Not All “Rs” Are Created Equal

What Russell and Burch Have To Teach D5 AALAS Members
53 Years Later

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Indiana Branch AALAS Meeting
May 11, 2012
Poll #1

Who has read the book?
Poll #2

What is the most important “R” for AALAS members?

- Working every minute of every working day in an animal research facility and/or
- Managing an animal research facility.

- R_{Replacement}?
- R_{Reduction}?
- R_{Refinement}?
Who Were Russell and Burch?

To answer this we need to take a trip back in time
Founded in 1926
- By Major C. W. Hume
- Charitable organization promoting humane treatment of animals
- Laboratory animal focus: after WWII
- 1947:
  - UFAW Handbook on the Care & Management of Laboratory Animals, 1st ed.

Major Charles Hume
(from UFAW)
Aim

- Promote humane behavior towards wild and domestic animals in Britain and beyond.
- Reduce the sum total of pain and fear inflicted upon animals by man.
- To do so using sound principles of science and scientific inquiry.
Science informs, motivates and facilitates advances in animal welfare by:

- providing a strong evidence base for changing attitudes and practices
- by creating practical and effective solutions to welfare problems.
UFAW Premise

- Scientists & animal care personnel desire to treat animals as humanely as possible.
  - Unanswered questions:
    - What is and is not humane?
    - How can humanity be promoted without confounding research aims?

- 1954: UFAW hired Russell & Burch
  - Project on humane technique in the lab.
Signs o’ the times?

“There were animal housing problems, problems with vendors who were selling animals of questionable quality, but most challenging were problems posed by antivivisectionists.”

Dr. Nathan R. Brewer, Recollections

In: 50 Years of Laboratory Animal Science, AALAS, 1999.
Russell and Burch Culmination

- “The Principles of Humane Experimental Technique”
  - Moral and ethical obligations toward animals
  - Appreciation of distress inherent in procedures
- 1959 publication
- 1992 reprinted
  - available in full text on the Altweb site at: http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc
Who were Russell and Burch?

William M. S. Russell, M.A., D.Phil
- Professor of Zoology & Comparative Anatomy
  - University College London
  - University of Reading
- 1925-2006

R.L. Burch
- Microbiologist
- 1926-1996
- Scientists!
- Not vets; not professional animal welfare activists.

Russell (l) and Burch (r)
(From UFAW)
The 3 R’s: In order of priority

1. **Replacement**
   - Substitution of conscious living higher animals with insentient material.

2. **Reduction**
   - Decrease in the number of animals used to obtain information of a given amount & precision.

3. **Refinement**
   - Any decrease in the incidence or severity of “inhumane” procedures applied to those animals which still have to be used.

Russell & Burch, p. 64.
Replacement

- **Absolute** (“Insentient models”)
  - Computer simulation, mathematical model
  - Immortal tissue culture
  - Higher plants, microorganisms, parasites

- **Relative** (“limited gains”)
  - Free-living invertebrates
    - Insects, octopus, horseshoe crabs
  - Animal use in distress-free experiments
    - Organ, tissue, cell harvest for *in vitro* use
Why not “Replacement”?

- Addressed at the lab and IACUC level
- Not a consideration when IACUC-approved work starts in the animal facility
Why the emphasis on “Reduction”?
It ’Ather Fatigue’on ‘Rs,’ Stupid!

1. **Replacement:**
   - inanimate models (computer simulation)
   - +/- phylogenetically lower species (snails, fish)

2. **Reduction:**
   - minimize numbers (statistics)
   - control variability

3. **Refinement:**
   - anesthesia/analgesia
   - endpoints
“In the early days... the **husbandry** of the animals was totally **makeshift**. The enclosures that held the animals varied from used shipping crates and glass jars to old horse stalls. The **bedding** used was far from sterile and often **was contaminated** with insects. There were **minimal** attempts at **lighting and temperature control**. In short, the conditions under which the research animals were kept often **caused stress, illness, and death** of the animals, leading to **questionably valid research results**, not to mention raising serious ethical issues...”

Dr. Nathan R. Brewer
1904-2009
“Inhumanity” Categories

- Direct
  - Unavoidable consequence of the procedure

- Contingent
  - Incidental, accidental or inadvertent by-product of the procedure
    - Imperfections in care & use

Russell & Burch, p. 54.

“The worst sin toward our fellow creatures is not to hate them, but to be indifferent to them: that’s the essence of inhumanity.”

George Bernard Shaw (1901)
“We” are the “environment”

- We provide the conditions where animals live & data is collected.
  - Consistent & wholesome
- We are the foundation for great science
  - Minimize environmental variability $\rightarrow \downarrow$ animals
- “Reduction” is the most ascendant “R”
Indiana and the Environment

- The Garner (Dr. Joseph P.)
- “.standardization…may generate spurious results, accounting for poor reproducibility…”
- The application of Dalton’s (Modified) Third Rule
  - We control the environment until it is time not to control the environment.
    - PI & IACUC
The Importance of “Good” Husbandry

Husbandry = “keeping the animals alive and healthy for long periods”

- an essential part of many experiments!
  - Unforeseen losses due to environment
    - Repetition of experiment + more animals

“This is why the contribution of animal technicians is so important for the progress of humane experimentation, even when they themselves do not carry out actual experimental procedures.”

Russell & Burch, p. 55.
Our Role in Research

- **Quality Control:**
  - Procure healthy animals
  - Maintain them in a wholesome, consistent environment

- **Quality Assurance**
  - Assess to verify no deviation
  - Intervene as necessary
Environmental Monitoring
Responsible & Efficient Data Management

Automate or

(Remember the process is automated, not automatic!)
Temperature

- Heat is one of the most important environmental factors affecting living organisms.

  - General and broad metabolic processes
    - 20 to 30% change in tissue and cellular function for each 1°C rise or loss in ambient temperature.
  - Food and water consumption
  - Enzyme activity
  - Disease resistance
  - Absorption of parenterally or topically applied drugs
  - Drug toxicity
  - Innumerable other biologic processes.
Excessive Heat (>30ºC)
Rodents

- Testicular atrophy *Lab Anim Sci* **27**: 76, 1977
- Embryonic mortality *J Reprod Fert* **30**: 71, 1972
- Teratogenesis *J Reprod Fert* **35**: 393, 1973
- Altered maternal care *Physiol Behav* **64**: 463, 1998
- Impaired lactation *J Reprod. Fertil* **27**: 369, 1971
- Immune dysfunction *Exp. Anim.* **48**: 9, 1999
- Death *Lab Anim Sci* **27**: 76, 1977
Chilling (5-20°C)

- Rodents:
  - Altered pup development *Physiol Behav* 64: 463, 1998
  - ↓ antibody production *J. Immunogenet* 5: 197, 1978
  - Elevated HR & BP *Am J Hyper* 15: 85, 2002

Goals:

- control temperature to a set point within a broad range of possibilities (59-85°F) to meet the needs of many species:
  - Tropical fish: > 80°F
  - Xenopus: ~ 65°F
  - Humans: 64-73°F
- manage variability around the mean as tightly as possible (+/- 1-2°F)
  - Reptiles may be the exception that may benefit from temperature fluctuation (i.e. gradients within the primary enclosure) throughout the day.
Is 18-26°C Best for Rodents?

- **Mice**
  - 28-29°C
    - Daylight *Physiol Behav* **65**: 255-62, 1998
    - Cardiovascular performance optimum
      - *Am J Regul Integr Comp Physiol* **287**: R391, 2004
  - 23-24°C: during the dark (active) period


<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>18</td>
<td>Human comfort</td>
<td></td>
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<tr>
<td>20</td>
<td>Rats</td>
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<td>23</td>
<td>Mouse</td>
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<td>28</td>
<td>The Guide</td>
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Monitoring Temperature

- Manual recording
  - High/low thermometers, sling psychrometers, infrared thermometer, thermohydrograph
- Automated recording & alarming via sensor
  - Edstrom Watchdog, Rees Centron, Siemens Apogee, HOBOs, etc.
Relative Humidity

- Biological Influences at Extremes (<10% or >90%)
  - Food and water consumption
  - Postnatal development
    - *Dev Psychobiol* **23**: 63, 1990
  - Transmission of infectious agents
  - Transcutaneous absorption of drugs
RH: Other Adverse Effects

- **High RH (> 50-55%)**
  - NH$_3$ production
  - Mold growth on feed
  - Food mite hatch
  - HVAC mold growth, condensation

- **Low RH (< 20-30%)**
  - Dust, allergens
  - Ringtail?
Sterling Bar Graph

http://www.natural-building.co.uk/images/nbt_images_tables/sterling_bar_graph_relative_humidity_and_health.gif

![Sterling Bar Graph](http://www.natural-building.co.uk/images/nbt_images_tables/sterling_bar_graph_relative_humidity_and_health.gif)
Relative Humidity

- Controlled within a range of 30-70% ("Guide", 2011)
  - Optimally within 10% of the set point on a year round basis.
  - 30-50% in rodent housing rooms
    - Ammonia production, mold retardation
    - Ringtail prevention?
  - Human comfort: 20-70%

- Monitoring
  - Decentralized & manual
    - Sling psychrometer
    - Thermohygrometer
    - Digital recorder
  - Centralized recording & **alarming** via sensor
Illumination

- **Components**: intensity, quality, photoperiodicity
  - Photostressors: light intensity, spectral quality, or photoperiod inconsistencies or failures.

- **Optimal L:D ratio has not been determined**
  - 12:12 cycles have traditionally been used.
  - 14:10 cycles may optimize breeding for long-day breeders (seasonally polyestrous)
  - Use caution with other ratios to not compromise science

- **30 foot-candles 1 m from the floor**
  - “Guide”, 2011

- **Importance**:
  - Not life-threatening
  - Among the greatest perils for the ruination of experiments
Illumination & Biological Effects

- Myriad influences:
  - Activity
  - Growth
  - Immunity
  - Physiology
  - Reproduction
  - Vision
Light Intensity Requirements

- Rodents:
  - Light period: \( \geq 5-10 \text{ lux (0.5-1 foot-candle)} \)
  - Dark period: \( \leq 0.15-0.2 \text{ lux (dim)} \)
    - \( > 0.2 \text{ lux} \) suppresses endogenous melatonin, alters circadian rhythms & enhance oncogenicity.
Effect of Light Contamination on Cancer In Rats

Infection Suppression Under Altered Lighting

*Pharmacol Biochem Behav* **51**: 947, 1995
Undetected Lighting Failures

- Continuous illumination from failed timers.
  - Lights remain on after operating hours when no one is around.
    - Can go on for extended periods before detected
- Manual activation during the dark period
- Cascading (repeat) alarms
  - Allows differentiation between transient (human-activated) & continuous lighting malfunction.
The Perception of Noise
Sound recordings in a mouse room

- Conversation
- Living Room
- Quiet garden
- Watch ticking

**Barking dogs**
- 90 dB
- 0.4-4 kHz

**Marmosets**
- 100 dB
- 12.5-70 kHz

Adapted from: *Physiol Behav* 53: 1067, 1993
Sources of Noise
Problem: excessive +/- unpredictable

- Personnel
  - Loud conversations
  - Overhead speakers
  - Radios
  - Husbandry & cleaning procedures
  - Mobile equipment movement, collisions and operation

- Vocalization and activity of animals
  - Barking dogs, squealing pigs, monkeys shaking cages

- Ventilation systems
- Fixed or research equipment
  - Aquatic pumps
  - Light fixtures and computer terminals
  - Security hardware
  - Fire alarms

- Construction or renovation/expansion projects
Biological Effects of Adverse Noise

- Development *Environ Res* **73**: 227, 1997
- Behavior *Brain Res* **1009**: 88, 2004
- Food consumption *Appetite* **26**: 193, 1996
- Neuroendocrine *J Anat* **200**: 159, 2002
- Wound healing *Adv Wound Care* **9**: 35, 1996
Noise As A Research Variable: Infant Tree Shrew Milk Consumption

From: Nature 251: 309, 1974
Noise and Neurochemical Effects

*Rats*

**Acoustical stress**
- 100 dB
  - “Automobile horn”
- 0 – 26 kHz

**Euthanasia immediate**
- After final exposure

**Adrenal gland assay**

**Conclusion**
- “Brief” = stressful
- “prolonged” = distress
  - Glandular fatigue
Goals for Noise

- Goal:
  - ≤ 55 db ambient

- Consider the value of:
  - White noise?
  - Background music?

- Monitoring?
“Refinement” in the AV/IACUC Bailiwick?

- Anesthesia & analgesia
- Supportive and nursing care
- Dose fine tuning
- Non-invasive or less invasive data collection
- Microarray technology
- Humane endpoints
- Pathogen substitution or attenuation
- Skill proficiency of (some) research personnel
Husbandry-related “Refinements”

- Standards of Care
- Modern Caging
- Regular Observation
  - Veterinary medical care
- Humane euthanasia
- Enrichment/exercise programs
  - Socialization
  - Species-typical behaviors
  - Science-based (IACUC?)

Fig. courtesy of CAAT
Fig. courtesy of Techniplast
Fig. courtesy of WNPRC
Fig. courtesy of CAAT
When we enrich the environment…

…we can add variability.

- Brain development
- Cocaine addiction
- Cognition in AD
- Neuroendocrine activity
- Onset of HD motor disorders
- Tumorigenesis
The Prescient
Russell & Burch

With a Warning Shot Across the Bow
Benefits of Large Scale Operations

- Affordability of automation
- Flexibility to experiment with new or alternative technology
- Specialization of talent
- Economy of scale
Benefits of Automation

- Diminishing legal resident labor pool
  - Concentrate precious human talent on things people do best:
    - decision making in novel or dynamic situations
    - non-programmed activities
    - small force application & ultra fine precision
    - teamwork

- Ergonomic advantages
  - Programmable, high through-put, consistent, repetitious +/- force work are best for machines.

- allergen exposure in cage wash rooms

- Cost containment
  - Life time automation costs < human labor
Automation: Many Applications

- Cage handling, processing & cleaning
- Cage routing (Tecniplast)
- Environmental control
- Environmental monitoring
- Floor cleaning
- Drinking water supply
  - Bottles
  - The greatest hazard for physical injury.
The Benefits of Automation

- ↓ Disturbance of animals
- ↓ Omissions/errors in care
  - e.g. failures to provide water
    - if ordinarily done manually
- ↑ Technical attention to:
  - Specific animals in need of monitoring
  - Researchers and their needs
- ↑ Professionalism & status of technicians.
- Lauded AWS, but…

Russell & Burch, p. 165.
...did not foresee the “drown” side.
DAR Monthly “Preventable” Mouse Mortality
January 1, 2011 – December 31, 2011
Average Daily Census: 65,607 mice

Losses per Month

0 5 10 15 20 25

AWS Flood
Premature weaning
Entrapment
AWS dehydration
Bottle flood
Fighting
Insufficient food
Bottle dehydration
Benefits of Large Scale Operations

- Flexibility to experiment with new or alternative technology
  - AWS valves, different caging types, etc.

- Specialization of talent
  - Business manager, customer service, maintenance, cage wash technician job titles, etc.
Economy of scale principles $\rightarrow$ $\downarrow$ costs

- Reduction of overhead
  - IACUC, veterinary, administrative expertise

- Animal purchase, animal *per diem*, & associated research personnel involvement are often the greatest lab cost.

- Some scientists consider animal care costs to be a greater threat to research than the ARM.
  - "As long as artificially high prices for mouse care exist, [this obstacle] not the right-to-life or animal-rights [movements], will be the major stumbling block for the transfer of molecular biology to humans." Dr. Irving Weissman: *Science* 288(5464): 254, 2000.
Prescient Warnings from Russell & Burch

- Risk of large scale/low cost operations:
  - “Big Pharma” & “Big Academe”
- Inherent contingent inhumanity
  - By spreading staff too thin
    - Russell & Burch, p. 65
  - Overzealous cost cutting
Prologue
(or what would R&B have us conclude?)

- Research animals respond to many factors, or changes in their environment, in many ways.
  - These responses may affect experimental results.
- Standardized conditions
  - Permit subject response to experimental manipulations w/o confounding variability.
  - Elementary charge of the animal resources program
It’s the Environment, Stupid!

2. **Reduction:**
   - minimize numbers (statistics)
   - control variability
The Animal Facility
The Foundation for Valid Animal Research

- Consistent, wholesome conditions
  - Minimizing the physiological variations associated with environmental changes
  - Mindful of possible effects of husbandry upon science.
  - Planned significant environmental changes should not be made without prior consultation with the investigators to ensure that minimal impact occurs to research programs.

- Monitoring is a key component of environmental control.
To fully control the variance of physiological responses…

… control phenotype
- Breeding methods +
- Environmental stability

... understand behavior:
- “…work on learning in animals is vitiated by failure to take into account the sort of social responses to the human experimenter which the circus trainer ignores at his peril.” Russell & Burch, p. 127

- Conundrum of studying nocturnal animals during the illuminated part of the photoperiod. Russell & Burch, p. 131
Continued “Reduction” Challenges

- Behavioral management
- Maintain SPF colonies
  - Challenges of MPV, MNV, fur mites
  - Enzootic helicobacteriosis, pasteurellosis, etc.
- Cage density standards
- Noise monitoring
  - Ultrasound, infrasound
- Drinking water quality
- Phototransition
- Ever ↑↑↑↑ scientist needs
Attempts to Standardize Remain Elusive

Group means (+/- SEM for n = 16 mice) for behavior videotaped for 5 min on elevated plus mazes having two open and two enclosed arms. (A) Total number of entries into any arm (defined as all four limbs in the arm). (B) Time (seconds) spent in the two open arms during the 300-s test. Smaller amounts of time indicate higher levels of anxiety.

Animal Resources:

Providing the Environment:
- Photoperiod
- Temperature/RH
- Food/water
- Sanitation
- et cetera

“Physiological uniformity is likely to be one of the great rewards of good husbandry.”
W.M.S. Russell and R.L. Burch, 1959
Russell and Burch

Champions of:
- The 3R’s

Advocates for:
- Animal Care
Special thanks to...

- ...Dr. Gerry Smith
- ...Deb Hickman