CYTOLOGY NOTES

Zoo 125

(Zoo 226)

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1942

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

Required Lab work:
4 drawings Golgi
8 drawings chondriosomes
in Bug spermgenesis.
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
Anasa tristis 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.

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.

Theory of Fixation

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.

Light as an abnormal factor.
Thanatology;

Strangeways & Canti QJMS 71
Belar Z IAV (1928)

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

Alexandrov Protopl 17:161 1932

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.

1/1/42

Dimensions of fibrillae, etc.?

Seifriz, Science 73 1931
Mudd, Science 84 1936
Bensley, Anat Rec. 72 1938
Banga & Szent-Györgyi, Sc. 92 1940
Pollister, Physiol Zool 14 1941
Wilson '24 pp 57-78
Symposium on the Structure of Protoplasm 1942.
Seifriz "Protoplasm"

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

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

granule defined as something angular.

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

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

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

Bowen La Cellule 39:123 (1929)

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

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

6

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

Bowen J. Morph 39:351 1924

Johnson Z. wiss Zool 140:115 1931

Pollister Biol Bull 65:529 1933

Fry Biol Bull 54, 56, 63, 65

" Anat Rec 46, 56

Destaining must be properly interpreted.

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.

In 1887 van Beneden and Boveri recognized its independent existence. The whole "region" (polar capsule) is called a centrosome (Wilson, p. 673..) The pole parts 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.

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.

8

10/9/42

Jordan Anat Anz 43 1913	Centrioles: characterized by position, form, staining.
Benda Arch Anat u Phys (Phys) 1901	Stain techniques are not too reliable.
Renyi Zeitsch. Anat 73:338 1924	Feulgen negative (Carothers?)
Walter Anat Rec 42 ✓ 1929	But preparation of cells and components usually is corre-
Kindred Anat Rec 34 ✓ 1927	lated 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. Induces 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.

Seminar 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?
corolary: can a ciliated cell divide?

Modified mitosis?

Jordan claims ciliated cells divide amitotically. Presumed that before ciliogenesis, basal granules can be found.
But Benda, Gurwitsch, Walter unmistakeably 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.

¿Arducaro's filaments?
ciliogenesis
protozoal analogues.

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; Vallowity in Salpa, Pollister... The case for genetic continuity is clear. But occasionally de novo formation ~~does app~~ occur: as in the spermatogenetic 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

Sturdivant J Morph 1934
Cleveland

product of something which is, like the nucleus.
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 Ioplax subcarinata, other pulmonates.

Perhaps because of physiological neutrality 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 cytes, there is one centrosphere, 1 or 2 grains. 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-

pated in late anaphase, 3rd spermatogonial division.

In cytaster formation (which may be diffusion artifact) 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

Chondriosomes

Schultze (1861) recognized granulations in protoplasm.

Altmann 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.

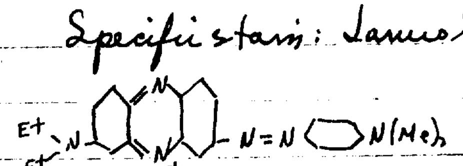
The development of techniques stimulated research. Mitochondria have survived from the bioblast theory. Benda developed specific methods and differentiated mitochondria from other granules. Now an enormous literature.

Universal occurrence probable.

Rods of uniform diameter; rounded ends. All similar in any one type of cell. Ought 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 .2 μ 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.

2 older reviews:

Duesberg Arch Zellf 6 (1910)

Cowdry, Carnegie 271, 1918 (None later)

Cowdry Gen. Cytology 1925.

Cowdry NH Biol Bull 33 (1917)

Lewis & Lewis Gen. Cytology '24

Rumjantzew Arch Zellf (1926)

Anitschkow Arch mikr Anat (1923)

Bensley & Gersh Anat Rec 57 (1933)

Michaelis Arch mikr Anat 55:558 Ehrlich's stud. 3

Cowdry Am J Anat 19:423 1916

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.

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

Portier Les Symbiontes 1918

Wallin Symbionticism... 1927

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

Claude CSH Q B Sym. 9 (1941)

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

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

10/23/41

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

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

Regaud CRSB 6:718 (1908)

Fauré-Fremiet, Archanatomie 11:457 (1910)

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

Mayer, Rathery & Schaeffer J de Phys & Path 16:607 (1914)

ibid 1929

Giroud Pr. 1:79 (1929)

Bensley & Hoerr An Rec 60:449 '34

Bensley & Hoerr An Rec 69:341 '37

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 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 Bioch 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 reflected. 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 Nouy & ... Anat Rec 34:313 '27

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

The lipoid acts as a semi-molecular solvent, and acts as an active surface for protoplasmic and enzymatic synthesis.

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

(after Langmuir) Robertson has shown an increase in the rate of resynthesis of proteins in tryptic solutions, when lipoid is added as emulsion.

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

de Nouy & Cowdry: measured various surfaces of formed components of pancreas cells. Assume, hypothesize orientation of semipolar compounds on a granular adsorption.
See Paper.

Hereditary factors?
Guillermond: 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 zymogenesis.

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.

Guilliermond '39 The Cytoplasm of the Plant Cell.

Guilliermond 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 ~~[illegible]~~ development of the egg.

Mitochondria in plant spermatogenesis??

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

Fate of nucleolus in oogenesis?

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.

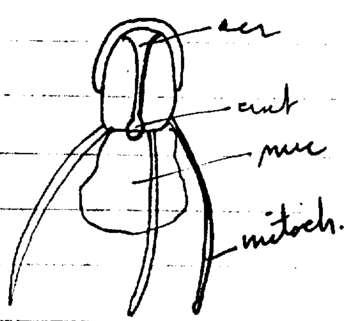
General features:

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

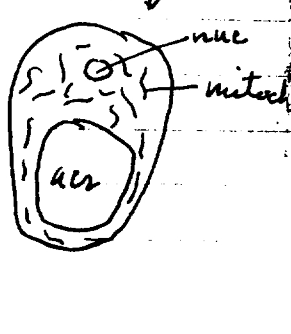
11/4/42

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