Assays and Techniques

Assays

• Measurement of hormone levels
  – Bioassays
  – Validation
    • Accuracy
    • Objective endpoint
    • Specificity
    • Precision
    • Sensitivity

• Chemical and Physical Assays
• Competitive binding Assays
• Immunoassays
  – RIA

• Start here 502

Cytological-Histological

• Early studies
  – Anatomical and descriptive using light microscopy
• Gross anatomy
  – Localization, size, blood supply, innervation
  – Hypertrophy /atrophy
• Immunocytochemistry
  – Indicator labeled antibody localization of hormone

Surgical Methods

• Ablation and replacement experiments
  – Removal of candidate tissue and observation of physiological changes
  – Ex. Berthold and the roosters
  – Compensatory hypertrophy
  – “ectomies”

Hormone Replacement Therapy

• Giving of exogenous hormone to replace lost hormone after the
ablation of endocrine tissues of glands
  – Ex menopause/ estrogen/progestin therapy

**Tissue Extracts/Purification**

- Crude extracts in early studies
  – Problems
- Purification
- Ex insulin
  – Ovine, porcine, bovine, now genetically engineered!

**Chemical Identification + Synthesis**

- Simple elemental analysis
- Primary amino acid sequence
- Secondary, tertiary, and quaternary structure
- Modifications
  – glycosylation

**Bioassays**

- Measuring the biological activity of a hormone of hormone extract
- Need living tissue that produces a detectable physiological response to the hormone
  - *in vivo* or *in vitro*
- Physiological vs. Pharmacological doses
- Ex Frog skin/MSH
- Epiphysial plates/ TSH

**Radio-isotope studies**

- Isotopes of I$^{125}$, Ca$^{45}$, S$^{35}$, P$^{32}$, Na$^{23}$, C$^{14}$, H$^{3}$ are incorporated into hormones and then tracked
- Can determine:
  – Metabolism, abundance, half- life, enzymatic rates
  – Ex. Radio immunoassay
Radio-isotope studies

- Radioreceptor Assays
  - Radiolabelled hormones bind to their normal membrane receptors, usually dose study
- Enzyme Assays
  - Enzyme activity ex. Adneylate cyclase activity
- Autoradiography
  - Classic – injection of tracer/to tissue, expose tissue to film, develop film to find tracer localization
    - Ex steroids and nuclear DNA
- Hybridization
  - Paring of nucleic acid fragments – tracer is attached to a probe (short radioactive DNA or RNA sequence), rest like autoradiography.

Electrophysiological Methods

- Excitable cells!
  - Monitoring of transmembrane charges with microelectrodes, detects depolarization and hyperpolarizations

Pharmacological Methods

- Use of drugs or chemicals to study physiological activity in cells
- Ex oubain inhibits the Na/K pump
- Inhibitors
  - Cochine- inhibits microtubule formation
- Actinomyosin –d
- Receptor agonist/antagonists

Genetic Engineering

- Microinjection of nucleic acid that incorporate in to the pronuclei of fertilized cells
- Examples
  - Insulin producing bacteria
  - Super salmon
- Transgenic species
Quiz 2

• Explain what parabiosis is and how it would help you determine the source of a hormone.