

Animal Models of Disease: Barking up the Right Tree

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DISCLOSURE

- Jeannette Wick has no relationships with ineligible companies

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Learning Objectives

- After completing this continuing education activity, learners will be able to
 - Discuss current legal and ethical positions on the use of animals in research
 - List the pros and cons of various animal models
 - Recall advantages and disadvantages for each animal model

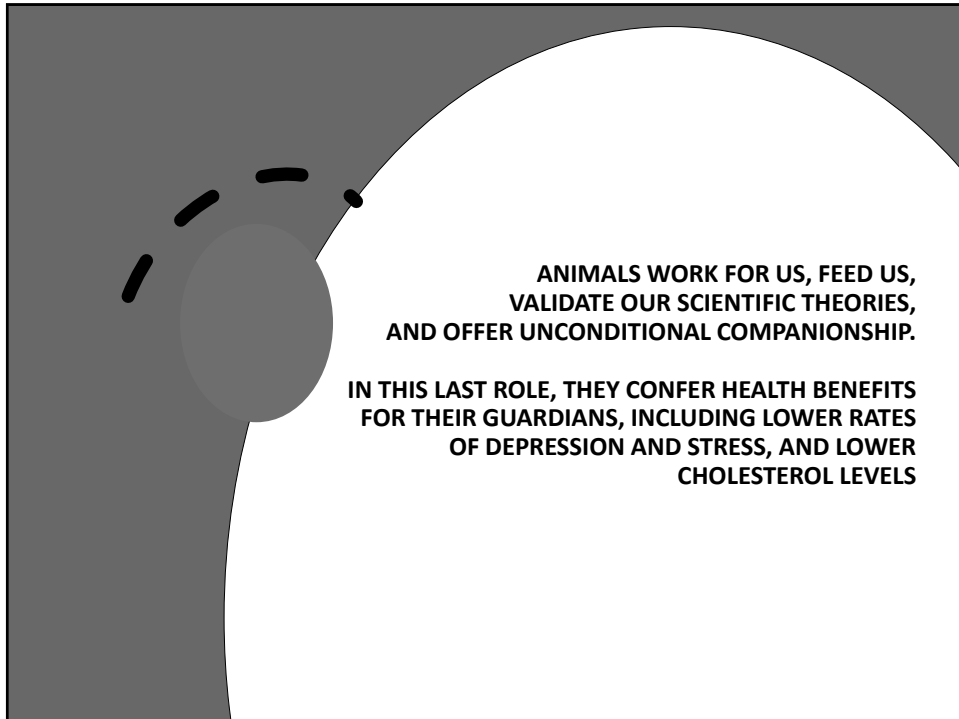
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History

- Aristotle, the “Father of Veterinary Medicine,” focused on physiology, comparative anatomy, and pathology in his *Historia Animalium, De Partibus Animalium*
 - Previous observers described an entire genus after examining one animal
 - Aristotle used multiple specimens, classified circulatory, organ, and reproductive systems

Thompson DW. The Works of Aristotle: Historia animalium, 1910

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Animals & Human Disease

- Sentinel Chickens – monitor for avian flu and mosquito-transmitted viruses
- Miners began using the yellow canary in 1911 for its sensitivity to CO.
 - They stop singing and eventually die as CO levels increase, warning their human coworkers
 - Only replaced by technology in the 1980s



Pollock C. *J Avian Med Surg*. 2016;30(4):386-391. Sentinel chickens. Volusia County, FL. Accessed March 25, 2024. <https://www.volusia.org/services/public-works/mosquito-control/sentinel-chickens.stm>

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Animals & Human Disease

- Species' similarities outweigh their differences
- Animals are used to test drugs and therapies
- Research objectives are only achieved when animal models
 - are analogues of human conditions
 - augment experimentation by providing an intermediate step between *in vitro* and human *in vivo* investigation

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. What are non-human animal models. North Carolina Association of Biomedical Research. Accessed March 25, 2024. http://www.ncabr.org/biomed/FAQ_general/faq_gen_4.html.

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Animals & Human Disease

- Animal testing
 - removes many behavioral complications
 - can be conducted more quickly because of animals' shorter life spans
- Animal models offer a high degree of experimental control, otherwise impossible with human testing
- Animal models = an integrated, complete biological system in which researchers can
 - examine similar diseases
 - induce a human disease or transplant organs
 - extrapolate findings to humans

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Turner AS. *Eur Cell Mater.* 2001;1:66-81.

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Animals & Human Disease

- Not all animal models are appropriate to study specific human conditions
- Need to know specific species' genetic composition and biological properties
 - Ferrets are obligatory carnivores = different drug metabolism
 - Pigs have a GI tract like humans



Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024

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Active learning!

Audrey is a pharmacy student who is studying adverse events (toxicity) associated with an injectable SGLT inhibitor that has suddenly become very popular with humans. Which proposal would the IRB be MOST likely to approve?

- A. Purchase 100 pigs, dose them at the maximum dose for the species, and measure outcomes in 6 months
- B. Recruit humans who are currently using the SGLTi for weight loss, and measure outcomes in 6 months
- C. Capture 100 mice, dose them at the maximum dose for the species, and measure outcomes every 7 days

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Animals and Human Disease

Millman I, et al. *Hepatology*. 1984;4(5):817-823.

- Other considerations when choosing a model
 - Ecologic impact
 - Animal availability
 - Cost
 - Bred in captivity = \$\$\$
 - Wild = cheaper, but may have parasites, disease
 - Ethical and social repercussions

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Animal Models of Disease

Disease or Pathology	Animal(s) Used
Accidents or paralysis	Primates
AIDS	Monkeys, primates
Alzheimer's disease	Mouse
Cancer	Zebrafish
CVD	Dog (prosthetic heart valves) Rabbit (HTN and atherosclerosis) Pig (restenosis)
Cystic Fibrosis	Mouse
Diabetes	Dog The Tally Ho mouse and non-obese diabetic mice
Esophageal sphincter dysfunction	Wild opossum

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Turner AS. *Eur Cell Mater*. 2001;1:66-81. Millman I, et al. *Hepatology*. 1984;4(5):817-823. Serreze DV, Chen YG. *Trends Immunol*. 2005;26(11):603-607. Sung YY, et al. *Biochem Biophys Res Commun*. 2005;338(4):1779-1787.

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Animal Models of Disease

Disease or Pathology	Animal(s) Used
Hepatitis B & D	Woodchuck
Influenza	Ferrets, Mice
Leprosy	Wild armadillo
Reproductive cycle	Mini or macro pig
Ovulation	Macaque
Parkinson's disease	Mouse
Wound healing	Pig

- U.S. law **requires** researchers to seek alternatives to animal experimentation
- Forbids unnecessary replication of animal experiments

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Animal Models

Face validity: model bears a similarity to the human disease that is visible immediately

Predictive validity: may look unlike the human disease, but targeted testing of some aspect of the disease translates reliably to human clinical care

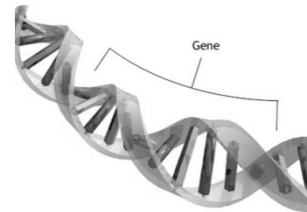
Construct validity = face + predictive validity **BEST**

Higgins GA, Jacobsen H. *Behav Pharmacol.* 2003;14:419-438.

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Animal Models

- To attain construct validity, scientists often target a portion of a gene locus (knocking it out with embryonic stem cells or actually implanting a new piece, called knock in)
- Forward genetic screening



Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Grippo PJ, Sandgren EP. *Methods Mol Med.* 2005;103:217-243

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Active Learning!

Dr. Virusopolous has received a grant to study hepatitis B and D. Which animal might he use, and what would be a limitation?

- A. The armadillo, but local availability is a concern
- B. A genetically engineered—but costly—rat model
- C. The wild woodchuck, but parasites are possible

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Hepatitis

- HBV infection is a prerequisite for HDV replication and transmission and unique to humans
 - Difficult to study in humans
 - Short peak viremic period, viremia levels highly variable
- HDV was characterized in costly primates that don't acquire the infection naturally
- Woodchuck naturally contracts woodchuck hepatitis virus (WHV), similar to human hepatitis
 - Smaller size
 - Less cost
 - GREAT availability
- Has construct validity in the laboratory



Gerin JL. *ILAR J.* 2001;42(2):103-106. Niro GA, et al. *Dig Liver Dis.* 2011;43 Suppl 1:S19-S24.

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Animal Models

- Necessary animal experimentation is highly regulated
- Many academic organizations and special interest groups have oversight committees and position papers guiding animal use
- Researchers must justify animal involvement
- Research review boards examine proposed procedures and care closely
- The DoA oversees animal experimentation in the US
 - Reports 95% of research performed on animals causes no pain or distress or is accompanied by relief medication

Research Facility Annual Usage Summary Report. USDA. Accessed March 24, 2024.
https://www.aphis.usda.gov/aphis/ourfocus/animalwelfare/sa_obtain_research_facility_annual_report/ct_research_facility_annual_summary_reports

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Which Animal?

- Public acceptance of animals chosen for experimentation is culturally based and influences animal selection
- Cats and dogs used only rarely because their prominent roles as pets makes their use socially unacceptable
- Rodents, which constitute more than 90% of research animals, generate little sympathy

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Research Facility Annual Usage Summary Report. USDA. Accessed March 24, 2024.
https://www.aphis.usda.gov/aphis/ourfocus/animalwelfare/sa_obtain_research_facility_annual_report/of_research_facility_annual_summary_reports




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Which Animal?

- A cattery identified a Maine Coon (called a *proband*, a first or index case) affected with what appeared to be hypertrophic cardiomyopathy
 - Several related cats with serious cardiac problems
- Resembles human familial hypertrophic cardiomyopathy (FHCM)
- Common cause of HF and sudden death caused by mutations in seven genes encoding muscle proteins
- Until this discovery, researchers suspected it was similarly heritable in pigs, but had no animal model

Kittleson MD, et al. *Circulation*. 1999;99(24):3172-3180.

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HCM

- HCM in cats is autosomal dominant, as in humans
- Homozygous individuals will die, often *in utero*
- Symptoms begin in childhood or adolescence and worsen progressively
- Males are affected more often than females
- All affected individuals will have symptoms by adulthood

Kittleson MD, et al. *Circulation*. 1999;99(24):3172-3180.

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Osteoporosis Model Issues

- Age at which peak bone mass occurs
- Patterns of age-dependent bone loss
- Reversibility of estrogen-induced bone loss
- Incidence of spontaneous fracture
- Magnitude of disease effect
- Cortical and cancellous bone involvement
- Confounding factors

Conn PM. *Animal Models for the Study of Human Disease*. 2nd ed. 2024. Turner AS. *Eur Cell Mater*. 2001;1:66-81.

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Active Learning!

Dr. Gno Bonesaboutit is studying osteoporosis and menopause and needs an animal model for observation only. Which species would be most likely to be a good model?

- A. Any long-lived species
- B. A long-lived mammal
- C. Any animal with bones

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Osteoporosis Model Issues

- Models may only cover some of the issues.




In baboons

- Peak bone mass occurs at ~ age 9
- Menopause occurs at ~ age 20
- Menstruate in a polyestrus pattern like humans
- Costly and difficult to handle
- Increased risk of zoonotic disease transmission

Researchers looking for other animal models

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Cox LA, et al. *JLAR J.* 2013;54(2):106-121.

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Osteoporosis Model Issues


- Cats and dogs: poor models for skeletal disorders
 - Although most female pets are ovariectomized (spayed) before age one, spontaneous fractures are rare, and calcium supplementation is unnecessary
 - Why? Cats and dogs follow a diestrus pattern, and their bones are resistant to estrogen-mediated bone loss
- Orchidectomy in beagles can cause osteoporosis, with bone growth patterns similar to humans
- Immobilization or a low calcium or high-phosphorus diet can cause osteopenia in cats, but other models are better
 - Minipigs weigh only 60 kg at maturity, menstruate in 18- to 21-day cycles and are omnivorous

Conn PM. *Animal Models for the Study of Human Disease*. 2nd ed. 2024. Cox LA, et al. *ILAR J*. 2013;54(2):106-121. Fukuda S, Iida H. *J Vet Med Sci*. 2000;62(1):69-73. doi:10.1292/jvms.62.69

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Menopause

- Lifelong reproduction would maximize reproductive success
- But human females cease reproduction several decades before life's end
 - 42% post-menopause compared to 20% in chimps
- Why?



Ellis S, et al. *Nature*. 2024;627(8004):579-585. Wood BM, et al. *Science*. 2023;382(6669):eadd5473.

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Menopause

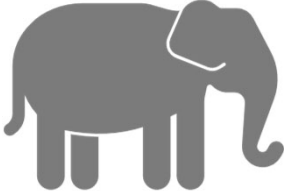
- Toothed whales are the only mammal in which menopause has evolved several times
- Extended lifespan without ↑'g reproductive lifespan
 - Called the 'live-long' or 'stop early' hypotheses
- ↑'g lifespan overlaps with granddaughter without ↑'g reproductive overlap = ↑'d opportunity for intergenerational help

REPRODUCTIVE SYSTEM. WHALES ONLINE. ACCESSED MARCH 26, 2024. [Reproductive System - Baleines en direct](#) Ellis S, et al. *Nature*. 2024;627(8004):579-585.

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Alzheimer's Disease

- Animal model would need three crucial components:
 - senile plaques
 - neurofibrillary tangles, and
 - selective neuronal cholinergic loss in the forebrain
- Elephants never forget
 - Not a good model for AD
- Polar bears develop AD like humans
 - Society would be really angry
- Wolverines develop AD like humans
 - Difficult to deal with

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Markowitz H, et al. *J Appl Behav Anal.* 1975;8(3):333-335. Kahle PJ, Haass C. *Expert Opin Ther Targets.* 2001;5(1):125-132. Selkoe DJ, et al. *Science.* 1987;235(4791):873-877.

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How do we know about polar bears?

- Polar-bear Alzheimer's was first discovered in 1987 in a 28-year-old zoo specimen that had been euthanized

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Active learning!

Why do researchers use mice in research so often?

- A. They live a long time so researchers can run long-term studies
- B. People who have mice as pets wish to rehome them (so they are cheap)
- C. Mice are small, inexpensive, and fairly easy to handle

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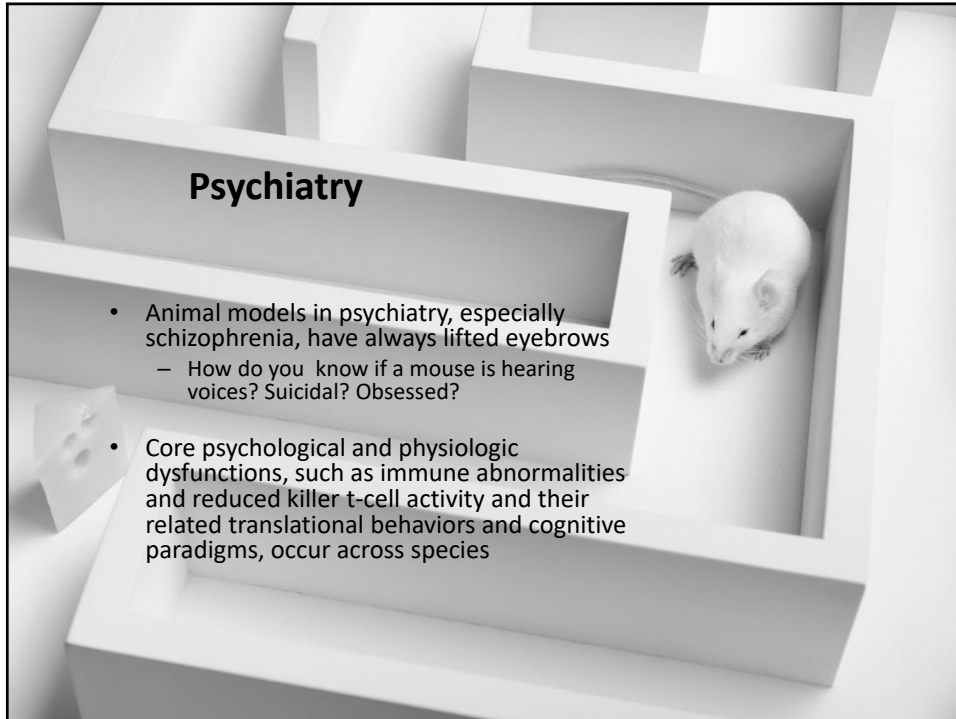
The Mouse

- Small, short-lived, inexpensive, and easy to handle
- More than 50 mouse models of AD
- Newest demonstrates how tau "spreads" through brain like poison in a river



Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Esquerda-Canals G, et al. *J Alzheimers Dis.* 2017;57(4):1171-11


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Psychiatry

- Animal models in psychiatry, especially schizophrenia, have always lifted eyebrows
 - How do you know if a mouse is hearing voices? Suicidal? Obsessed?
- Core psychological and physiologic dysfunctions, such as immune abnormalities and reduced killer t-cell activity and their related translational behaviors and cognitive paradigms, occur across species

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Other Matters of the Mind

- OCD has stereotypic behaviors (e.g., spinning, circling, sniffing, eating, and pacing)
- Markers of compulsion = excessiveness, resistance to extinction, and divorce from goal-directed activity
 - Cats will screech to a halt mid-run to lick a spot
 - Dogs may groom until they bleed = acral lick, similar to human trichotillomania

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Woods-Kettelberger A, et al. *Expert Opin Investig Drugs*. 1997;6(10):1369-1381.

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Other Matters of the Mind

- Flinders sensitive rat line used for depression
 - Partially resembles depressed individuals
 - Exhibits reduced appetite and psychomotor function, but normal hedonic responses and cognitive function
 - Supersensitive to cholinergic activity
 - Useful as an antidepressant screen because known antidepressants reduce swim test immobility when given chronically and psychomotor stimulants do not

Overstreet DH, et al. *Neurosci Biobehav Rev.* 2005;29(4-5):739-759.

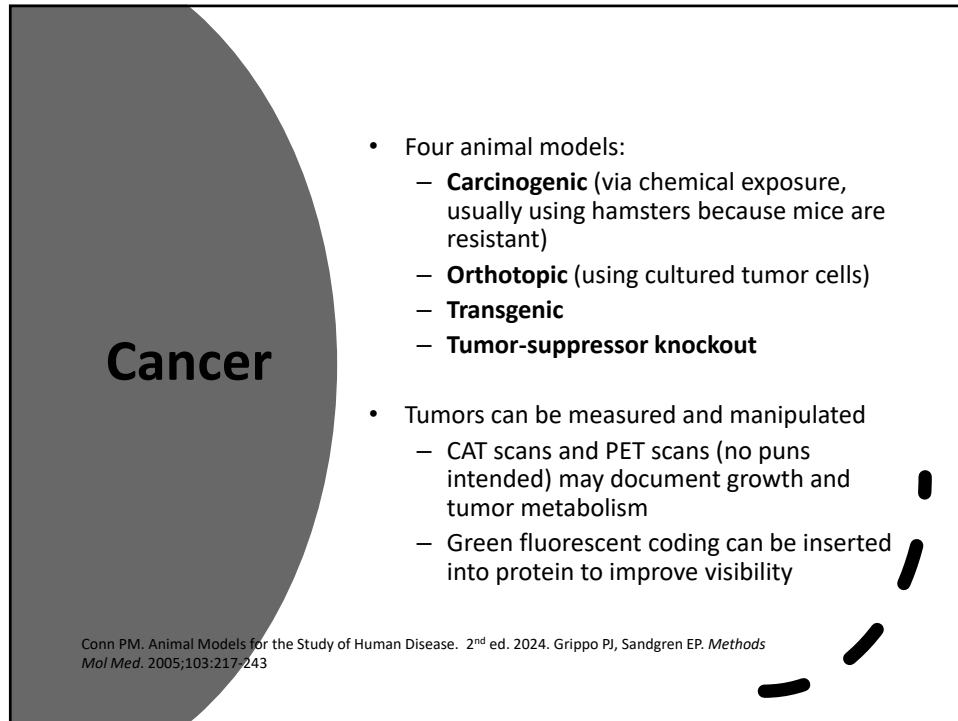
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Active Learning!

Which of the following animals is a GREAT model for cancer?

- A. The zebrafish because they develop tumors that are histologically similar to human neoplasms
- B. Members of the family *Mephitidae* because they are small mammals that are easy to handle
- C. The dog because their common cancers progress similarly to humans'

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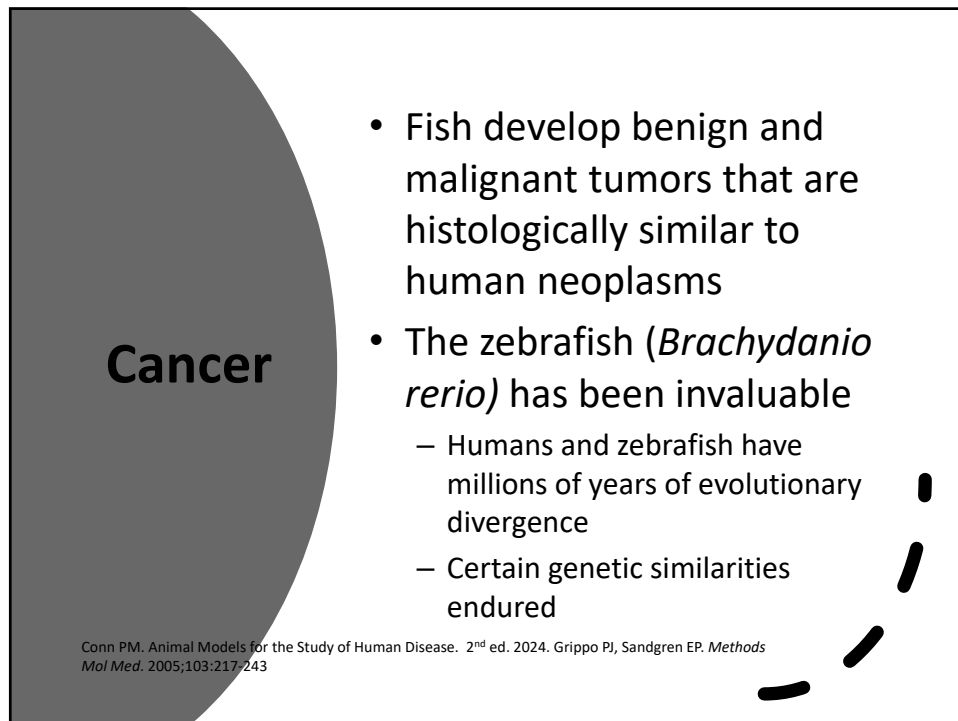


Cancer

- Four animal models:
 - **Carcinogenic** (via chemical exposure, usually using hamsters because mice are resistant)
 - **Orthotopic** (using cultured tumor cells)
 - **Transgenic**
 - **Tumor-suppressor knockout**
- Tumors can be measured and manipulated
 - CAT scans and PET scans (no puns intended) may document growth and tumor metabolism
 - Green fluorescent coding can be inserted into protein to improve visibility

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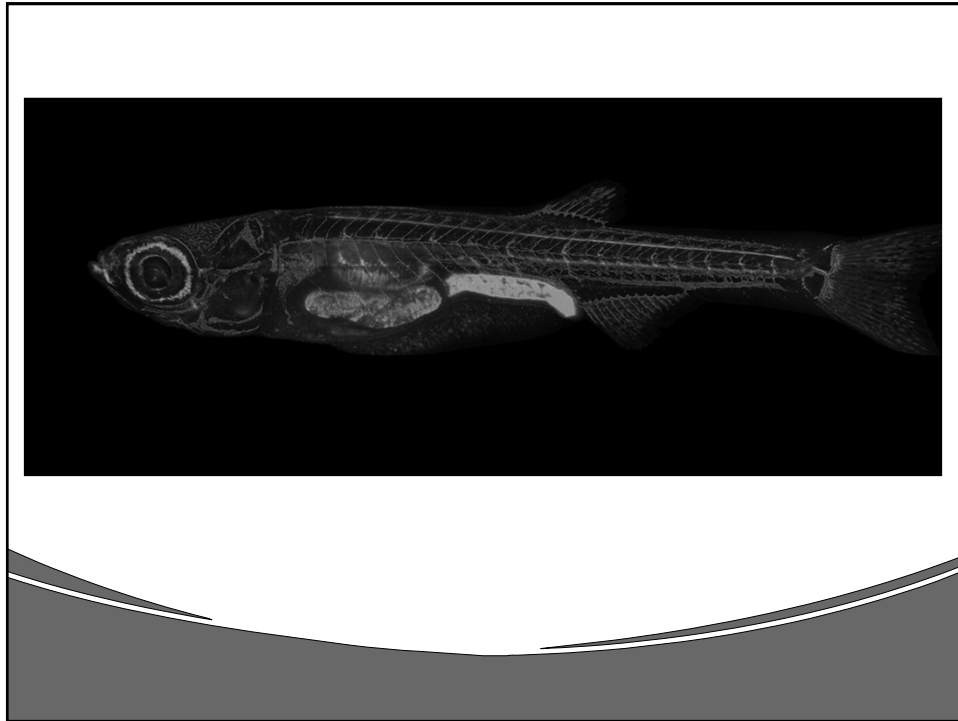


Cancer

- Fish develop benign and malignant tumors that are histologically similar to human neoplasms
- The zebrafish (*Brachydanio rerio*) has been invaluable
 - Humans and zebrafish have millions of years of evolutionary divergence
 - Certain genetic similarities endured

Conn PM. Animal Models for the Study of Human Disease. 2nd ed. 2024. Grippo PJ, Sandgren EP. *Methods Mol Med.* 2005;103:217-243

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Smoking

- Although “humans actively and religiously inhale cigarette smoke to satisfy extraordinary cravings for nicotine,” animals will do whatever they can to avoid it
 - Change their breathing patterns and flee if possible
 - Rodents are obligatory nose breathers that cannot inhale through the mouth

Sullivan MF, Willard DH. *Toxicol Appl Pharmacol.* 1978;45(2):445-462.

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Influenza

- Mouse models used
- Ferrets too
 - Pet ferret owners are warned to avoid people who have the flu
 - They acquire severe influenza without the need for viral adaptation
 - Clinical symptoms mimic human symptoms: fever, nasal discharge, and preferential infection of URT
- NEW MODEL: Guinea pigs

Belser JA, et al. *Microbiol Mol Biol Rev.* 2016;80(3):733-744. Published 2016 Jul 13. doi:10.1128/MMBR.00022-16
 Maher JA, DeStefano J. *Lab Anim (NY).* 2004;33(9):50-53.

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Miscellaneous Models

- Early researchers in erectile dysfunction used dogs, monkeys
 - Found rodents and lagomorphs (rabbits) better
 - Replaced sedated animals with conscious animals to avoid pharmacologic interference
- Dermatology researchers have models for dry skin that suggest psychosocial etiology
 - Isolated rodents develop terribly dry skin

Conn PM. *Animal Models for the Study of Human Disease.* 2nd ed. 2024. Burnett AL. *Int J Impot Res.* 2001;13(3):135-139.
 Schön MP. *J Invest Dermatol.* 1999;112(4):405-410.

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A to Z: Zoonoses

Avian flu

Diarrhea

Ebolavirus

Erlichiosis

Leptospirosis

MRSA

Roundworm

Toxoplasmosis

West Nile virus

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CONCLUSION

- With animal models of disease, three things are critical:
 - Following the rules
 - Knowing the pros and cons of animal research
 - Barking up the right tree!

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