SERIES
XLIX-I

THE HARVEY LECTURES

delivered under the auspices of
THE HARVEY SOCIETY OF NEW YORK
1953-1954

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THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF
The HARVEY SOCIETY of NEW YORK

1953–1954

UNDER THE PATRONAGE OF THE NEW YORK
ACADEMY OF MEDICINE

BY

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SERIES XLIX

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THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I

This Society shall be named the Harvey Society.

II

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the Society and who are neither Active nor Associate members. Associate and Honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV

The management of the Society shall be vested in an Executive Committee to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V

The Annual Meeting of the Society shall be held at a stated date in January of each year at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all meetings ten members shall constitute a quorum.

VI

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification to the members in writing.

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THE METABOLISM OF SULFUR AND ITS RELATIONS TO GENERAL METABOLISM*

CLAUDE FROMAGEOT

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris

Two classical fundamental notions have arisen among others from all the research made during the last thirty years concerning the behavior of living matter: one is the unity of the mechanisms of the main biochemical reactions, a unity which manifests itself in the higher animals as well as in the microorganisms; the other is the close interdependence which exists between the metabolisms of the substances belonging to the great groups of the carbohydrates, amino acids and fats. These notions apply to sulfur metabolism, or, to speak more accurately, to the metabolism of the molecules whose behavior is conditioned by the fact that they contain sulfur. Therefore, we shall see in the present paper that some fundamental reactions belonging to this metabolism are found equally in animals and in bacteria, and we will be able to discuss some new connections between sulfur metabolism and general metabolism in the living cell.

Broadly speaking, the interdependence between sulfur metabolism and the metabolism of other substrates might be more or less involved in the role of sulfur in the general metabolism of the cell. From this broad point of view, three distinct mechanisms correspond to such an interdependence:

1. Sulfur-containing molecules constitute, or belong to, catalytic systems, enzymes or coenzymes, playing a role in the general metabolism of the cell.

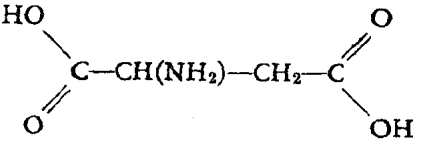
2. The metabolism of the sulfur-containing substances has some paths in common with the metabolism of other substrates.

3. Sulfur metabolism is under the influence of the same catalytic systems, such as coenzymes, which also play a role in the metabolism of other substrates.

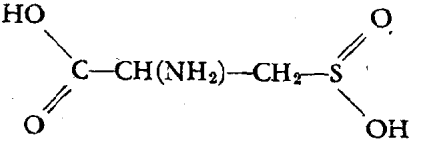
In a more limited way, I shall consider here only the mechanisms

* Lecture delivered September 24, 1953.

of interdependence which are defined under (2), with some additional remarks pertaining to (3). More particularly, I shall discuss the importance in sulfur metabolism of cysteinesulfinic acid, which, from the point of view of its chemical structure as well as from the point of view of its biochemical behavior, may be compared to aspartic acid:



Aspartic acid



Cysteinesulfinic acid

In the same way that aspartic acid plays a multiple role in the metabolism of amino acids and carbohydrates, cysteinesulfinic acid plays a multiple role in the metabolism of sulfur-containing amino acids and constitutes an important link between this metabolism and the metabolism of other amino acids and carbohydrates. Therefore it is not irrelevant to see what is the origin of cysteinesulfinic acid and what was known, until recently, of its possible role in animals and in bacteria.

The most important forms of organic sulfur that *animals* have to deal with are methionine, cysteine, and cystine. As far as sulfur is concerned, the biological degradation of all three leads to the elimination of the sulfur mostly as sulfate and taurine. The sulfur atoms of these three organic molecules partly follow some common path in their metabolism. Let us briefly recall how they arrive at this common path.

Considering first *methionine,* it is well known, particularly from the brilliant work of du Vigneaud and co-workers[1] that, in the mammalian body, a part of the methionine, acting as a donor of methyl groups, is transformed into homocysteine, and that through a transulfuration which implies the transitory formation

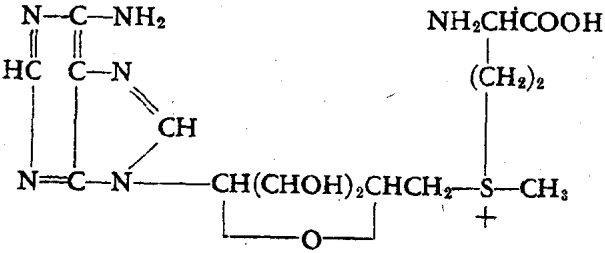
of cystathionine, the methionine sulfur is transfered to the carbon chain of serine, with the formation of cysteine:

$$HOOC{-}CH(NH_2){-}CH_2{-}CH_2{-}S{\cdot}CH_3 + AH \rightleftharpoons HOOC{-}CH(NH_2){-}CH_2{-}CH_2{-}SH + ACH_3$$

$$HOOC{-}CH(NH_2){-}CH_2{-}CH_2{-}SH + HO{\cdot}CH_2{-}CH(NH_2){-}COOH \rightarrow HOOC{-}CH(NH_2){-}CH_2{-}CH_2{-}S{-}CH_2{-}CH(NH_2){-}COOH + H_2O$$

$$HOOC{-}CH(NH_2){-}CH_2{-}CH_2{-}S{-}CH_2{-}CH(NH_2){-}COOH + H_2O \rightarrow HOOC{-}CO{-}CH_2{-}CH_3 + NH_3 + HS{\cdot}CH_2{-}CH(NH_2){-}COOH$$

The intimate mechanism of the activation of methionine as a methyl donor has been worked out quite recently in a very interesting way by Cantoni.[2] This author has shown that methionine reacts with ATP to give the compound



which is the "active methionine" in the reaction under consideration.

Considering *cystine*, we may assume that a great part of it is metabolized only after having been reduced to *cysteine*.[3] It appears, therefore, that cysteine may be considered a fundamental substrate as well as an intermediary product. We are then justified, in our limited study of sulfur metabolism, in starting with cysteine.

Definite knowledge of the pathway and of the enzymes involved in the transformations of cysteine into final products of metabolism in animals was until recently rather scanty.* Besides desulfhydra-

* More details and references are given in two reviews.[3,4]

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tion giving rise to hydrogen sulfide, ammonia, and pyruvic acid, cysteine has been shown to undergo extensive oxidation, particularly with liver preparations, with the possible formation of cysteic acid, as judged from oxygen utilization. Pirie[5] has demonstrated the formation of sulfate from cysteine (and also from cystine and methionine) in slices of rat kidney and liver and has postulated that both cysteine and cystine are oxidized to cysteinesulfinic acid, with the intermediary formation of cysteinesulfenic acid. The sulfinic acid was thought to be the point of desulfination and the inorganic sulfite the precursor of sulfate:

$$2R{\cdot}SH \rightarrow 2R{\cdot}SOH \rightarrow R{\cdot}SH + R{\cdot}SO_2H \rightarrow H_2SO_3 \rightarrow H_2SO_4$$

On the other hand, sulfinic acid may be further oxidized to cysteic acid; the latter does not form sulfate either *in vitro* with enzymic preparations or in the living animal. Among others, Medes[6] and Floyd[7] showed that cysteinesulfinic acid was a particularly good substrate for sulfate formation under the action of tissue slices, of homogenates, and even of extracts of rat tissues. In our laboratory,[8] we extracted an enzyme system from rabbit liver which transformed the sulfinic acid to alanine and inorganic sulfite. All these findings are in accord with the pathway earlier postulated by Pirie for the transformation of cysteine sulfur into sulfate. As for the formation of taurine, it was accounted for by a decarboxylation enzyme acting on cysteic acid,[9,10] and by another system, oxidizing and decarboxylating cystinedisulfoxide:[7]

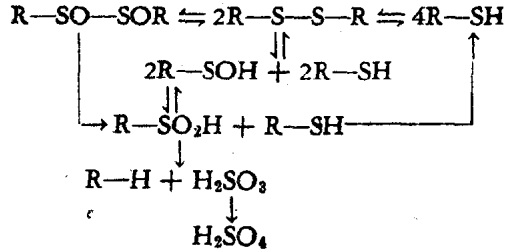
$$HOOC{-}CH(NH_2){-}CH_2{-}SO_3H \rightarrow CO_2 + CH_2(NH_2){-}CH_2{-}SO_3H$$

$$HOOC{-}CH(NH_2){-}CH_2{-}\overset{\overset{O}{\|}}{S}{-}\overset{\overset{O}{\|}}{S}{-}CH_2{-}CH(NH_2){-}COOH$$

$$+ 1\tfrac{1}{2}O_2 + H_2O \rightarrow 2CO_2 + 2CH_2(NH_2){-}CH_2{-}SO_3H$$

The metabolism of the three sulfur-containing amino acids in *bacteria* is very complicated, and each of these molecules should be considered separately. Without going into detail let us consider only what concerns cysteine (or cystine) in microorganisms. In this respect may be mentioned the pathway tentatively presented by Stahl and co-workers[11] concerning sulfur metabolism of

Microsporum gypseum, a fungus which is active in the degradation of wool and is able to oxidize cysteine sulfur into sulfate:



On the whole, this scheme is similar to the one suggested by Pirie concerning sulfur metabolism in mammals.

Curiously enough, cysteinesulfinic acid, which plays an important role in all these considerations, has been only recently characterized as such in an animal organ, viz., the brain of the rat.[12]

The uncertainty, or even the lack of knowledge, as to what really becomes of cysteinesulfinic acid up to the moment its sulfur is transformed into final degradation products has recently incited several workers in our laboratory to investigate the fate of this metabolite under different conditions. We shall therefore consider the reactions in which it takes part: *first*, in the presence of animal enzymic systems under anaerobic conditions; *second*, in living animals; *third*, in enzymic systems obtained from vitamin B_6-deficient animals; and *fourth*, when in the presence of oxygen it is oxidized by means of some bacterial cells and bacterial enzymes. And so we shall be able to get some idea of the whole metabolism of sulfur and of its relationships with other types of metabolism.

I. Anaerobic Reactions under the Action of Mammalian Enzymes

A. Desulfination

As mentioned above, when cysteinesulfinic acid is incubated (pH = 7.2, temp. 37° C.) for, let us say, 2 hours, with minced rat or rabbit liver or liver extract, one observes the formation of inorganic sulfite and alanine. This observation had led to the discovery of the so-called "desulfinicase."[8] The study of this new

enzymic system with purified preparations promptly showed that it is inhibited by CO reagents (Table 1)[13] and that it requires one or more factors, easily separable by dialysis and relatively thermostable.[14] Such factor(s), obviously present in the liver, is (are)

TABLE 1

INHIBITION OF ENZYMIC DESULFINATION OF CYSTEINESULFINIC ACID
(pH 7.2; temperature 35° C.)

Inhibitor, 10^{-3} M	SO_2 Formed after 2 Hours: Complete Enzymic System	SO_2 Formed after 2 Hours: Complete Enzymic System + Inhibitor	Inhibition
	μM.	μM.	per cent
KCN	83	9	89
Phenylhydrazine	83	14	83
Semicarbazide	66	18	73
Hydroxylamine	66	1	99

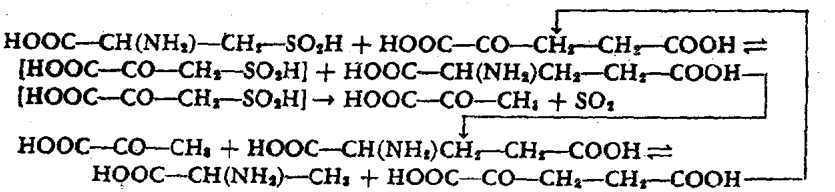
TABLE 2

SO_2 PRODUCTION FROM CYSTEINESULFINIC ACID
(Total volume, 25 ml.; M/30 phosphate buffer solution, pH 7.3; 2 hours at 35° C. in nitrogen)

Enzymic Preparation	Yeast Extract	α-Ketoglutaric Acid	Cysteinesulfinic Acid	SO_2 Formed
ml.	ml.	μM.	μM.	μM.
10	0	0	0	0
0	9	0	250	0
10	0	0	250	5
10	2	0	250	75
10	9	0	250	96
10	0	25	250	51
10	0	250	250	80
10	0	0	250	46

also present in large quantity in yeast. At first, it was thought that it was some coenzyme; no known coenzyme or combination of known coenzymes, however, is able to substitute for this factor. Finally, several experiments, of which Table 2 is an example, have

shown that this factor is α-ketoglutaric acid.[15] A transamination reaction was immediately suspected; the investigations, carried out quantitatively after the separation of the reaction products on adsorption columns, and qualitatively, using paper chromatography, have demonstrated that the desulfination reaction is an over-all reaction, resulting from the following more elementary reactions:



All the substances taking part in these reactions, with the exception of β-sulfinylpyruvic acid, have been at least qualitatively identified.*[16]

Several questions arise concerning these reactions. In the transamination reaction A, is α-ketoglutaric acid the only one able to participate? As a matter of fact, it appears from recent investigations of Singer and Kearney[17] that oxalacetic acid may play the same role. Is the transaminase acting between cysteinesulfinic acid and α-ketoglutaric acid or oxalacetic acid a specific one, or is it the same which acts also on aspartic acid? The equilibrium constant is known for reaction C[18] but not for reaction A; therefore, their ratio cannot yet be determined. However, one might assume that this ratio is such that for reaction A the system tends to proceed toward glutamic acid formation, whereas for reaction C it proceeds in the direction of alanine formation, this shift of the equilibrium position being due to the removal of the sulfinyl grouping from the pyruvic acid chain. Is it still necessary to assume the existence of a desulfinase? One might assume indeed that β-sulfinylpyruvic acid is quite unstable by itself and, either spontaneously or catalyzed by Mn^{++} ions, decomposes into inorganic sulfite and pyruvic acid, in a comparable way as does oxalacetic

* It is probable that at least a part of the sulfite formed reacts with the keto acids in the medium, giving the corresponding bisulfite derivates. But as such compounds are unstable in acidic conditions they do not interfere with the determination of SO_2 as it is done in the present case.

acid, which it closely resembles. These questions, as well as several others, require new investigations. In any case, the existence of these transamination reactions is in agreement with the strong inhibition of the desulfination reaction by most of the carbonyl group reagents. Furthermore, these transamination reactions obviously constitute a link between sulfur metabolism and the tricarboxylic acid cycle.

In the above equations, the reactions proceed without any oxidation. But one may imagine that should oxidations occur a new set of reactions would take place: the α-ketoglutaric acid which is used in reaction A might arise from glutamic acid through oxidative deamination; or, if the transamination happens to be between cysteinesulfinic acid and oxalacetic acid, the latter might be formed from malic acid.[17] Moreover, the glutamic acid or the aspartic acid formed as a result of the transfer of the amino group of cysteinesulfinic acid to the corresponding keto acids might be oxidized again to these keto acids without giving back their amino groups to pyruvic acid. Finally, one would expect that the liberated sulfite would be oxidized to sulfate; the enzymic system effective in this oxidation has been recently investigated.[19]

B. Decarboxylation

Quantitative investigations made with enzymic systems of animal origin have also shown that when cysteinesulfinic acid is incubated anaerobically with such systems the quantity of this acid which disappears is considerably larger than that which may be accounted for by the desulfination reaction. Investigations concerning this point have shown that such a disappearance corresponds to an enzymic decarboxylation of the substrate. Quantitative measurements of the CO_2 evolved and of the product which is formed have led to the following equation:

$$HOOC—CH(NH_2)—CH_2—SO_2H \rightarrow CO_2 + CH_2(NH_2)—CH_2—SO_2H$$

The aminoethanesulfinic acid since the beginning has been characterized by many reactions: behavior during paper chromatography, oxidation into taurine, reaction with ninhydrin, and reaction with chloroplatinate. To emphasize its relationship with taurine, it has been proposed[20] to call it hypotaurine (as one speaks

of hypoxanthine and xanthine). Recently, this substance has been isolated in nature from an enzymic medium by Awapara[21] and by Bergeret,[22] and its correct analysis has been given by the latter author. Moreover, it has been compared with the synthetic aminoethanesulfinic acid obtained independently by Awapara[21] and by Bricas,[23] and in all circumstances both substances, synthetic and natural, show exactly the same behavior. It appears, therefore, that

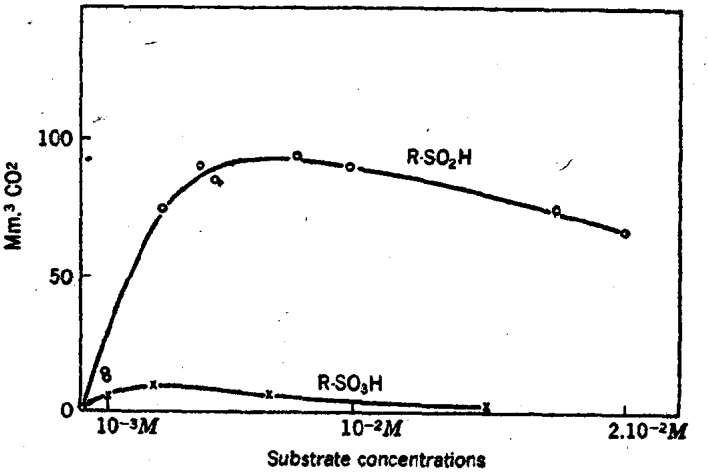


FIG. 1. Comparison of the decarboxylations of cysteinesulfinic acid and cysteic acid by rabbit liver.[24] Contents of Warburg vessels: phosphate buffer, 0.067 *M*; pH 6.8; liver homogenate, 185 mg. (dry weight); total volume, 3 ml.; temperature, 35° C.; duration, 60 minutes.

in rabbit and rat liver there exists, besides the desulfinating system, a carboxylase acting on cysteinesulfinic acid.

Moreover, because of the importance which up to the present time has been ascribed to decarboxylation of cysteic acid in the formation of taurine, it was of interest to compare the intensities of the decarboxylations of cysteic and of cysteinesulfinic acids under the same conditions by preparations from several tissues. Figure 1 shows that with a rabbit liver preparation the decarboxylation of cysteinesulfinic acid is considerably greater than the decarboxylation of cysteic acid. The same is true with kidney and brain preparations.[24]

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