# CYTOLOGY NOTES

## Zoo 125

(Zoo 226)

Joshua Lederberg
Columbia University

1942

See Fig. 6, p. 23 Wilson. The Cell, general view.

Required Lab work:
4 drawings Golgi
8 drawings chondriosomes
in Bug ♂ genesis.
Technique references:
Lee '37 X
Bowen An Rec 38 (1938)
Romeis '32 Taschenbuch
Benda gives nicest
results, but is difficult.
Murgantia sp. will be
sent up. Other sp.:
Nezara - large green bug, found on
blackgum trees with berries
Euschistus thistles
Brachymena fruit trees
Anasa squash

Orthoptera: Dissosteira
Mus

Do not anesthetize
Dissect and fix immediately

Alexandrov (1932). In vital staining at low pH there is a suppression of granule formation, a staining of the nucleus and a diffuse staining of the cytoplasm. The stain effects are probably through $C_H$ changes as pH is lowered at low pH.

Wilson, Introduction
Sharp pp.

Theory of Fixation

Light as an abnormal factor.
Thanatology;

Strangeways & Canti QJMS 71
Belar ZIAV (1928)

Alexandrow Protopl 17:161 1932

Cytology - history

Early work in embryology. - Wolf, Malpighi

1838 Schleiden and Schwann The cell is the unit of structure of the organism Formed by de novo crystallization

Mol, Nägeli botanical aspects cell division

Remak, Virchow 1852-55 omnis cellula e cellula

Modern Cytology, the basis of contemporary work, lies in 1880-1905: Van Beneden, the Hertwigs, Boveri, Wilson.

The basic questions in most fields have not really been answered but perhaps reformulated.

The early cell lineage studies on Salpa, etc., were a lot of work, but only descriptive, not expository. Schrader exhibits a rather cautious, cynical attitude to general fundamental progress. The feeling that "a little better microscope is the key to life" not well justified.

Since 1875, cytologists have been called "cell embalmers" because of work on fixed dead cells. There are various tricks. Vital staining:— some abnormality introduced by dye.

Usually cytoplasmic components are stained. Under conditions of high pH nucleus may stain somewhat in Chironomus

Even unstained cells must be examined cautiously. Sick cells are much more visible. Observations have repeatedly been made on sick cells. In tissue culture, optical conditions are never favorable.

It is amazing that anything comes out of fixation techniques

Chromosomes are easier of technique than chondriosomes.

Chondriosomes *may* never be globular.

The generalized picture of the cell — v. Wilson

All substances must pass through membrane; in some cases, only a plasma membrane; in others a definite cell membrane may be torn off.

Golgi usually in relation with central body.
In plants, no central body is present, but the spindle and chondriosomes act as if one were present.

Chromatin is defined as the material constituting the chromosomes, usually Feulgen positive.

## Ground substance of the cell

With development of immersion lenses, in the '80s activity began on the fundamental structure of cytoplasm. In living cells this is not clear, but there are appearances in fixed material.

*Fibrillar*: thready cytoplasm   complicated terminology.

A. Reticular (v. Beneden)   network or weave

B. Filar (Flem., Heidenh.)   discontinuous

*Alveolar* Butschli, Wilson   microsomes > 2 μ. microsomes. Living eggs seemed foam like. hyaloplasm seemed emulsoid. Also in some fixed preparations.

*But* In annelid and echinoderm egg are found yolk, pigment granules, etc., which are not particularly

2/4/42

Dimensions of fibrillae, etc.?

Seifriz, Science 73 1931

Mirsky, Science 84 1936

Bensley, Anat Rec. 72 1938 ✓

Banga & Szent-Gyorgyi, Sc. 92 1940

Pollister, Physiol Zool 14 1941 ✓

Wilson '24 pp 57-78 ✓

Symposium on the Structure of Protoplasm 1942. ✓

Seifriz "Protoplasm" ✓

essential. True alveoli are always < 2μ, and under certain technical conditions anything might be identified with them.

granule defined as something angular.

<u>Granular</u> The ultimate unit is a granule, the smaller ones are bioblasts (Altmann) many of which are now recognized as mitochondria. Continuous phase neglected.

<u>Schimper</u> had seen these divided and concluded: omne granula e granulae. The bioblasts hypothetically are the same as Darwin's pangenes and Weismann's biophores. When Altmann went insane his work was discounted. V. infra.

Wilson Am. Natur. 60

Interest has been renewed: ideas such as structural protein, etc. and long molecules. The dilated structure protein is thready... (long molecules).

See also: ~~Pitt~~ Picken "The Fine Structure of Biological Systems." ~~in~~ ~~(Perspectives of Biochem.)~~.
Biol Rev. 15:133 (1940)

Pollister indicates mitochondrial orientations parallel to direction of streaming and asters. This is believed to be due to the orientation of long molecules and the exclusion of mitochondria from the lines of flow. There are similar relations of "chondriosomes" and "dictyosomes" even in plant cells where the asters are not visible

Bowen La Cellule 39:123 (1929)

Schmidt Protoplasma Monogr. 11 (1937)

Birefringence phenomena studied; indicate orientation in asters, spindles, (chromosomes?) No orientation outside the aster.

Early considerations are superseded. New attack on a molecular level

General Cytology considers the standard equipment of the cell:

Nucleus
Centrioles
Golgi
Mitochondria
(Plastids)

10/7/42

Wilson '24 670-700

Heidenhain Plasma u. Zelle I

## Centrioles

The centriole is frequently found at the active center of the cell. It is most readily seen at metaphase, where the astral-spindle poles point it out.

Bowen J. Morph 39:351 1924

Johnson Z. wiss Zool 140:115 1931

Pollister Biol Bull 65:529 1933

In 1887 van Beneden and Boveri recognized (polar capsule) its independent existence. The whole "region" is called a centrosome (Wilson, p. 673..) The poleparts of the spindle and aster may stain more heavily and obscure the centriole. It is the centriole, the small granule at the center, that is the morphologically important and persistent structure. The centrosome is merely the confluence of astral rays.

Fry Biol Bull 54, 56, 63, 65

" Anat Rec 46, 56

Destaining must be properly interpreted.

The superficially obvious function of the centriole is as the spindle regulator in mitosis.

✓ see Heidenhain ← Rabl – all cells have 2 centrioles ∴ the function in non-dividing cells is as an organizing center.

In epithelial cells, polarity is frequently determined by the position of centrioles.

If the centriole has a generally constant position may it not have a general function?

~~(Similar to Golgi, see Bowen Hdb, 47:261 1928~~

Cohn (1897) If epithelial cells reverse their polarity, the centriole moves, as in the enamel organ.

Between 1890 and 1910.. a large literature on centrioles.

In epithelial cells, the distal centriole frequently bears a flagellum..

10/9/42

Jordan Anat Anz 43 1913
Benda Arch Anat u Phys (Phys) 1901
Renyi Zeitsch. Anat 73:338 1924
Walter Anat Rec 42 ✓ 1929
Kindred Anat Rec 34 ✓ 1927

Centrioles: characterized by position, form, staining. Stain techniques are not too reliable.

Feulgen negative (Carothers?)

But pepsinization of cells and components usually is correlated with nucleic acids being present.

May be protein; probably not lipoid

In intestinal epithelium, stains like cement substance, which is probably polysaccharide.

Still open question.

2 proved functions:

1. Organizes aster. In helicids with multiple centrioles, each develops an aster for the second division. Asters are rays of oriented protein molecules.

2. Blepharoplastic. Evidence flagellum formation. Uniflagellate cells rather widespread. Sperm flagellum is best-known.

Bowen J Morph 39:351 (1924...)
and previous ones.

Diverse centriole history in spermatogenesis, after 2nd maturation. Starts growth at Telophase II, then divides into p and d. The history of $d_1$ and $d_2$ does not yet seem rationalizable. Most commonly $d_2$ forms a ring. Possibly the distals are not entirely homologous.

Seen in living by Huettner,
Schrader.

Huettner Z. Zellf. 19:119
Henneguy Arch anat micr I ('98)

10/14 Blepharoplasts can act as division centers.
Henneguy & Lenhossek claim any vibratile processes arise from centriole; then basal bodies of cilia are homologous with centrioles. Can a centriole multiply?
corollary: can a ciliated cell divide?

Modified mitosis?

---

l'Arducaoro's filaments?
ciliogenesis
protozoal analogies.

Jordan claims ciliated cells divide amitotically. Presumed that before ciliogenesis, basal granules can be found.
But Benda, Friedel, Walter unmistakably showed mitosis in ciliated cells.
v. Mihalik '34, oviduct epithelium. Appearance of flagella in "subepithelial" cells, still attached to granules. These may be leucocytes and degenerate epithelial cells, instead of a progressive process.

Pollister doubts that basal granules are products of ~~ciliar differentiation, but are~~ centriolar multiplication but as products of the cilium, which is not necessarily homologous with a flagellum.

Can centrioles multiply autonomously?
Boveri denied de novo formation.
Huettner has traced them through mitosis in Drosophila eggs Belar, in leucocytes; Vallowitz in Salpa, Pollister... The case for genetic continuity is clear. But occasionally de novo formation does ~~app~~ occur: as in the spermogenetic divisions of bryophyta and pteridophyta. In II, this centriole acts as a blepharoplast and multiflagellate motile sperm are formed. Thus the centriole is not a self-perpetuating body, but the

product of something which is, like the nucleus.

Sturdivant J Morph 1934
Cleveland

In Ascaris spermatocytes, the centriole is intranuclear. In many protozoa, an aster is organized within the nucleus.

Pollister PNAS 25, 1940
" PNAS 39

10/16/42 Atypical spermatogenesis and meiosis: oligopyrene sperm as in Lioplax subcarinata, other pulmonates.

Perhaps because of physiological senility or profuseness, some cells are abnormal —

Only 2 chromosomes segregate normally. In interkinesis, acentrics degenerate, through peculiar vesicles. 1 small nucleus with 2 chromosomes. At Anaphase II 1 chromosome to each pole. Therefore there are only 4 centric chromatids per quartet. A small nucleus is formed and blepharogenesis follows.

In atypical ocytes, there is one centrosphere, 1 or in chain. As they break up they can be counted. They double in number at Anaphase I. They are not entirely apportioned to the spermatids. Counts in Telophase II or early spermatid are certain.

The extra centrioles are comparable to the acentric chromatids. Therefore, the supernumerary centrioles are the accumulated centromeres.

This emphasizes the centriole - centromere relationship. All good cases of centriolar division in somatic cells occur at metaphase or later — after it has been in relation to the chromosomes through the spindle. In Vivipara, the centriole is Feulgen-negative.

"centriole is a material formed by the chromosome"

Other cases give strong evidence for nuclear origin: algae, etc. In Marsilia, the aster and centriole appear, as antici-

[redacted]

pated in late anaphase, 3rd spermatogonial division.

In cytaster formation (which may be diffusion centers) centrioles may be present, but they do not occur before breakdown of germinal vesicle. v. Wilson.

Monasters?

Schrader Biol Bull 70 1936

In Amphiuma spermatocytes, a distinct granule is seen as the kinetochore; stained very similarly to centriole. Darlington proposes as much on theoretical grounds.

10/21/42

## <u>Chondriosomes</u>

Schultze (1861) recognized granulations in protoplasm.

<u>Altmann</u> bioblast theory, now known as mitochondria. Only granules are alive. Various shapes, sizes. Any stainable granules were included. Not all self-duplicating; many secretory structures.

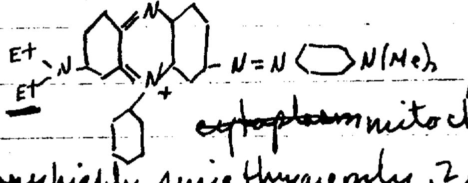
The development of techniques stimulated research. <u>Mitochondria</u> have survived from the bioblast theory. <u>Benda</u> developed specific methods and differentiated mitochondria from other granules. Now an enormous literature.

Universal occurrence probable

Rods of uniform diameter; rounded ends. All similar in any one type of cell. Might not to be really granular; may be misinterpreted as such. Very easily distorted by a poor fixation. Specific artifact studies

Shape of mitochondrium is reversibly affected by environment. Their orientation indicates a molecular structure of cytoplasm.

Specific stain: Janus Green B



Cells very rapidly absorb Janus Green B into the ~~cytoplasm~~ mitochondria. They must absorb very highly since they are only .2μ in diameter. Easily reduce the dye, anaerobically, through a rhodo and leuco form. The rhodo is diethyl safranine !! involving split molecule. The process is reversible!!!! Two ethyls are specifically required, and the phenosafranin.

<u>2 older reviews:</u>

<u>Duesberg Arch Zellf 6 (1910)</u>

<u>Cowdry, Carnegie 271, 1918</u>
<u>(None later)</u>

<u>Cowdry Gen. Cytology 1925.</u>

<u>Cowdry NH Biol Bull 33 (1917)</u>

<u>Lewis & Lewis Gen. Cytology '24</u> ←

<u>Rumjantzew Arch Zellf (1926)</u>
(1923)

<u>Oritschkow Arch mikr Anat</u>

<u>Bensley & Gersh Anat Rec 57 (1933)</u>

<u>Michaelis</u> Ehrlich's stud. I Arch mikr Anat 55:558

<u>Cowdry Am J Anat 19:423 1916</u>

Functional aspects:

Symbiotic organisms: Altmann
more recently by Wallin, Portier. Many morphological resemblances to bacteria. Some instances of incipient bacterial symbioses now known. Alterations over geological time have led to present forms.
They have never had any demonstrated significance.

Claude has reported ribonucleic acid in granules from centrifuging, which may be mitochondria.

Pollister et al., ultraviolet studies indicate no differential absorption at 2600 Å. Considerable refraction.

10/23/41

Chemistry: Lipid and protein components. The lipoids stain in the differential stain techniques.

Regaud: Rat testis Picro formalin; $CrO_3$ preserved them.
post-chrome required on basis of fat solubilities.

Fauré-Fremiet: similar results. Fat reaction on mitochondria found on cytolysis. Fats dissolved out; protein remains

analogies with fats: only unsaturated lipoids absorbed vital dyes. Scharlach Red is negative. Assume therefore a lipoprotein complex. Not determinable to be phospholipins. General statements to that effect may be erroneous. Various favorable evidences: Bensley

Russo: Lecithin injections increase oocyte mitochondria

Löwschin: Analogies with myelin structures in albumen, due to surface action. "phospholipid":

The melting point, density are higher than saturated fats in "UC" material. But protein condensation would account

Remarkable organization in some scorpions' spermatogenesis indicates some function! J.L.

Portier Les Symbiotes 1918
Wallin Symbionticism... 1927

Claude CSHQB Sym. 9 (1941)

Nebenkern may not be chemically identical with mitochondrial precursors. J.L.

Regaud CRSB 6:718 (1908)
Fauré Fremiet, Arch anat micr 11:457 (1910)
Mayer, Rathery & Schaeffer J de Phys & Path 16:607 (1914)
ibid 1929
Giroud Pr. 1:79 (1929)
Bensley & Hoerr An Rec 60:444 '34
Bensley & Hoerr An Rec 69:341 '37

for this.

Specificity?

Millon's test is positive.

Spermatozoa are rich in phosphatides – perhaps in sperm tail?

Bensley isolated "mitochondria" from liver cells, by slow centrifugation. Proximate analysis of mass:

40% fat 60% protein. dry weight

Detailed fat analyses: (1937) 100-200 mg samples

| | |
|---|---|
| Protein (and unknowns) | 64.67 |
| Lipoid: | 35.33 |
| as: glycerides | 28.88% |
| lecithin | 4.2 % |
| Sterol | 2.25% |

Some X-Ray studies indicate a periodic pattern in the mitochondrium.

Claude: analysis of granules: 60% protein
40% lipoid, largely phospholipins.

Bensley Science Oct 9 1942.

Most recent: Bensley –
Lecithin 45-58% of the lipid content of liver "mitochondria." Therefore, there is appreciable phospholipins.

## Mitochondrial Function:

Horning
Koehring J Morph

1. Enzymatic: Azine dyes pptd proteolytic enzymes. (Actually all large molecules.

Marston Biochem J 17:851 '23
" Aust J Exp Biol 3:233 '26
Robertson, ib., 3:97 1926

Marston - but the specificity of Janus Green B is not thus explained. Robertson showed ~~the~~ leuco-form does not precipitate enzymes, while mitochondria will react in leuco form, if oxygen is later readmitted.

Cowdry Amer Nat 60:157 '26
de Noüy + ... Anat Rec 34:313 '27

The Marston school has a lipoid orientation function; the mitochondria increase the surface (see Cowdry, de Noüy).

The lipoid acts as a semi-molecular solvent, and acts as an active surface for protoplasmic and enzymatic synthesis. (after Langmuir) Robertson has shown an increase in the rate of resynthesis of proteins in tryptic solutions, when lipoid is added as emulsion.

Kingsbury An Rec 6:39 (1912)
Joyet-Lavergne Pr. 6:84 ....

Hirsch Z. Zellf. 13:37 (1926)
Duthie PRS B114:20 ✓
Bowen Q. Rev Biol 4:488 1929 ✓
Bowen Z. Zellf 9 1929

de Noüy + Cowdry: measured various surfaces of formed components of pancreas cells. Assume, hypothesize orientation of semipolar compounds on a granular adsorption.
see Paper.

Hereditary factors?
Guilliermond: Animal mitochondria homologous with plant plastids. Conceivably factors in cytoplasmic inheritance. Question of self-perpetuation.

Meves, Benda, ... conceived a morphogenetic function, but this was carried too far: the pro-embryological basis of all fibrillae. Now abandoned

Hirsch, Duthie: vital observations on pancreas cells.
The earliest zymogen granules appear basally in the pancreas cell, at mitochondrial surfaces!
May have some function in myogenesis.

10/30. Homologies in plants –
In the meristematic cells, thread-like bodies appear which may be proplastids. Stain feebly with Janus Green B.
There are also "mitochondria" which do not become plastids.

Guillermond '39
The Cytoplasm of the Plant Cell.

Guillermond distinguishes between "active" mitochondria, the chondriome, which become plastids, and inactive mitochondria which are homologous.

Bowen '29, thought they could distinguish them ~~and~~ by stain reactions.

Plastids contain ribonucleoprotein.

# SPERMATOGENESIS

There is very great variation in spermatozoa. They are more species characteristic than any other cell, and perhaps most readily analyzable.

There are 4 constant morphological components, derived from:

nucleus → head...

Acroblast (Golgi) → acrosome, "perforatorium," refringent granules.

Centriole - centriolar apparatus (flagellum).

Mitochondrial Apparatus....

Primitively, flagellated, with head anterior.

Acrosome may be anterior, sometimes lateral (Lepisma) or even posterior. Usually very small, but in the hemipteran Notonecta it is very large. The dimensions of this sperm are:

Overall length - 1500μ
nucleus = 200μ
acrosome = 650μ
tail = 650μ

Significance of the 5th layer (mitochondrial) granules in fertilization: cortical reaction !! ?
See Bowen J Morph 1922
Studies in Insect Spermatogenesis
Wilson

The acrosome is not a perforatorium in function!!

Some mitochondria are always present, always posterior to the nucleus. May grow down to form a middle piece or a spiral organ. [May contribute to skeletal rods in head.]

Significance is not clear. The symbioticists would claim that the paranuclear fusion may be a syngamy, or loss of identity. [Characteristic onion structure of nebenkern] These mitochondria probably do not participate in ~~oogenesis~~ development of the egg.

Mitochondria in plant spermatogenesis??

Retzius: series of pictures....
Koltzoff...

Fate of nucleolus in Zinnia?

Mitochondrial studies on sperm material, after extraction of nucleoprotein?!!
Gatenby, Bowen

Non-flagellate sperms. (see Bowen's review.)

Homologies are difficult to establish. The topography is changed; they must be on a morphogenetic basis. While there is a tremendous modification, the morphological features still are recognizable. Amoeboid and non-flagellate, atypical sperms are secondarily derived from the primitive flagellate sperm, best represented in mammals or lower chordates.

General features:

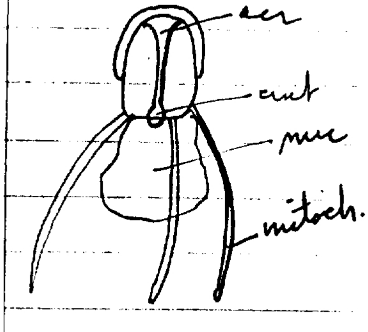
Acrosome from Golgi secretion; Golgi itself lost with cytoplasm. Formation variable in time and place.

Mitochondria fuse in spermatid

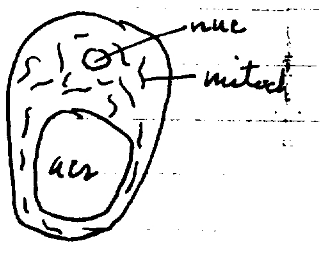
11/4/42

Worley, La Cellule 48 '39
Sturdivant J Morph '32
Bowen Anat Rec 31 '25

Various Decapoda: only part of the capsule is homologous with the acrosome: the stainable ring at the anterior end of the canal —



In Ascaris, non-motile, the "refringent body" is the Golgi derivative ↓



# Further notes on spermatogenesis

# The Golgi Apparatus

Study of these elements waited upon the development of special techniques for their demonstration. They are impossible to see except in living spermatocytes. Silver impregnation techniques demonstrate ~~them~~ that the axon is ~~not~~ ay an outgrowth of the cyton, and the same (... ) led Golgi to discover the internal apparatus now known as the Golgi apparatus. They were first seen in the nerve cells of the owl. This network never met the surface of the cell; on the outside there are nerve endings of a different character 1898

eview: Hirsch, G.C.
otoplasma Monographien
18 (1939). Form und
echsel der Golgi Apparat.
mplete bibliography, but
iased text.

irkman + Severinghaus –
Anat Rec 1938
uncritical

acobs Erg Biol II:357 (1927)

Negri discovered the apparatus in many types of cells, including non-nervous tissue.

Cajal (1914) modified the technique, surveyed the Golgi in many tissues.

Kopsch (1902) accidentally found the osmication technique. Applied to many different tissues by v. Bergen ('04), particularly vertebrates. All histologists now use it widely.

Hirschler investigated invertebrates 1912-1914.

Golgi techniques are rather capricious, variable. But the structures they do reveal, when they do, are rather consistent in structure, even throughout the animal kingdom –

Golgi described the apparatus as a series of strands broadening into plates and discs. Not reticular

Always a modified stack, according to Pollister.
An intermediate density of osmication is optimal for analysis; it permits 3-dimensional reconstruction.
lines, dots mean threads
areas, lines mean laminae

Nassonowa 1928: Hirudo
In invertebrates, they are usually organized into dictyosomes: cup-like structures

Pollister QJMS (1938)
In Amphibia, they are fundamental circled collars
Frequently the thickness can be approximated; this proves to be 25μ.

No Golgi rapid smear method

11/6/42
Transitions occur in invertebrate dictyosomes. In pulmonates they may run together. In each cell division the Golgi fragments, apparently diminishing in amount. In somatic early anaphase, the Golgi may almost disappear, reappearing at telophase.

Johnson
In late spermatogenesis, the Golgi is sloughed off, first breaking up. Each fragment is cup like. Hirschler misinterpreted this as a multiplication.

The cup may be considered as a fundamental sort of crystalline, orientation of the Golgi material.

Ultracentrifuge shows its specific gravity between oil and mitochondria.

Skepticism has frequently been expressed as to the real existence of Golgi material in vertebrates. The invertebrate dictyosomes are easily seen. The acroblastic homology of Golgi is therefore stressed.

Why do acids destroy
tty structures like the
ondriosomes and Golgi?

Hirschler Golgi as closed vesicles; osmophilic interior.

Hirsch Small granules a Golgi system. Synthesis of granular products. • ●◎... May be mistaken as fat droplets.

1928 Parat Neutral red accumulates in vacuoles, superficially resembling plant "vacuome" Osmium portion in the vacuoles. No organized Golgi apparatus. He concedes ~~now~~ however three types of formed components of cells: centrioles, chondriosomes and lipidosomes = Golgi.

Gatenby, Bowen The one proved function of Golgi is the formation of the acroblast which secretes the acrosome in spermatogenesis. The acrosome however is still quite mysterious. The acroblast may be a single large cup, or a group of smaller ones.

1923 Nassonowa In pancreas, secretory granules grow for most part at an osmophilic surface.

Cowdry Gen Cytology

The Golgi is usually quite polarized, and has been used to detect changes of polarity in enamel organ, thyroid, etc.

Trypan blue is absorbed in the Golgi region, or
Makarov Biol Bull 62 rather ends up there after absorption. Indicates vacuolar
Protoplasma 19 or excretory, secretory functions.

32

Plant homologues to Golgi

| | |
|---|---|
| Bowen, Zeitsch Zellf 6:689 1928 | Osmophil platelets, believed by Kiyohara to be |
| | stages of plastids |
| Weier A.J.B. 29 | ~~Pla~~ Plastids may have lamellar structure, are |
| | osmophile; participate in limosphere production in |
| v. Dec Adv Anatomy B/ch. | bryophytes. But mitochondria, proplastids |
| | are also homologizible. |

11/11/42

# CELL DIVISION

Amitosis no longer upheld by cytologists. No future in it! Even Amoeba, supposedly dividing by binary fission actually has a modified promitosis. Pathologists, working on relatively badly fixed material are the general supporters of amitosis, now conceded in only a very few cases.

Modified mitosis?

"Indirect division; karyokinesis Mitosis.

Define phases—

Interphase, 'resting' phase. loose, 'flocculent', almost homogeneous nuclei (chromatin) with ordinary fixatives.

Prophase - loose stuff into a thready form gradually forms chromosomes. Usually in this phase splitting appears obviously and suddenly.

Metaphase - chromosomes congress, coorient. Simultaneously the spindle appears, the nuclear wall disappears.

Anaphase - separation of chromosomes to opposite poles.

Telophase - chromosomes become diffuse nuclear wall is reformed.

Interphase—

Little agreement on time relations.

Spindle, in ideal zoological form, amphiastral.

In many cases, apparently anastral.

—But the presence or absence of centrioles is not so important as the existence of an aster or a fundamental astral configuration (chondriosomes in plants).

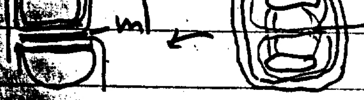
Cell Walls (after Wilson)

Plasma membrane – peripheral cytoplasm, membrane
"True" membrane – may be lacking; secretory prod

Plant Cell Division

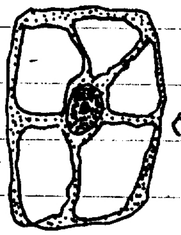
The phragmoplast is an organ derived from the interzonal spindle. By a running together of the fibres, a cell plate is formed which becomes continuous with the plasma membrane.

?. Precise relationships of the middle lamella, plasma membrane and the cellulose wall ??



Sinnott + Bloch PNAS 26 (1940)

Even vacuolated cells can divide, especially on wound stimulus. A strand of cytoplasm exists in the line of the cell plate.



← phragmosome. Orientation during prophase.

See Sharp.

Duration of Mitosis. Tremendous differences in opinion and species variation. In following Tabulation, Telophase and interphase are combined:

| | Minutes | Pr | Meta | Ana | Telo-Inter |
|---|---|---|---|---|---|
| Lewis + Lewis An Rec 13 (1917) | Mesenchyme, 39° | 35 | 5 | 3 | 50 |
| Zimmerman Z. Biol 15 (1923) | Sphacelaria | 10 | 7 | 4 | 9 |
| Laughlin Carn 265 | Allium | 55 | 1 | 1 | 35 |
| Rabinowitz J Morph 69 (1941) | Dros. eggs | 4 | .3 | 1.0 | 4.3 ! |

In general, one can say that there is considerable variation, but that meta and anaphase are generally the most rapid.

Chick Allantoic date, interpretation

v. Sha
K
Fr
St
Jollos
Schrad
EBHaw
Analog
Cell is a
Zool
Erlanger
McClendo
Robertso

v. Sharp
Kellicott
Friesner
Stafelt 1921

## Periodicity of Cell Division

do not confuse with spawning periods; has nothing to do with egg laying in birds.

Complex of causes. Various disagreement

Arbitrarily distinguish cell division and nuclear division

In most animals cell-division is largely cortical.

There is considerable independence of cell and nuclear division: Syncytial cleavages in fishes. Contrary to general opinion, centrolecithal eggs of insects have a total cytoplasmic cleavage; each nucleus has its own cytoplasm around it.

Jollos & Peterfi Biol Centbl 43 (1923)

Cell division may occur in the absence of nuclei. In the axolotl, removed ♀ pronucleus, ♂ pronucleus degenerates in hybrid crosses. Cleavage occurs nevertheless.

Schrader doubtful: fragmentation?
E B Harvey Biol Bull 71 (1936)

Parthenogenetic stimulation of merogonic eggs (from which the ♀ pronucleus has been removed by ultracentrifugation. ?

11/13/42

## CYTOPLASMIC DIVISION

Analogies work in simple systems
Cell is a very heterogeneous complex
See Wilson for diagrams....
Erlanger, Biol Centbl 17 (1897)
McClendon Arch Entwm 37 (1913)
Robertson Arch Entwm 35 (1913)

Mechanisms [Analogy analysis mainly]

1. Surface Tension [Butschli, Quincke] If a small surface on an oil drop is in contact with soap solution, currents are established inside, which tend to divide the drop. Erlanger presumed analogous currents in the Rhabditis egg. McClendon confirmed the oil drop experiment.

Spek Arch Entwm 44 (1918)

Robertson employed soaked linen thread; but the furrow appeared at the wrong place; he may have touched the drop and he used a floating drop of oil. Did not exist; negative conclusion.

Spek repeated earlier experiments; used solid NaOH. Critical of earlier technique. He noted analogous currents in various eggs.

Emphas.. Polar lobe formation, blebs at anaphase.

Just Am J Ph. 61 (1922)

Echinarachnius (In hypotonic) eggs burst at poles.

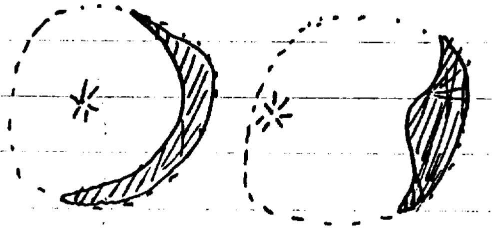
?? Reconstruction of cell wall at furrow

Dan, Yanagita, Sugiyama Pr. 28 '37

Marked egg surface with kaolin particles, and followed their movement.

2. Hyaloplasm The cleavage furrow is preceded by the hyaloplasm, "cleavage head" but correlates with monasters:

Painter, J. Exp. Zool 24 (1918)



Herbst Arch Entwm 9 (1900)

But, also.. There is no hyaloplasm in Ca-free seawater But such eggs do cleave.

3. Asters. a. No cleavage in monastral eggs. If there are more than two, furrows appear between all of them. No spindle is formed necessarily for cleavage. The size of cleaved cells is proportional to the size of the asters. But complete

Fry J Exp Zool 43 (1925)

cleavage is rare. The aster therefore is of some importance.

The aster is usually faintly visible in vivo.

Chambers J Exp Zool 23 (1917)

The aster may be produced by a system of centripetal lines of flow. In its growth, the homogeneous central material increases. [Echinoderm eggs.] Attempt to demonstrate this movement by injection of carmine particles, but this may have been seen between rays.

Detergent effects?

Forces of centriole movement.

Crenation of normal eggs?

Pollister '41: canals are not such, but the diffusion streams orient long molecules. Current flows between them.

Heilbrunn J Exp Zool 30 ('20)

Astral rays are gel strings. In centrifuge experiments the entire figure was displaced, bodily. ~~Exe~~ Corrugation of cell surface. (Rejuvenation of elastic mitotic hypotheses. W Heidenhain) What then moves the centrioles? There is some small movement of them sometimes, but in other cases not.?

Viscosity changes (Heilbrunn, Chambers; Fry + Parks)

Greatest increase just before the anaphase cleavage. As you increase the polar surface, division ensues.

Gray .... (BJ Exp Biol; Biol Rev 1: )

11/12/42 NUCLEAR DIVISION

1. The separation of chromosome halves.

Clearly splitting occurs very early in mitosis, possibly in preceding anaphase. Has no relation to the achromatic figure. The earliest steps of anaphase is autonomous of spindle and aster.

a. If a chromosome gets lost from its group. Possibly acentric chromosomes split autonomously also.

Geitler

Induced by heteroauxin; Greenleaf

b. Endomitosis (polysomaty): A division of chromosomes in the nucleus; halves never separate very far. In Gerris, some cells continue to endomitoses as indicated by the multiplication of pycnotic X.

Astragalus Vilkomerson

Culex, Spinacia Berger.

(Drosophila) ——

c. Monasters in Echinoderm eggs, after Mechanical disturbance.

d. "Deleterious conditions" cause disappearance of spindle and aster. the chromosomes continue to divide. (to 128 ploidy).

Wilson, Lillie

e. C-Mitosis

Levan, Federley....

This autonomous migration is very limited.

Does colchicine dissolve out spindles once formed? Yes??

Centers do not disappear under ether treatment; the centers divide, producing polycentric eggs: to 64 centers. After recovery, new asters form from centers.

See Harvey 128 (anoxia)

11/20/42. THEORIES OF MITOSIS

Electrostatic theory. Largely based on various attraction and repulsion, on the similarity of the mitotic figure to electrical lines of force. Asters would have to be ~~the~~ of different signs.

Fundamental objections:

1. Diagonal figures, quadri, tripolar figures
2. If asters are of different sign, they should attract each other.
4. The peripheral rays cross!

Various formulations

Gallardo

where?

Asters of like sign and chromosomes of the other sign. | But spindles occur without chromosomes. In many cases spindles form completely before breakdown of nuclear membrane.

Hartog

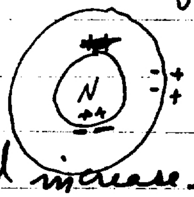
1 pole is more neutral than the other(s) in di and multipolars. | Only very small remnants of astral type figures.

Lillie, RS Am J Phys 15 1905
J Morph 22 1911
Hardy, McClendon, etc.

More thorough electrical theory of mitosis. Electrical analogues. Cells with high chromatin content cataphorese anodically

Churney & Klein Biol Bull 72 '37 Supports charge on salivary chromosomes. (+)

Lillie see paper. In interphase:



"What initiates mitosis is a local increase in permeability at the poles, neutralizing the dipole. Then the negative cytoplasm develops lines of force to the poles. The centers and chromosomes all have negative charges. At anaphase the charges reverse." The scheme has been adopted uncritically by various cytogeneticists.

Static or magnetic

Floating cork models.

But:

1 Magnetic fields have no known influence

Pease 1941 — 2. Hydrostatic pressure, although presumably not directly an electrical agent, does affect anaphase movement.

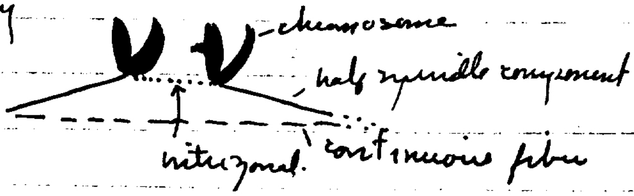
## Morphology of the Amphiaster.

1. (Asters). Easily seen in vivo. The spindle is only a clear zone, outlined by mitochondria, with no

Cooper PNAS 27:480 1941 — visible fibrillar structure, usually. But, see —— on Pediculopsis. In this case, there are neither centrioles, nor asters. The spindle is more viscous than the

Chambers & Sands J.G.P. 5 '23 — ~~F~~ cytoplasm.

Terminology



The continuous fiber is generally very fine and at the limit of visibility. Half-spindle fibers may attach to continuous fibers (by a chromosome fiber).

The interzonal generally seems structureless if there is a distinct half spindle.

Boveri: spindle derived from archoplasm; this is probably a Golgi remnant.
Bütschli: transformation of cytoplasmic alveoli

Thixotropy — Lillie, R.S. Polarization; orientation of long molecules.

Faure 1910 — In some cases (Arion, Amphibia) spindle is made of cytoplasmic materials, and may be formed before nuclear membrane breakdown. The centers are involved in spindle formation. The chromosomes migrate (congression) to the spindle fibers and attach to them (with coorientation).

More commonly, the centers are amphipolar before spindle formation, and the fibers grow toward each other, meet and fuse!! The nuclear membrane becomes corrugated by the active impushing of the fiber! They rupture, or lyse the nuclear wall, and penetrate, etc.

as in the figures of ✓ riffin J Morph 15 1899.

In Hemiptera heteroptera there is an outpushing of the nucleus at various points toward the centers at an early stage. The cytologist cannot distinguish astral inpushing from that of spindles. Cleveland upholds this view, on Protozoan material

✓ Science 81: 598 (1935)

hich?

There exist spindles without continuous fibers

There exist anastral spindles

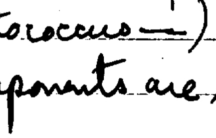
If asters formed spindles, the asters would have to connect only with chromosomes.

If there is an extra nuclear spindle, it consists only of continuous fibers until the nuclear wall breaks down.

Special cases:

✓ Belling J Gen 18 (1927) Univalents in heteroploids; form separate spindles

In Drosera (hybrids) in flattened cells, independent spindle components.



✓ Hughes-Schrader J Morph 39 (1924) (Llaveia, Neutococcus —) In late prophase

Z. Zellf 13 (1931) the half spindle components are independent. Later

Compound fiber with localized kinetochore? These orient so that chromosomes are on a plane.
These half spindle are intranuclear, clearly.

Thus, in Llaveia the chromosomes organize the half spindle components.

Bělař The interzonal is part of the continuous fiber, and not a neomorph. The chromosomes slide along the continuous fiber.

Ellenhorn Z.Zellf 20 The interzonal is the trail or track left in the nuclear material by the moving ~~[illegible]~~ chromosome.

Schrader (Particularly in Syromastes, other bugs): a hypothetical sheath about chromosome. This is drawn
Interzonals Feulgen positive out into a collapsed tube. In some cases, low density of medium, etc., there may persist a circular cross-section.

In toto: Cleveland maintains a cytoplasmic spindle
Wada a nuclear origin

1 In living figures, asters are visible; spindles not.
[ fibers can be seen in asters? in Protozoa ].

11/27/42 The Reality of Spindle Fibers.
Artifact opposition...

"Is there some morphological [not sub-microscopic] basis for spindle structure?"

Contra:

1. Invisible in vivo
Chambers, Gen Cyt 2. In microdissection, pulling in half-spindle should cause chromosomes to move; fibers cannot be

pulled out of the spindle. He neglected, however, to fix the material in final configuration.

Lewis, MR Bull Johns Hopkins Hospital 34 1923 — 3 In fibroblast mitosis, acid conditions cause a reversible denaturation of the spindle.

Gregoire — 4. In a "very good" fixation, fibres do not appear. [Stain reactions may be a factor.]

Pro:

Cooper, (Schrader) — 1. In some coccid, aphid oöcytes they can be seen in spindle at metaphase, but these may be semi-morbid.

Bělař Arch Entwm 118 437 (1929) — 2 a. In hypertonic media, spindles contract laterally, may bend. Shortening lines of force would not lead to a bending.

b. Spindles may be split, always longitudinal.

? — (The possibility of reversible coagulation must be considered).

?? — c. Brownian movement within spindles is limited to the longitudinal direction.

But very few cells recover if treated at metaphase.

Schrader Biol Bull (1934) — Centrifugation bending; species variation.

Schultze Chromosoma 1939 — Perhaps the final word on the matter is the birefringence: in echinoderm eggs.

Schmitt Coll Net 15 1940 — These deal with the ½ spindle component.

The interzonals persist after telophase, particularly in some Orthoptera, even for several divisions. Lines of force would not persist past the new nuclear wall.

[ — They stain somewhat differently. The interzonals are easily

Emphasizes difference | distorted.

gebn., Fortschr. d. Zool 8 (1935) | Hirschler has a fantastic theory of the persistence of spindle fibrillae.

12/2/42. | Further required laboratory: plasmosomes in mitosis
3 Feulgen slides.

Further on spindles.

# KINETOCHORE

Many synonyms: centromere, spd pt., kinomere and kinosome; primary constriction.

Schrader Biol Bull (1936) 1. If there is a well defined half-spindle fiber it goes to a particular spot on the chromosome.

2. at anaphase, this spot leads the chromosome poleward

3 In acentrics, the chromosomes cannot indefinitely persist mitotically. The loss of the chromosome need not occur immediately.

Not really

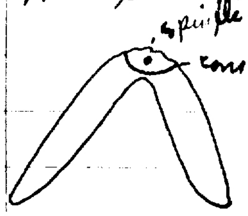
Most of our knowledge is recent. One "constriction" is always associated with the fiber. These are by definition primary. Others, called secondary, may be associated with nucleoli.

In some cases, the region is more chromatic.

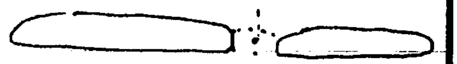
See Chromosoma 1 (1939) Analysis rather complete Chondriosomal techniques, with overfixation, demonstrate the kinetochore; with finest differentiation a granule can be seen within. The kinetochore is different from the rest of the chromosome.

Amphiuma, other Amphibia

Most easily demonstrated in arachnids, mammals.



At metaphase: (?)



Plant homologies obscure. In Tradescantia, and others, there is a projecting knob (sometimes double), which is Feulgen positive

Kopach & Chromosoma 1 '40

Iwata Jap J Bot '40 In Zea there is a large kinetochore at pachytene, a clear knob. At anaphase, there is a knob (Darlington) Consider that maize has no centriole.

Descr in pl — The spindle spherule divides first.

Tzenkowsky Z. Zellf 10 (1930)

In living grasshopper, there is a gap at the point.
In Oecanthus, there may be a centriole-like substance.

Rhoades Genet 21 25 1940 — No case known to persist with a "terminal" kinetochore. Invariably lost.

Cytological many organisms appear to have telomitic chromosomes. Ends of chromosomes are peculiar Telomeres.

Hinton & Atwood PNAS 1940

McClintock Genetics 23 (1938) — By X-Ray, the kinetochore can be split functionally. The high frequency of such splits is disturbing.

Darlington J. Genet. 37 (1939) — Misdivision of the centromere (Fritillaria spp., certain forms) leading to isochromosomes, branched chromosomes, etc. (The centromere may be pulled out.) Probably the essential part is fibrous, in a fluid matrix. Acc. Nebel: oriented micellae, permitted crystalline breaks.

?

Misdivision maybe origin of attached X.

Conception of diffuse kinetochore

There is (little) no experimental basis for a a kinosome-centriole relation, but a good, generalized idea. See Pollister....

Test for terminal ~~[illegible]~~ kinomere: coli → isochromosome temporarily.

Hughes-Schrader & Ris JEZ 1942 — Localized & diffuse kinetochore. → localized kinetochore diffuse into its region.

12/4

## WHY DO CHROMOSOMES MOVE

(No consistent hypotheses)

1. Chromosomes pulled to pole by half-spindle fibres.

Artefact — 2 ug fixer

a. As chromosomes move the fibres do not thicken

b. Where there is a large centrosome the chromosomes may be brought past the point of chromosome attachment.

c. Crinkled interzonals (should be in tension.

d. Establishment of metaphase: how?

[Wetaré proposes push: to equilibrium position: How anaphase? ]

See Rashevsky, Bull Math Biophys '42

2. Diffusion currents [many botanists]

An apparatus is essential for demarcation of currents.

Schaede Beitr Biol Pfl. 19 (1931)

The currents start at center, mid, and border.

Honeycomb spindle: But fibres are attached to chromosomes, particularly kinetochores

Bělař notes: If cytoplasmic currents are stopped, the chromosomes continue to move.

Stickiness of plant chromosomes.

Is there then, normally, a spindle current????

V-shapes in anaphase chromosomes.

Univalents X-chromosomes, move differently. If there are currents, should be no differential.

3. Lamb, Cannon... Hydrostatic waves, in resonance, induced by oscillation or pulsation of the centriole and possibly the kinomere. A change of density of the chromosomes after metaphase must be presumed. The kinomere must also vibrate if the forces are to be localized. Case of anastral, acentric spindles.

Drosophila centers are seen to move, but irregularly and slowly within the centrosome.

See Wassermann

4.

Handb. d. mikr. anat. d. Menschen. Vol II — Wassermann. Movement is due to "directed viscosity changes". *Physically untenable*.

Metz Biol Bull 1933 Science

Skand Arch Phys 77 1937
PNAS 21 1935 — Teorell Diffusion potentials can arise by known chemical situations; may modify function of chemical reactions.

12/9/42.

Arch Entwm 118:446-456 — Bělař

1. Internal chromosome division is autonomous.
2. Spindle contains only continuous fibers
3. Chromosomes are pushed into the equator by the growing out of fibers from both poles.
4. Kinetochore secretes some adherent substance and attaches to spindle. When attached, the chromosomes are pushed to the equatorial plane.
5. The secretion runs up the continuous fibers toward the poles. This secretion is called the Zugfaser.
6. First split and movement autonomous.
7. The Zugfaser slides with the chromosomes.
8. 3 mechanisms for further movement.
    a. Gliding along the Zugfaser.
    b. contraction of Zugfaser
    c. Expansion of continuous fibers = Stem

In Artemia, there is no expansion of the Stemmkörper, or in distance between centrioles.

*Lagging* of univalents, in grasshoppers with large Stemmkörper.

Spindles are not all continuous fibers (kinetics)

S B121 (1936)

Fittberg

Darlington: Electrostatic, after Lillie

1. Unsplit genes attract; split repel split. After splitting they repel; in meiosis chiasmata hold chromosomes together.

At late ~~metaphase~~ prophase the chromosomes are already split, repel any other chromosome. This accounts for the universal diakinetic repulsions.

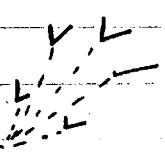
or paired.

The centres go polewise, mutually repelling. Spindle is established through a "redistribution of water. The chromosomes have kinetochores, but these do not split for a time. The first anaphase movement is the autonomous specific repulsion. The chromosomes reached the metaphase by centriole repulsion. Their charge now wanes, and the chromosomes go poleward.

Does not take expansion of Stemmkörper into account

Schrader

Anisolabis; 1 Chromosomes attracted to centres of diakinesis 2. When nuclear membrane breaks down, the chromosomes congress. The nuclear membrane must play some role. The metaphase is then set up quite orthodoxly. The ends of chromosomes must be rather peculiar for they are specifically attracted in the pachytene bouquet. (This is true also for chromosomes with subterminal kinetochore.)



The cases of Sciara and Micromalthus must be considered. Before division the chromosomes aggregate about the centriole. Then a monocentric mitosis; the V's are all pointed centrally; some chromosomes move away. If anything, the spindle fiber impedes the movement. Autonomous chromosome movement proposed.

Metz Cytologia 7 1936
Scott J Morph 59 1936
Korny Biol Bull 71:375 (1936)

## FURTHER ON MITOTIC MECHANISM

The Electrostatic hypothesis proposed by RS Lillie

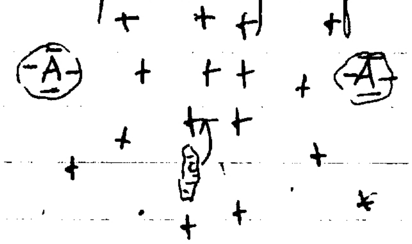
Lillie Am J Physiol 15:46-84 (1905)

The first to emphasize (if hastily + erroneously) the colloid character of protoplasmic substrates. His work must be considered in the light of modern knowledge of double layer phenomena, and of diffusion potentials. The Donnan equilibrium expressions had not yet been formulated.

$Ncl^\circ \rightleftarrows Ncl^- + ⊕$

One cannot assume a concentrated + ionic charge. Must assume a uniform distribution of mutually repelling ⊕ charges, even if they arise from chromatin dissociation. In this event, consider: reciprocal repulsion would not centralize a chromosome; reciprocal attraction is a system of high instability because of the $1/R^2$ law.

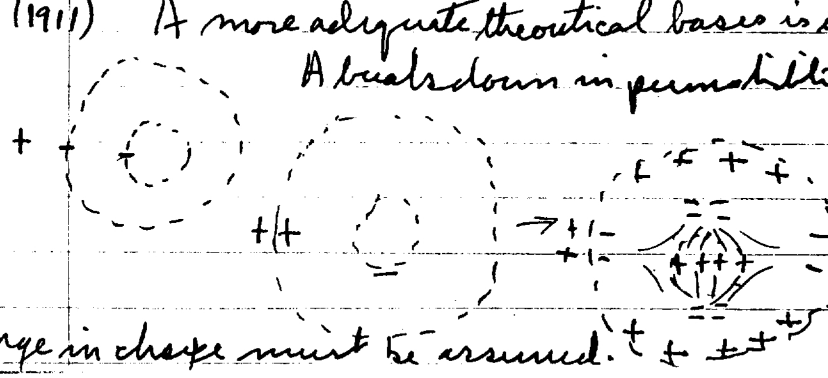
Chromatin, particularly chromosomes, are strongly electronegative, or acid. The appearance of chromosomes in the ~~central~~ equatorial region is due to the aster's being also negative and the highest concentration of electropositivity at equator.



The basic assumptions, therefore, at metaphase are polar repellent and interastral attractive field, with mutual chromosome repulsion. Models are reported of floating magnets, spheres, etc.

Lillie J Morph 22:695-730 (1911)

A more adequate theoretical basis is established. A breakdown in permeability equatorially



Then a change in charge must be assumed.

Mitotic mechanisms

What are the general conditions that must be satisfied by a theory of mitosis:

"Diffuse kinetochore" can be regarded as a limiting case of multiple kinetochores.

1. Localized application of force to the kinetochore region.
2. Stable equilibrium at metaphase
3. The existence of spindle fibers
4. A marked "Stemmung" in the interzonal in some cases; its absence in others.
5. Anastral, acentric mitosis
6. Specificity of action: precocious or lagging X.
7. The anomalous cases of Sciara, Micromalthus and, in meiosis
8. Body repulsion at diakinesis
9. Synaptic attraction; saturation
10. In Amoebae, the centriole-chromosome attraction
11. The division of the centromere.

ALSO

12. Autonomous split in c-mitosis, etc.
13. Pressure inhibition of chromosome movement
14 Anisotropy of the spindle
15. Coorientation and congression
16. Specificity of metaphase pattern, even in polyploids.
17. Ion rich cytoplasm. High Dielectric Constant.
1. Existence and orientation of multipolar spindles, and the chromosome movements resulting.

12/11/42

# NUCLEOLI

Plasmosomes and karyosomes.
↓ heteropycnosis of chromosome or part of it

Mottier '99
Gray

heavier than rest of nucleus. Generally visible in vivo. May be heterogeneous. Old rules of basophily are inadequate, particularly in oögenesis

The Feulgen-(Light Green) reaction is now employed. But almost certainly some Feulgen negative components exist in the chromosomes. In most animals there is no plasmosome at anaphase; reappearance in telophase May sometimes be lost in the spindle, and drawn out considerably. But there is no direct continuity of the plasmosome from generation to generation

In the lower vertebrate eggs, the plasmosome fragments into particles which may look like chromosomes, but are only lumpier threads than the latter. The plasmosomes here are Feulgen-negative.

Amphinucleoli In Arilus (Hemiptera) the karyosome increases and accretes the plasmosome; the chromosomes (compound X) breaking apart. Finally they leave the plasmosome for the spindle.

Agar QJMS 67 1923

In Marsupalia, a "mixed-up" amphinucleolus. Toward metaphase, the components segregate. Acc. to Agar, this is a fixation artifact, the fixation contracting the chromosome and forcing out a more liquid medulla of the chromosome.

Function:

1. Paragenoplastin — Too many cases of persistent nucleoli

2. Relation to chromosomes: In some animals the nucleolus is huge relative to the chromosomes which emerge.

3. Secretion, yolk formation —

Schreiner Arch mikr Anat 89; 92 (1916) Myxine slime cells, very active in the young. ("fish in an inevitable bucket," after McGilgar). Development traced. Young cells show budding of nucleolus; squeeze through nuclear membrane.

Similar phenomena in trichopterous insect — Caddis Fly larva; detailed account in deutogenesis, Limulus eggs —

Beams & Wu J Morph 47 (1929)
Gardiner J Morph 44 (1927)

After the ultimate gonium, no plasmosome. As egg grows, small irregular lumps in the cytoplasm; later, similar lumps in the nucleus; increase proportional to increase. Lumps fuse.

Chondriosomes then appear in the cytoplasm. Simultaneously plasmosome has budded, extruded in cytoplasm, chondriosomes & Golgi aggregate. Yolk spheres appear at the aggregates. "Test for plasmosomes!" During emission, high P content as shown by $(NH_4Mo)$ test.

Apparently not all plasmosomes are related specifically to chromosomes.

12/17/42

Relation to chromosomes. Zea Mays

Zirkle, C. Bot Gaz 86 (1928)

Plasmosome present at telophase; In early prophase the plasmosome connects with a chromosome. The plasmosome decreases in size; His interpretation as filling the inside of a hollow tubular chromosome. (See Fikry). But the chromosome is not a hollow tube; the spireme is not continuous.

Heitz ZIAV 70:405 (1935)
Planta 12: (1931)
Lorbeer Jahrb. w. Bot 80 (1934)

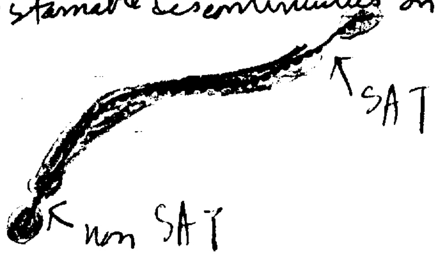
"Secondary constriction" Appearance varies. Usually nothing more than a gap. Satellites (SAT). In some cases association with the plasmosomes. In early telophase, the nucleolar globule increases. In a few cases, the plasmasome is formed as a collar at the SAT.

Balbiani - Chironomus
McClintock Z. Zellf 21 (1934)

By X-Ray split of the organizer at (VI), a heteropycnotic region, the relationship between nucleolus, organizer and matrix was established....

[Geitler opposes this interpretation].

Chromosome structure Gene string, with other stainable discontinuities on it (chromomeres)



There is more than one kind of plasmosome; do not generalize.

# THE GERM CELL

Darwin — Gemmulae (submicroscopic, hypothetic units) at some time the gemmules are circulated and gather in particular, which accumulated themself.

Weismann — "determinants" In differentiation, a germ cell is set aside, an undifferentiated cell.

Now recognized that all cells essentially have identical genotypes. Germ cells generally "immortal" Some somatic also (veget. propagation). Consider the parasitic role of the germ cell.

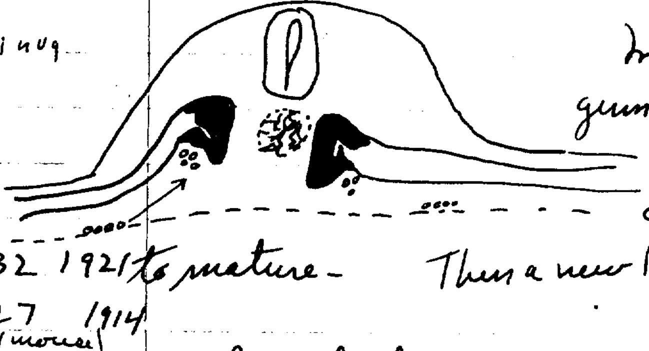
? point of optical differentiation of the germ in vertebrates Ceaseless, (futile) controversy!! Usually primordial germ cells have a large hypochromatic nucleus.

Waldeyer: - rather late differentiation, from epithelial cells.

Then ideas of migration; now accepted

Allen, on Dodd's goosefish, turtle

Heys QRB 6 1931
most recent review; nvq



In bull frog, the primary germ cells, do not form the testes; the primordials disintegrate after starting to mature. Then a new batch sets in.

Swingle JEZ 32 1921
Kingery Biol Bull 27 (mouse) 1914

In coelenterates any undifferentiated cell can give rise to the germ.

~~Boveri Festschr f Kupffer 1879~~
Hargitt J Morph 40 1925

<u>Boveri Festschr. f. Kupffer</u> ASCARIS MEGALOCEPHALA (BIVALENS.) First cleavage is normal; In the second cleavages, and five successives, one of the mitoses is normal, the other, giving rise to the somatic components is diminutory:

The ends of the long chromosomes break off from the middle region. This fragments into 60-70 small chromosomes. The unbroken pieces degenerate. A specific cytoplasmic material preventing diminution may occur, as indicated by centrifugation; 2 cells non-diminut.

In a diminution mitosis, the spindle fibers are localiz

Hogue Arch Entwm 29, 1910 at the center.

Boveri Arch Entwm 30, 1910 In polyspermics, 4 cells form at once, 2 non-diminut.

King & Beams ZEZ 77 (1938) Same conclusion in fact: distributed substance

Similar process in many nematodes

Kahle Zoologica 21 1908 MIASTOR [Diptera]. First 2 cleavages, 1 passes to one pole of the egg. At that pole; there are many granules, the cell there gives the germ cell. (mitochondria?)

beaut. plates; PHD's from Leipzig

Acc. Kahle the telophase nuclear wall cuts them long chromosomes, extruded half disintegrating. Repeated in 4th division. Other workers show whole chromosomes lost. The chromosome number in the somatic cells is 12, in germ 2n = 48. There is actually a reduplicated chromosome set.

Kraczkiewicz Folia Morph 6

Huettner Anat Rec 60 1934

<u>Reitberger Chromosoma 1 1940</u> (Olyuris)

Morgan & Bridges Carnegie 278 <u>Drosophila</u> A series of gynandromorphs. Non-disjunction. Assumed that the germ originate from a single cell. <u>No migration until after 7th cleavage; random</u>

Huettner J Morph 37 1923 migration. The polar nuclei varies from >5<11.
Rabinowitz J Morph 67 1941. But the gonads are mixed.

In embryology only a few polar cells include granular material; these yield germ cells.

No elimination in Drosophila

Kühn Zool Jahrb [Anat] 35 ('12) In Entomostraca (Daphnia...)

Groups of five cells in gonads; others are sacrificed to the one that develops. The nurse cell nuclei are pycnotic That mass lies at random. The cleavage furrows after the 3rd division include the nuclear mass (a Keimbahnkörper; Parakopulationskörper); cells containing this are the germ cells.

du Bois Z. Zellf 19 (1933) In Sciara:

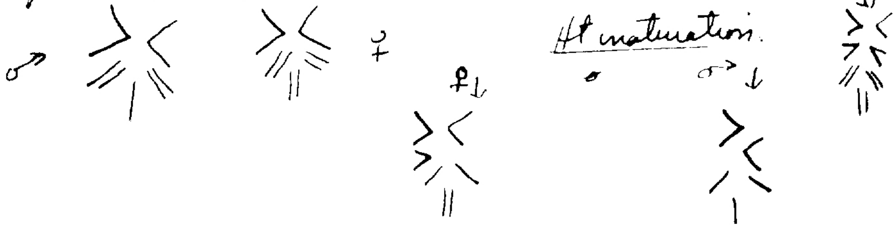
All fertilized eggs have 12 chromosomes:

1st five cleavages are normal.

In some ~~eggs~~ cells the > are extruded

In the 6th through 8th divisions, further elimination differences

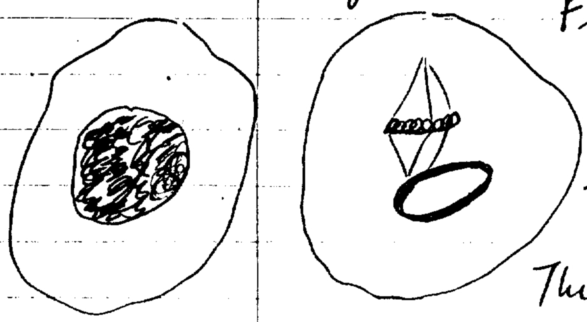
The germ cells lose only one >, /



1/13/42 Other presumed cases of germ cell determination:

Günthert Zool Jahrb (Anat) 30 1910

Dytiscus (water beetle, cytologically difficult) In ♀ in the last oogonial divisions, the germ nucleus differentiates one side becoming slightly pycnotic. The chromosomes come from the typical portion. The rest of the nucleus forms a ring: The ring is Feulgen positive. This occurs in 4 divisions, the ring segregating to 1 cell of 16. This forms the oocyte egg cell; the others are nurse cells

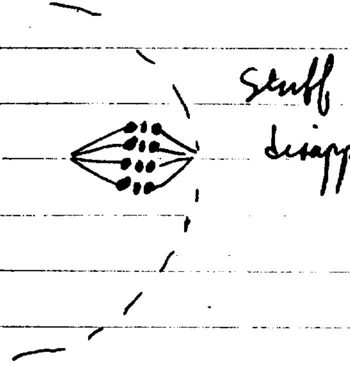


This is not a germ determination as such.

Seiler, Arch Zellf 13 (1915)

Cooper, KW Chromosoma 1 (1939) Arachnida

LEPIDOPTERA: Lymantria, Ephestia In maturation division. As metaphase tetrads appear and separate, some material was found between the chromosomes.



In Pediculopsis, this is found in both meiosis, and disappears the early cleavages of the zygote. Feulgen negative.

Summary:

1. True determination, may be extrachromosomal
    A. Pole plasm
    B. Nurse nuclei or adjacent cytoplasm
    C. Elimination; diminution
2. Pseudo A. Dytiscus
    B. Lepidoptera

## Fertilization

Controversy —

Must involve several consequent processes, but the essential task and purpose is syngamy, nuclear.

Large variation in phase of development of the fertilizable egg.

In Dinophilus (a rotifer like animal) the sperm enters long before maturation begins

Ascaris - just before the first prophase. As the sperm nucleus becomes diffuse, the egg matures.

In Coelenterata, Echinodermata, eggs are generally mature.

In Annelida, Insecta the meiosis has begun at the time of fertilization.

In Echinoderm eggs, fertilization may occur prematurely under experimental conditions. The sperm will not enter until the germinal vesicle has broken down; thereafter even the isolated cortex can be "fertilized."

Just Biol Bull 44 1923 If the cortex is removed (bolting cloth) completely no sperm will enter.

Violent currents in egg in relation to the sperm entrance. Fertilization cone; egg swallows sperm.

Path of pronuclei to "equilibrium" The ♂ may migrate directly to the ♀ pronucleus.

In some large eggs, the first path is rectilinear; then an orientation in respect to nucleus, the copulation path

see pp 82 et seq →

1/15/43 Partial Fertilization – gynogenesis No action of nucleus. May occur "accidentally" in nature (hybrid crosses, etc)

Bělař Z.Zellf 1 (1924) Rhabditis (nematode?) After 2 pb are given off the sperm enters at one pole, normally movements are as:

Under relatively anoxic conditions, the chromosomes arrange as usual, but the ♀ pronucleus does not migrate. In the neighborhood of the sperm an amphiaster forms. If oxygen is readmitted the egg nucleus migrates and "picks up" the amphiaster.

Packard Biol Bull 36 1918. Radium inactivated eggs, nuclei destroyed. The sperm alone will start with first cleavages up to blastulae

in normal life history activation of egg without the participation of sperm.

There may be a reverted division before cleavage.

Artificial Parthenogenesis

Hypertonic sea water → multipolar asters

Fertilization membrane not well separated

In double treatment, butyric – s.w. cytolysis checked by latter

2 steps in fertilization: cortical cytolysis + its check. But Just has shown hypertonic seawater is adequate if properly adjusted.

Herlant Arch Exp Zool 57 (1918) had actually proposed that each component of the double treatment gave rise to 1 aster. !!

In Amphibia –

Guyer Science 1925. Puncture egg with fine needle dipped in blood. Believed leucocytes active!

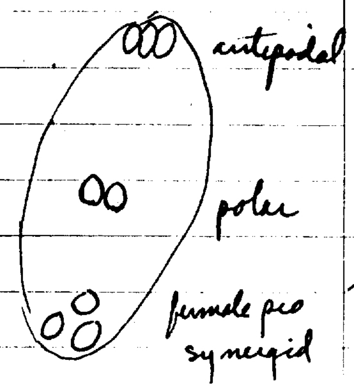
Bataillon CRAS 150 1910 In Toad eggs, only sterile puncture is required

Loeb J. Gen Phys 3 1921 In Rana, only needle is required

Parmentier J Exp Zool ca 1938-40 Various embryos. Only restored diploids survive. Mosaics. Both sexes!!! How explain?

Plant karyology

Progressive reduction of haploid phase. In maize only 8 nuclei in the female gametophyte.



The male gametophyte has 2 haploid germ nuclei. In double fertilization, triploid endosperm and diploid ~~[illegible]~~ zygotes are formed with consequent modification of Mendelism.

Early work on fertilization directed at centrioles.

Rable '84 – Fusion or syngamy of centers!!! Not sustained

"probably no definite rule of the centriole origin" The sperm brings in the center, usually.

In egg fragments division will occur
In "partial fertilization" only centriole participates
In polyspermy, numerous spindles.
In some cases, egg provides its own centrioles, as Gyrodactylus, Fasciola other trematodes.

Conklin Biol Bull 7 1904 Study of (parthenogenetic) Crepidula. Conservation of genetic continuity.

Wilson p445ff In Nereis the middle piece is left out. But this may have an elongate centriole, rod theory, the nucleus.

FIN DE LIVRE

4-13-43. C.L. Huskins on Chromosome Coiling.

Trillium first demonstrated this

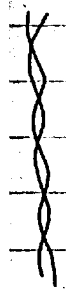
In Trillium there is spiralization in both divisions.

Relational coil shown.

At somewhat elevated temperatures, the spirals seem to iron out.

Reasons for difficulty:

1. Spiralization was first deductive; Darlington "has been completely disproven.
2. Variations in appearance: technique
   organisms, stage, eye domination.
3. Number of strands in a chromosome.
4. Visiblity
5. Optical artefact; psychological factors at limit of visibility (particularly)
6. 3 dimensional visualization difficult for many people. Stupidity



A: the relational coil: a twined electric wire is 2 spirals in the same direction. Right-hand spiral is right handed from any aspect.

Doubling of coil and bipartite coil are the same

see e.g. AJB '40

B. Direction of coil cannot be determined from photos. (MJD white)

Chromonemes vs. Spirals.

Strand Number: Kuwada & Nakamura hold 8 strands

Minor spiral fairly clear but may be really only the two cross strands.!!!!

If a spiral is coiled with ends fixed

If ends rotate, the spiral can fall apart.

But ends do not slip apart.

In Tradescantia: a clear minor spiral → major in 2nd division

In Trillium, the major persists; matrix contracts.
" " the matrix does not contract, chromonema elongates

Darlington proposes molecular spiral.

Huskins holds that in last premeiotic anaphase the relational coil is formed. Gene reproduction occurs during a coil stage. Pulling out causes twisting. For untwisting, relational spiral is produced.