THE NOMENCLATURE OF ENZYMES AND METHODS OF EXPRESSING RESULTS

BY

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The process of fermentation had been employed and appreciated for countless millennia before Payen and Persoz in 1833 obtained a precipitate of malt extract which was capable of converting starch into sugars. They termed this thermolabile fraction diastase, a word which became a generic term for similar catalysts or ferments such as trypsin and pepsin which were isolated in the same decade. In 1878 Kuhne introduced the word enzyme as the generic term for such substances which were named by their discoverers in haphazard fashion.

NOMENCLATURE

A systematic nomenclature was first proposed in 1898 by Duclaux who suggested that the last three letters of diastase be suffixed to the name of the substrate on which the enzyme acted. Since most of the earlier enzymes brought about similar chemical reactions, that is, hydrolysis or the splitting of substrates with addition of the elements of water, this scheme sufficed for some time and permitted of simple classification, such as the proteases, carbohydrases and lipases acting on proteins, carbohydrates and fats respectively.

However, when a particular substrate was acted upon by more than one enzyme and gave rise to different products it was realized that recognition must be given in the nomenclature to the chemical reaction catalyzed. Thus the enzyme which hydrolyzed glucose-6-phosphate to yield glucose and phosphoric acid still retained the name glucose-6-phosphatase. That which brought about isomerization to fructose-6-phosphate was called glucosephosphate isomerase. When the reaction was an oxidation, by removal of hydrogen, giving gluconolactone phosphate, the catalyst was called glucose-6-phosphate dehydrogenase.

This is the nomenclature which is familiar in clinical circles and for most purposes it is still sufficient. However, as the number of known enzymes increased and the mechanisms of the reactions catalyzed were elucidated inadequacies became apparent. For instance, two isocitrate dehydrogenases were known which oxidized isocitrate to 2-oxoglutarate through an intermediate, oxalsuccinate. One utilized nicotinamide adenine dinucleotide (NAD, formerly known as Coenzyme I or DPN) as coenzyme and did not decarboxylate oxalsuccinate. The other had a requirement for nicotinamide adenine dinucleotide phosphate (NADP, formerly known as Coenzyme II or TPN) and decarboxylated oxalsuccinate. Similar instances of different enzymes being called by the same name and of different names being given to the same enzyme had arisen. It was to end an increasing confusion that in 1955 the International Union of Biochemistry set up an Enzyme Commission “to consider the classification and nomenclature of enzymes and coenzymes, their units of activity and standard methods of assay, together with symbols used in the description of enzyme kinetics”. The report of this Commission (International Union of Biochemistry, 1961) put forward proposals for a systematic classification, numbering system and nomenclature which were later fully endorsed (International Union of Biochemistry, 1965) and accepted by an International Commission of Editors of Biochemical Journals.

SYSTEMATIC IDENTIFICATION

For a complete understanding the above publications must be consulted, but briefly the scheme devised is based upon the chemical reaction catalyzed and specifically identifies each enzyme by a systematic name and a code number of four integers separated by points. The first number indicates which of the six main types of reaction the enzyme catalyzes, that is, (1) oxidoreductases,
(2) transferases, (3) hydrolases, (4) lyases, (5) isomerases and (6) ligases.* The sub-class is given by the second number which indicates the chemical group, linkage or bond involved, while the third number specifies more precisely the nature of the reaction. The fourth number is the permanent individual number of the enzyme within its sub-sub-class. This scheme gives specific identification to every enzyme once its catalytic action is known but because of the complex nature of some substrates the systematic names are often too long for everyday use. As already adopted in other biological sciences it was recommended, therefore, that there also be a trivial or working name. Thus the enzyme, code number 1.14.3.1 whose systematic name is L-phenylalanine tetrahydropteridine:oxygen oxidoreductase (4-hydroxylating), is given the trivial name of phenylalanine-4-hydroxylase. It is absence of this enzyme which results in phenylketonuria, the commonly used term for phenylpyruvic oligophrenia.

WORKING (CLINICAL) NAMES

In most cases the recommended working name is that already in use unless it was considered to be misleading or already unnecessarily cumbersome. An example of the first of these failings is given by the enzymes involved in the hydrolysis of choline esters. In the past the esterase present at the motor endplates, synapses and in red cells has been referred to as “true” cholinesterase in distinction from the enzyme found in blood plasma which has been called “pseudo”-cholinesterase. In fact the former enzyme shows a specificity for acetic esters and of the choline esters actively hydrolyzes acetylcholine only. It is therefore correctly to be called acetylcholinesterase (EC 3.1.1.7). The latter enzyme, on the other hand, is effective only against esters of choline and therefore its recommended trivial name is cholinesterase (EC 3.1.1.8). Of the commonly used names already too complex, those of the aminotransferases are most obvious. Since glutamate almost fulfils the role of coenzyme in most transamination reactions, mention of it is superfluous and only the second amino-acid involved need be recorded. Hence the recommended trivial names for the two aminotransferases of clinical interest are aspartate transaminase (EC 2.6.1.1) (formerly known as glutamic-oxaloacetic transaminase) and alanine transaminase (EC 2.6.1.2) (formerly glutamic-pyruvic transaminase). Again the term “kinase” indicates a phosphate transfer from adenosine triphosphate to the named donor and the use of the term “phosphokinase” is unnecessarily repetitive, e.g. creatine kinase, not creatine phosphokinase, should be used.

ABBREVIATIONS

There are two further recommendations which are often disregarded in medical literature. First, where the substrate is present as an anion the name should end in the appropriate “-ate” not the acid suffix “-ic”, that is lactate dehydrogenase not lactic dehydrogenase. Secondly, it is stated that abbreviations for names of enzymes should be strongly discouraged. Initials such as MDH alone may be meaningless or confusing; they may represent any of the four known malate dehydrogenases, of the two mannitol dehydrogenases, even mannionate dehydratase or in the German literature milchsäure dehydrogenase, that is, lactate dehydrogenase. However, the recommended scheme for identification suggests that, where an enzyme is the main subject of a paper or abstract, the code number and systematic name be given at first mention in the text, or, where not the main subject, identified by code number only, and thereafter the trivial name used. If it is also clearly indicated what abbreviations are employed there seems little point in, far less hope of, Canute-like halting the increasing use of abbreviations. This is most certainly so when describing polymorphism in enzymes. There will be no ready acceptance of repeatedly referring to phosphoglucomutase a, phosphoglucomutase b, phosphoglucomutase c, etc., when PGMa, PGMb, PGMc may be used after appropriate explanation.

ISOENZYMES

No rulings regarding such multiple forms of enzymes were made by the International Union of Biochemistry (1961, 1965) but a sub-committee
on isoenzymes was constituted in 1964. This so far has only made recommendations regarding the numbering of multiple forms separated by electrophoresis and suggested that the term isoenzyme is to be preferred to isozyme (Webb, 1965). No definition of the term was attempted and certainly it would be difficult to formulate a definition which would embrace the multiple forms of, say, lactate dehydrogenase (different aggregation of sub-units), alkaline phosphatase (different tissue of origin) and cholinesterase (gene mutation) all of which have been referred to as isoenzymes. This is perhaps best with the present limited knowledge of protein architecture.

ENZYME MEASUREMENT
Enzymes are proteins and when isolated and purified may be estimated by any of the analytical methods for proteins and expressed in absolute quantities of mass and volume. However, this is not a practical concept for clinical purposes and indeed it is not in the protein character that interest is centred, but in the specific catalytic activity of the enzyme. And it is by means of this ability to accelerate a chemical reaction, which provides an amplification of, and under suitable conditions is proportional to, the concentration, that enzymes are usually measured. The results of such measurements are expressed in units of activity and in the past these have been arbitrarily defined by originators of assay procedures in terms of change in mass, equivalent volume, pH, optical density or other measurable parameter over time intervals ranging from 1 minute to several hours at reaction temperatures from 20°C to 55°C. The activity has then been referred to volumes varying from microlitre to litre.

INTERNATIONAL UNITS
As mentioned earlier, it was within the terms of reference of the Commission on Enzymes to define a standard unit and methods of assay. Accordingly, general recommendations for the conditions of measurement were put forward and the rulings given that “One unit (U) of any enzyme is that amount which will catalyze the transformation of 1 micromole of the substrate per minute under standard conditions” and “concentration of an enzyme in solution should be expressed as units per millilitre”. Use of the prefixes of the metric system such as milli-unit (mU) and kilo-unit (kU) was advocated where necessary to provide for convenient numerical values. Despite the fact that these decisions were taken after discussion with interested bodies “especially those concerned with clinical enzyme units” (International Union of Biochemistry, 1965) some authors have misguidedly decided to achieve convenient numbers by referring activity to the litre rather than the recommended millilitre.

At the time the task facing the Commission on Enzymes was probably impossible since it virtually demanded prescribing a single assay for each enzyme. Without this, definition of a unit of activity to be used for all assays has limited meaning; it does not permit of any more valid comparison of activities between enzymes and, more important, it does not lead to equality of results by different assay procedures for the same enzyme. Failure to appreciate this last point may well cause greater confusion than before, particularly for those not directly involved in enzymology.

THE EFFECT OF METHOD OF ASSAY
Basically there are two types of enzyme assay, those measuring the initial rate of reaction and those measuring the amount of change after a fixed time or, more rarely, the time required to achieve a fixed change. The first type is that which is recommended and is that which is capable—under optimal conditions of pH, substrate and cofactor concentrations—of yielding a true measure of enzyme concentration. Such procedures, in general, require a certain degree of technical skill and are not amenable to batch work. The second type of assay is theoretically less satisfactory and although it is more time-consuming for a single determination, a number of tests can readily be performed simultaneously. This is therefore the type of estimation most commonly employed in clinical laboratories. If results by both types of assay for the same enzyme are expressed in the same units, the values will not usually be the same; generally, if optimal conditions are employed in each, the initial rate of reaction procedure will yield higher values.

Again, for each type of assay outlined above, the measurement, in some cases, may be per-
formed from either side of the reaction catalyzed. In general, however, the velocities of the reaction in opposite directions will not be equal and therefore even valid initial rate measurements will give different results. For example, serum lactate dehydrogenase has a normal range of 30 to 120 mU/ml in the forward direction using a lactate substrate but in the reverse reaction, with a pyruvate substrate, the value will be 70 to 240 mU/ml.

**Effect of substrate.**

Even where the catalyzed reaction is not reversible, few enzymes have an absolute specificity, that is act upon one substrate only. Most are capable of affecting a change in a number of related compounds but usually at different rates. This is illustrated in table I (King, 1965) for alkaline phosphatase, from which it is seen that the results obtained vary with the substrate employed. Even with the same substrate, glycerophosphate, it is noted that a change from optimal conditions, in this case pH, results in a profound difference in the values obtained. A report, therefore, that a serum alkaline phosphatase is 35 mU/ml is meaningless, since by three of the methodologies shown in the table this would be normal and for the other two would indicate a pathological increase. In general, therefore, although use of the international unit is to be strongly advocated, it is not sufficient to report enzyme activities in such units without also recording the method employed or the normal range.

**Temperature.**

Another factor which should also be recorded is the reaction temperature. Historically, and for obvious reasons, most fixed time assays are performed at 37°C while the more recent initial rate procedures were at first conducted at room temperature which was loosely, and erroneously, equated to 25°C. In its first report (1961) the International Union of Biochemistry recommended adoption of 25°C as the standard temperature but later (International Union of Biochemistry, 1965) changed this to 30°C. It would seem unlikely that currently used methods at 37°C will be altered, nor has there been any general inclination, in clinical circles at least, to convert initial rate results to 30°C, although in many cases temperature correction factors do so are available. In any case it has been shown recently (King, 1967) that such factors are not always valid when the reaction temperature differs from the standard temperature by more than a few degrees. This last observation provides a further indication for the complete standardization of enzyme assays (Bergmeyer, 1966).

This awesome task of investigation, not to mention diplomatic compromise and co-operation, awaits the attention of a future International Commission.

**Table I**

Methods of estimating serum alkaline phosphatase activity (after King (1965), "Practical Clinical Enzymology", by permission of the publishers).

<table>
<thead>
<tr>
<th>Method</th>
<th>King and Armstrong (1934) modified King and Wootton (1959)</th>
<th>Bessey, Lowry and Brock (1946)</th>
<th>Klein, Read and Babson (1960)</th>
<th>Shinowara, Jones and Reinhart (1942)</th>
<th>Bodansky (1933)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defined unit</td>
<td>1 mg phenol in 15 minutes at 37°C</td>
<td></td>
<td></td>
<td></td>
<td>1 mg phosphorus in 60 minutes at 37°C</td>
</tr>
<tr>
<td>Normal range</td>
<td>3.5 to 13 units/100 ml</td>
<td>0.8 to 2.3 units/litre</td>
<td>0.6 to 4.0 units/100 ml</td>
<td>2.2 to 8.6 units/100 ml</td>
<td>1.5 to 4.0 units/100 ml</td>
</tr>
<tr>
<td>Ratio</td>
<td>1000×1/94=7.1</td>
<td>1000×1/15=6.7</td>
<td>318×1/30=1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original unit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1000×1/31=5.4</td>
</tr>
<tr>
<td>International unit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1000×1/6=5.4</td>
</tr>
<tr>
<td>Normal range in International units</td>
<td>25 to 92 mU/ml</td>
<td>13.4 to 38 mU/ml</td>
<td>0.6 to 4.2 mU/ml</td>
<td>15.1 to 46.4 mU/ml</td>
<td>8.2 to 21.8 mU/ml</td>
</tr>
<tr>
<td>Substrate</td>
<td>Phenyl phosphate</td>
<td>p-nitrophenyl phosphate</td>
<td>phosphatethalin phosphate</td>
<td>β-glycerophosphate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-glycerophosphate</td>
</tr>
</tbody>
</table>


REFERENCES

**BOOK REVIEW**


This is the third volume of digests of current literature on subjects of importance to anaesthetists, which have been prepared by members of the staff of the unit under Dr. Adriani's direction in New Orleans. There is no doubt that one of the very best methods of education is topic discussion in small groups composed of staff members and postgraduate students. One of the group prepares a digest of relevant literature, presents it and initiates the discussion. Such meetings can go into current problems in some depth and, if conducted in a spirit which ensures that neither staff nor students are afraid to reveal either superficiality in their knowledge or "blank spots", they are in the writer's opinion the best of all postgraduate seminars.

The subjects covered in these three volumes of digests are indeed eclectic and comprehensive. Throughout the three volumes there has been a gradual improvement in the degree of detail in which the subjects are reviewed and also in the presentation. They are of inestimable value to those preparing such discussions and wishing to be reminded of suitable topics. There is no doubt too that they have a useful role to play in keeping busy clinicians in line with the present position in respect of the numerous subjects reviewed.

The most recent volume contains fifty digests, covering subjects some of which are conventional and traditional, such as "Acute tracheal collapse after thyroidectomy" and "Pulmonary function tests" (three digests), to the more esoteric such as "Relationship of syndrome to choice of anesthetic" and "Mucoviscidosis and its implications in anesthesia and surgery". The vast bulk of the subjects considered are practical and of clinical importance. It is interesting to find sections on "Writing examination questions" and "What to teach residents in anesthesiology and what they should know".

It is inevitable that still the vast majority of references are to American work. The most recent references would appear to be to publications of 1966.

In his preface to the third volume, Dr. Adriani states that "the material may be considered 'old' as far as current journals are concerned but 'new' in regard to inclusion in textbooks in anesthesiology. These presentations are not intended to be exhaustive reviews . . . the subject matter is not directed towards research workers, those preparing exhaustive reviews or those who are interested in completeness and in massive detail". Within these terms of reference, these volumes are very useful and certainly the new one is recommended.

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