Fertilization:
Prevost & Dumas, 1824; Hermann Fol, 1876—early works on fertilization.

Fertilization: — Union of Egg & Sperm.

Results:
1. Activation
2. Hereditary Characters

Condition of the Gametes:
1. Must be mature. Complete maturity needed in case of the sperm.

Species arranged in 5 groups:
1. Obtains eggs fertilized in g.v. stage, circular.
   - Mecocaris, Helicletes, Platymonas, Sagitta,
   - Helosoma, Mactra.
2. Fast, with egg pronucleus at metaphase.
   - Cladocerus, Phoroslocoma, Amoebes, Mytilus
   - Conch, Phalusia.
3. One polar body given off before fertilization.
   Amphiprotis

4. Both polar bodies given off.
   Acicia.

5. Eggs which develop parthenogenetically.

Trust differentiates between maturation (nucleus) and cytoplasmic maturation.

It is necessary for the fertilization of the egg (release & pasteels).

Sperm should be diluted to activate them. There are exceptions to this rule, however. (Platynereis)

Gove et al. - paper on aging & sperm.

Cold Fresh - ... egg

Platynereis: Sperm enter through digestive tract and fertilize sperm inside body of f.

Cortes of egg only can be fertilized, endoplasm beneath cannot. (Chambers).

Helfi believes something in the egg cortex mixes with the sperm.

Pineal - Turbellaries, Squid, nematodes & insects. All...
possess micro pylori.

Polyspermia: When normal it is called "physiological polyspermia." Usually occurs in large eggs, devoid of tough membranes and micro pylori. Cortical changes move slowly over surface (sense of negativity).

In all cases of normal polyspermia only one sperm is used.

Pathological Polyspermia:
First studied by Bertwigs, Bower, Clark, etc.

Agents - Alcoholic, carbonic, nicotine, strychnine, salt solutions, chlorofom, ether, ethyl alcohol, ethyl ether, chlorhydraten.

Physical Agents - Mechanical injury.
Age of eggs, immersion of eggs, or insemination.

Pathological polyspermia due to slowing down of cortical response of egg - it is thought.

Specificity - Acidosis, tonicity.
Castle showed that young with 37 of part. an
in Ciura only with cross fertilization. Block to self fert., occurs at periphery of egg when "test cells" are located, thus prevent self fertilization — Morgan.

Selective fertilization in plants

Many pollen grains stigma $\rightarrow$ 8 sperm...

Due to diff. in rate of growth of 9.8.2 pollen grains. Application of NaOH to eggs surface aids hyaloid fertilization.

An egg activated by pothecogenie agents cannot be fertilized.

邵氏 first studied the effects of media and temp. on activity of the sperm of toad. Acid paralyzes sperm but they can be reactivated by alkali. CO$_2$ paralyses sperm, O$_2$ reactivates.

Core 19/8:

Egg at diff. pH.
Fertilized eggs contain no fertilin.
Blood and tissue inhibitors occupy zoonphilic group thus preventing fertilization.

Objectives of the theory:
1. Strongly concentrates eggs lose agglutinating power in diluted sea water and sperm can still be fertilized.

Lehrl and Wille both found this to be true.
Coute concluded that agglutinating substance similar to thymalin. Other view is that gliotoxin closely related.
Bovari - Centrosome theory of fertilization.
Hillie discredited this theory by work on Nerium.
Koech - Superficial Cytolysis Theory.
R. Struthers - Permeability Theory.
Chambers et al - Increase in Versatility Theory.

Antifragility of the Egg. (Research lecture).

Introduced in 1905 by E. P. Hyman.
Used by Morgan, Hillie, Cambelin, E.B. Wilson etc.
Was found that there is no mel. between axis of embryos and separation of stratifying egg.
Cambelin - rel between pigment & devel. in Styela.
Costello - surface properties change in egg during maturation so that it becomes more easily
(slightly) stratified by centrifugal force.
The jelly itself has nothing to do with the fertilization membrane.
Describes surface tension theory,
indicates that the vitelline membrane is
the same as the fertilization membrane
in absence of yolk, membrane in Asterias blastomeres
do not hold together.

Nereis

Centrifuged Nereis Egg

Stomatopodium persists many hours after centrifugation.
The heavy fragment of the egg may cleave if
it contains a germinal vesicle; a germinal
vesicle is the most important element in this
decentration.

Oelke found that if starfish egg cut before
breakdown of yolk, the fragment that contains
9.U. not fertilizable. Castello observed sperm entering heavy half but no fertilization to place.

![Diagram of an egg with labeled parts: oil, yolk, outer medulla, inner medulla, zona pellucida, egg, and AB CO.

"Doesn't seem possible to use cytolysed subcutaneous 9.U. to heavy fragment to obtain cleavage. Neither fragment did not produce any prototrochidia. Possible 2 explanations:
1. Altered nucleo-cytoplasmic ratio.
2. Yolk may be foodstuff, necessary for normal growth.
3. Yolk an interacting subcutaneous to produce a certain subcut.
4. May be basic subcutaneous that is not displaced by centrifuging. Not so good."

That prototroch disinherited mammalian pole and not displaced by centrifuging."
Side view opt. sect. of cell
Prot. Cresyl Violet
Blue (lower) acid
Red (upper) alkaline

Sphek 1534

alkaline (protoplasm, ectoderm)
acid granules (ectoderm)

acid yolk
acid "glossy" yolk
prototroch cells
somatoblasts.
Isolation of plastid enzymes.

A B all C D cell will both form prototrophic cells
A, B, C and D

Necessity of C, V for fertitization emphasized.
Cell lineage & Spiral Cleavage

First work done by Whitman

E. B. Wilson - Nereis 1901

F. R. Millie - Unio 1901

E. G. Calkin - Crepidula 1901

Goodwell - Podarke 1901

Child - Hemiola 1901

Walterschick - Polypodium 1903

Wronyseki - Physa 1905

Spiral cleavage found in aschaceans and mollusks

Polychaeta: Scoloplos, Nemertes (not in Cephalopods)

Spiral cleavage occurs at the cells at certain

cleavages.

2 cells, 8 cells in two tiers of 4.

Spiral cleavage of microsomes are oblique

Microsome lies at the upper right-hand corner

of the microsome from which it is derived

of this is true behavior clockwise or dehis-
trophic cleavage.
When it upper left we have counter-clockwise or levotrope cleavage.
Each group of microvessels given off to become as a
quartet or
A quadrant —
Distinction between microvessels and microvessels
is one of polarity those nearest the animal pole
being the microvessels.
An alternation of direction of cleavage
clockwise — counterclockwise — clockwise etc.

4-8 dorsal

8 - dorsal

8 - 16 dorsal

12, Dorsal

32, Ventral
In some forms spiral cleavage changes to a sort of bilateral cleavage with a transitional period in between.

Nereis. Spiral cleavage 1-38—all divisions transitional 1 38-58 bilateral 1 58-on.

Yolk lobe does not necessarily contain yolk.

Cephalic:

Anti post.

Left ABC

Post.

Right

Anteroclockwise

Axis of embryo determined at 1st cleavage.

1a 1b 1c 1d 1e 1f

16

The (see Cambellin)

End set of precursors given off before 1st set divides

1a 1b 1c 1d 1e 1f = tunet cells fail to divide for a while.
24 cell stage

4 apical cells
4 trumpet cells
12 belt cells
4 amnion cells

4th cell = primary mesoderm; in some forms
the 4th cell is called mesoblast, giving
rise to mesoderm and endoderm.
This constitutes the stage in development in which
cells have little influence on each other.
Pigmentation of Birds Experimental Studies.

Transplants from one embryo to another of a different breed and different coloration.

Transplants made at wing bud.

When skin ectoderm and some skin mesenchyme causes a larger and larger area of donor feathers. Feathers are similar in all features but pigment to other feathers of wing.

Donor pigment has come over from the donor.

Host furnishes the feather structure.

Fowl Robin pigment disk throughout the whole of Robin pigment changed at the nodes.

When Robin does not get Robin pigment but fowl distribution of granules.

Neoplastic transplants - feathers arise from host feather germ. Feather structure and pigmentation are two different things.

Granules of pigment of donor retain their identical shape in the host feathers.
72 hours, head skin pos. wing skin neg. 76 hours, both pos., as far as pigmentation is concerned.

Same with ect. or meso.

Transplant wing alone at 72 h, you get no pigment at all. Transplanting wing with neural crest, pigment develops. When no neural crest cells included in transplant there were negative results. Central half of spinal cord neg. while dorsal half is positive.

This explains why head ectoderm and mesoderm gives pos. results, i.e., includes neural crest. This seems to give evidence that neural crests are directly concerned with the origin of the pigment. There is probably migration of melanophores from neural crest.

Transplants from primitive streak blastoderm give positive results only from the area around the area.
Before death, if a morphological structure a cube area will produce pigment.

Note whole neural tube will give post results.

"dorsal" "only"

" neural crests"

A gradual narrowing down of the pigment.
Development of the Annelids

Annelida & Mollusca:

1. Spiral Cleavage.

2. Cell lineage, determinate cleavage. Organs can be traced back to one blastomere.

3. Have a trochophore larva.

Hydroidea (Eupomatus).

Trochophore

Bend 9 inviolate cell around equal - prototroch.
Divides larva into pre trochal and post trochal hemispheres.
An apical fold at anterior pole. Apical organ beneath contains nerve cells. Often in certain stages, a metatroch like band of cilia.
Bent lined by cilia.
One red eye spot always on right side.
Two statocysts on lower hemisphere.
Gut - stomach opens at an anterior side of ectodermal origin.
stomach of eutoderm

Intestine of eutoderm

Horns of proctodaeum, hind gut of eutoderm.

Bis.

Bilaterally symmetrical.

Feed on sponges etc.

Horned kidney has typical structure of a pronephridium - a closed tube containing a flagellum opening into the hind gut into the aorta.

Hornless muscle layer present. Main muscle from oviparal end to kidney (proephros).

Circular muscle around where metatroch appears.

Body cavity not surrounded by mesoderm.

It is nothing but the continued blastocel!

Is lined with eutoderm.

Hornless found in hydroids.

Hind of elamant mesoderm cells in vicinity of kidney have much importance in later level.

Hydroids of order Serpulida

Adults stationary.

Larvae swim and tend to distribute the form.
(Phylogeny of Annelida: polychaeta, annelid form, has a trochophore also)

Schelaria has long spines and is favorable for observation.

Prototroch and even prototroch is always in front of the mouth.

Metatroch always post. to prototroch.

Trochophore has played a very important role in phylogeny. Very famous.

Trochophore of the molluscs is called a Hetero veliger.

Annelids, molluscs, platyhelminthes (amoia, turbellaria thanannelid s larvae) 
Nonannelid (Bilateria, Rotifera, Bryozoa, Brachiopoda - all show forms similar to the trochophore.

One school of biologists considers the trochophore as the form which is the ancestor of all these classes and phyla. Called trochozoa - hypothetical ancestor.

Another school of zoologists considers the common ancestor Turbellarian like, primitive worm-like animal.
Embryology of the Isochrophone

Clawing of the spirid type.
Gastrolization by invagination.

Blastopore at first is an elongated slit. Point of invagination persists as the mouth. Just the opposite from Echinodermata & Caudata. Arms open up at lower part of slit of blastopore; thus many think arms also derived from blastopore, it opening up again to form the arms. Thus both arms and mouth originate from the same slit.

Protostomia - mouth, jaw, blastopore. Arthropods
Deuterostomata - arms. ... Vertebrates

Derivatives of Isochrophone:
Ectoderm - epidermis
Mesoderm - skeletal, organ.
Eye
Statocysts
Stambranch
Protostroch
Proctodaeum
Ectomesenchyme
Small connective tissue from outside
Ectoderm - 
Nervous Kidney
hernial arms.

Endoderm - Intestine
Endomesodermal bands of dormant cells are derived from cells in the gastrula, teloblasts
Gives off lines of cells which grows upon
Remains near arm. Mother cell always much larger than its derivatives.
Some of the descendants go to help form intestines and these are endodermal. Others remain
mesodermal. i.e. are endomesodermal cells
The cell in this type of phyla the embryological value of each cell important for the adult organism. Makes much difference whether there are 4 or 8 cells present etc.
Quite different from vertebrates; each cell counts here.
The actual fate of individual blastomeres is strikingly
similar in Annelids and Mollusca.
1st quartette - 1a - 1st ectoderm of upper hemisphere.
1a' 1st ectoderm
1a 2nd ectoderm
1a 3rd ectoderm
Clearage pattern very different
from 1a' 1a 2nd
1a 2nd of this moment these
1a' - 1a 2nd 1a 22nd cells stop dividing
1a 22nd form cilium and go to
form the protochord. Here
the mother of prototroch
Each blastomere has a definite fate, a definite
asymptotic figures etc.
Second Quartette - 2a - 2nd ectodermal
Form the lower hemisphere.
2 of cell forms a neutral globe, a thickening
From this cells almost the entire ectoderm
of the adult. = the "1st Somatopleur".
3rd Quartette - Ectodermal
Gives most of post trochal ectoderm.
Postoderm.
4th Quartette - Endoderm. It together with Macromeres, with exception of 4 d.
4 of cells gives rise to entomesoderm.
(Cheloblast in gastrula, ancestor of the mesoderm = 2d somatoblast)

Thus 1st quartettes are ectoderm.
4th "ectoderm"

4 d. mesoderm

Apical tuft
Apical organ
Ectomesoderm
Eye
Muscle
Prototroch

Mesoderm bands

Metamorphosis of Trachophore into Adult
Waltzoch studied Polygordius.
Mesoderm bands become segmented at a certain time and grow out posteriorly.
With it the ventral plate (from 2 d) grows out
with it. Teloblast remains post. end and any proliferates mesoderm off. A typical cellus formation starts.

Thickening of endoderm on neural plate, gives rise to neural mesencephalon.

Sudden radial outgrowth appears asph of spinal tuft from tentacles.

Endoderm collapses and muscles contracted being upper portion of upper hemispher in close contact with mesoderm. Stomach absorbed.

Eats itself up. Remaining cells reorganized into narrow tube.

Nasal organ persists as cerebral ganglia in adult. Most of upper hemispher is cast off.

Part of post-hoclinal hemispher between 1st somite and ciliated band (post troph) is cast off and is squeezed out. Kidney cast out.
Warm soils to bottom, gonads, adult kidneys
elev. from wells of coelum.
E. Injection of pituitary causes liberation of sperm within 24 hours in a frog.

Spam pass through 40 to 50 different tubules in kidney during heat.

Partioned germinals on surface of kidney draw pituitary extract into nervous system when injected into the eelam.

After injection sperm liberated from serosal cells into lumen of kidney.

Kidney pretty much given over to sperm transfer during the brief period of heat.

In adult frogs the seminiferous tubules are opened up even though no sperm were present.

X Rays:

No effect on embryos exposed prior to gastrulation.

Expose spermatozoa prior to insemination. Even if lowest possible irradiation.
No. of abnormal embryos in direct relation to amount of X-ray.
At 5,000 units, you get haploid embryos. 90% of eggs develop into genetically normal.
At 10,000 units practically all embryos die before hatching. From 15,000 upward, you get haploids and higher and higher nor hatching.
Spam cannot be inactivated as far as stimulating the egg is concerned.
Thus tissue seems to carry an induced lethal condition which causes the cells to die at a certain time.
Spam seems to have little influence on cleavage up to gastrulation.
Metamorphosis of Nereis.
Appearance of 3 pairs of seta sacs which form setae. 2 appear simultaneously. 
Traces place and day after fertilization.
Proto trochal cells become deeply segmented.
At 60 hours pericardia begin to appear. 3 pairs of seta sacs behind pericardia.
Sets themselves develop.
Segments develop with a pair of pericardia in each.
Beginning of cirri at anterior end.
Gradual transformation of tentacles into typical nematoid head.
Meta trochal bands appear at border lines of the segments.
On 4th day mandibles develop.

2d cell ➔ neural plate.
Nervous system
Seta sacs.
Body ectoderm
Some circular muscles
4d cell → Mesodermal bands
Cœlom
Septa
Long. muscles
Gonads
Myelidia
From cœlom → Special organ
One pair of eyes
Part of intestine
Nasal organs lost → Einbeinner
Kidneys
Bit of blastocoele
Experimental Embryology of the Protozoa & Mollusca.

Cell lineage studies similar to work of Vogt for Amphibia, i.e., yields only the prospective fate, the role in normal development. Classical work in this area by E. B. Wilson. Worked on Denticulina and Forilla.

E. B. Wilson:

Primary trochoblasts from 1a^2 - 12 cells
Secondary trochoblasts from 1a^12 - 2a^20
Isolate 1a^2 and a large group of isolated cells results in the cells of apical tuft attached.
1a^2 = 1d^2 - apical tuft
Isolation of one cell and you get the same but smaller primary trochoblasts and apical tuft cells.
Isolate 1a^1 and you get 4 primary trochoblasts alone.
1a^1 yields apical tuft cells.
1a^21 cell → 2 cells developing cilia - primary trochoblasts.
1a^21 develops cilia and is a primary protozoon.
Isolation of 1st Quartette in Patella
An embryological system of independent units.

Dentobium:

Isolate B, C, and you get two loops without spinal lift and no post. region.

Isolate C, D, and have abnormally large post. tail of region.

Contains full 2d metrid.

Isolation of A, B, and C.
Isolate D.

AB and A'B'C' fragments different from D cell. Even different in 1st cleavage, C D forming a polar lobe.

Problem whether nucleus or cytoplasm controls cleavage. Wilson cut polar lobe off.

If D is cut off →

D demonstrated that the determination is cytoplasmic.

If 3rd polar lobe is removed, larva is more complete.

Demonstrates that cleavage pattern is the method of determining organ forming properties.

If unfertilized eggs of 

Cheirolepis exposed to DC cleavage would not occur. If certain no.
characteristic differentiations develop in the nucleated egg.

Billie has demonstrated that cleavage itself is not responsible, but rather this flow of material in the developing egg is the cause. Cleavage is rather a stabilization of this flow which goes on in itself. Visible differentiations have nothing to do with this flow.

Problem — how early is the cytoplasm definitively localized. Whence Mesogenic fragments of an unperturbed egg fertilized and develop into

Fertilized lower half containing egg nucleus and you get a gastrula chorophore fairly normal.
Proved that there are early indications of determination in pre- cleavage stages.

Tyler (J. E. J., 57, 1932).
Compressed egg in pear shaped stage between 2 sides.

kept equal.
A-13 and E-D blastomeres.
Separate them then and you get 2 strong larvae.
This because each cell gets an equal amount of the differentiated cytoplasm.
If these two cells are left together you get strange double monsters.

Ceballos - you get the same results.
Navkoff - spallaria transplanted to almost any part.
Of egg, stuck for 10 hours, no results - no induction by polar fiber.
Self differentiation is all important here.
Cannot consider the polar body as an organizer in any sense of the word. We are dealing here with organ forming substances which act with the cells themselves and not on other cells. — Self differentiation.


A larvabulus performs res. similar to those in sea urchin. No double gradient found but strict determination of individual cells.

Typical mosaic development is present.

Difference between mosaic and regulative development is one merely of time of determination in mosaic (Annelids & Malacods) — early, in regulative (fish, amphibids etc.) — later.

Tadpole.

[Diagrams of tadpole and veliger stages]
Crepidula

- head
- tentacles
- mouth
- statocyst
- heart-kidney
- intestine
- shell

Dens always on right side.
Potencies in Regenerating Tissue:

Process of development consists in constant limitation of prospective potencies.

Development becomes more mosaic as it proceeds.

Regenerating tissue follows the order of the gastronomic process in its growth.

Transplant blastema of regenerating arm to another embryo's body well and it will develop into a perfectly normal limb.

Process is the same as in normal ontogeny.

Regenerative problems:

1. Causes of regeneration
   - Problem of general physiology
   - Proliferation
   - Involves the organism as a whole.

2. Origin of the material of the blastema.
   - Embryology
   - Exp of Paul Weiss proves that experimental
Methods are necessary.
Origin of blastema is not known yet.

3. Factors of Differentiation - Potencies

Deplancke we have to do regeneration.
Regeneration is expression of local potentialities.

Guizot and P. Weiss brought in Conception of anorganische Welt.

De George

Transplant to other animal will not differentiate.

Transplant and perfect tail differentiates.

A process of induction taking place here under the influence of the old tissue.
The lens is formed out of it.

Neural crest cells form the optic cup.

Plates of lens form around the optic cup.

Neural crest cells also form the mesenchyme.

Blastema is also present.

Regeneration depends on the type of tissue.

Differentiation is caused by interactions between blastema and surrounding tissues.

Regeneration is possible by dedifferentiation and reprogramming.
If the eye fields forming, do the tentum eye field can broaden out.

Lift flap of ectoderm from eye, remove lens and put flap back in. No death of cells. Remove flap and lens entirely, allow regeneration and a lens will develop.

Regenerating tissues of Amphibia show the same characters as embryonic tissue.
Embryology of the Crustacea

Fritz Müller, 1821-1897

Evolutionary significance of clad of Crustacea

"Für Darwin", 1864

Haeckel - Reciprocellular theory:

1. Gastraea theory that gastula formed by all.

New crustacea have total segmentation of the egg.

All eggs have centro-lateral drift of yolk.

(Only eggs of Aphelechinacea etc. are teloblastic)

Early cleavage - repeated nuclear divisions without cytoplasmic divisions, migration to periphery and formation of cell boundaries.
Astraeus. Embryology 1.

Zygote

Cells become thicker or more solid (ventral) because cell membranes are called ventral plate, forms nervous system.

Circles of cells develop in ventral plate.

Eyes: cephalic lobes.

Theoretical abdominal thickening.

Endodermal disk.
Cephalic lobes → eyes & cerebral ganglia.
Thoracic Abd. → thorax & abdomen.
Proteral disk → elementary germinal disk begins to invaginate to form entoderm.

Later joined by ectodermal invaginations, proto- & stomodeum.

Characteristic of all Crustaceans larvae.

Nauplius Stage.
Barnacle

J. V. Thompson discovered Nauplius in barnacles & thus included them in the Anthropods. 1835, 1843 Bornéipster found Nauplii & Cyprius stages. 1875 von Willewen, Scham found 6 diff. Nauplii stages, 1 cyprius stage. 1933 Nery - Bolamia carinata has 8 Nauplii & 1 cyprius stage. Develop takes 2 to 3 weeks.

Birghon 1902

Gonz 1894. - Cleavage in cyprius highly regular. Eggs of barnacle develop normally in mantle of mother.
yellow mass shifts away from animal pole at morula stage.

Cleavage meridional

Animal pole

from anim. pole

from veg. pole
At 8 cell stage, there are 1 macromere and 1 micromere.

16 an view

1 large macromere → endoderm
15 micromeres →
1 → mesoderm
14 → ectoderm

8 cell
Sagittal section.

16 cell
Sag. sect.
Gastrulation by epiboly - investment of the yolk by the blastoderm cells.
Balanus, 1st Nauplius.

Cypriis delt, from these stages, as attached.

8th Nauplius.
Histological Observations of certain embryological Processes in the living adult mammal.

As blood vessels enter macrophages clean up debris of fibrin, erythrocytes etc.
Near endothelium of blood vessels comes from red endothelium and reconnects with other endothelial cells of blood vessels.
As denuded, new capillaries proceed certain ones (8 out of 10) disappear.
Arteries and veins alternate.

Veins a secondary development.
Vascular pit denuded, if companion veins fibroblasts continue into wound with new blood vessels.
If companion veins denuded, they develop after arteries are well established.
A growth promoting environment. Question as to what causes this.
new cells growing inward.

degenerates 1st.

Degeneration starts at one end but before it proceeds all the way back at degeneration starts at the other end and proceeds forward. An indication of a secondary gradient. Hydranth end of planula is peak of gradient secondary gradient it assumes apical position. In one form planula lies on its side and hydranth comes out of center 90° from original polarity.

P.P. Matthews, 1903.

Showed that in some forms hydranth end was electro negative.

Both 34 a dem. that hydranth is — in tubuloria and Pennaria. Regeneration fastest at electroneg. end.
Child (baby) - demonstrated this in a west coast form.

bind, '21 showed that the hydranth is electro-positive in abelia.

Both in Eudendrium showed that the regenerating end is positive.

Rath, 11 x centrifuged eggs of hydactinia

Get a strong stratification.

Nucleus could lie anywhere in cytoplasm.

Purine & nucleus declined to yolk.

Where 1st cleavage would be.

Secundy cleavage resulted, but planulae were produced. Discription of polarity didn't seem to make very much difference.

Race, 01-05 - Suched 12 cell stage through yolk and got it so that the cells were aranged in a line. Inside of 5 hours this embryo was a morula and later formed a medusa with all its organs, but a little misshapen.

Polarity didn't seem to make much difference.

Zoja, Hort's '00, Raas '05, Counell '06 were all
Separated the blastomeres of cactilians. In most cases can form good planulae. Separation of 1st 16 cells of Cystida gave some normal planulae, denuel stopped here (?).

Therefore Cactilians can get away from original parent or replace it with care.
Child mis-treated various planulae with various chemicals. Found some that produced hydroids in several places and more than one often.

Adult hydroids

Graphing by Wetzel or reversed gradient of one form. Peebles' 005 by graphing hydroids together.

The cut off head end of our hydroids over and forms typical base and gradient is normal again.

Reverse exp. works also.

Controlling On supply, 1st done by P. H. Morgan in 1903. Cut off ends. Stick one spiral end in sand and lift basal end sticking up. Then basal formed hydranth 1st. Polarity thus reversed.

Booth '39, did same thing in more refined method. Flowed shear rate of spiral end by inserting it in a capillary tube.

Poisons - produce some sort of effect.
Child '27 - put hydranths on every centimeter of port.

Electricity -

1921, 1923, 1924. Improved Matthew's feeling. Put strong electricity in small current field and current may suppress regeneration as it is reverse to that of hydranth, then may go thriller on the opposite end.

Booth repeated exp. on other forms. Same results essentially.

A relation between electricity and regeneration here.
Pulverizing—Hydraulics. Done by H.V. Wilson. "I took stems, removed hydranth, washed then up in boiling salt solution, let cells drop through. Cells appeared rounded, started to clump together. When he could keep these clumps together they would form a polyp. Polypema and metanema would form sent out stalks and formed new hydraulics. Thus the cells were capable of reorganizing themselves into a new organism.

Child, '28. also this this and got the same results. A definite polarity to the new organism resulting if not disturbed. If disturbed the new form had greater difficulty forming and out forming polarity. 79% in 1st method, normal. 86% in 2nd method gets great forms with tentacles coming out in all directions.
Genes and Cytosol

Chloroplasts in plants are self-perpetuating bodies independent of the chromosomes.

Mitochondria may be self-perpetuating.

In general hybrids resemble mother rather than the father i.e. no maternal cytoplasmic inheritance here.

But there are some instances known where maternal inheritance is evident. Darwin like mother particularly in early stages responsible for the main pattern of development, it is thought.

Thought by some that cytoplasm responsible for the main pattern and genes for superficial characters.

This is somewhat disproved by genetics.

Bacons, hawk, and Carlklin were upholders of this cytoplasmic theory of inheritance.

In humans (Boyes & Devay) the skull is deeply coiled.

I crossed with inbred coiled the offspring are coiled like the mother. Further analysis showed it to be a genetic character carried on the chromosomes of the mother.
Can determine coil of shell by the spiral cleavage. In these cases, the cytoplasm influences development but is definitely but if it itself is caused by genes. It acts without chromosomes but was originally caused by eggs.

Specificity of cytoplasm depends upon the protoplasm. All cases in which specific cases have been traced through several generations are doubtful. Horder introduced cytoplasm of one individual into another but his results are doubtful.

Goldschmidt hypothesis. Showed that the sex of the individual depends on (M) & (F).

M - in chromosomes.

F - in cytoplasm transmitted materially. Complications may arise here due to its complexity therefore cannot be taken as clear.

There are as yet no clear cases of cytoplasmic inheritance known. It is quite clear that the cytoplasmic is not important and is itself highly specific, e.g. blood cells of mammals.
But this specificity is due to the chromosomes.

Idea of Goldenweiser that genes mean existence lacks evidence. - Statement.
Embryology of the Tunicates

1. Notochord
2. Nervous system formed by neural folds and dorsal hollow system.
3. Pharyngeal slits in alimentary canal.

Juniates, cephalaocardiates, hemiocardiates.

Pharyngeal slits in alimentary canal.

Embryology of Ascidians

Form a tadpole in one stage of metamorphosis. Swims loose from parent colony settles down and rapidly metamorphoses into an adult.

Pharynx with cilia

Endostyle

Bands of cuticle and glandular tissue used in food catching.
Other organs of the tunicate are reduced to essentials: Heart, kidneys, self sterility in reproduction. Stems and cellules around the animal, sexual and asexual reproduction by budding (Botylus, Bunamencium, Brophora).

Embryology of Styela.

A classical piece of work by Lovelace. Recently discussed more.

[Diagram of a circle with a ring of circles around it, labeled with: 'chlamyde', 'folllicle cells', 'egg'.]

Yellow pigment on the surface of the egg.

Motivation takes place at entrance of sperm causes clear protoplasmic cap on top of egg (grey protoplasm). Peripheral yellow pigment rises down over the cap surface of the egg.

[Circle with a ring of circles around it, labeled with: 'yellow', 'yello pigment'.]
Yellow pigment in a crescent at lower part of egg. Maturation completed by this time.
Sperm enters the lower pole of the egg, forms a penetration path, then changes in course and then forms acopulation path bringing it to "posterior" pole of the egg.

By this time all three axles of embryo have been formed.
Pigment now starts to stream to one side of the lower pole of the egg.

Center of crescent = post. pole of egg.
Egg nucleus now meets the sperm nucleus.
at posterior pole and then zygote nucleus moves to the center of the egg.
At thin lighter crescent visible for a time above the yellow crescent.
The yellow area is to form megoderm.
The lighter area is to form skin ectoderm.
Neural plate & corde mesoderm form ports on other side of egg.

1st cleavage cuts yellow crescent in two.

2nd cleavage...

y.c. split into cells.
A - do not have yellow c.
B - do have yellow c.
Cleavage: Regular cleavage.

Gastrulation: Similar to that of amphibians.

Materials distributed very precisely in the eggs.

Cell nuclei to believe that here we have a mosaic.

Centrifuged eggs indicating that pigment is not the
organ forming stuff but just an indicator of its
location. More centrifuging causes crazy mixtures
organ formed.

Chadry, '87 - Separated blastomeres in cell stage.

Cut 2 half embryos.

P. S. Shunt '34

Separated micromeres. Would go on dividing former
ectoderm, endoderm, sometimes mesenchyme, ectoderm.

Macromeres formed to normal looking larva, occasionally
found mid. plate. Ectoderm usually deficient

 Removed micromeres and turned them 90°, grew
put back on. Neural tissues sometimes displaced
on the embryos.
Indicate that much finity is present. But absolute mosaic pattern not performed.

Vanderbroek

Found a certain amount of freency in determination.
Found also cases of induction.
Antest reduced by underlying cuticle
Ostomyst also reduced by cuticle.
Dolgy has split unfertilized eggs in various ways.
Splits in 2 and both can develop to normal larvae.
Gets all sorts of organs out of these fissions.

Asexual Reproduction
1. Paebrodelial or Afree
   (a) found in Botrylus
   Ectodermal bud appears on each side of blastoderm cavitey. Ectoderm pushes out before it tissue from the outer wall of the animal. Ect. Portvin out of the animal.

level then into a new Botrylus exactly like the old one.
Sexual fusions of the two individuals exactly the same.
No metamorphosis from the budding, just forms an adult individual.

Oogonium → blastozoooid (but)
Oogonium never matures sexually, only blastozoooid does this. A true alternate train of generations here.

(b) Perophora

Similar to Protophora.
Ends off long stolons from which new blastozoooids develop.

d. Pharyngeal or Epicardial

1. Pharyngeal

Epicardium cut off in a massicle to form new individual. Comes from endoderm here. Old organism grows new epicardium.
Metamorphosis of the Ascidians:

Metamorphosis exhibited by species that live in more than one environment during their life history. It develops in an environment different from that of the adult.

A larva inhabits this first environment.

Metamorphosis in the Ascidians is very profound and sudden. It is stimulated in the frog by thyroxin.

Muscle system

Central nervous system

Cardiac and equilibrium

Adult action system

Central nervous system (different)

Lateral line canal

Metamorphosis is the destruction of all that has happened along the larval line.
Hemol
Notocard
Striated Muscles
Mantle

Adult
Atrium
Circulatory System
Hypophysis
Ovary & Testis

All these adult structures are instituted in the larva just before the beginning of the larval stage. Tetramers play the destruction of larval structures so that the adult structures may function.

In Botryllus, the free-swimming period is 24 to 36 hours. In some species it varies from 24 to 48 hours.

Out of too larval forms from parents you get nearly the same diversity.

Fig. 9: larval metamorphosis
larvae live longer with more space or volume of water. Variation in hatching is about 30 min.

Polyandrocopa tincta

Oriente to light & gravity (negative) plateau.

After about an hour the larvae have a negative reaction to light and then acquired a positive geotropism, larvae attack to bottom, untorpedoed larvae in water metamorphosis proceeds. Shaked the larvae once a minute, for an hour, larvae would attack to bottom reach termite, then swim around again with the light. Indicates that light has something to do with metamorphosis. But the real cause is something liberated within the organism.

All larvae when the hatch are not subject to metamorphosis but must be subjected to changes within the body; protozoa produces this internal stage by substance within the body with aging.
aging + activity \rightarrow 3rd substance
(substance) + (substance).

Substance = by product of neuromuscular activity.
Aging substance + activity substance which forms a
3rd substance which has a disrupting effect
on the central nervous system.

Stimulated neurons with certain substances.

In water solution of thyroxin (extract of thyroid
gland), also got 100% metamorphosis from
all tissue extracts of the ascidians. But there is
species specificity. This 3rd substance is
thus formed in the tissue of the individual.

Tissue extract without thyroid and protein will work.
Substance does not contain fat or protein and is stable
CaCl\textsubscript{2} \times 10^{-6} M produces rapid metamorphosis.

Therefore one of the metal salts is the
3rd substance that pulls the trigger in the
metamorphosis process. It catalysts
Metamorphosis is probably a kind of oxidation process.
Research lecture:

Work of Houssay has shown that swimming apparatus develops without nerve, i.e., without function.

Nerves kept from limb and it develops normally in the frog.

Effects:
1. Limbs without nerves one shorter.

On chick, transplanted limb and toward the neutral side, that the developing limbs will be normalless.

Normalless limbs on the chick lack fewer the sculpturing of the bone. Due to lack of functioning, also a fusion in the joint regions. This great morphogenesis, histological and macroscopic, is normal but details and perfect development depend upon the nervous system.