Deacon's Challenge No. 86 Answer

Lactate can be measured enzymatically by oxidation to pyruvate by lactate dehydrogenase (LDH) in the presence of NAD^+:

$$\text{L-lactate} + NAD^+ \overset{LDH}{\rightleftharpoons} \text{Pyruvate} + NADH + H+$$

NADH has a molar absorption coefficient of 6.22×10^3 $L.mol^{-1}.cm^{-1}$

Method

To 2.0 mL buffer, add 0.1 mL sample, 0.2 mL NAD^+ (?? mmol/L) and 30 µL LDH solution.

Result

The absorbance change (relative to a reagent blank, using a standard 1 cm cuvette) in the assay was 0.82.

Calculate the lactate concentration in this sample.

MRCPath, Autumn 2007

Assuming complete conversion of lactate to pyruvate, the amount of NADH formed is equal to the amount of lactate in the sample.

The relationship between absorbance and NADH concentration in the reaction mixture is:

$$A = \varepsilon.c.L$$

Where A = absorbance change = 0.82

ε = molar absorption coefficient for NADH = 6.22×10^3 $L.mol^{-1}.cm^{-1}$

c = NADH concentration (mol/L) = unknown

L = cuvette path length = 1 cm

Substituting these values and solving for c:

$$0.82 = 6.22 \times 10^3 \times c \times 1$$

$$c = \frac{0.82}{6.22 \times 10^3} = 1.32 \times 10^{-4} \text{ mol/L}$$

Since each mol of lactate produces 1 mol of NADH this is also the concentration of lactate in the reaction mixture. As lactate is usually reported in mmol/L, not mol/L, multiply by 1,000 (i.e. 10^3):

Lactate concentration = $1.32 \times 10^{-4} \times 10^3$ = 0.132 mmol/L

This is the lactate concentration in the final reaction mixture. To calculate the concentration in the sample multiply by the reaction volume and divide by the sample volume:

$$\text{Sample lactate (mmol/L)} = \frac{\text{Reaction mixture lactate (mmol/L)} \times \text{Reaction vol}}{\text{Sample vol}}$$

Reaction mixture consists of:

Buffer	=	2.0 mL
Sample	=	0.10 mL
NAD^+	=	0.2 mL
LDH	=	0.030 mL (= 30µL)
Reaction volume	=	2.33 mL

Sample lactate = $\frac{0.132 \times 2.33}{0.10}$ = **3.1 mmol/L** (2 sig figs)

Question 87

A 44-year old woman, who suffered a miscarriage of pregnancy four days previously, was found to have a serum β-HCG concentration of 578 IU/L. Given that the half-life of β-HCG at more than 48-hours after termination of pregnancy is 56 hours, in how many days time would you expect her serum β-HCG concentration to reach a level of 5 IU/L?

MRCPath, Autumn 2007