Deacon's Challenge No. 9 Answer

The absorbances of a solution containing NAD and NADH in a 1cm light path cuvette were 0.337 at 340 nm and 1.23 at 260 nm. The molar extinction coefficients are:

NAD: 1.8×10^4 at 260 nm, 1.0×10^{-3} at 340 nm
NADH: 1.5×10^4 at 260 nm, 6.3×10^3 at 340 nm

Calculate the concentrations of NAD and NADH in the solution.

(MRCPath Nov 1995)

Both NAD and NADH absorb at the two wavelengths used (260 nm and 340 nm). Absorbances are additive, therefore at either wavelength:

Total absorbance = Absorbance of NAD + Absorbance of NADH

At any wavelength the absorbance of NAD or NADH is given by:

Absorbance = Molar extinction coefficient x Molar concentration x Cell path

Therefore for each wavelength equations can be set up relating measured total absorbance to the sums of the individual absorbances of NAD and NADH:

Measured absorbance = (NADConc x NADCoeff) + (NADHConc x NADHCoeff)

At 340 nm: $0.337 = 1.0 \times 10^{-3}$ [NAD] + 6.3×10^3 [NADH] ....(i)

At 260 nm: $1.23 = 1.8 \times 10^4$ [NAD] + 1.5×10^4 [NADH] ....(ii)

(The cell path is 1 cm and can be ignored)

These form a pair of simultaneous equations that can be solved for [NAD] and [NADH] in the usual manner. However, solving a set of simultaneous equations can be a lengthy process and it is worth remembering that these calculations are designed to be simple! Therefore we should look for approximations and short cuts. In this particular example it is possible to simplify the calculation considerably. The

molar extinction coefficient of NAD at 340 nm is much lower than that of NADH (by a factor of approx. 10^{-6}) so that the contribution of NAD to absorbance at this wavelength can be ignored. Equation (i) can then be simplified to:

$$0.337 = 6.3 \times 10^3 \text{ [NADH]}$$

$$\text{[NADH]} = \frac{0.337}{6.3 \times 10^3} = 5.35 \times 10^{-5} \text{ M} = 53.5 \text{ mmol/L}$$

[NAD] can be calculated by substituting [NADH] = 5.35×10^{-5} into equation (ii):

$$1.23 = 1.8 \times 10^4 \text{ [NAD]} + (1.5 \times 10^4 \times 5.35 \times 10^{-5})$$

$$1.23 = 1.8 \times 10^4 \text{ [NAD]} + (8.03 \times 10^{-1})$$

$$1.8 \times 10^4 \text{ [NAD]} = 1.23 - (8.03 \times 10^{-1}) = 0.427$$

$$\text{[NAD]} = \frac{0.427}{1.8 \times 10^4} = 2.37 \times 10^{-5} \text{ M} = 23.7 \text{ mmol/L}$$

Question No. 10

A patient receiving total parenteral nutrition is receiving 11.8g nitrogen/24h as amino acids. Urinary urea excretion is 580 mmol/24h.

Indicating what assumptions you make, calculate whether she is in positive or negative nitrogen balance.

(MRCPath November 1999)