Letters

A Critique of Certain Parts of the 1961 Report of the Commission on Enzymes

The eminent enzymologists composing the Commission on Enzymes of the International Union of Biochemistry have rendered an important service by agreeing on the classification and nomenclature of enzymes and on some other matters of terminology. The recommendations in the 1961 report (*1*) have been published.

One of the recommendations is that the trivial name *diphosphopyridine nucleotide* for the substance also known as coenzyme I, or codehydrogenase I, be replaced by the more logical, trivial name *nicotinamide-adenine dinucleotide.* These names frequently are abbreviated to the symbols DPN and NAD, respectively. Added to this recommendation is another on the symbols to be used for the oxidant and the reductant of the DPN system (the NAD system). I have no first-hand knowledge of this system but while revising that part of a book which reviews the properties of this system I was amazed to find it impossible to make what is said in the report compatible with what seems to be the generally accepted, stoichiometric relation between the oxidant and the reductant. The discrepancy extends to Appendix E of the report where expressions of many reactions do not agree with those in current use. This critique is restricted to the stoichiometric aspect, and, in order to forestall possible misunderstanding, I emphasize that I am not now participating in the debate on the nature of the oxidation-reduction process except by a remark on a possible interpretation of a statement in the report.

Those who have used the DPN system (the NAD system) have recognized that within the range of *p*H where both the oxidant and the reductant are sufficiently stable for experiments in vitro there are no changes of the ionization of these species. Accordingly, it has become the custom to neglect the net charges and to focus attention on the nicotinamide residue. Then the + in the symbol DPN^+ (or NAD^+) refers to the quarternary nitrogen in the oxidized nicotinamide residue and the H in DPNH (or NADH) refers to the one H added in the course of reduction, as demonstrated by use of deuterium. The stoichiometric relation, expressed in the lowest terms, is:

$$\text{DPN}^+ + 2e^- + \text{H}^+ \rightleftarrows \text{DPNH},$$

or

$$\text{NAD}^+ + 2e^- + \text{H}^+ \rightleftarrows \text{NADH}$$

Two sets of data confirming this relation are reviewed by Clark (*2*): (i) the slope of the line relating the potentials of the DPN system at 50-percent reduction to the *p*H numbers of the solutions, as determined by Rodkey, and (ii) confirmation of Eq. 2, given later, in several specific cases. These two sets of data are for equilibrium states and are selected to avoid any difference of opinion regarding the nature of the oxidation-reduction process in the case at hand. For a thermodynamic purpose it is not necessary to know how a state of equilibrium was reached. It is important to preserve correct stoichiometric relations.

Instead of the widely accepted relation here briefly stated, the report offers two expressions, which can be distinguished by (A) and (B), of the relation of the reductant to the oxidant. I quote, (A) "$\text{NAD}\rightarrow\text{NADH}_2$, or (B) $\text{NAD}^+\rightarrow\text{NADH}+\text{H}^+$, but *not* $\text{NAD}\rightarrow\text{NADH}$."

Insertion of the last expression is virtually to make a premise and to promptly deny its validity. The purpose is obscure. In the text, $\text{NADH} + \text{H}^+$ is said to represent the reductant and it also is said that the H^+ pertains to a change of the medium. Both cannot be true. Thus two inconsistent symbols are offered for the reductant and two inconsistent symbols for the oxidant.

Embarrassment does not end here because relation (A) is used for one purpose and relation (B) for another purpose.

In Appendix E of the report are many expressions of reactions involving the NAD system and in all cases the symbols NAD and NADH_2 of relation (A) are used. For example, the first expression is:

"Alcohol + NAD =aldehyde or ketone + NADH_2"

Seeing this, a student might be led to write the equilibrium equation as Eq. 1 instead of Eq. 2.

$$\frac{[\text{Aldehyde, or ketone}]\,[\text{NADH}_2]}{[\text{Alcohol}]\,[\text{NAD}]} = K'' \qquad (1)$$

$$\frac{[\text{Aldehyde, or ketone}]\,[\text{NADH}]\,(\text{H}^+)}{[\text{Alcohol}]\,[\text{NAD}^+]} = K' \qquad (2)$$

It is true that in specific applications of Eq. 2 some authors have chosen to omit (H^+), the activity of the hydrated protons, but then they have recognized that the apparent equilibrium constant becomes *p*H-dependent. So far as I am able to recall, no experimenter has failed to recognize the involvement of (H^+). Therefore it is strange that no hint of this is given in the report. The experimentally confirmed stoichiometric relation is:

$$\text{Alcohol} + \text{NAD}^+ \rightleftarrows \text{aldehyde, or ketone} + \text{NADH} + \text{H}^+$$

Expression (B) is $\text{NAD}^+\rightarrow\text{NADH} + \text{H}^+$. Of this it is said: " . . . it is true that in neutral solution one of the two hydrogens [see my later remark on this] is released as a H^+ ion, and that for some purposes it is desirable to be able to show the production of one equivalent of acid during the reduction. In such cases it is permissible to write the reduced form [*sic*] as NADH + H^+."

It hardly can be doubted that this will be interpreted to be a statement of a generality. Let us submit it to tests. Because of the relatively low potential of the DPN system (the NAD system) at 50-percent reduction and *p*H 7, it is convenient to consider cases in which the dominant direction of change will be the oxidation of DPNH. Without changing the alleged generality in principle we restate it as: associated with the oxidation of DPNH, one equivalent of H^+ is removed from the medium per formula weight of DPN$^+$ formed. Each of the following reactions is to be regarded as occurring in neutral, aqueous solution, in vitro, with or without such a catalyst as may be needed for acceleration. The stoichiometry is based on known compositions of the substances. A single arrow designates the dominant direction of change.

Included in Appendix E of the report, but with other symbols, is reaction I.

$$\mathrm{DNPH} + \mathrm{TPN}^+ \rightleftharpoons \mathrm{DPN}^+ + \mathrm{TPNH} \quad \text{(I)}$$

In the next case the quinone has an ionization constant such that the charge is 1$^-$ at *p*H 7.

$$\mathrm{DPNH} + \text{2-hydroxy-1, 4-naphthoquinone}^{1-} + 2\mathrm{H}^+ \rightarrow \mathrm{DPN}^+ + \text{the neutral hydroquinone} \quad \text{(II)}$$

In considering the reaction with a cytochrome *c* I shall be naive in neglecting the evidence that different cytochromes of type *c* appear to differ in their states near *p*H 7 and in neglecting some other complications which recently have come to light. With this confession, one might be permitted, for the present purpose, to consider only the conventional charges allocated to the "iron" in the monomeric oxidant and reductant and to write:

$$\mathrm{DPNH} + 2\ \text{cytochrome}\ c^{3+} \rightarrow \mathrm{DPN}^+ + 2\ \text{cytochrome}\ c^{2+} + \mathrm{H}^+ \quad \text{(III)}$$

For low concentrations of an alcohol dehydrogenase:

$$\mathrm{DPNH} + \text{acetaldehyde} + \mathrm{H}^+ \rightarrow \mathrm{DPN}^+ + \text{ethanol} \quad \text{(IV)}$$

Of these four reactions only the last, IV, conforms with the alleged generality and it must be admitted that perhaps reactions of this type were in mind.

Incidentally in Appendix E (*1*) the reaction with a cytochrome *c* is written:

"$\mathrm{NADH_2}$ + oxidized cytochrome *c* = NAD + reduced cytochrome *c*"

Obviously this is wrong on two counts. There are many other errors of stoichiometry in Appendix E.

A suggestion of a reason for expressions (A) and (B) is found in the prefatory remark: "Like the flavin compounds, the coenzymes require two equivalents of hydrogen [*sic*] for their reduction . . . " Incidentally a study of the flavin adenine dinucleotide system (*2*) will show more contrast than likeness to the DPN system. The quoted statement sounds very much like a commitment to the concept of hydrogen transfer. If this was in mind, the expressions (A) and (B) may be related to half-reactions (a) and (b), respectively.

$$\mathrm{NAD} + 2\mathrm{H} \rightleftarrows \mathrm{NADH_2} \quad \text{(a)}$$
$$\mathrm{NAD}^+ + 2\mathrm{H} \rightleftarrows \mathrm{NADH} + \mathrm{H}^+ \quad \text{(b)}$$

As already noted, half-reaction (a) does not conform with certain facts. Half-reaction (b) is especially interesting when regarded exclusively in terms of stoichiometry. Then a proton may be eliminated from each side to yield the equivalent half-reactions (b′) and (b″).

$$\mathrm{NAD}^+ + \mathrm{H}^- \rightleftarrows \mathrm{NADH} \quad \text{(b')}$$
$$\mathrm{NAD}^+ + 2e^- + \mathrm{H}^+ \rightleftarrows \mathrm{NADH} \quad \text{(b'')}$$

Half-reaction (b′) is preferred by those who regard the reduction, when mediated by a dehydrogenase, to be by the transfer of a hydride ion, H$^-$, from a substrate such as ethanol.

Of course, half-reactions of the sort under consideration cannot be studied in isolation. They are written with an eye on complete reactions for which it is essential to preserve correct, stoichiometric relations. For this purpose it becomes a convenience to resolve each transferred hydrogen to its proton and electron so that, by the usual rule of combining half-reactions, eliminations can be made as is done in the foregoing treatment of expression (b). Such a treatment need imply no mechanism of the final reaction. With this understood, half-reaction (b″) is by far the more convenient when used with numerous other half-reactions each of which is expressed in the lowest terms. This system leaves open the question of the actual nature of a process until a decision thereon is supported by evidence.

The point of present interest is that the report does not mention the frequently used half-reactions (b′) and (b″), which seems to support my supposition that only hydrogen transfer was considered. This will be disturbing to teachers who instruct their students on important distinctions, especially on what a particular experimental method can and cannot reveal.

Although it may be doubted that any responsible investigator will be misled by the carelessness to which attention is called, the prestige of the International Union of Biochemistry and of its Commission on Enzymes may lead to the teaching of relations here criticized. If so, confusion could result.

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I suggest that if the Commission finally decides to have the symbols conform with the widely accepted stoichiometric relation between oxidant and reductant, it will suffice to state that they should be NAD^+ and NADH. By comparison with the currently used DPN^+ and DPNH the meanings will be well understood. Further comment of the sort injected into the 1961 report hardly can be regarded as germane to the principal subject of that report. One may also expect corrections of the errors in Appendix E.

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References

1. *Report of the Commission on Enzymes of the International Union of Biochemistry* (Pergamon Press, New York, 1961).
2. W. M. Clark, *Oxidation-Reduction Potentials of Organic Systems* (Williams and Wilkins, Baltimore, 1960).

The New Regulations Pertaining to Research Grants of the Public Health Service

During recent weeks, storms of criticism of the newly instituted regulations relating to the management of research grants of the National Institutes of Health (NIH), a division of the Public Health Service (PHS), have arisen among the staffs of many universities, institutes, and scientific societies, and among various individual scientists. Congressional Committees, the General Accounting Office, and the General Counsel of the Department of Health, Education and Welfare, recognizing the occasional lack of adequate responsibility by grantees of the PHS, have made demands that scientists not be treated as especially privileged citizens and that greater fiscal control should be exercised in the expenditure of the hundreds of millions of dollars awarded annually by the various agencies of Congress for the support of scientific research. It is important to note that the funds of the PHS are awarded on the basis of evaluations made by carefully selected scientists, who serve as consultants to the Surgeon General, and who carefully scrutinize each application for a grant, with respect to the objectives, the proposed means of