are avoided and a true concentration of dissolved O_2 may be found. Even so, care must be taken, as the oxygen electrode measures the activity of the dissolved O_2 rather than its concentration. The true concentration of dissolved O_2 may be determined empirically by, for instance, the method of Robinson & Cooper (1970).

Quoting concentration of dissolved gases in terms of partial pressures is a legacy of the time when measurements of quantities of gases were made by volumetric means and were often related to transport in the blood stream. In this particular context where most of the O_2 , for instance, is not in solution, but is bound to haemoglobin, quoting pO_2 values is still appropriate. I would like to suggest, however, that this is not true where kinetic measurements involving O_2 as substrate of an enzyme are concerned. The availability of oxygen electrodes, and the uncertainties in converting from partial pressures to concentrations in solution, argue for the recording of K_m values for O_2 in molar terms. Apart from any other consideration, this would remove from the literature a potential source of error in interpretation.

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Robin H. C. STRANG
Department of Biochemistry,
University of Glasgow,
Glasgow G12 8QQ, U.K.

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The structure of acetyldihydrolipoic acid

Lipoic acid occurs covalently bound in amide linkage in the acyltransferase component of 2-oxo acid dehydrogenase multienzyme complexes (Reed, 1960, 1974). In the course of action of these enzyme complexes the disulphide group of the lipoic acid is reductively acetylated and the acyl group is then transferred to coenzyme A. It is widely believed (e.g. Hageman, 1979) that the site of acylation of the enzyme-bound dihydrolipoyl group is its 6-mercapto group. This belief is based on the conclusion of Gunsalus *et al.* (1956) who found that exogenous dihydrolipoate could be acetylated by an extract of *Escherichia coli*. The methyl ester of this enzymically generated compound reacted smoothly with *N*-(diphenylmethylene)propionamide, which they thought suggested that it contained the primary $-CH_2SH$ group and so was the S^6 -acetyl compound. The chemically acetylated methyl dihydrolipoate did not react, so was assigned the S^8 -acetyl structure.

O'Connor (1979; for some of his earlier findings see Paukstelis *et al.*, 1977) used ^{13}C n.m.r. to examine the reaction product, and this study has cast doubt on the earlier view. He made S -[1- ^{13}C]acetyldihydrolipoamide from [1- ^{13}C]acetyl-CoA both enzymically, by the same method as Gunsalus *et al.* (1956), and non-enzymically. The ^{13}C n.m.r. spectrum showed signals for both S^8 -(1- ^{13}C)acetyldihydrolipoamide and S^6 -(1- ^{13}C)acetyldihydrolipoamide in a ratio of about 2.5:1, indicating that both compounds were formed in both reactions.

It seems to us that transfer of acyl groups between the thiol groups of dihydrolipoamide in neutral aqueous solution may be quite fast. Such acyl transfer occurs readily from S to O in the series $CH_3COS[CH_2]_nOH$ when n is 2 or 3, but not when n is 4 (Harding & Owen, 1954; Martin & Hedrick, 1962). The rate of transfer of acetyl groups between the primary and secondary groups of glycerol also rises with pH and is 0.056 min^{-1} at pH 7 and $37^\circ C$ (Wolfenden *et al.*, 1964). Transfer between two thiol groups may be faster because of the greater nucleophilicity of such groups at neutral pH, and may be slowed by the ring size of six instead of five in the intermediate, a decrease of 30-fold for the S→O transfer studied by Martin & Hedrick (1962).

The distribution of groups found by n.m.r. is therefore likely to represent equilibrium between S^6 - and S^8 -acetyl compounds. The ratio that may be inferred from signal strengths (2.5:1) is similar to that (5.6:1) measured by Wolfenden *et al.* (1964) for the distribution of acetyl groups between the primary and secondary hydroxyl groups of glycerol when correction is made for the presence in glycerol

of two primary groups. Equilibration did not occur in benzene (O'Connor, 1979).

If the proposed intramolecular transfer occurs, the existence of distinct n.m.r. absorption peaks indicates that it is not very fast; O'Connor (1979) calculated that at pH 7.5 and 5°C the lifetime of the acetyl group was more than 20 ms. Hence the lifetime at pH 7 and 30°C would be expected to be at least 10 ms. As this is comparable with the time (about 7 ms) required for a single turnover of the *E. coli* pyruvate dehydrogenase under the same conditions (Perham & Thomas, 1971), we cannot rule out spontaneous transfer (let alone enzyme-catalysed transfer) from being an obligatory step in the catalytic mechanism.

Presumably the acyl group is put specifically onto one of the thiol groups of enzyme-bound dihydrolipoic acid and is transferred specifically from one of them onto coenzyme A. If the same group participates in both reactions, this process could be faster than transfer between the two thiol groups, but it seems more likely that the acyl group is transferred back and forth between the thiol groups at a comparable rate. This view is supported both by the fact that the dihydrolipoyl group seems largely free in solution on the outside of the enzyme complex as it passes between the active sites (Ambrose & Perham, 1976) and by its rapid transfer between dihydrolipoyl groups on different polypeptide chains of the same complex (Danson *et al.*, 1978).

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Geoffrey HALE* and Henry B. F. DIXON
Department of Biochemistry, University of Cambridge,
Tennis Court Road, Cambridge CB2 1QW, U.K.
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* Present address: Department of Pathology, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.