

Articles

Oxidation Numbers in the Study of Metabolism

Received for publication, January 22, 2002, and in revised form, May 31, 2002

Ronald Bentley^{‡§}, James Franzen[‡], and Thomas G. Chasteen^{¶||}

From the [‡]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 and

[¶]Department of Chemistry, Sam Houston State University, Huntsville, Texas 77341

The calculation and use of oxidation numbers in the study of metabolic reactions are discussed for normal oxidations (alcohol dehydrogenase and the NAD⁺/NADH couple, propanediol dehydratase) and for enzymatic reactions with a “hidden” redox component (transamination, the coupled conversion of ethylamine to ethanol, and the biomethylation of arsenic and selenium).

Keywords: Oxidation number concept, metabolic redox reactions, hidden redox components, biomethylation of metalloids.

Biochemistry and molecular biology students easily comprehend the importance of energy-yielding oxidation processes. They are likely to know the definition of oxidation as loss of electrons and quickly become familiar with redox cofactors such as NAD⁺ and FAD.

In the detailed study of oxidation/reduction reactions one approach is to use the oxidation number concept. This useful method is apparently not generally used in teaching metabolism, because the indexes of several text books do not mention oxidation numbers; the main concern is with electrochemical potentials and free energy changes. However, one of us (J. F.) has introduced this concept in an upper level biochemistry course with good results. A similar conclusion has been reached recently by Halkides [1]. Consideration of oxidation numbers can indicate whether oxidation or reduction is involved in a metabolic reaction and can illuminate situations where oxidation or reduction is not immediately apparent. Because of the rather arbitrary way in which they are calculated, students do need careful coaching in their use (there are some pitfalls, admittedly), but once the general principles are well learned the use of these numbers can be rewarding and helpful. This article describes a different method for calculating oxidation numbers from that used by Halkides [1] and provides different metabolic examples of their use.

Oxidation Number, Definition, and Calculation

In organic chemistry, an oxidation number is a number assigned to a particular atom in a molecule to represent, either actually or notionally, the “ownership” or “control” of the electrons around it compared with that of the atom in

the pure (elemental) state. The oxidation number has two components; the sign indicates whether control has increased (negative value) or decreased (positive value), and the value gives the number of electrons over which ownership has changed compared with the valence state in the pure element. Atoms of pure elements have an oxidation number of zero; they have neither lost nor gained electrons, and the number of protons in the nucleus exactly balances the number of electrons. Oxidation numbers are usually written in capital Roman numerals.

In an electrovalent (ionic) compound of monatomic ions, e.g. NaCl, the concept is easily understood; the oxidation number of each atom is actually equal to the charge on the respective ion. For covalent compounds the charges under consideration are hypothetical or notional. Both electrons of a covalent electron-pair bond between non-identical atoms are assigned arbitrarily to the more electronegative element for which the values of interest are as follows: arsenic, 2.0; hydrogen, 2.1; selenium, 2.4; carbon, 2.5; sulfur, 2.5; nitrogen, 3.0; chlorine, 3.0; oxygen, 3.5 [2]. Thus in methane, CH₄, carbon is more electronegative than hydrogen; all the hydrogen atoms of methane are considered to have lost an electron and thus have oxidation numbers +I. The carbon is regarded as having acquired all eight of the valence electrons, four more than its normal value; hence it has oxidation number in methane of –IV. Depending on the electronegativities of the atoms attached to it, carbon oxidation numbers range from –IV (methane) to +IV (carbon dioxide).

For an uncharged, individual atom, X, the sign and value of the oxidation number, *n*, can be calculated from the following equation:

$$\Sigma (\text{number of electrons gained or lost by } X) + n = 0 \quad (\text{Eq. 1})$$

The value of zero represents the reference situation in the pure, elemental state where no electrons have been gained or lost. For a carbon atom linked to four other carbon atoms, C(C)₄, the oxidation state of the central

§ To whom correspondence should be addressed: 37 Thornwood Dr., Pittsburgh, PA 15228-2452. E-mail: rbentley@pitt.edu.

|| Supported in part by a departmental Robert A. Welch Foundation grant and by the Sam Houston State University Research Council.

carbon is zero. The numbers of electrons gained (lost) are derived as shown in Table I.

In the following ($X < Y$) is read as "X is less electronegative than Y," and ($X > Y$) is read as "X is more electronegative than Y." Methane has four carbon-hydrogen bonds; because hydrogen $<$ carbon, carbon gains four electrons. Hence $(+4) + n = 0$, and $n = -IV$ ($C^{-IV}H_4$). In methanol, oxidation numbers can be derived for both carbon and oxygen atoms ($H_3C^{-II}-O^{-II}H$) as shown in Table II.

TABLE I
Electron gain or loss

	Number of electrons gained or lost
For each bond to an identical atom	0
For each bond to a less electronegative atom	+1
For each bond to a more electronegative atom	-1

TABLE II
Calculation of oxidation numbers in methanol, $H_3C^{-II} - O^{-II}H$

Carbon Atom	Oxygen Atom
Three C-H bonds ($H < C$) +3	One O-C bond ($C < O$) +1
One C-O bond ($O > C$) -1	One O-H bond ($H < O$) +1
Hence: $(+3) + (-1) + n = 0$, and $n = -II$	And: $(+1) + (+1) + n = 0$, and $n = -II$

For a charged atom, the equation is as follows:

$$\Sigma \text{ electrons gained (lost)} + n$$

$$= \text{sign and value of charge (Eq. 2)}$$

If an atom has one positive charge, sign and value = +1; if an atom has one negative charge, sign and value = -1.

For the methylcarbocation (one positive charge), there are three carbon-hydrogen bonds (hydrogen $<$ carbon); hence: $(+3) + n = 1$, and therefore $n = -II$ leading to $(C^{-II}H_3)^+$. The carbon oxidation number for carbon in the methylcarbanion can easily be seen to be $-IV$ ($C^{-IV}H_3$)⁻. Oxidation numbers of atoms in many compounds of biochemical interest are listed in Table III.

For a polyatomic compound or ion, the oxidation numbers of all of the individual atoms sum to the charge on the compound or ion. Thus, for uncharged methane, CH_4 , the overall sum is $4 \times (+I) + (-IV) = 0$; for the methyl carbocation, CH_3^+ , the oxidation number of the carbon atom is $-II$ so that the sum equals the single positive charge, $3 \times (+I) + (-II) = +1$, and for the methyl carbanion, CH_3^- , the oxidation number of the carbon atom is $-IV$, and the sum equals the single negative charge, $3 \times (+I) + (-IV) = -1$.

Because oxidation is by definition the loss of electrons, an increase in oxidation number represents an oxidation,

TABLE III
Oxidation numbers useful in biochemistry

HYDROGEN ^a AND OXYGEN	
Elements	H^0-H^0
Superoxide anion	$O^0=O^{-I}\bullet$
Hydroperoxide anion	$(O^0=O^{-I}\bullet)^-$
Hydroxyl radical	$H^{+I}-O^{-I}\bullet$
Hydroxide ion	$H^{+I}-O^{-II}$
Water	$H^{+I}-O^{-II}-H^{+I}$
Hydrogen peroxide	$H^{+I}-O^{-I}-O^{-I}-H^{+I}$
CARBON (For methane, methanol, methylcarbocation, and methylcarbanion see text.)	
Ethane	$H_3C^{-III}-C^{-III}H_3$
Ethylene	$H_2C^{-II}=C^{-II}H_2$
Acetylene	$HC^{-I}\equiv C^{-I}H$
Formaldehyde	$H_2C^0=O$
Acetaldehyde	$H_3C-C^{+I}H=O$
Acetone	$H_3C-C^{+II}-O-CH_3$
Acetate ion	$H_3C-C^{+III}-O^-$
Carbon dioxide	$O=C^{+IV}=O$
Benzene	$C_6^{-I}H_6$
NITROGEN	
Element	$N^0\equiv N^0$
Nitric oxide	$N^{+II}=O^{-II}$
Nitrite ion	$O^{-II}=N^{+III}-O^{-II}$
Nitrate ion	$O^{-II}=N^{+V}-O_2^{-II}$
Ammonia	$N^{-III}H_3$
Hydrazine	$H_2N^{-II}-N^{-II}H_2$
Hydroxylamine	$H_2N^{-I}-O^{-II}H$
Methylamine (In the following, it is assumed that $N > C$ (see text).)	$H_3C^{-II}-N^{-III}H_2$
Methylammonium cation	$H_3C^{-II}-N^{-III}H_3^+$
Trimethylamine	$(H_3C^{-II})_3N^{-III}$
Trimethylammonium cation	$(H_3C^{-II})_3N^{-III}H^+$
Urea	$H_2N^{-III}-C^{+IV}-O^{-II}-N^{-III}H_2$
SULFUR	
Hydrogen sulfide	$H-S^{-II}-H$
Methanethiol	$H_3C-S^{-II}-H$
Dimethyldisulfide	$H_3C-S^{-I}-S^{-I}-CH_3$
Sulfurous acid	$H-S^{+IV}O_2-OH$
Methylsulfonic acid	$H_3C-S^{+IV}O_2-OH$
Sulfuric acid	$HO-S^{+VI}O_2-OH$

^a Note that in combination with metals, hydrogen is assigned the oxidation number $-I$. Moreover, to avoid the "unreasonable" situation that the oxidation number of a hydrogen atom bound to oxygen is the same as that bound to a carbon atom, it has been suggested that hydrogen be assigned oxidation number of 0 in combination with carbon, silicon, germanium and boron [7]. This suggestion has, however, not been widely adopted.

and conversely, a decrease in oxidation number represents a reduction. For instance, when methane is converted to methanol (for structures, see above), the oxidation number of the carbon atom increases from $-IV$ to $-II$ indicating an oxidative process.

Situations where two atoms have almost identical electronegativities may present some difficulties. For sulfur, the rounded-off electronegativity, 2.5, is identical to that of carbon, but at least one text of organic chemistry [3] regards sulfur as more electronegative than carbon. However, other authors, e.g. Hanselmann [4], regard the values as identical. The former method is used here, as exemplified by the case of methanethiol, $H_3C^{-II}-S^{-II}-H$, shown in Table IV. By whatever means the sulfur oxidation number is calculated, an increase in value still represents oxidation. Thus, for the simple oxidation process, $2H_3C-S^{-II}-H \rightarrow H_3C-S^{-I}-S^{-I}-CH_3$, the change in the sulfur oxidation number is from $-II$ to $-I$. The values for a number of other sulfur compounds are given in Table III.

Oxidation Numbers in Metabolic Redox Reactions

The NAD^+ /NADH Couple and Alcohol Dehydrogenase—Many biochemical redox reactions depend on the use of NAD^+ for oxidation and NADH for reduction. For the prototypical case of alcohol dehydrogenase, EC 1.1.1.1, the reaction (Scheme 1) can be diagrammed as a hydride transfer from ethanol to NAD^+ with the involvement of a general base, B:. Ethanol is, of course, oxidized, the oxidation number on C_1 increasing from $-I$ in ethanol to $+I$ in acetaldehyde. Instructors usually emphasize the cofactor changes by pointing to the fact that in NAD^+ , the nitrogen atom is quaternary and carries a positive charge; hence, the “chemically correct” abbreviation is NAD^+ , rather than NAD. Following the two-electron transfer, the reduced form, NADH, has a ternary nitrogen atom. However, examination of

changes in the oxidation numbers of nitrogen and C_4 and C_6 of the pyridine ring is more meaningful. The oxidation number of the nitrogen atom, $-III$, does not change during the $NAD^+ \rightarrow NADH$ conversion. It is the oxidation number changes on C_4 and C_6 that are crucial. At C_4 the change is from $-I$ in NAD^+ to $-II$ in NADH and at C_6 from I to 0 . Hence the reduction process concerns these carbon atoms, and the quaternary to ternary change at nitrogen is not significant. Because the pyridine ring is a resonance hybrid comprising two (Kekulé) structures, the process can be written as involving C_2 rather than C_6 (Scheme 1).

An Internal Redox Process (Propanediol Dehydratase)—Propanediol dehydratase, EC 4.2.1.28, converts propane-1,2-diol to propanol (propionaldehyde) by an internal oxidation/reduction process. Although evident from inspection of structures, the process is convincingly explained by oxidation number changes, $H_3C-C^0H(OH)-C^{-I}H_2-OH \rightarrow H_3C-C^{-II}H_2-C^{+I}H=O$.

For C_1 , the oxidation number increases from $-I$ to $+I$ indicating a two-electron oxidation. For C_2 the decrease in oxidation number from 0 to $-II$ indicates a two-electron reduction. One carbon atom is oxidized whereas the adjacent carbon atom is reduced.

A Coupled Process (Two-enzyme Conversion of Ethylamine to Ethanol)—Although some situations involving nitrogen may be ambiguous, because the electronegativity values are close (carbon, 2.5; nitrogen, 3.0), nitrogen is generally assumed to be more electronegative than carbon. Thus for ethylamine, the oxidation number of carbon is assigned as $-I$, and that of nitrogen is assigned as $-III$, $H_3C-C^{-I}H_2-N^{-III}H_2$. It is instructive to consider a conversion of ethylamine to ethanol using the enzymes amine dehydrogenase (EC 1.4.99.3) and alcohol dehydrogenase (EC 1.1.1.1) in two reaction steps. In the following, A (oxidized form) is an acceptor molecule for H^+ and H^- and is converted to the reduced form, AH_2 :

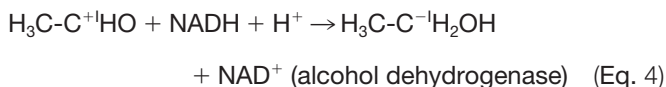
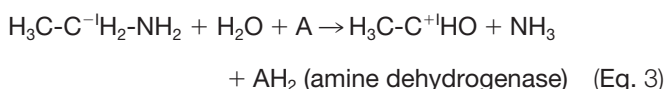
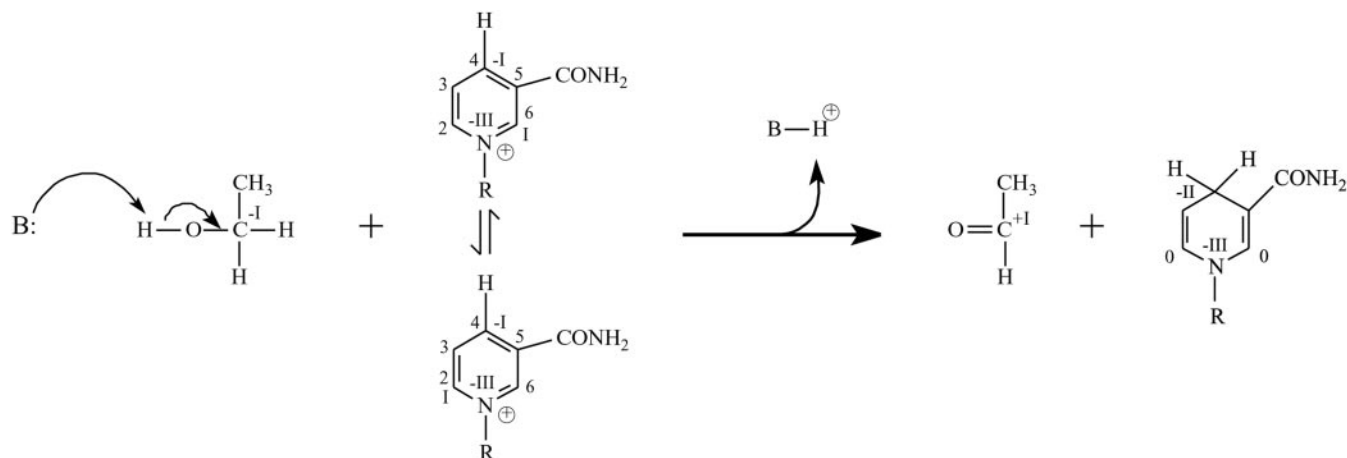
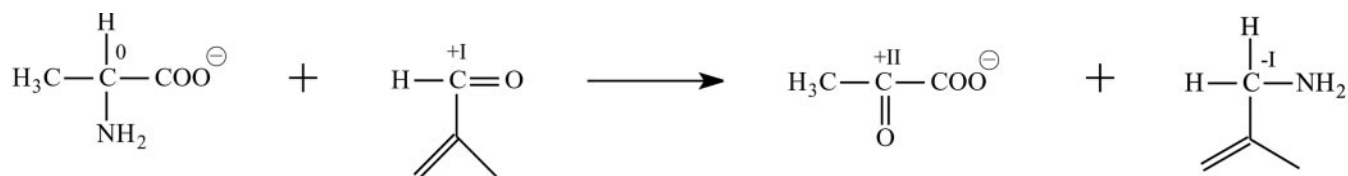


TABLE IV
Calculation of oxidation numbers in methanethiol, $H_3C^{-II}-S^{-II}H$

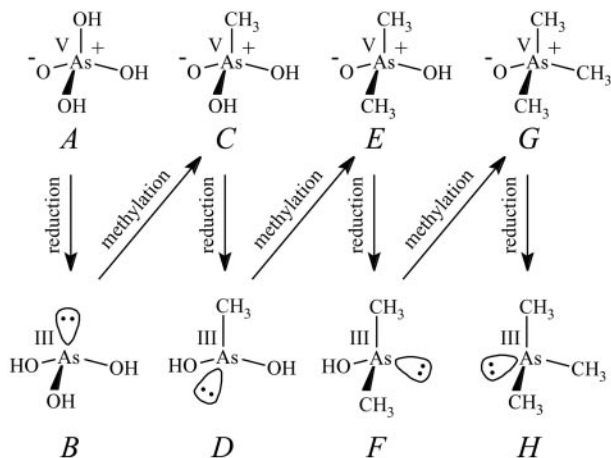
Carbon atom	Sulfur atom
Three C-H (H<C) +3	One S-C (C<S) +1
One C-S (S>C) -1	One S-H (H<S) +1
Hence: $(+3) + (-1) + n = 0$, $n = -II$	And: $(+1) + (+1) + n = 0$, $n = -II$



SCHEME 1. The alcohol dehydrogenase reaction. NAD^+ and NADH are shown in abbreviated structures where R = the remainder of the molecule.



SCHEME 2. **A partial transaminase reaction.** The conversion of alanine to pyruvate is shown, with abbreviated structures used for pyridoxal P and pyridoxamine P.

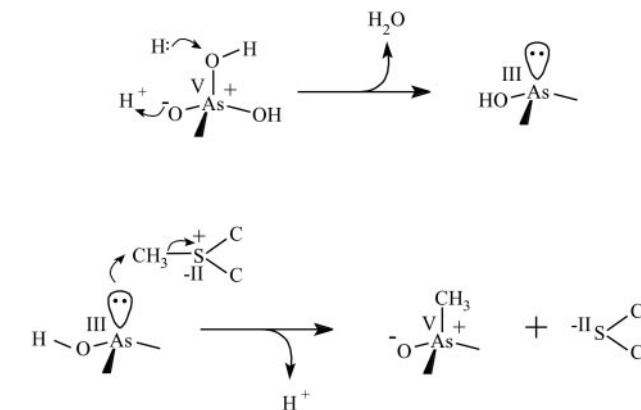


SCHEME 3. **The arsenate \rightarrow trimethylarsine conversion.** A, arsenic acid; B, arsenous acid; C, methylarsonic acid; D, methylarsonous acid; E, dimethylarsinic acid; F, dimethylarsinous acid; G, trimethylarsine oxide; H, trimethylarsine. In each step labeled *reduction*, the oxidation number of arsenic decreases, V \rightarrow III; in each step labeled *methylation*, the oxidation number of arsenic increases, III \rightarrow V.

Although it might be argued that the overall conversion of ethylamine to ethanol, $\text{H}_3\text{C}-\text{CH}_2-\text{NH}_2 \rightarrow \text{H}_3\text{C}-\text{CH}_2-\text{OH}$, is an oxidation after all oxygen is apparently added the oxidation number on C_1 of ethanol is the same, -1 , as that of C_1 of ethylamine. In fact, the overall process would be a coupled oxidation/reduction. In the first half-step (oxidation), the oxidation number on C_1 of ethylamine increases from -1 to $+1$ in acetaldehyde. In the second half-step (reduction) the oxidation number of C_1 of acetaldehyde, $+1$, decreases to -1 in ethanol. The conversion of ethylamine to ethanol by these two enzymes requires two coupled half-steps of oxidation and reduction; the overall reaction requires no net change in the oxidation number at C_1 . It is essentially a displacement process with the NH_2 group being replaced by OH. The first half-step presumably forms the imine, $\text{H}_3\text{C}-\text{CH}=\text{NH}$, as an intermediate.

Reactions with Hidden Redox Components

Transamination—For an enzyme such as alanine transaminase, EC 2.6.1.2, no redox cofactor is involved; the simple, overall process, $\text{L-alanine} + 2\text{-ketoglutarate} \rightarrow \text{pyruvate} + \text{L-glutamate}$, however, requires many reaction steps. The necessary cofactor, pyridoxal phosphate (pyridoxal P),¹ is concerned in a two-step process being converted in the first step to pyridoxamine phosphate (pyridoxamine P) and then back to pyridoxal P in a second step (mechanistically, each step is more complex than indicated here) as follows:



SCHEME 4. **A typical representation of the reduction and methylation of arsenic.** The *top line* shows the postulated reduction of arsenate to arsenite with a change in oxidation number from $+V$ to $+III$. The process results in an electron lone pair on arsenic. In the methylation, arsenite \rightarrow methylarsonate (*bottom line*), S-adenosylmethionine ($\text{CH}_3-\text{S}^+(\text{CH}_3)_2$) is converted to S-adenosylhomocysteine ($\text{C}-\text{S}-\text{C}$).

$\text{L-alanine} + \text{pyridoxal P} \rightarrow \text{pyruvate}$

+ pyridoxamine P (Eq. 5)

and

$2\text{-ketoglutarate} + \text{pyridoxamine P} \rightarrow \text{L-glutamate}$

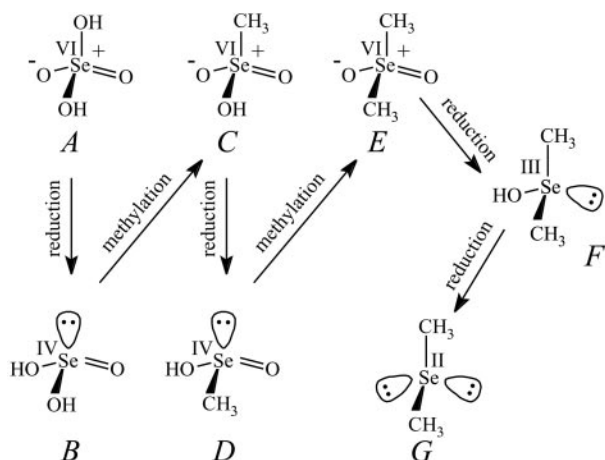
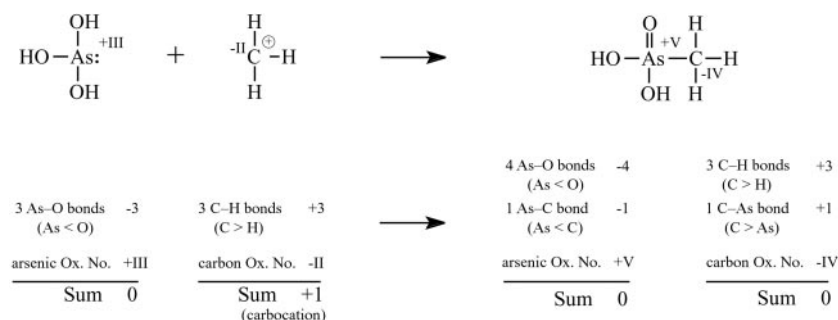
+ pyridoxal P (Eq. 6)

In the first half-step, the oxidation number of C_2 of alanine increases from 0 to $+II$ in forming pyruvate, indicative of an oxidation; for the aldehyde carbon in pyridoxal P the oxidation number decreases from $+I$ to $-I$ in forming pyridoxamine P, indicative of a reduction (Scheme 2). Similar relationships can easily be deduced for the second step ($2\text{-ketoglutarate} + \text{pyridoxamine P} \rightarrow \text{L-glutamate} + \text{pyridoxal P}$). Therefore, although transaminations do not use a redox cofactor, each half-step is a coupled oxidation/reduction process.

Biomethylation of Arsenic and Selenium—The microbial biomethylation of metal(loid)s has a very extensive literature. For instance, many microorganisms convert sodium arsenate to the volatile (and toxic) trimethylarsine; $\text{Na}_3\text{AsO}_4 \rightarrow \text{As}(\text{CH}_3)_3$. The fungus, *Scopulariopsis brevicaulis*, is very active in the volatilization of arsenic and has been investigated extensively [5]. The pathway from arsenate to di- and trimethyl arsenic derivatives (Scheme 3) involves a series of alternating reductions and methylations; the latter are accomplished with a methyl carbocation derived from S-adenosylmethionine [6]. The reductions, e.g. arsenate to arsenite, are simple in principle (see below) but complex in operation, usually involving the

¹ The abbreviation used is: P, phosphate.

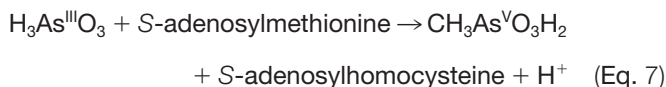
SCHEME 5. Changes in the oxidation numbers of arsenic and carbon during methylation with the methyl-carbocation. $X < Y$ is to be read as X is less electronegative than Y , and $X > Y$ is to be read as X is more electronegative than Y .



SCHEME 6. The selenate \rightarrow dimethylselenide conversion. For nomenclature of the oxyacids of selenium see Klayman and Günther [8]. A, selenic acid; B, selenous acid; C, methylselenonic acid; D, methylseleninic acid; E, dimethylselenone; F, dimethylselenenic acid; G, dimethylselenide. The overall process involves alternate reductions of selenium, $\text{VI} \rightarrow \text{IV}$, followed by methylation in which the oxidation number increases, $\text{IV} \rightarrow \text{VI}$. Dimethylselenone, compound E, is presumably reduced in a two-step process, $\text{VI} \rightarrow \text{III}$, $\text{III} \rightarrow \text{II}$.

participation of thiols such as glutathione. A reaction mechanism involving a hydride ion for the arsenate \rightarrow arsenite conversion ($\text{H}_3\text{AsO}_4 + \text{H}^+ + \text{H}^- \rightarrow \text{H}_3\text{AsO}_3 + \text{H}_2\text{O}$) is shown in Scheme 4. The other reduction steps (Scheme 3) are mechanistically similar.

At first sight, the methylation steps appear to be simple methyl transfer reactions; the methyl donor, S-adenosylmethionine, is converted to S-adenosylhomocysteine, and no redox cofactors are involved in the methylation (Scheme 4). However, examination of the oxidation numbers of the arsenic atoms reveals a more complex and interesting situation. In each methyl transfer, the oxidation number of arsenic increases from +III to +V (Scheme 3). Hence, there has been an oxidation, and the further implication is that there must also have been a concomitant reduction. From simple inspection of, e.g. the arsenite to methylarsonic acid conversion, it is not immediately apparent how a methyl transferase enzyme carries out an overall oxidation/reduction:



The demethylation, S-adenosylmethionine \rightarrow S-adenosylhomocysteine, involves no change in the oxidation number of the sulfur atom; it is -II in both compounds. Hence the reduction step must be sought elsewhere. In fact, the oxidation number of the carbon atom of the methyl group of S-adenosylmethionine is -II; in the product, methylarsonic acid, the oxidation number of this carbon atom is -IV (Scheme 5). Because the value has decreased, this carbon atom becomes more reduced during the methylation. Thus, the overall methyl transfer process contains a hidden redox component, not easily comprehended without consideration of the oxidation numbers.

A similar situation to that with arsenic occurs for the biomethylation of selenium where dimethylselenide is a common product from the action of microorganisms on selenate or selenite. Although there are likely multiple mechanisms for dimethylselenide biosynthesis, one possibility (e.g. with *S. brevicaulis*) is a pathway analogous to that postulated for arsenic with alternating reductions and methylations (Scheme 6). In the latter, the selenium atom is oxidized, because the oxidation number changes from +IV to +VI.

Not all S-adenosylmethionine methyl transfer reactions involve a hidden redox component. If trimethylamine is converted to the tetramethylammonium ion by the methyl carbocation, the oxidation number of nitrogen remains at -III, and that of the transferred carbon remains as -II. Arsenic is less electron-withdrawing than nitrogen, the electronegativities being, respectively, 2.0 and 3.0. Arsenic, therefore, can lose electrons to carbon on methylation, but nitrogen does not. If the examples given here and by Halkides [1] are considered, it is apparent that the application of the oxidation number concept can be very useful in clarifying aspects of many reaction mechanisms in metabolism.

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