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01-02 A human egg cell 1. H-E stain, x 250.





01-03 A human egg cell 2. Toluidinblue stain, x 200.





01-04 Mitosis 1. Prophase. x 400.





01-05 Mitosis 2. Prometaphase 1. x 500.





01-06 Mitosis 3. Prometaphase 2. x 500





01-07 Mitosis 4. Metaphase 1. x 500.





01-08 Mitosis 5. Metaphase 2. x 500.





01-09 Mitosis 6. Anaphase 1. x 640.





01-10 Mitosis 7. Anaphase 2. x 500.









01-12 Mitosis 9. Telophase 1. x 500.





01-13 Mitosis 10. Telophase 2. x 400.





01-14 Chromosomes of a human male. Giemsa stain, x 500.





01-15 Chromosomes of a human female. Giemsa stain, x 500.



(1) $(\mathbf{2})$

Real ranks 3 5 **** 12 10 X X 5 3 3 4 X 15 16 18 14 22 19 20 _____ 21 XY

Male chromosomes
Female chromosomes

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3 Development of male chromosomes

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01-17 Golgi-complex. Trigeminal ganglion, guinea pig, impregnated with OsO4, x 400.





01-18 Golgi-complex, Trigeminal ganglion, guinea pig, impregnated with OsO4, x 400.





01-19 Golgi-complex. Pancreatic acinar cells. Human, AgNO3-impregnation, x 400.





01-20 Golgi-complex. Ductus epididymidis. Rat, Da Fano's method, x 160.





01-21 Mitochondria. Distal convolution, mouse. iron-hematoxylin stain, x 400.





01-22 Mitochondria. Pancreatic acinar cells, mouse, iron-hematoxylin stain, x 400.





01-23 Mitochondria. Intestinal epithelium, mouse, iron-hematoxylin stain, x 400.





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01-24 Mitochondria. Proxymal and distal convolutions, mouse, epon section, toluidinblue stain, x 400.



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01-25 Basophilia of the cytoplasm. Pancreatic acinar cells, mouse, toluidinblue and eosin stain, x 225.

01-00 The Cell

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- The cell is, structurally as well as functionally, the smallest unit of all living beings, capable of independent existence. The size and form of cells are quite various but in general cells are colorless and small beyond the human sight.
- The cell is enclosed with a very thin cell membrane, and consists of two essential components, a spherical nucleus and surrounding cytoplasm.
- The nucleus is separated from the cytoplasm with a distinct nuclear membrane and consists of fine chromatin meshwork, deeply blue-violet stainable with hematoxylin, and contains a distinct nucleolus. The chromatin is composed of DNA, hereditary substance, and protein and controls all the functions of the cell. During the cell division chromatin forms a fixed number of chromosomes, each of that divides lengthwise into two and are distributed evenly into two daughter cells.
- The cytoplasm is stained with acid dye, for example, with eosin homogeneously pink and no structures would appear within the cytoplasm. Special staining methods reveal, however, several formed structures, morphoplasms, among that essentials to the cell activities, for example, mitochondria, Golgi-complex, centrosome (centrioles), endoplasmic reticulum and lysosomes are called cell organelles.

01-01 Scheme showing the cell structure. (1/3)



- In the center, a depiction of a cell observed by light microscope; around the periphery are drawings of the cell organelles as these appear in electron micrographs.
- (1) Cell membrane (plasma membrane).

Cell membrane is very thin, beyond the limit of resolving power of light microscope. In the electron micrographs, it appears as a thin dense line around the periphery of the cell. In higher magnification, it appears as two dense line (2.5³.0 nm) separated by a lucent intervening zone (3.5⁴.0 nm). The two dense lines are hydrophilic end of the phospholipid and the intervening pale zone represents their hydrocarbon chains. Except for the minor differences, all membranes of the cell have this same appearance so that this membrane is called unit membrane.

(2) Mitochondria (singl. Mitochondrion)

Mitochondria are slender rod-shape, $0.4 \ 0.8 \mu$ m in diameter, $4 \ 9 \mu$ m in length, and are distributed randomly in the cytoplasm. Mitochondria are stained with iron- hematoxylin of Heidenhain intensely blue and with acid-fuchsin deep red. They are also visible in epoxy resin sections stained with toluidinblue. In electron micrographs a mitochondrion is bounded by double i.e. inner and outer unit membranes and the inner forms thin folds projecting into the interior of the organelle. The folds are called the cristae mitochondriales, and serve for increasing the area of this enzyme-rich membrane. In mitochondria there are a lot of enzymes of oxidative phosphorylation, and with these mitochondria generate the energy from the nutrients (glucose and fatty acid), that the cell receives from the blood, in the form of ATP. ATP released from the mitochondria into the cytoplasm, is an ubiquitous store of energy that is needed for all synthetic processes and for mechanical work involved in motor activity of the cell.





(3) Centrosome and centrioles.

In the specimens adequately stained with iron-hematoxylin, a small spherical area with slightly different hue from that of the surrounding cytoplasm appears, usually in the vicinity of the nucleus. In its center there are two short rods, the centrioles, deeply stained dark blue. They are also perceivable by phase-contrast microscopy. In electron micrographs the centrioles are cylindrical structures, about 0.2 μ m in diameter and 0.5 \sim 0.7 μ m in length, with an electron-dense wall and electron-lucent central cavity. In the wall nine evenly spaced triplet microtubules are embedded. In the cross section each triplet is set at an angle of about 40° to its respective tangent. In each pair of the centrioles, the one is located at a rectangle to another. At the beginning of cell division, the two centrioles replicate, so that a new centriole develops in end-to-side relationship to a specific region on the wall of the preexisting centriole. After replication, the two members of the original diplosome move apart, and each, together with its newly formed daughter centriole arrives at opposite poles of the cell and there serve to develop the mitotic spindle. The centriole is not constructed with the unit membrane.

(4) Golgi-complex.

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Golgi-complex can be revealed by OsO4- or AgNO3-impregnation as black networks near the nucleus. This is especially conspicuous in the secretory cells and nerve cells. In electron micrographs, it appears as stack of 4 10 parallel flat sacks, enclosed by unit membrane, Golgi-lamellae. The lumen of these flat sacks, cisternae, is narrow but slightly expanded at their end. The Golgi-lamellae are often curved with a convex outer surface and a concave inner surface. This indicates the functional polarity of the Golgi-complex. Around the Golgi-complex numerous small vesicles (Golgi-vesicles), about 40 nm in diameter, and larger vacuoles (Golgi-vacuoles) are seen. Golgi-complex accepts precursors of many kinds of proteins from the endoplasmic reticulum, processes and finishes them to the final products. They are packed in the Golgi-vacuoles, and delivered to their respective destination.

(5) Endoplasmic reticulum (ER).

This is the complicate network system of tubules (canaliculi) or flat sacks (cisternae) bounded by the unit membrane and is found throughout the cytoplasm. This organelle is first detected by the electron microscopy. Two kinds of ER are identified: the rough surfaced ER (rER) and the smooth surfaced ER (sER). The two forms are continuous but their relative proportions vary in different cell types

01- heme showing the cell structure. (3/3)



(a) rER.

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This type of ER bears small dense particles on the outer surface of its unit membrane. These particles are very uniform in size, $20 \sim 25$ nm in diameter, consist of ribonucleo-protein and are called ribosomes. They also occur free in the cytoplasmic matrix. These particles of ribosomes are the site of synthesis of new protein in the cell. Free ribosomes are site of synthesis of protein necessary to sustain cell proliferation and for other uses within the cell. Ribosomes attached to ER membrane concern with synthesis of protein to be secreted by the cell. Ribosomes synthesize the protein according to the information coded in messenger RNA, formed in the nucleus in association with the DNA of chromosomes and carried to the ribosomes in the cytoplasm. Newly synthesized protein precursors are released into the lumen of cisternae, packed in small vesicles and pinched off from the rER to transport to the Golgi-complex, where they are concentrated and packaged into secretory granules.

(b)sER.

This type of ER is usually the complex network of tubules and less extensive than the rER. The sER is involved in the synthesis of fatty acids and other lipids. Highly developed sER is found in cells of steroid-secreting endocrine glands, for example, interstitial cells of testis, lutein cells, and cells of zona fasciculata of adrenal gland. Well developed sER is also seen in the hepatic cells and also in the striated muscle cells. Functional significance of the sER is still not uniformly clarified and will differ from one to another cell type.

(6) Lysosomes.

Lysosomes are the electron dense bodies, $0.2 \sim 1.0 \ \mu$ m in diameter, bounded with unit membrane and contain the enzymes of acid hydrolases. They vary in size, in form as well as in number even in the same cell type, but they are most abundant in polymorphonuclear leucocytes of the blood and macrophases in tissue, specialized cell types for phagocytosis. The most important role of the lysosomes is to destroy the invaded bacteria and also to eliminate cell organelles fallen into useless. To verify the lysosomes it is required the histochemical demonstration of acid phosphatase or other hydrolases in their interior.

Descriptions of the cell organelles are largely indebted to Dr. D. W. Fawcett, 1994.



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This is a human primordial egg follicle. In the center there is a large round nucleus consisting of fine meshwork of chromatin and containing a distinct nucleolus. In the cytoplasm surrounding the nucleus no formed structures (cell organelles) are visualized by this staining. The egg cell (the primary oocyte) is enclosed by flattened cells, follicular cells, and embedded in the ovarian stroma.



01-03 A human egg cell 2. Toluidinblue stain, x 200.



- In the center there is an egg cell, the primary oocyte, about 60 μ m in diameter, and encircled by a homogeneous band, zona pellucida. This egg follicle is slightly more developed than that of 01–02. This specimen was embedded in epoxy-resin mixture and cut with a thickness of about 1 μ m. Because of thinness of the specimen structures of cells, especially of surrounding follicular cells are clearly seen. But neither in nucleus nor in cytoplasm no special structures are recognized.
- Figures 01-04 to 01-13 show the mitotic figures in sequence from the beginning to the end. Materials are blastula of a fish, hybrid between *Caprinus carpio* L. and *Carassius carassius* (L.), generously supplied by Prof. Dr. Y. Ojima, Kwansei Gakuin auniversity.
- Sections were stained with Heidenhain's iron-hematoxylin.

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01-04 Mitosis 1. Prophase. x 400.



Two centrosomes moved apart and now arrived at each respective pole of the nucleus.

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01-05 Mitosis 2. Prometaphase 1. x 500.

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The chromosomal threads became thicker, the mitotic spindle started to form and the nuclear membrane now disappeared.



01-06 Mitosis 3. Prometaphase 2. x 500



The distinct mitotic spindle is now covering the chromosomes.

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01-07 Mitosis 4. Metaphase 1. x 500.

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Chromosomes are arranged on the equatorial plane of the cell and the mitotic spindle is very conspicuous.



01-08 Mitosis 5. Metaphase 2. x 500.



This is the polar view of the chromosomes arranged on the equatorial plane.



01-09 Mitosis 6. Anaphase 1. x 640.

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Two sets of sister chromosomes begin to move apart toward the respective pole, drawn by spindle fibers. This process advances rapidly, so that such figures are seldom obtained.



01-10 Mitosis 7. Anaphase 2. x 500.



Separation of two sets of sister chromosomes advanced further.



01-11 Mitosis 8. Anaphase 3. x 500.

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Two sets of sister chromosomes almost arrive at the respective pole but the nuclear membrane is still not appeared. The constriction appeared at the equatorial portion of the cell.



01-12 Mitosis 9. Telophase 1. x 500.

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Nuclear membrane appeared around each set of chromosomes and the constriction of two sister cells advanced further. Two sister cells are still connected with a bridge of cytoplasm.



01-13 Mitosis 10. Telophase 2. x 400.



Separation of two sister cells are almost completed



01-14 Chromosomes of a human male. Giemsa stain, x 500.



- These are photomicrographs of chromosomes of a Japanese male and a female. The number of chromosomes of human male is 46, consisting of 22 pairs of autosomes and an X and a Y, namely, 44 XY, whereas that of female consists of 22 pairs of outosomes and two X, namely, 44 XX.
- These preparations were made according to the Tjio and Puck's method.



01-15 Chromosomes of a human female. Giemsa stain, x 500.



- These are photomicrographs of chromosomes of a Japanese male and a female. The number of chromosomes of human male is 46, consisting of 22 pairs of autosomes and an X and a Y, namely, 44 XY, whereas that of female consists of 22 pairs of outosomes and two X, namely, 44 XX.
- These preparations were made according to the Tjio and Puck's method.



01-16 Analysis of the human chromosomes.

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Tjio, J. H. and T. T. Puck (1958) invented a method to demonstrate the mammalian chromosomes very precisely using the cultured leucocytes. By this method it is clear that human somatic cells of males have 22 pairs of autosomes and an X and a Y, whereas females have 22 pairs of autosomes and two X. Each of human chromosomes has been given a number, on the basis of its size, and they are arranged in groups, as shown in this figure.



01-17 Golgi-complex. Trigeminal ganglion, guinea pig, impregnated with 0s04, x 400.

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In these two figures Golgi-complex of nerve cells is demonstrated as blackened meshwork around the nucleus or blackened rods distributed throughout the cytoplasm.



01- Igi-complex, Trigeminal ganglion, guinea pig, impregnated with 0s0 0.

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In these two figures Golgi-complex of nerve cells is demonstrated as blackened meshwork around the nucleus or blackened rods distributed throughout the cytoplasm.



01-19 Golgi-complex. Pancreatic acinar cells. Human, AgNO3-impregnation, x 400.

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Blackened Golgi-complex is demonstrated between the nucleus and acinar lumen, namely, in the supranuclear region. This specimen was counterstained with Kernechtrot.



01-20 Golgi-complex. Ductus epididymidis. Rat, Da Fano's method, x 160.



Golgi-complex is also demonstrated in the supranuclear resion.



01-21 Mitochondria. Distal convolution, mouse. iron-hematoxylin stain, x 400.

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Mitochondria in the cells of distal convolution of kidney are rod-shaped and densely arranged in the basal area, perpendicular to the basal membrane.



01-22 Mitochondria. Pancreatic acinar cells, mouse, iron-hematoxylin stain, x 400.

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Mitochondria in the pancreatic acinar cells are fine threads and distributed throughout the cytoplasm. The deeply stained coarse granules are secretion granules.



01-23 Mitochondria. Intestinal epithelium, mouse, iron-hematoxylin stain, x 400.

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Mitichondria in the intestinal epitheliar cells are fine threads in the supranuclear region and fine granules in the basal area.



01-24 Mitochondria. Proxymal and distal convolutions, mouse, epon section, toluidinblue stain, x 400.

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In this epon section of about 1 μ m thickness, mitochondria in the distal convolutions, horizontally locating in the middle of the figure, are clearly identified both rod-like shape and perpendicular arrangement to the basement membrane, whereas that in the surrounding proximal convolutions are not clearly resolved, because they are very densely arranged.



01-25 Basophilia of the cytoplasm. Pancreatic acinar cells, mouse, toluidinblue and eosin stain, x 225.

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The basal region of the pancreatic acinar cells is very deeply stained with basic dye, for example, toluidinblue. This is because that this region is very rich in rER, active site of the protein synthesis. In the classical histology this basophilic region is called as ergastoplasm. The apical region of this pancreatic acinus is filled with coarse red granules. They are secretion granules.