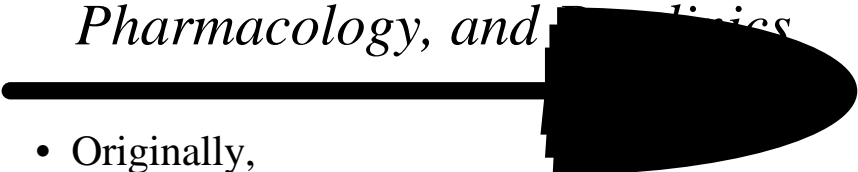


## *Drug Development / Testing*



- Preclinical Studies
  - Pharmacological profiling
    - *in vitro*
    - *ex vivo*
    - *in vivo*
  - Toxicology

## *The Blurring Line Between Discovery, Pharmacology, and Clinics*

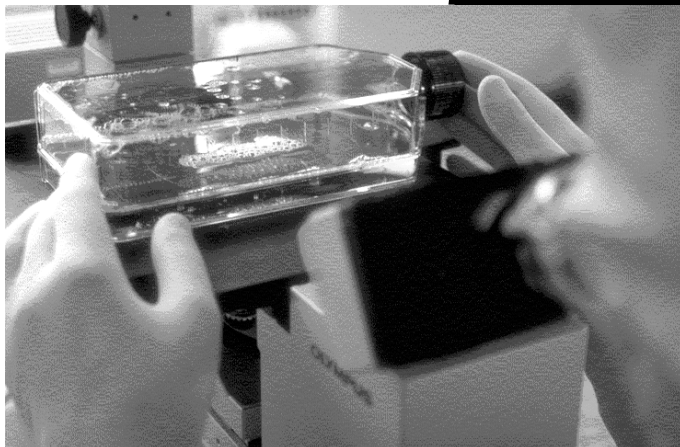


- Originally,
  - **Discovery** meant synthesis, screening, and in vitro profiling,
  - **Pharmacology** meant activity and side effect profiling in animals,
  - **Preclinics** meant dose finding in animals
- Current concepts involves an integrative approach with constant feedback

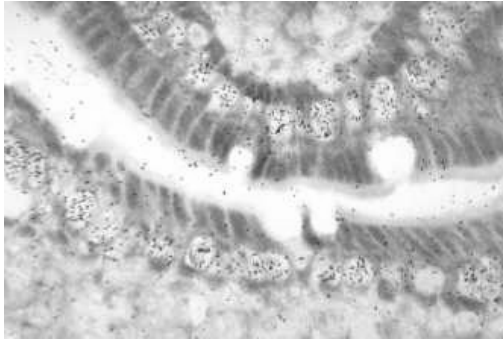
## *Why do Early-Stage Animal Experiments Fail?*

- Animal pharmacokinetic parameters (15%)
- Animal toxicity (11%)
- Human toxicity (9%)
- Given the ethical implications and the high cost of animal experiments, good in vitro models for ADME testing are called for

## *Cell Culture can Provide Surrogate Models for Some Animal Experiments*



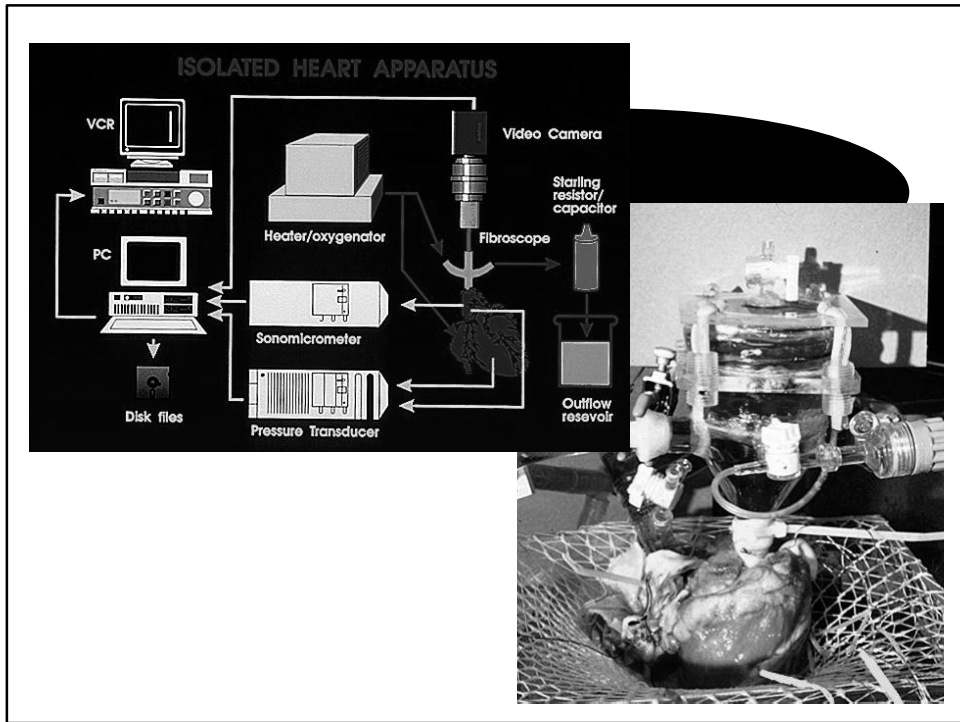
## *Ex vivo Receptor Models*



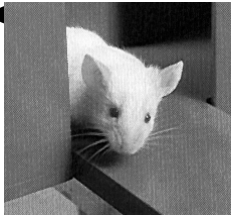
Labeled vitamin D receptors on rat duodenum

## *Isolated Organ Receptor Models*

- Guinea pig ileum
  - Muscarinic acetylcholine receptors (M3)
  - Histamine H1 and H3 receptors
  - Serotonine 5-HT3 receptors
  - Angiotensin AT2 receptors
- Rabbit jejunum
  - Alpha-2 adrenoceptors
- Rabbit aorta
  - alpha-1 adrenoceptors
  - Serotonine 5-HT2 receptors



*At some Point, in vivo Testing  
Becomes Unavoidable*



Albino rat



Marmoset



*ADME Profiling:  
A Major Objective for Toxicology*

- Absorption
- Disposition
- Metabolism
- Excretion



*Some Important Abbreviations*

- NOEL = No Observed Effect Level
- MED = Minimally Effective Dose  
(LAD = Lowest Active Dose)
- ED<sub>50</sub> = Effective Dose 50%
- NTEL = No Toxic Effect Level
- MTD = Maximal Tolerated Dose
- LD<sub>50</sub> = Lethal Dose 50%

## *Of Limited Value: In Vivo Testing*

- LD<sub>50</sub> results can vary widely between related species (e.g., mouse and rat)
- Predictive value limited to dose estimation in a particular animal strain, and for a particular route of administration
- Required by regulatory authorities



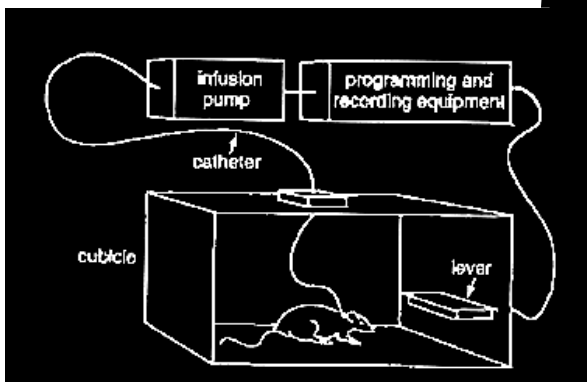
## *Behaviors Explored in Animal Testing*

- Reward-seeking → drug abuse potential
- Learning → cognitive potential
- Conflict → anti-anxiety potential
- Despair → antidepressant potential
- Aggression
- Socialization

## *The Irwin Test: The Simplest Behavioral Paradigm*

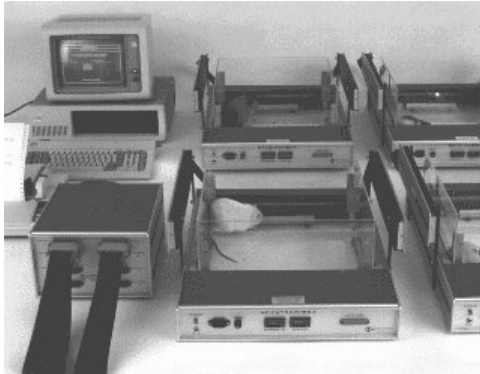
- After administration of the compound, the animal is observed for presence of pre-defined signs:
- Spontaneous motor activity
- Body posture, Straub tail, muscle tone, gait
- Tremor, writhing, convulsions
- Fear, aggression
- Reflexes (righting, pupil, ...)
- Salivation, lacrimation, defecation

## *Behavioral Testing: Abuse Potential*



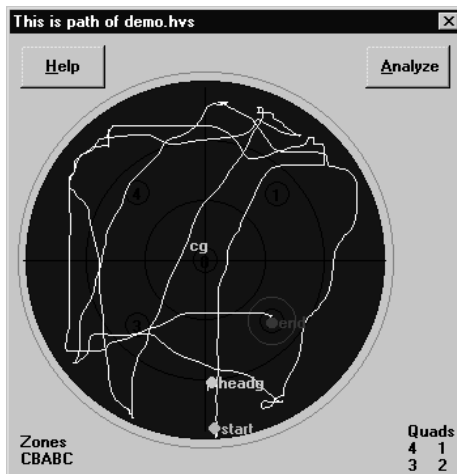
Model for self-administration of drugs

# *Behavioral Testing: General Activity Monitoring*



An array of beams and photodetectors detects animal movements

# *Cognitive Testing: Spatial Learning*



Training to find submerged platform and recall the position





## *Cognitive Testing: Spatial Learning*

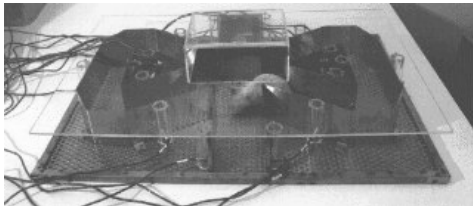
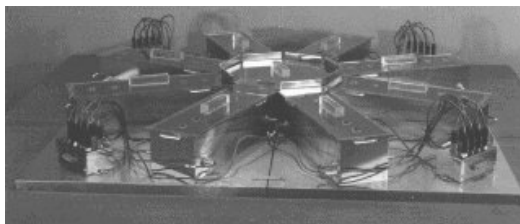
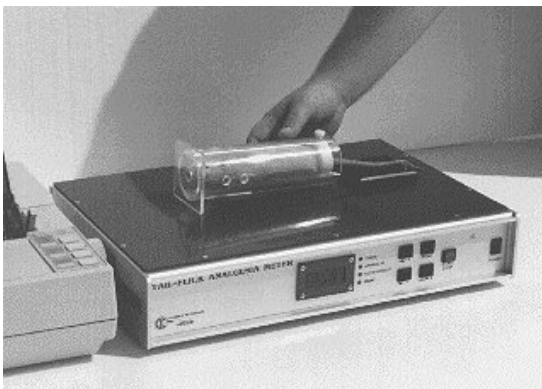


Figure-8 maze

Radial-arm maze

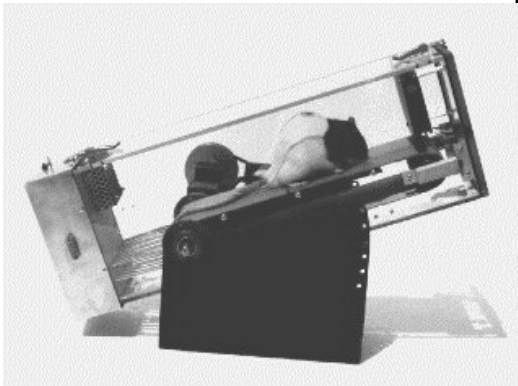


## *Analgesia: Tail-Flip Testing*



A photocell monitors the time lapsed until the animal flicks its tail away from a thermal light beam

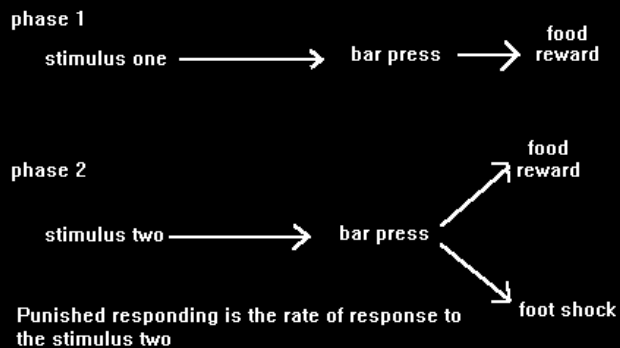
## *Exertion and Metabolic Testing*



Oxygen consumption and blood metabolites tested in a treadmill setup

## *Anxiolytics Increase Punished Responding*

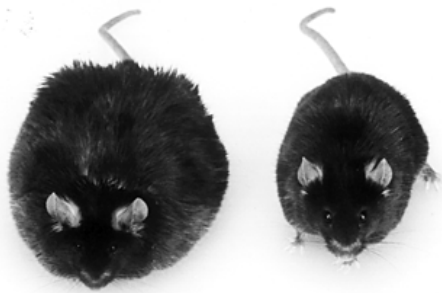
### Geller Conflict Test



## *Behavioral Despair: Forced Swimming and Tension*

- When confronted with an aversive situation without the possibility to escape, rodents become immobile after a predictable period (model for despair)
- Antidepressants and stimulants will prolong the escape-directed behavior while minor tranquilizers and neuroleptics will shorten it  
→ identification of different psychotropic classes

## *Mutant Animals for Specific Investigation*

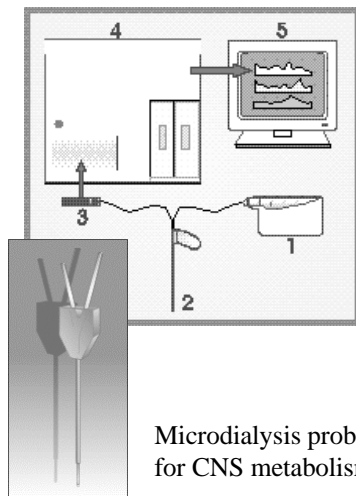


Leptin administration  
can attenuate obesity  
in mice with a genetic  
defect in the leptin gene  
(*ob* <sup>-/-</sup> knock-outs)

## *Microdialysis: Monitoring Metabolites in situ*

- A probe with a semipermeable membrane is perfused with metabolically neutral solution, is inserted into the tissue
- Net composition changes upon passage reflect the concentrations of metabolites in the interstitial space (depending on flow rate and MW)
- Dialysis solution can be “spiked” with metabolically active compounds
- Continuous, real-time monitoring

## *Microdialysis: Monitoring Metabolites in situ*



Microdialysis probe  
for CNS metabolism

The perfusion fluid is pumped from the microdialysis pump (1) through the catheter probe (2) into the microvial (3). The sample is transferred to the analyzer (4) which shows results on a display (5).

## *What is Toxicology*

- Derived from *toxicon* (gr.) = poison
- Paracelsus (1493-1541): “*Dosis facit venenum*” (the right dose differentiates a poison from a remedy)
- Defined as the science of adverse effects on biological systems
- Modern toxicology requires an integrated approach

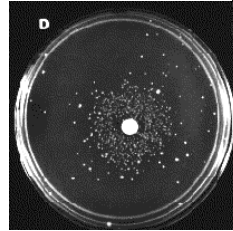
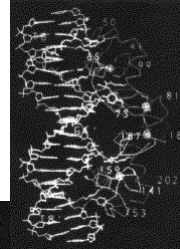
## *Issues in Toxicology*

- In vitro mutagenicity and genotoxicity
- Acute toxicity ( $LD_{50}$ )
- Subchronic and chronic toxicity
- Drug interaction toxicity
- Reproductive toxicity
- Developmental toxicity
- Ecotoxicity (environmental risk assessment)

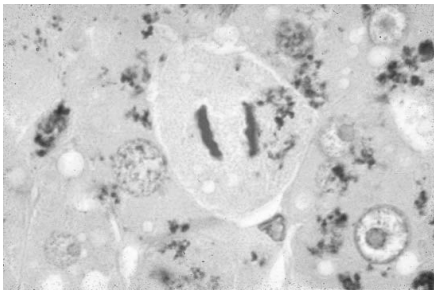


## Mutagenicity: The Ames Test

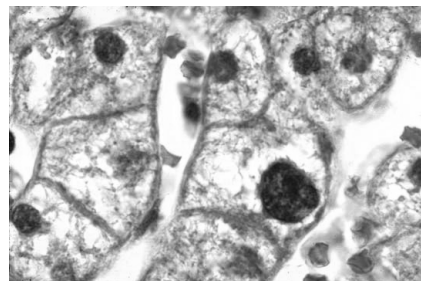
- Various mutant strains of *Salmonella typhimurium* are exposed to the test compound
  - Histidine synthesis deficiency
  - rfa strain: increased permeability
  - uvrB strain: defect in DNA repair
  - plasmid factor strains



## Mutagenicity: Looking for Polyploid Cells

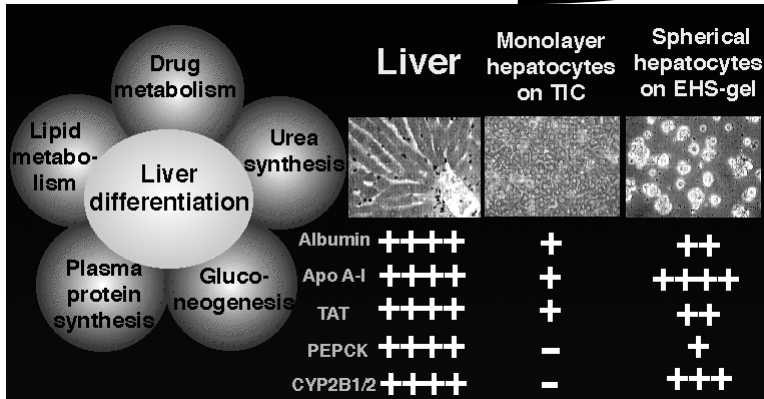


Normal mitosis  
in healthy liver cell

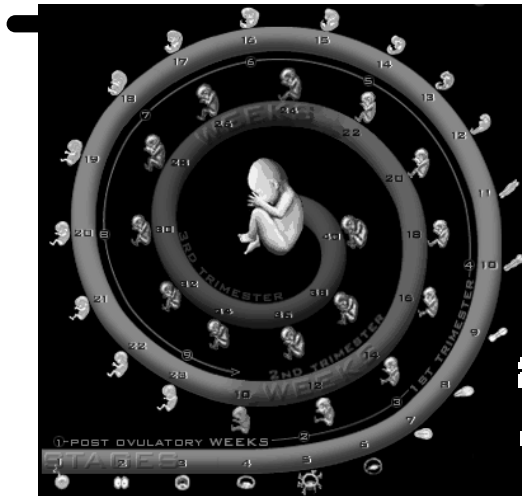


Polyploid liver cell  
after exposure to mutagen

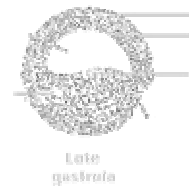
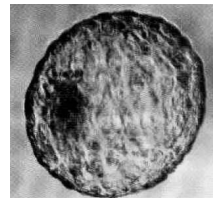
# The Liver: Great Metabolizer, and First Target of Toxicity



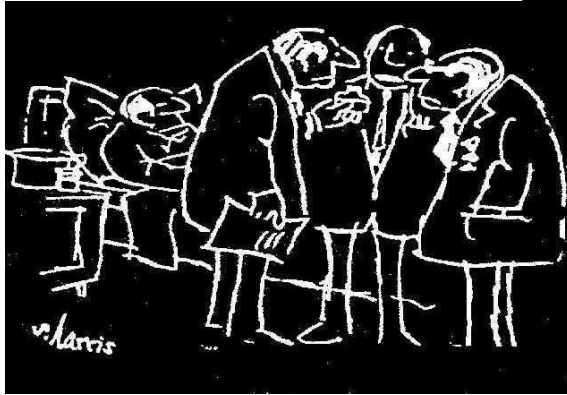
# Developmental Toxicology



Embryotoxicity



# *The Challenge After* ~~Tox~~



„Shall we risk it?  
After all, it has  
cured rats, and  
primates have  
survived...“