## D.  Growth and Differentiation of the Germ-cells

### 1.  *The Ovum*

(*a*) *Growth and Nutrition.* — Aside from the transformations of
the nucleus, which are considered elsewhere, the story of the ova-
rian history of the egg is largely a record of the changes involved in
nutrition and the storage of material.   As the primordial germ-cells
enlarge to form the mother-cells of the eggs, they almost invariably
become intimately associated with neighbouring cells which not only
support and protect them, but also serve as a means for the elabora-
tion of food for the growing egg-cell.   One of the simplest arrange-
ments is that occurring in cœlenterates, where the egg lies loose
either in one of the general layers or in a mass of germinal tissue,
and may crawl actively about among the surrounding cells like an
*Amœba*.   In such cases (hydroids) the egg may actually feed upon
the surrounding cells, taking them bodily into its substance or fusing
with them[1] and assimilating their substance with its own.   In such
cases (*Tubularia*, *Hydra*) the nuclei of the food-cells long persist in
the egg-cytoplasm, forming the so-called " pseudo-cells," but finally
degenerate and are absorbed by the egg.   It would here seem as
if a struggle for existence took place among the young ovarian cells,
the victorious individuals persisting as the eggs; and this view is
probably applicable also to the more usual case where the egg is
only indirectly nourished by its brethren.

In most cases, as ovarian development proceeds, a definite associa-
tion is established between the egg and the surrounding cells.   In
one of the most frequent arrangements the ovarian cells form a
regular layer or *follicle* about the ovum (Figs. 59, 79), and there is
very strong reason to believe that the follicle-cells are immediately
concerned with the conveyance of nutriment to the ovum.   A num-
ber of observers have maintained that the follicle-cells may actually
migrate into the interior of the egg, and this seems to be definitely
established in the case of the tunicates and mollusks (Fig. 75).[2]
Such cases are, however, extremely rare; and, as a rule, the material
elaborated by the nutritive cells is passed into the egg either in solu-
tion or in the form of granular or protoplasmic substance.[3]   An
interesting case of this kind occurs in the cycads, where, according
to Ikeno ('98), the egg-cell is connected with the surrounding cells
by broad protoplasmic bridges through which cytoplasmic material
flows directly into the egg-cell.

Very curious and suggestive conditions occur among the annelids
and insects.   In the annelids the nutritive cells often do not form

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[1] *Cf.* Doflein, '97.      [2] See Floderus, '95, and Obst, '99.      [3] *Cf.* p. 349.

a follicle, but in some forms each egg is accompanied by a single nurse-cell, attached to its side, with which it floats free in the body-cavity. In *Ophryotrocha*, where it has been carefully described by Korschelt, the nurse-cell is at first much larger than the egg itself, and contains a large, irregular nucleus, rich in chromatin (Fig. 76). The egg-cell rapidly grows, apparently at the expense of the nurse-cell, which becomes reduced to a mere rudiment attached to one side of the egg and finally disappears. There can hardly be a doubt, as Korschelt maintains, that the nurse-cell is in some manner con-nected with the elaboration of food for the growing egg-cell; and the intensely chromatic character of the nucleus is well worthy of note in this connection. Still more in-teresting are the conditions observed by Wheeler ('96, '97) in *Myzostoma*, where the young egg is accom-panied by two nurse-cells, one at either end. These cells fuse bodily with the egg, one having " some-thing to do in forming the vacuolated cytoplasm at the animal pole, . . . the other in forming the granu-lar cytoplasm at the vege-tative pole " ('97, p. 42). The polar axis thus deter-mined persists as that of the ripe ovum. This seems one of the clearest
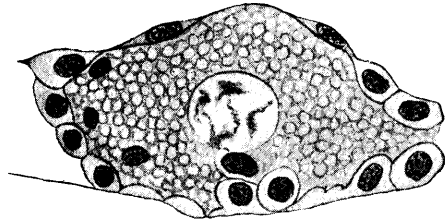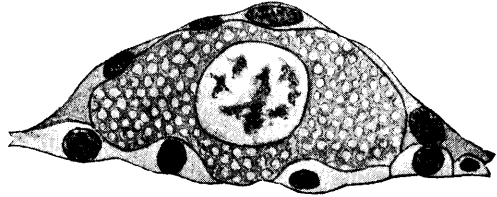


Fig. 75. — Ovarian eggs of *Helix*. [OBST.]

*A*. Earlier stage, surrounded by follicle. *B*. Later stage, showing inward migration and absorption of fol-licle-cells.

cases showing the establishment of the egg-polarity through the relation of the egg to its environment.[1]

Somewhat similar nurse-cells occur in the insects, where they have been carefully described by Korschelt. The eggs here lie in a series in the ovarian " egg-tubes " alternating with nutritive cells vari-ously arranged in different cases. In the butterfly *Vanessa*, each egg is surrounded by a regular follicular layer of cells, a few of which at one end are differentiated into nurse-cells. These cells are very large and have huge amœboid nuclei, rich in chromatin (Fig. 77, *A*). In the ear-wig, *Forficula*, the arrangement is still more remarkable, and recalls that occurring in *Ophryotrocha*. Here each

[1] *Cf.* p. 386.

egg lies in the egg-tube just below a very large nurse-cell, which, when fully developed, has an enormous branching nucleus as shown in Fig. 163. In these two cases, again, the nurse-cell is character- ized by the extraordinary development of its nucleus — a fact which points to an intimate relation between the nucleus and the metabolic activity of the cell.[1]

In all these cases it is doubtful whether the nurse-cells are sister- cells of the egg which have sacrificed their own development for the sake of their companions, or whether they have had a distinct origin from a very early period. That the former alternative is possible is shown by the fact that such a sacrifice occurs in some animals after the eggs have been laid. Thus in the earthworm, *Lumbricus terres-*
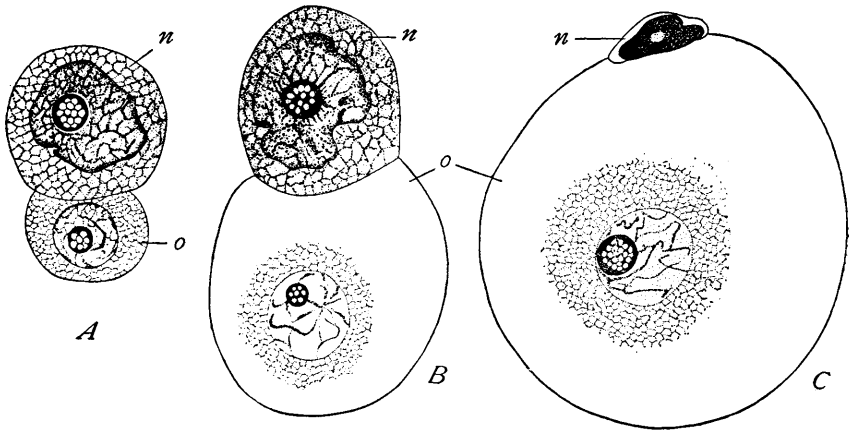


Fig. 76. — Egg and nurse-cell in the annelid, *Ophryotrocha*. [KORSCHELT.]
   *A.* Young stage, the nurse-cell (*n*) larger than the egg (*o*). *B.* Growth of the ovum. *C.* Late stage, the nurse-cell degenerating.

*tris,* several eggs are laid, but only one develops into an embryo, and the latter devours the undeveloped eggs. A similar process occurs in the marine gasteropods, where the eggs thus sacrificed may undergo certain stages of development before their dissolution.[2]

(*b*) *Differentiation of the Cytoplasm and Deposit of Deutoplasm.* — In the very young ovum the cytoplasm is small in amount and free from deutoplasm. As the egg enlarges, the cytoplasm increases enormously, a process which involves both the growth of the pro- toplasm and the formation of passive deutoplasm-bodies suspended in the protoplasmic network. During the growth-period a peculiar body known as the *yolk-nucleus* appears in the cytoplasm of many ova, and this is probably concerned in some manner with the growth

[1] See p. 338.                    [2] See McMurrich, '96.

of the cytoplasm and the formation of the yolk. Both its origin and
its physiological *rôle* are, however, still involved in doubt.

The deutoplasm first appears, while the eggs are still very small,
in the form of granules which seem to have at first no constant posi-
tion with reference to the egg-nucleus, even in the same species.
Thus Jordan ('93) states that in the newt (*Diemyctylus*) the yolk may
be first formed at one side of the egg and afterward spread to other
parts, or it may appear in more or less irregular separate patches
which finally form an irregular ring about the nucleus, which at this
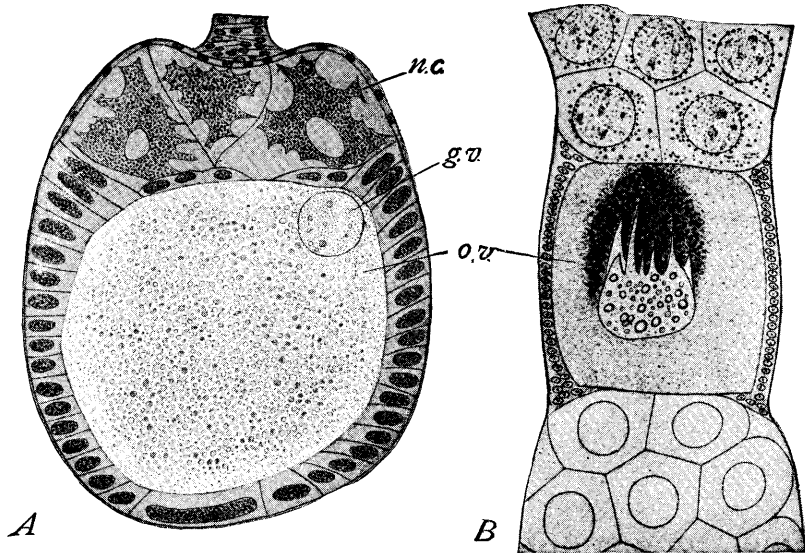period has an approximately central position. In some Amphibia



Fig. 77. — Ovarian eggs of insects. [KORSCHELT.]

*A.* Egg of the butterfly, *Vanessa*, surrounded by its follicle; above, three nurse-cells (*n.c.*) with
branching nuclei; *g.v.* germinal vesicle. *B.* Egg of water-beetle, *Dytiscus*, living; the egg (*o.v.*)
lies between two groups of nutritive cells; the germinal vesicle sends amœboid processes into the
dark mass of food-granules.

the deutoplasm appears near the periphery and advances inward
toward the nucleus. More commonly it first appears in a zone
surrounding the nucleus (Fig. 78, *C, D*) and advances thence toward
the periphery (trout, Henneguy; cephalopods, Ussow). In still others
(*e.g.* in myriapods, Balbiani) it appears in irregular patches scattered
quite irregularly through the ovum (Fig. 78, *A*). In *Branchipus* the
yolk is laid down at the centre of the egg, while the nucleus lies at
the extreme periphery (Brauer). These variations show in general
no definite relation to the ultimate arrangement — a fact which
proves that the eccentricity of the nucleus and the polarity of the

egg cannot be explained as the result of a simple mechanical displacement of the germinal vesicle by the yolk, as some authors have maintained.

The primary origin of the deutoplasm-grains is a question that involves the whole theory of cell-action and the relation of nucleus
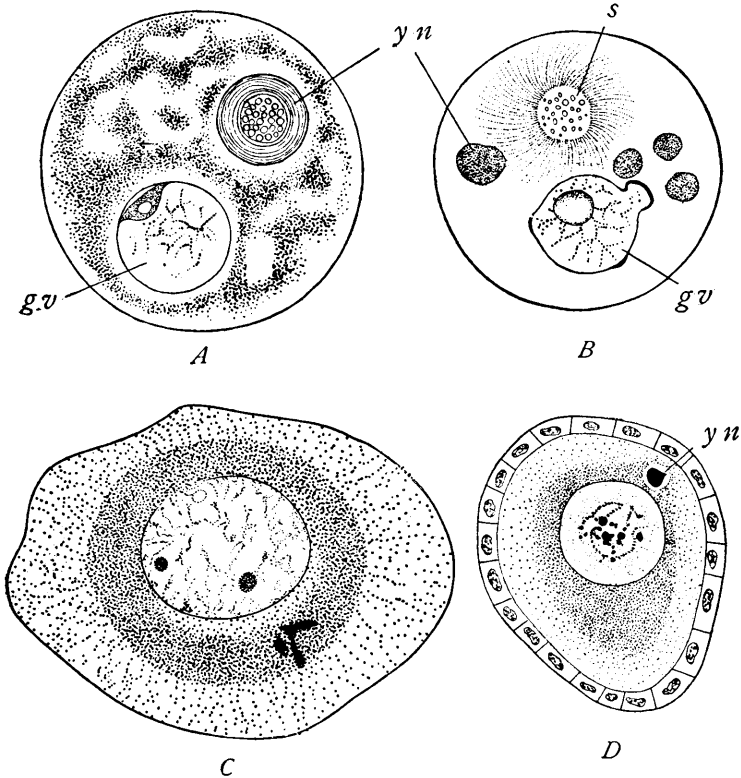


Fig. 78. — Young ovarian eggs, showing yolk-nuclei and deposit of deutoplasm.

A. Myriapod (*Geophilus*) with single "yolk-nucleus" (perhaps an attraction-sphere) and scattered deutoplasm. [BALBIANI.]

B. The same with several yolk-nuclei, and "attraction-sphere," *s.* [BALBIANI.]

C. Fish (*Scorpæna*), with deutoplasm forming a ring about the nucleus, and an irregular mass of "eliminated chromatin" (? yolk-nucleus). [VAN BAMBEKE.]

D. Ovarian egg of young duck (three months) surrounded by a follicle, and containing a "yolk-nucleus," *y.n.* [MERTENS.]

and cytoplasm in metabolism. The evidence seems perfectly clear that in many cases the deutoplasm arises *in situ* in the cytoplasm like the zymogen-granules in gland-cells. But there is now also a very considerable body of evidence indicating that a part of the egg-cytoplasm is directly or indirectly derived from the nucleus through the agency of the yolk-nucleus or otherwise; and the

subject can best be considered after an account of that body. It
may be mentioned here, however, that a large number of observers
have maintained a giving off of nuclear substance to the cytoplasm,
in the form of actual buds from the nucleus (Blochmann, Scharff,
Balbiani, etc.) as separate chromatin-rods or portions of the chromatin
network (Fol, Blochmann, Van Bambeke, Erlanger, Mertens, Calkins,
Nemec, etc.) or as nucleolar substance (Leydig, Balbiani, Will, Ley-
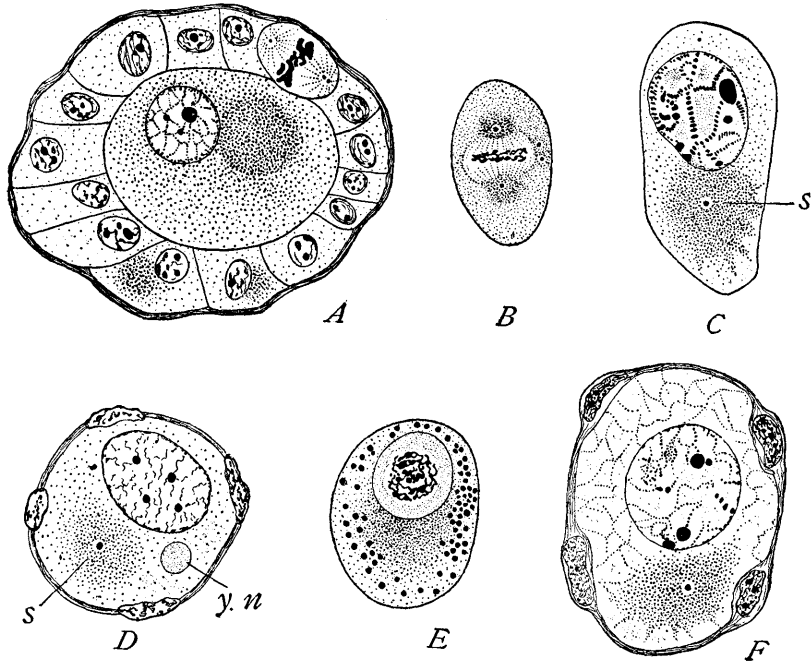dig, Henneguy), but nearly all of these cases demand reëxamination.



Fig. 79. — Young ovarian eggs of birds and mammals.   [MERTENS.]

A. Egg of young magpie (eight days), surrounded by the follicle and containing germinal
vesicle and "attraction-sphere." B. Primordial egg (oögonium) of new-born cat, dividing. C. Egg
of new-born cat containing "attraction-sphere" (s) and centrosome. D. Of young thrush sur-
rounded by follicle and containing besides the nucleus an attraction-sphere and centrosome (s),
and a yolk-nucleus (y.n.). E. Of young chick containing nucleus, attraction-sphere, and fatty
deutoplasm-spheres (black). F. Egg of new-born child, surrounded by follicle and containing
nucleus and attraction-sphere.

(c) Yolk-nucleus. — The term yolk-nucleus or vitelline body (Dotter-
kern, corps vitellin) has been applied to various bodies or masses
that appear in the cytoplasm of the growing ovarian egg; and it
must be said that the word has at present no well-defined mean-
ing. As originally described by von Wittich ('45) in the eggs of
spiders, and later by Balbiani ('93) in those of certain myriapods,
the yolk-nucleus has the form of a single well-defined spheroidal

mass which appears at a very early period and persists throughout the later ovarian history. In other forms there are several so-called "yolk-nuclei," sometimes of fairly definite form as described in the Amphibia by Jordan ('93) and in some of the myriapods by Balbiani ('93). In some forms the numerous "yolk-nuclei" are irregular, ill-defined granular masses scattered through the cytoplasm, as described by Stuhlman ('86) in the eggs of insects. In still others the "yolk-nucleus" or "vitelline body" closely simulates an attraction-sphere, being surrounded by distinct astral radiations and enclosing one or more central granules like centrosomes (*Geophilus*, Balbiani, '93, and *Limulus*, Munson, '98). Balbiani is thus led to regard the yolk-nucleus in general as being a metamorphosed attraction-sphere. Miss Foot ('96) has brought forward evidence to show that the polar rings, observed in the eggs of certain leeches and earthworms, are also to be regarded as "yolk-nuclei" (Fig. 102). Henneguy ('93, '96) finally compares the yolk-nucleus to the macronucleus of the Infusoria (!).

In the present state of the subject it is quite impossible to reconcile the discordant accounts that have been given regarding the structure, origin, and fate of the "yolk-nuclei", and from the facts thus far determined we can only conclude that the various forms of "yolk-nuclei" have little more in common than the name. It is, in the first place, doubtful whether the "yolk-nuclei" simulating an attraction-sphere have anything in common with the other forms; and Mertens ('93), Munson ('98), have shown that the young ovarian ova of various birds and mammals (including man) and of *Limulus* contain one or more "yolk-nuclei" in addition to the "attraction-sphere" ("vitelline body" of Munson). In the second place there seem to be two well-defined modes of origin of the yolk-nucleus. In one type, illustrated by Jordan's observations on the newt ('93), the "yolk-nuclei" arise separately *in situ* in the cytoplasm without direct relation to the nucleus. The same is true of the small peripheral "yolk-nuclei" of *Limulus* (Munson). In a second and more frequent type the "yolk-nucleus" first appears very near to or in contact with the nucleus, suggesting that the latter is directly concerned in its formation. The latter is the case, for example, in the eggs of *Cymatogaster* (Hubbard, '94) *Syngnathus* (Henneguy, '96), the earthworm (Calkins, '95, Foot, '96), *Polyzonium* and other myriapods (Nemec, '97, Van Bambeke, '98), *Limulus* (Munson, '98), *Cypris* (Woltereck, '98), and *Molgula* (Crampton, '99). In nearly all of these forms the yolk-nucleus first appears in the form of a cap closely applied to one side of the nucleus (Figs. 80, 81), sometimes so closely united to the latter that it is difficult to trace a boundary between them. At a later period the yolk-nucleus moves away from the nucleus and in.

most, if not in all, cases breaks up into smaller and smaller fragments which contribute, directly or indirectly, to the cytoplasmic growth. In all these cases the history of the yolk-nucleus is such as to indicate the participation of the nucleus in its formation.  Calkins ('95) endeavours to show that the yolk-nucleus in *Lumbricus* is directly derived from the nucleus by a casting out of a portion of the chro-
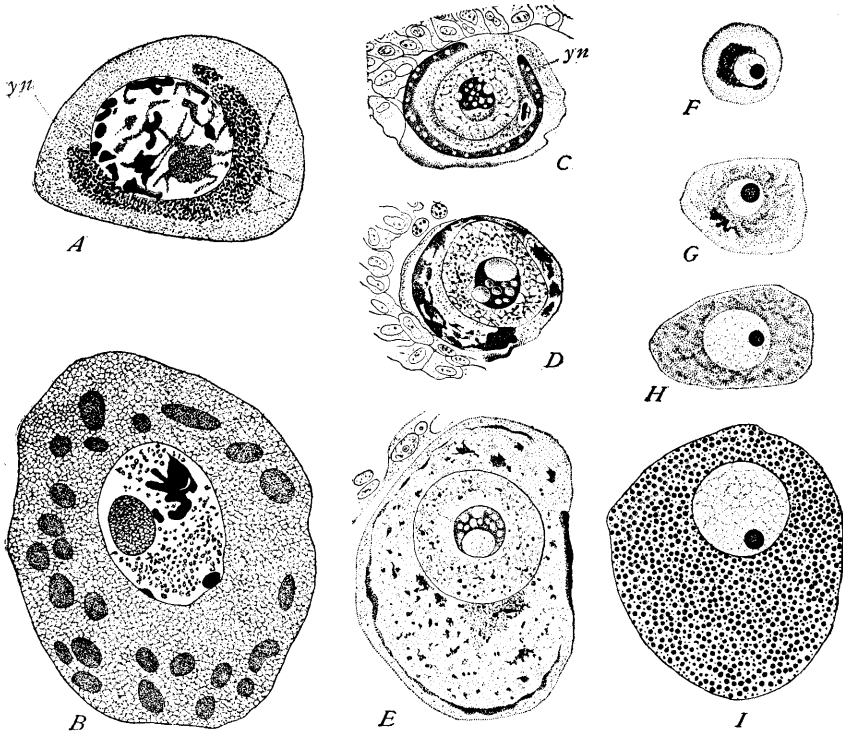


Fig. 80.—Yolk-nucleus in earthworm, spider, and ascidian.   [*A, B,* CALKINS;  *C–E,* VAN BAMBEKE;  *F–I,* CRAMPTON.]

  *A.* Early ovarian egg of *Lumbricus.*  *B.* Later stage; fragmentation of yolk-nucleus.  *C.* Ovarian egg of *Pholcus.*  *D.* Later stage; disintegration of yolk-nucleus.  *E.* Remains of the yolk-nucleus scattered through the cytoplasm.  *F.* Early stage of yolk-nucleus in *Molgula.*  *G–I.* Disintegration of the yolk-nucleus and enlargement of the products to form deutoplasm-spheres.

matin-reticulum — a result agreeing in principle with earlier observations on other eggs by Balbiani, Henneguy, Leydig, Will, and other observers.  This conclusion rests partly on the apparent direct continuity of yolk-nucleus and chromatin, partly on the staining-reactions.   Thus when treated with the Biondi-Ehrlich mixture (basic methyl-green, acid red fuchsin), the yolk-nucleus at first stains green like the chromatin, while the cytoplasm is red, and this is the case

even after the yolk-nucleus has quite separated from the nuclear membrane. Later, however, as the yolk-nucleus breaks up, it changes its staining power, and stains red like the cytoplasm. The later observations of Miss Foot ('96) give ground to doubt the conclusion that the yolk-nucleus is here actually metamorphosed chromatin, for by the combined action of lithium carmine and Lyons blue its substance is sharply differentiated from the chromatin. Still later studies by Crampton ('99) on *Molgula* demonstrate that in this case the yolk-nucleus is not directly derived from chromatin, but they nevertheless indicate clearly the formation of the yolk-nucleus by or under the immediate influence of the nucleus — a conclusion also reached on less satisfactory evidence by Hubbard, Van Bambeke, Woltereck, and Nemec. The general morphological history of the yolk-nucleus is here closely similar to that of *Lumbricus* (Fig. 80), except that no direct continuity between it and the nuclear substance was observed. Stained with methyl-green-fuchsin the yolk-nucleus and major part of the nuclear substance stain red, while the scattered nuclear chromatin-granules and the cytoplasm stain green. Millon's test, combined with digestion-experiments and the foregoing staining-reactions, proves that the yolk-nucleus and the red staining nuclear substance consist of albuminous substance and differ widely from the general cytoplasm, which probably consists largely of nucleo-albumins (*cf.* p. 331). These reactions give strong ground for the conclusion that the substance of the yolk-nucleus, which progressively accumulates just outside the egg-nucleus, is formed through the direct activity of the latter, perhaps arising within the nucleus and passing out into the cytoplasm. It is possible, further, that even the scattered "yolk-nuclei" that seem to be of purely cytoplasmic origin may arise in a similar manner, either, as Crampton suggests, through the early formation and breaking up of a single yolk-nucleus, or in some less obvious way.

Interesting questions are suggested by those "yolk-nuclei," such as occur in *Geophilus* and *Limulus*, that so closely simulate an attraction-sphere. Munson's observations show that this body ("vitelline body") first appears in the very young ova as a crescent applied to the nucleus precisely as in *Molgula* or *Lumbricus*, but containing one or more central granules (Fig. 81). In later stages it becomes spherical, moves away from the nucleus, and assumes the form of a typical radial attraction-sphere with concentric microsome-circles and astral rays. It is hardly possible to doubt that this body in *Limulus* is of the same general nature as the yolk-nucleus of *Lumbricus*, *Molgula*, *Cypris*, *Cymatogaster*, or *Pholcus ;* and if it be a true attraction-sphere in the one case we must probably so regard it in all. This identification is, however, by no means complete;

and even Munson's careful studies do not seem definitely to establish
its connection with the attraction-sphere or centrosome of the last
oögonium-division.    That a body simulating an attraction-sphere and
containing a central granule may arise *de novo* in the cytoplasm
is shown by Lenhossék's observations on the spermatids of the
rat (p. 170); and the central granule is in this case certainly not
a centrosome, since the true centrosomes are found in another
part of the cell.    It is quite possible that the "vitelline body" of
*Limulus* may have a similar origin.    Nemec ('97) finds in *Polyzonium*
in the earliest stages a single body applied to the nucleus and
later two bodies, one of which enlarges to form a cap-shaped yolk-
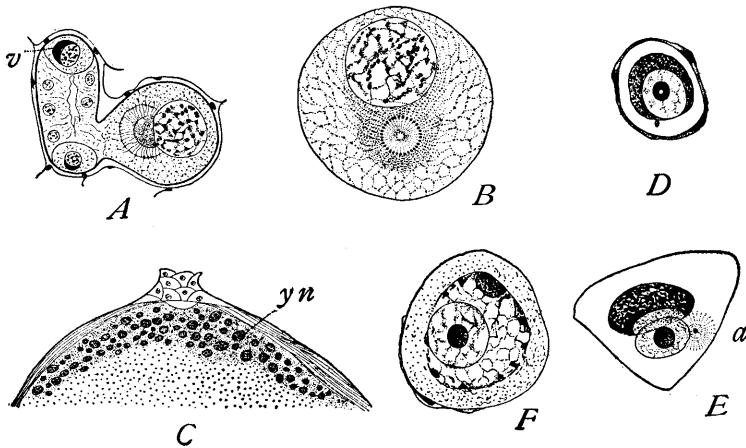


Fig. 81. — Forms of yolk-nuclei in *Limulus* and *Polyzonium*.  [*A–C*, MUNSON; *D–F*, NEMEC.]

   *A*. Very young ovarian eggs of *Limulus* ; at the left "vitelline body" (*v*) in the form of a cap
on the nucleus; at the right older egg showing astral formation.  *B*. Older stage of the same;
"vitelline body" in the form of an attraction-sphere with central granule.  *C*. Peripheral "yolk-
nuclei" (*y.n.*) in *Limulus*.  *D*. Very early ovarian egg of a myriapod, *Polyzonium*, with yolk-
nucleus.  *E*. Older egg with yolk-nucleus and astral body (*a*).  *F*. Still later stage, beginning
disintegration of the yolk-nucleus.

nucleus like those described above, while the other assumes the
structure of a radiating attraction-sphere containing a central
granule (centrosome?), and his observations suggest that the two
bodies in question may have a common origin (Fig. 81).    In none
of these cases do the astral radiations, surrounding this body, seem
to have any connection with cell-division, and it is probable that
a careful comparison of their physiological significance here, in
leucocytes, and in mitotic division, may give us a better understand-
ing of the general significance of astral formations in protoplasm.

   The fate and physiological significance of the yolk-nucleus are
still to a considerable extent involved in doubt.    In many cases it

breaks up into smaller and smaller granules (*Lumbricus, Molgula, Pholcus,* some myriapods, *Antedon*), which scatter through the cytoplasm and are believed by many observers (Balbiani, Mertens, Will, Calkins, Crampton, Nemec), following the earlier views of Allen Thomson, to become directly converted into deutoplasm-spheres (Fig. 80). Other observers (Van Bambeke, Foot, Stuhlman, and others) adopt the original view of Siebold, that the fragments of the yolk-nucleus are absorbed or converted into protoplasmic elements and thus only indirectly contribute to the yolk. In still other cases (*e.g.* the "vitelline body" of *Limulus*) the yolk-nucleus does not fragment, but seems to serve as a centre about which new deutoplasmic material is formed. A review of the general subject shows that we are justified only in the somewhat vague conclusion that the yolk-nucleus is probably involved in some manner in the general cytoplasmic growth; and that the facts strongly suggest, though they hardly yet prove, that at least some forms of yolk-nuclei are products of the nuclear activity and form a connecting link between that activity and the constructive processes of the cytoplasm. That the yolk-nuclei have no very definite morphological value, and that they are not necessary to growth, seems to be shown by Henneguy's observation, that in the eggs of vertebrates it is in some forms invariably present, in others only rarely, and in still others is quite wanting ('96, p. 162). If this be the case, we must conclude that the yolk-nucleus consists of material that contributes to the constructive process, but is not necessarily localized in a definite body. As to its exact *rôle* we are, as Henneguy has said, reduced to mere hypotheses.[1] The facts indicate that this material is a product of the nuclear activity, and that it may in some cases contribute directly to formed elements of the cytoplasm. It is probable, however, that beyond this the yolk-nucleus may supply materials, perhaps ferments, that play a more subtle part in the constructive process, and of whose physiological significance we are quite ignorant. The whole subject seems a most interesting and important one for further study of the actions of the cell in constructive metabolism, and it is to be hoped that further research will place the facts in a clearer light.

### 2. *Origin of the Spermatozoön*

(*a*) *General.* — The relation of the various parts of the spermatozoön to the structures of the spermatid is one of the most interesting questions in cytology, since it is here that we must look for a basis of interpretation of the part played by the sperma-

[1] '96, p. 170.

tozoön in fertilization. Obviously the most important of the questions, thus suggested, is the source of the sperm-nucleus and centrosome, though the relation of the other parts to the spermatid-cytoplasm involves some interesting problems.

Owing to the extreme minuteness of the spermatozoön, the changes involved in the differentiation of its various parts have always been, and in some respects still remain, among the most vexed of cytological questions. The earlier observations of Kölliker, Schweigger-Seidel, and La Valette St. George, already mentioned, established the fact that the spermatozoön is a cell; but it required a long series of subsequent researches by many observers, foremost among them La Valette St. George himself, to make known the general course of spermatogenesis. This is, briefly, as follows: From the primordial germ-cells arise cells known as *spermatogonia*,[1] which at a certain period pause in their divisions and undergo a considerable growth. Each spermatogonium is thus converted into a *spermatocyte*, which by two rapidly succeeding divisions gives rise to four spermatozoa, as follows.[2] The primary spermatocyte first divides to form two daughter-cells known as spermatocytes of the second order or sperm-mother-cells. Each of these divides again — as a rule, without pausing, and without the reconstruction of the daughter-nuclei — to form two *spermatids* or sperm-cells. Each of the four spermatids is then directly transformed into a single spermatozoön, its nucleus becoming very small and compact, its cytoplasm giving rise to the tail and to certain other structures. The number of chromosomes entering into the nucleus of each spermatid and spermatozoön is always one-half that characteristic of the tissue-cells, and this reduction in number is in most, if not in all, cases effected during the two divisions of the primary spermatocyte. The reduction of the chromosomes, which is the most interesting and significant feature of the process, will be considered in the following chapter, and we are here only concerned with the transformation of the spermatid into the spermatozoön.

All observers are now agreed that the nucleus of the spermatid is directly transformed into that of the spermatozoön, the chromatin becoming extremely compact and losing, as a rule, all trace of its reticular structure. It is further certain that in some cases at least the spermatid-centrosome passes into, or gives rise to, a part of the middle-piece, and that from it the axial filament grows out into the tail. The remaining structures arise, as a rule, from the cytoplasm, and both the acrosome and the envelope of the axial filament often show a direct relation to the remains of the achromatic figure ("ar-

[1] The terminology, now almost universally adopted, is due to La Valette St. George. *Cf.* Fig. 118.  [2] See Fig. 119.

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choplasm " or " kinoplasm ") which is found in the spermatid in the form of a sphere (sometimes an attraction-sphere) or " Nebenkern " or both.  Apart from the nuclear history, these facts have been definitely determined in only a few cases, and much confusion still exists in the accounts of different observers.  Thus a number of investigators (*e.g.* Platner, Field, Benda, Julin, Prenant, Niessing) have asserted that the centrosome passes into the acrosome, instead of
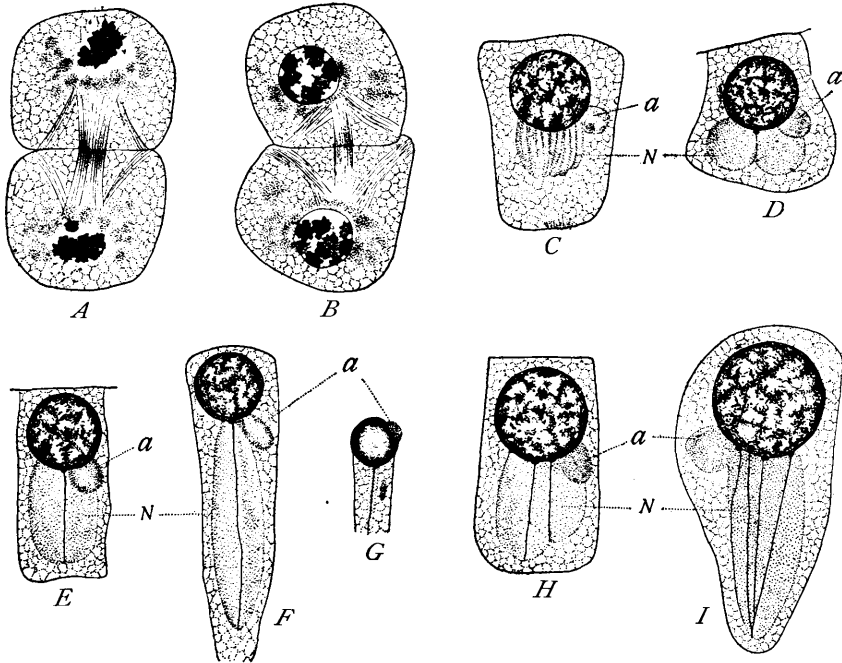


Fig. 82.— Formation of the spermatozoön in an insect, *Anasa*.  [PAULMIER.]
    *A*. Telophase of secondary spermatocyte-division, showing extra chromosome (small dyad of Fig. 127) below.  *B*. Reconstitution of the nuclei.  *C*. Spermatid with Nebenkern (*N*) and acrosome (*a*).  *D*. Nebenkern double, with centrosome between the two halves.  *E. F. G.* Elongation of the spermatid, outgrowth of axial filament, migration of acrosome.  *H*. Giant spermatid (double size) with two centrosomes and axial filaments.  *I*. Giant spermatid (quadruple size) with four centrosomes and axial filaments.

the middle-piece — a result which stands in contradiction with the fact that during fertilization in a large number of accurately known cases the centrosome arises from or in immediate relation to the middle-piece (Amphibia, echinoderms, tunicates, annelids, mollusks, insects, etc.; see p. 212).  The clearest and most positive evidence on this question, afforded by recent observations on the spermatogenesis of insects, annelids, mollusks, Amphibia, and mammals, leaves, however, little doubt that the former result was an error and that, as the facts.

of fertilization would lead us to expect, the centrosome of the spermatid passes into the middle-piece.

Accounts vary considerably regarding the origin of the acrosome, which according to most authors is of cytoplasmic origin, while a few describe it as arising inside or from the anterior part of the nucleus.

(*b*) *Composition of the Spermatid.* — The confusion that has arisen in this difficult subject is owing to the fact that the spermatid may contain, besides the nucleus and centrosome, no less than three additional bodies, which were endlessly confused in the earlier studies on the subject. These are the *Nebenkern*,[1] the attraction-sphere or *idiozome* (Meves), and the *chromatoid Nebenkörper* (Benda).

The Nebenkern (Fig. 82), first described by Bütschli ('71) in the spermatids of butterflies, was afterward shown by La Valette ('86), Platner ('86, '89), and many later investigators to arise wholly or in part from the *remains of the spindle* of the second spermatocyte division. Its origin is thus related to that of an attraction-sphere (which it often closely simulates), since the latter likewise arises from the achromatic figure. To the remains of the spindle, however, may be added granular elements, probably forming reserve-material ("centro-deutoplasm of Erlanger), that are scattered through the cytoplasm or aggregated about the equator of the spindle (Fig. 126). Thus the Nebenkern may have a double origin, though its basis is formed by the spindle-remains. The Nebenkern sometimes takes a definite part in the formation of the tail-envelopes and of the acrosome (insects), but in many cases it seems to be wholly wanting.[2] The idiozome is in some cases an undoubted attraction-sphere derived from the aster of the last division and at first containing the centrosome, *e.g.* in the earthworm as shown by Calkins ('95) and Erlanger ('96, 4), in the salamander and guinea-pig, Meves ('96, '99), and in *Helix* according to Korff ('99), though in later stages the centrosomes usually pass out of the body of the idiozome. In some cases, however (in the rat, according to Lenhossék, '99), the idiozome seems to arise independently through condensation of the cytoplasmic substance into a sphere having no relation to the centrosomes. In some cases the idiozomes of adjoining cells remain for a time connected by bridges of material (Fig. 7) representing the remains of the spindle, and hence corresponding to a Nebenkern (*e.g.* salamander, Meves, '96), and the distinction between Nebenkern and idiozome here fades away. The idiozome is usually concerned in the formation of the acrosome (Amphibia, mammals), but sometimes seems

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[1] The English equivalent of this should be *paranucleus*, but the latter word has already been used in various other senses, and it seems preferable to retain Bütschli's original German word.

[2] For critical discussion, see Erlanger, '97, 1.

to degenerate without contributing directly to the sperm-formation (*Helix*). The chromatoid Nebenkörper, finally, is a small rounded body, staining with plasma-stains, which appear always to degenerate without taking direct part in the formation of the spermatozoön. It is possibly an extruded nucleolus (Lenhossék), but its origin and meaning are not definitely known.

(*c*) *Transformation of the Spermatid into the Spermatozoön.* — In the works of earlier authors it is often impossible to distinguish
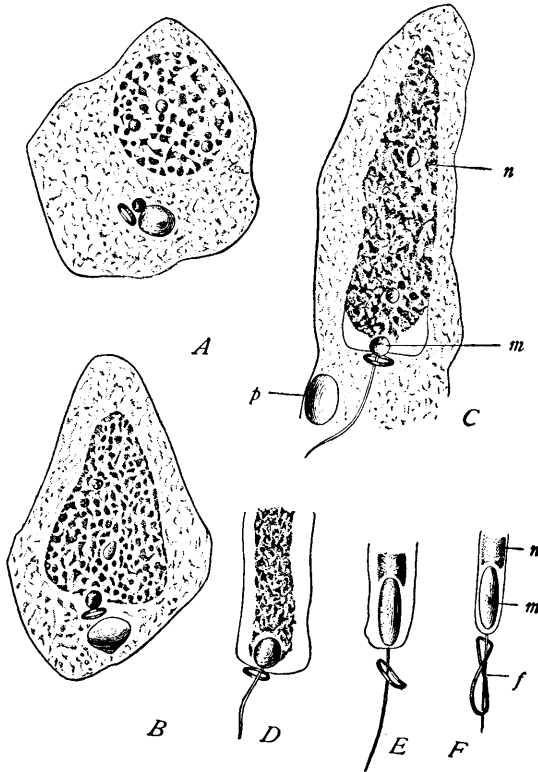


Fig. 83. — Formation of the spermatozoön from the spermatid in the salamander. [HER-MANN.]

*A.* Young spermatid, showing the nucleus above, and below the colourless sphere, the ring, and the chromatic sphere. *B.* Later stage, showing the chromatic sphere and ring at the base of the nucleus. *C. D. E. F.* Later stages, showing the transformation of the chromatic sphere into the middle-piece (*m*).

which of the various achromatic elements mentioned above have been under observation. We may therefore confine ourselves mainly to the latest works, in which these distinctions are clearly recognized. Owing to their great size, the spermatozoa of Amphibia have been the subject of most careful study; yet a clearer view of the subject

may, perhaps, be obtained by taking the spermatogenesis of annelids and insects as a basis of comparison. In the insects (butterflies), Bütschli showed, in 1871, that the tail is formed by an elongation of the cell-body, into which extends the elongated Nebenkern, now divided into two longitudinal halves (Fig. 82). Platner ('89), confirming this observation, further showed that the Nebenkern (in *Pygæra*) consisted of two parts, stating that one ("large mitosome") gives rise to the investment of the axial filament, the other ("small mitosome") to the middle-piece; while a third still smaller body, described as a "centrosome," passes to the apex. The later works of Henking ('91) and Wilcox ('95, '96) render it nearly certain that Platner confused the acrosome with the centrosome, the first-named observer finding in *Pyrrhocoris* and the second in *Caloptenus* that Platner's "centrosome" is derived from the Nebenkern, while Wilcox traced the centrosome directly into the middle-piece. Paulmier, finally, has shown in *Anasa* that the axial filament grows out from the centrosome,[1] proving that such is the case by the highly interesting observation that in giant spermatozoa, arising by the non-division of the primary or secondary spermatocytes, either two or four centrosomes are present, each of which gives rise to a single axial filament, though only one Nebenkern is present (Fig. 82). (The bearing of this important fact on the centrosome-question is indicated elsewhere.) These observations, made on three widely different orders of insects, seem to leave no doubt that in insects the centrosome lies in the middle-piece (*i.e.* at the base of the nucleus), while both the acrosome and the inner tail-envelopes are derived from the Nebenkern. The outer envelope of the tail is derived from unmodified cytoplasm.

In the earthworm the phenomena are slightly different, the middle-piece arising from an idiozome or attraction-sphere (Calkins, '95), in which lies the centrosome (Erlanger, '96), while the Nebenkern seems to have no part in the formation of either acrosome or tail-envelopes.[2]

We turn now to the Amphibia, elasmobranchs, and mammals, in which the same general result has been attained, though there is still some divergence of opinion regarding the exact history of the centrosome. Working on the basis laid by Flemming ('87, '88), Hermann ('89) traced the middle-piece in the salamander to a "Nebenkörper," which he believed to be not a Nebenkern but an attraction-sphere,

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[1] Moore ('95) seems to have been the first actually to describe the outgrowth of the axial filament from the centrosome, in the elasmobranchs. It has since been described by Meves ('97, 2) and Hermann ('97) in the salamander, by Lenhossék ('97), Meves ('98, '99), and Bardeleben ('97) in the rat, guinea-pig, and man; by Godlewski ('97) and Korff ('99) in *Helix*, and by several others.

[2] Calkins's preparations, which I have carefully examined, seem to leave no doubt that the middle-piece arises from a true attraction-sphere derived from the spindle-poles; but Erlanger believes that the granular "centrodeutoplasm" also contributes to the sphere.

consisting of three parts, lying side by side in the cytoplasm (Fig. 83). These are (*a*) a colourless sphere, shown by Meves's later researches to be probably an attraction-sphere; (*b*) a minute, intensely staining corpuscle, and (*c*) a small, deeply staining ring. The concurrent results of Hermann ('89, '92, '97), Benda ('93), and Meves ('96, '97, 2) have shown that the small corpuscle (*c*) *is one of the centrosomes of the spermatid*, and all these observers agree that it passes into or gives
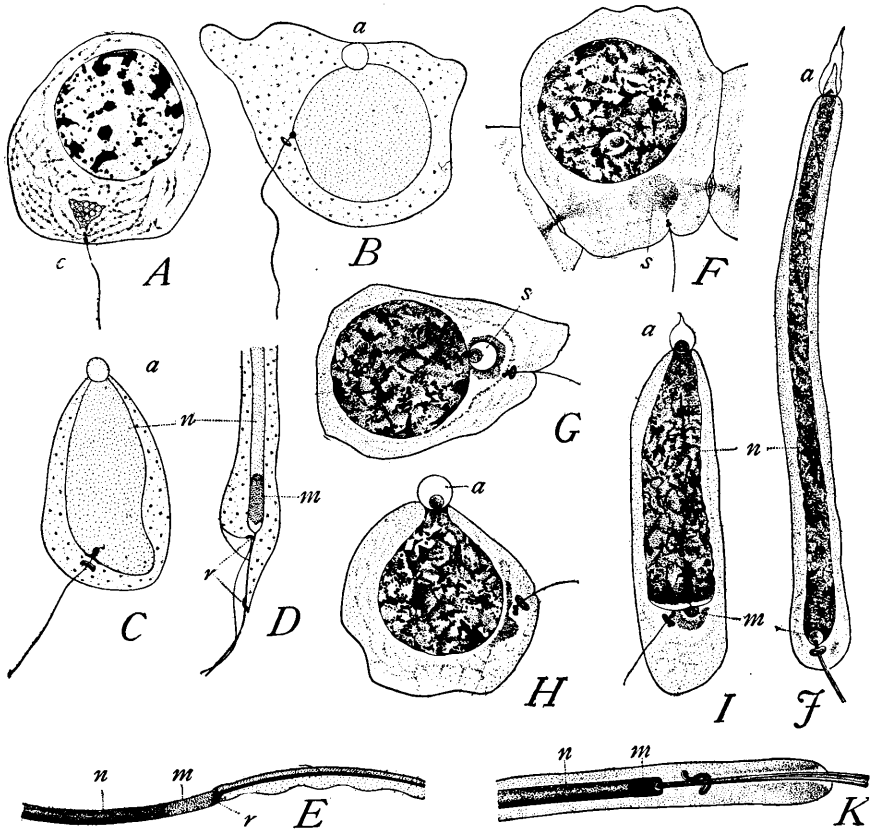


Fig. 84.—Formation of the spermatozoön in Amphibia. [*A–E. Salamandra*, MEVES; *F–K. Amphiuma*, MCGREGOR.]

*A.* Spermatid with peripheral pair of centrosomes lying outside the sphere, and axial filament. *B.* Centrosomes near the nucleus, outer one ring-shaped. *C.* Inner centrosome inside the nucleus, enlarging to form middle-piece. *D.* Portion of much older spermatid, showing divergence of two halves of the ring (*r*). *E.* Portion of mature spermatozoön, showing upper half of ring at *r*, and the axial filament proceeding from it.

*F.* Spermatid of *Amphiuma*, showing sphere-bridges and ring-shaped mid-bodies. *G.* Later stage; outer centrosome ring-shaped, inner one double; sphere (*s*) converted into the acrosome. *H.* Migration of the centrosomes. *I.* Middle-piece at base of nucleus. *J.* The inner centrosome forms the end-knob within the middle-piece, which is now inside the nucleus. *K.* Enlargement of middle-piece, end-knob within it; elongation of the ring.

rise to the middle-piece. According to Meves, who has most thor-
oughly studied the entire formation of the spermatozoön, the history
of these parts is as follows: In the young spermatids the two centro-
somes lie quite at the periphery of the cell (Fig. 84),[1] and from the
outer one grows out the axial filament. The two centrosomes, leav-
ing the idiozome by which they are first surrounded, now pass
inwards toward the nucleus, the outer one meanwhile becoming trans-
formed into the ring mentioned above, while the axial filament passes
through it to become attached to the inner centrosome. The latter
pushes into the base of the nucleus and enlarges enormously to form
a cylindrical body constituting the main body of the middle-piece.
The ring meanwhile divides into two parts, the anterior of which
gives rise to a small, deeply staining body at the posterior end of the
middle-piece identical with the "end-knob." The other half of the
ring wanders out along the tail, finally lying at the limit between
the main part of the latter and the end-piece. The envelope of the
axial filament, here confined to that side opposite the marginal fin
(*i.e.* the "ventral" side of Czermak), is formed by an outgrowth of the
general cytoplasm along the axial filament. The fin and marginal
filament are believed by Meves, as I understand him, to be formed
from the axial filament ('97, 2, p. 127).[2] The acrosome, finally, is
formed from the idiozome which wanders around the nucleus to its
anterior pole. McGregor's results on *Amphiuma* ('99) agree in their
broader features with those of Meves, but differ on two points, one of
which is of great importance. The acrosome here arises from only
a part of the sphere (idiozome), while a second smaller part passes to
the base of the nucleus and forms the main part of the middle-piece.
The inner centrosome passes into the middle-piece *to persist as the end-
knob* from which the axial filament passes out into the tail (Fig. 84).
The history of the sphere thus recalls the phenomena seen in the Ne-
benkern of the insect-spermatid; though the posterior moiety does not
contribute to the tail-envelope, while the history of the inner centrosome
is somewhat like that observed in the mammals, as described beyond.

In the elasmobranchs Moore ('95), Hermann ('98), Suzuki ('98), and
Benda ('98) likewise traced the spermatid-centrosome into the middle-
piece (Fig. 85), and Moore first showed that from it the axial filament
grows out.[3] Moore derived both middle-piece and acrosome from the

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[1] *Cf.* their position in epithelial cells, p. 57.

[2] Hermann ('97) gives a somewhat different account of the process, believing that the
ring is derived from the mid-body of the last mitosis. Meves and McGregor have, however,
shown that the ring and mid-body coexist in the early spermatids (Fig. 84), which seems
decisive against Hermann's conclusion.

[3] Hermann finds also the ring observed in the salamander, and believes it to be the mid-
body. The middle-piece is regarded by him as a product of the spindle-remains, but on
both these points he is contradicted by Suzuki.

"archoplasm" of the spermatid. Suzuki's studies clearly show, how-
ever, that the entire axial filament of the long middle-piece arises by
the elongation of the inner centrosome, while the outer centrosome,
from which the axial filament of the tail grows out, lies at the pos-
terior limit of the middle-piece (Fig. 85). A nearly similar result is
reached by Korff ('99) in the case of *Helix*. It was shown by God-
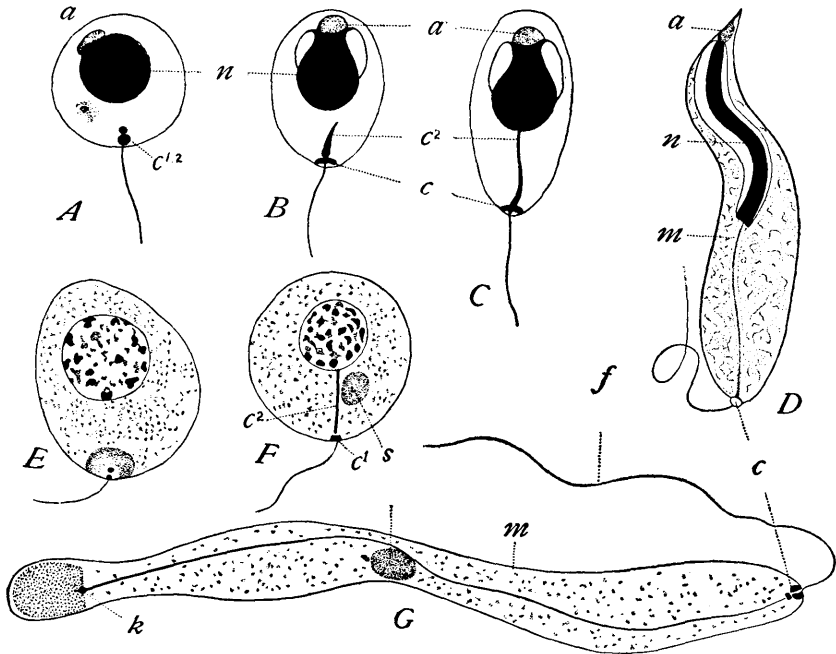lewski ('97) that in this form the axial filament likewise grows out



Fig. 85. — Formation of the spermatozoön in elasmobranchs. [*A–C*, SUZUKI; *D*, MOORE;
and in *Helix*, *E–G*, KORFF.]

*A–D.* Outgrowth of axial filament from peripheral centrosome ($c^1$), which persists at the
posterior limit of the middle-piece or connecting-piece ($m$). Elongation of inner centrosome ($c^2$)
to form the axial filament of the latter. *E–G* show similar phenomena in *Helix*, with casting off
of the sphere ($s$).

*a.* Acrosome; $c^1$. peripheral, and $c^2$. inner centrosome; *f.* flagellum; *k.* end-knob, derived
from inner centrosome.

from the centrosome. Korff's later studies show that here, exactly
as in the elasmobranch, the axial filament grows out from the periph-
eral centrosome and is afterward transformed into a ring (Fig. 85).
The inner centrosome elongates to form a rod, which afterward
becomes a long filament traversing the elongated middle-piece and
terminating in front in an end-knob at the base of the nucleus, while
the ring lies at its posterior limit. The idiozome (a true attraction-
sphere) degenerates without taking part in the formation of an acro-

some.    The envelope of the middle-piece is here formed out of the general cytoplasm.

In the mammals the recent work of Lenhossék on the rat ('98) and Meves on the rat, guinea-pig, and man ('98, '99) gives a result agreeing in its broader features with the forms already considered.    In all these mammals the young spermatids are closely similar to those of the salamander, containing two peripherally placed centrosomes, from the outer one of which the axial filament grows out (Fig. 86).    Meves
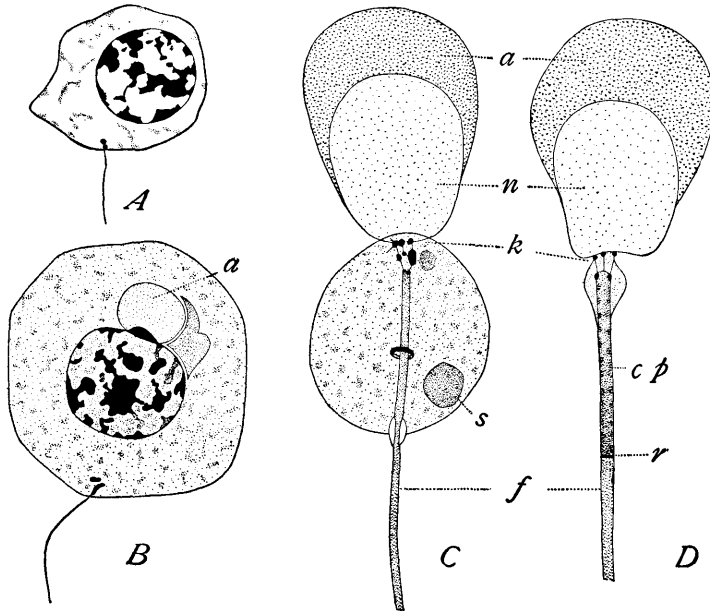


Fig. 86.— Formation of the spermatozoön in mammals.   [MEVES.]

*A.* Spermatid of man, showing centrosomes and axial filament.   *B.* Spermatid of guinea-pig, with acrosome.   *C.* Nearly mature spermatozoön, showing backward migration of the ring. *D.* Mature spermatozoön; *r.* final position of the ring.

*a.* Acrosome surrounded by cytoplasm of the cell-body, most of which is afterward thrown off; *c.* centrosomes; *c.p.* connecting-piece; *f.* flagellum; *k.* neck, containing end-knobs; *s.* remains of the sphere (idiozome).

and Lenhossék differ somewhat in their accounts of the later history of these centrosomes, though agreeing that both contribute to the formation of the middle-piece.    Lenhossék states that in the rat both centrosomes persist at the base of the nucleus to form the end-knob, which, as Jensen showed ('87), is double in this animal.    Meves finds the process to be more complicated, agreeing in the main with that observed by him in the salamander.    In man and the rat the inner centrosome passes to the base of the nucleus and flattens against it to form a small disc-shaped body.    The posterior centrosome divides

into two parts, of which the anterior gives rise to the end-knob, while the posterior is transformed into a ring, which wanders back to its final position at the posterior end of the so-called "connecting-piece." From this it follows that the latter body (Verbindungsstück) does not correspond to the middle-piece of the salamander (here represented by the small disc-shaped body at the base of the nucleus), but belongs to the flagellum proper. The origin of the axial filament and end-knob is, however, nearly the same in the two cases. In the guinea-pig the process is somewhat more complicated and is not quite cleared up by Meves; but the origin and fate of the ring is the same, and the end-knob passes into the neck of the spermatozoön as in the rat. Taken together, these observations conclusively show that in mammals and Amphibia the end-knob is a derivative of the centrosome, thus sustaining, though with some modifications, Hermann's earlier conjecture ('92) as to the nature of this body; and they overturn Niessing's result ('96) that the centrosome passes into the acrosome. As in the salamander, the acrosome is formed from an idiozome derived in the guinea-pig from the remains of the attraction-sphere (Meves), while in the rat, according to Lenhossék, it is independently formed in the cytoplasm without relation to the preceding mitotic figure or the centrosomes. Within the sphere appears a small, deeply staining body, resembling a centrosome, yet staining differently from the true centrosome, which enlarges to form the acrosome, while about it is formed a clear substance forming the "head-cap" (p. 139). In the rat the acrosome remains small ("Spitzenknöpfchen" of Merkel); in the guinea-pig it becomes nearly as large as the nucleus itself (Fig. 86). An interesting feature in the formation of the mammalian spermatozoön is the casting off of a portion of the spermatid-cytoplasm in the form of a "cytoplasmic vesicle" or "tail-vesicle," which degenerates without further use (Fig. 86). This process, described by Meves ('99) in the guinea-pig, is closely similar to that which occurs in the spermatozoid-formation in ferns (p. 144).

*Résumé.* In reviewing the foregoing facts we find, despite many variations in detail, three points of fundamental agreement, namely: (1) the origin of the sperm-nucleus from that of the spermatid; (2) the origin of a part at least of the "middle-piece" from the spermatid-centrosomes; and (3) the outgrowth of the axial filament from one of the spermatid-centrosomes. It is clear, however, that the term *middle-piece* has been applied to structures of quite different morphological nature, which agree only in lying behind the nucleus. Thus in the salamander the inner centrosome gives rise to the main body of the middle-piece; in the rat or in man it gives rise only to the small disc-shaped body lying in the "neck" in front of the so-called middle-

piece; while in *Helix* or the elasmobranch it is transformed into a long filament traversing a cytoplasmic "middle-piece" which forms a considerable part of the flagellum. The term *middle-piece* has thus become highly ambiguous and should only be employed, if at all, as a convenient descriptive term which has no definite morphological meaning.

A very striking fact in the origin of the spermatozoön is the prominent part played by the "archoplasm," *i.e.* substance in the form of idiozome or Nebenkern derived from the mitotic figure. Both the source and the fate of this material seem, however, to vary in different cases, the acrosome now arising from the Nebenkern (insects), now from the idiozome (salamander), the envelope of the flagellum being formed in some cases from the Nebenkern (insects), in others from unmodified cytoplasm (salamander, snail), while the idiozome may form the acrosome (salamander, mammal) or degenerate without apparent use (snail). We find here, I think, additional reason for regarding "archoplasm" not as a distinct and permanent form of protoplasm, but only as a phase in the general metabolic transformation of the cell-substance, which may or may not persist and play a definite morphological *rôle* in the cell according to circumstances. The close relation of this substance to the motor phenomena of the cell cannot, however, be overlooked.[1]

The outgrowth of the axial filament from the centrosome is a highly interesting fact, whether we compare it with the analogous phenomena in plants (p. 172) or with the facts observed in ordinary ciliated cells. In the latter case (Fig. 17), as has long been known, each cilium is attached to a small, highly refracting body known as the "basal knob" lying near the cell-periphery. These bodies stain intensely in iron hæmatoxylin, and it has been recently suggested by Henneguy ('98) and Lenhossék ('98) that they are of the same nature as centrosomes. The truth of this surmise must be tested by further study; but it seems highly probable that they are at least analogous to the spermatid-centrosome. Ishikawa ('99) has clearly shown that in the formation of the swarm-spores of *Noctiluca* the flagellum grows out from that end of the cell at which the centrosome lies, its substance apparently arising from the central spindle, while the centrosome lies at its base. A very interesting fact discovered by Moore ('95) in elasmobranchs, and confirmed by Meves ('97, 5) and Henneguy ('98) in the insects, is a more or less abortive attempt to form a flagellum by the spermatocytes, *i.e.* one or two generations before the spermatozoön. In the insects (Fig. 166) Henneguy has found the cilia actually attached to the centrosomes of the mitotic figure, thus removing every doubt as to their nature.[2]

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[1] *Cf.* 323.　　　　　　[2] *Cf.* Paulmier on giant spermatozoa, p. 165.

It is an important question whether the axial filament actually arises from the substance of the centrosome or is formed by differentiation from the cytoplasmic substance, after the fashion of an astral ray or spindle-fibre. Meves ('97, p. 117) accepts the latter alternative; but the observations of Korff on *Helix* and of Suzuki on elasmobranchs seem to show clearly that, in these cases at least, the inner centrosome elongates bodily to form an extremely long filament traversing the greater part of the flagellum, and apparently of the same nature as the true axial filament developed from the outer or distal centrosome. This seems to establish a probability in favour of the first of the above alternatives, and to show that contractile elements may be directly derived from the centrosome-substance. If this be true, this substance is itself nearly related with "archoplasm"; and the origin of a centrosome *de novo* may be brought under the same category with the formation of archoplasm.[1]

### 3. *Formation of the Spermatozoids in Plants*

While the origin of the spermatozoids has not yet been as fully investigated as that of the spermatozoa, recent researches have given good ground for the conclusion that essentially similar phenomena are involved in the two cases. All recent observers are agreed that the nucleus of the spermatozoid is directly derived from that of the spermatid, while the cytoplasm of the latter gives rise to the cilia and to certain other structures. The principal interest of the subject now lies in the origin of the cilia and their relation to the "archoplasmic" or "kinoplasmic" structures of the mother-cell. Belajeff ('92, '94) found that in *Chara* the cilia grow forth from a small, highly refracting body, taking an intense plasma-stain, that lies in the cytoplasma beside the nucleus. He afterward found the same body "which reminds one of a centrosome" in the developing spermatozoids of ferns and Equisetaceæ (Fig. 88), where it grows out into a band, lying in the anterior part of the spermatozoid, from which the cilia grow forth. Comparing these results with those of Hermann, Belajeff concluded "that the deeply staining corpuscle" (*i.e.* the centrosome) "in the spermatids of the salamander and the mouse corresponds completely to the deeply staining corpuscle in the spermatogenic cells of the Characeæ, ferns, and Equisetaceæ"; that, furthermore, "the middle-piece of the spermatozoön represents the band which bears the cilia of the plant spermatozoid, while the tail-like flagella[2] of the salamander or mouse represents the cilia."[3]

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[1] *Cf.* p. 321. For the function of the centrosome in fertilization, see p. 208.
[2] In the original "Fäden" perhaps meant to designate the axial filament.
[3] '97, 3.

This tallies with Strasburger's earlier conclusion that the cilia-bearing region consists of "kinoplasm" and corresponds to the middle-piece ('92, p. 139), but gives a still more definite basis of comparison.[1]

The history of the centrosome-like bodies (*blepharoplasts* of Webber, '97, 3) has been carefully followed out in *Zamia* and *Gingko* by Webber ('97), and in *Cycas* by Ikeno ('97, '98) with nearly similar results. In all these forms (Fig. 87) the blepharoplasts appear in the
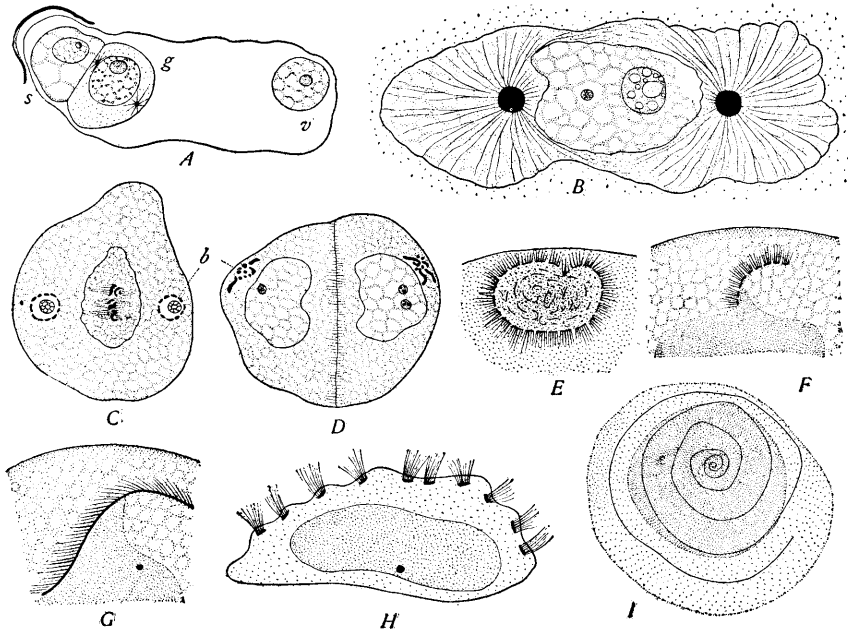


Fig. 87. — Formation of the spermatozoids in the cycads. [*A*, GINGKO; *B–D*, *Zamia*, WEBBER; *E–I*, *Cycas*, IKENO.]

*A*. Developing pollen-tube, showing stalk-cell (*s*), vegetative cell (*v*) and generative cell (*g*), the latter with two blepharoplasts. *B*. Generative cell, somewhat later, with blepharoplasts and asters. *C*. The same in the prophases of division, showing breaking up of blepharoplasts. *D*. The two spermatids formed by division of the generative cell; blepharoplasts fragmented; from these fragments arises the cilia-bearing band. *E*. Blepharoplast of *Cycas*, at a stage somewhat later than Fig. *C*; cilia developing. *F*. Later stage; ciliated band (derived from the last stage) attached to a prolongation from the nucleus. *G*. Cilia-bearing band continuous. *H*. Nearly ripe spermatozoid with nucleus in the centre; ciliated band, shown in section, forming a spiral. *I*. Slightly later stage, viewed from above, showing the spiral course of the band (cilia omitted).

penultimate cell-generation lying one on either side the nucleus, and in earlier stages surrounded by astral radiations very closely resembling those of a typical mitotic aster, and they lie opposite the poles

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[1] The "anterior" region of the spermatozoid thus corresponds to the "posterior" region of the spermatozoön, the confusion of terms having arisen from the fact that the former swims with the cilia-bearing region in front, the latter with the flagellum directed backward.

of the ensuing division-spindle. They seem, however, to have no part in the formation of the mitotic figure or in division, and both
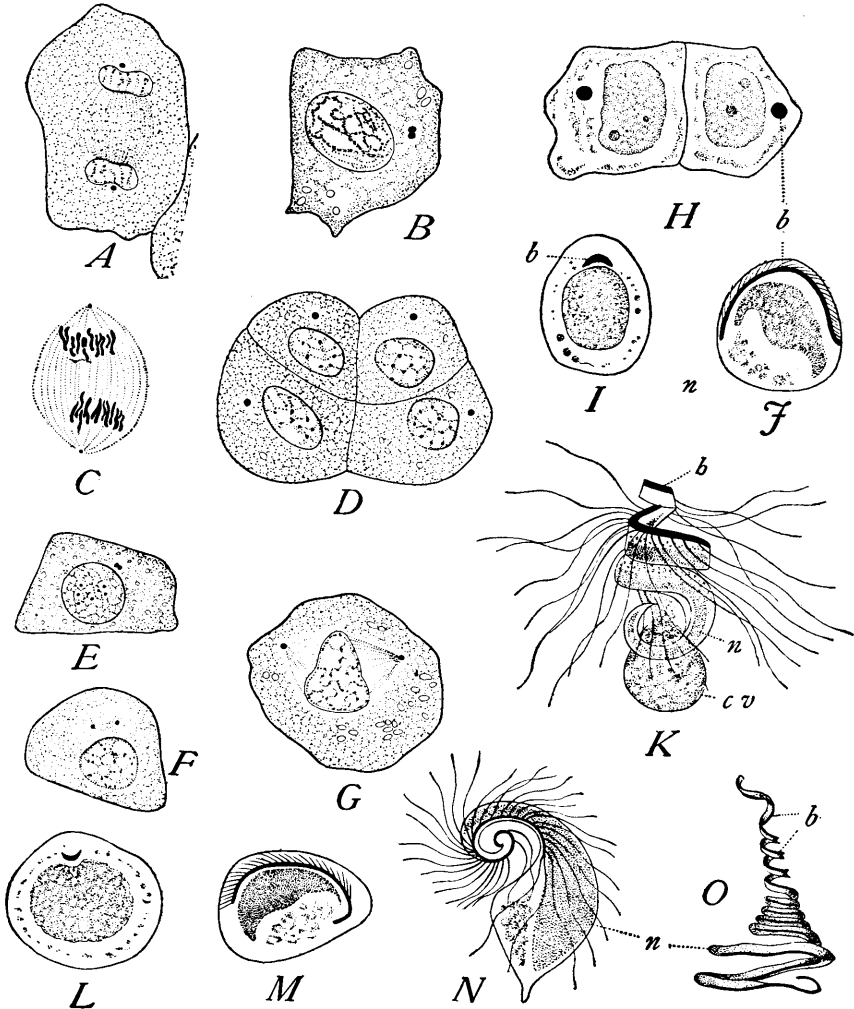


Fig. 88. — Formation of the spermatozoids in the vascular cryptogams, *Marsilia* (*A, D, E-G,* BELAJEFF; *B, C, O,* SHAW), *Gymnogramme* (*H-K,* BELAJEFF), and *Equisetum* (*L-N,* BELAJEFF).

*A.* Primary spermatogonium (two generations before the primary spermatocytes) in division, showing centrosomes. *B.* Primary spermatocyte with pair of "blepharoplastoids" (centrosomes). *C.* Spindle of primary spermatocyte (first maturation-division). *D.* Four of the eight secondary spermatocytes with blepharoplast. *E-G.* Prophase of second maturation-division. *H.* Pair of spermatids (*Gymnogramme*) with blepharoplasts. *I-J.* Formation of the ciliated band from the blepharoplast. *K.* Nearly ripe spermatozoid, showing ciliated band (*b*), nucleus, and "cytoplasmic vesicle" (the latter is ultimately cast off). *L. M.* Spermatids of *Equisetum. N.* Ripe spermatozoid from above, showing spiral ciliated band. *O.* Ripe spermatozoid of *Marsilia* with very long spiral ciliated band.

Webber and Ikeno have produced apparently strong evidence[1] that they arise separately and *de novo* in the cytoplasm. After the ensuing division (by which the two spermatids are formed) the astral rays disappear, and the blepharoplast gives rise by a peculiar process to a long, spiral, deeply staining band, from which the cilia grow forth. The later studies of Shaw ('98, 1) and Belajeff ('99) on the blepharoplasts in *Onoclea* and *Marsilia* leave no doubt that these bodies are to be identified with centrosomes. In *Marsilia* Shaw first found the blepharoplasts lying at the poles of the spindle during the anaphase of the first maturation-division and very closely resembling centrosomes. Each blepharoplast, at first single, divides into two during the late telophase, and during the prophases of the second division the halves diverge to opposite poles of the nucleus and lie at the respective spindle-poles. This account is confirmed by Belajeff, who shows further that during the prophases astral rays surround the blepharoplasts, and a central spindle is formed between them (Fig. 88). Belajeff also finds centrosomes in all of the earlier spermatogenic divisions. The blepharoplasts are thus proved to be, in one case at least, dividing organs which in every way correspond to the centrosomes of the animal spermatocytes; and the justice of Belajeff's comparison is demonstrated. Shaw believed that the primary blepharoplast, which by division gives rise to those of the two spermatids, arose *de novo*. He made, however, the significant observation that in *Marsilia* "blepharoplastoids," exactly like the blepharoplasts, appear at the spindle-poles of the preceding (antepenultimate) division, and that each of these divides into two in the late telophase. These are said to disappear, without relation to the blepharoplasts which at a slightly later period are found at the spindle-poles of the first maturation division; but in view of the demonstrated continuity of the blepharoplasts during the second division we may well hesitate to accept this result, as well as Webber's conclusion regarding the independent and separate origin of the blepharoplasts in *Zamia*. In any case the facts give the strongest ground for the conclusion that the formation of the spermatozoids agrees in its essential features with that of the spermatozoa, and for the expectation that the history of the achromatic structures in fertilization will yet be found to show an essential agreement in plants and animals.

### E. STAINING-REACTIONS OF THE GERM-NUCLEI

It was pointed out by Ryder in 1883 that in the oyster the germ-nuclei stain differently in the two sexes; for if the hermaphrodite

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[1] Dr. Webber has kindly given me an opportunity to look through his beautiful preparations.

gland of this animal be treated with a mixture of saffranin and methyl-green, the egg-nuclei are coloured red, the sperm-nuclei bluish green. A similar difference was afterward observed by Auerbach ('91) in the case of many vertebrate germ-cells, where the egg-nucleus was shown to have a special affinity for various red and yellow dyes (eosin, fuchsin, aurantia, carmine), while the sperm-nuclei were especially stained with blue and green dyes (methyl-green, aniline-blue, hæmatoxylin). He was thus led to regard the chromatin of the egg as especially "erythrophilous," and that of the sperm as "cyanophilous." That the distinction as regards colour is of no value has been shown by Zacharias, Heidenhain, and others; for staining-agents cannot be logically classed according to colour, but according to their chemical composition; and a red dye, such as saffranin, may in a given cell show the same affinity for the chromatin as a green or blue dye of different chemical nature, such as methyl-green or hæmatoxylin. Thus Field has shown that the sperm-nucleus of *Asterias* may be stained green (methyl-green), blue (hæmatoxylin, gentian violet), red (saffranin), or yellow (iodine), and it is here a manifest absurdity to speak of "cyanophilous" chromatin (*cf.* p. 335). It is certainly a very interesting fact that a difference of staining-reaction exists between the two sexes, as indicating a corresponding difference of chemical composition in the chromatin; but even this has been shown to be of a transitory character, for the staining-reactions of the germ-nuclei vary at different periods and are exactly alike at the time of their union in fertilization. Thus Hermann has shown that when the spermatids and immature spermatozoa of the salamander are treated with saffranin (red) and gentian violet (blue),[1] the chromatic network is stained blue, the nucleoli and the middle-piece red; while in the mature spermatozoön the reverse effect is produced, the nuclei being clear red, the middle-piece blue. A similar change of staining-capacity occurs in the mammals. The great changes in the staining-capacity of the egg-nucleus at different periods of its history are described at pages 338–340. Again, Watasé has observed in the newt that the germ-nuclei, which stain differently throughout the whole period of their maturation, and even during the earlier phases of fertilization, become more and more alike in the later phases, and at the time of their union show identical staining-reactions.[2] A very similar series of facts has been observed in the germ-nuclei of plants by Strasburger (p. 220). These and many other facts of like import demonstrate that the chemical differences between the germ-nuclei are not of a fundamental but only of a secondary character. They are doubtless connected with the very different character of the metabolic processes that occur in the history of the two germ-cells; and

[1] By Flemming's triple method.          [2] '92, p. 492.

the difference of the staining-reaction is probably due to the fact that the sperm-chromatin contains a higher percentage of nucleinic acid, while the egg-chromatin is a nuclein containing a much higher percentage of albumin.

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[1] See also Literature, V., p. 287.