Deacon's Challenge No. 80 Answer

75 mg of faeces were homogenised in 1 mL of concentrated hydrochloric acid, 3 mL diethyl ether added, mixed, 3 mL water added and mixed again. After centrifugation, the aqueous phase (volume 4.5 mL) was scanned in a spectrophotometer using a cell with a 1 cm pathlength. The peak height at 405 nm due to porphyrin, after applying a background correction, was 0.35 absorbance units. A separate 0.250 g portion of faeces was dried in a 100°C oven until its weight was constant (0.125 g). Given that the molar absorption coefficient of porphyrin is 2.75 x 10^5 L/mol/cm, calculate the porphyrin concentration in nmol/g dry weight of faeces.

$$A = \varepsilon \times c \times l$$

Where A = absorbance = 0.35

ε = molar absorption coefficient = 2.75 x 10^5 L/mol/cm

l = light path length = 1 cm

Rearranging and substituting for A, l and ε:

$$c = \frac{A}{\varepsilon \times l} = \frac{0.35}{2.75 \times 10^5 \times 1} = 1.27 \times 10^{-6} \text{ mol/L}$$

Divide by 1000 to determine the moles of porphyrin in 1 mL of extract, then multiply by the volume of extract (4.5 mL) to obtain the total porphyrin in the extract:

$$\text{Porphyrin content of extract} = \frac{(1.27 \times 10^{-6}) \times 4.5}{1000} \text{ mol}$$

This extract was prepared from 75 mg (= 0.075 g) of faeces. Divide by 0.075 to obtain the amount of porphyrin in 1 g of wet faeces:

$$\text{Porphyrin content of wet faeces} = \frac{(1.27 \times 10^{-6}) \times 4.5}{1000 \times 0.075} \text{ mol/g wet faeces}$$

Multiply by the ratio of wet to dry faecal weights used for the dry weight determination to give the porphyrin content in terms of faecal dry weight:

$$\text{Porphyrin content} = \frac{(1.27 \times 10^{-6}) \times 4.5 \times 0.25}{1000 \times 0.075 \times 0.125} \text{ mol/g dry faeces}$$

Finally multiply by $1x10^9$ to obtain the porphyrin content in nmol instead of mol:

$$\text{Porphyrin content} = \frac{(1.27 \times 10^{-6}) \times 4.5 \times 0.25 \times 10^9}{1000 \times 0.075 \times 0.125}$$

$$= \frac{1.27 \times 4.5 \times 0.25}{0.075 \times 0.125} = \textbf{152 nmol/g dry faeces}$$

Question 81

Haemochromatosis, a cause of abnormal liver function tests (LFTs), has a UK prevalence of 0.2%. Iron overload due to haemochromatosis is diagnosed by demonstrating raised serum transferrin saturation (TSat). A commoner cause of abnormal LFTs is non-alcoholic fatty liver disease (NAFLD), with a reported prevalence of 5%. Unfortunately, raised TSat has also been reported in 7.4% of patients with abnormal LFTs due to NAFLD (and there is no association between NAFLD and haemochromatosis). Assuming that there are no other causes of raised TSat, in what percentage of patients with abnormal LFTs will a raised TSat indicate haemochromatosis?

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