Radioimmunoassay (RIA) Experiment

In our experiment, a control blood sample was taken from an animal. Then a certain amount of angiotensin II was injected into the animal, and blood samples were collected at 2, 4, 6, and 8 hours after injection of angiotensin II. Angiotensin II stimulates cells of the zona glomerulosa of the adrenal cortex to secrete the hormone **aldosterone**. We will use the radioimmunoassay method to examing how the concentration of aldosterone in the blood changes over time. The analysis is broken up into two parts:

a) Calibration of the RIA

b) Determine the concentration of aldosterone in the blood samples taken at 2, 4, 6, and 8 hours after the injection.

A) Calibration

You can determine the calibration curve by carrying out experiments using the applet **riacal**. The link to this applet is next to this experiment description. In this applet, a fixed amount of "hot" hormone is placed in a test tube. The applet allows you to carry out experiments as you would in the lab: 1) You can adjust the amount of "cold" hormone that is added to the test tube, and 2) you can select if the tube has anti-bodies or not. When you click the record button, the number of counts/min is displayed. The Applet simulates a true gamma detector for which this count rate will is probabilistic and will be slightly different every time you record data.

The key idea in measuring the concentration of Aldosterone is based on the fact that the same amount of hot Aldosterone is in the tube each time. Using the following equation:

$$\frac{Concentration\ of\ Cold}{Concentration\ of\ Hot} = \frac{Fraction\ Bound_{Cold}}{Fraction\ Bound_{Hot}} = \frac{FB(cold)}{FB(hot)} \tag{1}$$

This gives FB(cold)/FB(hot) = 1/FB(hot) - 1 = (B_0 - NSB)/(B - NSB) -1 = (B_0 − B)/(B - NSB). Here B is the count rate, B_0 is the count rate with no "cold" hormone added, and NSB is the "Non-Specific Binding" count rate (i.e. no anti-bodies in the tube). Thus, the above equation becomes:

$$\frac{Concentration\ of\ Cold}{Concentration\ of\ Hot} = \frac{B_0 - B}{B - NSB} \tag{2}$$

Re-writing this equation, we have:

$$Concentration\ of\ Cold = (Concentration\ of\ Hot)\frac{B_0 - B}{B - NSB} \qquad (3)$$

which can be used for calibration as follows. First measure NSB and B_0. Then measure B for different values of added "cold" hormone. A graph of "Concentration of Cold" versus $(B_0 - B)/(B - NSB)$ should yield a straight line which can be used for calibration.

B) Determination of Hormone Concentration in the Plasma

To measure how the hormone concentration changes in time, you can use the applet **riasamp**. The link to this applet is next to the experiment description. In this applet, a fixed amount of "hot" hormone is placed in the tube, the same amount as in the calibration experiment. The applet allows you to select blood samples at hour-0 (before the injection of angiotestin II), at hour-2, hour-4, hour-6, and hour-8 after the injection. You can also choose to use a tube with and without the anti-bodies. When you choose the record button, the counts/min is displayed as in the calibration experiment. Your goal is to determine how the concentration of Aldosterone in the blood changes in time.

Laboratory Write-up

1. (2 point) Make (and turn in) a table of the data you took for the calibration graph.

2. (2 point) Plot of the "Concentration of Cold" versus $(B_0 - B)/(B - NSB)$ for your data.

3. (4 points) By using a "trendline" determine the best linear through the data points. What do you obtain for the "Concentration of Hot" Aldosterone that was put in the tube?

4. (2 points) Use the parameters obtained from the calibration fit in order to determine the Aldosterone concentration in the plasma samples.

5. (2 points) Make a plot of the plasma Aldosterone concentration as a function of time after the injection of Angiotensin II into the animal.