What is the single largest factor affecting research outcomes?

- Researcher bias:
  - How clean/pure should the glassware or reagents be?
  - What is (or is not) important to the research outcomes?

- 1st question researchers should ask:
  - What am I doing that may impact the laboratory also sharing this holding room, procedure room, or equipment?

FACTORS AFFECTING RESEARCH

INTRINSIC: Inherent to the animal

Genetics, age, sex, health, nutritional status, immune status, circadian rhythms, endocrine factors
INTRINSIC FACTORS AFFECTING RESEARCH

Genetics

- OUTBRED ANIMALS: Problems occur when:
  - Colony size insufficient to maintain heterogeneity
  - Management techniques do not ensure genetic variability

- INBRED ANIMALS: Problems occur when there are:
  - Incomplete inbreedings
  - Mismatings
  - Inadvertent outcrossings with other strains
  - Spontaneous mutations
  - Chromosomal aberrations
  - Residual heterozygosity

TAKE HOME MESSAGE: GENETIC INTEGRITY IS NOT GUARANTEED BY ITS NOMENCLATURE!

INTRINSIC FACTORS AFFECTING RESEARCH

Genetics

- Spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats:
  - Genetically disparate, while from the same parental Wistar stock.
  - WKY, used as controls, were discovered to share only approximately 50% of their DNA fingerprint bands in common with SHR.

- Heterogeneity between Dahl salt-sensitive rats (SS/Jr) and Dahl salt-resistant rats (SR/Jr) is small (80% common DNA).

- Variable metabolism due to qualitative and quantitative enzyme differences between species and strains:
  - Cytochrome P450 concentration and competing isoenzymes
  - Defective/absent enzymes or presence of unique enzymes

TAKE HOME MESSAGE: SELECT THE STRAIN CAREFULLY!

INTRINSIC FACTORS AFFECTING RESEARCH

Age

- Factor in rodent carcinogenicity studies.
- Neonates have immature body systems compared to adults.
- Older animals may develop deficiencies as previously normally functioning systems fail.
- On occasion the response may be strain related!

TAKE HOME MESSAGE: MATCH THE AGE TO THE NEED!
Marked differences in pharmacologic and toxicologic responses to xenobiotics has been demonstrated between male and female rats.

- Sex-related differences in DMBA-induced mammary tumors:
  - Wistar-Furth rats: 100% in females; 19% in males.
  - Copenhagen rats: No difference between males and females

Immunologic dysfunction, including hypersensitivity and allergy, autoimmunity and immunodeficiency may influence experimental outcome.

- Agents that alter immune function:
  - Age
  - Nutritional status
  - Chemicals
  - Drugs
  - Food additives
  - Metals
  - Microbes

Resistance to infectious agents may be dependent on the type of immune response that the host generates:
- CBA/J mice are highly resistant to L. major as they develop a cell-mediated response, vs. the humoral response of the non-resistant BALB/c
- 100% incidence of leukemia in response to murine leukemia virus by BALB.K mice (humoral) vs. resistance by B10.BR mice (cellular)
**INTRINSIC FACTORS AFFECTING RESEARCH**

**Nutritional Status**

- Nutritional status is dependent on:
  - Type of feed provided
  - Method and amount of feeding
  - Appetite
  - Age

- Various dietary conditions such as mineral, vitamin and protein deficiencies, lipid composition, and the composition of the diet alter the biotransformation of xenobiotics.

**Take Home Message:** KNOW WHAT YOU ARE FEEDING!

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**Circadian Rhythms**

- Many behavioral, biochemical and physiologic parameters (daily, rhythmic, minima and maxima) occur at specific times:
  - Blood counts and coagulation times
  - [CD-3 molecules] on T cell surface
  - T cell response to mitogens
  - Plasma steroid
  - Body temperature
  - Sensitivity to audiogenic seizure induction
  - Drug metabolism and toxicity
  - Susceptibility to neoplasia
  - DNA, protein synthesis

**Take Home Message:** BE CONSISTENT WITH DATA COLLECTION!

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**Endocrine Factors**

- Sex hormones are important determinants of cytochrome P450 enzyme activity.

- Testosterone administered to female rats increases their ability to biotransform xenobiotics

- Castrating male rats decreases the ability to biotransform xenobiotics.

**Take Home Message:** SEX IS IMPORTANT! CHOOSE WISELY.
**INTRINSIC FACTORS AFFECTING RESEARCH**

**Endocrine Factors**

- Gonadectomy, hypophysectomy and adrenalectomy will alter hormonal levels.
- Neonatal gonadectomy of CE and DBA strains of mice leads to high incidence of estrogen-secreting adrenal tumors.
- Postulated that hypersecretion of trophic pituitary hormones in gonadectomized ferrets may result in stimulation of the adrenal gland, producing adrenal gland tumors.

**TAKE HOME MESSAGE:** SEX IS IMPORTANT! CHOOSE WISELY.

**INTRINSIC FACTORS AFFECTING RESEARCH**

**Endocrine Factors**

- Prolactin-secreting pituitary adenomas may develop in nulliparous rabbits because of exposure of pituitary acidophils to high concentrations of plasma estrogens secreted by ovarian follicles.
- Prolactin-secreting tumors also develop in rats following prolonged administration of natural or synthetic estrogens.

**TAKE HOME MESSAGE:** SEX IS IMPORTANT! CHOOSE WISELY.

**FACTORS AFFECTING RESEARCH**

**EXTRINSIC:** External to the animal

Physical factors, chemical factors, microbial agents, stressors
EXTRINSIC FACTORS AFFECTING RESEARCH

- PHYSICAL
- CHEMICAL
  - Air
  - Water
  - Diet
  - Drugs
- MICROBIAL AGENTS
- STRESSORS

EXTRINSIC FACTORS AFFECTING RESEARCH

Physical Factors

- ENVIRONMENTAL
  - Macro vs. Micro Environment
  - Cage Design
  - Caging Accessories
  - Temperature
  - Humidity
  - Ventilation
  - Illumination
  - Noise

EXTRINSIC FACTORS AFFECTING RESEARCH

Physical Factors

- MICROENVIRONMENT
  - The physical environment immediately surrounding the animal
  - The primary enclosure

- MACROENVIRONMENT
  - The physical environment of the room, cubical, etc.
  - The secondary enclosure
**EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design**

- Amount of air, light and sound the animal receives.
- Amount of heat, humidity and gaseous waste dissipated into the macroenvironment.

**TAKE HOME MESSAGE:** RECORD CAGE DESIGN AS A VARIABLE!

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**EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design**

- Solid bottom caging for rodents
- Behavioral characteristics
- Recovery cages
- Mouse urologic syndrome
- Randomization of cage location

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**EXTRINSIC FACTORS AFFECTING RESEARCH Cage Design - Rack placement**

- Cage placement on the rack may be important!
- Rack vendors work hard to make all slots equal, but...
- Rack location may impact study outcomes.

**TAKE HOME MESSAGE:** ROTATE CAGE PLACEMENT ON RACKS!
EXTRINSIC FACTORS AFFECTING RESEARCH

Cage Design – a few facts …

• 68 F (20 C) air moving at 60 liner feet per minute has a cooling effect of 45 F (7 C).
• ACH has a wash-out effect upon pheromones
• Study: Effect of IVC air flow on rats:
  • 60 ACH did not impair the reproductive performance
  • Higher air flow increased the survivability of litters and …
    • those litters were more uniform in size and viability over the control in an open topped cage.
• Study: Comparing mice housing in static and IVC
  • IVCs had lower relative humidity, lower NH3 levels, lower CO2 levels, higher body weight gain, and higher water consumption.

EXTRINSIC FACTORS AFFECTING RESEARCH

Cage Design – a few more facts …

• Study: Measuring air quality in IVC versus static:
  • IVCs: Significantly lower ammonia, relative humidity, and carbon dioxide values.

• Study: Measuring noise:
  • The overall mean MICROENVIRONMENT decibel (dB) levels, as determined from a range of octaves, ventilated systems were associated with significantly higher mean noise levels, compared with room background levels.
  • There was no significant difference in noise detected among three vendor systems.

EXTRINSIC FACTORS AFFECTING RESEARCH

Cage Design – a few more facts …

• Study: Measuring MICRO and MACRO noise:
  • Significant differences between macro- and microenvironmental dB levels.
  • In addition, microenvironmental noise levels were significantly higher than room background levels in the majority of octaves tested for each caging system.

• Study: Effect of Failed Air Supply to IVCs (rats):
  • Greater than 30% mortality of rat pups of weaning age
  • Oxygen concentrations in the air in individual cages were rapidly depleted to less than 10% in 1 h.
  • Animals died within 60 min of loss of power to IVC.
EXTRINSIC FACTORS AFFECTING RESEARCH
Cage Design - a few facts ...

- 68°F (20°C) air moving at 60 liner feet per minute has a cooling effect of 45°F (7°C).
- ACH has a wash-out effect upon pheromones

TAKE HOME MESSAGE: IVCS ARE GREAT, BUT CAN HURT OUTCOMES!

EXTRINSIC FACTORS AFFECTING RESEARCH
Cage Design

- ACCEPTABLE PRIMARY ENCLOSURES
  - Normal physiologic and behavioral needs
  - Conspecific social interaction
  - Development of hierarchies within or between enclosures
  - Clean and dry
  - Adequate ventilation
  - Access to food and water
  - Secure environment
  - Free of sharp edges
  - Observe with minimal disturbance

TAKE HOME MESSAGE: BE CONCERNED WITH MICROENVIRONMENT!

EXTRINSIC FACTORS AFFECTING RESEARCH
Cage Bedding

- TYPES
  - Softwood
  - Hardwood
  - Corn cob
  - Paper products

- IDEAL
  - Dustfree
  - Nonpalatable
  - Absorbent
  - Free of contaminants
- Dependent upon species and experimental design

TAKE HOME MESSAGE: BEDDING IS A CONTROLABLE FACTOR!
**EXTRINSIC FACTORS AFFECTING RESEARCH**

**Cage Bedding**
- Autoclaving reduces fungal contamination risk
- Size and manipulability of bedding determine preference
- Bedding choices may influence the immune system
- Bedding choice influences ammonia level in the cage
  - (MOST): Aspen shavings > pine shavings > reclaimed wood pulp bedding > virgin pulp loose > bedding hardwood chip bedding > recycled paper bedding > virgin cellulose > pelleted paper bedding > corn cob bedding (LEAST)

**TAKE HOME MESSAGE:** BEDDING IS A CONTROLABLE FACTOR!

**EXTRINSIC FACTORS AFFECTING RESEARCH**

**Cage Accessories**

**MATERIALS**
- Nonreactive
- Uningestible
- Smooth and impervious
- Durable
- Corrosion resistant
- Sturdy - withstand rough handling
- Wood may be used
- Galvanized metal and rubber stoppers

**EXTRINSIC FACTORS AFFECTING RESEARCH**

**Enrichment**
EXTRINSIC FACTORS AFFECTING RESEARCH

**Watering**

- Too little water is not good, and ...
- Too much water is not good
- Water supply MUST be checked daily
- Are there advantages of auto water? Maybe
- Strain differences may affect wet bedding
- Cage design may affect wet bedding
- Enrichment devices may affect wet bedding

**TAKE HOME MESSAGE:** WATER MUST BE WATCHED CLOSELY!

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**EXTRINSIC FACTORS AFFECTING RESEARCH**

**Feeders**

- Type depends upon species, diet used and primary enclosure
- Animals must have easy access to food
- Minimize contamination with feces and urine
- Group housing considerations
  - Multiple feeding and watering points
- Diet Optimization

**TAKE HOME MESSAGE:** YOUR RESEARCH IS WHAT THEY EAT!

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**EXTRINSIC FACTORS AFFECTING RESEARCH**

**Temperature and Humidity**

- Depend upon husbandry practices and housing design
- Macro and microenvironment may differ due to:
  - Housing material, construction, and use
  - Forced ventilation (or not)
  - Filter tops
  - Number of animals per cage
  - Activity level of the animals
  - Cage location within the rack or room (if static)
  - Frequency of bedding changes
  - Bedding type
EXTRINSIC FACTORS AFFECTING RESEARCH
Temperature and Humidity

- Airborne particles, humidity and respiratory disease:
  - As RH increases, adhesiveness of particles increases leading to larger particle size
  - As RH decreases, electrostatic forces increase

EXTRINSIC FACTORS AFFECTING RESEARCH
Temperature and Humidity

- Lactating rats exposed to 95°F (35°C) for six hours daily produced less milk than rats housed at 72°F (22°C)
- Reproduction in rats decreases markedly at 90°F (32°C)
- High mortality and retarded testicular development in rats occurred after accidental exposure to high temps for prolonged periods
- Mice housed at 95°F (35°C) at 22% RH were more susceptible to influenza virus than mice housed at 97°F (35.6°C) and 90% RH
- Ringtail in rats, South African hamsters and mice is associated with the inability to control heat loss in environments of 40% RH or lower and results annular constrictions of the tail.

EXTRINSIC FACTORS AFFECTING RESEARCH
Temperature and Humidity

- Guide recommends RH between 30 and 70%
- Variations in RH are better tolerated at lower temps due to heat loss mechanisms of most animals
- Little evidence for strict RH control

TAKE HOME MESSAGE: WORK FOR STABLE 30%-70% HUMIDITY!
Species differences
No energy expended to either cool or warm itself
Does not necessarily equate to comfort
Measurements do not take into account differences in activity or metabolism
Exposure of unadapted animals to temperature >85°F or < 40°F without access to shelter may produce clinical effects which could be life threatening.

EXTRINSIC FACTORS AFFECTING RESEARCH
Thermal Neutral Zone

- Behavioral: If temp change is short / low magnitude
  - Huddling
  - Curling up
  - Nest building
  - Increased activity
- Physiological: Altered metab. rate, food/water consump., growth rate
  - Hibernation or estivation
  - Nonshivering thermogenesis
  - Peripheral vascular changes
  - Piloerection
- Morphological: Profound temperature changes for at least 14 - 21 days
  - Fat stores
  - Hair coat thickens
  - Heat radiating structures

EXTRINSIC FACTORS AFFECTING RESEARCH

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EXTRINSIC FACTORS AFFECTING RESEARCH
Ventilation

- Purposes
  - Supply adequate oxygen
  - Remove thermal loads
  - Dilute gaseous and particulate contaminants
  - Adjust moisture content
  - Create pressure differentials
- Guideline of 10-15 fresh air changes/hour
  - refers to macroenvironment
  - doesn't take into account heat load
Adequate room ventilation does not ensure adequate ventilation of microenvironment!
- Twelve air changes/hour (12 ach) equals:
  - Empty cage: Turnover rate is once every 4.5 minutes
  - Flexible film isolator cage: Turnover rate is every 6 minutes
  - Cage with Filter Top: Turnover rate is every 38 minutes

Excessive odor first indication to humans
- Concentrations capable of causing damage to animals are much lower (10 PPM) than human threshold level for ammonia (25 PPM)
- Best expressed in terms of volumetric air exchange rate/occupant versus room air changes per hour

Heat load dependent on:
- Species size
- Number of animals
- Type of bedding used
- Frequency of changing bedding
- Room dimensions
- Efficiency of air distribution from secondary to primary enclosure

Note influence of bio-safety cabinet and transfer station
EXTRINSIC FACTORS AFFECTING RESEARCH

Ventilation

- Filtered one-pass air is preferred, but ...
- Filtered recycled air: HEPA and charcoal if from animal facility
- Filtered recycled air: from non-animal use areas

EXTRINSIC FACTORS AFFECTING RESEARCH

Illumination

- Definitions:
  - Crepuscular: Active at dusk / dawn
  - Nocturnal: active at night
  - Light intensity = illumination
  - Foot candles (fc) = lumens/ft²
  - Lux = lumens/m²
  - Quality = color
  - Wavelength
  - Angstrom (Å)
  - Photoperiod or duration of light (L:D)
  - Hours of light (L) versus hours of dark (D) in a 24 hour day
  - Rodents in research: 12/12
  - Breeding rodents: 12/12 or 14/10

TAKE HOME MESSAGE: DARK CYCLE INTERRUPTIONS MOST DISRUPTIVE!

EXTRINSIC FACTORS AFFECTING RESEARCH

Illumination

- Albino rat is more susceptible to phototoxic retinopathy than other species
- Intensity and photoperiodicity can affect physiology, morphology, behavior
  - Hepatic enzyme activity
  - Metabolism of compounds
  - Rats given a fixed dose of hexobarbital:
    - Light cycle: longer sleep
    - Dark cycle: less sleep
- Lab housing MAY cause deviation from seasonal norms

TAKE HOME MESSAGE: LIGHT LEVELS & SEASONS MAY AFFECT RESULTS!
**EXTRINSIC FACTORS AFFECTING RESEARCH Illumination**

- Guide: light at cage level = 130 and 325 Lux
- Light levels of 325 Lux (30fc) about 1.0 m (3.3 ft) above the floor appear to be sufficient for animal care and do not cause clinical phototoxic retinopathy in albino rats
- Animal facilities should be windowless
- Cage position on rack is important
- Light intensity decreases with square of the distance from the light source
- Light at the top of a rack may be 80 times more intense than at the bottom of the rack

**TAKE HOME MESSAGE:** LOCATION, LOCATION, LOCATION!

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**EXTRINSIC FACTORS AFFECTING RESEARCH Illumination**

- Circadian rhythms are important
- Biorhythms are modulated by light
- Things YOU can do:
  - Rotate cage position
  - Provide hiding places
  - Choose colored manipulanda (yellow or red = dark)
  - Assure automatic light timers with variable intensity controls ARE WORKING!!!

**TAKE HOME MESSAGE:** DURATION AND INTENSITY MATTER!

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**EXTRINSIC FACTORS AFFECTING RESEARCH Illumination**

- Biorhythms types
  - Circadian: ~ 24 hours
  - Ultradian: < 24 hours
  - Infradian: > 24 hours
  - Circaseptadian: weekly

**TAKE HOME MESSAGE:** RESULTS MAY BE EFFECTED BY B-RHYTHM?
EXTRINSIC FACTORS AFFECTING RESEARCH

Noise

- Measurements
  - Frequency = hertz (Hz)
  - Pressure = decibels (dB)
  - Sounds > 20 kHz = ultrasound
- Most animals hear higher frequency sounds as compared to man
  - Rats - optimal hearing = 40 kHz
  - Mice - optimal hearing = 15-20 kHz

EXTRINSIC FACTORS AFFECTING RESEARCH

Noise

- Noise levels in animal facilities should not exceed 85 dB (human guidelines)
- No guidelines on frequency limits
- Auditory effects of noise at >85 dB
  - Destruction of sensory hairs and supporting cells
  - Mechanical damage in rats at 160 dB
  - Pain in rats at 140 dB
  - Inner ear damage in rats after prolonged exposure to 100 dB

EXTRINSIC FACTORS AFFECTING RESEARCH

Noise

- Eosinopenia
- Increased serum cholesterol, adrenal weights, adrenal ascorbic acid in rats exposed to 83 dB and intermittent sound of 114 dB
- Audiogenic stress due to pulsed noise exceeding 83 dB may cause reduced fertility in rodents
- Audiogenic seizures in gerbils, DBA/2, C57, AKR, BALB/c, and CBA.
EXTRINSIC FACTORS AFFECTING RESEARCH

Noise

• Radios, alarms, timers
• Guide - these devices should not be used unless part of an approved protocol or enrichment
• UFAW Handbook states use of artificial background noise may be useful in masking sudden unexpected noise
• Masking noise = one which is intense enough to render inaudible or unintelligible another sound which is simultaneously present - potential danger = adverse effect on animals

TAKE HOME MESSAGE: SOUNDS MAY CONFUSE OUTCOMES!

EXTRINSIC FACTORS AFFECTING RESEARCH

Noise

• Separate human and animal areas
• Separate noisy species from quieter species
• Loud, unexpected and unfamiliar sounds are probably more disruptive and harmful than constant sound

TAKE HOME MESSAGE: ACCLIMATE PRIOR TO USE!

EXTRINSIC FACTORS AFFECTING RESEARCH

Transportation

• Acclimation period after shipment
• Length of time required depends upon:
  ▫ Type and duration of transport
  ▫ Species
  ▫ Intended use
• Adolescent rats recovery period = 1 - 5 days
• Decrease stress by:
  ▫ Vendor supplying 1 - 2 weeks of food
  ▫ Use of similar cage and watering system

TAKE HOME MESSAGE: ACCLIMATE PRIOR TO USE!
EXTRINSIC FACTORS AFFECTING RESEARCH

Overcrowding & Isolation

- Effects behavior, reproduction, immune system, metabolism

- Cage size requirements depend upon:
  - Species
  - Size
  - Number of animals/cage
  - Breeding status

EXTRINSIC FACTORS AFFECTING RESEARCH

Overcrowding & Isolation

- Aggressive behavior can be strain specific

- Group housed mice show marked adrenal response that is directly proportional to the animal density

- When housed in pairs, the subordinate has the higher adrenal weight and plasma cortisol level

TAKE HOME MESSAGE: MAINTAIN ACCEPTABLE POPULATION DENSITY!

EXTRINSIC FACTORS AFFECTING RESEARCH

Social Ranking

- Consider species

- Social animals may fight if placed in new groups or new member placed in established group

- Breeding activity:
  - Can be significantly modified by group housing
  - Female mice
    - Group housed = anestrus
    - Male introduced = synchronized estrus

TAKE HOME MESSAGE: SOCIAL RANKING IS IMPORTANT!
EXTRINSIC FACTORS AFFECTING RESEARCH
Handling

- Regular handling may reduce stress
- Correct handling/restraint methods important
- Gentling studies in rats and rabbits
- Caretaking staff changes

TAKE HOME MESSAGE: HANDLE GENTLY AND SLOWLY!

EXTRINSIC FACTORS AFFECTING RESEARCH
Chemicals

- Sources
  - Air, feed, water, bedding, caging materials
  - Inherently toxic or metabolites are toxic
- Portals of entry
  - Skin, GI tract, respiratory tract
- May affect hepatic microsomal enzymes
  - Biotransformation of drugs and chemicals
  - Regulation of oxygen radical removal

EXTRINSIC FACTORS AFFECTING RESEARCH
Chemicals

- Serve as local irritants
- Produce generalized disease
- Alter immune functions
- Allergens
- Mutagens
- Teratogens
EXTRINSIC FACTORS AFFECTING RESEARCH
Chemicals

- Examples
  - Nitrates in drinking water - carcinogenic nitrosamines
  - Aflatoxins in feed - carcinogenic
  - Aromatic hydrocarbons - hepatic microsomal enzymes
  - Insecticides - lymphopenia
  - Heavy metals - alter immune response

TAKE HOME MESSAGE: KNOW WHAT IS BEING USED IN YOUR AREA!

EXTRINSIC FACTORS AFFECTING RESEARCH
Air Quality

- Air quality of macroenvironment affects the air quality of microenvironment
  - Ozone - depresses hepatic microsomal enzymes
- Microenvironmental contaminants
  - Ammonia - urease positive bacteria
  - Carbon dioxide

TAKE HOME MESSAGE: KNOW IF THERE HAVE BEEN FAILURES!

EXTRINSIC FACTORS AFFECTING RESEARCH
Air Quality

- Ammonia
  - Acceptable level < 25 PPM
  - M. pulmonis + ammonia enhances:
    - lesion severity
    - bacterial growth
    - bacterial adherence
EXTRINSIC FACTORS AFFECTING RESEARCH

Drinking Water

- Chemical impurities
  - Suspended solids, organic and inorganic solutes
- Trihalomethanes present in most chlorinated water
  - Potential carcinogens
- High levels of chlorine may affect immune system
- Nitrates - common contaminants
- pH, hardness, microbial or chemical

TAKE HOME MESSAGE: KNOW THE QUALITY BEING PROVIDED!

EXTRINSIC FACTORS AFFECTING RESEARCH

Diets

- Variations in quantity or quality of essential vitamins or minerals may:
  - Alter drug metabolizing systems
  - Affect membrane integrity
  - Predispose to effects of carcinogens
- Raw materials may contain:
  - Nitrates and amines - form nitrosamines in vivo
  - Fish meal high in nitrosamines

TAKE HOME MESSAGE: KNOW WHAT IS BEING FED!
EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – What’s Common?

- MHV – 2%
- Paroviruses
  - Mouse – 2%
  - Rat – 4%
- Rotavirus – 0.3%
- Norovirus – 3%
- RRV – 7%
- Theilovirus
  - Mouse – 0.3%
  - Rat – 1.4%
- Helicobacter spp. – 15%
- P. pneumotropica
  - Mouse – 15%
  - Rat – 5%
- C. bovis – 3%
- Pneumocystis carinii – 2%
- Pinworms –
  - Mouse – 0.3%
  - Rat – 1.3%
- Mites – 0.1%

TAKE HOME MESSAGE: IS THERE A SAFE DISEASE?

EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Mouse Hepatitis Virus (MHV)

✓ Degree of mortality can be strain related:
  - BALB/c mice more susceptible than C57BL/6 mice
  - IFN-γ KO BALB/c mice had higher mortality than IFN-γ KO C57BL/6 mice (polytropic)

✓ May contaminate in-vitro hepatocyte cultures

✓ Viral clearance:
  - Varies with strain of mouse and virus
    - 2–3 weeks in C3H (enterotropic)
    - 4 weeks in BALB/c (enterotropic)
    - 8 weeks in Hsd:ICR (polytropic)
  - Not cleared in many GM lines!

EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Mouse Hepatitis Virus (MHV)

✓ Prolonged immunologic effects:
  - MHV depletes NK cells through apoptosis and syncytia formation
  - T-cells, B-cells modulation
  - Infects monocytes, macrophages, bone marrow dendritic cells
  - Delayed allogeneic graft rejection

✓ MHV may confuse infection studies!
  - Co-infections on IFN-γ deficient (KO) mice on C3H background developed a wasting syndrome with granulomatous peritonitis
  - Co-infection with H. hepaticus and enterotropic MHV-G reduced mortality and lesion incidence and severity relative to MHV alone during first week, BUT had more severe hepatitis and meningitis at 28d!
EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Parvovirus (PV)

- Several variants (MPV-1, -2, -3, -4, -?)
- Cause persistent infection, even with seroconversion in some cases
- No anatomic lesions, even in scid mice
- C57BL/6 require 10-100x infective dose; DBA/2 only slightly better
- MPV-1a (cell culture adapted) modulates immune response
- Suppression of T cell response in vitro
- Cytokine- and antigen-induced T cell proliferation in vitro suppressed after exposure to MPV-1a
- Potentiates allograft rejection
- Induces isograft rejection

EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Parvovirus in Rats

- RV - Rat Virus (previously KRV, Kilham Rat Virus)
- Natural infections usually asymptomatic, but persistent
- Infects rapidly growing cells: vascular endothelium, lymphoreticular and hematopoietic tissues, developing cerebellum and liver
- Rare epizootic disease in fetal/neonatal rats: Cerebellar hypoplasia, anemia, thrombocytopenia
- Very rare disease in older rats: Hemorrhagic disease

EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Norovirus (MNV)

Impact upon research:

- Immunocompetent mice – No clinical signs, but ...
  - Minimal change in inflammatory cells in lamina propria of small intestine at 24 hours post infection
  - Increased nuclear staining in red pulp (spleen), no change cell nos.
  - Viral nucleic acid found in small intestine, spleen, mesenteric lymph nodes, liver
  - Anecdotal reports of decreased “stool contents” at 3 days
- Immunosuppressed mice:
  - Lethal infection in STAT1 +/- (with or without RAG2 and PKR), and IFN Raβy +/-.
  - Hepatitis, interstitial pneumonia
  - Encephalitis only with intracerebral inoculation
  - Virus present in dendritic cells
EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Helicobacter

- Chronic hepatitis (necrosis, hepatocytomegaly, biliary proliferation, nonsuppurative inflammation) in A/J, C3H/HeN, and some other immunocompetent strains
- Hepatitis may be particularly necrotizing in immunodeficient strains
- Hepatocellular carcinomas in A/J, C3H/HeN, SCID mice
- Colon carcinoma in SMAD-3 deficient mice
- Increased incidence of mammary carcinoma in RAG2⁻/⁻ Apc(min⁺/+) mice (secondary to inflammation)
- C57 resistant to disease, but can carry high level of colonization
- Proliferative typhlocolitis in athymic mice and rats
- Rectal prolapse
- Mild chronic hepatitis in immunocompetent mice (low incidence)

EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Pasturella pneumotropica

- Lesions in immunocompetent rodents:
  - Mastitis
  - Conjunctivitis / Dacryoadenitis
  - Metritis
- Lesions in immunodeficient rodents:
  - Necrotizing, suppurative dacryoadenitis
  - Suppurative dermatitis (abscesses)
  - Metritis

EXTRINSIC FACTORS AFFECTING RESEARCH
Diseases – Oxyurid nematodes (Pinworms)

- Induced Th2 response
- ↑ IgG, interleukins
- ↑ Ab to Sheep RBC (used in bench top tests)
- ↑ allergic response to ovalbumin challenge
- Rectal prolapse – did not exclude Citrobacter or Helicobacter
- Slow growth
- Altered electrolyte transport
- Depressed exploratory behavior