

## CHAPTER VI

### SOME PROBLEMS OF CELL-ORGANIZATION

“Wir müssen deshalb den lebenden Zellen, abgesehen von der Molecularstruktur der organischen Verbindungen, welche sie enthält, noch eine andere und in anderer Weise complicirte Structur zuschreiben, und diese es ist, welche wir mit dem Namen *Organization* bezeichnen.”  
BRÜCKE.<sup>1</sup>

“Was diese Zelle eigentlich ist, darüber existieren sehr verschiedene Ansichten.”  
HÄCKEL.<sup>2</sup>

THE remarkable history of the chromatic substance in the maturation of the germ-cells forces upon our attention the problem of the ultimate morphological organization of the nucleus, and this in its turn involves our whole conception of protoplasm and the cell. The grosser and more obvious organization is revealed to us by the microscope as a differentiation of its substance into nucleus, cytoplasm, and the like. But, as Strasburger has well said, it would indeed be a strange accident if the highest powers of our present microscopes had laid bare the ultimate organization of the cell. Brücke insisted more than thirty years ago that protoplasm must possess a far more complicated morphological organization than is revealed to us in the visible structure of the cell, repeating, though without accepting, an earlier suggestion of Henle's ('41) that the cell might be composed of more elementary vital units ranking between the molecule and the cell. Many biological thinkers since Brücke's time have in one form or other accepted this conception, which indeed lies at the root of nearly all recent attempts to analyze exhaustively the phenomena of cell-life. Without attempting to follow out the history of opinion in detail or to give any extended review of the various theories,<sup>3</sup> it may be pointed out that this conception was based both on theoretical *a priori* grounds and on the observed facts of cell-structure. On the former basis it was developed by Herbert Spencer<sup>4</sup> in his theory of “physiological units” by which he endeavoured to explain the phenomena of regeneration, development, and heredity; while Nägeli ('84) developed on the same general lines his theory of *micellæ* which

<sup>1</sup> *Elementarorganismen*, 1861, p. 386.

<sup>2</sup> *Anthropogenie*, 1891, p. 104.

<sup>3</sup> For an exhaustive review see Yves Delage, *La structure du protoplasma et les théories sur l'hérédité*. Paris, 1895.

<sup>4</sup> *Principles of Biology*, 1864.

has been so widely accepted by botanists. In the meantime Darwin<sup>1</sup> introduced a new element into the speculative edifice in his celebrated hypothesis of pangenesis, where for the first time appear the two assumptions of specific differences in the ultra-microscopic corpuscles ("gemmules") and the power of self-propagation by division. Darwin did not, however, definitely maintain that protoplasm was actually built of such bodies. The latter hypothesis was added by De Vries ('89), who remodelled the theory of pangenesis on this assumption, thus laying the basis for the theories of development which reached their climax in the writings of Hertwig and Weismann.

The views of Spencer and Darwin were based on purely theoretical grounds derived from the general phenomena of growth and inheritance.<sup>2</sup> Those of Nägeli, De Vries, Wiesner, Altmann, and others were more directly based on the results of microscopical investigation. The view was first suggested by Henle ('41), and at a later period developed by Béchamp and Estor, by Maggi and especially by Altmann, that the protoplasmic granules might be actually organic units or bioblasts, capable of assimilation, growth, and division, and hence to be regarded as elementary units of structure standing between the cell and the ultimate molecules of living matter. By Altmann, especially, this view was pushed to an extreme limit, which lay far beyond anything justified by the known facts; and the theory of genetic continuity expressed by Redi in the aphorism "*omne vivum ex vivo*," reduced by Virchow to "*omnis cellula e cellula*," finally appears in the writings of Altmann as "*omne granulum e granulo*"!<sup>3</sup>

Altmann's premature generalization rested upon a very insecure foundation and was received with just scepticism. Except in the case of plastids, the division of the cytoplasmic granules was and still remains a pure assumption, and furthermore many of Altmann's "granules" (zymogen-granules of gland-cells, etc.) are undoubtedly metaplasmic bodies.<sup>4</sup> Yet the beautiful discoveries of Schimper ('85) and others on the origin of plastids in plant-cells give evidence that these cells do in fact contain large numbers of bodies, other than the nuclei, that possess the power of growth and division. The division of the chlorophyll-bodies, observed long ago by Mohl, was shown by Schmitz and Schimper to be their usual if not their only mode of origin; and Schimper was able to trace them back to minute colourless plastids, scarcely larger than "microsomes," that are present in large numbers in the protoplasm of the embryonic cells and of the egg, and give rise not only to chlorophyll-bodies but also to the amyloplasts or starch-formers and the chromoplasts or pigment-bodies. While it still remains doubtful whether the plastids arise solely by division or also

<sup>1</sup> *Variation of Animals and Plants*, 1868.

<sup>2</sup> Cf. Introduction, p. 12.

<sup>3</sup> *Die Elementarorganismen*, Leipsic, 1894, p. 155.

<sup>4</sup> Cf. Lazarus, '98.

by new formation (as now seems to be the case with the centrosome), the foregoing observations on the plastids give a substantial basis for the hypothesis that protoplasm may be built of minute dividing bodies which form its ultimate structural basis. It was these facts, taken in connection with the phenomena of particulate inheritance and variation (Galton), that led De Vries and his followers to the fundamental assumption of "pangens," "plasomes," "biophores," and the like as final protoplasmic units;<sup>1</sup> but these were conceived not as the visible granules, plastids, etc., but as much smaller bodies, lying far beyond the limits of present microscopical vision, through the growth or aggregation of which the visible structures arise. This assumption has been harshly criticised; yet when we recall that in one form or another it has been accepted by such men as Spencer, Darwin, Beale, Häckel, Michael Foster, Nägeli, De Vries, Wiesner, Roux, Weismann, Oscar Hertwig, Verworn, and Whitman, and on evidence drawn from sources so diverse, we must admit that despite its highly speculative character it is not to be lightly rejected. In the present chapter we may inquire how far the known facts of cell-structure speak for or against this hypothesis, incidentally considering a number of detailed questions of cell-morphology which have not hitherto found a place.

#### A. THE NATURE OF CELL-ORGANS

The cell is, in Brücke's words, an *elementary organism*, which may by itself perform all the characteristic operations of life, as is the case with the unicellular organisms, and in a sense also with the germ-cells. Even when the cell is but a constituent unit of a higher grade of organization, as in multicellular forms, it is no less truly an organism, and in a measure leads an independent life, even though its functions be restricted and subordinated to the common life. It is true that the earlier conception of the multicellular body as a colony of one-celled forms cannot be accepted without certain reservations.<sup>2</sup> Nevertheless, all the facts at our command indicate that the tissue-cell possesses the same morphological organization as the egg-cell, or the protozoan, and the same fundamental physiological properties as well. Like these the tissue-cell has its differentiated structural parts or organs, and we have now to inquire how these cell-organs are to be conceived.

<sup>1</sup> The following list includes only some of the various names that have been given to these hypothetical units by modern writers: *Physiological units* (Spencer); *gemmules* (Darwin); *pangens* (De Vries); *plasomes* (Wiesner); *micella* (Nägeli); *plastidules* (Häckel and Ellsberg); *inotagmata* (Engelmann); *biophores* (Weismann); *bioblasts* (Beale); *somacules* (Foster); *idioblasts* (Hertwig); *idiosomes* (Whitman); *biogens* (Verworn); *microzymas* (Béchamp and Estor); *gemmæ* (Haacke). These names are not strictly synonymous, nor do all of the writers cited assume the power of division in the units.

<sup>2</sup> Cf. p. 58.

The visible organs of the cell fall under two categories, according as they are merely temporary structures, formed anew in each successive cell-generation out of the common structural basis, or permanent structures whose identity is never lost, since they are directly handed on by division from cell to cell.<sup>1</sup> To the former category belong, in general, such structures as cilia, pseudopodia, and the like; to the latter, the nucleus, perhaps also the centrosomes, and the plastids of plant-cells. A peculiar interest attaches to the permanent cell-organs. Closely interrelated as these organs are, they nevertheless have a remarkable degree of morphological independence. They assimilate food, grow, divide, and perform their own characteristic actions like coexistent but independent organisms, of a lower grade than the cell, living together in colonial or symbiotic association. So striking is this morphological and physiological autonomy in the case of the green plastids or chromatophores that neither botanists nor zoölogists are as yet able to distinguish with absolute certainty between those that form an integral part of the cell, as in the higher green plants, and those that are actually independent organisms living symbiotically within it, as is probably the case with the yellow cells of *Radiolaria*. Even so acute an investigator as Watasé ('93, 1) has seriously propounded the view that the nucleus itself — or rather the chromosome — should be regarded as a distinct organism living in symbiotic association with the cytoplasm, but having had, in an historical sense, a different origin. This rather fantastic view has not found much favour, and even were it true would teach us nothing of the origin of the power of division, which must for the present be taken as an elementary process forming one of the primary data of biology. Yet we may still inquire whether the power of division shown by such protoplasmic masses as plastids, chromosomes, centrosomes, nucleoli, and nuclei may not have its root in a like power residing in ultimate protoplasmic units of which they are made up. Could we accept such a view, we might much more easily meet some puzzling cytological difficulties. For under this assumption the difference between transient and permanent cell-organs would become only one of degree, depending on the degree of cohesion between their structural components; and we could thus conceive, for example, how such a body as a centrosome might form, persist by division for a number of generations, and finally disintegrate. In connection with this it may be pointed out that even such a typical permanent organ as the nucleus does not persist *as such* during the ordinary form of division; for it loses its boundary and many of its other structural characters, becoming resolved into a group of separate chromosomes. What persists is here not the structural unit, but the characteristic substance which forms its essential constituent, and

<sup>1</sup> Cf. footnote, p. 30.

a large part even of this substance may be lost in the process. The term "persistent organ" is therefore used in rather a figurative sense, and if too literally understood may easily mislead us.

With the foregoing considerations in mind let us turn to the actual structural relation of the cell-organs.

## B. STRUCTURAL BASIS OF THE CELL

In Chapter I. some of the reasons have been given for the conclusion that none of the obvious structural features of protoplasm (fibrillæ, alveoli, granules, and the like) can be regarded as necessary or universal; and we may now inquire whether there is any evidence that such structures may have such a common structural basis as De Vries's theory assumes. I shall here take as a point of departure my observations on the structure of protoplasm in echinoderm-eggs, already briefly reviewed at page 28. The beautiful alveolar structure of these eggs is entirely of secondary origin, and all the visible structural elements arise during the growth of the eggs by the deposit and subsequent enlargement of minute spherical bodies, all apparently liquid drops, in a homogeneous or finely granular basis which is itself a liquid. Some of these spheres enlarge to form the alveolar spheres, while the homogeneous basis or continuous substance remains as the interalveolar material. Others remain much smaller to constitute the "microsomes" scattered through the interalveolar walls; and these bodies, like the alveolar spheres, are perfectly visible in life, as well as in section; they are therefore not coagulation-products or artifacts. From these three elements arise all the other structures observed in these eggs, deutoplasm-spheres (*Ophiura*) and pigment-bodies (*Arbacia*) being formed by further enlargement and chemical alteration of the alveolar spheres, while astral rays and spindle-fibres are differentiated out of the inter-alveolar material and microsomes.<sup>1</sup> These various elements show a continuous gradation in size from the largest deutoplasm-spheres down to the smallest visible granules, the latter being the source of all the larger elements, and in their turn emerging into view from the "homogeneous" basis. Clearly, then, none of these bodies can be regarded as the ultimate structural units; for the latter, if they exist, must lie in a region at present inaccessible to the microscope. This fact, however, no more disproves their existence than it does that of molecules and atoms. It only shows the futility of such attempts as those of Altmann and his predecessors to identify "granules" or "microsomes" as final morphological units, and compels us to turn to indirect instead of direct evidence. It may, however, again be pointed out that it would be quite irrational to conclude that the small-

<sup>1</sup> Cf. Wilson, '99.

est visible granules first come into existence when they first come within view of the microscope. The "homogeneous" substance must itself contain or consist of granules still smaller. The real question is not whether such ultra-microscopical bodies exist, but whether they are permanent *organized* bodies possessing besides the power of growth also the power of division. This question can be only indirectly approached; and we shall find it convenient to do so by beginning at the opposite end of the series, through a reconsideration of the phenomena of nuclear division.

### C. MORPHOLOGICAL COMPOSITION OF THE NUCLEUS

#### I. *The Chromatin*

(a) *Hypothesis of the Individuality of the Chromosomes.* — It may now be taken as a well-established fact that the nucleus is never formed *de novo*, but always arises by the division of a preëxisting nucleus. In the typical mode of division by mitosis the chromatic substance is resolved into a group of chromosomes, always the same in form and number in a given species of cell, and having the power of assimilation, growth, and division, as if they were morphological individuals of a lower order than the nucleus. That they are such individuals or units has been maintained as a definite hypothesis, especially by Rabl and Boveri. As a result of careful study of mitosis in epithelial cells of the salamander, Rabl ('85) concluded that *the chromosomes do not lose their individuality at the close of division, but persist in the chromatic reticulum of the resting nucleus.* The reticulum arises through a transformation of the chromosomes, which give off anastomosing branches, and thus give rise to the appearance of a network. Their loss of identity is, however, only apparent. They come into view again at the ensuing division, at the beginning of which "the chromatic substance flows back, through predetermined paths, into the primary chromosome-bodies" (Kernfäden), which reappear in the ensuing spireme-stage in nearly or quite the same position they occupied before. Even in the resting nucleus, Rabl believed that he could discover traces of the chromosomes in the configuration of the network, and he described the nucleus as showing a distinct polarity having a "pole" corresponding with the point toward which the apices of the chromosomes converge (*i.e.* toward the centrosome), and an "antipole" (Gegenpol) at the opposite point (*i.e.* toward the equator of the spindle) (Fig. 22). Rabl's hypothesis was precisely formulated and ardently advocated by Boveri in 1887 and 1888, and again in 1891, on the ground of his own studies and those of Van Beneden on the early stages of *Ascaris*. The hypothesis was supported

by extremely strong evidence, derived especially from a study of abnormal variations in the early development of *Ascaris*, the force of which has, I think, been underestimated by the critics of the hypothesis. Some of this evidence may here be briefly reviewed. In some cases, through a miscarriage of the mitotic mechanism, one or both of the chromosomes destined for the second polar body are accidentally left

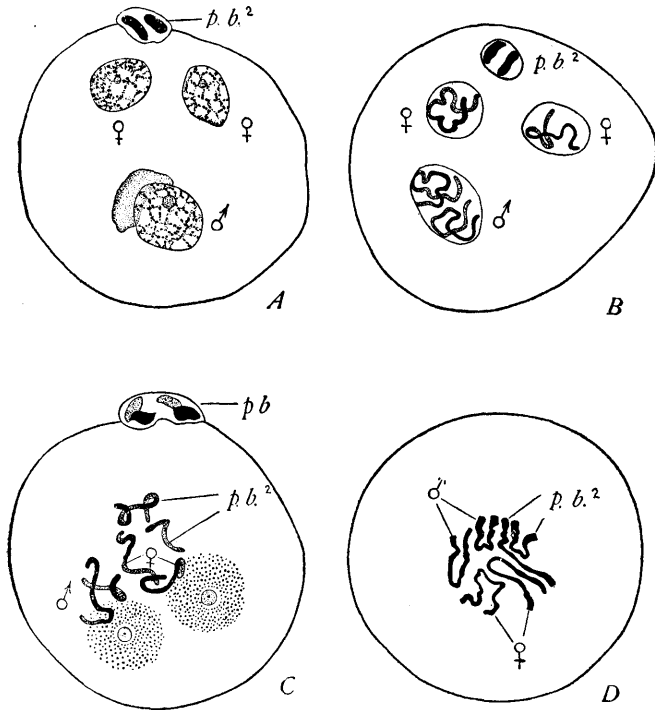


Fig. 143.—Evidence of the individuality of the chromosomes. Abnormalities in the fertilization of *Ascaris*. [BOVERI.]

*A.* The two chromosomes of the egg-nucleus, accidentally separated, have given rise each to a reticular nucleus (♀, ♀); the sperm-nucleus below (♂). *B.* Later stage of the same, a single chromosome in each egg-nucleus, two in the sperm-nucleus. *C.* An egg in which the second polar body has been retained; *p.b.²* the two chromosomes arising from it; ♀ the egg-chromosomes; ♂ the sperm-chromosomes. *D.* Resulting equatorial plate with six chromosomes.

in the egg. These chromosomes give rise in the egg to a reticular nucleus, indistinguishable from the egg-nucleus. At a later period this nucleus gives rise to the same number of chromosomes as those that entered into its formation, *i.e.* either one or two. These are drawn into the equatorial plate along with those derived from the germ-nuclei, and mitosis proceeds as usual, the number of chromosomes being, however, abnormally increased from four to five or six (Fig. 143,

C, D). Again, the two chromosomes left in the egg after removal of the second polar body may accidentally become separated. In this case each chromosome gives rise to a reticular nucleus of half the usual size, and from each of these a *single* chromosome is afterward formed (Fig. 143, A, B). Finally, it sometimes happens that the two germ-nuclei completely fuse, while in the reticular state, as is normally the case in sea-urchins and some other animals (p. 188). From the cleavage-nucleus thus formed arise four chromosomes.

The same general result is given by the observations of Zur Strassen ('98) on the history of giant embryos in *Ascaris*. These embryos arise by the fusion, either before or after the fertilization, of previously separate eggs, and have been shown to be capable of development up to a late stage. Not only in the first but also in some, at least, of the later mitoses, such eggs show an increased number of

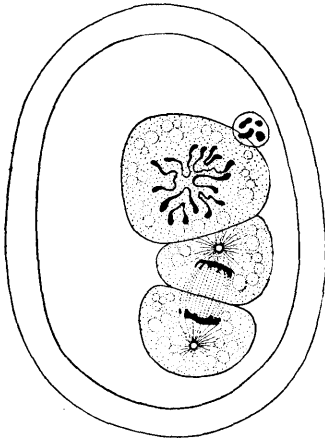


Fig. 144.—Giant-embryo of *Ascaris*, var. *bivalens*, arising from a double-fertilized double egg, showing eight chromosomes (Zur Strassen).

chromosomes proportional to the number of nuclei that have united. Thus in monospermic double eggs (variety *bivalens*) the number is six instead of four; in dispermic double eggs the number is increased to eight (Fig. 144).

These remarkable observations show that *whatever be the number of chromosomes entering into the formation of a reticular nucleus, the same number afterward issues from it*—a result which demonstrates that the number of chromosomes is not due merely to the chemical composition of the chromatin-substance, but to a morphological organization of the nucleus. A beautiful confirmation of this conclusion was afterward made by Boveri ('93, '95, 1) and Morgan ('95, 4), in the case of echinoderms, by rear-

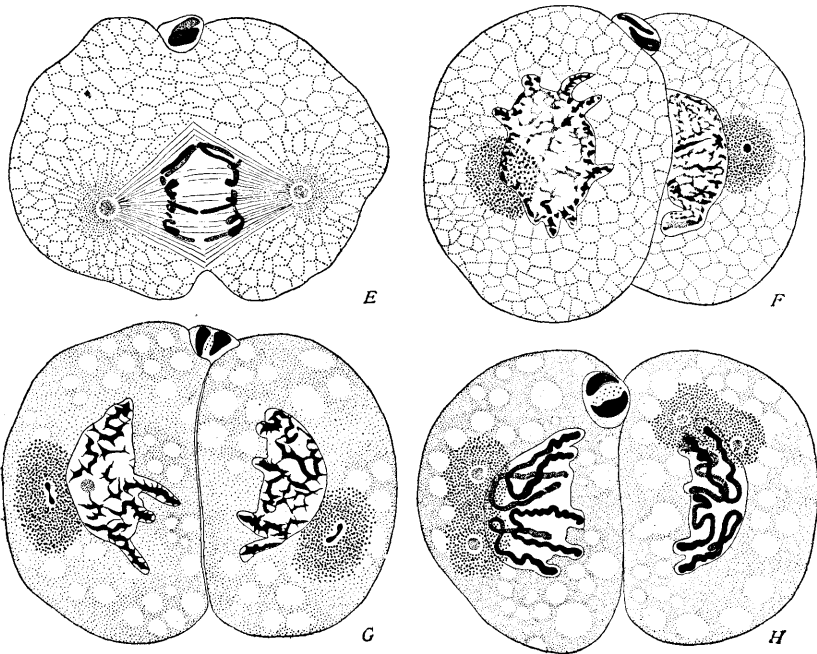
ing larvæ from enucleated egg-fragments, fertilized by a single spermatozoön (p. 194). All the nuclei of such larvæ contain but half the typical number of chromosomes,—*i.e.* in *Echinus* nine instead of eighteen,—since all are descended from one germ-nucleus instead of two!

Equally striking is the remarkable fact, described at page 275, that all of the cells in the sexual generation (oöphore) of the higher cryptogams show half the number of chromosomes characteristic of the sporophyte, the explanation being that while reduction occurs at the time of spore-formation, the spores develop without fertilization, the reduced chromosome-number persisting until fertilization occurs



long afterward. Attention may be again called to the surprising case of *Artemia*, described at page 281, which gives a strong argument in favour of the hypothesis.

In addition to the foregoing evidence, Van Beneden and Boveri were able to demonstrate in *Ascaris* that in the formation of the spireme the chromosomes reappear in the same position as those which entered into the formation of the reticulum, precisely as Rabl



**Fig. 145.**—Evidence of the individuality of the chromosomes in the egg of *Ascaris*. [BOVERI.]

*E.* Anaphase of the first cleavage. *F.* Two-cell stage with lobed nuclei, the lobes formed by the ends of the chromosomes. *G.* Early prophase of the ensuing division; chromosomes re-forming, centrosomes dividing. *H.* Later prophase, the chromosomes lying with their ends in the same position as before; centrosomes divided.

maintained. As the long chromosomes diverge, their free ends are always turned toward the middle plane (Fig. 31), and upon the reconstruction of the daughter-nuclei these ends give rise to corresponding lobes of the nucleus, as in Fig. 145, which persist throughout the resting state. At the succeeding division the chromosomes reappear exactly in the same position, *their ends lying in the nuclear lobes as before* (Fig. 145, *G, H*). On the strength of these facts Boveri concluded that the chromosomes must be regarded as “individuals” or “elementary organisms,” that have an independent existence in the

cell. During the reconstruction of the nucleus they send forth pseudopodia which anastomose to form a network in which their identity is lost to view. As the cell prepares for division, however, the chromosomes contract, withdraw their processes, and return to their "resting state," in which fission takes place. Applying this conclusion to the fertilization of the egg, Boveri expressed his belief that

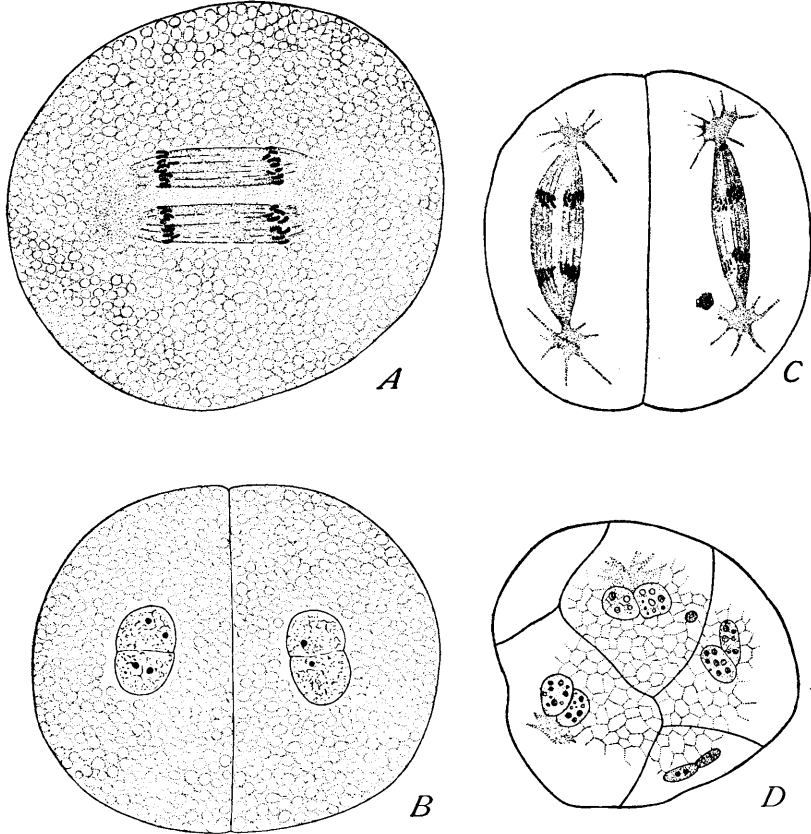


Fig. 146.—Independence of paternal and maternal chromatin in the segmenting eggs of *Cyclops*. [A-C, from RÜCKERT; D, from HÄCKER.]

A. First cleavage-figure in *C. strenuus*; complete independence of paternal and maternal chromosomes. B. Resulting two-cell stage with double nuclei. C. Second cleavage; chromosomes still in double groups. D. Blastomeres with double nuclei from the eight-cell stage of *C. brevicornis*.

"we may identify every chromatic element arising from a resting nucleus with a definite element that entered into the formation of that nucleus, from which the remarkable conclusion follows that in all cells derived in the regular course of division from the fertilized egg, one-half of the chromosomes are of strictly paternal origin, the other half of maternal."<sup>1</sup>

<sup>1</sup>91, p. 410.

Boveri's hypothesis has been criticised by many writers, especially by Hertwig, Guignard, and Brauer, and I myself have urged some objections to it. Recently, however, it has received a support so strong as to amount almost to a demonstration, through the remarkable observations of Rückert, Häcker, Herla, and Zoja on the independence of the paternal and maternal chromosomes. These observations, already referred to at page 208, may be more fully reviewed at this point. Häcker ('92, 2) first showed that in *Cyclops strenuus*, as in *Ascaris* and other forms, the germ-nuclei do not fuse, but give rise to two separate groups of chromosomes that lie side by side near the equator of the cleavage-spindle. In the two-cell stage (of *Cyclops tenuicornis*) each nucleus consists of two distinct though closely united halves, which Häcker believed to be the derivatives of the two respective germ-nuclei. The truth of this surmise was demonstrated three years later by Rückert ('95, 3) in a species of *Cyclops*, likewise identified as *C. strenuus* (Fig. 146). The number of chromosomes in each germ-nucleus is here twelve. Rückert was able to trace the paternal and maternal groups of daughter-chromosomes not only into the respective halves of the daughter-nuclei of the two-cell stage, but into *later cleavage-stages*. From the bilobed nuclei of the two-cell stage arise, in each cell, a double spireme and a double group of chromosomes, from which are formed bilobed or double nuclei in the four-cell stage. This process is repeated at the next cleavage, and the double character of the nuclei was in many cases distinctly recognizable at a late stage when the germ-layers were being formed.

Finally Victor Herla's ('93) and Zoja's ('95, 2) remarkable observations on *Ascaris* showed that in *Ascaris* not only the chromatin of the germ-nuclei, but also the paternal and maternal *chromosomes*, remain perfectly distinct as far as the twelve-cell stage — certainly a brilliant confirmation of Boveri's conclusion. Just how far the distinction is maintained is still uncertain, but Häcker's and Rückert's observations give some ground to believe that it may persist throughout the entire life of the embryo. Both these observers have shown that the chromosomes of the germinal vesicle appear in *two distinct groups*, and Rückert suggests that these may represent the paternal and maternal elements that have remained distinct throughout the entire cycle of development, even down to the formation of the egg!

Leaving aside all doubtful cases (such as the above suggestion of Rückert's), the well-determined facts form an irresistible proof of the general hypothesis; and it is one with which every general analysis of the cell has to reckon. I believe, however, that the hypothesis has received an unfortunate name; for, except in a few special cases,<sup>1</sup>

<sup>1</sup> Cf. p. 273.

almost no direct evidence exists to show that the chromosomes persist as "individuals" in the chromatin-reticulum of the resting cell. The facts indicate, on the contrary, that in the vast majority of cases the identity of the chromosomes is wholly lost in the resting nucleus, and the attempts to identify them through the polarity or other morphological features of the nuclear network have on the whole been futile. It is therefore an abuse of language to speak of a persistent "individ-

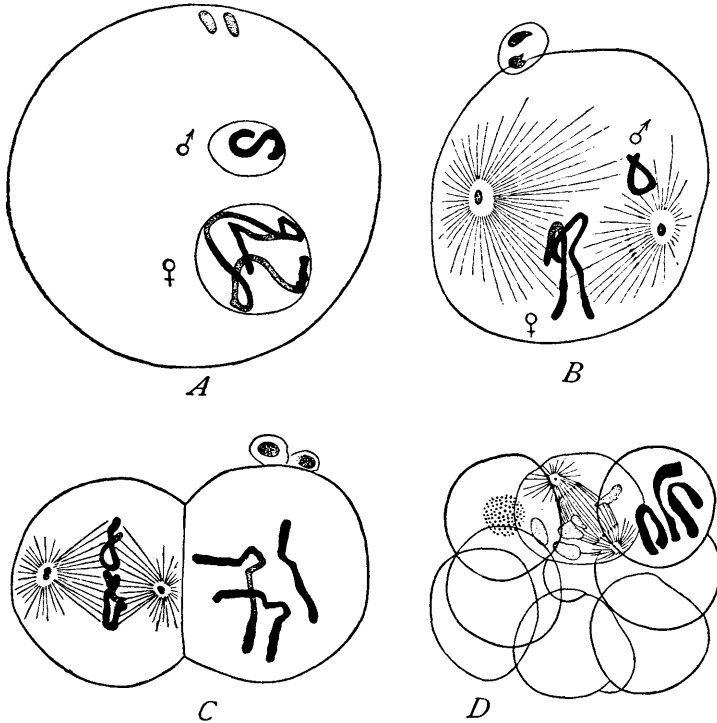


Fig. 147. — Hybrid fertilization of the egg of *Ascaris megalocephala*, var. *bivalens*, by the spermatozoön of var. *univalens*. [HERLA.]

A. The germ-nuclei shortly before union. B. The cleavage-figure forming; the sperm-nucleus has given rise to one chromosome ( $\sigma$ ), the egg-nucleus to two ( $\varphi$ ). C. Two-cell stage dividing, showing the three chromosomes in each cell. D. Twelve-cell stage, with the three distinct chromosomes still shown in the primordial germ-cell or stem-cell.

uality" of chromosomes. But this verbal difficulty should not blind us to the extraordinary interest and significance of the facts. It is difficult to suppose that the tendency of the chromatin to resolve itself into a particular number of chromosomes is directly due to its chemical or molecular structure, or is analogous to crystallization; for in the chromatin of the same species, or even in that of the same egg, this tendency varies, not with chemical, but with purely morphological

conditions, *i.e.* with the *number* of chromosomes that enter the nucleus. Neither can we assume that it is due merely to the total mass of the chromatin in each case; for this varies in different nuclei of the same species, or even in the nucleus of the same cell at different periods (as in the egg-cell), yet the same number of chromosomes is characteristic of all. Indeed, we seek in vain for an analogy to these phenomena and can only admit our entire inability to explain them. No phenomena in the history of the cell more clearly indicate the existence of a morphological organization which, though resting upon, is not to be confounded with, the chemical and molecular structure that underlies it; and this remains true even though we are wholly ignorant what that organization is.

(*b*) *Composition of the Chromosomes.* — We owe to Roux<sup>1</sup> the first clear formulation of the view that the chromosomes, or the chromatin-thread, consist of successive regions or elements that are qualitatively different (p. 244). This hypothesis, which has been accepted by Weismann, Strasburger, and a number of others, lends a peculiar interest to the morphological composition of the chromatic substance. The facts are now well established (1) that in a large number of cases the chromatin-thread consists of a series of granules (chromomeres) embedded in and held together by the linin-substance, (2) that the splitting of the chromosomes is caused by the division of these more elementary bodies, (3) that the chromatin-grains may divide at a time when the spireme is only just beginning to emerge from the reticulum of the resting nucleus. These facts point unmistakably to the conclusion that these granules are perhaps to be regarded as independent morphological elements of a lower grade than the chromosomes. That they are not artifacts or coagulation-products is proved by their uniform size and regular arrangement in the thread, especially when the thread is split. A decisive test of their morphological nature is, however, even more difficult than in the case of the chromosomes; for the chromatin-grains often become apparently fused together so that the chromatin-thread appears perfectly homogeneous, and whether they lose their individuality in this close union is undetermined. Observations on their number are still very scanty, but they point to some very interesting conclusions. In Boveri's figures of the egg-maturation of *Ascaris* each element of the tetrad consists of six chromatin-discs arranged in a linear series (Van Beneden's figures of the same object show at most five) which finally fuse to form an apparently homogeneous body. In the chromosomes of the germ-nuclei the number is at least double this (Van Beneden). Their number has been more carefully followed out in the spermatogenesis of the same animal (variety *bivalens*) by Brauer. At the time the chromatin-grains

<sup>1</sup> *Bedeutung der Kerntheilungsfiguren*, 1883, p. 15.

divide, in the reticulum of the spermatocyte-nucleus, they are very numerous. His figures of the spireme-thread show at first nearly forty granules in linear series (Fig. 120, *B*). Just before the breaking of the thread into two the number is reduced to ten or twelve (Fig. 120, *C*). Just after the division to form the two tetrads the number is four or five (Fig. 120, *D*), which finally fuse into a homogeneous body.<sup>1</sup>

It is certain, therefore, that the number of chromomeres is not constant in a given species, but it is a significant fact that in *Ascaris* the final number, before fusion, appears to be nearly the same (four to six) both in the oögenesis and the spermatogenesis. The facts regarding bivalent and plurivalent chromosomes (p. 87) at once suggest themselves, and one cannot avoid the thought that the smallest chromatin-grains may successively group themselves in larger and larger combinations of which the final term is the chromosome. Whether these combinations are to be regarded as "individuals" is a question which can only lead to a barren play of words. The fact that cannot be escaped is that the history of the chromatin-substance reveals to us, not a homogeneous substance, but a definite morphological organization in which, as through an inverted telescope, we behold a series of more and more elementary groups, the last visible term of which is the smallest chromatin-granule, or nuclear microsomes, beyond which our present optical appliances do not allow us to see. Are these the ultimate dividing units, as Brauer suggests (p. 113)? Here again we may well recall Strasburger's warning, and hesitate to identify the end of the series with the limits reached by our best lenses. Somewhere, however, the series must end in final chromatic units which cannot be further subdivided without the decomposition of chromatin into simpler chemical substances; and these units must be capable of assimilation, growth, and division without loss of their specific character. It is in these ultimate units that we must seek the "qualities," if they exist, postulated in Roux's hypothesis; but the existence of such qualitative differences is a physiological assumption that in no manner prejudices our conclusion regarding the ultimate *morphological* composition of the chromatin.

#### D. CHROMATIN, LININ, AND CYTOPLASM

What, now, is the relation of the chromatin-grains to the linin-network and the cytoplasm? Van Beneden long ago maintained<sup>2</sup> that

<sup>1</sup> Eisen ('99) finds that the chromosomes of the spermatogonia of *Batrachoseps* always consist of six "chromomeres," each of which consists of three smaller granules or "chromi-oles." The latter persist as the chromatin-granules of the resting nucleus; and it is through their successive aggregation that the chromomeres and chromosomes are formed.

<sup>2</sup> '83, pp. 580, 583.

the achromatic network, the nuclear membrane, and the cell-meshwork have essentially the same structure, all consisting of microsomes united by connective substance, and being only "parts of one and the same structure." But, more than this, he asserted that *the chromatic and achromatic microsomes might be transformed into one another, and were therefore of essentially the same morphological nature.* "They pass successively, in the course of the nuclear evolution, through a chromatic or an achromatic stage, according as they imbibe or give off the chromophilous substance."<sup>1</sup> Both these conclusions are borne out by recent researches. Heidenhain ('93, '94), confirmed by Reinke and Schloter, finds that the nuclear network contains granules of two kinds differing in their staining-capacity. The first are the basi-chromatin granules, which stain with the true nuclear dyes (basic tar-colours, etc.), and are identical with the "chromatin-granules" of other authors. The second are the oxychromatin-granules of the linin-network, which stain with the plasma-stains (acid colours, etc.), and are closely similar to those of the cytotreticulum. *These two forms graduate into one another, and are conjectured to be different phases of the same elements.* This conception is furthermore supported by many observations on the behaviour of the nuclear network as a whole. The chromatic substance is known to undergo very great changes in staining-capacity at different periods in the life of the nucleus (p. 338), and is known to vary greatly in bulk. In certain cases a very large amount of the original chromatic network is cast out of the nucleus at the time of the division, and is converted into cytoplasm. And, finally, in studying mitosis in sea-urchin eggs I found reason to conclude ('95, 2) that a considerable part of the linin-network, from which the spindle-fibres are formed, is actually derived from the chromatin.

From the time of the earlier writings of Frommann ('65, '67), Arnold ('67), Heitzmann ('73), and Klein ('78), down to the present, an increasing number of observers have held that the nuclear reticulum is to be conceived as a modification of the same structural basis as that which forms the cytoplasm. The latest researches indicate, indeed, that true chromatin (nuclein) is confined to the nucleus.<sup>2</sup> But the whole weight of the evidence now goes to show that the linin-network is of the same nature as the cell-meshwork, and that the achromatic nuclear membrane is formed as a condensation of the same substance. Many investigators, among whom may be named Frommann, Leydig, Klein, Van Beneden, Carnoy, and Reinke, have described the fibres of both the intra- and extra-nuclear network as terminating in the nuclear membrane; and the membrane itself is described by these and other observers as being itself reticular in structure, and by some (Van Beneden) as consisting of closely crowded

<sup>1</sup> *l.c.* p. 583.

<sup>2</sup> *Cf.* Hammarsten ('95).

microsomes arranged in a network. The clearest evidence is, however, afforded by the origin of the spindle-fibres in mitotic division; for it is now well established that these may be formed either inside or outside the nucleus, and at the close of mitosis the central portion of the spindle appears always to give rise to a portion of the cytoplasm lying between the daughter-nuclei. In such a case as that of the sea-urchin (see above) we have, therefore, evidence of a direct transformation of chromatin into linin-substance, of the latter into spindle-fibres, and, finally, of these into cytoplasm.

When all these facts are placed in connection, we find it difficult to escape the conclusion that no definite line can be drawn between the cytoplasmic granules at one extreme and the chromatin-granules at the other. And inasmuch as the latter are certainly capable of growth and division, we cannot deny the possibility that the former may themselves have, or arise from elements having like powers. But while we may take this as a fair working hypothesis, we should clearly recognize that the base of well-determined fact on which it rests is approached by a circuitous route; that in case of most of the cytoplasmic granules there is not the slightest evidence that they multiply by division; and that even though some of them may have such powers, we cannot regard them as the ultimate structural units, for the latter must be bodies far more minute.

#### E. THE CENTROSOME

From our present point of view the centrosome possesses a peculiar interest as a cell-organ which may be scarcely larger than a cytomicrosome, yet possesses specific physiological properties, assimilates, grows, divides, and may persist from cell to cell without loss of identity. Nearly all observers of the centrosome have found it lying in the cytoplasm, outside the nucleus; but apart from the Protozoa (p. 94) there is at least one well-established case in which it lies within the nucleus, namely, that of *Ascaris*, where Brauer made the interesting discovery that *in one variety (univalens) the centrosome lies inside the nucleus, in the other variety (bivalens) outside*—a fact which proves that its position is non-essential (*cf.* Figs. 120 and 148).

An intra-nuclear origin of the centrosome has also been asserted by Julin ('93) in the primary spermatocytes of *Stylopsis*, by Rückert ('94) in the eggs of *Cyclops*, Mathews ('95) in those of *Asterias*, Carnoy and Le Brun ('97, 2) in *Ascaris*, Van der Stricht ('98) in the eggs of *Thysanozoön*, by R. Hertwig ('98) in *Actinosphaerium*, Calkins ('98, 1) in *Noctiluca*, and Schaudinn ('96, 3) in spore-producing buds of *Acanthocystis*, though in the last-named form the centrosome of the vegetative forms is extra-nuclear (p. 92).



As already stated,<sup>1</sup> it is still undetermined whether a true centrosome may ever arise *de novo*, but the evidence in favour of such a possibility has of late rapidly increased. Carnoy ('86) long since showed that the egg of *Ascaris*, during the formation of the polar bodies, sometimes showed numerous accessory asters scattered through the cytoplasm. Reinke ('94) described somewhat similar asters in peritoneal cells of the salamander, distinguishing among them three orders of magnitude, the largest containing distinct centrosomes or "primary centres," while the smaller contained "secondary" and "tertiary" centres, the last named being single

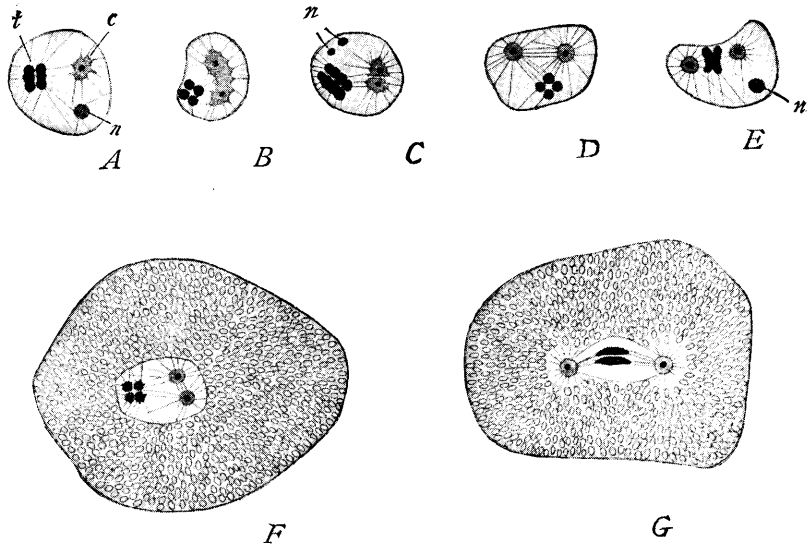


Fig. 148.—Mitosis with intra-nuclear centrosome, in the spermatocytes of *Ascaris megalocephala*, var. *univalens*. [BRAUER.]

A. Nucleus containing a quadruple group or tetrad of chromosomes (*t*), nucleolus (*n*), and centrosome (*c*). B. C. Division of the centrosome. D. E. F. G. Formation of the mitotic figure, centrosomes escaping from the nucleus in G.

microsomes at the nodes of the cytoreticulum. By successive aggregations of the tertiary and secondary centres arise true centrosomes as new formations. Watasé ('94-'95) also finds in the egg of *Macrobdeella*, besides the normal aster containing an undoubted centrosome, numerous smaller asters graduating downwards to such "tertiary asters" as Reinke describes with a microsome at the centre of each, and on this basis concludes that the true centrosome differs from a microsome only in degree and may arise *de novo*. Mottier ('97, 2) finds in pollen-mother-cells numerous minute "cyto-asters" having no direct relation to the spindle-formation (Fig. 133). Again Juel

<sup>1</sup> Cf. pp. 52, 214.

('97) finds that an isolated chromosome, accidentally separated from the equatorial plate (pollen-mother-cells of *Hemerocallis*), may give rise to a small vesicular nucleus which may subsequently divide by mitosis, though it is quite out of relation to the spindle-poles of the preceding mitosis (Fig. 149). Strong evidence of the same character as the last is given by the facts in the heliozoön *Acanthocystis*, as shown by Schaudinn ('96, 3), the ordinary vegetative cells containing a persistent extra-nuclear centrosome, while in the bud-formation of the swarm-spores a centrosome is formed *de novo*, without relation to that of the mother-cell, inside the nucleus of the bud (Fig. 41).

The strongest case in favour of the independent origin of centrosomes is, however, given by the observations of Mead on *Chatopterus* ('98) and the remarkable experiments of R. Hertwig ('95, '96) and

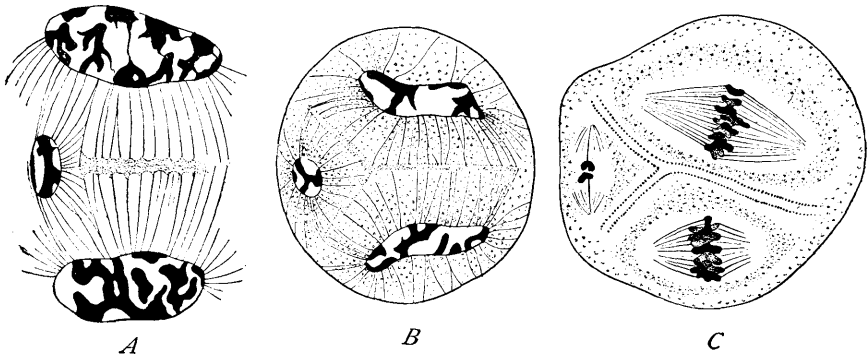


Fig. 149.—Abnormal mitosis in pollen-mother-cells of *Hemerocallis*, showing formation of small nucleus from one or two stray chromosomes and its subsequent division. [JUEL.]

Morgan ('96, 1; '99, 1) on the eggs of echinoderms and other animals. When eggs of *Chatopterus* are taken from the body-cavity and placed in sea-water, a multitude of small asters appear in the cytoplasm, two of which are believed to persist as those of the polar spindle, while the others degenerate (Fig. 150). Mead is therefore convinced that the polar centrosomes arise in this case separately and *de novo*.<sup>1</sup> R. Hertwig showed that when unfertilized eggs of sea-urchins (*Strongylocentrotus*, *Echinus*) are kept for some time in sea-water or treated with dilute solutions of strychnine the nuclei undergo some of

<sup>1</sup> A number of other authors (e.g. Griffin, *Thalassema*, Coe, *Cerebratulus*) have likewise found the first polar asters widely separated at their first appearance. On the other hand, Mathews ('95), whose preparations I have seen, finds the polar centrosomes in *Asterias* close together, and Francotte ('97, '98) has demonstrated that in *Cycloporus* and *Prostheceranus* they arise by the division of a single primary centrosome. The same is stated by Gardiner ('98) to be the case in *Polycharus*. It should be noted, further, that Mead could find no undoubted centrosomes save in the "primary" or definitive polar asters.

the changes of mitosis, the chromatin-network giving rise to a group of chromosomes and a spindle, or more frequently a fan-shaped half-spindle, arising from the achromatic substance. In some cases not only a complete spindle appeared but also asters at the poles, though no centrosomes were observed (Fig. 151). Morgan's experiments along the same lines were mainly performed upon the sea-urchin *Arbacia*, but included also the eggs of *Asterias*, *Sipunculus*, and *Cerebratulus* (Figs. 150, 151). In these eggs numerous asters may arise in the cytoplasm, if they are allowed to lie some time in sea-

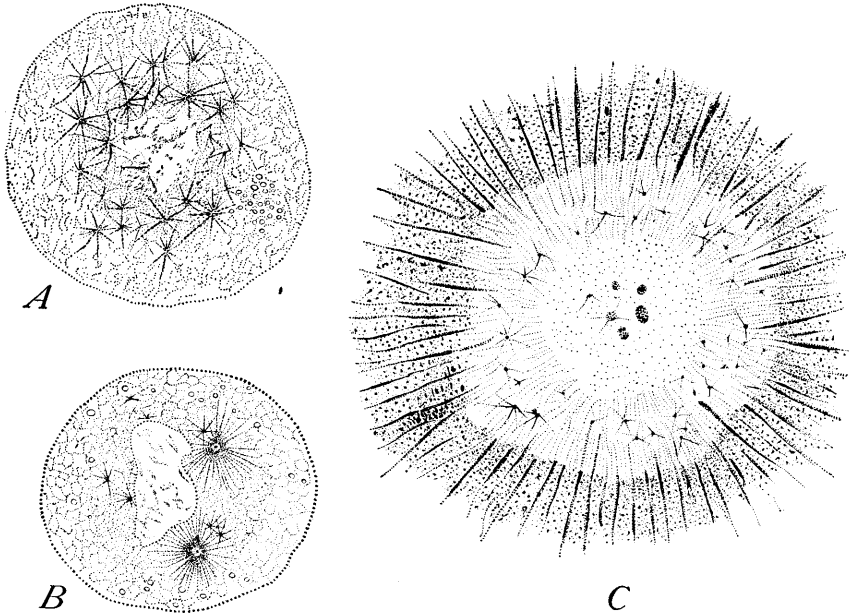
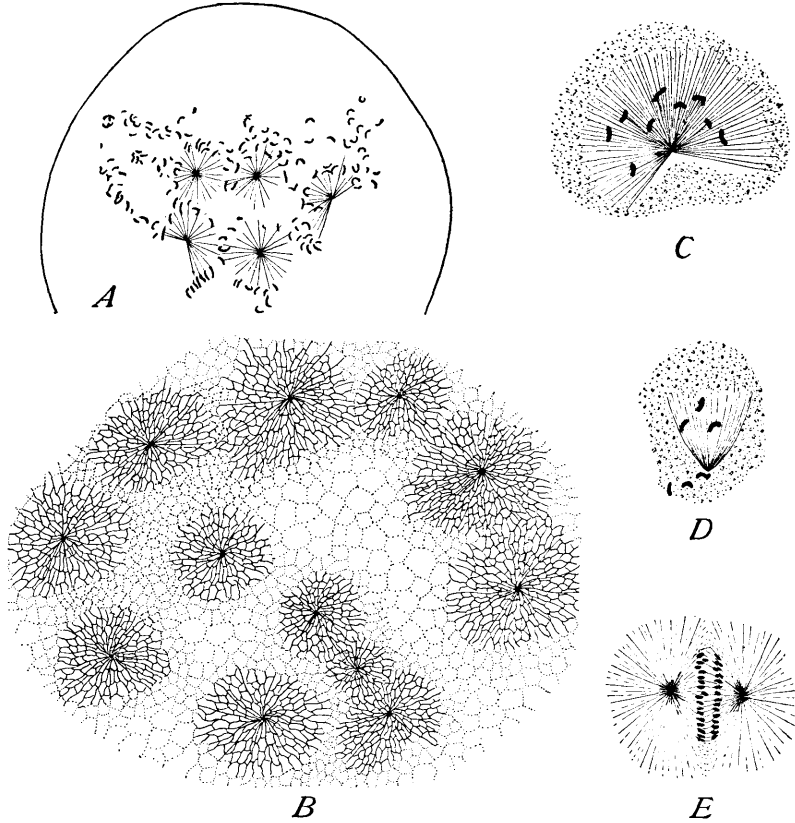


Fig. 150.—Formation *de novo* (?) of centrosomes. [A, B, MEAD; C, MORGAN.]

A. Unfertilized egg of *Chaetopterus* with "secondary asters" developed a few minutes after the egg is placed in sea-water. B. Slightly later stage with two definitive polar asters and centrosomes. C. Large "sun" (transformed polar aster) containing numerous small "secondary asters" and centrosomes, from unfertilized egg of *Cerebratulus* after 22 hours in 1.5% sodium chloride solution.

water or treated by weak solutions of sodium or magnesium chloride. These asters often contain deeply staining, central granules indistinguishable from the centrosomes of the normal asters; and, what is of high interest, such of them as lie near the nucleus take part in the irregular nuclear division that ensues, forming centres toward which the chromosomes pass. These divisions continue for some time, the chromosomes being irregularly distributed through the egg, and giving rise to nuclei of various sizes apparently dependent upon the number of chromosomes each receives. After a variable number of such

divisions the asters disappear, yet the irregular nuclear divisions continue, nuclear spindles with distinct centrosomes being formed at each division, but apparently without relation to the older asters, and they



**Fig. 151.**—Formation of centrosomes and asters in unfertilized echinoderm-eggs. [A, B, MORGAN ; C-E, R. HERTWIG.]

A. *Arbacia*, after 4½ hours in 1.5 % solution of sodium chloride, then 5 hours in sea-water; scattered chromosomes and asters. B. Asters formed after 6½ hours in NaCl. C-E. *Echinus* after treatment with 0.5 % strychnine-solution, showing various forms of astral formations (fan-shaped aster, half spindle, and complete mitotic figure).

are believed by Morgan to arise *de novo* from the egg substance.<sup>1</sup> In the meantime irregular cleavage of the egg occurs, though no embryo is produced.<sup>2</sup> Loeb, however, in the remarkable experiments

<sup>1</sup> '99, p. 479.

<sup>2</sup> Morgan makes the important observation, which harmonizes with that of Boveri, reported at page 108, that the divisions occur with respect to the number and position of the nuclei, not of the asters, concluding that the former must therefore play an essential rôle as centres of division, and that the activity of the asters is in itself not sufficient to account for division of the cytoplasm.