

**Chromidia and the Binuclearity Hypotheses :
A Review and a Criticism.**

By

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With 25 Text-figures.

SINCE the seed of the chromidia hypothesis was sown by Richard Hertwig in 1902, it has displayed such an amazing ability to absorb new or previously uncorrelated facts, for its own growth, that it now—in its more mature form—stands out as one of the most conspicuous objects in the whole wide field of cytology. And it has not—one may be allowed to think—merely flourished on the soil where none other could take root: it has also, in so doing, thrown into the shade many a less showy upgrowth. Yet it is not beyond the bounds of possibility that these smaller growths, being rooted in a firmer foundation of facts, may remain to ripen long after the chromidia hypothesis has fallen to the earth—from the sheer weight of its own overgrowth and the insecurity of the ground in which it grew.

The chromidia hypothesis took origin in protozoology. But it has since pushed out its roots so far that they now extend and ramify in other domains of zoology, and bacteriology. The result is that it is very difficult to view in its entirety.

A most important offshoot from the original conception of chromidia has been a hypothesis of the binuclear nature of

the cell—a hypothesis which has been most ably advocated by Goldschmidt. This hypothesis of binuclearity,¹ as I shall call it, does not stand alone. There is at least one rival hypothesis which also seeks to demonstrate the double nature of the cell nucleus.

Now to comprehend the chromidial hypothesis and its closely-connected conceptions of binuclearity² it is necessary to be familiar with a very large part of the modern literature of protistology, and also with much cytological research in general; because the branches of the chromidia hypothesis have become twisted and tangled among the branches of the neighbouring binuclearity hypothesis—so much so, in fact, that it is nearly impossible to find out where one ends and another begins. The only sure way is to trace the offshoots from the parent stem.

It will be my aim in this essay to set out briefly and baldly all the main facts regarding chromidia, and to make such deductions as seem justifiable; afterwards, to discuss the hypotheses based on these facts; and finally—as this will involve a discussion of one binuclearity hypothesis—to criticise the other binuclearity hypotheses which are at present often confused with the idea of chromidia. To this end I have endeavoured to discover and verify facts wherever possible for myself. But my main source of information has naturally been the immense cytological literature which has grown up in the last few years. From its very size it would, of course, be quite impossible to enter into details in a short space. But I shall try, by selecting the most important points, to place the essential facts side by side in such a way that the value of the hypotheses arising from them will become evident. I wish to show that prevailing opinions are

¹ I have used the word "binuclearity" as an English translation of the various expressions commonly used in Germany, e. g. "Doppelkernigkeit," "Kerndualismus," "Kernduplizität," "Kerndimorphismus," "Binuklearität."

² Already these hypotheses are occasionally honoured with the name of "theory"—and latterly even "law"!

not too firmly founded, and that a critical review of the facts does not justify all the inferences which have been drawn from them.

My object therefore is to discuss first the facts, secondly the speculations based upon them; endeavouring, by selecting the essential, to sacrifice detail for the sake of brevity.

TERMINOLOGY.

Before going any further I must define my terms. I shall use throughout only the two names introduced by Hertwig ('02), namely, chromidia and chromidial net (Chromidien, Chromidialnetz). Other terms are superfluous. By chromidia I understand any fragments of chromatin—irrespective of their shape or function—which lie freely in a cell,¹ without being massed together into a definite nucleus.² By chromidial net I understand any netlike arrangement of chromatin lying freely in the cytoplasm—regardless of its function. Both terms are purely morphological. It is sometimes convenient to speak of a whole system of chromidia—considered as a unit—in the singular number, as a chromidium.

Of other terms which have been used the following are the most important. Goldschmidt ('04a) employs the terms chromidia in the wider sense, for all chromidial structures of unknown function; chromidia (*sensu stricto*) for chromidia taking part in the vegetative functions of the cell; sporetia for chromidia which take part in forming gametes. This nomenclature has a physiological basis, and is difficult to use—except in a very few cases—owing to our present ignorance. Goldschmidt also introduced the term chromidial apparatus for any system of chromidia.

Mesnil ('05) uses a terminology which also has a physio-

¹ In the widest sense of the term.

² With Schaudinn I believe the nucleus should be defined morphologically. The above definition is not intended to embrace chromatin particles of extraneous origin (e. g. ingested bodies).

logical foundation; chromidia, used generally, like Goldschmidt's "chromidia in the wider sense"; trophochromidia, for chromidial structures of a vegetative function; idiochromidia, for chromidia which enter into the formation of gametes.¹

Schaudinn's ('05) three parallel terms are chromidia, somato-chromidia, gameto-chromidia. Other writers use various paraphrases of these, such as "somatic chromidia," "trophic chromidia," "vegetative chromidia"; and "gametic chromidia," "generative chromidia," "propagative chromidia," etc.

I will mention only one more term, used by Calkins ('05)—protogonoplasm. This unwieldy word is used to designate chromidia taking part in gamete formation. The self-explanatory term "distributed nucleus" is also used by this writer, though similar expressions (e. g. "diffuse nucleus") have long been in use.

I.

I will now endeavour to summarise the state of our knowledge regarding the existence of chromidia and their probable function in the Protista (Protozoa and Bacteria) and Metazoa. My aim here is to give facts, and to steer clear of hypothesis for the present.

(A) CHROMIDIA IN PROTOZOA.

(1) I will begin with the Heliozoa, as the chromidia hypotheses largely took root in this group. I refer, of course, to the magnificent researches of R. Hertwig on *Actinosphaerium*. From the immense mass of detail discovered by Hertwig and his school I select the following facts:

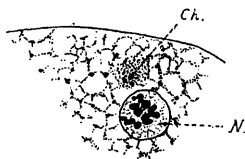
Hertwig ('99a) gave the first description of chromidia in

¹ Cf. Lubosch's ('02) terms, "trophochromatin" and "idiochromatin."

Actinosphaerium (text-fig. 1). They are in the form of chromatin strands or granules lying in the cytoplasm, and are formed from the nuclei. Their formation may be induced either by over-feeding or by starving the animal. They are simply metabolic products—explicable, perhaps, by Hertwig's "Kernplasmarelationtheorie" (cf. Hertwig, '03, etc.). Hertwig named them "chromidia" in 1902. He further found that, during degeneration, the nuclei of Actinosphaerium became enormously enlarged and hyperchromatic, and finally underwent fragmentation into chromidia (Hertwig, '00, '04; Howard, '08). These are the essentials.¹

(2) Let us pass on to the Thalamophora. Hertwig ('87)

TEXT-FIG. 1.



A portion of an Actinosphaerium in a chromidial condition.

N. nucleus; *Ch.* chromidia, formed from the nuclear chromatin. (The entire cytoplasm is filled with chromatin fragments lying in the walls of the alveoli.) (After R. Hertwig, '04.)

noted in *Arcella* an arrangement of extra-nuclear chromatin similar to that which he had already recorded in *Radiolaria* (vide infra). He described a "nuclear band" in addition to the vegetative nuclei.

Chromidia were discovered in *Polystomella* by Lister ('94, '95), but he was unable to decide upon their significance. Rhumbler ('94) probably observed chromidia in *Saccamina*, but was likewise unable to interpret their meaning. The chromidia in *Polystomella* were also seen by Schaudinn ('95a).

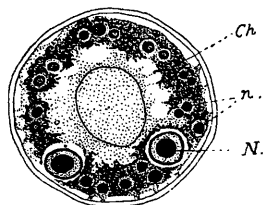
In 1899 Hertwig succeeded in fully tracing the develop-

¹ Similar processes occur in *Actinophrys* also (Distaso, '08).

ment of secondary nuclei from the chromidial mass—or, as he then called it, “the extra-nuclear chromatin net” of *Arcella* (text-fig. 2). And it has since been shown by Elpatiewsky ('07) that the macro- and micro-amœbæ, into whose formation the secondary nuclei enter, are gametes which conjugate in pairs.¹

When Hertwig ('02) introduced the name “chromidial net” for this extra-nuclear chromatin in *Thalamophora* its real meaning was still quite obscure. The riddle was solved by Schaudinn ('03). He found that the chromidial net (in *Polystomella*, *Centropyxis*, and *Chlamydothryx*) is a mass of chromatin—probably derived in the first instance

TEXT-FIG. 2.



Arcella vulgaris. *N.* primary nucleus; *Ch.* chromidium (extra-nuclear chromatin), in which the secondary nuclei (*n.*) are forming. (After R. Hertwig, '99.)

from the nucleus—which finally gives rise to the nuclei of minute gametes, which conjugate in pairs.

Other workers have extended Schaudinn's observations. In *Diffugia* (Zülzer, '04; Awerinzew, '06) the chromidia give origin to secondary nuclei,² which later enter into the

¹ Since this paper was written the interesting work of Swarczewsky ('08) on *Arcella* has appeared. In addition to confirming previous observations, this observer has found that a kind of conjugation (“chromidiogamy”) may take place between the entire chromidial masses of two individuals. A phenomenon to some extent parallel occurs in the giant sporic Bacteria, *B. bütschlii* (Schaudinn, '02) and *B. flexilis* (Dobell, '08a).

² And also form glycogen (Zülzer).

composition of gametes. A similar condition appears to prevail in *Euglypha*, *Trinema*, *Hyalosphenia*, *Nebela*, etc. (Awerinzew, '06).

Schaudinn's observations on *Polystomella* have been largely confirmed also in the case of *Peneroplis* (Winter, '07). Lister ('06) has already given a brief review of the nuclear phenomena in the Foraminifera.

Recently Doflein ('07) has re-examined many Thalamophora—namely, *Arcella* (2 species), *Platoum*, *Euglypha* (2 sp.), *Trinema*, *Gromiella*, *Lecquereusia*, *Nebela* (2 sp.), *Diffugia* (5 sp.), *Pseudodiffugia*, *Centropyxis*, *Cochliopodium*. A chromidial net was found in all, though its nuclear origin was not clearly made out. Its form shows great variation, being sometimes compact, sometimes diffuse. And it also varies considerably as regards the relative quantities of plastin and chromatin present in it. On the whole it seems that the chromidial net of the Thalamophora is a structure of nuclear origin whose chief purpose is to supply gamete nuclei.

(3) *Amœbina*.—Amongst the amœbæ three forms have received special attention—*Entamœba coli*, *Peloxyma*, *Amœba proteus*.

In the first, *Entamœba coli* Loesch, Schaudinn ('03) found that an autogamy takes place, in which chromidia play a part. Two daughter-nuclei in an encysted animal break up into chromidia, which are subsequently, in part, eliminated. The remaining chromidia mass themselves together to form two new nuclei, which, after each giving off two "polar bodies," become progamete nuclei. Each then divides, giving two gamete nuclei, which fuse in opposite pairs, to form two zygote nuclei.

It is unfortunate that the recent confirmation of much of this remarkable work by Wenyon ('07), in *E. muris*, has failed to corroborate the details of the history of the chromidia.

Entamœba histolytica (Schaudinn, '03) appears to have a chromidial net like that seen in the Thalamophora.

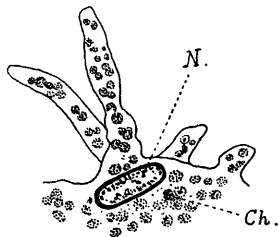
Chromidia were first found in *Pelomyxa* by Goldschmidt ('05). His discovery was confirmed by Bott ('06), who agreed that they were products of the nucleus, like those of *Actinosphærium*. They are produced when the animal hungers. But Bott was able to show further that chromidia play an important rôle in sexual reproduction. All the nuclei fragment, forming "somato-generative chromidia," of which a part degenerates and is cast out. The rest increase in size and form new nuclei, which—after eliminating more chromatin in the form of chromidia, and undergoing certain changes—give rise to gamete nuclei. Thus, in its essential points, gametogenesis in this creature resembles that of *Entamoeba coli*.

Chromidia have been described in *Amoeba proteus* by Calkins ('05). The nucleus was said to divide by mitosis,¹ until, after repeated division, a multinucleate condition of the cell resulted. These "primary nuclei" then broke up into "secondary nuclei" (by chromidia formation), and the "secondary nuclei" divided to form the hypothetical gamete nuclei. Since publishing this description Calkins has re-investigated the same material upon which these "evidences of a sexual cycle" were based. He now (Calkins, '07) comes to a quite different interpretation, and claims to have discovered the "fertilisation" of *Amoeba*. The "secondary nuclei" are now said not to divide, but to fuse in pairs—thus undergoing a kind of autogamy. I do not wish to enter into a long discussion of this matter, but I must point out—as the fate of the chromidia bears upon the present subject—that Calkins' account is, by his own showing, impossible to accept. Apart from the fact that the whole story is based upon only a few preserved specimens, there are serious discrepancies in

¹ The "mitosis," as far as one can judge from Calkins' figures, is quite unlike mitosis as usually understood. Awerinzew, moreover, has described and figured in detail the mitosis of this organism. Judging from my own impressions and from Awerinzew's description, I am inclined to believe that Calkins' figures do not represent division stages at all.

his two accounts. When he now desires to show that the secondary nuclei fuse and do not divide, he adduces as evidence—inter alia—the statements that “if the nuclei were dividing we should find dumb-bell shaped figures with the diameter of the nuclei drawn out at right angles to the plane of division. This is not the case. . . . We should expect to find connecting strands of chromatin substance between the recently divided karyosomes . . . but no such connecting strands exist. . . . We should expect to find the daughter-karyosomes elongated in the axis at right angles to the plane of division. . . . Such is not the case.” How

TEXT-FIG. 3.



Part of an *Amoeba proteus*, containing “chromidia” (gametes of *Allogromia*). *N.* nucleus; *Ch.* “chromidia.” (After Prandtl, '07.)

are we to accept such statements, when, to prove that the nuclei were dividing, he originally not only described but figured all these stages of which he now denies the existence? (See Calkins, '05, Pl. 3, fig. 23.) So sure was he of this division that he even called it “a modified mitosis,” and described the karyosome as a division centre, like the nucleolo-centrosome of *Euglena* (text-fig. 25).

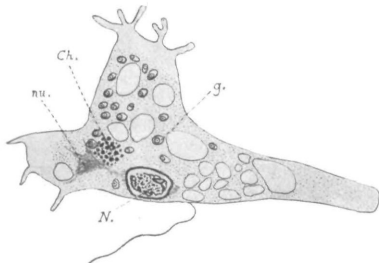
As Prandtl ('07a) has pointed out, Calkins' “gametes” of *Amoeba* are probably the gametes of parasites allied to *Allogromia*, whose remarkable life-history Prandtl carefully worked out. I cannot at all agree with Calkins in saying that if his secondary nuclei “are parasites, then the secondary

nuclei of *Arcella*, *Polystomella* and *Entamoeba* must likewise be parasites." Nor even from his description can I regard the "fertilisation" of *Amoeba proteus* as "strikingly similar to that of *Entamoeba coli*." The sexual phase—if it exist—in *Amoeba proteus* remains still unknown.

The facts about "chromidia" in *Amoeba* are therefore much too doubtful to allow of any profitable discussion at present.¹

(4) *Rhizomastigina*.—In the mastigamœbæ (*Masti-*

TEXT-FIG. 4.



Mastigella vitrea Goldschmidt, (a mastigamœba). *N.* nucleus; *Ch.* chromidia; *nu.* nucleolar substance; *g.* fully-formed gamete. (Modified from Goldschmidt, '07.)

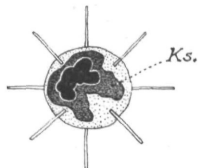
gella and *Mastigina*) we have one of the most carefully described cases of chromidia formation (Goldschmidt, '07). Chromidia—consisting of both "nucleolar substance" and chromatin—are extruded from the nucleus. In the cytoplasm they become aggregated at certain points and form gamete nuclei (text-fig. 4). The main nucleus remains behind, for a greater or less period, but in the end perishes.

(5) *Radiolaria*.—A structure like the chromidial net of *Thalamophora* was long ago described in *Acanthometrids* by

¹ Chromidia are described in *A. diploidea* (Hartmann and Nägler, '08) and some other species, but their significance seems to me to be very questionable.

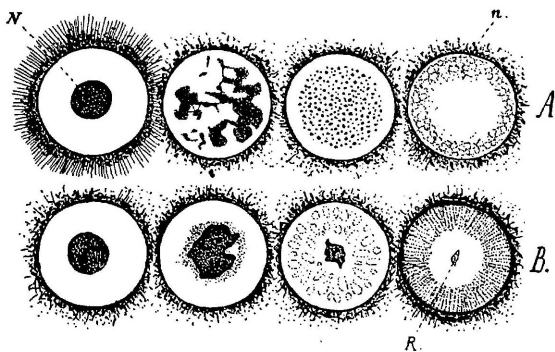
Hertwig ('79) as a "Kernrindenschicht" (text-fig. 5). Secondary nuclei (? gamete nuclei in all probability) are differen-

TEXT-FIG. 5.



A radiolarian, *Acanthochiasma krohnii*, showing the remarkable cortical layer ("Kernrindenschicht," *Ks.*) of the nucleus. This is probably the homologue of the chromidium of *Thalamophora*. (After R. Hertwig, '79.)

TEXT-FIG. 6.



Chromidia in a radiolarian—*Thalassicolla*. *A*, formation of isospores; *B*, of anisospores (probably gametes). In both cases the primary nucleus (*N*.) breaks up into chromidia, which give rise to secondary nuclei (*n*.) entering into the formation of the swarm-spores. In the formation of anisospores, a part of the nucleus remains behind (*R*.). The drawings are of the central capsule of the organism. (From Brandt, modified.)

tiated from it in subsequent development, just as in *Arcella*, etc. (Hertwig, Porta).

The formation of zoospores in Radiolaria was described by Hertwig, but in more detail by Brandt, whose results have become fully known during only the last few years ('02, '05). It appears from his researches (e. g. in *Thalassicolla*) that the entire nucleus fragments into chromidia, which later form the nuclei of isospores (asexual reproduction). But in the formation of anisospores (probably gametes) only a part of the nuclear material goes into chromidia, which subsequently form the nuclei of the swarmers. The nucleolus stays behind and perishes with the remains of the parent organism (cf. *mastigamœbæ*). (Text-fig. 6.)

This account has received confirmation from the work of

TEXT-FIG. 7.



Part of a plasmodium of *Plasmodiophora brassicæ*.
N. nucleus; C. chromidia. (After Prowazek, '05.)

Schouteden ('07), who was the first to bring these phenomena into line with the other work on chromidia.

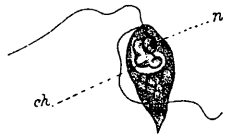
(6) *Mycetozoa*.—The chief work on chromidia in this group has been done by Prowazek ('04a, '05). He has found that the nuclei in the plasmodium of *Plasmodiophora* at one period in their development give up chromatin—in the form of chromidia—into the cytoplasm, and then after undergoing further changes give rise to gamete nuclei (text-fig. 7). Conjugation takes place as the spores are formed. Chromidia therefore take part in the vegetative existence of the organism. The sexual process in other *Mycetozoa* is not very well known. But recent work (Pinoy, '08) has shown that in one case at least (*Didymium*) there exist sexually differentiated plasmodia from the first.

(7) *Mastigophora*.—Chromidia have been described in

several flagellates. Prowazek ('03) recorded the presence of a "chromidium" in *Bicosœca*. He subsequently ('04) found a similar body in *Bodo lacertæ*. This structure lies near the nucleus, but it is difficult to see why it is called a "chromidium." Of its origin and fate nothing is known. It stains (in *Bodo*) with iron-haematoxylin but not with other chromatin stains, and perhaps consists of plastin¹ (text-fig. 8).

Prowazek has further described ('04) the formation of "chromidia" as a preliminary to a remarkable process of autogamy in *Bodo*. I will not discuss this further here as I have gone into the matter more fully elsewhere (Dobell, '08c). Suffice it to say that Prowazek probably mistook stages in

TEXT-FIG. 8.



Bodo lacertæ, from a preparation stained with hæmatoxylin and eosin. The so-called "chromidium" (*ch.*) is stained bright red, in striking contrast with the violet nucleus (*n.*). (Original.)

the development of yeast-like organisms for stages in the life-history of *Bodo*. The "chromidia" are reserve material. At all events the existence of chromidia in this animal is very doubtful.

Chromidia are said to play a part in the life-history of *Hæmoproteus* (*Trypanosoma*) *noctuæ*, (Schaudinn, '04, '05). They appear to be of a metabolic nature, as in *Actinosphærium* (cf. pp. 282, 283).

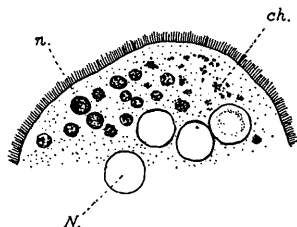
There are some other cases of chromidia recorded in flagellates, but they are not very satisfactory. In *Joenia*

¹ In Rhizopods the chromidial net may consist largely of plastin, and contain very little chromatin, so possibly this structure in *Bodo* is of a similar nature. Cf. Doflein ('07): "In *Trinema* conditions occur in which the chromidial body fills the apical part of the delicate shell as an almost compact, uniform mass of plastin."

(Grassi and Foà, '04), chromidia are described in the ordinary vegetative animal, but no particulars of their origin or function have been given. Perhaps they are really food bodies. Calkins ('98) has described the nucleus of *Tetramitus* as having its chromatin scattered through the cytoplasm during resting stages. This has never been confirmed, and I think it quite possible that the "chromidia" are here also merely ingested food masses, which often stain very strongly in such flagellates.

Awerinzew ('07) says that a part of the chromatin—in the

TEXT-FIG. 9.



Opalina: part of an individual which is preparing to form gametes. *N.* primary nucleus, which has given up most of its chromatin as chromidia (*ch.*). The latter, by aggregation at various points, give rise to the secondary nuclei (*n.*). (Modified from Neresheimer, '07.)

resting animal—is in the form of chromidia in *Chilomonas*. Prowazek ('07a) contests this, and believes Awerinzew's specimens were badly fixed. He himself ('03) found no chromidia in this animal.

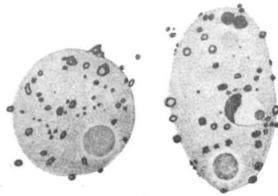
Quite recently Swellengrebel ('08) has found granules of "volutine" (A. Meyer) in *Trypanosoma*. He says: "It is evident these granules of volutine, from their nuclear origin, ought to be considered as chromidia." With this I cannot agree. They are not chromatin, therefore to my mind they are not chromidia.

On the whole the chromidia of flagellates are at present of

too doubtful a nature to allow of any profitable discussion regarding them.

(8) *Ciliata*.—The best instance of chromidia playing a part in the life-cycle of a ciliate is to be seen in *Opalina*, (Neresheimer, '07) (text-fig. 9). At a certain period in its development *Opalina* extrudes chromidia from its nuclei into the cytoplasm. The chromidia then collect themselves at various points, and so build up new nuclei—the original nuclei perishing. These secondary nuclei, after undergoing a chromatin reduction, become the nuclei of gametes. The history of the chromidia in this animal is therefore rather like a multiple version of that in *Thalamophora*.

TEXT-FIG. 10.



Degenerating fragments of *Opalina*, with nuclei in a chromidial condition. (The large bodies surrounded by a pale area are "eosinophil" bodies.) (After Dobell, '07a.)

Chromidia are also formed in *Opalina*—as in many other Protozoa—during degeneration (Dobell, '07a) (text-fig. 10).

Gonder ('05) has given a description of remarkable chromidial phenomena in *Opalinopsis* and *Chromidina*. I have re-investigated these forms (Dobell, '08d) and arrived at a very different conclusion from Gonder's. There is no complicated series of chromidial changes in *Opalinopsis* during division. The nucleus is in the form of a network ("chromidial net" if one likes to call it so, though there is no evidence that it is in any way homologous with the chromidial net of *Thalamophora*), and remains so during division. In *Chromidina* the nucleus is also in the form of

a net (text-fig. 11). The "chromidia" in these two forms are in part ingested food material and in part appearances due to imperfect fixation—artifacts. As I have already discussed the matter elsewhere I will say no more about it here.

The only other case of chromidia which need be considered in this group is that of *Cryptochilum*. It is stated by Russo and Di Mauro ('05a) that there is a chromidial net in the posterior region of this holotrichous infusorian. But they have also described ('05) the fragmentation and digestion of the macro-nucleus in the same region. Is the "chromidium" merely the degenerated and broken-up macronucleus? It is impossible to say from their account. Further, they have described ('05b) the conjugation of this animal, but without

TEXT-FIG. 11.



Chromidina elegans, an infusorian having its nuclear apparatus in the form of a network. (Original.)

enlightening us as to the rôle of the chromidium—which is neither mentioned nor figured. It may be that it is either a worn-out remnant of the macronucleus, or possibly a mass of ingested food bodies. It is useless to attempt to argue about it before we have more definite data.

(9) Sporozoa.—There are some good examples of chromidia formation in this class of Protozoa. I select the following. In *Eimeria schubergi* (Schaudinn, '00) the nucleus of the micro-gametocyte undergoes an analysis into chromidia, which become aggregated at various points at the periphery of the organism and so synthesise the chromatin microgametes. A similar process takes place in *Adelea* (Dobell, '07) (text-fig. 12), but here a chromidial network is formed. In this form also, formation of macromerozoites from a macroschizont is accompanied by a series of nuclear changes analogous to those just noticed in *E. schubergi* (Siedlecki, '99, Dobell, 07).

The formation of secondary nuclei from chromidia has been described in *Lymphocystis* (see Awerinzew, '08). The same kind of nuclear phenomenon has, in addition, been described by Siedlecki ('98) in *Aggregata* (*Klossia*, *Eucoccidium*, etc.), during the formation of sporoblasts and microgametes. Recently this has been challenged by Moroff ('08), who has described most remarkable chromidial formations, centrosomes, etc., and based a number of speculations thereupon. I have been engaged in studying these parasites for some time past, and hope to be able to consider Moroff's work in detail later. For the present I will merely

TEXT-FIG. 12.

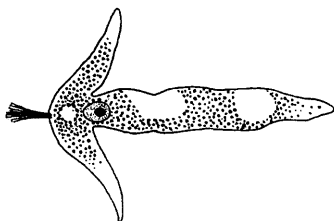
Formation of microgametes in *Adelea ovata*. (After Dobell, '07.)

say that, in most respects, my work so far confirms and amplifies that of Siedlecki. Moroff's "chromidia," etc., are to my mind in great part artifacts, due to defective cytological methods.

The Gregarines furnish many examples of chromidia. Chromatin particles in the cytoplasm have been noticed by many observers, in many different species, for a long time past. They vary greatly in amount. A very good instance has been described and figured by Cecconi ('03) in *Anchorina*, but he was unable to discover their origin or significance (text-fig. 13).

According to Drzewiecki ('03) most remarkable nuclear phenomena occur in *Monocystis*. In the vegetative period of development the nucleus is said to undergo complete fragmentation into chromidia. A new nucleus is then gradually

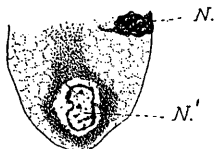
TEXT-FIG. 13.



Anchorina sagittata, a gregarine. The protoplasm is filled with "chromatophile granules" (chromidia). (After Cecconi, '05.)

built up from new chromidia, which make their appearance in the cytoplasm—the first-formed chromidia disappearing (text-fig. 14). Drzewiecki ('07) has lately described a similar phenomenon in *Stomatophora*, introducing new terms into his

TEXT-FIG. 14.



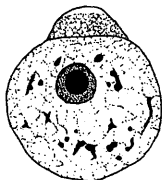
Posterior end of a gregarine, *Stomatophora coronata*. The original nucleus (*N.*) has broken up, and a new nucleus (*N'.*) is in process of formation from chromidia in the cytoplasm (?). (After Drzewiecki, '07.)

description ("nucleoids," "chromatogens," etc.). His account is based entirely on the study of fixed and stained specimens—in the second paper, on the study of a single preparation stained by Heidenhain's method! The results have been regarded with some scepticism already (e. g. by

Lühe, '04), and I think it is almost certain, from the recent work of Kuschakewitsch ('07), that Drzewiecki has arrived at his results by combining a series of degeneration phenomena. At all events, Drzewiecki's account stands in need of confirmation, and cannot be accepted at present.

It appeared from the work of Léger ('04) and others, that the chromidia of gregarines were probably the same sort of thing as those of *Actinosphaerium*. But the most careful recent work—that of Comes ('07)—has put the matter in a different light. Comes studied *Stylorhynchus* and *Stenophora* (text-fig. 15). He observed the chromidial changes

TEXT-FIG. 15.



A small *Stenophora juli*, showing deeply stained particles (chromidia) in the cytoplasm. (From a borax-carminé preparation. [Original.])

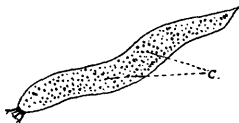
which occurred with change of nutrition, temperature and season. The important fact brought out by this study is that the chromidia are not of nuclear origin—they are metabolic products in the cytoplasm. Their part is played in the vegetative life of the organism. In view of these facts it is obvious that the chromidia of gregarines require cautious consideration in relation to the nucleus.

Before passing to the bacteria, I may here note the nuclear apparatus of a very remarkable, and as yet unclassifiable, organism—*Siedleckia nematoides* (Caullery and Mesnil, '98, '99). I have lately studied this parasite, from a new host, *Aricia foetida*. *Siedleckia* contains small chromatin

masses, whose number varies according to the size of the animal, and which multiply by a simple division (text-fig. 16). They cannot properly be called nuclei. They should be regarded, I think, as composing a nuclear apparatus consisting of scattered fragments of chromatin—a chromidial system—as in some bacteria (e. g. *B. flexilis*, Dobell, '08a). In large animals they are present in immense numbers, but at no period do they—individually—possess the attributes of a formed nucleus.

In some Protozoa nuclear reduction by chromidia formation takes place in a gamete preparatory to conjugation (e. g. macrogametocyte of *Adelea* (Siedlecki, '99), and in

TEXT-FIG. 16.



Large *Siedleckia nematoides* (from *Aricia fœtida*).
C. chromatin fragments in the cytoplasm. (Original.)

Monas (Prowazek, '03). Their meaning is bound up with the general problem of nuclear reduction, and I shall say no more about it here.

(B) CHROMIDIA IN BACTERIA.

In spite of the great discussion which has raged—and still rages—round the problem of the bacterial nucleus, there is a large and growing body of evidence to show that some, at least, of the granular inclusions in bacteria consist of chromatin (cf. Guilliermond, '07). In part, however, the granules ("metachromic granules," "red granules," "volutine granules," etc.) probably consist of some reserve material (cf. Guilliermond, '06, '07). It can hence be said that certain bacteria¹

¹ And probably also Cyanophyceæ.

have their chromatin in a chromidial condition. (Cf. also the morphology of *Achromatium*, as carefully studied by Schewiakoff, '93.)

In large bacteria which have been carefully studied, the chromidia are seen to come together to form a nucleus-like body during spore formation (cf. Schaudinn '02, '03a; Dobell, '08; Guilliermond, '08, etc.) (text-fig. 17).

It appears equally certain, however, that some bacteria—or organisms at present classified as such—possess a well-differentiated nucleus, and not chromidia (Vejdovský, Mencl, etc.). The nucleus may sometimes be in the form of a filament or otherwise modified.

So much for the true bacteria. We may here consider, as an appendix to them, that interesting little group of protists,

TEXT-FIG. 17.



Bacillus flexilis. The nuclear apparatus is seen to consist of chromatin particles scattered through the cytoplasm. (Original)

the spirochæts. In some, at least, of these the chromatin appears to be arranged, wholly or in part, in the form of chromidia. I will give *Spirochæta plicatilis* as an instance. In this organism, "The nuclear apparatus consists of a thread-like structure running in the long axis . . . whilst the vegetative nuclear mass surrounds this thread in the form of granular chromidia" (Schaudinn, '05a).

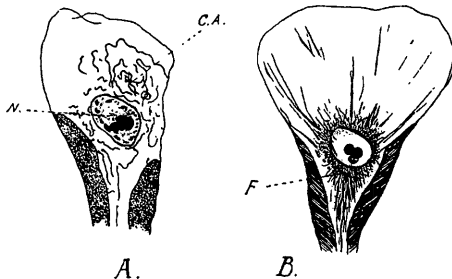
(c) CHROMIDIA IN METAZOA.

Descriptions of free chromatin particles in metazoan cells—homologized with the chromidia of the Protista—are not few. The two most important cases—the two which I shall chiefly discuss here—are the chromidia of the tissue-cells of nematodes, and the chromidia in the gametogenesis of gastropods. These are the mainstays of the arguments, in favour of the chromidia hypotheses, derived from multicellular organisms.

The Chromidia of Nematodes.—Goldschmidt ('04, '04a), has described at considerable length certain curious chromatin strands, which occur in various tissue-cells—especially muscle-cells—of *Ascaris*. These structures he calls the chromidial apparatus of the cell. Upon them Goldschmidt's binuclearity speculations are largely founded.

The chromidial apparatus is said to consist of chromatin extruded from the nucleus when the cell is in a state of activity—the amount of chromatin being an index of the

TEXT-FIG. 18.



- A. A muscle-cell of *Ascaris lumbricoides*, after one hour's tetanus, showing the "chromidial apparatus" (C.A.), which is supposed to have come from the nucleus (N.). (In the original figure—from a hæmatoxylin preparation—the nucleus is coloured violet, the "chromidia" black.) (After Goldschmidt, '05.)
- B. A muscle-cell of *Ascaris ensicaudata*, showing the supporting framework (F.) in the cytoplasm. (After Vějdovský '07.)

Both figures are from transverse sections, so that only a part of the cytoplasmic structures is seen.

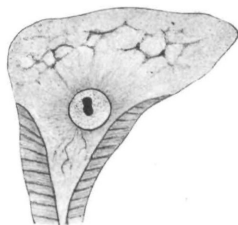
degree of activity of the cell. Thus, when an *Ascaris*¹ is stimulated to violent muscular movement, the chromidial apparatus is found more strongly developed in the cell (text-fig. 18).

Leaving out of the question for the moment the vast edifice of speculation which Goldschmidt has erected on these obser-

¹ *A. lumbricoides* and *A. megaloccephala* were used.

vations, we must inquire, "What is this chromidial apparatus?" The evidence that it is chromatin from the nucleus is not—to me—convincing, but it has been widely accepted. The most important evidence yet brought forward in opposition to Goldschmidt is that of Vejdovský ('07). This investigator—and his opinion is of special weight, owing to his long experience in matters of vermian cytology—has examined another species of *Ascaris* (*A. ensicaudata*) with this result. He finds¹ remarkable fibrillar structures, which "must be regarded as only a supporting framework" of the cell. He believes that Goldschmidt's "chromidia" are merely broken

TEXT-FIG. 19.



Muscle-cell of *Ascaris lumbricoides*, showing structure of cytoplasm in a fixed and stained cell. (Original.)

up parts of this fibrillar system—in reality artifacts due to the methods employed. (Cf. fig. 18.) As he himself concisely expresses it, "The chromidial apparatus described by Goldschmidt represents the strands of the 'normal' fibrillar framework—much damaged and torn as a result of the violent action of the reagents employed—which is probably derived from the original ray-system of the centroplasm." (Vejdovský, '07, p. 89, and cf. Fig. 19.) With regard to the staining reactions of these fibrils, Vejdovský further adds that the strands of the "primary centroplasm" in *Fridericia* also

¹ These supporting fibrils have been long known to cytologists—including, of course, Goldschmidt.

stain (with iron-hæmatoxylin or brasilin) just like the nuclear chromatin.

The increase of chromidia with increased activity is thus explained: the more prolonged and violent the stimulus, the greater the damaging and tearing of the fibrils, and hence the greater the number of "chromidia."

Whether Goldschmidt or Vejdovský ultimately prove to be correct, it is important to note for the present that the "chromidia" of *Ascaris* may be really nothing more than much modified derivatives of centroplasmic rays (cf. p. 303).

The Chromidia in the Gametogenesis of Gastropods.—The advocate for chromidia in the development of gastropod¹ eggs and sperms is Popoff. According to him ('07) chromidia are formed in the spermatocytes and oocytes at certain stages of development (cf. text-fig. 20A). They are extruded from the nucleus as chromatin granules. Personally I am far from being convinced of the nuclear origin of the "chromidia," either by his figures or his description.

Now the "chromidia" are really nothing more than the "pseudochromosomes," "Nebenkern," etc., already long known from the work of Meves, Platner, Bolles Lee and others (cf. Meves, '00). But for Popoff, "the observations (i. e. Popoff's on *Helix*) . . . show that the structures described by various authors under the names mitochondria, pseudochromosomes, archoplasm, ergastoplasm, Nebenkern, idiozome (only in certain cases) and idiozome remains, are referable to different isolated stages of one and the same developmental series of the chromidia." He considers his work to be an "undoubted proof" of this.

As a great deal has been written on this matter, I will content myself with citing the opinion of three other investigators of the same structures.

Murray ('98) found centrosomes in the Nebenkern of *Helix*. And he concluded that the Nebenkern was really the attraction sphere, and that in it "no structures exist in any way comparable to chromosomes." This conclusion was

¹ *Paludina* and *Helix*.

accepted by Boveri ('00), in whose laboratory the observations were made.

Ancel ('02) has given a most exhaustive account of the same structures. He believed that the pseudochromosomes and Nebenkern were stages in the development of the same thing, but that they were not formed from nuclear chromatin, being "the product of transformation of differentiated cytoplasmic filaments."

Bolles Lee ('02) says the Nebenkern in *Helix* is nothing but a degenerating bunch of spindle rays. He "can affirm that the Nebenkern is derived from the spindle with as much certainty as one can affirm that an oak is derived from an acorn."

In face of these assertions regarding the "chromidia" of *Helix* it is surely necessary for Popoff to bring some further proofs forward before we can accept his interpretation.¹

Attempts have been made to homologize various structures (mitochondria, etc.) in nerve-cells with chromidia, (e. g. by Goldschmidt, '04a; Popoff, '06, etc.) But the evidence is even less convincing than in the two cases already given. It seems not unlikely that they, like the "chromidia" of *Ascaris* and *Helix*, are really nothing more than the remains of centropasmic fibres. It is significant that this same result should have been arrived at in these different cases by quite independent observers.

Chromidia have been described in several other multicellular organisms, e. g. in dicyemids (Hartmann, '07). They are here said to play a part in the vegetative life of the animal, but the observations require confirmation. And this, indeed, may be said of most cases of chromidia in the Metazoa.²

¹ According to Wassilieff ('07) similar structures (mitochondria) in the spermatocytes of *Blatta germanica* originate from the nucleus, but are "no special kind of chromatin, but only superfluous chromatin."

² An interesting chromidial condition appears to occur also in sponges, e. g. in the gastral actinoblasts of *Clathrina cerebrum*, as described by Minchin ('98). I am indebted to Prof. Minchin for kindly calling my attention to the fact.

Now let us consider all these facts about chromidia, regardless of any hypotheses which have already been introduced to "explain" them.

First, it seems to me that the evidence at present is strongly in favour of the view that in the Metazoa most of the so-called chromidia are really scattered remnants of centropasmic fibrils or their derivatives—properly speaking, not nuclear chromatin at all. Consequently, I believe that any hypothesis which is based upon the assumption of their nuclear nature¹ has a very insecure foundation. But before we have more facts to go upon it seems to me premature to argue the matter further.

Secondly, I believe that certain facts regarding the Protista are sufficiently well established to permit of generalisations being made.

It is perfectly evident that under the name chromidia at least four quite distinctly different things are comprised, whose morphological resemblance alone allows of their sharing a common title. Physiologically they are quite different. First, chromidia may represent the normal condition of the chromatin in a vegetative cell which has no formed nucleus (e. g. in Bacteria, Siedleckia, etc.). Secondly, chromidia may be the products of cell metabolism—either of the nucleus (e. g. Actinosphaerium)² or of the cytoplasm (e. g. Stenophora).³ Thirdly, chromidia may be decomposition products of the nucleus, due to degeneration or death of the cell (e. g. degenerating Opalina).⁴ And fourthly, chromidia may represent one stage in a process of multiple nuclear division (e. g. Mastigella).⁵ This process of nuclear division occurs frequently—though not exclusively (cf. isospores of Radiolaria, p. 289)—in the formation of gametes.

¹ I do not mean to imply that the centrosome and centropasm were not originally themselves derived from the nucleus. On the contrary, I regard this as highly probable.

² See p. 282.

³ See p. 297.

⁴ See p. 293.

⁵ See p. 288.

I will consider this last case in more detail, as it is the basis of much theorizing.

Whatever theoretical value we may give to the chromatin itself, it cannot be denied that chromidia represent an intermediate stage in the simultaneous formation of a number of nuclei from a single nucleus. The reason why we find this method of multiple division so frequently occurring in gametogenesis is, to my mind, quite obvious. It is an adaptation to ensure the formation of a number of gametes at the same time. From the very nature of the life-conditions of many Protozoa it is absolutely necessary for a large number of gametes to be formed at once; for a large number must usually, like the sperms of Metazoa, fail to fulfil their duty.

There are few accurate accounts of other methods of multiple nuclear division, but it has been studied carefully in at least one protozoon—*Calcutuba* (Schaudinn, '95). Except for the fact that all the events take place inside the nuclear membrane, it is exactly comparable with the method by chromidia formation as seen in *Aggregata*, etc.

In *Thalamophora*, *Radiolaria* and *Rhizomastigina*, where the chromidium remains for some time as a permanent organelle during the vegetative life of the cell, we see merely a device by which, through the independent growth of the chromidia, a larger brood of gametes can be eventually produced than by the sudden multiple division of a single nucleus.

The multiple nuclear division in *Opalina* is cloaked by the fact that the cell is originally multinucleate. This applies also to *Pelomyxa*. And here, apparently, nuclear reduction and multiple division occur at the same time, so that they obscure one another.

There is one other interesting conclusion which may be drawn from the facts regarding chromidia. It is that an actual cell death exists in the "immortal" Protozoa. Consider the following instances. In many of the rhizopods the primary nucleus and the remains of the cytoplasm are left

behind when the brood of gametes swims off to conjugate. The whole of this residuary mass then dies. The same fate overtakes the remains of the microgametocyte in coccidiids—e. g. *Adelea ovata*, *Eimeria schubergi*, etc. This does not indicate that the cell must be regarded as by nature containing two kinds of chromatin—somatic and generative—any more than it indicates that the cell by nature contains two sorts of cytoplasm. It simply shows us that a cell, or part of a cell, can get worn out with its life-activities and die. The residuum (Restkörper) is the corpse.

This same idea has already occurred to R. Hertwig ('06a), amongst others.

II.

And now to the hypotheses connected with chromidia. As Hertwig's original conceptions of chromidia began with *Actinosphærium*, and have been woven into his hypothesis of the karyoplasmic relation,¹ I will begin with this.

The hypothesis states that "the relation of nucleus to protoplasm, the quotient $\frac{k}{p}$ —that is, the mass of nuclear substance divided by the mass of protoplasm—is a constant, whose magnitude is of fundamental importance for all vital processes influenced by the nucleus, for assimilation and organising activity, for growth and division." Now, if nucleus and cytoplasm do not grow at the same rate, the nucleus may become too large for the cell, a condition which may lead to degeneration and death. The nucleus, however, may reduce its size by giving up part of its chromatin—as chromidia—and so re-establish the normal relation $\frac{k}{p}$. The chromidia are thus a means for regulating the karyoplasmic relations.

The formation of chromidia by the macrogametocyte of the

¹ I use this expression as an English equivalent of Hertwig's term, "Kernplasmarelation."

malaria parasite, in a recurrence of malaria—explained by Schaudinn ('02a) as a kind of sexual process—is also accounted for by Hertwig ('06) as a process which corrects the karyoplasmic relations.

The basis of this hypothesis is now so wide that it will be quite beyond the scope of this essay to discuss the large mass of literature relating to it. There are already many striking experimental facts in favour of the correctness of the hypothesis, and even if it is not destined to take its place as one of the fundamental theories of cytology it will have served as a working hypothesis of the very greatest importance.

The other hypothesis which sprang from the facts concerning chromidia is the hypothesis of binuclearity. It gradually took shape in the later work of Schaudinn, but has found its most ardent advocate in Goldschmidt ('04a, '05). From his work on nematodes (cf. p. 300), and a consideration of chromidia in the Protozoa, Goldschmidt came to the following conclusions:

“(1) Every animal cell is by nature¹ binucleate; it contains a somatic and a propagatory nucleus. The former presides over somatic functions, metabolism and movement . . . The propagatory nucleus contains especially the hereditary substances, which also possess the ability to generate a new somatic nucleus.

“(2) Both kinds of nucleus are usually united into a single nucleus—the amphinucleus. Separation may take place to a greater or less extent . . .

“(3) Complete separation of the two kinds of nucleus can be seen in only a few cases, in connection with reproduction in Protozoa and also in oogenesis and spermatogenesis of Metazoa.

“(4) In tissue cells the separation may be quite unnoticeable. . . . An almost complete separation may occur in ganglion and muscle cells. The somatic nucleus lies in the cytoplasm as the chromidial apparatus . . .

¹ “Ihrem Wesen nach.”

"(5) Cells with only a propagatory nucleus, but which can, of course regenerate a somatic, are found only in the gametes of protozoa, and in certain nutritive cells of the ovary—possibly also in many sorts of spermatozoa."

"(6) Cells with only a somatic nucleus are also possible: the residuum of gregarines, the reduced cells of *Ascaris*, certain muscle cells."

In the first place, it must be noted that the term "binuclearity" ("Doppelkernigkeit") is not a happy one. The conception is not of two nuclei but of two kinds of chromatin, and in this it differs from the other binuclearity hypothesis (Schaudinn-Prowazek-Hartmann, cf. p. 311). The idea would be more exactly expressed by a word such as "dichromaticity" ("Dichromatizität"). An actual somato-reproductive binuclearity exists only in such forms as the Infusoria, where somatic nucleus (meganucleus) and propagatory nucleus (micronucleus) are often completely separate. This arrangement—which, for Goldschmidt, shows a resolution of the nucleus into its primary parts—is, for me, merely a mark of the high degree of differentiation which the Infusoria exhibit in so many other ways besides. To my mind it is a specialisation, not a simplification. There is, moreover, some evidence to show that even here the two nuclei do not necessarily consist of two essentially different kinds of nuclear substance. For, as has been abundantly proved, the micronucleus can form a meganucleus after conjugation; and conversely, the meganucleus can probably form a micronucleus (Le Dantec, '97).

It is further to be noted that the "propagatory nucleus," far from being entirely concerned with reproduction, can in certain cases exhibit independent powers of metabolism and growth.¹ It is as unjustifiable to maintain that the micro-

¹ Cf. Mastigina, "The sporetia . . . must indeed nourish and reproduce themselves independently. And we must assume the same for the . . . chromidial net of the shelled rhizopods" (Goldschmidt, '07). And further, in *Arcella*: "The generative function (i. e. of the chromidial net) is indubitable. On the other hand, it is equally

nucleus (or its homologue the chromidial net¹) plays no part in the vegetative life of the protozoan cell, as it is to maintain that the germ cells of a metazoan individual play no part in its general somatic metabolism.

In that the nuclear conditions seen in Infusoria are specialised, and not primitive—to my mind—they show no more that the cell is by nature binucleate than a metazoan containing n different organs—each with its specialised nuclei—shows that the cell was originally by nature n -nucleate.

To say that the cell nucleus possesses the two distinct functions of growth and reproduction is a platitude. But to say that these two functions are restricted to special parts of the nuclear material is not warranted by the facts known to us at present. All the facts appear to me to point to the conclusion that growth and reproduction—somatic and propagative functions—are united in the same living nuclear molecule. One or other may come to preponderate, but that is the necessary result of cellular differentiation.

I think a great deal of error has crept into the chromidial hypothesis of binuclearity through the unfortunate application, originally, of a similar name to two quite different things—the chromidia of *Actinosphærium* (products of metabolism or disintegration) and the chromidial net of *Thalamophora* (a reproductive organ). When Goldschmidt added to these the chromidial apparatus of ascarids—again quite a different thing (cf. p. 302)—confusion was complete, and hence the deductions which at first sight appear so legitimate. To my mind, the facts by no means allow of the conclusions drawn by Goldschmidt (p. 307).

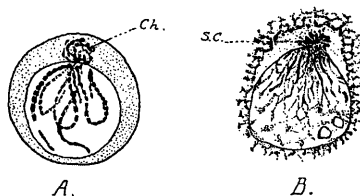
Starting from these—to me—false premises, Goldschmidt

certain that the chromidium fulfils trophic functions” (Elpatiewsky, '07). “That there exist pure gametochromidia, entirely without admixture of somatic nuclear matter, is improbable” (Schaudinn, '05).

¹ Cf. Swarczewsky, '08. From his work it appears that in *Arcella* the chromidial net gives rise to secondary nuclei, which enter not only into the gametes but also into asexual buds; so that here at least there is no justification for regarding the chromidium as purely gametic.

and Popoff ('07) have greatly extended the original chromidial hypothesis. For them, the "sphere" of *Noctiluca* (Ischikawa, Calkins, Doflein), and the "spongy

TEXT-FIG. 20.

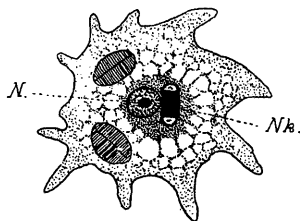


A. Formation of "chromidia" (*Ch.*) in the oocyte of *Paludina vivipara*. (After Popoff, '07.)

B. Formation of the "spongy centrosome" (*S.C.*) from the nucleus in *Actinosphærium*. (After R. Hertwig, '98.)

centrosome" of *Actinosphærium* (Hertwig, '98), correspond to the "chromidia" of *Paludina* (cf. p. 302), all being chromidial structures. Further, the nucleolo-centrosome of

TEXT-FIG. 21.



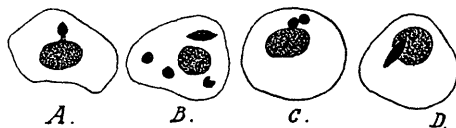
Paramœba eilhardi. *N.* nucleus; *Nk.* Nebenkörper. The latter stains deeply with chromatin stains, and functions as a cytocentre. According to Goldschmidt and Popoff it represents a "chromidial apparatus." According to Hartmann and Prowazek, a kintonucleus. (After Schaudinn, '96.)

Euglena (Keuten, '95) and the Nebenkern of *Paramœba* (Schaudinn, '96) are each regarded by them as constituting a "chromidial apparatus" (text-figs. 21, 25).

Such speculations, to my mind, greatly exceed the limits of legitimate inference. Yet it has come to be the fashion of late to repeat that a binuclearity of this kind exists in all accurately-investigated Protozoa (e.g. Enriques, etc.).

One of the most striking pieces of evidence in favour of somato-generative binuclearity is seen in the life-history of the remarkable infusorian, *Ichthyophthirius* (Neresheimer, '08). The nucleus (amphinucleus) buds off a smaller nucleus, which divides into two. The latter then undergo two reduction divisions each, and finally fuse—thus enacting an autogamy. The zygote nucleus then re-enters the original nucleus and so reconstitutes a fresh amphinucleus (text-fig.

TEXT-FIG. 22.

*Ichthyophthirius.*

- A. The originally single nucleus gives off a micronucleus.
 B. The micronucleus undergoes two divisions. Three of the four resulting nuclei degenerate—the fourth divides once more (spindle).
 C. The spindle gives rise to a pair of nuclei which fuse (autogamy).
 D. After fusion, the nuclei re-enter the original nucleus and fuse with it.
 (After Neresheimer, '08—schematic.)

22). It certainly appears as though we were here dealing with two different kinds of chromatin—trophic and gametic—united into one nucleus.

Entamoeba coli also seems to furnish strong evidence in favour of this view. In neither of these cases, however, is the evidence conclusive, and both stand in need of confirmation.

The second binuclearity hypothesis—which has, to a considerable extent, been confused with the one already discussed—is more properly so-called, for it has, as its basis,

the conception of an originally doubly nucleate cell. This hypothesis is much older than the chromidial idea, and is intimately bound up with speculations regarding the origin of the centrosome. I will try to sketch its history as briefly as possible, and then say something about its most recent developmental phase.

In 1891 Bütschli noticed a chromatin-staining centrosome in the diatom *Surirella*, and suggested that it might possibly be homologized with the micronucleus of an infusorian. A somewhat similar view was advanced by R. Hertwig ('92). He said that the ordinary nucleus of a metazoan cell might be regarded as a nucleus with little or no active substance, but rich in chromatin—the centrosome, however, as a nucleus which had lost its chromatin but retained its activity. This would thus presuppose the original cell to contain two nuclei.

Lauterborn ('93), continuing Bütschli's work on diatoms, also pursued the ideas which the latter had started. Before he had given a complete exposition of the result at which he had arrived, however, Heidenhain ('94) published an elaboration of Bütschli's original conception. He regarded the condition seen in the Infusoria—a cell containing two nuclei—as a primitive condition, and regarded the nucleus and centrosome of a metazoan cell as derived from the infusorian meganucleus and micronucleus respectively. As Lauterborn pointed out, this is in the highest degree improbable, as the arrangement seen in the Infusoria is a highly specialised one, and not primitive.

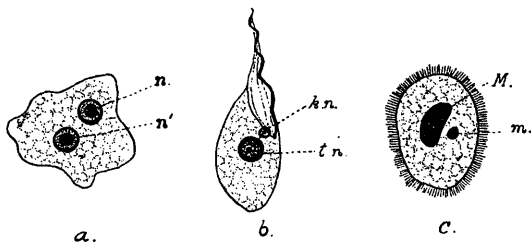
Lauterborn himself gave a full exposition of his views in 1896. As a starting point he takes, not the specialised binuclear condition seen in Infusoria, but a cell containing two exactly similar nuclei, *Amœba binucleata* Gruber (Schaudinn, '95b). From this primitive condition the meganucleus and micronucleus of Infusoria, and the nucleus and centrosome + central spindle of Metazoa, are supposed to have been collaterally evolved. Lauterborn supposes that in diatoms also the centrosome + central spindle represents one original nucleus, *Paramœba eilhardi* (Schaudinn, '96)

with its nucleus and Nebenkörper representing a stage intermediate between the diatom and the *A. binucleata* condition.

These views were all very clearly expressed and are the parents of the existing binuclearity hypothesis of Schaudinn and his followers.

Schaudinn's ('96a, '05, etc.) conception of binuclearity was chiefly based upon his observations on *Acanthocystis* and *Hæmoproteus* (*Trypanosoma*) *noctuæ*. In the latter we see an organism which is actually binucleate, there being a

TEXT-FIG. 23.



Illustrating three different kinds of binuclearity which actually exist in three different groups of Protozoa:—

a, in the Rhizopoda, *Acanthocystis binucleata*, an organism with two exactly similar nuclei (*n. n'*).

b, in the Flagellata, *Hæmoproteus noctuæ*, which has two differentiated nuclei—kinetic (*k.n.*) and trophic (*t.n.*).

c, in an Infusorian. Here the nuclei are differentiated into a somatic (meganucleus, *M.*) and sexual (micronucleus, *m.*).

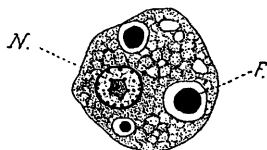
The three figures also serve to illustrate the starting points of the three binuclearity hypotheses,—namely those of Lauterborn, Schaudinn, Hartmann and Prowazek, and Goldschmidt—respectively.

second nucleus (kinetonucleus) in addition to the main nucleus (trophonucleus). Both nuclei take part in conjugation, and at certain periods in the life-cycle they may be united into a single nucleus (synkaryon). The kinetonucleus (blepharoplast) is specially concerned with the locomotor functions of the cell.

Now it is this second nucleus—the kinetonucleus—which is

supposed to be the homologue of the metazoan centrosome. We thus have a conception of binuclearity which starts neither from the somato-gametic nuclear differentiation of infusoria (Goldschmidt), nor from a condition in which the cell contained two equivalent nuclei (Lauterborn); but it presupposes the primitive condition to have been a tropho-kinetic binuclearity. These views of Schaudinn have been much elaborated by Hartmann and Prowazek ('07),¹ who have pushed them to their extremest limit. According to these two writers, other protozoan cells are really binucleate in just the same way as the trypanosomes, the only difference being that we usually find the kinetonucleus boxed up—as a karyosome—inside the trophonucleus. The encased nucleus

TEXT-FIG. 24.



Entamoeba tetragena Viereck. *N.* nucleus, inside which is a "karyosome" with a "centriole" and "a kind of nuclear membrane." This is supposed to represent an encased nucleus. *F.* ingested body. (After Hartmann and Prowazek, '07.)

assumes many different forms, and it is said in some cases actually to show all the morphological features (centriole, nuclear membrane,² etc.) of a free nucleus (text-fig. 24).

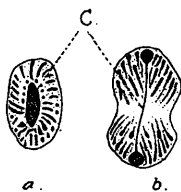
This kinetic nucleus is said to be recognisable in a variety of Protozoa. It is represented by the Nebenkörper of *Paramecium*, by the Centralkorn of Heliozoa, by the nucleolo-centrosome of *Euglena*, by the karyosome in coccidia, etc. It is even suggested that the encased nucleus is visible in a form like *Amoeba limax* (cf. Vahlkampf, '04) but I cannot persuade myself that this is so—with the best will.

¹ They are also held apparently by Keysselitz ('08) and others.

² E. g. in *Entamoeba buccalis* and *E. tetragena*.

This encasement hypothesis is, in face of the facts, to my mind exceedingly far fetched: and moreover, were it true, would not shed any light on the fundamental problem involved. For it is obvious that by assuming the original presence of a separate kinetic nucleus—ancestor of the centrosome—in the cell, we have merely put the problem a little further out of reach. What gives the kinetic nucleus itself the ability to divide? Its centriole? Then is the centriole another kinetic nucleus within the kintonucleus? And has its own kintonucleus again inside that, and so on, in an unending box-within-box system? One is forcibly reminded of the “scatulation theory” of the preformationists.

TEXT-FIG. 25.



Sections through the nucleus of *Euglena*: *a*, resting; *b*, in division; *C*, the so-called “nucleolocentrosome.” (After Keuten, '95.)

It is curious to note how a structure like the nucleolocentrosome of *Euglena* can be regarded on the one hand (Goldschmidt, Popoff) as a chromidial apparatus, and on the other (Hartmann, Prowazek) as an actual independent, encased nucleus (text-fig. 25).

There can be little doubt that the karyosome is really a structure of physiological significance in many cases, and, as such, a structure which cannot be homologized throughout the Protozoa. This has been very clearly brought out by Siedlecki ('05) in his admirable study of the coccidian *Caryotropha*. The karyosome, he maintains, is “an amplification of the whole nuclear apparatus.” For him, “we have in a

protozoan cell—no matter whether we see in it a primary nucleus and chromidial mass, or a vegetative karyosome in the nucleus, or even a separate vegetative and generative nucleus—in each case, but a single and simple nuclear apparatus before us.” The physiological nature of the karyosome is also well seen in the case of *Actinosphaerium* (cf. Hertwig, '98a). In well-fed animals the karyosome consists almost entirely of plastin, but in ill-fed individuals it comes to be largely composed of chromatin; and so on. Its different behaviour in different organisms is also to be noted. For example, in *Eimeira schubergi* the macrogametocyte casts out the karyosome before fertilisation, whereas in *E. lacazei* it is retained.

That the trypanosome blepharoplast is homologous with the centrosome I have elsewhere ('08b) endeavoured to show. But I cannot in the least agree with the homologization of the blepharoplast with the karyosome. The centrosome, I believe, is an organ of nuclear origin, but originally not a nucleus. The facts regarding the Protozoa and Metazoa¹ all appear to me to point in this direction.

With Boveri ('00) “I fully agree with R. Hertwig in that I do not hold a binucleate condition as the necessary starting point for the phylogenetic origin of the centrosome.”

Phylogenetically, the centrosome probably arose, not from an originally present kinetonucleus, but as a differentiation of part of an original single nucleus—in a manner indicated by Hertwig ('95), Boveri, Calkins, etc. Hertwig himself believed the centrosome to be a specialisation of the central spindle, so that the spindle of Protozoa (e.g. *Paramecium*) is equivalent to centrosome + spindle of the Metazoa. In many groups of Protozoa it is possible to trace a fairly perfect series of nuclear types, from simple amitotic nuclei up to nuclei

¹ Cases of centrosomes appearing in the cytoplasm independently of the nucleus are of course known. But here there is no proof that they did not originally come from the nucleus. (E.g. cf. Yatsu, '05, who admits that the centrosomes do not appear until the nuclear membrane has disappeared.)

dividing by a complex mitosis (e. g. in Flagellata, as I have elsewhere shown, '08).

As Schaudinn ('05) and Prowazek and Hartmann¹ ('07) have pointed out, there can be no doubt that Goldschmidt ('04a) is in error when he describes the blepharoplast and nucleus of *Trypanosoma* respectively as somatic and gametic nuclei. This binuclear condition must, for Goldschmidt, be a secondary one, independent of the real binuclearity (somatogametic). And conversely, the binuclearity of Infusoria must appear to Hartmann and Prowazek in the same light—as a mere coincidence, having nothing to do with the real trophokinetic binuclearity of the cell. I believe that neither trypanosome nor infusorian represents a primitive condition—both being results of cell differentiation, but along different lines.

Schaudinn's conceptions ('05) did not stop at a trophokinetic binuclearity. He tried to show that there co-exists in the trypanosome cell a sexual binuclearity. There is thus "a double nuclear dimorphism" in these organisms. "The blepharoplast is chiefly male, the large nucleus chiefly female. The dimorphism of both nuclei is hence a sexual dimorphism. The indifferent *Trypanosoma* is hermaphrodite." In *Trypanosoma* the maleness and femaleness find expression in the katabolic nature of the kinetonucleus and the anabolic nature of the trophonucleus (cf. the Geddes-Thomson theory of sex). We thus arrive at a conception of the cell as an entity which is partly male and partly female—a conception at which embryologists (Minot, van Beneden, Balfour, etc.) long ago arrived. Schaudinn pointed out that the micronucleus of *Didinium* (Prandtl, '06) must also be regarded as hermaphrodite; and the same is true for Para-

¹ It may be remarked, however, that the occurrence of forms without a trophonucleus in five-day cultures of *Leishmania* no more indicates the function of the blepharoplast than the occurrence of enucleate *Amoebæ* (Prandtl, '07) or gregarines (Kuschakewitsch, '07) proves that the cell does not require a nucleus. In both cases we are probably dealing with degeneration phenomena.

mecium (Calkins and Cull, '07) and probably for other Infusoria. This kind of hermaphroditism must be a very deep-rooted phenomenon if we agree with Schaudinn and his followers (e. g. Prowazek) that sexuality is a fundamental attribute of living matter—a belief which I by no means share.

A view similar to that of Schaudinn regarding the trypanosome cell has been put forward by Salvin-Moore and Breinl ('07), who suggest that the nucleus and blepharoplast are differentiated gamete nuclei in one and the same individual. With Minchin ('08) I believe that this view is "far-fetched and misleading in the highest degree."

In connection with this matter mention may be made of a very remarkable binucleate protozoan, *Amœba diploidea*, recently described by Hartmann and Nägler ('08). The animal contains two nuclei, lying side by side, which a study of the life-cycle has shown to be the two gamete nuclei, which have not fused, from a previous conjugation. Fusion to form a zygote nucleus only occurs before the next conjugation. We have here an organism in which the "paternal" and "maternal" chromatin remain separate all through the vegetative existence. Truly this is a most extraordinary state of affairs. It appears that *A. diploidea* is formed from two incompletely fused organisms, just as *A. binucleata* is formed from two incompletely divided ones.

Finally, I will summarise the conclusions to which the foregoing considerations have led me. They are that the facts relating to chromidia are not yet sufficiently strong to bear the weight of the binuclearity hypothesis which rests upon them: that, therefore, this binuclearity hypothesis, however suggestive it may be as a working hypothesis, is far from being a "law," as some would have it called: and that the tropho-kinetic binuclearity hypothesis is equally unworthy to rank as a cytological truth. The real significance of chromidial structures has been greatly distorted by viewing them from a theoretical standpoint.

The most important inference, however, is that we require

many, many more facts and unbiased observations before we can hope to unravel the tangled skein of cytological problems of which the foregoing form but a small part. But in the study of the complex simplicity of the Protista we have already found a beginning.

CAMBRIDGE,
August, 1908.

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