Pharmacy Exam Guide

Step II

Practical Book

-1st Edition

(P2C5)

PHARMACY EXAM GUIDE

STEP II PRACTICAL BOOK 1ST EDITION (P2C5)

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The authors, editors, and publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accordance with the current recommendations and practice at the time of publication.

DEDICATED TO OUR PARENTS

AND TEACHERS

ACKNOWLEDGEMENT

The future belongs to those who believe in the beauty of their dreams.

The preparation of this book "**Pharmacy Exam Guide**" was just a dream of some students of **Doctor of Pharmacy, University of Central Punjab**, which could not be fulfill without the help and support of our teachers and parents.

We appreciate the tireless efforts of ***Our Teachers*** who encouraged us always to achieve our endeavor, no matter, how hard they can be.

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PHARMACEUTICS

PREPARATION OF SIMPLE SYRUP (B.P AND U.S.P)

THEORY

Syrups are concentrated aqueous preparation of sugar or sugar substitute with or without adding flavoring agent and medicinal substances.

Components of a syrup:

- Sugar usually sucrose or sugar substitute
- Antimicrobial preservative
- Flavorants
- Colourants
- Solubilizing agents
- purified water

METHODS

- Solution with heat or hot method
- Agitation without heating
- Percolation

Simple syrup

When purified water alone is used in making the solution of sucrose, the preparation is known as simple syrup.

Quantities

According to B.P, it is 66.7%(w/w)

According to U.S.P, it is 85%(w/v)

APPARATUS

- Stirrer
- Beaker
- Weighing balance
- Measuring cylinder

CHEMICALS

- Sugar
- water

PROCEDURE

- Wash, clean and dry all the glassware
- Weigh sugar and dissolve per 100g of purified water by continuous stirring
- Heat the mixture on water bath slowly to dissolve sugar completely
- Cool the syrup and determine its specific gravity by pycnometer

PHARMACEUTICAL APPLICATIONS

- Vehicle for other drug substances

- Flavoring agent
- Sweetening agent
- Demulcent
- Masking agent for nauseous drugs

Simple Syrup B.P.

25ml

Composition: Sucrose 66.67% w/w

Uses: It is used as sweetening agent and pharmaceutical vehicle.

Store in a well closed container at cool and dry place.

Keep out of the reach of children.

Mfg. Date: 1/10/2013 Exp. Date: 10/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Simple Syrup U.S.P.

25ml

Composition: Sucrose 85% w/v

Uses: It is used as sweetening agent and pharmaceutical vehicle.

Store in a well closed container at cool and dry place.

Keep out of the reach of children.

Mfg. Date: 1/10/2013 Exp. Date: 10/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Camphor Water

Theory

These are clean, saturated aqueous solutions containing volatile oils or other aromatic or volatile substances. These are also known as “medicated waters”.

Taste and odour of aromatic waters are similar to the drugs or the volatile substances from which they are prepared.

Aromatic waters should be free from smoky conditions and should be free from unpleasant odours.

All substances used in the preparation of the aromatic water should be of pharmacopeia quality.

Methods of preparation

- Solution method
- Distillation method

Camphor water

Camphor is a ketone obtained from the plant *cinnamomum camphora*. It occurs as white, crystalline solid and is highly volatile in nature. It readily burns with smoky flames. It is soluble in 800 parts of water and in 1 part of ethanol. So, for the preparation, ethanol is used as distributing agent.

APPARATUS

- Beaker
- Burner
- Measuring cylinder
- Weighing balance

INGREDIENTS

For 1000 ml camphor water

- Camphor. 1g
- 90% Ethanol 2ml
- Distilled water . . . . . q.s to make 1000ml

PROCEDURE

- Wash, clean and dry all the glassware
- Calibrate the weighing balance
- Weigh camphor and dissolve in ethanol
- Add alcoholic solution drop wise in distilled water
- Shake well until camphor is completely dissolved
- Preserve in a closed container
- Label it properly

USES

- Flavoring agent
- Carminative
- Anesthetic
- Pharmaceutical aid
- Upper respiratory tract treatments
- Vehicle for preparations

STORAGE

Preserve in a closed container to protect from heat and light

PRECAUTIONS

- All the glassware should be clean
- Weighing should be proper
- Order of reaction should be followed

- Add alcoholic solution drop wise to ensure completion of reaction
- Preserve in a cold container

Camphor Water B.P.

100ml

Composition: Camphor 0.1 % w/v

Uses: It is used as carminative and pharmaceutical vehicle.

Store in a well closed container at cool and dry place.

Keep out of the reach of children.

Mfg. Date: 8/10/2013 Exp. Date: 18/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Double Strength Chloroform Water

Theory

These are clean, saturated aqueous solutions containing volatile oils or other aromatic or volatile substances. These are also known as “medicated waters”.

Taste and odour of aromatic waters are similar to the drugs or the volatile substances from which they are prepared.

Aromatic waters should be free from smoky conditions and should be free from unpleasant odours.

All substances used in the preparation of the aromatic water should be of pharmacopeia quality.

Chloroform water

Chloroform acts as an anesthetic when inhaled through mouth. It has a pleasant taste and causes sedation. It is used in the preparation of single strength chloroform water, double strength chloroform water.

Ingredients (B.P)

- Conc.chloroform 5ml
- Purified water freshly boiled and cooled..... 1000ml
- Conc.chloroform water..... 2.5 chloroform+100ml water

Procedure

- Wash, clean and dry all the glassware
- Dissolve the concentrated chloroform water by mixing and then make up the volume that is required with continuous stirring
- Shake well
- Preserve in a container
- Check that no chloroform remains un-dissolved at the bottom of the container

Precautions

- All the glassware should be clean
- No chloroform should remain un-dissolved

Uses

- Sweetening agent
- Carminative
- preservativ

Result

Double strength chloroform water is prepared

Chloroform Water B.P.

25 ml

Composition: Chloroform 2.5% v/v

Uses: It is used as sweetening agent, carminative and preservative.

Store in a well closed container at cool and dry place.

Keep out of the reach of children.

Mfg. Date: 22/10/2013 Exp. Date: 30/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Double Strength Chloroform Water B.P.

55 ml

Composition: Conc. Chloroform Water 0.5% v/v

Uses: It is used as sweetening agent, carminative and preservative.

Store in a well closed container at cool and dry place.

Keep out of the reach of children.

Mfg. Date: 22/10/2013 Exp. Date: 30/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Iodine Tincture

Theory

Tinctures are alcoholic or hydro-alcoholic solutions of medicinally active ingredients obtained from vegetable materials or from chemical substances.

Tinctures can prepare by following process:

- Maceration
- Percolation
- Simple solution

Ingredients

- Iodine 25g
- Potassium iodide 25g
- Water 25ml
- 90% Ethanol. . . . . q.s to make 1000ml

Procedure

- Wash, clean and dry all the glassware
- Dissolve potassium iodide in distilled water by continuous stirring and adding small amount each time
- Add iodine and shake it well
- Then make the volume up to 1000ml with 90% ethanol
- Shake well and then store in a closed wall container
- Label it properly

Indications

- Act as anti-septic and disinfectant for minor cuts, wounds and abrasions
- To sanitize surface water fro drinking

Dose

- Topically as prescribed by the physician

Direction for use

- Apply to the affected part with cotton wool or a small brush

Auxiliary labels

- For external use only
- Avoid prolonged use
- Do not cover
- Do not use on open wounds
- Protect from light
- Keep out of reach of children

Storage

- Store in a closed container to protect from heat and light

Precautions

- All the glassware should be clean
- Handle iodine carefully as it had staining property

- Dissolve potassium iodide by adding small amount each time
- Add iodine pinch by pinch to dissolve completely

ROLE OF INGREDIENTS

- Iodine----------anti-septic and disinfectant
- Potassium iodide----------to enhance solubility of iodine
- Distilled water----------as drug carrier
- 90% Ethanol----------as an anti-septic and to make volume up to the mark

Iodine Tincture B.P.

25ml

Composition: Iodine 2.5% w/v
Potassium iodide 2.5% w/v

Uses: It is used as an antiseptic for minor cuts

Dose: Tropically as prescribed by the physician. Avoid prolonged use.

Directions: Apply to the affected part with cotton wool or a small brush

Store in a well closed container at cool and dry place.

For External Use Only
Do Not Use On Open Wounds
Keep out of the reach of children.

Mfg. Date: 29/10/2013 Exp. Date: 09/11/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

PREPARATION OF CONCENTRATED PEPPERMINT EMULSION (B.P)

THEORY

Concentrated peppermint emulsion is a 2% v/v dispersion of peppermint oil in a suitable vehicle containing a non-ionic surface active agent.

An emulsion is a biphasic liquid dosage form of medicaments in which two immiscible liquids are made miscible by the addition of emulsifying agent.

Emulsions are widely used in pharmacy and medicines. They are used internally as well as externally.

Types of Emulsions

- Oil in water (o/w)
- Water in oil (w/o)

Methods of preparation

- Wet gum method
- Dry gum method
- Bottle method
- Nascent soap method

INGREDIENTS

- Peppermint oil 20ml
- Polysorbate 20. . . . . 1ml
- Double strength chloroform water. 500ml
- Purified water boiled and cooled. 1000ml

PROCEDURE

- Wash, clean and dry all the glassware
- Shake the peppermint oil with polysorbate 20
- Continually add double strength chloroform water, after each addition shake it well
- After sufficient stirring, make up the volume up to 1000ml with water
- Place the emulsion in a suitable container and label it properly

USES

- Flavoring agent
- Used in digestive problems

STORAGE

- Store in a well closed container

AUXILIARY LABEL

- Shake well before use
- For oral use only
- Keep out of reach of children

ROLE OF INGREDIENTS

- Peppermint oil----------as flavoring agent, and active ingredient for treatment of digestive system

- Polysorbate 20----------as an emulsifying agent and stabilizer
- Double strength chloroform water------as preservative and sweetener

Concentrated Peppermint Emulsion B.P.

25ml

Composition: Peppermint oil ... 2%v/v

Uses: It is used as flavoring agent for some other preparation
Use in digestive problems.

Store in a well closed container at cool and dry place.

Shake well before use
For oral use only
Keep out of the reach of children.

Mfg. Date: 1/10/2013 Exp. Date: 10/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

PREPARATION OF CHLORAL HYDRATE ELIXIR (B.P)

AIMS

- To understand the concept of elixir
- To prepare chloral hydrate elixir

THEORY

Elixirs are clear, sweetened hydro alcoholic solutions intended only for oral use and usually flavored to enhance their palatability.

Non-medicated elixirs are employed as vehicles and medicated elixirs are used for therapeutic effect. Eg:phenobarbital elixir and theophylline elixir

Compared with syrups, elixirs are usually less sweet and less viscous because they contain a low-proportion of sugar. However, because of their hydro alcoholic character, elixirs are better able then syrups to maintain both water soluble and alcohol soluble components in solution.

COMPONENTS

- Vehicle
- Colouring agent
- Flavoring agent
- Stabilizer
- Preservatives

INGREDIENTS

- Chloral hydrate. 40g
- Blackurant syrup. 200ml
- Purified water 20ml
- Alcohol. 4ml
- Simple syrup 1000ml

PROCEDURE

- Dissolve chloral hydrate in hydro alcoholic solution
- Gradually add blackcurrant syrup and dissolve it
- Then add simple syrup and dissolve it to make volume up to 1000ml
- Place it in a well closed container and label it properly

INDICATION

- It is used for short term treatment of severe insomnia

STORAGE

- Store in a tight container

DOSE

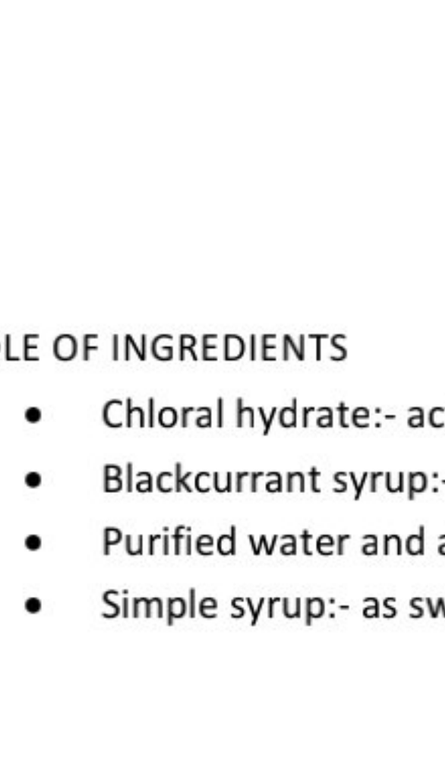
- For children up to 1 yeat = 2.5-5ml
- For adults =5-10ml daily in to two divided doses

AUXILIARY LABELS

- For oral use only
- Keep away from reach of children

ROLE OF INGREDIENTS

- Chloral hydrate:- active ingredient for treatment of insomnia
- Blackcurrant syrup:- for flavoring agent
- Purified water and alcohol:- to prepare hydro alcoholic solution, as preservative
- Simple syrup:- as sweetening agent and vehicle



Chloral Hydrate Elixir B.P.

25ml

Composition: Chloral hydrate... 4%w/v

Uses: Sever Insomnia

Store in a well closed container at cool and dry place.

Dose: For Children up to 1 year = 2.5-5 ml daily
For Adults= 5-10ml daily in two divided dose
OR as prescribed by the doctor

Shake well before use
For oral use only
Keep out of the reach of children.

Mfg. Date: 1/10/2013 Exp. Date: 10/10/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

PREPARATION OF PEPPERMINT SPIRIT (B.P 2012)

THEORY

Spirits are the solutions of volatile oils in alcohol (90%). Spirits differ from tinctures in being alcoholic solutions of volatile substances. Formerly they were all prepared by distillation, but many of them are now simple solutions of volatile oils in alcohol.

INGREDIENTS

- Peppermint oil. 100ml
- Ethanol (90%). . . . . q.s to produce 1000ml

PROCEDURE

- Dissolve peppermint oil in ethanol
- Add sufficient ethanol to produce 1000ml
- If the solution is not clear, shake with previously sterilized purified talc or pulp filter paper

INDICATION

- As carminative agent in digestive problems
- As a flavoring agent

DOSAGE

- 0.3-2ml in diluted form

AUXILIARY LABELS

- Keep out of reach of children
- For oral use only

STORAGE

- It should be stored in a well closed container and at a cool place

Peppermint Spirit B.P.

25ml

Composition: Peppermint oil 10% v/v

Indication: Carminative and Flavoring Agent

Dose: 0.3-2ml in Diluted form; or as prescribed by the physician

Store in a well closed container at cool and dry place.

For Oral Use Only

Keep out of the reach of children.

Mfg. Date: 19/11/2013 Exp. Date: 29/11/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

PREPARATION OF FERROUS SULPHATE SYRUP

THEORY

Syrups are concentrated aqueous preparations of sugar or sugar substitute with or without adding flavoring agent and medicinal substances.

Ferrous sulphate syrup is an iron supplement, which is formulated for children. Iron plays a vital role in production of hemoglobin, which is the oxygen0transporting substance found in red blood cells. if the diet does not contain enough iron, the body cannot make enough red blood cells. this results in iron deficiency anemia, with symptoms such as tiredness, palpitations, shortness of breath, dizziness and fainting.

To treat iron deficiency anemia, iron supplements such as this one are needed. The increased iron intake allows the body to increase its production of red blood cells.

The absorption of iron from the gut is decreased it is taken at the same time as coffee, teas, eggs or milk. Its absorption is enhanced if it is taken at the same time as foods or supplements containing vitamin C (ascorbic acid).

INGREDIENTS

- Ferrous sulphate. 40g
- Citric acid 2.1g
- Peppermint spirit. 2ml
- Sucrose 825g
- Purified water . . . . . q.s to make 1000ml

PROCEDURE

- Dissolve the ferrous sulphate, citric acid, peppermint spirit and 200g of the sucrose in 450 ml of purified water and filter the solution until clear
- Dissolve the remainder of the sucrose in the clear filtrate and add purified water to make 1000ml
- Mix, and filter it necessary through a pledge of cotton

USES

- As an iron supplement

AUXILIARY LABELS

- For oral use only
- Keep out of reach of children

Ferrous Sulphate Syrup B.P.

25ml

Composition: Ferrous Sulphate.... 4% w/v

Uses: Iron Supplement

Dose: As prescribed by the physician

Store in a well closed container at cool and dry place.

For Oral Use Only

Keep out of the reach of children.

Mfg. Date: 26/11/2013 Exp. Date: 06/12/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

PREPARATION OF LUGOL'S SOLUTION

THEORY

Solutions are homogenous mixtures of two or more kinds of different molecules or ionic substances.

Lugols iodine, first made in 1829, is a solution of elemental iodine and potassium iodide, named after the French physician G.A.Lugol.

Lugols solution is available in different potencies of 1%, 2%, or 5% iodine. The 5 % solution consists of 5% (w/v) iodine and 10% (w/v) potassium iodide (KI) mixed in distilled water.

Potassium iodide renders the elementary iodine soluble in water through the formation of tri-iodide ion. It contains no alcohol.

INGREDIENTS

- Iodine. 50g
- Potassium iodide. 100g
- Purified water. . . . . q.s to make 1000ml

PROCEDURE

- Wash, clean and dry all the glassware
- Dissolve potassium iodide in distilled water by continuous stirring and adding small amount each time
- Add iodine in small amount and stir well
- Then make the volume up to 1000ml with distilled water
- Shake well and then preserve in a closed container
- Label it properly

INDICATIONS

- As an anti-septic for wounds
- As a disinfectant for drinking water
- As a mordant when performing grams staining
- As an indicator for the presence of starches in organic compounds

PRECAUTIONS

- All the glassware should be clean
- Handle iodine carefully as it has staining property
- Preserve in a closed container

Lugol's Iodine Solution B.P.

25ml

Composition: Iodine.....5% w/v

Uses: Antiseptic for wounds
Disinfectant for drinking water
Indicator for starch in organic compound

Store in a well closed container at cool and dry place.

For External Use Only
Keep out of the reach of children.

Mfg. Date: 10/12/2013 Exp. Date: 18/12/2013

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

COMPOUND NACL MOUTH WASH (B.P 1980)

THEORY

A mouth wash is an aqueous solution with a pleasant taste and odour, used for rinsing, refreshing or anti-septic action.

It may contain alcohol, glycerin, sweetening agent, surface active agent, flavoring and coloring agent. Medicated mouth wash containing astringents, anti-microbial agents or other are also used but under the supervision of the dentist. The container should be labeled with direction for diluting mouthwash before use.

TYPES

- Cosmetic
- Special purpose

INGREDIENTS

- Sodium bicarbonate. 10g
- Sodium chloride 15g
- Conc.peppermint emulsion 25ml
- Double strength chloroform 500ml
- Distilled water q.s 1000ml

PROCEDURE

- Wash, clean and dried all the glassware
- Weigh the ingredients accurately
- Triturate them in pestle and mortar
- Mix them with the help of small volume of H_2O
- Now add double strength chloroform in water and conc.peppermint emulsion in the solution
- Make up the volume up to required volume by water
- Label it properly

USES

- Used as anti-septic

EXPIRATION

- 4 weeks

DOSE

- Four tale spoons four times a day

Compound NaCl Mouth Wash B.P.

25ml

Composition: Sodium Chloride ... 1.5% w/v
Sodium Bicarbonate ... 1% w/v

Uses: As an antiseptic

Dose: Take four table spoon four times a day or as prescribed by physician

Direction of Use: Dilute with equal volume before using

Store in a light resistant tightly closed container.

Do not swallow in large amount
Keep out of the reach of children.

Mfg. Date: 17/12/2013 Exp. Date: 14/01/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

PREPARATION OF SALICYLIC ACID COMPOUND DUSTING POWDER

AIMS

- To understand the concept of compound dusting powder
- To prepare salicylic acid dusting powder

THEORY

Medicinal powders are mixture of medical substances usually in an inert base such as talcum or starch

The powder should be small enough to ensure against grittiness and skin irritation. Powders are more frequently applied topically to relieve conditions such as diaper rash, chafing and athletes foot.

Medicated powders for external use are dusted on the affected area from a container or applied from a powder aerosol.

INGREDIENTS

- Salicylic acid 0.3g
- Boric acid 0.5g
- Talc (sterilized) 10g

PROCEDURE

- Triturate the ingredients and pass them through sieve no.20
- Weigh the required quantity of all ingredients
- Mix them uniformly in pestle and mortar
- Dispense in suitable container

USES

- As an anti-infective

DOSE

- Apply on affected area as prescribed

STORAGE

- Preserve in well closed container protected from moisture

AUXILIARY LABEL

- For external use only

Salicylic acid Compound Dusting Powder

10.7g

Composition: Salicylic acid... 2.8% w/w
Boric acid ... 4.6% w/w

Uses: As an anti-infective

Dose: Apply on affected area as prescribed

Preserve in a well closed contained protected from moisture

For external use only
Keep out of the reach of children

Mfg. Date: 24/12/2013 Exp. Date: 30/12/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Effervescent Granules

Theory

Effervescent granules contain a medicinal agent in a dry mixture usually composed of sodium bicarbonate, citric acid and tartaric acid.

When added to water, the acids and the base react to liberate CO_2, resulting in effervescence.

Using combination of citric acid and tartaric acid rather then either alone avoids certain difficulties. When tartaric acid is used as the sole acid, the resulting granules readily lose their firmness. Citric acid alone results in a sticky mixture difficult to granulate.

The ratio of the effervescent ingredients is 1:2:3.4 for the citric acid : tartaric acid : sodium bicarbonate

Methods of preparation

- Dry or fusion method
- Wet method

Ingredients

- Citric acid 1.55g
- Tartaric acid 2.16g
- Sodium bicarbonate 4.08g
- Ammonium citrate 0.43g

Procedure

- Place the porcelain dish on water bath
- Weigh amount of all ingredients and mix
- Put all the powder in hot porcelain dish
- Stir the mixture by bone spatula till the mixture has formed damp coherent mass
- Quickly press the mixture through sieve
- Spread the granules on a tray in a warm place and dry for 2-3 hours
- Fill it in clean container and label it properly

Uses

- As a gastric antacid
- Urinary alkalizing agent

Dose

As a prescribed (4-8g in half a glass of water, 2-3 times daily taken on an empty stomach and followed with additional water)

Storage

- Preserve in a well closed container away from moisture

Precautions

- Drink after effervescence

Auxiliary label

- Keep out of reach of children
- For oral use only

Effervescent Granules

Composition: Citric acid ..... 1.55 g
Tartaric acid ..... 2.16 g
Sodium bicarbonate ..... 4.08 g
Ammonium citrate ..... 0.43 g

Uses: As an gastric antacid
and Urinary Alkalizing agent

Dose: As prescribe (4 -8 g in a half glass water, 2-3 times daily taken on an empty stomach and followed with additional water)

Prevention: Drink after effervescence

Preserve in well closed container, away from moisture

For oral use only
Keep out of the reach of children

Mfg. Date: 31/12/2013 Exp. Date: 08/01/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Oral Rehydrating Salts

Aims

- To understand the concept of oral rehydrating salts
- To prepare oral rehydrating salts

Theory

ORS are oral powders, containing either anhydrous glucose, sodium chloride and potassium chloride and sodium citrate or sodium bicarbonate. After being dissolved in the required volume of water, they are intended for the treatment of dehydration due to diarrhea including maintenance therapy. It is recommended by diarrheal disease control, program of WHO and UNICEF.

Ingredients

- Sodium chloride 2.6g
- Potassium chloride 1.5g
- Sodium citrate 2.9g
- Anhydrous glucose 13.5g

Procedure

- Wash, clean and dry all the apparatus
- Weigh each ingredient properly
- Triturate them in pestle and mortar and mix properly
- Label it

Indication

- Prevention and treatment of dehydration

Dose

- Depending upon the age and severity of dehydration

Infants and children

1-2 litres (5-10 glasses) over a period of 24 hours

Adults

2-4 litres (10-20 glasses) over a period of 24 hours

Directions for use

Dissolve in one litre of water to be taken orally and continue treatment until diarrhea stops. Solutions to be used within 24 hours.

Storage

- Store in a cool and dry place

Precautions

- Use with caution in impaired renal function or intestinal obstruction
- Close tightly after every use and keep away from moisture

Result

The ORS has been prepared and labeled properly

Oral Rehydration Salt B.P.

20.5g

Composition: Sodium Chloride 02.6g
Potassium Chloride 01.5g
Sodium Citrate 02.9g
Anhydrous glucose 13.5g

Uses: Prevention and treatment of dehydration

Dose: Depending upon the age and severity of dehydration.
Infant: 1-2 liters (5-10 glasses) over a period of 24 hours
Adult: 2-4 liters (10-20 glasses) over a period of 24 hours

Direction of Use: Dissolve in one liter of drinking water to be taken orally

Store in cool and dry place

For oral use only
Keep out of the reach of children.

Mfg. Date: 07/01/2014 Exp. Date: 14/01/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Calamine Lotion (B.P.)

Theory

Lotions are usually liquid or liquid suspension containing one or medicaments intended to be applied uniform on skin without friction.

They are either dubbed on skin or applied with the help of some absorbent material like wool or cotton.

They may be prepared by triturating the ingredients to a smooth paste than gradually adding the remaining liquid phase. A wide variety of ingredients are employed in the preaparation to produce better dispersion that show good cooling, soothing, drying or protective nature of lotion. Following substances are use in preparation of lotions.

Bentonite → Suspending agent

Glycerin → Keep the skin moist for considerable period of time

Alcohol → Use to accentuate action like drying or cooling etc.

Lotion can be used for delivery to the skin of medication such as antibiotics, antiseptics, antifungal , corticosteroids, soothing, smoothing, moisturizing or protective agent, such as calamine.

Apparatus/ Equipment

- Weighing balance

- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

INGREDIENTS

- Calamine … 8g
- Zinc Oxide … 8g
- Glycerin … 2ml
- Bentonite Magma… 25 ml
- Calcium hydroxide 1% … q.s. to make 100 ml

PROCEDURE

- Dilute bentonite magma with equal volume of calcium hydroxide solution
- Triturate calamine and zinc oxide in a mortar
- Mix the content of the mortar with glycerin and add about 10 ml of bentonite magma
- Triturate the solution and add remaining bentonite magma in it
- Dilute solution with 1% Calcium Hydroxide Solution to make 100ml

INDICATION

- For the relief of sunburns rough dry skin.
- Chapped and tender skin
- Anti-parotic (anti-itching)

DIRECTION

Apply gently on the affected area when required

STORAGE

Store it in a well closed container at room temperature

AUXILIARY LABEL

- For External Use Only
- Content should be shake well before use
- Keep out of reach of children

Expiry: After two weeks

ROLE OF INGREDIENTS

- Calamine → Active ingredient, that acts as soothing and protective agent
- Zinc Oxide → as protective coating against irritation and antiseptics
- Glycerin → Keep the skin moist
- Calcium Hydroxide 1% → as vehicle and preservative

Calamine Lotion

25ml

Composition: Calamine ..... 8% w/v

Indications: For the relief of sunburns, rough and dry skin
Chapped and tender skin
Anti-pruritic

Direction of Use: Apply gently on the effected area when required

Store in a well closed container at room temperature.

For External Use Only
Contents should be shake before use
Keep out of the reach of children.

Mfg. Date: 04/03/2014 Exp. Date: 18/03/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Salicylic Acid Lotion (B.P.)

Theory

Lotions are usually liquid or liquid suspension containing one or more medicaments intented to be applied uniform on skin without friction.

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Salicylic Acid ... 20g
- Castor oil ... 10 ml
- Ethanol .. q.s. 1000 ml

Procedure

- Wash, clean and dry all the glassware
- Calibrate the weighing balance and weigh the required amount of ingredients
- Dissolve the salicylic acid in a portion of ethanol
- Add the castor oil with continuous stirring
- Add sufficient ethanol to form the required volume (i.e. 1000ml) and mix it well

Indication

Keratolytics:- Removing excess skin in certain condition , including psoriasis and warts

Direction to apply

Wash the affected area with soap and water. Completely dry the affected area. Apply a thin film of medicine to the affected area and gently massage until the medicine is evenly distributed

STORAGE

Preserve it in a well closed container away from sunlight and heat

AUXILIARY LABEL

- Avoid contact with eyes
- Keep away from the reach of children
- For topical use only

Salicylic Acid Lotion **(B.P)**

20ml

Composition: Salicylic acid..... 2% w/v

Indications: As Keratolytic

Direction of Use: Wash the affected area with water and dry it. Apply thin film of medicine to that area with gentle massage

Store in a well closed container away from sunlight and heat

Avoid contact with eye
For topical use only
Keep out of the reach of children.

Mfg. Date: 11/03/2014 Exp. Date: 25/03/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Camphor Liniment (I.P)

Theory

Liniments are alcoholic or oleaginous solution or emulsion of various medical substance intended to e rubbed on the skin

Liniments with an alcoholic or hydro alcoholic vehicles are useful when rubefacient, counter irritant or penetrating action is desired. Oleaginous liniments are employed primarily when massage is desired.

Liniments are not applied to skin areas that are broken or bruised because excessive irritation might result

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Camphor … 125g
- Turpentine oil … 5ml
- Ammonia solution strong … 250ml
- Alcohol q.s. to make … 1000 ml

Procedure

- Wash, clean and dry all the glassware
- Dissolve camphor and turpentine oil in 600 ml alcohol
- Add measured quantity of strong ammonia solution with frequent shaking
- Make up the volume by adding alcohol and mix it
- Fill it a clean and dry container
- Label it properly

Uses

As a counter irritant

Direction to Apply

Apply with rubbing on the affected area

Storage

Preserve in a well closed container

Auxiliary Label

- For topical use only
- Should not be applied to skin that is broken or bruised

Role of Ingredients

- Camphor → Counter irritants
- Turpentine oil → Counter irritant

- Ammonia solution Strong → Rubefacient
- Alcohol → Penetration enhancer, preservative and as a vehicle

Camphor Liniment (I.P)

20ml

Composition: Camphor.....12.5 % w/v

Indications: As Counter Irritant

Direction of Apply: Apply with rubbing on the affected are

Store in a well closed container away from sunlight and heat

Should not be applied to broken or bruised skin
For topical use only
Keep out of the reach of children.

Mfg. Date: 18/03/2014 Exp. Date: 01/04/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Brilliant Green and Crystal Violet Paint

Theory

Paints are solution or dispersion of one or more medicaments intended for application to skinor in some cases mucous membrane

They may contain volatile solvent that evaporates quickly to leave a dry or resinous film of medicaments.

Throat paints are more viscous due to high content of glycerin which being sticky adheres to the affected site and prolong the action of medicaments.

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Brilliant Green … 0.5g
- Crystal Violets … 0.5g
- Alcohol … 50 ml
- Purified water q.s. .. 100 ml

Procedure

- Dissolve weighed quantity of brilliant green and crystal violets in measure volume of alcohol.
- Add sufficient purified water to make 100 ml.
- Filter the solution through a clean wet cloth
- Fill in a clean and dry bottle
- Label it properly

Uses

- Antibacterial and antifungal
- Used as an antiseptic
- It is used in hospital for the treatment of serious heat burns and other injuries of the skin and gum

Storage

Preserve in a well closed container

Auxiliary Label

- For external/ topical use only
- Keep away from reach of children

Role of Ingredients

- Brilliant Green → Antiseptics
- Crystal Violets → Antibacterial, antifungal
- Alcohol →Solvents
- Purified water → Vehicle

Brilliant Green and Crystal Violet Paint (B.P)

20ml

Composition: Brilliant Green.....0.5 % w/v
Crystal Violet.....0.5 % w/v

Indications: As antibacterial and antifungal
As antiseptic
For serious heat burns

Store in a well closed container away from sunlight and heat

For topical use only
Keep out of the reach of children.

Mfg. Date: 25/03/2014 Exp. Date: 08/04/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Kaolin Poultice

A poultice also called cataplasm is a viscous or paste like preparation intended for application to the skin whilst hot

Lotion pulta and Greek Poltos → Porridge; Greek Kataplasso →Spread over

Uses for boils, infection and inflammation

Can be applied directly to the skin and covered with a piece of clean cloth

When poultice is applied on the skin, drugs are absorbed into the skin and the healing process starts.

The poultice increase the supply of blood to affected area

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Heavy Kaolin … 52.7g
- Boric acid… 4.5g
- Thymol … 50 mg
- Methyl Salicylate … 0.2ml
- Peppermint oil … 0.05 ml
- Glycerol … 42.5g

Procedure

- Mix heavy kaolin and boric acid with the glycerol
- Heat the mixture at 120'C for one hour along with continuous stirring
- Allowed to cool (A)
- Dissolve thymol and methyl salicylate in separate container (B)
- Add B in A
- Add peppermint oil and mix thoroughly

Uses

- Anti-infective
- Anti-rheumatic

Storage

In a tight container

Auxiliary Label

For External Use Only

Role of Ingredients

- Heavy Kaolin → Absorbent
- Boric Acid → Bactericidal
- Thymol → Bactericidal
- Methyl Salicylate → Anti-rheumatic
- Peppermint oil → Flavoring agent
- Glycerol → Vehicle; Hygroscopic

Kaolin Poultice 50g

Composition: Kaolin 26.35g
Methyl Salicylate ..... 0.1 ml

Uses: Anti-infective
Antirehumatic

Store in a well closed container

For external use only
Keep out of the reach of children.

Mfg. Date: 08/04/2014 Exp. Date: 22/04/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Tragacanth Jelly (B.P.)

Theory

Pharmaceuticals gels are semi-solid system that are being increasingly used as pharmaceutical topical preparation

Jellies are a class of gels in which the structural coherent matrix contains a high proportion of liquid usually water

They are usually formed by adding a thickening agent such as Tragacanth or carboxymethylcellulose solution of drug substance

The resultant product is usually clear and uniformly distributed

Jellies are subject to bacterial contamination and growth so most are preserved with anti-microbial agent

Jellies should be stored in a tight container because water may evaporate leaving a dry product

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Ichthamol … 2g
- Tragacanth … 5g
- Alcohol 90% … 10 ml
- Glycerol … 2g
- Water … 100g

Procedure

- Prepare Tragacanth mucilage using 35ml of water
- Mix the glycerol, ichthamol in 10 ml of water, add this to mucilage and shake well
- Add alcohol with continuous shaking
- Adjust the weight with water and shake well.

Uses

Anti-psoriatic agent

Storage

Stored in air tight container in a cool place

Auxiliary Label

For external use only

Role of Ingredients

- Icthamol → anti-psoriatic agent

- Tragacanth → gelling agent
- Alcohol 90% → Preservative
- Glycerol → Hygroscopic
- Water → Vehicle

Tragacanth Jelly B.P

20g

Composition: Ichthammol ..... 2% w/w
Tragacanth.....5% w/w

Uses: As an anti-psoriatic agent

Store in a closed container in cool place

For external use only

Keep out of the reach of children.

Mfg. Date: 15/04/2014 Exp. Date: 29/04/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Simple Ointment (B.P.)

Theory

Ointments are formulated to provide preparation that immiscible, miscible or emulsifable with skin secretion

Ointments are used for their emollient and protective agent on the skin

The absorption of medicaments by tissues from the ointments applied to the skin depends upon number of factors

- Properties of the drug incorporated
- Properties of the base used in formulation
- Condition of the patient skin
- Site of application
- Duration of action

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Wool Fat … 50g
- Hard Paraffin … 50g
- Cetostearyl alcohol … 50g
- White soft paraffin … 850g

Method

- Mix all the ingredients
- Heat them together well over a water bath gently with stirring until melt
- Keep on stirring until cold

Indication/ Uses

- Used as an emollient
- Used as a vehicle for other medicaments

Dose

As per requirements

Storage

It should be stored in a well closed wide mouth jar

Auxiliary Label

For External Use Only

Role of Ingredients

- Wool fat → for water proofing or occlusive dressing
- Hard Paraffin → as an emollient or stiffening agent
- Cetostearyl Alcohol → as an emollient and co-emulsifier
- White Soft Paraffin → As an emollient

Simple Ointment B.P

25g

Composition: Wool fat 1.25g
Hard Paraffin.....1.25g
Cetostearyl Alcohol.....1.25g
White Soft Paraffin.....21.25g

Uses: As an emollient
Used as a vehicle for other medicaments

Dose: As per required

Store in a closed wide mouth jar

For external use only

Keep out of the reach of children.

Mfg. Date: 06/04/2014 Exp. Date: 20/04/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Sulphur Ointment (B.P.)

Theory

Ointments are formulated to provide preparation that immiscible, miscible or emulsifable with skin secretion

Ointments are used for their emollient and protective agent on the skin

The absorption of medicaments by tissues from the ointments applied to the skin depends upon number of factors

- Properties of the drug incorporated
- Properties of the base used in formulation
- Condition of the patient skin
- Site of application
- Duration of action

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Sulphur … 100g
- Simple Ointments … 900g

Method

- Triturate sulphur with a portion of simple ointment until smooth
- Gradually add the remainder of simple ointment
- Mix it thoroughly until smooth and free from grittiness

Indication

- Used as scabicides
- Used as fungicide
- Use for the treatment of eczemas

Dose

It should be applied on the affected area 2-3 times daily

Storage

It should be stored at a cool place and in a well closed wide mouth jar

Auxiliary Label

For external use only

Role of Ingredients

- Sulphur →active ingredients fused as scabicide, fungicide and treatment of eczemas

- Simple ointment → as a vehicle

Sulphur Ointment **B.P**

10g

Composition: Sulphur..... 10 % w/w
Simple Ointment.....90% w/w

Indication: Use as scabicide
Use as fungicide
Use for treatment of eczemas

Dose: It should be applied on the affected area 2-3 times daily

Store in a well closed wide mouth jar in a cool place

For external use only

Keep out of the reach of children.

Mfg. Date: 13/05/2014 Exp. Date: 27/05/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Zinc Oxide Paste (B.P.)

Theory

Paste are semi-solids preparation meant for application to the skin

They differ from ointments that they contain a large amount of finely divided powdersolids such as zinc oxide, starch, calcium carbonate

Due to presence of high solid content they become quite thick and stiff than ointments but are less greasy than ointments

Paste are stiff they do not melt at body temperature thus holding a protective covering over the areas to which they are applied

It is easier to stick the paste to diseased area rather than ointment which are generally less viscous than paste and tend to spread on healthy skin which may result in sensitivity reaction.

Pastes are generally prepared by levitating process.

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Zinc oxide, finely divided … 250g
- Starch … 250g
- White Soft Paraffin … 500g

Procedure

- Melt the white soft paraffin in a small beaker over a water bath
- Generally, incorporated zinc oxide and starch with continuous mixing until smooth

Indication/ Uses

- As a protectant
- As an antiseptic
- As an astringent

Dose

Apply 1-2 times daily

Storage

Store it in a suitable container at a cool place

Auxiliary Label

- For External Use Only
- Do Not Apply on Broken or Inflammed Skin

Role of Ingredients

- Zinc Oxide → Active ingredient
- Starch → act as filler
- White Soft Paraffin → as a vehicle

Zinc Oxide Paste **B.P**

10g

Composition: Zinc oxide..... 25 % w/w

Indication/ Uses: As a protectant
As an antiseptic
As an astringent

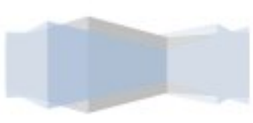
Dose: Apply 1-2 times daily

Store in a suitable container in a cool place

For external use only
Do not apply on broken or inflamed skin
Keep out of the reach of children.

Mfg. Date: 20/05/2014 Exp. Date: 03/06/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.



Preparation of Potassium Permagnate Gargles

Theory

Gargles are the aqueous solution used for treating pharynx and nasopharynx by forcing air from the lungs through gargle that is held in the throat

- Gargles are generally dispensed in concentrated form
- They must be diluted with water prior to use
- Gargles are pleasant flavoured and medicated than mouth watches
- Many mouth watches are use as garlges, either as such or diluted with warm water
- Gargles should not be swallowed but should be thrown out after cleansing oral cavity

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Potassium permagnate … 25mg
- Purified water q.s. 100ml

Procedure

- Grind weigh amount potassium per magnate with water
- Remove un-dissolve per magnate y filtration and add sufficient amount of purified water to produce 100 ml.

Indication

Astringent

Dose

As prescribed by physicians, diluted with equal amount of water

Direction for use

Gargle in back of throat for 15-20sec and spit out

Storage

Store it in a well closed container

Auxiliary Label

- Do not swallow
- Keep out of reach of children

Role of Ingredients

Potassium Per magnate → astringent

Potassium Permagnate Gargle

100ml

Composition: Potassium Permagnate .... 0.025% w/v

Indication: As an astringent

Dose: As prescribed by physician, diluted with equal amount of water

Direction for use: Gargle in back of throat for 15-20 sec and spit out

Store it in a well closed container

Do not swallow

Keep out of the reach of children.

Mfg. Date: 03/06/2014 Exp. Date: 17/06/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Alkaline Nasal Wash

Theory

Nasal Preparations

- Contains adrenergic agents….decongestant activity on the nasal mucosa
- Most preparations are in solution form (nose drops or sprays)
- Packaged in dropper bottles or plastic spray bottles usually 15-30 ml of medications
- Patient should be counseled to discard the solution if it becomes discolored or contains precipitated matter

Nasal decongestant solutions are:

- Aqueous
- Isotonic to nasal fluids (0.9% NACL)
- Buffered to maintain drug stability
- Preserved by antimicrobial preservative
- Pediatric preparations have half strength of adult conc.
- Intended for treatment of rhinitis of common cold, allergic rhinitis including hay fever and sinusitis

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Ingredients

- Sodium bicarbonate…50gm
- Sodium chloride…50 gm
- Sodium borate(borax).. .50 gm

Procedure

- Weigh each ingredient, then grind them to fine powders, then sieve them
- Add the powders to each other gradually and mix them well.
- Take 2gm of the mix powder to be dissolved in 120 ml of warm water and used as directed

Indications

Used as nasal wash in nasal congestion and allergic rhinitis.

Dose

2 gm to be dissolved in 120 ml of warm water and used as directed

Auxiliary Label

- For nasal use only.
- Keep out of the reach of children.

Storage

Keep at dry place, in a closed container

Alkaline Nasal Wash

10g

Indication: Use as nasal wash in nasal congestion and allergic rhinitis

Dose: 2gm to be dissolved in 120 ml of warm water and use as directed

Store at dry place in a cool container

For nasal use only

Keep out of the reach of children.

Mfg. Date: 27/05/2014 Exp. Date: 09/06/2014

Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

Preparation of Pilocarpine Eye Drops

Theory

Ophthalmic preparations (eye preparations) are sterile, liquid, semi-solid, or solid preparations that may contain one or more active pharmaceutical ingredient(s) intended for application to the conjunctiva, the conjunctival sac or the eyelids.

The different categories of ophthalmic preparations include drops consisting of emulsions, solutions or suspensions, and ointments.

Ophthalmic drops

Ophthalmic drops (eye drops) are sterile aqueous or oily solutions, suspensions, or emulsions intended for instillation into the conjunctival sac.

Ophthalmic drops should be clear and practically free from particles.

The preparation of aqueous ophthalmic drops requires careful consideration of the need for isotonicity

Ideally, the pH of ophthalmic drops should be equivalent to that of tear fluid, which is 7.4.

Water for injection should be used in the manufacture of aqueous ophthalmic drops. The addition of antimicrobial agents and/or antioxidants may also be required.

Pilocarpine is an alkaloid obtained from the leaves of *Pilocarpus microphyllus.*

It is primarily indicated in glaucoma.

Ingredients

- Pilocarpine Nitrate ……. 50 mg
- Sodium Chloride…….. 34 mg
- Solution for eye drops…….. 5 ml

<u>Solution for eye drops</u>

- Methyl hydroxybenzoate ……. 22 mg
- Propyl hydroxybenzoate……. 11.4 mg
- Purified water, q.s……. 100 ml

Apparatus/ Equipment

- Weighing balance
- Measuring cylinder
- Beakers
- Glass rod / Stirrer
- Pippette
- Gloves
- Mask

Procedure

- Dissolve sodium chloride in solution of eye drops
- Add pilocarpine nitrate and dissolve it by shaking
- Make up the final volume

- Clarify/Sterile by filtration
- Fill in a clean and dry container (plastic containers with a fixed built-in dropper)
- Label properly
- Preparation must be performed in aseptic area.

USES

In the treatment of Glaucoma

DOSE

2-3 drops 8 hourly

STORAGE

Preserve in well-closed container protected from light

PRECAUTIONS

For ophthalmic use only.

ROLE OF INGREDIENTS

- Pilocarpine Nitrate …….. To treat Glaucoma
- Sodium Chloride …….. Tonicity modifier
- Methyl hydroxybenzoate ……. Preservative
- Propyl hydroxybenzoat ……. Preservative
- Purified water, q.s……. Solvent

Pilocarpine Nitrate Eye Drop **(B.P)**

5ml : 50mg

Composition: Pilocarpine nitrate…… 50mg

Indications: In treatment of glaucoma

Dose: 2-3 drops 8 hourly

Store in a well closed container away from sunlight and heat

For ophthalmic use only

Keep out of the reach of children.

Mfg. Date: 01/04/2014 Exp. Date: 15/04/2014

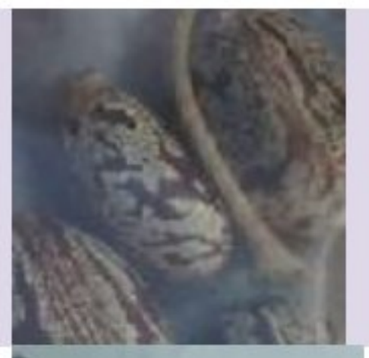
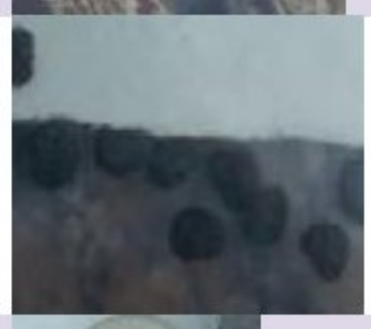
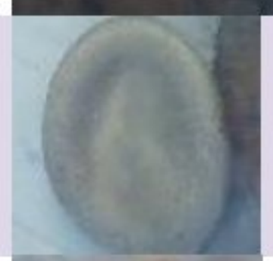
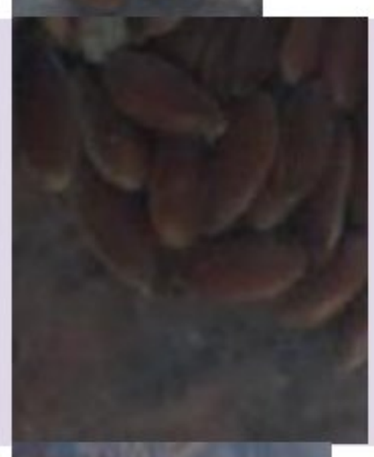
Manufactured By: White Collar Pharmaceuticals Pvt. Ltd.
12 Km, Defence Road Lahore.

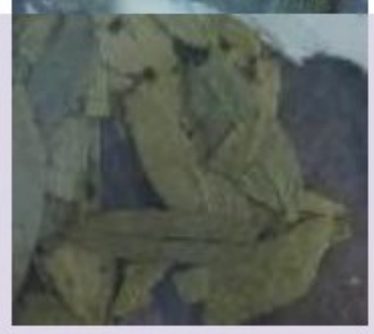
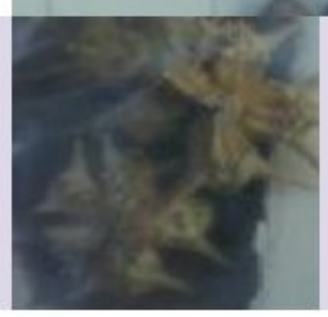
PHARMACOGNOSY

ORGANOLEPTIC FEATURES

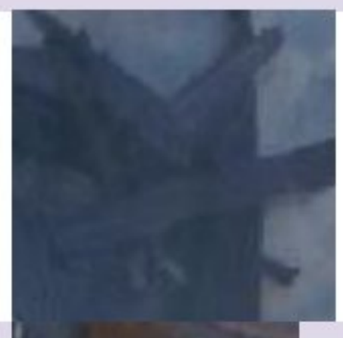
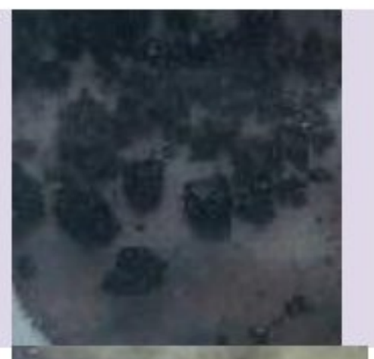
ORGANOLEPTIC / MACROSCOPIC EVALUATION

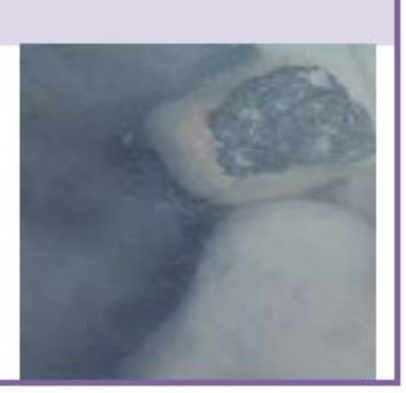
Organoleptic means impression on organ. It means evaluation by the help of organ or senses. It is based upon size, shape, colour, taste, odour, texture and sound and type of fracture. E.g. Ribbon shape characteristic of Tragacanth, disc shape of nux-vomica, aromatic order of umbelliferae fruit, sweet taste of lliquorice, and pungent taste of ginger are important diagnostic characteristics of these drugs. Fracture can be hard, weak, soft and brittle. External marking like ridges, furrows, wrinkles, annulation, fissures, or scars are also important. Taste can be sweet, bitter, salty, acrid etc. Odor can be characteristic, pleasant, unpleasant, spicy, aromatic, liliaceous, garlic like etc.

Common Name English Name	Scientific Name	Family	Constituent	Uses	Diagram
SEEDS					
Arund Caster bean	*Ricinus communis*	Euphorbiaceae	Fixed oil (Ricinoleic acid),Protein (Ricin)	Purgative	
Datura Thorn apple	*Datura stamonium*	Solanceae	Hyoscine, -Hyoscyamine, +-hyoscyamine (atropine)	Asthama, Antispasmatic, Neuralgia	
Kuchla Dog button	*Stychnos nux-vomica*	Loganiaceae	Alkaloids (Stychnine, brucine)	Cardiac and respiratory stimulant	
Mathi Bird's foot	*Trigonella foenum-grecum*	Papilionaceae	Trigonella wax, mucilage, volatile oil, starch	Condiment, tonic, laxative	
Alsi Flax/lin seed	*Linum usitatissimum*	Linaceae	Fixed oil, protein, mucilage cyanophore, glycosides	Demulcent, laxative for chronic constipation	
Ispaghul Spogel	*Plantago ovata*	Plantaginacea e	Mucilage (D-arbinose, glactose, galactoronic acid, rhaminose)	Demulcent, laxative for chronic constipation, diarrhoe	
LEAVES					
Gurrh-maar-booti Gymnema	*Gymnema sylivester*	Asclepiadanceae	Volatile oil, gumnemic acid	Antidiabetic Expectorant diuretics	

Tezpat Cinnamon leaf	*Cinnamomum zeylanicum*	Lauraceae	Volatile oil (Cinnamic aldehyde, cinnamic acid), tonic, sugars	Diphorectic, carminative, diureticm dyspepsia	
Podina Peppermint	*Mentha piperita*	Labiatae (laninaceae)	Volatile oils (menthol, menthone, menthofurain)	Aromatic, stomachic, carminative, local anesthetic/ analgesic	
Senna maki Senna leaf	*Cassia angustfolia*	Leguminosae (fabaceae)	Antraquinone glycosides (senosides A and B) flavonoids, mucilage	Purgative, anodyne	
Burg-e-gaozuban Borage leaf	*Onosoma bracteatum*	Boraginaceae	Proteins, mucilage, carbohydrates	Bronchitis, asthma, tonic, demulcent	
FRUITS					
Soaf Indian sweet soaf	*Foeniculum vulgare*	Umbelliferae	Essential oil (fenchone, anethole, phenol)	Carminative, aromatic, stimulant	
Sofaid Zera Cumin seeds	*Cuminum cyminum*	Umbelliferae	Essential oil (Cinamic aldehyde, pinene)	Carminative, aromatic, stimulant, condiment, astrigent	
Kali mirch Black pepper	*Piper nigram*	Piperaceae	Alkaloids (pipernine, piperidine), Vit B1, B2, C	Carminative, condiment, hypotension, sore throat	
Chota Gokhroo Small Calotropis	*Tribulus terrestris*	Zygophylaceae	Alkaloid, sugar, resins, nitrate, essential oil	Demulcent, calculus, aphrodisiac, dysuria	

Aalu Bukhara Plum	*Prunus domestica*	Rossaceae	Malic acid, citric acid, sugar, albumin, pectin	Demulcent, laxative, piles, enlarged liver	
Bail gree Bail tree	*Aegle marmeloe*	Ruteceae	Essential oil (mameolsin), Vit A, C	Astringent, diarrhea, laxative, anthelmintic	
Ajuwain Ajowan	*Trachysperm um ammi T.Copticum*	Umbelliferae	Volatile oil (Thymo, carvocol, camphor)	Colic, carminative, diuretic, antimicrobia l, antispasmod ic	
Anisoon Sweet fennel	*Pimpinella anisum*	Umbelliferae	Volatile oil (Anathole, fenchone), fixed oil	Aromatic, stimulant, carminative, colic, expectorant	
Reetha Soap nut	*Sapindus trifoliatus*	Sapindaceae	Fat, glycosides, pectin, saponins, tannins	Purgative, painkiller, expectorant, emetive	
FLOWERS					
Gul-e-banafsha English violet	*Viola odorata*	Violacea	Flavoroids, carotenoids, alkaloids (violin)	Expectorants , bronchitis, emollient, bitters, emetic	
FLOWER BUD					
Long Clove	*Eugenia carophyllus*	Myrtaceae	Volatile oil (eugenol), acetyl euginol, camphor	Aromatic, antiseptic, analgesic, anesthetic	
ROOTS					

Mulethi Glycyrrhiza	*Glycyrrhiza glabra*	Leguminosae	Saponins (Glycyrrhizin), Glycyrrhetic acid, mannitol	Demulcent, ulcer, anti-tussive, expectorant	
RHIZOME					
Adrak Ginger	*Zingiber officinale*	Zingiberaceae	Volatile oil (Zingiberene, zingibenol, phellandrene, oleoresin mainly gingerol	Carminative, Stomachic, aromatic, stimulant, sialagogue	
BARK					
Ratanjot Red bush	*Onosoma echioides*	Boraginaceae	Anthraquinone glycosides	Cardiac stimulant, purgative, hair growth stimulant	
Daarchini Cinnamom bark	*Cinnamomum zeylonicum*	Lauraceae	Essential oil (cinnamic aldehyde, eugenol, cinnamic acid), tannins, mucilage	Diuretic, diaphoretic, carminative, condiment	
EXUDATE					
Aloe Aloe	*Aloe indica*	Lilliaceae	Anthraquinone glycosides (alum, barbaloin), resin	Purgative, burns, anthelmintic, stomachic	
Loban Benzoin	*Styrax benzoin*	Straceae	Cinnamic acid, benzoic acid and their esters	Expectorant, antiseptic, lotion, tincture, cosmetic	
Kiker ki gond Acacia gum	*Acacia Arabica*	Leguminosae (fabaceae)	Carbohydrate, Arabic acid, tannins	Demulcent, binding agent, emollient, emulsifier, tonic	

Gond kateera Tragacanth	*Astragalus gummifer*	Leguminosae (fabaceae)	Tragacanthin (water soluble) , Basorin (water insoluble), galactose, galactronic acid	Demulcent, suspending agent, binder	
EXTRACT **Katha** Pale catechu	*Uncaria gambier*	Rubiaceae	Tannins, catechutannic acid, gambrtannins	Astrigent, tanning agent, burns, diarrhea, for wounds	

Name..... Drug.....

Pharmacognostic Features

Scientific Name.....

Family.....

Common Name.....

English Name.....

Parts Used.....

Constituents.....

Uses.....
.....

Organoleptic features of Crude Drug:

Texture.....

External marking.....

Internal Marking.....

External Colour.....

Internal Colour.....

Shape.....

Odour.....

Taste.....

Fracture.....

Signature.....

Date Checked.....

Microscopic Evaluation

Microscopic means evaluation of drug by using microscope. It is based upon histological character. It is mainly used for powdered drugs and section cutting of a drug (used for organized drug). As a powdered drug has few macroscopic features for identification, the microscopic characteristics become important. Transverse section shows specific arrangement and shape of cells on the basis of which a drug can be identified. Presence of calcium oxalate crystals, starch, cell wall, fibers, and vessels can be indicated or studied by a microscope. For example, powdered clove does not contain sclerosis or calcium oxalate crystals both are present in powdered clove stalk.

Different reagents or stains can be used to distinguish various cellular structure e.g. phloroglucinol, and concentrated HCl produce red color with lignin, iodine solution produce blue colour with starch, purple with dextrin and orange red with glycogen.

Quantitative microscopy may involve following parameters

Stomatal Number

Which is defined as average number of stomata per square inch.

Palisade Ratio

Average number of palisade cell beneath each epidermal Cell.

Section Cutting

Cutting of a piece of leaf, bark or any other part at right angle to its axis is called crossed section or transverse section cutting.

Procedure

- Cut a fine section of the given suspension by means of sharp blade and place it in a petri dish containing water
- Transfer the section into a watch glass containing 10% alcohol (ethanol) for 2 minutes.
- After 2 minutes, transfer the section to another watch glass containing 30% alcohol.
- After 2 mins transfer the section into another watch glass containing 50% alcohol.
- After 2 mins transfer the section into a watch glass containing safranin red and leave it for 5 mins.
- After 5 mins transfer the section into another watch glass containing 60% alcohol for 2 mins.
- Transfer it into 70% alcohol for 2 mins.
- Transfer it into a watch glass containing malachite green for 3-5mins
- Transfer the section in a watch glass containing 90% and 100% alcohol for 2 mins in each concentration.
- Remove the section and place it in clove oil for 1 minute, after which place it on a clean glass slide and add a drop of Canada Balsam
- Place the cover slip and observe it under microscope.

Histology of Ginger

Cork

The cork tissue is differentiated into outer zone of irregularly arranged cells and inner zone of radially arranged cell produced by tangential division of cortical cells.

Cortex

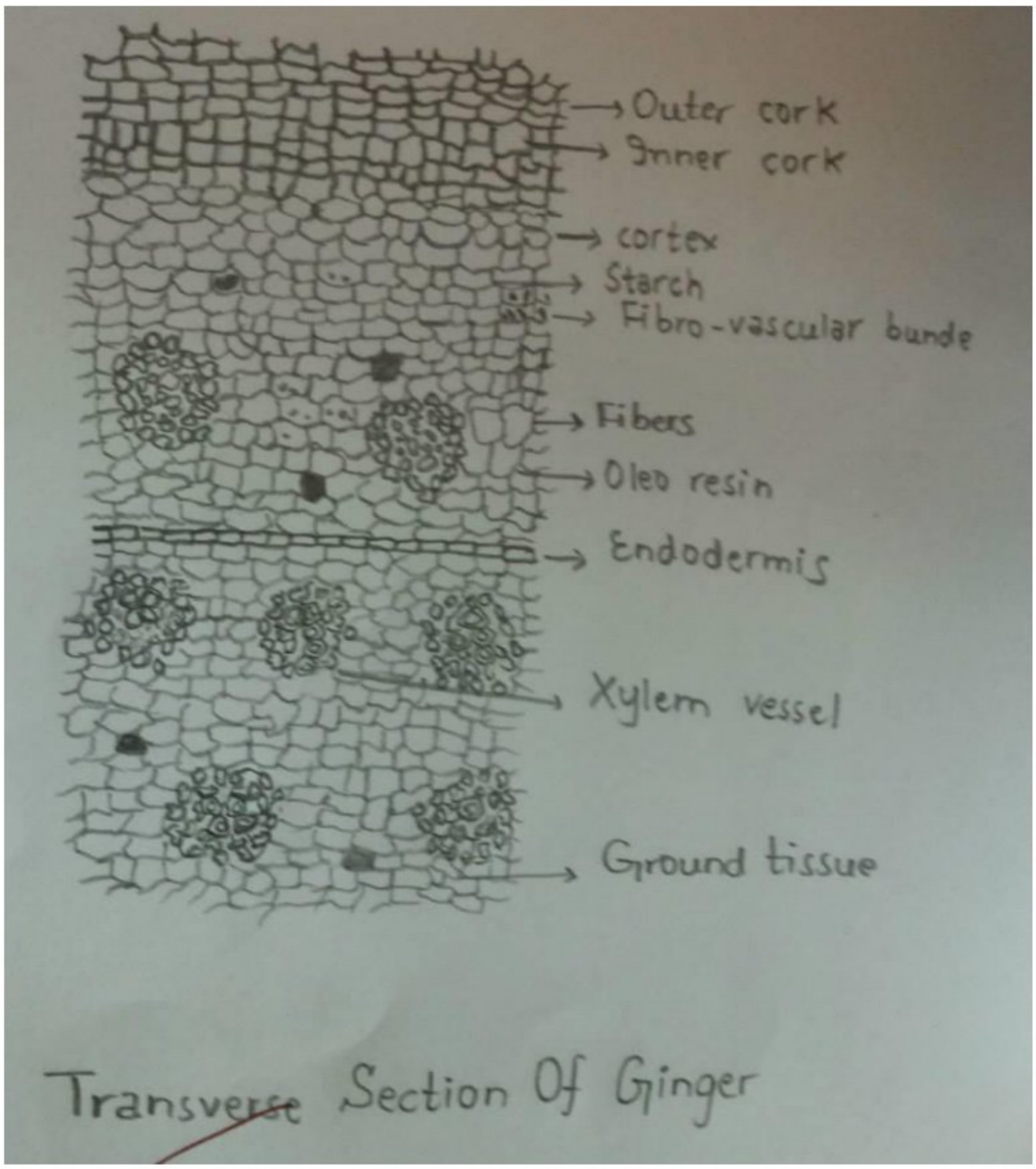
Below the cork is cortex; cortex is also divided into an outer zone and inner zone consisting of parachymatic cells. Cortical cells contains starch grains, and volatile oil and resins. Starch grains are ovoid or sac shape. Oil cells are yellow in colour.

Vascular Bundles

The inner cortical zone usually contain about 3 rings of collateral bundles, each vascular bundle contain phloem showing well marked sieve tubes and a xylem

Endodermis

It is composed of cells, present as a single layer of cells. It marks the inner limit of cortex.



Histology of Peppermint Leaves

Upper Epidermis

It is composed of large epidermal cells, having stomata and cyanogen pigments

Palisade Parenchyma

It consist of columnar cells which contain chlorophyll and are involved in photosynthesis

Spongy Parenchyma

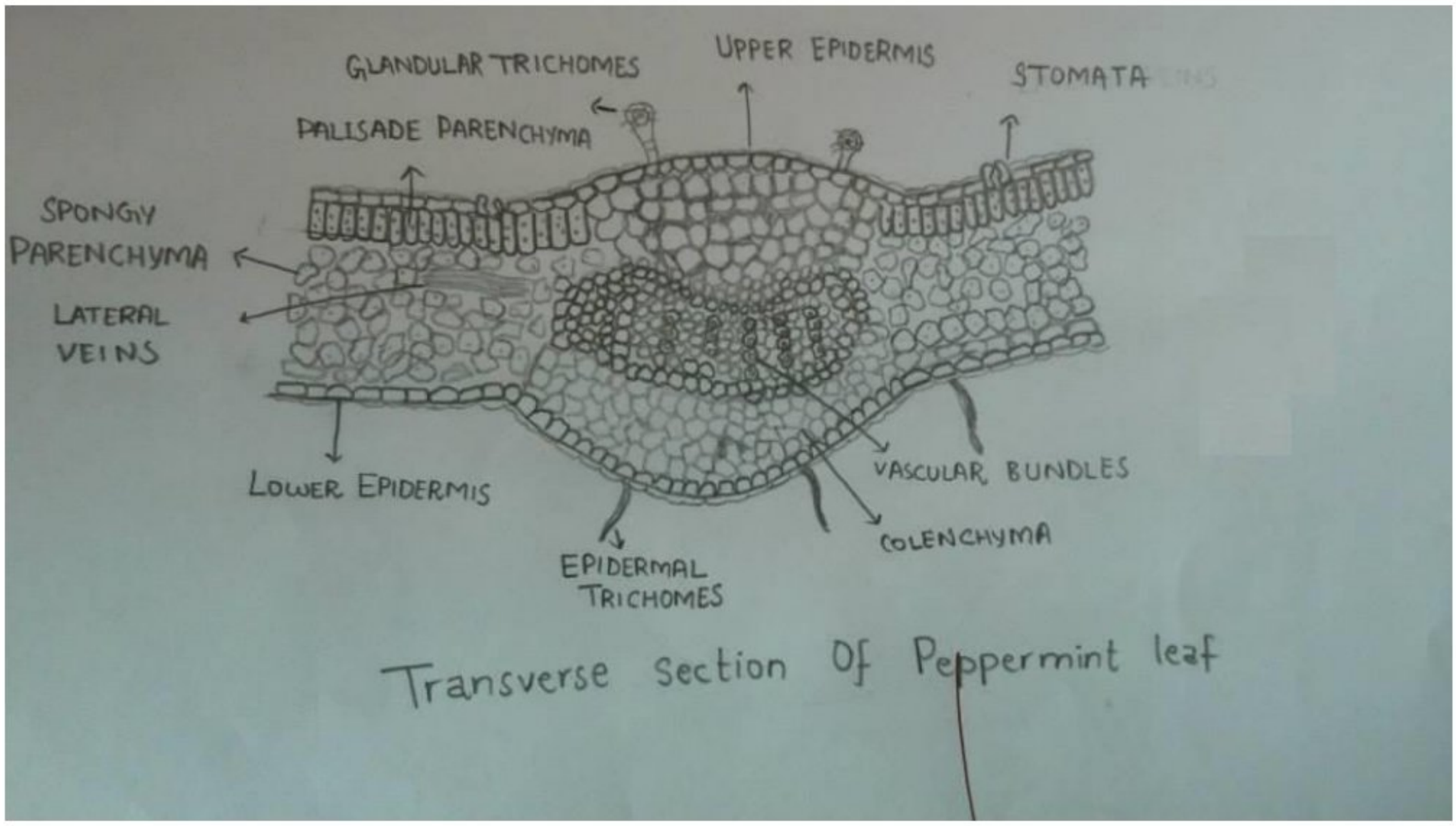
It consists of spherical cells, also contain chlorophyll. Intracellular spaces are present

Lower Epidermis

Epidermal cells with numerous glandular and non-glandular hair are present. Glandular hair produce volatile oil

Vascular Bundles

Collateral vascular bundles are present



Histological Characteristic of Eucalyptus Leaf

Upper Epidermis

Consisting of a single layer of cells

Palisade Parenchyma

Consists of 2-3 layers of columnar cell. It is present under both upper and lower epidermis

Spongy Parenchyma

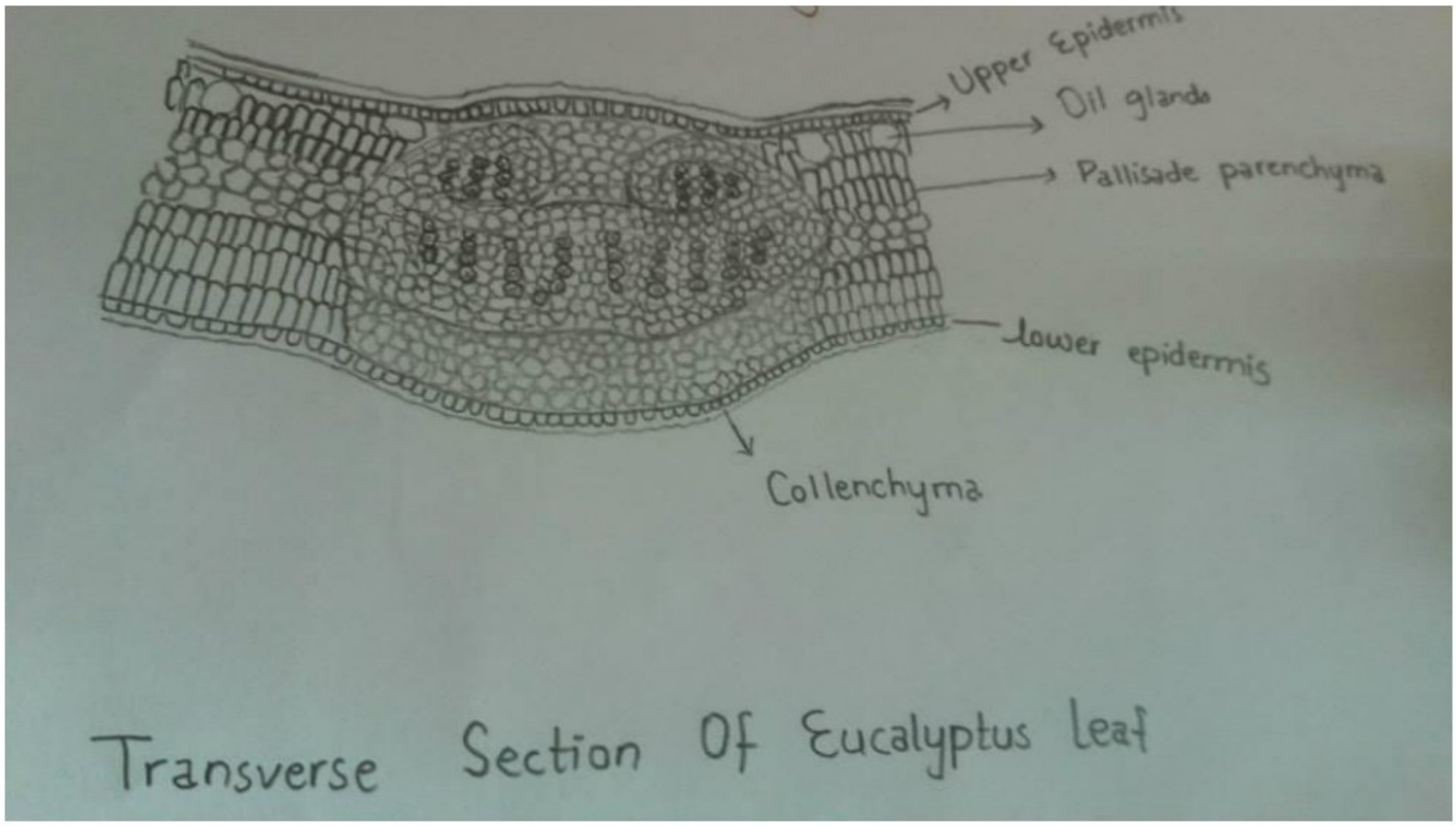
Consisting of almost spherical cells

Vascular Bundles

Xylem fibers are quite prominent

Lower Epidermis

Consists of single layer of epidermal cells



Histological Study of Cinnamon Bark

Cork and Cortex

Occasional oatches of cork are present. Parenchymatic cells (cortex) are also present occasionally.

Sclerenchyma Cells

Sclerenchyma cells are thickly lignified consisting of stone cells constituting pericycle fibers

Xylem

Xylem is absent

Phloem

Phloem fibers are present, transverse by medullary rays

Tannins containing cells are also present. Volatile oil glands are also present

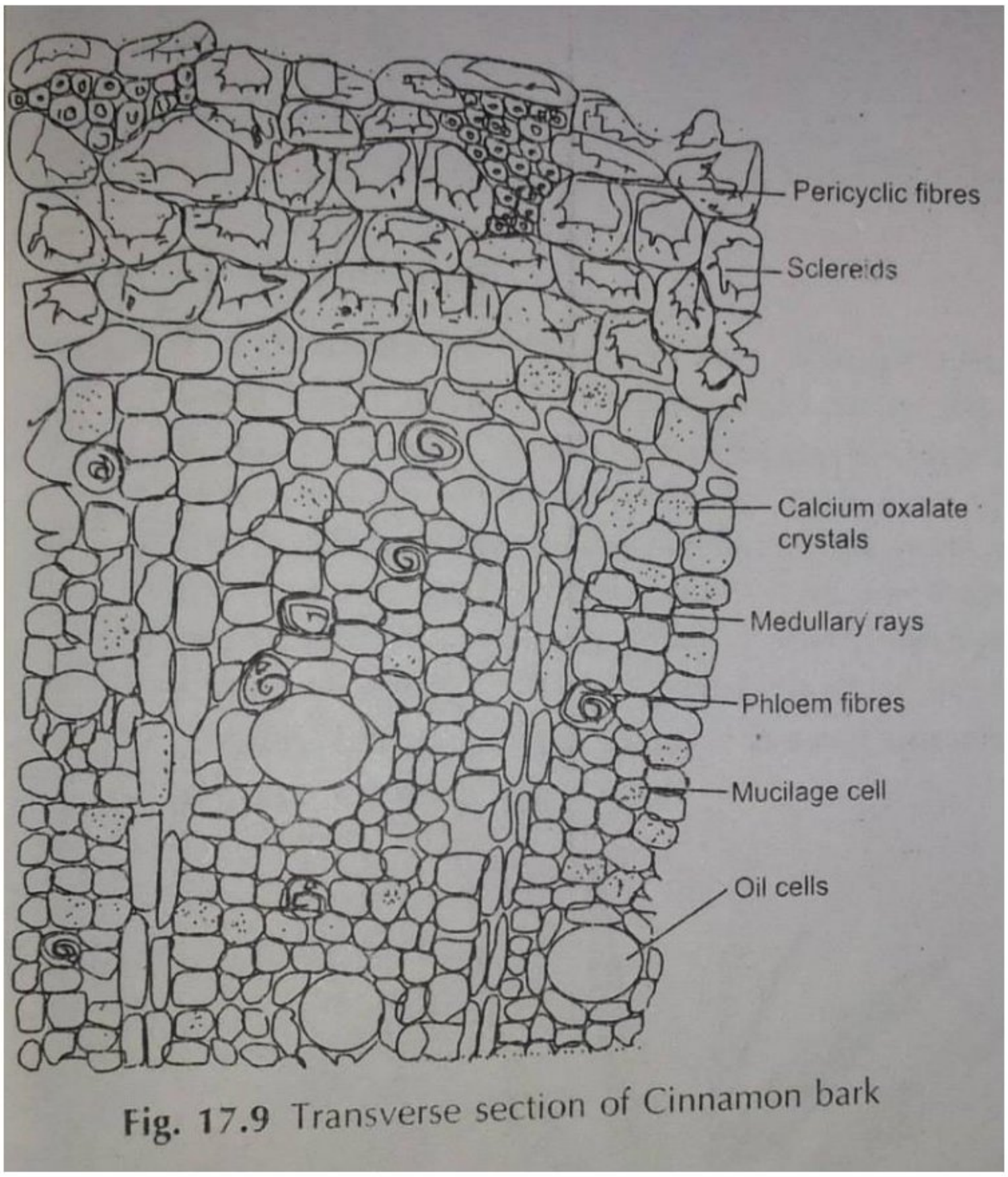


Fig. 17.9 Transverse section of Cinnamon bark

Histological Characteristic of Clove

Epidermis

Heavily centralized epidermis is present with flat cell walls

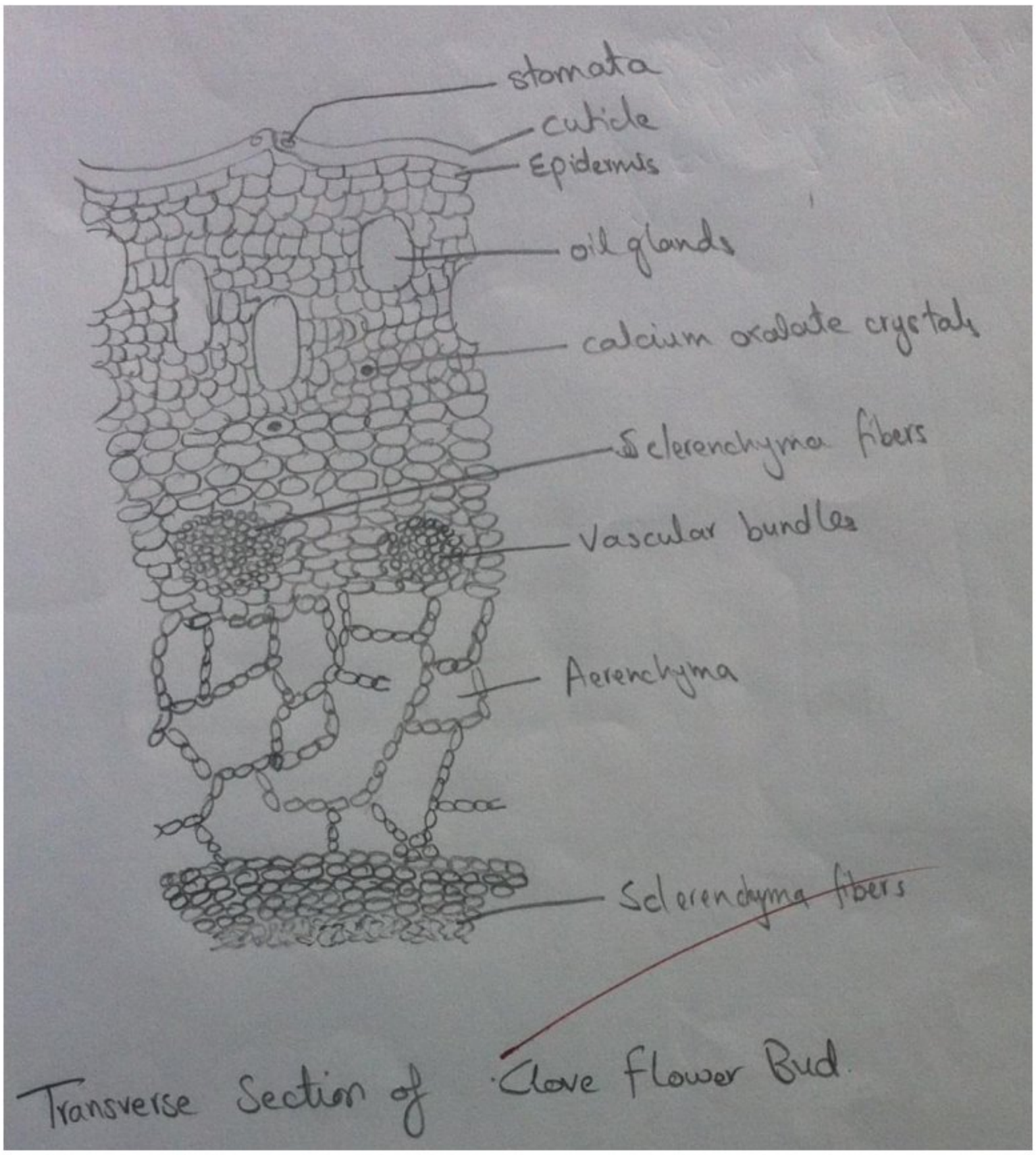
Cortex

Cortex consist of 3-regions or zone; outer zone contain 2-3 layer of elliptical oil reservoir (schizolysogenic oil glands)

Vascular bundles and crystals of calcium oxalate are present in some parenchymatic cells

Middle region contains biolateral vascular bundles

Third zone consists of arenchyma with plenty of air spaces. These cells are also known as chain cells.



Histological Characteristic of Glycyrrhiza

Cork

Cork consists of upto 10 layers of radially arranged rectangular dead cells

Cortex

Below cork is cortex, first five layer of which are collenchymatus while rest if the layers are parenchymatas

Phloem

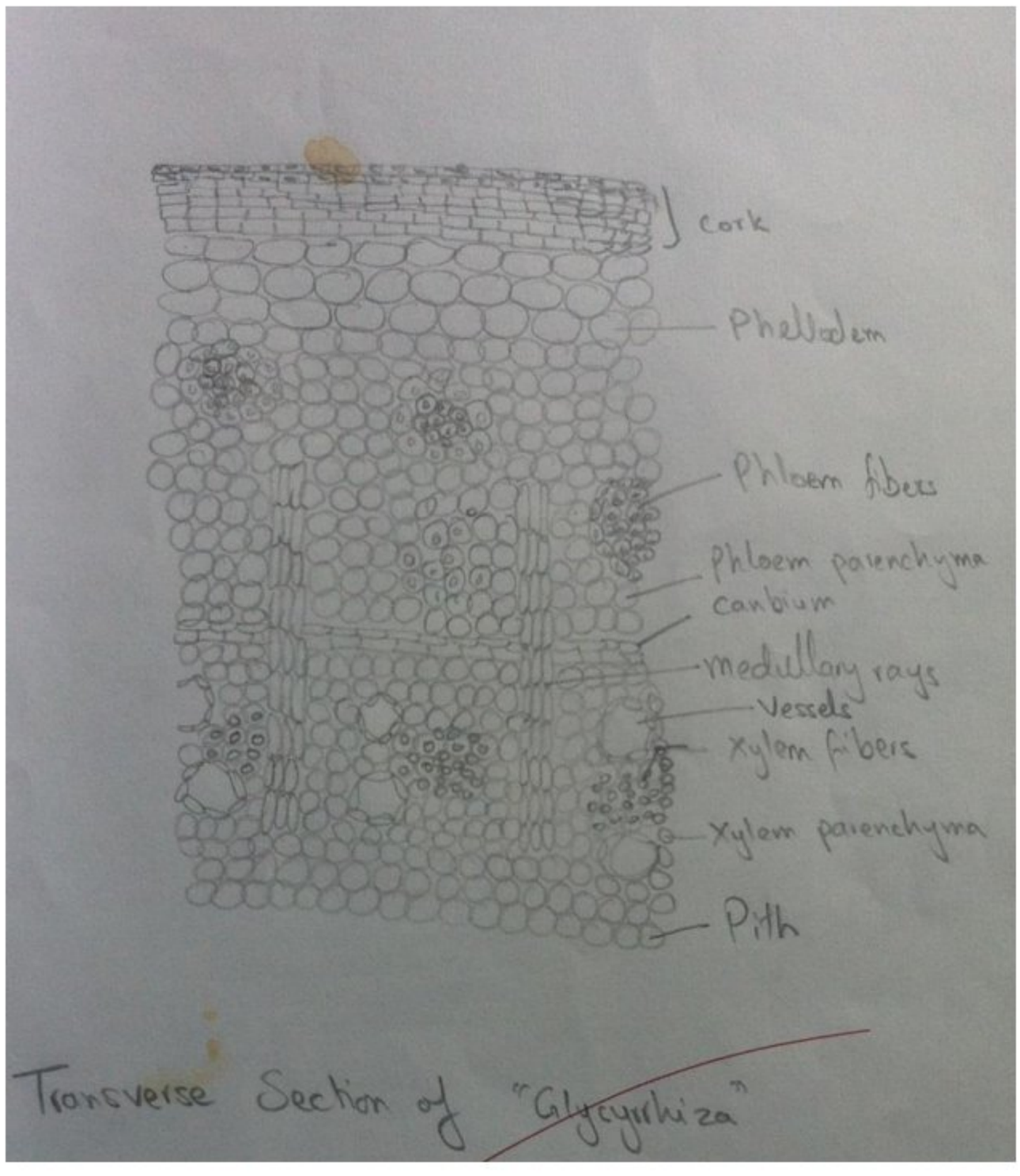
Alternate hard and soft bast patches of phloem are present. Hard phloem fibers are thick walled and lignified while soft bast consist of phloem parenchyma

Xylem

Broad zone of secondary xylem is present below the cambium consisting of large xylem vesels, wood fibers and wood parenchyma. Xylem tissues are serpated by medullary rays

Pith

Pith is central region of the rhizome consisting of parenchymata cells.



Microscopic Evaluation of Powder Drugs

PHARMACOGNOSY LAB.

COURSE ID. PHMD4121

MICROSCOPIC EVALUATION OF POWDER DRUGS

DATE:_____ **DRUG:**_____

Pharmacognostic Features of Drug	Main Diagnostic Features	Other Features
Scientific Name: _____ Family: _____ Local Name: _____ English Name: _____ Part Used: _____ Constituents: _____ _____ Actions and Uses: _____ _____		
Organoleptic Features		
Colour: _____ Odour: _____ Taste: _____		

CLOVE

Syzygium aromaticum (L.) Merrill and L.M. Perry — Myrtaceae
[(Eugenia caryophyllus) (C. Spreng.) Bullock and Harrison]

Caryophyllum, Cloves

A dark brown powder with a characteristic, spicy odour and an aromatic, pungent and slightly astringent taste.

The diagnostic characters are:

(a) The abundant fragments of the *hypanthium in surface view.* The *epidermis* is composed of small, polygonal cells with slightly thickened walls; large, almost circular *anomocytic stomata* are fairly numerous; the underlying tissue contains abundant, very large, brown, ovoid *oil glands* and occasional cluster crystals of calcium oxalate. Fragments of the hypanthium also occur in sectional view and these show the presence of a very thick *cuticle.*

(b) The very abundant yellowish-brown *parenchyma of the hypanthium* in which the oil glands are embedded; the cells are frequently unevenly thickened and appear collenchymatous; they contain numerous small cluster crystals of calcium oxalate.

(c) The *cluster crystals of calcium oxalate,* which are abundant in the parenchymatous tissue but are rarely found scattered; they vary in size and are usually composed of a large number of small, sharply pointed components.

(d) The occasional *fibres,* which are found singly or in groups of two or three cells; they are rather short and broad with bluntly pointed ends which are occasionally notched; the walls are lignified, usually strongly thickened and show faint striations and few pits; the lumen is sometimes filled with brown contents. These fibres may be found associated with small groups of vessels or with parenchymatous cells.

(e) The fragments of the *filaments of the anthers.* In surface view the epidermis is composed of longitudinally elongated cells with thin, slightly sinuous walls and a striated cuticle. In sectional view the fragments show the presence of a central vascular strand containing small, lignified *vessels* with spiral or annular thickening; the thin-walled parenchymatous tissue underlying the epidermis contains numerous cluster crystals of calcium oxalate, particularly in the cells adjacent to the vascular strand; occasional oil glands occur embedded in the parenchyma.

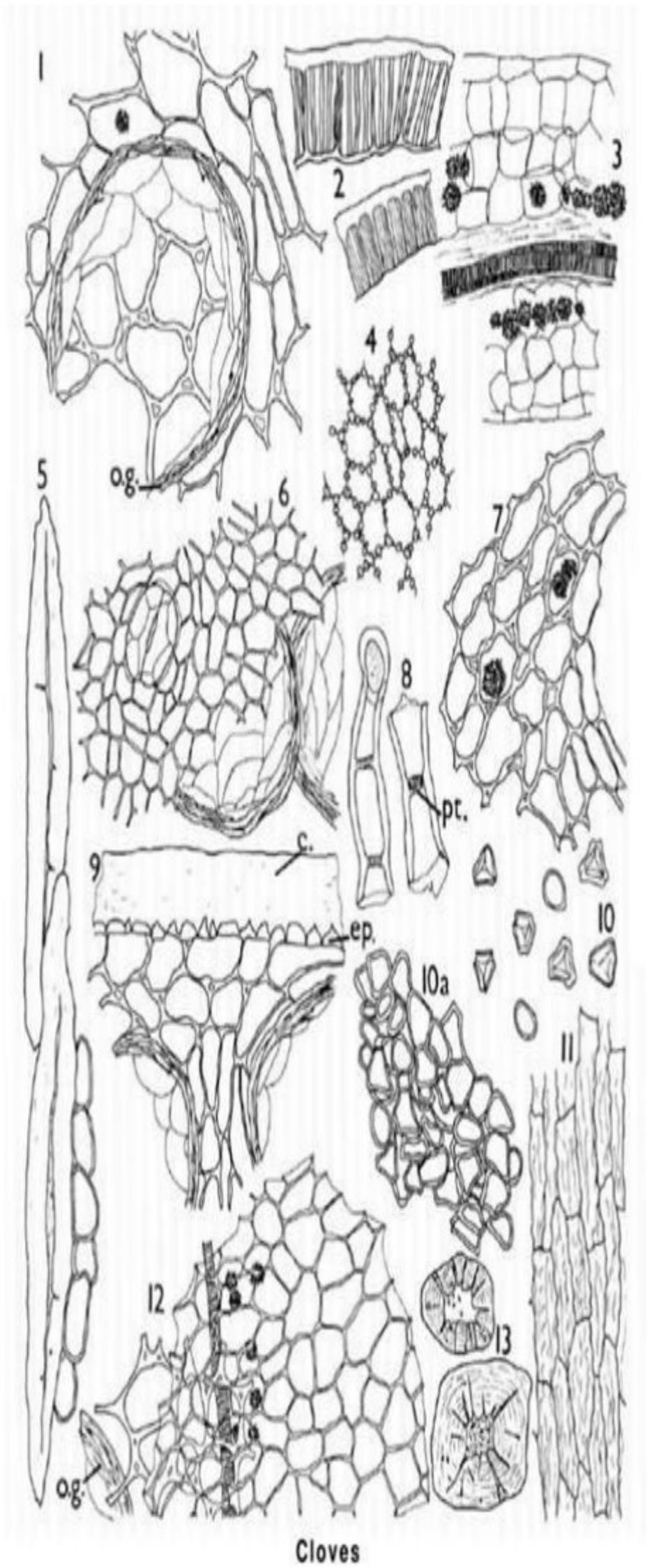
(f) The fragments of the *fibrous layer of the anther* composed of rather small cells; in sectional view the lignified thickening on the side walls of the cells appears as closely packed longitudinal bands and these are seen as small beads in surface view.

(g) The abundant *pollen grains* which are small, biconvex with a rounded, triangular outline and a smooth exine. A number of immature pollen grains also occur and these may be found in closely packed masses, frequently enclosed in the pollen sacs.

(h) The fragments of the *petals in surface view.* The epidermis is composed of slightly thickened, polygonal cells, larger than those of the hypanthium and stomata are absent. The underlying tissue consists of parenchymatous cells containing cluster crystals of calcium oxalate with occasional oil glands and small groups of vascular elements.

(i) The occasional fragments of the *aerenchyma of the hypanthium* composed of chains of two or three parenchymatous cells with moderately thickened walls; the contiguous walls of adjacent cells are traversed by numerous very small pits.

(j) The very occasional *sclereids* from the stalk; they are oval to subrectangular in outline with strongly thickened and striated walls which have numerous simple or branched pits; the lumen is frequently filled with brown contents.



Cloves

x330

1 Parenchyma of the hypanthium showing an oil gland (o.g.).
2 Fibrous layer of the anther in sectional view.
3 Part of the filament of the anther in longitudinal section, showing the central vascular strand and parenchymatous cells containing cluster crystals of calcium oxalate.
4 Fibrous layer of the anther in surface view.
5 Fibres and associated parenchymatous cells.
6 Epidermis of the hypanthium in surface view showing a stoma and underlying oil glands.
7 Parenchyma of the hypanthium with cluster crystals of calcium oxalate.
8 Fragments of aerenchyma from the hypanthium
9 Part of the hypanthium in sectional view showing the thick cuticle (a), epidermis (ep.) and underlying parenchyma with oil glands.
10 Mature pollen grains.
10a Part of a group of immature pollen grains.
11 Epidermis of the filament of the anther in surface view.
12 Epidermis of a petal in surface view with underlying tissue composed of parenchymatous cells containing cluster crystals of calcium oxalate, part of an oil gland (o.g.) and part of a vascular strand.
13 Sclereids from the stalk.

LIQUORICE

Glycyrrhiza glabra and varieties of *Glycyrrhiza glabra* — Leguminosae

Liquorice Root

A pale, yellowish-brown powder with a faint, characteristic odour and a sweet taste.

The diagnostic characters are:

(a) The abundant *starch granules*, most of which are simple; they are rather small, spherical to ovoid and slightly flattened; a slit-shaped hilum is visible in some of the larger granules. A few compound granules are present with two, three or four components.

(b) The very abundant *fibres* which occur in groups surrounded by a calcium oxalate prism sheath. Individual fibres are very thick-walled with few, small pits; the different layers in the walls are sometimes clearly seen and only the middle lamella and primary wall give a reaction for lignin; frequently no lumen is visible.

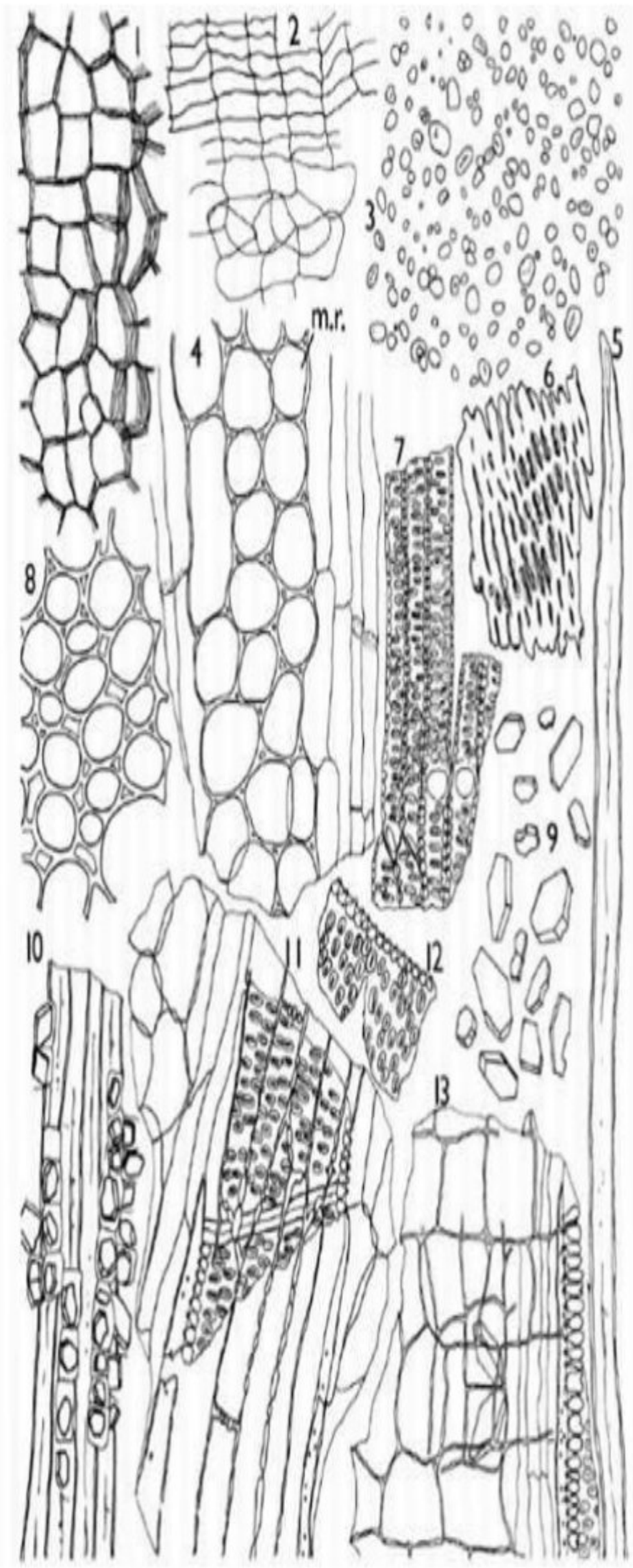
(c) The *vessels*, which are found singly or in small groups; some of the individual vessels are very large and are frequently found fragmented. They are lignified and usually bordered pitted, although in some of the larger vessels the pits are very much elongated and the borders are difficult to discern. Many of the smaller, narrower vessels show a single perforation in the somewhat oblique end walls. The larger vessels are usually accompanied by lignified xylem parenchyma composed of moderately thin-walled cells, square to elongated rectangular in outline with variably pitted walls.

(d) The *prisms of calcium oxalate*, the majority of which are fairly uniform in size and occur in the cells forming the crystal sheath surrounding the fibres. In addition a few larger prisms occur; they are present in some of the parenchymatous cells of the medullary rays and pith and may be found in these cells or, more usually, scattered in the powder.

(e) The fairly abundant fragments of orange-brown *cork* composed of thin-walled cells; in surface view the cells are polygonal and fairly regular in outline.

(f) The abundant thin-walled *parenchyma from the cortex, medullary rays* and *pith*, the cells vary from rounded to rectangular in outline and are usually filled with starch granules. Occasional groups of sieve tissue, composed of very thin-walled cells with faint sieve areas, may be found associated with the medullary rays. A small amount of collenchyma is also present.

Liquorice powder prepared from the *peeled drug* is more yellowish in colour and contains very infrequent cork fragments.



Liquorice

x330

1 Cork in surface view.
2 Part of the cork and cortex in sectional view.
3 Starch granules.
4 Part of a medullary ray (m.r.) in tangential longitudinal section with associated sieve tissue.
5 Part of a single fibre.
6 Fragment of a large vessel with elongated pits.
7 Part of a group of smaller vessels with bordered pits.
8 Collenchyma from the cortex.
9 Prisms of calcium oxalate.
10 Part of a group of fibres with incomplete calcium oxalate prism sheath.
11 Lignified xylem parenchyma with part of an underlying bordered pitted vessel and adjacent thin-walled parenchyma.
12 Fragment of a bordered pitted vessel.
13 Part of a medullary ray in radial longitudinal section with underlying thin-walled parenchyma and part of a bordered pitted vessel.

CINNAMON

Cinnamomum zeylanicum Lauraceae

Cinnamon Bark, Ceylon Cinnamon

A reddish-brown powder with a characteristic, pleasant and aromatic odour and taste.

The diagnostic characters are:

(a) The abundant *sclereids*, which occur singly or, more frequently, in small groups; they show considerable variation in size and shape but are usually more or less isodiametric; the walls of most of the cells are moderately thickened and often the outer wall is less thickened than the others; occasional cells have very thick walls with a small lumen; pits are numerous and conspicuous, and striations are usually visible.

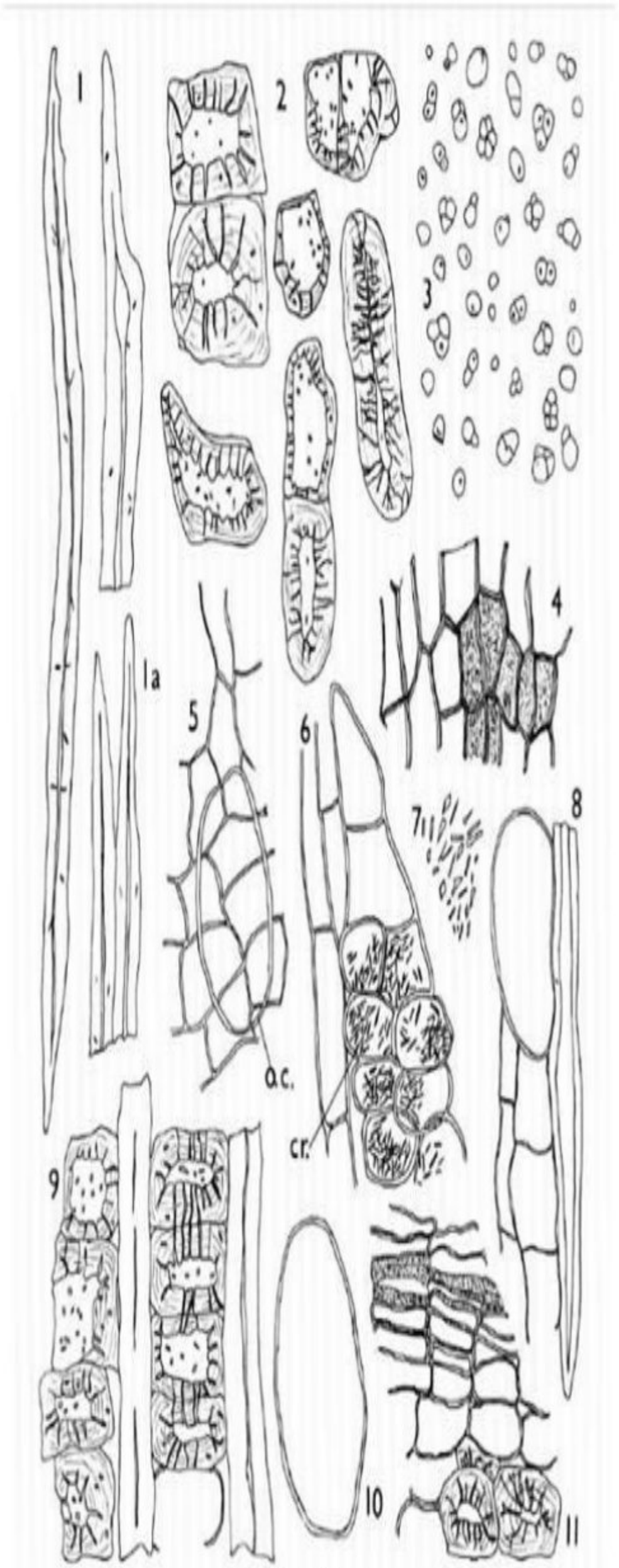
(b) The abundant *fibres*, which usually occur singly; they are thick-walled and lignified with a small, somewhat uneven lumen and few, inconspicuous slit-shaped pits. Occasional fibres are found associated with the sclereids of the pericycle; others occur associated with the oil cells and the parenchyma of the phloem.

(c) The abundant *starch granules*, which are found scattered and in the parenchymatous tissues and in some of the sclereids; they are rather small, simple or compound with up to four or more components; a rounded or slit-shaped hilum is visible in some of the larger granules.

(d) The thin-walled *oil cells*, frequently found associated with the parenchyma or fibres of the phloem; the cells are large, ovoid, and usually occur singly.

(e) The thin-walled *parenchyma* and *medullary rays* of the phloem; the medullary ray cells frequently contain numerous small, *acicular crystals of calcium oxalate*.

(f) The very occasional fragments of *cork*. In surface view the cells are thin-walled and polygonal; in sectional view occasional fragments show the cell layers arranged in alternating bands of thin-walled cells and thicker-walled, rather indistinct, lignified cells.



Cinnamon

1 Fibres.
1a Part of a small group of fibres.
2 Sclereids.
3 Starch granules.
4 Cork in surface view.
5 Phloem parenchyma and an oil cell (o.c.).
6 Part of a medullary ray with some of the cells containing acicular crystals of calcium oxalate (cr.), and associated phloem parenchyma in tangential longitudinal section.
7 Calcium oxalate crystals.
8 Part of a fibre with an associated oil cell and phloem parenchyma.
9 Part of a group of fibres and sclereids from the pericycle.
10 A single oil cell.
11 Part of the cork and cortex in sectional view.

HENNA

Lawsonia inermis L. (*Lawsonia alba*) Lythraceae

Henna Leaf

A yellowish-green powder with a slight odour and a faintly bitter taste.

The diagnostic characters are:

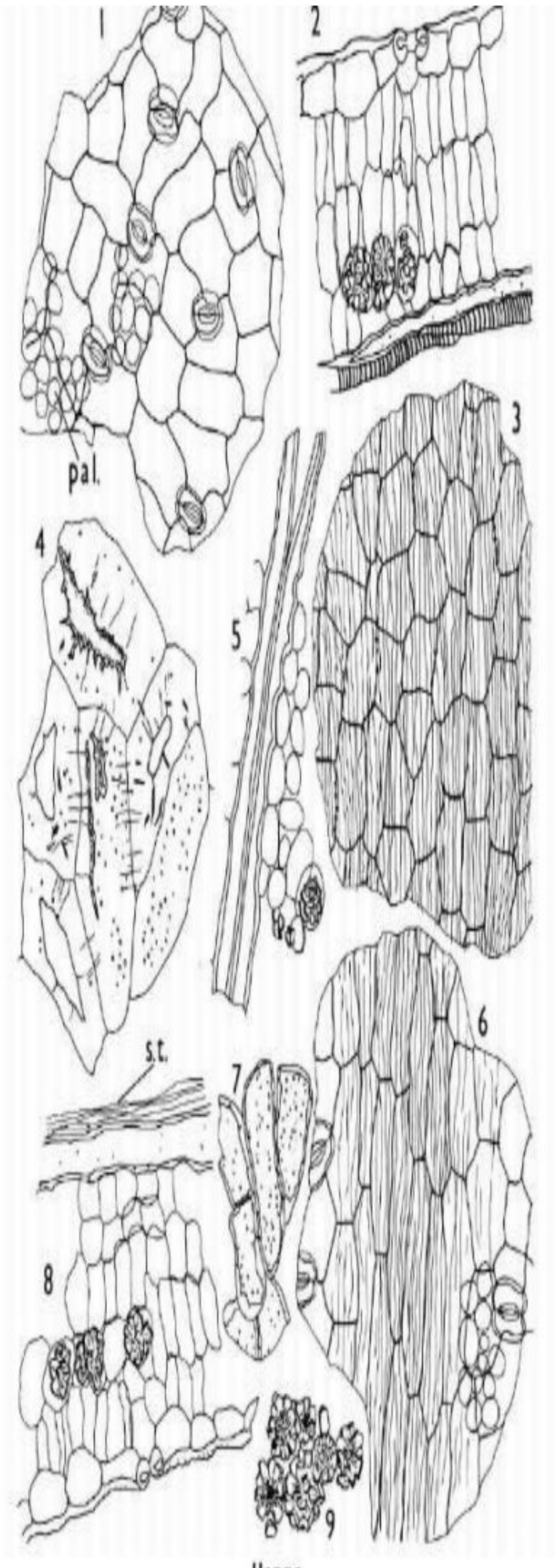
(a) The fragments of the *lamina in surface view*. The leaf is isobilateral and both *epidermises* are similar in appearance being composed of polygonal cells with thin, straight or slightly sinuous walls which may occasionally show slight thickening at the corners; *anomocytic stomata* are fairly numerous on both surfaces. On the lower surface the cells of the epidermis over the veins are more elongated and have a striated cuticle. Fragments of the *epidermis of the petiole* also occur in which the cells are similar to those of the epidermises of the lamina but the walls are slightly beaded; the *cuticle* is strongly striated and stomata are absent.

(b) The *cluster crystals of calcium oxalate*, which are found scattered and in the cells of the spongy mesophyll; they are fairly large and sometimes show a dense brown centre.

(c) The lignified *fibres* from the pericyle of the midrib and larger veins; they occur in small groups and are moderately thick-walled with few pits; the outer fibres in a group frequently show distinctly dentate walls.

(d) The fragments of the *lamina in sectional view* showing the two-layered palisade under both epidermises; the palisade cells under the lower epidermis are slightly shorter than those under the upper epidermis. Many of the cells of the spongy mesophyll contain cluster crystals of calcium oxalate.

(e) The occasional *sclereids* from the pericarp of the fruits; these occur in groups and are of two types—large and very thick-walled with simple or branched pits—or smaller with thinner walls and numerous simple pits.



Henna

1 Epidermis of the lamina in surface view with anomocytic stomata and part of the underlying palisade (pal.).

2 Part of the lamina in sectional view showing the upper epidermis, underlying two-layered palisade and spongy mesophyll cells containing cluster crystals of calcium oxalate.

3 Epidermis of the petiole in surface view showing cuticular striations.

4 Large, thick-walled sclereids from the pericarp.

5 Pericycle fibres and adjacent mesophyll cells containing cluster crystals of calcium oxalate.

6 Lower epidermis of the lamina in surface view showing anomocytic stomata, underlying palisade cells and the region over a vein with cuticular striations.

7 Smaller, thinner-walled sclereids from the pericarp.

8 Part of the lamina in sectional view showing the lower epidermis, palisade, spongy mesophyll with cluster crystals of calcium oxalate, part of a fibre and crushed sieve tissue (s.t.).

9 Cluster crystals of calcium oxalate.

NUX VOMICA

L *Strychnos nux-vomica* Loganiaceae

Nux Vomica Seeds

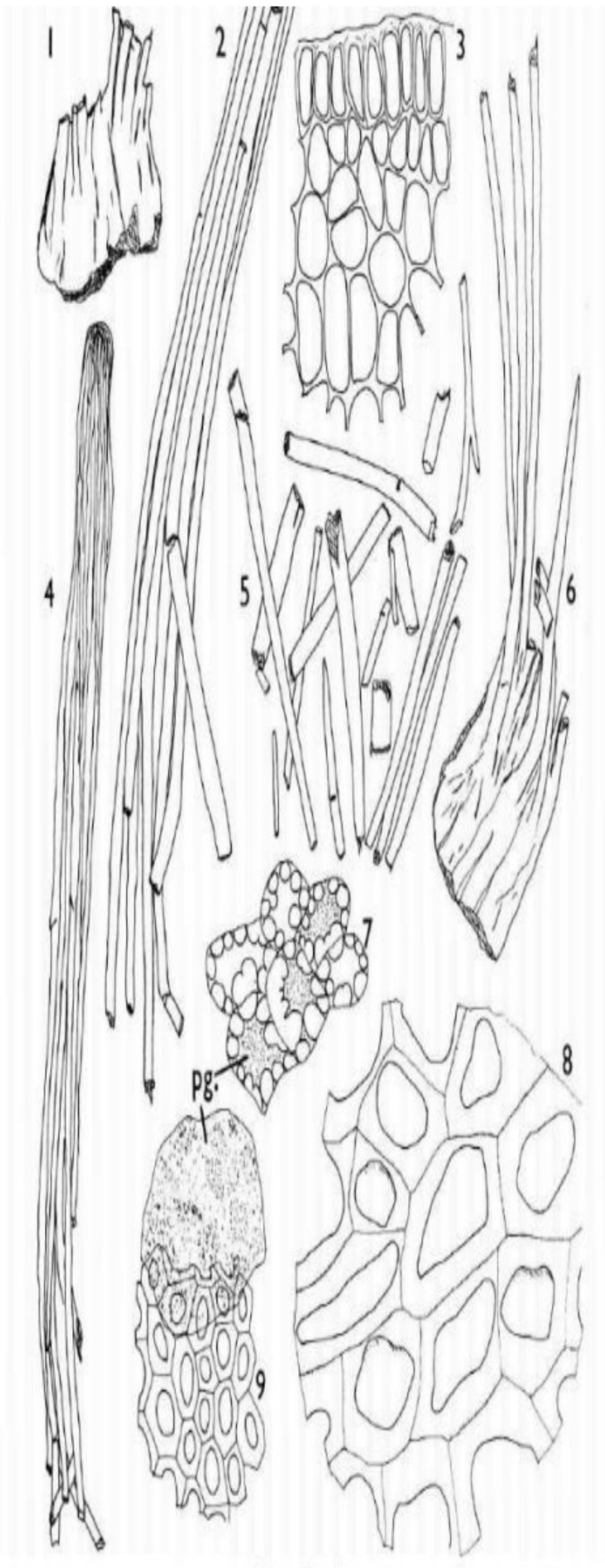
A yellowish-grey to brownish-grey powder with a slightly fatty and rancid odour and an intensely bitter and persistent taste.

The diagnostic characters are:

(a) The *sclerenchymatous epidermis of the testa* composed of a single layer of yellowish-brown cells each of which is extended to form a trichome; the walls of the epidermal cells are strongly thickened and pitted and each trichome has about ten narrow, lignified rods running longitudinally; the trichomes are usually broken off and the broken ends of the lignified rods are seen attached to the epidermal cells. This layer is nearly always found in side view; very occasional fragments are seen in surface view with the almost circular bases of the lignified rods of the trichomes visible around the periphery of each cell.

(b) The very abundant fragments of the *lignified rods of the trichomes;* they are cylindrical and vary considerably in length and thickness. Occasionally more complete fragments of the trichomes are found, composed of up to about ten lignified rods aggregated to form a cylindrical structure

(c) The abundant fragments of the *endosperm.* Those from the outer layer are composed of small thick-walled cells, polygonal in surface view and slightly elongated radially in sectional view; these fragments are often found associated with the pigment layer of the testa, composed of a layer of rather indistinct cells containing orange to brown pigment. The greater part of the endosperm is composed of large cells with very thick walls and a small lumen; occasional cells show faint plasmodesmata in the walls.



Nux Vomica

1 Part of the sclerenchymatous epidermis of the testa in side view, with broken-off remains of the trichome rods attached.
2 Part of a large trichome.
3 Outer part of the endosperm in sectional view.
4 An almost complete trichome showing the rounded apex.
5 Fragments of trichome rods.
6 Part of the sclerenchymatous epidermis of the testa in side view, with parts of the trichomes attached.
7 Sclerenchymatous layer of the testa in surface view showing the bases of the lignified rods and pigment (pg.) in some of the cells.
8 Endosperm cells from the central region.
9 Outer layer of the endosperm in surface view, with associated pigment layer of the testa (pg.).

Microbiology

To Study Parts of Microscope

Microbiology

The study of of organisms of microscopic size (microorganisms) including their culture, economic importance and pathogenicity etc.

Microbes

Any microscopic organism, an microorganism e.g. bacteria, fungi, virus, algae and protozoa etc.

Theory

It is an important scientific tool which is used for studying small objects that are not visible to naked eyes. The resolution power of a microscope is its extent to which it can make information or details of an object clearly visible. Different microscopes have different resolution power.

Types of Microscope

There are four types of microscope

- Simple microscope
- Compound microscope
- Dissecting microscope
- Electron microscope

Simple Microscope

It is modified form of hand lens, consisting of a convex lens like magnifying glass. It was invented by Anton van Leevwenhoek.

Compound Microscope

It consists of two lens. It was invented by Galileo Galilei. Its resolution power is 2mm.

Dissecting Microscope

It is also a high power microscope with special arrangements. Dissections of very small objects can be seen under this microscope.

Electron Microscope

It is used to see very small object in which electron beams is used to produce image. It can magnified object to 50,000 X times and in some cases 500,000 X times. It is used to study the structure of cell organelles. Its resolving power is 2-4 Å.

Parts of Microscope

It has two main parts

- The Stand
- The Body

The Stand

It is further divided into two parts

Base/Foot

It is horse-shoe shaped and made up of some heavy metal. It supports the entire weight and keeps microscope in standing position.

Pillar/Arm

It is curved and metallic; and connects the foot to main part. It is used to support the microscope when carried and holds the body tube, nose piece and objective lens

The Body

It is further dived into following parts

Body Tube

It is long metallic tube which maintains a proper working distance between eye piece and objective and can be drawn up and down. It holds the objective lenses and the ocular lens at the proper distance

Ocular Lens/ Eye Piece

It is a combination of two convex lenses on the upper end of the draw tube. It has different power i.e. 5X, 10X, 15X and magnified the image up to 5, 10, 15 times respectively.

Resolving Nose Tube

It is projecting part present below the body tube. It can be rotated and thus allows interchange of objective lenses of different magnification

Objectives

These are two or three metallic tubes containing lenses of different magnification

Stage

It is a circular or square flat form supported by a stand. The slide can be placed on it

Stage Clips

Two clips are moveably fix on stage to firmly hold the slide in place

Diaphragm

It is attached below the stage hole and regulates the passing light through the object

Mirror

A moveable plane concave mirror fixed at some distance below the stage. In some microscope electric light is fitted instead of mirror.

Coarse Adjustment Knob

It moves the stage up and down for focusing image.

Fine Adjustment

This knob moves the stage slightly to sharpen the image.

Procedure

- Carry the microscope with one hand under the base while grasping the arm with the other hand
- Rotate the nose piece; and obtain proper objective lens
- Look through the eye piece and witch on the lamp
- Place slide on stage; center specimen over the space
- Locate specimen on low power using coarse adjustment knob
- Carefully switch to medium or high power

- Focus image with fine adjustment knob

APPLICATION

EDUCATION

It is widely used in education in scientific lab. In different fields to study the small objects which are not seen y naked eyes.

HEALTH RESEARCH

The study of different disease and the biological materials which are the reason for their cause are due to microscope.

BOTANIST

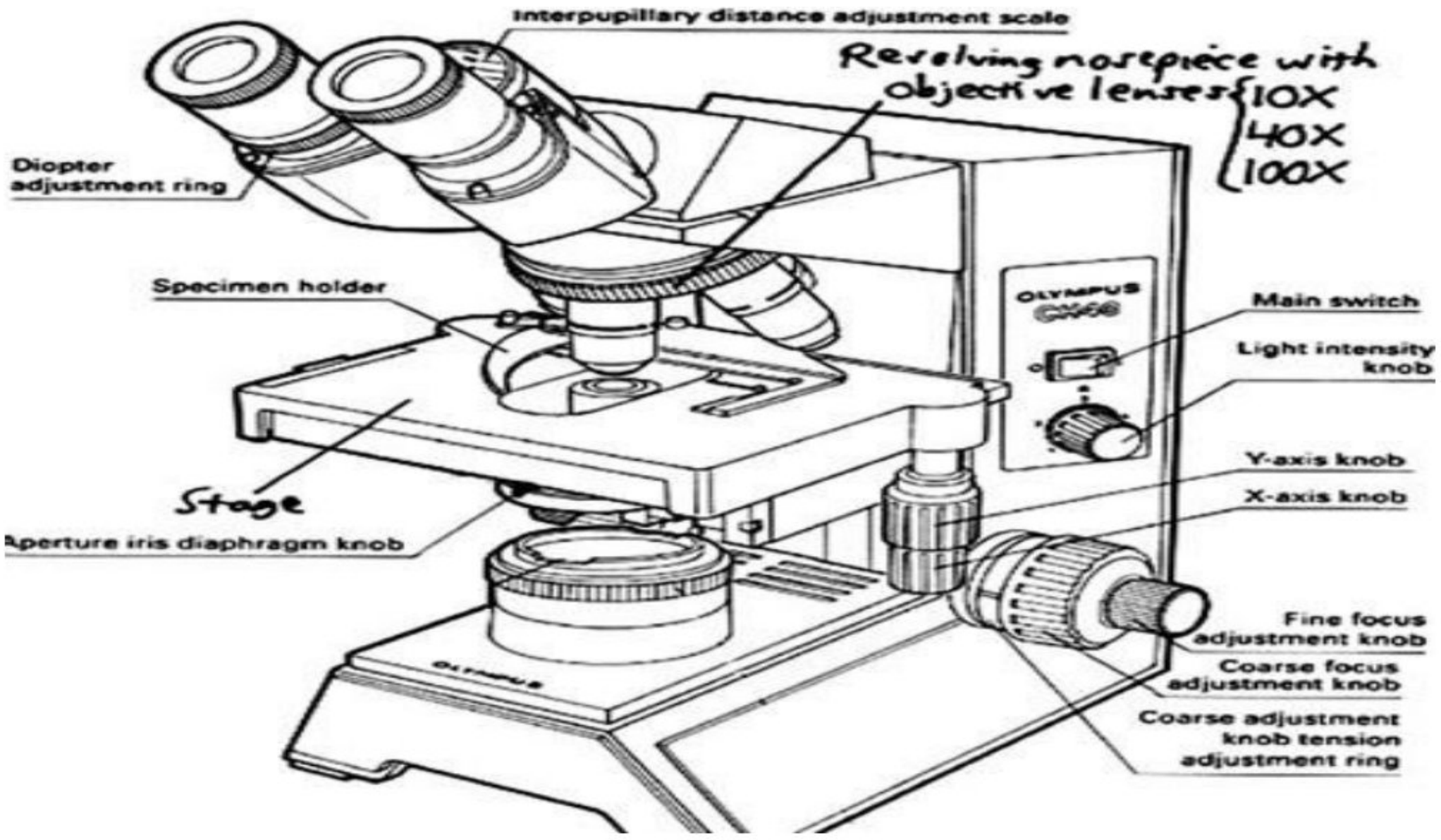
Botanist use microscope for the study of different section of plant.

FORENSIC

In forensic science microscope is of common use. It used in the study of small materials which cannot be seen without an aided eyes.

MISCELLANEOUS

It is also used in the study of genetic, tissue, cell and organ etc.



Simple Staining of Microbes of Buccal Cavity (In Between the Teeth)

Theory

The microbial flora of the buccal cavity varies from the individual to individual. A person suffering from a disease may show a disease causing microbe in their buccal cavity. However, each individual has certain types of microbes present in their buccal cavity.

These microbes are generally present and labeled "natural flora". The microbes which are present in harmonious manner without causing any disease, sickness or problem are called natural flora.

Apparatus

- Toothpick
- Microscopic slide
- Burner

Chemicals required

- Safranin red
- Crystal violet
- Methylene blue

Procedure

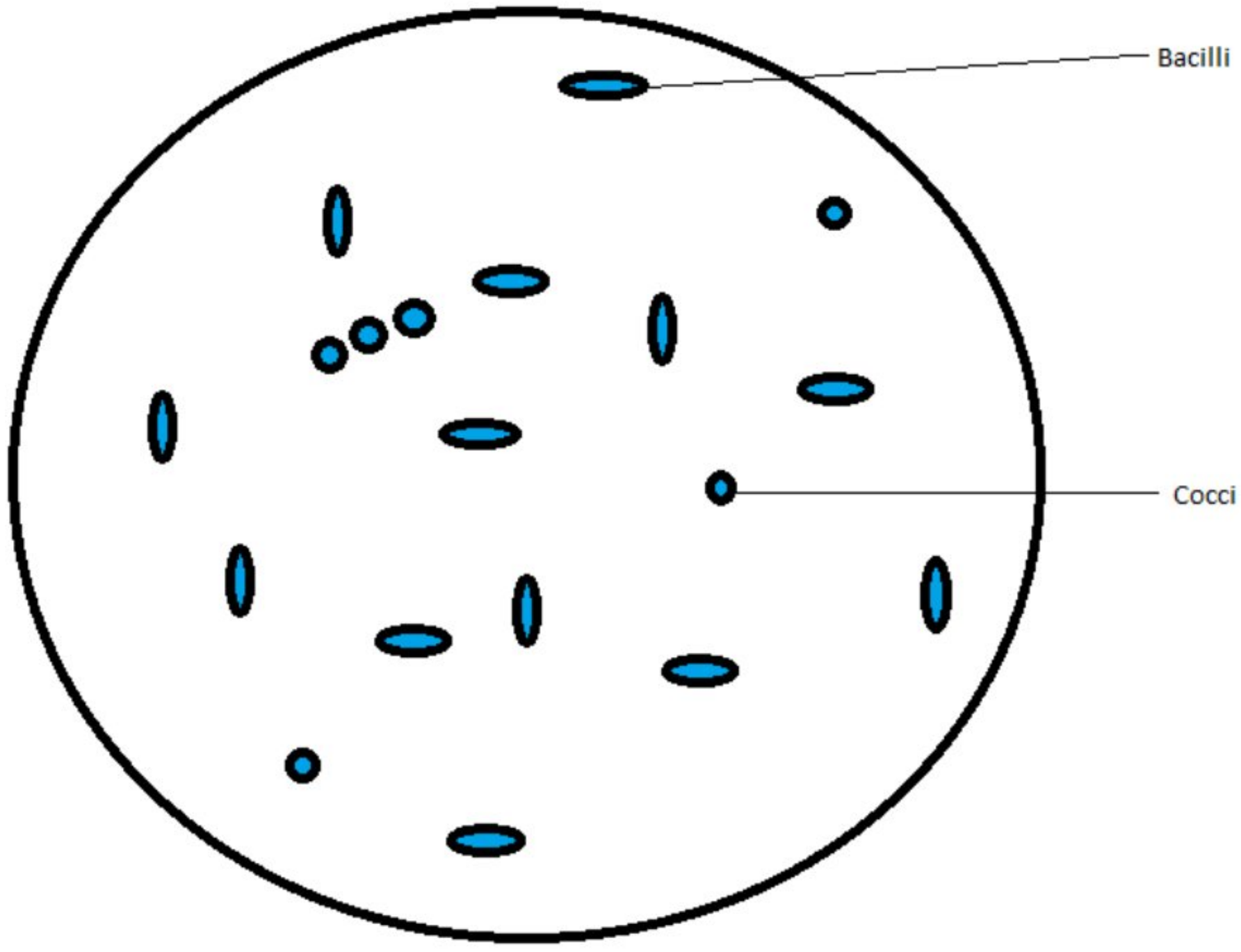
- Wash the slide
- Slide must be washed thoroughly twice or thrice by washing soda by rubbing
- Air dry the slide
- Slide must be passed on the flame frequently to remove the glaziness
- Mark the slide on one side with roll no. using permanent marker and fix it by passing on flame.
- Use toothpick to get sample from buccal cavity on the slide having a drop of water.
- Preparation of smear
- The sample is spreaded out on the slide evenly as thin as possible which is called smear
- Air dry the smear
- Fix the smear by passing the slide over flame
- Dip the slide in the given staining solution, for a minute
- Wash the slide immediately with distilled water
- Air dry and observe the fixed smear

Precautions

- Do not use contaminated toothpick
- Do not leave the slide on top of the flame for long
- Use blue flame of the burner
- Do not **blow** air to dry the slide
- Prepare the smear at one attempt (meaning…one you touch the wire loop to the slide, only turn it in one direction, and once you have raised, do not touch it to the smear again, that would result in layers forming in the smear which would decrease its clarity)

Result

Few clusters along with many single pair coccus and bacillus were observed after simple staining along with a few food particles.


Bacilli
Cocci

Simple Staining of Common Yeast

Theory

The fungi are heterotrophic microbes ranging from unicellular to multicellular macroscopic organisms. The most important species of the fungi include yeast, a unicellular having a number chromosomes varying from 8-16. At the time of reproduction, the number of chromosomes would be double. The way of reproduction also varies from asexual to sexual, thus most commonly budding and conjugation.

The fungi must be studied from top as well as bottom of the petri dish without inverting it.

Study of fungi is mycology and infection caused by it is called mycoses.

Importance and classification of yeast

True yeast

Most important and mostly used in daily life for preparation of baking products. Sometimes used as digestive agent. Its commercial preparation is available in markets. It can be produced in a media. The most common media used for the growth of fungi is known as Saborauds Dextrose Agar media. (SDA) when yeast is added, it is called (SDA+Y).

False yeast

The yeast that are pathogenic are commonly false yeast. Eg: Candida albicans

Bottom yeast:

Used particularly for preparation of low grade wine. Eg: beer

Top yeast:

Used for preparation of high grade wine

Requirement

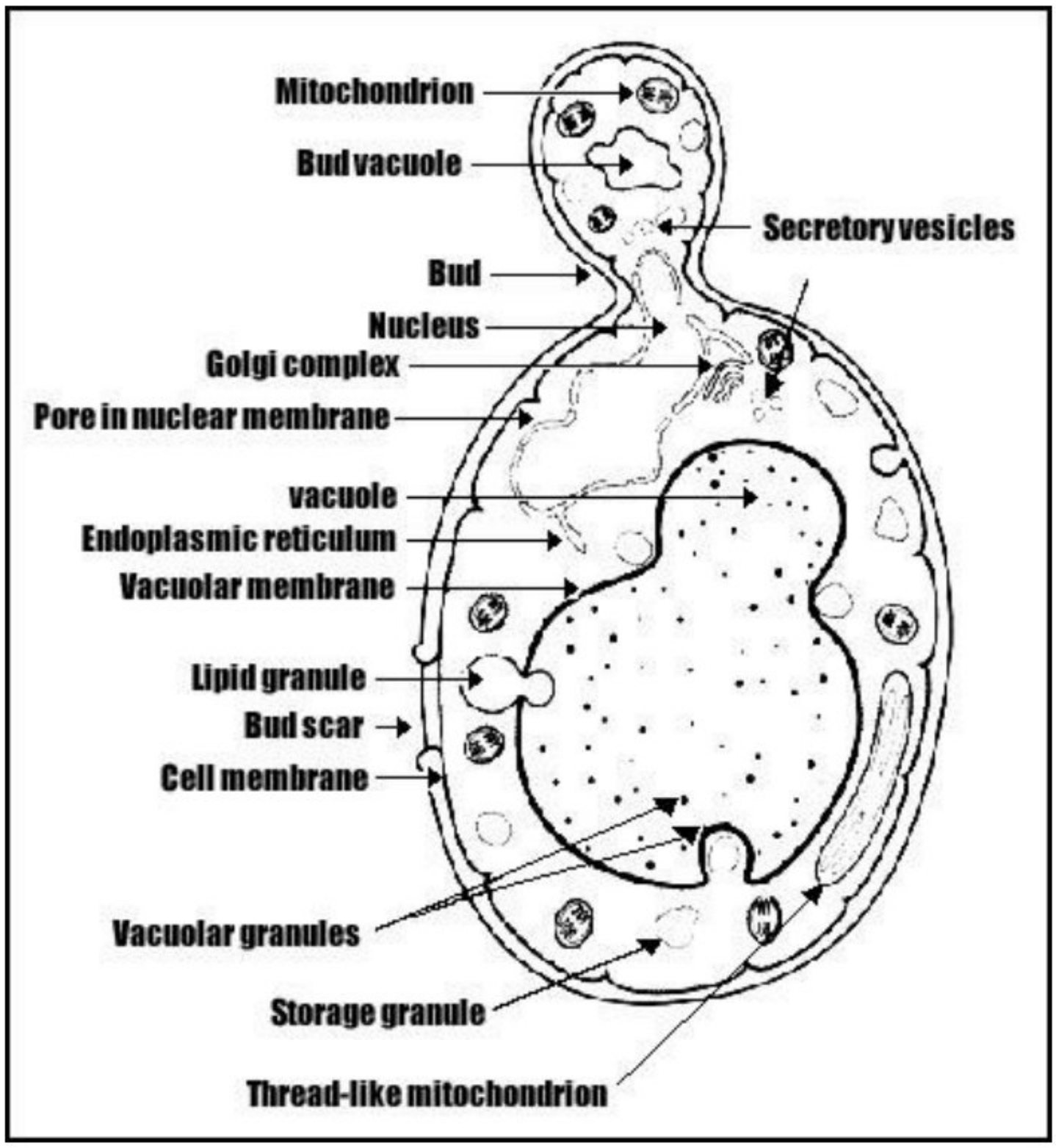
- Culture of yeast in the form of lyophilized granules
- Wire loop
- Burner
- Match box
- Slide
- Dyes

Procedure

- Wash the slide
- 1 or 2 granules of yeast is placed on a small drop of water on slide
- Wait for 3-5 mins
- Smear is prepared by minimum quantity of sample evenly spreaded out on an area of $1cm^2$ in one direction in one attempt
- Air dry the smear and remove the excessive granules which is present in the corner of the smear
- Fix the smear
- Simple stain the slide and observe

Result

Common yeast was observed under microscope with few having buds as well.


Mitochondrion
Bud vacuole
Secretory vesicles
Bud
Nucleus
Golgi complex
Pore in nuclear membrane
vacuole
Endoplasmic reticulum
Vacuolar membrane
Lipid granule
Bud scar
Cell membrane
Vacuolar granules
Storage granule
Thread-like mitochondrion

GRAM STAINING OF PURE CULTURE OF BACTERIA

APPARATUS

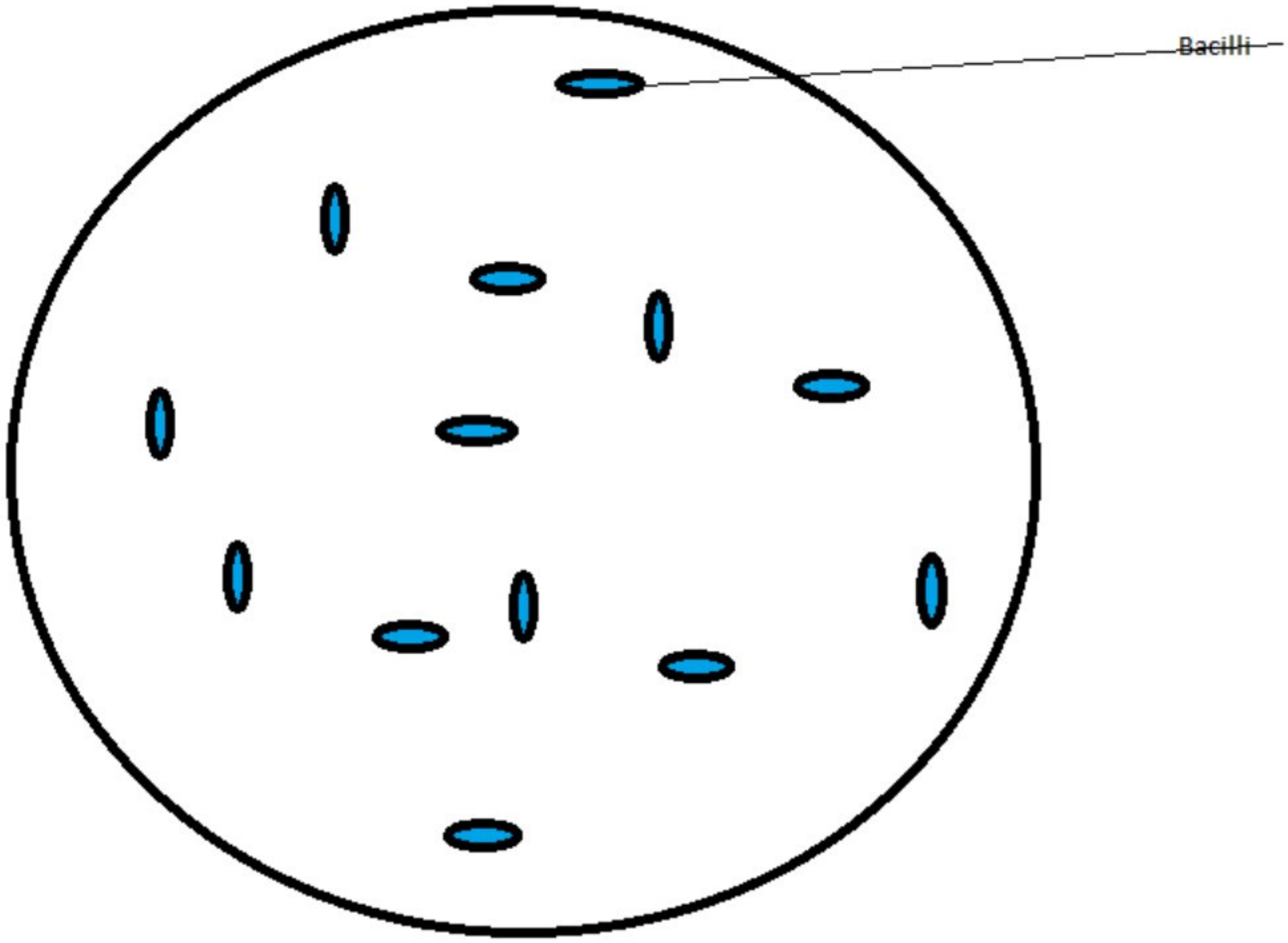
- Wire loop
- Burner
- Microscope
- Slide
- Marker
- Chemical dyes

PROCEDURE

- Wash the slide as described in previous experiment
- Put a drop of water on slide
- Prepare the smear by distributing minimum quantity of the sample evenly on an area of about $1cm^2$ in each direction in one attempt
- Air dry and fix the smear
- Gram stain the slide by following method:
- Dip the slide in violet solution for 1 minute
- Directly dip the slide in gram iodine solution for I minute and wash the slide in slow stream of water
- Dip the slide in decolourizer for 15 seconds or 3 dips and immediately wash the slide
- Dip the slide in safranin red for 1 minute
- Wash the slide
- Fix the slide and dry
- Observe it

RESULT

Gram positive bacillus was observed, few were new, most were more then a day old, so were stained purple.


Bacilli

To Observe the Motility of the Given Bacterial Culture (In Broth) by Hanging Drop Method

Theory

The bacteria which can move through their flagella are called motile and bacteria who have no flagella are called non-motile. Gram negative bacteria are mostly motile.

Flagella

Flagella arises from cytoplasm, just beneath the cytoplasmic membrane and cross up through cell wall. Their length is greater than the cell. Filament is composed of flagellum.

Classification

A motile bacteria can be classified according to number and position of flagella as following:

Monotrichous:

Bacteria having single flagella eg: *Vibrio Cholerae*

Amphitrichous:

Bacteria having single flagellum at each end of bacteria or cluster of flagella at both end. Eg: *Spirilum serpens*

Lophotrichous:

At one end of bacteria, there will be cluster of flagella. Eg: *Pseudomonas fluorescens*

Peritrichous:

The flagella are present throughout the body of bacteria. Eg: *Escherichia coli* and *Salmonella typhi*

Movement

The movement of bacteria comprises of two phases:

1. Run
2. Tumbling

Requirement

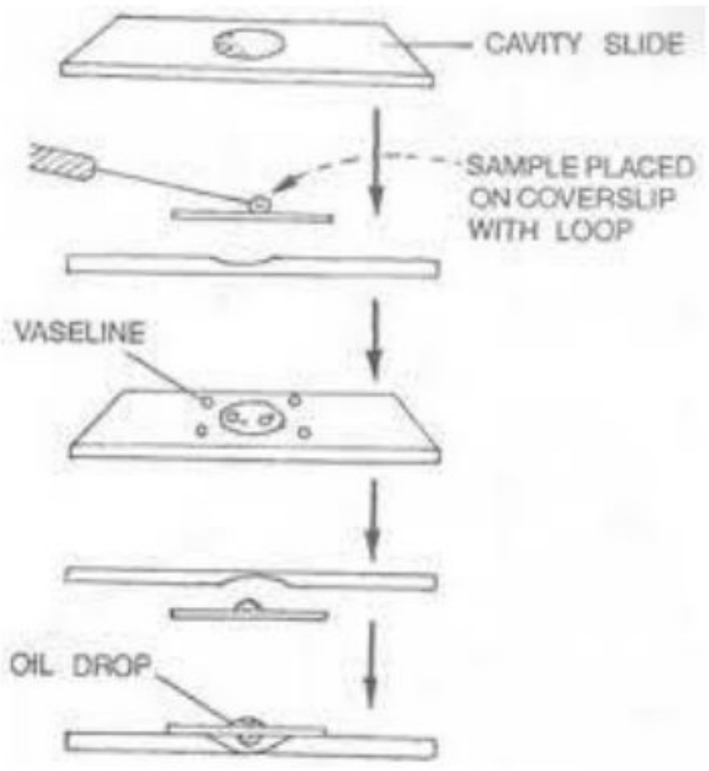
- Broth culture
- Safranin red
- Cavity slide
- Cover slip
- Wire loop

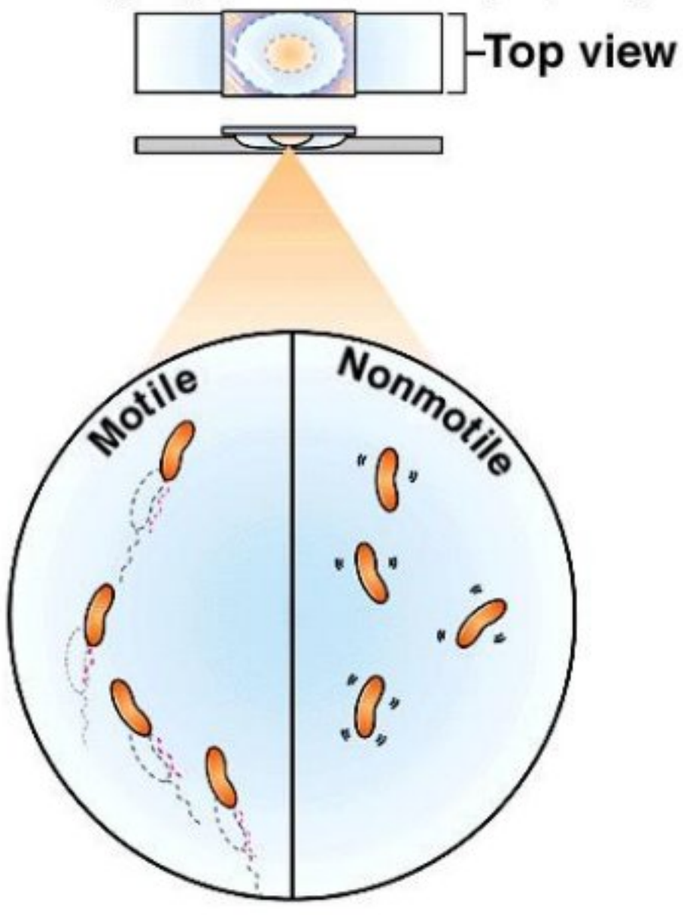
Procedure

- Take the cavity slide and make a boundary around the cavity by using soft petroleum jelly by means of a toothpick or match stick
- The boundary should be slightly raised and equally elevated
- After inverting the slide, place the slide very carefully on the cover slip, so that the boundary of slide attaches to cover slip.
- Invert the slide carefully again to its original position.
- The microbial preparation in the form of suspension is allowed to hang in the cavity.
- Observe it under microscope

Observations and Result

Some cells show no movement, some show jerky movement, some show Brownian movement, while a few cells move till the end of the microscopic field, proving to be motile.





NORMAL STAINING OF BACTERIAL SPORES

THEORY

Bacterial spores are resistant bodies which appear during normal life cycle of spore forming bacteria, however, the culture may show a large number of spores if these are not germinated because of depletion substrate.

Spores require fresh media/food to germinate directly into bacterial cell, thus the bacterial spore is considered as" vegetative" which means without reproduction. However, each and every bacterial cell will show the sporulation. Thus, the bacterial cell show reproduction and then spores.

On the other hand, fungal spores are considered as "reproductive" i.e prior to germination. The fungal spore divide into 4,6 or 8 cells. After reproduction, the fungal spore will show reproduction and in this way about 8 cells are produced.

The bacterial spores are usually formed and remain inside the cell and these are labeled as endospores. The spores are only liberated outside after destruction of cell, but the ideal condition of spore forming cell is bacterial cell having or showing spore inside.

REQUIREMENT

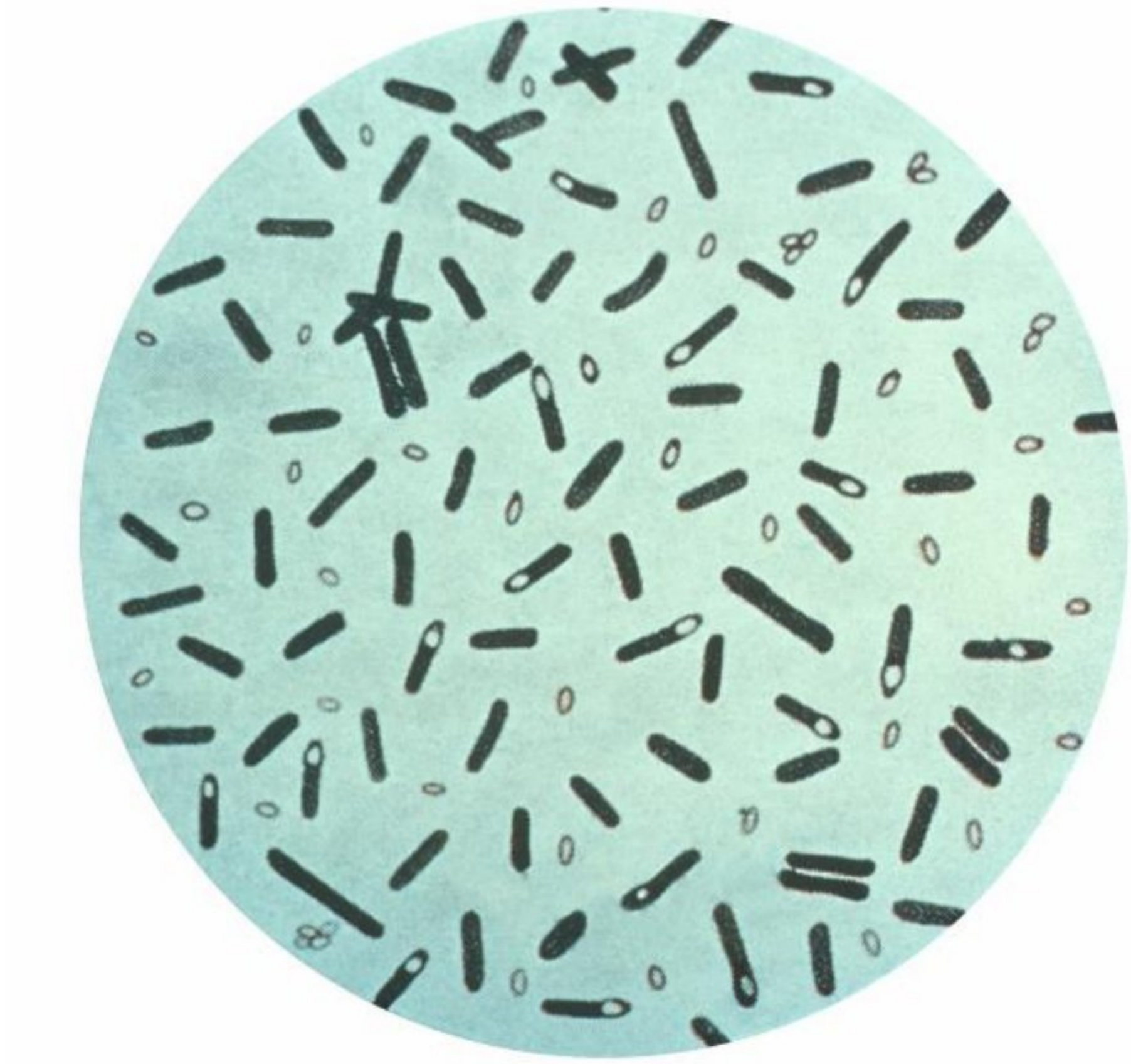
- Spore forming culture
- Microscope
- Wire loop
- Slide
- Burner
- Chemical dyes

PROCEDURE

- Smear is prepared by taking a loop full of broth on a clean, washed microscopic slide by normal method.
- Air dry and fix the smear.
- Pour and flood the slide placed on glass rod with malachite green.
- Heat the slide by passing flames for 10 minutes, atleast till the dye easily penetrates the spore.
- Let the slide cool down, and then pour 5% solution of glacial acetic acid (used as decolourizer) for 15-60 seconds.
- Immediately wash the slide with low stream of water.
- Apply safranin red for 1-3 minutes
- Finally wash the slide.
- Slide should be cleaned by decolorizer, leaving behind the smear intact.

RESULT

Green spores surrounded by red cytoplasm are observed under microscope.



Determination of the Length (Internal Dimension) of Given Bacteria

Theory

In order to determine the size of the bacteria, negative staining is performed. That is a technique through which the background is coloured and the organism should appear bright and colourless.

The dye is selected which cannot penetrate into bacterial cell wall or the cytoplasm, because the dye is negatively charged and known as acid dyes. (nigrosine black)

It is noted that the bacteria cell also pocess negative charge, so they repel the acidic dye and remain colorless. The dye, will be observed in the background.

If the internal dimension of the bacteria is to be determined, simple staining can be used, but simple staining does not cover the cell wall and capsule.

Requirement

- Broth of bacterial culture
- Ocular micrometer
- Stage micrometer
- Wire loop
- Slide
- Microscope
- Chemical dye

Procedure

- Ocular micrometer is cleaned and placed in eye piece
- Focus the ocular micrometer so it is visible clearly
- Place the stage micrometer on stage
- Calibrate the actual micrometer by the help of stage micrometer
- Wash, clean and air dry the microscopic slide
- Remove the glaziness
- Stain the wire loop and use it to take a loop full of broth
- Prepare the smear by using method, minimum quantity of the sample, evenly homogenously spread out on an area of about $1cm^2$ in one direction at one attempt
- Air dry the smear and focus the slide on the same microscopic power
- Calculate the size of the bacterial cell by help of divisions of ocular micrometer
- Find out the average size of the bacterial cell

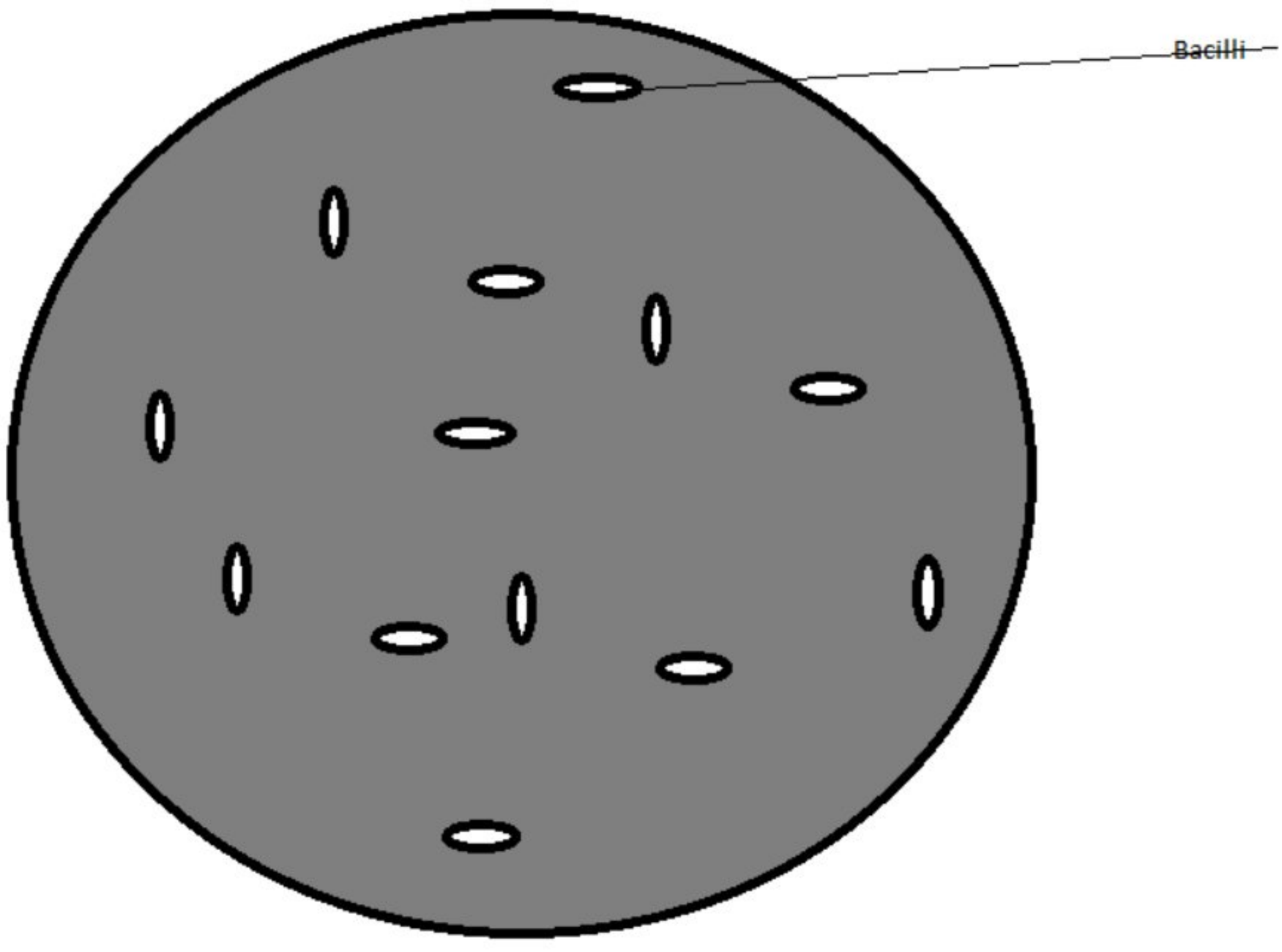
Observation and Calculation

Supposed Calculation

- 1 division of stage = 0.1mm = 100 um
- 40 Division of ocular = 1 divison of stage @ 40X
- 1 division of ocular = 1/40 divison of stage = 1/40 x 100 = 2.5 um
- 1 division of ocular micrometer = 2.5 um at high power (i.e. 40X)

Result

The average size of bacterial cell wall was 9.8 µm.


Bacilli

CAPSULE STAINING BY MANEVAL METHOD

THEORY

Capsules are the outermost covering or envelope of capsule forming bacteria, eg: *Streptococcus pneumoneae*. There are certain bacteria having no capsule and termed as non-capsulated bacteria. The normal composition of capsule is the presence of polusacharide.there may be one type of sugar throughout the capsule which means the capsule is made up of homo-polysacharide, eg*: Streptococcus mutans.* Capsules showing different types of sugars, show the presence of heteropolysacharides, eg: *Klebseilla pneumoneae*.

However, there are certain people having different chemical nature i.e polypeptide eg: *Bacillus anthracis*

In order to promote the capsule formation, milk may be added to the media.

The normal capsule is thick, conjusted and have a protective layer of the cell. It completely resists the phenomena of phagocytosis.

The presence of capsule is a sign of virulence. When capsule layer is loose, non conjusted, it will be known as slime layer. If capsule is thin layered, it is known as microcapsule.

METHODS

1. Maneval method
2. Anthony method
3. Welch method
4. Negative staining

REQUIREMENT

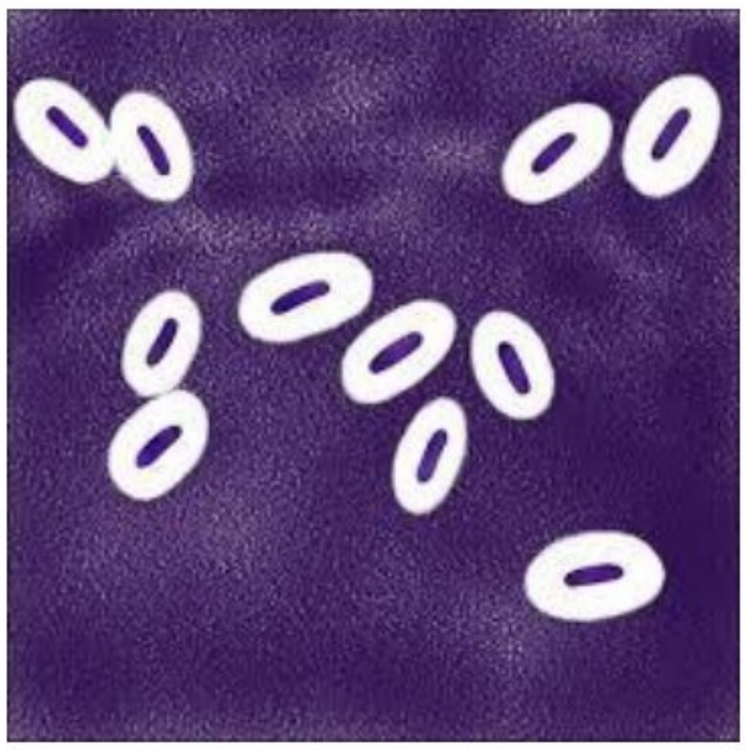
- Maneval solution
- 1% congo red solution
- Microscope
- Wire loop
- Dropper
- Given broth culture

PROCEDURE

- Wash, clean and air dry the slide
- Pen down, your roll no. on slide
- Place a small drop of congo red solution on slide
- Prepare smear directly in congo red solution, after staining the wire loop and taking a loop full of culture
- Smear should be prepared by the minimum quantity of the sample, evenly, homogenously spread out on an area of about 1cm^2 in one direction at one attempt
- Air dry the smear and avoid fixing of smear
- Dip the slide in manevals solution for 1-3 minutes
- Wash the slide very carefully, and clean up
- Air dry the slide and observe it under microscope

RESULT

The capsule of the given culture was observed under the microscope.



Preparation of Nutrient Broth

Theory

In order to grow the microorganism on a lab, a typical media is provided having proper nutrition value, so the desired organism could grow properly.

The beef extract is a complex substance containing almost all important required substances which are necessary for the growth such as carbohydrates, proteins, amino acids, vitamins and some lipids.

The media or a complex mass showing various types of ingredients in different concentrations, is labeled as complex mass or media. Peptone is a source of protein, for the proper growth, reproduction and easy division. Sodium chloride is a source which can be used for the growth of particularly marine microbes.

Ingredients

- Beef extract. 0005.0g
- Peptone. 0010.0g
- Sodium chloride. 0005.0g
- Distilled water q.s.. . . . . .1000.0g

Procedure

- Wash, clean and dry all the apparatus
- Weigh the amount of the ingredients as per needed and collect them in mortar
- Use the pestle and mortar to grind the ingredients into powder like fine particles
- Gradually add water with continuously mixing to obtain a thin paste
- Pour it into a flask and make up the volume by adding water
- Heat the solution with continuous stirring to make sure no particle remains undissolved
- Plug the cotton at the mouth of the flask in such a wau that the air can pass through but dust carrying microbes cannot.
- Best way to do it is to plug the cotton like a screw. Cotton should be tight enough to be able to lift the flask and loose enough to be able to rotate like a screw. (it probably take some practice to be done right)
- Autoclave the nutrient broth to sterilize it
- Nutrient broth is prepared, labeled and ready to use.

Result

Nutrient broth was prepared and labeled properly.

Preparation of Nutrient Agar

Theory

In order to grow the microorganism on a lab, a typical media is provided having proper nutrition value, so the desired organism could grow properly.

The beef extract is a complex substance containing almost all important required substances which are necessary for the growth such as carbohydrates, proteins, amino acids, vitamins and some lipids.

The media or a complex mass showing various types of ingredients in different concentrations, is labeled as complex mass or media. Peptone is a source of protein, for the proper growth, reproduction and easy division. Sodium chloride is a source which can be used for the growth of particularly marine microbes.

Agar is added as solidifying agent, because no bacteria can use agar as substrate till now. The concentration of agar varies from season to season. (15-25g)

Ingredients

- Beef extract. 0005.0g
- Peptone. 0010.0g
- Sodium chloride. 0005.0g
- Agar. 0020.0g
- Distilled water q.s. 1000.0g

Procedure

- Wash, clean and dry all the apparatus
- Weigh the amount of the ingredients as per needed and collect them in mortar
- Use the pestle and mortar to grind the ingredients into powder like fine particles
- Gradually add water with continuously mixing to obtain a thin paste
- Pour it into a flask and make up the volume by adding water
- Heat the solution with continuous stirring to make sure no particle remains undissolved
- Plug the cotton at the mouth of the flask in such a wau that the air can pass through but dust carrying microbes cannot.
- Best way to do it is to plug the cotton like a screw. Cotton should be tight enough to be able to lift the flask and loose enough to be able to rotate like a screw. (it probably take some practice to be done right)
- Autoclave the nutrient broth to sterilize it
- Nutrient broth is prepared, labeled and ready to use.

Result

Nutrient agar was prepared and labeled properly.

Negative Staining of Given Bacterial Culture

Theory

Negative staining is technique through which the background is coloured and the organism, should appear bright and colourless. The dyes are so selected which cannot penetrate into the bacteria cell or the cytoplasm, because the dye is negatively charge and known as acid dyes like sudan black or dorners nigrosine black.

It is noted that the bacteria cell also possess negative charge, so they repel acidic dye and remain colourless. The dye and its coloured will be observed in the background.

REQUIREMENT

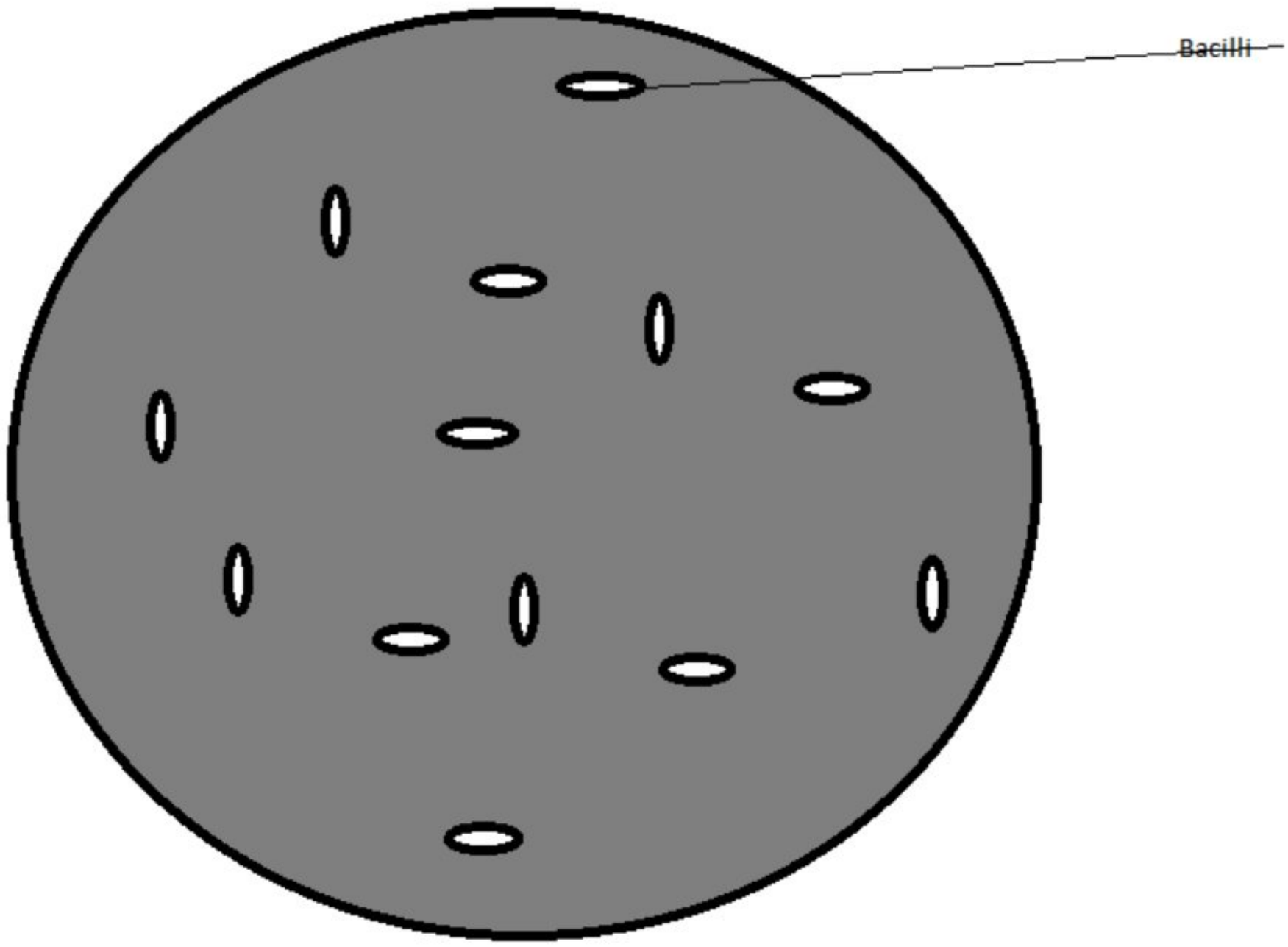
- Pure culture
- Wire loop
- Nigrosine black
- Burner
- Slide

PROCEDURE

- Wash the slide as mentioned in previous experiments (air drying really does work better)
- Fix the slide by passing on flame
- Pen down roll no. on one side of slide and fix it
- Stain the wire loop
- Place a small drop of nigrosine black on the slide
- Unplug the container containing the culture near the burner. Use wire loop to take the sample and prepare the smear directly on the nigrosine black
- Observe the slide under microscope

RESULT

Microbes were colourless, unstained while the background was coloured so that the microbes appear bright with dark background.



Capsule Staining by Dorner's Nigrosine Solution

Theory

Negative staining is technique through which the background is coloured and the organism, should appear bright and colourless. The dyes are so selected which cannot penetrate into the bacteria cell or the cytoplasm, because the dye is negatively charge and known as acid dyes like sudan black or dorners nigrosine black.It is noted that the bacteria cell also possess negative charge, so they repel acidic dye and remain colourless.

Requirements

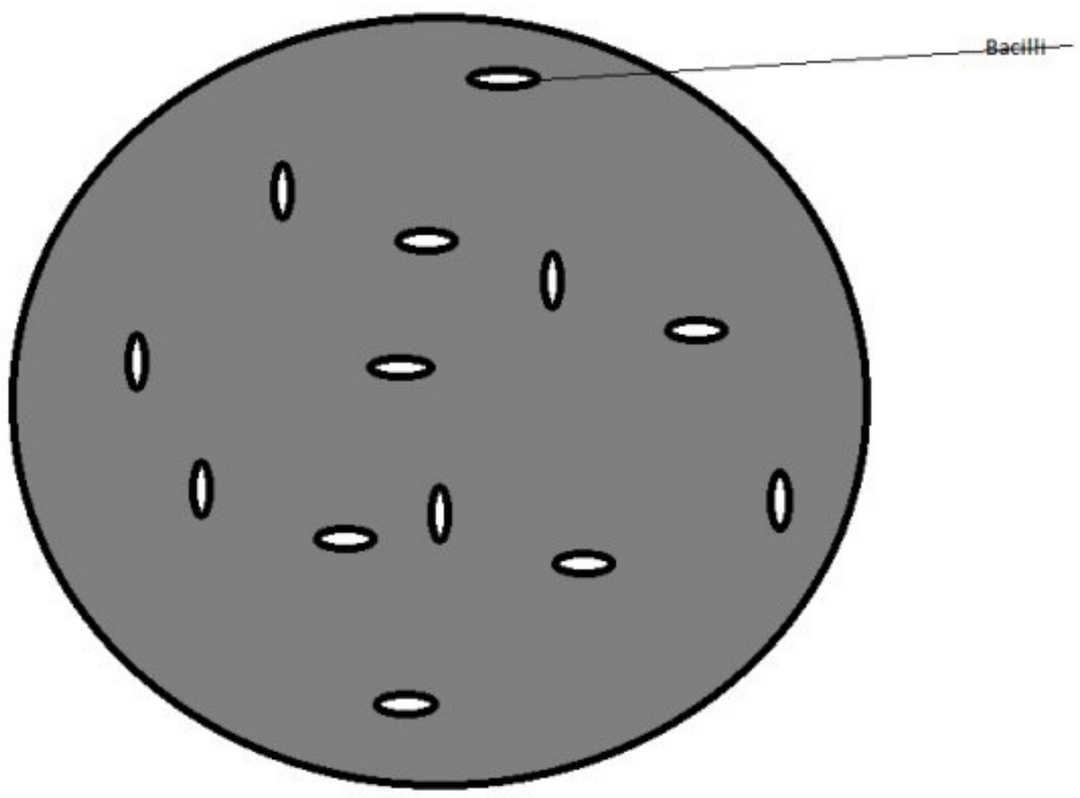
- Pure culture (solid)
- Wire loop
- Burner
- Slide
- Nigrosine black

Procedure

- Was the slide as mentioned in previous experiments
- Fix the slide, write roll no.
- Stain the wire loop
- Place a small drop of nigrosine black on the slide
- Unplug the container containing the culture near the burner. Use wire loop to take the sample and prepare the smear on the nigrosine black
- Observe it under microscope

Result

Microbes were colourless, unstained while the background was coloured so that microbes appear bright with dark background.



Capsule Staining by Anthony's Method

Theory

Capsules are the outermost covering or envelope of capsule forming bacteria, eg: *Streptococcus pneumoneae*. There are certain bacteria having no capsule and termed as non-capsulated bacteria. The normal composition of capsule is the presence of polusacharide.there may be one type of sugar throughout the capsule which means the capsule is made up of homo-polysacharide, eg*: Streptococcus mutans.* Capsules showing different types of sugars, show the presence of heteropolysacharides, eg: *Klebseilla pneumoneae*.

However, there are certain people having different chemical nature i.e polypeptide eg: *Bacillus anthracis*

In order to promote the capsule formation, milk may be added to the media.

The normal capsule is thick, conjusted and have a protective layer of the cell. It completely resists the phenomena of phagocytosis.

The presence of capsule is a sign of virulence. When capsule layer is loose, non conjusted, it will be known as slime layer. If capsule is thin layered, it is known as microcapsule.

Methods

1. Maneval method
2. Anthony method
3. Welch method
4. Negative staining

Requirements

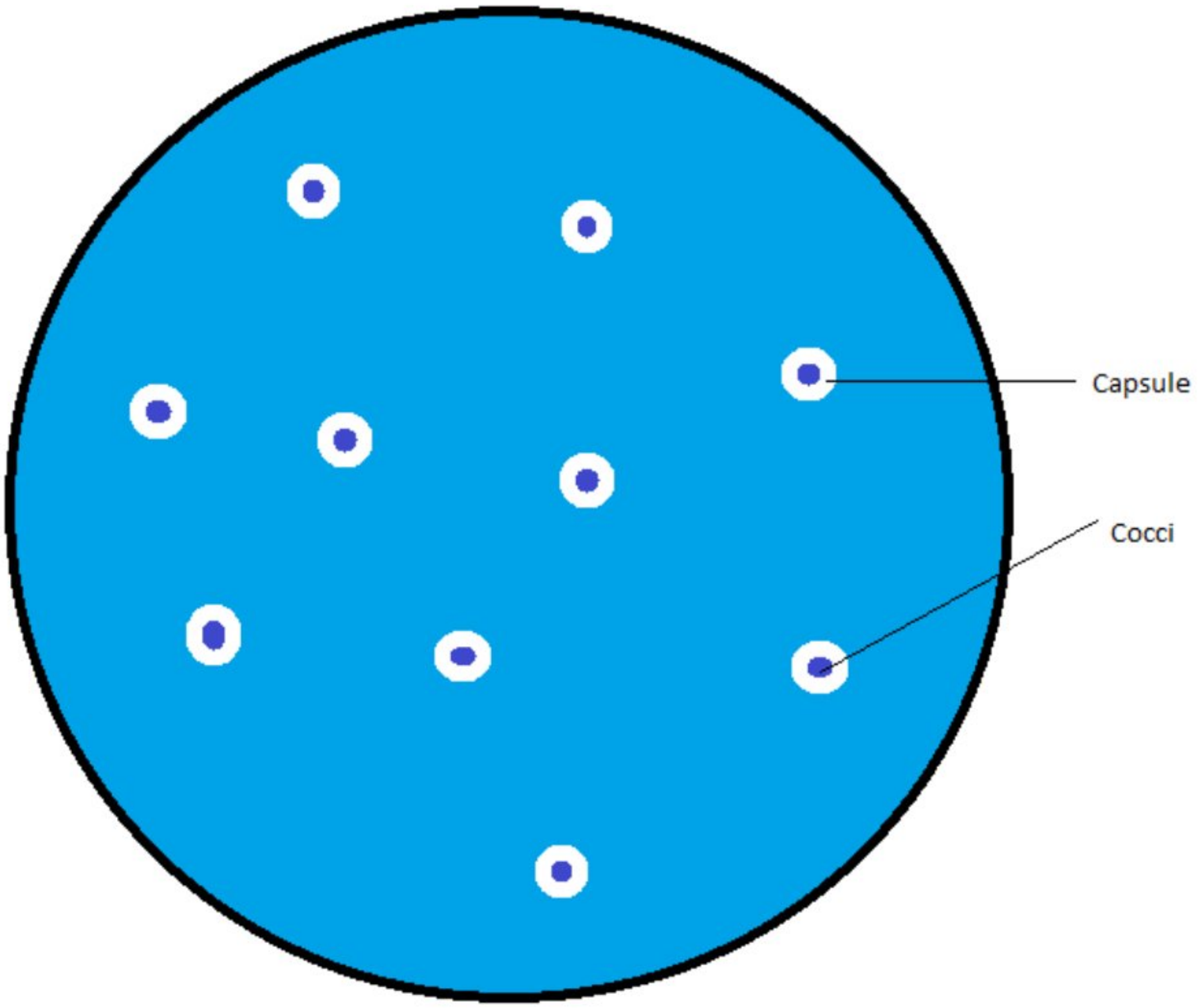
- Copper sulphate 20% soltion freshly prepared
- Crystal violet 1% solution
- Broth culture
- Slide
- Wire loop
- Burner
- Dropper

Procedure

- Wash, clean and air dry the slide
- Prepare the smear on the slide
- Stain the slide by dipping it in 1% crystal violet solution for 3-7 minutes
- Wash the slide with freshly prepared 20% copper sulphate solution
- Observe the slide under microscope

Result

The capsule of given culture was observed under the microscope as uncoloured with the blue background and violet bacterial cell cytoplasm.


Capsule
Cocci

Study of Fungi by Wet Mount Method

Theory

Fungi are basically decomposers, they are identified on the basis of their spores which are already colored.

These spores may be exospores (conidia) or endospore and they are reproductive. Typical examples of exospore forming fungi:

- *Penicillium*
- *Methylosthus trichosporium*
- *Aspergillus*

Typical example of endospore forming fungi:

- *Mucor*
- *Yeast*
- *Rhizopus*

Some fungi with the colour of their spore are mentioned below:

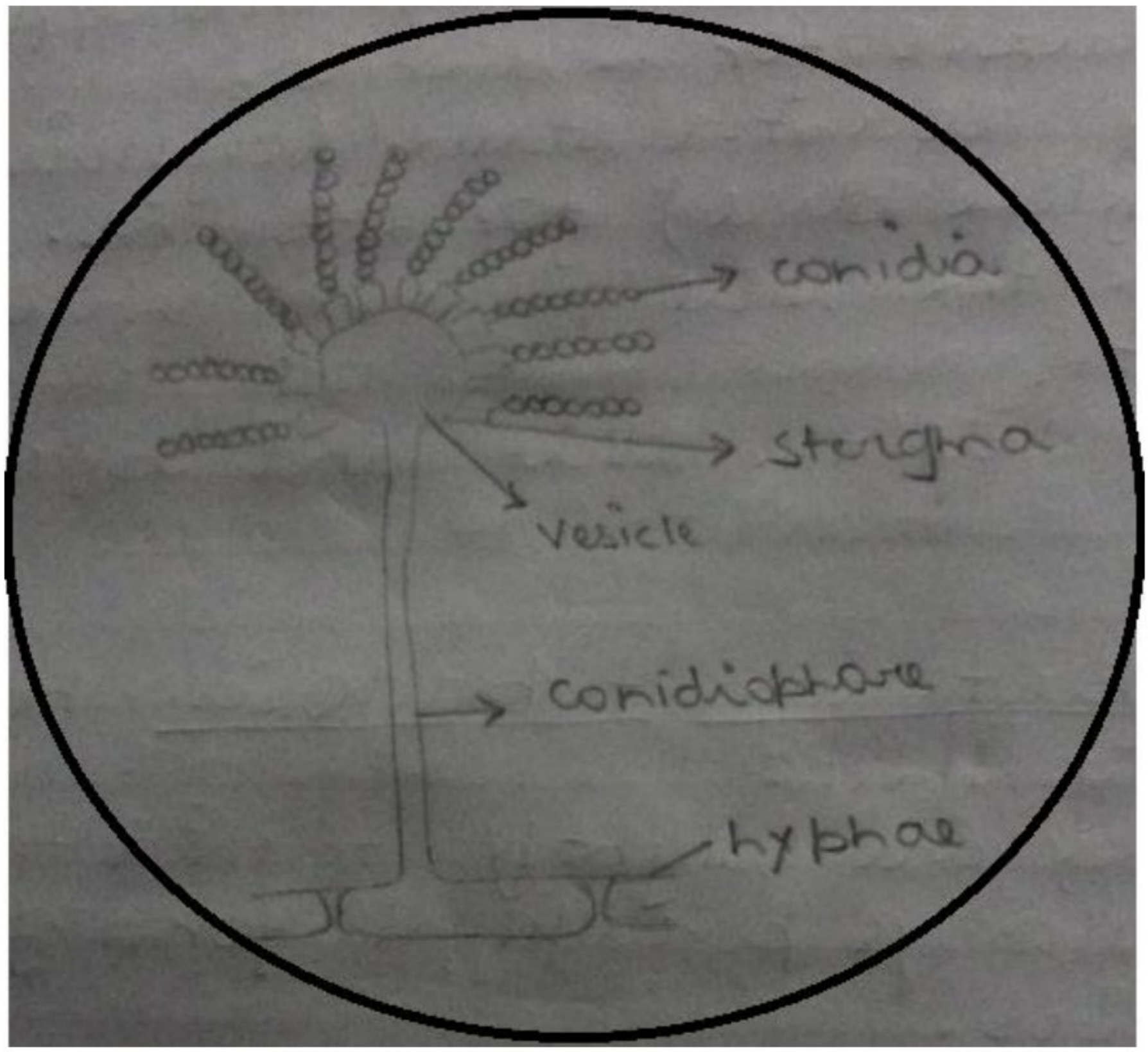
- *Aspergillus niger* -------black spores
- *Aspergillus flavus*------green spores
- *Penicillium*----------yellow spores

Requirements

- Microscope
- Slide
- Cover slip
- Cellulose liquor
- Inoculating needle
- Filter paper
- Lactophenol blue staining solution
- Given culture

Procedure

- Microscopic slide is washed, dried and fixed
- Sterile and cool inoculating needle is used to take the sample from the border of the given growth culture
- Sample is collected by twisting the needle around the growth
- Lactophenol drop is placed on the slide
- The sample containing the growth on inoculating needle is teased on lactophenol drop.
- The drop is covered with the help of cover slip in such a way that no air bubble is retained
- The slide is passed frequently on flame so that the stain can penetrate into the hyphae. There is no need of heating if kept for 24 hours
- Excessive stain is removed by the help of filter paper, and boundary is cleaned
- The cover slip is sealed around the boundary by the help of cellulose liquor.
- It is then observed under the microscope


conidia
sterigma
vesicle
conidiophore
hyphae

PREPARATION OF THIOGLYCOLATE BROTH

THEORY

Thioglycolate broth contains sodium thioglycolate, a reducing agent that creates anaerobic conditions when it reduces molecular oxygen to water.

It permits growth of anaerobic bacteria. Rosazurin is used as visual indicator of presence of oxygen. Rosazurin is pink when oxidized and colorless when reduced. The pink band near the top of the broth results when oxygen diffuses in. Strict aerobes will grow only in pink band. Nierophiles will grow near the bottom of the band.

The absence of pink in the rest of the tube indicates absence of oxygen and a suitable environment for strict anaerobes.

INGREDIENTS

1. Pancreatic digest of casein (trypticase). 15.0g
2. L-cystine. 0.5g
3. Dextrose. 5.0g
4. Yeast extract. 5.0g
5. NaCL. 2.5g
6. Sodium thioglycolate 0.5g
7. Rosazurin 0.001g
8. Agar 0.75g
9. Water q.s 1000.0g

PROCEDURE

- Take all ingredients
- Put them all in the flask, shake it, stir it, plug it, and you are done.
- Just kidding…things are never that simple in microbiology.
- Ok so…take all ingredients.
- Ass small amount of water to make paste of all ingredients in pestle and mortar
- Convert paste into suspension by passing small quantity of water
- Transfer into flask and make up the volume
- Carefully plug the container with cotton and label it
- Autoclave the flask

RESULT

Thioglycolate broth was prepared and labeled properly. Media was checked and found sterile.

PREPARATION OF SABORAUD'S DEXTROSE AGAR MEDIA

THEORY

Saboraud's Dextrose Agar is media containing peptones. It is used to cultivate the fungi.dextrose concentration supports the growth of fungi. It only allows fungi to grow on medium.

INGREDIENTS

(A)

1. Peptone. 10g
2. Beef extract. 04g
3. Agar. 15g
4. Distilled water q.s. 900g

(B)

1. Dextrose/glucose 40g
2. Distilled water q.s 100ml

PROCEDURE

- Take peptone, beef extract, agar and small amount of water to make paste in pestle and mortar
- Convert it into suspension by adding water
- Transfer in beaker A and make up the volume
- Now take dextrose and water, make paste, convert it into suspension in separate beaker B and make up the volume
- Now transfer both solution in different flasks and heat it till it becomes clear
- Carefully plug container with cotton
- Autoclave at low pressure and then mix near burner

PRECAUTION FOR CARBOHYDRATE MEDIA

- It should not be autoclaved at high temperature
- They are prepared separately and after autoclaving are mixed near burner

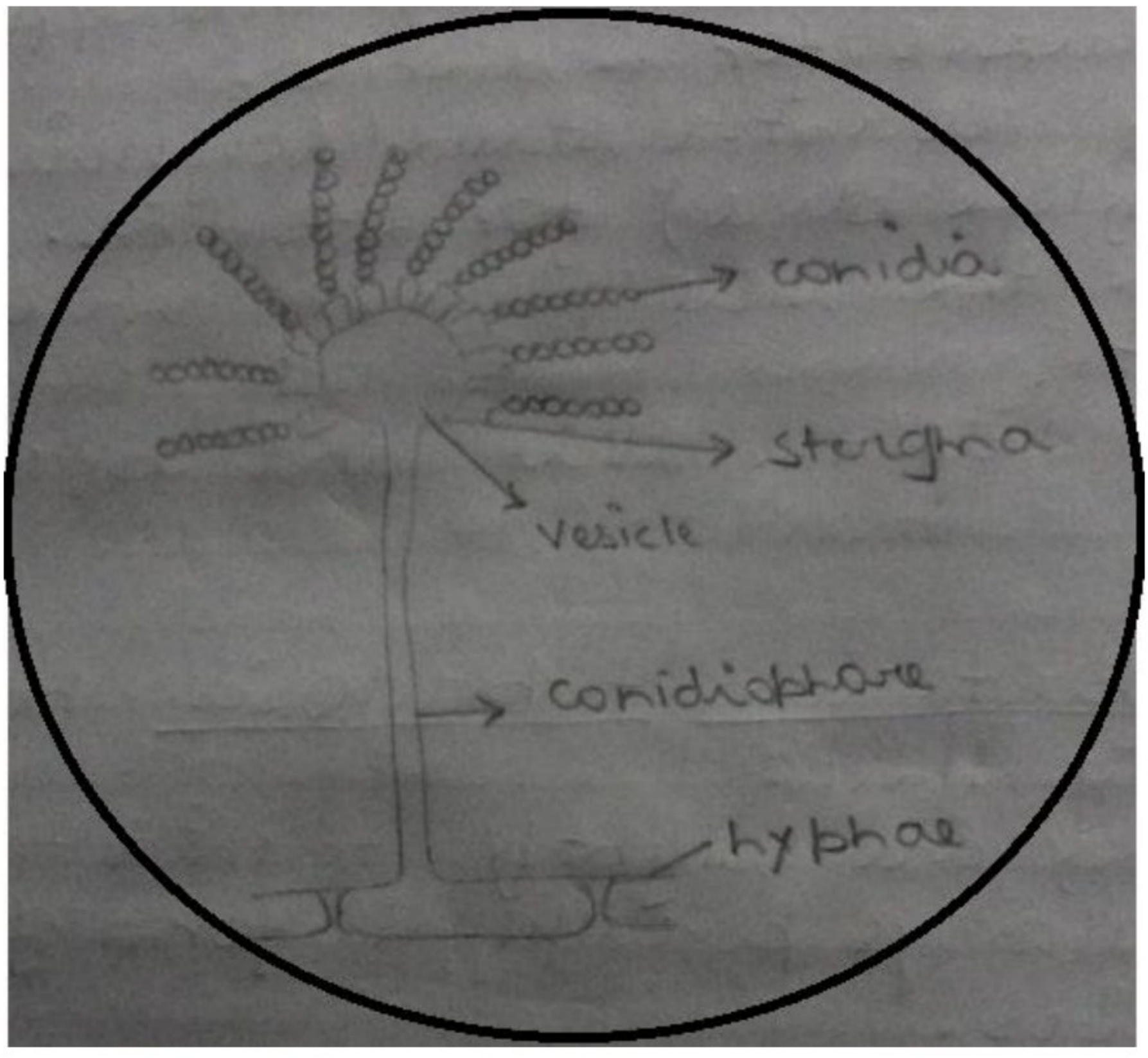
Study of Fungi by Slide Culture Technique (Riddel's Culture Technique)

Theory

Fungi are identified mostly by close examination of its morphology and the characteristics it possesses. In slide culture, we are growing the fungi directly on the slide on a thin film of agar. By doing this, there is no need to remove a position of the fungi of the fungus from a culture plate and transfer it to slide. So there is less chance of damaging the features. It is also the rapid method of preparing fungal colonies for examination and identification.

Procedure

- Wash, clean and dry the given slide and write roll no. on one side as mentioned in previous experiments
- Use solidified SDA , to cut a cube of about 1cm^2 by the help of blade
- Use forceps to lift the agar cube and place it in the centre of the slide
- By the help of inoculating needle take sample from old fungal culture and inoculate it on the four corners of the cube
- Place clean cover slip over the top of cube and incubate the slide at room temperature for 48 hours
- After 48 hours, remove the cover slip from the slide culture and discard the agar block
- Place a drop of lactophenol blue blue stain on clear microscopic slide
- Place the cover slip over the drop, and seal it and heat it.
- Examine the slide under microscope



Staining of Bacterial Flagella

Theory

Flagella, the bacterial organ for locomotion, are very thin and delicate in structure. There presence cannot be revealed by ordinary staining procedure, because even a slight agitation and mechanical force results in their destruction. Thus, special staining procedure with utmost care has been deviced.

Requirements

- Slide
- Burner
- Wire loop
- Carbol Fuchsin
- Grays flagella staining solution (mordant)

(A)

1. Potash alum (sat.soln) 50ml
2. Tarmac acid solution(20%) 10ml
3. Mercuric chloride (sat.aq.soln). 10ml

(B)

1. Basic Fuchsin (sat.soln). 03ml

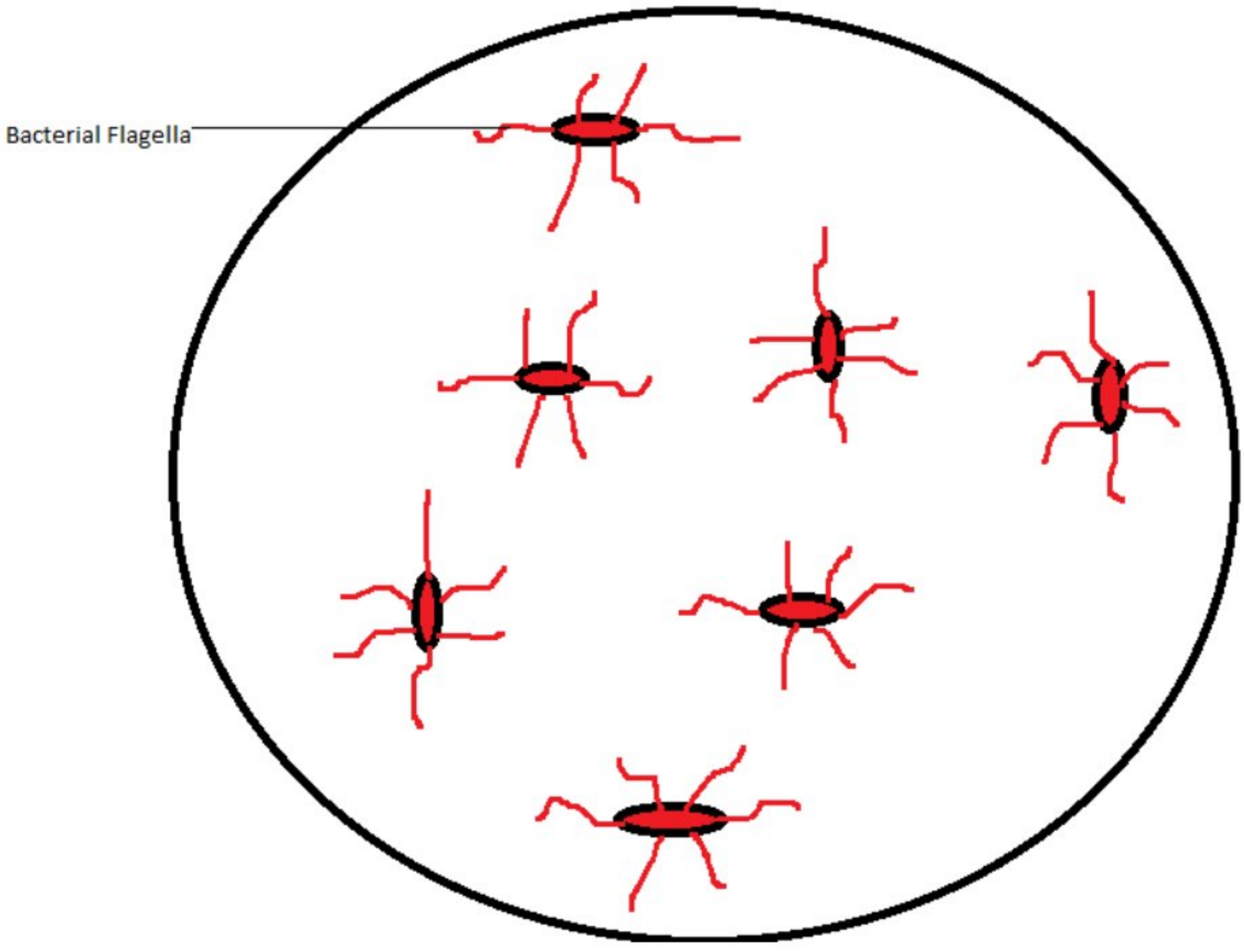
Note: A and B should be kept separately, mix them freshly before use.

Procedure

- The culture is suspended in water blank or nutrient broth, respectively, without jerking and incubated at 37°C for 20-30 minutes, to untangle the flagella of the bacteria.
- Place a drop of suspension at one end of very clean, unglazed and dried slide.
- Spread the drop over the slide using strip of paper very carefully.
- Air dry the smear rapidly
- Flood the slide with Grays flagellar staining solution for 10 mins.
- Wash the slide carefully with water.
- Add Carbol Fuchsin solution for 5 minutes.
- Wash it with water and air dry
- Observe it under high power, then finally seal the slide by using cellulose liquor

Result

Red colour flagella and bacterial cell was observed.


Bacterial Flagella

Gram Staining of the Bacteria of Buccal Cavity

Theory

Gram in 1884 devised a differential staining method, by passing this process, the bacteria can be grouped into two categories which are gram negative and gram positive. The gram positive bacteria can retain the primary colour because of a complex formation commonly known as crystal violet-iodine complex. The complex would be compact, thick and stable in case of gram positive bacteria and is thin in case of gram negative bacteria.

Examples

Shape	Gram positive	Gram negative
bacillus	*Bacillus subtilis* *Lactobacillus acidophillus*	*Salmonella typhi* *Escherichia coli*
Coccus	Neisseria sp.	Staphylococcus sp.
Helical/vibroid	Spirillum sp.	

Requirement

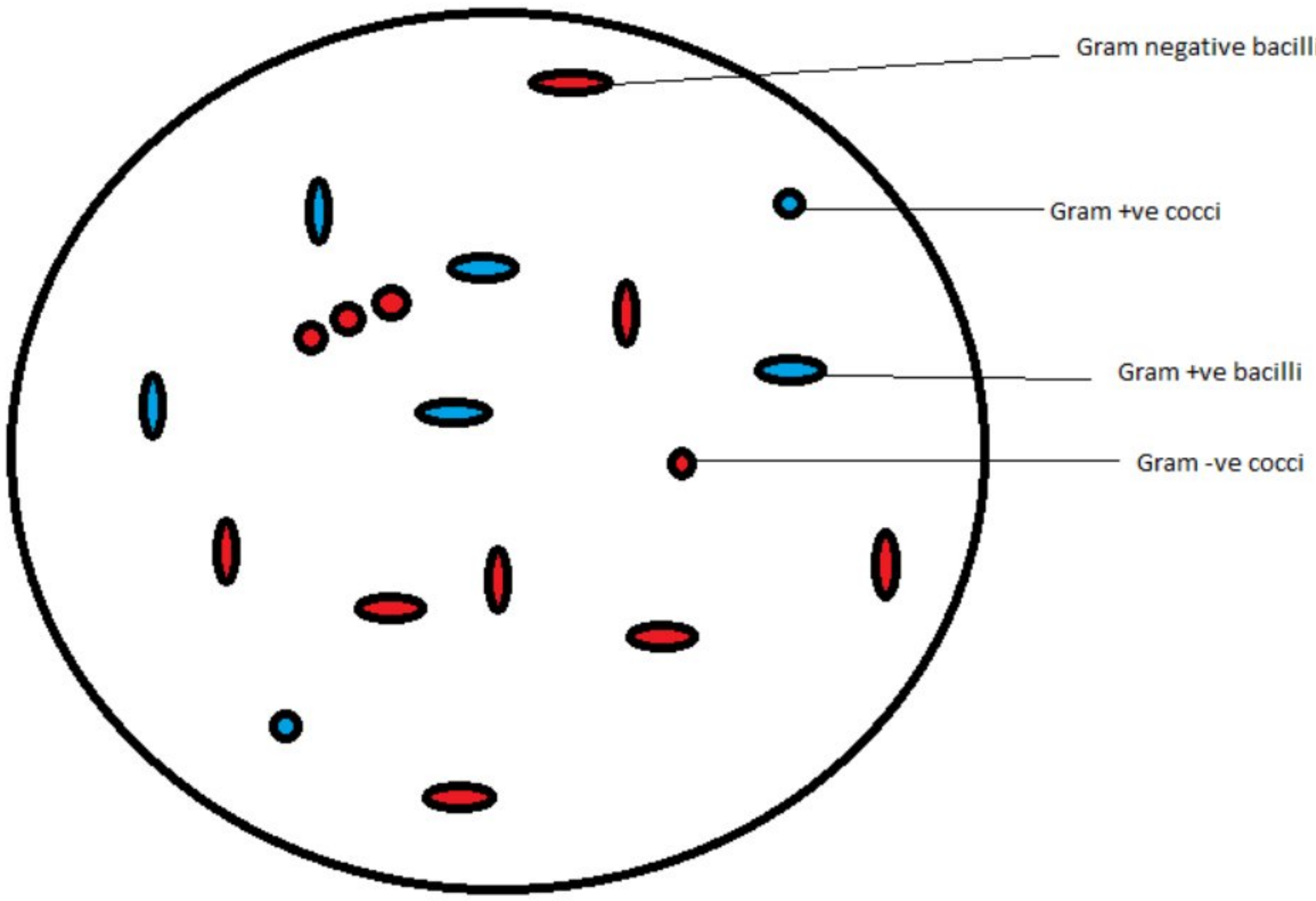
- Wire loop
- Burner
- Slide
- Muslin cloth
- Safranin red
- Crystal violet
- Lugols iodine solution
- Decolourizer

Procedure

- Wash the slide as described in previous experiment
- Put a drop of water on slide
- Use a toothpick to get sample from buccal cavity
- Make a smear
- Air dry and fix the smear
- Gram stain the slide by following method:
- Dip the slide in violet solution for 1 minute
- Directly dip the slide in gram iodine solution for I minute and wash the slide in slow stream of water
- Dip the slide in decolourizer for 15 seconds or 3 dips and immediately wash the slide
- Dip the slide in safranin red for 1 minute
- Wash the slide
- Fix the slide and dry
- Observe it

Result

Violet colour gram positive bacterial cells and some red colour gram negative bacterial cell were observed under the microscope.


Gram negative bacilli
Gram +ve cocci
Gram +ve bacilli
Gram -ve cocci

Gram Staining of Pure Culture of Bacteria

Theory

Gram in 1884 devised a differential staining method, by passing this process, the bacteria can be grouped into two categories which are gram negative and gram positive. The gram positive bacteria can retain the primary colour because of a complex formation commonly known as crystal violet-iodine complex. The complex would be compact, thick and stable in case of gram positive bacteria and is thin in case of gram negative bacteria.

Examples

Shape	Gram positive	Gram negative
bacillus	*Bacillus subtilis* *Lactobacillus acidophillus*	*Salmonella typhi* *Escherichia coli*
Coccus	Neisseria sp.	Staphylococcus sp.
Helical/vibroid	Spirillum sp.	

Apparatus

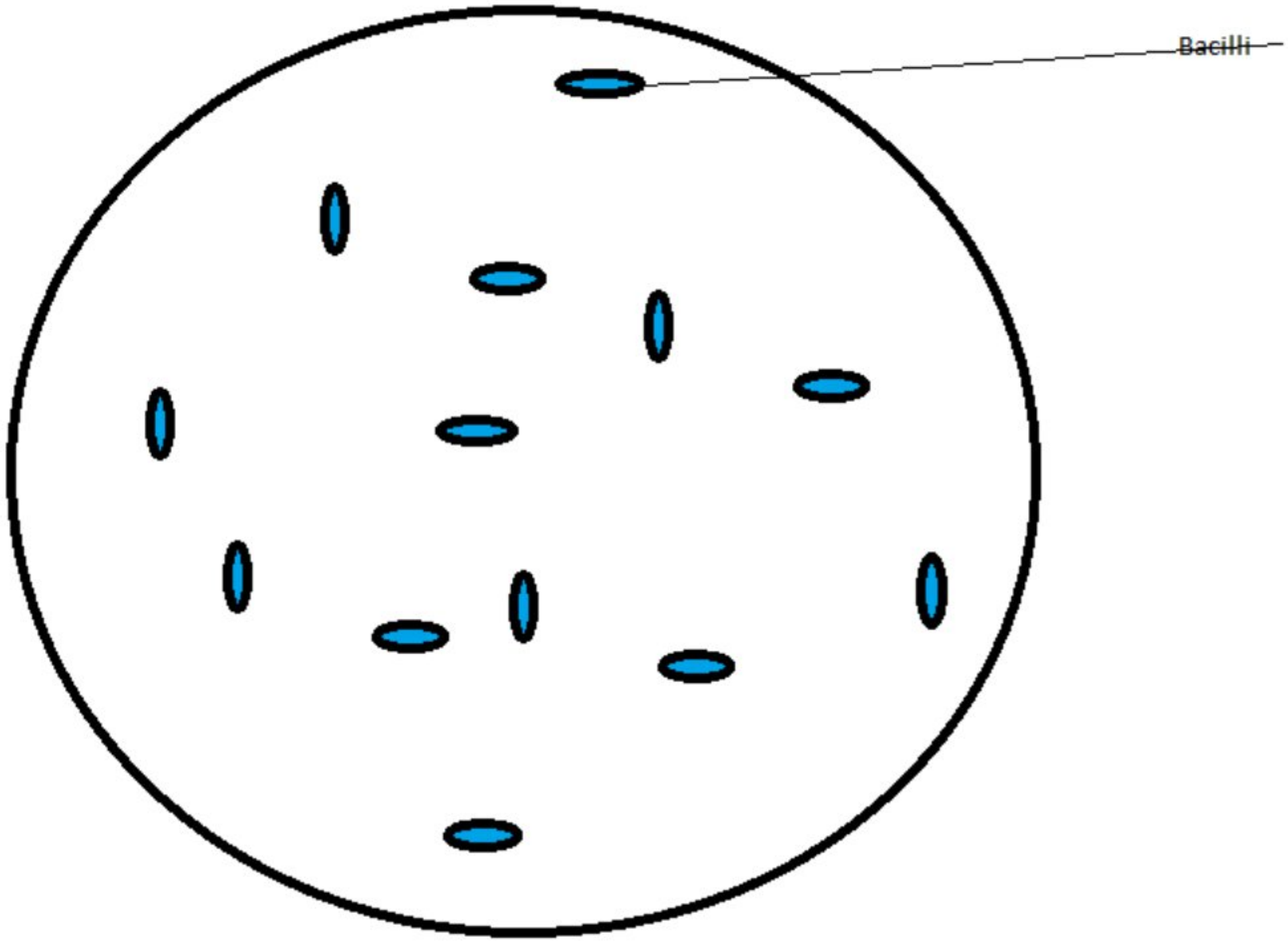
- Wire loop
- Burner
- Microscope
- Slide
- Marker
- Chemical dyes

Procedure

- Wash the slide as described in previous experiment
- Put a drop of water on slide
- Prepare the smear by distributing minimum quantity of the sample evenly on an area of about $1cm^2$ in each direction in one attempt
- Air dry and fix the smear
- Gram stain the slide by following method:
- Dip the slide in violet solution for 1 minute
- Directly dip the slide in gram iodine solution for I minute and wash the slide in slow stream of water
- Dip the slide in decolourizer for 15 seconds or 3 dips and immediately wash the slide
- Dip the slide in safranin red for 1 minute
- Wash the slide
- Fix the slide and dry
- Observe it

Result

Gram positive bacillus was observed, few were new, most were more than a day old, so were stained purple.


Bacilli

Determination of Microbial Flora of Air

Theory

Air is not the media for microbes but air is completely filled with dust particles, which carries microbes. The microbes travel through the dust particle and use these particles for transportation. The dust particle of air are allowed to trap mostly on a solid agar media by settling plate method.the intensity of the microbes can thus b estimated by counting them per minuteof the exposure of the petri dish containing agar medium. Microbial flora varies from place to place.

Requirement

- 4 sterile petri dishes containing molten media
- Wire loop
- Incubator
- Slide
- Burner
- Crystal violet
- Safranin red
- Decolourizer (alcohol and acetone 7:3)

Procedure

- Molten media is poured into 4 sterile petri dishes near the burner
- The media containing agar in petri dishes is allowed to be solidified
- The petri dish are labeled as A,B,C,D
- A is kept closed throughout the experiment and is marked as control
- The other 3 petri dishes are opened in the area of which microbial flora is to be determined
- Petri dish are moved from one corner to another corner diagonally as much to cover the whole area
- Petri dish B is closed after 5 minutes, C is closed after 10 minutes and D is closed after 15 minutes.
- All petri dishes are incubated for at least 24 hours

Counting:

- After incubation the colonies formed in each petri dish are counted
- The colony count of control petri dish A is subtracted from the count of other petri dish
- Count of each petri dish is divided by the time it was kept opened to obtain per minute count
- Average per minute count is found out
- Colony characters which are most dominant and also not present in control petri dish are noted down.
- Gram staining is done of the sample taken from the selected dominant colony

Calculation

Average Colony Present in Control

	Reading 1	Reading 2	Reading 3	Average Reading
Control Count				

Colony Count

Petri Dish	Reading 1	Reading 2	Reading 3	Average Reading	Colony Count per min

5 mins
10 mins
15 mins

Average per minute count = Sum of Colony Count per min/ 3

RESULT

The result of this experiment is not constant, hence it is not written. Mention the average reading of colony count per minute and also the gram nature of the selected colony.

MICROBIOLOGY OF WATER (DETERMINATION OF MICROBIAL FLORA OF WATER)

THEORY

The water which is free from pathogenic microbe and any chemical substances particularly injurious to health is considered as potable water. It is fit for drinking. The water which is not fit for drinking is called non-potable water. In general, the water should be colorless, tasteless, odorless and it should not be hard.

The most important and common water contaminant is *Escherichia coli.* this organism indicates the contamination of drinking water with fecal matter. Thus, the E.coli is the indicator microbe for the contamination of water and means presence of other pathogens in the water.

REQUIREMENT

- 4 sterile test tubes filled with water blank
- 4 petri dish
- 1cc sterile injection
- Wire loop
- Crystal violet
- Safranin red
- Lugols iodine solution

PROCEDURE

Collection of sample and dilution

- Water should be allowed to run for few seconds before collecting it in a sterile test tube about 5ml of sample is collected without letting the test tube touch the tap
- The sample is then diluted serially by taking 1ml from the original test tube and pouring it in 9ml blank test tube to obtain 1:10 dilution. It is labeled as A.
- For further dilution 1ml from sample A is poured in 9ml blank to obtain 1:100 diluted sample labeled as B
- Similarly test tube C with dilution 1:1000 is prepared by using sample from test tube "B"
- The molten media is poured in 4 sterile petri dishes near the burner
- 1ml of the last dilution C Is first poured into a petri dish labeled as C
- Similarly 1ml of dilution B and A are poured turn by turn in petri dishes containing molten media and labeled as B and A respectively
- All four petri dishes are incubated with the last one labeled as control petri dish containing only one molten media
- Before incubation, the petri dishes are mixed by moving them in one direction on plain surface at one attempt and allow to be solidified
- After incubation, colonies will be obtained in three forms due to pour plate method, in which colonies are formed on surface, at bottom and some embedded in media
- Colony count is performed on all test tubes and the number of colonies of control is subtracted from the colony count of others
- The average colony count is calculated per ml^3
- A colony which is dominant is selected for gram staining and growth characters of the selected colony are noted down

CALCULATION

Average Colony Present in Control

	Reading 1	Reading 2	Reading 3	Average Reading

Control Count

Colony Count

Petri Dish (Dilution)	Reading 1	Reading 2	Reading 3	Average Reading	Colony Count per ml
10^{-3}					
10^{-4}					
10^{-5}					

Average per ml count = Sum of Colony Count per ml/ 3

Acid Fast Staining of Sample from Buccal Cavity

Theory

The acid fast bacteria are resistant to acid. These bacteria also possess an acid "mycolic acid" in cell wall that is why genus mycobacterium is considered as acid fast bacteria.

In 1882, Ehrlich introduced and developed a method of acid fast staining and was modified by Ziehl and Nelson by introducing special staining solution ZNCF (Ziehl-Nelson Carbol Fuchsin).

Certain species of mycobacterium are also present in buccal cavity, which are non-pathogenic. The waxy mycolic acid containing cell wall of mycobacterium are impermeable to ordinary staining technique. So , these bacteria can be stained by special staining solution. Using drastic measures such as application of heat and phenol. Heat softens the wax in cell wall and allow the stain to enter into the cell. The fuchsin dye is more soluble in phenol than in alcohol or water.

The cell once stained, resists to decolorizer by weak acid (20% H_2SO_4 which is now replaced by 3% HCL in alcohol)

Phenol acts as mordant. The mycobacterium retains the primary stain. The background would be decolorized and will take up the colour of counter stain (methylene blue).

Mycobacterium tuberculosis have specialty that:

- They are slightly turned
- Always parallel, in the form of pairs

Requirements

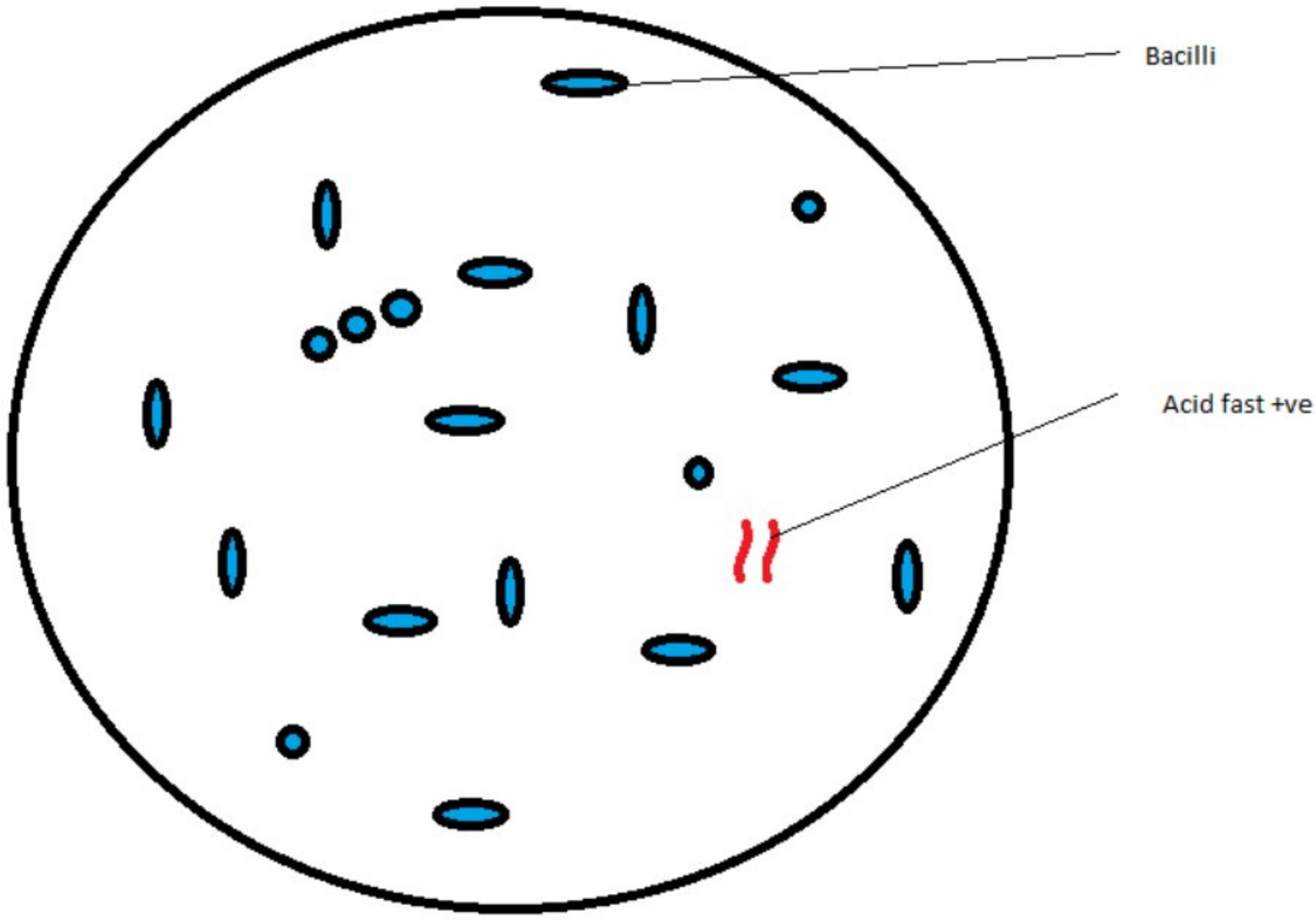
- Slide
- Tooth pick
- Burner
- Dropper
- ZNCF solution
- Decolorizer
- Methylene blue

Procedure

- Cleaning and fixing of slide is done as mentioned in previous experiments
- The smear is prepared from the material from buccal cavity by standard method
- Place the slide on rods
- Flood the slide with ZNCF solution, and heat it for 5-7 minutes.
- Move burner frequently there should be no boiling.
- Cool the slide
- Wash the slide with water and apply decolourizer for 15 seconds
- Apply counter stain (Loefflers methylene blue) for 2-3 minutes
- Wash the slide leaving the smear intact
- Air dry the slide and observe it

Result

Few clusters, single and paired coccus and bacillus, and food particles were observed along with acid fast bacteria.


Bacilli
Acid fast +ve

Determination of Microbial Flora of Soil Sample by Contact Slide Method (Buried Slide)

Theory

Soil is a region where biological and geological systems meet together. (deep)

Soil constitutes various particles of soil along with certain chemical substances of organic and inorganic in nature. The organic masses are used as substrate by the microbes and end up with a sterile black coloured mass known as humos which is a sort of fertilizer for the vegetative part of soil.

Various biological activities are carried out in the soil through the agency of microbes in the form of C,N,S and P etc cycles. (the letters are initials for elements…in case you didn't get it…I didn't). the microbial flora of soil can be studied by various method including:

- Serial dilution
- Contact slide
- Direct gram staining

The soil contain maximum number and types of microbes which may be upto level of $1x10^9$/gm of soil.

Requirements

- Slides
- Lactophenol blue
- Burner
- Microscope
- Lugols iodine solution
- Methylene blue
- Safranin red
- Decolourizer

Procedure

- Slides must be washed, clean, air dried and fixed
- It must not be glazed, and roll no. should be written at the corner
- Two slides are joined together and buried in soil near the root sphere and only $1/4^{th}$ of the slide is exposed to air on which roll number is written
- Slides are placed in soil by making a slit by means of sterile spatula or knife
- Let the slide have contact with the soil for about 7-10 days
- After 7 days the slides are removed and the area which is exposed to soil is washed in low stream of water, it is then dried and fixed
- Slide A is selected for gram staining while slide B is simple stained for fungi by lacto phenol blue
- Slides are observed under microscope

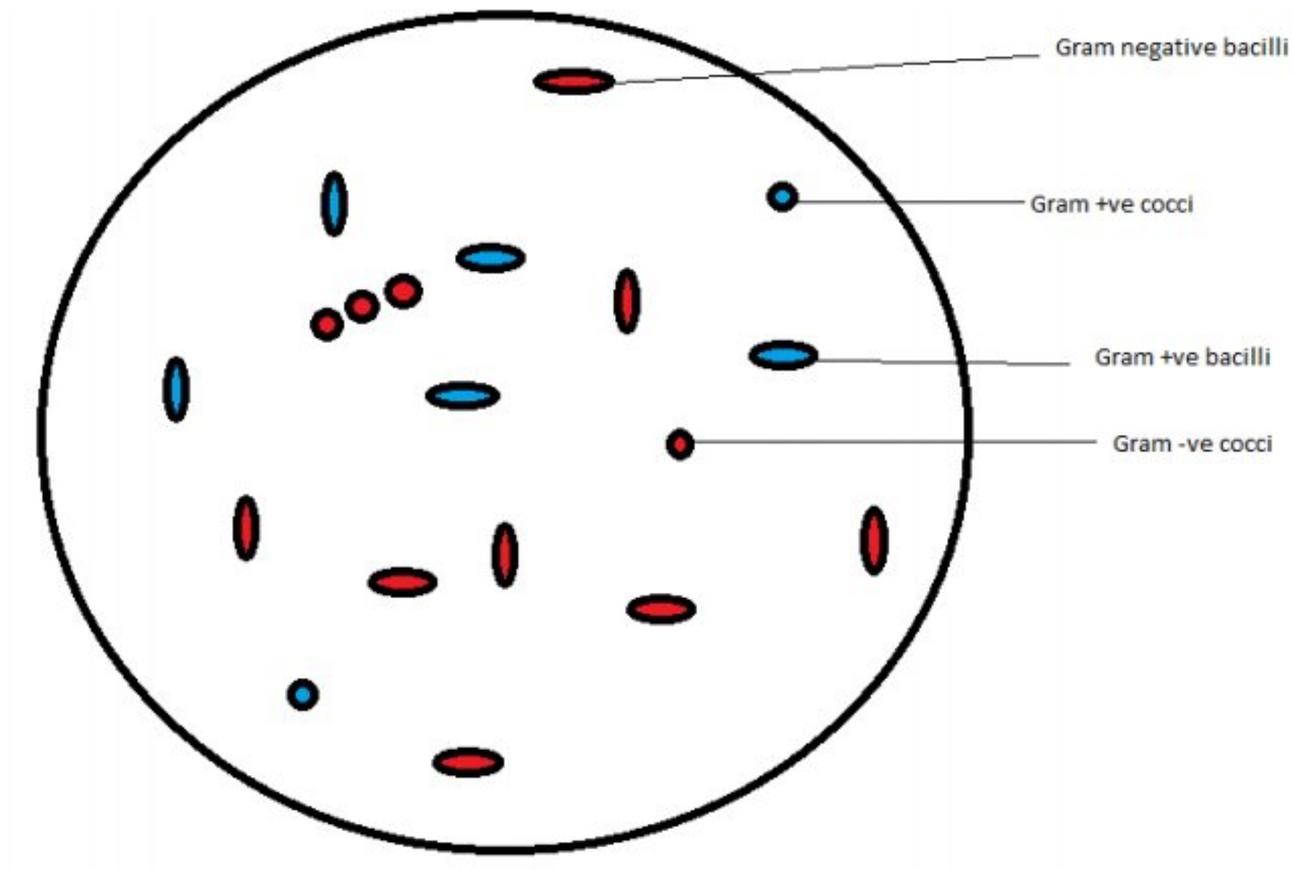
Result

Slide A was gram stained and it contains mostly gram positive cocci and bacilli and a few gram negative cocci along with soil particles

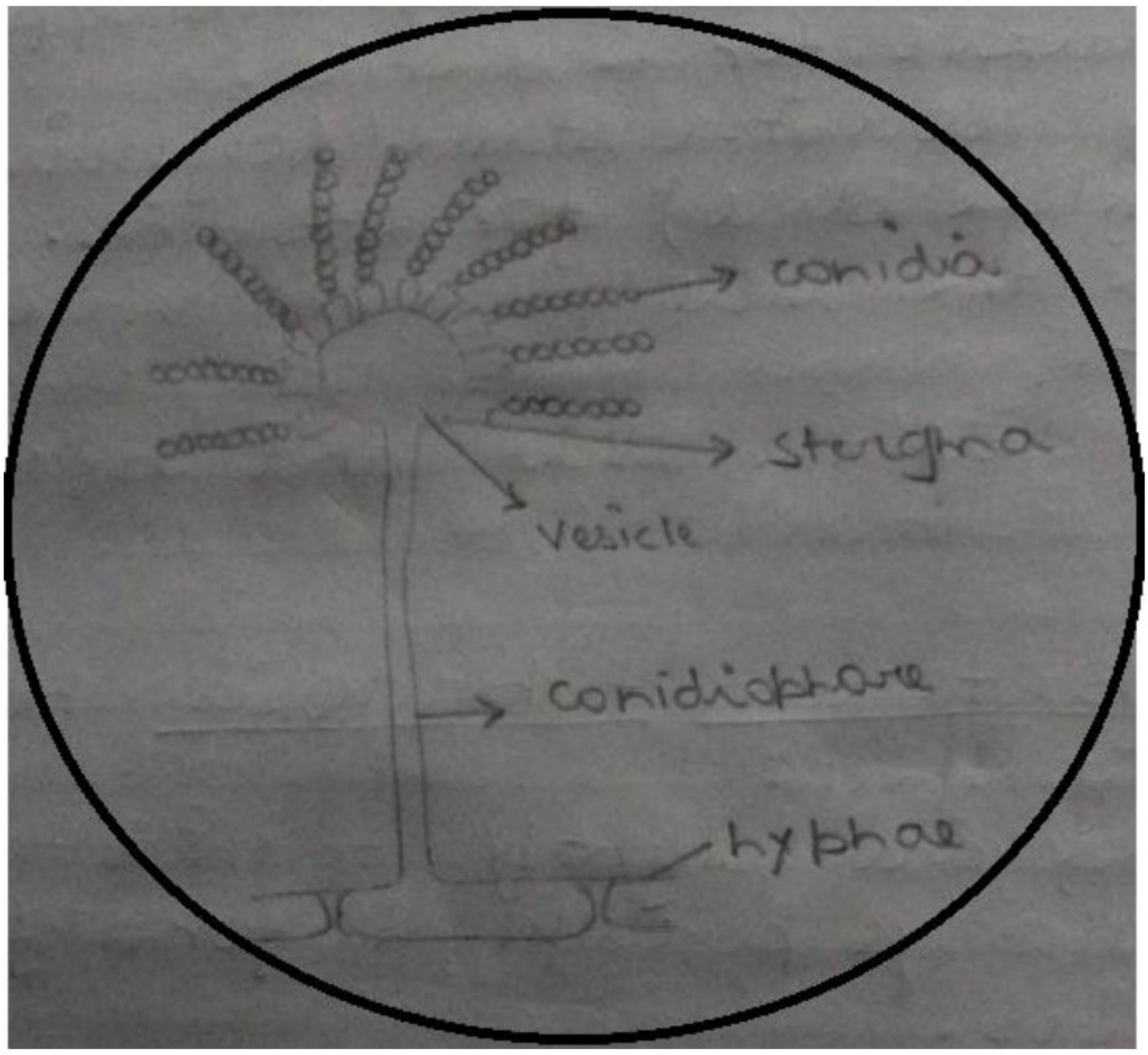
Slide B was simple stained for fungi and contained *Aspergillus niger* stained blue with black conidia

Note: results in your experiment could be different

Slide A


Gram negative bacilli
Gram +ve cocci
Gram +ve bacilli
Gram -ve cocci

Slide B


conidia
sterigma
vesicle
conidiophore
hyphae

Study of Microbial Flora of Soil Qualitatively and Quantitatively by Direct Staining Method

Theory

Soil is a region where biological and geological systems meet together. (Deep)

Soil constitutes various particles of soil along with certain chemical substances of organic and inorganic in nature. The organic masses are used as substrate by the microbes and end up with a sterile black coloured mass known as humos which is a sort of fertilizer for the vegetative part of soil.

Various biological activities are carried out in the soil through the agency of microbes in the form of C,N,S and P etc cycles. (the letters are initials for elements…in case you didn't get it…I didn't). the microbial flora of soil can be studied by various method including:

- Serial dilution
- Contact slide
- Direct gram staining

The soil contain maximum number and types of microbes which may be upto level of $1x10^9$/gm of soil.

Requirements

- Slide
- Syringe
- Micrometer
- Test tube with water blank
- Methylene blue, safranin red, decolourizer and lugols iodine solution

Procedure

- About 50g of soil is collected from root sphere, which is not so dried and not so wet as well, by the help of sterile spatula
- The sample collected is mixed and 1g of the sample is weighed on watch glass
- The sample is diluted to 1:10 by adding it into water blank and allowed to stand
- Syringe is calibrated to know the number of drops required for placing 0.05ml of diluted sample on slide. The diluted sample is collected from the center of the test tube with least number of soil particles
- Smear is prepared at exact area of $1cm^2$
- Slide containing 0.05ml of diluted sample is fixed and gram stained
- Diameter of microscopic field is calculated by the help of stage micrometer at high power
- Area of 1 microscopic field is found out by the help of diameter
- Number of microscopic field in $1cm^2$ area is found out
- Slide is observed under microscope and average microbial count from 5 different microbial field is calculated
- Qualitative analysis of microbes is also performed
- Total count of microbes in 1g of soil sample can be calculated by the average microbial count in one microbial field and number of microbial fields in $1cm^2$ area

Supposed Calculation

- 1 division of stage micrometer = 0.1 mm
- 5 division of stage micrometer = diameter of 1 microbial filed
- Diameter of 1 microfield = 5 x 0.1 = 0.5mm

- Radius of 1 microfield = 0.5/2 = 0.25 mm
- Area of 1 microfield = πr^2 = 0.2 mm^2
- Area of smear = 1 cm^2 = 100 mm^2
- Total number of microfield = area of smear / area of microfield = 100/0.2 = 500
- Total number of microfield in smear = 500

Total count of microbes in microfield

Mircrofield	Number of microbes
1	2000
2	800
3	300
4	1200
5	1400

- Average number of microbes in 1 microfield = 5700/5 = 1140
- 1 microfield have 1140 number of microbes
- 500 microfield have 1140 x 500
- 1 smear has 570000 microbes
- Smear consist of 0.05 ml
- 0.05 ml = 570000 microbes
- 1 ml – 570000 / 0.05 = 1,140,0000 microbes
- Smear is diluted to 1:10
- 11400000 x 1 = 114000000 microbes = 11.4 x 10^7 microbes/g

Result

Given Soil sample contains 11.4 x 10^7 microbes/g

Study of Microbial Flora of Milk Sample Qualitatively and Quantitatively by Direct Acid Fast Staining Method

Theory

Milk is supposed to be excellent food containing all the necessary nutrition i.e carbohydrates, proteins, fats and lipids, certain vitamins, enzymes and a system analogous to phagocytosis.

The milk fit for drinking is known as palatable milk and must be free from mycobacterium tuberculosis. As this bacterium is considered as an indicator microbe for milk. This bacterium is killed at the temperature of 62°C for 30 minutes or 72°C for 15 seconds. After passing the milk to this temperature, the milk is considered as pasteurized milk.

Requirement

- Given raw milk sample
- Slide
- Wire loop
- Burner
- ZNCF
- Decolourizer
- Loefflers Methylene blue

Procedure

- Cleaning and fixing of slide is done
- On the opposite side of slide, $1cm^2$ area box is made with the help of marker
- Smear is prepared by taking milk sample on that $1cm^2$ area
- The syringe is calibrated and 0.05 ml of milk sample in form of number is drawn from the given sample to make the smear
- Smear is allowed to air dry and is fixed by passing on flame
- Acid fast staining of the smear is performed
- Diameter of microscopic field is calculated by the help of stage micrometer at high power (40x)
- Area of 1 microscopic field is found out by the help of diameter
- Number of microscopic field in $1cm^2$ is also calculated
- 4 corner and a center microscopic field of smear selected for microbial count
- The average microbial count in one microscopic field is used to calculate the total number of microbes in per ml of the given sample
- Milk smear is also qualitatively checked for the presence of any acid fast positive microbe

Supposed Calculation

- 1 division of stage micrometer = 0.1 mm
- 5 division of stage micrometer = diameter of 1 microbial filed
- Diameter of 1 microfield = 5 x 0.1 = 0.5mm
- Radius of 1 microfield = 0.5/2 = 0.25 mm
- Area of 1 microfield = πr^2 = 0.2 mm^2
- Area of smear = 1 cm^2 = 100 mm^2
- Total number of microfield = area of smear / area of microfield = 100/0.2 = 500
- Total number of microfield in smear = 500

Total count of microbes in microfield

Mircrofield	Number of microbes

1	600
3	450
3	700
4	630
5	520

- Average number of microbes in 1 microfield = 2900/5 = 580
- 1 microfield have 580 number of microbes
- 500 microfield have 580 x 500
- 1 smear has 290,000 microbes
- Smear consist of 0.05 ml
- 0.05 ml = 290000 microbes
- 1 ml = 290000 / 0.05 = 5800000 microbes/ ml = 5.8×10^6 microbes/ ml

RESULT

Milk sample contain 5.8×10^6 microbes/ ml

Determination of Microbial Flora of the Given Sample of Soil by Serial Dilution Method

Theory

Soil is a region where biological and geological systems meet together. (Deep)

Soil constitutes various particles of soil along with certain chemical substances of organic and inorganic in nature. The organic masses are used as substrate by the microbes and end up with a sterile black coloured mass known as humos which is a sort of fertilizer for the vegetative part of soil.

Various biological activities are carried out in the soil through the agency of microbes in the form of C,N,S and P etc cycles. (the letters are initials for elements…in case you didn't get it…I didn't). the microbial flora of soil can be studied by various method including:

- Serial dilution
- Contact slide
- Direct gram staining

The soil contain maximum number and types of microbes which may be upto level of $1x10^9$/gm of soil.

Requirement

- Soil sample
- Test tube
- Water blank
- Safranin red
- Crystal violet
- Decolourizer
- Lugol iodine solution

Procedure

- An amount of 50g soil is required from a representative area (mostly from root area i.e rhizophore)
- Proper mixing of the soil by that area, by means of sterile spatula
- The sample is brought to lab for further study
- The representative sample must be mixed again and an amount of 1g of the sample is suspended in 10 ml of the water blank and thoroughly shaken, without touching the cotton plug, and allowed to stand the test tube for a minute or more so the particles settle down and the light particles will be allowed to float, to obtain the first dilution which is 1:10
- 1ml of the sample is removed from this tube and diluted into 9ml water blank
- This procedure is continued until the dilution up to 10^{-9} is formed which is 1:1,000,000,000
- Last three test tube which are $10^{-9,}$ 10^{-8} and 10^{-7} are used to prepare petri dish by taking 1 ml of sample from each into different petri dishes.
- Molten agar media is added in these petri dishes and mixed in one direction at one attempt in a plane surface
- A control petri dish with no sample but only media is also prepared.
- All the petri dishes are incubated for at least 24 hours
- After incubation, viable colony count is performed and the average number of microbes/gm of soil sample is calculated.
- A colony is selected for gram staining and its characteristics are also noted.

Calculation

Average Colony Present in Control

	Reading 1	Reading 2	Reading 3	Average Reading
Control Count				

Colony Count

Petri Dish (Dilution)	Reading 1	Reading 2	Reading 3	Average Reading	Colony Count per gm
10^{-7}					
10^{-8}					
10^{-9}					

Average per gm count = Sum of Colony Count per gm/ 3

Determination of Microbial Flora of the Given Sample of Milk by Serial Dilution Method

Theory

Milk is supposed to be excellent food containing all the necessary nutrition i.e carbohydrates, proteins, fats and lipids, certain vitamins, enzymes and a system analogous to phagocytosis.

The milk fit for drinking is known as palatable milk and must be free from mycobacterium tuberculosis. As this bacterium is considered as an indicator microbe for milk. This bacterium is killed at the temperature of 62oC for 30 minutes or 72oC for 15 seconds. After passing the milk to this temperature, the milk is considered as pasteurized milk.

Requirement

- Given raw milk sample
- Water blank
- Wire loop
- Safranin red
- Crystal violet
- Lugols iodine solution
- Decolourizer
- Media
- Syringe

Procedure

- After proper shaking of given milk sample, 1ml of the sample is drawn and added into test tube containing 9ml water blank. It is shaken thoroughly without touching the cotton plug to obtain 1:10 dilution
- 1ml of this dilution is added and mixed with 9ml water blank, to obtain 10^{-2} dilution
- 1ml of 10^{-2} dilution is added into 9 ml of water blank to obtain 10^{-3} dilution
- 1ml of each dilution 10^{-3},10^{-2},10^{-1} is added into separate sterile petri dishes and molten media is poured
- The petri dish are mixed by moving it in one direction at one attempt on a smooth surface
- A control petri dish containing only media is also prepared
- All the petri dishes are incubated for at least 24 hours
- After incubation viable colony count is performed to check number of microbes/ml of the given sample
- A selected colony is gram stained and its characteristics are also noted down

Calculation

Average Colony Present in Control

	Reading 1	Reading 2	Reading 3	Average Reading
Control Count				

Colony Count

Petri Dish (Dilution)	Reading 1	Reading 2	Reading 3	Average Reading	Colony Count per ml

10^{-3}
10^{-2}
10^{-1}

Average per ml count = Sum of Colony Count per ml/ 3

Effect of Temperature on the Given Bacterial Culture Exposed for 5, 10 and 15 Minutes

Theory

Environment and physical factors affecting the activity of bacterial enzymes. Temperature is one of the most important physical factors affecting microbes. The bacterial cell lacks homeostatic functions, so enzyme system are directly and readily effected by environmental factors including temperature.

Low temperature nearly inactivates the enzyme but is generally less damaging than high temperature, which denatures the protein causing irreversible, and total enzyme destruction which leads to the death of the cell.

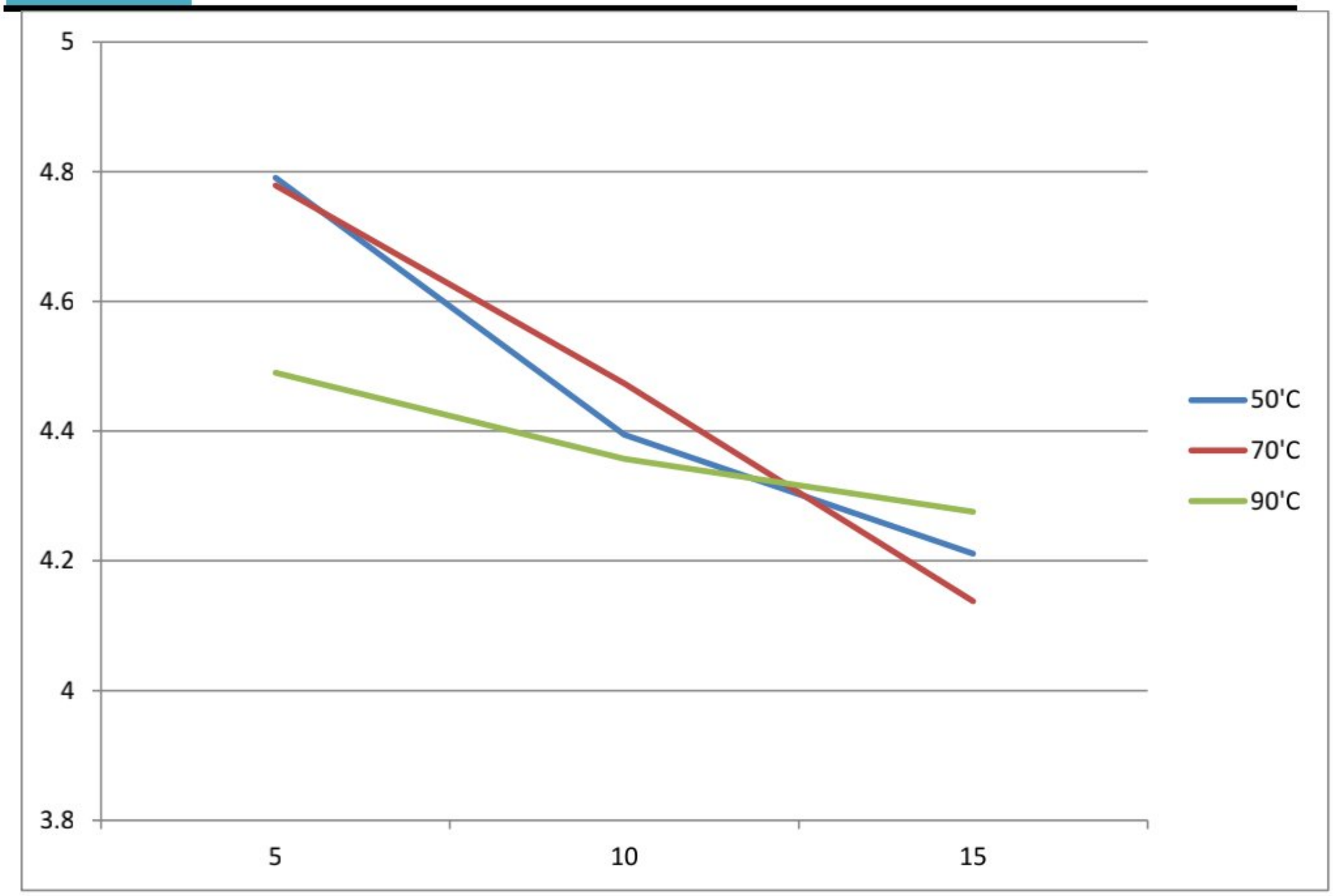
Requirement

- Given culture "A" spore former
- Given culture "B" non-spore former
- Oven fixed for temperature at 50°C, 70°C and 90°C
- Incubator
- Sterile petri dish
- Water blank

Procedure

- The bacterial suspension are prepared uniformly homogenously with a given optical density of turbidity
- A part of the standard suspension is used for the viable counting without exposing to the temperature and treated as control
- 1ml of suspension is added into 9ml water blank to make dilution 10^{-1}
- 1ml of dilution is than added into 9ml water blank to get 10^{-2} diluted suspension
- Repeat the process to get suspension diluted till 10^{-5}
- Last three test tubes are kept while the rest are discarded
- 1ml of each of these test tubes are poured into different sterile petri dishes and molten media is poured
- Labeled as viable count with no exposure of heat
- To check the effect of temperature, the standard undiluted suspension is divided minimum 2ml in 9 different test tubes
- Three of these 9 test tubes are kept at 50°C while the other 6 are kept similarly at 70°C and 90°C, three each
- After 5 minutes of exposure at a given temperature one out of the three suspension kept at that temperature is removed
- Similarly after 10 and 15 minutes of exposure, other two suspensions are also removed
- These suspension are than diluted by the addition of water blank till we get 10^{-3} dilution
- 1ml of 10^{-3},10^{-2} and 10^{-1} diluted suspension with labeled time of exposure, temperature and sample is added into different petri dishes each and media is poured into it
- All the labeled petri dishes are incubated for at least 24 hours
- Effect of temperature is noted by calculating the colonies after different exposure time and temperature
- Similar procedure is performed for sample A and B
- Data is recorded in tabular and graphical form

	10^{-1}	10^{-2}	10^{-3}	Average Reading	Reading/ Min	Log_{10}
Viable Count						
50'C						
Time	`					
5 mins						
10 mins						
15 mins						
70'C						
Time	`					
5 mins						
10 mins						
15 mins						
90'C						
Time	`					
5 mins						
10 mins						
15 mins						



Effect of Radiation UV Light against the Given Bacterial Culture Exposed for Different Period of Time (5, 10, 15 minutes) at Two Variable Distances (1ft and 1 Meter)

Theory

The physical factors effecting the bacterial growth also include radiation. We can control the physical condition in a limited environment to kill, inhibit or remove microorganism.

The UV source is used as sterilizing device to reduce the number of bacteria i.e. cell must be in direct path of radiation.

UV rays may also be converted to heat as they strike glass or water. The killing efficiency is related to both the distance between organism and UV light as well as the time of exposure. Prolong radiation causes irreversible damage to hereditary molecules. These changes may be lethal or not. in this way mutant offspring can also be produced.

Radiation is used medicinally to change or destroy malignant cells which are reproduced rapidly than normal cells.

Precautions

- Do not look at rays coming from the UV source which can damage our eyes by sun burning even with a short exposure of time. (there is actually a superhero called “daredevil” who was blinded in his childhood, due to that all his other senses became pro-active and he transformed into a superhero...cool right? Don’t get any ideas though)
- Bacteria are more susceptible to radiation then fungi

Requirement

- UV radiation chamber
- sterile Petri dishes
- molten media
- wire loop
- given bacterial culture (its only “required” if after its given, you lose it...but that happens very rarely)

Procedure

- the bacterial suspension is prepared uniformly homogenously with a given optical density of turbidity
- molten media is poured into 8 petri dishes and allowed to solidify
- Out of these 8 petri dishes, 1 petri dish with only media is set as control. (if you ever find bacteria in your control dish after incubation, it just means the bacteria had too much fun and went out of control)
- 1ml of bacterial suspension is added into other 7 petri dishes
- The sample is spread over the media by tilting petri dish or by a sterile glass rod
- Another control is set by not exposing one of these petri dishes to radiation. This control contains the media and culture, and is actually viable count of bacterial suspensions
- Rest of the 6 petri dishes are exposed at different distance and for different period of time
- Three of these petri dishes are exposed at 1 ft distance while three other are exposed at 1 meter distance
- Out of these three petri dishes, the time of exposure is 5,10 and 15 minutes for different petri dishes
- After exposure at different distance and time, petri dishes are incubated for at least 24 hours

- Effect of radiation is observed by counting the colony at different exposure and recording the data in tabular and graphical form

Calculation

1 Foot

Time	Number of Microbes/ml	% Survival
5 mins		
10 mins		
15 mins		

1 Meter

Time	Number of Microbes/ml	% Survival
5 mins		
10 mins		
15 mins		

Sensitivity of the Given Bacterial Culture against Various Antibiotics by Disc Plate Method

Theory

We will study the antimicrobial effect of chemicals in the form of antibiotics. Antibiotics are different form of disinfectant or antiseptic as these are biosynthesized which refer to the production of a substance from a living organism, these substances are of no use to the microbe that produce them, so we can also call them by-product of microbial metabolism.

The important genera that produce the largest number of antibiotics and useful for human are:

- Bacillus
- Penicillium
- Streptomyces

Antibiotics are also different from disinfectant in a way that these are administered internally and travel via blood stream to most parts of the body. This means the antibiotics should have less toxicity towards the host cell, while it should be toxic or destructive to the concerned microbes.

Antibiotics must be used to control the infection and showing inhibition to the growth of causative agent, we can determine this effort in vitro by simple lab test. Bacterial sensitivity to the antibiotics is used for diagnostic purposes because it is standardized test and therefore, give most accurate test.

In summary, we can determine the most effective antibiotic to be used in treating the patient, so antibiotics sensitivity test is performed by using a culture obtained from the patient and study its sensitivity against various antibiotics.

Antibiotics kill bacteria in several ways eg. Penicillin inhibits the synthesis of cell wall, while streptomycin competes with PABA (para aminobenzoic acid) as a substrate for an enzyme reaction. The drug enter the reaction in place of PABA thereby blocking the synthesis of essential cell component.

Some broad spectrum antibiotics interfere with enzyme synthesis.

The death of bacteria/microbes is manifested by the zone of inhibition around impinged disc.

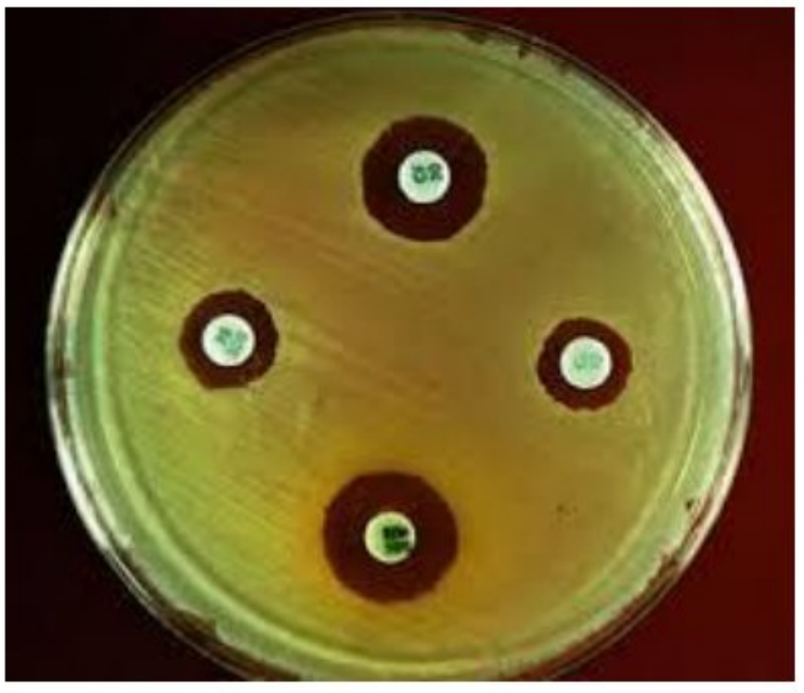
Requirement

- Sterile petri dish
- Culture
- Media
- Given antibiotics

Procedure

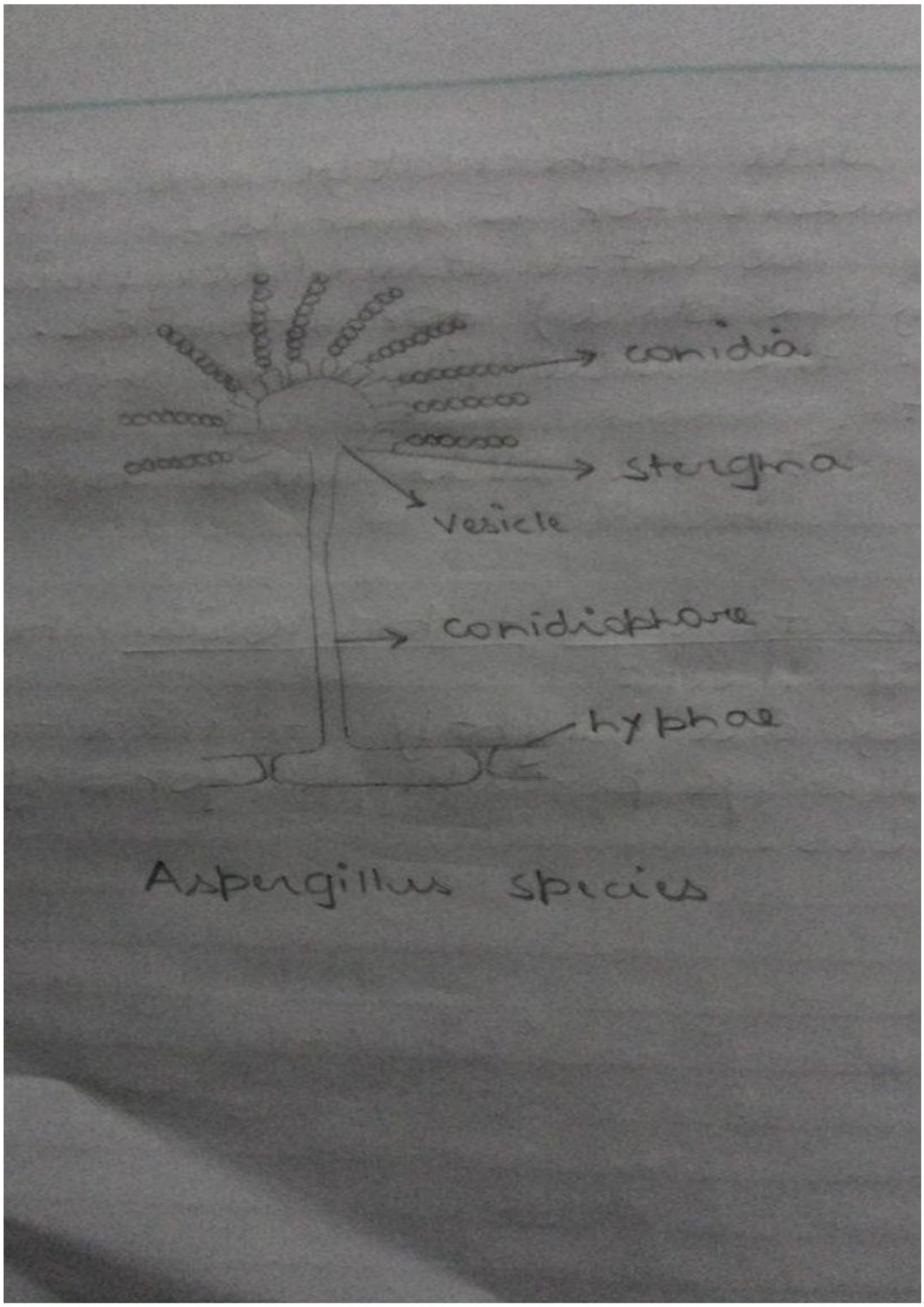
- A petri dish containing only media is marked as control
- Another petri dish is divided into four equal portions
- Each quarter is labeled with the number of the antibiotics being used
- 1ml of bacterial culture is added into the petri dish
- By using pour plate method, media is added
- Petri dish are rotated at one direction in one attempt on a smooth surface, and allowed to cool down
- A disc of antibiotics is impinged in the specified labeled quarter
- The fourth quarter has no antibiotic and is labeled as control with culture

- Both petri dishes are incubated for minimum 24 hours and maximum 48 hours, after which there are chances of bacteria to become resistant (enough is enough!)
- Vernier calipers is used to determine the diameter in (mm) at different angle from the centre of the disc of the zone of inhibition
- Usual size of the disc is 6mm to 8mm, so the minimum size of zone of inhibition for ascentive bacteria should be more that 10mm or it will be considered as resistant.

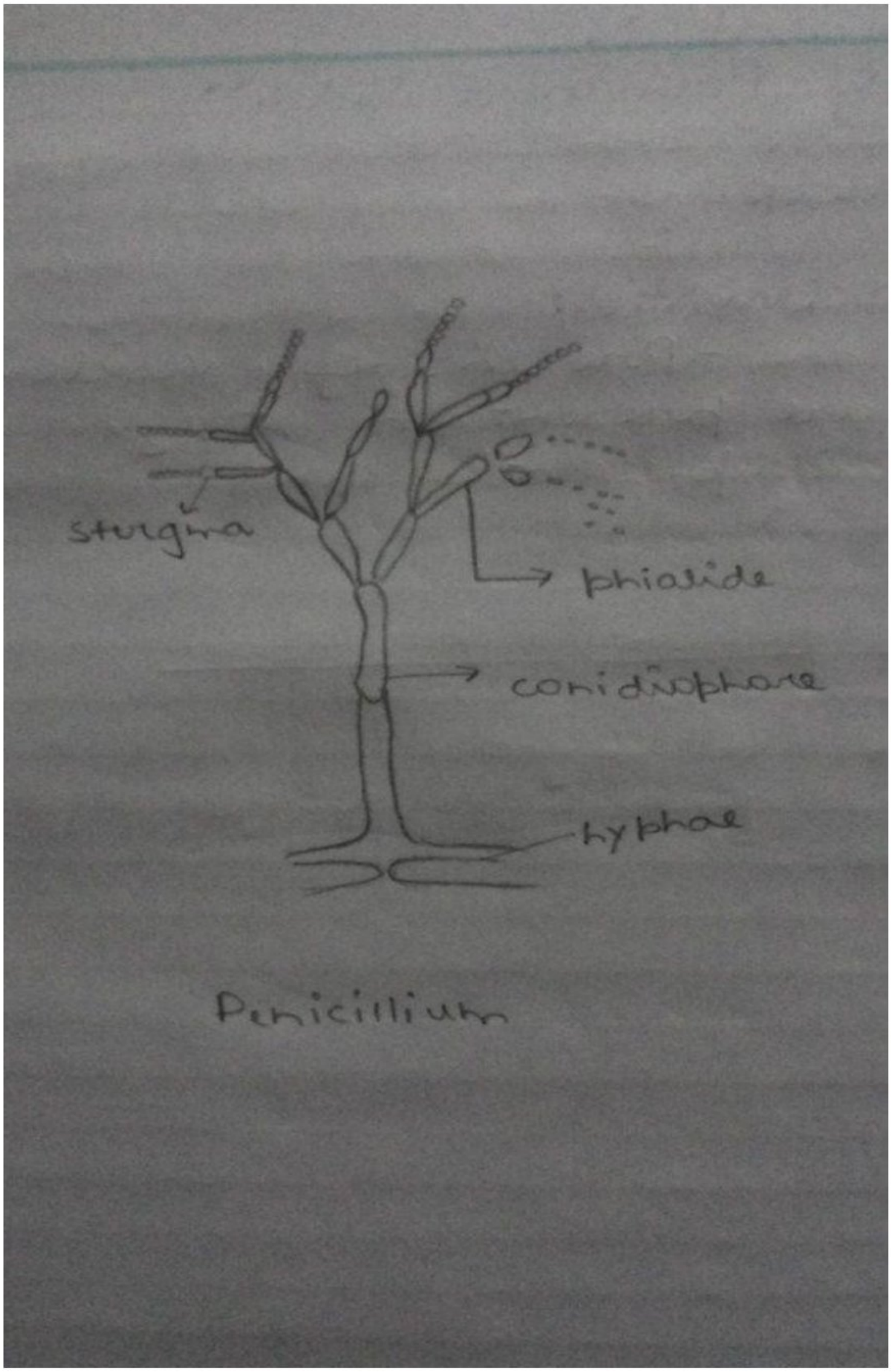


Microbiological Slides

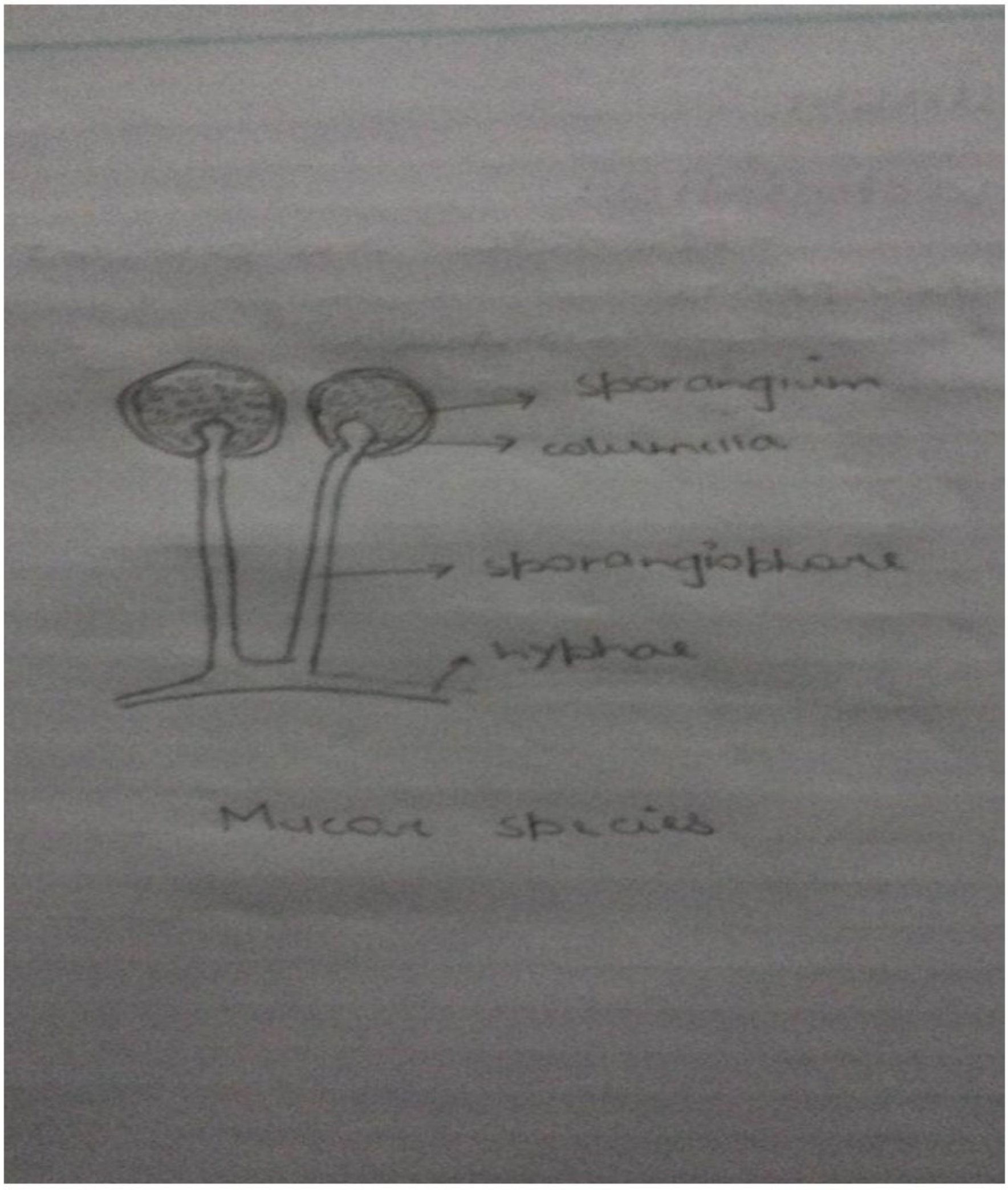
Aspergillus Species



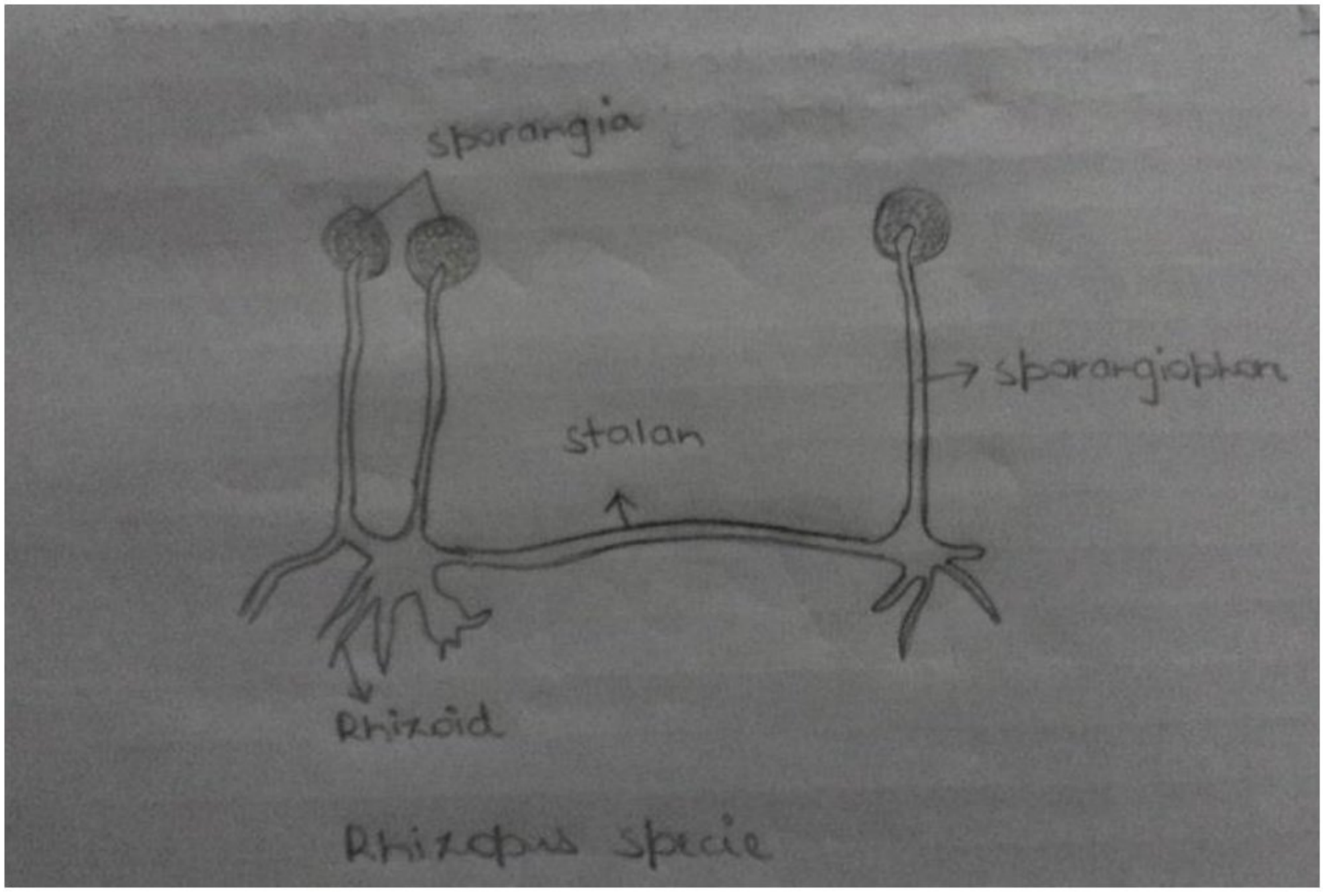
PENICILLUM



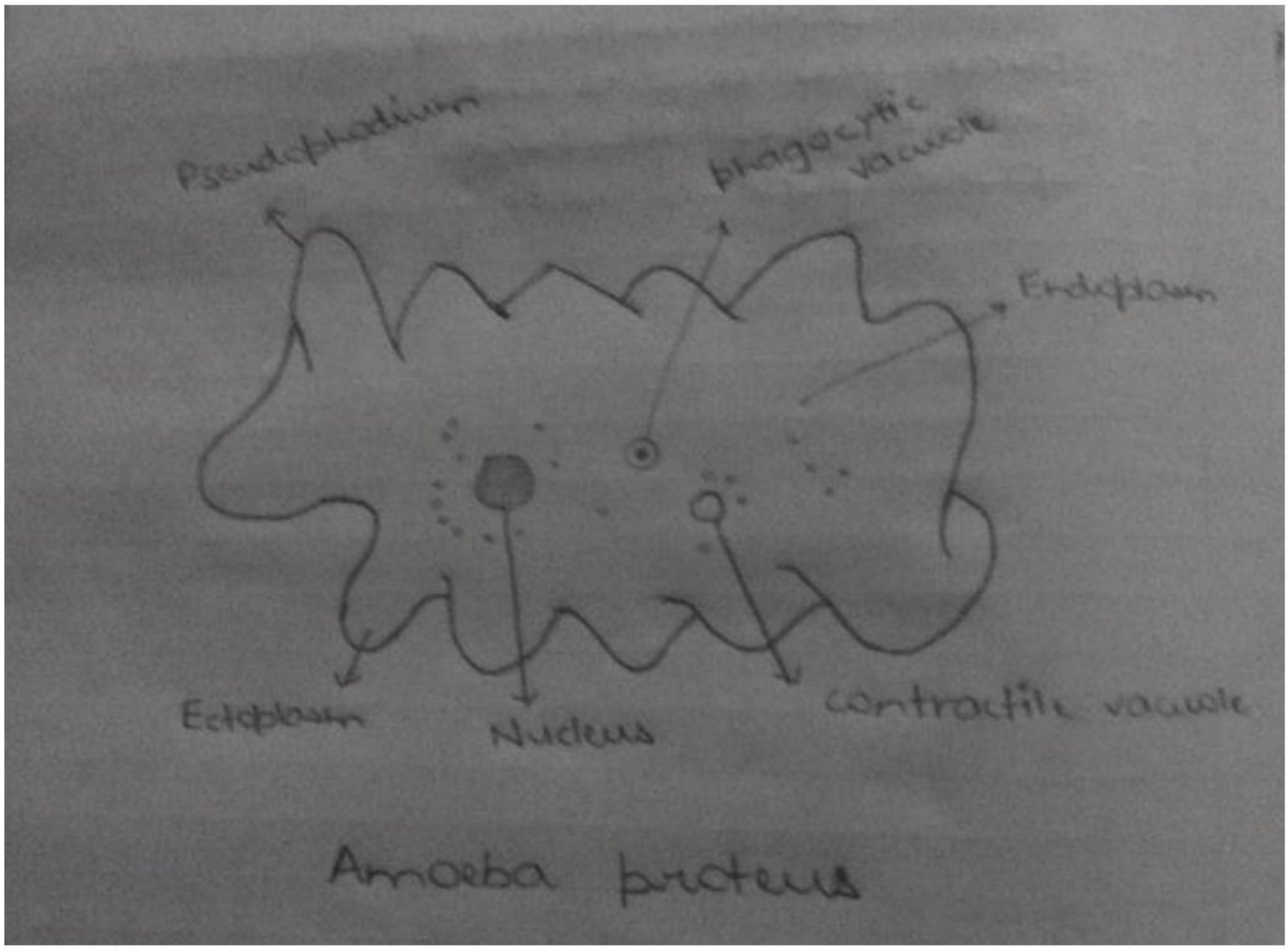
Mucor Species



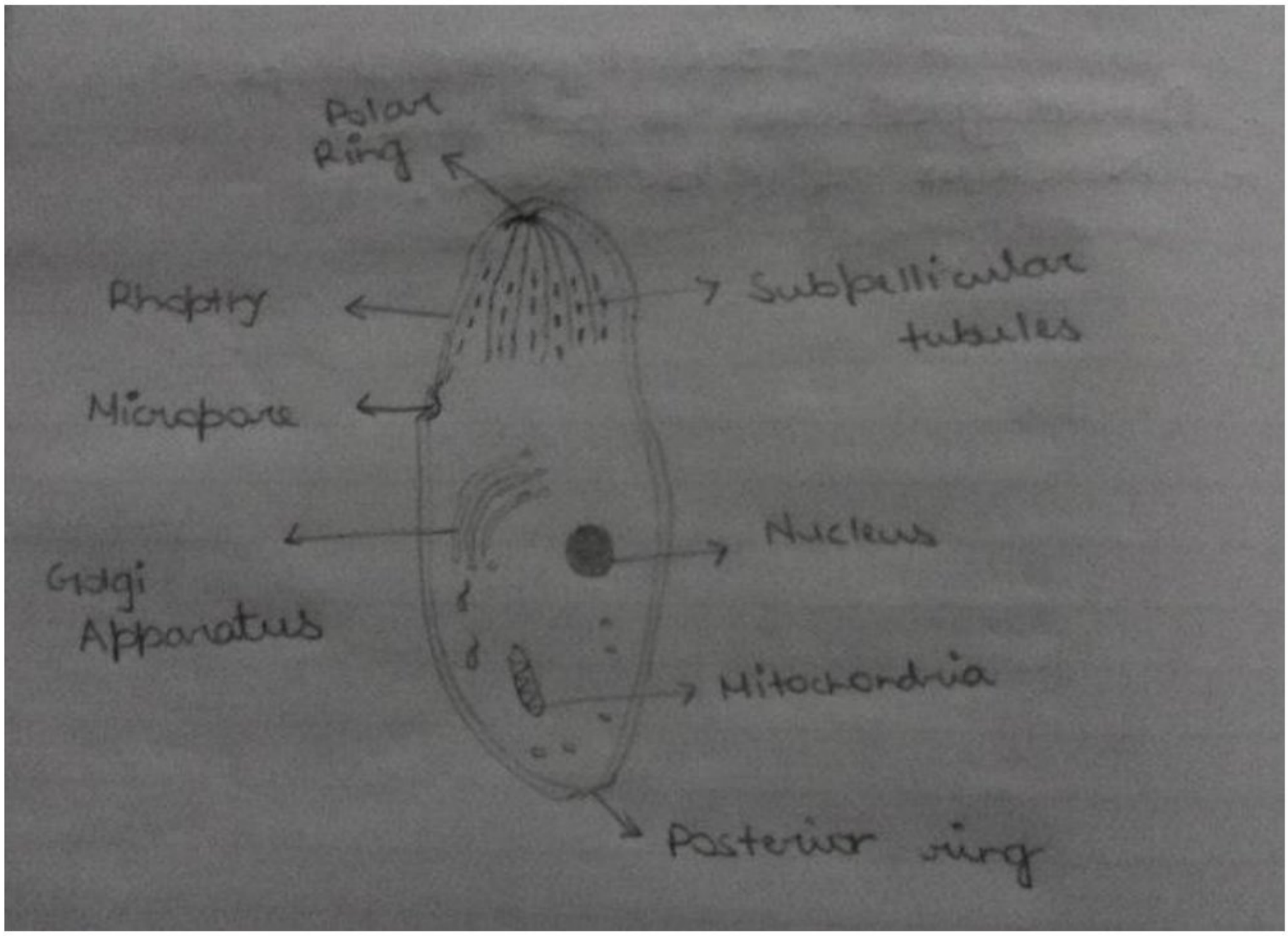
Rhizopus Species



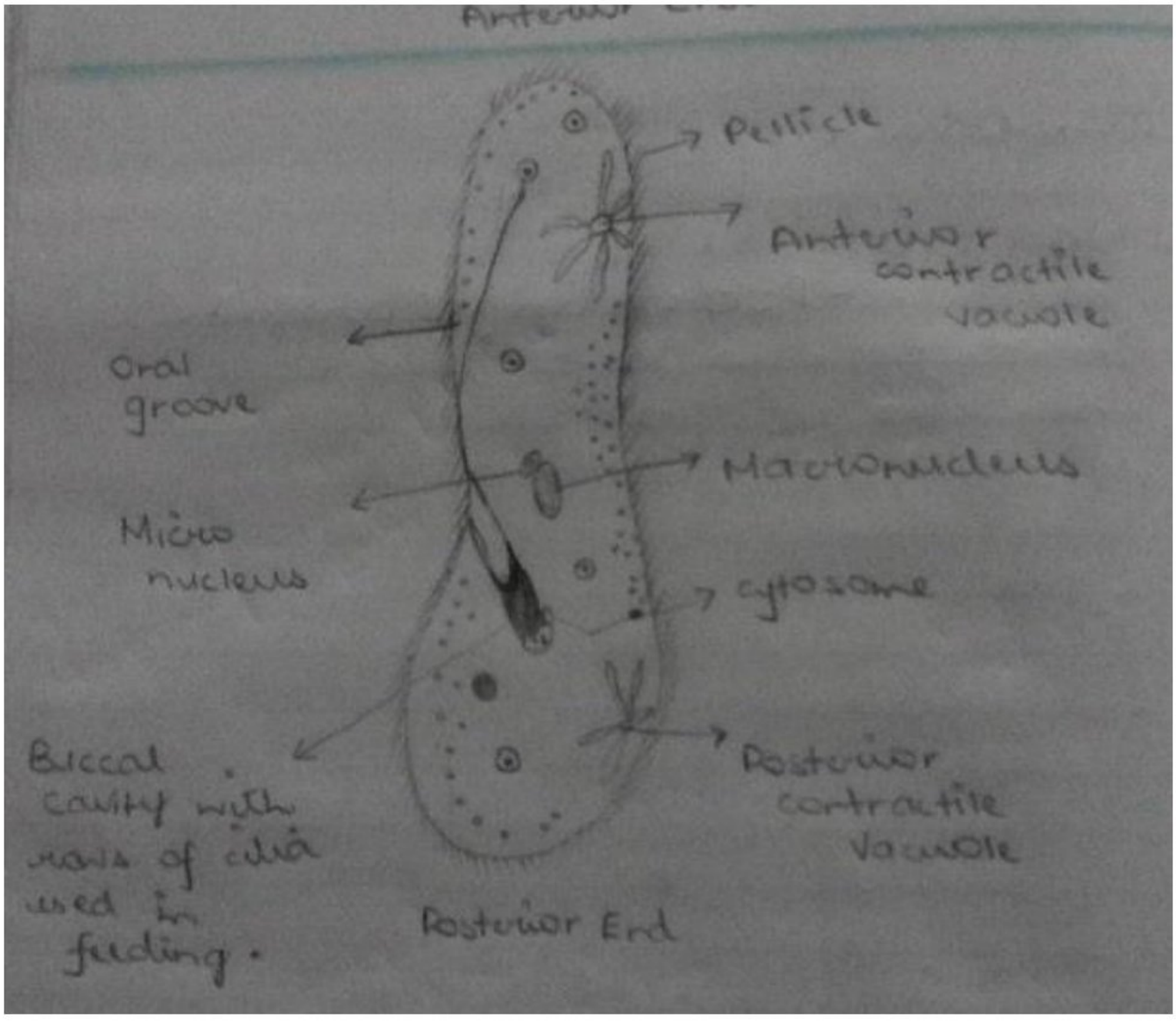
AMOEBAE


Pseudopodium
phagocytic vacuole
Endoplasm
Ectoplasm
Nucleus
contractile vacuole
Amoeba proteus

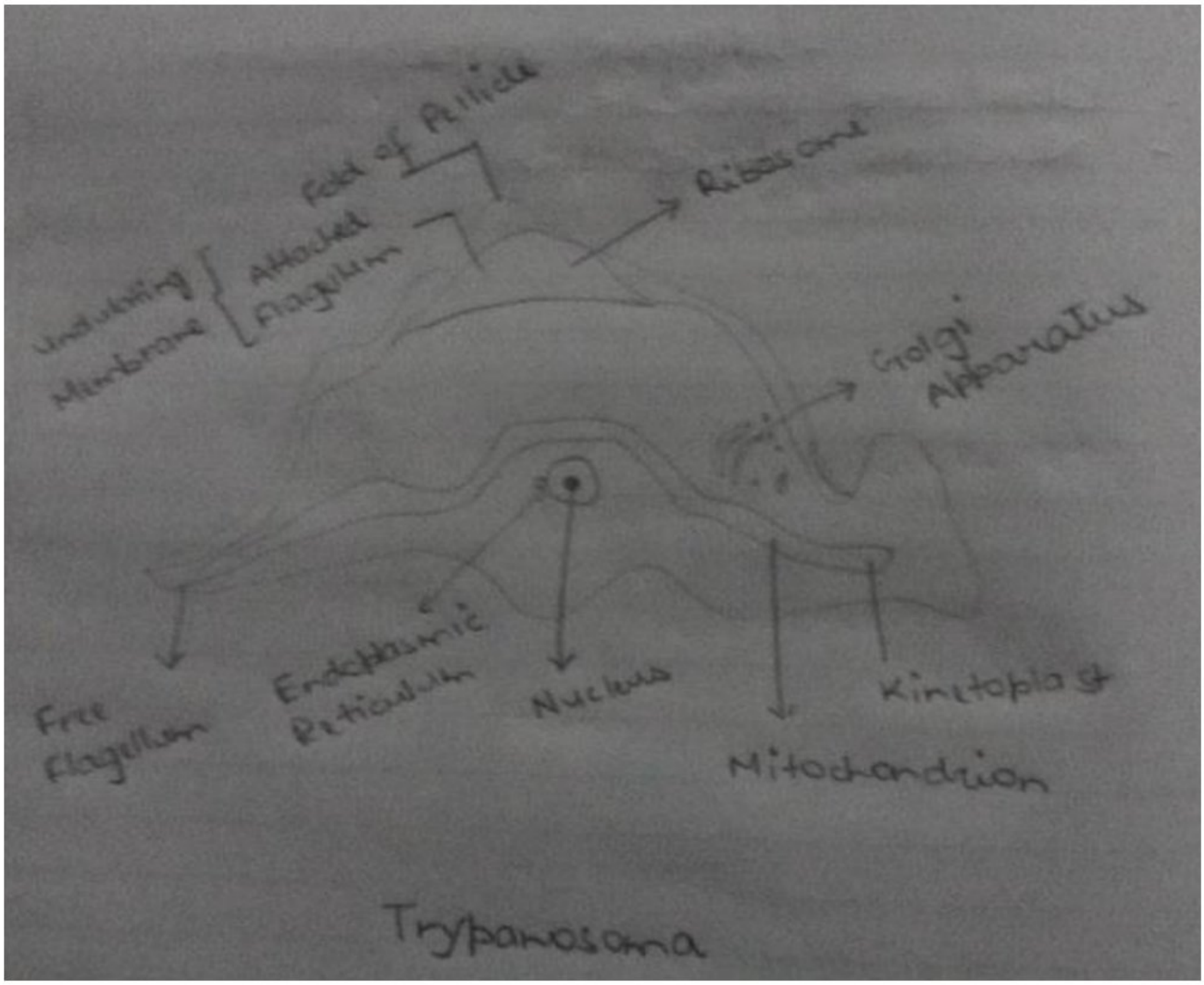
PLASMODIUM


Polar Ring
Rhoptry
Subpellicular tubules
Micropore
Golgi Apparatus
Nucleus
Mitochondria
Posterior ring

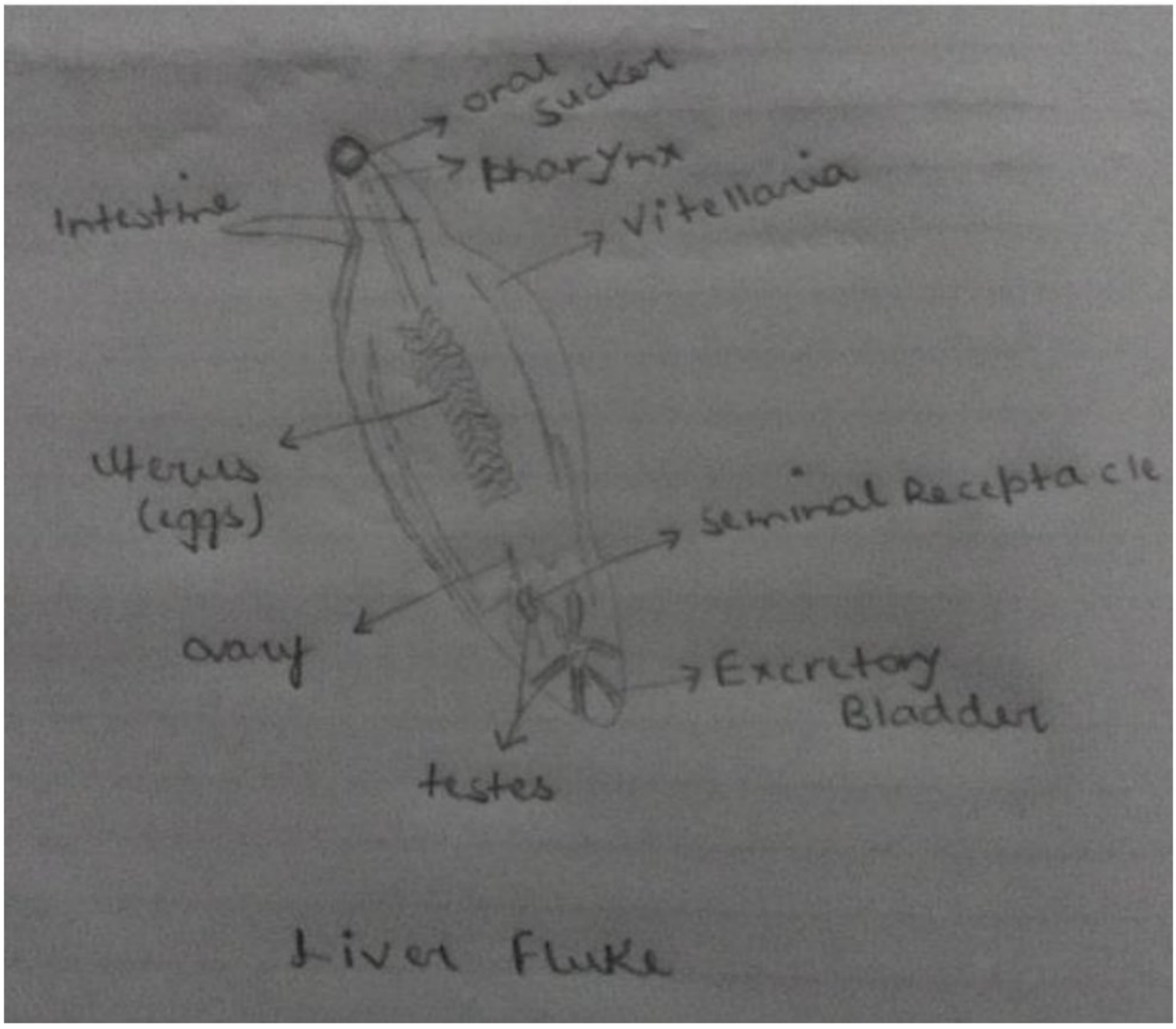
PARAMECIUM


Pellicle
Anterior contractile vacuole
Oral groove
Macronucleus
Micro nucleus
cytosome
Buccal cavity with rows of cilia used in feeding.
Posterior contractile Vacuole
Posterior End

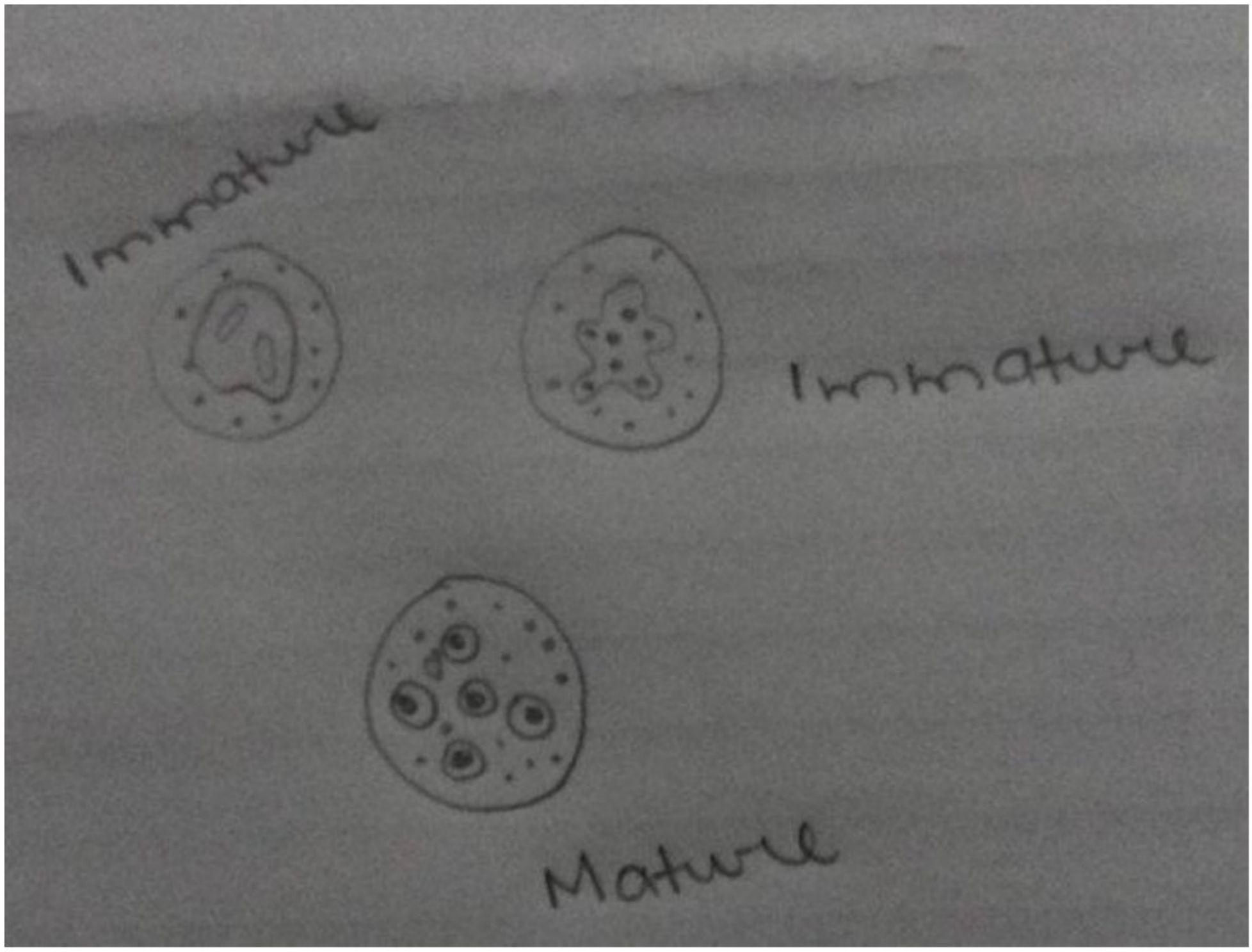
TRYPANOSOMA



Liver Fluke


oral Sucker
Pharynx
Intestine
Vitellaria
Uterus (eggs)
Seminal Receptacle
ovary
Excretory Bladder
testes
Liver Fluke

SCHIZONT(MALARIAL PARASITE)



PHARMACOLOGY

PREPARATION OF NORMAL SALINE SOLUTION

THEORY

Phrase solution of 0.9% w/v of NaCl

APPLICATION OF NORMAL SALINE SOLUTION

- N.S. is used frequently in intravenous drips for patient when cannot take fluids orally and have developed or in danger of developing dehydration.
- Normal saline is used for aseptic purpose. It is used to flush wounds and skins abrasions.
- N.S. is typically the firs fluid used when hypovolemia is serve enough to threaten the adequacy of blood circulation.

APPARATUS REQUIRED

- Beakers
- Measuring Flask
- Weighing Balance
- Meauring Cylinder
- Stirrer
- Pipette

METHOD OF PREPARATION

The normal saline solution is simply prepared by dissolving 9 grams of NaCl dissolved in water to ta toal volume of 1000ml in clean apparatus.

RESULT

Normal Saline Solution was prepared and labeled properly.

Preparation of Physiological Salt Solution (Tyrode's Solution)

Various isolated issues such as intestinal, tracheal and uterine smooth muscle, cardiac and skeletal muscles isolated from body of freshly killed animals, remains viable and responsive to the drugs, when these are suspended in a suitable physiological salt solution.

These solution provide nutrition (glucose), essential ions, requsite pH, temperature and oxygen for activity of the isolated tissues.

Apparatus Required

- Beakers
- Measuring Flask
- Weighing Balance
- Meauring Cylinder
- Stirrer
- Pipette

Composition of Tyrode's Solution (Quantities for 10 Litres)

Chemical	Quantity
NaCl	80g
KCl 10%	20ml
$MgSO_4.7H_2O$ 10%	26ml
$NaH_2PO_4.2H_2O$ 5%	13ml
Glucose	10g
$NaHCO_3$	10g
$CaCl_2$ (Molar)	18ml
Aerating gas	O_2 or Air

Procedure

- Wash, clean and dry the apparatus.
- Prepare 10% KCl solution in a beaker and label it.
- Similarly prepare MgSO4 and NaH2PO4 10% and 5% respectively by dissolving in distilled water.
- Prepare solution A by mixing the given amount of NaCl, KCl, MgCl, glucose and NaHPO4 in require amount of water.
- Prepare solution is by dissolving Na2CO3 in specific amount of water require to dissolve it.
- Prepare solution C by dissolving CaCl2 in require amount of water
- Prepare solution C by dissolving CaCl2 in require amount of water.
- Prepare solution N by dissolving/mixing solution A and Solution B.
- Tyrode's Soution is preapared by mixing solution N with Solution C and making the volume up to 10 liters.

Application of Tyrode's Solution

Tyrode's solution is used for the study of isolated intestine tissue study by making it responsive and functional.

Prevention

Do not mix CaCl with NaHCO3 directly or undissolve calcium bicarbonate will be formed which is undesirable.

Result

Tyrode's Solution was prepared and labeled.

Preparation of Physiological Salt Solutions (Frog's Ringer and Kreb's Solution)

Theory

Various isolated tissues from the body of freshly killed animals remain viable and responsive to drugs, when these are suspensed in a suitable physiological salt solution.

These solution provide nutrition (glucose), essential ions, requisite pH, temperature and oxygen for activity of isolated tissue.

Apparatus Required

- Beakers
- Measuring Flask
- Weighing Balance
- Meauring Cylinder
- Stirrer
- Pipette

Composition of Physiological Salt Solution

Chemicals	Frog's Ringer	Kreb's
NaCl	65g	69g
KCl 10%	14ml	35ml
$MgSO_4.7H_2O$ 10%	-	29ml
$NaH_2PO_4.2H_2O$ 5%	1.3ml	-
KH_2PO_4 10%	-	16ml
Glucose	20g	20g
$NaHCO_3$	4g	21g
$CaCl_2$ (Molar)	Air	25.2ml
Aerating gas	Air	O_2 +5% CO_2

Methods

- Wash, clean and dry all the apparatus
- Prepare 10% solution of KCl, MgSO4 and KH2PO4.
- Prepare 5% solution of NaH2PO4
- Prepare Frog's Ringer Solution by mixing the chemical and solution in correct proportion and make up the volume up to 10L.
- Prepare Kreb's Solution similarly
- Label both the solution.

Precaution

Mix the chemicals in correct way, avoiding the formation of salts, bicarbonates or any undesirable reaction.

Application

- For amphibian tissue use frog's ringer solution.
- For mammalian or aian skeletal muscle use Kreb's Solution.

Result

Frog's Ringer Solution and Kreb's Solution were prepared and labeled properly.

Assembly of Kymograph and Organ Bath

The Isolated Organ Bath

Isolated organ bath is equipment used in experiments on isolated tissues and organs. Various parts of the organ bath, along with their functions are summarized below:

Organ Bath

Organ bath is conical hollow glass tube that is connected with inlet and outlet tubes and is fixed in the center of outer water bath. It may have capacity varying from 10-50ml.

Outlet Pipe

It is attached with the base of organ bath and stop cork in it. When opened, it drains out the fluid from the Organ Bath.

Inlet Pipe

It enters the water ath and gain the coiled tube form around the organ bath

Thermostat and Heater

It is provided with in the water bath to maintain the temperature of the tissues in the organ bath at 35-37'C.

Kymograph

Drum

It is a metallic cylinder. It is 15.2 cm in diameter and 15.2 cm in height. It is covered with paper for recording activity.

Spindle

It is a metallic rod at the top of kymograph that hold the drum and the shaft.

Contact Arm

It is a triangular projection is two halves at the base of the spindle, present in those units that have a built in stimulator.

Kymograph Arm

It is a moveable adjustable arm like structure on the top of kymograph

Gear and Speed Regulator

It is used to adjust the speed of the drum

Start/ Pause Lever

It is a small lever concerned with the drum. If it is at the start position drum moves freely, and if it at pause position, the drum takes one rotation and then stop.

Assemble the kymograph and organ bath as shown by the teacher in the class

Precautions

- Writing needle or pen should be balanced
- Tissue hanging lever should not touch the walls of the organ bath
- Arms should be perpendicular to writing lever
- Arms should not touch the smoke drum

Effect of Local Anesthetic on Rabbit's Eye

Theory

An anesthetic is a drug that causes anesthesia-reversible loss of sensation. They contrast with analgesics which relieve pain without elimination sensation. These drugs are generally administered to facilitate surgery. Local anesthetics are agents that prevent transmission of nerve impulses without causing unconsciousness e.g. procaine, cocaine, amethocaine and dibucaine etc.

Requirements

- Rabbits
- Cotton
- Torch
- Scale
- Animal weighing Balance
- Stop Watch
- Stethoscope
- Therometer
- Given Drug

Procedure

- Vital signs of rabbit are observed are recorded which include weight, body, temperature, heart rate, respiratory rate, hunger and thirst.
- Corneal reflex is observed and noted by placing a thin cotton near Rabbit's eye
- Light reflex is observed and recorded by applying light through torch on both eyes.
- 2 drops of drug X are administered in to Rabbit's eye
- Corneal and light reflex after each 5, 10, 15 and 20 minutes are observed and noted.

Result

Drug X causes the local anesthetic effect on the left eye of Rabbit

	Reading 1	Reading 2	Reading 3	Average Reading
Weight (kg)				
Body Temp. ('C)				
Heart Rate (beat/min)				
Resp. Rate (breaths/min)				
Hunger				
Thirst				
Both Eyes				
Corneal Reflex				
Light Reflex				
Pupil Size (cm)				
After Instillation of Drug X				
Corneal Reflex on left eye				
After 5 mins				
After 10 mins				
After 15 mins				
After 20 mins				
Corneal Reflex on Right eye				

After 5 mins	
After 10 mins	
After 15 mins	
After 20 mins	

To Study the Effect of Given Drug on The Rabbit's Eye

Theory

A wide variety of drugs can produce changes in the eye. These includes changes in size of the pubil, intraocular pressure, acomadation, light reflex and corneal reflex.

Iris contain two types of muscle fibers i.e. circular and radial fibers. The circular fibers are innervated by parasympathetic nerous system through oculmotor nerve. They are far more prominent as compared to the radial fiers and produce pupillary constriction or contraction.

The radially arranged muscle fibers, few in number are innervated by sympathetic nerves and contain alpha-1 receptor on them. On stimulation they produce incomplete pupillary dilation.

Requirements

- Rabbits
- Cotton
- Torch
- Scale
- Animal weighing Balance
- Stop Watch
- Stethoscope
- Thermometer
- Given Drug

Procedure

- Vital signs of Rabbits are observed and recorded which includes
    - Weight
    - Body temperature
    - Heart rate
    - Respiratory Rate
    - Hunger and Thirst
- Corneal reflex is observed and recorded by placing a thin cotton near rabbit eyes
- Light reflex is observed and recorded by applying light on both eyes through torch.
- Initial reading of pupil size is taken by the help of scale which is placed just near the Rabbit's eye
- Given drug is administered drop wise (2 drops).
- After 5, 10, 15 and 20 mins of drugs administration the reading of pupul size is observed and recorded.

	Reading 1	Reading 2	Reading 3	Average Reading
Weight (kg)				
Body Temp. ('C)				
Heart Rate (beat/min)				
Resp. Rate (breaths/min)				
Hunger				
Thirst				
Both Eyes				

Corneal Reflex
Light Reflex
Pupil Size (cm)
After Instillation of Drug X
Corneal Reflex on left eye
After 5 mins
After 10 mins
After 15 mins
After 20 mins
Corneal Reflex on Right eye
After 5 mins
After 10 mins
After 15 mins
After 20 mins

RESULT

The given drug causes the constriction of the pupil of rabbit's eye.

To Study the Antagonistic Effect of Drugs on Rabbit's Eyes

Theory

An antagonistic drug effect is when 2 days negate each other. An example would be a drug that causes the dilation of the pupil of rabbit's eye while the other causes constriction. Both the drugs are considered to be antagonistic with regard to pupil of the rabbit's eyes.

Requirements

- Rabbits
- Cotton
- Torch
- Scale
- Animal weighing Balance
- Stop Watch
- Stethoscope
- Thermometer
- Given Drug

Procedure

- Vital signs of Rabbits are observed and recorded which includes
    - Weight
    - Body temperature
    - Heart rate
    - Respiratory Rate
    - Hunger and Thirst
- Corneal reflex is observed and recorded by placing a thin cotton near rabbit eyes
- Light reflex is observed and recorded by applying light on both eyes through torch.
- Initial reading of pupil size is taken by the help of scale which is placed just near the Rabbit's eye
- 2 drops of drug X are administered in to Rabbit's eye
- Reading is noted after 5, 10 and 15 minutes of administration
- After it the drug is washed with water for 5 minutes
- Drug Y is administered in to Rabbit's eye
- Reading after 5, 10 and 15 minutes is noted down

Precautions

After using a drug sufficient time should be allowed before applying the next drug so that the eye recovers from the action of previous drug.

	Reading 1	Reading 2	Reading 3	Average Reading
Weight (kg)				
Body Temp. (‘C)				
Heart Rate (beat/min)				
Resp. Rate (breaths/min)				
Hunger				
Thirst				
Both Eyes				
Corneal Reflex				

Light Reflex	
Pupil Size (cm)	
After Instillation of Drug X	
Corneal Reflex on left eye	
After 5 mins	
After 10 mins	
After 15 mins	
After 20 mins	
Corneal Reflex on Right eye	
After 5 mins	
After 10 mins	
After 15 mins	
After 20 mins	

RESULT

Drug X and Y are antagonistic with regard to the pupil of rabbit's eye

To Study the Contraction and Relaxation of Rabbit's Intestine

Theory

The intestinal wall is composed of outer longitudinal and inner circular muscle coat. The contraction and relaxation of these muscles can be observed by freshly cut isolated rabbit's intestine in a specific oxygenated solution placed in isolated organ bath and attached to assemble kymograph. Kymograph helps in representing the contraction and relaxation of muscles in form of graph.

Requirements

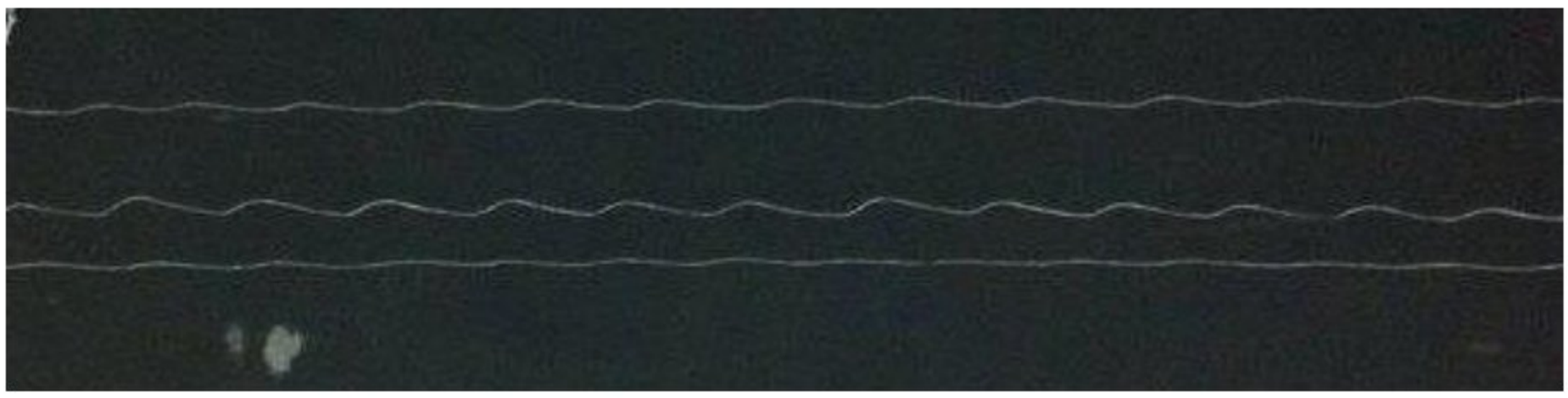
- Isolated Rabbit's intestine
- Solution
- Assembled Kymograph and Organ bath
- Smoke paper

Procedure

- Sacrifice an overnight fasting rabbit. Take out small intestine by recognizing the caecum and cut down 2 cm piece of Rabbit's ileum. Pass a thread at both ends and transfer to isolated organ bath containing solution at 35-37^{0}C.
- One end of a piece of tissue is attached with hook by means of thread while the other is connected to frontal writing lever.
- The lever moves with the contraction of tissues which are recorded on drum with the help of kymograph.
- A record of normal movement is taken.

Precautions

The temperature of the solution should be maintained throughout the experiment between 35-37 ^{0}C.



To Study the Normal Contraction and Relaxation of Frog's Heart Muscle

Theory

Efficiency of the heart as a mechanical pump depends on auto-rhythmicity, conductivity and contractility which are inherent properties of myocardium but also under the influence of autonomic nervous system.

Parasympathetic Inversion

The preganglionic fibers arise from the vagal nuclei in the medulla and pass via tha vagus nerves and their cardiac branches to the cardiac plexuses. From the cardiac ganglia postganglionic fibers go out to the cells in the sino-atrial (SA) and atrio-ventricular (AV) nodes and the bundle of HIS. No parasympathetic fibers are present the ventricle. The post synaptic parasympathetic receptors in heart are of M2 Type, which are stimulated by acetylcholine released from the nerve ending.

Anticholinergic drugs like atropine facilitate AV conduction and increase the heart rate by blocking the vagal activity.

Sympathetic Innervation

The preganglionic fibers of sympathetic nerve originate from the cells in the inter-mediolateral gray column of the 4^{th} and 5^{th} segments of thoracic spinal cord.

Stimulation of sympathetic nerve causes a great increase in both the rate and force of contraction of heart e.g. propranolol, atenolol etc.

Effects of various ions and drugs on the cardiac activity, various ions also effect the myocardial activity. An increase in serum potassium level results in slowing of heart rate and decrease in conduction velocity.

Calcium is an essential ion for excitation-contraction coupling in cardiac muscle. It also plays an important role in depolarization and conduction of impulse through AV node. Those which directly increase the force of cardiac contraction are like Na+/K+ ATPase (sodium pump) inhibition in cardiac cell membrane and called cariotonic such as cardiac glycosides (e.g. digoxin and digitoxin).

Direct acting cardiac depressant include quinidine, procainamide and lignocaine tat act by blocking sodium channel. Calcium channel blocker like verapamil and diltiazem are also cardio-depression.

Procedure

Stun and pith the frog and fix it on the frog board by pins. Expose the heart by cutting sternum. Fix the hook of the lever in the apex of the heart. Lever moves with normal cardiac contraction which are recorded on the revolving drum of the kymograph. Take the normal tracing.

Precaution

- Take a fresh normal tracing before using every drug and compare the effects of subsequently used drug with it.
- Lever must be attached properly at 90' angle
- Temperature of organ bath and Ringer's solution must be maintained