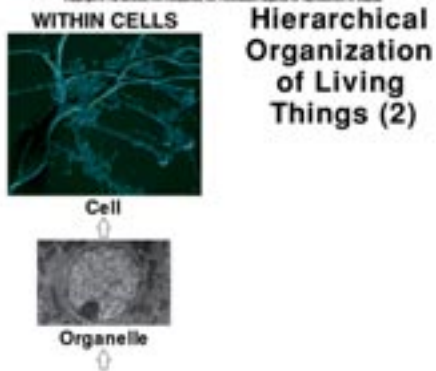
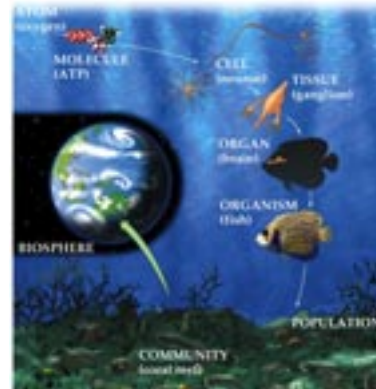


Characteristics of Life

- Order
 - Atom, molecules, organelles, cells, tissues, etc.
- Sensitivity
 - Respond to stimuli
- Growth, development and reproduction
 - Crystals? viruses?
- Regulation
- Homeostasis
 - Constant internal environment



The Scientific Method

- Observation
- Establishing a model
- Formulate a hypothesis
- Experimentation
- Collecting and evaluating results
- Drawing conclusions
- Reporting what has been found



Observations



- Physical or chemical basis
 - Looking
 - Hearing
 - Smelling
 - Touching
 - Measuring
 - Reading about previous studies
 - Serendipity
- Why not metaphysical, supernatural, existence of God
- Science is amoral

Models



- A simplified view of how the components of a system operate.
 - Must be consistent with previous scientific knowledge.
 - Must offer new insight.
- May compare a process that is not understood to one that is understood

Hypothesis



- Is **NOT** an educated guess.
- Someone can devise an experiment to **disprove** the hypothesis if it were incorrect.
 - Can you truly prove an hypothesis correct??
- A hypothesis is valuable only if it is **testable**.

Two forms of hypothesis



- Null hypothesis (H_0)
 - States that what is observed or measured is **not** unusual from what is usually observed, or from what is seen in the control experiment.
- Alternative hypothesis (H_a)
 - States that what is observed or measured is unusual from what is usually observed.

Experiments



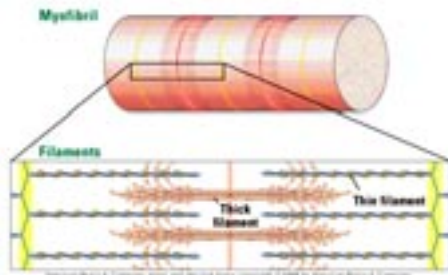
- Procedures carried out under conditions controlled by the scientist
- Experiments include **controls** and **treatment variables** designed and implemented to **prove the hypothesis false** if possible
 - Controls?
 - Treatment variables

Biceps and triceps operate the main movement of the arm



Reductionism: understanding the whole in terms of parts

The value of the reductionist approach to science



Collecting and Evaluating Results



- Collect experimental data.
- Computer and statistical analysis.

Conclusions



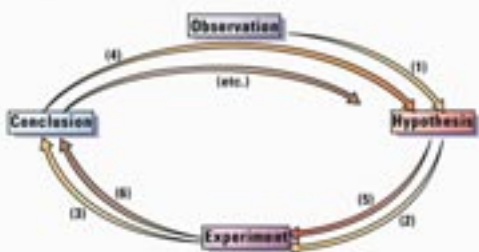
- Evaluate the results.
 - Statistics -- collecting and analyzing numerical data
- Refine hypothesis or test alternative hypothesis.

Reporting



- Inform other scientists.
- Permits scrutiny of scientific community.
- Informs the public.

Scientific method



Theory

- A set of related hypotheses that consistently resists scientists efforts to disprove them.

Problem

- Coccidioidal meningitis is a devastating disease
- No known cure
 - Current drugs control, do not cure the disease
- Better chance of being cured of cancer

Observations

- IFN γ favors Th-1 responses
- IFN γ activates NO producing cells -- peripheral macrophages, microglia, and astrocytes
- IFN γ activates oxygen metabolite producing cells -- neutrophils, monocytes
- Therapy for certain intracellular infections
- Enhances in vitro effector-cell antifungal activity against *Coccidioides immitis*

Model



Hypotheses?

Objective

- Determine the therapeutic efficacy of IFN γ alone and in combination with fluconazole for the treatment of experimental coccidioidal meningitis.

Methods

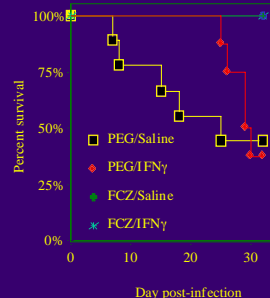
- NZW Rabbits, 3-4 kg, male
- Hydrocortisone acetate, I.M. Days -1, 0, 1, 2, 3
- *Coccidioides immitis* challenge, 4.4×10^4 & 6.0×10^4 arthroconidia, intracisternally, day 0
- Treatment - 21 days starting on day 5
 - PEG-200 orally Q.D. & Saline SQ Q.O.D. (control)
 - PEG-200 orally Q.D. & IFN γ (1×10^6 U/kg) SQ Q.O.D.
 - FCZ (40 mg/kg/day) orally Q.D. & Saline SQ Q.O.D.
 - FCZ (40 mg/kg/day) orally Q.D. & IFN γ (1×10^6 U/kg) SQ Q.O.D.
 - Uninfected animals - PEG-200 orally Q.D. & IFN γ (1×10^6 U/kg) SQ Q.O.D.

Methods

- Daily clinical evaluation
- CSF and serum sampled every 7 to 12 days
- Buprenorphine was given as needed to alleviate pain and discomfort
- Moribund animals were euthanized
- Euthanize surviving animals 7 or 8 days after last treatment

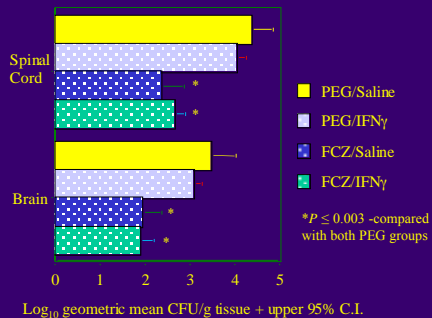
Time to Euthanasia

Treatment	n	Mean time to euthanasia (days)
PEG/Saline	9	22.3
PEG/IFN γ	8	29.4
FCZ/Saline	8	32.0*
FCZ/IFN γ	8	32.0*



* $P \leq 0.02$

Recovery of *C. immitis* from Spinal Cord and Brain



Conclusions

- IFN γ appeared to have a modest effect on survival and tissue CFU reduction, however, it was not significant.
- Fluconazole was effective at controlling coccidioidal meningitis and reducing *C. immitis* in the CSF and tissues.
- At the dosages tested, it is not clear if IFN γ -FCZ combination therapy has an advantage over fluconazole alone.