**, 913.
51. CHATAGNER, F., BERGERET, B., SÉJOURNÉ, T. and FROMAGEOT, C. (1952). *Biochim. biophys. Acta*, **9**, 340.
52. TABECHIAN, H., BERGERET, B. and CHATAGNER, F. (1953). *Bull. Soc. Chim. biol., Paris*, **35**, 615.
53. SINGER, T. P. and KEARNEY, E. B. (1954). *Biochim. biophys. Acta*, **14**, 570.
54. CHAPEVILLE, F. and FROMAGEOT, P. (1954). *Biochim. biophys. Acta*, **14**, 415.
55. FROMAGEOT, C. and ROYER, A. (1945). *Enzymologia*, **11**, 361.
56. AWAPARA, J. and WINGO, W. J. (1953). *J. biol. Chem.* **203**, 189.

57. BLANCHARD, M., GREEN, D. E., NOCITO, V. and RATNER, S. (1944). *J. biol. Chem.* **155**, 421.
58. BLANCHARD, M., GREEN, D. E., NOCITO, V. and RATNER, S. (1945). *J. biol. Chem.* **161**, 583.
59. CAMMARATA, P. S. and COHEN, P. P. (1950). *J. biol. Chem.* **187**, 439.
60. ARNSTEIN, H. R. V. and CRAWHALL, J. C. (1953). *Biochem. J.* **55**, 280.
61. BUTTS, J. S., BLUNDEN, H. and DUNN, M. S. (1938). *J. biol. Chem.* **124**, 709.
62. MEDES, G. and VITANZA, I. (1939). *Biochem. J.* **33**, 1570.
63. STÖHR, R. (1938). *Biochem. Z.* **299**, 242.
64. HESS, W. C. (1949). *J. biol. Chem.* **181**, 23.
65. BAUMANN, E. and PREUSSE, C. (1879). *Ber. dtsh. chem. Ges.* **12**, 806.
66. JAFFÉ, M. (1879). *Ber. dtsh. chem. Ges.* **12**, 1092.
67. BAUMANN, E. and SCHMITZ, P. (1895). *Hoppe-Seyl. Z.* **20**, 586.
68. YOUNG, L. and ZBARSKY, S. H. (1944). *J. biol. Chem.* **154**, 389.
69. AZOUZ, W. M., PARKE, D. V. and WILLIAMS, R. T. (1955). *Biochem. J.* **59**, 410.
70. PARKE, D. V. and WILLIAMS, R. T. (1955). *Biochem. J.* **59**, 415.
71. JONDORF, W. R., PARKE, D. V. and WILLIAMS, R. T. (1955). *Biochem. J.* **61**, 512.
72. BOURNE, M. C. and YOUNG, L. (1934). *Biochem. J.* **28**, 803.
73. BOYLAND, E. and LEVI, A. A. (1936). *Biochem. J.* **30**, 1225.
74. ZBARSKY, S. H. and YOUNG, L. (1943). *J. biol. Chem.* **151**, 487.
75. WOOD, J. L. and FIESER, L. F. (1940). *J. Amer. chem. Soc.* **62**, 2674.
76. STEKOL, J. A. (1938). *J. biol. Chem.* **124**, 129.
77. STEKOL, J. A. (1942). *J. biol. Chem.* **138**, 225.
78. BRAY, H. G., JAMES, S. P. and THORPE, W. V. (1957). *Biochem. J.* **67**, 607.
79. ROBERTS, J. J. and WARWICK, G. P. (1957). *Nature, Lond.* **179**, 1181.
80. ZBARSKY, S. H. and YOUNG, L. (1943). *J. biol. Chem.* **151**, 217.

81. STEKOL, J. A. (1937-38). *J. biol. Chem.* **122**, 333.
82. GUTMANN, H. R. and WOOD, J. L. (1951). *J. biol. Chem.* **189**, 473.
83. STEKOL, J. A. (1940). *Proc. Soc. exp. Biol., N.Y.*, **43**, 108.
84. STEKOL, J. A. (1948). *J. biol. Chem.* **176**, 33.
85. STEKOL, J. A. (1941). *J. biol. Chem.* **140**, 827.
86. BOYLAND, E., SIMS, P. and SOLOMON, J. B. (1957). *Biochem. J.* **66**, 42P.
87. KNIGHT, R. H. and YOUNG, L. (1957). *Biochem. J.* **66**, 55P.
88. MULDOON, J. A., SHIPLE, G. J. and SHERWIN, C. P. (1924). *J. biol. Chem.* **59**, 675.
89. SHIPLE, G. J., MULDOON, J. A. and SHERWIN, C. P. (1924). *J. biol. Chem.* **60**, 59.
90. ROSE, A. R., SHIPLE, G. J. and SHERWIN, C. P. (1924). *Amer. J. Physiol.* **69**, 518.
91. COOMBS, H. I. and HELE, T. S. (1927). *Biochem. J.* **21**, 611.
92. LAWRIE, N. R. (1931). *Biochem. J.* **25**, 1037.
93. MARSDEN, C. M. and YOUNG, L. Unpublished observations.
94. ADAMS, D. R. (1930). *Brit. J. Ophthal.* **14**, 49.
95. TSUJI, T. (1932). *J. Biochem., Tokyo*, **15**, 33.
96. NAKASHIMA, T. (1934). *J. Biochem., Tokyo*, **19**, 281.
97. WHITE, A. and JACKSON, R. W. (1935). *J. biol. Chem.* **111**, 507.
98. STEKOL, J. A. (1939). *J. biol. Chem.* **127**, 131.
99. WHITE, J. and WHITE, A. (1939). *J. biol. Chem.* **131**, 149.
100. MEDES, G. (1937). *Biochem. J.* **31**, 12.
101. LEWIS, H. B. (1932). *Ann. intern. Med.* **6**, 183.
102. BRAND, E., CAHILL, G. F. and HARRIS, M. M. (1935). *J. biol. Chem.* **109**, 69.
103. DENT, C. E. and ROSE, G. A. (1951). *Quart. J. Med.* **20**, 205.
104. YEH, H. L., FRANKL, W., DUNN, M. S., PARKER, P., HUGHES, B. and GYÖRGY, P. (1947). *Amer. J. med. Sci.* **214**, 507.
105. STEIN, W. H. (1951). *Proc. Soc. exp. Biol., N.Y.* **78**, 705.
106. DENT, C. E., HEATHCOTE, J. G. and JORON, G. E. (1954). *J. clin. Invest.* **33**, 1210.
107. DENT, C. E., SENIOR, B. and WALSH, J. M. (1954). *J. clin. Invest.* **33**, 1216.
108. GARROD, A. E. (1923). *Inborn Errors of Metabolism*. 2nd Edn, London: Henry Frowde and Hodder and Stoughton.

109. CANTONI, G. L. and SCARANO, E. (1954). *J. Amer. chem. Soc.* **76**, 4744.
110. ERICSON, L.-E., WILLIAMS, J. N. jun. and ELVEHJEM, C. A. (1955). *Acta chem. scand.* **9**, 859.
111. DU VIGNEAUD, V., LORING, H. S. and CRAFT, H. A. (1934). *J. biol. Chem.* **105**, 481.
112. SHEN, C.-W. and LEWIS, H. B. (1946). *J. biol. Chem.* **165**, 115.
113. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1937). *J. biol. Chem.* **119**, 697.
114. RACKER, E. (1955). *J. biol. Chem.* **217**, 867.
115. DYER, H. M. and DU VIGNEAUD, V. (1935). *J. biol. Chem.* **109**, 477.
116. ROSE, W. C. and RICE, E. E. (1939). *J. biol. Chem.* **130**, 305.
117. BOROOK, H. and DUBNOFF, J. W. (1947). *J. biol. Chem.* **169**, 247.
118. DU VIGNEAUD, V., WOOD, J. L. and IRISH, O. J. (1939). *J. biol. Chem.* **129**, 171.
119. BOREK, E. and WAELSCH, H. (1941). *J. biol. Chem.* **141**, 99.
120. FROMAGEOT, C. and DESNUELLE, P. (1942). *C.R. Acad. Sci., Paris*, **214**, 647.
121. KALLIO, R. E. (1951). *J. biol. Chem.* **192**, 371.
122. CANTONI, G. L. (1955). *A Symposium on Amino Acid Metabolism*, p. 601. Ed. by W. D. McElroy and B. Glass. Baltimore: The Johns Hopkins Press.
123. WAELSCH, H. and RITTENBERG, D. (1941). *J. biol. Chem.* **139**, 761.
124. WAELSCH, H. and RITTENBERG, D. (1942). *J. biol. Chem.* **144**, 53.
125. ANDERSON, E. I. and MOSHER, W. A. (1951). *J. biol. Chem.* **188**, 717.
126. JOHNSTON, R. B. and BLOCH, K. (1951). *J. biol. Chem.* **188**, 221.
127. YANARI, S., SNOKE, J. E. and BLOCH, K. (1953). *J. biol. Chem.* **201**, 561.
128. SNOKE, J. E. and BLOCH, K. (1952). *J. biol. Chem.* **199**, 407.
129. SNOKE, J. E., YANARI, S. and BLOCH, K. (1953). *J. biol. Chem.* **201**, 573.
130. SNOKE, J. E. (1955). *J. biol. Chem.* **213**, 813.
131. SNOKE, J. E. and BLOCH, K. (1955). *J. biol. Chem.* **213**, 825.

132. OLSON, C. K. and BINKLEY, F. (1950). *J. biol. Chem.* **186**, 731.
133. BINKLEY, F. and OLSON, C. K. (1951). *J. biol. Chem.* **188**, 451.
134. HIRD, F. J. R. and SPRINGELL, P. H. (1954). *Biochem. J.* **56**, 417.
135. HANES, C. S., HIRD, F. J. R. and ISHERWOOD, F. A. (1950). *Nature, Lond.* **166**, 288.
136. KINOSHITA, J. H. and BALL, E. G. (1953). *J. biol. Chem.* **200**, 609.
137. FODOR, P. J., MILLER, A., NEIDLE, A. and WAELSCH, H. (1953). *J. biol. Chem.* **203**, 991.
138. FRUTON, J. S. (1950). *Yale J. Biol. Med.* **22**, 263.
139. NEUBERG, C. (1913). *Biochem. Z.* **49**, 502.
140. DAKIN, H. D. and DUDLEY, H. W. (1913). *J. biol. Chem.* **14**, 155.
141. DAKIN, H. D. and DUDLEY, H. W. (1913). *J. biol. Chem.* **14**, 423.
142. LOHMANN, K. (1932). *Biochem. Z.* **254**, 332.
143. HOPKINS, F. G. and MORGAN, E. J. (1948). *Biochem. J.* **42**, 23.
144. CROOK, E. M. and LAW, K. (1950). *Biochem. J.* **46**, xxxvii.
145. RACKER, E. (1950). *Fed. Proc.* **9**, 217.
146. RACKER, E. (1951). *J. biol. Chem.* **190**, 685.
147. KRIMSKY, I. and RACKER, E. (1952). *J. biol. Chem.* **198**, 721.
148. RACKER, E. and KRIMSKY, I. (1952). *J. biol. Chem.* **198**, 731.
149. COLOWICK, S., LAZAROW, A., RACKER, E., SCHWARTZ, D. R., STADTMAN, E. and WAELSCH, H. (Editors) (1954). *Glutathione*. New York: Academic Press.
150. LYNEN, F. and REICHERT, F. (1951). *Angew. Chem.* **63**, 47.
151. LIPMANN, F. (1954). *Science*, **120**, 855.
152. HOAGLAND, M. B. and NOVELLI, G. D. (1954). *J. biol. Chem.* **207**, 767.
153. LEVINTOW, L. and NOVELLI, G. D. (1954). *J. biol. Chem.* **207**, 761.
154. NOVELLI, G. D. (1953). *Physiol. Rev.* **33**, 525.
155. GOVIER, W. M. and GIBBONS, A. J. (1951). *Arch. Biochem. Biophys.* **32**, 349.
156. JONES, M. E., LIPMANN, F., HILZ, H. and LYNEN, F. (1953). *J. Amer. chem. Soc.* **75**, 3285.

157. LYNEN, F. (1953). *Fed. Proc.* **12**, 683.
158. LYNEN, F. (1954). *Nature, Lond.* **174**, 962.
159. GREEN, D. E. (1954). *Biol. Rev.* **29**, 330.
160. LYNEN, F. (1955). *Annu. Rev. Biochem.* **24**, 653.
161. REED, L. J. (1953). *Physiol. Rev.* **33**, 544.
162. GUNSALUS, I. C. (1953). *J. cell. comp. Physiol.* **41**, 113.
163. GREEN, D. E. (1952). *Science*, **115**, 661.
164. SANADI, D. R., LITTLEFIELD, J. W. and BOCK, R. M. (1952). *J. biol. Chem.* **197**, 851.
165. SCHWEET, R. S. and CHESLOCK, K. (1952). *J. biol. Chem.* **199**, 749.
166. TANRET, C. (1909). *J. Pharm. Chim., Paris*, **30**, 145.
167. BARGER, G. and EWINS, A. J. (1911). *J. chem. Soc.* p. 2336.
168. HUNTER, G. and EAGLES, B. A. (1925). *J. biol. Chem.* **65**, 623.
169. BENEDICT, S. R., NEWTON, E. B. and BEHRE, J. A. (1926). *J. biol. Chem.* **67**, 267.
170. NEWTON, E. B., BENEDICT, S. R. and DAKIN, H. D. (1926). *Science*, **64**, 602.
171. HUNTER, G. and EAGLES, B. A. (1927). *J. biol. Chem.* **72**, 123.
172. LEONE, E. and MANN, T. (1951). *Nature, Lond.* **168**, 205.
173. MANN, T. and LEONE, E. (1953). *Biochem. J.* **53**, 140.
174. HEATH, H., RIMINGTON, C., GLOVER, T., MANN, T. and LEONE, E. (1953). *Biochem. J.* **54**, 606.
175. MELVILLE, D. B., HORNER, W. H., OTKEN, C. C. and LUDWIG, M. L. (1955). *J. biol. Chem.* **213**, 61.
176. MELVILLE, D. B., OTKEN, C. C. and KOVALENKO, V. (1955). *J. biol. Chem.* **216**, 325.
177. MELVILLE, D. B., HORNER, W. H. and LUBSCHEZ, R. (1954). *J. biol. Chem.* **206**, 221.
178. MELVILLE, D. B. and EICH, S. (1956). *J. biol. Chem.* **218**, 647.
179. BALDRIDGE, R. C. and LEWIS, H. B. (1953). *J. biol. Chem.* **202**, 169.
180. MELVILLE, D. B., EICH, S. and LUDWIG, M. L. (1956). *Fed. Proc.* **15**, 314.
181. MELVILLE, D. B. and HORNER, W. H. (1953). *J. biol. Chem.* **202**, 187.
182. HEATH, H. (1953). *Biochem. J.* **54**, 689.
183. WINGO, W. J. and LEWIS, H. B. (1946). *J. biol. Chem.* **165**, 339.

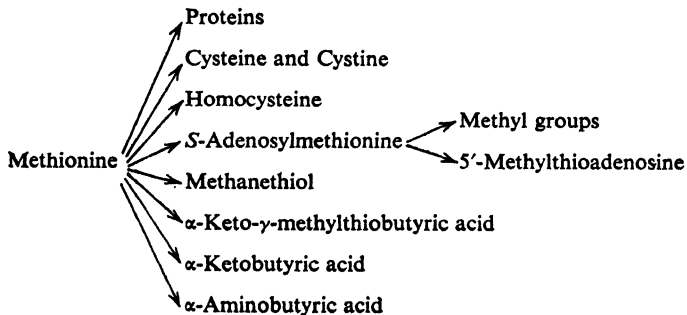
184. HILL, R. M. and LEWIS, H. B. (1924). *J. biol. Chem.* **59**, 557, 569.
185. WESTERMAN, B. D. and ROSE, W. C. (1928). *J. biol. Chem.* **79**, 423.
186. WESTERMAN, B. D. and ROSE, W. C. (1927). *J. biol. Chem.* **75**, 533.
187. WESTERMAN, B. D. and ROSE, W. C. (1928). *J. biol. Chem.* **79**, 413.
188. CANELLAKIS, E. S. (1952). *Fed. Proc.* **11**, 194.
189. CANELLAKIS, E. S. and TARVER, H. (1953). *Arch. Biochem. Biophys.* **42**, 446.
190. BLACK, S. and WRIGHT, N. G. (1956). *Fed. Proc.* **15**, 222.
191. SMITH, W. J. (1894). *Pflüg. Arch. ges. Physiol.* **57**, 418.
192. SNOW, G. A. (1956). *Biochem. J.* **63**, 9P.
193. PETERS, R. A., STOCKEN, L. A. and THOMPSON, R. H. S. (1945). *Nature, Lond.* **156**, 616.
194. WATERS, L. L. and STOCK, C. (1945). *Science*, **102**, 601.
195. YOUNG, L. (1946). *Science*, **103**, 439.
196. STOCKEN, L. A. and THOMPSON, R. H. S. (1949). *Physiol. Rev.* **29**, 168.
197. PETERS, R. A., SPRAY, G. H., STOCKEN, L. A., COLLIE, C. H., GRACE, M. A. and WHEATLEY, G. A. (1947). *Biochem. J.* **41**, 370.
198. SIMPSON, S. D. and YOUNG, L. (1950). *Biochem. J.* **46**, 634.
199. TAMBOLINE, B., MATHESON, A. T. and ZBARSKY, S. H. (1955). *Biochem. J.* **61**, 651.
200. STOCKEN, L. A. and THOMPSON, R. H. S. (1946). *Biochem. J.* **40**, 548.
201. SPRAY, G. H., STOCKEN, L. A. and THOMPSON, R. H. S. (1947). *Biochem. J.* **41**, 362

CHAPTER III

Thioethers

METHIONINE

Although methionine is one of the more recently discovered amino acids, much information has accumulated concerning its role in living organisms. This knowledge has not only transformed our ideas of sulphur metabolism, but it has had an important bearing on the understanding of other aspects of intermediary metabolism. The chief metabolic reactions which L-methionine is known to undergo, are summarized in the following diagram:



Methionine is incorporated into proteins, and in addition it participates in the formation of cysteine and cystine. It is also a source of the methyl groups required in the synthesis of many methylated compounds, such as choline, creatine and adrenaline. Furthermore, by its conversion to cysteine, methionine participates in several other reactions which have been described in the preceding chapter. Among these are the formation of taurine

and sulphate. In the animal, apart from protein synthesis, quantitatively the most important of the processes shown above are cysteine formation and the transfer of the *S*-methyl group. The formation of compounds such as methanethiol, α -ketomethylthiobutyric acid and α -ketobutyric acid has been observed under special conditions, although under normal conditions this probably occurs only to a small extent. The catabolites produced may, nevertheless, be of importance to the organism, and the relative significance of these reactions is an aspect of methionine metabolism which requires further investigation.

Desulphuration and transsulphuration

There appear to be at least four possible pathways by which sulphur can be removed from the methionine molecule: (i) transfer of sulphur to form cysteine and cystine, (ii) conversion to hydrogen sulphide, (iii) conversion to methanethiol, and (iv) transfer of the thiomethyl group to 5'-methylthioadenosine.

Cystine formation and transsulphuration. The conversion of methionine to cystine was the first aspect of methionine metabolism to be studied in detail. In a series of growth studies on rats Osborne and Mendel [1] in 1915 established the importance of cystine in animal nutrition, and for many years this amino acid was believed to be an indispensable constituent of the diet. The discovery of methionine as a sulphur-containing component of proteins was made by Mueller [2] in 1922, and its structure was established by Barger and Coyne [3] in 1928. Three years later Jackson and Block [4] made the important observation that methionine could support the growth of rats on the low-cystine diet of Osborne and Mendel as efficiently as cystine itself, and this was subsequently confirmed by other workers [5, 6]. This finding seemed at the time to indicate that in a nutritional sense the two amino acids were interchangeable. As a result of the work of Womack, Kemmerer and Rose [7], however, it is now recognized that whereas methionine is nutritionally indispensable, cystine need be present in the diet only if the amount of methionine is below a certain level. In these circumstances, cystine does become an essential dietary constituent. Cysteine formation is one of the principal metabolic reactions of methionine, and on account of this, the presence of cystine in the diet can have a

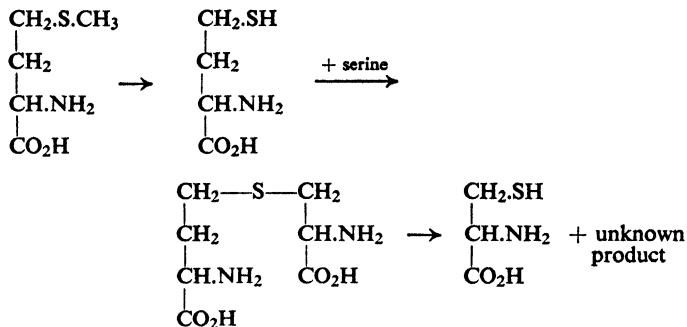
'methionine-sparing' action, in that it reduces the nutritional need for methionine [8].

Direct proof of the conversion of methionine to cystine was obtained by Tarver and Schmidt [9] in 1939, when they showed that cystine isolated from the newly-grown hair of rats fed [^{35}S]methionine contained significant amounts of the isotope. Some years later du Vigneaud, Kilmer, Rachele and Cohn [10] administered methionine labelled with a stable isotope of sulphur, ^{34}S , over a period of 35 days to rats fed on a cystine-free diet. From the hair grown during this period they were able to isolate [^{34}S]cystine in amounts suggesting that about 80% of the cystine had been synthesized from the methionine administered. Other evidence for this conversion includes the effect of methionine administration in increasing the cystine excretion of cystinurics [11, 12, 13] and in raising the cystine content of plasma [14]. Furthermore, Rose and Wood [15] found that the cystine content of the tissues of rats maintained on cystine-free diets supplemented with methionine was the same as that of rats fed on a cystine-containing diet. Methionine has also been shown to take part in the synthesis of taurocholic acid and mercapturic acids in animals [16, 17].

Various explanations have been advanced to account for the formation of cystine from methionine. Several groups of workers have suggested that demethylation is a preliminary step in the process. In support of this, du Vigneaud, Dyer and Harmon [18] showed that in the growing rat the cystine of the diet could be replaced by homocystine as effectively as by methionine. Brand and his coworkers [19] in 1936 made the suggestion that cystine formation might take place by demethylation of methionine to homocysteine, followed by transfer of the sulphur of homocysteine to a three-carbon compound, α -aminoacrylic acid, $\text{CH}_2\text{:C}(\text{NH}_2)\text{CO}_2\text{H}$. The possibility that α -aminoacrylic acid might be the source of the carbon chain of cysteine was first suggested by Nicolet [20]. Toennies [21] proposed an alternative scheme whereby methionine and serine react to give an intermediate sulphonium compound which breaks down to give cysteine.

The mechanism accepted at the present time embodies features from the schemes of both Brand and Toennies. It is considered that methionine is first demethylated to homocysteine, which

then reacts with serine to form an intermediate thioether. This compound is then split by a mechanism which is either reductive or hydrolytic, and yields cystine together with a second product:



In this way the sulphur of methionine is transferred to the three-carbon skeleton of serine, a process which has been described by Binkley [22] as transsulphuration. Stetten [23] obtained convincing evidence for the participation of serine in the reaction, for he found that when ^{15}N -labelled serine was fed to rats, the ^{15}N content of the cystine of the body proteins was 5% of that of the serine administered. This was five times as great as the ^{15}N content of the body glutamic acid, so that transfer of the isotope by an indirect process such as transamination could be discounted. Proof that the sulphur of methionine but not its carbon chain is used directly in cystine synthesis, was provided by the experiments of du Vigneaud, Kilmer, Rachele and Cohn [10]. These workers found that after administering $[\beta\text{-}, \gamma\text{-}^{13}\text{C}, ^{34}\text{S}]$ methionine to rats, the cystine of the freshly-grown hair was labelled with ^{34}S but contained negligible amounts of the carbon isotope.

The intermediate thioether, cystathionine, was synthesized in 1941 by Brown and du Vigneaud [24], and the L-isomer was found to substitute for cystine in the diet of the rat [25]. Du Vigneaud and his coworkers [26] subsequently demonstrated the conversion of L-cystathionine to cystine by showing that the cystine in the newly-grown hair of rats fed on the ^{35}S -labelled thioether was radioactive. Furthermore, in a cystinuric patient the ^{35}S -labelled thioether gave rise to radioactive cystine in the

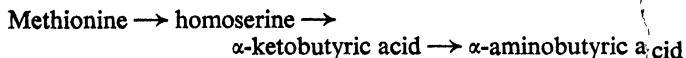
hair and in the urine [27]. By means of paper chromatography, [^{35}S]cystathionine and [^{35}S]cystine have since been identified in the livers of animals injected with [^{35}S]methionine [28]. The participation of this thioether in cystine synthesis would therefore seem to be established.

Methionine and homocysteine and certain of their derivatives (see p. 72) appear to be the only organic sulphur compounds which can act in the animal as precursors of cysteine. Several homologues of methionine, namely *S*-methylcysteine [29, 30], homomethionine, $\text{CH}_3\text{.S.[CH}_2\text{]}_3\text{.CH(NH}_2\text{).CO}_2\text{H}$ [31], and hexomethionine, $\text{CH}_3\text{.S.[CH}_2\text{]}_4\text{.CH(NH}_2\text{).CO}_2\text{H}$ [32], have been tested as substitutes for cystine in the diet of the rat, but they have proved to be quite ineffective.

Much of the information concerning cystine formation in the intact animal has been confirmed by experiments with tissue slices and extracts. Binkley and du Vigneaud [33] observed that cysteine was formed in small amounts when methionine was incubated with liver slices. There was an increased synthesis when homocysteine was substituted for methionine, and this was greatly enhanced by the further addition of serine. The reaction proceeded under anaerobic conditions and only took place when the *L*-isomers of the two amino acids were used. The cysteine formed was isolated as cystine and shown to have the *L*-configuration.

L-Cystathionine has also been shown to give rise to *L*-cysteine in liver slices and extracts [34]. The nature of the second cleavage product has not, however, been established with certainty. It seems probable that hydrolytic breakdown occurs rather than reduction, and that homoserine is formed rather than α -aminobutyric acid. The grounds for believing this are that the anaerobic incubation of cystathionine with rat-liver extracts leads to the formation of α -ketobutyric acid, and that under these conditions homoserine is converted to this keto acid, whereas α -aminobutyric acid and other possible reaction products, such as threonine and homocysteine, are not [35]. It is of interest that Matsuo and Greenberg [36] have shown that homoserine is a metabolite of methionine and is excreted in the urine. A further metabolite was identified as α -aminobutyric acid, which had previously been reported by Dent [37] to be a constituent of human blood and urine, and to be excreted in increased amounts after methionine

administration. This compound might be formed from methionine in the following manner:



Binkley [38, 39] has made a study of the cystathionine-splitting enzyme in rat liver and also the enzyme which promotes the synthesis of the thioether from homocysteine and serine. He has obtained evidence that these enzymes are activated by pyridoxal phosphate [40]. If this vitamin has a role in transsulphuration, then its absence from the diet of an animal might be expected to decrease the overall rate of cysteine synthesis from methionine. In these circumstances, there might be an accumulation and even an excretion of intermediary metabolites formed in those stages of the process not requiring pyridoxine. It is therefore of some interest that in the urine of pyridoxine-deficient rats, Chatagner and her associates [41] have detected a compound which behaves chromatographically like cystathionine.

In animals the transsulphuration reaction appears to be virtually irreversible [42]. A completely different situation exists in micro-organisms, however, many of which normally synthesize their methionine requirement from cystine or cysteine (see Chapter IX).

Other pathways of methionine desulphuration. The formation of hydrogen sulphide from methionine may possibly occur in animals, for if the demethylation of methionine to homocysteine can take place, then hydrogen sulphide might be expected to result from the action of the enzyme homocysteine desulphydrase (see p. 40).

The formation of methanethiol from methionine in mammalian tissue preparations was first reported by Binkley [43] and was later verified by Canellakis [44] using methionine labelled with ^{14}C in the methyl group and with ^{35}S . The α -keto analogue of methionine was found to give the thiol much more readily, and this suggested that the desulphuration of the amino acid might first involve a deamination to the corresponding α -keto acid. On the other hand, the reaction may well resemble the breakdown of cystathionine in that it may be a hydrolytic cleavage, giving rise to homoserine. Challenger and Walshe [45] have

recently identified methanethiol in the urine of a subject suffering from massive hepatic necrosis. The appearance of the thiol was accompanied by a marked rise in the level of plasma methionine, a condition which has been observed in other cases of severe parenchymal disease [46]. The formation of the thiol might be expected to take place when the main pathways of methionine metabolism, namely transmethylation and cysteine formation, are overloaded or defective, and when the concentration of methionine in the blood and tissues is markedly above normal.

A sulphur transfer reaction giving rise to 5'-methylthioadenosine takes place in yeasts, and is believed to involve the intermediate formation of 'active methionine' or *S*-adenosylmethionine. This process is discussed in more detail on page 91. Whether it occurs in animals is not known.

Demethylation and transmethylation

Research into the interrelationships between methionine and cystine led to the recognition of another important role of methionine in biochemistry, namely the indispensability of its *S*-methyl group in nutrition and metabolism. From this finding has developed the concept of transmethylation, or the biological transfer of intact methyl groups from one compound to another. Transmethylation has been discussed in detail in several reviews by du Vigneaud [27, 47, 48], whose research school has been responsible for setting it on a sound experimental basis.

In 1932, Butz and du Vigneaud [49] obtained the amino acid homocystine by the chemical demethylation of methionine, and they suggested that demethylation might be a preliminary step in the utilization of methionine by the animal. Demethylation was also considered as a likely catabolic step by Brand [11] and by Lewis [50], who proposed that the *S*-methyl group might not be removed oxidatively but might be used in the synthesis of other compounds, such as creatine.

The importance in metabolism of the methyl group of methionine became apparent from the observation of du Vigneaud and his coworkers [51, 52, 53] that methionine could not be replaced in the diet of the growing rat by homocysteine or homocystine unless certain other compounds were also present. These included the quaternary ammonium derivatives choline and glycine-betaine, and the conclusion was drawn that they were acting as

sources of methyl groups for the conversion of homocysteine to methionine. Furthermore, in view of the fact that rats could grow on methionine-supplemented diets which were free of choline and betaine, it was suggested that synthesis of these *N*-methylated compounds was taking place in the animal, with methionine supplying the necessary methyl groups. This carried the implication that the transfer of methyl groups between methionine and choline and betaine was biologically reversible [47].

Proof of methyl transfer from methionine to choline and other compounds was obtained by du Vigneaud and his coworkers [54] with the aid of trideuteromethionine,



which was added at a level of 1.4% to a diet containing purified amino acids in place of protein. Groups of rats were maintained on this diet and then killed. The choline and creatine of their tissues and the creatinine of their urine were isolated as derivatives, and were found to contain deuterium in the *N*-methyl groups in approximately equal amounts. The extent to which the isotope was incorporated into these compounds increased with the length of time the animals were maintained on the experimental diet. After 94 days, 87–89% of the *N*-methyl groups of the three compounds were found to have been derived from the deuteromethionine. This transfer of deuterium was considerably greater than would have occurred had the methyl groups first been oxidized to an intermediate such as formaldehyde and then been resynthesized to form choline- and creatine-methyl groups. This would have entailed the loss of at least one deuterium atom per trideuteromethyl group, and the incorporation of the isotope would not have exceeded 67%, as indicated below:



If formic acid had been the intermediate, the maximum possible incorporation of deuterium would have been even lower. Conversion of the methyl group to methanol was excluded by experiments with the labelled alcohol, which revealed negligible transference of its methyl group as such [55]. Clear proof of the biological transfer of the methionine-methyl group as an entity was obtained by Keller, Rachele and du Vigneaud [56], who fed a mixture of [*methyl*-¹⁴C]methionine and trideuteromethionine

to rats. They found that the ratio of deuterium to ^{14}C in the *N*-methyl groups of the tissue choline and creatine isolated agreed with the corresponding isotopic ratio in the methionine administered.

It is significant that transmethylation from methionine to choline occurs even when there is ample choline in the diet. The reaction is thus not a pathway of methionine catabolism brought into play in order to enable the animal to cope with unphysiological amounts of the amino acid, but is a normal and continuous metabolic process. The reverse reaction, namely the transfer of methyl groups from choline to methionine, has been demonstrated by the administration to rats of choline labelled with deuterium in the methyl groups, and the isolation from the tissue proteins of methionine labelled with deuterium in the *S*-methyl group [57].

Other *S*-methyl and *N*-methyl compounds which occur in animal tissues or are excreted in the urine, also appear to derive their methyl groups from methionine. For example, [*methyl*- ^{14}C] methionine injected into rats has been shown to give rise to [*methyl*- ^{14}C]adrenaline [58], and anserine containing deuterium in its *N*-methyl group has been isolated from the muscle of rabbits to which deuteromethionine had been orally administered [59]. Transmethylation has been demonstrated not only in the rat and rabbit but also in man [60], and there is abundant evidence that it operates in the dog, guinea pig and chick, as well as other species.

In recent years it has become apparent that methyl groups can be synthesized in the animal from various precursors, one of these being the amino acid serine. The β -carbon atom of serine can be metabolized to a one-carbon compound, probably formaldehyde, formic acid, or some closely related compound, which then forms a methionine-methyl group. This occurs only to a limited extent, however, and is unable to satisfy completely the needs of a growing animal for methyl groups.

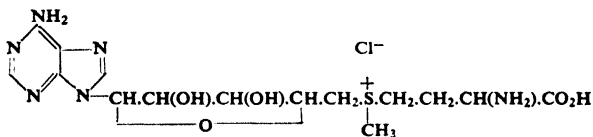
Important advances have resulted from a study of transmethylation *in vitro*. Borsook and Dubnoff [61] in 1940 showed that creatine was formed in small amounts from glycocyamine in the presence of rat-liver slices under aerobic conditions. This synthesis was enhanced by the addition of methionine or a mixture of choline and homocysteine to the medium [62]. It was also found

that in the presence of guinea pig-liver suspensions the formation of creatine from glycocyamine and methionine was more than doubled by the addition of either adenosine triphosphoric acid (ATP) or a source of this compound [63]. It was assumed that aerobic conditions were needed to ensure the continuous provision of ATP, which was involved in some way in the trans-methylation reaction. Nicotinamide has also been shown to undergo methylation by methionine in the presence of liver slices, and this reaction, in common with creatine synthesis, is dependent on the presence of oxygen [64].

A further development has come from the work of Cantoni and his coworkers, who found that ATP participates in the methylation of nicotinamide by methionine. Cantoni [65] showed, however, that ATP does not act as a phosphorylating agent under these conditions, but that it takes part in the conversion of methionine to a biochemically-active form before the methyl group is transferred from the amino acid. An enzyme system occurring in the liver of various animal species is capable of bringing about this activation according to the following equation [66]:



The presence of glutathione and magnesium ions is necessary for full activity of the enzyme. The compound, 'active methionine', contains no phosphorus, and from a study of its properties, Cantoni [67] concluded that it is a sulphonium compound, *S*-adenosylmethionine, with the following structure:



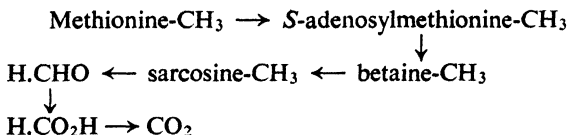
The correctness of this formula was established when *S*-adenosylmethionine was synthesized [68] and the synthetic product was shown to have the properties of the isolated material. Cantoni [69, 70] has shown that *S*-adenosylmethionine is able to bring about the direct methylation of both glycocyamine and nicotinamide in the presence of the appropriate enzymes (see p. 90). Before this work appeared it had been difficult to envisage the

mechanism by which methionine so readily gives up its methyl group. The finding that the active form of the amino acid is a methylsulphonium compound has greatly clarified the concept of transmethylation (see Chapter IV).

Oxidation of methionine-methyl groups

When methionine labelled with ^{14}C in the *S*-methyl group is administered orally to rats there is rapid oxidation of the *S*-methyl group and radioactive carbon dioxide soon makes its appearance in the expired air. Mackenzie and his coworkers [71, 72] found that almost one-third of a dose of 200 mg. of the labelled amino acid given to a rat underwent oxidation in this manner within 52 hours. Possible pathways for the reaction are that the methionine-methyl group might be oxidized directly while still attached to the amino acid, or alternatively after it has been split off as methanethiol. A more likely possibility is that oxidation occurs after the methyl group has been transferred to some other compound, for example choline or betaine, the methyl groups of which are known to be rapidly oxidized to carbon dioxide in the rat [73]. Further work by Mackenzie suggests that the formation of betaine is a preliminary step in the process, and that sarcosine, formaldehyde and possibly formic acid are also intermediates. By means of ^{14}C -labelling, the methyl groups of both methionine and betaine have been shown to give rise to the methyl group of sarcosine in the rat [74]. Furthermore, in the intact rat and in rat-liver preparations, sarcosine itself is converted to formaldehyde and formic acid and is oxidized ultimately to carbon dioxide [75].

These observations are embodied in the following scheme:



Work on the metabolism of formaldehyde and formic acid has revealed that these two compounds participate in a variety of synthetic reactions, including the biosynthesis of serine and glycine, and the formation of purines, porphyrins and other

compounds. In view of the diverse roles played by formaldehyde and formic acid, the fact that they are catabolites of methionine greatly increases the importance which must be attributed to this amino acid in intermediary metabolism.

Deamination and transamination

In common with the majority of other essential amino acids, methionine is capable of undergoing deamination and has been shown to serve as a substrate for the L-amino acid oxidases of rat liver and kidney [76] and of snake venom [77]. Waelsch and Borek [78, 79] and Bernheim, Bernheim and Gillaspie [80] have demonstrated that deamination of the amino acid occurs in kidney preparations with the formation of α -keto- γ -methylthiobutyric acid, $\text{CH}_3\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}\cdot\text{CO}_2\text{H}$, which they characterized as its 2:4-dinitrophenylhydrazone. This keto acid was the main metabolite produced in these particular experiments. It has also been found in the urine of rats fed large amounts of methionine [81].

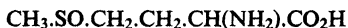
In recent years several groups of workers have confirmed the original observation of Braunstein and Kritzmann [82] that transfer of amino groups to various keto acids can occur from the majority of amino acids, including methionine. In extracts of heart muscle, liver and kidney from the rat and rabbit, methionine has been shown to donate its amino group to α -ketoglutaric acid and to pyruvic acid [83, 84]. Rowsell [85], using washed preparations of rat liver, has demonstrated transamination between methionine and pyruvic acid or α -keto-*n*-butyric acid, but not between the amino acid and either oxaloacetic acid or α -ketoglutaric acid. It is evident that several transaminases as well as L-amino acid oxidase itself may be involved in the deamination of methionine, and in fact, they are probably responsible for the major part of the deamination which has been observed *in vivo*.

Oxidation of the sulphur of methionine

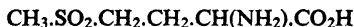
The two main end-products formed in the catabolism of the sulphur of methionine are taurine and sulphuric acid. In addition to being a component of bile acids, taurine is found in small amounts in urine. It appears to be derived only indirectly from methionine, the more immediate precursor being cysteine (see

Chapter II). The conversion of the amino acid to taurine was demonstrated by the appearance of [^{35}S]taurine in the bile after the administration of [^{35}S]methionine to dogs [86]. This confirmed the earlier experiments of Virtue and Doster-Virtue [87] (see Chapter V).

The oxidation of methionine to sulphate was reported by Mueller [88] soon after his discovery of the amino acid. He showed that the excretion of urinary inorganic sulphate increased after the administration of the compound to man, and similar results have been obtained by numerous other workers in experiments with animals of various species. A major part of administered methionine appears to be metabolized by this pathway. If direct oxidation of the thioether linkage were to take place, such intermediates as the sulfoxide and the sulphone of methionine,



and

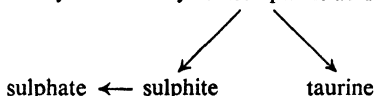


respectively, and possibly methanesulphonic acid, $\text{CH}_3.\text{SO}_3\text{H}$, would be formed, and these compounds would themselves be converted to sulphate in the animal. There is evidence to suggest that this is not the case. Neither methionine sulphone nor methanesulphonic acid when administered causes an increase in urinary sulphate excretion [89, 90], and the slight increase which the sulfoxide produces [91] is most probably the outcome of its preliminary reduction to methionine.

It is more likely that sulphate formation is a consequence of the transmethylation and transsulphuration of methionine, for these two reactions result in the formation of cysteine, which in turn is readily oxidized to sulphate in the liver. In rat-liver slices the oxidation of methionine to sulphate is markedly slower than that of its demethylation product, homocysteine, and slower still than that of cysteine [92]. The transfer of methyl groups is a slow process under these conditions, and it would seem to be the rate-limiting step in the oxidation. It is significant that Borek and Waelsch [79], when studying the metabolism of methionine in isolated tissues, found that the amount of the amino acid participating in the methylation of glycocyamine in rat-liver slices corresponded closely with the amount oxidized to sulphate under similar experimental conditions.

The oxidation of the sulphur of methionine may be represented as taking place through the following series of reactions:

Methionine $\rightarrow$ homocysteine $\rightarrow$ cysteine $\rightarrow$ cysteinesulphinic acid



Sulphate might also result from the oxidation of hydrogen sulphide formed in the desulphuration of homocysteine and cysteine, and also from the oxidation of methanethiol, a possible metabolite of methionine. However, there is no indication at present that these last two reactions are more than minor pathways in the oxidation of methionine-sulphur.

DERIVATIVES OF METHIONINE AND OTHER THIOETHERS

The importance of the L-isomer of methionine in biochemical processes has prompted many studies of the specificity of the amino acid in its various metabolic roles and the extent to which its chemical structure may be altered without loss of its biological activity. Attention has been given to the corresponding D-isomer and to related compounds such as the α -keto and α -hydroxy analogues, the corresponding sulfoxide and sulphone and the S-ethyl analogue, ethionine, as well as to a variety of S-substituted cysteines and homocysteines. Much of this work has provided an insight into the metabolism of L-methionine itself.

D-Methionine

In early nutritional studies on methionine, the racemic amino acid was found to show little difference in behaviour from the L-form. Growth experiments with the D-isomer have since shown that it may substitute for L-methionine in the diet of several species [93, 94, 95], although Bennett [96] has concluded that it is not utilized in rats quite as efficiently as the natural form. The ability of D-methionine to function in place of the L-isomer may be explained on the assumption that it undergoes optical inversion in the body. α -Keto- γ -methylthiobutyric acid is believed to be formed as an intermediate by the action of liver D-amino acid oxidase, for which D-methionine is known to be a good substrate [97]. The α -keto analogue acts as a dietary substitute for methionine [98] and as a methyl donor in creatine synthesis [99], pre-

sumably because it is reaminated to L-methionine. This also applies to the corresponding α -hydroxy acid,



which is believed to be oxidized first to the α -keto acid [100, 101].

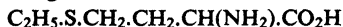
N-Substituted methionines

Substitution in the α -amino group of methionine does not necessarily destroy the biological activity of the amino acid. *N*-Methyl-DL-methionine, for example, possesses the growth-promoting properties of L-methionine [102] and is also extensively oxidized to sulphate in the animal [103]. This is undoubtedly due to the ability of both the D- and the L-isomers of this substituted methionine to act as substrates for D- and L-amino acid oxidases [104].

The metabolism of *N*-acyl derivatives of methionine is, however, dependent not only upon the nature of the substituent group, but also upon the optical configuration of the amino acid moiety. As an illustration of this, neither the D- nor the L-isomer of *N*-benzoylmethionine is metabolized to sulphate in the rabbit [105] and they are therefore unlikely to act as substitutes for methionine in the diet. *N*-Formyl-L-methionine is able to replace methionine in the diet of the rat [93] but *N*-formyl-D-methionine is unable to do so, and since D-methionine is known to be readily converted to the L-isomer *in vivo*, this must mean that the enzyme system responsible for the hydrolytic removal of the formyl group acts only on the L-derivative. Other examples of the utilization of *N*-acyl derivatives of an L-amino acid by animals and their failure to metabolize the corresponding *N*-acyl-D-isomers are also known [106, 107].

Ethionine

The replacement of the *S*-methyl group of methionine by an ethyl group produces a profound change in physiological properties. The resulting analogue, ethionine,

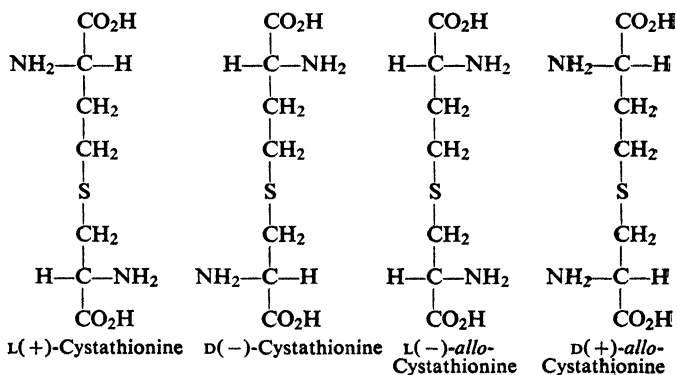


is incapable of replacing methionine in the diet [108], and its presence in the food causes a diminution in the growth rate of rats [109]. Furthermore, it has recently been shown to produce a rapid increase in the fatty acid content of the livers of fasted female rats [110]. These effects are counteracted by the

simultaneous administration of methionine, and this suggests that ethionine is acting as a metabolic antagonist. Stekol and Weiss [111], fed [*ethyl*- ^{14}C , ^{35}S]ethionine to rats, and found that the labelled carbon appeared in the creatinine fraction of the urine and the choline and creatine fractions of the tissues. Whether these reactions involved an actual transethylation, with the formation of the ethyl analogues of creatine and choline, or whether the *S*-ethyl groups were converted to methyl groups, is not known. The sulphur of ethionine is to some extent available for cysteine synthesis, since administration to rats of the labelled compound, together with bromobenzene, has led to the isolation from the urine of *p*-bromophenylmercapturic acid labelled with ^{35}S . The conversion of ethionine to cysteine must, however, be too slow to satisfy the normal cysteine requirements. There is some evidence that ethionine is incorporated into proteins, and that the abnormal proteins so formed accumulate in the liver [112].

Isomers of cystathionine and related compounds

A comparison of the biochemical properties of the four isomers of cystathionine [113], the structures of which are shown below, is of interest.

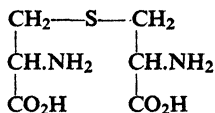


When added to a diet low in methionine and free of cystine, only *L*-*allocystathionine* in addition to *L*-cystathionine, was found to support the growth of rats. Furthermore, of the four isomers,

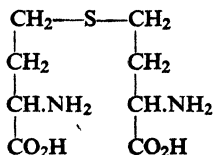
L-allocystathionine alone was able to support growth when added together with choline to a diet deficient in methionine. The results of these growth studies have been interpreted as indicating that *L-cystathionine* is metabolized in the animal to cysteine, whereas *L-allocystathionine* is converted to homocysteine.

With the aid of enzyme preparations from liver, Anslow and du Vigneaud [114] showed that while *L-cystathionine* was split to yield *L-cysteine*, the *D*-isomer underwent only very slight breakdown, a finding in harmony with the previous animal experiments. *L-allocystathionine* was cleaved almost quantitatively to homocysteine. In view of the configuration of the original thioether, this fission product must be *D*-homocysteine, which is known to be capable of replacing cystine in the diet [115] and probably of replacing methionine when choline has been added to the diet. The reason for the growth-promoting properties of *L-allocystathionine* is therefore clear. Liver extracts also brought about extensive breakdown of *D-allocystathionine*, but in this case the main product was cysteine. This compound would be expected to have the *D*-configuration. *D*-Cysteine and *D*-cystine, however, are incapable of replacing their *L*-isomers in the diet of the rat [116], and this provides a reasonable explanation for the biological inactivity of *D-allocystathionine*.

By treatment of wool with sodium sulphide, Küster and Irion [117] in 1929 obtained a thioether which would now appear to have been identical with cystathionine. Some years later a second compound of this type, to which the name lanthionine was given, was isolated by Horn, Jones and Ringel [118] from the products of acid hydrolysis of wool. The *meso* form of this symmetrical thioether was found to be incapable of replacing cystine in the diet of the rat [119]. On the other hand, the *DL*-form was able to replace cystine for growth purposes [120], the most probable explanation being that the *L*-isomer can undergo cleavage to *L*-cysteine.



Lanthionine



Homolanthionine

Homolanthionine, a homologue of lanthionine, also takes part in sulphur metabolism [121, 122], and in the diet of the rat is able to replace cystine, but surprisingly enough, not homocystine.

Eight *S*-substituted cysteines and five *S*-substituted homocysteines have been examined by the rat-growth technique [30, 123], but none of these compounds is able to replace dietary cystine. There is clearly a fairly high degree of structural specificity among the compounds capable of giving rise to cysteine and homocystine. Binkley [43] has investigated the metabolism of these thioethers using a partially purified preparation of the cystathionine-splitting enzyme, which he has named 'thionase'. Almost all the cysteine derivatives tested were acted upon to some extent by the preparation, and in general it was the bond between the sulphur and the cysteine-carbon chain which was broken with the formation of thiols, a finding which explains the behaviour of the thioethers in the animal. *S*-Methyl-L-cysteine, for example, was split to yield methanethiol and not cysteine. L-Cysteine itself gave hydrogen sulphide and, on account of this, it has been suggested that thionase is identical with cysteine desulphydrase. In the presence of larger amounts of the enzyme preparation, many *S*-substituted homocysteines were broken down [124]. Thus methionine and ethionine were converted to the corresponding thiols, homocystine itself gave hydrogen sulphide, and *S*-carboxymethyl-, *S*-carboxyethyl- and *S*-carboxypropyl-homocystine also gave breakdown products containing sulphydryl groups.

Deamination and acetylation of *S*-substituted cysteines and homocysteines

Certain of the *S*-substituted cysteines and homocysteines which have been tested as dietary substitutes for cystine have been found to increase the α -keto acid content of the urine. These include ethionine, *S*-phenylcysteine, *S*-benzylcysteine and *S*-benzylhomocystine [103]. When the last two compounds were fed to rabbits, the corresponding α -keto acids appeared in the urine. There is evidence that amino acid oxidases participate in the formation of these keto derivatives, for *S*-ethyl-L-cysteine and *S*-benzyl-L-cysteine will serve as substrates for the L-amino acid oxidases of rat kidney and liver [125], and *S*-ethyl-DL-cysteine

is acted upon by the D-amino acid oxidase from the same source [97]. On the other hand, S-carboxymethylcysteine does not give a significant increase in keto acid excretion when administered to rabbits, and is acted upon only to a slight extent by amino acid oxidases *in vitro*.

As described in Chapter II, various aromatic compounds undergo conjugation in the animal body to form N-acetyl-S-substituted L-cysteines, or mercapturic acids. The S-substituted L-cysteines are themselves acetylated in the organism, and mercapturic acid formation may therefore be regarded as another metabolic reaction of thioethers.

Stekol [126] and later, du Vigneaud, Wood and Binkley [127] demonstrated the occurrence of this reaction following the administration of S-(*p*-bromophenyl)-L-cysteine to rats, and S-phenyl-L-cysteine [128] and S-(*p*-chlorophenyl)-L-cysteine [129] have also been shown to undergo acetylation in the rat. Some indication of the general nature of the reaction is apparent from the work of West and Mathura [130], who reported the formation of mercapturic acids from a further twelve S-substituted L-cysteines. The process has also been shown to take place in man [131]. Furthermore, it is not confined to L-cysteine derivatives, for S-benzylhomocysteine is known to be acetylated in the rat and the rabbit [132, 133].

An interesting aspect of the reaction concerns the fate of various S-substituted derivatives of D-cysteine and D-homocysteine. A number of these compounds, in addition to being acetylated, undergo optical inversion, while others retain their configuration either partially or completely. During a study of the utilization of D-amino acids by the rat, du Vigneaud, Wood and Irish [133] found that S-benzyl-D-cysteine and S-benzyl-D-homocysteine were converted mainly into N-acetyl-S-benzyl-L-cysteine and N-acetyl-S-benzyl-L-homocysteine respectively. These transformations were considered to take place as the result of an initial deamination to give the corresponding α -keto acid, followed by an asymmetric reamination coupled with an acetylation to give the corresponding L-amino acid derivative. The S-benzyl-D-cysteine was also excreted to a small extent as the racemic acetyl derivative. When S-(*p*-bromophenyl)-D-cysteine was fed or injected [127], the corresponding N-acetyl derivative formed was predominantly of the D-configuration. It is clear, therefore,

that in addition to inversion by way of deamination, direct acetylation of a D-isomer can take place.

Binkley, Wood and du Vigneaud [134] further showed that *S*-phenyl-D-cysteine resembles the corresponding benzyl derivative in that it mainly undergoes inversion, whereas *S*-(*p*-bromobenzyl)-D-cysteine resembles the *S*-(*p*-bromophenyl) compound in being acetylated directly. On the other hand, *S*-(*p*-bromobenzyl)-D-homocysteine is almost completely inverted. Structural factors thus play an important role in the metabolism of these compounds. It is possible that the retention of configuration in a D-isomer, whenever it occurs, is an outcome of a slow rate of deamination, whereby direct acetylation has ample opportunity to take place.

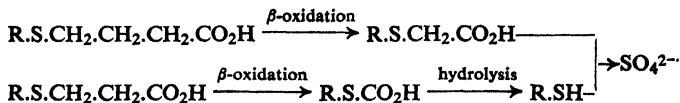
Oxidation of thioethers to sulphate

Several thioethers have been examined to determine whether they are metabolized to sulphate. In addition to methionine and certain isomers of cystathionine and lanthionine, a number of thioethers containing methylthio and ethylthio groups, when administered to animals, cause a substantial increase in urinary sulphate. These compounds include *S*-methyl-L-cysteine [135, 136], β -hydroxyethyl methyl sulphide [137], methylthioacetic acid, $\text{CH}_3\text{S}\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$, and ethylthioacetic acid, $\text{C}_2\text{H}_5\text{S}\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$ [137]. Simple sulphides, such as dimethyl and diethyl sulphides, do not appear to be oxidized [137, 138]. This is also true of other *S*-substituted cysteines and homocysteines which have been tested [103, 135, 139], as well as thiodiglycollic acid, $\text{S}(\text{CH}_2\cdot\text{CO}_2\text{H})_2$ [140], and phenylthioacetic acid, $\text{C}_6\text{H}_5\text{S}\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$ [92].

Those isomers of cystathionine and lanthionine which form sulphate do so most probably as a result of an initial fission of the thioether linkage leading to the formation of L-cysteine. With the other thioethers which are oxidized, there is again reason to believe that bond fission, leading to the formation of a more labile catabolite, takes place. The oxidation of *S*-methyl-L-cysteine, for example, does not proceed through the formation of cysteine or cystine, since the compound is unable to replace cystine in the diet of the rat [29, 30], but according to Binkley [43], it can be split enzymically by thionase to give methanethiol. The occurrence of this reaction as a preliminary catabolic step could well account for sulphate formation, as methanethiol has

been shown by Canellakis and Tarver [141] to be oxidized to sulphate.

It is possible that methylthioacetic acid and ethylthioacetic acid, both of which are oxidized by the intact rat and by rat-liver slices [92, 137], are also converted to the corresponding thiols by thionase. Methylthioacetic acid is unable to lose its methyl group by transfer to methyl acceptors *in vivo* [142], but there remains the possibility that the compound undergoes oxidative demethylation with the formation of formaldehyde and formic acid, and at the same time gives rise to thioglycollic acid or one of its partially-oxidized derivatives. Brown and Scholefield [143] showed that injection of the sodium salts of benzylthiopropionic acid and ethylthiopropionic acid into mice resulted in the appearance of phenylmethanethiol (benzyl mercaptan) and ethanethiol, respectively, in the expired air. Sodium benzylthioacetate and sodium ethylthioacetate, however, did not give rise to these thiols under the same conditions. By labelling the carboxyl group with ^{14}C , the carbon moieties of ethylthio fatty acids were shown to be oxidized by way of the tricarboxylic acid cycle. The oxidation was inhibited by fatty acids such as acetic acid, propionic acid and butyric acid, and also by malonic acid, fluoroacetic acid and 2:4-dinitrophenol. To account for the formation of thiols from alkylthiopropionic acids, as distinct from alkylthioacetic acids, the following scheme for the catabolism of thiofatty acids has been proposed [143]:



The idea that β -oxidation participates in the process was derived from the observation that, in the presence of mitochondrial preparations from guinea pig liver, ethylthioacetic acid forms a coenzyme A derivative [144].

REFERENCES

1. OSBORNE, T. B. and MENDEL, L. B. (1915). *J. biol. Chem.* **20**, 351.
2. MUELLER, J. H. (1922). *Proc. Soc. exp. Biol., N.Y.* **19**, 161.

3. BARGER, G. and COYNE, F. P. (1928). *Biochem. J.* **22**, 1417.
4. JACKSON, R. W. and BLOCK, R. J. (1931). *Science*, **74**, 414.
5. ROSE, W. C., KEMMERER, K. S., WOMACK, M., MERTZ, E. T., GUNTHER, J. K., McCOY, R. H. and MEYER, C. E. (1936). *J. biol. Chem.* **114**, lxxxv.
6. WHITE, A. and BEACH, E. F. (1937). *J. biol. Chem.* **122**, 219.
7. WOMACK, M., KEMMERER, K. S. and ROSE, W. C. (1937). *J. biol. Chem.* **121**, 403.
8. WOMACK, M. and ROSE, W. C. (1941). *J. biol. Chem.* **141**, 375.
9. TARVER, H. and SCHMIDT, C. L. A. (1939). *J. biol. Chem.* **130**, 67.
10. DU VIGNEAUD, V., KILMER, G. W., RACHELE, J. R. and COHN, M. (1944). *J. biol. Chem.* **155**, 645.
11. BRAND, E., CAHILL, G. F. and HARRIS, M. M. (1935). *J. biol. Chem.* **109**, 69.
12. TARVER, H. and SCHMIDT, C. L. A. (1947). *J. biol. Chem.* **167**, 387.
13. REED, L. J., CAVALLINI, D., PLUM, F., RACHELE, J. R. and DU VIGNEAUD, V. (1949). *J. biol. Chem.* **180**, 783.
14. BROWN, B. H. and LEWIS, H. B. (1941). *J. biol. Chem.* **138**, 717.
15. ROSE, W. C. and WOOD, T. R. (1941). *J. biol. Chem.* **141**, 381.
16. STEKOL, J. A. (1937). *J. biol. Chem.* **117**, 147.
17. STEKOL, J. A. (1937). *J. biol. Chem.* **118**, 155.
18. DU VIGNEAUD, V., DYER, H. M. and HARMON, J. (1933). *J. biol. Chem.* **101**, 719.
19. BRAND, E., BLOCK, R. J., KASSELL, B. and CAHILL, G. F. (1936). *Proc. Soc. exp. Biol., N.Y.* **35**, 501.
20. NICOLET, B. H. (1938). *J. Wash. Acad. Sci.* **28**, 84.
21. TOENNIES, G. (1940). *J. biol. Chem.* **132**, 455.
22. BINKLEY, F. (1944). *J. biol. Chem.* **155**, 39.
23. STETTEN, D. jun. (1942). *J. biol. Chem.* **144**, 501.
24. BROWN, G. B. and DU VIGNEAUD, V. (1941). *J. biol. Chem.* **137**, 611.
25. DU VIGNEAUD, V., BROWN, G. B. and CHANDLER, J. P. (1942). *J. biol. Chem.* **143**, 59.
26. RACHELE, J. R., REED, L. J., KIDWAI, A. R., FERGER, M. F. and DU VIGNEAUD, V. (1950). *J. biol. Chem.* **185**, 817.

27. DU VIGNEAUD, V. (1952). *A Trail of Research in Sulfur Chemistry and Metabolism and Related Fields*. Ithaca, New York: Cornell University Press.
28. TARVER, H. and TABACHNICK, M. (1953). *Fed. Proc.* **12**, 279.
29. BLOCK, R. J. and JACKSON, R. W. (1932). *J. biol. Chem.* **97**, cvi.
30. ARMSTRONG, M. D. and LEWIS, J. D. (1951). *J. biol. Chem.* **189**, 461.
31. DYER, H. M. and DU VIGNEAUD, V. (1935). *J. biol. Chem.* **108**, 73.
32. JONES, C. B. and DU VIGNEAUD, V. (1937). *J. biol. Chem.* **120**, 11.
33. BINKLEY, F. and DU VIGNEAUD, V. (1942). *J. biol. Chem.* **144**, 507.
34. BINKLEY, F., ANSLOW, W. P. jun. and DU VIGNEAUD, V. (1942). *J. biol. Chem.* **143**, 559.
35. CARROLL, W. R., STACY, G. W. and DU VIGNEAUD, V. (1949). *J. biol. Chem.* **180**, 375.
36. MATSUO, Y. and GREENBERG, D. M. (1955). *J. biol. Chem.* **215**, 547.
37. DENT, C. E. (1947). *Science*, **105**, 335.
38. BINKLEY, F. and OKESON, D. (1950). *J. biol. Chem.* **182**, 273.
39. BINKLEY, F. (1951). *J. biol. Chem.* **191**, 531.
40. BINKLEY, F., CHRISTENSEN, G. M. and JENSEN, W. N. (1952). *J. biol. Chem.* **194**, 109.
41. CHATAGNER, F., TABECHIAN, H. and BERGERET, B. (1954). *Biochim. biophys. Acta*, **13**, 313.
42. ARMSTRONG, M. D. and BINKLEY, F. (1948). *J. biol. Chem.* **174**, 889.
43. BINKLEY, F. (1950). *J. biol. Chem.* **186**, 287.
44. CANELLAKIS, E. S. (1952). *Fed. Proc.* **11**, 194.
45. CHALLENGER, F. and WALSH, J. M. (1955). *Biochem. J.* **59**, 372.
46. WALSH, J. M. (1953). *Quart. J. Med.* **22**, 483.
47. DU VIGNEAUD, V. (1942-43). *Harvey Lect.* **38**, 39.
48. DU VIGNEAUD, V. (1948). *Proc. Amer. phil. Soc.* **92**, 127.
49. BUTZ, L. W. and DU VIGNEAUD, V. (1932). *J. biol. Chem.* **99**, 135.
50. LEWIS, H. B. (1935). *J. Nutr.* **10**, 99.

51. DU VIGNEAUD, V., CHANDLER, J. P., MOYER, A. W. and KEPPEL, D. M. (1939). *J. biol. Chem.* **128**, cviii.
52. DU VIGNEAUD, V., DYER, H. M. and KIES, M. W. (1939). *J. biol. Chem.* **130**, 325.
53. DU VIGNEAUD, V., CHANDLER, J. P., MOYER, A. W. and KEPPEL, D. M. (1939). *J. biol. Chem.* **131**, 57.
54. DU VIGNEAUD, V., COHN, M., CHANDLER, J. P., SCHENK, J. R. and SIMMONDS, S. (1941). *J. biol. Chem.* **140**, 625.
55. DU VIGNEAUD, V., VERLY, W. G. L., WILSON, J. E., RACHELE, J. R., RESSLER, C. and KINNEY, J. M. (1951). *J. Amer. chem. Soc.* **73**, 2782.
56. KELLER, E. B., RACHELE, J. R. and DU VIGNEAUD, V. (1949). *J. biol. Chem.* **177**, 733.
57. SIMMONDS, S., COHN, M., CHANDLER, J. P. and DU VIGNEAUD, V. (1943). *J. biol. Chem.* **149**, 519.
58. KELLER, E. B., BOISSONNAS, R. A. and DU VIGNEAUD, V. (1950). *J. biol. Chem.* **183**, 627.
59. SCHENK, J. R., SIMMONDS, S., COHN, M., STEVENS, C. M. and DU VIGNEAUD, V. (1943). *J. biol. Chem.* **149**, 355.
60. SIMMONDS, S. and DU VIGNEAUD, V. (1942). *J. biol. Chem.* **146**, 685.
61. BORSOOK, H. and DUBNOFF, J. W. (1940). *J. biol. Chem.* **132**, 559.
62. BORSOOK, H. and DUBNOFF, J. W. (1945). *J. biol. Chem.* **160**, 635.
63. BORSOOK, H. and DUBNOFF, J. W. (1947). *J. biol. Chem.* **171**, 363.
64. PERLZWEIG, W. A., BERNHEIM, M. L. C. and BERNHEIM, F. (1943). *J. biol. Chem.* **150**, 401.
65. CANTONI, G. L. (1951). *J. biol. Chem.* **189**, 203.
66. CANTONI, G. L. (1951). *J. biol. Chem.* **189**, 745.
67. CANTONI, G. L. (1953). *J. biol. Chem.* **204**, 403.
68. BADDILEY, J. and JAMIESON, G. A. (1954). *Chem. & Ind.* p. 375.
69. CANTONI, G. L. (1952). *J. Amer. chem. Soc.* **74**, 2942.
70. CANTONI, G. L. and VIGNOS, P. J. jun. (1954). *J. biol. Chem.* **209**, 647.
71. MACKENZIE, C. G., CHANDLER, J. P., KELLER, E. B., RACHELE, J. R., CROSS, N., MELVILLE, D. B. and DU VIGNEAUD, V. (1947). *J. biol. Chem.* **169**, 757.

72. MACKENZIE, C. G., CHANDLER, J. P., KELLER, E. B., RACHELE, J. R., CROSS, N. and DU VIGNEAUD, V. (1949). *J. biol. Chem.* **180**, 99.
73. FERGER, M. F. and DU VIGNEAUD, V. (1950). *J. biol. Chem.* **185**, 53.
74. HORNER, W. H. and MACKENZIE, C. G. (1950). *J. biol. Chem.* **187**, 15.
75. MACKENZIE, C. G. (1950). *J. biol. Chem.* **186**, 351.
76. BLANCHARD, M., GREEN, D. E., NOCITO, V. and RATNER, S. (1945). *J. biol. Chem.* **161**, 583.
77. ZELLER, E. A. and MARITZ, A. (1944). *Helv. chim. acta*, **27**, 1888.
78. WAELSCH, H. and BOREK, E. (1939). *J. Amer. chem. Soc.* **61**, 2252.
79. BOREK, E. and WAELSCH, H. (1941). *J. biol. Chem.* **141**, 99.
80. BERNHEIM, F., BERNHEIM, M. L. C. and GILLASPIE, A. G. (1936). *J. biol. Chem.* **114**, 657.
81. WAELSCH, H. (1941). *J. biol. Chem.* **140**, 313.
82. BRAUNSTEIN, A. E. and KRITZMANN, M. G. (1937). *Enzymologia*, **2**, 129.
83. CAMMARATA, P. S. and COHEN, P. P. (1950). *J. biol. Chem.* **187**, 439.
84. AWAPARA, J. and SEALE, B. (1952). *J. biol. Chem.* **194**, 497.
85. ROWSELL, E. V. (1951). *Nature, Lond.* **168**, 104.
86. TARVER, H. and SCHMIDT, C. L. A. (1942). *J. biol. Chem.* **146**, 69.
87. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1937). *J. biol. Chem.* **119**, 697.
88. MUELLER, J. H. (1923). *J. biol. Chem.* **58**, 373.
89. WINGO, W. J., SMITH, R. A. and WOOD, J. (1953). *Arch. Biochem. Biophys.* **47**, 307.
90. MAW, G. A. (1953). *Biochem. J.* **55**, 37.
91. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1941). *J. biol. Chem.* **137**, 227.
92. MAW, G. A. (1954). *Biochem. J.* **58**, 665.
93. JACKSON, R. W. and BLOCK, R. J. (1937-38). *J. biol. Chem.* **122**, 425.
94. ROSE, W. C. (1938). *Physiol. Rev.* **18**, 109.
95. GRAU, C. R. and ALMQUIST, H. J. (1943). *J. Nutr.* **26**, 631.

96. BENNETT, M. A. (1939). *Biochem. J.* **33**, 885.
97. KREBS, H. A. (1935). *Biochem. J.* **29**, 1620.
98. CAHILL, W. M. and RUDOLPH, G. G. (1942). *J. biol. Chem.* **145**, 201.
99. HANDLER, P. and BERNHEIM, M. L. C. (1943). *J. biol. Chem.* **150**, 335.
100. BLOCK, R. J. and JACKSON, R. W. (1932). *J. biol. Chem.* **97**, cvi.
101. AKOBE, K. (1936). *Hoppe-Seyl. Z.* **244**, 14.
102. PATTERSON, W. I., DYER, H. M. and DU VIGNEAUD, V. (1936). *J. biol. Chem.* **116**, 277.
103. SHEN, C.-W. and LEWIS, H. B. (1946). *J. biol. Chem.* **165**, 115.
104. HANDLER, P., BERNHEIM, F. and KLEIN, J. R. (1941). *J. biol. Chem.* **138**, 203.
105. VIRTUE, R. W. and LEWIS, H. B. (1934). *J. biol. Chem.* **104**, 59.
106. DU VIGNEAUD, V., SEALOCK, R. R. and VAN ETEN, C. (1932). *J. biol. Chem.* **98**, 565.
107. DU VIGNEAUD, V., DYER, H. M. and JONES, C. B. (1937). *J. biol. Chem.* **119**, 47.
108. DYER, H. M. (1938). *J. biol. Chem.* **124**, 519.
109. STEKOL, J. A. and WEISS, K. (1949). *J. biol. Chem.* **179**, 1049.
110. JENSEN, D., CHAIKOFF, I. L. and TARVER, H. (1951). *J. biol. Chem.* **192**, 395.
111. STEKOL, J. A. and WEISS, K. (1950). *J. biol. Chem.* **185**, 577.
112. LEVINE, M. and TARVER, H. (1951). *J. biol. Chem.* **192**, 835.
113. ANSLOW, W. P. jun., SIMMONDS, S. and DU VIGNEAUD, V. (1946). *J. biol. Chem.* **166**, 35.
114. ANSLOW, W. P. jun. and DU VIGNEAUD, V. (1947). *J. biol. Chem.* **170**, 245.
115. DYER, H. M. and DU VIGNEAUD, V. (1935). *J. biol. Chem.* **109**, 477.
116. DU VIGNEAUD, V., DORFMANN, R. and LORING, H. S. (1932). *J. biol. Chem.* **98**, 577.
117. KÜSTER, W. and IRION, W. (1929). *Hoppe-Seyl. Z.* **184**, 225.
118. HORN, M. J., JONES, D. B. and RINGEL, S. J. (1941). *J. biol. Chem.* **138**, 141.
119. JONES, D. B., DIVINE, J. P. and HORN, M. J. (1942). *J. biol. Chem.* **146**, 571.

120. JONES, D. B., CALDWELL, A. and HORN, M. J. (1948). *J. biol. Chem.* **176**, 65.
121. STEKOL, J. A. and WEISS, K. (1948). *J. biol. Chem.* **175**, 405.
122. STEKOL, J. A. and WEISS, K. (1949). *J. biol. Chem.* **179**, 67.
123. STEVENS, C. M., JOHNSON, C. A. and WATANABE, R. (1955). *J. biol. Chem.* **212**, 49.
124. BINKLEY, F. (1951). *J. biol. Chem.* **192**, 209.
125. BLANCHARD, M., GREEN, D. E., NOCITO, V. and RATNER, S. (1944). *J. biol. Chem.* **155**, 421.
126. STEKOL, J. A. (1937-38). *J. biol. Chem.* **122**, 333.
127. DU VIGNEAUD, V., WOOD, J. L. and BINKLEY, F. (1941). *J. biol. Chem.* **138**, 369.
128. ZBARSKY, S. H. and YOUNG, L. (1943). *J. biol. Chem.* **151**, 217.
129. WEST, H. D., MATHURA, G. R. and BLACK, L. A. (1951). *J. biol. Chem.* **193**, 133.
130. WEST, H. D. and MATHURA, G. R. (1954). *J. biol. Chem.* **208**, 315.
131. STEKOL, J. A. (1946). *J. biol. Chem.* **164**, 651.
132. STEKOL, J. A. (1939). *J. biol. Chem.* **128**, 199.
133. DU VIGNEAUD, V., WOOD, J. L. and IRISH, O. J. (1939). *J. biol. Chem.* **129**, 171.
134. BINKLEY, F., WOOD, J. L. and DU VIGNEAUD, V. (1944). *J. biol. Chem.* **153**, 495.
135. PIRIE, N. W. (1932). *Biochem. J.* **26**, 2041.
136. DU VIGNEAUD, V., LORING, H. S. and CRAFT, H. A. (1934). *J. biol. Chem.* **105**, 481.
137. MAW, G. A. (1953). *Biochem. J.* **55**, 42.
138. SMITH, W. J. (1894). *Pflüg. Arch. ges. Physiol.* **55**, 542.
139. BLOOD, F. R. and LEWIS, H. B. (1941). *J. biol. Chem.* **139**, 407.
140. HILL, R. M. and LEWIS, H. B. (1924). *J. biol. Chem.* **59**, 557, 569.
141. CANELLAKIS, E. S. and TARVER, H. (1953). *Arch. Biochem. Biophys.* **42**, 387.
142. MAW, G. A. and DU VIGNEAUD, V. (1948). *J. biol. Chem.* **176**, 1037.
143. BROWN, W. T. and SCHOLEFIELD, P. G. (1954). *Biochem. J.* **58**, 368.
144. AVIGAN, J. and SCHOLEFIELD, P. G. (1954). *Biochem. J.* **58**, 374.

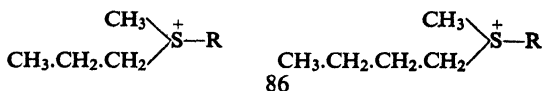
CHAPTER IV

Sulphonium Compounds, Sulphoxides and Sulphones

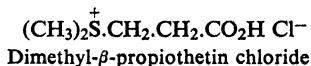
SULPHONIUM COMPOUNDS

It is only within the last few years that the biochemistry of sulphonium compounds has been given serious attention, although it has been known for a considerable time that these compounds possess certain of the pharmacological properties of the quaternary ammonium salts [1, 2, 3]. Several sulphonium salts have been isolated from plant and animal sources, and enzymes have been shown to exist which are concerned specifically with their utilization and catabolism. Some years before they were shown to occur naturally, Toennies [4] suggested that sulphonium compounds might be synthesized in cells, and he postulated the formation of a sulphonium derivative of methionine as an intermediate in cysteine synthesis. Although this particular reaction has not been substantiated experimentally, the views of Toennies on the biological significance of the sulphonium structure have nevertheless proved correct.

In 1895, Abel [5] reported that dog urine when treated with hot alkali gave rise to a volatile sulphur compound which he concluded was diethyl sulphide. A reinvestigation of this substance by Challenger and his associates [6], however, revealed that it was a mixture of methyl *n*-propyl sulphide and methyl *n*-butyl sulphide. These thioethers appear to be the breakdown products of two sulphonium compounds present in the urine. Their cations probably have the following structures, in which one of the substituent groups still remains to be identified:

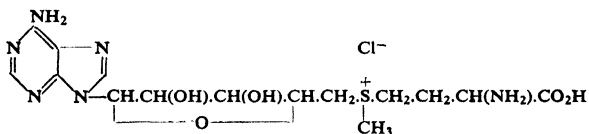


The first sulphonium compound to be isolated in pure form was obtained by Challenger and Simpson [7] from the red alga *Polysiphonia fastigiata*, and it proved to be a thetin, dimethyl- β -propiothetin.



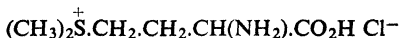
This substance was subsequently isolated from the green algae *Enteromorpha intestinalis* and *Spongomorpha arcta* by Bywood and Challenger [8].

In 1951, Cantoni [9, 10] came to the conclusion that methionine, before participating in transmethylation reactions, is converted to an active derivative by interaction with adenosine triphosphoric acid (see p. 68). Cantoni [11, 12] presented convincing evidence that the product formed possesses a sulphonium structure and is a salt of *S*-(5'-deoxyadenosine-5')-methionine, or *S*-adenosylmethionine.



S-(5'-deoxyadenosine-5')-methionine chloride

This structure has since been verified by synthesis of the compound [13]. By making use of the reaction whereby sulphonium compounds yield dimethyl sulphide when treated with hot sodium hydroxide, Challenger [14] demonstrated the wide distribution of these compounds in plants. One such substance, isolated from a variety of plants by Shive and his coworkers [15] and from asparagus by Challenger and Haywood [16], was shown to be a methionine methylsulphonium salt,



This compound also appears to play a role in bacterial metabolism [17].

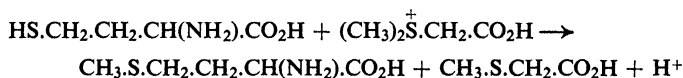
The participation of sulphonium compounds in transmethylation
Interest in the biochemistry of sulphonium compounds was an outcome of the study of biological transmethylation, a subject

which has been discussed in Chapter III. Moyer and du Vigneaud [18] found that a small number of compounds could act, like choline, in promoting the growth of rats fed on a 'methyl-deficient' diet supplemented with the amino acid homocystine. These compounds also prevented the appearance and development of the characteristic deficiency symptoms exhibited by animals fed on such a diet. One of these compounds was glycine betaine, and a second was its sulphur analogue, dimethylthetin chloride, $(\text{CH}_3)_2\overset{+}{\text{S}}\cdot\text{CH}_2\cdot\text{CO}_2\text{H Cl}^-$. Choline and glycine betaine were subsequently shown to transfer methyl groups in the animal to homocystine, probably after its reduction to homocysteine [19], and it was assumed that the thetin was behaving as a methyl donor in the same manner [20].

The ability of other sulphonium compounds to substitute for choline in the diet was investigated by Maw and du Vigneaud [21]. It was found that the naturally-occurring dimethyl- β -propiothetin salt also possessed this property, whereas diethylthetin chloride, $(\text{C}_2\text{H}_5)_2\overset{+}{\text{S}}\cdot\text{CH}_2\cdot\text{CO}_2\text{H Cl}^-$, which lacks the necessary methyl groups, did not. Ethylmethylthetin chloride, $(\text{C}_2\text{H}_5)(\text{CH}_3)\overset{+}{\text{S}}\cdot\text{CH}_2\cdot\text{CO}_2\text{H Cl}^-$, with only one available methyl group, showed some activity, in that it was able to promote growth in some but not all test animals, while trimethylsulphonium chloride, $(\text{CH}_3)_3\overset{+}{\text{S}}\text{ Cl}^-$, proved to be quite inactive. A rather surprising finding was that the sulphonium analogue of choline, sulphocholine, $(\text{CH}_3)_2\overset{+}{\text{S}}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH Cl}^-$, was unable to promote the growth of rats and was, moreover, a toxic substance. It was able, nevertheless, to prevent the characteristic liver and kidney lesions of methyl-deficiency, and appeared to be incorporated into the liver lipids, possibly in the same manner as choline itself [22].

The ability of methylthetins to act as methyl donors for methionine synthesis in animal tissues is due to the presence of a specific enzyme system concerned with the methyl transfer. This was first demonstrated by Dubnoff and Borsook [23], who showed that in the presence of suspensions of liver from the rat, pig and guinea pig, both dimethylthetin chloride and dimethylpropiothetin chloride were able to methylate homocysteine

under anaerobic conditions. Preparations of the transmethylyase catalysed the transfer of one methyl group from each molecule of thetin. The reaction taking place under physiological conditions, with dimethylthetin chloride as the methyl donor, is believed to be:



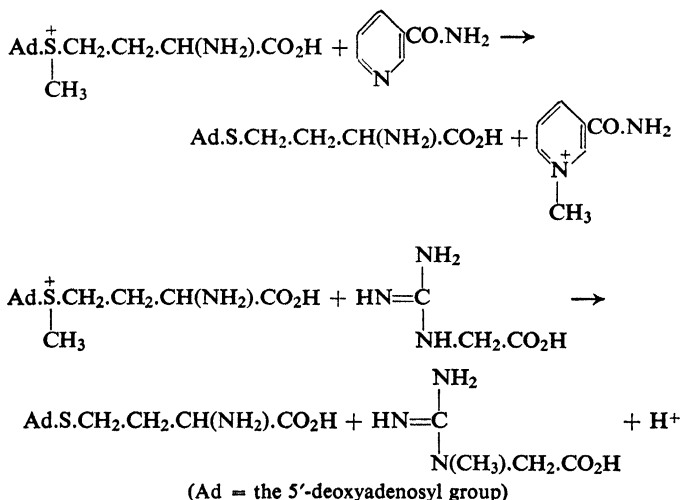
Methylthioacetic acid is formed as the demethylation product of the thetin. The liberation of a hydrogen ion is of some significance, for it means that when the reaction is carried out in a bicarbonate buffer medium, carbon dioxide is evolved and the course of the reaction may be followed manometrically. This procedure has been used by Maw [24] to study the properties of the thetin-homocysteine transmethylyase of rat liver.

The sulphonium derivatives of methionine

The position of methionine methylsulphonium salts in mammalian metabolism is far from clear. The balance of evidence is in favour of the conversion of these compounds to methionine, but this has not yet been shown to be an enzymic process. Bennett [25] found that methionine methylsulphonium chloride could promote the growth of rats on a methyl-deficient diet as efficiently as methionine itself, but that there was an initial period during which growth did not take place. The occurrence of this lag period was taken by Bennett to indicate the development of a mechanism in the rat for the demethylation of the sulphonium compound to methionine. The findings, however, might also be explained by the development of such a mechanism in the intestinal flora of the animals. According to Handler and Bernheim [26], methionine methylsulphonium chloride acts as a methyl donor to glycocyamine in liver slices, although Cohen [27] did not find this to occur in the presence of liver suspensions. In micro-organisms there is some evidence of the participation of methionine methylsulphonium salts in transmethylation, for in *Aerobacter aerogenes* and in the yeast *Torula utilis* these compounds appear to act as methyl donors to homocysteine [28, 29].

The work of Cantoni has revealed that *S*-adenosylmethionine is an important intermediate in transmethylation reactions in

the animal, and that it acts as a direct methyl donor to nicotinamide and glycocyamine, as shown in the following equations:

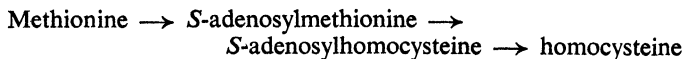


Each of these reactions requires an enzyme, a methyltransferase, which is present in mammalian liver and is specific for the particular methyl acceptor [11, 30]. Synthetic *S*-adenosylmethionine, first prepared by Baddiley and Jamieson [13], was found to have only half the activity of the natural material in these reactions [31], and this is probably attributable to the racemic nature of the synthetic compound.

Methionine methylsulphonium salts and also *S*-adenosylmethionine may be regarded as methyl- γ -butyrotethins, and they are therefore akin in structure to dimethylthetin and dimethylpropiothetin. This relationship is of considerable interest, for it means that all the known biological methyl donors are of the same chemical type, namely onium compounds. It explains the lability of the *S*-methyl group in methionine and it suggests that the methylation by methionine of nicotinamide, glycocyamine and other compounds is, in reality, very similar in mechanism to the methylation of homocysteine by thetins and by glycine betaine. All these reactions may be classified as substitu-

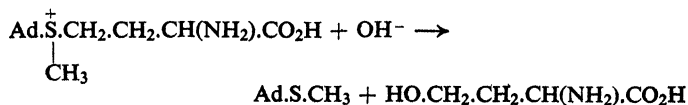
tion reactions occurring at a methyl carbon atom, a concept originally proposed by Challenger [32], and one which has since been discussed by Cantoni [33].

The demethylation product of *S*-adenosylmethionine has been identified as *S*-adenosylhomocysteine [34], and this compound has been shown by Ericson, Williams and Elvehjem [35] to be broken down in rat-liver preparations to homocysteine. The conversion of methionine to homocysteine, previously thought to be a simple demethylation, may now be regarded as taking place in at least three main steps:



Methionine may be formed to some extent in the animal by a reversal of this series of reactions, with formaldehyde and formic acid serving as a source of methyl groups for the methylation of *S*-adenosylhomocysteine.

S-Adenosylmethionine is also believed to participate in the formation of 5'-methylthioadenosine, a nucleoside known for some considerable time to be a metabolic product of yeasts [36, 37]. Recent work has shown that the accumulation of 5'-methylthioadenosine in yeasts is dependent upon the presence of an adequate supply of methionine in the culture medium [38, 39]. Furthermore, by the use of methionine labelled with ^{14}C and ^{35}S , Schlenk and Smith [40] found that the $\text{CH}_3\text{S}-$ group of the amino acid is incorporated into the nucleoside molecule, a process which these authors have termed transthiomethylation. Schlenk and Smith have proposed a mechanism for this reaction whereby *S*-adenosylmethionine is first formed as an intermediate, and then undergoes hydrolytic fission to the nucleoside and homoserine:



Baddiley, Cantoni and Jamieson [41] were able to bring about the hydrolytic step by careful treatment of *S*-adenosylmethionine with alkali.

The oxidation of sulphonium compounds

Studies have been made of the fate of sulphonium-sulphur in the animal. Maw [42] has shown that certain sulphonium salts when administered to rats are oxidized and give rise to an increase in the inorganic sulphate of the urine, although this is not a general mode of metabolism for this type of compound. For example, the chlorides of dimethylthetin, dimethyl- β -propiothetin, ethylmethylthetin and sulphocholine undergo oxidation to sulphate, whereas the chlorides of diethylthetin, trimethylsulphonium, triethylsulphonium and ethyldimethylsulphonium are not broken down in this way. There appears to be a correlation between the demethylation of these compounds and their catabolism to sulphate. In consequence, the loss of a methyl group to an acceptor, such as homocysteine, has been suggested as an initial step in their oxidation, as it is in the case of methionine [42]. Confirmation of this was obtained from the observation that dimethylthetin chloride does not give rise to sulphate when incubated aerobically with rat-liver slices unless homocysteine is also present [43]. Methylthioacetic acid, the demethylation product of the thetin is, however, readily oxidized under these conditions in the absence of homocysteine.

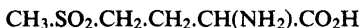
Further information on the metabolism of thetins has been obtained by labelling their methyl groups with ^{14}C . When dimethylthetin chloride or dimethylpropiothetin chloride, labelled in this way, is injected intraperitoneally into rats, the isotope rapidly appears in the carbon dioxide of the expired air [44]. The methyl groups are probably not oxidized while they are still part of the thetin molecule, but are catabolized after they have become incorporated in methionine and other compounds such as betaine (see p. 69).

SULPHOXIDES, SULPHONES AND RELATED COMPOUNDS

Thioethers may be oxidized in the laboratory to sulfoxides and sulphones, but this reaction does not appear to take place in biological systems. At one time the sulfoxide and sulphone of methionine were considered to be intermediates in the catabolism of methionine, but there is no proof that enzymes exist capable of forming or degrading these two compounds. According to Bennett [45], methionine sulfoxide, $\text{CH}_3\cdot\text{SO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$,

is able to replace methionine in the diet of the rat. This can be interpreted as indicating a reduction of the compound to methionine. The ease of oxidation of the compound to sulphate in the dog [46] and its ability to act as a precursor of taurine for conjugated bile acid formation [47] may also be explained in this way. The conversion has not been achieved with tissues *in vitro*, for in rat-liver slices the sulfoxide is not oxidized to sulphate [48], nor is it used as a methyl donor for creatine synthesis [26]. Bennett [49] also established that the disulphoxide of cystine, $(-\text{SO}.\text{CH}_2.\text{CH}(\text{NH}_2).\text{CO}_2\text{H})_2$, can substitute to some extent for dietary cystine, and a reduction of the sulfoxide group may again be postulated. There might be an alternative mechanism in this case, for the sulfoxide can decompose non-enzymically with the formation of cysteinesulphinic acid and cystine [50]. The compound is oxidized extensively to sulphate in the whole animal [51] and in tissue slices [52], and in dogs with biliary fistulae it has been shown to give rise to taurine [53].

The sulphone of methionine,



appears to be resistant to enzymic breakdown. A study of the fate of the ^{35}S -labelled compound in the rat by Wingo, Smith and Wood [54] has revealed that it is not oxidized to sulphate to any significant extent, but that it is excreted in the urine in at least three forms, one of which is a conjugate of glutamic acid. The sulphone had previously been found to be unable to replace methionine in the diet [25], or to act as a methyl donor in tissue preparations [26]. The absence of a mechanism in the animal for converting it to methionine has often been exploited in nutrition experiments, for if the protein component of an experimental diet is treated with hydrogen peroxide, the methionine present is oxidized to the sulphone, and the diet is thereby rendered methionine-deficient [55].

Several aliphatic disulphones, including sulphonal, trional and tetronal, were for some time used as hypnotics.



Sulphonal



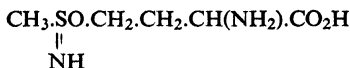
Trional



Tetronal

According to Baumann and Kast [56], who made a study of these compounds, there is some correlation between the pharmacological properties of the disulphones and their susceptibility to metabolic breakdown. Those with the general structure $\text{CH}_2(\text{SO}_2.\text{R})_2$ have virtually no hypnotic activity and are excreted mainly in the unchanged form. Those with the structure $\text{CRR}'(\text{SO}_2.\text{R}'')_2$ have greatest hypnotic activity and are extensively metabolized to compounds of unknown nature, while disulphones of the form $\text{CHR}(\text{SO}_2.\text{R}')_2$ occupy an intermediate position. Several aromatic sulphones are of value as antibacterial agents, and some are used in the chemotherapy of leprosy. These compounds undergo metabolic changes in the body, but the reactions concerned involve the ring structures and not the sulphone grouping.

Considerable attention has recently been centred on the sulphoximine of methionine.



Methionine sulphoximine

This compound is formed by the action of nitrogen trichloride on prolamines and is the toxic factor in agenzized flour. In the animal it is not converted to the parent amino acid and is not oxidized to any significant extent to inorganic sulphate [57]. There are good reasons for believing that the compound acts as a metabolic antagonist of methionine.

REFERENCES

1. CRUM BROWN, A. and FRASER, T. R. (1872). *Trans. roy. Soc. Edinb.* **7**, 663.
2. ING, H. R. and WRIGHT, W. M. (1931). *Proc. Roy. Soc. B.* **109**, 337.
3. HUNT, R. and RENSHAW, R. R. (1932). *J. Pharmacol.* **44**, 63.
4. TOENNIES, G. (1940). *J. biol. Chem.* **132**, 455.
5. ABEL, J. J. (1895). *Hoppe-Seyl. Z.* **20**, 253.
6. CHALLENGER, F., LEAVER, D. and WHITAKER, M. I. (1954). *Biochem. J.* **56**, ii.
7. CHALLENGER, F. and SIMPSON, M. I. (1948). *J. chem. Soc.* p. 1591.
8. BYWOOD, R. and CHALLENGER, F. (1953). *Biochem. J.* **53**, xxvi.

9. CANTONI, G. L. (1951). *J. biol. Chem.* **189**, 203.
10. CANTONI, G. L. (1951). *J. biol. Chem.* **189**, 745.
11. CANTONI, G. L. (1952). *J. Amer. chem. Soc.* **74**, 2942.
12. CANTONI, G. L. (1953). *J. biol. Chem.* **204**, 403.
13. BADDILEY, J. and JAMIESON, G. A. (1954). *Chem. & Ind.* p. 375.
14. BYWOOD, R., CHALLENGER, F., LEAVER, D. and WHITAKER, M. I. (1951). *Biochem. J.* **48**, xxx.
15. McRORIE, R. A., SUTHERLAND, G. L., LEWIS, M. S., BARTON, A. D., GLAZENER, M. R. and SHIVE, W. (1954). *J. Amer. chem. Soc.* **76**, 115.
16. CHALLENGER, F. and HAYWARD, B. J. (1954). *Chem. & Ind.* p. 729.
17. McRORIE, R. A., GLAZENER, M. R., SKINNER, C. G. and SHIVE, W. (1954). *J. biol. Chem.* **211**, 489.
18. MOYER, A. W. and DU VIGNEAUD, V. (1942). *J. biol. Chem.* **143**, 373.
19. SIMMONDS, S., COHN, M., CHANDLER, J. P. and DU VIGNEAUD, V. (1943). *J. biol. Chem.* **149**, 519.
20. DU VIGNEAUD, V., MOYER, A. W. and CHANDLER, J. P. (1948). *J. biol. Chem.* **174**, 477.
21. MAW, G. A. and DU VIGNEAUD, V. (1948). *J. biol. Chem.* **176**, 1037.
22. MAW, G. A. and DU VIGNEAUD, V. (1948). *J. biol. Chem.* **176**, 1029.
23. DUBNOFF, J. W. and BORSOOK, H. (1948). *J. biol. Chem.* **176**, 789.
24. MAW, G. A. (1956). *Biochem. J.* **63**, 116.
25. BENNETT, M. A. (1941). *J. biol. Chem.* **141**, 573.
26. HANDLER, P. and BERNHEIM, M. L. C. (1943). *J. biol. Chem.* **150**, 335.
27. COHEN, S. (1953). *J. biol. Chem.* **201**, 93.
28. SHAPIRO, S. K. (1955). *Biochim. biophys. Acta*, **18**, 134.
29. SCHLENK, F. and DEPALMA, R. E. (1955). *Arch. Biochem. Biophys.* **57**, 266.
30. CANTONI, G. L. and VIGNOS, P. J. jun. (1954). *J. biol. Chem.* **209**, 647.
31. CANTONI, G. L. (1955). *A Symposium on Amino Acid Metabolism*, p. 601. Ed. by McElroy, W. D. and Glass, B. Baltimore: The Johns Hopkins Press.

32. CHALLENGER, F. (1945). *Chem. Rev.* **36**, 315.
33. CANTONI, G. L. (1952). *Phosphorus Metabolism*, **2**, 129. Ed. by McElroy, W. D. and Glass, B. Baltimore: The Johns Hopkins Press.
34. CANTONI, G. L. and SCARANO, E. (1954). *J. Amer. chem. Soc.* **76**, 4744.
35. ERICSON, L.-E., WILLIAMS, J. N. jun. and ELVEHJEM, C. A. (1955). *Acta chem. scand.* **9**, 859.
36. SUZUKI, U. (1914). *J. Tokyo chem. Soc.* **34**, 1134.
37. SUZUKI, U., ODAKE, S. and MORI, T. (1924). *Biochem. Z.* **154**, 288.
38. WEYGAND, F., JUNK, R. and LEBER, D. (1952). *Hoppe-Seyl. Z.* **291**, 191.
39. SMITH, R. L. and SCHLENK, F. (1952). *Arch. Biochem. Biophys.* **38**, 167.
40. SCHLENK, F. and SMITH, R. L. (1953). *J. biol. Chem.* **204**, 27.
41. BADDILEY, J., CANTONI, G. L. and JAMIESON, G. A. (1953). *J. chem. Soc. p.* 2662.
42. MAW, G. A. (1953). *Biochem. J.* **55**, 42.
43. MAW, G. A. (1954). *Biochem. J.* **58**, 665.
44. FERGER, M. F. and DU VIGNEAUD, V. (1950). *J. biol. Chem.* **185**, 53.
45. BENNETT, M. A. (1939). *Biochem. J.* **33**, 1794.
46. PIRIE, N. W. (1932). *Biochem. J.* **26**, 2041.
47. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1941). *J. biol. Chem.* **137**, 227.
48. MEDES, G. and FLOYD, N. (1942). *Biochem. J.* **36**, 259.
49. BENNETT, M. A. (1937). *Biochem. J.* **31**, 962.
50. LAVINE, T. F. (1936). *J. biol. Chem.* **113**, 583.
51. MEDES, G. (1937). *Biochem. J.* **31**, 1330.
52. MEDES, G. (1939). *Biochem. J.* **33**, 1559.
53. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1939). *J. biol. Chem.* **127**, 431.
54. WINGO, W. J., SMITH, R. A. and WOOD, J. (1953). *Arch. Biochem. Biophys.* **47**, 307.
55. TOENNIES, G. (1942). *J. biol. Chem.* **145**, 667.
56. BAUMANN, E. and KAST, A. (1890). *Hoppe-Seyl. Z.* **14**, 52.
57. ROTH, J. S. (1954). *Arch. Biochem. Biophys.* **52**, 269.

Sulphonic Acids

Some of the aliphatic sulphonic acids, such as the α -aminosulphonic acids, are unstable, and their breakdown in the organism may well be an outcome of their instability rather than the operation of enzymic processes. A number of sulphonic acids which are chemically stable appear not to be metabolized when they are administered parenterally to animals, although when given by mouth they may be broken down by micro-organisms in the intestinal tract. The sulphonic acids to which biochemists have paid most attention are cysteic acid and taurine, and these two compounds are treated separately in the present chapter.

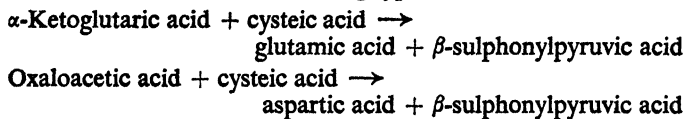
CYSTEIC ACID

Interest in cysteic acid, $\text{HO}_3\text{S}.\text{CH}_2.\text{CH}(\text{NH}_2).\text{CO}_2\text{H}$, has centred mainly around the question of whether this compound is an intermediate in the conversion of cysteine and cystine to taurine and to sulphate. The idea that cysteic acid and taurine might be derived in the animal body from cysteine and cystine arose from the work of Friedmann [1, 2] who, in 1902, in the course of establishing the structure of cysteine, prepared cysteic acid by the oxidation of cysteine with bromine, and then converted it to taurine by the action of heat. While it has been established that cysteine can be converted to taurine *in vivo*, recent findings suggest that cysteic acid plays only a minor role in this process.

Evidence that cysteine can give rise to cysteic acid was obtained in 1939 by Bernheim and Bernheim [3] and by Medes [4] who showed that enzyme preparations from rat liver bring about this oxidation. Although early attempts to demonstrate that cysteine-sulphinic acid is an intermediate in this process yielded conflicting results [4, 5], it has been reported more recently that direct oxidation of cysteine-sulphinic acid to cysteic acid does occur on a small scale in kidney [6].

The decarboxylation of cysteic acid was studied by Virtue and Doster-Virtue [7] who gave cysteic acid and cholic acid by mouth to dogs with biliary fistulae in experiments similar to those devised by Bergmann (see p. 99). Under these conditions there was a marked increase in the secretion of taurocholic acid. Dog liver was later shown by Blaschko [8] to contain an enzyme, cysteic acid decarboxylase, which brings about the conversion of cysteic acid to taurine under anaerobic conditions, and a similar enzyme was found by Medes and Floyd [9] in rat kidney. Notwithstanding these various findings there are some objections to the acceptance of cysteic acid as a major precursor of taurine. The chief of these is that although cysteic acid decarboxylase has been found in the liver of the rat, guinea pig and pig, it does not appear to be present in the liver of the cat, rabbit and cod, all of which, nevertheless, form taurocholic acid. This led Blaschko [10] to suggest that an alternative pathway of taurine synthesis might exist. The likelihood of this was increased by the finding that rabbit-liver suspensions and preparations from other tissues decarboxylate much more cysteinesulphinic acid than cysteic acid in a given period [6]. The decarboxylation of cysteinesulphinic acid gives β -aminoethanesulphinic acid, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SO}_2\text{H}$, and this compound has come to be regarded as a more important precursor of taurine than is cysteic acid.

Various experiments [9, 11, 12, 13] have been carried out in which cysteic acid has been administered to man and animals by mouth or by injection. It has been concluded that the small increased excretion of sulphate which is observed after cysteic acid has been given orally is a consequence of the activity of intestinal micro-organisms, for no increase is detected when the compound is injected. It is generally accepted, therefore, that cysteic acid is not an intermediate in the oxidation of cysteine and cystine to sulphate. In muscle preparations cysteic acid has been shown to take part in transamination reactions [14]. These are believed to be of the following type:



Little is known about the metabolism of β -sulphonylpyruvic acid,

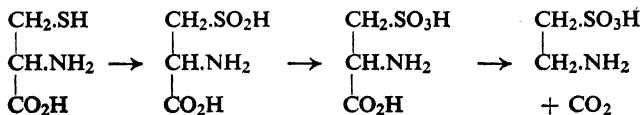
$\text{HO}_3\text{S}.\text{CH}_2.\text{CO}.\text{CO}_2\text{H}$, in animals. It does not appear to give rise to sulphate formation, and there are some indications that it may be excreted unchanged in the urine [9, 11].

TAURINE

Free taurine occurs in various types of organism, often in quite considerable amounts [15, 16, 17]. In mammals it appears in the bile in conjugation with bile acids [18], and it is also present in normal urine and faeces [19]. It is generally assumed that taurine is an end-product of sulphur metabolism, for there is no evidence to show that it is broken down in the tissues of man and higher animals, although it may be acted upon by intestinal micro-organisms [11, 12].

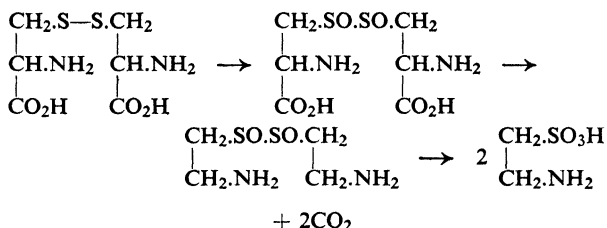
Friedmann's [1, 2] suggestion that cystine might serve as a precursor of taurine *in vivo* received support within a short time from the work of Bergmann [20]. Dogs with biliary fistulae were depleted of taurine by feeding them with cholic acid, and under these conditions taurocholic acid secretion almost ceased. Bergmann was able to restore the production of taurine by the administration of cystine. This finding was confirmed and extended by Foster [21, 22, 23] and by Virtue and Doster-Virtue [7, 24, 25] who showed that in experiments of the Bergmann type the biliary secretion of taurocholic acid was increased by the administration of methionine, cystine, cysteinesulphinic acid, cysteic acid, cystine disulphoxide, and homocysteine, but not homocystine. Proof of the conversion of cysteine to taurine was obtained by Awapara and Wingo [26] who injected [^{35}S]cysteine intravenously into rats and showed the presence of [^{35}S]taurine in the liver.

For many years the hypothesis that cysteic acid is the immediate precursor of taurine went unchallenged, and with the discovery that cysteinesulphinic acid can serve as a precursor of cysteic acid and also of taurine [7], the following series of reactions came to be considered the chief pathway of taurine formation:

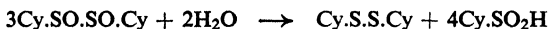


The evidence in support of this hypothesis has already been considered, as well as the objections to it (see p. 98).

The finding that cystine disulphoxide can give rise to taurine formation [7] indicated that this compound might possibly be an intermediate in the conversion of cystine to taurine [5, 9]. Decarboxylation of the disulphoxide would be expected to yield cystamine disulphoxide, and this latter compound was found to be readily oxidized by an enzyme preparation from liver, whereas cystamine itself was not oxidized [5]. This led Medes and Floyd [5, 9] to suggest the following route for the formation of taurine:



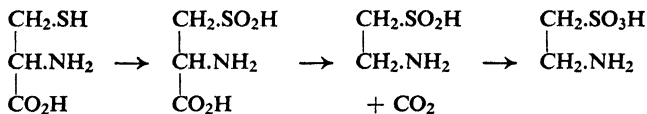
In his chemical studies of cystine disulphoxide, Lavine [27] found that this compound undergoes dismutative reactions with the formation of cysteinesulphinic acid and cystine, both of which are precursors of taurine in the animal body. The overall reaction can be represented as follows:



While it is uncertain whether reactions of this type occur *in vivo*, it is known that cystine disulphoxide is capable of substituting for cystine in the diet of rats [28, 29]. In these circumstances it seems likely that the formation of taurine from this disulphoxide occurs as a result of its conversion to cystine and possibly cysteinesulphinic acid rather than to cystamine disulphoxide.

Ideas concerning the mode of formation of taurine have undergone revision as a result of the discovery that β -aminoethanesulphinic acid is a product of cysteine metabolism. In 1951, in a study of the decarboxylation of cysteinesulphinic acid by rabbit-liver preparations, Chatagner and Bergeret [30, 31] observed the formation of a compound which they concluded had the formula $\text{H}_2\text{N.CH}_2\text{.CH}_2\text{.SO}_2\text{H}$. This compound, β -aminoethanesulphinic

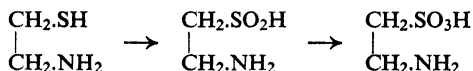
acid, to which they gave the name 'hypotaurine', was also found to be present in the livers of rats dosed intravenously with cysteine-sulphinic acid [32], and in 1953 it was reported to be capable of being oxidized to taurine [33]. In the same year, Awapara [34] described the isolation of β -aminoethanesulphinic acid from the livers of rats to which he had given large doses (400 mg.) of cysteine by intravenous injection, and he established the identity of the compound by comparison with a synthetic sample. More recently Cavallini and his coworkers [35] have succeeded in isolating β -aminoethanesulphinic acid in pure form from the urine of rats fed on a diet supplemented with cystine. The formation of the sulphinic acid has been shown by Awapara and Wingo [26] to occur in rats dosed intravenously with [^{35}S]cysteine. Ten minutes after the compound had been administered, the livers of these animals contained [^{35}S]cysteine, but labelled taurine and β -aminoethanesulphinic acid were not present in detectable amounts. After 20 minutes [^{35}S] β -aminoethanesulphinic acid was present, and [^{35}S]taurine appeared within 30 minutes. [^{35}S]Cysteic acid was not detected in the liver in these experiments. From these and other observations it has been concluded that in the rat taurine can be formed by the oxidation of β -aminoethanesulphinic acid, which arises from the decarboxylation of cysteinesulphinic acid:



These findings do not altogether exclude cysteic acid as a precursor of taurine. Awapara and Wingo [26] found that when a dose of 400 mg. of cysteic acid was injected into a rat it did give rise to taurine, and there is some likelihood that the formation of cysteic acid provides an alternative pathway for the conversion of cysteine to taurine. Oxidation of cysteinesulphinic acid to cysteic acid does not occur readily, however, and furthermore cysteinesulphinic acid is decarboxylated by tissue preparations more rapidly than cysteic acid under the same conditions [6, 36]. While there is no doubt that in some animals, at least, taurine can be formed from cysteic acid, it now appears probable that

β -aminoethanesulphinic acid is a more important precursor of this compound.

Work by Eldjarn [37, 38] on the metabolism of cysteamine and cystamine suggests that these compounds may also have a role in taurine synthesis. In experiments in which he administered [^{35}S]cysteamine to rats he found that [^{35}S]taurine was excreted in the urine. Although labelled cysteamine and cystamine were both converted to sulphate, about half the ^{35}S of the urine was present in the organic sulphur fraction. Similar findings with [^{35}S]cysteamine have been reported for the dog [39]. The formation of [^{35}S]taurine from [^{35}S]cystamine was shown by Eldjarn [37] to occur in rat-liver slices, and by the use of radiochromatographic methods he was able to demonstrate that [^{35}S] β -aminoethanesulphinic acid was also produced. He has suggested that cysteamine may be liberated from coenzyme A and oxidized to β -aminoethanesulphinic acid which undergoes further oxidation to yield taurine:



The formation of taurine in the rat appears to be dependent on the presence of vitamin B₆ in the diet. In rats on a diet deficient in pyridoxine the liver does not show cysteic acid decarboxylase activity [40, 41] and the decarboxylation of cysteinesulphinic acid by the tissues is also suppressed [6]. These findings offer an explanation for the absence of taurine from the urine of rats on a vitamin B₆-deficient diet, whereas it is present in the urine when the animals are on a normal diet [41].

An interesting development in the study of taurine metabolism has been the discovery that inorganic sulphate-sulphur can give rise to taurine-sulphur. In 1952 Boström and Åqvist [42] reported that after injecting ^{35}S -labelled sodium sulphate intraperitoneally into adult rats, ^{35}S was present in the taurine isolated from the liver, but there was no evidence that ^{35}S had been used in the synthesis of cystine and methionine. The amount of ^{35}S incorporated into the taurine, although significant, was small, and it would appear that inorganic sulphate is not an important source of taurine-sulphur in the rat. In the developing chick embryo, however, the situation is very different. Machlin, Pearson and

Denton [43] showed recently that about 65% of the ^{35}S administered as inorganic sulphate to a 24-hour embryo appeared as taurine-sulphur in the day-old chick whereas none was present in cystine, methionine and cysteic acid. Lowe and Roberts [44] studied the distribution of ^{35}S at intervals between 30 minutes and 24 hours after the injection of [^{35}S]sulphate into chick embryos, and they found that it was incorporated rapidly into taurine and a number of other substances, but not into cystine, methionine and glutathione. The mechanism of the process whereby sulphate-sulphur becomes incorporated into taurine has yet to be established, but the fact that this occurs without sulphate-sulphur appearing in the sulphur-containing amino acids and cysteic acid lends considerable interest to this problem.

OTHER ALIPHATIC SULPHONIC ACIDS

Salkowski [45, 46, 47] reported that sulphate and thiosulphate were formed from sodium isethionate, $\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{SO}_3\text{Na}$, aminomethanesulphonic acid, $\text{H}_2\text{N}\cdot\text{CH}_2\cdot\text{SO}_3\text{H}$, and taurine, when these compounds were given orally to dogs and rabbits. Aminomethanesulphonic acid is unstable, however, and in an aqueous medium at 37° it readily breaks down with the formation of sulphite and sulphate [48]. Furthermore, Schmidt and Clark [11] were unable to confirm that sodium isethionate and taurine are oxidized in the dog, and Maw [48] found no evidence for the oxidation of these compounds to sulphate when they were administered subcutaneously to the rat. It seems probable that the oxidation of these compounds in Salkowski's experiments was the result of the activity of the intestinal flora. No increase in sulphate excretion occurred when sodium *n*-octanesulphonate was given to dogs by mouth and it was possible to isolate the acid from the urine as its barium salt [49]. Similar results were obtained by Maw [48] after injecting rats subcutaneously with the sodium salts of methanesulphonic acid, iodomethanesulphonic acid, sulphoacetic acid and β -sulphopropionic acid. These compounds appeared to be excreted for the most part unchanged, and three of them were recovered from the urine in about 70% yield in the form of their phenylhydrazine salts.

In a study of the oxidation of aliphatic sulphonc acids by rat-liver preparations, Heimberg, Fridovich and Handler [50] found that among the compounds resistant to oxidation were cysteic

acid, taurine, *N*-methyltaurine and the bisulphite derivatives of *n*-propionaldehyde and acetone. Under the same conditions a number of α -hydroxy-, α -amino-, and *N*-substituted α -amino-sulphonic acids were oxidized. The results obtained suggested that there was a preliminary enzymic cleavage of the compounds with the formation of carbonyl compounds and bisulphite, and that the bisulphite was then oxidized enzymically to sulphate. It has been suggested [50] that the desmolytic enzyme might be needed in the organism to bring about the breakdown of addition compounds formed from bisulphite produced during cysteine metabolism, and from carbonyl compounds which arise in other metabolic processes.

AROMATIC SULPHONIC ACIDS

The information at present available indicates that the sulphur of the aromatic sulphonic acids is not oxidized to sulphate in the animal body. Salkowski [51] described the isolation of unchanged benzenesulphonic acid from the urine of dogs injected with this compound. The phenolic group of *p*-hydroxybenzenesulphonic acid does not appear to undergo conjugation *in vivo* [52] and the unchanged compound is excreted in the urine [53]. The outcome is different when the *p*-substituent is an amino group, for sulphanilic acid, like its carboxy analogue, is converted to an acetyl amino derivative in the animal body [54]. It has been reported that 1-hydroxy-2-amino-, 1-amino-2-hydroxy-, and 1-amino-3-hydroxy-benzene-4-sulphonic acid are probably excreted unchanged when administered to the rabbit [52].

REFERENCES

1. FRIEDMANN, E. (1902). *Beitr. chem. Physiol. Path.* 2, 433.
2. FRIEDMANN, E. (1903). *Beitr. chem. Physiol. Path.* 3, 1.
3. BERNHEIM, F. and BERNHEIM, M. L. C. (1939). *J. biol. Chem.* 127, 695.
4. MEDES, G. (1939). *Biochem. J.* 33, 1559.
5. MEDES, G. and FLOYD, N. (1942). *Biochem. J.* 36, 259.
6. FROMAGEOT, C. (1953-54). *Harvey Lect.* 49, 1.
7. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1939). *J. biol. Chem.* 127, 431.
8. BLASCHKO, H. (1942). *Biochem. J.* 36, 571.

9. MEDES, G. and FLOYD, N. (1942). *Biochem. J.* **36**, 836.
10. BLASCHKO, H. (1945). *Advanc. Enzymol.* **5**, 67.
11. SCHMIDT, C. L. A. and CLARK, G. W. (1922). *J. biol. Chem.* **53**, 193.
12. WHITE, F. R., LEWIS, H. B. and WHITE, J. (1937). *J. biol. Chem.* **117**, 663.
13. MEDES, G. (1937). *Biochem. J.* **31**, 1330.
14. COHEN, P. P. (1940). *J. biol. Chem.* **136**, 565.
15. AWAPARA, J., LANDUA, A. J. and FUERST, R. (1950). *Biochim. biophys. Acta*, **5**, 457.
16. WALKER, D. M. (1952). *Biochem. J.* **52**, 679.
17. AWAPARA, J. (1956). *J. biol. Chem.* **218**, 571.
18. HASLEWOOD, G. A. D. (1955). *Physiol. Rev.* **35**, 178.
19. STEIN, W. H. (1953). *J. biol. Chem.* **201**, 45.
20. VON BERGMANN, G. (1904). *Beitr. chem. Physiol. Path.* **4**, 192.
21. FOSTER, M. G. and HOOPER, C. W. (1919). *J. biol. Chem.* **38**, 355.
22. FOSTER, M. G., HOOPER, C. W. and WHIPPLE, G. H. (1919). *J. biol. Chem.* **38**, 379.
23. FOSTER, M. G., HOOPER, C. W. and WHIPPLE, G. H. (1919). *J. biol. Chem.* **38**, 421.
24. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1937). *J. biol. Chem.* **119**, 697.
25. VIRTUE, R. W. and DOSTER-VIRTUE, M. E. (1939). *J. biol. Chem.* **128**, 665.
26. AWAPARA, J. and WINGO, W. J. (1953). *J. biol. Chem.* **203**, 189.
27. LAVINE, T. F. (1936). *J. biol. Chem.* **113**, 583.
28. MEDES, G. (1935). *J. biol. Chem.* **109**, lxiv.
29. BENNETT, M. A. (1937). *Biochem. J.* **31**, 962.
30. CHATAGNER, F. and BERGERET, B. (1951). *C.R. Acad. Sci., Paris*, **232**, 448.
31. BERGERET, B. and CHATAGNER, F. (1952). *Biochim. biophys. Acta*, **9**, 141.
32. BERGERET, B., CHATAGNER, F. and FROMAGEOT, C. (1952). *Biochim. biophys. Acta*, **9**, 147.
33. TABECHIAN, H., BERGERET, B. and CHATAGNER, F. (1953). *Bull. Soc. Chim. biol., Paris*, **35**, 615.
34. AWAPARA, J. (1953). *J. biol. Chem.* **203**, 183.

35. CAVALLINI, D., MONDOVI, B. and DEMARCO, C. (1955). *J. biol. Chem.* **216**, 577.
36. HOPE, D. B. (1955). *Biochem. J.* **59**, 497.
37. ELDJARN, L. (1954). *Scand. J. clin. Lab. Invest.* **6**, Supp. 13.
38. ELDJARN, L. (1954). *J. biol. Chem.* **206**, 483.
39. VERLY, W. G. and KOCH, G. (1954). *Biochem. J.* **58**, 663.
40. BLASCHKO, H., CARTER, C. W., O'BRIEN, J. R. P. and SLOANE STANLEY, G. H. (1948). *J. Physiol.* **107**, 18P.
41. BLASCHKO, H., DATTA, S. P. and HARRIS, H. (1953). *Brit. J. Nutr.* **7**, 364.
42. BOSTRÖM, H. and ÅQVIST, S. (1952). *Acta chem. scand.* **6**, 1557.
43. MACHLIN, L. J., PEARSON, P. B. and DENTON, C. A. (1955). *J. biol. Chem.* **212**, 469.
44. LOWE, I. P. and ROBERTS, E. (1955). *J. biol. Chem.* **212**, 477.
45. SALKOWSKI, E. (1873). *Virchows Arch.* **58**, 460, 580.
46. SALKOWSKI, E. (1876). *Virchows Arch.* **66**, 315.
47. SALKOWSKI, E. (1918). *Biochem. Z.* **89**, 178.
48. MAW, G. A. (1953). *Biochem. J.* **55**, 37.
49. FLASCHENTRÄGER, B., BERNHARD, K., LÖWENBERG, C. and SCHLÄPFER, M. (1934). *Hoppe-Seyl. Z.* **225**, 157.
50. HEIMBERG, M., FRIDOVICH, I. and HANDLER, P. (1953). *J. biol. Chem.* **204**, 913.
51. SALKOWSKI, E. (1871). *Pflüg. Arch. ges. Physiol.* **4**, 91.
52. SAMMONS, H. G., SHELSWELL, J. and WILLIAMS, R. T. (1941). *Biochem. J.* **35**, 557.
53. BAUMANN, E. and HERTER, E. (1877). *Hoppe-Seyl. Z.* **1**, 244.
54. ZEHENDER, F. (1943). *Helv. chim. acta*, **26**, 1338.

CHAPTER VI

Sulphuric Acid Esters

For many years the only sulphuric acid esters studied from a metabolic standpoint were the arylsulphuric acids, and even at the present time there are still some types of sulphuric acid ester, such as the alkylsulphuric acids and the dialkyl sulphates, about whose metabolism almost nothing is known. Partly as a result of the availability of ^{35}S attention is now being paid to the metabolism of the sulphomucopolysaccharides and the steroid sulphates, and in the present chapter an account is given of the metabolism of these compounds together with that of the arylsulphuric acids.

ARYLSULPHURIC ACIDS

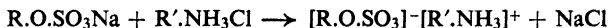
Biosynthesis

In 1876 Baumann [1, 2] established that formation of arylsulphuric acids takes place in the animal body. He isolated phenylsulphuric acid as its potassium salt from human urine, and he then went on to show that excretion of arylsulphuric acids follows the administration of various phenols as well as compounds which give rise to phenols *in vivo*.



The synthesis of arylsulphuric acids appears to have been observed in every species of higher animal in which it has been tested. The formation of these compounds provides an important pathway for the removal of phenols from the body, for in addition to being less toxic than their parent phenols, they pass readily into the urine. The difference between the amounts of inorganic sulphate and total sulphate in the urine has come to be known as the 'ethereal sulphate' content of the urine. It has been found that the small ethereal sulphate fraction in normal urine includes

tyrosine-*O*-sulphate [3] together with various sulphuric acid conjugates formed from phenolic compounds produced by intestinal bacteria. The magnitude of this fraction is usually increased after the administration of an aromatic compound, and this type of observation forms the basis of most of the claims to be found in the literature for the metabolic conversion of aromatic compounds to sulphuric acid esters. This arises from the fact that the isolation of arylsulphuric acids from urine is not readily accomplished. One difficulty encountered is the ease with which many of these compounds undergo hydrolysis when in the free acid form, and this led early workers to attempt to isolate them as their sodium or potassium salts. Laughland and Young [4] drew attention to the fact that arylsulphuric acids can be separated from urine in the form of their salts with certain organic bases such as *p*-toluidine, and Barton and Young [5] showed that salts of this type can be precipitated from an aqueous medium by exploiting the following reaction:



This reaction has since been used for the isolation from urine of steroid sulphates [6] as well as various arylsulphuric acids [7, 8]. One arylsulphuric acid which can be obtained in the free acid form is 2-amino-1-naphthylsulphuric acid, and this compound has been isolated from the urine of dogs [9] and rats [10, 11] dosed with the carcinogenic agent 2-naphthylamine.

The mode of formation of arylsulphuric acids has been a source of much controversy. Baumann [1] was of the opinion that they arise by the combination of inorganic sulphate and phenols, and this view was supported later by Hele and his co-workers [12]. On the other hand, Sherwin [13] suggested that mercapturic acids might serve as intermediates in the formation of ethereal sulphates, and Shipley, Muldoon and Sherwin [14] presented experimental evidence in support of this hypothesis. This work was severely criticized by Hele [12] and recent experiments by Marsden and Young [15] with ³⁵S-labelled mercapturic acids have revealed no significant oxidation of these compounds to arylsulphuric acids in the rat. The question of whether inorganic sulphate can participate in arylsulphuric acid synthesis was studied by Laidlaw and Young [16, 17] who showed that when ³⁵S-labelled sodium sulphate was administered to rats by

injection, a small proportion of the ^{35}S was excreted in the ethereal sulphate fraction of the urine. A much higher proportion of the ^{35}S was converted to ethereal sulphate when there was simultaneous administration of 2-naphthol (which is known to form 2-naphthylsulphuric acid) or 2-naphthylamine (which gives rise to 2-amino-1-naphthylsulphuric acid). Furthermore, these arylsulphuric acids were isolated and shown to be labelled with ^{35}S . Dziewiatkowski [18] in experiments with rats dosed with ^{35}S -labelled sulphate observed that when phenol was administered it was converted to ^{35}S -labelled phenylsulphuric acid. While these various experiments show conclusively that administered inorganic sulphate can take part in ethereal sulphate formation, it does not follow that dietary sulphate is the normal source of the sulphate used for this purpose. Binkley [19] found that the addition of cystine to the diet of rats receiving *p*-bromophenol brought about a resumption of growth and an increased excretion of ethereal sulphate, whereas the addition of inorganic sulphate to the diet produced neither of these effects. It seems likely that sulphate derived from cystine is the main source of the sulphate normally used in arylsulphuric acid synthesis. As Binkley has pointed out, this is not incompatible with the results of the experiments in which ^{35}S -labelled sulphate was administered.

In the course of their kinetic studies of the metabolism of foreign organic compounds, Thorpe and his coworkers [20] investigated the conjugation of phenols with sulphuric acid. They found that in fasting rabbits ethereal sulphate formation proceeded at a rate which was independent of the amount of phenol present above a certain level in the body. When a sulphate precursor, such as sodium sulphite or cystine, was administered simultaneously with the phenol, the percentage of the phenol conjugated with sulphate was increased and the kinetics of the conjugation process became those of a first-order reaction. These observations indicate that the availability of sulphate limited the rate of sulphate conjugation, and they point to the participation of sulphate in the conjugation process. When the velocity constants for sulphate conjugation of various phenols were determined, it was found that the effect of the substituent groups did not appear to be related to their size or electronic influence.

As long ago as 1902 Embden and Glaessner [21] demonstrated ethereal sulphate formation in the liver and kidney of the dog

by means of perfusion experiments. More recently this process has been shown to occur in rat-liver slices [22] and suspensions [23, 24, 25, 26], chick embryo-liver slices [27] and rat-kidney slices [28]. The conversion of *m*-aminophenol to *m*-aminophenylsulphuric acid by rat-liver preparations has been studied by Bernstein and McGilvery [24, 25]. By differential centrifugation they obtained a liver fraction which provided energy for the reaction, and this led to the recognition of the role of adenosine triphosphoric acid (ATP) in ethereal sulphate synthesis. Bernstein and McGilvery [25] investigated whether ATP serves to create an active form of phenol or of sulphate. The results obtained showed that it is the inorganic sulphate rather than the phenol which is activated, and that at least two enzymes participate in the esterifying mechanism. One of these requires ATP and activates sulphate. The other condenses the phenol with the active intermediate. Similar conclusions have been reached by De Meio [26, 29] and by Segal [30]. Microsomes were found by De Meio and his coworkers to inhibit phenylsulphuric acid synthesis by rat-liver preparations, and they attributed this inhibition to the adenosine triphosphatase activity of the microsomes. They found that two enzymes participate in the synthesis of arylsulphuric acids by rat liver. One of these gives rise to an active sulphate intermediate, the other is concerned with the transfer of the sulphate from the intermediate to the phenolic substrate. Work on sulphate activation has recently been carried forward by Lipmann and his coworkers [31, 32, 33], who have made the important finding that 'active sulphate' is adenosine-3'-phosphate-5'-phosphosulphate.

Arylsulphatase

Of the various enzymes concerned with the hydrolysis of sulphuric acid esters, that now known as arylsulphatase has been studied most extensively. For the detection of this enzyme and the measurement of its activity, various methods have been devised which are based on the use of gravimetric [34], colorimetric [35, 36, 37, 38] and manometric [39] procedures, and also ultraviolet spectrophotometry [40, 41]. The tissues of several animal species have been found to contain arylsulphatase [36, 42, 43]. In the rat the enzyme has been detected in all the major tissues and appears to be present in the greatest concentration

in the liver [36, 41]. It is now apparent that the arylsulphatase system of mammalian tissues is of a complex nature. Roy [44, 45] established the presence in liver of two enzymes which he designated sulphatase A and B, and this finding has been supported by the observation of Dodgson, Spencer and Thomas [46] that the activity of the mitochondria of rat liver can be separated into two fractions comparable to Roy's sulphatases. These workers also found that there is an arylsulphatase localized in the microsomes of rat liver, and a comparable enzyme, sulphatase C, has been shown by Roy [47] to be present in ox liver. Arylsulphatase activity appears to be absent from cell nuclei [38, 48]. Soda and Egami [49] showed that arylsulphatase is present in tropical marine molluscs, where it is located mainly in the digestive glands, and the most active preparations of the enzyme so far obtained have come from molluscs [50]. The enzyme is present in *Aspergillus oryzae* [34] and certain other fungi [51] but it does not appear to be widely distributed among bacteria. Whitehead, Morrison and Young [37] examined 212 strains of a wide variety of micro-organisms for arylsulphatase activity. They detected it in some but not all of the strains of salmonellae and mycobacteria tested, and from *Mycobacterium piscium* they made cell-free aqueous extracts containing the enzyme. Recently, three other micro-organisms have been shown to contain arylsulphatase [52, 53, 54].

The function of arylsulphatase is obscure. Although the enzyme is widely distributed in mammalian tissues, experiments [55, 56] in which arylsulphuric acids have been administered to animals indicate that almost the whole of the dose is excreted unchanged in the urine. As all these experiments were based on changes in the ethereal sulphate content of the urine, however, they could not be expected to reveal with certainty a small degree of hydrolysis. Experiments by Hawkins and Young [57] in which the potassium salts of [^{35}S]phenylsulphuric acid and [^{35}S]1- and [^{35}S]2-naphthylsulphuric acid were administered at a dose level of 0.05 m-mole per rat by stomach tube or by intraperitoneal injection showed that most of the compound was quickly excreted unchanged, but that a small amount (4–12%) did undergo hydrolysis in the organism. Hanahan and Everett [58] administered sodium oestrone [^{35}S]sulphate to rats and found that a large proportion of the dose was hydrolysed. Similar findings

have been obtained by other workers [59, 60, 61]. In view of these results it is noteworthy that Roy [62] has found that his sulphatase A has a greater affinity for sulphates containing a polycyclic ring system than for the simpler aryl sulphates.

Although the distribution of arylsulphatase in liver cells appears to be different from that of the arylsulphuric acid synthesizing system, the possibility cannot be ruled out that the normal role of arylsulphatase is that of a transferring enzyme in the synthesis of sulphuric acid esters. Roy [63], in considering the functions of the sulphatases, has made the comment that their action *in vivo* may be 'only distantly related to the commonly used assay methods'.

SULPHURIC ACID ESTERS OF STEROIDS

Among the metabolic changes which steroid hormones and their derivatives undergo *in vivo* are conjugation with glucuronic acid and with sulphuric acid. The significance of these conjugative reactions in relation to the biological activity of the steroid hormones has yet to be elucidated. The first naturally-occurring steroid sulphate to be isolated was oestrone sulphate, which was obtained from the urine of pregnant mares by Schachter and Marrian [64]. Subsequently sulphates of various non-phenolic steroids were separated from urine, such as the *allopregnenolone* sulphate obtained by Klyne, Schachter and Marrian [65]. Dehydroepiandrosterone occurs in human urine as a sulphate and it is claimed that it is present almost entirely in this form [66]. Rat-liver preparations have been shown to bring about the synthesis of oestrone sulphate [30] and dehydroepiandrosterone sulphate [67], and the system involved resembles that which brings about the formation of sulphuric acid esters of phenols. In a recent study of the enzymic synthesis of steroid sulphates, Schneider and Lewbart [68] incubated 32 steroids in a system which consisted of a microsome-free supernatant fluid prepared from rabbit liver, ATP, sulphate and magnesium ions. They found that 14 of the steroids were conjugated with sulphuric acid and these included testosterone and deoxycorticosterone.

Mammalian tissues show arylsulphatase activity and various workers have reported that administered oestrone sulphate undergoes hydrolysis in the body (see p. 111). Although the occurrence of a sulphatase capable of bringing about the hydrolysis of

alcohol sulphates has not been demonstrated in the tissues of higher animals, *Helix pomatia* and certain other molluscs have been shown to contain an enzyme which brings about the hydrolysis of urinary steroid sulphates not acted upon by arylsulphatase [69, 70, 71]. The steroid sulphatase of *Patella vulgata* has been studied by Roy [72] and that of *Otala punctata* by Savard, Bagnoli and Dorfman [73], and it has become apparent that this enzyme is not a general alkylsulphatase, but that it is highly specific and hydrolyses only the 3β -sulphates of the 5α - and Δ^5 -steroids. The limitation which this sets on the application of the enzyme to the hydrolysis of steroid sulphates in urine has been discussed by Roy [72] and by Stich and his coworkers [74, 75].

Sulphuric acid derivatives occur among the bile acids of certain species, for steroid alcohols with molecules containing between 27 and 29 carbon atoms have been found as conjugates with sulphuric acid in the bile of sharks, rays and certain other fish, as well as toads and frogs [76].

SULPHOMUCOPOLYSACCHARIDES

The mucopolysaccharides which contain sulphuric acid ester groups are widely distributed in biological systems and include such compounds as chondroitin sulphuric acid, mucoitin sulphuric acid and heparin. The nature of these compounds and the still unsolved problems concerning their structures have increased the difficulties surrounding the study of their metabolism. Some progress in this field has been made in recent years, however, mainly as a result of the application of isotopic tracer techniques employing ^{35}S .

When sodium [^{35}S]sulphate is administered to an animal, most of the ^{35}S is rapidly excreted in the urine [16, 77]. Some of it is retained, however, for whereas the ^{35}S content of the blood, liver and brain of the dosed animal falls rapidly, there is a marked uptake followed by slow elimination of ^{35}S in the case of bone, bone marrow and cartilage. Dziewiatkowski, Benesch and Benesch [78] suggested that some of the [^{35}S]sulphate was incorporated in the chondroitin sulphuric acid of the cartilage. Dziewiatkowski [79] obtained support for this possibility by dosing rats with sodium [^{35}S]sulphate and then isolating ^{35}S -labelled chondroitin sulphuric acid from cartilage after the addition of

the non-radioactive compound. He also used autoradiographic techniques to study the incorporation of [^{35}S]sulphate into cartilage and bone. Similar observations were made by Campbell [80] and by Davies and Young [81].

Boström [82] separated chondroitin sulphuric acid from the rib cartilage of groups of adult rats at different intervals after they had been dosed with sodium [^{35}S]sulphate by intraperitoneal injection. He was able to show that the ^{35}S content of this compound reached a maximum at 24 hours and then decreased slowly to half this amount by about the 17th day after dosing. Whereas the incorporation of ^{35}S into chondroitin sulphuric acid in these experiments was high, the amounts of ^{35}S in other compounds, such as taurine, methionine and cystine, was either low or insignificant, findings which support the belief that the administered sulphate which is retained is mainly in the form of sulphomucopolysaccharide.

The fixation of [^{35}S]sulphate by tissues has also been shown to occur *in vitro*. This was first observed by Layton and his co-workers [83, 84]. Boström and Månsson [85] incubated slices of calf costal cartilage in Krebs-Ringer medium containing sodium [^{35}S]sulphate, separated the chondroitin sulphuric acid, and found that there was a progressive increase in its ^{35}S content up to 24.5 hours, the longest period studied. There was no corresponding uptake of ^{35}S if the slices were kept in a boiling aqueous medium before exposure to the radioactive sulphate or if the experiment was carried out in an atmosphere of nitrogen at 0° . No exchange of ^{35}S occurred when sodium [^{35}S]sulphate was incubated for 24 hours with pure chondroitin sulphuric acid.

Studies of sulphate exchange in rats injected with sodium [^{35}S]sulphate have shown that ^{35}S incorporation occurs to a greater extent in skin than in cartilage [86]. In skin the maximum uptake of ^{35}S occurred in 24 hours and the biological half-life of the sulphate group of the chondroitin sulphuric acid was 8–9 days. Autoradiographic studies [87, 88] of the skin showed that mast cells are very active in taking up sulphate and it has been suggested [88] that this is due to exchange of ester sulphate groups of heparin and its precursors. Autoradiography has also been used to study the incorporation of radioactive sulphate in different tissues and organs of adult rats and rabbits [89]. The aorta, which contains chondroitin sulphuric acid, had a high

uptake of ^{35}S . Various epithelial regions of the gastrointestinal tract also showed a high ^{35}S uptake, some of which was apparently in the sulphomucopolysaccharides of the mucus [90].

Boström [91] has shown that sulphate fixation in cartilage is an enzymic process, and in recent work [92] he has found that the reaction is accelerated by glutamine. The question arises of whether the uptake of sulphate which occurs is the result of a synthesis of chondroitin sulphuric acid or a sulphate exchange in this compound [91, 93]. Schiller, Mathews and Dorfman [94, 95, 96] have studied the incorporation of [^{14}C]acetate into the mucopolysaccharides of rabbit skin. The results obtained indicated a half-life of 7–8 days for chondroitin sulphuric acid. While this is close to the half-life (8–9 days) found earlier by Boström and Gardell [86] for the sulphate group of this compound in rat skin, it would seem premature to suggest that these values represent turnover of the chondroitin sulphuric acid molecule as a whole rather than single parts of it [97]. Direct evidence that rats are able to split the ester linkage between sulphate and carbohydrate in administered chondroitin sulphuric acid has been obtained by Dohlman [98] and by Dziewiatkowski [99], for after injecting ^{35}S -labelled chondroitin sulphuric acid into rats, both these workers observed the excretion of ^{35}S as inorganic sulphate in the urine.

Layton [93, 100] observed that the capacity of different tissues for fixing sulphate was decreased after the administration of cortisone and suggested that this was the outcome of an inhibitory effect of cortisone on sulphomucopolysaccharide synthesis. Similar observations were made by Boström and Odeblad [101] in their study of sulphate incorporation in the chondroitin sulphuric acid of cartilage and skin in intact animals. They were also able to produce an inhibition of [^{35}S]sulphate uptake by the action of cortisone on calf-cartilage slices *in vitro*. These findings are especially interesting in view of the association between collagen and chondroitin sulphuric acid in the body [102, 103] and the action of cortisone in collagen diseases.

REFERENCES

1. BAUMANN, E. (1876). *Pflüg. Arch. ges. Physiol.* **12**, 63, 69; **13**, 285.

2. BAUMANN, E. (1878-79). *Hoppe-Seyl. Z.* **2**, 335.
3. TALLAN, H. H., BELLA, S. T., STEIN, W. H. and MOORE, S. (1955). *J. biol. Chem.* **217**, 703.
4. LAUGHLAND, D. H. and YOUNG, L. (1942). *Trans. roy. Soc. Can. Series III*, **36**, 166.
5. BARTON, A. D. and YOUNG, L. (1943). *J. Amer. chem. Soc.* **65**, 294.
6. KLYNE, W. and MARRIAN, G. F. (1945). *Biochem. J.* **39**, xlv.
7. BERENBOM, M. and YOUNG, L. (1951). *Biochem. J.* **49**, 165.
8. DODGSON, K. S., ROSE, F. A. and SPENCER, B. (1955). *Biochem. J.* **60**, 346.
9. WILEY, F. H. (1938). *J. biol. Chem.* **124**, 627.
10. DOBRINER, K., HOFMANN, K. and RHOADS, C. P. (1941). *Science*, **93**, 600.
11. MANSON, L. A. and YOUNG, L. (1950). *Biochem. J.* **47**, 170.
12. HELE, T. S. (1931). *Biochem. J.* **25**, 1736.
13. SHERWIN, C. P. (1922). *Physiol. Rev.* **2**, 238.
14. SHIPLE, G. J., MULDOON, J. A. and SHERWIN, C. P. (1924). *J. biol. Chem.* **60**, 59.
15. MARSDEN, C. M. and YOUNG, L. Unpublished work.
16. LAIDLAW, J. C. and YOUNG, L. (1948). *Biochem. J.* **42**, 1.
17. LAIDLAW, J. C. and YOUNG, L. (1953). *Biochem. J.* **54**, 142.
18. DZIEWIATKOWSKI, D. D. (1949). *J. biol. Chem.* **178**, 389.
19. BINKLEY, F. (1949). *J. biol. Chem.* **178**, 821.
20. BRAY, H. G., HUMPHRIS, B. G., THORPE, W. V., WHITE, K. and WOOD, P. B. (1952). *Biochem. J.* **52**, 419.
21. EMBDEN, G. and GLAESSNER, K. (1902). *Beitr. chem. Physiol. Path.* **1**, 310.
22. DE MEIO, R. H. and ARNOLT, R. I. (1944). *J. biol. Chem.* **156**, 577.
23. DE MEIO, R. H. and TKACZ, L. (1952). *J. biol. Chem.* **195**, 175.
24. BERNSTEIN, S. and MCGILVER, R. W. (1952). *J. biol. Chem.* **198**, 195.
25. BERNSTEIN, S. and MCGILVER, R. W. (1952). *J. biol. Chem.* **199**, 745.
26. DE MEIO, R. H., WIZERKANIUK, M. and FABIANI, E. (1953). *J. biol. Chem.* **203**, 257.
27. MACHLIN, L. J., DENTON, C. A., PEARSON, P. B. and BIRD, H. R. (1952). *Fed. Proc.* **11**, 448.

28. LOWENSTEIN, J. M. (1953). Ph.D. Thesis, University of London.
29. DE MEIO, R. H., WIZERKANIUK, M. and SCHREIBAM, I. (1955). *J. biol. Chem.* **213**, 439.
30. SEGAL, H. L. (1955). *J. biol. Chem.* **213**, 161.
31. HILZ, H. and LIPMANN, F. (1955). *Proc. nat. Acad. Sci., Wash.* **41**, 880.
32. ROBBINS, P. W. and LIPMANN, F. (1956). *Fed. Proc.* **15**, 338.
33. ROBBINS, P. W. and LIPMANN, F. (1956). *J. Amer. chem. Soc.* **78**, 2652.
34. NEUBERG, C. and KURONO, K. (1923). *Biochem. Z.* **140**, 295.
35. MORIMOTO, K. (1937). *J. Biochem., Tokyo*, **26**, 259.
36. HUGGINS, C. and SMITH, D. R. (1947). *J. biol. Chem.* **170**, 391.
37. WHITEHEAD, J. E. M., MORRISON, A. R. and YOUNG, L. (1952). *Biochem. J.* **51**, 585.
38. RUTENBURG, A. M., COHEN, R. B. and SELIGMAN, A. M. (1952). *Science*, **116**, 539.
39. LOWENSTEIN, J. M. and YOUNG, L. (1952). *Biochem. J.* **52**, xxv.
40. DODGSON, K. S. and SPENCER, B. (1953). *Biochem. J.* **53**, 444.
41. DODGSON, K. S., SPENCER, B. and THOMAS, J. (1953). *Biochem. J.* **53**, 452.
42. NEUBERG, C. and SIMON, E. (1925). *Biochem. Z.* **156**, 365.
43. HOMMERBERG, C. (1931). *Hoppe-Seyl. Z.* **200**, 69.
44. ROY, A. B. (1953). *Biochem. J.* **53**, 12.
45. ROY, A. B. (1954). *Biochem. J.* **57**, 465.
46. DODGSON, K. S., SPENCER, B. and THOMAS, J. (1955). *Biochem. J.* **59**, 29.
47. ROY, A. B. (1956). *Biochem. J.* **64**, 651.
48. DODGSON, K. S., SPENCER, B. and THOMAS, J. (1954). *Biochem. J.* **56**, 177.
49. SODA, T. and EGAMI, F. (1933). *J. chem. Soc. Japan*, **54**, 1069.
50. DODGSON, K. S. and SPENCER, B. (1953). *Biochem. J.* **55**, 315.
51. HARADA, T., KONO, K. and YAGI, K. (1954). *Mem. Inst. Sci. Res. Osaka Univ.* **11**, 193.
52. HARADA, T. (1954). *J. agric. chem. Soc. Japan*, **28**, 840.

53. HARADA, T. and KONO, K. (1954). *J. agric. chem. Soc. Japan*, **28**, 608.
54. DODGSON, K. S., MELVILLE, T. H., SPENCER, B. and WILLIAMS, K. (1954). *Biochem. J.* **58**, 182.
55. RHODE, H. (1922). *Hoppe-Seyl. Z.* **124**, 15.
56. GARTON, G. A. and WILLIAMS, R. T. (1949). *Biochem. J.* **45**, 158.
57. HAWKINS, J. B. and YOUNG, L. (1954). *Biochem. J.* **56**, 166.
58. HANAHAN, D. J. and EVERETT, N. B. (1950). *J. biol. Chem.* **185**, 919.
59. DAVIS, M. E., KELSEY, F. E., FUGO, N. W., LOUCKS, J. E., HORNER, E. N. and VOSKUIL, P. (1950). *Proc. Soc. exp. Biol.*, N.Y. **74**, 501.
60. LEWISON, E. F., LEVI, J. E., JONES, G. S., JONES, H. W. jun. and SILBERSTEIN, H. E. (1951). *Cancer*, N.Y. **4**, 537.
61. RAKOFF, A. E. and GROSS, M. L. (1951). *Fed. Proc.* **10**, 107.
62. ROY, A. B. (1953). *Biochem. J.* **55**, 653.
63. ROY, A. B. (1955). *Biochem. J.* **59**, 8.
64. SCHACHTER, B. and MARRIAN, G. F. (1938). *J. biol. Chem.* **126**, 663.
65. KLYNE, W., SCHACHTER, B. and MARRIAN, G. F. (1948). *Biochem. J.* **43**, 231.
66. LIEBERMAN, S., MOND, B. and SMYLES, E. (1954). *Recent Progr. Hormone Res.* **9**, 113.
67. DE MEIO, R. H. and LEWYCKA, C. (1955). *Endocrinology*, **56**, 489.
68. SCHNEIDER, J. J. and LEWBART, M. L. (1956). *J. biol. Chem.* **222**, 787.
69. HENRY, R., THEVENET, M. and JARRIGE, P. (1952). *Bull. Soc. Chim. biol., Paris*, **34**, 897.
70. HENRY, R. and THEVENET, M. (1952). *Bull. Soc. Chim. biol., Paris*, **34**, 886.
71. STITCH, S. R. and HALKERSTON, I. D. K. (1953). *Nature, Lond.* **172**, 398.
72. ROY, A. B. (1956). *Biochem. J.* **62**, 41.
73. SAVARD, K., BAGNOLI, E. and DORFMAN, R. I. (1954). *Fed. Proc.* **13**, 289.
74. STITCH, S. R., HALKERSTON, I. D. K. and HILLMAN, J. (1956). *Biochem. J.* **63**, 705.

75. STITCH, S. R. and HALKERSTON, I. D. K. (1956). *Biochem. J.* **63**, 710.
76. HASLEWOOD, G. A. D. (1955). *Physiol. Rev.* **35**, 178.
77. DZIEWIATKOWSKI, D. D. (1949). *J. biol. Chem.* **178**, 197.
78. DZIEWIATKOWSKI, D. D., BENESCH, R. E. and BENESCH, R. (1949). *J. biol. Chem.* **178**, 931.
79. DZIEWIATKOWSKI, D. D. (1951). *J. biol. Chem.* **189**, 187.
80. CAMPBELL, D. (1951). *Nature, Lond.* **167**, 274.
81. DAVIES, D. V. and YOUNG, L. (1954). *J. Anat., Lond.* **88**, 174.
82. BOSTRÖM, H. (1952). *J. biol. Chem.* **196**, 477.
83. LAYTON, L. L. (1950). *Fed. Proc.* **9**, 195.
84. LAYTON, L. L., FRANKEL, D. R. and SCAPA, S. (1950). *Cancer, N.Y.* **3**, 725.
85. BOSTRÖM, H. and MÅNSSON, B. (1952). *J. biol. Chem.* **196**, 483.
86. BOSTRÖM, H. and GARDELL, S. (1953). *Acta chem. scand.* **7**, 216.
87. BOSTRÖM, H., ODEBLAD, E. and FRIBERG, U. (1953). *Acta path. microbiol. scand.* **33**, Fasc. 4.
88. JORPES, E., ODEBLAD, E. and BOSTRÖM, H. (1953). *Acta haemat.* **9**, 273.
89. ODEBLAD, E. and BOSTRÖM, H. (1952). *Acta path. microbiol. scand.* **31**, 339.
90. DAVIES, D. V. and YOUNG, L. (1954). *Nature, Lond.* **173**, 448.
91. BOSTRÖM, H. (1953). *Ark. Kemi*, **6**, 43.
92. BOSTRÖM, H., RODÉN, L. and VESTERMARK, A. (1955). *Nature, Lond.* **176**, 601.
93. LAYTON, L. L. (1951). *Proc. Soc. exp. Biol., N.Y.* **76**, 596.
94. SCHILLER, S., MATHEWS, M. B. and DORFMAN, A. (1954). *Fed. Proc.* **13**, 290.
95. SCHILLER, S., MATHEWS, M. B., JEFFERSON, H., LUDOWIEG, J. and DORFMAN, A. (1954). *J. biol. Chem.* **211**, 717.
96. SCHILLER, S., MATHEWS, M. B., GOLDFABER, L., LUDOWIEG, J. and DORFMAN, A. (1955). *J. biol. Chem.* **212**, 531.
97. HORECKER, B. L. and MEHLER, A. H. (1955). *Annu. Rev. Biochem.* **24**, 207.
98. DOHLMAN, C.-H. (1956). *Acta physiol. scand.* **37**, 220.
99. DZIEWIATKOWSKI, D. D. (1956). *J. biol. Chem.* **223**, 239.

100. LAYTON, L. L. (1951). *Arch. Biochem. Biophys.* **32**, 224.
101. BOSTRÖM, H. and ODEBLAD, E. (1953). *Ark. Kemi*, **6**, 39.
102. JACKSON, D. S. (1953). *Biochem. J.* **54**, 638.
103. JACKSON, D. S. (1954). *Biochem. J.* **56**, 699.

CHAPTER VII

Miscellaneous Organic Compounds of Sulphur

Although the preceding chapters deal with the metabolism of most of the main types of sulphur compound, they do not include a number of miscellaneous compounds of sulphur which are of biochemical interest. An account of the metabolism of some of the more important of these is given below.

Thiamine

Higher animals require thiamine, and it has been shown that in man and certain other animals this need is met, in part at least, by the synthesis of the vitamin by intestinal bacteria [1]. Micro-organisms differ widely in their ability to synthesize thiamine. Some appear to be able to bring about its complete synthesis, others must be supplied with one or both of the heterocyclic components of the molecule, and there are some which must be provided with preformed thiamine. Its formation in *Neurospora* has been studied by Harris [2] who found that a precursor of thiazole reacts with the pyrimidine component to form an intermediate which is then converted to thiamine. More is known about the conversion of thiamine to its pyrophosphate, cocarboxylase. This compound acts as a coenzyme in the decarboxylation of α -keto acids. From various sources, such as yeast and the intestinal mucosa and liver of the rat, enzyme preparations have been obtained which bring about the formation of thiamine pyrophosphate from thiamine when adenosine triphosphoric acid (ATP) and Mg^{2+} are present [3, 4, 5]. Experiments with ^{32}P -labelled ATP have shown that this compound participates in the synthesis of thiamine pyrophosphate in the intact rat [6], and it is possible that the transfer of a pyrophosphate group from

ATP to thiamine takes place. It is noteworthy that the phosphorylation of thiamine is depressed in diabetic animals [7], and that the administration of insulin brings about an increase in the cocarboxylase levels in the blood of diabetic patients [8] and in the livers of diabetic rats [9].

The metabolism of thiamine in rats has been studied by the use of thiamine labelled in the thiazole ring with ^{14}C in position 2 [10] or with ^{35}S [11]. In each series of experiments about two-thirds of the radioactive isotope was excreted in the urine by normal animals. Very little radioactivity was present in the faeces. Almost all the ^{35}S in the urine was present as neutral sulphur and only a small proportion was excreted as sulphate. In several respects the results are similar to those obtained earlier by Borsook and his coworkers [12] from experiments in which [^{35}S]thiamine was administered to man. When [^{14}C]thiamine was administered to rats, an examination of their urine by paper chromatography showed the presence of a variety of ^{14}C -labelled compounds [10, 13]. These included thiazole derivatives, phosphorylated forms of thiamine, and a number of compounds which showed a thiamine response when tested microbiologically.

An enzyme, thiaminase, which brings about the breakdown of thiamine, has been obtained from various sources. Its discovery by Fujita and his coworkers [14, 15] was the outcome of the observation that thiamine is absent from certain shellfish and is not recovered when it is added to them [16, 17]. At about the same time Green and his coworkers [18, 19], in their study of the paralysis which had been observed in foxes fed on raw fish, showed that the disease was brought about by a factor present in carp. Further work led to the recognition of this factor as thiaminase [20, 21]. The enzyme was also found to be present in certain bacteria [22] and in ferns [23, 24]. An important aspect of the occurrence of 'antithiamine' activity in foodstuffs is that the consumption of diets containing such foodstuffs is liable to give rise to thiamine deficiency. For example, the eating of ferns by livestock can cause 'bracken disease' [23] and this is related to the presence of an antithiamine factor which according to Fujita [25] consists of thiaminase and a thermostable factor. When extracts containing thiaminase were prepared from carp viscera and injected into mice they produced a condition similar to that observed in thiamine deficiency [26]. Krampitz and

Woolley [27] obtained evidence that the thiaminase of fish splits thiamine into its pyrimidine and thiazole components. Certain aromatic amines markedly stimulate the activity of thiaminase [28] and under these conditions the action of the enzyme may be envisaged as bringing about an exchange reaction whereby the pyrimidine moiety of thiamine is conjugated with an organic base in place of the thiazole component. There are indications that proline may be a natural cofactor for the enzyme, for it is present in active preparations from bracken, and it serves as a cofactor for the bracken thiaminase system *in vitro* [29]. The physiological significance of thiaminase is not known.

Biotin

d-Biotin occurs in the free state and in bound forms, one of which is biocytin. This latter compound has been isolated by Wright and his coworkers in minute amounts from 25 tons of yeast and has been shown to be ϵ -*N*-biotinyl-L-lysine [30]. *d*-Biotin-*l*-sulphoxide is also a metabolic product of certain micro-organisms [31]. Biotin is required only in extremely minute quantities by animals and these needs can readily be met by the synthetic activities of their own intestinal bacteria (see Chapter IX). In order to produce a state of biotin deficiency, it is therefore necessary to prevent this bacterial synthesis by incorporating in the diet an anti-bacterial agent which is active in the intestinal tract. Another procedure has been to add to the diet raw egg white containing the protein complex, avidin, which combines with biotin and prevents it from becoming available to the animal.

Experiments with biotin-requiring micro-organisms and with tissues taken from biotin-deficient animals indicate that the vitamin is implicated in a number of metabolic processes [32]. The mode of action of biotin, however, has yet to be established. It may require to be converted to a more complex molecule with coenzymic properties. Several workers have suggested the possibility that it is concerned in the synthesis of enzymes rather than that it participates directly in metabolic reactions [33, 34, 35]. Biotin is involved in the incorporation and removal of carbon dioxide from various carboxylic acids. It is believed that it has a role in the synthesis of various four-carbon acids, such as oxaloacetic acid and aspartic acid from pyruvic acid [36, 37].

Thus bacteria and yeasts grown on a biotin-deficient medium show decreased growth and a reduced capacity to use carbon dioxide for the formation of the carboxyl groups of aspartic acid, probably as a result of a block in the formation of oxaloacetic acid [38]. The addition of aspartic acid to the medium circumvents the metabolic block, and growth is thereby restored [39, 40].

In animals, one aspect of biotin deficiency is an increased excretion of ammonia. This is assumed to be a consequence of hyperacidity brought about by a reduction in the carboxylation of pyruvic acid, and the accumulation of this compound in the blood. The carboxylation of propionic acid to succinic acid, which has been demonstrated in rat-liver mitochondria, is also markedly reduced in biotin deficiency [32]. Biotin appears to be linked with the decarboxylation of such acids as acetoacetic, oxaloacetic, succinic, malic and aspartic acid, and there is evidence of its participation in the oxidation of *isovaleric* acid to acetoacetic acid [41]. Diminution in the rate of synthesis of citrulline from ornithine also occurs in biotin deficiency [42], and there is reason to believe that the step in the process which is affected is that concerned with the conversion of glutamic acid to carbamylglutamic acid. Furthermore, it has been suggested that biotin plays a role in the deamination of certain amino acids [43], and in the synthesis of nicotinic acid [44], oleic acid [45] and purines [46].

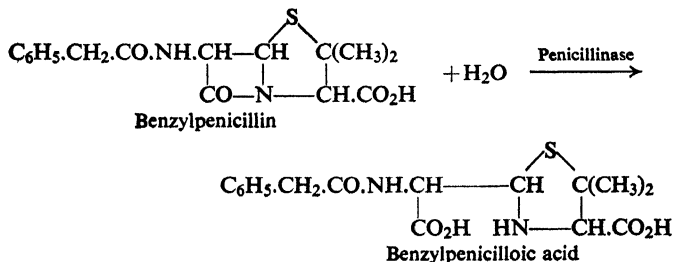
Some attempts have been made to study the fate of biotin in the body. When relatively large amounts are administered to rats by injection, at least 40% is excreted in the urine. Fraenkel-Conrat and Fraenkel-Conrat [47] used biotin labelled with ^{14}C in the ureido group and found that the compound was excreted partly in a biologically-inactive form. The reaction which occurred could not have involved the breakdown of the ureido group, for no radioactive carbon dioxide was detected in the expired air of the experimental animals. Baxter and Quastel [48] with the aid of [*carboxy*- ^{14}C]biotin, showed that the vitamin was degraded aerobically by guinea pig-kidney slices to biologically-inactive products and labelled carbon dioxide. These authors suggested that oxidation of the side chain had occurred, but considered that the enzyme system responsible was distinct from that concerned with the oxidation of the lower fatty acids. In *Aspergillus niger* there is evidence that oxidation of biotin to the

corresponding sulfoxide can take place [49]. There is no evidence that the sulphur of biotin undergoes any other change.

It has been established that the blood contains a hydrolytic enzyme, biocytinase, which splits biocytin into biotin and lysine [50], and which also hydrolyses biotinamide. This enzyme may be responsible for the appearance of biotin in the urine of animals dosed with biocytin, and it may be of nutritional significance in making biotin available from bound forms. Biocytin is utilized as a source of biotin by some bacteria, and the ability of a particular species to make use of the compound seems to be coupled with its ability to bring about the hydrolysis to biotin. The presence in liver of a second enzyme which acts on biocytin and bound forms of biotin has been reported. It has been given the name biotinidase [51].

Penicillins

The penicillins are produced by over thirty species of *Penicillium* and *Aspergillus*, although in the large-scale production of these compounds, only certain strains of *P. notatum* and *P. chrysogenum* are used. The various penicillins differ in the nature of the *N*-acyl group attached to the β -lactam ring (see p. 14). The β -lactam ring structure is an unstable one and is readily split by a variety of reagents, including alcohols, amines, thiols and water. The hydrolytic fission is catalysed by the presence of certain metal ions and it may also be brought about by the bacterial enzyme penicillinase [52], giving rise to penicilloic acids, which are biologically inactive:



This enzymic reaction results in the formation of a free carboxyl group, and it may be followed in a bicarbonate medium by the

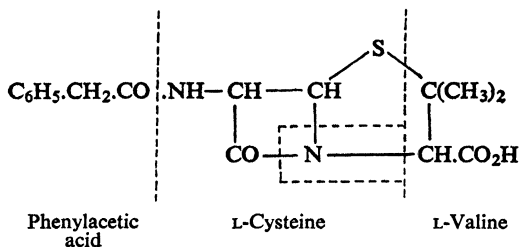
manometric estimation of the carbon dioxide evolved [53]. Acid hydrolysis breaks down penicillins to carbon dioxide, an aldehyde and penicillamine, $(\text{CH}_3)_2\text{C}(\text{SH})\cdot\text{CH}(\text{NH}_2)\cdot\text{CO}_2\text{H}$. Penicillamine may be regarded as a derivative of cysteine or of valine.

The penicillins depress the oxygen consumption of growing bacteria [54]. Gale and Taylor [55] found that these compounds can also markedly reduce the assimilation of glutamic acid by *Staphylococcus aureus*, and they suggested that this was the primary action of penicillins. An alternative theory, put forward by Krampitz and Werkman [56], is that these compounds produce a dislocation of nucleic acid metabolism in susceptible organisms. Cooper [57, 58] has recently shown, however, that penicillins become firmly bound to lipid-containing material in the region of the bacterial cell wall, and that this precedes the appearance of any metabolic effects. It would appear that the biochemical changes observed are secondary phenomena, and that the initial effect of penicillin is on the osmotic characteristics of the cell.

Biosynthesis of penicillins. At an early stage in the development of penicillin production it was noted that the addition of phenylacetic acid, β -phenylethylamine or phenylacetamide to culture media resulted in a significant increase in the yield of benzylpenicillin [59]. In the absence of these compounds *n*-heptylpenicillin was the main penicillin formed. The role of phenylacetic acid and related compounds as precursors of the benzyl group in benzylpenicillin has been verified by the use of these compounds suitably labelled with deuterium [59]. *p*-Hydroxyphenylacetic acid has also been shown to be a precursor of the *p*-hydroxybenzyl group in *p*-hydroxybenzylpenicillin [59], and in its absence from the culture medium, only very small amounts of this penicillin are obtained.

More recently the biosynthesis of the ring structures of the penicillins of *Penicillium chrysogenum* has been studied by Arnstein and Grant, and by Stevens and his coworkers. It had previously been shown that the sulphur of the thiazolidine ring arises from the inorganic sulphate of the culture medium [60, 61]. Since micro-organisms readily convert inorganic sulphate to the sulphur-containing amino acids, these latter compounds were considered as possible metabolic intermediates. Arnstein and Grant [62, 63], using L-cystine labelled with ^{14}C , ^{35}S and ^{15}N ,

showed that this amino acid was incorporated into the penicillin molecule probably with no structural change other than reduction to L-cysteine. The carbon chain of L-valine was also found to provide part of the penicillamine moiety [64]. Stevens and his coworkers [65, 66, 67, 68] have obtained very similar results using labelled cystine, valine and other compounds. The contribution of these amino acids and of phenylacetic acid to the benzylpenicillin molecule is indicated below:



Glycine, L-serine and formic acid act as sources of the carbon atoms in the β -lactam ring [63, 69]. This is probably the result of the ability of these compounds to act as precursors of L-cysteine. The activity of methionine and glutathione in penicillin synthesis may be explained in the same way. Acetic acid acts as a precursor of both cysteine and valine, and it is consequently incorporated into the β -lactam ring and the penicillamine moiety. Penicillamine itself has been shown not to participate in penicillin synthesis.

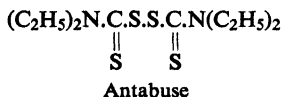
Fate of administered penicillins. When administered to animals, penicillins diffuse readily into most tissues, but there is little penetration into the central nervous system, and negligible amounts are found in the cerebrospinal fluid [70]. The compounds are rapidly excreted in the urine, for about 40–50% of a parenterally-injected dose is eliminated by this pathway within the first hour. At least 80% of the urinary excretion takes place through the kidney tubules and this can be reduced by the simultaneous administration of other substances, for example, sodium *p*-aminohippurate or caronamide (4'-carboxyphenylmethanesulphonanilide), which are excreted in the same manner. Some excretion of the penicillins also occurs in the bile.

Penicillins, especially *n*-heptylpenicillin, undergo some breakdown in the body. Decomposition is brought about by acid hydrolysis in the stomach and by bacterial action in the intestine. Some inactivation probably occurs in the blood by hydrolysis and also by reaction with proteins and amino acids. Furthermore, several workers have claimed that serum contains a heat-labile factor which causes loss of penicillin activity (71, 72). Penicillins are liable to break down in the presence of tissues *in vitro* [73], but it has not been established that this is an enzymic reaction.

Various bacteria, including Gram-positive as well as Gram-negative species, produce the enzyme penicillinase and are therefore able to inactivate penicillins. In the case of bacteria of the genus *Bacillus*, such as *B. subtilis*, the enzyme passes out of the organism into the surrounding medium, whereas in some other organisms it remains as an intracellular component. Penicillinase has been shown by Abraham and Chain [52] and others to be absent from animal tissues.

Antabuse

Tetraethylthiuram disulphide, or antabuse, was found by Hald, Jacobsen and Larsen [74] to resemble cyanamide and certain other compounds in rendering man and animals hypersensitive to the effects of ethanol.



Antabuse is toxic to bacteria and lower organisms, probably on account of its marked ability to form complexes with metals, such as copper. It has, on the other hand, a low toxicity in higher animals and is proving of value in the treatment of alcoholism. The administration of ethanol to human subjects and animals treated previously with antabuse results in the accumulation of acetaldehyde in the blood and tissues [75, 76], and most of the associated symptoms can be attributed to the presence of acetaldehyde, for they can be induced by administration of this substance [77]. The site of action of antabuse appears to be the liver, where the compound exerts an inhibitory action on the metabolism of acetaldehyde [78, 79]. Confirmation of this has been

obtained from *in vitro* experiments by Kjeldgaard [80], who has found the compound to be a powerful inhibitor of rabbit-liver aldehyde oxidase.

According to Hald, Jacobsen and Larsen [81], antabuse disappears rapidly from the blood of experimental animals. One metabolite which has been recovered in significant quantities from the urine of dosed rabbits is diethyldithiocarbamic acid, $(C_2H_5)_2N.CS_2H$ [82]. In a study of the metabolism of antabuse labelled with ^{35}S , Eldjarn [83] found that when administered orally to rats or to man, about 80% of the compound was absorbed from the intestinal tract. Part of this was excreted in unchanged form together with some diethyldithiocarbamic acid, while a large proportion was oxidized to sulphate. Eldjarn noted that there was considerable retention of ^{35}S in the tissues, and that in rats this occurred mainly in the adrenals, liver and spleen. There was, moreover, a similarity between the distribution of antabuse-sulphur in various tissues and that of administered carbon disulphide, which suggested a connection between the metabolism of these two substances. It has since been shown that rat-liver preparations break down antabuse to carbon disulphide [84]. An initial reduction to diethyldithiocarbamic acid in the presence of triphosphopyridine nucleotide is believed to take place, followed by conversion of the intermediate to carbon disulphide and diethylamine. Carbon disulphide has also been identified in the breath of rats given oral doses of antabuse.

Carbon disulphide

Carbon disulphide is a toxic substance which can have a damaging effect on nervous tissues. The rates of absorption and elimination of carbon disulphide by men, dogs and rabbits were studied by McKee, Kiper, Fountain, Riskin and Drinker [85]. They showed that little of the carbon disulphide retained after a period of exposure to the vapour appeared in the expired air, and that most of it was excreted in the urine as inorganic sulphate and as unidentified organic sulphur compounds. More recently, Strittmatter, Peters and McKee [86] studied the fate of ^{35}S -labelled carbon disulphide in guinea pigs and mice, and found that about 30% of the retained ^{35}S was excreted in the urine, mostly as inorganic sulphate.

Thioacetamide

The prolonged administration of thioacetamide to rats produces liver damage [87, 88] and cancer of the bile ducts [89]. The ^{35}S -labelled compound was prepared by Nygaard, Eldjarn and Nakken [90], who showed that when this compound was injected subcutaneously into rats, most of the ^{35}S was excreted in the urine in the first 24 hours after the injection. The greater part of the thioacetamide was metabolized, for only 25% of the compound was excreted unchanged in the urine and the sulphur of almost all the rest of the compound appeared in the urine in the inorganic and total sulphate fractions.

Thiourea

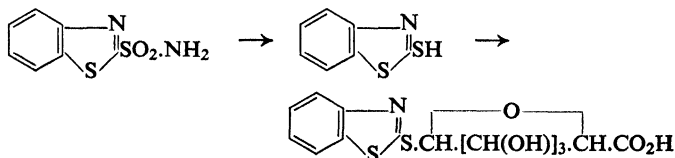
Early work on the metabolism of thiourea in the rabbit showed that most of the compound was excreted unchanged in the urine and there was no evidence of oxidation of the sulphur to sulphate [91]. The injection of [^{35}S]thiourea into rats revealed that while most of the compound was excreted as such, a small proportion of the dose [12%] was present in the sulphate fractions of the urine [92]. In these experiments concentration of ^{35}S was observed to occur in the thyroid, and it was later found [93] that oxidation of thiourea to sulphate occurs in this gland.

Attention has been drawn to the presence of a substance with a sweetish odour in the breath of patients receiving thiourea during the treatment of thyrotoxicosis [94]. A similar effect is not produced by the administration of 2-thiouracil or 4-methyl-2-thiouracil, and Challenger and Charlton [95] have suggested that the odorous compound produced from thiourea is probably dimethyl sulphide.

Sulphonamides

Following the discovery of the chemotherapeutic properties of *p*-aminobenzenesulphonamide and a number of related sulphonamides, numerous studies were made of the metabolism of these compounds in man and animals [96]. The chief changes found to occur were acetylation of aromatic amino groups, and the formation of phenols which subsequently underwent conjugation to yield sulphuric acid and glucuronic acid derivatives. In a study of the metabolism of [^{35}S]sulphapyridine in the rabbit by Thorpe

and his coworkers [97], almost all the ^{35}S administered was found to be excreted in the urine within three days, but no significant amount was present in the inorganic or total sulphate fraction. From these various findings it became generally accepted that aromatic sulphonamide groups do not undergo metabolic change. This does not appear to apply, however, to sulphonamides of the type $\text{R}.\text{SO}_2.\text{NH}_2$, where R is a heterocyclic ring structure. Clapp [98] has recently shown that the intravenous administration of 2-benzothiazolesulphonamide to dogs is followed by the excretion of a glucuronic acid derivative of 2-mercaptobenzothiazole :



This is a very interesting finding, for neither the metabolic reduction of a sulphonamide to a thiol, nor the conjugation of a thiol with glucuronic acid, had been demonstrated previously.

REFERENCES

1. NAJJAR, V. A. and HOLT, L. E. jun. (1943). *J. Amer. med. Ass.* **123**, 683.
2. HARRIS, D. L. (1953). *Fed. Proc.* **12**, 214.
3. CHEVILLARD, L. and THOAI, N. VAN (1951). *Bull. Soc. Chim. biol., Paris*, **33**, 1147.
4. LEUTHARDT, F. and NIELSEN, H. (1952). *Helv. chim. acta*, **35**, 1196.
5. EICH, S. and CERECEDO, L. R. (1954). *J. biol. Chem.* **207**, 295.
6. ROSSI-FANELLI, A., SILIPRANDI, N., FASELLA, P., SILIPRANDI, D. and SALVETTI, M. (1954). *Experientia*, **10**, 73.
7. FOA, P. P., WEINSTEIN, H. R., SMITH, J. A. and GREENBERG, M. (1952). *Arch. Biochem. Biophys.* **40**, 323.
8. SILIPRANDI, D. and NAVAZIO, F. (1952). *Acta med. scand.* **142**, 147.
9. SILIPRANDI, D. and SILIPRANDI, N. (1952). *Nature, Lond.* **169**, 329.

10. IACONO, J. M., WOLF, G. and JOHNSON, B. C. (1953). *Fed. Proc.* **12**, 223.
11. MCCARTHY, P. T., CERECEDO, L. R. and BROWN, E. V. (1954). *J. biol. Chem.* **209**, 611.
12. BORSOOK, H., BUCHMAN, E. R., HATCHER, J. B., YOST, D. M. and McMILLAN, E. (1940). *Proc. nat. Acad. Sci., Wash.* **26**, 412.
13. IACONO, J. M. (1954). Doctoral Thesis, University of Illinois, Urbana, Illinois.
14. FUJITA, A. and NUMATA, I. (1942). 18th Annu. Meeting, Jap. Biochem. Soc., Oct. 7.
15. FUJITA, A. and NUMATA, I. (1944). *Seikagaku*, **18**, 63, 325, 339.
16. FUJITA, A. and MATSUKAWA, D. (1942). *J. Biochem., Tokyo*, **35**, 89.
17. DOHI, K. (1942). *J. Biochem., Tokyo*, **35**, 419.
18. GREEN, R. G., CARLSON, W. E. and EVANS, C. A. (1941). *J. Nutr.* **21**, 243.
19. EVANS, C. A., CARLSON, W. E. and GREEN, R. G. (1942). *Amer. J. Path.* **17**, 79.
20. SEALOCK, R. R., LIVERMORE, A. H. and EVANS, C. A. (1943). *J. Amer. chem. Soc.* **65**, 935.
21. SEALOCK, R. R. and WHITE, H. S. (1949). *J. biol. Chem.* **181**, 393.
22. SHIBATA, J. and CHANG, C. (1948). *Vitamins, Japan*, **1**, 209.
23. WESWIG, P. H., FREED, A. M. and HAAG, J. R. (1946). *J. biol. Chem.* **165**, 737.
24. EVANS, E. T. R. and EVANS, W. C. (1949). *Biochem. J.* **44**, ix.
25. FUJITA, A. (1954). *Advanc. Enzymol.* **15**, 389.
26. WALSH, L. B., GRIEFF, D. and PINKERTON, H. (1952). *Fed. Proc.* **11**, 432.
27. KRAMPITZ, L. O. and WOOLLEY, D. W. (1944). *J. biol. Chem.* **152**, 9.
28. SEALOCK, R. R. and DAVIS, N. C. (1949). *J. biol. Chem.* **177**, 987.
29. WATKIN, J. E., THOMAS, A. J. and EVANS, W. C. (1953). *Biochem. J.* **54**, xiii.
30. WRIGHT, L. D., CRESSON, E. L., SKEGGS, H. R., WOOD, T. R., PECK, R. L., WOLF, D. E. and FOLKERS, K. (1952). *J. Amer. chem. Soc.* **74**, 1996.

31. WRIGHT, L. D., CRESSON, E. L., VALIANT, J., WOLF, D. E. and FOLKERS, K. (1954). *J. Amer. chem. Soc.* **76**, 4163.
32. LARDY, H. A. and PEANASKY, R. (1953). *Physiol. Rev.* **33**, 560.
33. LICHSTEIN, H. C. and UMBREIT, W. W. (1947). *J. biol. Chem.* **170**, 329.
34. OCHOA, S., MEHLER, A., BLANCHARD, M. L., JUKES, T. H., HOFFMANN, C. E. and REGAN, M. (1947). *J. biol. Chem.* **170**, 413.
35. MOAT, A. G. and LICHSTEIN, H. C. (1954). *Arch. Biochem. Biophys.* **48**, 300.
36. LARDY, H. A., POTTER, R. L. and ELVEHJEM, C. A. (1947). *J. biol. Chem.* **169**, 451.
37. SHIVE, W. and ROGERS, L. L. (1947). *J. biol. Chem.* **169**, 453.
38. LARDY, H. A., POTTER, R. L. and BURRIS, R. H. (1949). *J. biol. Chem.* **179**, 721.
39. KOSER, S. A., WRIGHT, M. H. and DORFMAN, A. (1942). *Proc. Soc. exp. Biol., N.Y.* **51**, 204.
40. STOKES, J. L., LARSEN, A. and GUNNESS, M. (1947). *J. biol. Chem.* **167**, 613.
41. PLAUT, G. W. E. and LARDY, H. A. (1950). *J. biol. Chem.* **186**, 705.
42. MACLEOD, P. R., GRISOLIA, S., COHEN, P. P. and LARDY, H. A. (1949). *J. biol. Chem.* **180**, 1003.
43. LICHSTEIN, H. C. and UMBREIT, W. W. (1947). *J. biol. Chem.* **170**, 423.
44. SHANMUGA SUNDARAM, E. R. B., TIRUNARAYANAN, M. O. and SARMA, P. S. (1953). *Curr. Sci.* **22**, 211.
45. WILLIAMS, W. L., BROQUIST, H. P. and SNELL, E. E. (1947). *J. biol. Chem.* **170**, 619.
46. MACLEOD, P. R. and LARDY, H. A. (1949). *J. biol. Chem.* **179**, 733.
47. FRAENKEL-CONRAT, J. and FRAENKEL-CONRAT, H. (1952). *Biochim. biophys. Acta*, **8**, 66.
48. BAXTER, R. M. and QUASTEL, J. H. (1953). *J. biol. Chem.* **201**, 751.
49. WRIGHT, L. D. and DRISCOLL, C. A. (1954). *J. Amer. chem. Soc.* **76**, 4999.
50. WRIGHT, L. D., DRISCOLL, C. A. and BOGER, W. P. (1954). *Proc. Soc. exp. Biol., N.Y.* **86**, 335.

51. THOMA, R. W. and PETERSON, W. H. (1954). *J. biol. Chem.* **210**, 569.
52. ABRAHAM, E. P. and CHAIN, E. (1940). *Nature, Lond.* **146**, 837.
53. HENRY, R. J. and HOUSEWRIGHT, R. D. (1947). *J. biol. Chem.* **167**, 559.
54. CHAIN, E., FLOREY, H. W., ABRAHAM, E. P. and HEATLEY, N. G. (1949). *Antibiotics*, Vol. II, p. 1137. London: Oxford University Press.
55. GALE, E. F. and TAYLOR, E. S. (1947). *J. gen. Microbiol.* **1**, 314.
56. KRAMPITZ, L. O. and WERKMAN, C. H. (1947). *Arch. Biochem.* **12**, 57.
57. COOPER, P. D. (1955). *J. gen. Microbiol.* **13**, 22.
58. COOPER, P. D. (1954). *J. gen. Microbiol.* **10**, 236.
59. CHAIN, E. (1949). *Antibiotics*, Vol. II, p. 965. London: Oxford University Press.
60. HOWELL, S. F., THAYER, J. D. and LABAW, L. W. (1948). *Science*, **107**, 299.
61. LESTER SMITH, E. and HOCKENHULL, D. J. D. (1952). *J. appl. Chem.* **2**, 287.
62. ARNSTEIN, H. R. V. and GRANT, P. T. (1953). *Biochem. J.* **55**, v.
63. ARNSTEIN, H. R. V. and GRANT, P. T. (1954). *Biochem. J.* **57**, 353, 360.
64. ARNSTEIN, H. R. V. and CLUBB, M. E. (1955). *Biochem. J.* **60**, xxxiv.
65. STEVENS, C. M., VOHRA, P., INAMINE, E. and ROHOLT, O. A. jun. (1953). *J. biol. Chem.* **205**, 1001.
66. STEVENS, C. M., VOHRA, P., MOORE, J. E. and DE LONG, C. W. (1954). *J. biol. Chem.* **210**, 713.
67. STEVENS, C. M., VOHRA, P. and DE LONG, C. W. (1954). *J. biol. Chem.* **211**, 297.
68. STEVENS, C. M., INAMINE, E. and DE LONG, C. W. (1956). *J. biol. Chem.* **219**, 405.
69. MARTIN, E., BERKY, J., GODZESKY, C., MILLER, P., TOME, J. and STONE, R. W. (1953). *J. biol. Chem.* **203**, 239.
70. FLOREY, H. W. and JENNINGS, M. A. (1949). *Antibiotics*, Vol. II, p. 1254. London: Oxford University Press.
71. HOLMES, L. F. and LOCKWOOD, J. S. (1944). *Amer. J. med. Sci.* **207**, 267.

72. WATTS, P. S. and McLEOD, D. H. (1946). *J. comp. Path.* **56**, 170.
73. FLOREY, H. W. and JENNINGS, M. A. (1949). *Antibiotics*, Vol. II, p. 1267. London: Oxford University Press.
74. HALD, J., JACOBSEN, E. and LARSEN, V. (1948). *Acta pharm. tox., Kbh.* **4**, 285.
75. HALD, J. and JACOBSEN, E. (1948). *Acta pharm. tox., Kbh.* **4**, 305.
76. LARSEN, V. (1948). *Acta pharm. tox., Kbh.* **4**, 321.
77. ASMUSSEN, E., HALD, J. and LARSEN, V. (1948). *Acta pharm. tox., Kbh.* **4**, 311.
78. JACOBSEN, E. and LARSEN, V. (1949). *Acta pharm. tox., Kbh.* **5**, 285.
79. HALD, J., JACOBSEN, E. and LARSEN, V. (1949). *Acta pharm. tox., Kbh.* **5**, 298.
80. KJELDGAARD, N. O. (1949). *Acta pharm. tox., Kbh.* **5**, 397.
81. HALD, J., JACOBSEN, E. and LARSEN, V. (1949). *Acta pharm. tox., Kbh.* **5**, 179.
82. DOMAR, G., FREDGA, A. and LINDERHOLM, H. (1949). *Acta chem. scand.* **3**, 1441.
83. ELDJARN, L. (1950). *Scand. J. clin. Lab. Invest.* **2**, 198, 202.
84. JOHNSTON, C. D. and PRICKETT, C. S. (1952). *Biochim. biophys. Acta*, **9**, 219.
85. McKEE, R. W., KIPER, C., FOUNTAIN, J. H., RISKIN, A. M. and DRINKER, P. (1943). *J. Amer. med. Ass.* **122**, 217.
86. STRITTMATTER, C. F., PETERS, T. and McKEE, R. W. (1950). *Arch. industr. Hyg.* **1**, 54.
87. FITZHUGH, O. G. and NELSON, A. A. (1948). *Science*, **108**, 626.
88. AMBROSE, A. M., DeEDS, F. and RATHER, L. J. (1949). *J. industr. Hyg.* **31**, 158.
89. GUPTA, D. N. (1955). *Nature, Lond.* **175**, 257.
90. NYGAARD, O., ELDJARN, L. and NAKKEN, K. F. (1954). *Cancer Res.* **14**, 625.
91. BLOOD, F. R. and LEWIS, H. B. (1941). *J. biol. Chem.* **139**, 413.
92. SCHULMAN, J. jun. and KEATING, R. P. (1950). *J. biol. Chem.* **183**, 215.
93. SCHULMAN, J. jun. (1950). *J. biol. Chem.* **186**, 717.
94. ASTWOOD, E. B. (1943). *J. Amer. med. Ass.* **122**, 78.

95. CHALLENGER, F. and CHARLTON, P. T. (1947). *J. chem. Soc.* p. 424.
96. WILLIAMS, R. T. (1947). *Detoxication Mechanisms*. London: Chapman & Hall.
97. BRAY, H. G., FRANCIS, G. E., NEALE, F. C. and THORPE, W. V. (1950). *Biochem. J.* **46**, 267.
98. CLAPP, J. W. (1956). *J. biol. Chem.* **223**, 207.

Inorganic Forms of Sulphur

In this chapter a description is given of the metabolism, in animals, of elementary sulphur and the inorganic ions, sulphide, thiosulphate, tetrathionate, sulphite, sulphate and thiocyanate. The microbiological aspects of the subject are discussed in Chapter IX and are considered here only when the metabolism of an inorganic form of sulphur is bound up with the activity of micro-organisms in the alimentary tract.

Elementary sulphur

The small absorption which occurs when powdered sulphur is fed to animals is thought to result from its conversion to hydrogen sulphide by intestinal micro-organisms [1, 2, 3]. In colloidal form, sulphur is said to be absorbed readily [3, 4] and Greengard and Woolley [3] claimed that colloidal sulphur administered to human subjects in doses of 0.50 to 0.75 g. was completely absorbed and excreted quantitatively in the urine as sulphate. They observed a marked increase in the urinary excretion of sulphate within two hours of administering the sulphur. Preparations of colloidal sulphur are liable to contain polysulphides and polythionates, however, and the possibility exists that these compounds may have been sources of sulphate. When Tarver and Schmidt [5] fed 1 mg. doses of elementary sulphur labelled with ^{35}S to rats they found that the greater part of the ^{35}S appeared in the urine in the form of sulphate. Less than 0.035% of the ^{35}S administered was present in the proteins of the internal organs and it was concluded that the rat probably cannot use elementary sulphur for the synthesis of sulphur-containing amino acids. In ruminants, on the other hand, the activity of micro-organisms in the alimentary tract results in the utilization of inorganic forms of sulphur, and in the lamb, for example, a consequence of this

is that elementary sulphur can be used in the synthesis of sulphur-containing amino acids [6].

Sulphide

Hydrogen sulphide is a highly toxic substance and exposure to an atmosphere containing 0.6 mg. of hydrogen sulphide per litre for 30 to 60 minutes is followed by fatal illness in man [7]. Its effect on tissues appears to be selective, for Smythe [8] found that whereas the oxygen consumption of rat liver and kidney was not sensitive to hydrogen sulphide, this compound markedly inhibited the oxygen consumption of brain. Haggard [9, 10] observed, however, that quite considerable amounts of sodium sulphide could be injected intravenously into dogs without producing toxic effects, and he showed that oxygenated blood can bring about the oxidation of sulphide-sulphur. It is known that sulphide when administered intravenously to dogs is oxidized to sulphate [11], and when Dziwiatkowski [12] gave small doses of sodium [^{35}S]sulphide to rats by mouth (1.66 mg. sulphide-sulphur) or by intraperitoneal injection (0.2 mg. sulphide-sulphur) he found that about half the dose was excreted in the urine as sulphate on the first day after dosing.

There is an enzyme present in bacteria and in animal tissues which can bring about the breakdown of cysteine to pyruvic acid, ammonia and hydrogen sulphide. This enzyme, cysteine desulphydrase or desulphurase, has been the subject of reviews by Smythe [13] and by Fromageot [14]. Other desulphydrases are known, and these include a homocysteine desulphydrase which catalyses the production of hydrogen sulphide from homocysteine [15], and an exocystine desulphydrase which liberates hydrogen sulphide from peptides with terminal cystine groupings [16]. The mode of formation of hydrogen sulphide from cysteine is discussed in Chapter II.

It has been established that in addition to undergoing oxidation to sulphate in the animal body, sulphide-sulphur can to a small extent give rise to cystine-sulphur. When rats were given an intraperitoneal injection of 1 mg. of sodium [^{35}S]sulphide daily for 11 days and then killed, the cystine isolated from their hair, skin, muscle and liver showed a small but definite content of ^{35}S . A low specific activity was also shown by the *p*-bromophenylmercapturic acid obtained after each of the rats had been

dosed with 0.15 ml. of bromobenzene daily for three days during the experiment [17]. Conversion of sulphide-sulphur to cysteine-sulphur might occur as a consequence of the reversibility of the desulphydrase system, and this possibility was examined by Smythe and Halliday [18]. They incubated cysteine and sodium [^{35}S]sulphide with a cysteine desulphydrase preparation and stopped the reaction before all the cysteine had broken down. The cysteine which remained was separated, and after careful purification was found to have a low content of ^{35}S . Formation of cysteine from pyruvic acid, ammonia and sulphide was not found to occur, however, and although these experiments demonstrated the formation of cysteine-sulphur from sulphide-sulphur the mechanism was not established. While it appears unlikely that the sulphide ion plays a significant part in cysteine formation in animal tissues, it may have a major role in the synthesis of sulphur-containing amino acids by micro-organisms such as those which occur in the rumen [19].

Thiosulphate

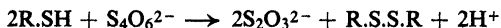
The presence of thiosulphate in the urine of cats and dogs was demonstrated in 1867 by Schmiedeberg [20], by isolation of the barium salt. Since then, thiosulphate has been shown to be a normal constituent of the urine of man and a variety of animals [21]. In early attempts to determine the amount of thiosulphate in urine the analytical procedures used were lacking in specificity. In 1932, however, triethylenediamine nickel nitrate was introduced as a specific precipitant for thiosulphate [22], and was subsequently applied to its determination in urine [23, 24, 25]. This reagent was used by Gast, Arai and Aldrich [25] who found thiosulphate invariably present in the urine of healthy adults and in amounts ranging from 2 to 17 mg. of thiosulphate-sulphur per day. The urinary output of thiosulphate probably gives little indication of the amount formed during the course of metabolism, for it is known that thiosulphate is oxidized to sulphate in the intact animal [26, 27, 28], and this has also been shown to occur in tissue slices [29]. Thiosulphate is also capable of serving as a sulphur donor in the synthesis of thiocyanate by the enzyme rhodanese (see p. 143), but the extent to which it normally participates in this process is unknown.

It is generally agreed that thiosulphate is derived in the body

from the breakdown of cystine and methionine [21, 25], but there have been different opinions expressed concerning the nature of the process which brings this about. A number of workers, including Pirie [29], have favoured the view that thiosulphate formation is dependent on the activity of the intestinal flora. According to Vassel, Partridge and Crossley [24] the excretion of thiosulphate by dogs ceases when they are fasted and begins again 24 hours after feeding is resumed. On the other hand, Fromageot and Royer [21, 30] have reported that animals, irrespective of whether they are carnivorous or herbivorous, excrete more thiosulphate during starvation than when they are being fed normally, and these workers have concluded that thiosulphate is formed in the tissues and not by the intestinal bacteria. It is noteworthy that Baxter, Van Reen and Pearson [31] have recently reported the presence in rat liver of a system which catalyses the oxidation of sulphide to thiosulphate and which shows markedly increased activity when the rats have been fasted for 24 hours.

Tetrathionate

The metabolism and nephrotoxic action of tetrathionate have been the subject of a study by Gilman, Philips, Koelle, Allen and St. John [32]. These workers observed that the injection of moderate doses of tetrathionate into dogs is quickly followed by the appearance of a lesion of the kidney tubules which may bring about complete anuria within an hour. They established that when tetrathionate is injected intravenously into rabbits and dogs it is converted rapidly to thiosulphate. It can be reduced by various thiols, and Gilman and his coworkers showed that with compounds such as cysteine and 1-thiosorbitol the following reaction takes place:



They were able to protect rabbits against the nephrotoxic action of tetrathionate by the prior administration of cysteine or 1-thiosorbitol, and it appears not unlikely that the toxic effects of tetrathionate, as well as its reduction to thiosulphate in the organism, can be related to its reaction with thiol groups. This is supported by the findings of Goffart and Fischer [33] who have shown that tetrathionate poisoning brings about a decrease in

the reduced glutathione content of tissues which is most marked in the kidney. The redox equilibrium in the cells is thereby disturbed and this in turn affects the thiol groups of the intracellular enzymes.

Sulphite

Interest in sulphite metabolism has increased since evidence was obtained that in the metabolic oxidation of sulphur-containing amino acids to sulphate there is formation of cysteinesulphinic acid, and that this compound gives rise to β -sulphinylpyruvic acid which breaks down to sulphur dioxide and pyruvic acid (see Chapter II). A reversal of this process has been achieved by Chapeville and P. Fromageot [34] who showed that formation of [^{35}S]cysteinesulphinic acid occurred when a preparation from rabbit kidney was incubated under anaerobic conditions with a mixture of pyruvic acid, glutamic acid and ^{35}S -labelled sulphite.

Sulphite is autoxidized in the presence of traces of polyvalent metal ions, but this reaction is unlikely to occur in biological systems, for it is inhibited by proteins. The conversion of sulphite to sulphate was shown to occur *in vivo*, however, by Schmidt and Clark [35]. More recently, Heimberg, Fridovich and Handler [36] have obtained evidence for the occurrence in mammalian liver and kidney, and in certain bacteria, of an enzyme which catalyses the oxidation of sulphite to sulphate, and which appears to be a flavoprotein [37]. It has also been reported that hypoxanthine serves as a cofactor in the oxidation of sulphite by dog-liver preparations [38] and that the first step in the process may be a reaction between sulphite and the disulphide group of lipoic acid [39].

Sulphate

Most studies of sulphate metabolism have been concerned with the sulphuric acid esters and an account of this work is given in Chapter VI. Certain other aspects of the metabolism of sulphate are considered below.

When small doses of sodium [^{35}S]sulphate are administered to man [40] and rats [41, 42, 43, 44] by mouth or by injection, most of the ^{35}S is excreted in the urine as inorganic and ethereal sulphate, and the small proportion which is retained appears to be

mostly in the form of sulphomucopolysaccharide. Considerable interest attaches to the question of whether sulphate-sulphur can be used in the animal body in the synthesis of sulphur-containing amino acids. While there is little evidence to suggest that animal tissues can bring this about, there are good grounds for believing that it does take place in animals, and that its occurrence is dependent on the activity of micro-organisms in the alimentary tract. It has been stated that all the amino acids which have been found to be essential components of the diet of the rat can be synthesized in the sheep, provided the diet supplies sufficient urea and inorganic sulphate [45, 46]. In cows [47] and goats [48] the utilization of sulphate-sulphur in the synthesis of cystine and methionine occurs rapidly, and within three hours of the ingestion of [^{35}S]sulphate it is possible to detect ^{35}S in the amino acids of the milk proteins. The steps whereby this is brought about are uncertain, but the protein of the rumen bacteria would appear to be a probable source of these ^{35}S -labelled amino acids. Reduction of sulphate has been shown to occur in the rumen of the sheep [19], and ^{35}S -labelled cystine and methionine have been found to be components of the protein present in the rumen of this animal after feeding [^{35}S]sulphate [47, 48].

The conversion of sulphate-sulphur to cystine-sulphur is not confined to ruminants, for it has been shown to occur in the growing chicken [49], the laying hen [50] and the rat [51]. In the hen the administration of carrier-free $\text{H}_2^{35}\text{SO}_4$ by mouth or by intramuscular injection leads to the production of eggs in which the cystine, but not the methionine, is labelled with ^{35}S . Although earlier workers [52, 53] were unable to obtain evidence of the utilization of sulphate-sulphur in the synthesis of cystine by adult rats, Dziewiatkowski [51] has recently shown this to take place in growing rats following the intraperitoneal injection of carrier-free $\text{Na}_2^{35}\text{SO}_4$. While this occurred after the injection of inorganic sulphate, the amount of sulphate-sulphur involved was very small, and some of it may have passed into the intestinal tract and there been acted upon by bacteria.

In the chick embryo, unlike the growing chicken and the hen, administered sulphate-sulphur is not used in cystine synthesis [54, 55]. While some of this sulphate-sulphur is used by the embryo in the synthesis of sulphomucopolysaccharides [56, 57], much of it is incorporated into taurine. Lowe and Roberts [55]

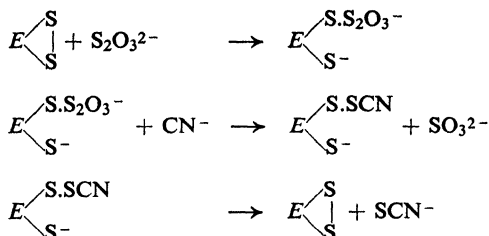
found this latter reaction to occur within 30 minutes of the injection of ^{35}S -labelled sulphate into the embryo, and Machlin, Pearson and Denton [54] showed that about 65% of the ^{35}S injected as sodium sulphate into a 24-hour embryo was recovered as taurine in the day-old chick. This type of change is not confined to embryonic tissues, for Boström and Åqvist [53] found that a small proportion of the ^{35}S administered to adult rats as $\text{Na}_2^{35}\text{SO}_4$ could be detected in taurine separated from the liver. The mechanism of the process whereby sulphate-sulphur is used in the synthesis of taurine has yet to be elucidated.

Thiocyanate

Lang [58, 59] showed that cyanide is converted to thiocyanate as a result of the activity of an enzyme, rhodanese, which occurs in liver and other tissues. Small amounts of thiocyanate are present in blood, urine and saliva, and it is thought that they originate from traces of cyanide produced by metabolic processes within the animal or liberated from dietary sources such as the cyanogenetic glycosides. In smokers the concentration of thiocyanate in the blood is higher than in non-smokers [60, 61], and inhalation of tobacco smoke has been shown to be followed by a sharp increase in the thiocyanate level of the saliva [62]. Cyanide administration leads to an increased excretion of thiocyanate in the urine [63], and a similar effect has been observed in animals dosed with organic nitriles, such as acetonitrile, which give hydrocyanic acid when metabolized [64]. Boxer and Rickards [65] found that after injecting ^{14}C -labelled cyanide into a dog the radioactive carbon appeared in the urine as free cyanide, vitamin B_{12} -bound cyanide and thiocyanate.

Rhodanese is widely distributed in mammalian tissues [58, 66]. In the presence of a suitable sulphur donor, such as thiosulphate [58], the enzyme readily brings about the conversion of cyanide to thiocyanate. Other sulphur compounds, including several aliphatic and aromatic thiosulphonates have been found to serve as sulphur donors in the reaction [67]. The enzyme has been separated from beef liver and obtained in crystalline form by Sörbo [68, 69], who has proposed a reaction mechanism in which one or more disulphide groups of the enzyme [E] combine with thiosulphate. This product then reacts with cyanide so as to

split off sulphite and give a labile enzyme-thiocyanate complex which breaks down with the liberation of thiocyanate [67, 70].



Fiedler and Wood [71] have obtained evidence that the rhodanese system of Lang contains not only the enzyme for which thio-sulphate serves as the sulphur donor in thiocyanate formation (thiosulphate-cyanide transsulphurase) but also another transsulphurase which uses the sulphur of β -mercaptopyruvic acid for this purpose.

Thiocyanate itself is liable to undergo breakdown in the body. After administering ^{14}C -labelled thiocyanate to rats, Boxer and Rickards [65] observed the oxidation of about 30% of the thiocyanate carbon to carbon dioxide. In experiments by other workers in which ^{35}S -labelled thiocyanate was injected into rats, the major part of the dose was excreted unchanged, and less than 5% of the thiocyanate-sulphur was excreted as sulphate in the urine [72]. Cyanide has been detected in the blood of dogs following the intravenous injection of toxic doses of sodium thiocyanate [73], and there have been several reports [74, 75, 76] of the presence of an enzyme in human and animal erythrocytes which brings about the conversion of thiocyanate to cyanide.

REFERENCES

1. DENIS, W. and REED, L. (1927). *J. biol. Chem.* **73**, 51.
2. ANDREWS, J. C. (1937-38). *J. biol. Chem.* **122**, 687.
3. GREENGARD, H. and WOOLLEY, J. R. (1940). *J. biol. Chem.* **132**, 83.
4. MAILLARD, L.-C. (1911). *C.R. Acad. Sci., Paris*, **152**, 1583.
5. TARVER, H. and SCHMIDT, C. L. A. (1942). *J. biol. Chem.* **146**, 69.

6. STARKS, P. B., HALE, W. H., GARRIGUS, U. S. and FORBES, R. M. (1952). *J. Anim. Sci.* **11**, 776.
 7. RUMPEL, W. (1950). *Mitt. chem. ForchInst. Ind. Wien*, **4**, 113.
 8. SMYTHE, C. V. (1943). *Arch. Biochem.* **2**, 259.
 9. HAGGARD, H. W. (1921). *J. biol. Chem.* **49**, 519.
 10. HAGGARD, H. W., HENDERSON, Y. and CHARLTON, T. J. (1922). *Amer. J. Physiol.* **61**, 289.
 11. DENIS, W. and REED, L. (1927). *J. biol. Chem.* **72**, 385.
 12. DZIEWIATKOWSKI, D. D. (1945). *J. biol. Chem.* **161**, 723.
 13. SMYTHE, C. V. (1945). *Advanc. Enzymol.* **5**, 237.
 14. FROMAGEOT, C. (1951). *The Enzymes*, chap. 41, p. 1237. Ed. by Sumner, J. B. and Myrbäck, K. New York: Academic Press.
 15. FROMAGEOT, C. and DESNUELLE, P. (1942). *C.R. Acad. Sci., Paris*, **214**, 647.
 16. GREENSTEIN, J. P. and LEUTHARDT, F. M. (1944). *J. nat. Cancer Inst.* **5**, 209.
 17. DZIEWIATKOWSKI, D. D. (1946). *J. biol. Chem.* **164**, 165.
 18. SMYTHE, C. V. and HALLIDAY, D. (1942). *J. biol. Chem.* **144**, 237.
 19. LEWIS, D. (1954). *Biochem. J.* **56**, 391.
 20. SCHMIEDEBERG, O. (1867). *Arch. Heilk.* **8**, 422.
 21. FROMAGEOT, C. and ROYER, A. (1945). *Enzymologia*, **11**, 361.
 22. SPACU, G. and SPACU, P. (1932). *Z. anal. Chem.* **89**, 192.
 23. GAST, J. H. (1940). Publ. No. 214, University Microfilms, Vol. 2 (No. 2).
 24. VASSEL, B., PARTRIDGE, R. and CROSSLEY, M. L. (1944). *Arch. Biochem.* **4**, 59.
 25. GAST, J. H., ARAI, K. and ALDRICH, F. L. (1952). *J. biol. Chem.* **196**, 875.
 26. TRACHTENBERG, W. (1861). Dissertation, Dorpat.
 27. NYIRI, W. (1923). *Biochem. Z.* **141**, 160.
 28. ZÖRKENDÖRFER, W. (1935). *Biochem. Z.* **278**, 191.
 29. PIRIE, N. W. (1934). *Biochem. J.* **28**, 1063.
 30. FROMAGEOT, C. and ROYER, A. (1944). *C.R. Acad. Sci., Paris*, **219**, 37.
 31. BAXTER, C. F., VAN REEN, R. and PEARSON, P. B. (1956). *Fed. Proc.* **15**, 215.
 32. GILMAN, A., PHILIPS, F. S., KOELLE, E. S., ALLEN, R. P. and ST. JOHN, E. (1946). *Amer. J. Physiol.* **147**, 115.
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33. GOFFART, M. and FISCHER, P. (1947-48). *Arch. int. Physiol.* **55**, 258.
34. CHAPEVILLE, F. and FROMAGEOT, P. (1954). *Biochim. biophys. Acta*, **14**, 415.
35. SCHMIDT, C. L. A. and CLARK, G. W. (1922). *J. biol. Chem.* **53**, 193.
36. HEIMBERG, M., FRIDOVICH, I. and HANDLER, P. (1953). *J. biol. Chem.* **204**, 913.
37. FRIDOVICH, I. and HANDLER, P. (1954). *Fed. Proc.* **13**, 212.
38. FRIDOVICH, I. and HANDLER, P. (1956). *J. biol. Chem.* **221**, 323.
39. FRIDOVICH, I. and HANDLER, P. (1956). *J. biol. Chem.* **223**, 321.
40. BORSOOK, H., DEASY, C. L., HAAGEN-SMIT, A. J., KEIGHLEY, G. and LOWY, P. H. (1949). *J. biol. Chem.* **179**, 689.
41. SINGHER, H. O. and MARINELLI, L. (1945). *Science*, **101**, 414.
42. DZIEWIATKOWSKI, D. D. (1949). *J. biol. Chem.* **178**, 197.
43. EVERETT, N. B. and SIMMONS, B. S. (1952). *Arch. Biochem. Biophys.* **35**, 152.
44. LAIDLAW, J. C. and YOUNG, L. (1953). *Biochem. J.* **54**, 142.
45. LOOSLI, J. K., WILLIAMS, H. H., THOMAS, W. E., FERRIS, F. H. and MAYNARD, L. A. (1949). *Science*, **110**, 144.
46. THOMAS, W. E., LOOSLI, J. K., WILLIAMS, H. H. and MAYNARD, L. A. (1951). *J. Nutr.* **43**, 515.
47. BLOCK, R. J. and STEKOL, J. A. (1950). *Proc. Soc. exp. Biol., N.Y.* **73**, 391.
48. BLOCK, R. J., STEKOL, J. A. and LOOSLI, J. K. (1951). *Arch. Biochem. Biophys.* **33**, 353.
49. MACHLIN, L. J., JACKSON, J. T., LANKENAU, A. H. and PEARSON, P. B. (1954). *Poult. Sci.* **32**, 234.
50. MACHLIN, L. J., PEARSON, P. B., DENTON, C. A. and BIRD, H. R. (1953). *J. biol. Chem.* **205**, 213.
51. DZIEWIATKOWSKI, D. D. (1954). *J. biol. Chem.* **207**, 181.
52. TARVER, H. and SCHMIDT, C. L. A. (1939). *J. biol. Chem.* **130**, 67.
53. BOSTRÖM, H. and ÅQVIST, S. (1952). *Acta chem. scand.* **6**, 1557.
54. MACHLIN, L. J., PEARSON, P. B. and DENTON, C. A. (1955). *J. biol. Chem.* **212**, 469.
55. LOWE, I. P. and ROBERTS, E. (1955). *J. biol. Chem.* **212**, 477.

56. LAYTON, L. L., FRANKEL, D. R. and SCAPA, S. (1950). *Cancer*, N.Y. **3**, 725.
57. LAYTON, L. L. and FRANKEL, D. R. (1951). *Arch. Biochem. Biophys.* **31**, 161.
58. LANG, K. (1933). *Biochem. Z.* **259**, 243.
59. LANG, K. (1933). *Biochem. Z.* **263**, 262.
60. TRASOFF, A. and SCHNEEBERG, N. G. (1944). *Amer. J. med. Sci.* **207**, 63.
61. STØA, K. F. (1951). *Medd. norsk farm. Selsk.* **13**, 93.
62. ARMENIO, G., LAFORGIA, P. D. and BUONSANTO, M. (1953). *Boll. Soc. ital. Biol. sper.* **29**, 152.
63. BODANSKY, M. and LEVY, M. D. (1923). *Arch. intern. Med.* **31**, 373.
64. LANG, S. (1894). *Arch. exp. Path. Pharmac.* **34**, 247.
65. BOXER, G. E. and RICKARDS, J. C. (1952). *Arch. Biochem. Biophys.* **39**, 7.
66. ROSENTHAL, O. (1948). *Fed. Proc.* **7**, 181.
67. SÖRBO, B. H. (1953). *Acta chem. scand.* **7**, 1137.
68. SÖRBO, B. H. (1953). *Acta chem. scand.* **7**, 238.
69. SÖRBO, B. H. (1953). *Acta chem. scand.* **7**, 1129.
70. SÖRBO, B. H. (1951). *Acta chem. scand.* **5**, 1218.
71. FIEDLER, H. and WOOD, J. L. (1956). *J. biol. Chem.* **222**, 387.
72. WOOD, J. L., WILLIAMS, E. F. jun. and KINGSLAND, N. (1947). *J. biol. Chem.* **170**, 251.
73. GOLDSTEIN, F. and RIEDERS, F. (1951). *Amer. J. Physiol.* **167**, 47.
74. PINES, K. L. and CRYMBLE, M. M. (1952). *Proc. Soc. exp. Biol.*, N.Y. **81**, 160.
75. GOLDSTEIN, F. and RIEDERS, F. (1953). *Amer. J. Physiol.* **173**, 287.
76. BRIN, M. (1955). *Fed. Proc.* **14**, 186.

Microbiological Aspects of Sulphur Metabolism

Important advances in our knowledge of sulphur compounds and their metabolism have resulted from studies with micro-organisms. Investigations of this kind led, for example, to the discovery of methionine, biotin and lipoic acid, and have contributed much to our understanding of the roles of thiamine and coenzyme A. The main features of the metabolism of sulphur compounds in micro-organisms form the subject of the present chapter.

Sulphur requirements of micro-organisms

While micro-organisms resemble animals closely in the nature of their constituent sulphur compounds, they differ from animals in showing a remarkably wide variation in their nutritional requirements for sulphur.

A number of bacteria, including many of the saprophytic species found in soil, are able to utilize purely inorganic forms of sulphur, in particular sulphate, as their main source of this element. They include the nitrogen-fixing *Azotobacter* and *Rhizobia*, as well as species of *Pseudomonas*, *Proteus* and *Neisseria*. Sulphate can act as the sole sulphur source for certain of these bacteria, but the majority also require the presence of biotin in the medium, and others need thiamine. Other species have more exacting demands, and require cystine or methionine, or both these compounds. Examples of such bacteria are *Clostridium botulinum* which requires methionine, some strains of *Lactobacillus casei* and *Leuconostoc mesenteroides* which require cystine but not methionine, and *Lact. arabinosus* which requires both amino acids. These bacteria also differ in their need for

biotin and thiamine [1]. Another growth factor which plays an important role in bacterial metabolism is α -lipoic acid. Before its chemical identity was established, this compound was known as the 'acetate replacement factor' and as 'protogen'. It has been shown to stimulate the growth of *Streptococcus faecalis*, *Escherichia coli* and other organisms. It can, however, be replaced in culture media by acetate or by intermediates of the tricarboxylic acid cycle, while for some organisms even bicarbonate will serve as a substitute.

The presence of a particular sulphur compound in the medium may not be essential for the growth of an organism, yet it may produce an increased rate of growth. This may be taken to indicate that the compound is synthesized, but at a rate inadequate for maximal growth. The growth-stimulating effect of methionine on *Staphylococcus aureus*, of cystine on *Brucella tularensis*, and of biotin on *Proteus vulgaris* are examples of this. Another aspect of bacterial nutrition is that even individual strains of the same organism may differ in their requirements, for some need to be supplied with more compounds than others, as a result of being devoid of certain synthetic abilities. These more exacting strains may often be 'trained' to utilize simpler nutrients if they are subcultured several times in a suitable medium. For example, some strains of *Corynebacterium diphtheriae* require cystine and methionine for growth, but they can be trained to use inorganic sulphide in place of these amino acids. In a similar manner the ability to synthesize thiamine may be developed in *Propionibacteria* and *Staphylococci* which normally require this growth factor. Bacteria are biologically so labile that it is evident that any rigid classification of their nutritive requirements may be misleading. An important point which has emerged from these various studies, however, is that cystine, methionine, biotin and thiamine play essential parts in the metabolism of micro-organisms.

In general, it would appear that other micro-organisms have the same range of nutritional requirements as bacteria. Most yeasts, moulds and fungi, and also many protozoa, can synthesize the sulphur-containing amino acids from sulphate, although some appear to have lost this ability. The yeast *Mycoderma vini* and the mould *Allomyces arbuscula*, for example, require cystine for growth, and the yeasts *Schizosaccharomyces* and *Candida*

albicans, and moulds of the genus *Blastocladia* all require methionine. The extent to which yeasts can synthesize biotin and thiamine varies, and in consequence these compounds often stimulate growth. Among the first to show that thiamine is concerned in the metabolism of micro-organisms was Schopfer [2] who established that it is a growth factor for the fungus *Phycomyces blakesleeana*. Biotin is essential in the nutrition of *Neurospora* and also of *Aspergillus niger*. In the case of the wood-rotting fungi of the genus *Schizophyllum*, both biotin and thiamine have been found essential for growth.

The protozoa vary markedly in their sulphur requirements [3]. Whereas most of them need thiamine, some, such as the flagellate *Chilomonas paramecium*, can grow when only inorganic salts are provided. *Tetrahymena geleii*, which has been the subject of extensive biochemical study, requires methionine but not cystine, and its growth is stimulated by thiamine, which it can synthesize only slowly [4]. It also has a requirement for α -lipoic acid, although this can be replaced in part by acetic acid.

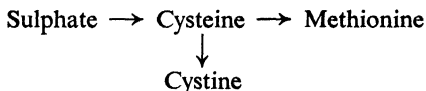
Knowledge of the nutritional requirements of micro-organisms has found application in the development of methods for the assay of amino acids, the B vitamins and other compounds. Several methods of this type, employing the bacteria *Lact. fermenti*, *Lact. arabinosus*, *Leuc. mesenteroides* and *Strep. faecalis*, have been described for the estimation of methionine [5, 6, 7]. Addition of the amino acid, or material containing it, to cultures of these organisms in a methionine-deficient medium produces a growth response which may be evaluated in terms of the increased opacity of the culture or the amount of lactic acid produced. *Lact. lycopersici*, *Lact. buchneri* and *Leuc. mesenteroides* have been used for the assay of cysteine and cystine [8]. Biotin may be assayed with *Lact. casei* or *Lact. arabinosus*, and thiamine by its effect on the fermentation rate of various yeasts [9].

Synthesis of sulphur-containing amino acids

Information about these processes has been obtained from experiments on the nutritional requirements of micro-organisms, and more particularly from the use of mutants produced by irradiating bacteria and moulds with X-rays or ultra-violet light, or by treating them with chemical mutagenic agents, such as mustard gas and the nitrogen mustards. The utilization of ^{35}S -labelled

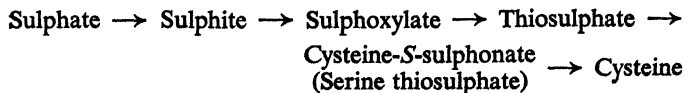
compounds by organisms under various conditions has also provided valuable quantitative data.

The fact that many bacteria, moulds and fungi grow and synthesize protein when inorganic sulphate is their sole source of sulphur, is clear evidence that these organisms form sulphur-containing amino acids from inorganic sulphur. This has been demonstrated directly in the case of *Esch. coli* grown in a medium containing radioactive sulphate. The organism accumulated considerable quantities of the isotope in the methionine and cystine of the cellular proteins [10]. For most, if not all micro-organisms, the overall reaction is believed to be:

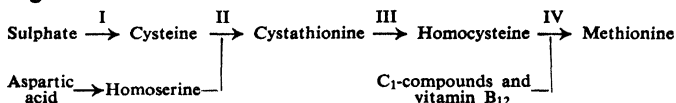


The various steps in the process will be considered separately.

Conversion of sulphate to cysteine. Little more than a beginning has so far been made in elucidating the mechanism of cysteine synthesis. It clearly involves the reduction of the sulphate-sulphur and also its conversion to an organic form. There is general agreement that sulphate is first reduced to sulphite, but the intermediates beyond this stage are not known with any certainty. It has been suggested that reduction to sulphide occurs, and that the hydrogen sulphide formed interacts with pyruvic acid or alanine to give cysteine by a reversal of the cysteine desulphydrase reaction (see Chapter II). Apart from some strains of *Pasteurella pestis*, however, few organisms have been found to utilize hydrogen sulphide in this way. A further possibility is the interaction of sulphite and pyruvic acid to give β -sulphinylpyruvic acid, followed by a transamination and a reduction to yield cysteine. The first step is analogous to the Wood-Werkman reaction. From growth studies with mutants of *Neurospora crassa*, Horowitz [11] has concluded that thiosulphate is another intermediate in the synthesis, and Hockenhull [12] has suggested that the following series of reactions is operative in *Aspergillus nidulans*:



Conversion of cysteine to methionine. The main steps in the formation of methionine in *N. crassa*, *B. subtilis*, *Esch. coli* and other organisms, are believed to be as follows:



The various stages have been deduced from studies of the nutritional requirements of mutant strains of these organisms [11, 13, 14]. Mutants of *N. crassa* with differing sulphur requirements have been produced by exposure of cultures of the mould to X-rays. Those which cannot make use of inorganic sulphate, but which require preformed cystine for growth, are described as 'cystineless' mutants. Others, which require methionine, are known as 'methionineless' mutants. In certain of the latter group, methionine can be replaced by homocysteine or by cystathionine. The cystineless mutants are unable to bring about stage I of the synthesis, although if provided with cysteine or cystine they can carry out the succeeding steps. Of the methionineless mutants, those which can use cystathionine have lost the ability to bring about stage II, and they can only carry out stages III and IV. Those which can use homocysteine, but not cystathionine, cannot achieve stage III, while the mutants which have an absolute requirement for methionine, have lost the ability to carry out stage IV. It is of interest that in the case of the protozoon, *Tetrahymena geleii*, the nutritional requirement for methionine is absolute, and it cannot be satisfied by homocysteine, even in the presence of vitamin B₁₂ and suitable methyl donors. This organism appears unable to carry out stage IV of the synthesis.

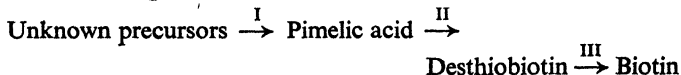
It is probable that in *Aerobacter aerogenes* and in the yeasts *Saccharomyces* there exists a second pathway of methionine synthesis, in which the nucleoside, 5'-methylthioadenosine, is an intermediate. This compound appears to be capable of transferring its thiomethyl group to a four-carbon compound, such as α -aminobutyric acid, to give methionine. This has been confirmed with the aid of 5'-methylthioadenosine labelled with ¹⁴C and ³⁵S [15]. The origin of the sulphur of the nucleoside is not known.

Conversion of methionine to cysteine. A large number of micro-organisms which normally require either sulphate or cystine as

their main sulphur source can make use of methionine, and this can be interpreted as indicating that they can convert methionine to cysteine. The reaction is believed to take place by a mechanism akin to that occurring in animals (see Chapter III). By no means all bacteria can achieve the conversion, however, and this is often the result of their inability to carry out the initial demethylation of methionine. For example, strains of *Lact. casei*, *Lact. arabinosus* and *Leuc. mesenteroides*, which normally require cystine for growth, will not grow when this amino acid is replaced by methionine, but will do so in the presence of a mixture of homocysteine and serine [16]. These bacteria are therefore unable to form homocysteine, but they can convert it to cystathionine and thence to cysteine. In experiments with *Esch. coli*, Cowie, Bolton and Sands [17] observed that the uptake of [³⁵S]methionine was less than half that of labelled cystine or inorganic sulphate, and that it was unaffected by the presence of a wide variety of sulphur compounds in the medium. The methionine taken up by these bacteria was probably incorporated directly into cell proteins without being catabolized to any great extent.

Synthesis of biotin, thiamine and lipothiamide

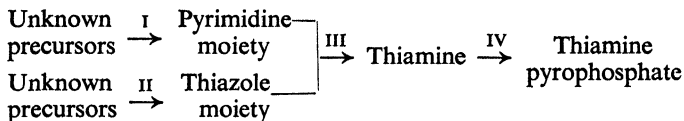
In the period between the recognition of biotin as a microbial growth factor and the determination of its chemical structure, pimelic acid was found to stimulate the growth of certain strains of *Corynebacterium diphtheriae*. It was subsequently shown that in these organisms biotin can substitute for pimelic acid, and it is now believed that the latter compound is a precursor of biotin. A related compound, desthiobiotin, is considered to be another intermediate in biotin formation. The main steps in the synthesis are now thought to be as follows:



Micro-organisms differ markedly in their ability to synthesize biotin. Whereas *B. subtilis* is able to form the growth factor from simple precursors, *Lact. casei* is incapable of performing any of the steps in the process, and its requirement for biotin is absolute. *C. diphtheriae* can perform stages II and III, but not I, and it requires preformed pimelic acid. *Leuc. mesenteroides* can

carry out only stage III, for it can make use of desthiobiotin, but not pimelic acid.

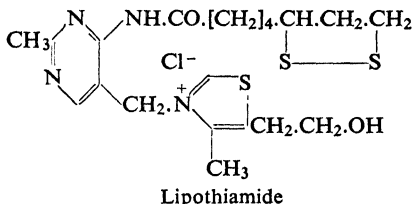
Studies of the ability of different micro-organisms to synthesize thiamine, or to use related compounds in its place, have revealed some of the steps in the formation of this substance:



C. diphtheriae and *Neisseria gonorrhoeae* are two organisms which show wide differences in their ability to synthesize thiamine. The former organism can apparently perform all these reactions, for it requires neither preformed thiamine nor related compounds. *N. gonorrhoeae*, on the other hand, is not only unable to form thiamine, but is even incapable of converting it to its pyrophosphate (stage IV). The last step can be carried out by *Strep. salivarius*, but this organism can bring about none of the others, and it is therefore dependent on an external supply of the growth factor. Other organisms occupy intermediate positions. *Staph. aureus*, while unable to synthesize the separate heterocyclic portions of the molecule, can bring about their combination (stages III and IV). The thiazole moiety, in the form of certain thiazole derivatives, is required by *Mucor ramannianus* and this mould can achieve stages I, III and IV, whereas the yeast *Endomyces vernalis* requires the pyrimidine moiety, and can perform stages II, III and IV of the reaction sequence. The types of compound which can act as precursors of the thiazole and pyrimidine components of the thiamine molecule have been discussed in detail by Knight [1] in his extensive review of microbial growth factors.

α -Lipoic acid is known to influence the growth of a number of micro-organisms and may well play a role in the metabolism of all of them. Reed and his coworkers [18] have shown that commercial yeast extracts contain several forms of lipoic acid in which the compound appears to be linked to thiamine. A certain mutant of *Esch. coli* was found to grow in the presence of 'bound' lipoic acid, but was unable to utilize the free compound. *Strep. lactis*, however, was shown to grow on a mixture of the free acid

and thiamine and to produce a combined form which facilitated the growth of the *Esch. coli* mutant. It has been suggested by Reed [19] that the metabolism of lipoic acid involves its reaction with thiamine to form lipothiamide. The results of further study of this possibility will be awaited with interest.



Synthesis of sulphur compounds by intestinal and rumen bacteria

No account of the synthetic activities of bacteria would be complete without reference to those inhabiting the alimentary tract. These bacteria are able to synthesize most of the B vitamins and amino acids in amounts which are of great significance in the nutrition of the host [20]. The microbial synthesis of biotin in the intestine of the rat is sufficient to supply the total needs of the animal for this vitamin, and it is therefore not possible to bring about biotin deficiency in the rat simply by feeding it on a biotin-free diet. In fact, rats on such a diet not only grow normally but also excrete considerable quantities of biotin. If, however, one of the less soluble sulphonamides is incorporated in the diet, biotin synthesis in the intestine is diminished or ceases, and the symptoms of biotin deficiency develop.

The main sites of thiamine synthesis in several species of birds and animals have been shown to be the colon and caecum. In general, the extent of synthesis is insufficient to meet the entire needs of the host. In human faeces, Najjar and Holt [21] found appreciable amounts of thiamine of bacterial origin, and this faecal excretion decreased during a nutritional deficiency of the vitamin, as a result of an increased absorption of the compound from the intestine. It also decreased when the activities of the intestinal bacteria were inhibited by sulphonamide therapy.

In ruminants, synthesis of growth factors occurs not only in the colon and caecum but also in the upper parts of the gut. This

leads to a more extensive overall synthesis of these factors and a greater opportunity for them to become available to the host. It is not surprising, therefore, that ruminants are largely independent of a dietary supply of many of the growth factors required by other animals. The rumen is an important site for the synthesis of biotin and thiamine, and the amounts of the vitamins present in this organ increase several-fold within a few hours of the ingestion of food. In the sheep, goat and steer, the essential amino acids, including methionine, are all synthesized by the rumen micro-organisms. The extent of this synthesis may be judged from the finding of Gallup and his coworkers [22] that in the lamb, microbial formation of methionine from inorganic sulphate is sufficient to meet the animal's needs for this amino acid. A valuable review of ruminant nutrition has been written by Huffman [23].

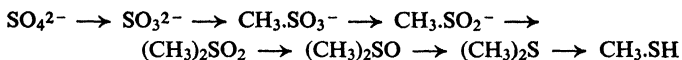
Catabolism of sulphur compounds by micro-organisms

Many of the catabolic changes which sulphur compounds undergo in animal tissues also occur in micro-organisms. Some of these processes are considered in the following sections.

Methionine. As described in a previous section, the demethylation of methionine is achieved by some micro-organisms. The product is assumed to be homocysteine, but the possible role of 'active methionine' as an intermediate in the methyl transfer, with *S*-adenosylhomocysteine as the demethylation product, has not yet been adequately explored. Various bacteria, moulds and fungi are able to convert methionine to methanethiol, dimethyl disulphide and in some cases, dimethyl sulphide. Among these are the *Pseudomonads* and the moulds *Aspergillus niger* and *Scopulariopsis brevicaulis*. The *Pseudomonads* act only on the amino acid itself [24], whereas other bacteria are known which also break down the corresponding α -keto acid. Kallio [24] has applied the name dethiomethylase to the enzyme system responsible for the formation of methanethiol from methionine.

Methanethiol and dimethyl sulphide are also metabolic products of the wood-rotting fungus *Schizophyllum commune*, but in this organism the source of the sulphur of these compounds appears to be inorganic sulphate. A combined reduction and

methylation of sulphate has been suggested to account for the formation of dimethyl sulphide, as follows [25]:

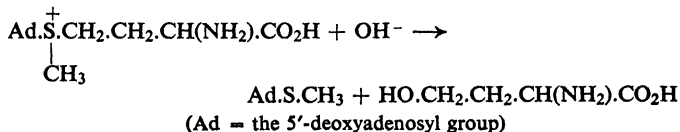


Amino acid oxidases and transaminases present in bacteria and moulds bring about the conversion of the D- and the L- forms of methionine to α -keto- γ -methylthiobutyric acid,



The transaminases require the presence of an amino-group acceptor, such as α -ketoglutaric acid, and the mechanism of the reaction is probably similar to that occurring in the animal.

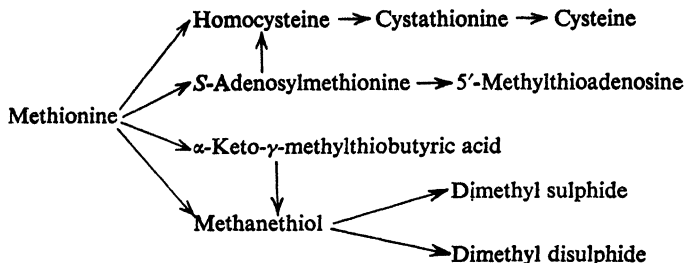
Schlenk and Smith [26] have shown that methionine is used in the biosynthesis of 5'-methylthioadenosine in yeasts. This nucleoside has been known for some considerable time to be a constituent of these organisms [27]. Its accumulation in yeast cells was shown to be dependent on the presence of an adequate supply of methionine in the culture medium, and by the use of the amino acid labelled with ^{14}C and ^{35}S it was established that the thiomethyl ($\text{CH}_3\text{S}-$) group, and not the attached carbon chain, was incorporated into the nucleoside molecule. The process has been termed transthiomethylation, and Schlenk and Smith have proposed that the initial conversion of methionine to S-adenosylmethionine is followed by hydrolytic fission of this intermediate to methylthioadenosine and homoserine:



Baddiley, Cantoni and Jamieson [28] have been able to bring about the chemical counterpart of the latter step by the careful alkaline hydrolysis of the sulphonium compound.

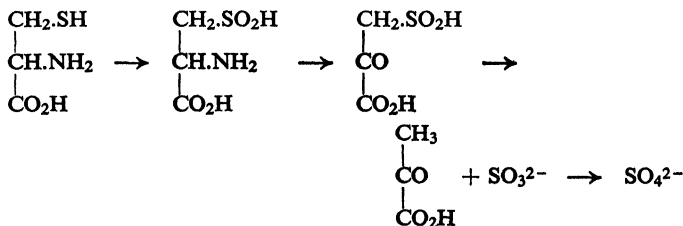
A summary of some of the reactions of methionine which are believed to occur in micro-organisms is given in the diagram on the following page.

Recent experiments with ethionine, the ethyl analogue of methionine, indicate that it can undergo transthioethylation in



yeasts, and that in *Tetrahymena geleii* it is even incorporated into cell proteins.

Cysteine and cystine. A number of the steps in the oxidation of cysteine by *Proteus vulgaris* have been examined in detail by Kearney and Singer [29, 30]. The reaction sequence appears to be identical with that occurring in animals (see Chapter II). The oxidation is believed to involve the intermediate formation of cysteinesulphinic acid, followed by transamination of this compound with either oxaloacetic acid or α -ketoglutaric acid to give β -sulphinylpyruvic acid, which on desulphination forms sulphite and pyruvic acid:

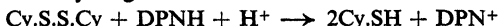


Cysteine is known to be oxidized to sulphate by other bacteria and by moulds and fungi, and the same mechanism may well be operative.

The formation of hydrogen sulphide from cysteine, cystine and related compounds, is brought about by a wide variety of micro-organisms [31]. As in animals (see Chapter III), two pathways for the conversion of cysteine-sulphur to hydrogen sulphide have been proposed. One is a direct desulphydration, leading to

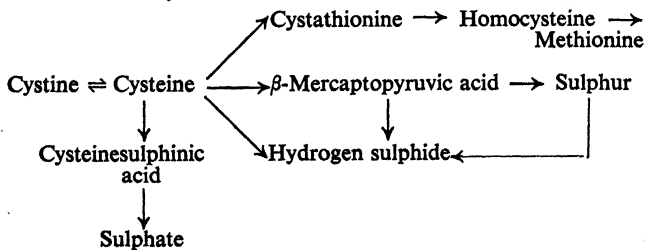
the formation of hydrogen sulphide, pyruvic acid and ammonia [32]. There is some evidence that β -mercaptopyruvic acid may be first formed in this process [33], and it is noteworthy that in yeasts hydrogen sulphide production from this keto acid is more rapid than from cysteine itself [34]. The other mechanism is a transaminative conversion of the amino acid to β -mercaptopyruvic acid, followed by the breakdown of this compound to pyruvic acid and elementary sulphur [35]. The sulphur so formed is then believed to be reduced to hydrogen sulphide by interaction with thiol groups. In support of the occurrence of this catabolic pathway, Meister and his coworkers have demonstrated the formation of sulphur from β -mercaptopyruvic acid by preparations from *Esch. coli* and *Aerobacter aerogenes*. Furthermore, it is known that elementary sulphur is formed from cystine by *Achromobacter cystinovorum* [36]. As β -mercaptopyruvic acid appears to be an intermediate in both of these suggested pathways of cysteine desulphuration, the question arises of whether they are, in fact, distinct mechanisms.

The reduction of cystine to cysteine takes place in many micro-organisms. Various yeasts can bring about this reduction, and they possess specific reductases requiring diphosphopyridine nucleotide as hydrogen carrier [37]:



Racker [38] has recently obtained from baker's yeast a glutathione reductase for which both diphosphopyridine nucleotide and triphosphopyridine nucleotide can act as hydrogen carriers.

Cysteine is used by micro-organisms for the formation of glutathione and coenzyme A, as well as compounds such as penicillin which are specific to certain organisms. The microbial metabolism of cysteine is summarized in the following scheme:



Aliphatic sulphides and disulphides. Challenger and Charlton [25] have shown that some moulds, including *Scopulariopsis brevicaulis*, *Aspergillus niger* and *Penicillium notatum*, act on a number of thioethers and convert them to thiols. *S*-Methyl-, *S*-ethyl- and *S*-propyl-cysteine are metabolized in this way, with the formation of methane-, ethane- and propane-thiol respectively. The reduction of many dialkyl disulphides to the corresponding thiols is also brought about by these organisms. The thiols formed from thioethers and disulphides are generally accompanied by the corresponding alkyl methyl sulphides, and the methyl groups arising in this step have been shown by ¹⁴C-labelling to be derived from such compounds as choline and formic acid [39], as in methylation reactions in animals.

Sulphonium compounds. Certain moulds are able to catabolize sulphonium compounds possessing *S*-carboxyalkyl groups [40]. These groups are split off from the sulphonium pole and a thioether is formed. *P. notatum*, for example, breaks down dimethyl- β -propiothetin and methionine methylsulphonium salts to dimethyl sulphide, although it has no action on dimethylthetin. *S. brevicaulis*, on the other hand, has no action on either dimethylthetin or dimethylpropiothetin, but it converts diethylthetin to diethyl sulphide. There is evidence that methionine methylsulphonium salts can act as methyl donors to homocysteine in micro-organisms, for a mixture of a methionine methylsulphonium salt and homocysteine has been reported to support the growth of *Aerobacter aerogenes*, whereas the compounds separately are ineffective [41]. A similar finding has been obtained with the yeast *Torula utilis*. The participation of the methionine methylsulphonium ion in bacterial metabolism is also apparent from the observation of Shive and his coworkers [42] that this derivative of methionine is more effective than the amino acid itself in promoting growth and in reversing the antibacterial activity of sulphanilamide. It may well have a similar role to *S*-adenosylmethionine as a biologically-active form of methionine.

Metabolism of sulphur compounds by autotrophic bacteria

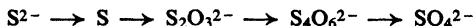
Most heterotrophic bacteria can metabolize inorganic forms of sulphur, but for certain autotrophs the metabolism of elementary

sulphur and inorganic sulphur compounds serves as their primary source of energy. The various reactions which take place are coupled with the reduction of carbon dioxide to other one-carbon compounds, such as formaldehyde. Autotrophic organisms of this type are very widely distributed in nature, for they are found in soils, fresh water and sea water, and have adapted themselves to a wide range of conditions [43]. Several species are found in hot mineral springs at temperatures up to 80° C, and others can tolerate sulphuric acid in concentrations as high as 10 per cent. The term 'sulphur bacteria' is often used to describe these organisms collectively, although in its strictest sense it refers only to those which produce either intracellular or extracellular elementary sulphur. The sulphur bacteria may be grouped as follows: (a) the colourless sulphur bacteria, which oxidize sulphide-sulphur to intracellular elementary sulphur; (b) members of the genus *Thiobacillus*, which oxidize sulphide to extracellular sulphur, and which can further oxidize this sulphur, as well as other inorganic sulphur compounds, to sulphate; (c) the photosynthetic, coloured sulphur bacteria, which carry out many of the reactions achieved by groups (a) and (b); and (d) the sulphate-reducing bacteria, which reduce sulphate and also sulphite and thiosulphate to sulphide.

Colourless sulphur bacteria. The two best-known genera in this large group of organisms, *Beggiatoa* and *Thiothrix*, were first systematically investigated by Winogradsky [44]. These are strict autotrophs which require aerobic conditions and a source of carbon dioxide. They bring about the oxidation of sulphide-sulphur to elementary sulphur, and this is accumulated in the form of granules within the cells. When the external supply of sulphide diminishes, the energy required by the cells is provided by the oxidation of the granules to sulphate. During rapid growth, some strains of these bacteria can oxidize up to four times their weight of hydrogen sulphide per day [43].

Thiobacilli. Oxidation of sulphide to free sulphur is also brought about by this group of bacteria. The sulphur generally accumulates in the medium rather than in the cells. Two related species, the aerobes *Thiobacillus thio-oxidans* and *Thiob. thioparus*, can carry the oxidation to sulphate, and they can also oxidize

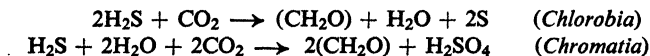
thiosulphate and tetrathionate. The oxidation of sulphide is thought to proceed as follows [45]:



None of the steps beyond the free sulphur stage has yet been established experimentally. A complicating factor in the study of the intermediary metabolites is the likelihood of purely chemical interactions between the compounds used as nutrients and the products formed from them. Thus, the appearance of free sulphur in media containing thiosulphate might be the result of a reaction between thiosulphate and any tetrathionate formed as an intermediate product, or it might arise through the decomposition of thiosulphate by the acid formed.

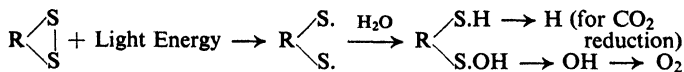
It is significant that the growth of *Thiob. thio-oxidans* is prevented by certain compounds known to be inhibitors of cytochrome systems [46], and there is evidence that the oxidation of sulphur compounds is associated with the appearance of organic phosphates within the cell [47, 48]. Furthermore, chemical examination of this bacterium has revealed the presence of compounds such as adenosine triphosphoric acid, glucose phosphates and diphosphopyridine nucleotide [49, 50]. It is highly probable that *Thiobacilli* possess many of the biochemical characteristics of heterotrophic organisms, but differ from them in the initial mechanisms used to generate labile phosphate compounds.

Coloured sulphur bacteria. These are photosynthetic organisms which generally require a strictly anaerobic environment, and they are facultative autotrophs. The two best-known genera are the green-coloured *Chlorobium* and the purple *Chromatium*, both of which have been studied in detail by van Niel [51]. *Chlorobium thiosulphatophilum*, a member of the former group, according to Larsen [52], oxidizes sulphide to intracellular granules of sulphur and converts this and other inorganic sulphur compounds to sulphate. The purple *Chromatium*, like the *Thiobacilli*, produce extracellular elementary sulphur. The energy-yielding reactions are as follows:



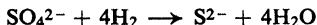
These photochemical reactions are at present far from being understood. Calvin [53] has suggested that in plant photosyn-

thesis α -lipoic acid (RS_2) acts as a carrier of H and OH radicals, following the fission of its disulphide link:

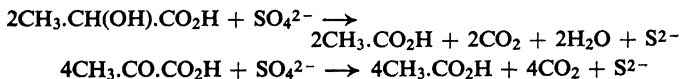


According to this hypothesis, the primary energy-yielding process involves an interaction between lipoic acid and water. Lees [45] has raised the possibility that in the photosynthetic sulphur bacteria, the OH radicals might be destroyed by reaction with such compounds as hydrogen sulphide, sulphur and thiosulphate. The function of inorganic sulphur compounds thus becomes that of electron donors to the unwanted OH radicals. The energy arising from their oxidation is thought not to be available to the organism.

Sulphate-reducing bacteria. In this group, *Desulphovibrio desulphuricans*, an anaerobic facultative autotroph, has been intensively studied on account of the various problems resulting from its prolific production of hydrogen sulphide in soils and stagnant water. This organism reduces inorganic sulphate, sulphite, thiosulphate and tetrathionate to sulphide. It is able to grow autotrophically by using hydrogen gas as an electron donor [54], according to the following equation:



A variety of organic compounds can also act as reducing agents, for example, lactic acid and pyruvic acid:

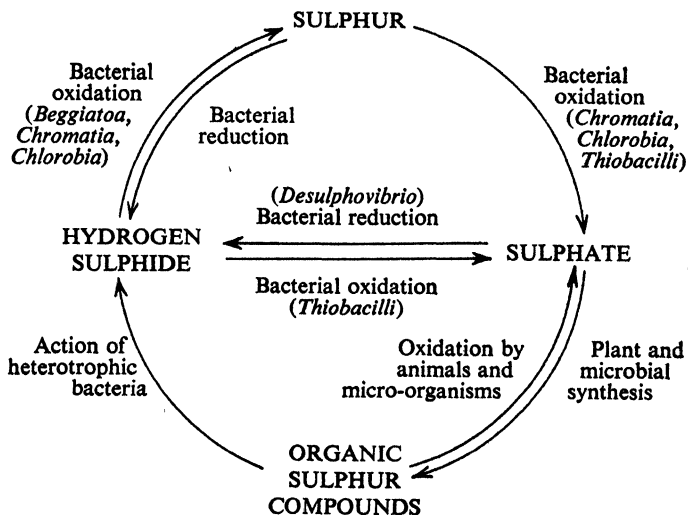


Detailed studies by Postgate [55] indicate that sulphite is an intermediate in the reduction. Thiosulphate and tetrathionate may also be formed, although there is probably a separate mechanism for the metabolism of these compounds, by which they are depolymerized to intermediate ions containing one sulphur atom.

The sulphur cycle in nature

Plants and micro-organisms are responsible for the greater part of the interconversion of sulphur compounds in nature, for the

contribution of animals to this process is quantitatively a small one. A scheme embodying the main biological transformations of sulphur has been put forward by Bunker, Butlin and others [43, 56, 57]. A modified form of this is shown below:



As indicated in the lower half of the diagram, inorganic sulphate is converted by plants and micro-organisms to organic forms of sulphur, the chief of which are the sulphur-containing amino acids. This organic sulphur is to some extent reconverted to sulphate by catabolic reactions in animals and micro-organisms, but most of it is liberated as hydrogen sulphide as a result of bacterial action on plants and animals after their death. This hydrogen sulphide then undergoes oxidation to sulphate both non-enzymically and by the action of various bacteria, and so becomes available once more for absorption by plants.

The upper half of the diagram, which is concerned with transformations between hydrogen sulphide, sulphur and inorganic sulphate, represents a second and subsidiary cycle, although a no less interesting one. Its operation has many effects on our every-

day life, and it is largely because of this that the biochemistry of the organisms responsible has been examined so thoroughly. The production of hydrogen sulphide by the sulphate-reducing bacteria gives rise to widespread problems, for it leads to the pollution of water in refuse and gravel pits, canals, and even certain coastal waters. It is responsible for the death of fish and damage to the paintwork of ships, and it causes corrosion of iron pipes laid through soils which support the growth of these bacteria.

Conditions which lead to the production of hydrogen sulphide by bacteria may also favour the proliferation of *Thiobacilli*, with the result that formation of sulphuric acid takes place. Unless precautions are taken, this can occur in sewers and water towers to such an extent that it brings about rapid disintegration of concrete and stonework. The presence of sulphate-reducing bacteria in the Black Sea is responsible for the characteristic colour of the water, for the hydrogen sulphide produced is converted to finely-divided iron sulphides. In certain areas, however, *Thiobacilli* exist in symbiosis with the sulphate-reducing organisms, and as a result of their oxidation of sulphide to sulphate, colourless zones are found in the water.

A striking example of symbiosis among sulphur bacteria and the operation of the sulphur cycle is provided by the observations of Butlin and Postgate [58] on the formation and deposition of sulphur which takes place in certain lakes in Cyrenaica. These lakes and the springs feeding them contain sufficient sulphate to support the growth of *Desulphovibrio desulphuricans*. On the fringes of the lakes are to be found large colonies of photosynthetic bacteria, including *Chlorobia*, which metabolize the hydrogen sulphide formed by the sulphate-reducing organisms. The sulphur produced by the *Chlorobia* passes into the water and settles as a thick sediment. The photosynthetic bacteria probably supply the small amount of organic matter needed by the *Desulphovibrio* for the reduction of sulphate. The formation of sulphur in one lake of about half a million gallons was estimated to be approximately one hundred tons annually. It is possible that the extensive deposits of sulphur found in Louisiana, Texas and Sicily owe their origin to similar bacterial associations [57]. The microbiological production of sulphur and hydrogen sulphide on a large scale is now being investigated [57], and the results

obtained offer the hope that such processes may become a valuable source of supply in the not unlikely event of a world shortage of elementary sulphur.

REFERENCES

1. KNIGHT, B. C. J. G. (1945). *Vitam. & Horm.* **3**, 105.
2. SCHOPFER, W. H. (1934). *Arch. Mikrobiol.* **5**, 511.
3. KIDDER, G. W. (1951). *Annu. Rev. Microbiol.* **5**, 139.
4. KIDDER, G. W. and DEWEY, V. C. (1951). *The Biochemistry of the Protozoa*. New York: Academic Press.
5. DUNN, M. S., CAMIEN, M. N., SHANKMAN, S. and BLOCK, H. (1946). *J. biol. Chem.* **163**, 577.
6. RIESEN, W. H., SCHWEIGERT, B. S. and ELVEHJEM, C. A. (1946). *J. biol. Chem.* **165**, 347.
7. LYMAN, C. M., MOSELEY, O., BUTLER, B., WOOD, S. and HALE, F. (1946). *J. biol. Chem.* **166**, 161.
8. HUTCHIN, M. E., HARPER, H. A., MARGEN, S. and KINSELL, L. W. (1950). *J. biol. Chem.* **185**, 839.
9. The Association of Vitamin Chemists (1951). *Methods of Vitamin Assay*. New York: Interscience Publishers.
10. BOLTON, E. T., COWIE, D. B. and SANDS, M. K. (1952). *J. Bact.* **63**, 309.
11. HOROWITZ, N. H. (1950). *Advanc. Genet.* **3**, 33.
12. HOCKENHULL, D. J. D. (1949). *Biochim. biophys. Acta*, **3**, 326.
13. TEAS, H. J. (1950). *J. Bact.* **59**, 93.
14. SIMMONDS, S. (1948). *J. biol. Chem.* **174**, 717.
15. SCHWARTZ, M. and SHAPIRO, S. K. (1954). *J. Bact.* **67**, 98.
16. HIFT, H. and WALLACE, G. I. (1949). *J. biol. Chem.* **177**, 927.
17. COWIE, D. B., BOLTON, E. T. and SANDS, M. K. (1951). *J. Bact.* **62**, 63.
18. REED, L. J., DEBUSK, B. G., JOHNSTON, P. M. and GETZEN-DANER, M. E. (1951). *J. biol. Chem.* **192**, 851.
19. REED, L. J. (1953). *Physiol. Rev.* **33**, 544.
20. NAJJAR, V. A. and BARRETT, R. (1945). *Vitam. & Horm.* **3**, 23.
21. NAJJAR, V. A. and HOLT, L. E. jun. (1943). *J. Amer. med. Ass.* **123**, 683.
22. GALLUP, W., POPE, L. S. and WHITEHAIR, C. K. (1949). *J. Anim. Sci.* **11**, 572.

23. HUFFMAN, C. F. (1953). *Annu. Rev. Biochem.* **22**, 399.
24. KALLIO, R. E. and LARSON, A. D. (1955). *A Symposium on Amino Acid Metabolism*, p. 616. Ed. by W. D. McElroy and B. Glass. Baltimore: The Johns Hopkins Press.
25. CHALLENGER, F. and CHARLTON, P. T. (1947). *J. chem. Soc.* p. 424.
26. SCHLENK, F. and SMITH, R. L. (1953). *J. biol. Chem.* **204**, 27.
27. SUZUKI, U., ODAKE, S. and MORI, T. (1924). *Biochem. Z.* **154**, 288.
28. BADDILEY, J., CANTONI, G. L. and JAMIESON, G. A. (1953). *J. chem. Soc.* p. 2662.
29. KEARNEY, E. B. and SINGER, T. P. (1953). *Biochim. biophys. Acta*, **11**, 270.
30. KEARNEY, E. B. and SINGER, T. P. (1953). *Biochim. biophys. Acta*, **11**, 276.
31. TARR, H. L. A. (1933). *Biochem. J.* **27**, 1869.
32. SMYTHE, C. V. (1945). *Advanc. Enzymol.* **5**, 237.
33. TAMIYA, N. (1951). *J. chem. Soc. Japan*, **72**, 118.
34. GARREAU, Y. (1943). *C.R. Soc. Biol., Paris*, **137**, 176.
35. MEISTER, A., FRASER, P. E. and TICE, S. V. (1954). *J. biol. Chem.* **206**, 561.
36. BARBER, H. H. and BURROWS, R. B. (1936). *Biochem. J.* **30**, 599.
37. NICKERSON, W. J. and ROMANO, A. H. (1952). *Science*, **115**, 676.
38. RACKER, E. (1955). *J. biol. Chem.* **217**, 855.
39. CHALLENGER, F., LISLE, D. B. and DRANSFIELD, P. B. (1954). *J. chem. Soc.* p. 1760.
40. CHALLENGER, F. and LIU, Y. C. (1950). *Rec. Trav. chim. Pays-Bas*, **69**, 334.
41. SHAPIRO, S. K. (1955). *Biochim. biophys. Acta*, **18**, 134.
42. McRORIE, R. A., GLAZENER, M. R., SKINNER, C. G. and SHIVE, W. (1954). *J. biol. Chem.* **211**, 489.
43. BUNKER, H. J. (1936). *A Review of the Physiology and Biochemistry of the Sulphur Bacteria*. London: H.M.S.O.
44. WINOGRADSKY, S. (1887). *Bot. Ztg.* **45**, 489.
45. LEES, H. (1955). *Biochemistry of Autotrophic Bacteria*. London: Butterworths Scientific Publications.
46. VOGLER, K. G., LEPAGE, G. A. and UMBREIT, W. W. (1942). *J. gen. Physiol.* **26**, 89.

47. VOGLER, K. G. and UMBREIT, W. W. (1942). *J. gen. Physiol.* **26**, 157.
48. UMBREIT, W. W. (1954). *J. Bact.* **67**, 387.
49. LEPAGE, G. A. and UMBREIT, W. W. (1943). *J. biol. Chem.* **147**, 263.
50. LEPAGE, G. A. and UMBREIT, W. W. (1943). *J. biol. Chem.* **148**, 255.
51. VAN NIEL, C. B. (1954). *Annu. Rev. Microbiol.* **8**, 105.
52. LARSEN, H. (1953). *K. norske vidensk. Selsk. Skr.* 1.
53. CALVIN, M. and BARLTROP, J. A. (1952). *J. Amer. chem. Soc.* **74**, 6153.
54. BUTLIN, K. R. and ADAMS, M. E. (1947). *Nature, Lond.* **160**, 154.
55. POSTGATE, J. R. (1951). *J. gen. Microbiol.* **5**, 725.
56. BUTLIN, K. R. (1953). *Research, Lond.* **6**, 184.
57. BUTLIN, K. R. and POSTGATE, J. R. (1953). *Symposium on Microbial Metabolism, Rome*, p. 126. Istituto Superiore di Sanita, Rome.
58. BUTLIN, K. R. and POSTGATE, J. R. (1954). *Biology of Deserts*, p. 112. Ed. by J. L. Cloudsley-Thompson. Institute of Biology, London.

Conclusion

It will have become apparent from the preceding chapters that the chemical changes which sulphur compounds undergo in living organisms are not only numerous, but are also very varied in character. The electronic constitution of sulphur is such that the element exhibits several valency states and is able to give rise to inorganic and organic compounds of many different types. Not only do many of these compounds occur in nature, but the ease with which they undergo oxido-reductive and other changes helps to explain the great diversity of reactions which are encountered in the study of their metabolism.

In higher animals on the one hand and in plants and micro-organisms on the other, it is possible to detect two quite different metabolic trends. In animals the sulphur present in the diet is chiefly in the divalent form and its metabolism is attended by a movement towards a higher oxidative state, with sulphate as the chief end-product. In plants and micro-organisms, however, the opposite type of change can occur. This latter process is coupled with an ability to convert inorganic sulphur to organic forms and leads eventually to the production of those organic sulphur compounds which are essential components of the diets of higher animals. Sulphur metabolism, therefore, provides yet another example of the ultimate dependence of animals upon plants and micro-organisms.

In a field of research as active as that described in this book it is inevitable that important advances will have been made in the period between the preparation of the book and its passage into the hands of the reader. Rapid progress is now being made, for example, in the investigation of the metabolism of various types of sulphuric acid ester. As with other aspects of sulphur metabolism this is being aided greatly by the exploitation of ^{35}S as a tracer isotope. This approach is proving especially valuable in the study of the sulphomucopolysaccharides, a group

of substances which do not readily lend themselves to metabolic experiments. Furthermore, the great step forward made when 'active sulphate' was shown to exist and to be adenosine-3'-phosphate-5'-phosphosulphate is already stimulating research on the biological synthesis of sulphuric acid esters. Increasing interest in the sulphatases has recently led, among other things, to the discovery of a steroid sulphatase. Sulphuric acid esters, whether they be sulphomucopolysaccharides or steroid sulphates, are substances of considerable interest to other workers besides biochemists, and it seems not unlikely that an increased understanding of the synthesis and breakdown of these compounds in living organisms will have repercussions elsewhere, for example in clinical research. Thiol groups have long been known to play important roles in biological systems. The isolation of coenzyme A, itself a thiol, has made possible a better understanding of many aspects of intermediary metabolism, and in spite of the great volume of work which has already appeared, it is certain that thiol esters of the type formed from coenzyme A will continue to attract much attention. It also appears likely that the further study of lipoic acid will show this compound to be of great significance in metabolic processes. Lastly, it is clear that certain aspects of the metabolism of sulphur compounds are being studied in a new context, for in an age which is becoming increasingly preoccupied with the hazards of exposure to ionizing radiations, attention is now being devoted to sulphur compounds in relation to radiation injury and to their potentialities as protective agents.

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