Hunt Institute for Botanical Documentation
5th Floor, Hunt Library
Carnegie Mellon University
4909 Frew Street
Pittsburgh, PA 15213-3890
Telephone: 412-268-2434
Email: huntinst@andrew.cmu.edu
Web site: www.huntbotanical.org

The Hunt Institute is committed to making its collections accessible for research. We are pleased to offer this digitized item.

Usage guidelines
We have provided this low-resolution, digitized version for research purposes. To inquire about publishing any images from this item, please contact the Institute.

Statement on harmful and offensive content
The Hunt Institute Archives contains hundreds of thousands of pages of historical content, writing and images, created by thousands of individuals connected to the botanical sciences. Due to the wide range of time and social context in which these materials were created, some of the collections contain material that reflect outdated, biased, offensive and possibly violent views, opinions and actions. The Hunt Institute for Botanical Documentation does not endorse the views expressed in these materials, which are inconsistent with our dedication to creating an inclusive, accessible and anti-discriminatory research environment. Archival records are historical documents, and the Hunt Institute keeps such records unaltered to maintain their integrity and to foster accountability for the actions and views of the collections' creators.

Many of the historical collections in the Hunt Institute Archives contain personal correspondence, notes, recollections and opinions, which may contain language, ideas or stereotypes that are offensive or harmful to others. These collections are maintained as records of the individuals involved and do not reflect the views or values of the Hunt Institute for Botanical Documentation or those of Carnegie Mellon University.

About the Institute
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.

Hunt Institute was dedicated in 1961 as the Rachel McMasters Miller Hunt Botanical Library, an international center for bibliographical research and service in the interests of botany and horticulture, as well as a center for the study of all aspects of the history of the plant sciences. By 1971 the Library's activities had so diversified that the name was changed to Hunt Institute for Botanical Documentation. Growth in collections and research projects led to the establishment of four programmatic departments: Archives, Art, Bibliography and the Library.

B381SJ

1

Introduction - smear techniques

(1) clean slides + covers

① Jars of sulphuric acid - potassium bichromate
② put in as many slides as will hold + about 20 covers
③ after meal J + B. have to stay + finish lab work begun Thursday - you will wash each slide bef. in running water + then store slides in alcohol. ~~to be used~~ as you need slides take from alc, rinse in water + wipe dry with a cloth

Monday

(2) Toxler - dissection

(3) " - lab

(4) Interesting again connected class in microtech - 20's large classes - during depression - cut down on courses said to be ~~[illegible]~~ less essential (absolutely? unessential). Long time needed + glad again set.

(5) As cyt. watched + was concerned with techniques of preparing pl. tissue for exam. under the micros. for almost 40 yrs. Followed each improvement or mod. of standard techniques + tried them out in relation to the mat. involved on my own special problems. Originally concerned with both "live" + "cyt" techniques - for last 25 yrs concerned exclusively with "cyt" ones + part. those which bear dem. on structure + behavior.

(6) Of recent years been real help of skilled technicians - technique will des. + you will try to refine full time attention - did not have full time to give. For some yrs Dr. Bradley has been studying the most mod. cyt. techniques + applying them to cytogen problems in which both interested. You are fortunate to have Dr. B's help in the lab work dealing with smear techniques because she has as intimate knowledge of them as anyone in the country.

(7) when I began cyt. xs. the par. method was the only techn. available for study of nuclear phenomena. The sources of error in preparing microbrutalis & reproductive tissue for micros. were ~~too~~ serious + a long series of modifications ~~in the process of~~ each step in process up to final mounting under cover glass were suggested. Equip. was far less effective than at present - rocking microtome - minimum pace

(8) All our background picture of ~~the~~ mit. & meiosis has been constructed ~~by~~ from study of prep. made by par. method. And up to recently knowledge of Structure ch. # & behavior was similarly based upon what was seen in "par. sections"

(9) ~~To~~ Today 95% of all modern cyt. involves use of an entirely different techn. - by mod. cyt. I mean ch. cyt. or cyt gen which occupies the attention of the vast maj. of those who today call themselves cytologists.

(10) This mod. technique is called the smear method or the smear squash technique. With its present day refinements more adv. in fund. know. of ch. structure & behavior has been in 10 years than was made in the 30-40 years when par. method alone was available to the cyt. Indeed, so many errors of interpret. were produced by ~~par~~ evidence from "par section" that today an article based upon evidence from such sections is autom. viewed with suspicion.

(11) Smear technique as designation implies involves the spreading of the tissue on the slide or cover glass + carrying on all steps in killing + fixing, dehydrating, clearing, staining + mounting by trans. slide or cover thru succ. solutions. Obv. smears can ~~be made but~~ most readily be made of tissues in which the cells are free from each other - microsporocytes higher plants & cells of testes in animals

(12) By pretreatment of any tissue not too hard - meristems for ex - with fragments dis. mid. lam. very effective smears can be made + carried through sub. operations. In future this mod. of human tech. (Gram. Smear tiss. based squash tested. Prob. gradually intruded + worn par. to hard tissues)

ref. to John. (Sum entires inadequate & will appear in monograph)

~~[illegible]~~ imp. & adv. re sm. techniques share by biblio — ? any on particular

so as to hist. sketch ~~& biblio~~

Smear and Squash Methods

Use of these methods -

For cytological work sections have now been largely replaced by smears and squashes. One of the advantages is the rapidity of handling without embedding. Another advantage is the rapidity of fixation, since smears are fixed instantaneously, and squashes are fixed by rapid-acting acetic-alcohol combinations.

or animal

Any plant/tissue which is undergoing cell division, such as leaves, petals, tendrils, or glumes, can be fixed and treated as squashes for chromosome study. By adapting fixatives and macerating methods for cytological studies other than thoseconcerned with chromosomes, the squash methods can also be used.

Smearing consists of the direct spreading of the cells in a semi-fluid tissue, such as PMC. over the surface of a slide, followed by the immersion of the slide in fixative, or it consists of spreading the cells in a drop of fluid which fixes and stains at the same time.

The squash method is used when the tissue to be studies is composed of cells held together by middle lamellae. After the material has been fixed, it is treated in such a way that the middle lamellae break down, allowing the cells to separated and be come dispersed in a single layer.

Historical -

Smears of bacteria and of blood have been known since 1877. In that year Salmonsen added a drop of stain to a drop of putrescent blood on a slide, and noticed that not only did the bacteria stain, but that the different types of blood cells stained differentially. Koch, in that same year, smeared bacterial suspensions on cover glasses to fix the bacteria in 1 plane, and then stained the preparation.

Schneider in 1880 was the first to use aceto-carmine, the fixative-stain used most commonly in smears and squashes today. With this stain he studied cell divisions in germinating eggs. Belling in 1921 adapted this stain-fixative for chromosome studies in plants. He improved upon this early technique and in 1926 published what has become the standard iron-aceto-carmine method used in the study of PMC. It is now equally widely used for animal material. Improvements to the method were later published by the following: McClintock, who in 1929 gave a method for reducing the staining of the cytoplasm and of making smears permanent; Bridges, in 1935 introduced a briefer method for making smears permanent, which is suitable for PMC of some plants, but not for others; Nebel in 1940 found that compound staining using chlorazol black E combined with carmine is better for differentiating small chromosomes than carmine alone; and Zirkle in 1940 combined aceto-carmine with water- or fat-soluble mounting media, thus giving a method for smearing and permanently mounting the material in one step.

The Feulgen technique, which is actually a microchemical test for thymonucleic acid (found only in the chromosomes) was first developed by Feulgen and Rosenbeck in 1924. It depends on Schiff's aldehyde reaction, mild hydrolysis liberating the aldehyde groups of the nucleic acid so that they can enter into a chemical reaction with leuco-basic fuchsin, thus giving a violet color to the chromosomes. Improvements to the Feulgen technique have been suggested by Bauer 1932 in reference to length of hydrolysis after different fixatives, by Coleman in 1938 in obtaining decolorized fuchsin, Semmens and Bhaduri 1939 gave a method for counterstaining nucleoli after staining chromosomes by the Feulgen method, and Hillary 1940 simpli-

fied this counterstaining ~~of~~ method.

Other techniques used in staining smear and squash preparations are: the crystal violet method of Newton 1927, with modifications given by Johansen 1932, La Cour 1937 and Stockwell ? ; the brazilin method of Belling 1928 and Capinpin 1930; the aceto-orcein method recommended by La Cour 1941; and the aceto-lacmoid (resorcin blue) technique of La Cour 1942.

SMEAR TECHNIQUES

Oct. 1st. Lecture on smear techniques
Fix PMC - 1) Nicotiana, 2) ?
Fix root tips - Vicia faba
Fix leaves
Smear fixed material of Agapanthus PMC and leave slides in alcohol vapor jars, to be madepermanent at next lab period

Oct. 3rd. Mount Agapanthus PMC in euparal
Smear PMC fixed on Oct. 1st.
Make slides permanent by longer method than alcohol-vapor method
Make tempor ry mounts
Smear pollen-divisions if time allows

Oct. 8th. Smear fresh PMC - temporary mounts
Smear pollen divisions, ifnot done previously
Smear root tips and make permanent by either or both methods

Oct. 10th. Root tip smears - temporary or permanent
Leaf smears - temporary or permanent

BOTANY 155

Required material
to be purchased by student

1 box microslides
1/2 oz. no. 7020 square cover glasses (22 mm #1)
1/2 oz. no. 7022 circular cover glasses (22 mm #1)
1/2 oz. 24 x 60 cover glasses (#1)
1 pkg. lens paper
1 cloth
1 dissecting set
1 slide box (100 slides)
2 camels hair brushes
1 box slide labels
1 notebook cover
ruled paper

Required text

J E Sass. Elements of botanical microtechnique

"Simpler" techniques

--oooOooo--

Microtec. - Example special techniques

Tissue culture - " " "

Smear - study without sectioning (referred only to simplicity & time saving, not to improved results)
referred to - Aceto carmine

(pollen grain divs - not only convenient (= see a lot of material)
but whole cells, etc.

(assigned - Ch. V Sass)
Can watch killing, fixing, staining & have confidence that
little or no distortion has occurred.

Materials needed for smear techniques

Smearing implements - tooth-brush handles ground down, or spear-point needles
Dissecting needles
Grease pencil
Forceps
Cloth - not linty (old silk, rayon or linen)
Slides and cover glasses

General equipment

Vials - 4 or 5 per person
Coplin jars - 6 for each table
Alcohol burners - 1 for each table
Dropper bottles for aceto-carmine - 1 per table
Balsam jars for Euparal - 1 per table
Sealing medium - 1 container per table
Sealing tools - 1 per table
Alcohol-vapor jars - wide-mouthed, covered jars lined with paper towel saturated with alcohol - 1 per table
Oblong dishes, each with 2 pieces of glass rod the length of the shorter inner diameter of the dish - 1 dish per table
Paper towels

Reagents

100 % ethyl alcohol
Glacial acetic acid
50% acetic acid (in dropper bottles) - 2 or 3 for the lab
10% acetic acid
Chloroform
Euparal (green)
50% hydrochloric acid
Distilled water

fold in
BV 153

BOTANY 155

Laboratory Schedule

September 24 and 26

1. Organization of course
2. Killing and fixing material for paraffin method

October 1 and 3

1. Introduction to aceto-carmine smear technique
2. Practice with anthers of Nicotiana

October 8 and 10

1. Continue practice with anthers and pollen-grain divisions
2. Root tips of Vicia; leaf primordia of Tradescantia or Nicotiana

October 15 and 17

1. Free-hand sections and staining
2. " " " " "

October 22 and 24

1. Maceration techniques
2. " "

October 29 and 31

1. Demonstration and practice in casting paraffin molds
2. Practice in casting paraffin molds. Demonstration of use of rotary microtome (incl. mounting of ribbons).

→ DEHYDRATION, CLEARING, and INFILTRATION of fixed materials

November 5 and 7

1. Clearing techniques
2. Practice in serial sectioning of imbedded material.

Remaining 16 Periods

1. Preparation and staining of various serial paraffin sections
2. Further practice in maceration and clearing techniques.

NOTE: Slide collection to be turned in the week of January 20.

****