

## APPENDIX

## Continuous Coupled Assay for Acetyl-Coenzyme A Production: Correction of Observed Rates

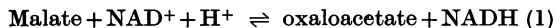
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Buckel & Eggerer (1965) and Pearson (1965) have shown that assays for acetyl-CoA with malate dehydrogenase and citrate synthase do not yield NADH stoichiometrically, but that values must be corrected to give true acetyl-CoA contents. The former authors also extended the argument to the measurement of citrate synthase and, by implication, to the assay of any system producing acetyl-CoA when malate dehydrogenase is used as an auxiliary enzyme. I append the following derivation, which is slightly different from that of Buckel & Eggerer (1965).

The reaction:



lies to the left at equilibrium and, for small changes at constant pH, all the substances on the left can be considered to have constant concentration. Therefore:

$$[\text{Oxaloacetate}][\text{NADH}] = K'$$

At zero time the production of acetyl-CoA is started. Malate, NAD<sup>+</sup> and malate dehydrogenase are already present and the equilibrium of eqn. (1) is established. Using subscripts 0 and *t* for zero time and time *t*:

$$[\text{Oxaloacetate}]_0 = [\text{NADH}]_0$$

Therefore:

$$[\text{Oxaloacetate}]_t [\text{NADH}]_t = K'$$

$$= [\text{oxaloacetate}]_0 [\text{NADH}]_0 = [\text{NADH}]_0^2$$

$$[\text{Oxaloacetate}]_t = \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t}$$

$$[\text{Oxaloacetate}]_0 - [\text{oxaloacetate}]_t$$

$$= [\text{NADH}]_0 - \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t}$$

and in volume *v*:

$$(\text{Oxaloacetate})_0 - (\text{oxaloacetate})_t$$

$$= v \left( [\text{NADH}]_0 - \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t} \right) \quad (2)$$

where (oxaloacetate) indicates amount of oxaloacetate.

During the period *t*, the overall decrease in the amount of oxaloacetate will be equal to the difference between (a) the formation of oxaloacetate from malate, accompanied by an equivalent formation of NADH, and (b) the disappearance of oxaloacetate through combination with acetyl-CoA (it is assumed that acetyl-CoA is removed completely). Then:

$$\text{Formation of oxaloacetate} = v[\text{NADH}]_t - v[\text{NADH}]_0$$

and

$$\text{Disappearance of oxaloacetate} = v(\text{acetyl-CoA})_p$$

where (acetyl-CoA)<sub>*p*</sub> is the amount of acetyl-CoA produced/unit volume in time *t*.

Net disappearance of oxaloacetate

$$= v(\text{acetyl-CoA})_p - v[\text{NADH}]_t + v[\text{NADH}]_0$$

$$= (\text{oxaloacetate})_0 - (\text{oxaloacetate})_t$$

From eqn. (2):

$$v \left( [\text{NADH}]_0 - \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t} \right)$$

$$= v(\text{acetyl-CoA})_p - v[\text{NADH}]_t + v[\text{NADH}]_0$$

Therefore:

$$(\text{Acetyl-CoA})_p = [\text{NADH}]_t - \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t}$$

$$\frac{d(\text{Acetyl-CoA})_p}{d[\text{NADH}]} = 1 + \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t^2}$$

or

$$\frac{d(\text{Acetyl-CoA})_p}{dt} = \frac{d[\text{NADH}]}{dt} \left( 1 + \frac{[\text{NADH}]_0^2}{[\text{NADH}]_t^2} \right) \quad (3)$$

Thus when [NADH]<sub>*t*</sub> is approximately equal to [NADH]<sub>0</sub>, early in the reaction, the rate of appearance (or removal) of acetyl-CoA. The correction given by Buckel & Eggerer (1965) also leads to this conclusion, but their correction term is

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$1 + [\text{NADH}]_0/[\text{NADH}]_t$ , so that the observed slope would approach the true rate more slowly than is predicted by the correction derived here; when the NADH concentration has doubled, the observed rate will be 20% low by my correction, but 33% low by theirs.

If NADH is added initially (e.g. if NADH is present in a preparation whose enzyme activity is being tested), the initial NADH concentration will be greater than  $[\text{NADH}]_0$ , the equilibrium value defined above. Thus  $[\text{NADH}]_t$  at  $t=0$  will be

greater than  $[\text{NADH}]_0$  (eqn. 3) and the initial rate will be more than half the true rate of acetyl-CoA production. Under these circumstances the difference between the factor of Buckel & Eggerer (1965) and the one given here becomes crucial for initial rates.

#### REFERENCES

- Buckel, W. & Eggerer, H. (1965). *Biochem. Z.* **343**, 29.  
Pearson, D. J. (1965). *Biochem. J.* **95**, 23c.