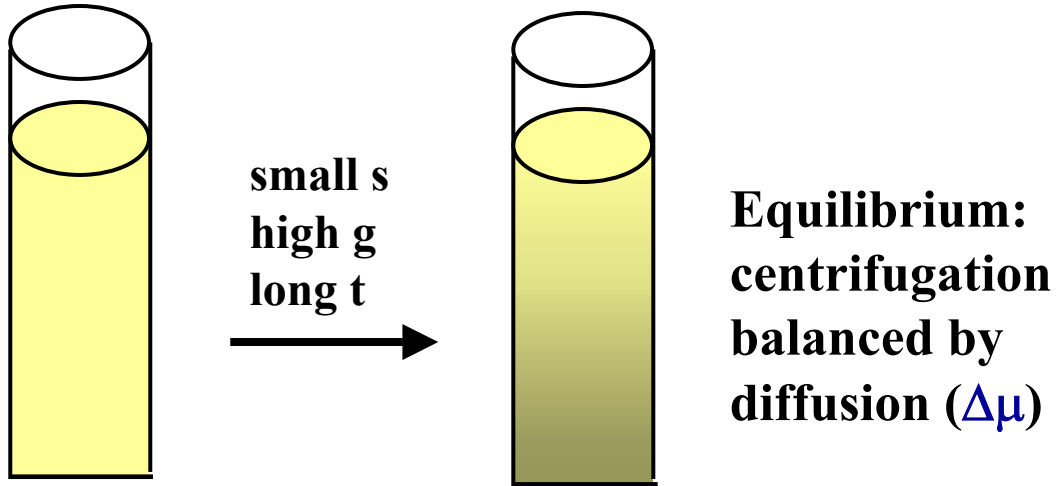


Centrifugation was performed on **5–25% sucrose gradients** containing **0.1M NaCl + 50 mM sodium phosphate buffer, pH 8.0**. SW41 Ti rotor (Beckman), 26 h, $160,000 \times g$. Fractions (0.3 ml) were collected and assayed for AChE activity. The molecular forms correspond to tetramers, dimers, and monomers. (Mendelson *et al.*, Bovine acetylcholinesterase: cloning, expression and characterization, *Biochem J.* 334: 251-259, 1998.)

Isopycnic centrifugation (sedimentation equilibrium)
(cf. isoelectric focusing)

- **protein MW determination**
- **separation of molecules of very similar ρ**
(DNA, RNA in CsCl gradients)



$$\Delta\mu = RT \ln (C_2/C_1)$$

Sedimentation equilibrium - theory

$$\Delta\mu = RT \ln (C_2/C_1) \quad \ln (C_2/C_1) = \Delta\mu / RT$$

At equilibrium:

$$\begin{aligned} \Delta E &= \int_{r_1}^{r_2} F(r) dr = \int_{r_1}^{r_2} m_0(1-\bar{v}\rho) \omega^2 r dr \\ &= \frac{1}{2} \omega^2 (r_2^2 - r_1^2) m_0(1-\bar{v}\rho) \end{aligned}$$

$$\therefore \ln \left(\frac{C_2}{C_1} \right) = \frac{\omega^2 (r_2^2 - r_1^2) m_0(1-\bar{v}\rho)}{2RT}$$

$$\ln \left(\frac{C_2}{C_1} \right) = \frac{\omega^2 (r_2^2 - r_1^2) m_0 (1 - \bar{v} \rho)}{2RT}$$

**If \bar{v} can be measured (or estimated),
the slope of a plot of $\ln C$ vs. r^2 gives $M (= m_0 N)$**

**In contrast to the Svedberg equation, this
analysis does not require measurement of f or D .**