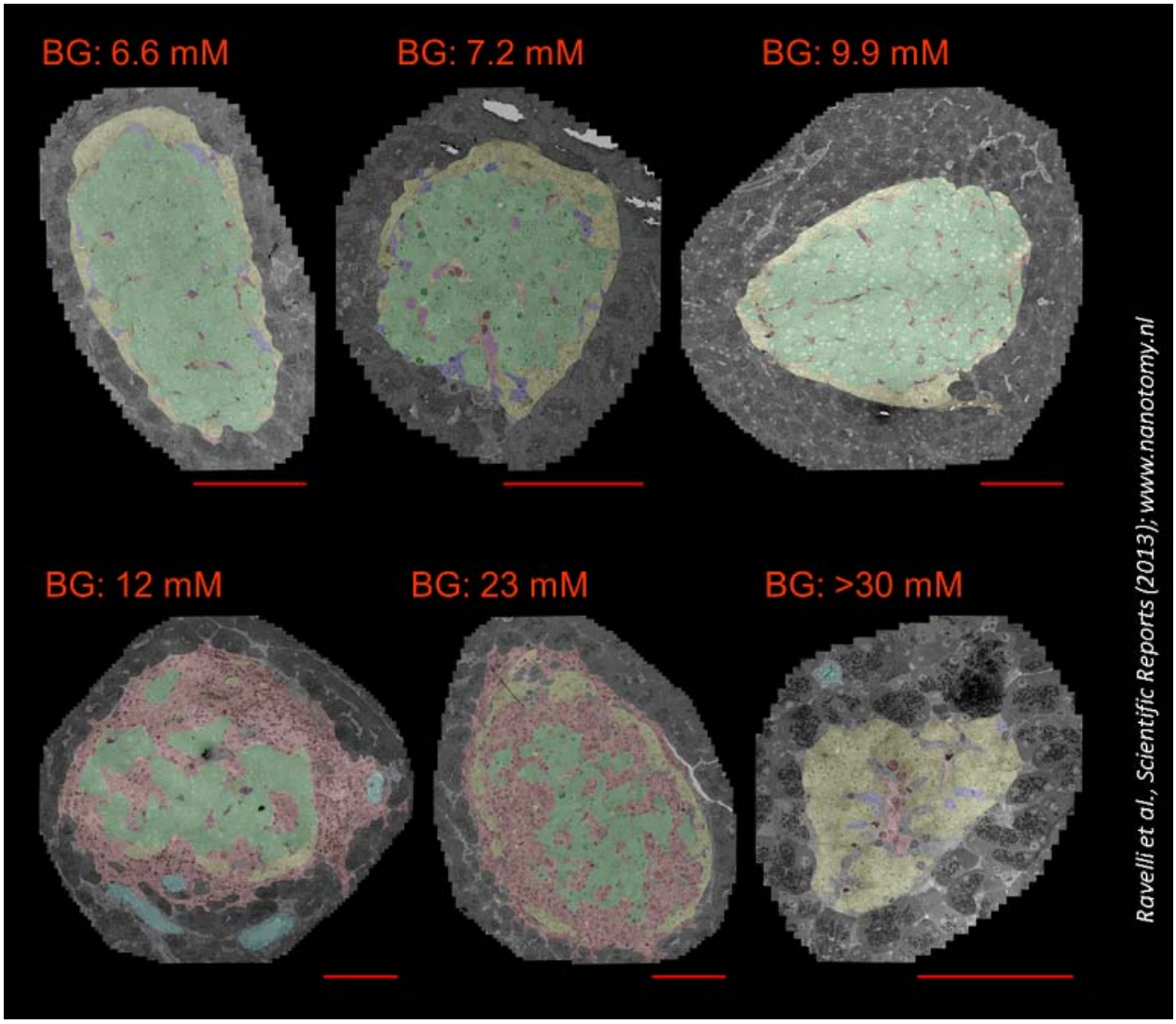
Cells under the microscope

Electron microscopy



Lecturers: Jack Fransen and Huib Croes
Department: Cell Biology and Microscopic Imaging Centre, RUMC

Preparation

Reading

- Ravelli et al. SREP01804 (2013) accessible via: http://www.nature.com/srep/2013/130508/srep01804/full/srep01804.html
- The assignments in this manual
- *Essential Cell Biology*, Alberts et al., (Garland, 4th ed. 2013).
  - Chapters 1 & 15
- *Functionele histologie* [Functional Histology, in Dutch], Junqueira (Reed, 14th ed. 2014)
  - Chapters 1, 2, 3 & 19: pp. 566-568

To do

- Execute assignments 1-4 during the self-study on day one
- Assignments 5-7 will be handed out and performed during the second day
- Ensure you are on time for the practicals

Acknowledgement

This workshop is adapted from the Nanotomy workshop originally developed by Ben Giepmans, Dept. of Cell Biology, UMCG, and set up in close collaboration with him.

In addition to this workshop you may be interested in this link: http://www.ronaldschulte.nl/

Objectives

After this workshop, students should be able to:

1. recognize and interpret electron microscopic images, with regard to tissue characteristics, cell types, organelles, and macromolecular complexes
2. explain how functional information about cell function, e.g. regarding secretion, can be determined using nanotomy
3. describe the structure of the cell, cell organelles and macromolecular complexes and name their functions
4. use the information from 1-3 for pathophysiological analysis, in this workshop primarily with regard to type 1 diabetes

Assignment 1: Electron microscopy structure/function

A cell contains organelles that are essential for its function. Depending on cellular function, one type of cell will have a higher number of certain organelles than others. To check if you know the various cell organelles, examine the following schematic drawing of a cell (an exocrine cell).

a. Identify the various cell organelles by placing the right number at the right line
b. State the main function(s) of the organelle in the table

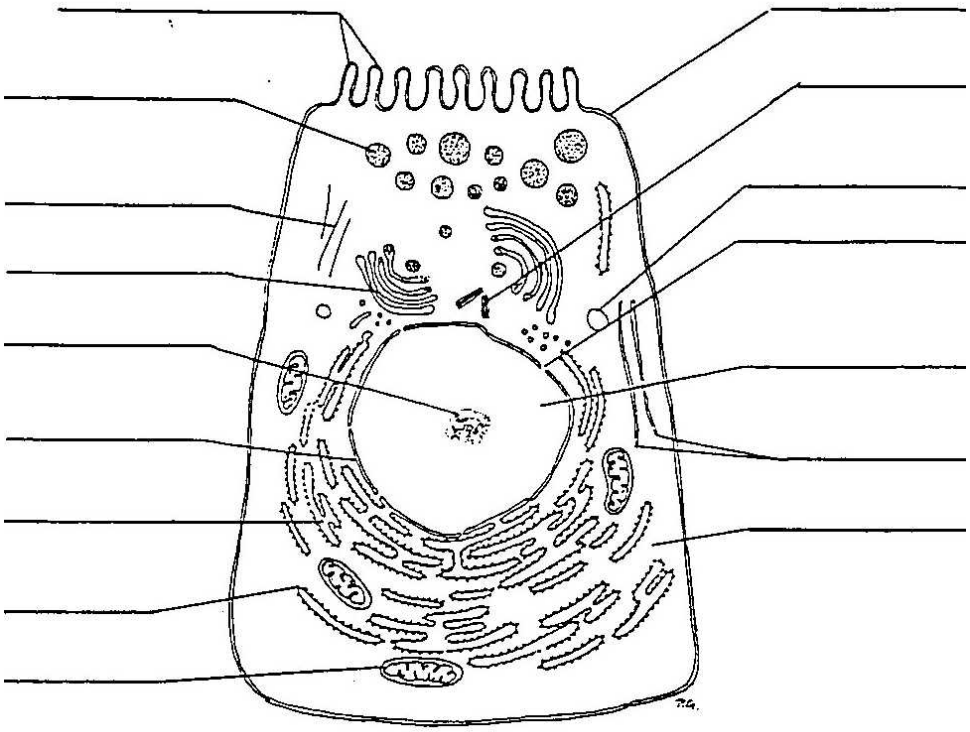


Fig. 1. Source: *Laboratory Manual of Histology,* Pappas. (W. C. Brown, 1990)

Structure	**Function**	**Structure**	**Function**
1. centriole		9. microtubules	
2. cytosol		10. mitochondria	
3. Golgi complex		11. microvilli	
4. nucleus		12. nucleolus	
5. nuclear envelope		13. plasma membrane	
6. nuclear pore		14. ribosomes	
7. lysosome		15. rough endoplasmic reticulum	
8. microfilaments		16. secretion drops	

Assignment 2: Studying electron microscopy

In ***transmission EM (TEM)***, a high voltage generated between a heated cathode (incandescent filament) and an anode produces a beam of electrons. One or more condenser lenses focus this beam onto the plane of focus of the objective lenses, where an ultrathin specimen section which can be irradiated has been placed. The objective lenses create a magnified image of the object which is shown on screen or captured by camera. A standard TEM provides magnification of up to 300,000 times, with a resolution of ~ 2 nm, of specimen sections which are ~ 60 nm thick, with a maximum diameter of 3 mm.

The biological material present in the ultrathin section mainly comprises C, H, N and O and does not scatter electrons sufficiently to provide an image, which is why the specimen must be stained with heavy metals, which do scatter electrons. The most common contrast medium is osmium tetroxide (OsO_4), which binds particularly easily to double bonds of lipids, fixing them by creating cross-links, making membranes visible.

In ***scanning EM (SEM)***, the electron beam is focused by the condenser and objective lenses in the same manner as in TEM. Here, too, the specimen is placed in the focal point. The primary electron beam is not stationary, like in TEM, but scans it in a grid-like fashion. The electron beam scans the specimen surface line for line, releasing secondary electrons in the sample or having the electrons reflected (backscatter electrons). These are both used to create an image of the specimen surface. If the specimen section is ultrathin, the electrons will of course pass through it. By placing a detector underneath, a TEM image can be transformed into an SEM image. This is known as ***STEM: scanning transmission EM.***

Also read: Essential Cell Biology, Alberts et al., (Garland, 4th ed. 2013) page 11.

Assignment 3: Studying membrane transport

Membranes form compartments. The plasma membrane is the boundary between the cytoplasm and the extracellular side. This boundary is dynamic, with various transport processes allowing substances to pass in and out of the cell. Some processes are exemplified using glucose-induced insulin secretion (Fig. 2). Note that various molecules and substances can be transported across the membrane in regulated fashion. In the figure below, indicate what type of transport process is involved (encircle one).

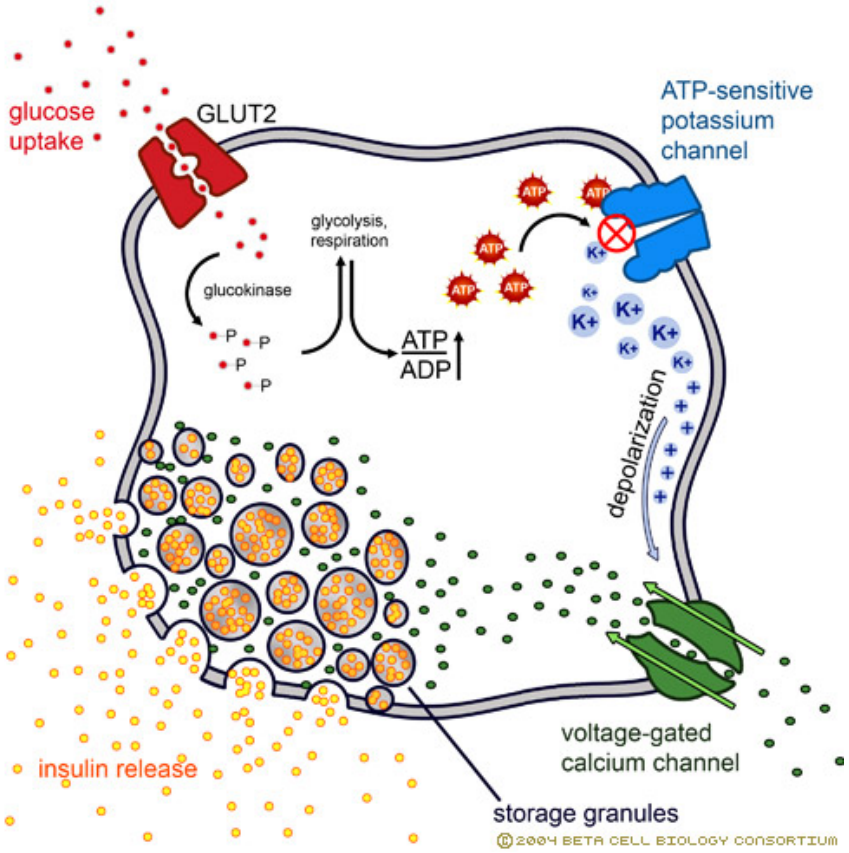


Fig. 2. Insulin secretion in beta cells caused by the increasing blood sugar levels.
Uptake of glucose by GLUT2 and glycolytic phosphorylation of glucose causes the ATP:ADP ratio to rise. This inactivates the potassium channel which depolarizes the membrane so that a voltage-dependent calcium channel opens. The increase in the calcium concentration leads to the release of insulin
Source: www.betacell.org

1. Glucose uptake by GLUT2 is primarily dependent on:
Exocytosis / Concentration / Voltage-dependent influx / Efflux inhibition

2. The ATP-sensitive K^+ pump is a type of:
Exocytosis / Concentration / Voltage-dependent influx / Efflux inhibition

3. The voltage-gated calcium pump is a type of:
Exocytosis / Concentration / Voltage-dependent influx / Efflux inhibition

4. Insulin secretion is a type of:
Exocytosis / Concentration / Voltage-dependent influx / Efflux inhibition

Assignment 4: From tissue to molecular complexes

Microscopy allows studying samples at different magnifications. Figs. 1 and 2 showed models. Figure 3 is a histological depiction of the islets of Langerhans. In assignment 5, we will look at a section of a single islet.

1. Try to estimate the resolution by drawing a scale bar in figure 3, indicating the estimated dimensions. Are you able to give scale bars in figures 1and 2? If you cannot, return to it after completing Assignment 5.

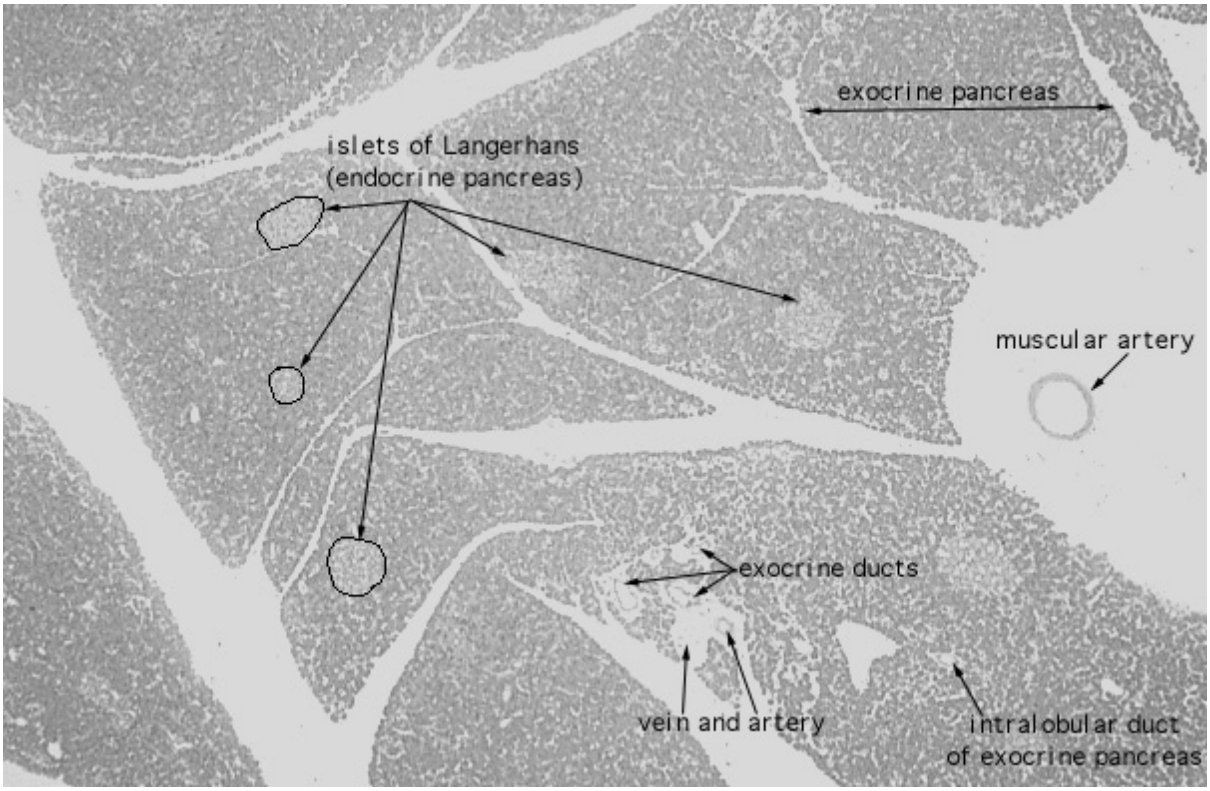


Fig. 3. The beta cells grouped in the islets of Langerhans in the pancreas. The rest of the pancreas consists of exocrine tissue where digestive enzymes are produced. The endocrine tissue – the islets of Langerhans – produces other hormones besides insulin.
Source: www.bu.edu/histology/p/10401loa.htm

Please fill in and hand in after finishing the assignments
Names:

Assignment 5: Nanotomy: EM of tissues, cells, organelles and macromolecules

Type 1 diabetes (T1D) is an auto-immune disease results in degradation of the insulin-producing beta cells (Fig.2), which are located in the islets of Langerhans in the pancreas (Fig.3). A cure does not exist; patients depend on lifelong insulin therapy. Moreover, the trigger that causes the disease is also unknown. Finding alternatives for insulin therapy and making advances in etiology of T1D benefits from a full structural and functional insight into Islets of Langerhans. EM can visualize Islet morphology at the highest possible resolution; however, conventional EM only provides biased snapshots and lacks context.

Nanotomy is an innovation in EM that allows studying tissues, cells, organelles and macromolecules in Google-Earth-like fashion. Here, nanotomy has been used in an animal model for Type 1 diabetes: Ravelli et al. (2013); www.nature.com/srep/2013/130508/srep01804/full/srep01804.html.

Study the ultrastructure of an islet of Langerhans. Go to www.nanotomy.nl and click on the largest islet (grey). This dataset can be studied in the same way you view a landscape in Google Earth. Click on the IIP icon at top left for extra instructions if necessary. Go through the annotations and answer the questions below.
The numbers correspond to the annotations: 1A refers to 1, for example. If you place your cursor on 'Supracellular', for example, a submenu will appear, including A, Islet. ***Before you begin, drag the menu at bottom left up a bit and the scale will appear.***

1. Islets of Langerhans in recent-onset type 1 diabetes (rat)

The annotation menu can be dragged to allow the scale to show.

1A. Name the clearest differences which distinguish the islets of Langerhans from the exocrine pancreas.

What are the various functions?

1B. Which cell is in the capillary? What is approximately its size?

1C. Which two cell types do you recognize in the vein? What are the most obvious differences? Can they always be seen?

1D. The centroacinar lumen belongs to the:

a. endocrine pancreas and contains enzymes
b. endocrine pancreas and contains hormones
c. exocrine pancreas and contains enzymes
d. exocrine pancreas and contains hormones

1E. Depicted here is a cross-section of a bundle of unmyelinated axons. The bundle was discovered more or less by chance: the electrons cause the axons to be slightly lighter. These contain round tubules and light-grey filaments. How many axons do you see here? What are these filaments?

1F. Some exocrine cells do not show a nucleus. Why?

2A. The exocrine cell contains a lot of rough ER for protein synthesis. The content will be secreted with cytoplasmic vesicles and eventually end up in the:

a. blood
b. digestive tract

2B. The alpha cell produces glucagon, which is visible in the dark vesicles. Glucagon secretion ensures that the blood sugar levels:

a. increase
b. decrease

2C/D. The depicted beta cell in 2C is in bad shape: the rat has diabetes. Later you will compare the differences with a healthy rat. Only a few granules with hormones are visible, in particular to the bottom left of the nucleus. The crystal-like shape is typical and is more pronounced in human beta cells. Which hormone is it?

2E. Is the centroacinar cell important for protein production, or the structure of the efferent ducts? Is this visible in this picture?

2F. The indicated cell, known as a pericyte, separates the hormone-producing endocrine pancreas from the enzyme or proenzyme-producing exocrine pancreas.

2G. Inflammatory cells are present because there is an ongoing immune response to the islets in the rat. Shown here is a lymfocyte. How can you recognize this?

2H. What is the grey matter around this erythrocyte?

2I. What is typical of the nucleus of a monocyte?

2J. This phagocyte is very active, because it contains a lot of material. Do you recognize what this cell has taken up via phagocytosis?

2K/L/M Examples of different leucocytes.

3A. The rough ER's main function is:

The black spots measure approximately ..... nm. These are ..... on the inside / outside of the rough ER.

3B. A mitochondrion is easily recognized by:

3C. The cell nucleus contains:

In the nucleus you see light gray and dark grey parts. How are they called?

With regard to function, this reflects the process that we call:

3D. The Golgi apparatus can be distinguished from the ER by:

The main functions of the Golgi apparatus are:

4A. Zymogen granules contain enzymes and proenzymes in the ..... cells.

4B. Insulin is produced by the ……..... cells.

4C./4D./4E. Different phases of fusion of insulin granules.

Is the process of insulin secretion a form of regulated secretion or constitutive secretion? How can you see this?

4F. Glucagon is produced by the ……..... cells.

4G. Somatostatin is produced by the ……..... cells.

5. Structure / function of vesicles

5A. The dense bodies are:

5B. Lysosomes play an important role in:

5C. What type of cell is this which is swarming with caveolae (the small vesicles)?

5D. These are about the smallest vesicles in existence. What is their diameter?

5E. They gray dots are lipid droplets, what is the diameter?

5F. Which two characteristics allow the structure in the middle to be recognized as an early endosome?

5G. Clathrin-coated pits are characteristically involved in (a) endocytosis ***or*** (b) exocytosis.

5H. Multi-vesicular bodies can fuse with the plasma membrane and being secreted. How are these secreted multi-vesicular bodies nowadays called?

6A. Crystae are typical structures of:

6B. Technical question; which atom is accumulated in this membranous mass?

6C. From which cell is this a detail (zoom in and out for orientation) and how are the structures in the middle also called? These structures enable:

6D. The basement membrane depicted here is between two types of cells, which are:

6E. Here, the basement membrane forms part of a complex structure. This is still the diabetic rat. Morphologically speaking, both cells containing nuclei clearly appear to be leukocytes. However, these are in different locations. The leukocyte on the left is in the .... , while the other clearly is not. Explain what may be going on.

7. Macromolecules are just barely discernible at these image settings. There are certain characteristics which allow the various macromolecules to be recognized.

7A. How many nuclear pores can you distinguish in the ENTIRE cross-section of the nuclear membrane?

7B. This is the tip of the nucleus where nuclear pores can also be distinguished. How many are there?

7C. The numerous polysomes shown here consist of:

7D. Sketch a model of a single polysome with 5 ribosomes. If possible, indicate the 5'UTR and 3 'UTR and sketch also the peptide chains herein.

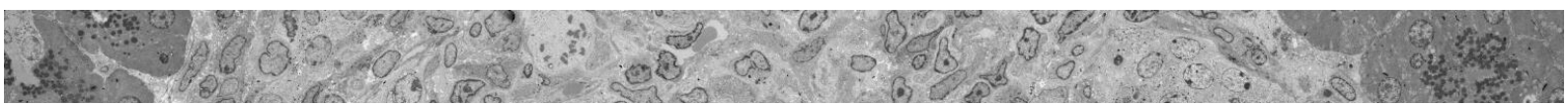
7E. Desmosomes are specialized cell-cell contact points, which in particular are important for (a) tissue strength ***or*** (b) forming a barrier.

7F. Tight junctions are specialized cell-cell contact points, which in particular are important for forming a barrier. Which barrier has been created here?

7G./H. Collagen is (a) cytoplasmic ***or*** (b) extracellular and serves particularly to:

7I./7J. Centrioles are often found perinuclearly and are mainly made up of:

7K. Every cell has a pair of centrioles. Explain why we so so little centrioles then in this dataset.



Assignment 6: Islets during type 1 diabetes

Following this introduction to the EM of cells, organelles and macromolecules, the focus will now turn to the effect of type 1 diabetes in the rat model. Return to the homepage (nanotomy.nl) and compare Dataset 1 (control) with Dataset 5 (diabetes).

1. What is the blood sugar level of the healthy animal? And that of the animal with diabetes?

2. This is caused by a deficit in:

3. This is caused by the breakdown of beta cells. Insulitis clearly exists, since Dataset 5 shows many more:

4. The beta-cell destruction is clearly recognizable due to the following characteristics (name at least 3):

5. People with diabetes will benefit from the following treatment:

6. Too much treatment leads to .... and can be compensated by:

Comatose patients benefit from:

Two stages have now been shown. Time permitting; knowledge can be further tested by studying the other stages. This can also be done at home.


Assignment 7: Testing newly acquired knowledge on a healthy islet (Dataset 1)

Distinguish 9 different types of cells. Which characteristics can be used in doing so?

1.
2.
3.
4.
5.
6.
7.
8.
9.

Sketch 4 different organelles and indicate their characteristics.

Sketch 4 different macromolecules and macromolecular complexes, and name a function.

---------- end of practical ----------