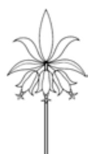

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The Hunt Institute for Botanical Documentation, a research division of Carnegie Mellon University, specializes in the history of botany and all aspects of plant science and serves the international scientific community through research and documentation. To this end, the Institute acquires and maintains authoritative collections of books, plant images, manuscripts, portraits and data files, and provides publications and other modes of information service. The Institute meets the reference needs of botanists, biologists, historians, conservationists, librarians, bibliographers and the public at large, especially those concerned with any aspect of the North American flora.

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Chapts. 6 + 15.

Suffixes for
order – ales
Suborder ineae
Family aceae
Sub " oideae
Tribe – eae
Syn – inea
Genus –
Sp –

6/29 – Taxonomic treatment of bact.

1. Basis – activity more than form.
2. Necessity – an understanding.

Bergey's system is presently accepted – manual edited by many specialists.

Only used in U.S – does not apply in countries outside of US.

Classification of Schizomycetes
Pl. Kingd., Thallophyta – Algae + Fungi (subphylla)
Class Schizomycetes (Fission fungi)

1st Requirement of classification – rigidity of cell

Rigid Orders
1. Eubacteriales
2. Actinomycetales
3. Chlamydobacteriales
↓ cells enclosed in sheaths
+ 3. | – 1. 2.
↓ Branching or mycelial cells
+ 2. | – 1.

Non Rigid Orders
4. Myxobacteriales
5. Spirochaetales
↓ (spiral cells)
+ 5. Spiral | – 4. non spiral

Starting w/ 1-Eubacteriales – contains most org. considered as true bact. – suborders based on morphological + physiological charac.
↓ Photosynthetic pigments w/ some cells containing free Sulfur
+ Rhodobacterineae | – 1. Eubacterineae (true bact.) 2. Caulobacterineae
↓ Cells attached to substrate by a stalk –
+ 2. | – 1.

The Eubacterineae (true bact., largely studied in lab.)
13 families.

Caulobacterineae – a single spherical or kidney shaped cell supported by a stalk attached to substrate by holdfast
Stalk made of $Fe(OH)_3$ – the prepondarant group, in natural habitat of cool, iron containing waters, a nuisance in H_2O pipes

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Conidia - special struct. available for reproduction.
as cellular material grows out into mycelium, ~~septae formed~~
~~as~~ hyphae produce a bulbous structure (conidiophore)
which produces ~~large~~ numbers of hyphae within which
septae are formed, w/ production of large nos. of conidia.

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Rhodobacteriineae - photosynthetic bacteria, not considered true bact., but the only group w/ this character - have similar physiological reactions (respir. + fermentation) as true bact.
Resemble bact., but contain a pigment of chlorophyll-like subst. + carotene (
Growth under anaerobic conditions in presence of light - in H_2O w/ sulfur compds. occurring particularly in the canals of Holland - because of this occurrence, Van Niel - Delft school worked on group. $H_2S + CO_2 \rightarrow (CH_2O) + H_2O + S$.
This equation worked out by Van Niel important because here found ~~that~~ O_2 not the most important, but H_2.
van Niel divided this group into 3 subgroups on basis of morph. + physiol. charact.

↓ Green pigment

+	−
Chlorobacteriaceae	1. Thiorhodaceae / Sulfur + or −, purple (Thio indicates Sulfur)
	2. Athiorhodaceae - always w/o sulfur. Purple or brown, prefer organic source of H_2

↓ prefer organic source of H_2

+	−
2. Athio	1. ~~Thiorhodo~~

⟹ Order Actinomycetales - stepping stones from bact. to fungi.
- org. forming elongate cells sometimes branching or mycelia, multiply by splitting, spores or conidia

↓ Tangled mycelial masses w/ production of conidia at end of hyphae

+	−
Streptomycetaceae	1. Mycobacteriaceae
	2. Actinomycetaceae

↓ tendency toward branching + breaking off to form new filaments or cells

+	−
2.	1.

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Streptomycetaceae - most organisms used today to prepare antibiotics.

Actinomycetaceae - contain a considerable no. of pathogens.
Mycobacteriaceae - causative organism of T.B.

Order Chlamydobacteriales - sheathed bacteria, cellulose material surrounding cells, aquatic habitat.

↓ 1. Sulfur granules + or -
2. No false branching

\+ | -

Beggiatoaceae

1. Chlamydobacteriaceae
2. Crenothrichaceae

↓ Type of cellular division
1. Capable of division in 3 planes

\+ | -
2 | 1.

↓ may or may not show false branching + may show deposition of ferric hydroxide in their sheaths.

div. in 3 planes
cells spherical
capable of conidia prod.

capable here of 3 plane division

sheath of $Fe(OH)_3$ or polysaccharides

apparently organism does not require Fe for metabolism

Beggiatoaceae - non photosynthetic sulphur bact.

July 2 — Myxobacteriales - called slime bacteria
Thin, irregularly shaped spreading colonies in swarm stage -
Change in fruiting bodies - cysts formed.
Able to decompose cellulose (a complex sugar) so are valuable in decomposition of pl. material in soil.

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Genus *Cytophaga* - some capable of decomposing agar.

Order Spirochaetales - entirely motile by serpentine or spinning motion

When seen by mike, one plane only seen so look: ∿∿∿ but actually ꝏꝏ ꝏꝏ.

Best observed by dark field mike - below resolution of ordinary mike.

Best stained w/ silver to impregnate flagella.

Families

1. Spirochaetaceae - aside from protoplasmic make up, have a lateral "crysta" on one side. Some have an axial center -.

2. Treponemataceae - w/o any of additional features of "1".

Pathogenic

Genus *Treponema* - strict anaerobes, parasites, poorly stained - cause syphilis.

Borrelia

Leptospira ∿∿∿ loops over an end.

Observation of *Treponema* - grace w/ which it swims, and no. of curves useful in identification. An org. from syphilis lesion pretty good change of being *Treponema*.

Further discussion of suborder Eubacteriineae -

True bact., common in lab, pathogens, water contaminants - the typical organisms - thirteen families.

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Books to be purchased

1. Plant Biochemistry - Bonner, 1950
   Academic Press, Inc.
   N.Y. 10.

2. Topley + Wilson - Principles of Bacteriology + Immunity.
   2 Vols. William Wilkins, N.Y.

3. "Methods & Materials for Teaching Biological Sciences": Miller + Blades 1938.
   McGraw-Hill.

4. Dictionary of Genetics - R. L. Knight R 575.03
   Chronica Botanica, Waltham, Mass. K 747d

Rex D. Pearce
Moorestown, N.J., for Desmodium gyrans + sensitive plant.

Manual of Methods for Pure Culture Study of Bacteriology.
Biotech Publications Box 269, Geneva, N.Y.
Price $2.00

Manual of Dehydrated Culture Media + Reagents.
Difco Laboratories, Detroit Mich.

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Bact - Clifton - Introd. to the Bact. - McGraw-Hill,

Chapt 11 - Clifton - Growth requirements.

Classific on food requirements

1. Autotrophic - large no. of soil + H_2O bact. self nourishing cap. of util. inorg. salts, CO_2 from air + using the C. Cap. of using N from NH_3 salts. cap. of growing on inorganic media. Agar is sometimes inhibitory.

2. Heterotrophic - req. organic mater furnished - saccharides - amino acids (building blocks of proteins)
process AA → Proteoses → Peptones → Proteins.

Proteins very imp. in metabolism
Amino acid

NH_2 + COOH
amino grp. organic acid.

Ex. of heterotrophs - pathogens, parasites.

Classif. on energy deriv.

1. Photosynthetic
2. Chemosynthetic - chem. oxidations (exch. of electrons gain - oxidat, loss of elect. - reductions

Classif. on oxigen req.

1. Obligate aerobes + facultative.
2. Microaerophils - req. small amt. O_2.
3. Anaerobes -
4. Facultative anaerobes - cap or incap. of O_2 consumption prefer w/O O_2.

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In cultivat. 5 major requirements.

Water
Regulatory substances (adjustable subst.
1. Osmotic Press
2. Permeability of Cell Mem.
3. H-ion conc.
4. Oxid- Reduc. Potential
Sources of Energy
" of Bldg. Mater.
Accessory Growth Factors.

Water is basic -
Removal of heat of oxidation - only pt. of heat used by cell.

Regulatory subst.
Over or under conc. is dangerous.
A Hypertonic soln - high osmotic soln will cause dehydration
A Hypotonic soln - low osmotic press.

Hypertonic

cell

Isotonic soln. ideal.

Permeability of membrane - must consider in preparing media to be sure of optimum. Not much known about

H ion conc. controlable - organism optimum pH 7.

Media on autoclaving will drop the pH.

Organism on growth will reduce the pH so

must have some sort of buffer soln.

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Oxid-Reduct. Pot.-

Methods of det.n

1. Methylene blue - a H_2 acceptor. oxidized ~~white~~ blue, and not suitable for anaerobic growth, white - reduced, not suitable for aerobic growth

2. ??

Sources of energy -

1. Autotrophs other than photosynthet require (chemosynthetic). NH_3, N, CO_2 + others.
   Nitrobacter rec. energy by oxid of $NO_2 \Rightarrow NO_3$
   Nitrosomonas } " " " of ~~NH~~ $NH_3 \Rightarrow NO_2$ -
   Nitrosococcus }
   High energy phosphate bonds - provides energy for transf. of elect.

Sources of building material.
Either simple or complex compds or elements may be utilized.

Accessory growth factors - vitamins, substances not produced by org. but is necessary. ~~The~~ Are not food materials but are enzymes.

First found that access growth substances needed when a small amt. of an inoculum (define) would not grow, but incr. of inoculum in media increases ability to growth. The dead cells in the inoculum ~~food~~ contains necessary growth factor + produces increased growth.

Microbiological assays to determine requirements of organisms ~~for~~ certain growth factors

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Influence of media on bacterial flora.

Organisms found depends on kind of media ~~agar~~ provided.

a protein media will provide food only for protein bact.
a carbohydrate " " " " " " carbohydrate.

Pure cultures by enrichment cultures??

Development of media–

Determination of water contamin. - check only for E. coli. since + in fecal material.
Method - 1 cc H_2O in lactose broth - then grow in a selective media (Endo's or EMB) to determine + of E. coli.

Dilution for pure culture not satisfactory.

Better method of pure culture prod. is to streak out organism on agar plate, remove this organism to sterile culture by examinat. under micro w/ micro-manipulator.

Colony formation–

1. Considered a post fission movement - immediately after fission cells move to certain position - movement ~~cau~~ unknown.

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Bact. 6/14 Chapt 8 Clifton - Energetics

Metabolism var of complex chem react util. to cont. viability of cell.

1. Selective environments -

2. Requirements - H_2O -
   Temperature - optimal
   $0°$-$30°$C. Psychrophilic (cold lovers)
   optimum 10-20
   $5°$-$45°$ Mesophyllic
   opt. $25°$-$37°$
   $40°$-$80°$ Thermophilic
   optimum 60-$65°$

Nutrients - building material + energy
Autotrophs - inorganic CO_2, $CaCO_3$, NH_3, NH_4Cl.
Photosynthetic or
Chemosynthetic

Photosynthetic: $6CO_2 + 12H_2S +$ light energy $\rightarrow$
$6(CH_2O) + 6H_2O + 12S^{-}$
Microbes req. S or some compd. of S.

(CH_2O) genl compd of simple sugar, this incorporates some of the energy of the sun.
The H^+ bond is weakest bond in any organic compd., all of which have more H_2 than anything else.

Chemosynthetic:

$6CO_2 + 48H_2S + 15O_2 \rightarrow 6(CH_2O) + 36H_2O + 48S$

CO_2 red. to sugar
H_2S oxid. to mole. S.
energy released in process & packed in sugar.
Same sugar is product as w/ photosynthetic.

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Nutrients (cont.)
Heterotrophic
Photosynthetic:
$6CO_2$ + organic material + light energy → $6(CHO)$ + oxidized organic material

Chemosynthetic: more involved - how?

In all cases reducing materials to get sugars & oxidizing others - whether free O_2 is present or not.

Oxidation does not require presence of O_2
Oxidation $Fe^{+++}Cl \rightarrow Fe^{++}Cl$
Reduction $Fe^{++}Cl \rightarrow Fe^{+++}Cl$.

lactic acid — pyruvic

CH_3 | OH-CH | C-OH (=O) + O_2 → CH_3 | C=O | C-OH (=O) +

another reaction giving same product w/o addition of any "air"
lactic + fumaric → pyruvic + succinic.

OH-C=O | CH ‖ C-H | C-OH (=O)

these bonds weakened →

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① $CH_3C^{H}=O \xrightarrow[\text{hot platinum needle}]{O_2} CH_3C^{OH}=O$

acetaldehyde ... acetic acid

found that same reaction w/ any compd. which can accept + donate H^+.

② $CH_3-\overset{H}{C}=O + H_2O$ (or (HOH)) → intermediate $CH_3-\overset{H}{\underset{OH}{C}}-OH$ + Paladium Black or methylene blue

↓

$CH_3\underset{OH}{C}=O + MBH_2$

The = bond capable of opening out

$-\overset{H}{\underset{|}{C}}-O$ (bond open)   =O needs one bond

C–O–(open bond)

this compd same as w/ addition of O_2 in 1st reaction, only no free O_2

The hydrogen atoms of the H_2O molecule is most important.

After production of the sugar molecule, regardless of how the sugar was produced, ~~the~~ all organisms react as chemosynthetic heterotrophs.

The enzymes + coenzymes determine what reactions will occur in utilization of the sugar provided.

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6/18 - Sect. on Respiration -

Enzymes - complex proteins - structure unknown, named for material acted on. (oxidase, reductase, etc.).
Rated on chem. activity. - temp, pH, conc., substrate.

When trying to govern or set up optimum environment for bact. cell, actually setting up system for best activity of the enzyme system.

Coenzyme makes H^+ transfer from substrate possible.
the " is the key in the lock of the enzyme.

Substrate - material on which enzyme acts.

Method of determination of the reactions + substances involved in respiration:
Stop enzyme activity at one point w/ inhibitors + see what piles up in ~~__~~ reactions up to this point.

High energy bonds -
The H bond is weakest, + empty spaces on molecules usually accept the H atom.
The PO_4 bond, by contrast, is very strong + takes much energy to separate the PO_4 from molecule. Coenzyme I contains high PO energy bonds -
Adenosine Triphosphate (ATP) → A diphosphate (ATP)

The point in cycle where pyruvic acid is produced is cross roads of respiration.
1. In anaerobic respiration

(3 carbon)
pyruvic acid → loss of CO_2 + prod. of acetaldehyde

acetaldehyde reduced → ethyl alc.
(2 carbon)

2. In aerobic ~~acid~~ respirat. → Krebs cycle (p. 194) which has the purpose of removing CO_2 + H_2O + producing products for synthesis.

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Two types of end products of glucose fermentation useful in determination of type of organism.

Lab - ~~take~~ make transfers from stock culture of E. coli + Sarcina lutea to nutrient broth.
Place stock cultures in refrigerator
" transfers in incubator.

6/19 -

Mikroscopy - Tube length - all Am. scopes have tube length of 160 mm.
The Leitz scope (Germany) has Tube length = 170 mm.

$$X = \frac{(\text{tube length})(\text{ocular } x)}{\text{focal length of tube}}$$

Measurements - methods
1. Micrometer Disc in ocular. + stage micrometer (a slide, accurately calibrated) for calibration of ocular.
2. Filar stage micrometer
3. Camera lucida - place a specimen of known size on stage,

Varieties of microscopal morphology -

1. Rod shaped (actually cylindrical) bacilli are the majority of bacter. - usually independent or ⊂⊃⊂⊃ (diplobacillus) caused by failure of complete division or separation. If farther division fails to sever chain, produces Streptobacillus.

division always at rt > to long axis

Bacillus ⊂⊃
↓
Vibrio ⌣ (cholera organism)
↓
Spirillum ∫ → Spirochaetes ≶ (not strict - bacteria) see next p.

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but between protozoa + bact. — sensitive to arsenic (arsenicals)

Coccus O cocci. division in any plane.

diplococci never have a single organism, but always at least 2. Can mechan. separate.

OOOOO — if no separation, a chain of cocci — Streptococci.

These divisions in one plane gives the chain-like structure.

Tetrads w/o separation — division in 2 planes

division in 3 planes — Sarcinae.

Staphylococcus — a grape like ~~culture~~ cluster. w/ division in all planes.

No depth of vision possible since bact. so small. These not circles, but spheres.

~~The Gram stain~~ — Weigert - 1875 advanced generally the general staining techniques. + the development of the Abbe condenser helped Koch's work w/ development

In staining, the staining ability seems to be an ion exchange.

After gram stain — know that
1. bact are either gram + or —   gram + = blue stain
" — = red ".
2. Morphology +

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Botany SS 311 Bacteriology

1st Exam. June 22nd, 1951

1. Assuming that some bacteria do have flagella, discuss them in a paragraph or two, telling all you can about them. (12 points)

1. Alternative. Explain how microorganisms can be used to determine the amount of vitamin B-1 present in "Cream of Wheat"

2. Make a drawing of a bacterial cell and identify the cellular structure which could be observed with 100,000 times magnification. (18)

3. If you wish to start a culture from a single bacterial cell, what three methods are available for your research. (14)

4. What would you do in the way of setting up the necessary environment in the laboratory for the growth of the following organisms:
   a, A psychrophylic, photosynthetic, autotroph.
   b, An aerobic, thermophylic, chemosynthetic, gram-positive, spore-forming heterotroph.
   c, Beta-type streptococcus (Causative organism of scarlet fever) (18 points)

5. Give a reasonable account for the possibility of oxidation occurring in the absence of molecular oxygen. Use words, or equations , or diagrams. (9 points)

6. Which is more (efficient) useful in terms of cellular economy, aerobic or anaerobic respiration? Why? (10 points)

7. Name a technique, method, or instrument which would expedite handling of the following problems:
   a, How to examine living material whose dimensions are less than the lower limits of resolution of the microscope.

   b, How to demonstrate motility in a microorganism.

   c, How to measure the dimensions of a bacillus. (Total value, 9)

7-Alternative. In determining the complete growth curve for a bacterial culture, the method of tube dilution and plating is more satisfactory than either volume or dry weight measurements. Give two reasons why the volume measurements are less desirable. Two reasons why the dry-weight measurements are less desirable.

8. There are two good possibilities as to why bacterial cultures exhibit an "Initial Stationary Phase" What are these hypotheses and what experimental evidence supports each? (10 points)

Choose either (1) or (1-Alternative). Do not do both !!
Treat question seven (7) in the same manner.

Turn in this paper with your answer booklet.

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These disinfectants tested by a comparison w/ Phenol activity + expressed as phenol coefficient - Number of ~~organism~~ disinfectants which do not act like phenol, so phenol comparison not too specific. These tested on chick embryos.

Other techniques of testing - plate + broth

6/26 - Chapt. 14 - Microbial variation.

1. Capable of change, usually an adaptation to environment. Example of a typical change - work done in 1930 by Karstrom w/ Leuconostoc mesenteroides - a lactic acid fermenter.
   Steps.
   1. ~~G~~Grow on nutrient A + Glucose - fermentation. Transferred to Nut. Br + Glucose - fermentation.
   2. NA + lactose (after washing w/ normal saline) → fermentation
   3. Going back to step 1, grew cells on NA w/o Glucose, washed cells, added glucose → fermentation.
   4. NA —wash→ NB + lactose → no fermentation.

   If lactose + in original medium → ferment.
   " " — " " " → no ferment.

   So that the organism adapts itself to environment + will ferment, but will not adapt to ferment. if lactose — in original.

   Constituent enzymes + at all times in organism.
   Adaptative " + only when occasion demands.

   1. Adaptive enzymes possibly + but in small quantities
   p. 310 2. Cells produce enzyme when needed.
   3. The enzyme is a suppressed character - liberated

The adaptive enzymes is one of variety of methods by which organisms vary.

Organisms always in state of flux

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Variation during growth.

1. Length of time from formation to divis. is av. 20 min.
   The change youth → maturity → div. also causes change in activity + constituents of org.
   Differences in cells taken from diff. places on growth curve
   " " staining, flagella, motility - in log. increas phase may get uniform stains, whereas death ~~ph~~ (decline) ununiform staining Even gram + organ. in decline may stain gram −.
   Change in content.
   ~~" " form~~

2. Change due to mutation.

3. Another type of adaptive change -
   Org. Listeria monocytogenes
   1. NA + Blood → small, uniform, ~~ph~~ discreet colonies, the cells flagellated only in early growth phase.
   2. NA + Glucose →(partial anaerobiosis) long, microscopic, curved rods. flagellation same as before.

Illustrates
1. Flagellation -
2. Variation due to environment.
Cells adapts to meal served.

4. W/ E. coli
   an organism in 36 hr. old environment → irregular size + shape.
   The short rods due to high surface tension.
   " long " " " low " " ,

No. of ions important.
The ions of Fe causes toxins increase, influences sporulation, capsule formation, flagella formation, colony appearance.
Flagellated cells tend to form a spreading colony (Called the "H form")
Non flagellated colonies produce a small discreet "O" form ~~fo~~ colony.

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Pigment formation influenced by + of O_2.
The ions + molecules in media influence the operation of enzyme systems.

Some adaptive changes become permanently altered.

Staphylococcus org. in presence of sulfanilamide of sublethal doses

staph. — PABA subst. sulfa- for PABA

will start to produce its own PABA and ∴ increase growth. The org. becomes highly resistant. This is not a transitory change.
Known as "drug fast" organisms - a big problem in chemotherapy.

The organism causing meningitis Meningococci - when subjected to small doses of streptomycin become so adapted to streptomycin that it requires " for growth.

Variation by Natural Selection.
a more permanent change-
An org. exactly like E. coli (except lactose fermenting ability), E. coli mutabilis.

dye + lactose
at pH
if colony ferments lactose → red.
" " does not ferment " → no color. white.
however E. coli mutable → white
but within few days colony will have little red parts.
W/ endose media, transf. white part → white at first and red dots.
" " " " red part → red colonies which indicates fermentation of lactose is going on.

So E. coli mutable mutates to E. coli

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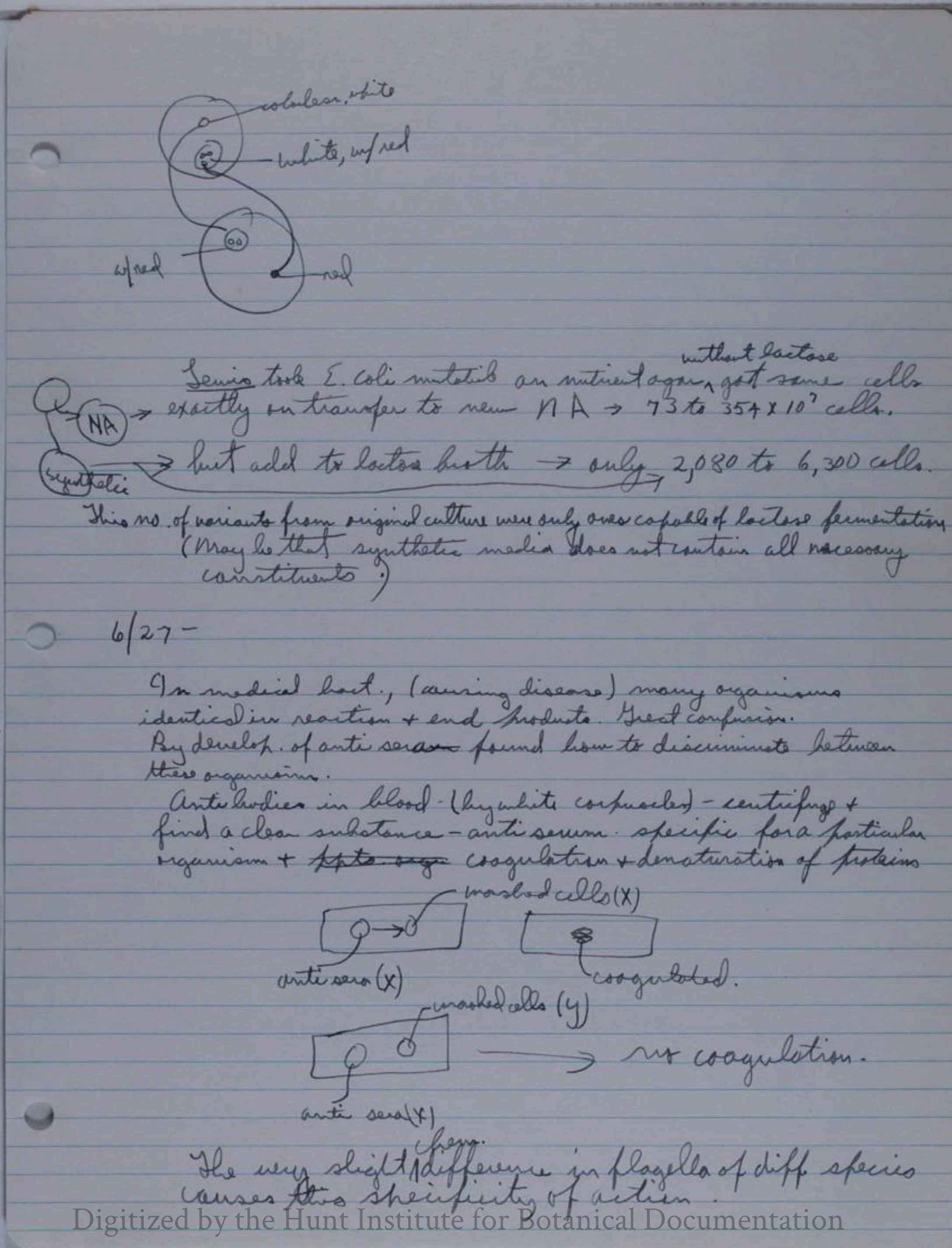

Lewis took E. coli mutabile on nutrient agar without lactose got same cells exactly on transfer to new NA → 73 to 354 x 10^7 cells.
but add to lactose broth → only 2,080 to 6,300 cells.
This no. of variants from original culture were only ones capable of lactose fermentation.
(May be that synthetic media does not contain all necessary constituents.)

6/27 –

In medical bact., (causing disease) many organisms identical in reaction + end products. Great confusion.
By develop. of anti sera found how to discriminate between these organisms.
Antibodies in blood - (by white corpuscles) - centrifuge + find a clear substance - anti serum - specific for a particular organism + ~~ppts. org.~~ coagulation + denaturation of proteins

The very slight chem. difference in flagella of diff species causes this specificity of action.

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Lab. supplies in student desk-

1. Soap + 1 needle
1. Sponge
1. Nike light
1. Bunsen burner
1. tripod
1. asbestos screen
1. pad lense paper
1. hand towel
1. grease or china marking pencil.

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Ideas to be included in lectures—

1. Students of the life sciences, (biology) probably more aware of trials & tribulations of life than any other group of scientific or non-scientific workers. These students must study the ecology, interrelationships of organisms, but does not yet know how to. In Bacter. for ex, the ecology has not developed, because all the answers now available are for pure cultures, w/artificial separation from the normal conditions, and no answers are to be had from interrelated studies w/the organisms which are the constant associates of the indiv. in nature

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BOTANY 311 BACTERIOLOGY JULY 6, 1951

1. If the following items were to be sterilized, describe how you would do the job and why you chose the particular method.

a. A five gallon capacity glass flask containing one gallon of nutrient agar (flask too large for autoclave).

b. A broth made up of complex sugars capable of being destroyed at 95° C.

c. The end of a solid steel cylinder 1 mm. in diameter.

(30 points)

2. Discuss briefly the importance of time, temperature and cellular individuality in disinfection.

(15 points)

3. Differentiate between adaptative and constitutive enzymes. Under what conditions might each be activated or deactivated.

(20 points)

4. Explain how bacteria maintain the nitrogen cycle. Why is such a cycle of importance to individuals not directly concerned with soil bacteriology?

(20 points)

5. Describe fully either of the two techniques generally used to secure a pure culture from a mixed suspension of bacteria.

(10 points)

6. Of what significance is the Gram stain?

(5 points)

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6/21 Exercises 13 + 15 -

1. Pour plates, let harden
2. Discussion of Gram stain
3. Streak plates
4. Pour plates let harden
5. Do the gram stain

Lab - Gram stain

Gram stain
Organisms

	+	—
1. Huckers crystal violet	Blue	Blue
2. Gram's iodine	Blue	Blue
3. Decolorizing	Blue	Colorless
4. Safranin (counterstain)	Blue	Red

If an organism is Gram +, the mordant fixes stain in organism. A chem. react. The Gram - organisms not effected, since no chem. present in organism to react w/ the mordant.
Decolorizing agent will wash off any dye not fixed in the cell

Never say an organism is gram + until you have made the complete gram procedure.

Divide slide into 3 areas   1 S. lutea 2 E. coli 3 E. coli + S. lutea
loopful of culture

mix 2 organisms on slide

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lab. instructions 6/26 - Ex 13 cont.

Using best pl. find yellow + white colonies well separated
Examine thru back of plate, circle + no. Use streak rather
pour plate for ease in use
After marking, make a smear of each on slide
(both on one slide)

Gram stain - use 2 min. crystal violet.
1 " grams. iodine
20 sec. alc.
10 " safranin
rinse between each stain.

Determine morphology - examine for homogeneity
of morphology
When satisfied is a pure cult., go back
to original colony - merely touch needle to
colony + transfer. to agar slants.

This technique used as process of identification
of unknowns later.

Ex. 14 - Relief or negative staining.
Use only tartar from teeth.

Ex 21 - Demonstration of motility
2 Techniques possible, this one is best.

Aside from Brownian movement, motility demonstr. by
a determined course.

Technique
Semi-solid media (agar w/ H_2O)
Inoculate tube w/ stab.

liquified

This not good because agar at the temp. prevailing
may not show liquid from non-liquid portions.

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lab 6/28 - Ex 16, 18, 19

16. K. pneumonia - do not heat
place where no drafts can blow - a virulent org.

18. B. subtilis } on same slide
B. mesentericus }
prepare smear, air dry + heat fix.

19. Mycobacterium phlei } mix org. in one place on
S. aureus } slide, dry in air + heat fix

These (expts.) not of general use

16. Most pathogens form capsules, but others too.
Some capsules are polypeptides rather than polysaccharides
so stains do can't always take.

18. Possible to see spores w/ gram stain, where just wall of
spore takes stain, but here actually staining the spore.
Variety of methods of spore staining, but phenolic
stains enter easily. heat required to get stain into cells.
Malachite green is one of best.
Use. B. cereus organism.

19. Primary stain is Ziehl's carbol fuchsin, is a phenolic
compd capable of penetrating the fats.
The cells are difficult to stain, but when stained,
difficult to remove stain.
Most organisms not acid fast.
Mycobacterium + Actinomycetes amongst few
organisms which are acid fast.

In steaming - keep slide wet w/ stain - add stain as
necessary - always place new stain in wet place +

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Lab. 7/3 – Fermentation
Method
1. Nutrient broth + glucose + ~~ot~~ indicator (for acid formation).

Always use a control (one w/ no inoculum).

2. Inverted tube in broth catches gases from fermentation

Use following media –

1 cc per 1000 cc.

1 L Glucose broth w/ phenol red indicator
1 L lactose " " " "
1 L sucrose " " " "
1 L Nutrient agar for slants.
500 cc EMB "
200 cc Starch agar

For broth, use 8 gms. Nutrient Broth
5 gms. of particular carbohydrate
1000 cc H_2O
1 cc (20 drops) indicator.
(10 cc. per tube)

For nutrient agar 8 gms. Nut. Broth
20 gms. agar
1000 cc H_2O

EMB agar dissolve (suspend) 18 gms. in 500 cc H_2O
heat to boiling.
(15 cc per tube)

Starch agar		
	Nut. Broth	1 g.
	Soluble starch	2 g.
	Agar	4 g
	H_2O (dist.)	200 cc.

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Ex 39-

Lab. 7/5/51 - Taxonomic study of Various Groups of Bacteria.
Parts A; B; C.

Part A — a taxonomic study of cocci
use following bact:
Sarcina lutea
Gaffkaya (Micrococcus) tetragenes -
Staphylococcus aureus
" albus

Make tests to determine, although each is pretty distinctive-

Inoculate ea of types of media w/ ea. of 4 organisms.
Use 1 control for ea

In addition melt up agar, pour petri plate
divide plate

Take needle + inoculate w/ S lutea on ½, S. aureus on other, same for 2 other organisms.
This in order to enable one to recognize type of growth of organism, so that when one gets unknown, will have some idea of the organisms worked with.

Also inoculate agar slant of each organism.

Part B. Most organisms here are small gm - rods.

Indole uses a trypton broth.
H_2S - test with iron citrate agar
EMB - inoculate plates instead of media.

To inoculate iron citrate - take needle, flame, gather some inoculum, make a stab - do not melt the agar.

observe cultures prepared last time
Other types for nut. broth.
on 1st A org. notice
type of scum around the top.
other things - fluorescence
pigmentation, flaky on top
pellicle formation

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Part C – organisms p. 56 –

necessary on all broths ~~to~~ to keep a control; on such things as agar, no control necessary since it is simply growth + or –

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Sect. July 9 - Water Bacteriology

Its study similar to soil bact. - habitat not controllable. But study more adv. because of tie up betw. H_2O + disease - where man is affected, usually a speed up in study.

The N. cycle in water
Azotobacter agiles is imp. org. maintaining.

Types of H_2O invironments.
1. Atmospheric H_2O
2. Surface "
3. Ground "
4. Stored "

In practise not so easy to so characterize.

Atmos. H_2O - in rain + snow - not such a large specific group - little importance.

Surface H_2O - rain H_2O contaminated w/ soil, developing into streams - H_2O which drains surface, up to point it flows into lake or ocean. Generally org. found at surf. of soil. Kinds of org. deter. by kind of surface involved - shifting of flora w/ kind. The type + no. of org. controlled to large extent to organic matter + silt present. Purification of streams by filtering down + out. Amts. of O_2 (part of non-photosynthetic types) influences the no. of org.
(B.O.D) The Biochemical Oxygen Demand - the amt. of O_2 req. to completely oxidize a sample.
If anaerobic condition +, poor fishing, + presence of large quantities of anaerobes.
In slow moving streams, ultra violet rays of sun may cut down no. of org.
In fast moving streams the UV is not effective, because of lack of penetration - also aeration caused by
The competitive growth of higher plants helps inhibit bact. growth because of O_2 demand.

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Ground H_2O - found deep in ground. the deeper, the fewer org.
apparently a good filtration technique.
In springs, iron + sulfur bact.

Stored waters - ponds, reservoirs, lakes, oceans, each of which is a separate problem.
Lake H_2O - good bit of experimental work here. Zobel (Chron. Bot.) wrote a text of marine microbiology.
Lake work done at Univ. of Washington
Classification of lakes.
1. Eutrophic
2. Oligotrophic
3. Dystrophic.

Eutrophic - essentially well nourished, fed by streams from fertile lands.
Oligotrophic - poorly nourished - arid + swampy source.
Dystrophic - abnormally nourished, + of much iron in lake - detrimental to fish.

The work done not too good, because we are accustomed to growing ~~on~~ mostly heterotrophic organisms ~~for~~ in controlled environments. Those occurring in lakes cap. of filtering tremendous amts. of material from surrounding media. Little done in lab. study.
Methods - a trot line - suspend glass slides, examine the bact. found there. Meas. a certain area of slide, mult. by proper fraction, found from 900 - 30,000 per cc. depending on lake type.
Variable from time to time. Culturing very difficult.
Found, partic. in swamp H_2O, the methane group of bact. red. $CO_2 \rightarrow CH_4 \nearrow$
Critical work done on sea water - a thesis from U of Palestine in Dead Sea - organisms in salty water "Halophilic" - those capable of tolerating considerable range of salt conc. - "halotolerant".
A variety of org. occurred, more at surface + near banks than deeper + middle.
H_2O contained 23 - 33 gms. of salts per ??
a large conc. - spectacular ~~as~~ that organisms can tolerate such large amts. of salt.

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See "The Microscopy of Drinking H_2O" by Geo. Whipple, revised. 1927 - authority on drinking H_2O.

Manual of Methods for Pure Culture Study of Bacteriology by Soc. of Am. Bacteriologists.

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+ acid, + gas ⊕
− alkaline

7/10 – Notes on lab. exercises – Signs

Prod. of acid noted by change of indicator red → yellow (+) for acid

Pick up control tube + check + inoculated broth cultures of glucose, etc
A break down of the carbohydrate, w/ form. of CO_2

Starch plates
Starch → dextrine → maltose → sugars
Same org. stop on intermediate break down substance.
Test: Iodine + starch → blue color
Iodine − starch (no cleavage) no blue color

not blue where organism is growing.
blue

Indole product.
Considerable no. of org. in intestinal tract capable.

Test: Kovac's reagent red color, indole produced.
no " " not " .

Reduction of Nitrates → NO_2

Litmus milk is a complex reaction
red → blue indicates alkalinity
red → red " acidity

Continuation of H_2O bacteriology.
Water supplies.

To decide H_2O is okay – "Examine source of supply – what type of organisms may be expected. A privy above would certainly cause suspicion.
(2) Chem. analyses – a history of H_2O (not if pathogens + at moment) but helpful in giving a clue
a high BOD – much organic matter available, so if pathogens +, good chance of survival.
Chlorides: an increase suddenly in supply indicates sewage contamination (presence of urine, high chlorides)

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tests for compds of NH_4 -
a. Combined NH_3 (NH_3 in compds).
b. Free "
c. Presence of NO_3 + NO_2. All H_2O has some of these +, but sudden rise in quantity is indicator.

b + c balance w/ a.

Free ammonia probably from decomposition of urea. + indicates recent pollution.

NH_3 eventually converted → NO_2 eventually to NO_3. So if free NH_3 + in quantity, very recent polution.

+ of NO_2 – fairly recent polution.

+ of NO_3 – polution but not recent.

These are indirect tests, not for actual organism, but the possibility of their occurrence.
So bact. obs. is best.

~~To be sure~~, The pathogens (undesirable) usually from feces of animal + man.
Must look for + of E. coli, since this org. + in 95% of all stools. A normal person defecates 2 hundred billion organisms (E. coli) per day. Pathogens may produce slower, but the indication from E. coli makes one suspicious.
Another org. Aerobacter aerogenes + in 10% of stools, so somewhat useful in contamination detection.
Either E. coli or A. aerogenes causes suspicion.
E. coli's natural habit is in feces, not much elsewhere.

"Standard Methods for Exam. of Water + Sewage" establisheds techniques

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Tests for coliform bact. (all essentially same).
All ferment lactose w/ prod. of acid + gas.
Culture in lactose rich media, isolate + identify

3 stages of testing.
1. Presumptive.
2. Confirmed
3. Completed

1. Collect water aseptically.
2. inoculate in lactose broth tubes.
3. Incubate 24 hrs.
If acid + gas formed – a positive presumptive test. Actually inconclusive since a few other organisms produce acid + gas, or a synergistic effect – 2 different organisms, 1 produces gas, other gas.

If no acid + gas after 24 hrs., reincubate, + if then acid + gas, positive presumptive, but if simply acid, or if no (+), a negative presumptive, but if less than 10% gas in vial, a ~~doubtfl~~ doubtfull presumptive.

If positive or doubtful, tests must be carried further.
Must determine whether organism is one which is of interest. Streak from orgin. culture on a lactose + dye media ~~to determine~~ (Endose media) E. coli is metalic on Endose. on EMB, pathogens colorless, E. coli → a green metalic sheen, A. aerogenes → a gummy growth.

Also may plates of organisms known to be found, and compare w/ organisms found
(Difficult to have knowledge of ~~to~~ appearance of all org.).

If found + on lactose + dye, a positive confirmed test.

" colonies do not ferment + change color, a negative confirmed.

but if some growth + dye change a doubtful confirmed, hence a final test (completed).

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7/24 - For lab.
3000 cc. Nutrient Agar
10 cc/tube
2 large baskets - sterile tubes
20 Flasks containing 150 cc. H_2O
500 cc Nutrient Broth
10 cc/tube

Flow sheet for activities of Group B organisms (Ex. 39).

Unknown
↓
○ — Plate
↓
// — agar slants (one in reserve, one for sub-cultures)
↓
gram stain
↓
+ : Rods | Cocci
− (Rods) — Grp B - gram - Rods.
Best to find a reac. dividing organisms in ½

↓ Glucose

⊕	+	−
1. E. coli	P. vulgaris	A. fecalis
2. A. aerogenes	S. marcescens	
4. K. pneumon.	Sl. + P. fluorescens	
5. Sal. schott.		

on basis of gas prod.

1, 2, 4, 5
↓ tryptone broth for indol formation.

+	−
1.	2, 4, 5

↓ prod. of H_2S.

+	−
5	2, 4 - These 2 have same reaction, test by stringing out

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The acid formers w/o gas

3. P. vulgaris
8. S. marcescens - red
6. P. fluorescens - fluoresces.

3, 6, 8
↓ H_2S

+	−
3	6, 8

Lab 7/24
Ex. 33, 34

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Droplet infec. becoming to fore as a means of explaining.
Sneeze, 20,000 bact. expelled, 10 μ - 1 mm. sizes expelled
15 ft. - Larger particles projected farthest, but fall before evap.,
but smaller " stay longer in air, but travel smaller
distant (2-4 ft.)
Large - curved traj. 15 ft. fall to ground before evap.
Small - low rate of drop 1-3 ft per hour in abs. still
air. W/disturb., remain suspended indefinitely
Exptl. evid. showing S. marcescens, in mouth,
sneezed, + found that all people in room, 15-20
min. to 1 hr. had org. from throat cultures.
In hallway, spread to all rooms in bldg.
Frenchman in Pasteur inst., w/ same org. found
100 m. from source.

Coughing not so much expelled. The pushed letters in
speaking - to 1 ft.

How many organisms pass from medium to air
Flora of mouth different for each of us.
How to count no. org. in air - question of how to culture
to find out - plain media not useful for all, nor will any
particular media suffice.
Attempts made, however in 3 diff. ways.
1. Impingement devices -
2. Bubbling
3. Atomizing.

Impingement catches things on agar.
Varieties 1. simple agar plate.
2. Centrifuging + filtering
thin agar film
certain amt. air.

3 Funnel device

4. metal shield w/ slits over plate
above this a single slit

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a crank turns above top slit, allowing air + org. to fall on a certain part of petri plate. Either sample all spots, or 1 spot.

Bubbling device – air bubbled into broth.

← air (particular vol.)

glass beads

culture + count.

atomizing – a layer of air around ea. bacteria in broth, spray this on to a plate. Difficulty is that may have several bact. stuck together, so colony started not from one, but several org.

Control:

1. Ultraviolet (U.V) light. How effective? Dust particles distort rays. Dust on lamp will distort the rays. Used in hospitals, use a deflector to keep rays out of eyes so organisms must be at top of room – not effective.

2. Useful, but not practical, is use of bactericidal vapors, i.e. propylene or triethylene glycol – these actually bactericidal, odorless, tasteless, not poisonous, hard to vaporize. Big difficulty is dependence upon rel. humidity best at 40% rel. hum. + 75°F.

Knock out gusts + dusts, little trouble

Contamination of inoculums controlled by inoculating rooms or chambers previously sterilized w/ phenol spray – since phenol is toxic must wait for a while.

Just as well to try to remove dust by oil or water spray which will filter air + bring down many organisms.

After rain, air seemingly cleaner.

In many situations, is it really worthwhile?

UV in factories, offices, okay, but outside contacts remove value.

Good in operating rooms, etc.

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No. Antiseptics for Ex 41
(all working soln, except 8 & 13.

1. Streptomycin
2. Tincture methaphen
3. Campho Phenique
4. Mercurochrome
5. Tincture merthiolate
6. 5% Phenol
7. Bactine
8. Creolin
9. Dioxygen
10. Tincture of Iodine
11. S.T. 37
12. C.N.
13 Zonite
14. 1:1000 $HgCl_2$
15. Lysol
16. Glyphen

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Air conditioning not too effective for small rooms, but if whole air in a building is treated, then is more effective.

Glass impregnated w/ ~~oil~~ oil (filters) & good in inoculating rooms.

Lab. 7/12 – Ex 41
(Types of disinfection – whether org. will grow.
Test (Staph. albus), E. coli, B. subtilis.
Take org. + inoc. tube of nut agar, mix well, pour in petri plate.
Take disinficitant, so patches of sterile filter paper, pick up w/ sterile forceps, dip + remove excess, place so that 3-4 pieces on ea plate. Growth → α organism,
Width of clear zone around paper indicates strength.
One group work w/ one organism. Place 3 loopfuls to inoculate tubes of broth, from this mixture, inoculate (3 loopfuls) the melted agar + pour.
16 in all – 5 plates.

Using same org. + broth Ex 48, ~~inoculate~~ pour that plate.

Clean coin in H_2SO_4, wash in tap H_2O

Ex 30 Bact. antagonism start today

Grow one org. on plate, then grow another against it.
Use B. subtilis, another group. P. fluorescens, Penicillium notatum
Streak from agar slant

Take petri plate, uninoculated agar, streak as in illustr. one streak.

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Bacteriology Exam July 19, 1951

1. What are the laboratory problems concerned with the measurement of ~~contaminated~~ air ? (8)

2. Why a-re "Endo" and "E.M.B." media useful in wa ter bacteriology? (8)

3. As bacteriologists, outline a set of controls (to prevent the spread of disease) to be used by food handlers. Outline a similar plan for grocery stores. (20)

4. Name four pathogens which may be found in unpasteurized milk. Name one disease with which each is associated. (4)

5. Give two examples of self-purification(organisms being directly responsible for their destruction or removal). (8)

6. Give two reasons why a good milk supply is often harder to attain than a good water supply. (12)

7. What is the chemical significance of meaning of a high B.O.D.? What is the bacteriological significance? (8)

8. What are the conditions necessary ~~xxxxxxxx~~ for use of a water supply without purification ? (8)

9. Explain how the use of an enzyme can help determine the efficacy of a germicidal process. (15)

10. What is the bacteriological significance of chemical analyses of water with respect to various nitrogenous components ? (9)

Special for Romanko

1. Discuss the importance of enzymes in microbial variation.

2. ~~Dis~~ Which of the following may be used to define pasteurization:
Bacteriostat, Disinfectant, Antiseptic, Prophylactic?

3. Explain the cyclic nature of Carbon, Nitrogen, Phosphorus and Sulfer in soil , showing the part played in each by microorganisms.

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7/23 - Industrial bact. - use of organisms for conversion of one to another commercially valuable product.

Brewing, wine making alc. production.

How is champaign prepared?

Important factor is grape: 2 types - red / white.
Ordinary wines prepared by mashing grapes - certain amt sugar +, fermented by organisms → alc. + CO_2↑
Generally organism is + on grape.
Squashing → grape juice + sugar + sunlight → wine.
Usually sufficient organisms → satis. alc. content; bottle, cap & store.

grape → grape juice → wine
ferment.

Alc. content usu. 12% by vol.

Sweet or dry wines produced by stopping ferment. at desired level by pasteur. or centrifug.

Allowing complete ferment. → little sugar left, dry.

Referment wines, do not allow CO_2 to escape → carbonated wines.
At this point, blending to get certain taste.
Many wines blended → one wine, sugar gone.
Add sugar, inoculate w/ organisms, bottle.
A "natural" wine means fermented in bottle.
Organism involved, a yeast - Saccharomyces ellipsoideus.

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7/25 - Lect. Wine making.

1. Grape is right. item - best grape. - must be handled carefully - stemming + crushing must be
   Grape juice treated by sulfides to remove acetic acid & bact., wild yeasts, etc.
   Ferment:
   1. selection of yeast: Sacch. ellipsoideus + several variants var-tokay,.
   2. Start ferment w/ an inoculum (in big wineries) to get right org.
   3. Grape juice after ferment (the "must") pasteurize, + desired organism remains, used as inoculum 2-5 % of total volume.
   4. Sugar added or diluted, depending on natural content. Sugar will decide how much alc. prod.
   5. Occasionally NH_4SO_4 or phosphates used in control of yeast growth - increase yeast population.
   6. Good amt of aeration - prod. by stirring.
   7. Temp. imp. in wining - careful control, best below 85° F. or 29°C. Too high alters bouquet.
   8. 3-5 days req. for ferment., but may last longer.
   9. Stored in casks - some further fermentation
   10. "Racking" - draw off from sediment "lees".
   11. Storing + aging - to clear + to develop flavor - cheap wines filtered, rather than allowing to clear by settling.
   12. Clarification "fining" agents - casein, tannin, heat, or refrigeration for fine wines.
   13. Packaging, drinking.

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Brewing.

Important item is the barley grain in preparation of malt beverages (ale + beer).

Steps: 1. Preparation of malt.
2. Mashing
3. Boiling of wort with hops
4. Fermentation
5. Finishing operation

Malt prep. by soaking barley grains w/ 45-47 % moisture uptake - called steeping - to allow germinations.

Morpholog. Formation of plumule + rootlets
Physiol: Proteins, starches, etc. broken by enzymes.
At certain size, enzyme activity at its height.
Dry, slowly + carefully, not to destr. enzyme.
5 % moist. left.
Two imp. enzymes
a + β amylase
Effect change: starch → sugar (maltose).
No. of other enzymes +.

The "malt" ground, cereal adjuncts (corn, rice, wheat) added, steeped in hot water - the mashing process.
Enzymes + digest the substances (prot. + starch).
Soluble products dissolved in hot H_2O - the "wort" -
Filtered, the residual mass used as cattle + hog feed - rich, also used in various synthetic products.

The wort is boiled w/ hops (a plant) best grown in Czech., Poland, - domestic not so good.
Hops aid in flavoring + antiseptic action (prevent. lactic acid bacteria develop.)
Boiled wort called hop wort -
"Pitching" - inoculation w/ pure cult. of Saccharomyces cerevisiae
Ferment. strictly anaerobic process.
Two classes yeast
top - prod. of ale.
bottom - " " beer.

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Temp. of 0-6°C at beginning, but allowed later 6-12°C.

6-10 days ferment for beer.
5-7 " " " ale.

Prod. is called green or young beer

Aging is next, to lose harshness, mellowing, debitterizing - from 2 weeks - 3 months.

Matured beer carbonated, displacing O_2 w/ CO_2 - the great CO_2 cont., the better the beer - has much to do w/ keeping the head.

CO_2 beer is cooled, filtered racked, pasteurized 20 min at 61°C., bottled.

Prod. of ethyl alc.
Org. S. cerevisieae
Method - depends on raw mater.
Molasses best, but can use starchy materials i.e. corn after breaking starch → sugar -
Molasses method.
1. Mash (molasse + inorg. salts) treated w/ Hot H_2O
, pH adjusted -
a yeast starter mixed intimately in tanks.
Anaerobic ferment. Pipe in mash + inoculum under press. to properly mix,
Stop stirring to prevent aerobic cond.
A 250,000 gal. tank - 11,000 gal. starter (yeast cells)
Once ferment. started, a rapid process w/ good amt. of CO_2↗ (a by-product).
4 n 50 hrs. ferment. compl. - the prod. "beer" distilled in a continuous still.

continuous addition of material

alc. + vol. compds

arm back to still for continuous

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Alc. purified by rectifying columns (temp. control + other techniques).
The quality of alc. depends on kind of grain.

Rum - from molasses
Bourbon - 85% corn.

Prod. of penicillin
Org. Penicillium notatum - now use P. chrysogenum.

Since 1940, when 1st used, prod. incr. at phenomenal rates.

Nut. involved proc.
1. Cornsteep liquor (resid. from corn starch prod.)
2. Sugar-lactose.
3. inorg. salts, PO_4, SO_4
4. Ferment 60-75 hrs. a decent yield.
(See Mo Bot Gard Bull. No. 10, 1949

Bacterial fermentations much the same as these, but not so important

Importance of fermentation
1. Jub. Vol. of A. van Leeuwenhoek - Jour. of Micr. ~~+ Serology~~ microbiology. 12: 1-4, ~~1944~~ 1947. p. 267.
Microbiological experiences in Japanese camps for PW. By G. Giesberger

7/26/51 - Medical Bact. - read introd. to parasitology in some such text., Topley + Wilson.
Principles
Diseases of man in 2 categories.
1. Specific for man as host
2. Disease caused by accidental infection.

Group 1 - Considered as living org.
Ecology - interdependence - man eats animals, plants for food, clothing, house
Civilization causes moroys in manner of survival, method of procuring money, dependent on others

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for source of income - this a form of parasite -

Parasites may be
1. Predatory
2. Commensal (no harm to org.)
3. Symbiotic (mutual aid)

Symbiotic system most efficient - living, growing, nursing + prod. products useful to
Commensal - not too inefficient.
Predatory - poorly adjusted to host, destroying host - either destroying itself, or causing death of host.
2 reasons of parasitism.
1. Hasn't been w/ host long enough to be commensal.
2.

4 essentials to perpetuate
1. must have method of gaining entrance -
Orifices (nose, throat) to internal structures - intestinal tract, mucous membrane.
Gaining entrance thru food, fluid or air.
" " " urinary + genital tract, eye
(but these flushed w/ special products, so organism has hard time, or covering of parts).
The skin - usually a protection, but breaks in skin, hair glands, sweat follicles, or insect
⇒ mere physical entrance not enough, but must establish a residence - transport + penetrate these superficial protections. Capacity of org. to become estab. in
→ host constitutes infectivity

2. Multiplication + adaption - med. bacter. employed here. If invader established w/o injury becomes part of "normal flora" - of vagina, nasal passages - a saprophyte + benign (non spreading. But if invader causes injury + reaction of host is called disease, and invader is a pathogen.
Severity of invasion or injury is measure of virulence.
If a few cause serious injury, highly virulent - i.e. Clostridium tetani - very few produce suff. toxin to cause death.
Staph. aureus, strep[illegible], ~~[illegible]~~ low virulence or normal flora

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At this point of invasion, the host rallies defenses, + at same time the parasite meets these defenses w/ its own, causing a contest - either one or other killed, or a stalemate, or this broken if either org. breaks or relaxes its defenses. Encounters + blocks may be intermittent activity, Chronic diseases are such - w/ periods of quiescence, but if either org. loses defenses, one or other will be destroyed.
The death of host by the parasite causes trouble for org.

So:

3. Must have satis. portal of exit - generally same as entrance (part. in respiratory disease; also insect carriers. Generally a single avenue of escape - Pub. health people interested in these avenues in order to prevent spread.

4. Effective mechanism for transfer to new host.
Parasite requires continued environment -
Move from one host to another of same species + same environment, i.e. respir, food handlers, g.u., contaminated foods
or
intermediate hosts - vectors - insects

Although procedure of transf. pretty stand., doesn't mean standard activity in new host - ~~host~~ new host not as active as the parasite at first - example polio (some have continued)
This not so w/ a community of hosts - for ex., colds. to which we become more or less accustomed.
If a community is subject to a "new" organism, ex men among mice) the parasite will cause a v. dangerous disease, freq. giving rise to a new, different type of reaction than in normal environment. Ex.

This is example of "accidental"

Brucelosis - abortion in cattle, undulent fever in man.
Nos. of rats - increase may cause plague, but org. is normal among rats.
If org. becomes establ. in new environment - this is evolution, and shows that there is probability that new disease will occur.

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Feb. 7/26/51 Ex 30 + 31 -

Make up tube of Nut. broth cult. of B. subtilis for next lab. - leave cult. in incubator

Plating techniques Ex. 55 - Bact. exam. of H_2O.

Technique by

10 ml sample of drinking H_2O in 25 ml. lactose broth (gas tubes).
(Make 4 other tubes of same thing as check.

1 ml. sample in melted agar - plate out.

24 hrs. check gas prod.; the +, transfer to Brilliant Bile Green media (specific media for H_2O bact.) if positive, is a positive, confirmed test.

Dilution

1 cc of distribution sample in 9 cc. of steril H_2O
1:10 dil.

Mix thoroughly - inoculate 5 tubes of lactose broth w/ 1 cc of the 1:10 dilution.

take 1 cc of the 1:10 dil., place in 9 cc of steril H_2O, for 1:100 dilution

Mix thoroughly inoculate lactose broth as above.

Make 1:1000 dilution, same as above + inoculate

By use of tables, able to estimate how much of coliform bact. + in samples.

Sample bottles must be sterile, then w/ sodium thiosulfate soln,

Ex 56 - Use only pasteurized milk. Glucose tryptone skim milk agar.

1 cc milk, inoculate plate
Pipette 1 cc into 9 cc steril H_2O blank 1:10
[illegible] pipette, inoculate plate, 1 cc, make 1:100 dilution

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Bact lect.

1. Announcement:
   Text: Frobisher elements Bact.
   Lab. Man. McClung -
   Meetings - lect. Sat 10:30 Wed, labs 1:30 M + W.

(bracketed in margin: last)

2. What are the objectives of this course
   A Immediate - functions, structure, ~~form~~ inheritance, of large gp. of org. - Classificat. - Develop techniq.
   B ~~Long Range~~ - of handling, familiarity w/ procedure, methods of thoroughness in bact. - how to look up references;

   B. Long range - something of nature of life + of other forms around him, Modern concept of how + why of behavior bact. + other organisms. Descriptive bact. - not amassing voluminous factual detail.

3. Modern bact -
   no longer merely descriptive, but an effort to bact. + behavior on basis of modern biological physical + biochem concepts.
   Biochem. princ. enrich understanding in recent years

4. Condensation - today's knowledge is spread over many publications - to sift them + present only essential material of the what, why + how of these organisms is a difficult job.
   This will be as simple + brief as possible, but still be consistent w/ a level of fundamental understanding of ~~the~~ ~~to~~ bact. + related forms.
   Some biochemical

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Cytology - 42[illegible]

List of slides in loan collection

1. Necturus blood smear - Wright's blood stain
2. Salamander tadpole tail, whole mount - B-3, Mayer's Haemalum
3. Salamander tadpole tail, cross section - Bouin's,Hematoxylin andEosin
4. Salamander lung, cross section - Bouin's Hematoxylin and Eosin
5. Salamander Kidney (Necturus) Bouin's, Iron-Alum-Hematoxylin
6. Salamander kidney (Amphiuma), for mitochondria - Regaud, Aniline-Fuchsi[n]
7. Salamander kidney (Amphiuma), for Golgi - Mann-Kopsch method
8. Salamander liver (Amphiuma), for mitochondria - Formol-Zenker, IAH
9. Salamander liver (Amphiuma), Golgi and mitochondria - Champy, Osmic; Acid Fuchsin and Aniline Blue
10. Salamander small intestine (Amphiuma), Golgi & mitochondria - Champy, Osmic, Acid Fuchsin, Methyl Green
11. Salamander pancreas (Amphiuma), Golgi & mitochondria - same as above
12. Mitosis in whitefish (Coregonus) blastula
13. Mitosis in cleavage of Clypeaster (echinoderm)
14. Oogenesis and mitosis in Ascaris (roundworm)
15. Bone marrow sections, cat or goat - Bouin's, H. and F
16. Pollen mother cells, corn - Acetocarmine
17. Cockroach (Periplaneta) ovary, sections - Bouin's IAH
18. Salivary gland chromosomes (Sciara) - Acetocarmine
19. Salivary gland chromosomes (Drosophila) - Acetocarmine
20. Kitten ovary
21. Maturation in the Palolo worm egg (Annelid)
22. Rat testis - Bouin's IAH
23. Rat testis - Regaud, IAH
24. Grasshopper testis
25. Squash bug (Anasa) Testis

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7.6 - coincidence

91 - stage
66 - ocular

66) 910 (13.8   546
66   546
250   6006
198
520
528

66
10
660

26

3.25
80) 260.
240
200
160
400
400

16 mm = 13.8 μ, .0138 mm

HD = 3.25 μ, .00325 mm.

Oil = 1.48 μ, .00148 mm.

1.48
81) 120.00
81
390
324
660
648
12

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Zoology 426 - Cytology Spring, 1951

Lecture and Laboratory Schedule

Lecture	Week beginning	Laboratory
	January 29	Use of microscope; calibration; measurement
I. Introduction II. Terminology	February 5	Acetocarmine smears; grasshopper cells
III. Historical	February 12	Living grasshopper cells
IV. Technical Methods	February 19	Tadpole tail; living and fixed
V. Golgi-bodies and Mitochondria	February 26	Triturus tail
VI. Nucleus	March 5	Golgi and Mitochondria
VII. Mitosis	March 12	Mitosis
VIII. Mitosis	March 26	Amitosis; nucleolus
IX. Chromosomes	April 2	Salivary gland chromosomes
QUIZ	April 2	
X. Endopolyploidy	April 9	Onion root tips
XI. Chromosome cycles	April 17	Kitten ovary
XII. Meiosis	April 23	Oogenesis
XIII. Syngamy and Apomixis	April 30	QUIZ; Student projects
XIV. Changes in chromosome number	May 7	Student projects
XV. Chromosome aberrations	May 14	Student projects
XVI. Evolution of Genetic Systems	May 21	Student projects

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Parcenter

High Dry – from low 8-9 o'clock

Oil from high dry 11 o'clock

26

cell 40 divisions

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D. J. Rogers, 400 4 26

See end for comment

1. Some of the facts:

Hämmerling's work with Acetabularia spp. is one of the most significant experiments ~~th~~ showing that the nucleus is fundamental to extended life of cells.

Acetabularia, a unicellular alga of the Siphonales group is differentiated into rhizomes, stalk, and a reproductive fruiting cap. The single nucleus is usually ~~By removal~~ found in one of the two rhizome branches at the base of the plant.

By excising a portion of the stem, Hämmerling demonstrated that the nucleus is the important factor in regeneneration of a new cap by taking the excised portion of the stem of one species, grafting this part onto the base (with the nucleus) of another species, and producing a cap which is identical to that produced normally at the tip of the plant onto which the excised portion of the other spp. had been grafted. This was done after having tested the excised portions of the stems, ~~sege~~ to show that they (the excised portions) normally produce the same type cap as one would expect for the species.

Conclusion: the nucleus is the controlling factor in the determination of form of the plant, no matter whether these has been the introduction of other cytoplasmic material than normally encountered or not.

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Another bit of factual evidence of the fundamental importance of the nucleus may be found in the experiments with the centrifuged sea urchin egg. By ultra-centrifugation a normal egg, with all nuclear and cytoplasmic material present is divided first in 2 halves, the "light" half and the "heavy" half, and by further centrifugation into 4 parts.

In the first division, the nuclear material is found in the light half, while the heavier cytoplasmic structures were found in the heavy half. The "light" half produced a normal embryo when sperm was injected, but the "heavy" half produced an embryo which developed only to the blastula stage, and this, of course parthenogenetically.

In the second division, into four parts, the only portion which developed was the lightest, and the only one which contained nuclear material.

From this work, it is farther evident that the nucleus is important for normal development of the embryo, although some development occurs in the cytoplasmic material. This seems to show that the nucleus is the primary body for development, but that some influence is also shown by the cytoplasm, of a secondary import.

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Although I know of no actual experimental work on the work with cells in tissue culture, it seems to me a significant fact that cells, when ~~mate~~ maintained for some time in tissue culture, show a tendency to revert to a general type of cell rather than retaining their original state. In this view, it would seem that there is some material ~~of~~ of a supra-cellular nature which coordinates the normal development of tissues, and in whose absence, there is no ability of the cell to maintain its normal cycle of development. This is, of course, mere hypothesis on my part - I am not able to judge the real significance. Certainly "in vitro" studies cannot be considered normal conditions, but if the nucleus were the *only* director of normal development, it seems to me that there should be no regression or atavistic tendency demonstrated.

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I would like to have seen a better employment of genetic principles to support one or both of these ideas. You seem a little reluctant to cut across course lines and draw on your more general information. Do you know the Neurospora work on biochemical genetics?

Nucleic acid cycles also provide evidence of function.

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Zoology 426 - Cytology - Quiz - April 3, 1951

NOTE: Undergraduates should answer three of the four questions. Graduate students are asked to answer question 1, but need not write on any of the others unless they have time and want to.

1. It is generally concluded that the nucleus, or nuclear material, is of fundamental importance to the extended life of the cell. Consider the evidence for this by discussing the facts 1) on which this conclusion is based, and 2) which indicate what the function of the nucleus is.

2. Discuss the mitochondria, with special reference to methods of study, structure and distribution in various types of cells.

3. Suppose someone contended that the Golgi apparatus is merely an artifact of fixation and thus is of no significance. Organize and present a concise statement of the evidence that this is not so.

4. In a short paragraph, distinguish between:

    a. microsome and centrosome
    b. coenocyte and XXXXXXX energid
    c. nucleolus and chromocenter
    d. growth and proliferation
    e. hyaloplasm and deutoplasm

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Required Text: DeRobertis, E. D. P., et. al. General Cytology
Saunders, 1948.

Supplies: 1 pair small forceps
1 pair small scissors
2 teasing needles: good teasing needles can be made from the ordinary wooden-handled dissecting needles supplied with most dissecting kits. Cut off the needle point so that it is about 1/2" long, then grind them down with a stone to an abrupt sharp point
Small box Kleenex or similar grade tissue.
1 clean cloth (old handerkchief or shirt-tail)

Drawing materials:

1 4H drawing pencil
1 #2 ordinary pencil
eraser
millimeter ruler
drawing paper
manila folder
small piece of sand paper
charcoal stumps (if obtainable)

Bit 8 - Sept 23

I. THE USE OF THE MICROSCOPE

Cells are such extremely small objects that in order to see their fine details, as you will be required to do, you must maintain optimal microscope conditions at all times. This is easier said than done, and expertness with the microscope comes only after considerable practice. Whereas you may have gotten by so far in your studies with the microscope without paying particular attention to such things as scrupulously clean lenses and critically adjusted mirror, diaphragm and condenser, you must now train yourself to always do these things, and to do them automatically.

A. Observation and drawing of a microscopic object

After verbal instructions, test your microscope to see whether it is parcenter and parfocal and practice the use of the oil immersion lens, using slide #1 which is a stained smear of [illegible]r blood. Make note on any individual pecularities which your microscope may have.

Select an isolated and well-stained red blood cell, bring it to oil immersion and adjust the microscope in such a way that it is illuminated optimally. When you are sure it is just right, call me over to criticize it. Now make a drawing of it. The type of drawing required in this course calls only for pains-taking attention to proportion and detail. Faithfulness to detail should be photographic, all small granules, lumps and blobs, even if they seem not to mean much, must be put in just as they are. Make a light outline of the cell, about 100 mm long, watching the proportions carefully. Scratch a soft pencil on a piece of paper or card. Shade in the lighter areas first, then the darker areas, using a charcoal stump as a paint-brush.

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Sharply-defined dark granules or areas may be finished off with either the hard or the soft pencil. Label fully, stating actual length in microns (if you have not done B below, record the length in ocular micrometer divisions; this can be converted into microns later) and the number of diameters enlargement that your drawing represents. If a drawing is ten times as large as the specimen in any one dimension, it is said to be magnified 10 diameters or simply 10X. This means that it is 10 times as long or wide as the specimen; it is much greater than 10 times the size of the specimen, which is three-dimensional.

B. THE MEASUREMENT OF MICROSCOPIC OBJECTS

This requires calibration of the microscope. Two scales are necessary, the ocular micrometer, which is a glass disc bearing an arbitrary scale of 50 or 100 divisions and a stage micrometer, which is a slide etched with an absolute scale 2 mm. long, subdivided into units of 0.1 mm and 0.01 mm. (10 microns).

1. Procedure for calibration:

Extra oculars containing ocular mictometer discs are provided. When measuring objects in the future, always use the micrometer to which your microscope is now to be calibrated. Bring your microscope into focus on the blood smear with the 16 mm. objective. Substitute the ocular containing the micrometer. The ocular scale obviously remains unchanged when different objectives are used, and must be calibrated with each objective. For this purpose the stage micrometer is used. Substitute this slide for the blood smear and focus carefully, with light cut down, on the absolute scale. The smallest divisions on this scale represent exactly 0.01 mm (10 microns). This scale occupies the position of objects to be measures later. Both ocular and stage micrometers are now visible. Adjust the scales so that they are super-imposed upon or parallel to each other and a division near the end of one scale coincides with one on the other. As far as possible (why?) from this point of coincidence, now find another point at which the divisions coincide. Count the number of spaces on each scale between these points, and multiply the number of spaces on the stage scale by the length of each space. This product, divided by the number of spaces counted on the ocular micrometer, gives the distance measured by one space on the ocular scale.

The following is an example of an actual calibration of a 16 mm. objective:

50 spaces on the ocular scale = 36 stage spaces, each 10 microns (0.01mm)
or 360 microns (0.36mm).
1 space on the ocular scale = 7.2 microns (0.0072mm).

Note: Calibrations are accurate only to tenths of microns.

Proceeding as before, calibrate the 4mm and oil immersion objectives and record the values obtained. Due to the thickness of the divisions on the stage micrometer when highly magnified, [illegible] coinciding

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with divisions on the ocular scale. The calibration thus obtained is accurate only when the microscope is used with the same ocular and same objectives.

Record the calibrations for all three objectives on a 3 x 5 card according to the following outline:

~~Name~~ ~~Rogers, David~~
Microscope No. 251 Ocular ~~Micrometer~~ No. 5

Objectives	Length measured by 1 space
16 mm (low power)	.0138 mm or 13.8 microns
4 mm (high dry)	.00326 mm or 3.26 microns
1.9 mm (oil immersion)	.00148 mm or 1.48 microns

2. Measurement of the actual size of objects

Measure and record the actual size (diameter or length in microns) of the following: (measure 4 or 5 of each, and average the results) ~~a) Necturus erythrocyte, b)~~ human erythrocyte. ~~c) Paramecium~~. Memorize the size of the human erythrocyte; it is a classic object for size comparison. ~~Paramecium is just visible to the naked eye.~~

~~Calibration records and measurements should be copied on 3 x 5 cards and handed in along with the drawing of the Necturus erythrocyte.~~

References:

Allen, R. M., The Microscope. 1940
Belling, The Use of the Microscope. 1930
Munoz, F. J., The Microscope and its Use. 1943
Richards, O., The Effective Use of the Microscope. 1941

II. LIVING CELLS AND THE ACETOCARMINE TECHNIQUE

The purpose of this exercise is to compare cells which have been fixed and stained with those that are living. The material consists of the testis cells of the grasshopper [Chortophaga viridifasciata]. Insect testes, especially those of the grasshopper, are classic objects for study because the cells are large, easily dissociated from one another and usually some cells are undergoing division.

16x3
16x4
16x3

A. Acetocarmine smears

220x41.4

This simple, efficient cytological technique is useful primarily for the study of the nucleus and the chromosomes; it may be used on a wide variety of plant and animal material. The dye, carmine, is dissolved in 45% acetic acid, which acts as the fixing agent. Acetocarmine thus fixes and stains the tissue at the same time. Two other dyes, orcein and lacmoid, are also widely used in the same way. For further details, see Darlington and LaCour, The Handling of Chromosomes.

5
4
5
5

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1. Temporary smear preparations. Obtain several follicles from a grasshopper testis which has been removed and placed in deep-welled slide full of Acetocarmine for 15-60 min. Place in a small drop of Acetocarmine on a clean slide. Tease and crush the follicles thoroughly. Cover with a clean coverslip before the stain has formed a dried ring around the drop. Place the slide on the table and cover with a blotter, applying slight pressure to the cover with the fingertips through the paper. Press vertically, so that the cover does not slip sideways. Seal the edges with gum mastic-paraffin mixture. The preparation will overstain slowly at room temperature, but may be kept a week or more in the refrigerator.

2. Permanent smear preparations. Prepare several good permanent preparations according to the following schedule. Use material fixed and stained as above.

a. Prepare a covership as follows: Clean and dust until lint-free. Place a minute dab of Meyer's albumen in the center of the cover and spread evenly with the little finger. The film must be very thin and practically invisible. Dry over an alcohol flame, 1-2 seconds.

b. Crush and tease 4 or 5 follicles in a fresh drop of stain on a very clean slide. Do not allow evaporation to occur. Drop the albumonized cover vertically onto the drop containing the teased follicles. Blot lightly.

c. Pass the slide quickly over an alcohol flame 5-6 times; overheating will ruin the preparation; judge the heat by drawing the slide over the back of your hand. It should be no hotter than your skin can stand!

d. Invert slide on glass rods in a petri dish containing 10% acetic acid. The coverslip, with the cells attached, should separate from the slide after 5-20 min.

e. Keeping the cell surface up, and handling the cover with forceps, place in clean 95% alcohol for 2-3 min.

f. Fresh 95% alcohol 2-3 min.

g. Mount in Diaphane (turn cell surface down!)

Study both the temporary and permanent mounts. A variety of cells will be seen; most of these are germ cells in various stages of maturity although the cells of the wall of the follicle are also present. The latter do not dissociate in such a preparation. The various germ cell types may be recognized by their size; the average diameter of each in the flattened state is approximately as follows:

spermatogonia	20 microns
primary spermatocytes	30 microns
secondary spermatocytes	[illegible] microns
spermatids	[illegible] microns

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Spermatozoa, with intensely-staining thread-like nuclei and long tails will also be found. Concentrate attention on the largest cells (primary spermatocytes) which are well-stained and isolated. Find nucleus, nuclear membrane, cytoplasm, cell membrane, chromonemata and chromomeres and a darkly-staining chromocenter (the X chromosome). The nucleoli are difficult to observe in these cells. What structures have the greatest affinity for the stain? Can you see any structural detail in the cytoplasm? Survey the preparation under low power looking for dividing cells in which the chromosomes will be prominent. These will be studied in detail later.

Make a drawing of a single, isolated and well-stained spermatocyte, and label fully. Measure and record the size of the nucleus and cytoplasm.

B. Living cells in paraffin oil

The study of living (or surviving) cells has many pitfalls; severe degenerative changes set in almost immediately when a cell dies. Careful attention to the details of the following technique is thus absolutely necessary. Prepare a spotlessly clean new slide and coverslip by breathing on them and polishing with Kleenex or a clean cloth. Obtain several testis follicles in a small drop of Ringer's solution. Rupture the follicles with clean instruments, ring with paraffin oil and cover immediately so that no evaporation of the Ringer's solution can occur. If properly made, the cells may stay alive and dividing for 3-6 hours.

Locate all the cell types seen in the stained preparation, and compare in detail the aspects of the various components of the primary spermatocytes (they average 35-40 microns in dia. in the unflattened state). Can you see the chromonemata and chromomeres? Is the chromocenter visible? What are the visible characteristics of the membranes? Often very large "cells" with two or more nuclei within one cytoplasmic mass will be seen. How can you explain this? Give particular attention to the mitochondria, which appear as thread-like bodies in the cytoplasm. Are these preserved after acetocarmine treatment? Dead and dying cells may be recognized in that the chromatin of the nuclei is much easier to see. This is caused by a change in refractive index at death. The cell is probably dead when the mitochondria have clumped into irregular masses.

As you are going over the preparation look carefully for any cells giving evidence of mitotic division. Make a note under low power of where these are. The chromosomes of *Chortophaga* and some other grasshoppers are visible in the living condition and in a good preparation the complete mitotic cycle may be observed. If you find such cells, make sketches of individual cells throughout the period.

The mitochondria of the spermatids aggregate into a refactile body, the nebenkern. This behavior is found in the spermatids of many animals. These will be studied later in the course.

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Make a drawing of a living spermatocyte and compare with the one drawn from the AC preparation. Record additional observations by making a table of three columns headed respectively "cell feature", "appearance after AC" and "appearance in the living cell". Record all details, including size, shape, membranes, chromocenter, nucleus, chromonemata, chromomeres, mitochondria, etc.

References

Acetocarmine technique

Darlington, C. D. and L.F. LaCour 1942 The Handling of Chromosomes. Allen and Unwin Ltd. London
Painter, T. 1939 Science 90: 307-308. (application to mammalian tissues)

Living cells of the grasshopper testis: Belar, K. 1929 Roux" Archiv. Entwick. Org. 118: 359-484.

III. Living and fixed cells of the tadpole tail

A. Survey slide #2 (fixed and stained whole mount of a salamander tail) under the dry objectives. Note character and position of the following cells; find and be able to recognize the nuclei and the extent of the cytosome of each type: a) epithelium of the epidermis (only the nuclei stain; the cell boundaries are indistinct) b) large pigment cells (chromatophores) c) fibroblasts (connective-tissue cells of the dermis) d) muscle cells and e) blood vessels composed of endothelial cells and containing blood cells. When you are familiar with the general arrangement and appearance of these cells in the tail, proceed to B.

B. Obtain a small tadpole, place in a watch glass in 1:3000 MS 222 (an anaesthetic). Allow to stand until the animal is quiet, then place it in the well of a clean depression slide in several drops of the anaesthetic solution. Orient as directed and cover the tail with a clean cover slip. Do not allow the preparation to dry. Examine with the dry objectives and try to identify all cells seen in the stained preparation. Make sketches of each type, noting what can and cannot be seen in the living condition. Toward the end of the period, after your observations on the living are completed, remove the coverslip, blot off all the excess anaesthetic with Kleenex and add several drops of Acetocarmine. Cover and examine. What is the effect on the cells, especially the processes of the fibroblasts and the striated muscle? Look for mitoses in the epithelium.

C. From slide #2 and from the living, make large careful drawings of the following; (notes under each drawing should be made as to what features are drawn from the living animal).

1. an epithelial cell from the epidermis
2. a fibroblast
3. a chromatophore
4. a portion of a muscle fiber

Label each drawing carefully. State actual size of each cell and the diameter enlargement.

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noting the action of the ectoplasm and endoplasm. This type of cell movement is widely found; for instance some spermatozoa, most embryonic cells, and many vertebrate cells in tissue culture as well as normally in the body (leucocytes, macrophages) move by amoeboid action.

Make a large drawing of a single advancing psuedopod representing the cytoplasmic inclusion accurtely. Make also separate drawings of the nucleus viewed from different angles. When you have completed observations on the living animal, stain the nucleus with acetocarmine or methyl green. This is best done by adding a drop of the stain to one side of the coverslip and drawing it under the cover by absorbing the water at the opposite edge with a piece of Kleenex. If possible, observe under the microscope the reaction of the animal and its structures, as the stain comes in contact with it.

References: Chalkley, A. W. 1936 (Mitosis in A. proteus). J. Morph., 60: 13-29.
DeBruyn, P. P. 1947 Quart. Rev. Biol., 22: 1-24 (Theories of Amoeboid movement)

V. Mitochondria and Golgi-bodies

1. Supravital staining of mitochondria

Obtain a few grasshopper testis follicles in a drop of Ringer's solution on a slide which has been smeared with a thin film of Janus Green B in absolute alcohol and evaporated to dryness. Tease and cover. Examine immediately. In a short time, the mitochondria will be stained, but they soon lose their color as the stain becomes reduced. Study the various forms assumed and look especially for dividing cells with mitochondria undergoing division (chondriokinesis). Note the smaller cells (spermatids) and look for the large mitochondrial mass, the nebenkern. What becomes of the nebenkern as the spermatid metamorphoses into a mature sperm?

2. Mitochondria and Golgi-bodies in the superficial epithelium of the intestinal mucosa.

Slide #10 is a thin (4 microns) section of the mucosa and adjacent connective tissue of Amphiuma intestine. It is fixed in modified Nassanow's solution, post-osmified in 2% osmic acid and stained with acid fuchsin and aniline blue. The golgi is black, mitochondria, red and the mucigen of the goblet cells blue. Do not confuse the spherical black blobs, which are fat droplets, with the golgi, which is in the form of delicate threads. Concentrate attention on a portion of the section where the epithelium has been cut vertically, and the golgi shows clearly. Is the form and position of the golgi constant? Is its form different in the goblet cells? Do the mitochondria show any regular distribution in the cells? Do they vary in form? Draw a single absorptive cell and an adjacent goblet cell which show these structures clearly. Label fully.

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D. Study slide #3, a cross section of a salamander (Triturus) tail. The arrangement of cells is similar to that seen in the living tadpole tail, and in slide #2. Orient the section so that the dorsal side is up. Find and note the position of: epidermis, dermis, nerve cord, notochord, muscles, caudal artery and vein, capillaries containing blood cells, epidermal glands and chromatophores. As this is a section of a much older animal than the tadpoles studies, considerable differentiation has occurred. What changes have occurred in the epidermis? What is the origin of the epidermal glands? What is their position? What changes have occurred in the dermal connective tissue cells? What is the distribution of the pigment cells? How can you tell caudal artery from caudal vein? Draw several selected small areas in careful detail under high power; label fully.

Fixative: Bouin's Fluid; stains: Hematoxylin and Eosin (H & E)

References: Speidel, 1932 J.E.Z., 61: 279-331 (Growth of nerve fibers in living tail)
Dawson, 1920 J. Morph., 34: 487-589 (Integument of Necturus)
Dawson, 1937 J. Morph., 61: 385-397 (Epidermal glands)
DuShane, 1943 Quart. Rev. Biol., 18: 109-126 (Pigment cells)

IV THE CYTOLOGY OF AMOEBA

Amoeba proteus (Phylum Protozoa, class Sarcodina) is the object of this study. Recall the discussion in lecture which stressed the fact that it is ill-advised to consider the Protozoa rigidly as primitive, single-celled organisms. We are studying amoeba in order to get a better idea of protoplasm as the basic living substance, but it must be remembered that the amoeba is fully as specialized, in its own way, as any vertebrate cell is in its way.

Prepare a spotlessly clean slide and coverslip and receive a little of the material containing Amoeba. Support the coverslip by means of small fragments of coverglass. Examine under low power with faint illumination at first then use the higher power. Add water to the side of the cover if the preparation begins to dry.

Locate an amoeba, and look for the following cell characteristics: cell membrane, cytosome, ectoplasm and endoplasm, nuclear membrane, nucleus. Observe the latter carefully as it is moved through the endoplasm. What is its shape? Also present are less widely distributed cell characters such as the contractile vacuoles, (Is the interval between contractions regular?) food vacuoles, and ergastic substance in the form of crystals. What is the shape of the latter? Is more than one type present?

Study carefully the pseudopodial locomotion of the animal,

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Before doing 3, 4, and 5 it is suggested that the histology of liver and pancreas be reviewed in Maximov and Bloom, Textbook of Histology.

3. Mitochondria in liver cells

Sections are provided of Amphiuma liver (slide 8). Fixative: Formol-Zenker, followed by a mordant; stain: Iron-alum-Hematoxylin (IAH). Identify liver cells, bile canaliculi and sinusoids containing blood cells. The mitochondria in the cells stain black. Groups of heavily pigmented cells are present.

Pollister (1932, Amer. Journ. Anat., 50: 170-199) found that the mitochondria in the liver cells vary from a spherical shape to short rods, but considers this variation to be due to fragmentation of filamentous types. The orientation of the mitochondria, according to Pollister, is such that the long axes of the mitochondria are along the line passing from the sinusoids to the bile canaliculi. Mitochondria are often more abundant near the nucleus, especially on the side towards the bile canaliculus. See if you can confirm these observations on this and the following slide

4. Mitochondria and Golgi in Amphiuma liver cells

Use slide #9. The technique is the same as that used for the intestine (above) except that methyl green was used instead of analine blue. Mitochondria are red; Golgi black; note staining reaction of nucleus and nucleolus. Again, the large spherical fat droplets have been blackened. Study particularly the distribution of the Golgi in the liver cells. Does it show a constant arrangement? Significance? Draw a small group of cells, including a bile canaliculus. (Refer to Pollister, loc. cit.).

5. Mitochondria and Golgi in Amphiuma pancreas cells

Slide #11. Technique similar to 4. Mitochondria are red, Golgi black and zymogen granules orange. Find an acinus which has been cut in direct cross or longitudinal section and in which the duct is clearly seen. Study the distribution of the cytoplasmic inclusions. What is the significance of the arrangement of the Golgi? Zymogen granules? Draw one or two cells showing these features and indicate the position of the lumen of the duct.

VII. Mitosis

1. Amphiastral mitosis in the cells of the whitefish blastula (slide #12)

a) Study sections through the blastula of the whitefish (Coregonus clupeiformis). All stages of mitosis will be found but many are obliquely cut and therefore show an incomplete picture. Analyze carefully all of the favorable figures on your slide as to stage of mitosis and view represented. In this survey, note particularly changes in the shape of the spindle, the breakdown and reformation of the nuclear membrane, changes

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in the chromosomes and cytokinesis by furrowing. What percentage of the nuclei are undergoing mitosis? What is an "interphase" nucleus?

2. Study slide #13, which shows mitosis in the early cleavage stages of the eggs of the echinoderm Clypeaster. Compare with Coregonus, especially as regards the achromatic figure. Examine the demonstration of a tripolar spindle in an early cleavage stage of Clypeaster.

3. Mitosis in the cleavage of Ascaris (slide #14).

Ascaris megalocephala is a nematode parasite of the intestine of the horse. There are two races: A.m. bivalens (diploid chromosome number, as seen in early cleavages, = 4) and A.m. univalens (diploid number = 2). The slides provided are of bivalens material. Each slide contains 5 longitudinal sections through various regions of the uterus of a female worm; each section shows the heavy uterine wall and the central lumen containing numerous eggs.

The stages of mitosis of the early cleavages are located on the last of these sections. The other sections show meiotic divisions in the maturation of the egg, and will be studied later. The mitoses are best shown in the first and second cleavage divisions. Identify the very thick shell (fertilization membrane), shrinkage (perivitelline) space, cell membrane and pronuclei.

Find a zygote showing two pronuclei. One of these is the sperm nucleus, the other the egg nucleus. Try to find a zygote in which the chromosomes of the pronuclei are delicate elongated prophase threads. (see demonstration).

DRAW (large) the following stages:

a) a zygote or blastomere in metaphase, polar view
b) a zygote or blastomere in metaphase, lateral view
c) an anaphase or a telophase, lateral view.
Label fully. Give actual size.

4. Anastral mitosis in the onion root tip.

Examine a longitudinal section through a root tip of the onion (Allium cepa (slide #15)). Under low power, note that the region of active mitosis (meristem) extends back from the tip for only a distance of one millimeter or so. Proximal to this region, non-dividing differentiated cells are found. Art the cells at the very tip of the root dividing?

Study the resting nuclei carefully. To what extend does the number of nucleoli very? Do the nucleoli have a constant position in the nucleus? Heterochromatic chromocenters are observable in most resting nuclei; they are small darkly-stained areas located principally adjacent to the nucleolus. Study dividing cells. Note particularly the orientation of the chromosomes at metaphase; prophases in which the somatic coil of the chromosomes may be seen; telophases in which the reappearance

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of the nucleoli may be observed. What is the shape of the metaphase and anaphase chromosomes? What are the characteristics of the centromere region? Can you find a chromosome showing a secondary (nucleolar) constriction? Can you find polar views of metaphase? What features characterize an anastral mitosis such as this as opposed to amphiastral mitosis in the whitefish blastula? Make notes and sketches.

5. Rapid differential staining of onion root-tip cells. Modified technique from Kurnick & Ris

The material consists of 24-hour old root tips from onion sets placed in damp sand or of root tips which were fixed in 1:3 glacial acetic acid and absolute alcohol for 24 hours and stored in 70% alcohol.

1. Excise a fresh tip and fix in a vial containing 3:1 absolute alcohol: glacial acetic acid (Use 30 drops alcohol to 10 drops acetic).
2. Let stand 1/2 hour.
3. Place in a vial containing 2:1 absolute alcohol: concentrated HCl (Use 20 drops alcohol to 10 drops HCl). Maceration
4. Let stand 5 minutes (no more!).
5. Transfer tip to vial of 70% alcohol 5-10 min.
6. Transfer tip to clean slide in large drop of acetic-orcein-fast green.
7. Tease thoroughly with needles.
8. Add more stain if necessary and float a coverslip on the preparation.
9. Let stand 1/2 hour.
10. Squash through blotter with heavy vertical pressure with thumbs.
11. Seal with gum-mastic paraffin.

A good preparation resembles closely one which has been prepared by the more selective techniques for the differential staining of ribose- and desoxyribose nucleic acids. The RNA of the nucleolus and cytoplasm are stained by fast green (note that the elongated differentiated cells have very lightly-staining cytoplasm). Orcein, which resembles the Feulgen technique in its specificity for DNA under these conditions, stains the chromosomal components. Examine this preparation closely, especially for chromonemata coils in prophase and metaphase chromosomes.

References: Darlington and LaCour, The Handling of Chromosomes.
Kurnick and Ris, Stain Technology, 23: 1948 (Aceto-orcein-fast green).

VIII. The "Resting" nucleus and nucleoli.

A. The megakaryocyte nucleus (slide 15).

Study sections of the bone marrow of either the cat or goat. Identify the megakaryocytes by their large size and large lobed nuclei. (Compare with the classical illustration in Wilson). The centrioles are difficult to see in an ordinary hematoxylin preparation. The large nucleus appears to arise by repeated mitoses without accompanying cytokinesis. After each mitosis, the daughter nuclei fuse together. These cells are phagocytic, and commonly engulf leucocytes and worn-out erythrocytes.

B. Examine the demonstration of the ciliate protozoan. Dileptus, showing "distributed" nucleus: refer to Visscher, J. Morph. 44: 1927.

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C. Prophase changes in nucleoli in corn (slide 16).

Study permanent AC preparations of the prophase of dividing pollen mother cells. Diploid number in corn is 20, but in these cells the homologues have united in synapsis so that at metaphase only 10 elements are visible. Some slides show early prophases, others late. Some show metaphases. Draw two cells, one in early prophase and one in late prophase, showing the changes in the nucleolus and its relation to a particular chromosome pair (#VI). Refer to Sharp, Introduction to Cytology, pp. 118-121 for a discussion of the relation of the nucleolus to the chromosome matrix. See also McClintock, Zeit. Wiss. Biol., 21: 1934.

D. Amitosis

Study the slide of Periplaneta (Cockroach) ovary (slide 17). The developing eggs are enclosed by follicle cells which are responsible for the production of yolk and other substances for the maturing egg. For a while, these divide mitotically, but later, in the follicle cells surrounding the larger eggs, amitosis appears to set in. Draw a mitotic cell from the follicle wall of a young egg and several cells in which the nuclei show evidence of amitosis. Reference: Murray, Biol. Bull. 50: 1926.

IX. Nuclear Phenomena in fly larvae.

As discussed in lecture, mitosis continues in certain insect tissues (e.g. brain and ganglia) throughout larval life, whereas in others (salivary glands, midgut, Malphigian tubules, etc.) mitosis ceases at a very early time and further growth is by increase in cell and nuclear size only. The purpose of this exercise is to prepare and study examples of each of these types of tissues, and to study examples of giant salivary gland chromosomes.

1. Cell and nuclear size in the salivary glands and brain of the larvae of Drosophila robusta

   a. Place a full-grown larva in the center of a clean slide.
   b. Under the dissecting mircorsope, pull off the head as directed.
   c. Add immediately a generous drop of acetocarmine.
   d. Allow to stand about 2 min., then cover with a coverslip. Do not apply pressure to the preparation, or seal it. Add more stain to the side of the coverslip if necessary.

Survey the preparation and look for the following structures: brain, salivary glands, oesophagus, oesophageal bulb, midgut, Malphigian tubules and fat bodies. Study the nuclei in all of these structures, especially the salivary glands. Refer to the diagram in Darlington and LaCour, Handling of Chromosomes, p. 60. Draw several cells of the latter and measure and record the diameters of a number of the largest nuclei.

48.3 max diam

When you have finished the above, prepare a second slide as

13.8
3.5
690
414
48.30
3
L.P 3
4
3
4
4

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follows: Pull off the head as before, and add acetocarmine. Locate salivary glands and brain and carefully dissect them free from the rest of the structures. DO NOT ALLOW DRYING TO OCCUR. After at least 10 min. in the stain, draw these structures out into a diverticulum of the drop. Wipe off excess debris and stain with a rolled-up Kleenex. Cover with a clean coverslip and apply firm, even pressure to the cover through a blotter. If successful, seal with gum mastic-paraffin.

In this preparation, the salivary gland cells should be flattened and the giant chromosomes spread out; the brain also should be flattened sufficiently to allow study of the very small cells. Study the brain and look for mitoses. Note the size of the chromosomes and their arrangement in pairs (somatic pairing) at metaphase. 2n in Drosophila robusta is 8. Draw a metaphase or sketch the one on demonstration.

Measure and draw in outline 4 or 5 of the diploid nuclei from the brain and compare the size with that of the salivary gland nucli. The volume of the nucleus approximately doubles with each doubling in valence (polyteny) or number (polyploidy) of the chromosomes. On the basis of measurements of diameters of somewhat flattened spheres, such as you have made, it has been calculated that for each doubling in volume, the diameter increases by a factor of about. 1.45. On this basis, a nucleus with a diameter of about 4 microns would attain the following diameters after a number of doubling cycles:

#of doublings	diameter in microns
5	25.4
6	36.8
7	53.4
8	77.4

Considering the individual chromosome of a diploid cell to consist of two chromonemata, how many chromonemata would be present in a single salivery gland chromosome. To what degree of polyploidy would this be comparable? Due to the many sources of error, for which it is difficult to correct, such figures should be used only as rough approximations of the situation.

2. Identification of a chromosome end. Using your own preparation or slide 19, find a nucleus in which the chromosomes are well spread out. As the chromosomes may twist on themselves or overlap other chromosomes, focus carefully. Howm many chromosomes should there be? Why? Note the chromatic discs arranged at intervals along the cylindreical chromosomes. These are Feulgen positive (see demonstration). The arrangement of these discs is constant for an individual chromosome, enabling one to recognize that chromosome in all individuals of the species. Using the maps and drawings on the table, study the banding pattern of the end of Chromosome 2, left arm, then locate it in your smear. Draw the pattern of the first 8 or 10 discs. If time allows, identify as

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many of the other ends as you can.

References: Structure of giant chromosomes:
Metz, Symposium on Quant. Biol. v. 9: 1941
Painter, (same)

XI. Meiosis

Fundamentally, meiosis consists of an elongated and special prophase (auxocyte stages) followed by two specialized mitoses, the maturation or meiotic mitoses. The details of the process vary considerably in different organisms, although the essential features, inclusing synapsis, chromatid exchange and random segregation, are well-nigh universal.

1. Meiotic prophase (primary oocytes)in the kitten ovary (slide #20).

The periphery of the ovary is occupied by the germinal eipthelium from which the oogonia arise by proliferation. As it matures, each oocyte becomes surrounded by follicle cells, which are differentiated from the connective tissue stroma of the organ. In general, the earlier oocytes will be found toward the periphery and the more advanced ones toward the center. Before starting your drawings find all the following stages and be sure you understand the changes undergone by the chromosomes. Auxocyte stages are exceedingly difficult to interpret, and considerable time should be spent in learning to recognize cells which are favorably cut and stained.

Make drawings of the following:

a. late oogonial mitosis; peripherally located. 2n in the cat=36.
b. primary oocyte in leptotene (lepto=slender, tene=thread) chromonemata very slender, showing chromomeres. Orientation towards idiosome may have begun.
c. primary oocyte in zygotene (zygo=union) chromonemata in characteristic "boquet", oriented to idiosome. Synapsis has begun.
d. primary oocyte in synizesis a clumping of zygotene chromosomes which is partly due to poor fixation.
e. primary oocyte in pachytene (pachy=thick). Synapsis is complete; chromosomes shorter and thicker; half the number of earlier stages. Orientation to idiosome is retained for a while.
f. primary oocyte in diplotene (diplo=double) chromosomes (tetrads) shorter, not oriented and visible double, perhaps showing tetrapartite nature. Opening out and chiasma formation begins. Nucleus considerably larger.

Following diplotene, a pseudo-resting stage of long duration ensues, during which the oocyte increases enormously in size and is enveloped in follicle cells. (Graafian follicle). See semonstration of section of adult ovary. Diakinesis and MI occur shortly before and as the oocyte is liberated from the Graafial follicle.

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h. diakinesis

For the reason above, this important stage is not shown in the kitten ovary slides. Study slide #24 or the demonstration of diakinesis tetrads from a grasshopper spermatocyte. Draw several tetrads showing chiasmata. How can a chiasma be told from a simple twisting of the homologs over one another? Model a diakinesis tetrad as interpreted by the one-plane theory, using different colors of plasticene to distinguish the homologues. Have your model approved. What becomes of the chiasmata as metaphase of MI is reached?

2. <u>The meiotic mitoses in Ascaris megalocephala.</u> (slide 14)

Draw the following stages and space so that they will supplement and precede your drawings of mitosis of the first cleavage.

a. an oogonium into which a sperm is just entering showing also the poorly preserved tetrads in the center of the cell. How many tetrads? Why? Is a fertilization membrane present at this time? Ascaris sperm are not typical of those generally found in animals.

b. a primary oocyte in some stage of the first meiotic division. Select this stage carefully, trying to find a side view metaphase which shows all four chromatids of each tetrad.

c. a secondary oocyte in second maturation division. The first polar body has separated from the egg and lies against the inner surface of the fertilization membrane. Your drawing should show dyad chromosomes in the secondary oocyte.

d. ootid stage. Monad chromosomes remain to form the nucleus of this stage. These monads rather quickly form the female pronucleus, while the sperm nucleus swells at this time to form the male pronucleus. Compare with drawing a. of mitosis.

3. The meiotic mitoses in the eggs of the Palolo worm, <u>Leodice</u>. (slide #21).

As in Ascaris, meiosis is initiated by sperm entrance, 12-14 minutes after insemination, metaphase of MI is reached. MII occurs about 15 minutes later.

Draw at least three of the following stages (do not draw the whole egg, and omit cytoplasmic details , but be sure you see all of them.)

a. primary oocyte in metaphase of MI (note tetrads, fertilization membrane and yolk).

b. primary oocyte in anaphase of MI (note dyads separating)

c. primary oocyte in telophase of MI (note formation of 1st polar body).

d. secondary oocyte in metaphase or anaphase of MII (note orientation of spindle and dyads, or monads separating).

e. ovum ready for fertilization (note nuclear reconstitution by vesicle formation). Can you find the sperm pronucleus?

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Rogers
200426
2/1/51

Necturus erythrocyte 50.05 X 27.95 (High Dry)
Human erythrocyte 7.4 µ (O.I)
Paramecium 220 X 41.4 µ (LP) OK

Objectives	Length measured by 1 space
16 mm.	.0138 mm or 13.8 microns
4 mm.	.00325 mm or 3.25 (3.3) microns
1.9 mm.	.00148 mm or 1.48 (1.5) microns

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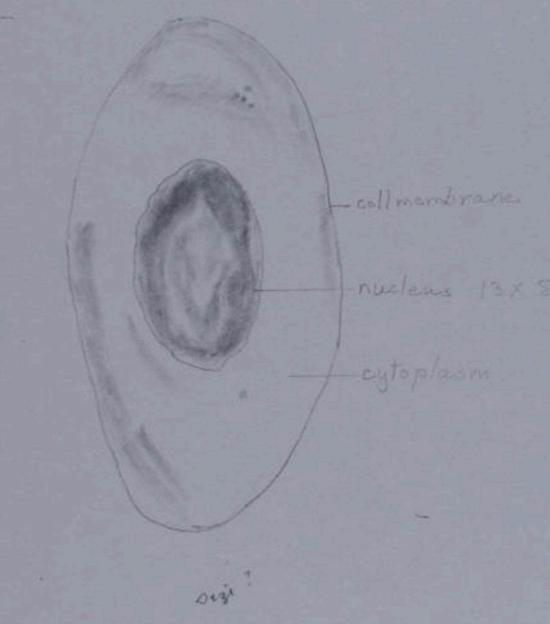
Necturus erythrocyte

Rogers



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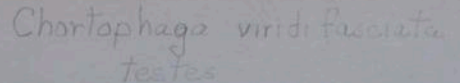
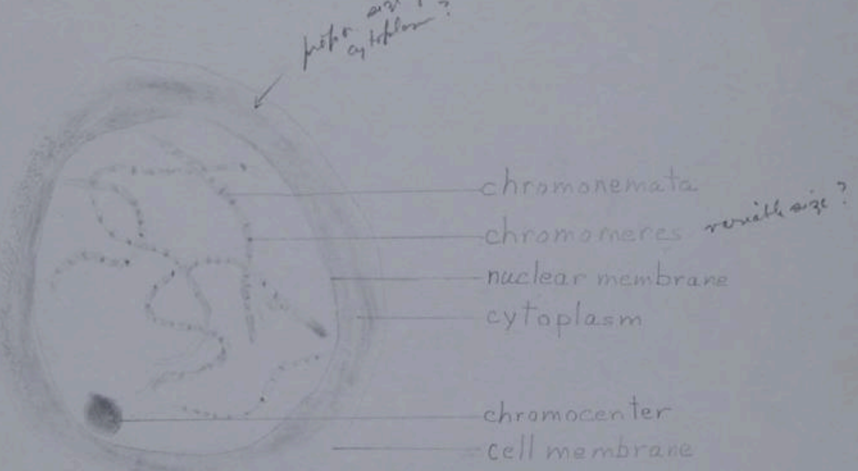
Rogers





nucleus 23.68 μ (approx.)
celldiameter ca. 27.45 μ

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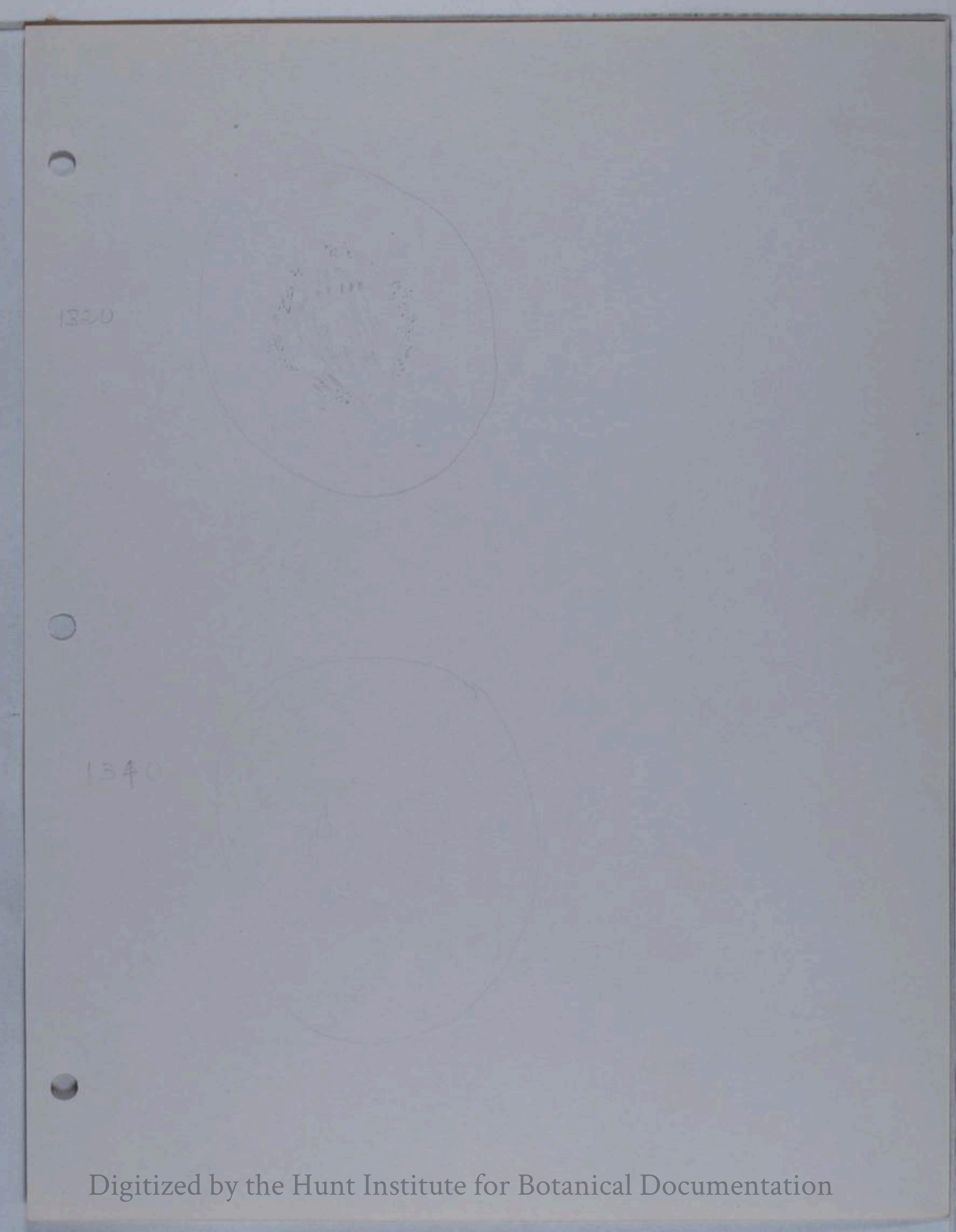

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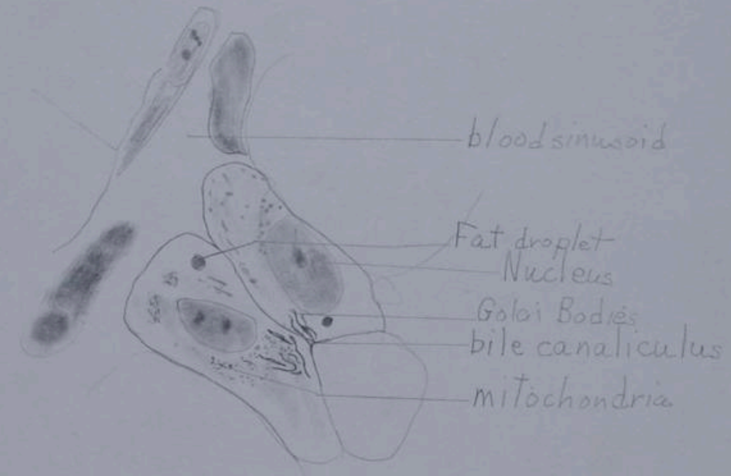
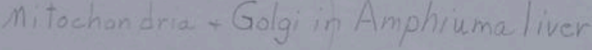
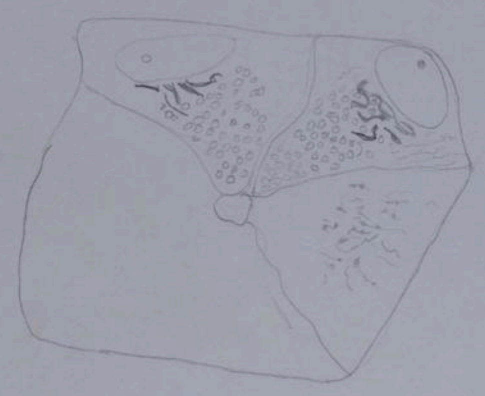



Mitochondria + Golgi in Amphiuma liver



Mitochondria + Golgi in Amphiuma pancreas

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Triturus Tail

Rogers

gland

mucilage

nucleus of epidermis

chromatophore

Epidermis w/gland

vertebra

notochord

muscle fiber

caudal vein 43 μ

caudal artery 30 μ

chromatophore

x 10

nerve chord

185 μ

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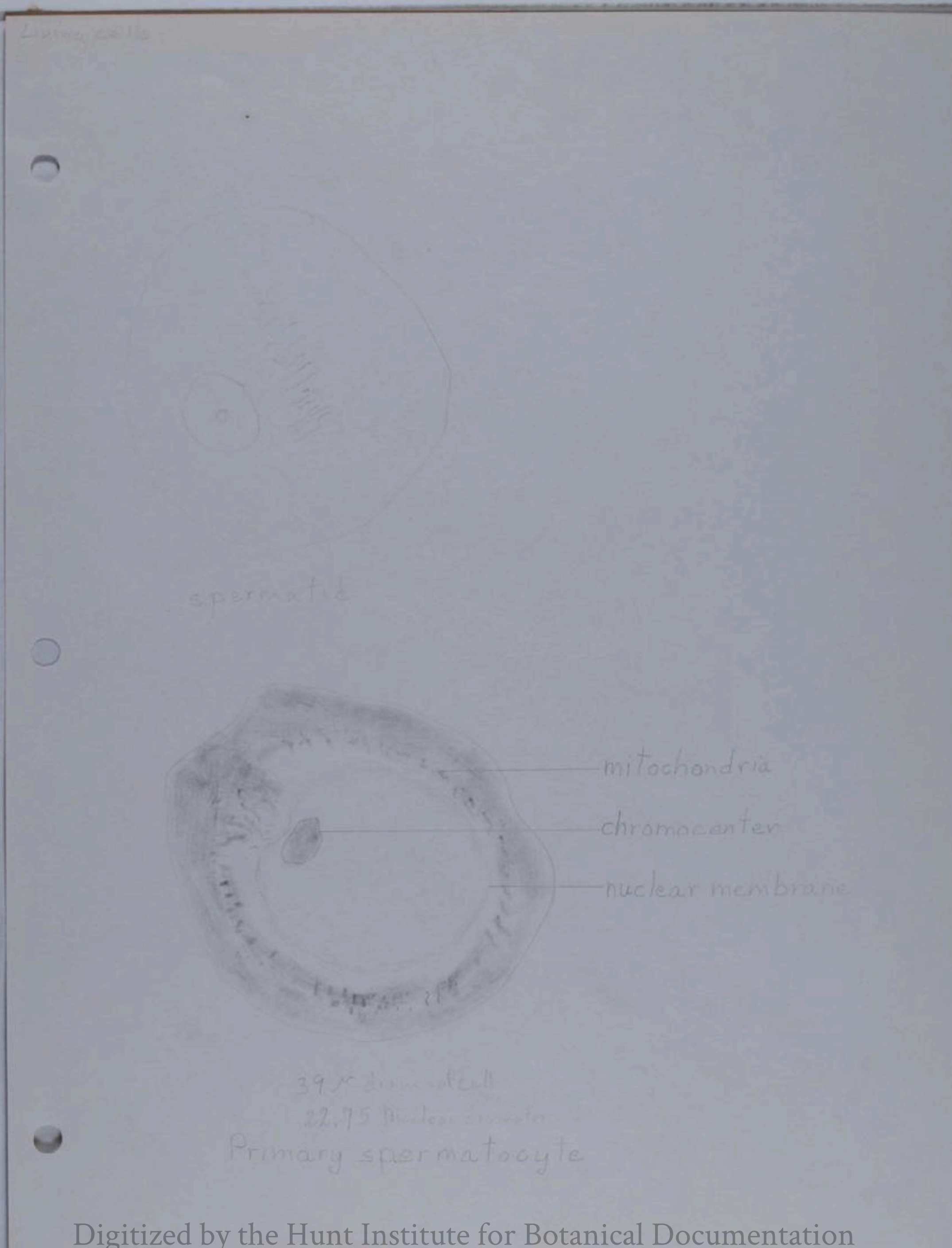

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Ambylostemma sp.

Rogers

position of nucleus

yolk

Epithelial cell
(living)

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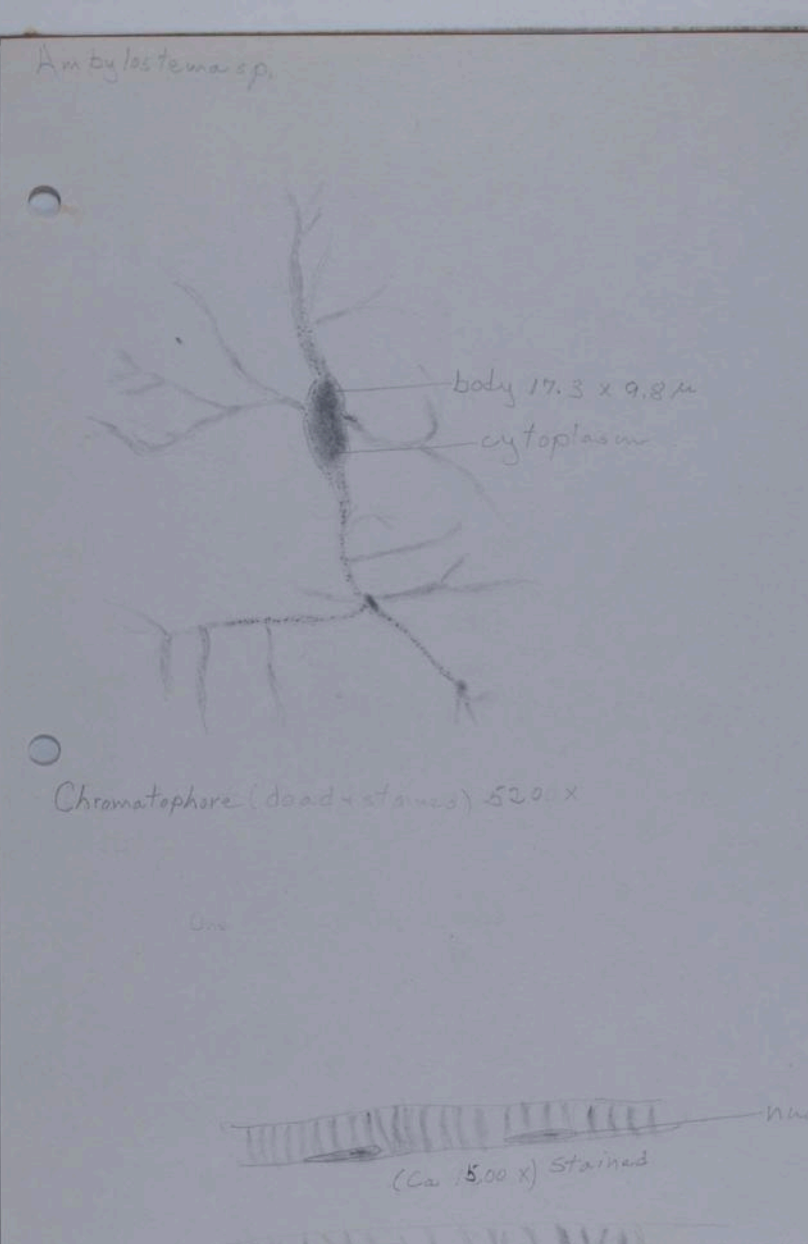
Ambylostema sp. Rogers



Chromatophore (dead + stained) 520 x

(Ca 500 x) Stained

Living muscle fiber

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Ambylostema sp.

Rogers

Epithelial cell - wall undistinct

Ca 580 X

Nucleus 8.9 X 5.9 μ

Body 10.7 X 4.4 μ

Fibroblast (Stained)

Ca 600 X

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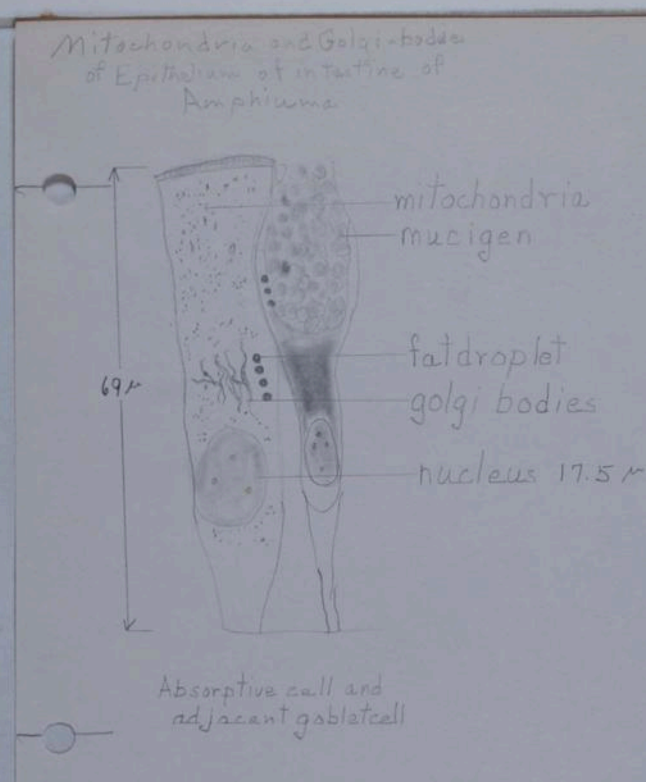
Mitochondria and Golgi-bodies of Epithelium of intestine of Amphiuma



Absorptive cell and adjacent gobletcell

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Zoo 426 2/5/51

I Introduction
A. The course
B. Cytology + related sciences.
1. Fundamental studies of cell structures, its functions in plant + animal
2. Cellular physiology - w/techniques experimented w/chem + physics; genetics - hereditary studies; embryology - development of special functions, the embryo differentiation - these are some of the related fields.
3. The narrow sense of cytology - a microscopic observation science, based on what is seen under mike. Biological, by inference, comparison w/other disciplines.
4. The studies of:
morphology - the whole organism (except protozoa)
anatomy - organ systems
organs } - Histology
tissues }
cells - - cytology

C. Sources of information
1. Sharp - Fundamentals of cytology 1943
" - Introduction to " 1934 - Source of names given - w/all sides of a question examined, the opposing ideas.

Mitochondria well disc. in Fundamentals.

2. Wilson - The Cell, 1926 - the classical reference work as a guide to older references

3. Darlington - Recent Advances in Cytology 1937.
Cytoplasm hardly touched - mostly nuclear

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4. Riley - Introduction to Gen. + Cytogen.
Nuclear

5. White - Animal Cytology + Evolution

II The cell: its terminology.
A. The problems of definitions
1. Definitions confined + cause boundaries to exist in mind which do not exist in nature.
2. Definitions should be useful generalizations -

B. Conditions difficult to classify as cellular -
1. Example from muscle cell or fiber - dozens of nuclei in one cell - different from definition of a cell, viz. a nucleus surr. by protoplasm

a syncytium - cells together (a no. of fused cells). with the nuclei within one cell membrane.
Or - may be multinucleate by division of nucleus within same membrane
known as a coenocyte, or plasmodium

2. Insect egg is another ex. of coenocyte.

In frogs, embryo makes clear cleavages of cells + nucleus. but in insects, embryo becomes multinucleate thru nuclear division within one cell, and later differentiate into individual cells

This breaks down def. of cell division - the nucleus + cytoplasm dividing at same time.

Other ex. of coenocytes - incomplete cell division, leaving a connection between 2 cells this in vertebrates, plants (as plasmodesma). Another in osteoblast in the bone.

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Feb. 2/6

Grasshopper

Testes

Head

thorax

Testes

Follicles

follicle

cells

2/8

Changes in growth of testes correspondents to developmental stages of grasshopper

young testes

Distal

spermatogonia

proximal

2 zones of testes (cysts)
in 1, cells in grps w/ very immature cells "spermatogonia" at very tip
in 2 — larger cells, primary spermatocyte w/ large nucleus + smaller cytoplasm.

Mature testes

2

1

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cyst 2 - largely filled w/ mature sperm.
" 1 - primary

intermediate stage testes

1 primary sperm

spermatids

2ndary spermatocytes

2ndary spermatocyte.

tail

spermatids
metamorphosis to spermatozoa

nucleus

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Feb. 13, '51 - Zoo 426. Lecture.

II The Cell: Its Terminology -

In paramecium, 2 types of nuclei -

1. macronucleus
2. micronucleus

May have 1, or several of these 2 types of nuclei in different paramecia.

Cells w/o nuclei - vertebrate red-blood cell is ex.

In history of prod. of red-blood cell, the "mother" cell has a nucleus, but mature corpuscle is only haemaglobin. The nucleus is lost somewhere along line. Red blood cell is very short-lived.

Cells w/o protoplast - hair is ex. cork, xylem, skin, etc.

The nuclear material of bacteria is scattered, but is definite - called "Chromidia."

A paramecium Dileptus has many spots of nuclear structure

Virus - a self-duplicating nuclear body - parasitic on cytoplasm of other cells.

Concept of energid - in a multinucleate body - each particle is a nucleus, w/a sphere of influence on the nearby cytoplasm.

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Clifford Dobell - protozoologist vociferous in argument against cell theory.
an Englishman, working on his own, no institution.

In 1911, Arch. for Protist. 23: 1911.
biologists use "cell" for 3 things.
1. Whole organism - amoeba.
2. a part of a whole org. - liver cell.
3. a potential whole org. - fertilized egg.

Wants to realize that 3 entirely diff. phenomena.
This a rather extreme view, but points up fact that definitions are too definitive.

Dobell would consider # 2 only for cell definition
Considers protozoa as an org. w/o compartments as compared w/ multi-cellular org.
Simpson "Meaning of Evolution" supports this view.

Acceptable terminology

centrosome — centrosphere
centrioles
golgi apparatus
dictyosome.
mitochondria (dots - chondriomites
chondriosomes (rods - " conts
ectoplasm (plasmagel)
endoplasm (plasmasol)
nucleoplasm
nucleus
1. Cell membrane (animal)
5 nuclear membrane
2 cell wall (plant) cellulose
plastids - chloroplasts, leucoplasts
vacuoles
3 protoplasm
6 cytosome (region)
yolk
crystal
oil droplet
starch grain
pigment

deutoplasms - non living ergastic substance
metaplastic

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acceptable term + synonyms

1. cell membrane
   plasma "

3. protoplasm — nucleoplasm
   — cytosome

ectoplasm
  cortex
  cortical layer
  cortical cytoplasm

vacuoles + golgi apparatus same?

The nucleus

chromocenter
in resting condition, fine threads - chromonema(ta)
chromomeres
nuclear sap
nucleoli - definite no + position

Chromatin - a loose term for some part of this system.

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Lab. 2/13

Living cells in paraffin oil.

1. Obtain completely clean slide + cover

Grasshopper testes in deep well slide in

Belar soln. (a Ringer soln.) autoclaved + buffered.

Place a ring (bright) of paraffin oil, with a center clear

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III Historical aspects of cell concept.

A. The early microscopists (1650-1700).

B. The cell theory 1800-1840

C. The foundation period (1840-1870).

D. Emergence of cytology as a distinct science (1870-1900)

E. Cytogenetics 1900.

Seneca - 1st cent. A.D. - saw that water in a curved bottle magnified objects.

1. Late 16th cent. use of curved glass begun - ground lenses for spectacles.
   Leonardo DaVinci - studied principle of optics - no ~~[illegible]~~ conclusion.
   16th cent.
   Jensen bros (Dutch) made compd mikse w/2 lenses.

   R. Hook - 1665 - 1st to recog. use of mike as a research tool. Published "Mikrographia, etc." includes discussion of cork structure, 1st use of cell. Observed fresh material, that the cells contained a juice.

   Van Leeuwenhoek - son of a weaver, no formal education. Microscopy a hobby - made his own mikes ~~&~~ mounted them. Simple lenses of remarkable accuracy. Up to 300 diameters, practically w/o chromatic aberration.

1st to see Protozoa in pond water.
- Bacteria
- Spermatozoa
- Circulation in a fishes tail
- Muscle fiber
- Capillaries

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Leeuwenhoek used techniques of illumination which were similar to the dark field.

Descriptions are accurate - meas. by comparison to width of a hair. Good on detail, poor in theory.

After Leeuwenhoek, not much done until 19th century.

The theory that the cell has a life of ~~his~~ its own as well as in connection w/ life of other cells is a part of the cell theory.

Schleiden + Schwann - 1838

The main interest of Schleiden was the origin of the cell - Started out as a lawyer, turned to botany after failure at law.
Plants composed of independent cells; there is a duality to cellular life, i.e. the independent cell is a part of the multicellular body.

Schwann - similar to Schleiden, agreed w/ ~~Schleiden's~~ theory - thought cells begun w/ a crystal,

cytoplasm
nucleus
crystal (nucleolus)

Mirbel 1808 - Plants are made up of cells - w/o mention of dual

Dutrochet - 18~~38~~ - All animal tissue is differentiated cells.

Turpin 1826 -

These precede Schleiden + Schwann, so cell theory ~~not~~ begun entirely by these 2.

See Biolog. Symp. VI 1938.

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Protoplasm, tho ~~the~~ seen by Hook, not recognized until 1835 by Dujardin as the basis of life - called it "sarcode", working w/ amoeba.

Protoplasm as an adjunct of cell theory not recognized by 1865.

Feb. 27, 1951 -

IV Technical methods

A. General

Three types of studies of living cells.

1. In vivo - not removed from normal environment
2. In vitro - removed, placed in cultural media where they can grow - tissue culture.
3. supravital - surviving, but not reproducing, cells.

Fin tail of amphibian larvae is found a good place to see vertebrate cells in vivo.

Speidel studied living nerve growth, pathways, etc in tadpole tail.

Clark + Clark - studied living cells in ears of rabbit.

Marine eggs furnish good material for in vivo studies.

Chambers (Columbia) developed microdissection apparatus for operations under the objective lense.

Grasshopper testes - study is of supra-vital nature. Cells in abnormal state, hard to correlate w/ actual conditions.

"Vital" stains not really vital - bound to alter some of the processes in cells.

Disadvantages of fixed material -

Material is dead.

Advantages

Change of refractive index provides much more detail to be seen than in life.

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Possibility of artifacts -
In preparation of Necturus erythrocytes, many cracks in cytoplasm appear. Although an artifact, shows something of the nature of the cytoplasm

Buchsbaum studied living cell, then fixed & stained the same cell, showing that fixatives didn't produce any artifacts.

In vitro studies: tissue culture under sterile conditions. Specialized types lose their peculiarity of function in tissue culture.

Hardy & Fischer, (ca. 1849) operations stimulated by observations of other biologists of their day on protoplasm. Before these 2 men, people were preparing protoplasm so as to produce certain effects, so that people were producing theories as to nature of protoplasm i.e. alveolar, granular theory, etc. due to the methods of fixing. These 2 men showed that these theories were not real, but due to fixing methods. Had a good effect of biologists.

Fixatives - properties necessary.
1. kill instantly
2. render components insoluble.
3. produce strong differences in refractive index.

Health is an important feature in preparations.

Classes of fixatives.
1. Nuclear fixes - anything with acetic acid.
   a. aceto-carmine - produces granular effect in protoplasm wipes out mitochondria, but good for the nucleus.
2. Particular cytoplasm fixes -

Fixing by freezing & dry.

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Staining – nuclear + cytoplasmic stains.
Little actual experimental method in development of particular techniques until recently – just trial + error.

Microscopes
1. Resolution – ability to see detail.
2. 2 μ is highest magnification which light microscope will resolve.
3. Ultra-microscope – ultraviolet light, using only quartz, have to photograph, brings resolution down to .075 μ.
4. Dark field micro. esp. useful for studying things in great dispersion in a fluid – saliva, bacteria, etc.

   In dark field, a disc-like stop in center of substage condenser, producing cone of light going to condenser; beams crossed at point of focus, + light does not enter objective lense, but catches objects at crossing point.
5. Electron micro – X 21,000 + resolution to that.
   1. Bombardment of object w/ electrons.
   2. Electrons won't pass glass.
   3. Have to have a complete vacuum.
   4. Material must be exceedingly thin.

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Lab. 2/27

capillary elements

tail fin

ventral blood vessel

notochord (skeletal rod)

nerve cord

connective tissue

epidermis

tadpole Amblystoma sp.

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~~Feb~~ March 6, 1951 - Cytology lect.

V The Golgi bodies + Mitochondria
A. Protoplasm + its formed inclusions.
B. The Golgi-bodies
1) Distribution + technique
2) morphology
3) function
C. The mitochondria
1 as above.
2
3

Read chapters 2 + 4 in text of physico-chem. prop. of proto.

A complex colloid substance is basis of protoplasm.
The sea urchin egg centrifuged is good place to see structure.

Normal sea urchin egg

mitochondria (grading bet. dots + filaments)
granules which take up neutral red dye
echinochrome pigment
spheres of yolk comp. of protein + ~~fat~~.
oil or fat droplets

Centrifuged cells

centripetal
oil droplet cap
nucleus
hyaloplasm (clear, not inclusions) (considered highly basic to cells) optically homogeneous.
mitochondria (to the constriction)
yolk
neutral red granules
pigment
centrifugal

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Mrs. Harvey, exp. w/ seaurchin eggs

Continued cent. until egg splits in 2.

Light Half.

Heavy half

put sperm in ~~heavy~~ light half, development is normal.
The heavy half will also develop, not as well as light ½,
but produces a blastula. Develops parthenogenetically,
but not beyond blastula stage.

Further centrif. break egg into ¼'s.

1 cont. nucl
1 " mito.
1 " yolk
1 " pig.

If inject sperm, some
development in 1st.

Importance is to determ. essent. material
{1. Must have nucleus or some nucleo protein.
{2. Hyaloplasm - always +.
these most sign.

Mitochondria + golgi-bodies nearly universal.

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The Golgi-apparatus.

1. Presence.

All, except very old cells, old nerve cells, or cells w/ very short life (i.e. erythrocytes).
Plant cells do I have Golgi-apparatus.
In vacuoles, neutral red stains the vacuome same as in certain animals.

Observations:

1. Difficult in living cells, w/ all kinds microscopy.
2. Possible, with cert. tricks, to centrifuge + see by surrounding golgi-a. with granules.
3. Neutral red, + methylene blue dye the g-a. in vital studies.
4. Most study done w/ dead + stained mat.
5. Dest. by fixatives which dissolv. fats.
   Dissolved by alcohol, acetic acid.
   Indicates it is lippo-protein compd.

Techniques:

1. Originally, impregnated w/ metalic Ag.
2. Mann-Kopsch tech.
   Fix 1% osmic acid. 1:1
   1 sat. $AgCl_2$

   ½ hr. treatment, wash in dist. H_2O

   Placed in lam dish, barely covered w/ 2% osmic acid for 10 days-2 weeks.

Trouble is that osmic acid blackens fat. This avoided by placing in soln. of turpentine to dissolve out the fat.

Appearance:

near nucleus, filaments in vertebrate cells; well developed

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Young cells, + esp. invertebrates
Golgi app. appears as peculiar ½ moon shaped body which is osmophilic, + ½ which is osmophobic.

Function + significance.
1. Connected w/ process of secretion.
Secreting cells show polarity

lumen
mucigen
goblet secreting cell
polarity
nucleus
golgi-apparatus between nucleus + secreted substance

Pouse, 1938

In thyroid gland

blood stream
secretory subs. (colloid)
nuc.

X-section of follicle

Using golgi-technique

golgi-ap.
nucl. outside, except in 1 out of 500, where nucleus is near other end
colloid

but when demand for thyroxin is great, 1 in 10 nuclei have reversed polarity.

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so, in normal activity thyroxin is stored in center, but in abnormal "", thyroxin excreted outward.

In pancreas

x sect.

lumen

long.

pancreatic enz normally pass to lumen, then to duct

The zymogen granule

zymogen granule in blood

proenzyme granules when traced to origin, found to come from golgi-app.

one cell.

<u>Bowen</u> says that mitochondria form the granules of mucigen, zymogen, etc.

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3/13/51 Zoo. 426 Sect.

Series representing regeneration of secretory substance in polarity cells (Pancreas)

(1) Golgi presubstance

(2) externum (osmophilic)
internum (osmophobic)

(3) acidophylic staining material

(4) externum decreases
internum increases

(5) zymogen granules

zymogen.

(6)

This is process of Hirsch, for prod. of zymogen granules

Worley - snail embryos w/ m. Blue stains osmophilic particles, showing increase of protein yolk; a localized polarized synthesis w/in the cell, but physiology not understood.

In other cells, same process occurs, but can't see this same product, since secreta won't stain.

No studies include study of nuclear activity, since staining techn. for golgi destroys nucleus.
There is circumstantial evidence that nucleus goes thru some changes during process of secretion.

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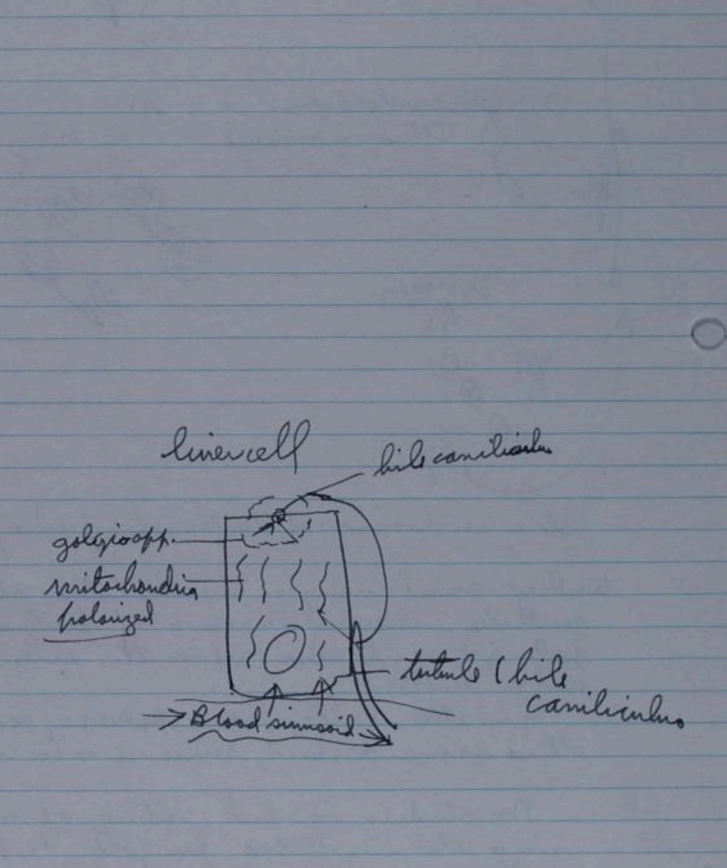

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In cytoplasm of pancreas cell, a strong basic reaction to staining (also in nucleus) shows some changes in these organs, a close relation to synthesis, esp. of proteins, and particularly ribo-nucleic acids.

So, visible evid. that golgi-app. concerned w/ synthesis of protein.

Mitochondria —
Like golgi-app., universal in cells, w/ few exceptions i.e blood cells, old cells.

V. similar in plants + animals.

Stains - janus green B in great dilut., 1/11,000 in supra vital studies will selectively stain mitochondria, no other objects take. This only in living condition, for in dead cells, all organs take stain. After long period, color changes thru yellow-green to rose color. This reduction only in living cells.

Mitoch. destroyed by usual fixatives w/ acetic acid. Will take osmic acid, but weakly, therefore structure of a lipid nature.

~~In fit.~~ Attempts to isolate, particularly by Claude; w/ liver cells ground up + centrifuge rapidly, at certain speed + time provides a fraction similar to mitochondria.

Contents of mito.
- 25% lipids - simi. to golgi app
- 75% protein

Indirect evid. of large amts. of enzymes.

Function of:
Facts:
- embryonic cells - mito. extremely numerous in cytoplasm, showing not specific position & generally scattered.
- in differentiated cells, (liver cells [which shows polarization]) mitochondria polarized.

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In pancreas cell

golgi

mitochondria

Circumstantial evidence that mitochondria assist in synthesis, that it is a body for transport of materials.

epitheleal cells in gut
evidence for transport

lining of gut

mitochondria

cyclic behavior. Certain cells synthesizing fats.

fibroblast accumulate fat droplets until fat takes up allmost all area of cell.

Mitochondria numerous in young cells, decreases as fat increases.

This true of starch in plant cells.

No direct evidence of ford these.

In case of plastids in plants, claimed to see mitochondria actually changing to starch.

Some close connections to respiration + enzyme systems.

Vitamins precursors of respiratory enzymes, + mitochondria considered as store houses of resp. enzymes, and this good hypotheses because of distribution.

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In some cases mitochondria assoc. w/ enzymes.
In Paramecia, some role in elaboration of digestive enzymes.

Isolated mitochondria contain substances which are important factors for cell respiration.
The janus green reduction to rose color is evidence that reducting systems are present.

A metabolic gradient is noted in the reducing series of janus green.

Mitoch. esp. abundant in cells actively respiring.

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Lab 3/15

Histology of liver-

Cells in genl occur in chords

portal system → blood sinusoid → no wall here

bile canaliculi

bile duct

Materials + functions of liver

1. Glucose → glycogen storage
2. $O_2 - CO_2$
3. amino acid → urea
4. Bile production

Pancreas

Many acinus in each pancreas

lumen

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Mar. 27, 1951 - Zool 426. Lecture.

VI Introduction to nuclear phenomena.
  A. General
  B. Structure of resting nucleus.
    1. form
    2. nucleoproteins.

Methods of study of n.

1. Genetics - studying effects of heredity. indirect.
2. Observ. by microscope.

Gene cannot ~~out~~ live w/o the environment of cytoplasm.

Operation of genes studied thru biochemical ~~S~~ activities, as in Neurospora.
Nucleus universal - apparently similar in diverse forms.
Life has two characters
  1. Continuity
  2. Change - evolution.

Functions -
1. Reproduction of protoplasm.
2. W/o nucleus, cytoplasm soon dies (pull off nucl. in amoeba.
3. Large quantities of cytoplasm dispensible to cell.
4. How much of nuclear material required for normal function?
  By use of x-rays, remove pieces of nucl. (or chromosome) - most cases lethal!
  In mature cells, w/ no further reproduction, nucleus removed w/o harm, but no further development.
  But nucl. must be generally intact in cells for normal function.

Read !! this paper →

Hämmerling, w/ unicellular alga Acetabullaria sp. mediterrana has made studies to show the requirement of nucleus to reprod. + life.
Very significant work for showing importance of nucleus.

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Structural basis of nucleus.
1. Interkinetic (between divisions) - capable of further mitosis.
2. Fundamental type cell

All structures here of 2 kinds of nucleo-proteins.

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Sect. Zoo 426 3/30/51

B. Structure of resting nucleus
1. Form - a great deal of what is known has been inferred from dividing nucleus.

Very fine basophilic threads: = chromonemata

Cross connections (anastomoses) denied by some.
Chromomeres claimed by some to be nothing but spiraled sections
Heterochromatin mostly assoc. closely with nucleoli.
Almost all this material basophylic.

2. Nucleoproteins.
Nucleic acids in cells always conjugated w/ a protein (protamines + histones). Nucleic acid itself consists of PO_4 combined w/ desoxyribose or ribose type sugar, + purine or pyramidine.
Original studies by Mischer on salmon sperm, pus, thymus, etc. On account of sperm structure, it was originally that ~~that~~ DNA and RNA only in nuclei.
Yeast + thymus nucleic acid both almost universal in cells.
1924 Feulgen + Rossenbeck. 1st specific microchem. test. Fix-treat in cold 1N HCl, then warm 1N HCl, Schiff's reagent. RNA is completely dissolved by reaction, so doesn't stain.
Stains chromomeres + chromonemata intensely. Chromocenters very intensely stained, but nucleolus does not stain (may be located by closely applied ~~[illegible]~~ heterochromatic areas.
Brachet + others developed meth. of enzyme digestion of RNA. Basic stain then employed.

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Toluidin blue in control will stain fairly intense areas about nucleus, nuclear structures including nucleolus. But in enzyme treated sections, no staining of cytoplasm or of nucleolus.

Probably fair amt. RNA in heterochromatin, but none in euchromatin.

Other simpler methods may be substituted. Purified methyl green almost foolproof for DNA., toluidin blue stains both DNA + RNA: used pretty widely in studies of distrib. of nucleic acid in cytoplasm.

Methyl green - pyronin - chromocenter stains purplish-red; indicates presence of both nucleic acids. (After ribonuclease treatment, stains green like chromomeres.

Now possible to stain with methyl green-pyronin and draw conclusions about nucleic acids.

Orcein - fast green same reaction as m-g-py.

Chromidia = Granules of RNA in cytoplasm. Claude obtained fraction of very fine granules of almost pure nucleic acid. Called them microsomes; apparently same thing.

Nissl bodies of vertebrate nerve cells = masses of RNA in cytoplasm.

Protein synthesis always correlated with deeply staining nucleolar RNA + cytoplasm heavily laden w/ same.

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Sect. Zoo 426 Apr. 5, 51

chromonemata
chromomeres
anastomoses

1

Resting stage Prophase

1/2 is chromatid

chromosome

3

2

metaphase plate

spindle fiber

daughter chromosome

4

VII Nuclear reproduction: Mitosis
Strictly a nuclear process - sep. from cytoplasm.
Most fundamental & universal process.

A Gen'l review
1. "Mitosis" is "cell division"
2. Essen. features
3. Constancy of chrom.

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B. The mitotic prophase + The Coiling Cycle.

C. Meta - ana + telophase.

Constant + finite no. of chromosomes in any one organism, w/exceptions or aberrations, but essentially, not.

Somatic mitosis - characteristic of somatic (body) tissue - division of a cell gives the same no. of chromosomes.

The prophase.

In resting stage, chromosomes present, greatly expanded, uncoiled - remain unchanged. Chromomeres variable on chromonema - constant on individual.

In prophase -
1. Anastomoses disappear -
2. Chromonema begins forming in "gyres"
3. After anastomoses disappear, no evidence that there is a div. of chromonema, but chromomeres seem to be double, at least lobed, some not.
4. Chromonema shortens + becomes thicker
5. " begins doubling or reduplicating almost as soon as mitosis begins - a very significant time. Fundamental doubling prob. begins earlier, but can't be seen.
6. Threads fall apart, the 2 parts twisted around one another
→ Much as 2 coiled springs called the *plectonemic coil* (fig 1.)
this actually left from previous condition.
7. This coil unwinds, the threads begin a new coiling cycle superposed on previous coil. Called the major (somatic) coil (fig 2).
At this stage, very evident that thing is 2-parted.

8. Major coils get tighter, along side one another but independent (fig 3.) Effect is shortening, changing

good illustration!

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chromomeres much closer together, blending them closer together. The chromomeres begin to show doubleness (diff.) from that of previous (fig. 3)

A new material, DOR nucl. acid. accumulates around + obscures the chromonema - ~~call~~ called matrix

Nuclear material RNA changing to DNA.

As coiling continues nos. of gyres decrease. (Fig. 4)
Diameter of chromosome due to no. of gyres.

At one point on chromonema is a structure acting diff. from all other points - the centromere, which stay in close association, (tho divided into 2) up to last moment before div.
The matrix covers all parts except the centromere, often called the primary constriction. The spindle fiber makes attachment ~~to~~ at centromere + this is 1st point to move away.
Point of attachment or position of centromere on chromomere variable, but never at tip end of chromonema.

Because of density of matrix, impossible to determine detail of coils.
Possible to dissolve matrix w/ hot water

After metaphase, daughter chromosome is formed, + is double in anaphase - probably before - at metaphase.

Telophase is reversal of prophase, back to resting nucleus.

The plectonemic coil occurs in daughter chromosome, and doesn't separate again until the next mitosis, and is called the relic coil.

Plectonemic coils of the 2 chromatids
Paranemic coils of the individual chromatids.

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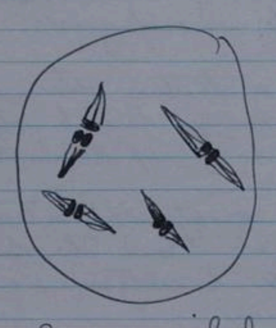

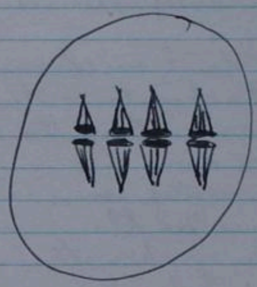

Super-coiled chromosomes.

Mitosis in coccids (meally bugs). No apparent centromere, but of a multiple spindle fiber attachment region - each chromosome moves on own fibers w/o any activity from asters.

Even after ~~xx~~ xray w/ fragments, still move in orderly manner.

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Cytology 426 Apr 10, 1951.

VIII Nuclear reprod. (Mitosis, cont.)
A. The Achromatic figure
B. The centriole
C. Amphiastral vs. anastral mitosis.
D. Changes in nuclear membrane
E. Duration of cycle.
F. Theories of mitotic mechanism.

The basic self duplication is in self-duplication of the chromonema in the essence of all mitosis, but there is a tremendous variations on method of accomplishing this.
The non-chromosomal participants in process of mitosis.
1. The achromatic figure - most conspicuous "fibers" - not fibers but lines of force - never stain - no proteins, merely "fibers" for convenience.
Four kinds.
1. A form w/ a clear centriole - astral rays.

astral rays
gelated protoplasm
centriole

In white fish blastula, no dot like struct at center.

2. Central spindle fibers, not attached to any chromosomes

3. Half spindle-fiber (traction fiber) attached to chromos.

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Bacteriology references.

1. Manual of Methods for Pure Culture Study of Bact.
   Ed. By Comm. on Bact. Techn. + the Am. Bact. Soc.

2. Difco Manual - Man. of Dehydrated Cult. Med. + Reag.

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4. interzonal fiber trailing behind ends of chromosomes.

Chambers (1924) injected vital dyes + proved an actual flow of material of less viscosity than surrounding cytoplasm, indicating lack of fibrous structure.

Continuous (2) fibers not connected to chromosomes so similar to astral rays. Fixing techniques show at least some sort of organization here.

In most plants, rays absent, but some plants have.

Frequently astral rays may be seen attached to nuclear membrane.

Interest in movements of chromosomes - half-spindle fiber attaches to centromere - may grow to the chromosome (centromere) from aster, or vice versa. Some cases partly from each.

Traction fibers may be attached to central spindle fibers or indirectly to asters.

See fig on previous page. - Shows diversity of method.

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In many asters - find a central body - the centriole. Really not a widespread phenomenon. Frey considers them as pure artifacts. Said that when there is a concentration of molecules as at center of rays, there is bound to be a darker area - compression of fibers.

However, seems to be a real structure, visible frequently in resting nucleus.

In certain grasshoppers, instead of being a dot, is a V-shaped body

Little doubt then there is such a structure.

A Protozoan (name??) in gut of wood-eating roaches - (large protozoa w/ big nucleus) - show very definite centrioles

Close relation to certain granules occurring at base of flagella in cert. Protozoa.

blepharoplast divides into 2 - nucleus, the blepharos migrate to edge, maintaining the flagella

In certain cases, centriole gives rise to flagella.

In others, the centriole arises from nucleus,

+ division occurs within nuclear mem. - this so for many Protozoa

nuclear membrane

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Another feature of centrioles:

Possible to induce production of them in Cerebratulus. — a ~~sea~~ phosphorescent marine annelid —

sperm

Sperm + egg each furnish one ~~centr~~ centriole —

if taken in unfert. state, chemical treatment (hypertonic sea water).

produces asters (cytasters) all over cytoplasm — up to 20 in one egg, and actually function — have no centriole.

cytasters

Sect. 4/17/51

Amphiastral mitosis - cell w/ astral rays

Flat metaphase plate.

Chromosomes at anaphase funnel toward aster.

Anastral - only spindle + chromosomes

only centromere region reaches metaphase plate.

No funneling to a discrete point at anaphase.

No obvious asters, but a region.

Nuclear membrane formed from vesicles at or near telophase.

These vesicles formed from the matrix of the chromosomes.

Eg - chromosome forms a vesicle, these increase in size, finally fuse to form the nucleus.)

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Duration of mitosis is extremely variable, from a few minutes to years in arrested processes.

Theories of mechanisms of mitosis.

Most theories over-simplified.

1. Movement of chromosomes –
a. Anaphase movement – a universal process.
Hypothesis – a fiber pulling the chromosome (a contraction of spindle fiber). As the fiber shortens, should thicken, but no observed facts for this.
How did chromos. get to center? This alone doesn't expl.

Boveri found astral ray attachment to nuclear membrane helped pull away chromos. at anaphase.
The pushing hypothesis – dependent on assumption of (a ~~[illegible]~~ connecting fibers for migrating chromosomes – again no facts.

Two factors in movement of chrom from

1. fiber shortening
2. actual movement of poles or spindle elongation.

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Autonomous movement of chromosomes-

1. Experimental work.
   a. Colchicine treatment - destroys spindle, but no harm to c-some. But c-some does not come to plate at metaphase, continues to shorten due to super-coiling of chromonema. Chromatids divide & move apart, - not an anaphase movement, & thought by some to show the autonomous
   b. Colchicine fragments chromosome - the fragments both come to plate, w/ a splitting, but no normal movement to poles.
2. Natural selective X some movement.
   1. Sciara - notes - 2n = 7

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Lect. 4/19/51 - Zoo 426.

IX Nuclear reproduction - chromosomes
- A. Individuality
- B. Homologous chromosomes
- C. Structure
- D. Nucleolar forming chromosomes
- E. Heterochromatin vs. euchromatin.

Individuality
1. Each chromosome (in any organism) has its own individuality - w/ indiv. characteristics, in all similar organisms.
2. Ind. recognized by
   - a. Constant size + shape.
     Makes possible construction of ideogram (showing only 1 member of pair)

D. melanogaster
Polar view

   - b. Position of centromeres.
   - c. Type of banding
   - d. Behavior pattern in mitosis.
   - e. Observ. of chromosome which form the nucleolus.
   - f. Always occur in pairs - homologous.

Structure - primarily based on work of Kuwada

centromere
enclosing matrix
coiled chromonema

Origin of matrix
1. Synth. by chromonema - ignores relat. between chr. matrix + nucleolus

2. Nucleolus loses size uniformly as matrix formed + vice versa
[illegible]

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Matrix largely DNA - Chromonema largely RNA.

B. McClintock - experiments shows clearly in nucleolar organising chromosomes of corn (chr. #6).

knob

nucleolar organiz. region

centromere

Pollen mother cell.

Nucleolus clearly from region known as nucleolar org. reg.

X-rayed chromosomes, break thru nucleolar org. reg.

giving a portion of n. org. reg. which produces much smaller nucleolus, which is disorganized

In another break, removed complete n. org. region, leaving heterochromatic region gone

nucleolar material completely scattered in nucleus, no organization.

Where does nucleolar material come from - arise directly from surface of chromosome - directly from matrix.

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Lab on onion root tip rapid differential staining.

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Apr. 24. Zoo 426

I Nuclear reprod. - Endopolyploidy & related phenomena.
A. Endopolyploidy -
B. Endomitosis
C. Mitotic blockage & experimental polyploidy.
D. Polyteny
E. Amitosis
F. Gen'l review of nuclear growth.

Heterochromatin - a chromosome "out of phase" so far as staining capabilities of other parts during mitosis is concerned.

chromocenter

centr.

heterochromatin - lighter staining at metaphase, heavily stained at ~~meta~~ resting stage.

Euchromatin takes stain at metaphase.

Heterochr. has no major gene loci.

Sometimes entire chromosome is heterochromatin as in Corn - being an additional chromos. to a normal 2n no. - called "B" chromosomes.
Chromosome "knobs" are heterochromatin.
Randolph in corn added up to 34 heter. chr. somes to 2n no. w/o disturbing the process.
Up to now little evidence as to use of heterochromat.

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Endopolyploidy -

Haploid-diploid cycle -

Normal diploid 2n in somatic.
In gonads at synapsis ⇒ Haploid

Not always the somatic cells have the diploid no, but frequently these "doubled up" - polyploid cell,

$n = 5$
$2n = 10$
$4n = 20$

Evidence that polyploidy is norm. shown by Jacobi, 1925 - in liver, different size nuclei, the distribution of which is discontinuous.

Observed that the larger ch-somes had more nuclear material

Variation of nuclear size was discontinuous, w/relatively few nuclei between the 4 peaks.

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In development of larvae → insect

Many mitoses, w/ very small cells, but final increase in size (enormous) not with mitosis.

○→○→○→○→○→○→◯

Berger, studying mid-gut of mosquito found $2n = 6$ in cells where mitosis was active, but in mid-gut of adult, these mitotic process showed 24, 48, 96.

Various types of Endopolyploidy comp. w/ mitosis: hypothetical 2n chrom. # of 6.

1. polysomaty (mosquito midgut)

zygote

Embryo

young larva

old larva

48-96 ch.

2. "endomitosis" (water spider)

12

24

48

96

1. Multiple chromosomes of old larva comes from polytene synapsis in somatic cells of the dipters.
2. Doubling due to situa. looking like mitotic prophase, but nuclear membrane doesn't break + no spindle development, but ea. of 6 chrom. splits long. → 12 chromosomes. + w/ continued cyclic doubling – in 1. chromosomes don't split apart until final (polyploidy) step, but in 2. doubled chromosomes [illegible] visibly

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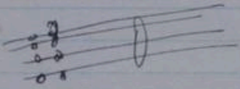
3. Polyteny (Drosophila salivary gland)

?

6 giant c-somes

Not known how c-somes become giant in size. Apparently a laying down of ~~number~~ numerous polytene strands, and each time chromos. doubles in size, length doubles, + each strand doubles itself. Each chromocenter also doubles so that as c-some doubles itself, these chromocenters appear next to each other to give the picture of the bands on the giant c-somes of Drosoph.



4 Ways of determining polyploidy.

1. Counting metaphase c-somes.
2. " chromocenters when there is a small enough no. to be sure that all can be seen.
3. Count nucleoli (normally 2 in ea. diploid cell, + should be 4 in tetraploid).
4. Rhythmical (rythmical) ~~s~~size doubling.

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Experimental induction of polyploidy.
1. By colchicine (C-mitosis).
Colchicine selectively wipes out spindle, so that c-somes don't go to plate, but coil up in situ. after centromere divides, the c-somes split apart, the new cell walls are formed + voila! double.

Sect. 5/1/51

XI The cytology of reproduction
1. Meiosis + chromosome cycles.
A. Asexual vs. sexual reproduction
B. Meiosis + syngamy.
C. Chromosome cycles ~~showing~~ involving:
1. gametic meiosis
2. sporic "
3. zygotic "

Amitosis – a pinching in ½ w/o form. of chromosomes of the nucleus.
In most cases, cells never seen to divide again.
Freq. products un= in size.
No evidence that these nucl. participate in inheritance.
A much rarer phenomenon than previously thought.
True amitosis occurs in:
1. very specialized tissues w/o perpetuation.
2. in pathological conditions
3. in degenerating tissues.
4. In tissue culture cells, large quant. of amitosis – no future to these cells.
5. Macronucleus of many ciliates.

but w/ mitotic division in micronucleus.

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Original derivation of macronucl. is from micronucl.

Another instance of amit. in specialized tissue is in yolk cells of embryos in insects.

Amitosis is most important as a physiological process, not imp. to nuclear reproduc.

XI The cytology of reproduction.

Asexual reprod is simplest method.
Resulting offspring identical w/ parent -
Provides no method of hereditary variation, (except thru mutation or other alteration of the c-somes.
The products of strict asex. reprod. called a clone. In a biological sense, the same individual.
This process is extremely rare as the only means of reproduction. Potentiality to change is done away with.
Evolution based on descent with change.
Sexual reproduction essential to change. Ex= evolution of horse.
Sexual reprod. produces immense amt of variation, but maintaining at same time a stable product.
Processes of meiosis + syngamy ~~gent~~ are the ones responsible. (fusion)
Syngamy is larger term for fertilization to include all kinds (same sized reprod. gametes + diff. size gametes). — Anisogametes + isogametes.
Meiosis is cytol. proc. involv. c-somes occurring only in reproductive organs. in the germ cells.
Consists of
1. Proc. where certain cells begin complicated prophase, very diff, (but w/ similarities) to mitosis - this process lasts for years, followed by 2 rapid mitotic divisions

meiotic divis M_1
meiotic " M_2

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Result = gamete capable of fusion w/another individual (syngamy).
Meiosis initiated in diploid cells 2n + reduced to 1n number. Real significance is in prophase process. Reduction in no. not haphazard. Cells (2n) have paired nuclei at random in nucleus, and reduction produces 4 gametes, each w/ 1 of each pair of 2n c-somes in it.

$2n = 8, 1n = 4.$

Position of meiosis in lifecycle -

XII The cytology of reproduction.
II Meiosis in detail

A. The meiotic prophase
1. leptotene
2. zygotene
3. pachytene
4. diplotene
5. diakinesis
6. Metaphase MI

B. Diakinesis + the significance of chiasmata
1. The two-plane theory
2. " one-plane "
3. Chiasma terminalization

C. The meiotic mitoses (MI, MII)

D. Significance of meiosis.

1 — idiosome; nucleus; cell mem. — Telophase, last oogonial div.

2 — homologous c-some — leptotene

3 — b, b', a, a' — late leptotene — ⁘ = chromomeres

4 — c, c', b, b', d, d', a, a' — Zygotene, begin.

5 — late zygotene

6 — pachytene

7 — Diplotene

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Fundamentals of meiosis.
1. Primitive germ cells - div. by mitosis (normal) - referred to as
gonia ♀ oogonia
♂ spermagonia

After meiosis initiated called
♀ oocyte or microsporocyte
♂ spermatocyte or megasporocyte.

2. Main phases
1 long prophase
2 rapid metaphases.

3. Processes or stages vary in occurrence - omitted, telescoped or delayed -

C-some in "1" seems to have terminal centromere which thru all stages remains attached to nuclear membrane

appearance of all chromosomes at early stage (

In leptotene, the c-some lengthen considerably, bending back on themselves.
Not able to see split nature of c-some at leptotene - appears single.

zygotene - c-somes begin to pair (synapsis) chromomere for chromomere. - homologous pairing. Highly specific attraction, a force working over quite a distance.
late zygotene - synapsis complete c-somes
As soon as zygotene finished, polarity lost, + c-somes pull away from idiosome.

Pachytene - threads thickened + ch-somes lie close together.

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at Diplotene - c-somes appear definitely as 2 chromonemata so-called Bivalents or tetrad.

Diakinesis - 4 strands fall appart giving a cross-configuration
apart movement

1 2 3 4

Chiasma.

referred to as a chiasma (a fig. in tetrad) where members of pair trade partners.

chromatids

"sisters"

homologous chromatid.

Geneticists worked out details of crossing over
Normally expect only 2 types gametes from following

A | a → A B | a b but frequently find
B | b

A | a
b | B

crossing over

Two plane theory - synapsis on 2 planes

So nothing to do w/ chiasmata.

~~One plane theory - requires strands breaking & rejoining of sister~~

One plane theory -

One sister ½ of one ½ of other at time tetrad opens out.

and so ~~to~~ break somewhere

in leptotene or zygotene.

The chiasmata appears because pairing already occured.

so crossing over occurs earlier than at diakinesis.

∴ they say that no. of chiasma indicate ~~no. of~~

amt. of crossing over.

Chiasma somewhat proportional to genetic crossing over.

Evidence from interlocking c-somes that splitting

only on synaptic plane + not on the sister plane -

so, only one plane -.

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Chiasma may slip towards end of c-somes "terminalization"

Reductional + equational division

Homologous centromeres separated at 1st div. - so reductional, but because of frequency of crossing over, practically never find reductional or ~~either~~ equational divisions occurring exclusively.

5/8/51 -

E. The significance of meiosis.

1. Factors producing hereditary heterogeneity among offspring
   a. crossing over
   b. random segregation of chromatids.
   c. Chance recombination at syngamy.
2. Evolutionary aspects

Homologous c-somes from ♀ + ♂ parents, because of the large no. of gene loci may have ~~genes~~ genes which are slightly different tho homologous, which direct same function.

New combinations + genetic variability caused by

1. crossingover.
2. Random segregation of chromatids at metaphase of M I. depends on way chromatids lie at plate as 2 whether 2 or 4 different combinations in the gametes. 2^n ~~~~ " depending on no. of tetrads which take part. For ex. in human, w/ 48 c-somes, no of diff. combinations due to segreg of chromatids, ~~~~ = 2^{24} = 16,777,216.

So, meiosis essentially scrambles the genetic material of an

3. Chance recombination at syngamy doubles the factor of #2 above.

Meiosis doesn't ~~~~ create differences, but merely taking differences already present + reorganizing into new recombinations

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Biological significance - struct. on which evolution is built. Allows selective advantage.

XIV Ch-some changes + Evolution.
1. Change in c-some no.
A. Gen. aspects of c-some variability
B. Polyploidy
C. Aneuploidy
D. Breakage & fusion at the centromere.

Evolution of: species divergence is crux of modern study.

As groups diverge, certain c-some differences arise + any permanent change is accompanied by perm. diff. in c-some.

What are the kinds of changes + importance + consequence to organisms.
Broadly 2 kinds: A Invisible under micro-ex mutations.
B Visible " "

Mutations not at all visible - a genetic change w/ no vis. effect on c-some - level of molecules.

Visible changes in 2 categories.
1. "Breakage" - some broken part to linear organization of c-some, causing change in arrangement of genetic material. Possible that such changes perpetuated.

c-some aberrations:
a. Inversions (180°)
b. Translocations
c. deletions
d. duplication

2. Changes in no. - involve whole c-somes w/o breakage. Simplest one is doubling of no. to give tetraploid (4n).

In Triticum, 3 groups

x = basic no.

1	2	3
n = 7 (2n = 14) diploid (2x)	n = 14 (28) tetrapl.	n = 21 (42) hexaploid.

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[illegible] - [illegible] doubling of basic no.

Aneuploidy - nos. not exactly = or double the basic no.
2n-1 or more commonly 2n+1, 2n+2.

Polyploidy more general in plants than in animals - reason is that doubling of c-somes w/ sex determining c-somes (XX XY) when polypl. the XXXX XXYY disturbs the sex determining mechanism. balance.
Most frequent in places w/ high % of parthenogenesis as in cert. insects, crustacea
Not a significant phenomenon in evolution as far as animals.
Fankhouser (Princeton) on salamanders is most significant ploidy work in animals -
A small % of animals will be polyploid, but do not establish polyploid race -

Origin of polyploidy:
1. Failure of formation of cell wall between mitoses. (somatic doubling)
2. Failure of meiosis to go from 2n to n - occasional 2n gametes due to some sort of somatic doubling previous to production of gametes.
3. Fusion of 2 nuclei ?? not proven.

In plants
autopolyploidy

$2n^a$ → n^a + n^a → $2n^a$ → $4n^a$

easy to establ. by colchicine

allopolyploidy

Species A $2n^a$ → n^a
Species B $2n^B$ → n^B
$n^a + n^B$
colchicine →
$2n^A$ $2n^B$

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Autoploids high° of sterility because of 4 homologs must pair - + no of gametes may be unbalanced.

(2n) (n) → (3n)

A A' A''

A A' A''

at synapsis 2 may pair but one does not

may have a trivalent A A' A''

Allopolyploids more fertile the farther apart their parents. 2 closely related species not so fertile due to competition among c-somes.

May 22, 1951.

Aberrant c-some nos. evolved by fusion of sep. c-somes at the centromere - evidence from Dros. virilis group in which the ancestral form is D. neomexicana w/ six sep. c-somes + gives rise to 2 other spp. D. americana + D. texana, each w/ 4 c-somes, the arms of which may be compared w/ the sep. c-somes of the ~~ancestral~~ primitive type.

C-some breakage effected by N. mustard gas, x-ray, peroxide treatment.

Certain regions of c-some susceptible to breakage.

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Inversion involves 2 breaks in c-some arm, the pieces rotated 180° + rearrangement of genes on the arm.

ABC | DE | FG

ABCED FG

Inversion may produce an acentric c-some (this known only after X-radiation) one w/ 0 centromere

F E B A
C D
or
F E D A
C D

Pericentric inversion around the centromere
Paracentric " one side of " .

F A
B E
C D

F A acentric fragment
B
centric ring → C E
c-some
D

Paracentric inversion difficult to detect unless able to make detailed banding pattern study.

Cross over in meiosis - if one long c-some w/ 2 centromeres, one at each end, plus a fragment occurs at anaphase.

possible to infer a cross over within the inversion.

Translocation - arises from same event as inversion -

Homologous pairs

translocation of non-homologous c-somes -

dicentric

acentric

These inversions cause genes to stick together causing a stability in hereditary structures, rather than providing raw material for new combinations. Evolutionary activities may proceed apace w/o the use of inversions.

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