Deacon's Challenge No. 33 Answer

An assay mixture for the measurement of lactate dehydrogenase constituted 2.7 mL of buffered NADH and 100 µL of serum. The reaction was started by adding 100 µL of sodium pyruvate. The absorbance change over 5 minutes was 0.150 when measured in a 0.5 cm light path at 340 nm. Assuming the molar absorbtivity of NADH at 340 nm is 6.30×10^3 $Lmol^{-1}\,cm^{-1}$, calculate the enzyme activity.

LDH activity = µmol substrate consumed/min/L plasma

The reaction is monitored by following the fall in absorbance at 340 nm due to the oxidation of NADH as pyruvate is consumed:

$$CH_3C0.COO^- + NADH + H^+ \rightarrow CH3CH(OH)COO^- + NAD^+$$

First calculate the amount of NADH oxidized over the reaction period of 5 min:

$$\Delta \text{ Absorbance} = \Delta[NADH]\ mol/L \times \varepsilon_{NADH}\ (Lmol^{-1}cm^{-1}) \times \text{Cell path (cm)}$$

$$0.150 = \Delta[NADH] \times 6.30 \times 10^3 \times 0.5$$

$$\Delta\,[NADH] = \frac{0.150}{6.30 \times 10^3 \times 0.5}\ mol/L/5\ min$$

To covert from mol to mmol multiply by 1,000,000, and from 5 min to 1 min divide by 5:

$$\Delta\,[NADH] = \frac{0.150 \times 1{,}000{,}000}{6.30 \times 103 \times 0.5 \times 5}\ \mu mol/L/min$$

This is the LDH activity per litre of reaction mixture not plasma.

Since 100 µL of plasma was diluted to a final volume of 2.9 mL (i.e 100 µl plasma + 2.7 ml NADH/buffer + 100 µL of substrate) the activity (mmol/min/L plasma) is obtained by multiplying this result by 2.9 and dividing by 0.1:

$$\text{LDH activity} = \frac{0.150 \times 1{,}000{,}000 \times 2.9}{6.30 \times 10^3 \times 0.5 \times 5 \times 0.1} = 276\ mmol/min/L$$

Question No. 34 **Christmas Special**

What is the pH of a 1.0×10^{-8} molar solution of hydrochloric acid?
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allan.deacon@bedhos.anglox.nhs.uk