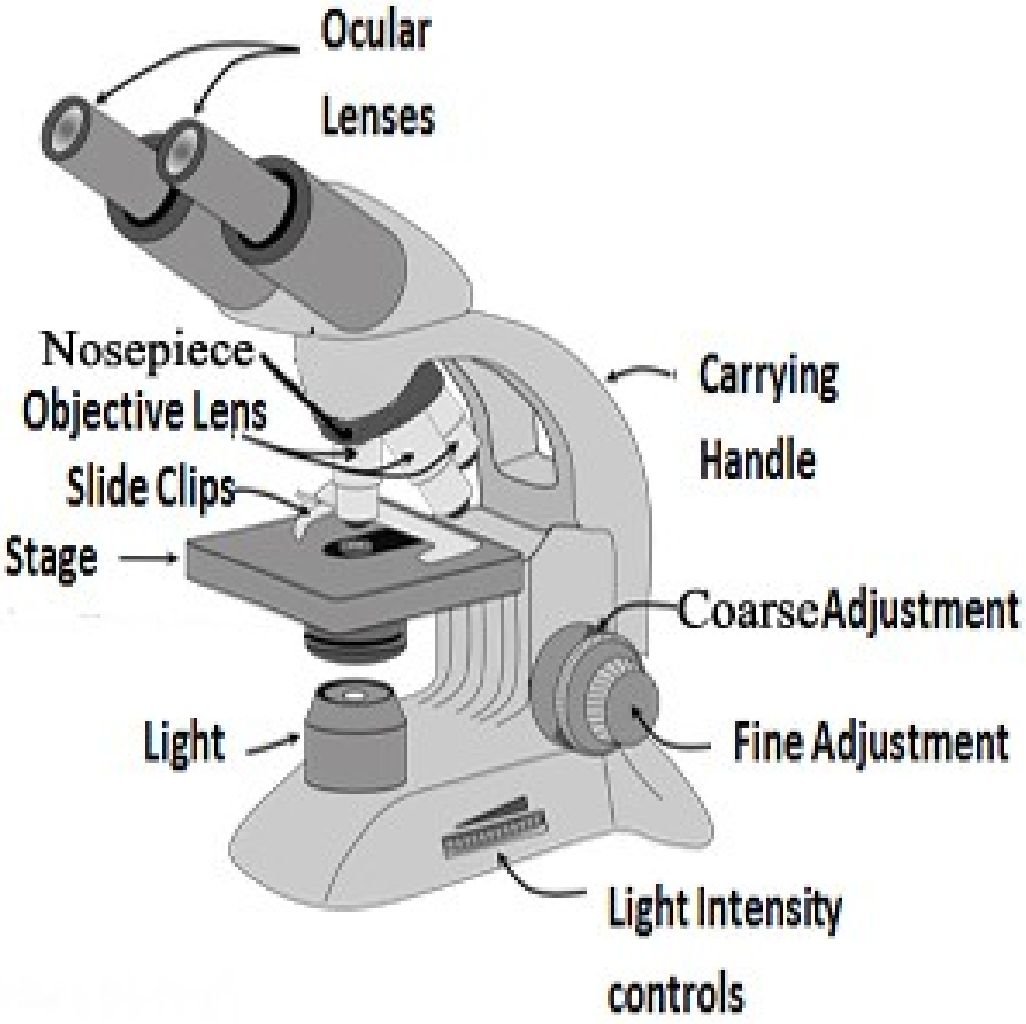
Cells and Organelles

- **The purpose of the work**:
- familiarity with the device of the microscope and the rules of work in the laboratory of botany.
- **Tasks:**

1) familiarize yourself with the safety rules when working in a botanical laboratory;
2) to study the device of the microscope and the procedure for working with it.
3) master the methods of manufacturing drugs

Microscope Structure and Function



- **Eyepiece Lens:** the lens at the top of the microscope that you look through. They eyepiece is usually 10x or 15x power.
- **Tube:** Connects the eyepiece to the objective lenses.
- **Arm:** Supports the tube and connects it to the base of the microscope.
- **Base:** The bottom of the microscope, used for support.
- **Illuminator:** A steady light source (110v) used in place of a mirror. If your microscope has a mirror, it is used to reflect light from an external light source up through the bottom of the stage.
- **Stage:** The flat platform where you place your slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it forward and back.
- **Removing Nosepiece or Turret:** This is the part of the microscope that holds two or more objective lenses and can be rotated to easily change power (magnification).
- Coarse adjustment: Brings the specimen into general focus.

- **Fine adjustment:** Fine tunes the focus and increases the detail of the specimen.
- **Nosepiece:** A rotating turret that houses the objective lenses. The viewer spins the nosepiece to select different objective lenses.
- When focusing the microscope, be careful that the objective lens doesn't touch the slide, as it could break the slide and destroy the specimen.
- **Stage clips:** Metal clips that hold the slide in place.
- **Stage height adjustment (Stage Control):** These knobs move the stage left and right or up and down.
- **Illumination:** The light source for a microscope.
- **Condenser:** Gathers and focuses light from the illuminator onto the specimen being viewed.
- **Base:** The base supports the microscope and it's where illuminator is located.

- Razor blades, Petri dishes, slides, coverglasses, needles, forceps, Pasteur pipettes with rubber bulbs , and a large water bottle.

- Other supplies such as filter papers, lens paper and lens cleaner for slides and microscope lens are available in the laboratory.

Free hand sectioning methods

Free hand section

- is the simplest method of preparing specimens for microscopic viewing. This method allows one to examine the specimen in a few minutes.
- It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs.
- The fixation of materials is generally not required for temporary preparations.

Procedures:

- 1. Obtain a new double edge razor blade. To minimize the risk of cutting oneself, cover one edge of the razor blade with masking tape. Rinse the blade with warm tap water to remove traces of grease from the surface of the blade if necessary.
- 2. Hold the plant material firmly. The material should be held against the side of the first finger of the left hand (or right hand) by means of the thumb. The first finger should be kept as straight as possible, while the thumb is kept well below the surface of the material out of the way of the razor edge (see Figure 1).
- Flood the razor with water. This will reduce the friction during cutting as sections can float onto the surface of the blade.
- Take the razor blade in the right hand (or left hand) and place it on the first finger of the left hand (or right hand), more or less at a right angle to the specimen.

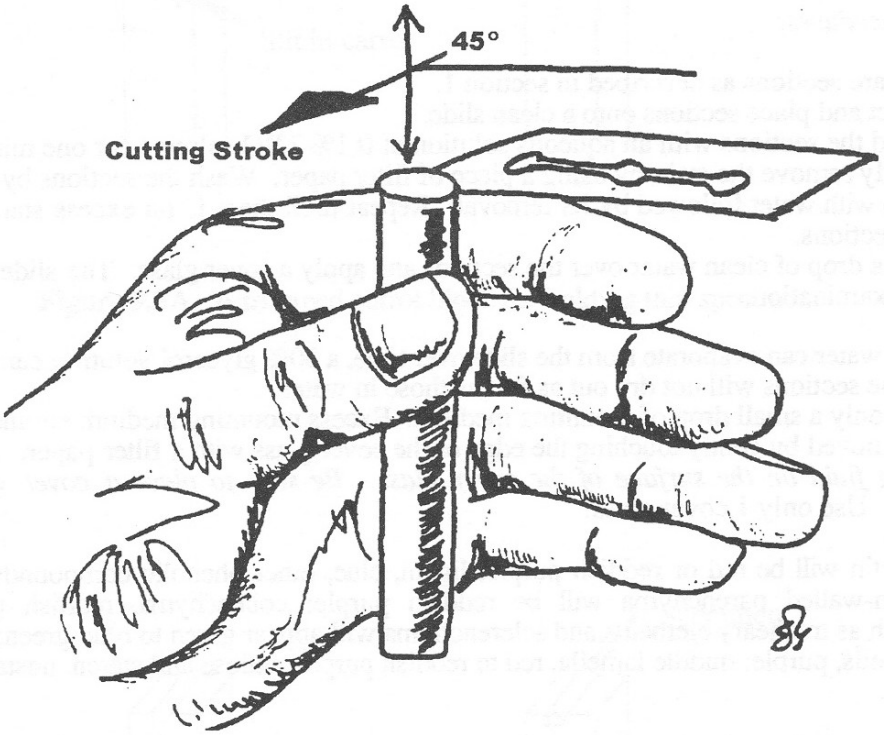


Figure 1

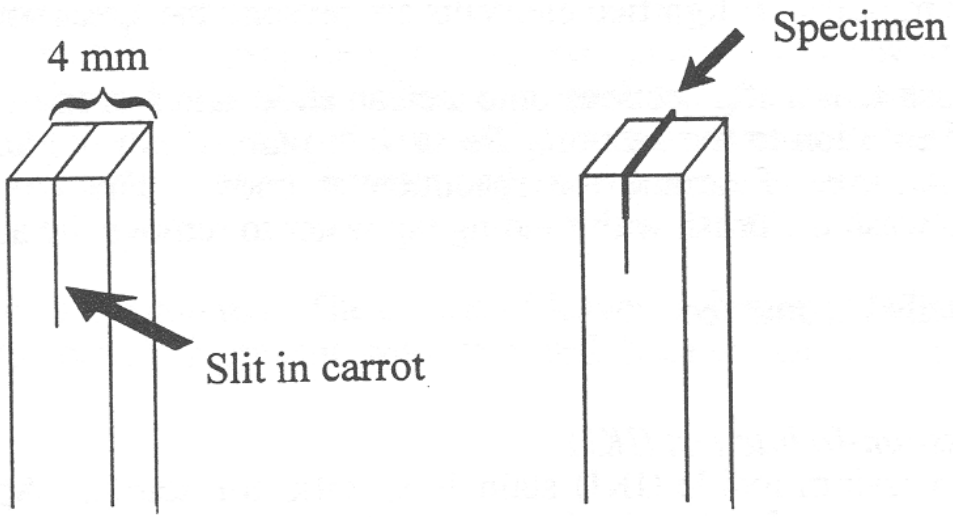


Figure 2A.

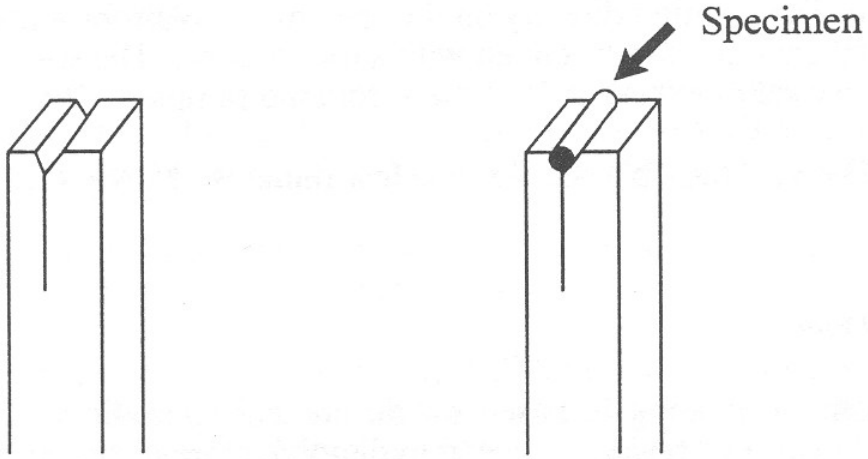


Figure 2B.

- Draw the razor across the top of the material in such a way as to give the material a drawing cut (about 45∞ in the horizontal direction). This results in less friction as the razor blade passes through the specimen. Cut several sections at a time. Sections will certainly vary in thickness. However, there will be usable ones among the "thick" sections.
- Transfer sections to water .
- Select and transfer the thinnest sections (the more transparent ones) onto a glass slide.
- For delicate and hard to hold specimens such as thin leaves and tiny roots, additional support can be used to facilitate hand sectioning. The following methods will allow for the sectioning of thin leaves and small, soft specimens such as roots. As shown in Fig. 2A, tissue pieces can be inserted into a small piece of pith such as a carrot root. Once the tissue is firmly in place, the hand sectioning technique can be applied.
- Longitudinal sections are also difficult to obtain by hand without supporting material as small stem and root pieces are difficult to hold with one's finger. However, by cutting a v-shaped notch into the pith support (Fig. 2B), it is possible to hold the tissue firmly for free hand sections.

Laboratory work 1

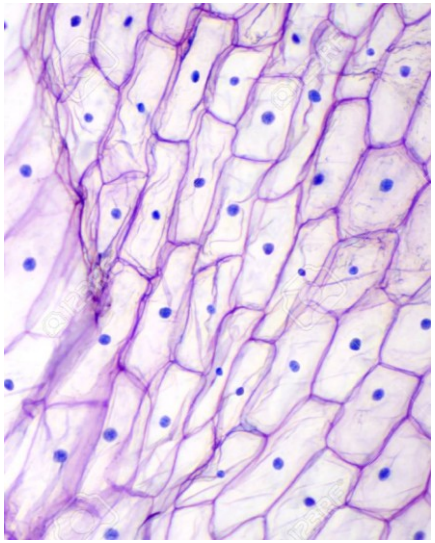
The structure of the plant cell; cytoplasmic streaming, plastids

Material:
onion bulb (*Allium cepa* L.), iodine solution;
Elodea leaves (*Elodea canadensis* Rich.), fresh tomato fruit (*Licopersicum* sp.)
Tradescantia leaf (*Tradescantia* sp.); pear fruit (*Pyrus* sp.).

Objective: to study the structural features of the plant cell**.**

Tasks of work: to consider a plant cell with its constituent parts - the membrane, cytoplasm, nucleus, plastids, get acquainted with the location of the cytoplasm and cytoplasmic streaming.

- Make wet mount (temporary mount) of epidermis onion bulbs. To study the structure of the cell at high magnification first in a drop of water, and then in iodine in iodine solution. Draw one or two cells and designate their main parts (Fig. 1).



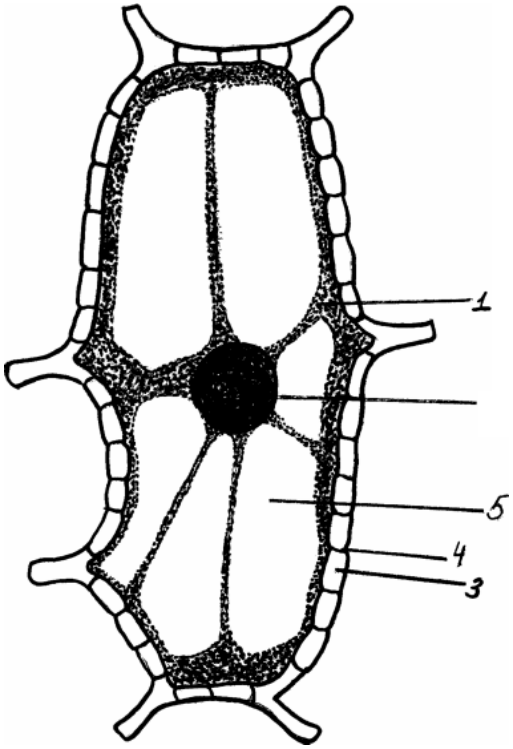


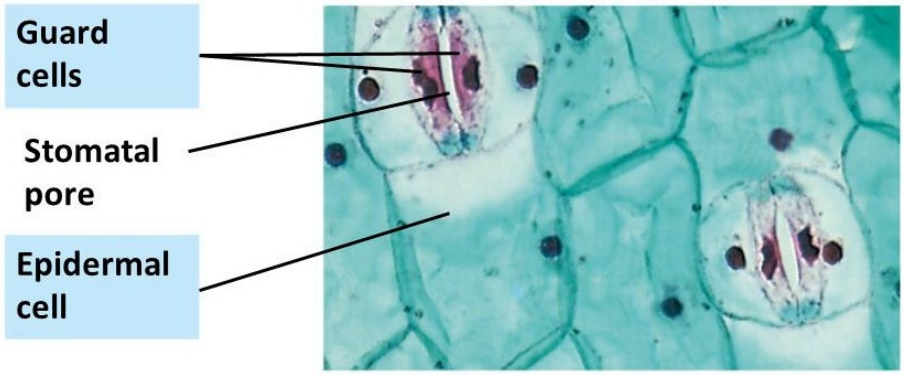
Fig. 1. Epidermal cells of onion (*Allium cepa*):
1 - cytoplasm; 2 - the nucleus; 3 – wall cells; 4 - pore; 5 - vacuole

- Place a small leaf of the aquatic plant *Elodea* in a drop of water on a slide and cover with a cover glass.
- Note an individual cell with 1 cell wall, a large central vacuole (the membrane of which is usually invisible), and peripheral chloroplasts (Fig. 2).

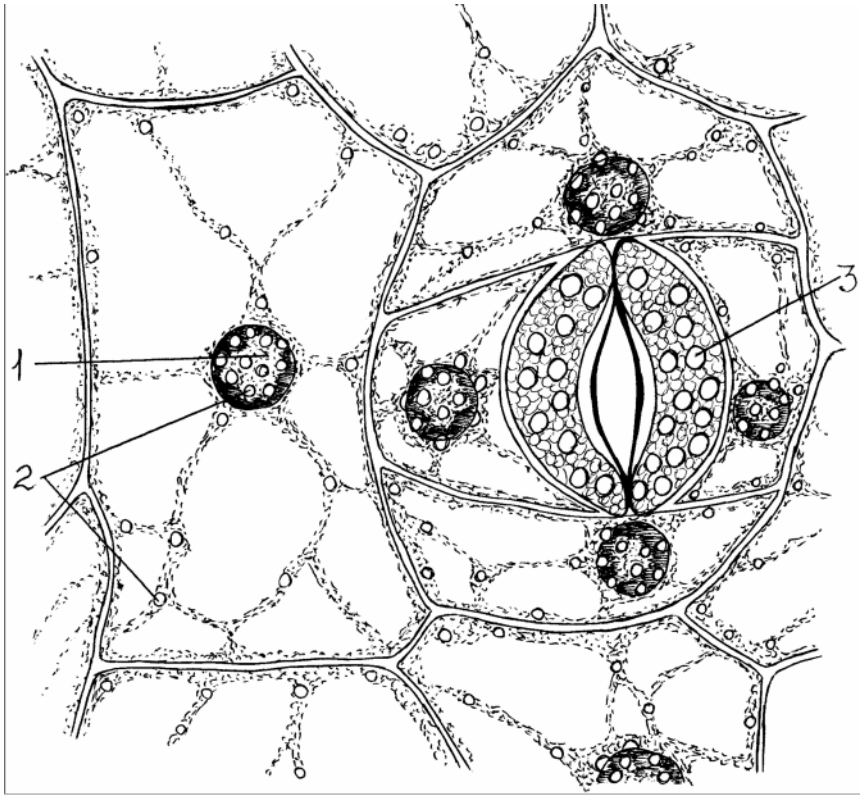
Fig. 2

- Note the cytoplasmic streaming (cyclosis). Draw.
- Add a drop of salt solution (hypertonic solution) to the side of the coverslip and observe the cell shrinking (optional).
- When the salt solution is added, the salt ions outside the cell membrane cause the water molecules to leave the cell through the cell membrane causing it to shrink into a blob in the centre of the cell wall.
- Plasmolysis is the phenomenon of the contraction of cytoplasm from the cell wall caused due to withdrawal of water when placed in a strong(hypertonic) solution.

- Make a wet mount of a small piece of fresh tomato fruit. Observe under high (40x) magnification and draw the chromoplasts, containing variably shaped carotenoid deposits that impart color to the tomato fruit. Draw and designate their main parts.
- Make a wet mount of the lower epidermis of the Tradescantia leaf. Observe under high magnification and find leucoplasts. Draw the 1-2 cells and designate their main parts (Fig.3) .



(b) Surface view of a spiderwort (*Tradescantia*) leaf (LM)

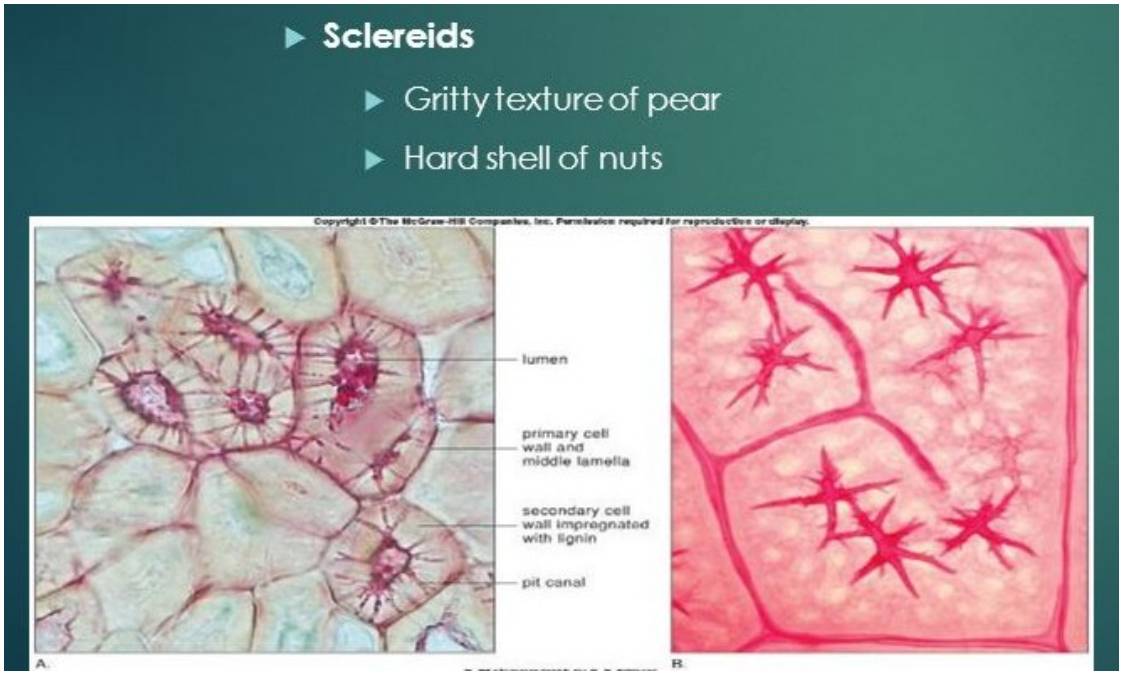


1 - the core; 2 - leucoplasts; 3 - chloroplasts

Fig.3

Cell walls

- Growing plant cells produce primary cell walls composed predominantly of polysaccharides. Some cell types produce a **secondary cell wall** that may become impregnated with an aromatic polymer called **lignin.** Cells with primary cell walls and those with lignified secondary cell walls can be observed in the flesh of pear fruit. Obtain a small piece of pear flesh and place it on a slide**.** Place a coverslip over the material and press gently to spread the cells. Draw a diagram that distinguishes cells with primary cell walls only from those with secondary cell walls (Pear sclereid or stone cell).



Laboratory work

Ergastic Substances of plant cells

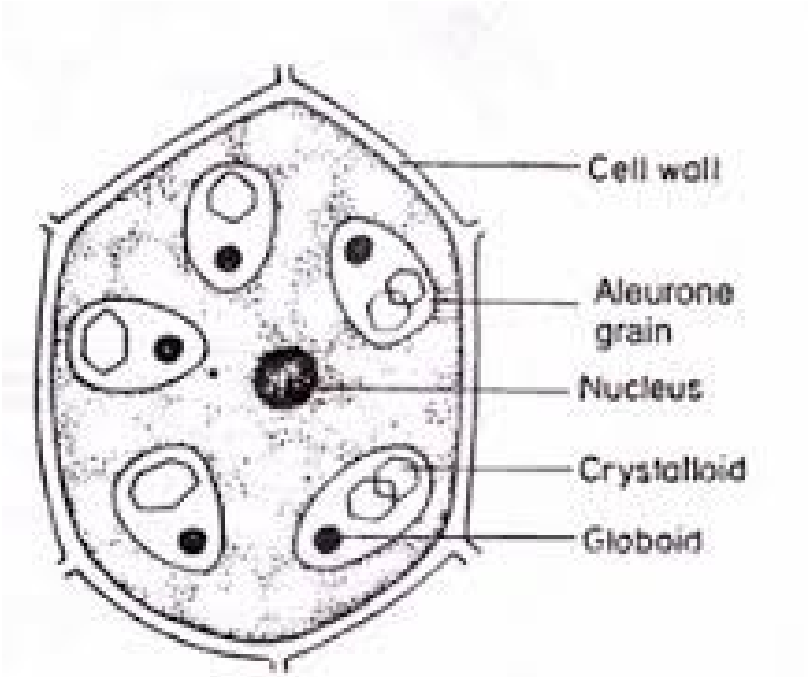
Material:
potato tuber (*Solanum tuberosum* L.), wheat grains (*Triticum aestivum* L.), maize (*Zea maize* L.), oats (*Avéna satíva*), petioles the leaves of begonia (*Begonia* sp.), tradescantia (*Tradescantia* sp.); iodine solution, glycerin.
Objective: to show the diversity of reserve substances and crystals of mineral salts formed in plant cells
Tasks of work: to consider the forms of starch grains in plants, the deposition of proteins in seeds and the structure of crystals.

Amyloplasts (starch grains): Scrape a small piece of potato (*Solanum tuberosum*) both in a drop of water and in a drop of iodine solution and mount with a cover slip. Observe the lamellate starch grains, often having a distinctive morphology for each species. The iodine solution stains starch blue to black in color. Draw complex and simple grains of starch .

Starch grains of various plant species:
1 - potatoes; 2 - peas; 3 - wheat; 4 - corn; 5 - oats

Aleurone grain.

Scrape a small piece from the surface pre-wetted seeds of pea (*Pisum sativum*) or lentil (*Lens culinaris)* both in a drop of water and in a drop of iodine solution and mount with a cover slip. Observe the aleurone grains. Draw cells with aleurone grains. Iodine solution stains aleurone grains in golden yellow color. In most seeds, the aleuron grains contain three morphologically distinct regions: the matrix, crystalloid, and globoid.

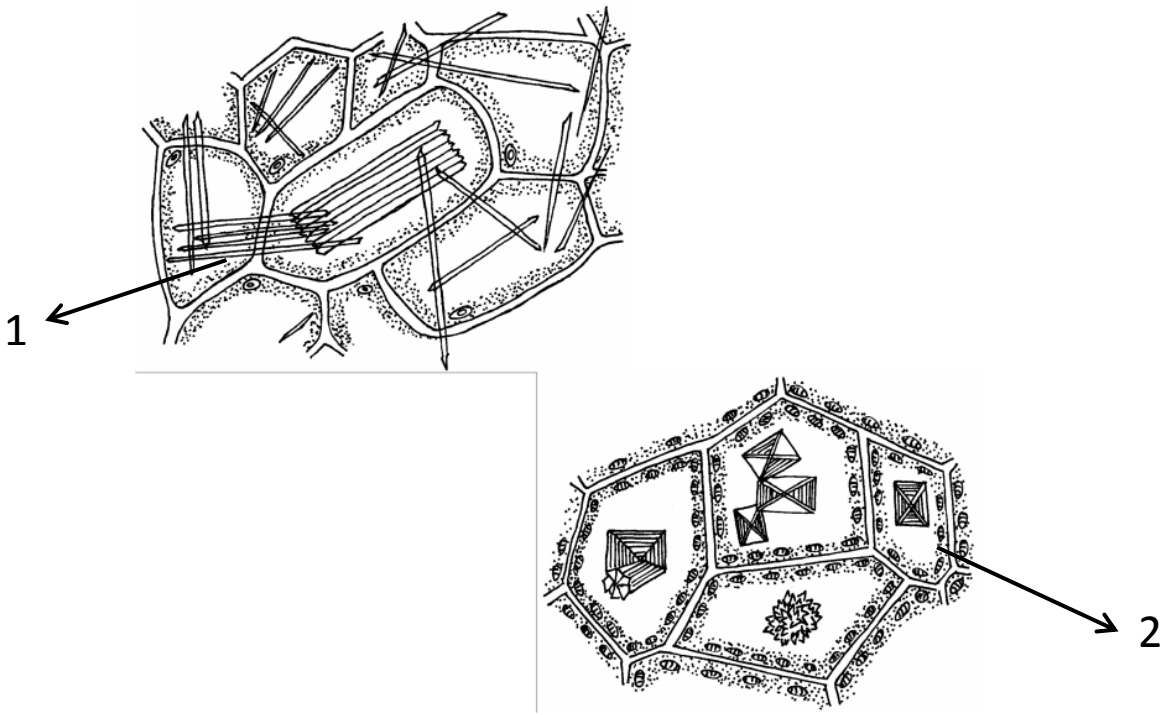


Aleurone grains of Casterbean (Ricinus sp.)

Crystals:

Make wet mount of a cross-section of the

petiole begonia and tradescantia. Observe calcium oxalate crystals. Draw.



Crystals of calcium oxalate:
1 – tradescantia, 2 - begonia