

Rack, Rum, or Brandy — A Biochemical History of Fluid Preservation

John E. Simmons

Museologica

and

Earth and Mineral Sciences Museum & Art Gallery

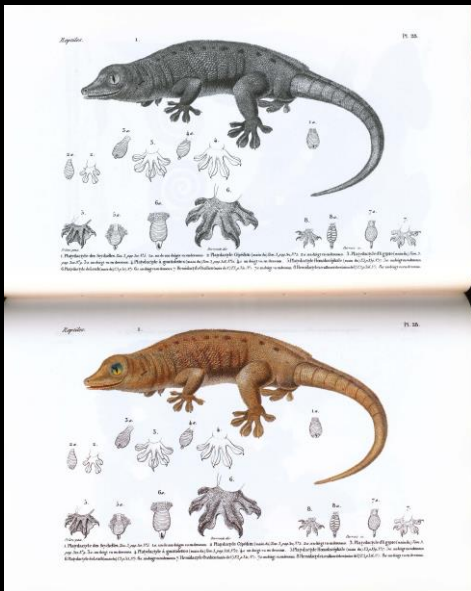
The Pennsylvania State University

“Qui ne sait la vaste part que notre illustre Cuvier accordait, dans le progrès des sciences naturelles , à celui qui le premier eut l'idée de conserver les pieces dans l'alcool?”

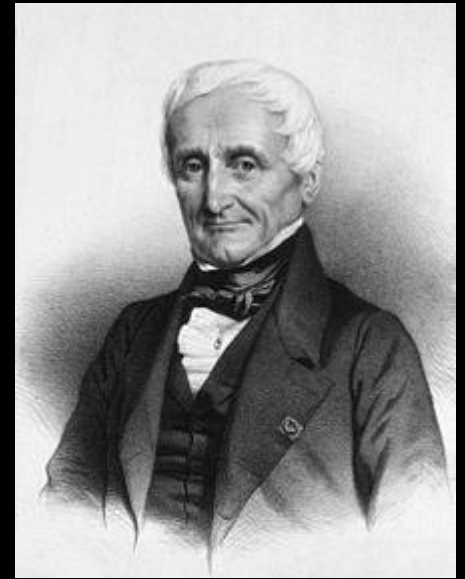
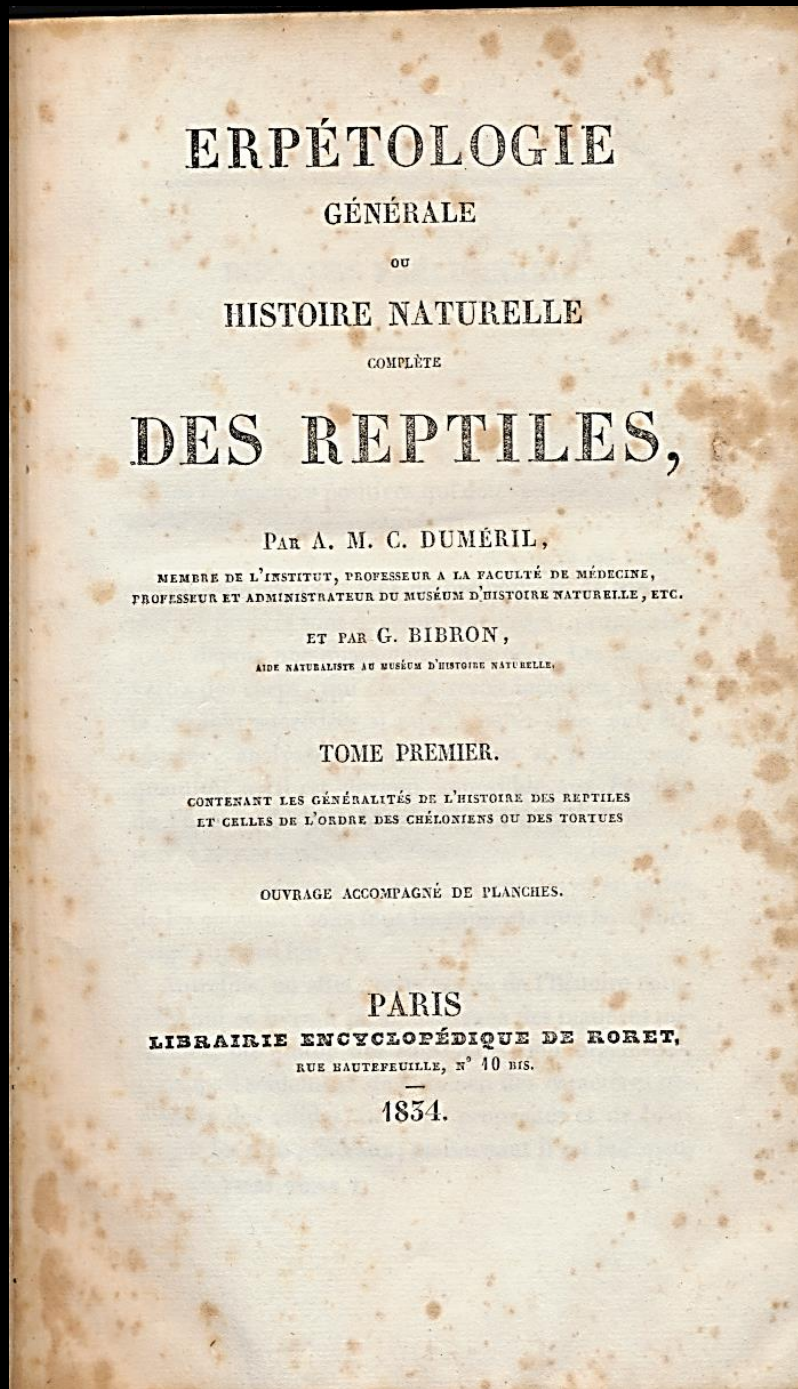
[“Who does not know the vast importance to which our illustrious Cuvier attributed the progress of the natural sciences to him who first conceived the idea of preserving objects in alcohol?”]

—Jean-Nicolas Gannal (1791-1852)

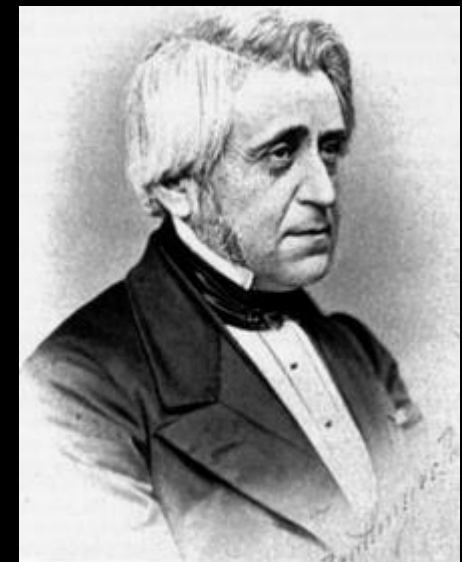
Histoire des embaumements et de la preparation des pieces d'anatomie normale, d'anatomie pathologique et d'histoire naturelle; suivie de procédés nouveaux (1838)



G. Bibron



C. Duméril



A. Duméril

Biochemical effects of preservatives

- 356 years of preservation in fluids
- 2015—reviewed 1,042 published references
- Biochemical effects of preservation are not well understood



FLUID PRESERVATION

A COMPREHENSIVE REFERENCE

JOHN E. SIMMONS

History of preservation

- Brine, vinegar, honey, and oil used to preserve food
- Herodotus (484-425 BCE):
 - Egyptians ate fish preserved in brine
 - Babylonians preserved the dead in honey
- Greek and Roman references to fish preserved in vinegar



Ethyl alcohol, C_2H_5OH

- Oldest known organic chemical
- Accessible from natural fermentation processes
- Beer—fermented grain
- Mead—fermented honey
- Wine—fermented grapes (or other fruit)

Sumerian beer recipe



Ethyl alcohol, C_2H_5OH

- 9,000 year-old alcoholic beverage recipe from China
 - Fermented rice, honey, and hawthorn fruit
- Jiahu (Neolithic village)
- Fermentation yields 12-15% ethyl alcohol

Bottle label for Chateau Jiahu
from Dogfish Head Brewery



Distillation

- Higher concentration requires distillation
- Originated in Middle East or North Africa ca. 3500 to 3000 BCE
- Alchemical tradition
- Miriam (the sister of Moses)

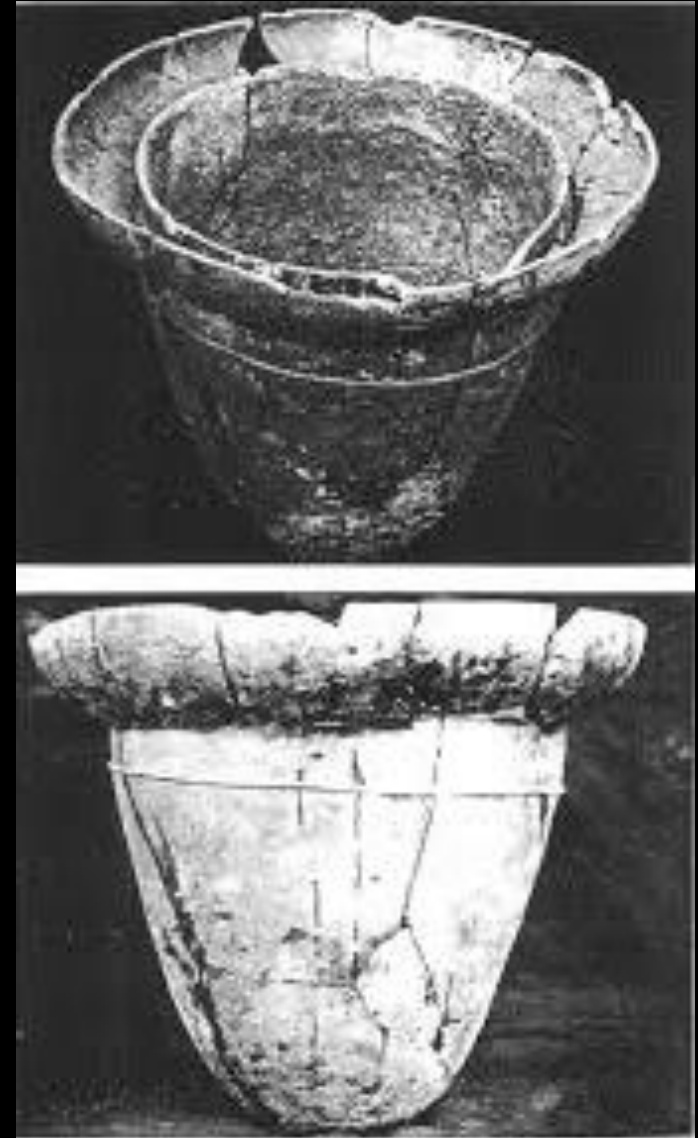


Miriam the Alchemist

Distillation

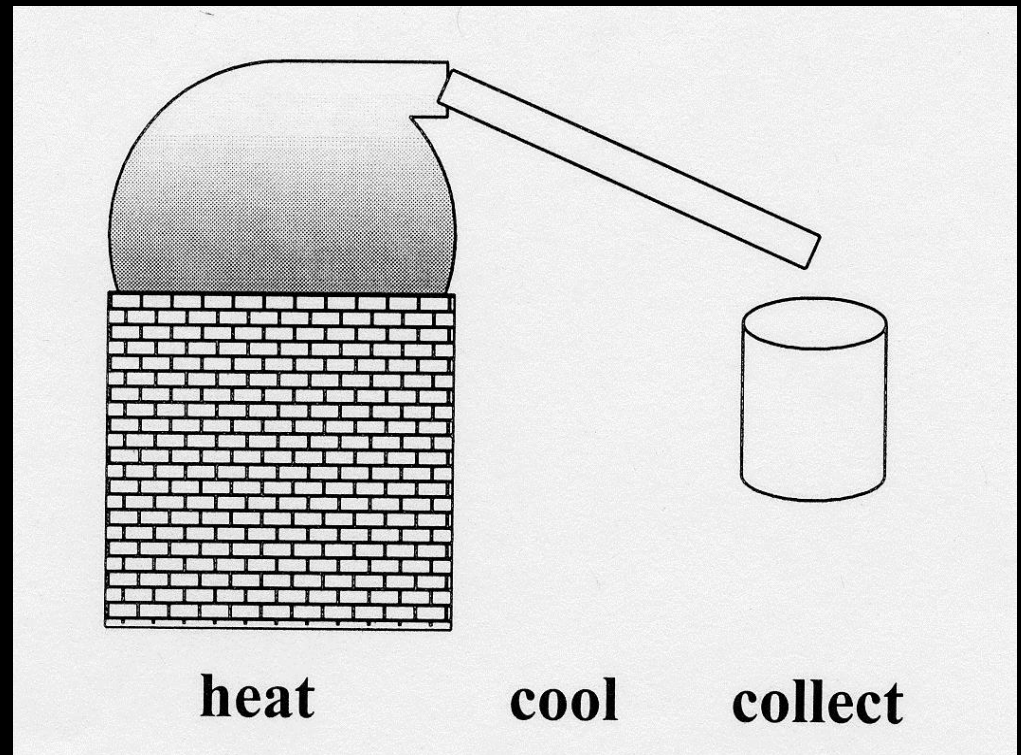
- Extraction pots from Mesopotamia, 3500 BCE
- Chinese distilled rice wine ca. 800 BCE
- Pliny the Elder (50 BCE), “wine that can be ignited”
 - *Solum vinorum flamma accenditur*)

Double-rim
extraction pot



Distillare (“to drip down”)

1. Heat the liquid
 2. Cool the vapors
 3. Collect the condensate
 4. Repeat
- Ethyl alcohol boils at 78.5°C
 - Water boils at 100.0°C



95.6% ethyl alcohol

- Alcohol forms a binary azeotrope with water
- 100% alcohol must be chemically dehydrated
- *Aqua ardens* (50 to 60.0%)
- *Aqua vitae* (95.6%), water of life



A . Signifieth the place, where the ashes rest.

B. Doth here represent the grate bearing the fire.

C. Doth here manifestly shew the place, where the fire is made.

D. Doth expresse the high & narrow furnace, woorkmanly made.

E. Sheweth the long and byg vessell, receyving the great quantitie of wyne.

F. Representeth the most narrow synt.

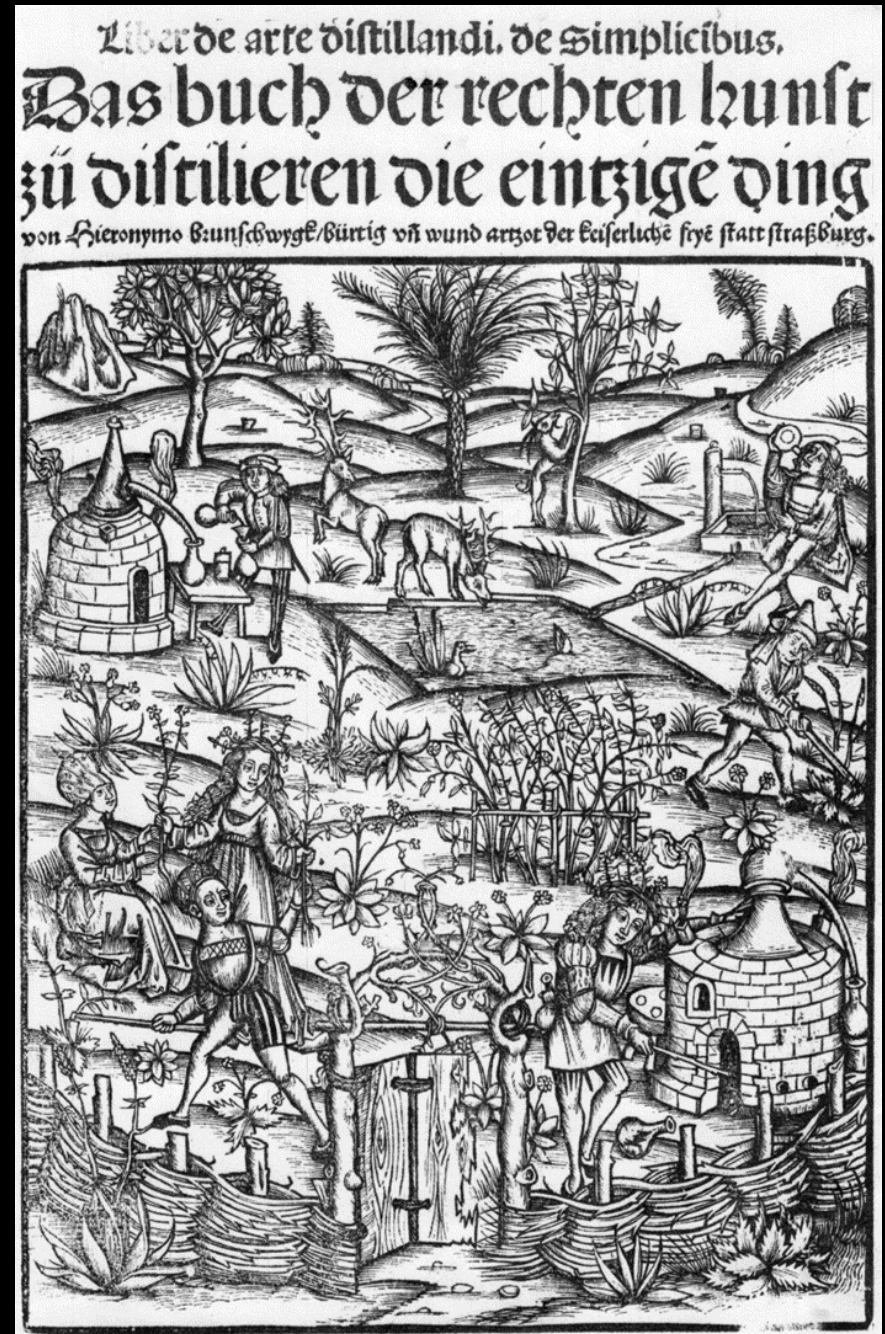
G. the pype, by which the wine is poured in.

H. doth instructe the way, by which the vapors ascende.

I. The nose of the heade, to which the mouth of the recepuer is set & fastned. K. Doth here represent the bucket or other vessell, filled with colde water, and cooling the head. L. Doth shew the cane or pype, by which the colde water ascendeth or ryseth up into the bucket. M. Representeth the nose or pype of the bucket, by which the hote water is drawne forth. N. Signifieth the barrell or hogges heade made long, and filled with colde water.

An instrument which is so formed, that the water by sucking, is forced to ryse up and run forth: as the lyke practise is often used, in pittes of water, or welles. And by this instrument with a little fire, maye a great quantitie of the water of lyfe be dystilld and gatherd.

- Improvements in distillation
- Hieronymus Brunswyck (1450-1512)
- *Liber de arte distillandi de simplicibus* (1500)
- Widely circulated in Europe



Expense of ethyl alcohol

- Production is time-consuming
- Distillation has high energy requirements
- Ethyl alcohol is usually taxed



Other fluids

- Preservative chemicals

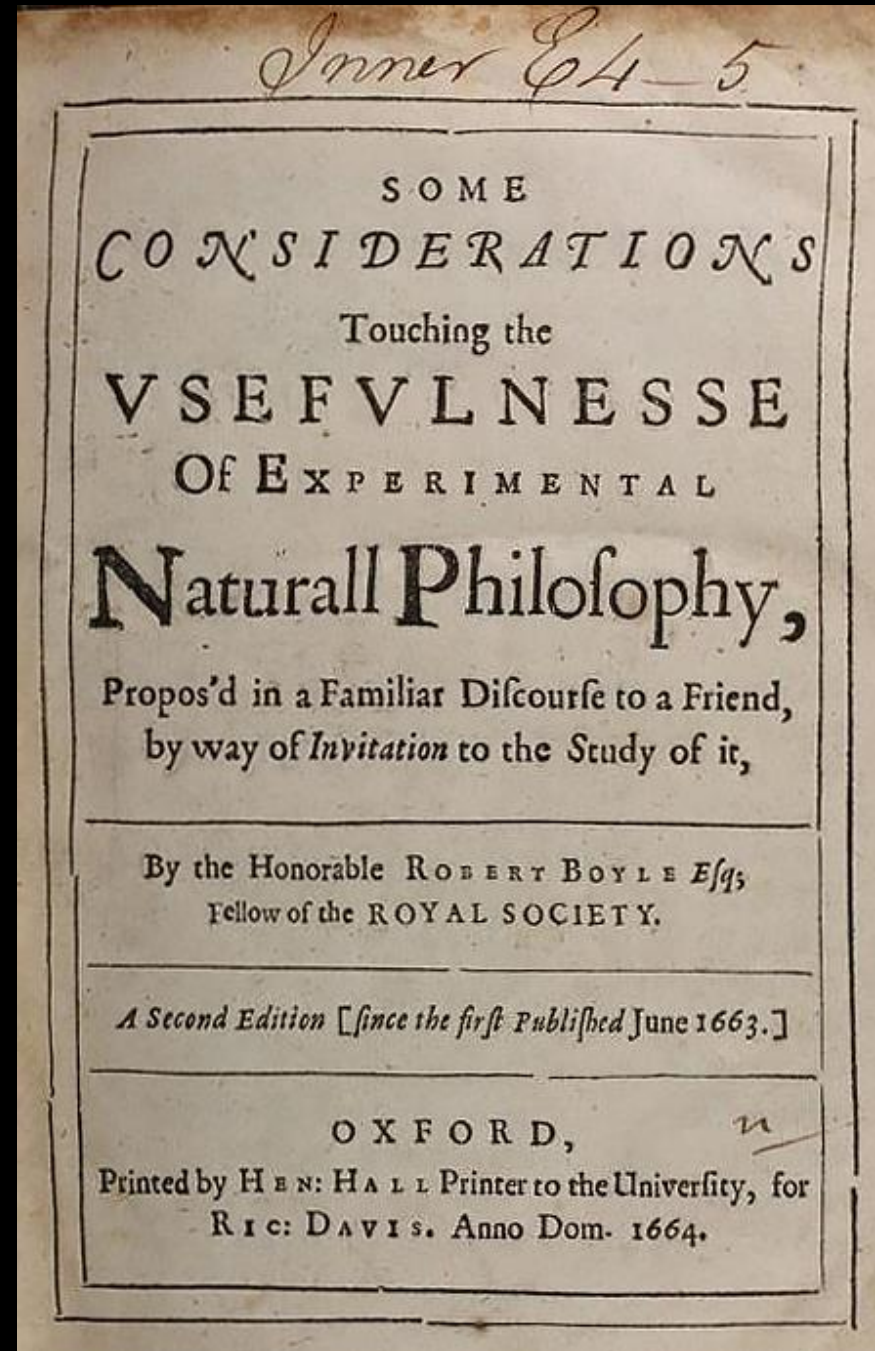
- Vinegar
- Turpentine
- Alum (alun)
- Mercuric chloride
- Brine
- Oils

- Robert Boyle (1627-1691) experimented with “oyl of spikes” as a preservative



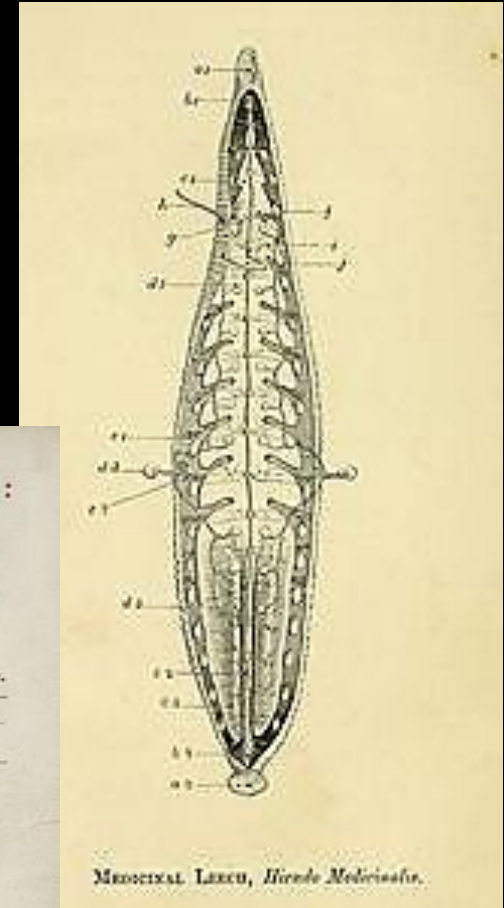
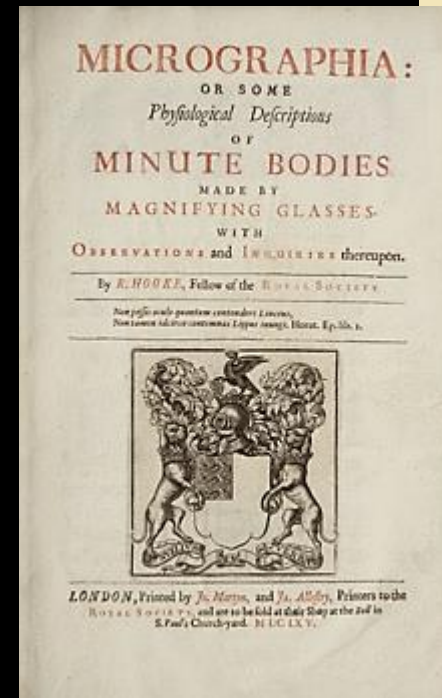
Boyle, 1664

“...it cannot but be a great help to the student of anatomy to be able to preserve the parts of human bodies, and those of other animals...”



Other preserving fluids

- Robert Hooke (1635-1703)
 - Leeches in vinegar
- 1838—Jean-Nicolas Gannal (1791-1852)
 - Aluminum sulfate
 - Alum (alun)



William Croone

- 04 June, 1662
- Royal Society of London
- Preserved two dog embryos in "spirit of wine"



Mr. CROONE produced two embryos of puppy-dogs, which he had kept eight days, and were put in spirit of wine in a glass-vial sealed hermetically.

Mr. BOYLE promised to make the like experiment in rabbits.

The lord viscount BRONCKER was desired to bring to the next meeting his paper of experiments, to be registered.

Dr. WILKINS brought two dead insects, called death-watches, which Dr. ENT had promised to produce.

International Old Croone Day

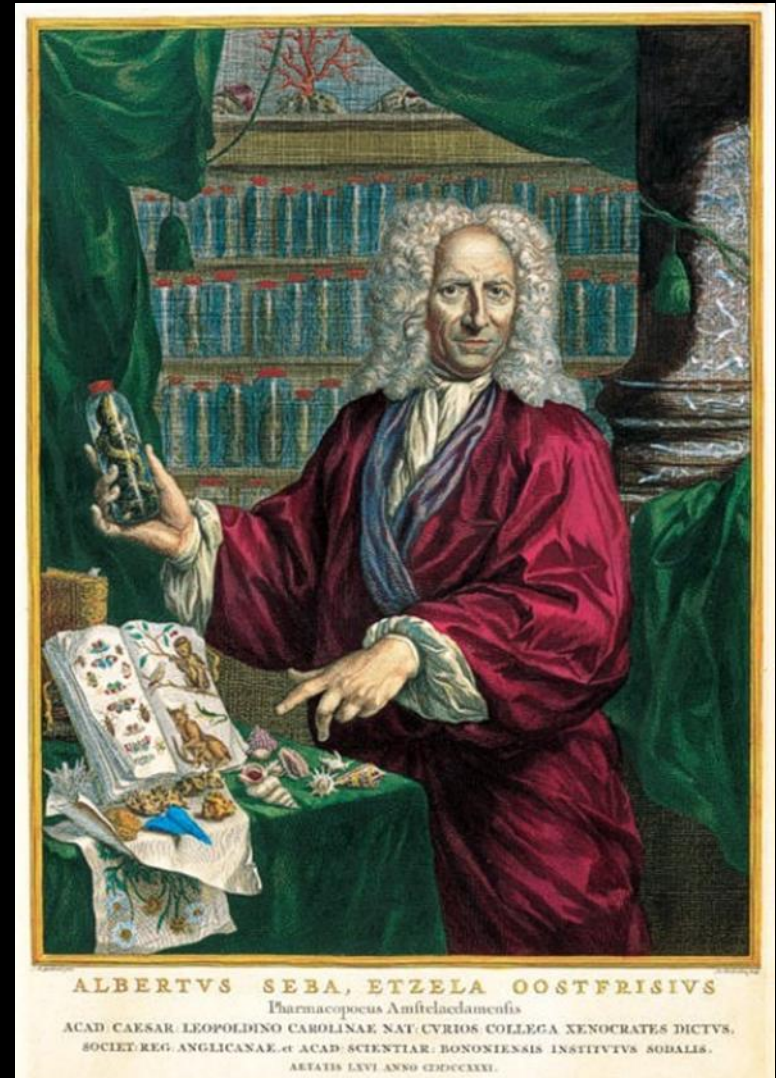
- 04 June
- Toast Dr. Croone
- Beverage of your choice



Expense of alcohol

- Expense of alcohol and containers
- Boyle's experiments
 - Change fluid after initial preservation
 - Test with flame

Albertus Seba (1665-1736)
with jars of fluid preserved
specimens sealed in red wax



Expense of alcohol

- Beverage alcohol often substituted
- 1884—British Museum
 - 56% alcohol for fish
 - Goadby's solution (salt, alum, mercuric chloride, and water)



Specimens from the
Museum Adolphi
Friderici collection,
Stockholm



Rack, rum, or brandy

- James Petiver (1635-1718)
- Brief Directions for the Easie Making and Preserving Collections of all Natural Curiosities (ca. 1700)

Brief Directions for the Easie Making and Preserving Collections of all NATURAL Curiosities. For JAMES PETIVER Fellow of the Royall Society LONDON.

All small Animals, as Beasts, Birds, Fishes, Serpents, Lizards, and other Fleehy Bodies capable of Corruption, are certainly preserved in Rack, Rum, Brandy, or any other Spirit; but where these are not easily to be had, a strong Pickle, or Brine of Sea Water may serve; to every Gallon of which, put 3 or 4 Handfulls of Common or Bay Salt, with a Spoonfull or two of Allom powder, if you have any, and so send them in any Pot, Bottle, Jar, &c. close stopp'd, Cork'd and Rejsin'd. N.B. You may often find in the Stomachs of Shaaks, and other great Fish, which you catch at Sea, divers strange Animals not easily to be met with elsewhere; which pray look for, and preserve as above.

As to Towls, those that are large, if we cannot have their Cases whole, their Head, Leggs, or Wings will be acceptable, but smaller Birds are easily sent entire, by putting them in Spirits as above, or if you bring them dry, you must take out their Entrails; which is best done by cutting them under their Wing, and then stuff them with Ockam or Tow, mixt with Pitch or Tar; and being thoroughly dried in the Sun, wrap them up close, to keep them from Moisture, but in long Voyages, you must Bake them gently, once in a Month or two, to kill the Vermin which often breed in them.

All large pulpy moist Fruit, that are apt to decay or rot, as Apples, Cherries, Cowcumpers, Oranges, and such like, must be sent in Spirits or Pickle, as Mangoes, &c. and to each Fruit, its desired you will pin or tie a sprig of its Leaves, and Flowers.

All Seed and dry Fruit, as Nutts, Pods, Heads, Huiks, &c. these need no other Care, but to be sent whole, and if you add a Leaf or two with its Flower, it will be the more instructive, as also a piece of the Wood, Bark, Root, or Gum of any Tree or Herb that is remarkable for its Beauty, Smell, Uie, or Vertue.

In Collecting PLANTS, Pray observe to get that part of either Tree, or Herb, as hath its Flower, Seed, or Fruit on it; but if neither, then gather it as it is, and if the Leaves which grow near the Root of any Herb, differ from those above, be pleas'd to get both to Compleat the Specimen; these must be put into a Book, or Quire of Brown Paper stich'd (which you must take with you) as soon as gathered; You must now and then shift these into fresh Books, to prevent either rotting themselves or Paper. N.B. All Gulph-Weeds, Sea-Mollies, Coralls, Corallines, Sea Feathers, Spunges, &c. may be put altogether into any old Box, or Barrel, with the Shrimps, Prawns, Crabs, Crawfish, &c. which you will often find amongst the Sea-weeds, or on the Shoar with the Shells, which you may place in layers, as we do a Barrel of Colchester Cysters. All SHELLS may be thus sent as you find them, with or without their Snails in them, and wherever you meet with different sizes of the same sort; pray gather the fairest of all Magnitudes; the Sea shells will be very acceptable, yet the Land, and Fresh-water ones, are the most rare and desirable. In Relation to INSECTS, as Beetles, Spiders, Grasshopper, Bees, Waips, Flies, &c. these may be Drowned altogether, as soon as Caught in a little wide Mouth'd Glass, or Vial, half full of Spirits, which you may carry in your Pocket: But all Butterflies and Moths, as have mealy Wings, whose Colours may be rub'd off, with the Fingers, these must be put into any small Printed Book, as soon as caught, after the same manner you do of Plants.

All Metals, Minerals, Ores, Chrystals, Spars, Colow'd Earths, Clays, &c. to be taken as you find them, as also such formed Stones, as have any resemblance to Shells, Corals, Bones, or other parts of Animals, these must be got as entire as you can, the like to be Observ'd in Marble'd Flints, Slates, or other Stones, that have the Impression of Plants, Fishes, Insects, or other Bodies on them: These are to be Found in Quarries, Mines, Stone or Gravel Pits, Caves, Cliffs, and Rocks, on the Sea shore, or wherever the Earth is laid open. NOTE If to any ANIMAL, PLANT, MINERAL &c. you can learn its Name, Nature, Vertue, or Use, it will be still the more Acceptable.

N.B. As amongst Foreign Plants, the most common Grass, Ruth, Moss, Fern, Thistle, Thorn, or wildg Weed you can find, will meet with Acceptance, as well as a scarcer Plant; So in all other things, gather whatever you meet with, but if very common, or well known, the fewer of that Sort, will be acceptable to

Your most Humble Servant

Aldersgate street
LONDON.

JAMES PETIVER.

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Directions for Collecting Natural Rarities. 6.

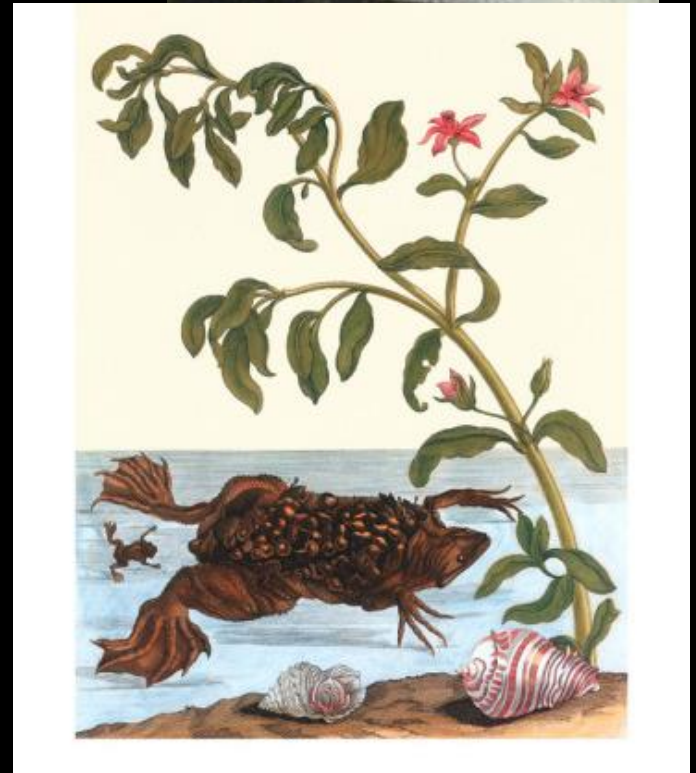
Such as Buy 5 of each, to have a 6th Gratis. PETIVER

“All small Animals, as Beasts, Birds, Fishes, Serpents, Lizards, and other Fleshy Bodies capable of corruption, are certainly to be preserved in Rack, Rum, Brandy, or any other Spirits; but where these are not easily had, a strong Pickle, or Brine of Sea Water may serve; to every Gallon of which, put 3 or 4 Handfulls of Common or Bay Salt, with a Spoonful or two of Allom powdered...”

- Rack = arrack (coconut or rice)
- Rum (sugar cane)
- Brandy (distilled wine)
- Strong pickle (vinegar), brine (salt)
- Allom = alum

Maria Sybilla Merian (1647-1717)

- Surinam, 1699-1701
- “...everything I did not need to paint (in Surinam) I brought with me, such as butterflies and beetles and everything I could steep in brandy...”

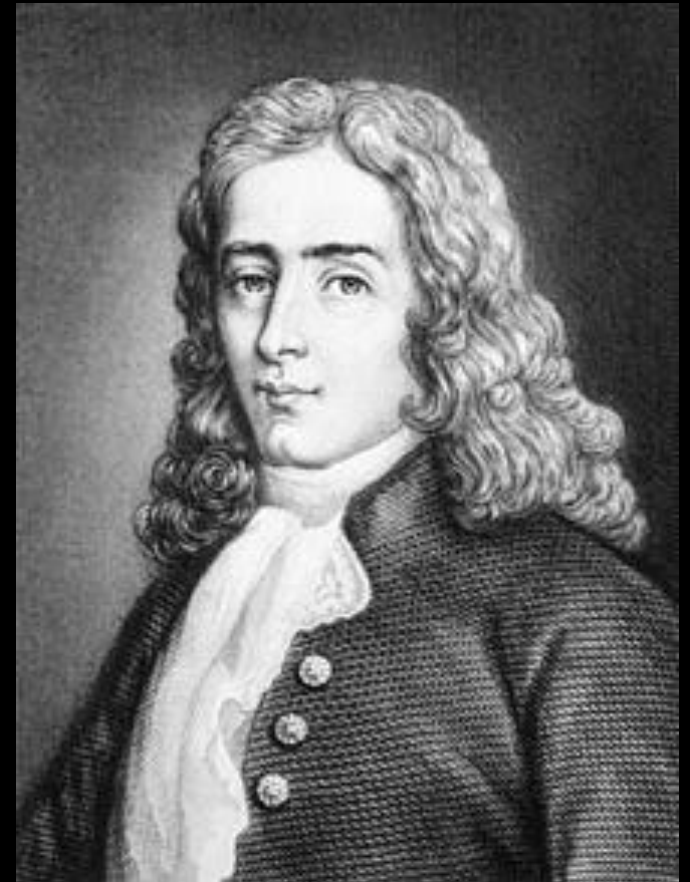


Very old fluid specimens

- Cook expeditions, 1668-1771
- Joseph Banks (1743-1820)
- Preserved in rum
- Oldest extant fluid-preserved bird specimens?
 - *Vestiaria coccinea*, Hawaii
 - *Creadion carunculatus*, New Zealand

Specimen preservation

- René-Antoine Ferchault de Réaumur (1683-1757)
- 1748—Described four most common methods of preserving specimens
 - Stuffed and dried
 - Embalmed with spices
 - Dried in an oven
 - Preserved in alcohol



“There is no great Skill required for putting one or several [specimens] into a Vessel full of Spirit of Wine, or very strong Brandy. It has been usual for a long time to make use of those Liquors with Success for preserving the Flesh of dead Animals...”

“...you are only to keep them in Brandy; the stronger the better it will be for producing the intended Effect: Spirit of Wine is even preferable. As for the rest, it is indifferent whether the Brandy be distilled from Wine, Corn, or Sugar”

Additives to alcohol

- Alum
- Ammonium chloride
- Arsenic
- Acids
- Glycerin
- Mercuric chloride
- Salts

Tarsius tarsier, preserved
prior to 1777



Additives to alcohol

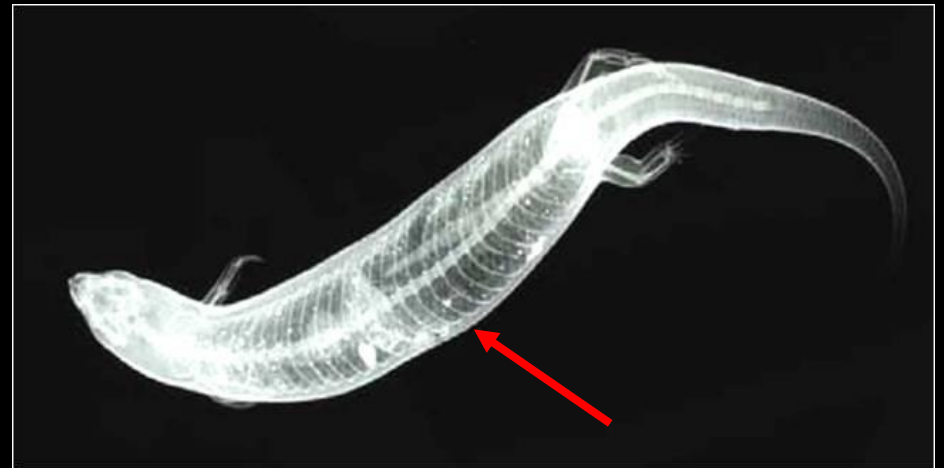


“History can build up
in specimen jars.”
—Dirk Neumann



Additives to alcohol

- Preserved 1893-1914
- Mercuric chloride, HgCl_2
- Reduction reaction with ethanol



Chalcides ocellatus

Fixation

A. Physical methods

1. Dehydration
2. Heat

B. Chemical methods

1. Cross-linking chemicals
2. Metal salts
3. Acids

C. Combined methods

Physical fixation

- Dehydration
- Heat
- Effects
 - Removes water from tissues
 - Precipitates proteins
 - Aggregates cellular components

Chemical fixation

- Coagulants
 - Alcohols
 - Acetone
 - Acids
 - Salts
 - Precipitate proteins
 - Coagulate proteins

Chemical fixation

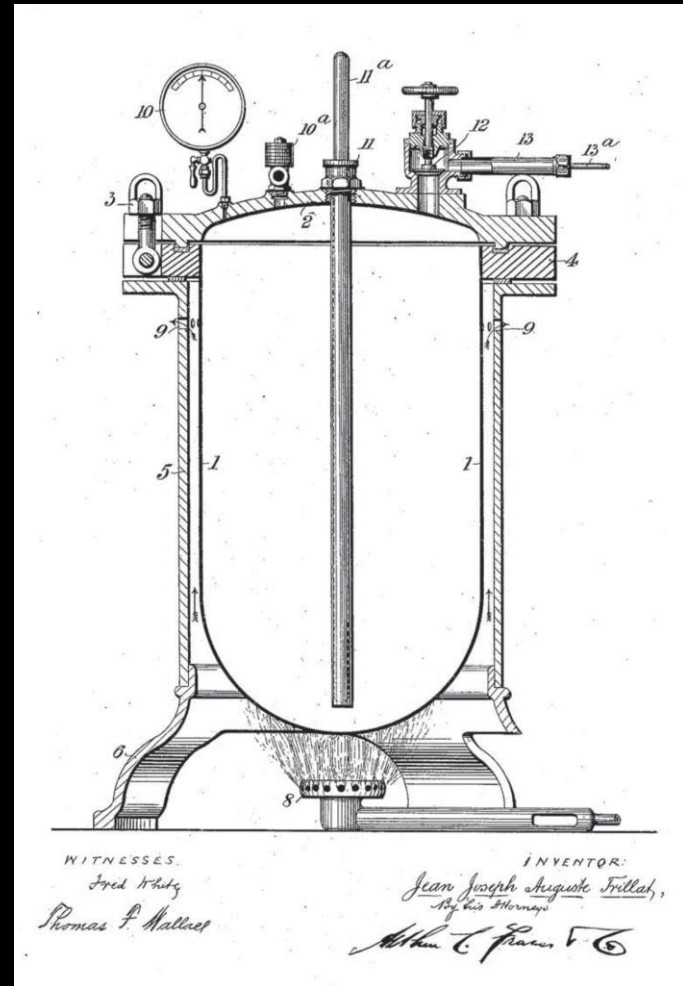
- Non-coagulants
 - Cross-link proteins
 - Aldehydes
 - Formaldehyde
 - Gluteraldehyde
 - Glyoxal (OCHCHO)
 - Chloral hydrate
 - Metal salts (mercuric chloride, zinc chloride)
 - Oxidizing agents (e.g., osmium tetroxide)

History of fixatives

- 1663—Robert Hooke (1635-1703)—olive oil and vinegar
- 1666—Marcelo Malpighi (1628-1694) boiled kidney slice, coated surface with ink
- Fixation more common with increased use of microscopes
- 1833—chromic acid to harden tissues

Formaldehyde history

- 1858—discovery of gas by Alexander Butlerov (1828-1886)
- 1868—Aqueous solution by August Wilhelm von Hofmann (1818-1892)



Formaldehyde history

- 1888—patent for manufacture by Jean-Auguste Trillat (1861-1944)
- 40% (saturated solution)

Trillat patent filed in the United States in 1897; patent granted 1899

UNITED STATES PATENT OFFICE.

JEAN JOSEPH AUGUSTE TRILLAT, OF PARIS, FRANCE, ASSIGNOR TO LA SOCIÉTÉ CHIMIQUE DES USINES DU RHÔNE, ANCIENNEMENT GILLIARD, P. MONNET ET CARTIER, OF LYONS, FRANCE.

FORMALDEHYDE SOLUTION.

SPECIFICATION forming part of Letters Patent No. 628,502, dated July 11, 1899.

Application filed November 16, 1897. Serial No. 658,754. (No specimens.)

To all whom it may concern:

Be it known that I, JEAN JOSEPH AUGUSTE TRILLAT, of Paris, France, have invented certain new and useful Improvements in Disinfectants, (patented in Great Britain, No. 20,622, September 17, 1896,) of which the following is a specification.

bodies are insoluble in the aforesaid ethers, it is necessary to dissolve them first in an appropriate solvent of low boiling-point. Methylic alcohol has been proved to be very appropriate for this purpose, as a very small quantity is required to dissolve the disinfecting agent. To this solution is added the re-

Formaldehyde history

- Ferdinand Blum (1865-1959)
- Diluted 40% formaldehyde 1:9 = 4%
- 1892—hardened the epidermis of his own fingers

Der Formaldehyd als Härtungsmittel.

Vorläufige Mittheilung

von

Dr. F. Blum,

praktischer Arzt in Frankfurt a. M.

Dem Formaldehyd in wässeriger Lösung kommt, wie ich neulich gezeigt habe², die merkwürdige Eigenschaft zu, selbst in ziemlich concentrirten Lösungen nur langsam, aber auch äusserst verdünnt mit grosser Sicherheit Mikroorganismen abzutöden. Diese langsame, sichere Desinfection scheint auf einer eigenthümlichen Umwandlung der organischen Materie zu beruhen, bei welcher die Gewebe — welcher Bestandtheil derselben, möge heute vollständig unerörtet bleiben — aus ihrem festweichen Aggregatzustand in eine wesentlich resistenterere, härtere Modification übergehen.

Formaldehyde

“Laboratories have been using a 4% solution ever since, not because of any scientific evidence, but because that was the dilution of the commercial product used by Dr. Blum when he fixed his fingers.”



(Fox and Benton 1987)

Isopropyl alcohol

- First made in 1855
- 1920—commercial production
- 1922—used as preservative
- Less expensive than ethyl alcohol
- Less regulated than ethyl alcohol
- Twice as toxic as ethyl alcohol
- Causes more shrinkage than ethyl alcohol

Other preservatives

- Phenols and glycol (1950s-1960s)
 - Less hazardous
 - Short-term holding fluids
- Glycerin
 - Additive to alcohol
 - Preservative
 - Absorbs moisture
 - Good medium for microorganism growth



Phenol solution with specimen



Left = bacterial bloom in glycerin

Processes and chemicals

- Collection, relaxation, euthanasia
 - Other chemicals
- Preservation methodology
 - Time between death and chemical treatment
- Preservation directly in alcohol
 - Syneresis (distortion from rapid shrinkage of cellular contents)
 - Generally results in more shrinkage than with a fixative

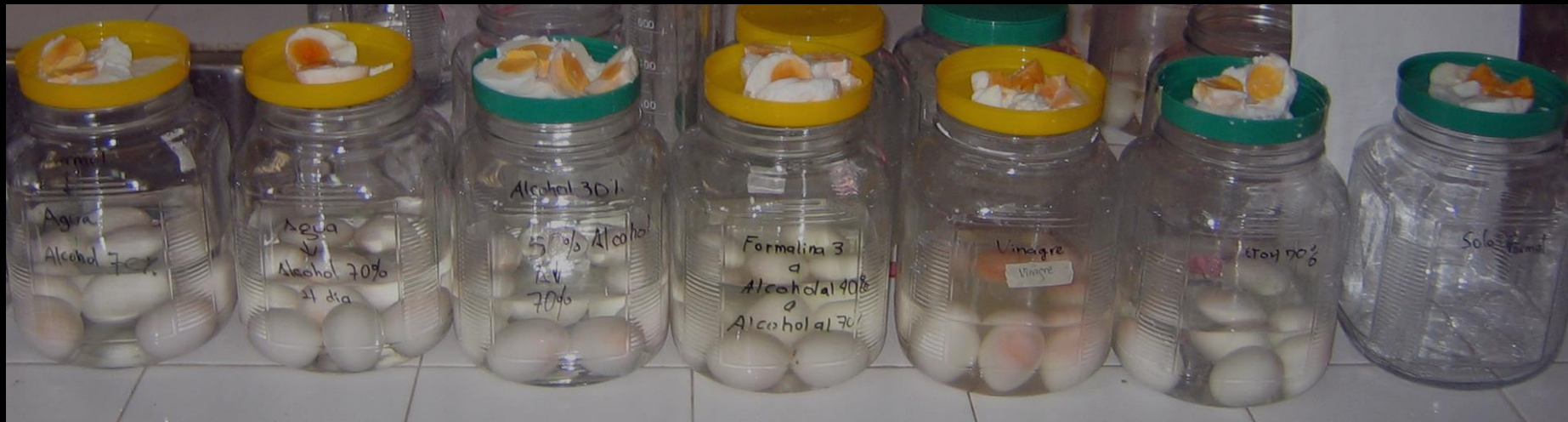
Effects of chemicals

- Chemical modifications
 - Proteins
 - Lipids
 - Cells and cell contents
 - Loss of minerals and metals
- Morphometric (dimensional) changes
- Alterations of colors



Proteins

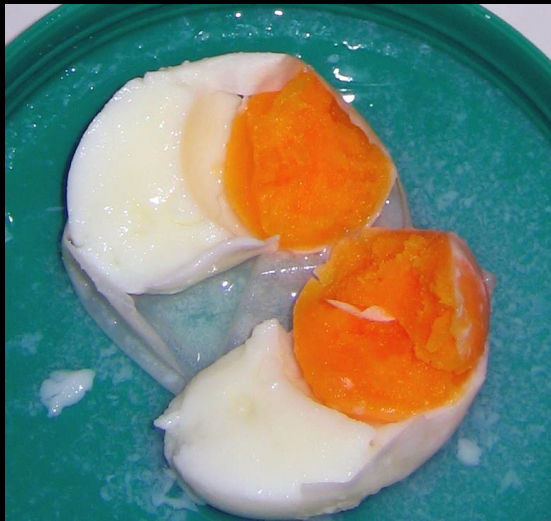
- Hydroxymethyl groups form crosslinks in tissues
- Other complex reactions
 - Formation of methylene glycol alters cellular membranes and mitochondrial organization
- Little research on particular tissues



Vinegar used to remove shells; eggs preserved in vinegar, formaldehyde, 70% alcohol, 40% to 70% alcohol, or formaldehyde-to-70% alcohol



Vinegar



Formaldehyde



70% ETOH

von Endt (1994)

- Loss of general and structural protein
- Keratin and collagen samples (hair and feathers)
- 70% ETOH at 180°C for 1-2 days
- 50-55% isopropyl alcohol at 180°C for 1-2 days
- Within and without 1% formaldehyde
- Dry samples for controls



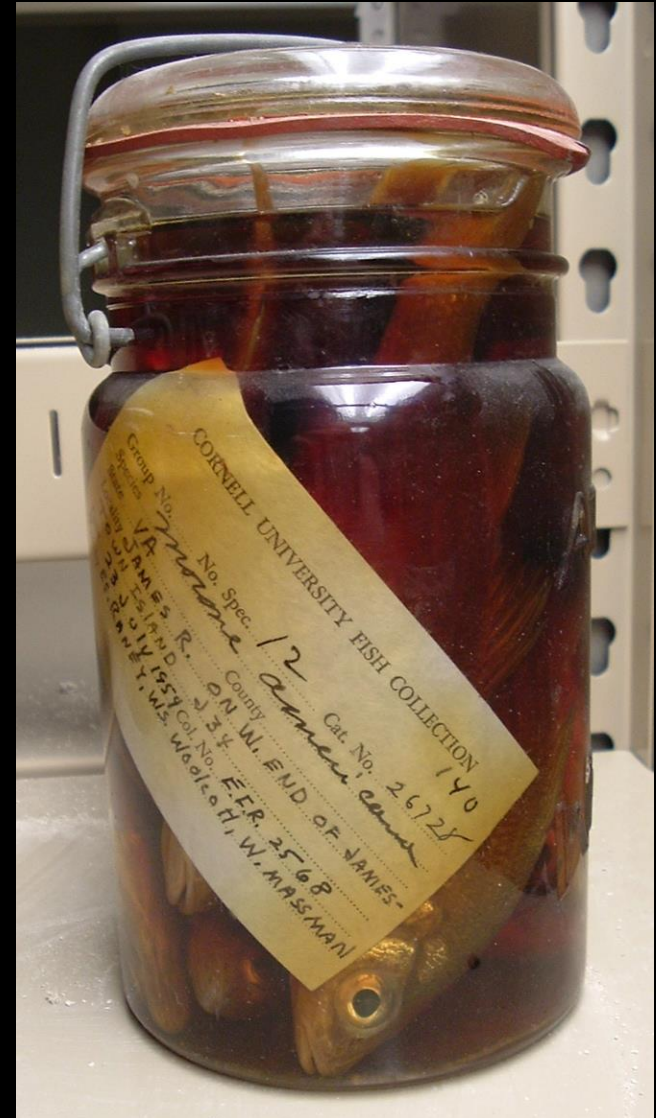
von Endt (1994)

- Collagen dissolves more quickly than keratin
- Isopropyl causes more damage than ethanol
- Keratins unstable at high temperature
- Feathers less stable than hair
- Dry keratin more stable than in fluid
- Formaldehyde promotes deterioration of keratin



Lipids

- Lipids are hydrolized
- Migrate within specimens
- Removed by alcohol
- May form fatty acids that affect preservative quality



Other modifications

- Coagulation and dispersal of cell contents
- Loss of mineral content
- Damage to otoliths
- Loss of muscle definition
- Changes in skin texture
- Deposits on specimens



Dimensional (morphometric)

- Invertebrates:
 - Swelling
 - Shrinkage
 - Weight loss
 - Color changes
 - Embrittlement
 - Loss of structural integrity
 - Extraction of minerals and metals



Benthic animals	3-10% weight loss
Littoral benthos	1-18% weight loss or 1-7% weight gain
Plankton	15-87% shrinkage
Zooplankton	37-43% weight loss; 59-69% weight loss
Planktonic hydromedusae	67-85% shrinkage
Sycphomedusa	7-10% shrinkage; 15-30% umbrella shrinkage
Medusae	26% shrinkage; 70% weight loss
Copepods	20-38% weight loss
Ctenophores	5-80% weight loss, 20-31% shrinkage
Salps, doliolids	39-52% weight loss; 86-93% shrinkage
Tubificid worms	10-38% weight loss
Crayfish	4-16% weight loss
Mayflies	5-17% shrinkage

Dimensional (morphometric)

- Vertebrates:
 - Morphometric variation in fish
 - Shrinkage is variable
 - Less shrinkage in methyl alcohol
 - More shrinkage in isopropyl alcohol



Fish	2-5% weight gain 4-9% weight gain 2% weight loss 3.5-7% shrinkage 5-14% shrinkage 9-16% shrinkage 12-18% shrinkage 30-43% shrinkage
Anurans	1-6% shrinkage
Lizard eggs	34-64% weight increase
Lizards	3-16% shrinkage
Snakes	2-3% shrinkage

Morphometric changes

“The effects of preservatives on fish morphometrics are difficult to predict because of variance related to the type of preservative, duration of preservation, origin of species (marine or freshwater fish), species, life stage, and others”

—Sagnes 1997

Colors and patterns

- Formaldehyde darkening (formaldehyde pigment)
 - Formic acid
 - Blood-rich tissues
 - Below pH 6
 - Reaction with hematin



Colors and patterns

- Alteration or extraction of pigments
- Changes in reflection or refraction of light
- Both



Leaching of xanthophores by alcohol + alteration of iridophores by dehydration

Summary

- The preservative fluid contains components extracted from the specimen.
- Understanding chemical changes in specimens is fundamental to use of specimens in research.
- Much more research needs to be done.

Thank you

