Reciprocal **A**ffinity **D**epletion (RAD) Method to find proteomic differences between normal and disease

Normal tissue total proteins

Immunize chickens

Chicken Abs against normal tissue proteins

Make a reusable affinity column

Pass diseased tissue proteins through anti-normal column

Proteins unique to diseased tissue & with low abundance pass through; Common & antigenic proteins are retained on the column

Reciprocal Affinity Depletion

Diseased tissue total proteins

Immunize chickens

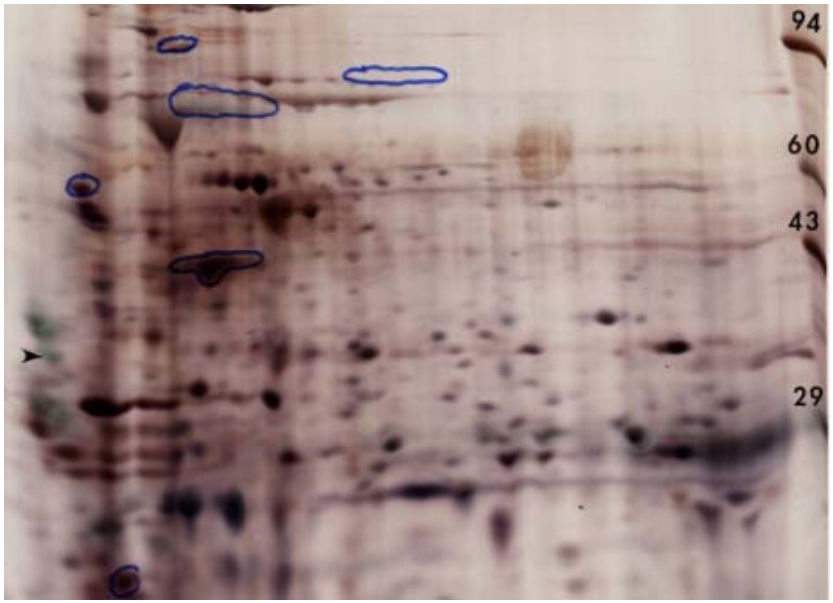
chicken Abs against diseased tissue proteins

Make a reusable affinity column

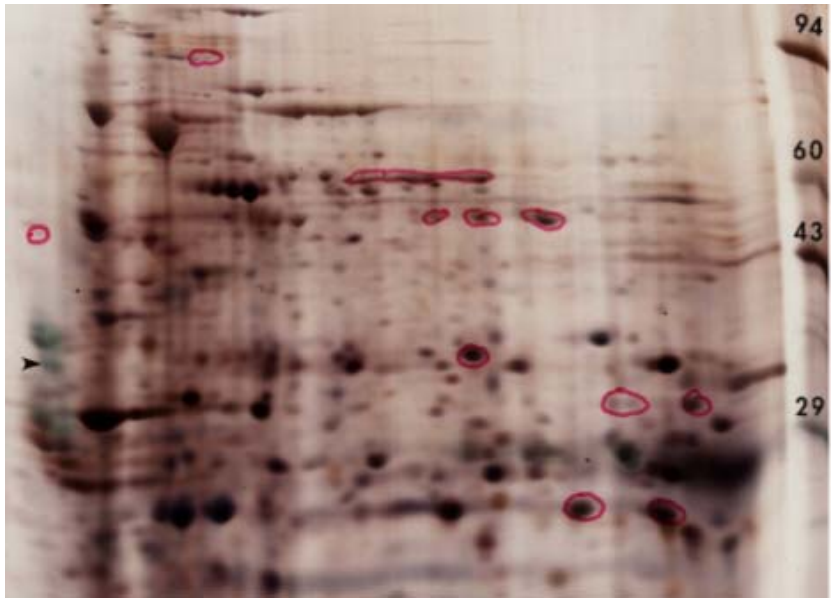
Pass normal tissue proteins through anti-diseased column

Proteins unique to normal tissue & with low abundance pass through; Common & antigenic proteins are retained on the column

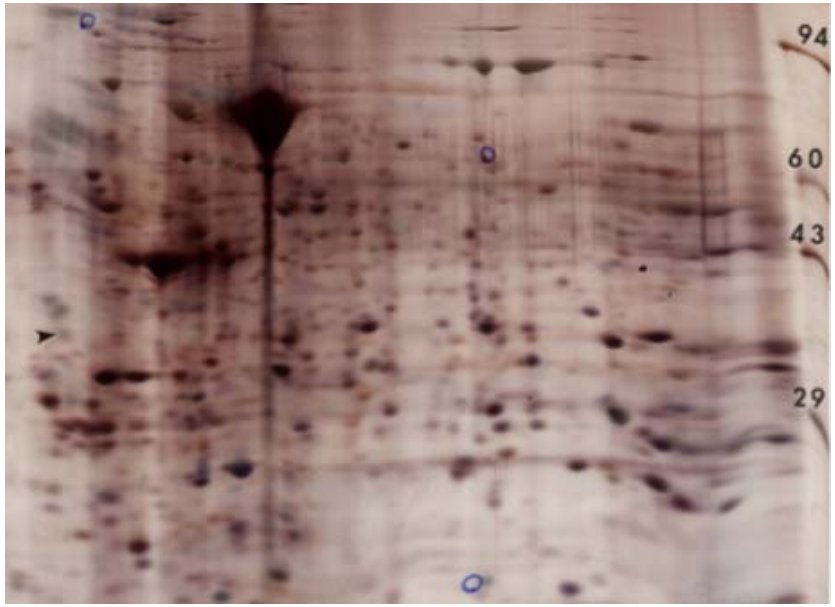
The eluents are greatly enriched in proteins differing between the tissues.
Comparison by 2D gel electrophoresis to find all-or-nothing and quantitative differences becomes straightforward. The proteins of interest may be subsequently identified by mass spectrometry.



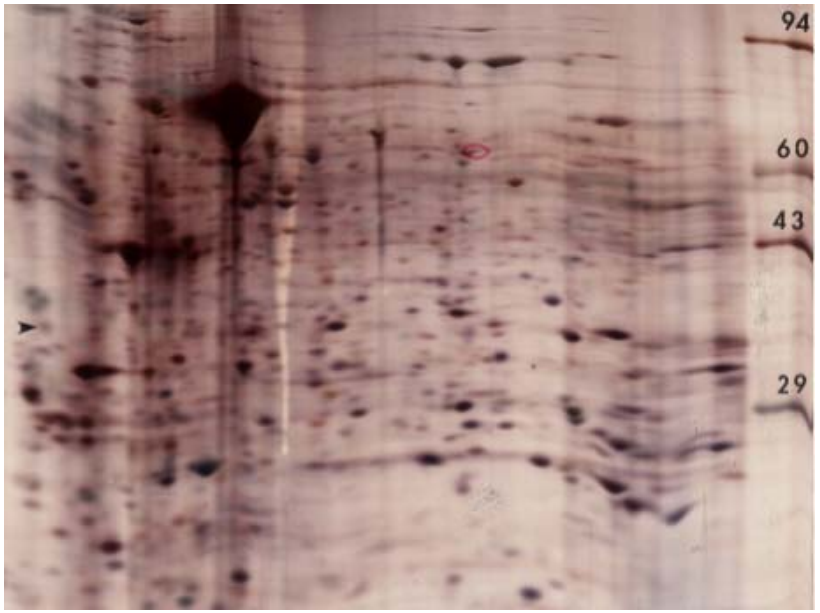
RAD Control Tissue Homogenate



RAD Diseased Tissue Homogenate



Control Tissue Homogenate before RAD



Diseased Tissue Homogenate before RAD