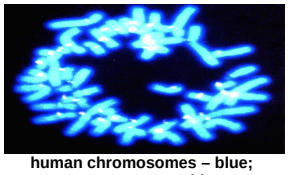
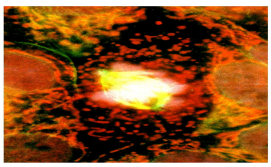
3.

Practical class: Cell division.

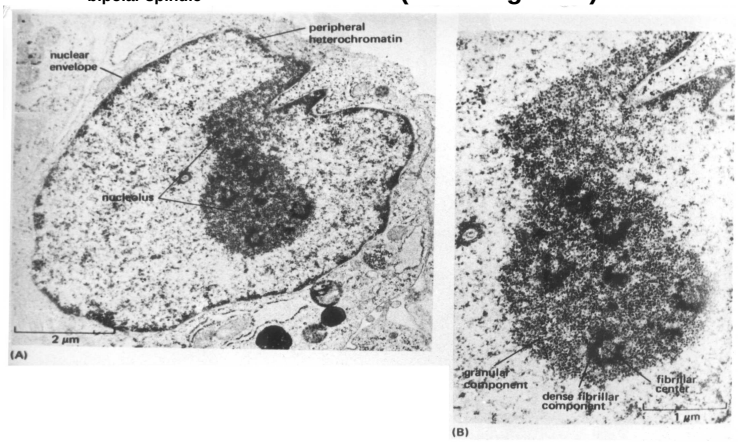


human chromosomes – blue; centromeres – white



bipolar spindle

- **mitosis in sections of limb obtained from 16.5-day-old mouse fetus (slide # 4),**
- **mitosis in in vitro cultured cells (slide # 1),**
- **nucleus and nucleolus (EM # 52),**
- **nucleosomes and nucleofilaments (EM # 231),**
- **sex chromatin (fig. # 30),**
- **motor proteins – dynein and kinesin (fig. # 11),**
- **hypothetical mechanism of chromosome movement during anaphase (fig. # 3, 4),**
- **microtubules attached to the kinetochore and schematic drawing of a chromosome (fig. & EM # 29),**
- **human metaphase chromosomes visualized by various methods (fig. # 132),**
- **inborn deformations caused by abnormal number or structure of chromosomes (text & fig. # 89)**



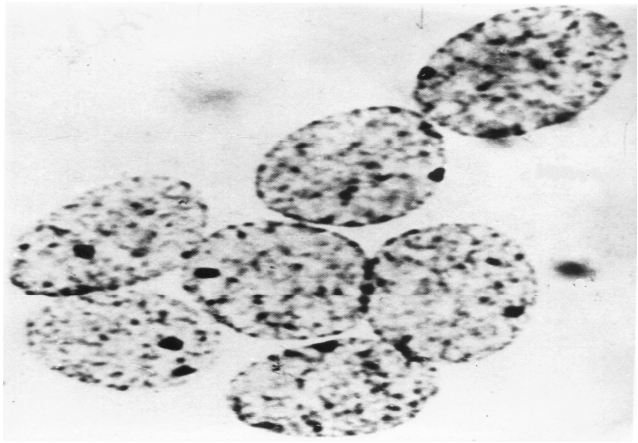
EM - 52.

Nucleus and nucleolus.

Nuclear envelope; peripheral chromatin; granular com-ponent; dense fibrillar component; fibrillar center.

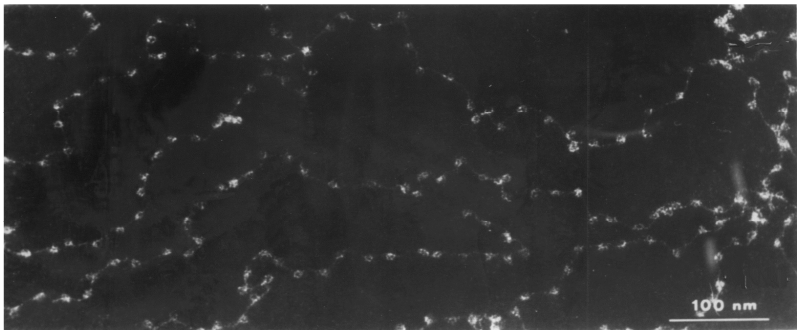
Nucleolus contains ribosome precursors which bind to each other and form an extensive network. Fibrillar center contains DNA, which is not trans-cribed; dense fibrillar component contains DNA and RNA in the process of trans-cription and the granular component corresponds to maturing ribosomes.

In man genes encoding ribosomal RNA are pre-sent in the terminal segments of 5 different chromo-somes (i.e. in 10 chromosomes in diploid cell, con-taining 46 chromosomes). The nucleoli disappear at the onset of mitotic division, in prophase, and reappear in telophase, as small units near the end of chro-mosomes called nucleolar organizing regions. These tiny nucle-oli enlarge and usually fuse forming one or more large nucleoli typical for interphase cells.



EM. 30.

Sex chromatin (Barr bodies) in epithelial cells lining the



EM - 231.

Nucleosomes

Chromatin fibers are about 20 nm in diameter and con-sist of straight smooth areas of chromatin interspersed with nucleosomes.

Each nucleosome is 10 nm in diameter and consists of a tight DNA spiral of 146 base pairs wrapped around the octameric core of histones.

In chromatin nucleosomes are arranged as beads on a string.

Fig. 132.
Human methaphase chromosomes vizualized by various methods

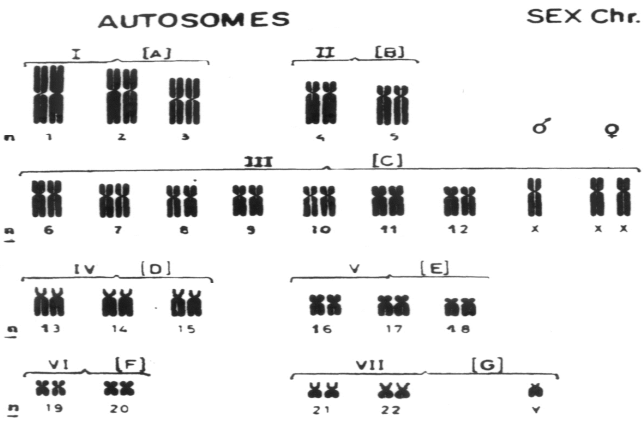


Fig. 11

MOTOR PROTEINS - DYNEIN AND KINESIN

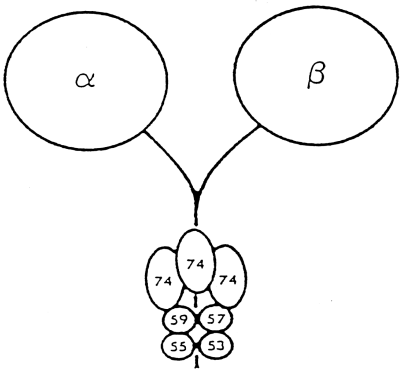


Fig.11a

Cytoplasmic dynein (microtubule-associated protein, MAP 1 C molecule) is an enzyme responsible for movement of cytoplasmic organelles towards the minus-end of microtubules (the end associated with centrosome or basal body). Dynein is ATPase, i.e. it requires ATP for activity). Cytoplasmic dynein contains two heads, a stalk and a base. Heads and stalk contain two heavy chains, while three intermediate chains and four light chains are probably also in the base. Heads are thought to be responsible for ATP-dependent microtubule binding and force production. The base is the likely locus for interaction of MAP 1C with a membranous organelle or a chromosome.

Fig. 11b

The second motor protein - kinesin - is a tetramer composed of two heavy chains and two light chains. In the cell, the kinesin powers a plus-end directed organelle transport. Kinesin is an elongated molecule with two globular heads at one end, a long stalk in the middle, and a tail at the opposite end. The heads display ATPase activity and bind to microtubules; the tail probably binds to the organelles.

Fig. no 3 & 4

HYPOTHETICAL MECHANISM OF CHROMOSOME MOVEMENT DURING ANAPHASE

Microtubules during mitosis attach to the kinetochore situated in the centromere region of a chromosome. Thus microtubules connect chromosomes with centrosomes containing centrioles. Other microtubules extend from the centrosome region towards and slightly beyond midzone of the mitotic spindle. In metaphase chromosomes form a metaphase plate, located in the midzone of the mitotic spindle. During anaphase the chromosomes proceed towards the spindle poles.

Anaphase occurs in two stages. Anaphase A is thought to involve the specific shortening of kinetochore-associated microtubules, while during anaphase B the whole mitotic spindle is elongated and the cell poles move apart.

The discovery of the protein motors was essential for understanding of chromosome movement. It appears that heads of the dynein molecules present in the kinetochores attach to microtubules and move the chromosome along it. Kinesin (and other similar proteins belonging to the kinesin superfamily) seems to be involved in formation of a spindle. It appears that during prometaphase and metaphase kinesin is bound to kinetochores, but at anaphase it translocates to associate with microtubules in the mid-zone. It may drive antiparallel microtubule-microtubule sliding and thus drive anaphase B spindle elongation.

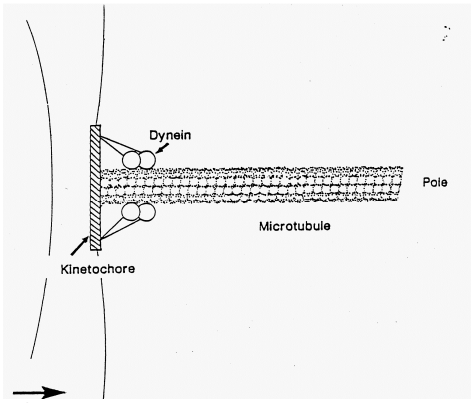


Fig. 3
Possible arrangement of dynein in the kinetochore. Two-headed dynein molecules are shown tugging on the end of a microtubule, whose disassembly could govern rate of movement.

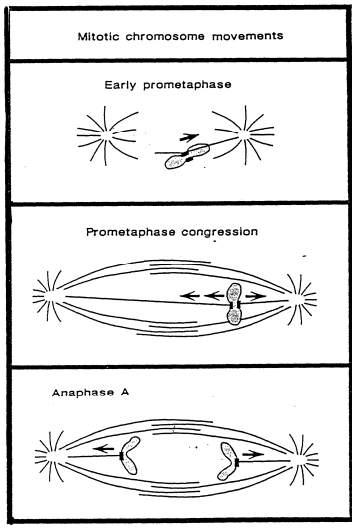
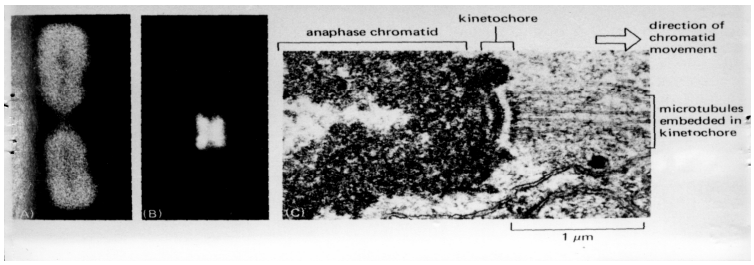
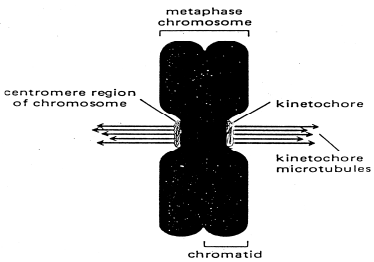


Fig. 4.
In promethaphase, the chromatid pair is first drawn to the spindle pole, then migrates towards the centre of the spindle. At the onset of anaphase, the chromatids separate and migrate slowly toward the spindle poles.

EM – 29 Microtubules attached to the kinetochore and schematic drawing of a chromosome





Text 89

GENETIC DISORDERS RELATED TO ABNORMAL NUMBER OR STRUCTURE OF CHROMOSOMES

Numerical chromosome aberrations

Numerical anomalies may concern both somatic and sex chromosomes. Loss of one chromosome is called monosomy while the presence of an additional one is called trisomy. The most common are trisomy of the 21st, 13th, and 18th chromosome pair as well as X chromosomes.

Trisomy 21syndrome (Down's syndrome) *89a: upper photos*

This trisomy is usually (95% of cases) a result of chromosome nondisjunction during meiosis, particularly during oogenesis. The frequency of this disorder increases with the age of parents. In women younger than 30 yr the risk is 1 per 1000 children. In older women, *e.g.* over 40-yr-old the risk may reach 1 per 100 births. Typical clinical manifestations of Down's syndrome are craniofacial malformations (upslanting of the eye slits, skin folds at the inner sides, flatness of the bridge of the nose, the tongue protrusion), growth inhibition, mental retardation, hypotonia and congenital cardiac disorders.

Trisomy 13 syndrome (Patau's or trisomy D syndrome) *89a: middle photo*

The frequency of this disorder is around 1 per 15,000 births. The main clinical manifestations are mental retardation, cleft lip and palate, deafness, multiple eye abnormalities (colobomas - structural defecrs of an eye ball, microphtalmia or anophtalmia), and cardiac disorders. The children usually die by the age of 3 mo.

Trisomy 18 syndrome (Edwards-Patau or trisomy E syndrome)

The frequency of this syndrome is about 1 per 5000. The main clinical manifestations are mental retardation, low-set malformed ears, micrognathia and other skeletal malformations, eye abnormalities, and hypertonicity. Children usually die by the age of 2 mo.

Turners syndrome 89a:lower photo

The 75% of cases is related to X0 karyotype that may be due to chromosome nondisjunction during meiosis in the course of spermatogenesis. The rest 25% may be related to structural X chromosome disorders including chromosomal mosaicism. The patients are phenotypic females displaying short stature, sexual infantilism and lack of ovaries.

Other disorders that are related to sex chromosome aberrations may also include Klinefelter's syndrome (XXY or XXXY karyotype) and chromosome X trisomy syndrome.

Structural chromosome aberrations

Structural chromosomal aberrations usually result from physical breaking or disruption of one or more chromosomes or their arms. Very often this may lead to loss of chromosome fragment (microdeletion or deletion) that usually results in serious congenital disorders depending on the function of the lost genes.