58TH ANNUAL MEETING & ToxExpo · MARCH 10–14, 2019

Continuing Education Course PM10

Sunday, March 10 | 1:15 PM to 5:00 PM

Beauty of the Skin Is in the Eye of the Beholder: A Basic Course on Dermal and Ocular Toxicology

Chairpersons:

Michael F. Hughes
Neera Tewari-Singh

Presenters:

Michael F. Hughes
Neera Tewari-Singh
Marion Gordon
Patrick McNutt
Nicole C. Kleinstreuer

Chairpersons

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Primary Endorser

Dermal Toxicology Specialty Section

Other Endorser

Association of Scientists of Indian Origin Special Interest Group

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Beauty of the Skin Is in the Eye of the Beholder: A Basic Course on Dermal and Ocular Toxicology

1:15 PM–1:30 PM	**Welcome and Course Introductions**	
1:30 PM–2:05 PM	**Dermal Absorption of Xenobiotics: Skin Anatomy, Factors That Affect Absorption, and Methods to Assess Absorption** Michael F. Hughes, US EPA, Research Triangle Park, NC	5
2:05 PM–2:40 PM	**Dermal Toxicity: Hazardous Chemical Exposure Assessment and Animal Models** Neera Tewari-Singh, Michigan State University East Lansing, MI	24
2:40 PM–3:00 PM Part 1	**Ocular Anatomy and Manifestations of Ocular Toxicity** Marion Gordon, Rutgers, The State University of New Jersey, Piscataway, NJ	41
3:00 PM–3:30 PM	**Break**	
3:30 PM–3:50 PM Part 2	**Ocular Anatomy and Manifestations of Ocular Toxicity** Marion Gordon, Rutgers, The State University of New Jersey, Piscataway, NJ	
3:50 PM–4:25 PM	**Tissue-Specific Aspects of Corneal Injury: The Cornea Is Not Merely a Window to the Soul** Patrick McNutt, US Army Medical Research Institute of Chemical Defense, Fallstom, MD	71
4:25 PM–5:00 PM	**Advances in Nonanimal Alternatives to Dermal and Ocular Toxicity Testing** Nicole C. Kleinstreuer, NIEHS/NICEATM, Research Triangle Park, NC	88

Course Participant Agreement

SOT | Society of Toxicology

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Dermal Absorption of Xenobiotics: Skin Anatomy, Factors That Affect Absorption, and Methods to Assess Absorption

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Conflict of Interest Statement

I have no conflicts of interest to disclose.

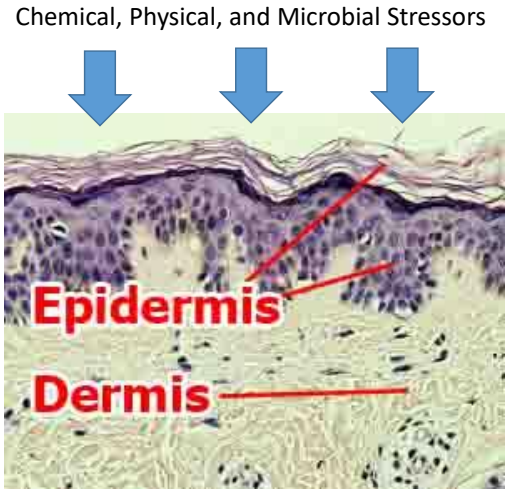
The contents of this presentation do not represent the views and policies of the US Environmental Protection Agency.

Abbreviations

- BW – body weight
- T.B. – total body
- J_{max} – maximal flux
- J_{SS} – steady-state flux
- K_p – permeability coefficient
- Log P – log octanol:water partition coefficient
- FBS – fetal bovine serum
- TEWL – transepidermal water loss
- MW – molecular weight

Outline

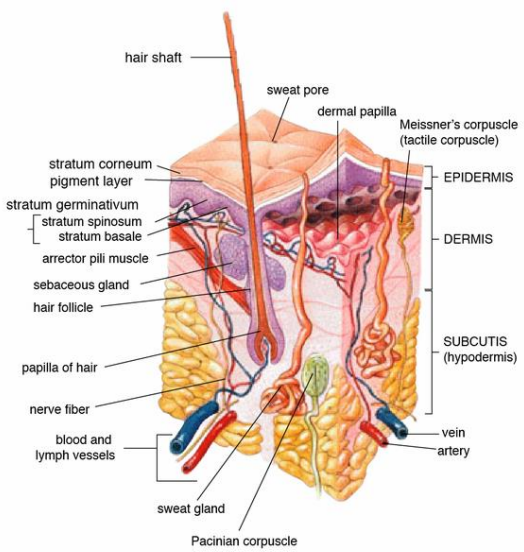
- Function
- Anatomy of skin
- Metabolism
- Barrier properties
- Percutaneous absorption
  - Theory and barrier properties
  - *In vivo* methodology
  - *In vitro* methodology
  - Prediction of human absorption



https://commons.wikimedia.org/wiki/File:Epidermis_y_dermis.jpg

Skin

- Part of the body's enveloping membrane
  - Integument—skin, hair, nails, glands
- Largest organ in the body by weight (ca. 10% BW)
- Surface area—20 square feet (adult)
- Epidermis (derived from ectoderm)
  - avascular
- Dermis (derived from mesoderm)
  - Blood and lymph vessels, nerve fibers present
  - 90% thickness of skin
  - 15–20% T.B. weight; 18–40% T.B. water
- Hypodermis or subcutis
  - fat



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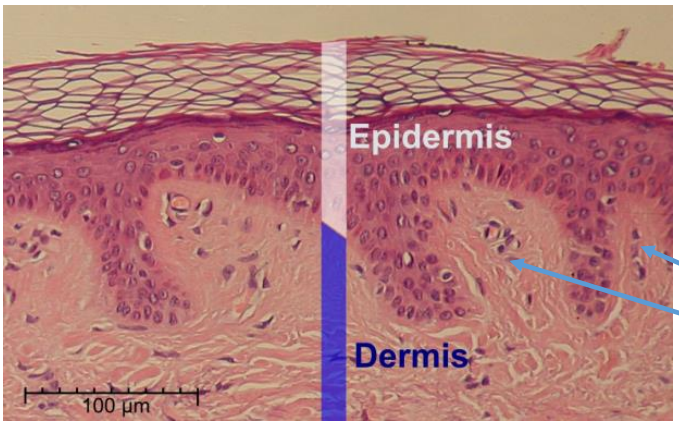
Functions of the Skin

- Regulates body temperature
- Prevents loss of body fluids
- Impedes penetration of xenobiotics
- Protects body from ultraviolet radiation (i.e., sunlight)
  - Melanin
- Mechanical support
- Sensory organ (touch, temperature, emotional senses)
- Synthesis of Vitamin D
- Immunological function
- Excrete (toxic) substances via sweat

Anatomy of Human Skin

Basement membrane (not visible in this picture, but between epidermis and dermis)



Stratum corneum

Dermal papillae

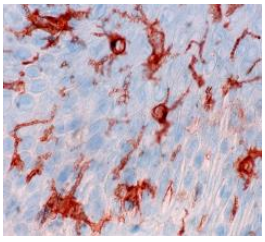
This is a hematoxylin and eosin stained slide at 10x of normal epidermis and dermis

https://upload.wikimedia.org/wikipedia/commons/8/84/Epidermis-delimited.JPG

Cells of the Epidermis

- Basal cells—innermost single layer of stem cells that constantly divide by mitosis
- Keratinocytes—squamous epithelial cells; most abundant of the epidermal cells; contains the protein keratin
- Merkel cells—mechanoreceptor cells for touch
- Langerhans cells—immune (dendritic) cells that have a role in antigen presentation
- Melanocytes—synthesize melanin
  - Melanin—a skin-darkening pigment that protects skin from ultraviolet light; some immune function

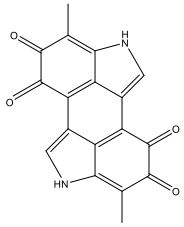
Langerhans cells



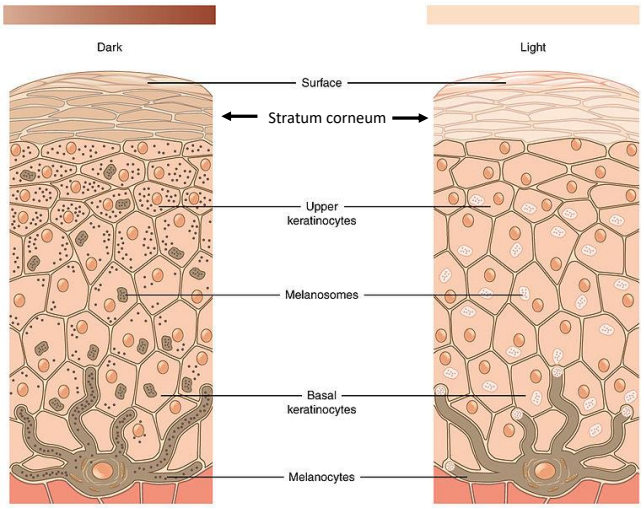
https://commons.wikimedia.org; courtesy of Ed Uthman from Houston, TX, USA - Langerhans Cells in Normal Epidermis, CD1a Immunostain

Melanocytes

- Ultraviolet light stimulates melanocytes to synthesize melanin, a natural pigment
- Melanin formed from tyrosine, which is oxidized then polymerized, packaged in melanosomes
- Types of melanin
  - Eumelanin (brown and black), pheomelanin, and neuromelanin
  - Skin, hair, iris, brain
- Protects skin from exposure to ultraviolet light
- Melanoma—cancer of melanocytes



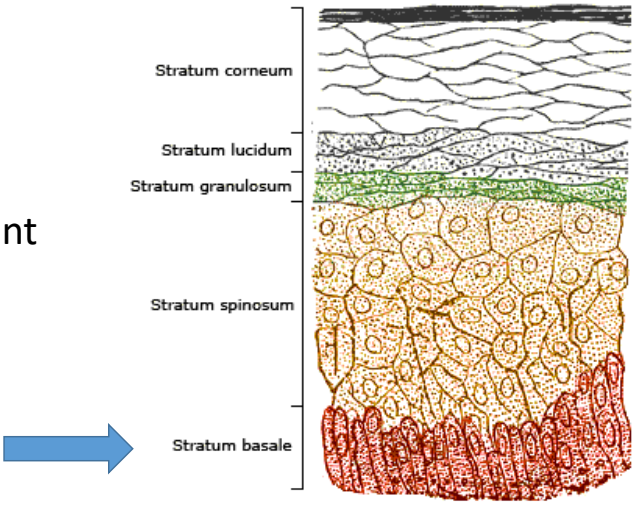
melanin



https://commons.wikimedia.org/wiki/File:504_Melanocytes.jpg via http://cnx.org/content/col11496/1.6/

Epidermis—Stratum Basale (Germinativum)

- A single layer of stem cells
- Overlays basement membrane
- Divide by mitosis and push cells up
- Merkel cells and melanocytes present
- Basal cell carcinoma



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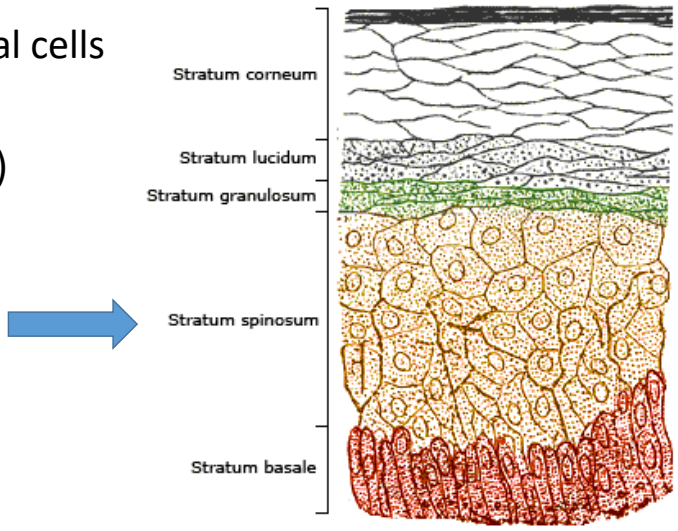
Epidermis—Stratum Spinosum

- 8–10 layers of squamous epidermal cells
- Connected by desmosomes
- Keratin synthesized (keratinocytes)
- Langerhans cells present
- Squamous cell carcinoma



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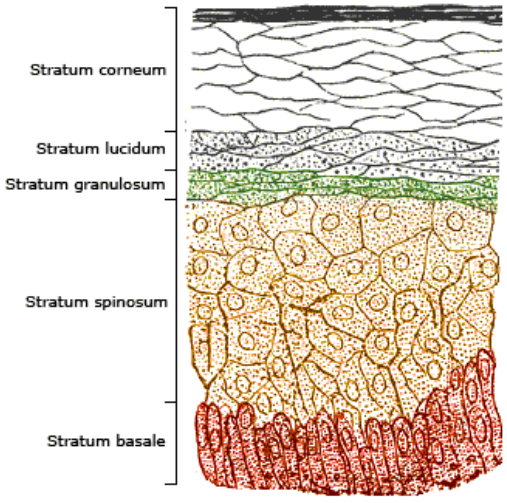
Epidermis—Stratum Granulosum

- 3–5 layers of epidermal cells
- Flattened cells
- Cell membrane thickens
- Tight junction-associated proteins
- Lamellar bodies become apparent
  - Fuse with cell membrane
  - Secrete lipids, keratohyalin, and other biochemicals
  - Upper layers of skin become more waterproof



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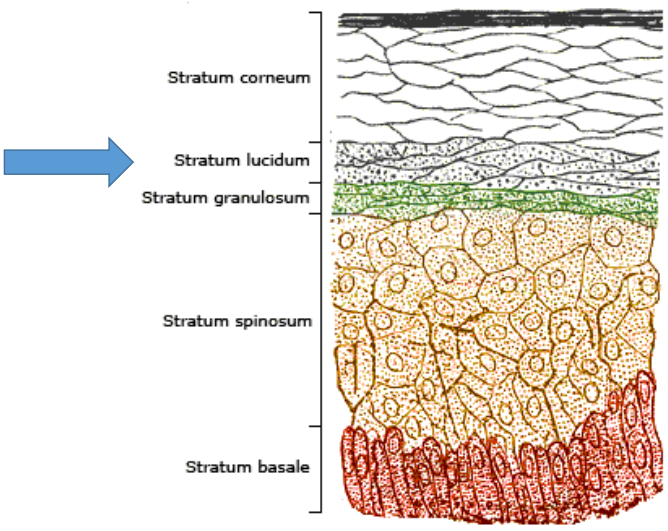
Epidermis—Stratum Lucidum

- 2–3 layers of epidermal cells
- Found in areas where skin is thick
  - Soles of feet and toes
  - Palms of hands and fingers
- Cells contains eleidin
  - Waterproofs the skin
- Cells are dead or are dying



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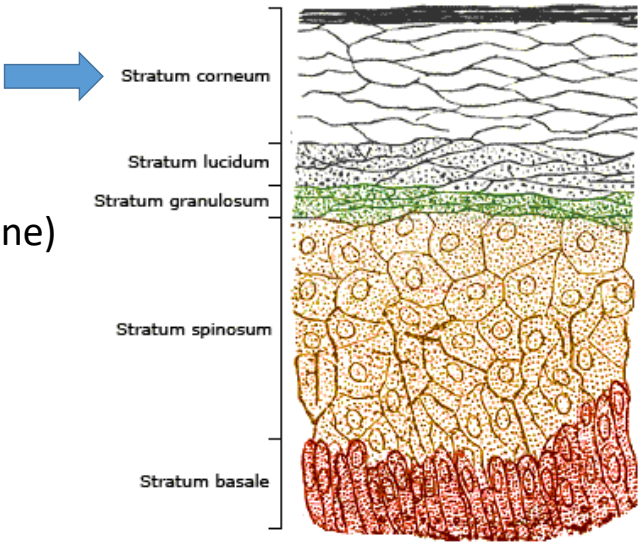
Epidermis—Stratum Corneum

- 15–30 layers of dead epidermal cells
- Highly keratinized and dry
- Main barrier to absorption of xenobiotics (i.e., rate limiting membrane)
- Desquamation
- Turnover—4 weeks



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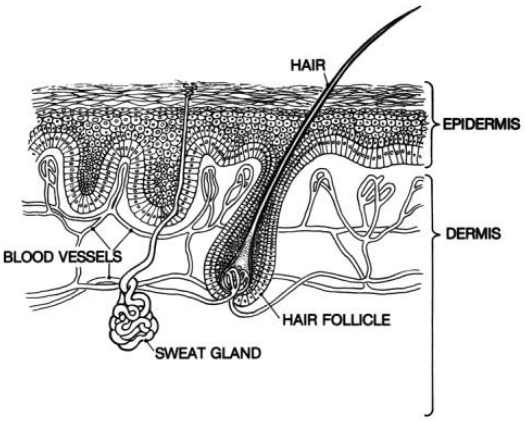
Basement Membrane

- Thin membrane between dermis and epidermis
  - Basal lamina (lamina lucida and lamina densa)
  - Lamina reticularis
- Originates from stratum basale
- Contains collagen fibrils (III, IV, and VII), integrins, nidogens, and others
- Function—anchor epidermis to dermis, mechanical barrier between dermis and epidermis

Dermis

- Cell types
  - Fibroblasts
  - Macrophages
  - Adipocytes
- Papillary layer
  - Uppermost layer—collagen fibers
  - Terminal networks of blood capillaries
- Reticular layer
  - Thicker than papillary layer
  - Collagen, elastic and reticular fibers—strength, extensibility, elasticity
  - Hair roots, sebaceous and sweat glands, blood vessels



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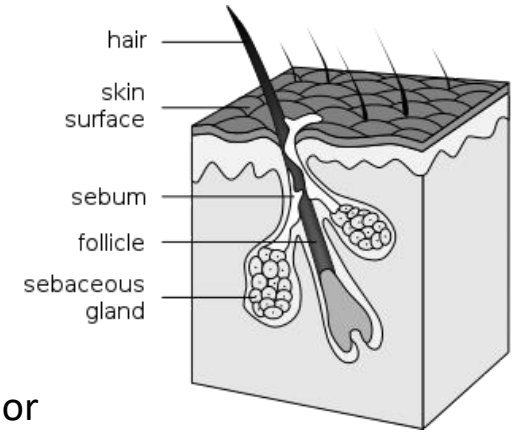
Appendages

- Hair follicles
- Sweat glands
- Sebaceous glands
- The appendages constitute a small percentage of the skin surface area. Generally thought that absorption via appendages is minor relative to skin



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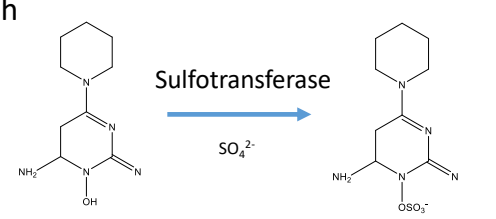
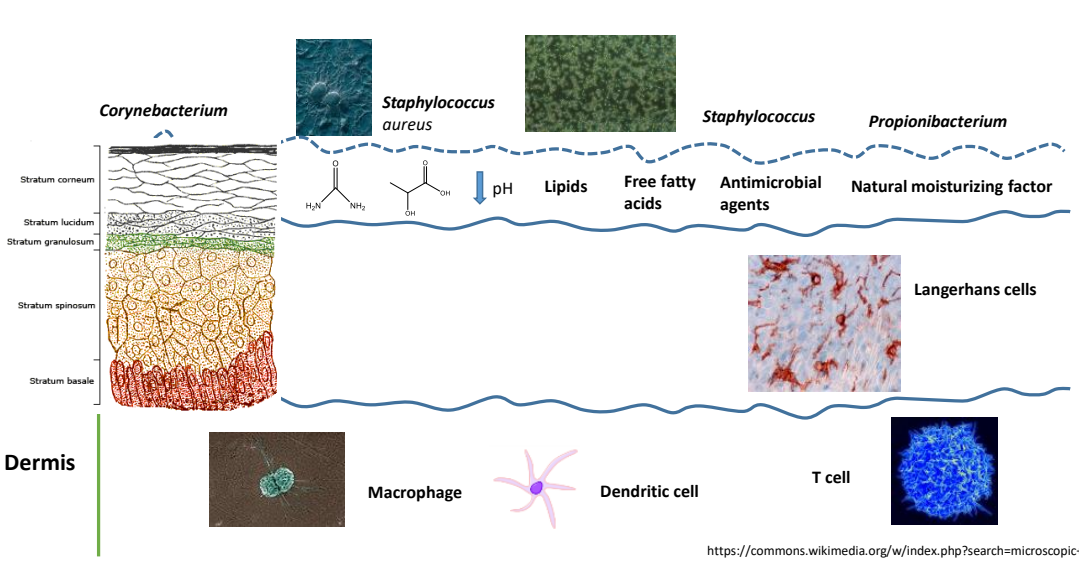
Metabolism in Skin

- Metabolism (Phase I)
  - Oxidative, reduction, esterase, hydrolytic activities
  - CYP450, FMO, COX—activities present, but less than in liver and other tissues
  - CYP450s inducibles—polycyclic aromatic hydrocarbons, TCDD
  - Role in sensitization with hapten formation
- Conjugation (Phase II)
  - Glucuronidation, sulfotransferase, glutathione transferase present
  - Minoxidil—sulfation required to stimulate hair growth
- Transporters (Phase III)
  - Detected
  - Research is developing
- Activities principally in epidermis



Minoxidil | Minoxidil Sulphate

Interconnected Barriers of the Skin



Barrier

Microbiome
Wards off "bad" bacteria

Chemical
Maintains hydration state and pH of skin; lipids hinder water loss

Physical
Stratum corneum and tight junctions between cells

Immune
Senses microbial danger signals, recruitment of immune cells, inflammation, and repair

Adapted from Eyerich et al., 2018

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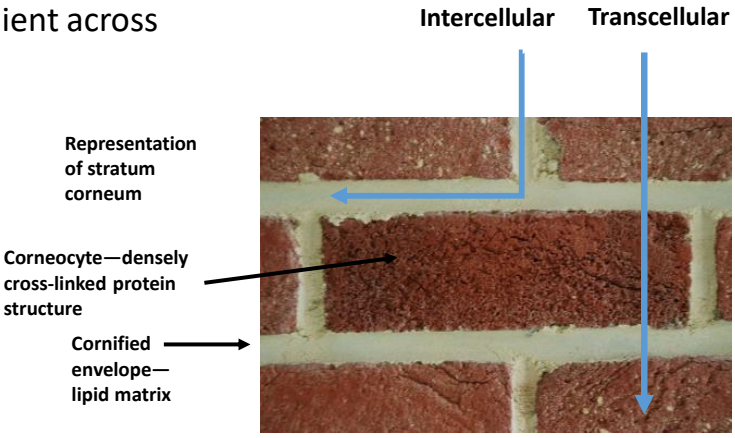
Percutaneous Absorption—Mechanism

- Diffusion
  - Driven by concentration gradient across the skin
    - Fick's 1st Law of Diffusion
    - At steady state:

$$J_{ss} = A*K_p*\Delta C$$

- Transcellular route
- Intercellular route
- Appendages

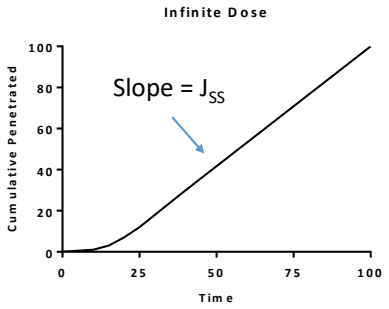


Fick's 1st Law of Diffusion

- $J_{SS} = A*K_p*\Delta C$
- J_{SS}—flux at steady state; A = area of exposed skin; K_p = permeability coefficient; ΔC = change in concentration between donor and receptor
- With K_p and contact time known, can estimate body burden

$M = A*K_p*C*T$ (M = mass absorbed; A = area exposed; C = chemical concentration on skin; T = time exposed)

- Caveats
  - Infinite dose technique—⇧ skin hydration results in ⇧ absorption
  - Most occupational or environmental exposures do not involve infinite dose concentrations

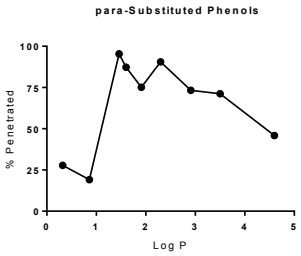


Percutaneous Absorption/Penetration

- Absorption—generally refers to xenobiotic that has diffused into skin and is detected there
- Penetration—generally refers to xenobiotic detected in blood, tissues, or excreta for *in vivo* dermal exposures; detected in media for *in vitro* dermal exposures
- Note—other investigators use different terminology. It is important to know investigator's terminology

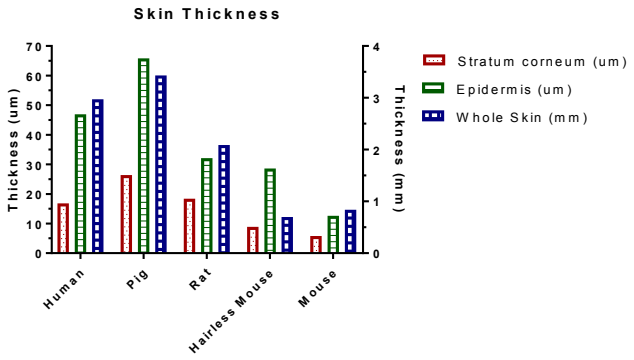
Factors That Affect Percutaneous Absorption

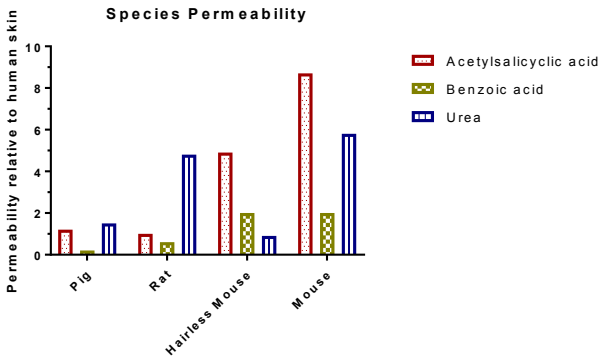
- Age of the exposed person—preterm infants (hexachlorophene); aged?
- Physicochemical characteristics of xenobiotic
  - Molecular weight, Log P, pH
  - Ionization
  - Binding properties
  - Solid or liquid
- Physicochemical characteristics of vehicle/matrix (e.g., soil)
- Hydration state of the skin— ↑ hydration → ↑ absorption
- Physical condition of the skin—damaged skin (cut, eczema) → ↑ absorption
- Potential for biotransformation
- Body site of exposure (skin thickness; thinner in scalp than palms of hands)
- Temperature— ↑ hydration → ↑ absorption
- Blood flow — ↑ circulation → ↑ absorption
- Contact duration, frequency of dosing, area of exposed skin, concentration of chemical



Hughes et al., 1993

Species Comparison for Absorption/Penetration





In general terms, rank order of species permeability: mouse > rat > pig ~ human

Bronaugh et al., 1982

Percutaneous Absorption—*In Vivo*

- Select species/strain
  - Rat and mouse are common, guinea pig has been used
- Chemical-specific
  - Is physical form of chemical solid, semisolid, liquid? Radiolabel (^{14}C preferred); if non-radiolabeled, method for detection? Dose? Vehicle? Volume of vehicle?
- Precondition animals in metabolism cages
- Clip dorsal hair on anesthetized animals
- 24 h later, anesthetize animal, dose a specific area on dorsal area (e.g., 1 cm^2), cover dosed area (nonocclusive, metal cap)
- Return animals to metabolism cages. Collect excreta over time

Percutaneous Absorption—*In Vivo*

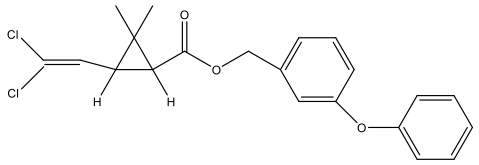
- End of experiment, euthanize animal, wash dose site, remove dosed skin, collect tissues for analysis, a day later tape strip dosed skin (e.g., 10 strips)
- Analyze collected excreta, tissues, wash, tape strips, dosed skin; can analyze for radiolabel, parent, metabolites
  - Unabsorbed dose—wash, tape strips 1–2 (1–3?)
  - Absorbed dose—tape strips 3–10, skin
  - Penetrated dose—excreta, tissues

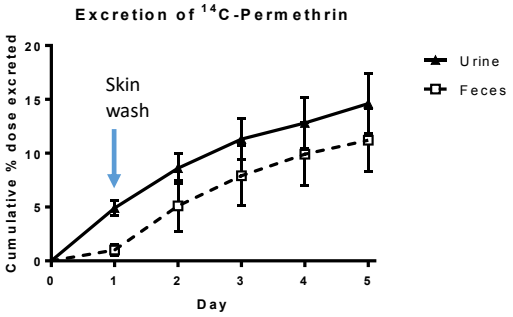
} Bioavailable

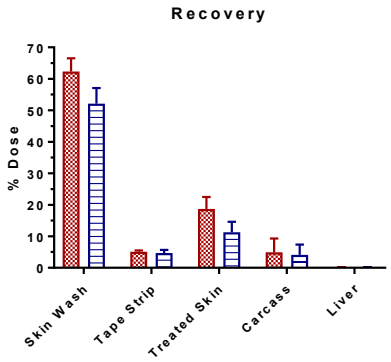
Guidelines to consider:
OECD 2004a
US EPA 1991

Percutaneous Absorption—*In Vivo*

Permethrin ^{14}C; 312.5 nmole/cm^2
MW—391.3 g/mol; Log P 6.5
Male Long-Evans rats; dorsal
Skin wash at 24 hr post-exposure
Euthanasia at 24 or 120 hr



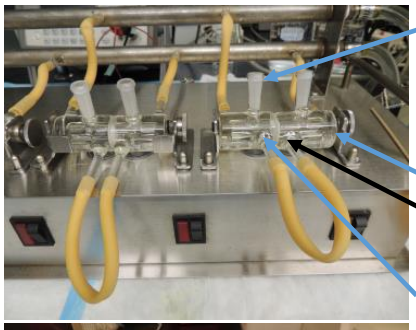




% dose in lung, brain, kidney, fat lower than in liver, except for fat at 120 hr, 0.18%

Hughes and Edwards 2016

Percutaneous Absorption—*In Vitro*



Sampling port

Side-by-side diffusion cells

Donor cell

Skin (epidermal side faces right)

Receiver cell

Flow-through cells (Bronaugh)

Sampling port

Franz (static) cell

Skin (epidermal side up)

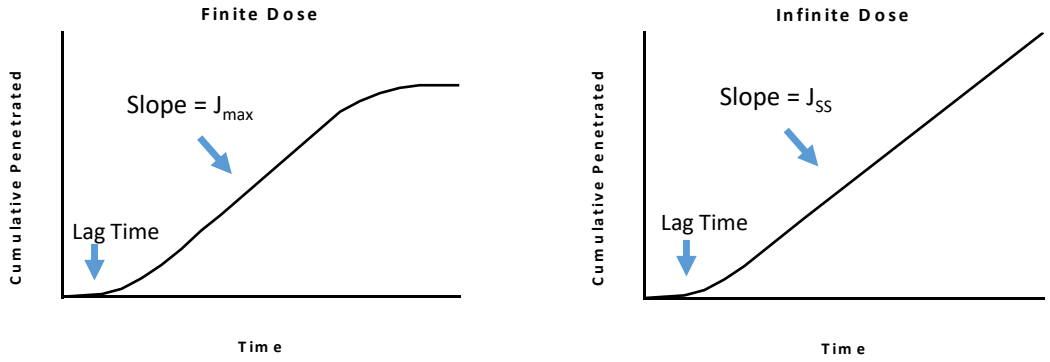
Fraction collector for flow-through cells

Peristaltic pump

Heated block for cells

Percutaneous Absorption—*In Vitro*

- Finite dosing—limited amount of chemical available
- Infinite dosing—constant amount of chemical available



Percutaneous Absorption—*In Vitro*

- Select method of dosing and apparatus type
  - Infinite—side-by-side, flow through, and static
  - Finite—flow-through or static diffusion cells
  - Assure test chemical soluble in receptor fluid (sink conditions); add FBS to maintain viability for metabolism; add FBS or other chemical to aid in solubility in receptor fluid
- Select species/strain
  - Rat, mouse, guinea pig are common; human skin can be used (cadaver or surgical donation)
- Chemical-specific
  - Is physical form of chemical solid, semisolid, or liquid? Radiolabel (^{14}C preferred); if non-radiolabeled, method for detection? Dose? Vehicle?
- Clip dorsal hair on anesthetized animals
- 24 h later, euthanize animal, removed clipped skin, dermatome**, and mount in apparatus with epidermal surface facing donor side

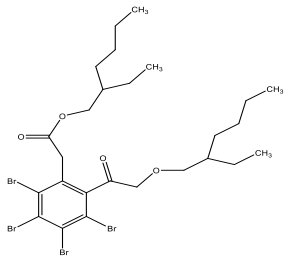
Percutaneous Absorption—*In Vitro*

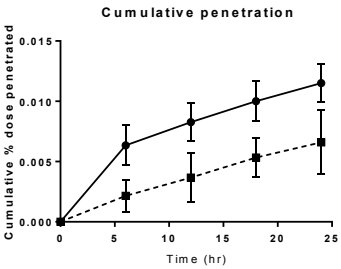
- Check (human) skin for barrier integrity—^3H_2O, electrical resistance, TEWL
- Dose skin (or donor cell in infinite study) and collect media (receiver) samples over time
  - Finite—minimize vehicle volume (ca. 10 µl/cm^2)
- End of experiment
  - Infinite—can stop when steady state has been attained over a period time
  - Finite—wash skin and cell top, a day later tape strip dosed skin (e.g., 10 strips); wash cell body
- Analyze media samples, wash, tape strips, dosed skin; can analyze for radiolabel, parent, metabolites
  - Infinite—analyze media samples to determine cumulative amount penetrated, calculate J_{ss}
  - Finite
    - Unabsorbed dose—skin wash, cell top wash, tape strips 1–2 (1–3?)
    - Absorbed dose—tape strips 3–10, skin, cell body
    - Penetrated dose—media

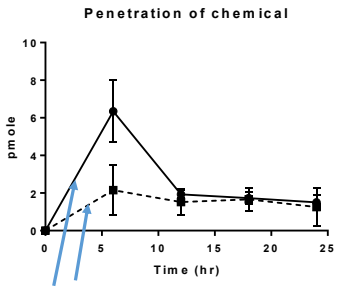
Guideline to consider: OECD 2004b

Percutaneous Absorption—*In Vitro* (Finite)

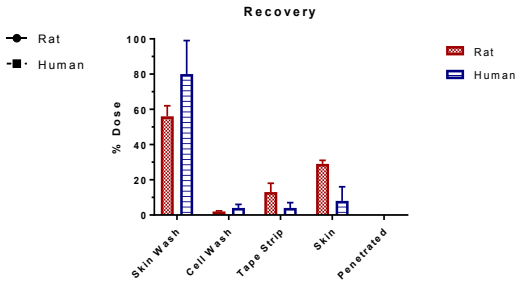
Bis(2-ethylhexyl) tetrabromophthalate (^{14}C)
MW = 706.1 g/mol
Log P = 11.95 (estimated)
Dose = 100 nmole/cm^2; area = 0.64 cm^2
Flow-through system







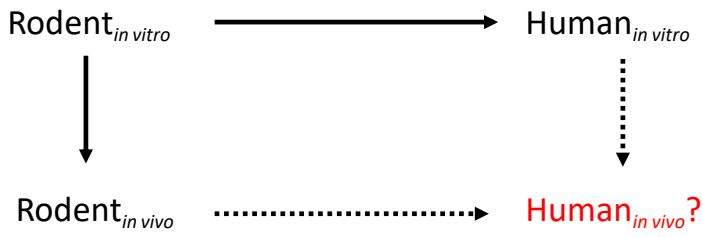
Calculate slope to determine J_{max}



Knudsen et al., 2016

Parallelogram Approach

- A method to estimate human *in vivo* percutaneous absorption



$$Human_{in\ vivo} = (Rodent_{in\ vivo}/Rodent_{in\ vitro}) \times Human_{in\ vitro}$$

Parallelogram Approach—Estimated Penetration of Permethrin

	% Dose penetrated		
Species/study	Permethrin—2 µg/cm²	Permethrin—20 µg/cm²	Permethrin—200 µg/cm²
Human/*in vitro*	1.3 ± 1.1	2.7 ± 3.6	2.1 ± 1.7
Rat/*in vitro*	20 ± 9	18 ± 5	24 ± 8
Rat/*in vivo*	22 ± 8	22 ± 9	29 ± 9

$$Human_{in\ vivo} = (Rodent_{in\ vivo}/Rodent_{in\ vitro}) \times Human_{in\ vitro}$$

2 µg/cm²: $Human_{in\ vivo} = (22/20) \times 1.3 \times 100 = 1.4\%$

20 µg/cm²: $Human_{in\ vivo} = (22/18) \times 2.7 \times 100 = 3.3\%$

200 µg/cm²: $Human_{in\ vivo} = (29/24) \times 2.1 \times 100 = 2.5\%$

Estimated human penetration of permethrin: 1–3% of applied dose

0.5% dose of permethrin (1250 mg) medically applied to skin excreted in urine over 48 h

Ross et al., 2011
Van der Rhee et al., 1989

Summary

- The skin is a complex organ with living and dead cells that have a role in its overall function
- Chemicals that contact the skin have the potential for absorption and penetration into the systemic circulation. However, there are many exposure, chemical, and biological factors that determine the extent of the absorption/penetration
- Stratum corneum is the main barrier to chemical absorption/penetration
- Dermal absorption/penetration is a process of diffusion
- Experimental *in vivo* and *in vitro* methods are used to estimate human *in vivo* dermal penetration
  - Rodent skin tends to be more permeable to chemicals than human skin

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Dermal Toxicity: Hazardous Chemical Exposure Assessment and Animal Models

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Conflict of Interest Statement

- I have no conflicts of interest to disclose
- The contents of this presentation represent the views of the presenter

Presentation Overview

- Introduction to chemical threat agents and a wide variety of occupational, accidental, or intentional exposures
- Overview of the dermal toxicity from direct chemical exposures as well as dermal effects from systemic exposures
- Biomarkers of skin chemical agents' exposure
- Animal and *in vitro* tissue models of skin chemical exposure

In the present world scenario, with technological advances and increasing industrialization, we are constantly under threat

Mammalian skin acts as the first line of defense, providing a physical barrier that protects the body from the deleterious effects of noxious chemicals

https://health.mo.gov/living/environment/hazsubstancesites/healtheffects.php

Occupational/accidental/deliberate exposure of skin to hazardous chemical agents

Chemical Agents

Environmental Agents: Dermal Toxicity

- **Air Pollution**
  - Ultraviolet radiation: *skin aging and skin cancer*
  - Particulate matter, cigarette smoke (polycyclic aromatic hydrocarbons), gases (ozone, etc.): *premature aging, increase in psoriasis, acne, and skin cancers, pigmentation changes, and cancer*
  - Volatile organic compounds (organic solvents in paints, varnishes [aliphatic hydrocarbons, ethyl acetate, glycol ethers, methylene chloride, and acetone], environmental tobacco smoke, stored fuels, exhaust from cars [benzene] and emissions from industrial facilities [tetrachloroethylene]): *atopic dermatitis and skin burns*

Chemical Agents

Environmental Agents: Dermal Toxicity

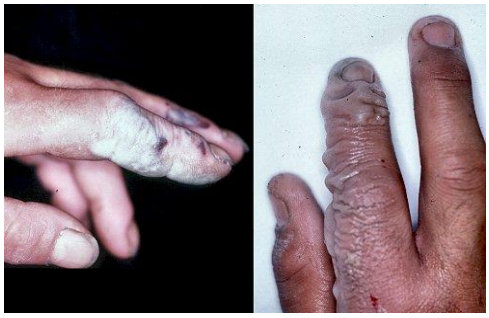
- **Household products and chemicals**
  - formaldehyde, acids, chlorine, ethylene glycol, sodium hypochlorite, ammonia, etc. (cleaners, etc.): *contact and irritation dermatitis, skin burns, scarring*
  - Cosmetics
- **Water pollution**
  - chlorine, other chemicals, acids, etc. (fertilizers and pesticides; sewage; industrial and hazardous chemical waste): *chloracne, skin burns, corrosion*
- **Pesticides**
  - Organophosphates, glyphosate, chloropicrin, parathion, maneb: *irritant or allergic contact dermatitis*

https://en.wikipedia.org/wiki/Environmental_impact_of_agriculture

Chemical Agents

Industrial Agents: Dermal Toxicity

- **Accidental Exposure**
  - Ammonia (fertilizer, petroleum, mining, food, cold storage, etc.): *skin irritation, corrosive injury, and burns*
  - Hydrogen fluoride (refrigerators, air conditioners, glass, petroleum, etc.): *corrosive burn*
  - Other acids and alkali: *skin burns*
  - Chemicals (metals, epoxy and acrylic resins, rubber additives, and chemical intermediates): *contact dermatitis or urticaria*



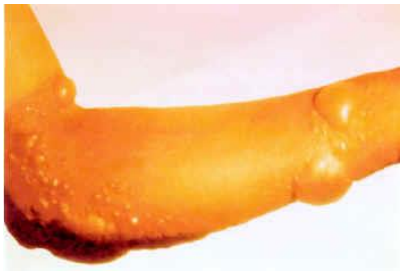
https://commons.wikimedia.org/wiki/File:HF_burned_hands.jpg

Chemical Agents

Chemical Warfare Agents: used as potential weapons of mass destruction to cause a catastrophic medical disaster

- **Vesicating Agents**
  - Strong alkylating agents that affect skin, eyes, mucus membranes, and internal organs (sulfur mustard, nitrogen mustard [HN-1, 2, and 3], lewisite [L], phosgene oxime [CX])

Direct skin effects
Edema, erythema, severe blistering, and damage to skin leading to long-term effects
Systemic effects: Leukopenia → secondary infections → higher mortality rates and carcinogenic effects



https://en.wikipedia.org/wiki/File:Blister-arm.jpg

Dermal Toxicity from Direct Skin Exposure and Chemical Absorption

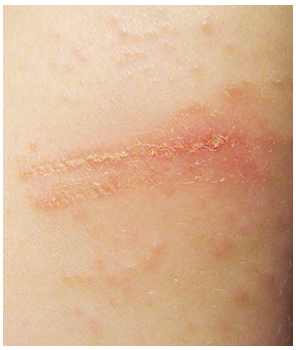
- **Direct skin effects and immune-mediated skin effects**
  - Contact dermatitis
  - Urticaria **(possible skin effect from systemic toxicity)**
  - Psoriasis
- **Skin effects from systemic exposure** (arsenic exposure)
- **Systemic effects**
  - Health problem in the body due to absorption of chemical through the skin
    - Toxicity to the immune system, nervous system, or respiratory system
    - Bladder, liver, or kidney damage
- **Cancer**

Contact Dermatitis

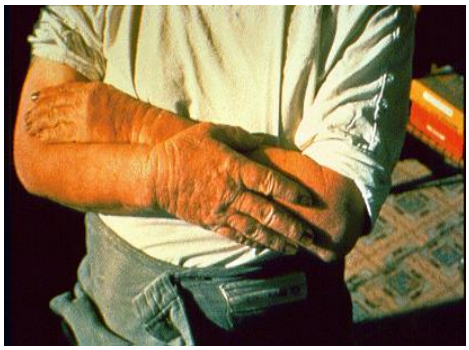
- **Irritant contact dermatitis**
  - Most common
  - Develops quickly when skin comes in contact with a strong irritant/chemical
  - Skin is repeatedly exposed to a mild irritant
  - Skin barrier breaks, causing inflammation
- **Allergic contact dermatitis**
  - Occurs when skin becomes sensitive to a certain substance (allergen)
  - A delayed skin reaction that typically develops 12 to 72 hours after exposure
  - More common in women due to nickel and acrylate allergy
  - Especially common in metal workers, hairdressers, beauticians, health care workers, cleaners, painters, and florists

Contact Dermatitis: Mechanism and Symptoms

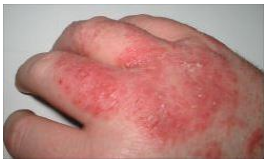
- Due to skin barrier disruption, epidermal cellular changes and release of mediators of inflammation (cytokines) predominantly from epidermal cells (keratinocytes)
- **Acute dermatitis**
  - Red, irritated skin (erythema)
  - Itching
  - Swelling (edema)
  - Bumps or blisters
  - Hot or tender skin
  - Scaling
- **Chronic dermatitis**
  - lichenification
  - hyperkeratotic scale
  - fissures or ulcerations



https://en.wikipedia.org/wiki/Contact_dermatitis



https://upload.wikimedia.org/wikipedia/commons/a/af/Hands_damaged_by_kerosene.jpg



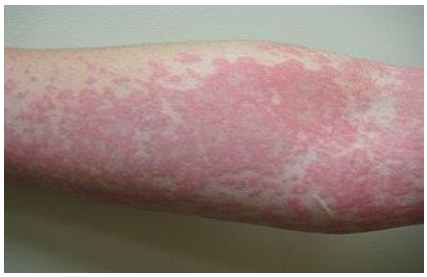
https://commons.wikimedia.org/wiki/Category:Dermatitis

Contact Dermatitis: Diagnosis and Treatment

- Patch test to identify the allergen
- Removal of etiologic agent
- Wet dressings with Burow's solution
- Large vesicles can be drained
- Topical steroid creams
- Oral corticosteroids to reduce inflammation
- Antihistamines to relieve itching
- Antibiotics to fight a bacterial infection

Urticaria

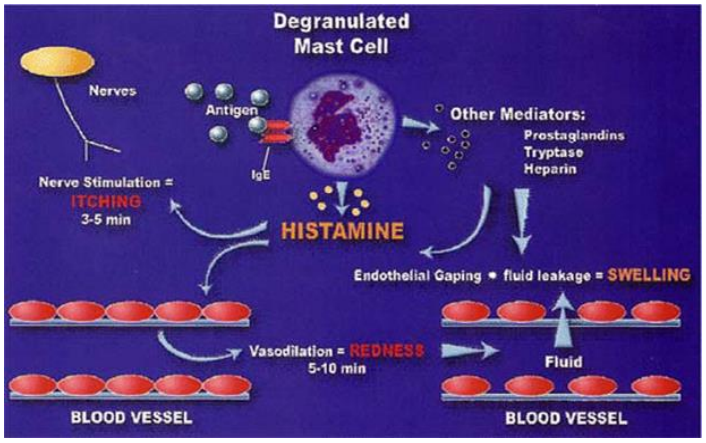
- Urticaria ("hives") may affect up to 20% of population at some time
- Acute urticaria—more common in children, chronic urticaria—more common in adults
- It can be triggered by various chemical exposures or allergies, including food, medications, stings, plants, etc.
- Starts as an itchy patch of skin that turns into swollen red wheals with clear edges
- Blanching of hives and angioedema
- Can be accompanied with **anaphylaxis,** a rare, potentially life-threatening reaction that impairs breathing and can send the body into shock



https://en.wikipedia.org/wiki/Solar_urticaria

Urticaria: Mechanism and Treatment

- **Mechanism**
  - Vasodilatation
  - Increased vascular permeability
  - Following release of mediators by mast cells
  - Individual lesions could resolve within 24 hours



https://www.clspectrum.com/issues/2007/april-2007/treating-allergy-in-contact-lens-patients

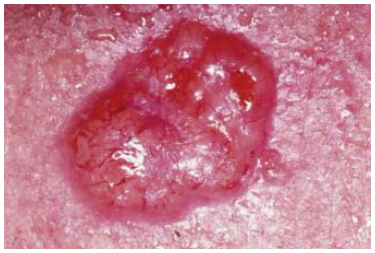
- **Treatment**
  - Antihistamines
  - Intranasal corticosteroid nasal sprays
  - Combination therapies
  - Adrenaline (epinephrine)—as a first-aid treatment in anaphylactic shock

Skin Cancer

- Caused by chemical exposures, including arsenic, coal tars, mineral oils, sunlight (UV radiation)
- DNA damage in cells can lead to cancer
- **Major Types:**
  - basal-cell skin cancer (BCC)—the most common type of skin cancer; is caused by damage to basal epidermal cells that sit just below the skin's surface
  - squamous-cell skin cancer (SCC)—cancer of the flat cells on the epidermis (surface) of the skin
  - melanoma—damage to melanocytes, the cells that produce melanin and give color to the skin

Skin Cancer

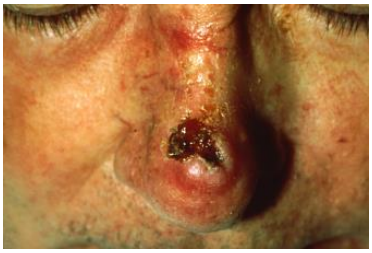
Basal Cell Carcinoma



https://commons.wikimedia.org/wiki/Category:Basal-cell_carcinoma#/media/File:517_Basal_Cell_Carcinoma.jpg

- Rarely fatal
- Pink bumps
- Pearly or waxy appearance
- Sunken center
- Irregular blood vessels on surface
- Bleed easily after injury

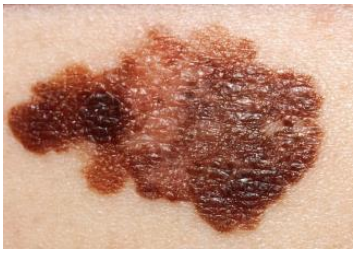
Squamous Cell Carcinoma



https://commons.wikimedia.org/wiki/File:Squamous_Cell_Carcinoma.jpg

- May spread or reoccur
- Raised, dull-red skin lesion
- Thick crusted scale
- Ulcerated appearance

Melanoma



https://commons.wikimedia.org/wiki/Category:Melanoma#/media/File:Melanoma.jpg

- Most dangerous
- If undiagnosed, can spread
- A change in an existing mole
- The development of a new pigmented or unusual-looking growth on skin

Skin Cancer: Diagnosis and Treatment

- **Diagnosis**
  - Noninvasive photography techniques
  - Biopsy
  - Histopathological examination
- **Treatment**
  - Chemotherapy (Imiquimod)
  - Radiation
  - Photodynamic therapy
  - Cryotherapy
  - Surgery
  - For metastatic melanoma: biologic immunotherapy agents ipilimumab, pembrolizumab, and nivolumab; BRAF inhibitors, such as vemurafenib and dabrafenib; and a MEK inhibitor, trametinib

Biomarkers of Dermal Toxicity

- Identify the progress of dermal injury
- Identification of medical countermeasures
- Evaluation of the potential toxicity of a substance
- Analysis of the risk presented by exposure
- Understanding of the mechanisms that underlie the associations environmental exposures and development of injury and disease

Important biomarker groups:

- Biomarkers of exposure (provide information regarding the extent and relative toxicant dose)
- Biomarkers of effect (physiological effects: histological/ultrastructural)

Biomarkers of Dermal Toxicity

- Structural and distinct physiological changes induced by numerous dermal toxins have been well characterized
  - **Clinical**
    - Draize scoring of edema, erythema, skin-bi-fold thickness, lesions, necrosis, ulceration, scarring
  - **Biological**
    - Histopathological analysis
- Biochemical events accompanying the progression of dermal toxicity remain to be characterized
- Identification of molecular markers released from the injured tissue is helping to understand the complex toxicity events
  - **Molecular**
    - Immunologic markers (Cytokine profiles, etc.)
    - Metabolic markers (Oxidative stress and inflammatory mediators)

Biomarkers (important study endpoints) following chemical threat agent nitrogen mustard exposure of mice and their optimum injury time

Primary Endpoints	Time of Optimum NM skin injury
1. Epidermal thickness	24, 72 and 120 h
2. Microvesication	12, 24, 72 and120 h
Secondary Endpoints	
1. MPO assay	24 and 72 h
2. DNA damage (H2A.X phosphorylation)	12, 24 and 72 h
3. Inflammatory mediator (COX-2)	120 h
Other Important Endpoints	
1. Erythema and edema (clinical scores)	24 h
2. Wounding , pigmentation change and xerosis (clinical scores)	72 and 120 h
3. Epidermal death and denuding	72 and 120 h
4. Outgrowths or Scab formation	72 h
5. Parakeratosis	72 and 120 h
6. Hypercornification, acanthosis and re-epithelialization	120 h, 120 h, 72 and 120 h
7. Dermal necrosis	72 h
8. Apototic cell death (TUNEL)	12 and 24 h
9. Epidermal cell proliferation (Ki 67)	12 and 24 h
10. Mast cells	12, 24 and 72 h
11. Macrophages	12 and 24 h
12. Phospho-ERK MAPK (western)	12, 24 and 120 h
13. Phospho-AKT (Western)	12, 24 and 120 h
14. TNF alpha levels (western)	12, 24 and 120 h
15. MMP-9 levels (western)	72 h

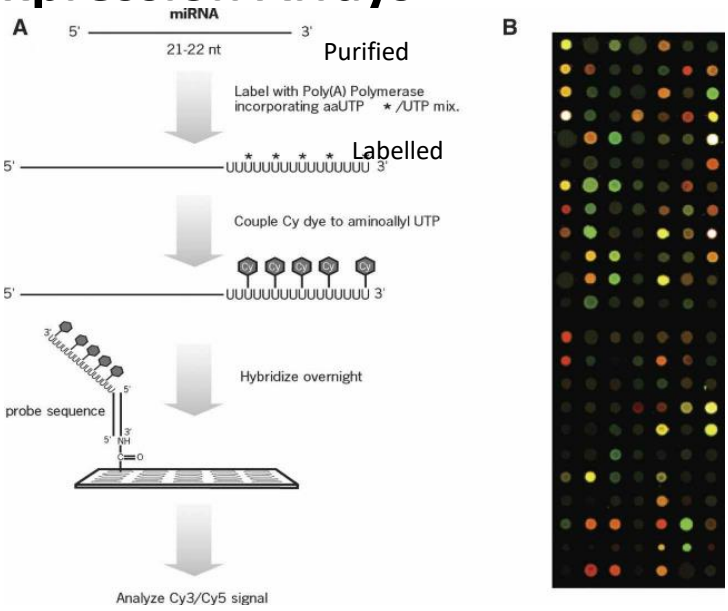
"High-Throughput" Technologies to Identify Biomarkers of Skin Chemical Exposures

Reflect biochemical events underlying the continuum of injury to healing, including initial cellular damage effects, water loss and pH changes inflammatory and debridement stages, the resolution of inflammation, repair, and tissue regeneration

- Toxin-responsive genes at the whole genome level with gene expression microarrays—**microRNA expression arrays**
- Effects of toxins on the:
  - **Transcriptome**
  - **Proteome**
  - **Metabolome**
  - **Epigenome**
  - **Analysis of the microvesicles released following toxic injury**

microRNA Expression Arrays

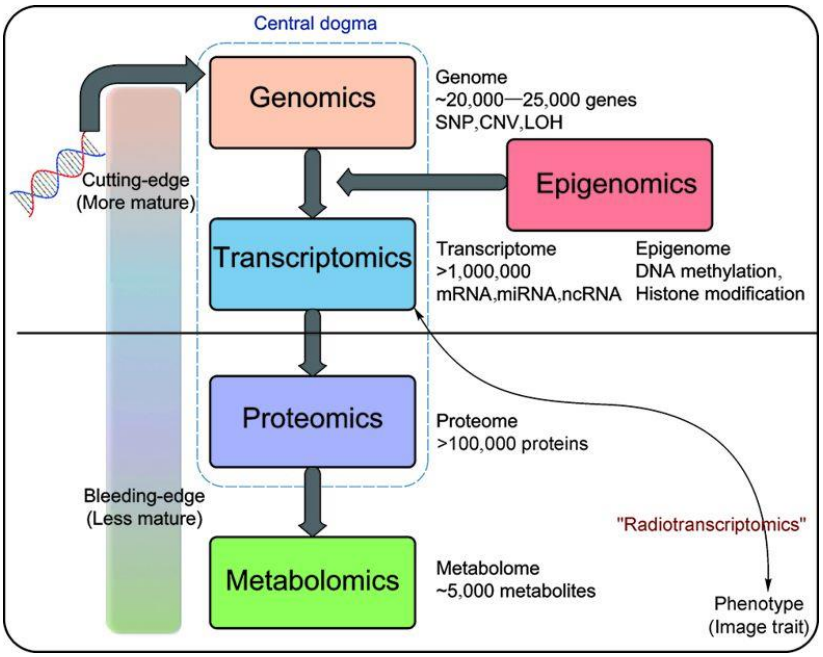
- Microarray technology is a powerful high-throughput tool capable of monitoring the expression of thousands of small non-coding RNAs at once within tens of samples processed in parallel in a single experiment
- GeneChip™ miRNA Arrays are powerful tools for studying the role of small non-coding RNA (miRNA, snoRNA, and scaRNA) in complex diseases, including skin toxicity



https://www.researchgate.net/figure/Overview-of-the-miRNA-microarray-procedure-RNA-species-smaller-than-40-nt-are-purified_fig1_7701176

Overview of the miRNA microarray procedure.

"'Omics Tools"



'Omics technologies are primarily aimed at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) in a specific biological sample

https://link.springer.com/article/10.1007/s40484-016-0061-6

Systems Biology Approach and Biomarker Discovery

Effective in prognosis or diagnosis, assessment of severity and response to therapy in a number of clinical disease states as well as therapeutic drug monitoring

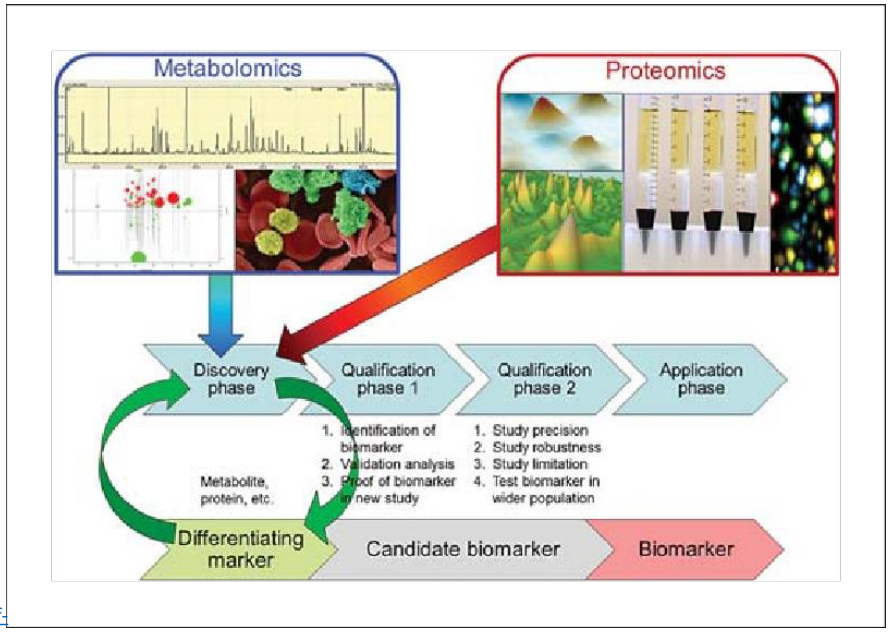
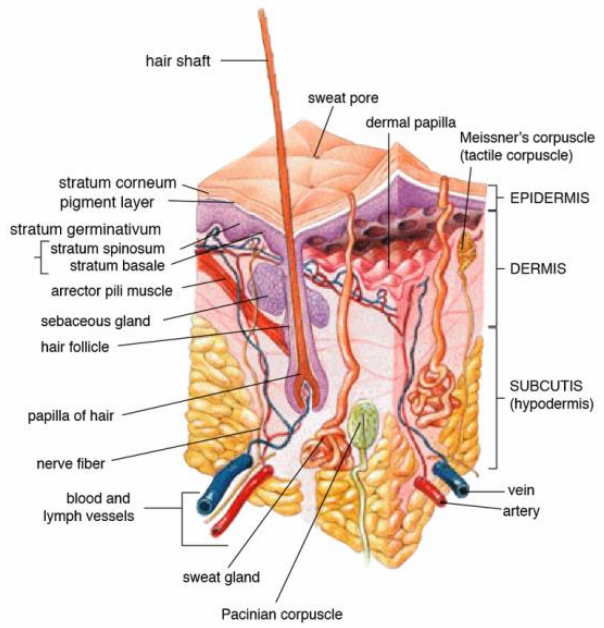


Figure adapted from Koulman et al., https://www.researchgate.net/figure/Application-of-omics-techniques-in-biomarker-discovery-Omics-technologies-are-primarily_fig4_266476687

Application of 'omics techniques in biomarker discovery

Toxicology studies employing animals and *in vitro* cellular or tissue preparations have been used to study the toxic effects and mechanism of action of chemicals on human skin, develop biomarkers, and determine the effective and safe dose of drugs in humans and the risk of cutaneous toxicity from chemical exposures

Human Skin



https://en.wikipedia.org/wiki/Sebaceous_gland#/media/File:Skin.png

Dermal Toxicity: *In Vivo* Models

- *In vivo* models have been and will continue to be used in the study of chemical absorption, skin toxicity studies, and development of therapeutic strategies to protect against chemical exposure
- *In vivo* studies remain practical experimental alternatives because they are easier to obtain, less subject to regulation, and have less intersubject variability due to inbred animals
- To obtain most predictive data of human skin toxicity—animal model's physiology, biochemistry, and anatomy of skin should be similar to humans
- Animal models:
  - Monkeys
  - Rats
  - Rabbits
  - Guinea pigs
  - **Pigs**
  - **Mice (haired and hairless)**

Pig Animal Model

- Appropriate animal model for human skin studies, both *in vivo* and *in vitro*
- Similarities between porcine and human skin:
  - Histological appearance of epidermis
  - Follicular structure and dermis
  - Filament density and epidermal-dermal junction
  - Number, size, distribution, and communications of the dermal blood vessels
  - Architecture and thickness of collagen fibers and fiber bundles
  - Biochemical similarities
  - Enzyme patterns
  - Thickness of skin layers

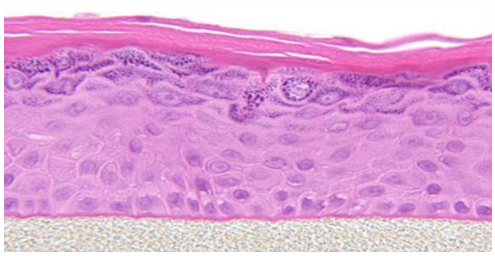
Rodent Models

- Hairless guinea pig
  - Has a multilayer epidermis similar to human skin
  - Has normal numbers of large follicular units with sebaceous glands, hairs are not produced
- Hairless mouse
  - Outbred SKH1 mice are the most widely used in dermatologic research
  - Unpigmented and immunocompetent mice allow for ready manipulation of the skin, application of topical agents, and exposure to UVR, as well as easy visualization of the cutaneous response
  - Wound healing, acute photobiologic responses, and skin carcinogenesis have been extensively studied in SKH1 mice and are well characterized

In Vitro Skin Models

- EpiDerm, EpiDermFT, and MelanoDerm *in vitro* human skin tissue equivalents can be used to assess the toxicity of chemicals and safety of topically applied products

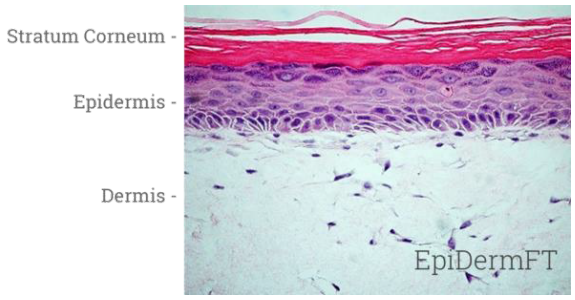
EpiDerm



https://www.mattek.com/products/epiderm/

EpiDerm is a ready-to-use, highly differentiated 3D tissue model consisting of normal, human-derived epidermal keratinocytes (NHEK) cultured on specially prepared tissue culture inserts

EpiDermFT



https://www.mattek.com/products/epidermft/

Consists of normal, human epidermal keratinocytes (NHEK) and normal, human dermal fibroblasts (NHFB) cultured to form a multilayered model of the human dermis and epidermis

Summary

- The skin is the body's largest organ and is maximally exposed to chemical threats
- Chemical agents are the main cause of accidental, occupational, and deliberate skin diseases
- Dermal exposure to chemicals can result in a wide range of other adverse health effects, including direct skin effects and immune-mediated effects
- Biomarker development and studies in relevant skin toxicity models are very important in the diagnosis, in toxicity studies, and in countermeasure development

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Thank You

- Dermal Toxicology Specialty Section
- Association of Scientists of Indian Origin Special Interest Group
- Alexander Suvorov and Kevin Merritt: CE Committee Liaisons
- SOT CE Committee

Ocular Anatomy and Manifestations of Ocular Toxicity

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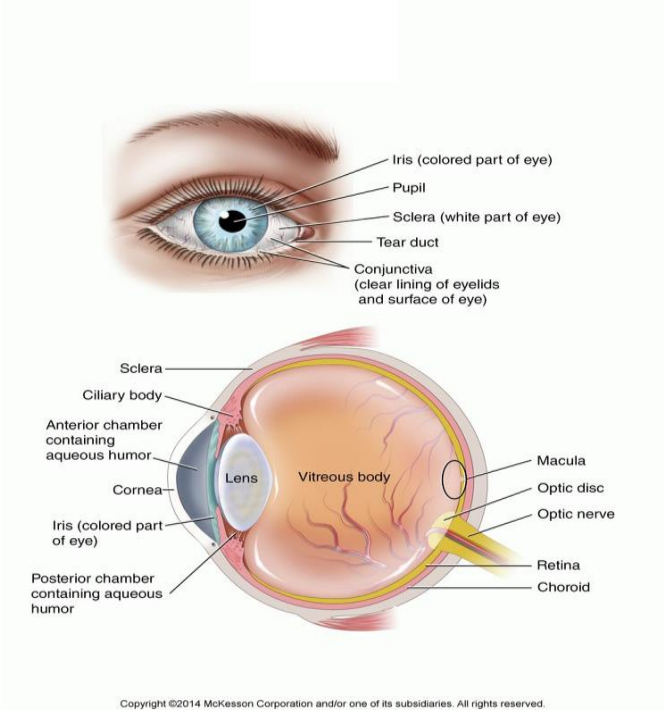
Conflict of Interest

- The presenter declares no conflict of interest

The Eye



Aqueous Humor

Aqueous humor: optically clear, slightly alkaline liquid that occupies the anterior and posterior chambers of the eye (the space in front of the iris and lens and the ring-like space encircling the lens).

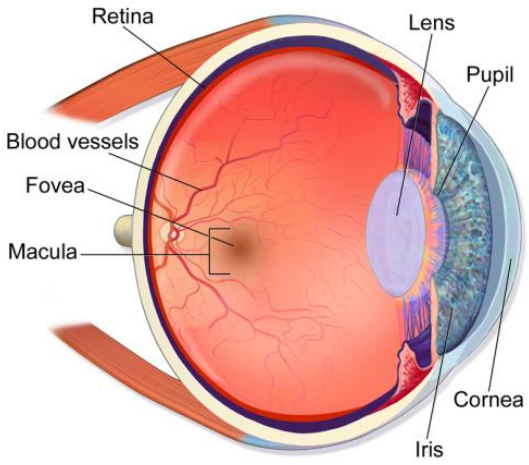
The aqueous humor resembles blood plasma in composition but contains less protein and glucose and more lactic acid and ascorbic acid. It provides these nutrients (as well as oxygen) to eye tissues that lack a direct blood supply (such as the lens) and also removes their waste products. In addition, it provides an internal pressure, known as intraocular pressure, that keeps the eyeball (globe) properly formed.

Aqueous humor is formed from the blood by filtration, secretion, and diffusion through the ciliary body, a muscular structure located behind the iris that controls the curvature of the lens. Aqueous humor leaves the eye through the porous trabecular meshwork and flows into Schlemm's canal, a ring-like passageway around the outer angle of the anterior chamber in front of the iris. From the canal the liquid enters the veins.

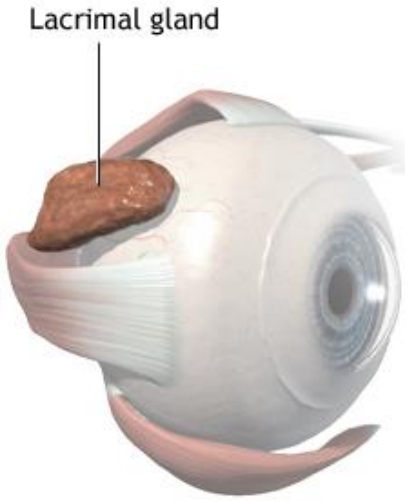
Vitreous Humor

The vitreous humor has a viscosity two to four times that of water, giving it a gelatinous consistency. This humor is a stagnant (immobile) fluid that is not served by any blood vessels and is not actively regenerated or replenished. (This is in contrast to the aqueous humor, which fills the anterior chamber in front of the lens.) Light rays pass through this dense, transparent, gel-like substance, which fills the globe of the eyeball and helps the eye hold its spherical shape. Problems with the vitreous humor may ultimately lead to detachment of the retina from the back wall of the eye, which may require surgery. Retinal detachment can result in permanent loss of vision.

Anatomy of the Eye

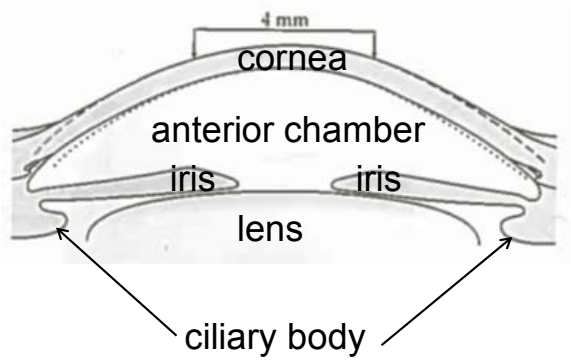


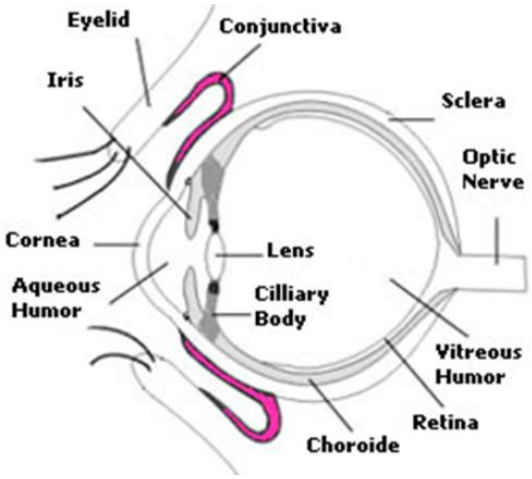
Eye Anatomy



Anatomy of the Eye

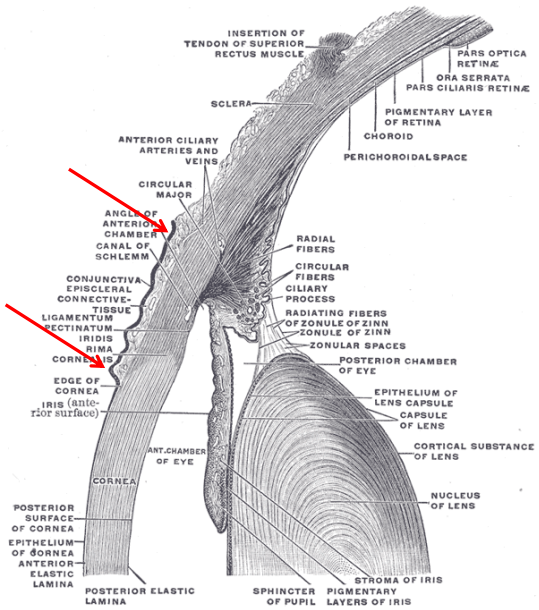
- Conjunctiva and Lacrimal gland
- Cornea
- Iris
- Lens
- Retina
- Optic Nerve



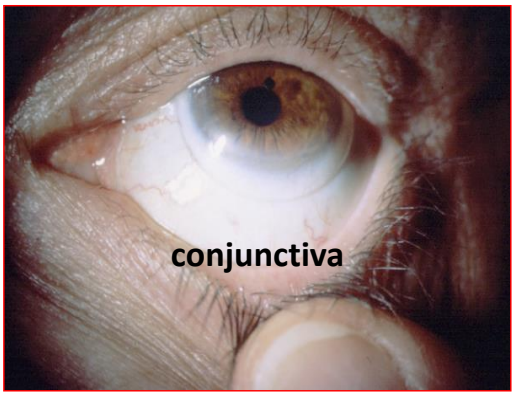


The central 4 mm of the cornea is the "optical zone." It has a near constant radius curvature.

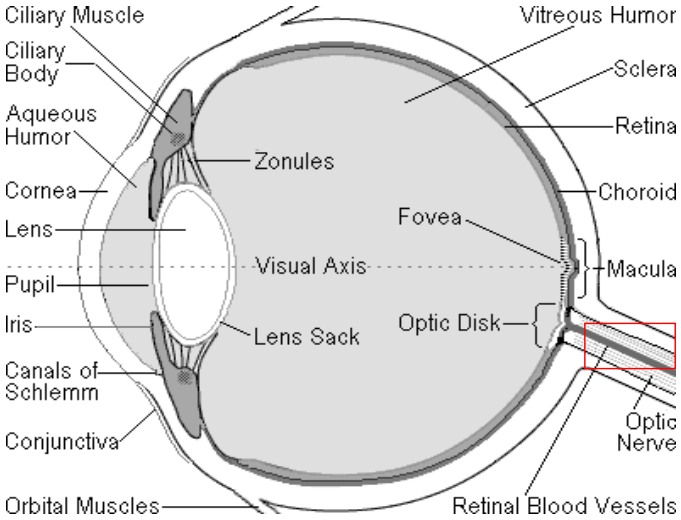
The Conjunctiva



The conjunctiva lines the inside of the eyelids and covers the sclera (white part of the eye). It is composed of nonkeratinized, stratified columnar epithelium with goblet cells.

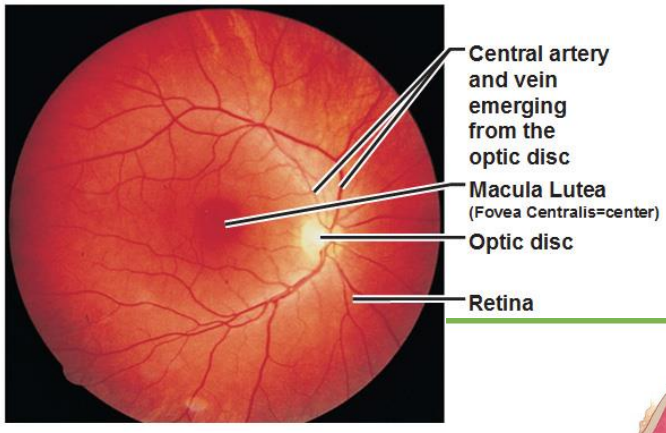


Anatomy of the Human Eye



The fovea is located in the center of the macula of the retina, an oval-shaped pigmented area near the center of the retina.

The fovea (Latin for *pit*) is a small, central pit in the eye composed of DENSELY packed cones (with no rods).



Macula means “spot”

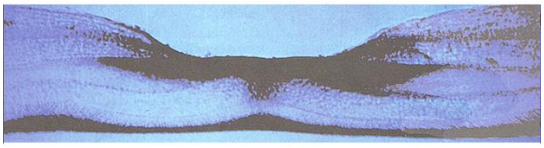
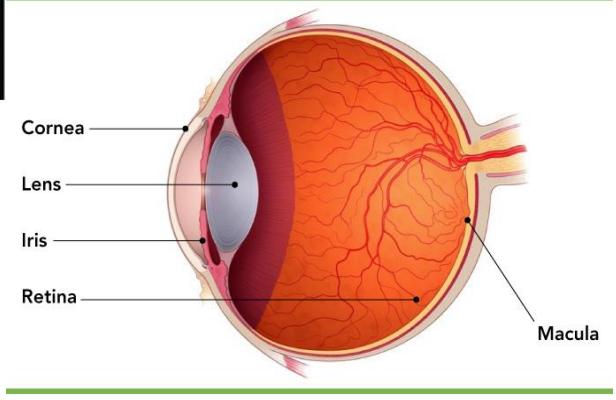
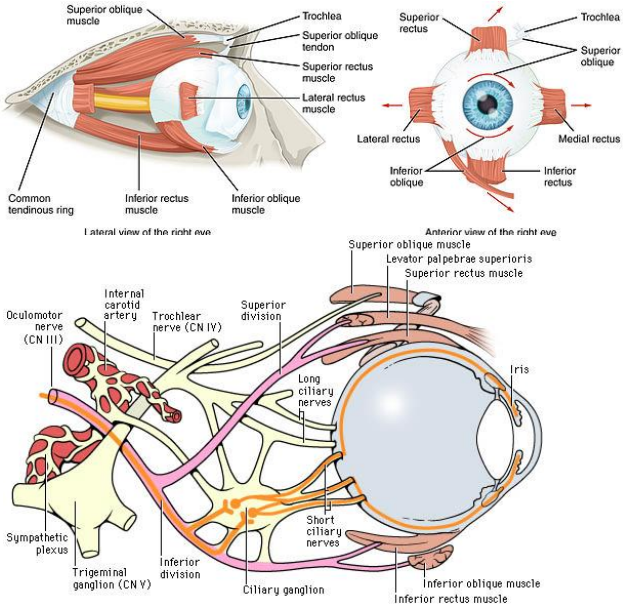


Fig. 15. Vertical section through the monkey fovea to show the distribution of the macula lutea (black). From Snodderly et al., 1984.



Supporting Structures

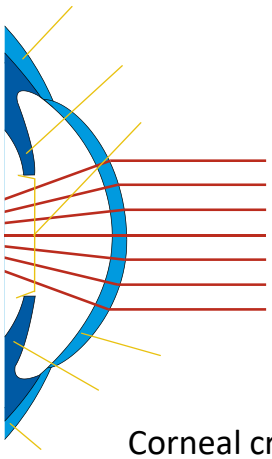


<u>Muscles</u>
Involuntary (e.g., iris muscles change pupil size)
Voluntary (e.g., extraocular muscles move eyes)

<u>Blood vessels</u>
Transport nutrients and oxygen in and waste out
But remember the cornea has no blood vessels

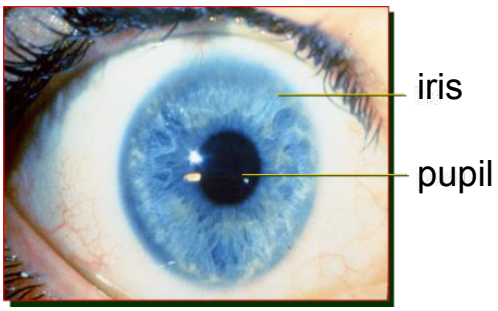
<u>Nerves</u>
Corneal nerves are extremely sensitive to touch—3–4x more sensitive than our fingertips

The Cornea



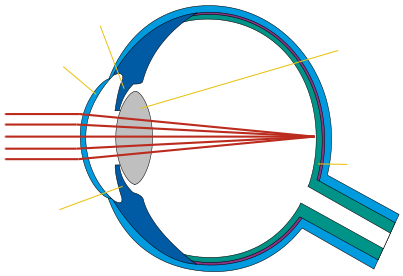
- Front outer surface of the eye
- Transparent tissue
- Refracts (bends) light—it does ~75–80% of the refraction

Corneal crystallins (enolase and aldolase) have enzymatic functions in other tissues. When densely packed into the cornea, they do not act as enzymes, but instead confer transparency to the cornea. The enolase and aldolase gene promoters have HIF-1 binding sites.

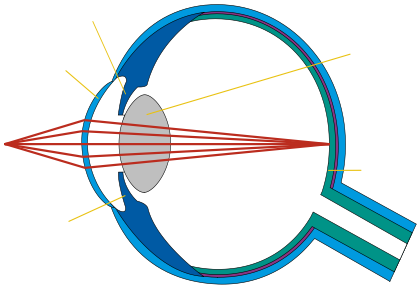


The Crystalline Lens

DISTANCE VISION

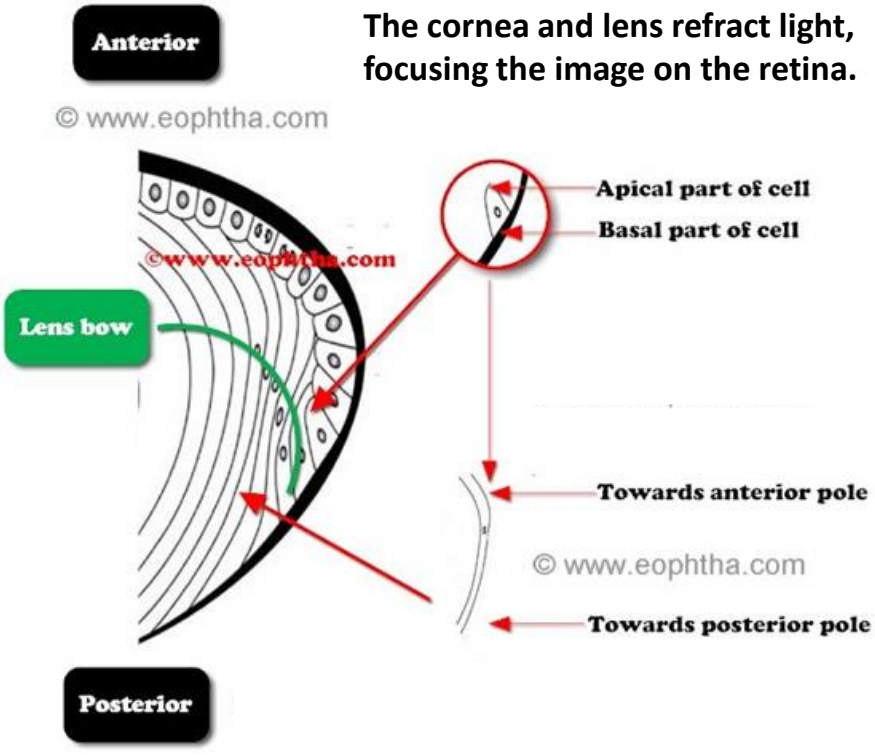


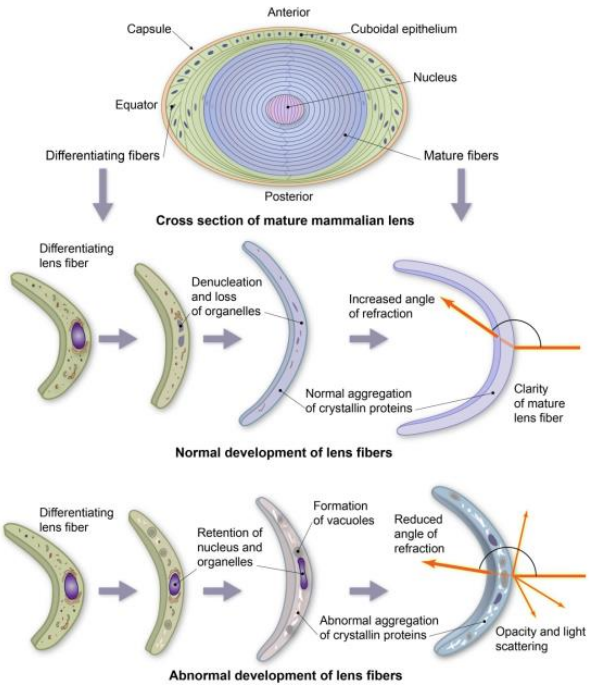
NEAR VISION

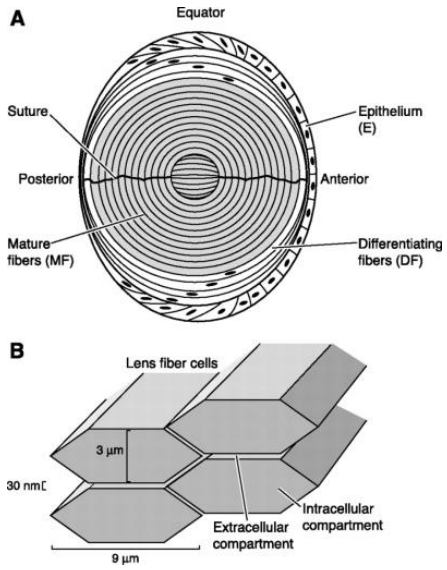


The lens is located behind the iris. It develops in the first months of pregnancy. Lens crystallins are densely packed proteins used to refract light. The cornea does ~75% of the light refraction; the lens fine-tunes our sight by doing the rest of the refraction. (Diagrams rarely show this well.)

The cornea and lens refract light, focusing the image on the retina.







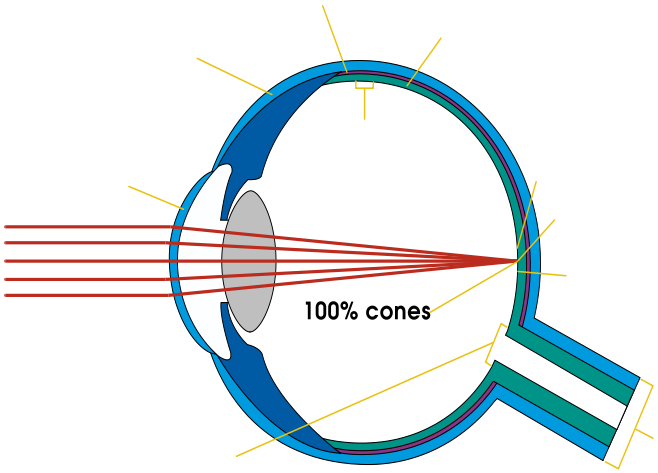
The Lens

- Opacity: Lens cataracts = lens opacity
  - Edema
  - Xenobiotic interactions
  - Protein precipitation
  - Aging

  Loss of Acuity

  Luckily we can replace the lens—RLE = refractive lens exchange. A significant development in intraocular lens surgery is the US FDA approval of multifocal and accommodating IOLs, which provide vision at multiple distances and reduce or eliminate the need for glasses or contact lenses.

The Retina

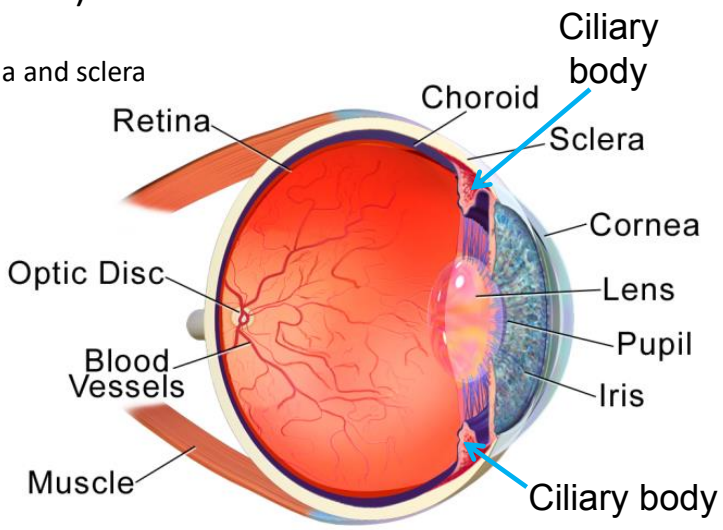


Retina

A Complex Neurological Network of the CNS

- Rods and cones (photoreceptors)
- Choroid coat—the vascular layer of the eye containing CT between retina and sclera
- Optic nerve
- Ganglion cells
- LOF→ retinopathy
- Reduced visual acuity

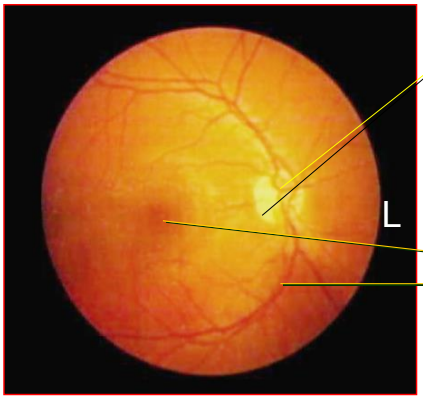
The optic disc or optic nerve head is the exit point for ganglion cell axons leaving the eye.



Anatomy of the Eye

The Fundus

The Part of the Eye Opposite the Pupil
(General Definition: Part of a Hollow Organ Farthest from Its Mouth)



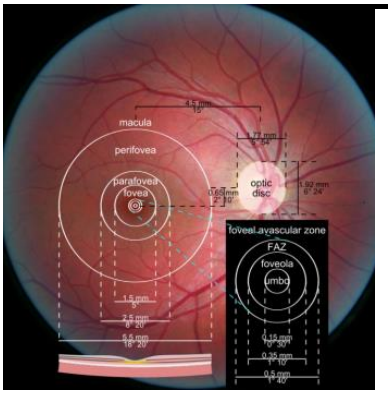
Optic disk where blood vessels and the optic nerve head exit the eye

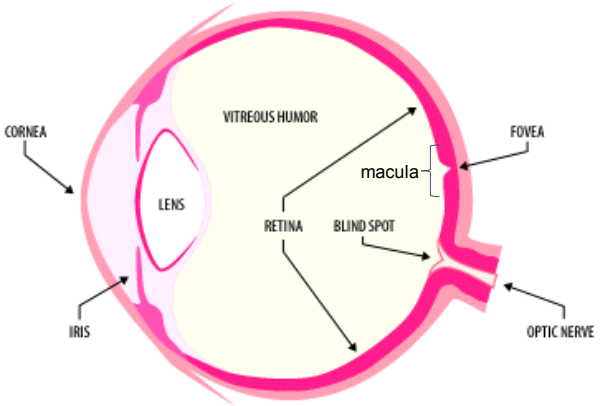
Macula

Retinal blood vessels

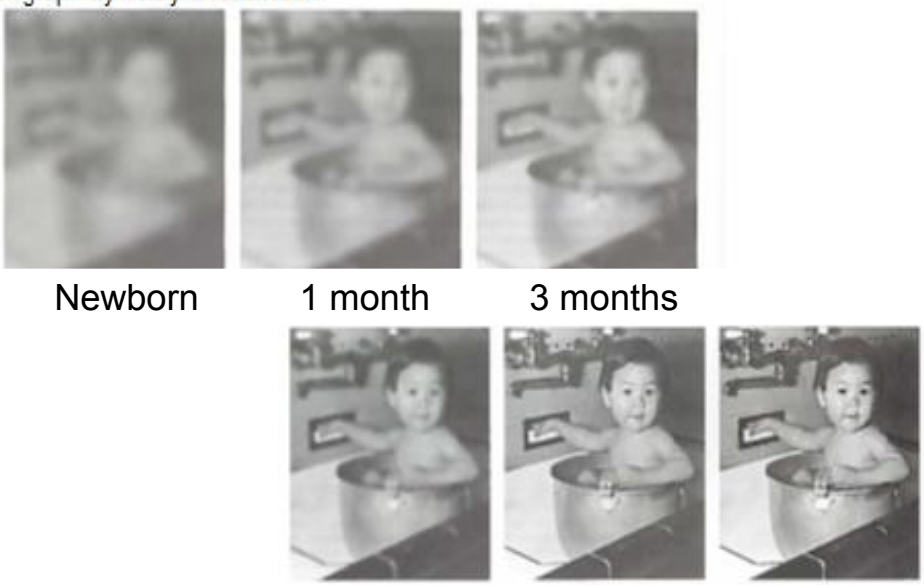
Fundus: the interior lining of the eye, including the retina, macula, and optic disc (the hole where the optic nerve enters the eye). **The macula is the area in the retina where cones are concentrated**, providing high-resolution vision. The fovea is a tiny pit in the macula, providing the clearest vision.

Additional Eye Diagrams



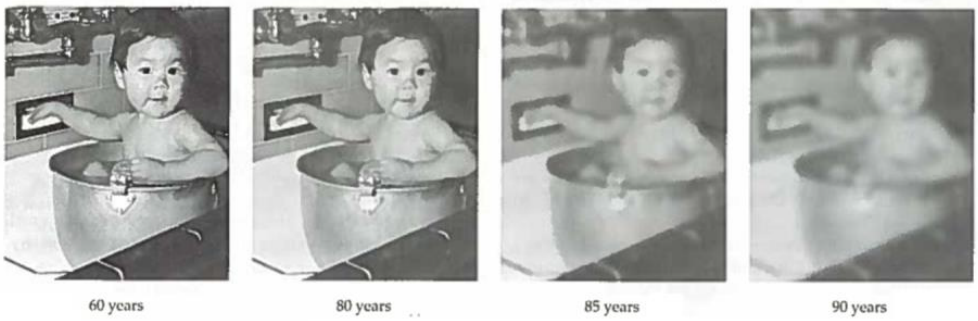


The Appearance of a Visual Scene to Infants of Different Ages: Newborn infants have very poor visual acuity. Acuity improves to about 20/200 at 3 months of age and to about 20/80 at 6 months. At 3 years of age, acuity will be about 20/40 and will require another 2 or 3 years to reach the adult level. Photograph by Henry M Takahashi.



Newborn 1 month 3 months

6 months 3 years adult



60 years 80 years 85 years 90 years

The Appearance of a Visual Scene to Older Adults of Different Ages
Compared to the average visual acuity at age 60, which is better than 20/20, acuities at later ages are reduced. The views for ages 80, 85, and 90 correspond roughly to acuities of 20/40 (6/12), 20/80 (6/24), and 20/200 (6/60), respectively. These views represent only the effects of deterioration in the eye and take no account of any perceptual compensation or of any deterioration in the perceptual apparatus.

Ocular Manifestations of Toxicity

- Cornea—inflammation, infection = keratitis → very acute pain, edema, thickening opacity < acuity
- Conjunctiva—inflammation, infection → acute pain, redness (injection), edema
- Tear film—LOF → dry eye and keratitis
- Ciliary body—neurotoxicants → reduced accommodation and change in IOP (steroids can change IOP also)
- Iris—infection, edema, LOF → < reflex
- Aqueous humor (anterior) > IOP glaucoma, infections → reduces visual field

Ocular Manifestations of Toxicity

- Lens: opacity (cataracts)—blindness
- Vitreous humor: (posterior chamber)—distorted vision and retinal damage if vessels leak blood into it
- Choroid: choroiditis from surgery, trauma, or inflammatory disease
- Retinopathies: LOF with decreased visual acuity
- Retinal ganglion cell death from glaucoma—these cells cannot regenerate in mammals
- Optic nerve: pressure on it from glaucoma also injures the optic nerve. The optic nerve has no regenerative capacity

Cornea/Conjunctiva

- Acids—(H^+) ionic strength x time
- Alkali—(OH^-) pH; residual damage
- Organic solvents—lipid dissolution = m/o
- Bacterial and fungal infections
- Alkylating chemical agents (e.g., mustards)
- Detergents—cationic > anionic > nonionic
    - Irritation, necrosis, scarring, neovascularization

Cornea/Conjunctiva

- Gases and vapors
    - Mustard gases—alkylating agents
    - Lewisite-alkylating agents
    - Tear gas, α-acetophenone (lacrimators)
    - Smog, airborne irritants, allergenic agents
- Ultraviolet light (270 nm)—cataracts
- Poison Ivy, airborne irritants, and allergens

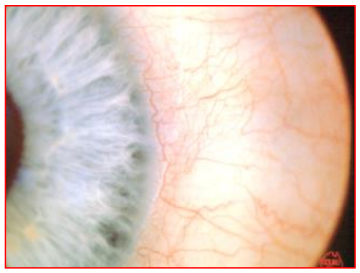
  (antihistamines and nonsteroidal anti-inflammatory eyedrops)
- Drugs: chemo, chloroquine
- Contact lenses (mostly older ones)

Conjunctivitis

- Inflammation of the conjunctiva
- Immune reaction to foreign substances
  - Blood vessel enlargement
  - Increased blood flow
- "Red Eye"

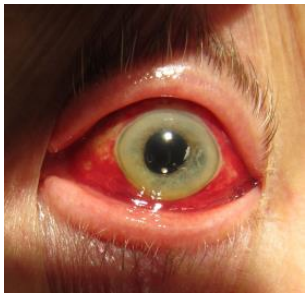
Mild "Red Eye"

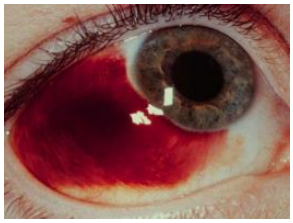
Chronic redness—many causes
- Allergies
- Alcohol overindulgence
- Smoky environments
- Smog, etc.
- Dirty contact lens



Severe "Red Eye"

Potentially serious: (e.g., infection) Contact ophthalmologist! Patient should avoid whitening drops until cause known (e.g., NO brightening drops).





Broken blood vessel
(subconjunctival hemorrhage

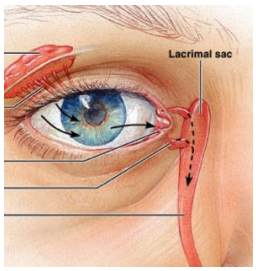
Bloodshot eye
Can be from infection

Uveitis is inflammation of the middle layer of the eye, i.e., the uvea and surrounding tissues, but can be inflammation of other parts of the eye as well. It is not a single disease, and it has different causes.

Uveitis

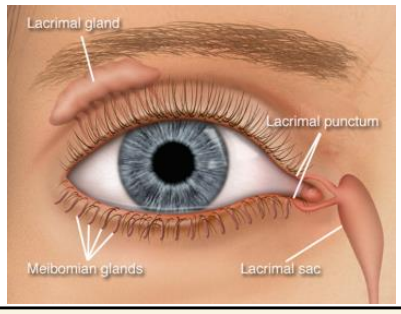
The Lacrimal Apparatus

- Tears produced by a system of glands
- Keeps eyes lubricated and clean
  - Eyelid = windshield wiper
  - Tears = windshield washer fluid
- Drainage via puncta, which are openings of tear ducts



Meibomian Glands

aka tarsal glands
Synthesize and secrete meibum, which is oily and prevents evaporation of the tear film

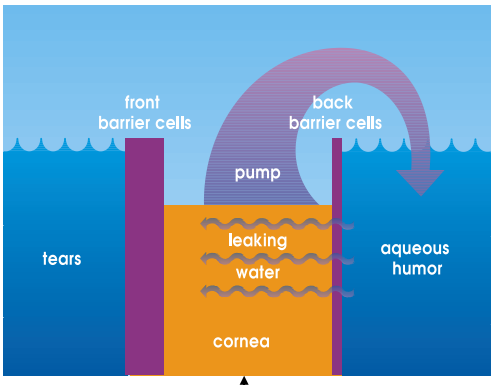


Composition of the Tears

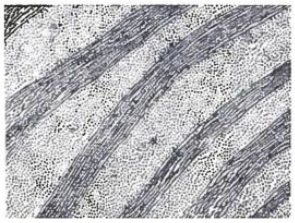
- White blood cells
- Fluid is mostly water (99%), with muscins, lipids, lysozyme, glucose, lactoferrin, immunoglobulins, urea, Na+, and K+
- Substances for preventing evaporation and for facilitating uniform spreading
- Nutrients and oxygen

Corneal Transparency

- Water content is strictly controlled to be less than in the surroundings regions. The cornea needs to be relatively dehydrated for transmission and refraction of light. Hydration disrupts the tight organization of collagen fibrils that set up the structure allowing light transmission.
- Anterior and posterior cells form water barriers.
- Back barrier leaks, but pumps water out.

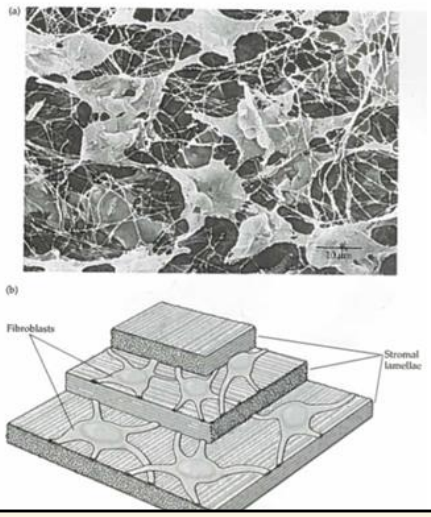


As for drug absorption into the eye, the corneal epithelium is the main barrier restricting this.

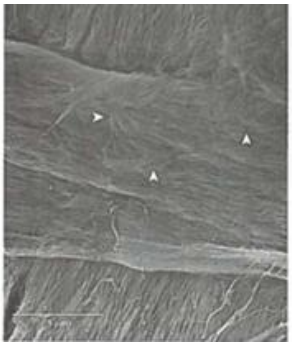


Chick cornea

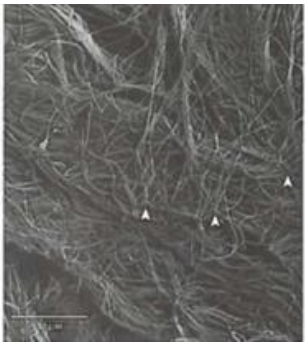
The organization of collagen fibrils in the cornea allows the cornea to be transparent.



Human cornea

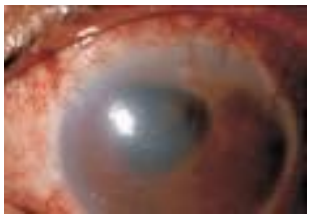


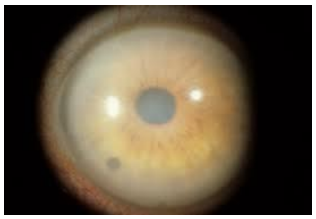
Human sclera



Corneal Edema

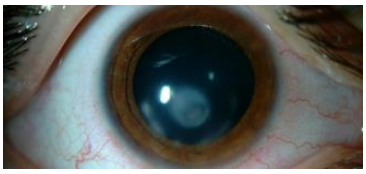
Swelling of the cornea following ocular surgery, trauma, infection, inflammation, as well as a secondary result of various ocular diseases. Corneal edema can also occur following over-wear of certain types of contact lenses.
(water accumulation)

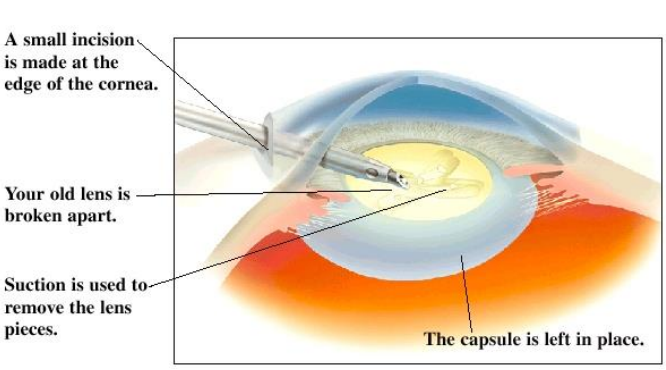


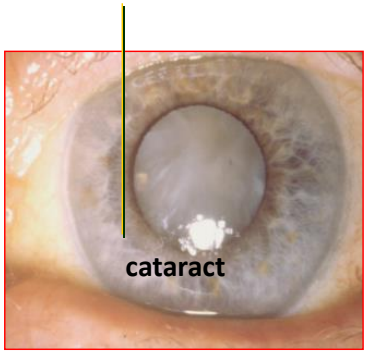


Cataract

- Clouding of crystalline lens
- Eventually leads to blindness
- Age-related is the most common
- UV radiation
- It is observed with a biomicroscope
- It causes refractive error changes

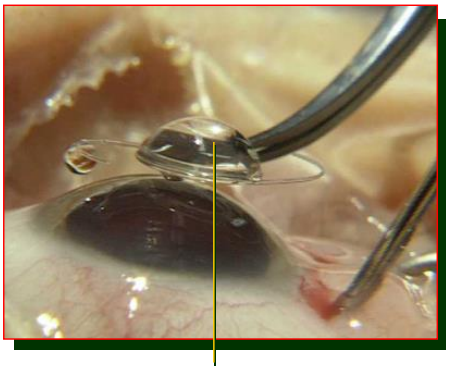






Cataract Surgery

- Remove crystalline lens
- Replace with IOL
- Most common surgery performed in US
- Cataracts are the largest cause of blindness in the world



Lens (Cataracts)

More Opacity Means Less Acuity

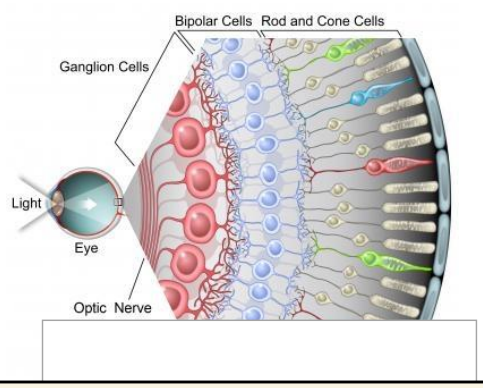
- Xb-induced
  - 2,4-DNP (2 4-dinitrophenol)
  - 2,6-DCNA (2,6-dichloro-4-nitroaniline)
  - Corticosteroids: cortisone, prednisone, dexamethasone
  - ChE inhibitors
  - Chlorpromazine+
  - Naphthalene
  - Triparanol (MER-29)
  - Aging + UVR
  - Disease states: diabetes mellitus, galactosemia, chronic infections, hexose sugars

Retinopathies

- Damage to retinal tissue; choroid; optic nerve; ganglion layer; cranial nerve
- Chloroquine, phenothiazines, digoxin, indomethacin, phenothiazines, high O_2 pressure, UV radiation
- Cytotoxic chemotherapies: 5-FU, cisplatin, methotrexate
- PDE inhibitors: sildenafil, etc.
- Tamoxifen at high dosages

Retinopathies
via Nerve Impulse Transmission

- Methanol
- Ethanbutol (for tubercular and nontubercular mycobacterial disease)
- Carbon disulfide
- Nerve gases and other OPs
- Quinine and quinidine derivatives
- Methylmercury
- Lead, As^{5+}
- Organic solvents



Ocular Signaling

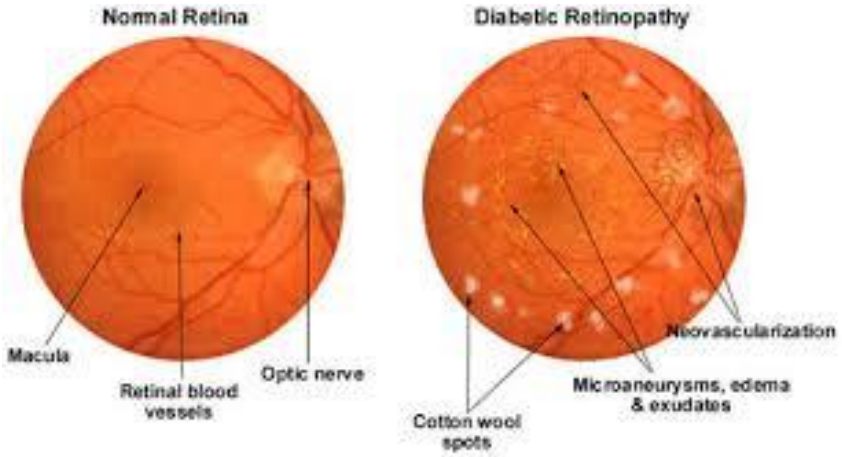
For ocular signaling to a nerve impulse, cyclic GMP is required to open Na^+ channels in the plasma membrane. In the dark, cyclic GMP is abundant and these channels stay open. Sodium cations enter freely into the rod cell, because the cell typically has a lower potential (is more negative) than the external environment, thus attracting the positively charged ions. However, when cyclic GMP is hydrolyzed (gains an H_2O and breaks a bond) by activated phosphodiesterase, it is no longer available to keep the Na^+ channels open.

Sodium cations can no longer enter the cell freely, and so the cell's potential suddenly becomes even lower relative to the external environment. A large charge difference across the membrane is built up; this is known as hyperpolarization.

The large potential difference travels as an electrical impulse down the rod cell to the synaptic terminal, and is then transferred to an adjoining nerve cell. The nerve cell carries this impulse all the way to the brain. The brain then determines where the nerve impulse originated, and interprets the image.

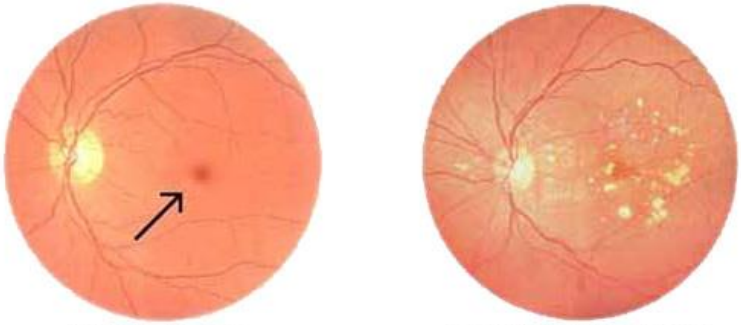
Diabetic Retinopathy

- Diabetes = high blood sugar
- Serious retinal effects
- Blood vessels leak
- Macular swelling
- Leads to blindness
- Painless
- Detect with ophthalmoscope



Diabetic changes to retina

Macular Degeneration



Normal Macula | Macular Degeneration

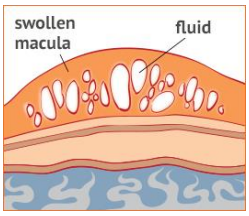
- Disease of macula area of retina
- The leading cause of blindness in US for people over 65
- Cause unknown, no known treatment

Age-Related Macular Degeneration

Recently, much progress has been made in gene discovery and mechanistic studies, which clearly indicate that AMD involves the interaction of multiple genetic and environmental factors. The identification of genes that have a substantial impact on the risk for AMD is not only facilitating the diagnosis and screening of populations at risk but is also elucidating key molecular pathways of pathogenesis. Pharmacogenetic studies of treatment responsiveness among patients with the "wet" form of AMD are increasingly proving to be clinically relevant; pharmacogenetic approaches hold great promise for both identifying patients with the best chance for vision recovery as well as tailoring individualized therapies.

Potential risk factors are smoking, oxidative stress, antioxidant depletion, alcohol consumption, sun exposure, hypertension, etc.

Yuhong Chen, Matthew Bedell, and Kang Zhang, Age-related Macular Degeneration: Genetic and Environmental Factors of Disease. *Mol Interv*. 2010 Oct; 10(5): 271–281.



Age-Related Macular Degeneration

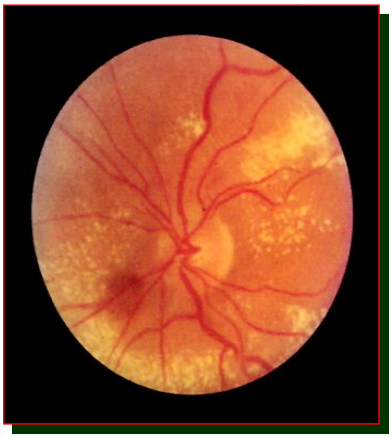
IVI-intravitreal Injection

In AMD, the blood vessels leak and fluid collects in the macula and the retina. Before injection of an anti-vascular endothelial growth factor (VEGF) drug, the eye is numbed and a speculum may be put in place to keep the eyelids out of the way.

Two anti-VEGF drugs have been approved by the US Food and Drug Administration (US FDA) for the treatment of wet macular degeneration. The one most widely used **is Lucentis (ranibizumab—a VEGF antibody).** In the studies that evaluated Lucentis, the results were more favorable than for any other previously approved treatment. Instead of only slowing the rate of vision loss, the drug appeared to stop disease progression in most people for as long as two years. The first anti-VEGF drug to be approved by the US FDA for the treatment of wet macular degeneration was Macugen (pegaptanib sodium). It works in a similar manner to Lucentis, but is not as effective. This is most likely because it acts against only one form of the VEGF protein, called VEGF-165, whereas Lucentis targets all forms of VEGF.

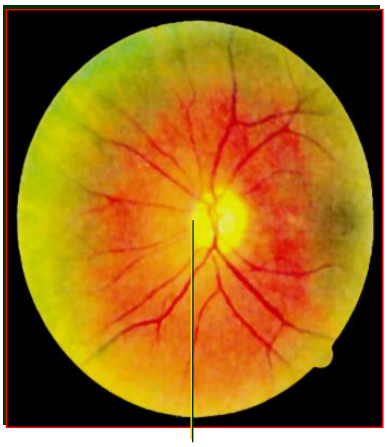
Hypertensive Retinopathy

- High blood pressure
- Causes retinal changes
- It's best to catch HBP early



Multiple Sclerosis

- Disease that attacks myelin
- Myelin surrounds some nerves like insulation does a wire
- Multiple remissions and relapses
- Eye effects:
  - Eye tremors
  - Double vision
- Papillitis: optic nerve inflammation in the optic disc
  - Reduced central vision
  - Pain in back of eye, especially upon eye movement
  - Often unilateral



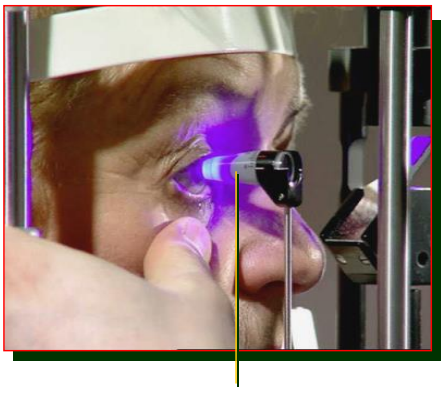
Glaucoma

- Elevated IOP (>22 mm Hg) [normal is 12–22 mm Hg]
- Can be hereditary
- Slowdown in flow of aqueous humor out of eye
- Destroys retinal nerve fibers
- Gradual blindness
- Early detection difficult

- Acute close angle IOP 60–100, severe pain
- Chronic glaucoma
  - Usually painless
  - Does not affect central vision until advanced

Glaucoma

- Diagnosis
  - Measure IOP
  - Visual fields
- Closed angle glaucoma
  - Acute aqueous blockage
  - Medical emergency
- 1.5 million in US
  - 50% don't know
  - Runs in families



Ocular Toxicity of Drugs

- Latesse/brimatoprost
- Hydrochloroquine
- Ketek (telithromycin)
- Glitazones (DM-II)
- Sildenifil (PDE-5&6)
- Topomax (epilepsy)

- Inflammation/pigment
- Retinal pigmentation
- Double/blurred vision
- Macular edema
- Optic nerve, ischemia
- Glaucoma, acute myopia

Acids and Bases

Acids

- pH + [C] dependent
- pH > 3.5 not irritating
- Conjunctivitis and red eye
- Photophobia
- Iritis
- Keritits
- +/- lens clouding

Bases

- pH + [C] dependent
- pH < 10 not irritating
- Conjunctivitis and atrophy
- Iritis
- Lens clouding
- Keritits irreversible
- Neovascularization
- Blindness

Consumer Products

- **Hair Care**
- Shampoos, rinses, and dyes
- pH- and time-dependent
- Conjunctivitis and red eye
- Discharge
- Iritis—many dyes
- **Household cleansers**
- Similar to ionic [+/-] surfactants and alcohols
- **Skin Care, Emollients**
- **OTC acne products**
- Keritits + conjunctivitis
- **Depilatories**
- Kerititis, conjunctivitis, moderate to severe red eye and stinging/corneal tearing
- Sensitization
- Photophobia

Systemic Ethanol

Acute
- Diplopia
- Nystagmus
- Mydriasis
- Accommodation
- Color vision and dark adaptation
- Depth perception
- Visual hallucinations
- Blindness

Chronic
- Extraorbital muscle paralysis
- Nystagmus
- Miosis
- Accommodation
- Color vision and dark adaptation
- Visual hallucinations
- Blindness

Methanol

- Acute ingestion
- *Effects delayed 18–24 hrs*
- Loss of VF and VA
- Mydriasis
- Papilledema
- Retinal edema
- Blindness
- Rx *Ethanol???*

Surfactants

- Cationic (precipitate proteins [benzalkonium Cl, cetylpyridinium Cl, etc.])
- Anionic (lysis of cells) (soaps, SDS, alkyl, Triton X-30)
- Nonionic (Brij, Tween 80, Triton X-155) (mild reactions)
- [C] dependent
- Causes loss of the epithelium and corneal permeability
- Stinging, lacrimation, keratitis
- Conjunctival swelling and discharge
- Iritis and potential allergic sensitivity

Insecticides

- ChE Inhibitors
  - Miosis, blurred vision
  - Spams of ciliary body
- Organochlorines (DDT, etc.)
  - Conjunctivitis
  - Rarely keratitis
  - Lacrimation
- Pyrethroids
  - Mild conjunctivitis and erythema (red eye)

Silver

- TOPICAL
  1) Argyrol (silver protein)**
  2) silver nitrate
  3) silver iodide
- Antimicrobial
- Conjunctivitis
- Corneal clouding*
- Argyria and argyrosis**

*Systemic administration

Copper

- **Topical copper salts**
  - Corneal opacity
  - Conjunctivitis
- **Systemic**
  - Discoloration of cornea
  - Discoloration of lens
  - Photophobia

Lead

- **Topical lead salts**
  - Corneal cloudiness
  - Conjunctivitis
- **Systemic**
  - Onset months to years
  - Loss of VA (usually reversible)
  - Visual cortex, optic nerve, retina, IO/EO muscles, lens
  - *Rx—**penicillamine, Ca EDTA?***

Mercury

Topical

- *Thimerosal*—in vaccines, nonirritating at therapeutic levels

Systemic toxicity of organic Hg

- Discoloration of cornea and lens
- Eyelid tremors and nystagmus
- Conjunctivitis
- Photophobia

Treatment: Chelation

Mechanism is generally deposition from overdose

Mercury is found in many rocks, including coal. When coal is burned, mercury is released into the environment. Coal-burning power plants are the largest human-caused source of mercury emissions to the air in the US, accounting for more than 40% of all domestic human-caused mercury emissions. The US EPA has estimated that about 25% of US emissions from coal-burning power plants are deposited within the contiguous US and the remainder enters the global cycle.

References

- Yuhong Chen, Matthew Bedell, and Kang Zhang, Age-related Macular Degeneration: Genetic and Environmental Factors of Disease. *Mol Interv*. 2010 Oct; 10(5): 271–281.

Tissue-Specific Aspects of Corneal Injury: The Cornea Is Not Merely a Window to the Soul

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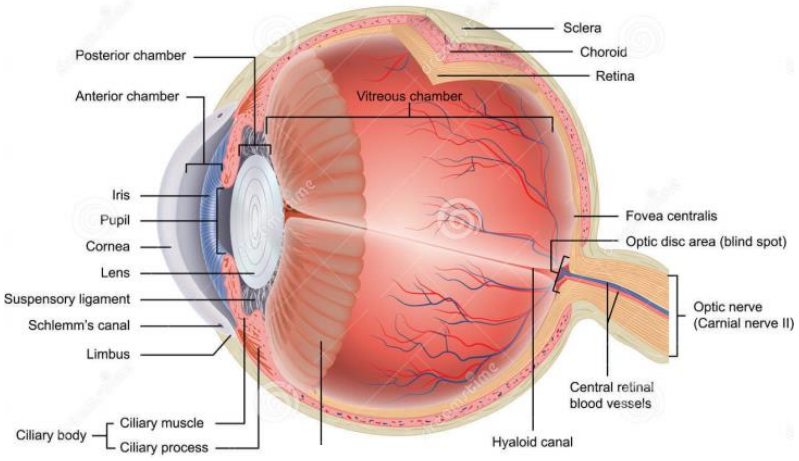
Disclaimer

- The views expressed in this abstract are those of the presenter and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government
- I have no conflicts of interest to declare

Outline

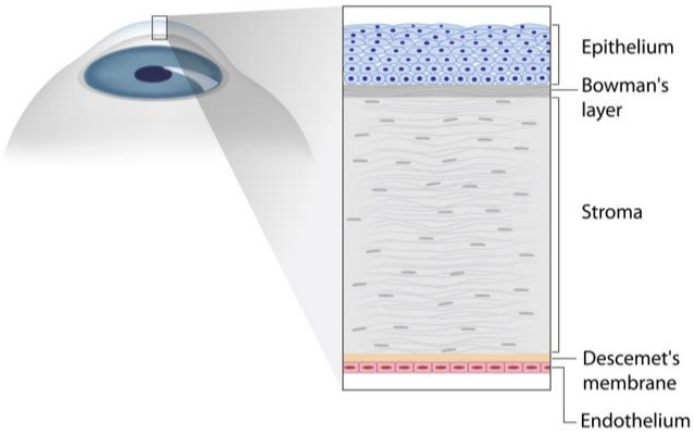
- Role of the cornea in vision
- Overview of corneal structure and composition
- Basics of corneal development: a demonstration of inductive complexity
- Structure, function, and regeneration of the corneal epithelium
- Structure, function, and regeneration of the corneal stroma
- Structure, function, and regeneration of the corneal endothelium
- Contributions of vascularization and nerve innervation to corneal function
- Integrated perspective on corneal injury and healing
- Mechanisms of corneal toxicities
- Methods to assess corneal toxicity
- Factors involved in corneal drug delivery, today and tomorrow
- References

The Eye



- The globe (eye) is the most complex organ other than the brain
- Cornea and sclera form a fibrous tunic that surrounds the globe
- Cornea's primary functions are (1) to protect eye against microorganisms and (2) to support proper transmission and refraction of light
- Cornea provides 70% of the eye's resolving power and scatters ≤1% of light
- Cornea is a physically accessible therapeutic target for topical drug administration, but its complex physicochemical properties render drug delivery difficult

Corneal Structure



- Precorneal tear film lubricates epithelium, transports oxygen and nutrients to the cornea, provides smooth surface for light refraction, and has wound-healing properties
- Corneal epithelium serves as impermeable barrier to fluids and microorganisms
- Avascular stroma has specialized arrangement of collagen fibrils that enables transparency and proper light refraction
- Endothelium actively maintains stromal hydrostasis and corneal nutrition
- The limbus is a highly vascularized region where cornea and sclera merge that is source of new corneal epithelial cells
- Cornea is innervated by rich plexus of nerve fibers that influence corneal function

Gross Corneal Anatomy

- Hemisphere ~11.7 mm in diameter
- Surface area is ~1.3 cm^2 (1/6 of globe)
- Radius of curvature is 7.8 mm (anterior surface) and 6.5 mm (posterior surface)
- Refractive power = 43.1 Diopter
  - Air-tear interface = 43.6 Diopter
  - Tear-cornea interface = 5.3 Diopter
  - Cornea-aqueous humor interface = -5.8 Diopter
- Cornea responsible for 70% of resolving power of eye
- Refractive index = 1.376

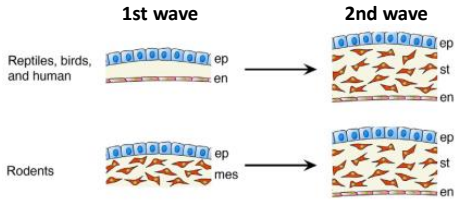
Corneal Composition

- 78% H_20
- 15% collagen
  - Type I: 50–55%
  - Type III: 1%
  - Type IV: 8–10%
  - Type VI: 25–30%
  - Descemet's: Type IV and VIII
- 5% other proteins
- 0.7% keratan sulphate
- 0.3% chondroitin/dermatan sulphate
- 1% salts
- Trace quantities of hyaluronic acid

Corneal Development

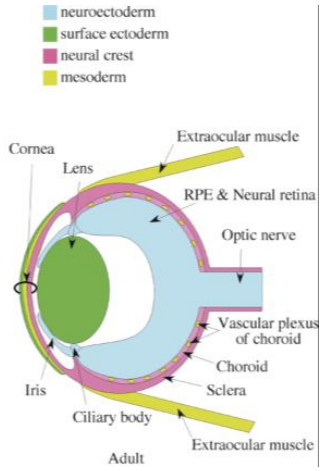
- Induced by lens and optic cup at 7 weeks
- 1st wave neural crest cells form endothelium in humans
- 2nd wave neural crest cells form keratoblasts/keratocytes
- Extracellular matrices are secreted by corneal cells

Corneal tissue	Developmental origin
Corneal epithelium/limbus	**Surface ectoderm**
Basal lamina	Synthesized by basal epithelial cells
Keratocytes	**Periocular neural crest cells**
Corneal stroma	Synthesized by keratocytes
Bowman's membrane	Synthesized by keratocytes
Corneal endothelium	**Periocular neural crest cells**
Descemet's membrane	Synthesized by endothelium
Corneal nerves	**Neural crest vice trigeminal ganglion**

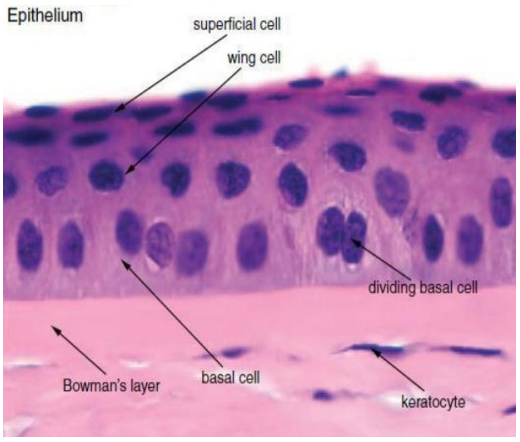


(Lwigale, 2015)

(Harada et al., 2007)

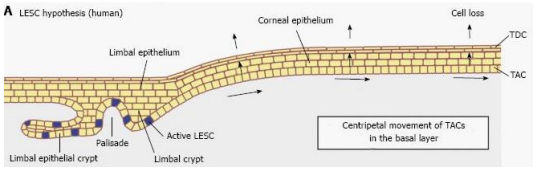


Corneal Epithelium

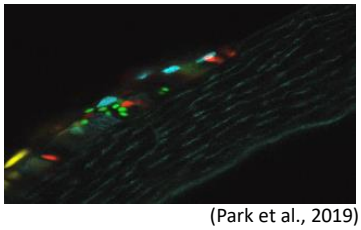


- Stratified, squamous, and nonkeratinized
- Connected to conjunctival epithelium via limbus
- 50–90 µm in thickness
- Contains 5–6 layers of cells, divided into three cell types:
  - Basal epithelial cells: transient amplifying cell population; associated with Bowman's layer via hemidesmosomes
  - Suprabasal epithelial cells (aka, wing cells)
  - Superficial epithelial cells: 2–3 cell layers, hexagonal in shape, exhibit surface microvilli, regularly sloughed
- Adhesion by tight junctions and desmosomes (superficial); desmosomes (wings); and desmosomes and hemidesmosomes (basal)
- Basal epithelial cells secrete basal lamina (0.5–1 µm thick) with context-specific composition
- Highly regenerative cell population, evolutionarily designed to rapidly recover from epithelial trauma

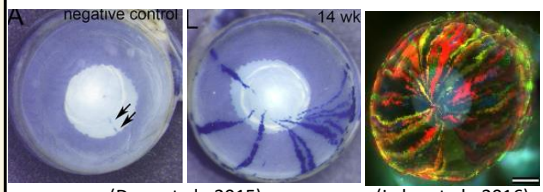
Homeostasis of the Corneal Epithelium



(West et al., 2015)



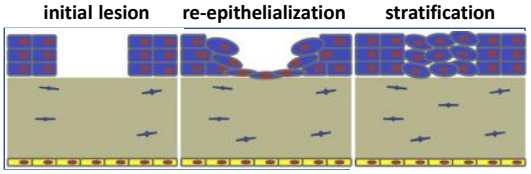
(Park et al., 2019)



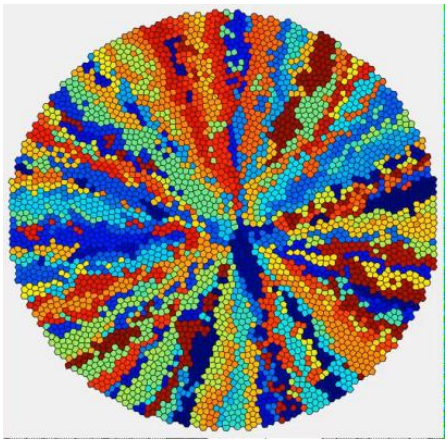
(Dora et al., 2015) (Lobo et al., 2016)

- Corneal epithelium constantly undergoes sloughing and regeneration
- Germinative layer is located in specialized niches in limbus at corneal margin (limbal epithelial stem cells; LESCs)
- Asymmetric and stochastic LESC proliferation produces transient amplifying cells (TAC) that migrate centripetally
- TACs turn into basal epithelial cells, which undergo limited proliferation to produce suprabasal epithelial cells, which in turn become terminally differentiated superficial epithelial cells
- Thoft and Friend (1983) proposed that corneal epithelial homeostasis was maintained by a balance among loss of superficial epithelial cells from the corneal surface, cell division in the basal epithelium, and renewal of basal epithelial cells by centripetal migration of TACs originating from LESCs

Corneal Epithelial Response to Injury



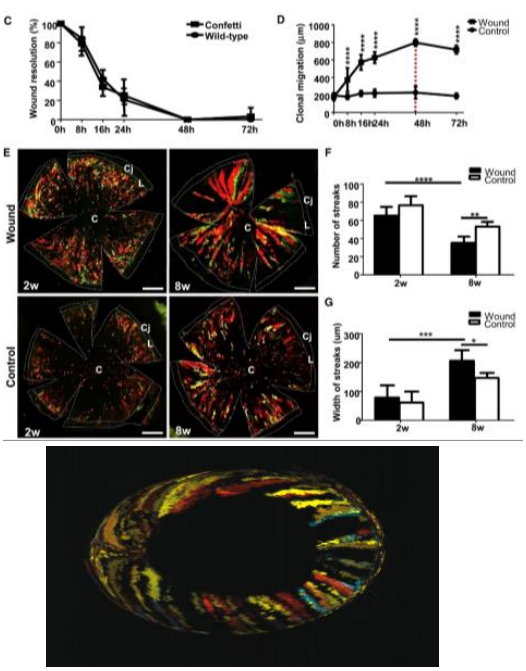
(modified from Liu et al., 2015)



(Park et al., 2019)

- The corneal epithelium is evolutionarily designed to undergo robust and rapid recovery from lesions
- Homeostatic mechanism go into overdrive to repair significant injury to the corneal epithelium
- Cells at wound edge delaminate and travel laterally at 60–80 µm/h to cover the defect, with migration terminated by contact inhibition (see Dr. Gordon's talk)
- Following reestablishment of an impermeable cap, epithelial stratification and differentiation occurs following mitosis of basal cells
- Surface adhesions and hemidesmosomal plaques are r-established within 7–14 d after injury
- Penetrating corneal lesions involve a stromal repair component that interlaces with epithelial injury repair

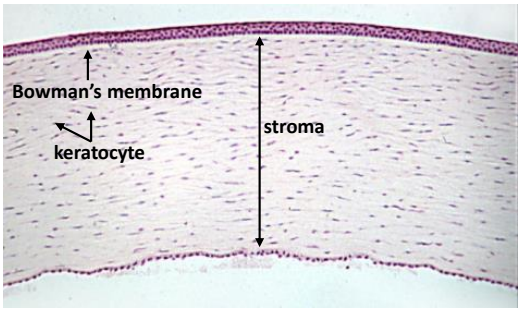
Corneal Epithelial Response to Injury

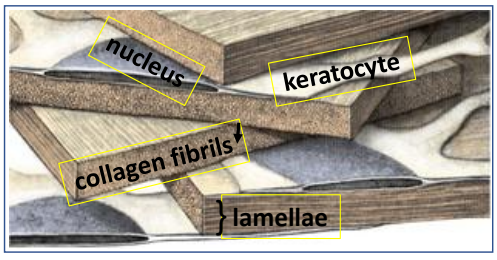


(Park et al., 2018)

- Epithelial injury evokes increased LESC proliferation and production of increased numbers of TACs
- Following injury, newly created TACs exhibit accelerated rate of centripetal migration
- Newborn TACs backfill for depleted basal epithelial cells and support ongoing regeneration of a stratified epithelial layers
- Loss of LESCs compromises ability to renew and regenerate corneal epithelium under homeostatic and injury conditions, leading to recurring corneal lesions, persistent corneal edema, and inability to heal
- Traumatic elimination of limbus and epithelium results in conjunctivalization of the corneal surface, neovascularization, and chronically disrupted vision

Corneal Stroma

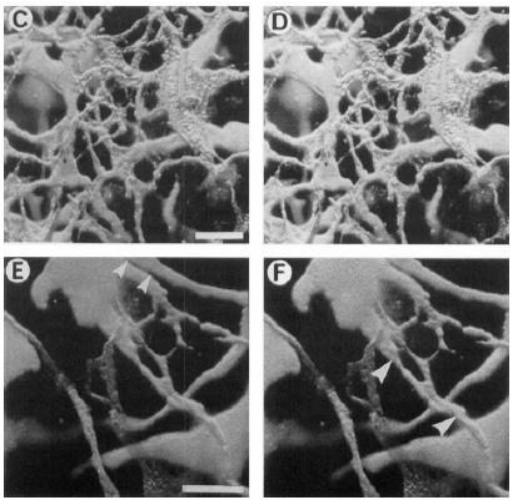




(modified from Hogan et al., 1971)

- Carefully organized to optimize transparency
- Anterior margin is Bowman's membrane, which is a modified region of acellular stroma that is 8–14 µm thick and organized to protect against structural trauma
- Stroma is about 500 µm thick (90% of corneal thickness)
- Consists of 300 (central) to 500 (peripheral) layers of collagen bundles oriented orthogonally, with each layer ~ 1.15–2 µm thick
- The high degree of organization among collagen fibrils within lamellae and among lamellae are critical for transparency
- Stromal hydrostasis is critically important for ensuring transparency and light refraction
- Stroma is maintained in a relatively dehydrated state (78 ± 5%) by corneal epithelium and corneal endothelium

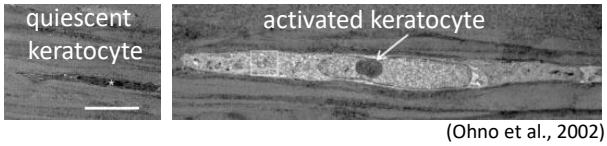
Corneal Stroma



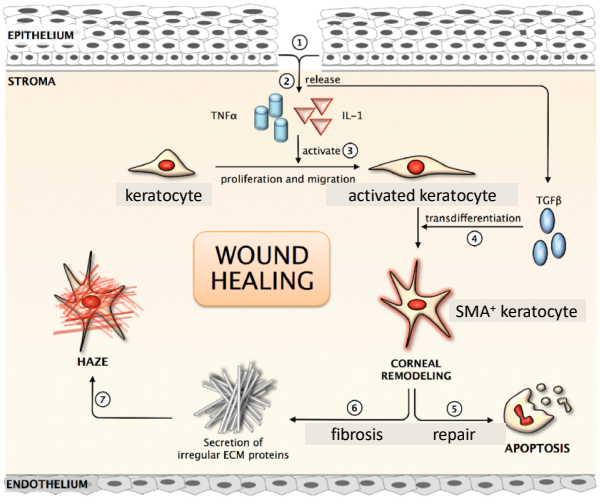
(Poole et al., 1996)

- Stroma is sparsely populated by a specialized form of proliferative fibroblasts called keratocytes, which occupy 2–5% of total stromal volume
- Keratocytes synthesize and remodel stromal matrix
- Keratocytes are long, thin cells (≤ 2 µm thick) between the stromal lamellae that extend lengthy stellate processes and form gap junctions with neighboring keratocytes in the same horizontal plane
- Largely dormant under unperturbed conditions
- Activated after corneal injury and play critical remodeling role during stromal repair
- Physiological state of keratocytes is regulated by several factors, including epithelial protein secretion, inflammatory cell activity, tear film infiltration, and aqueous humor infiltration

Stromal Injury and Repair



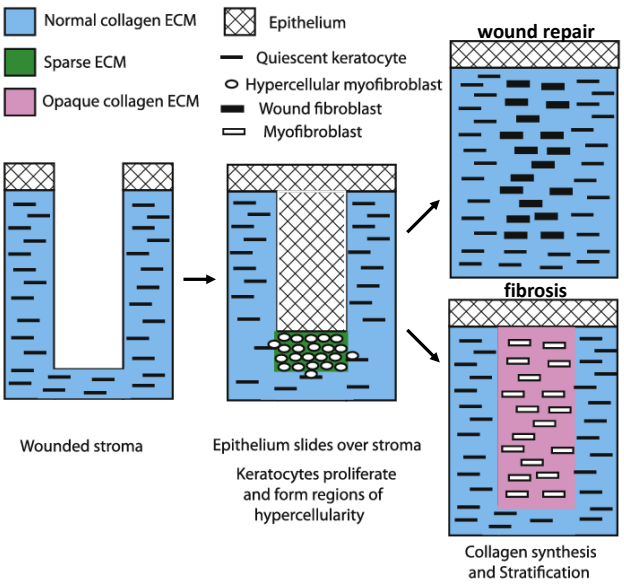
(Ohno et al., 2002)



(modified from Chaurasia et al., 2015)

- Following minor corneal injury (scrape or incision), proximal keratocytes undergo apoptosis or proliferation in response to influx of tear film and inflammatory mediators into the stroma
- Activated keratocytes produce α-smooth muscle actin and low levels of extracellular matrix (ECM)
- Depending on the severity of the injury, activated keratocytes become either wound fibroblasts (repair phenotype) or myofibroblasts (fibrotic phenotype)
- Myofibroblasts produce high levels of ECM but little keratan sulfate, resulting in a disorganized ECM that causes opacity
- Wound fibroblasts produce high levels of collagen and keratan sulfate, producing an organized ECM that eventually restores stromal transparency

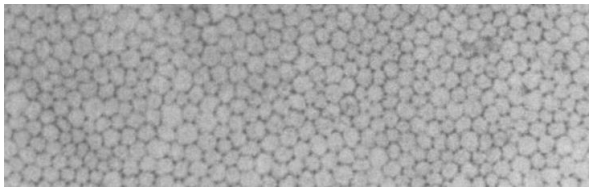
Stromal Injury and Repair



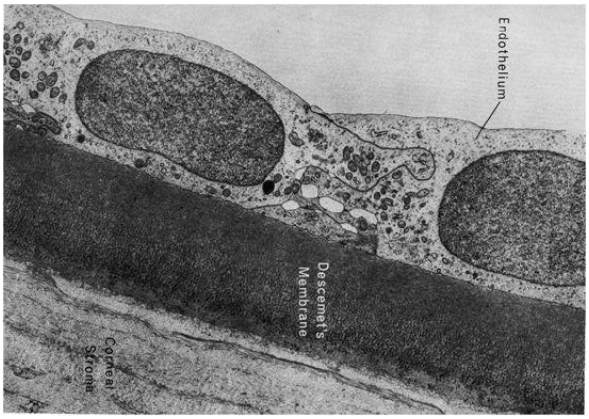
(modified from Hassell and Birk, 2010)

- Following an incisional wound, the corneal epithelium migrates over wound site
- Activated keratocytes accumulate and differentiate to wound fibroblasts (repair phenotype), which produce relatively normal collagen fibril, or myofibroblasts (fibrotic phenotype), which produce disorganized, light-scattering fibril
- Migration, proliferation, and inflammatory responses of activated fibroblasts compromise corneal clarity
- Clarity then slowly improves during wound repair, but remains compromised in the fibrotic response
- Fibrotic response can be remodeled to improve transparency, but occurs in a protracted and delayed fashion

Corneal Endothelium



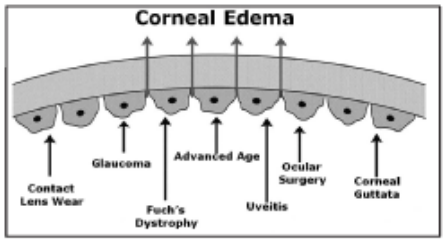
(Geroski, 1989)



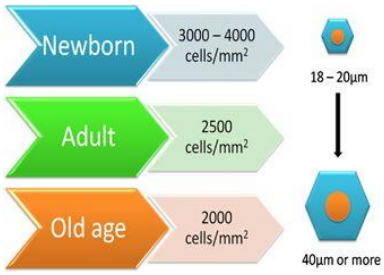
(with permission of Cell Image Library CIL: 10944)

- The corneal endothelium is a monolayer of specialized, predominantly hexagonal, uniformly sized, mitochondria-rich cells on the posterior margin of the cornea
- Governs fluid and solute transport across the posterior surface of the cornea, actively maintaining the cornea in a slight dehydrated state necessary for optical transparency
- Allows leakage of nutrients from aqueous humor into stroma, followed by active pumping in the opposite direction to remove fluid
- Corneal endothelial integrity is critically important for corneal function, and chronic disruption of the corneal endothelium results in corneal degeneration and impaired/lost vision

Corneal Endothelium Injury and Repair



(Thomas, 2009)

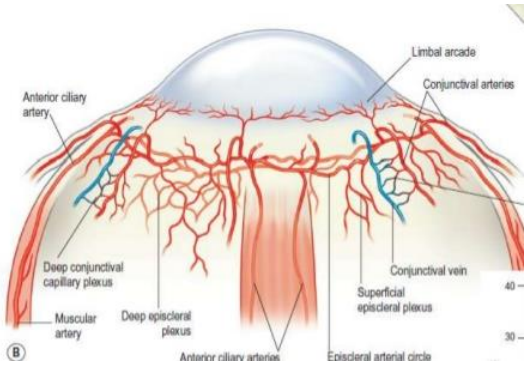


(Zavala et al., 2013)

- Postnatal endothelium is post-mitotic and a stem cell population has not been identified in humans
- Consequently a regenerative mechanism is not available for severe endothelial lesions (which are rare in nature)
- Following endothelial cell injury, neighboring cells slide to cover exposed Descemet's membrane, resulting in decreased cell density and increased polymegathism (cell size) and polymorphism
- Normal density in adults is about 2,500 cells/mm^2; if cell density drops below 500–1,000 cells/mm^2, the endothelium can no longer maintain corneal hydrostasis. The resulting edema causes decreased transparency and elicits secondary keratopathies
- Endothelial failure can only be therapeutically treated via corneal transplant

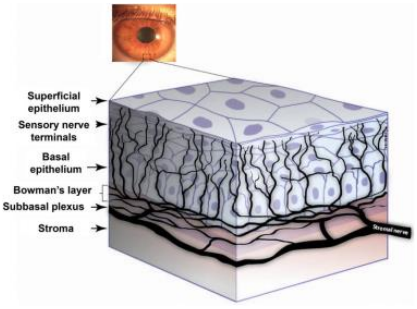
Blood Supply

- Under physiological conditions the cornea is avascular
- Dense vasculature in the limbus richly supports limbal epithelial stem cell niches
- Neovascularization of cornea via limbus is pathological and reduces transparency
- Corneal oxygenation occurs through tear film



Nerve Supply

- Cornea is richly innervated by sensory nerves originating from the ophthalmic division of the trigeminal nerve
- Anterior ciliary nerves form a pericorneal plexus that passes myelinated fibers into the cornea
- Fibers pass through stroma, form plexus subjacent to Bowman's layer, and penetrate to epithelium



(Yang et al., 2018)

Integration of Corneal Injury and Healing

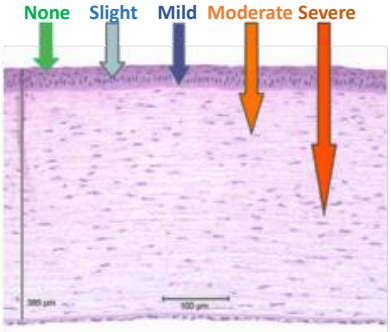
- Corneal function is strongly dependent on its structure, and even mild toxic events can have irreversible long-term effects on vision
- Corneal injury responses are highly dynamic and manifest in tissue-specific compartments on different time scales, resulting in a complex injury progression
- Collectively the cornea has a limited capacity to respond to toxic insults
  - Functionally essential tissues have poor regenerative capacity
  - Balance between healing and fibrosis is fraught; epithelial scrapes can normally be easily healed, but moderate corneal incision or endothelial toxicity may not be repairable
- Tissue-specific regenerative capacity is a key factor influencing toxicity and injury progression
  - Corneal epithelium is highly regenerative via LESC niches, but …
  - Limbal niches are highly sensitive to inflammatory toxicity
  - Corneal endothelium has little regenerative capacity (largely driven by morphological changes in cell shape as opposed to cell proliferation)
  - Keratocytes are highly proliferative, but susceptible to fibrotic pathways under severe injury conditions

Integration of Corneal Injury and Healing

- Chronic disruption of the corneal epithelium or endothelium leads to sustained corneal edema with diverse and severe secondary effects
  - Persistent proinflammatory condition that can promote fibrotic transformations in the corneal endothelial and keratocyte cell populations
  - Corneal infiltration by highly reactive immune cells can cause cytotoxic and structural damage
  - Damage to LESC niches eliminating corneal epithelial homeostasis
  - Toxic metabolic stress to endothelium
  - Hydrostatic stress on epithelium (creating bullous keratopathy, damaging basal lamina)
  - Structural damage to corneal ECM (activity of matrix-active enzymes, disruption of collagen fibrils and lamellae, destruction and/or disorganization of basement membranes)
- Chronic corneal edema causes irreversible pathological changes in corneal structure that permanently impair vision; at long time frames chronic edema can result in loss of vision
- Corneal transplants may be the only therapeutic option, but often have poor outcomes in the context of a persistent inflammatory state
- New drugs are needed to influence and improve outcomes following corneal injuries

Corneal Toxicity

- Corneal toxicity results in damage to the cornea that can vary from irritation and inflammation to irreversible blindness
- Most often results from chemical trauma or iatrogenic causes
- Clinical signs of corneal toxicity are relatively nonspecific
  - Include corneal infiltration, keratopathies (band, focal, punctate, idiosyncratic, filamentary), drug deposition, neovascularization, opacity, epithelial defects, and corneal failure
  - Usually associated with variable degrees of conjunctival involvement
  - Depth of injury model largely predicts recovery from toxic insults (but not limbal injury)



Depth of injury model: Epithelial damage alone is associated with full recovery provided the basal lamina is intact. Deeper injury into the stroma has more serious consequences to recovery, while full thickness injury including damage or loss of the endothelium is predictive of severe injury with poor prognosis Jester, 2001

"Regardless of the process leading to tissue damage, extent of initial injury is the principal mechanistic factor determining the outcome of the ocular irritation"
Maurer et al., 2002

Chemical Corneal Toxicity

- Chemical toxicity usually occurs by topical exposure, but can also occur in response to systemic exposure (e.g., chemotherapy agents or heavy metals)
- Chemical injuries to the eye represent between 11.5%–22.1% of ocular traumas (Clare et al., 2012)
- Usually caused by alkali or acid agents:
  - Alkali: liphophilic, saponify fatty acids, destroy collagen and elicit proteolytic enzymes. Typical examples are NH_3, KOH, NaOH, $Mg(OH)_2$, and $Ca(OH)_2$
  - Acids: denature and precipitate proteins, delimiting injury (with the exception of HF). Typical examples include H_2SO_4, H_2SO_3, HF, CH_3COOH, and HCl
  - Prognosis for corneal burns established using Roper Hall and Dua classification methods
- Noncaustic cytotoxins (sulfur mustard, chemotherapy agents, preservatives, or alcohols) can elicit complex manifestations depending on the depth of injury and damaged corneal populations. These agents rarely cause direct damage to corneal matrix material
- Detergents/surfactants cause cell death and may disrupt corneal matrix
- Chemical toxicity can manifest immediately (caustic agents) or after a latent period (cytotoxins)

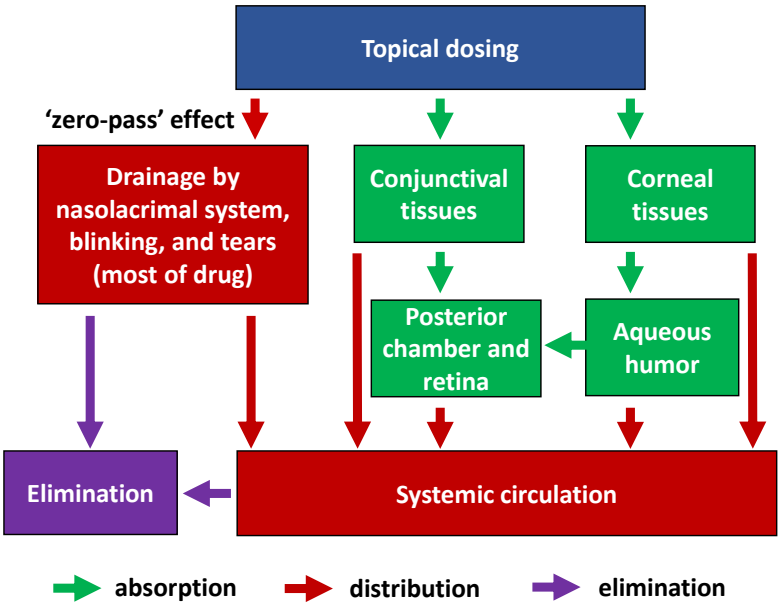
Iatrogenic Corneal Toxicity

- Most iatrogenic toxicity occurs in patients as a result of short-term or long-term use of topical medications
- Iatrogenic disease is usually an exacerbating factor in ocular surface disorders and rarely the sole cause of ocular manifestations
- In a large clinical study, 134/1024 (13.1%) of ophthalmology patients developed iatrogenic disease (Wilson 1983)
  - 119/134 exhibited corneal symptoms, with 114/119 developing epithelial defects
  - Resolution could take 17+ weeks (median 4 weeks)
  - Toxic components were most commonly preservatives (e.g., benzalkonium chloride) and excipients (e.g., EDTA) in drug formulations rather than the active pharmaceutical ingredient
- Recovery time after withdrawal of toxic drugs is often prolonged: usually 2–6 weeks to improve, and 4–12 weeks to resolve. Persistent epithelial defects may require secondary treatments to heal
- While ocular toxicity testing helps to reduce iatrogenic disease, some toxic reactions are idiosyncratic and can only be evaluated using clinical methods

Methods to Assess Corneal Toxicity

- The *in vivo* Draize test in rabbits remains the international standard of corneal toxicity
  - Application of 0.1 mL or 0.1 g test substance on cornea and conjunctiva for up to 72 h, followed by 21 d clinical monitoring for behavioral markers of pain and distress and ocular signs
  - The only assay formally accepted and validated to assess the full range of irritation severity
  - No full-thickness, humanized corneal model exists for toxicity testing
- *Ex vivo* methods to study ocular irritation
  - Generally organotypic models are best suited for short-term toxicity testing (~4 h)
  - Can involve whole eye or isolated corneas from cows, rabbits, chickens, etc.
  - Reduced ethical concerns and reduced costs; however, suffer from interspecies differences and do not account for extracorneal or systemic contributions to corneal toxicity
  - Usually involve measures of cornea edema and epithelial integrity, although corneal opacity and permeability tests also measure light transmission
- Corneal epithelial models
  - *In vitro* toxicity models developed using stratified, cultured corneal epithelial cells
  - Retain many *in vivo* epithelial repair mechanisms
  - MatTek and SkinEthic have each developed standardized 3D cultures with high validity
  - Recent models based on human LESC-derived cultures under consideration (Katoh 2013; Jung 2011)

Factors Affecting Drug Delivery to the Cornea



- Topical drug delivery to the cornea often has poor pharmacokinetics because of physicochemical constraints
- Typical corneal bioavailability is ≤5%, while posterior segment bioavailability is ≤1%
- Absorption is mostly by diffusion, although drug transport can occur
- Metabolism of drugs may occur in each compartment
- Intracameral injection may offer better cornea access, but incurs risk of infection and trauma and has poor compliance with limited repeatability
- Systemic exposures can also occur via bloodstream to aqueous humor

How to Improve Corneal Dosing?

- Getting sufficient drug into the selected compartment and retaining it there is difficult
- Corneal epithelium and stroma and conjunctival epithelium and stroma are formidable barriers for hydrophilic and hydrophobic drugs, respectively
- Traditional routes of corneal drug administration have limited efficacy
  - Topical: noninvasive, dilution in tear film, cornea acts as barrier, <5% penetration
  - Subconjunctival: depot formation and sustained delivery, high degree of vascular distribution
  - Intracameral: high drug levels in anterior chamber, invasive, can result in endothelial toxicity
  - Oral/systemic: corneal bioavailability very low except via aqueous humor, must pass two cell layers of the blood-aqueous barrier
- Drug parameters heavily influence drug uptake
  - Solubility, molecular size, weight, ionic form, and concentration of the drug
  - pH and tonicity of the solution
  - Surface active agents that metabolize, bind, or inactivate drugs
  - Pro-drug form can exploit transporters or prevent biotransformation

New Options for Corneal Drug Delivery

- Several options for corneal drug delivery may increase anterior segment bioavailability
- Bandage contact lens for sustained, noninvasive, topical delivery
- Colloidal formulations, such as liposomes, nanoparticles, microemulsions, and nanoemulsions, to modify drug physicochemical properties and improve retention and penetration
  - Provide sustained and controlled drug release at the targeted site to overcome poor absorption (reduce dosing frequency)
  - Colloid efficacy influenced by size, charge, and functionalization of nanoparticles
  - Can be functionalized to promote retention (e.g., by adding ECM adhesion capability)
- Fibrin sealant to form gel-based semisolid structure that provides long-term delivery
- Anterior segment transplant/implant/gene delivery
- Ultrasound-mediated drug delivery (e.g., 880 kHz for 5 min or 20 kHz for 1 h)
- Ocular iontophoresis to enhance corneal penetration

Summary: Corneal Complexity Is Both Boon and Bane

- The cornea is a developmentally, structurally, and functionally unique structure that plays a critical role in controlling and focusing the entry of light into the eye
- The cornea consists of five layers (corneal epithelium, Bowman's layer, avascular stroma, Descemet's membrane, and corneal endothelium) and accessory structures (tear film, keratocytes, limbus, and corneal nerves). The functions of these tissues are rigorously orchestrated to optimize corneal transparency and ensure proper vision
- Each corneal tissue has a distinct and essential role in corneal function. As a result, toxic manifestations are complex and, in turn, can elicit secondary keratopathies
- Each corneal tissue has a unique regenerative capacity that operates on divergent time scales. Thus, the pace and success of corneal healing mechanisms are influenced by the specific nature of the toxic insult (i.e., corneal target(s) and severity of injury)
- The complex corneal structure and extracorneal interactions limit the utility of *ex vivo* and *in vitro* corneal models. While relevant *in vitro* models of human corneal epithelium are available, a functionally relevant, full-thickness corneal model is not
- Noninvasive drug delivery to the cornea is complicated by physicochemical limitations

Questions?

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Advances in Nonanimal Alternatives to Dermal and Ocular Toxicity Testing

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Conflict of Interest Statement

Neither myself nor any of my coauthors, including members of our immediate families, have any financial interest or affiliation with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.

Abbreviations

- AMCP – Antimicrobial Cleaning Products
- AOP – Adverse Outcome Pathway
- ARE – Antioxidant Response Element
- BCOP – Bovine Corneal Opacity and Permeability
- CPSC – Consumer Product Safety Commission
- DA – Defined Approach
- DoT – Department of Transportation
- US EPA – US Environmental Protection Agency
- DPRA – Direct Peptide Reactivity Assay
- US FDA – US Food and Drug Administration
- UN GHS – United Nations Globally Harmonized System of Classification and Labeling of Chemicals
- GPMT – Guinea Pig Maximization Test
- h-CLAT – human Cell Line Activation Test
- IATA – Integrated Approach to Testing and Assessment
- ICATM – International Cooperation on Alternative Test Methods
- ICCVAM – US Interagency Coordinating Committee on the Validation of Alternative Methods
- ICE – Isolated Chicken Eye
- IIVS – Institute for In Vitro Sciences
- ITS – Integrated Testing Strategy
- KE – Key Event (in an AOP)
- LLNA – Local Lymph Node Assay
- MTT – Tetrazolium dye (cytotoxicity measurement)

Abbreviations

- NTP – National Toxicology Program
- NICEATM – NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
- NRR – Neutral Red Release
- ODIWG – Ocular/Dermal Irritation Working Group
- OECD – Organisation for Economic Co-operation and Development
- OSHA – Occupational Safety and Health Administration
- QSAR – Quantitative Structure-Activity Relationship
- Rh(C)E – Reconstructed human (Corneal) Epithelium
- SSWG – Skin Sensitization Working Group
- STS – Sequential Testing Strategy
- TER – Transcutaneous Electrical Resistance
- TG – Test Guideline
- WoE – Weigh of Evidence

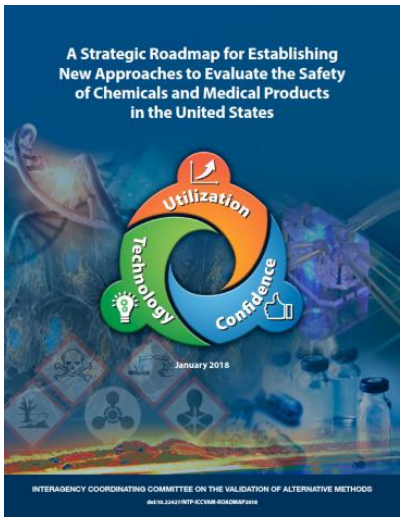
Presentation Outline

National Strategic Roadmap Framework

- Dermal Toxicity Testing
  - Regulatory Needs
  - Skin Sensitization
    - Adverse Outcome Pathway
    - Alternatives evaluation
    - International harmonization
  - Skin Irritation
    - OECD guidelines
    - 3D tissue models
    - AMCP project
- Ocular Toxicity Testing
  - Regulatory Needs
  - Eye Irritation/Corrosion
    - Available alternatives
    - OECD guidance
    - Prospective testing
- Non/Scientific Challenges

US National Strategic Roadmap and Implementation Plans

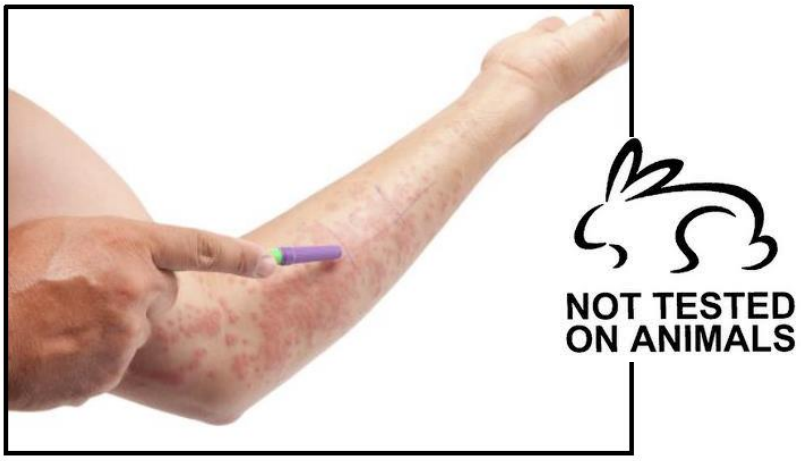
- Coordinate activities via ICCVAM work groups
- Draft a scoping document to identify US agency requirements, needs, and decision contexts
- Coordinate efforts with stakeholders
- Identify, acquire, and curate high-quality data from reference test methods
- Identify and evaluate nonanimal alternative approaches
- Gain regulatory acceptance and facilitate use of nonanimal approaches



https://ntp.niehs.nih.gov/go/natl-strategy

Skin Sensitization

"Allergic Contact Dermatitis"



Accounts for 10–15% of all occupational disease (Anderson et al., 2010)

Major testing requirement for cosmetics, pesticides, industrial chemicals, etc.

US Regulatory Requirements/Considerations

		Reference Animal Method	Classification Criteria
US EPA	Pesticides Industrial Chem.	LLNA	NS S → Hazard
CPSC	Household Products	LLNA	NS S SS → Potency
US FDA	Dermatological Products	GPMT	Potency*

*preference

Historical Accuracy of Animal Tests against Human Data

LLNA

Hazard	Potency (GHS)
72%–82%	54%–60%

GPMT / Buehler

Hazard	Potency (GHS)
~72%	~60%

Reproducibility of Multiple Tests

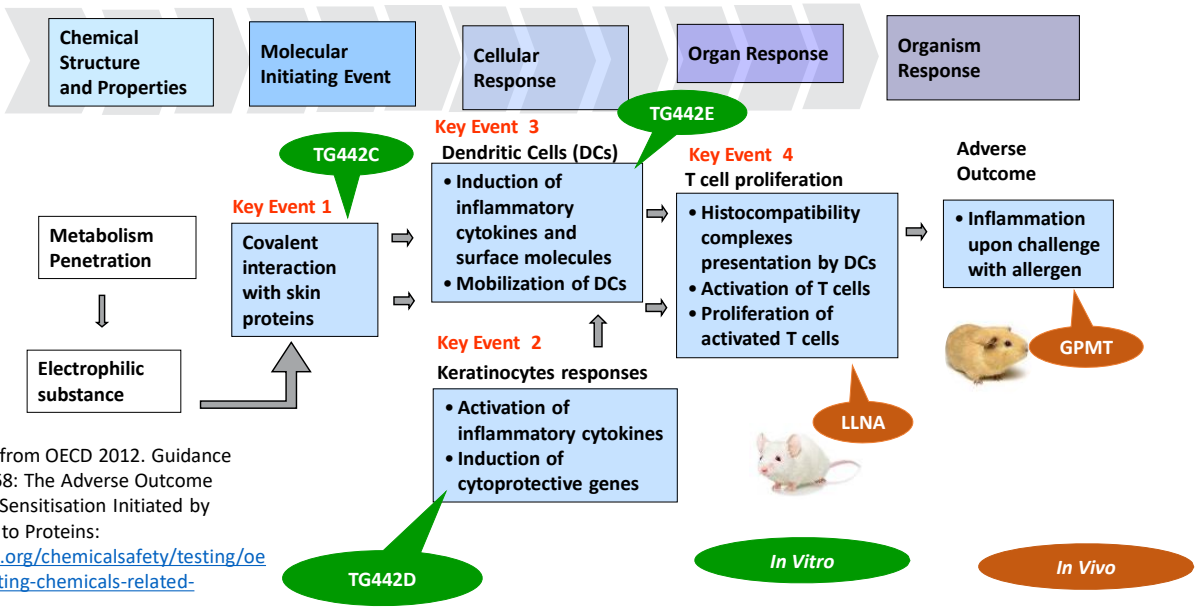
Hazard	Potency
70%–80%	60%–65%

ICCVAM. 1999. NIH Publication No. 99-4494
ICCVAM. 2010. NIH Publication No. 11-7709
Urbisch et al., 2015. *Reg Tox Pharm* 71:337-351
Dumont et al., 2016. *Tox In Vitro*
Hoffmann et al., 2018. *Crit Rev Tox*

Available Alternatives for Regulatory Use

- Direct peptide reactivity assay (DPRA)
  - OECD TG 442C
- ARE-Nrf2 Luciferase assay (KeratinoSens™)
  - OECD TG 442D
- Human cell line activation test (h-CLAT)
  - OECD TG 442E
- All are recommended for use in integrated strategies rather than as stand-alone tests
  - However, positive results may be accepted (alone) by some regulatory authorities

Adverse Outcome Pathway (AOP): Test Methods



Graphic adapted from OECD 2012. Guidance Document No. 168: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins: http://www.oecd.org/chemicalsafety/testing/oecd-guidelines-testing-chemicals-related-documents.htm

Global Skin Sensitization Project

- Objective: analysis of available nonanimal defined approaches (DAs)
- Collaboration with Cosmetics Europe; 128 substances selected
- Curation/generation of
  - *in vivo* LLNA and human data
  - *in vitro* cell-based data that maps to AOP
  - *in silico* computer predictions, chemical, structural features and properties
- Qualitative and quantitative evaluation of OECD-submitted DAs
- Fully transparent approach (i.e., build open-source code packages)
- Evaluate performance against LLNA and human hazard/potency categories

Hoffmann et al., 2018
Kleinstreuer et al., 2018

Defined Approach Evaluation

Most nonanimal testing strategies evaluated so far perform **better** than the LLNA at predicting human skin sensitization **hazard and potency**

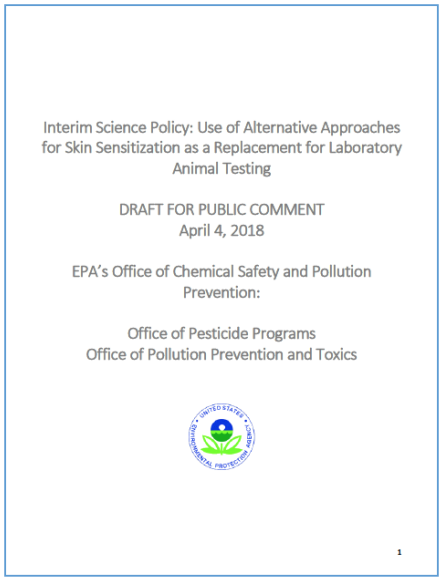
Hazard: 74% (mouse) vs. 75–85% (DAs)

3-Class Potency: 59% (mouse) vs. 55–69% (DAs)

(And when compared to the LLNA are equivalent in performance to the LLNA at predicting itself)

US EPA Acceptance of Defined Approaches

Interim Science Policy released April 2018


Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

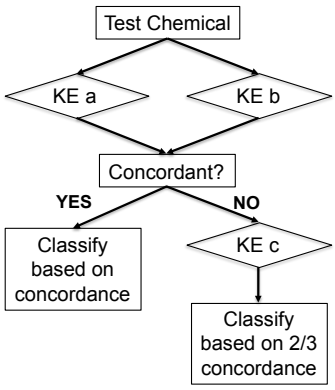
DRAFT FOR PUBLIC COMMENT
April 4, 2018

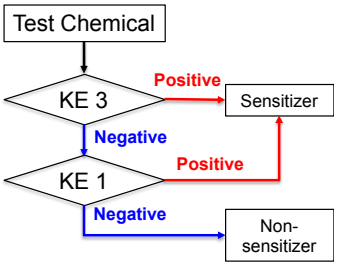
EPA's Office of Chemical Safety and Pollution Prevention:

Office of Pesticide Programs
Office of Pollution Prevention and Toxics

1

Defined Approaches (AOP WoE and KE 1/3 STS) accepted by US EPA based on comparison to LLNA data





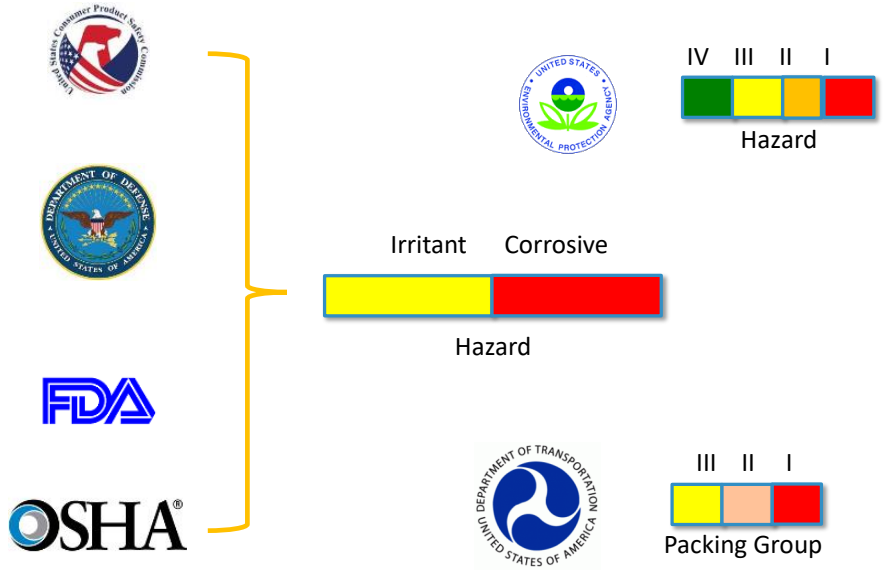
International Harmonization

- OECD proposal co-led by US, EU, and Canada
  - Create an international performance-based test guideline (GL) for nonanimal defined approaches (DA) to skin sensitization testing
  - Achieve widespread replacement of mouse test
- Special sessions of the OECD national coordinators held in December 2017 and 2018 to review progress and discuss next steps
  - Achieved consensus on evaluation framework for DA assessment
  - Formed expert group on skin sensitization DAs, including subgroups on uncertainty and applicability domain
  - Expert review of simple, rule-based DAs complete (June 2018)
  - DA GL drafted (September 2018) and revised (February 2019)

Skin/Eye Irritation and Corrosion: US Statutes and Regulations

US Statute/Regulations	Agency
Federal Hazardous Substances Act (FHSA) (1960): 16 CFR 1500.3: **Consumer Products** Poison Prevention Packaging Act (1970): 16 CFR 1700: **Hazardous Household Substances**	**CPSC**
Federal Hazardous Material Transportation Act (1975): 49 CFR 173.132, 49 CFR 173.137: **Transported Substances**	**DOT**
Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6): 40 CFR 156, 40 CFR 158.500, 40 CFR 158.2140, 40 CFR 158.2230, 40 CFR 159.165: **Pesticides**	**US EPA**
Toxic Substances Control Act (TSCA; 1976): 40 CFR 720.50: **New or Imported Chemicals**	**US EPA**
Federal Food, Drug, and Cosmetic Act (1938): 21 CFR 807.92(b)(1): **Biologics other than those regulated by CDER**	**US FDA/CBER**
Federal Food, Drug, and Cosmetic Act (1938): **All routes of administration for small molecule drugs, protein therapeutics, and monoclonal antibodies**	**US FDA/CDER**
Federal Food, Drug, and Cosmetic Act (1938): **Medical devices and radiation-emitting products**	**US FDA/CDRH**
Federal Food, Drug, and Cosmetic Act (1938): 21 C.F.R. §170, 21 C.F.R. §73, 21 C.F.R. §74, 21 C.F.R. §700, 21 C.F.R. §701, 21 C.F.R. §710, 21 C.F.R. §720, 21 C.F.R. §740: **Food ingredients and cosmetics**	**US FDA/CFSAN**
Occupational Safety and Health Act (1970): 29 CFR 1910.1200: **Workplace materials and hazards**	**OSHA**

US Agencies Using Skin/Eye Irritation Data



Skin Irritation/Corrosion—State-of-the-Science

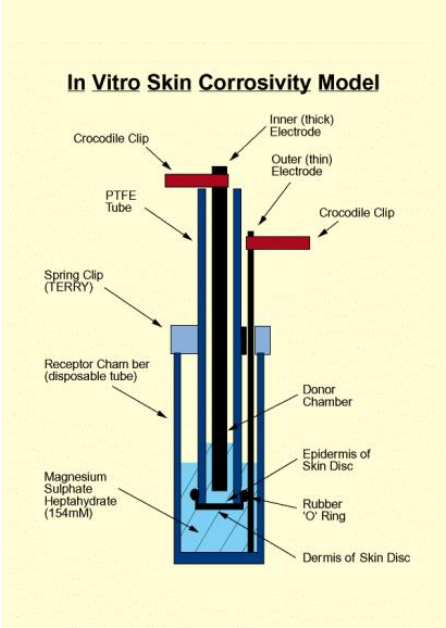
- OECD test guidelines
  - Alternative animal methods
    - TG 430, Transcutaneous electrical resistance test, adopted 2004; updated 2015
      - Corrosives and noncorrosives
      - Limitations: gases and aerosols (not evaluated in validation study)
      - Cannot separate UN GHS subcategories
    - TG 431, *In vitro* skin corrosion: reconstructed human epidermis test (e.g., EpiDerm SCT), adopted 2004; updated 2015
      - Corrosives and noncorrosives
      - Limitations: gases and aerosols (not evaluated in validation study)
      - Can separate UN GHS subcategory 1A and subcategories 1B/C
    - TG 435, Membrane barrier test for skin corrosion, adopted 2006; updated 2015
      - Corrosives and noncorrosives
      - Limitations: identified based on a compatibility test
      - Can separate all 3 UN GHS subcategories
    - TG 439, *In vitro* skin irritation: reconstructed human epidermis test (e.g., EpiDerm SIT), adopted 2010; updated 2015
      - Irritants (i.e., GHS Category 2), but not mild irritants (GHS Category 3)
      - Limitations: gases and aerosols (not evaluated in validation study)

Skin Irritation/Corrosion—State-of-the-Science

- No adverse outcome pathway available
- Integrated approaches—OECD Guidance Document on IATA for skin corrosion and irritation
- No ongoing pilot projects
- Alternative methods in the pipeline
  - *In vitro*—Skin+ and epiCS RhE irritation methods, expedited review based on performance standards in TG 439 (proposed as additions); LabCyte EPI-MODEL24 RhE corrosion method under consideration to add to TG 431
  - *In silico*—OECD Toolbox, along with many published models

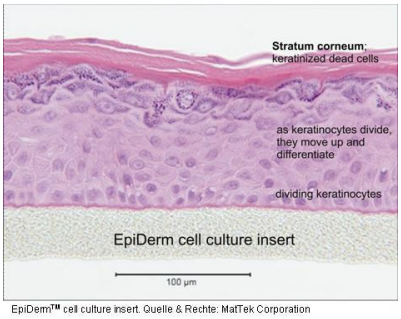
Skin Corrosion: Rat Skin TER

- Utilizes rat skin discs
- Corrosive materials identified by their ability to produce a loss of normal stratum corneum integrity and barrier function measured as a reduction in TER
- Dye-binding step confirms positive results
- Limitations:
  - Does not allow testing of gases or aerosols
  - Not applicable to UN subcategories

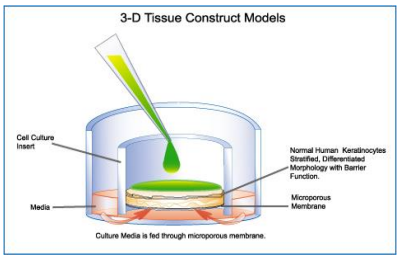


Skin Corrosion: 3D RhE Tissue

- Uses a biomembrane and chemical detection system that changes color when in contact with corrosive substances
- Applicable to UN subcategories
  - EpiSkin: 1A vs. 1B-1C vs. NC
  - EpiDerm, SkinEthic: 1 vs. 1B-1C vs. NC (high overprediction of 1A)
- Limitations:
  - Test chemicals and chemical mixtures that interfere with the cell viability measurements (e.g., direct MTT reducers, color interference)
  - Gases and aerosols



EpiDerm™ cell culture insert. Quelle & Rechte: MatTek Corporation

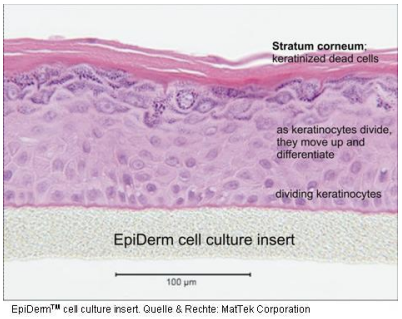


Skin Corrosion: Membrane Barrier Test

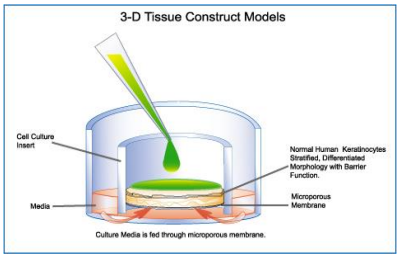
- Uses a biomembrane and chemical detection system that changes color when in contact with corrosive substances
- Applicable to UN subcategories (1A vs. 1B vs. 1C)
- Limitations:
  - Test chemicals and chemical mixtures not causing a detectable change in the compatibility test

Skin Irritation: 3D RhE Tissue

- Similar systems to those used for skin corrosion; modified protocols for irritation testing
  - EpiSkin™, EpiDerm™ SIT, SkinEthic™ RHE, LabCyte EPI-MODEL24 SIT
- Applicable to GHS Category 2 (irritant)
  - Not applicable to optional Category 3 (mild irritants)
- Limitations:
  - Test chemicals and chemical mixtures that interfere with the cell viability measurements (e.g., direct MTT reducers, color interference)
  - Gases and aerosols



EpiDerm™ cell culture insert. Quelle & Rechte: MatTek Corporation

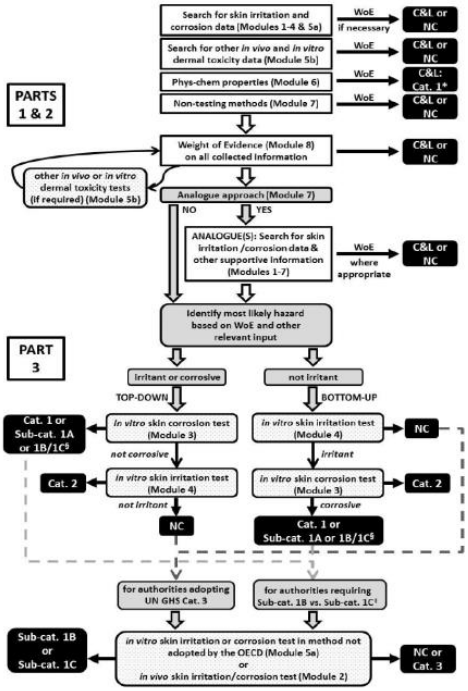


Skin Irritation: Private-Public Partnership

- Optimization of 3D skin model for testing antimicrobial cleaning products (AMCPs)
- Companies donated AMCPs
- Optimization/testing ongoing at IIVS
- Regular stakeholder teleconferences to discuss updates, data needs, etc.
  - Industry, government agencies, and animal welfare groups

IATA for Skin Irritation: An International Effort

- OECD Guidance Document 203 (US and EU co-led project)
- Three parts:
  1. Existing and available information (physchem properties QSAR, read-across, bridging)
  2. Weight of evidence
  3. New testing (*in vitro* and/or *in vivo*)



Eye Irritation/Corrosion—State-of-the-Science

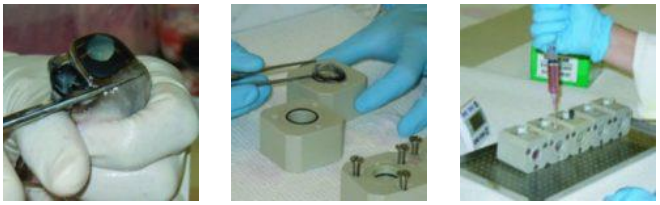
- OECD test guidelines: Alternative (nonanimal) methods
  - TG 437, Bovine Corneal Opacity and Permeability Test Method; adopted 2009; updated 2017
    - For identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
  - TG 438, Isolated Chicken Eye Test Method; adopted 2009; updated 2018
    - For identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
  - TG 460, Fluorescein Leakage Test Method; adopted 2012; updated 2017
    - For identifying water soluble ocular corrosives and severe irritants (GHS Cat 1)
  - TG 491, Short Time Exposure Test Method; adopted 2018
    - For identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
  - TG 492, Reconstructed human Cornea-like Epithelium (RhCE) test method; adopted 2017
    - Only for identifying chemicals not requiring classification and labeling for eye irritation or serious eye damage
- All TGs: Cannot delineate UN GHS categories 2/3 for moderate/mild irritants
- Limitations: gases and aerosols, strong acids/bases, highly volatile chemicals
- Inclusion of histopathology as optional endpoint (TG 437, 438)

Eye Irritation/Corrosion—State-of-the-Science

- No adverse outcome pathway available
- Data collection efforts underway—partnership between NICEATM and Cosmetics Europe
- Integrated approaches—OECD Guidance Document on IATA for eye irritation
- Prospective testing ongoing—agrochemical formulations
- Alternative methods in the pipeline
  - *In vitro*—Vitrigel EIT undergoing final TG approval; current OECD proposal for membrane barrier methods TG (e.g., Irritection, Optisafe); MCTT-HE method proposed as addition to TG 439
  - *In silico*—OECD Toolbox, along with many published models
  - Defined approaches—Under development by multiple groups (e.g., ICCVAM ODIWG, Cosmetics Europe)

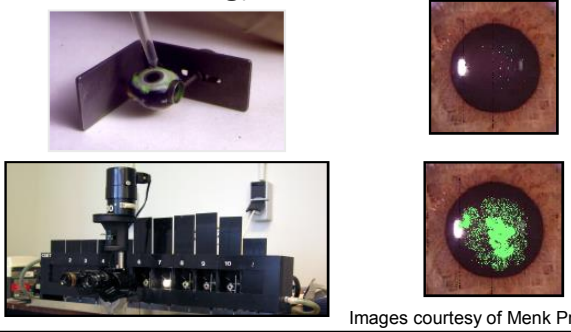
Organotypic Models: e.g., BCOP, ICE

- *Ex vivo* corneal models assess corneal injury (*in vitro* irritation score) based on:
  - Opacity (measure the amount of light transmitted through the cornea)
  - Permeability (measure the amount of fluorescein dye that penetrates through the cornea)
- Taken from animals used in food production (bovine, porcine)
- Porcine model (PORCORA) developed to assess reversibility of corneal effects based on fluorescein retention



http://iivs.org/testing-services/assays/ocular/bcop/

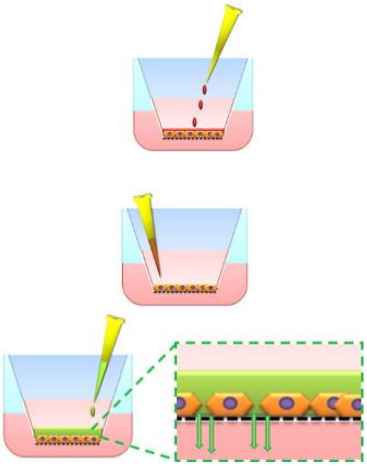
- *Ex vivo* whole eye models assess corneal injury based on:
  - Disruption of the corneal epithelium
  - Swelling of the cornea
  - Corneal opacity
- Evaluate effects *ex vivo* using whole eyes taken from animals used for other purposes (e.g., food production; other research testing)



Images courtesy of Menk Prinsen

Monolayer Culture Systems: e.g., FLT, STE

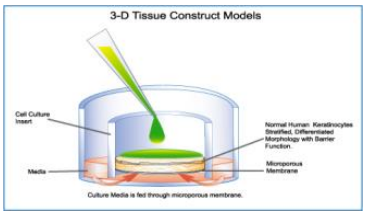
- Assess cell damage
  - Epithelial cells (primary cells or immortalized cell lines)
  - Keratinocytes, corneocytes, kidney epithelial cells
  - Cultured on plate or culture inserts
- Some measure cytotoxicity (% viability relative to control)
- Some measure permeability to a marker dye (disruption of tight junctions)
- Assumes that chemicals causing eye damage/irritation will induce cytotoxicity or disrupt epithelial barrier function in the corneal epithelium and/or conjunctiva

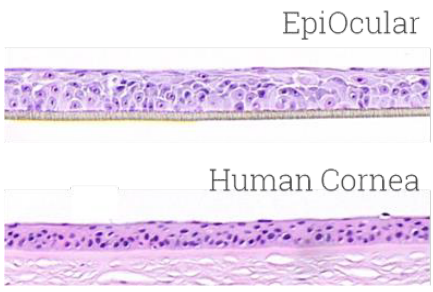


Images adapted from Wilson et al., 2015

3D Tissue RhCE Models

- Similar systems as those used for skin irritation; using corneal epithelial cells
  - EpiOcular, SkinEthic, Vitrigel
- Only applicable to classify GHS Category NC (nonirritant)
- Limitations:
  - Test chemicals and chemical mixtures that interfere with the cell viability measurements (e.g., direct MTT reducers, color interference)
  - Gases and aerosols



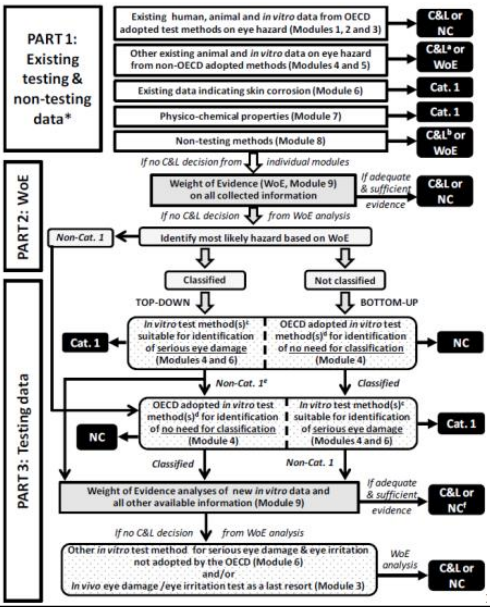


Eye Irritation: Private-Public Partnership

- Analysis of paired *in vivo* and *in vitro* data for ~200 agrochemical formulations provided by 5 companies
- Prospective testing—BCOP, ICE, NRR, EpiOcular (time-to-toxicity and TG492 protocols), PorCORA (21 days—can detect reversibility)
  - Phase 1: small number (n=6) tested in all assays to demonstrate proof of concept
  - Phase 2: comprehensive assessment of applicability with a larger set (n=40)
- Regular stakeholder teleconferences to discuss updates, data needs, etc.
  - Industry, government agencies, and animal welfare groups

IATA for Eye Irritation: An International Effort

- OECD Guidance Document 263 (US and EU co-led project)
- Three parts:
  1. Existing and available information (physchem properties QSAR, read-across, bridging)
  2. Weight of evidence
  3. New testing (*in vitro* and/or *in vivo*)



Key Need: Defined Approaches

- ICCVAM ODIWG tasked with developing DAs for eye and skin irritation/corrosion prediction
- How to benchmark performance?
  - Animal data unreliable/irreproducible
  - Compare to human data/human biology
    - Use consensus of validated *in vitro* methods as "gold standard" for training models?

Reproducibility of Animal Data

Ocular Potency Categorization

Conditional probability of Draize evaluations given a previous test result

491 substances with at least two Draize studies and extractable eye irritation category in REACH registrations 2008–2014

Prior Type	1	2A	2B	Non	Total
1	73%	16.1%	0.4%	10.4%	46
2A	4.2%	32.9%	3.5%	59.4%	138
2B	0.2%	4%	15.5%	80.2%	86
Non	1.1%	3.5%	1.5%	93.9%	400

Leuchtefeld et al., 2017

Scientific and Nonscientific Challenges

- Animal methods currently provide the reference data for evaluating alternatives
  - Results are variable
  - Need to identify summary metric and characterize uncertainty
- Data requirements vary across US and global regulatory authorities and are often ambiguous
- Overcoming regulatory and institutional inertia
  - Education and training, communication with method/model developers

Acknowledgments

- David Allen and ILS/NICEATM
- Cosmetics Europe Skin Tolerance Task Force/Eye Irritation Task Force
- ICCVAM SSWG
- ICCVAM ODIWG
- ICATM partners
- IIVS
- PISC
- CropLife America

Questions?

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