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# COMPARATIVE CYTOLOGICAL STUDIES, WITH ESPECIAL REGARD TO THE MORPHOLOGY OF THE NUCLEOLUS.

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## I. INTRODUCTION.

THE following studies are based upon animal cells, both egg cells and somatic cells having been investigated. They were made, primarily, with a regard to the morphology of the true nucleoli (plasmosomes), though numerous other points in ontogenetic cellular development have been considered. In connection with these observations the zoölogical literature upon the subject of nucleoli has been reviewed as thoroughly as possible, and, less completely, the literature from the botanical standpoint as well; reviews are given of these observations of previous writers. No attempt has been made to review the literature from the pathological standpoint. Under the caption "General Comparisons and Conclusions" are compared together the more important deductions from my own observations, and these are compared with those of previous investigators.

The nucleoli are cellular structures which have been studied to much less extent than other constituents of the cell, and though there are numerous observations upon them, these are so scattered through works of more general import that it is well-nigh impossible to collect together all the previous investigations upon the subject. I hope that this explanation may be taken as an apology by any authors whose papers I have chanced to overlook.

At the laboratory of the Fish Commission at Woods Holl, the following species were collected by me: *Montagua*, *Amphiporus glutinosus*, *Tetrastemma catenulatum*, *Zygonemertes*, *Lineus*, *Polydora*, and *Piscicola*. At Sea Isle City, at the laboratory of the University of Pennsylvania: *Tetrastemma elegans*, *Doto*, and certain of the species found at the former locality. *Stichostemma* was collected in the aquaria of the University



of Berlin; and the preparations of the siphonophore *Rodalia* were kindly placed at my disposal by Dr. E. G. Conklin.

*Doto* and *Montagua* belong to the family of the *Acolidiidae*; *Amphiporus*, *Tetrastemma*, *Zygonemertes*, and *Stichostemma* are *Metaemertini*; and *Lineus* and *Cerebratulus* are *Heteronemertini*; *Polydora* is a *Polychacte*; and *Piscicola* a rynchobdellid leech.

The present paper was sent to Dr. Whitman, editor of the *Journal of Morphology*, on Feb. 3, 1897; on receiving the MSS. again in March, 1898, I was able to incorporate in the text reviews of the literature of the whole year 1897. No other changes of importance, however, were then made in the original text, except brief mention of observations which I had made in the past year. It is my intention to follow this paper by others on nucleolar structures, particularly on structures which have received but little consideration in the present paper, namely, "double" nucleoli and chromatin nucleoli.

## II. REVIEW OF THE LITERATURE UPON NUCLEOLI.

In this review shall be considered separately, first, those papers from the zoölogical, and, second, those from the botanical standpoint. The references from zoölogical papers I have endeavored to make as complete as possible, while my citations from the observations of botanical observers are much less numerous, though even in this I have consulted the more important papers from 1880 to the present time. In referring to the zoölogical papers, I have taken them up in chronological order; and in doing so, shall treat separately the periods 1781-1860, 1861-69, 1870-79, and from the year 1880 to the present time I shall treat the literature for each year separately, in order that the reader may more conveniently be able to turn to the citations from a given paper. Under each year papers are reviewed according to the alphabetical sequence of the authors' names. The botanical literature, on the other hand, shall simply be treated in chronological order, without regard to any division into periods. The full titles of the papers referred to are to be found on

page 542, where their arrangement is according to the alphabetical order of the authors' names, both the zoological and botanical papers being in this one list. A certain number of contributions dealing with nucleoli are entered into the literature list, which I was unable to find in the libraries at my disposal; all such papers have been distinguished by an asterisk (\*); the contents of some of the latter I have reviewed from the citations of other writers.

Literature reviews are here given of all papers, with the object of furnishing a reference library on the subject; in Chapter IV, consequently, brief allusions only are made to the views of particular authors, and readers can compare their views by referring to the present section. This arrangement of the literature appears the most practical.

#### A. ZOÖLOGICAL LITERATURE.

1781-1860.

Fontana (1781, cited by Carnoy, '84) was the first to figure the nucleolus in the nucleus, which he describes as "un corps oviforme, pourvu d'une tache en son milieu."

The discoverer of the nucleolus in germinal vesicles is R. Wagner ('35), and he termed it "Keimfleck" or "macula germinativa." He notes that the germinal vesicle of *Unio* and *Anodonta* "zeigt constant zwei Flecke in Form von Kreisen, welche sich schneiden, selten finden sich Abweichungen; der grössere derselben möchte eine gewisse Aehnlichkeit mit dem Keimfleck haben." In his "Nachtrag" to the same paper, he states: "Der Keim ist bei seinem ersten Auftreten eben das, was ich Keimfleck genannt habe. Es ist eine Schicht körniger Masse, welche bald einfach (Säugethiere, Schnecken, Insekten etc.) als Fleck erscheint, bald mehrere zerstreute Kügelchen bildet (Flusskrebs, Fische, Batrachier), . . . die an der inneren Wand des Keimbläschens angeheftet sind." In two subsequent communications ('36, '37) he notes the occurrence of nucleoli in the germinal vesicles of *Coryna*, *Lucernaria*, *Cyanca*, *Chrysaora*, *Asterias*, and *Insecta*, and finds in *Melolontha vulgaris* one large and one small nucleolus. Finally he remarks: "Viel-

leicht bildet das Material des Keimbläschens und der Keimfleck die Grundlage zum serösen Blatt und zum Fruchthof der Keimhaut." (Jones, '35, '37, does not mention the nucleolus; accordingly, he is not the discoverer, as is claimed by Bischoff.)

Valentin ('36, cited by Carnoy, '84) describes the nucleolus as a "rundes Körperchen, welches eine Art von zweitem Nucleus bildet." (On this historical ground Carnoy considers the term "nucleolus" should be limited to his "nucléole-noyau.")

Valentin ('39, mentioned by Carnoy, '84) introduces the terms "nucleolus" and "Kernkörperchen"; the latter term was proposed also by Schwann ('39) in the same year.

Bischoff ('42) found in the egg of the rabbit one nucleolus, "ein schwach granulirtes Körnchen," which he considers to be a "Zellenkern."

Vogt ('42) found several nucleoli (six to twelve) in the ova of *Coregonus*; these subsequently migrate into the yolk to form the first cells of the blastoderm.

Leydig ('49) describes in the germinal vesicle of *Nepheleis* one nucleolus, in *Clepsine* one or numerous ones, in *Piscicola* two to four, while in *Haemopsis* "der Keimfleck war einfach, 8-förmig oder doppelt."

Kölliker ('49) studied numerous Gregarines, and concludes that the nucleoli ("Körnchen") "bei manchen Gregarinen gewisse bestimmte Entwicklungen durchlaufen, nämlich bei jungen Individuen einfach vorhanden sind, bei älteren allmählig in zwei, drei oder mehr Körner zerfallen." In *G. terebellae*, *clavata*, *saenuridis*, and *enchytraei* there is a single nucleolus; *G. sipunculi* has from one to six; *G. heeri*, six to eighteen; *G. sieboldii*, one to seven, which are either homogeneous or vacuolar, or else only one or two are present, and each of these is composed of a mass of smaller ones; *G. brevirostra* has from six to nine nucleoli.

Lovén ('49) studied the eggs of *Modiolaria*, *Cardium*, *Patella*, and *Solen*, and found that during the process of fecundation the nuclear membrane ruptures, and the nucleolus passes out through the vitelline membrane. (It is very probable that he confused the nucleolus with a pole body.)

Quatrefages ('49) found that preceding the first maturation division of *Teredo* the nucleolus dissolves in the nucleus.

v. Wittich ('49) found that in the germinal vesicles of *Lycosa*, *Theridium*, *Epeira* the "Keimfleck" first appears "als ein matt gelblicher, nicht immer scharf begrenzter, aber durchaus homogener Fleck, wird immer entschiedener rund, verliert seine Homogenität, indem er hie und da den Schein von unregelmässig rundlichen Aushöhlungen bietet, und neben ihm treten zuletzt zerstreut ungleich geformte Körperchen auf, die dem ersteren sehr ähnlich, an Zahl immer mehr zunehmen, je mehr sich das Bläschen [Kern] seinem gänzlichen Schwinden nähert." In *Gasterosteus aculeatus* the number of the "Keimflecke" increases with the size of the egg. In the youngest germinal vesicles of *Fringilla* there is at first no nucleolus, later a single large, excentric one.

Leydig ('50) finds that in the ovarian egg of *Paludina vivipara* there are two widely separated nucleoli, while in the ripe egg they are in contact with each other: "so muss wohl angenommen werden, dass der achterförmige Keimfleck des reifen Eies durch Aneinanderrücken und theilweises Verschmelzen der früher getrennten Körperchen entstanden sei."

Leydig ('52), ovum of *Synapta digitata*: there is a single nucleolus with a vacuole; "was aber als eigenthümlich hervortritt, ist, dass er constant an einem Pol des Keimbläschens liegt und zwar in einer tellerförmigen Grube desselben."

Leuckart ('53) states: "Der Keimfleck bildet eine zusammenhängende Masse von feinkörniger Beschaffenheit und opakem Aussehen, die unter dem Deckgläschen mancherlei Formen annimmt und ohne Umhüllungshaut ist. Nicht selten lassen sich im Innern auch einzelne grössere Moleküle ganz deutlich unterscheiden. In manchen Fällen nehmen diese Moleküle an Zahl und Selbständigkeit in einem solchen Grade zu, dass der ganze Keimfleck eine haufenförmige Aggregation von Körnern darstellt."

Hessling ('54) finds in the youngest eggs of *Unio* a single large nucleolus; in larger ova there is a larger and a smaller nucleolus, the latter having divided off from the former, and showing a different reaction to acetic acid.

Lacaze-Duthiers ('54) finds in the eggs of *Lamellibranchs* either one nucleolus, or when two are present they are of unequal size.

Leydig ('55a) says that in the egg of *Cyclus* "der Keimfleck hat constant die Bisquitform." In a second paper of the same year ('55b) he makes the following notes on the ova of *Rotatoria*: in *Notommata myrmecio* there are about 100 finely granulated nucleoli; in *N. sieboldii* "Die Keimflecke erscheinen als Haufen von kleinen, hellen Kügelchen," and disappear in the ripe egg; in *N. centrura* and *Brachionus bakeri* there is a single large nucleolus.

Agassiz ('57) in studying the egg of the turtle introduces the following terms: "ectoblast" for cell membrane, "mesoblast" for nucleus, "entoblast," or "Wagnerian vesicle," for the nucleolus, and "entosthoblast," or "Valentinian vesicle," for the body sometimes enclosed in the latter. In the youngest ova the nucleoli are absent, later they become numerous and large, though they disappear in the ripe egg. The excentric vacuole ("Valentinian vesicle") of the nucleolus "increases in size at a greater proportionate rate than its parent, the "Wagnerian vesicle," till at its final stage it oftentimes occupies three-fifths of the diameter of the generating medium."

Lacaze-Duthiers ('57), ovarian egg of *Dentalium*: at first there is but a single nucleolus, later a second one appears and apposes itself to the former; the volumes of the two are different. (Cf. Fol, '89.)

Remak ('58), blood cells of *Gallus*: "Es kann kaum einem Zweifel unterliegen, dass die Theilung der Blutzellen mit der Theilung des Kernkörperchens beginnt. . . . Die Regel ist, dass das Kernkörperchen sich in zwei Theile abschnürt, und ebenso der Kern in zwei Kerne. Wie es aber zuweilen vier Kernkörperchen giebt, so finden sich auch zuweilen vier Kerne in einer Zelle."

1861-69.

Pflüger ('63) found one nucleolus in the egg of the calf. While in the "Urei" of the cat he makes the interesting observation that after a division of the nucleus, whereby one

of the daughter-nuclei retained the original nucleolus, in the other a new nucleolus soon appeared, first in the form of a granular mass.

In the paper by Balbiani ('64) movements of nucleoli are described for the first time, and these observations were made upon the living eggs. The first kinds of movements which he distinguishes are exhibited by the eggs of spiders: "ces mouvements de la tache germinative sont caractérisés par la production de prolongements transparents ayant presque toujours la forme de lobes arrondis qui s'allongent et se rétractent alternativement." The second kind of movements is shown in the egg of *Phalangium*, where there is a single large, spherical nucleolus, which appears spongy, owing to the presence of a number of vacuoles, some of which "s'élèvent plus ou moins au dessus de la surface en soulevant sous forme d'une ampoule la couche la plus externe de la substance du corpuscule. . . . Lorsqu'un porte son attention sur une de ces vésicules superficielles, on ne tarde généralement pas à la voir grossir insensiblement, en même temps que la couche de substance qui forme sa paroi extérieure se soulève en s'amincissant de plus et plus; puis, assez brusquement, cette paroi se rompt comme sous la pression d'un liquide intérieur, et ses bords se rétractent vers la base adhérente de l'ampoule qui se trouve ainsi transformée en une petite cupule ou excavation superficielle, . . . et bientôt il ne reste plus aucune trace de l'ampoule ni de l'excavation qui lui a succédé." All the peripheral vacuoles discharge themselves thus in succession, while at the same time the smaller central vacuoles increase in size and wander towards the periphery to take the place of the preceding. Balbiani compares these movements to those of the contractile vacuoles of the *Rhizopoda*, but notes this difference: in the latter forms the vacuole always forms itself at the same place again. In the eggs of *Geophilus* and of *Helix pomatia* he finds that the vacuole discharges through a small orifice.

Balbiani ('65b) describes some remarkable structures in germinal vesicles, all studied in life. In *Geophilus longicornis* there is an external infundibular canal extending from the sur-

face of the nucleus to the surface of the vitellus, its larger opening being apposed to the nucleus. A smaller inner infundibular canal extends from the nucleolus into the outer canal. The numerous vacuoles of the nucleolus are contractile, and empty into the inner canal. He believes that these canals disappear at the time that the nucleolus does. "Dans les ovules de la Chienne, après la séparation des follicules primordiaux, la vésicule et la tache germinative offrent chacune un prolongement canaliculé dont l'un est intérieur à l'autre, comme chez le Géophile. . . . Chez la Raie, où les ovules renferment généralement d'un à quatre petits corpuscules germinatifs creusés d'une vacuole centrale, chacun de ceux-ci émet un nombre variable de petits canaux, ordinairement de deux à quatre, lesquels traversent dans différentes directions la cavité de la vésicule, percent sa paroi et vont se perdre dans le vitellus ambiant. . . . Chez les poissons osseux et les Batraciens, dont les œufs renferment . . . un grand nombre de taches germinatives adhérentes à la paroi interne de la vésicule, celle-ci est entourée d'un système de canaux rayonnants vers la surface de l'œuf, légèrement flexueux et de longueur inégale suivant le trajet qu'ils ont à parcourir pour atteindre cette surface. Chaque canal est en rapport avec un des corpuscules précédents, et présente un calibre correspondant au diamètre de ce dernier. . . . Quelquefois, ainsi que je l'ai observé chez quelques Crustacés (Ecrevisse, *Cancer moenas*), ces taches multiples s'ont paru en outre réunies, dans l'intérieur de la vésicule, par des canaux qui s'étendaient de l'une à l'autre. . . . Chez plusieurs Annélides, Turbellariés, Mollusques et Acalèphes, dont j'ai examiné les œufs, ceux-ci ne renfermaient pour la plupart qu'une tache germinative simple, souvent assez volumineuse, en rapport avec un canal unique renfermé dans l'intérieur d'un deuxième canal émanant de la vésicule germinative." In the germinal spots of *Helix*, *Vortex*, and *Prostomum* he noticed one or several contractile vacuoles.

Schrön ('65) finds in the eggs of the cat and rabbit one or two "Körner" in the nucleoli of the larger eggs, though not in those of the smaller eggs. He considers the "Körn" dif-

ferent in structure and substance from the rest of the nucleolus, and that it is characteristic for a certain stage of the cell.

Stepanoff ('65) describes for the youngest germinal vesicles of *Cyclus* two nucleoli which are unequal in size, while in more mature ova there are usually two (seldom one) large ones. He figures, further, in one nucleus a smaller nucleolus in contact with a larger one.

La Valette St. George ('66) studied in iodized serum the germinal vesicles of various animals. In the egg of the kitten there is one large nucleolus, either homogeneous or finely granular, containing sometimes a large vacuole. In that of the embryo of a sheep he noticed one or several nucleoli, with slight differences in size, finely granular in structure, and containing each a clear vacuole. In the egg of a larva of *Libella* there was a small and a large nucleolus, the latter being darker and more refractive, and spherical or irregular in form; "seine Substanz war entweder homogen oder zeigte je nach der Einstellung des Mikroskopes hellere oder dunklere Flecken von sehr verschiedener Zahl und Grösse, von unmessbarer Kleinheit bis zu zwei Drittel des Keimfleckes. . . . Anfangs war der grosse Keimfleck unregelmässig geformt fast viereckig und zeigte in der Mitte eine hellere Stelle, etwa ein Drittel so gross wie der ganze Keimfleck und daneben ein zweites kleineres Fleckchen. . . . Nach einer Viertelstunde hatte er seine Form geändert, der kleinere Fleck war verschwunden, der grössere nach der Spitze zu gerückt. Nach Verlauf einer halben Stunde war er kuglig geworden und jene helle Stelle verschwunden." (In this last stage the nucleolus touches the nuclear membrane, according to his Fig. 2c.) In the egg of *Porcellio scaber* the nucleolus is an irregular granular mass, and later becomes a massive body; "zuweilen stellt er einen nach einer Seite geöffneten Ring dar, oft auch eine ausgehöhlte Kugel." By these observations he believes he has proved what Schrön termed a solid granule ("Korn") to be a vacuole.

Ransom ('67), egg of *Gasterosteus*: young eggs with numerous peripheral germinal spots, which are spherical and homogeneous. He supposes these "are soluble in some of the constituents of the yolk, and we may thus explain their disap-



pearance in ripe ova." A 1.5% solution of NaCl gives rise to vacuoles in the nucleoli (this antedates the observation of Morgan, '96).

Van Beneden ('69) studied *Gregarina gigantea*: "Le nombre de nucléoles varie à chaque instant; quelques-uns disparaissent, tandis que d'autres se forment; ils apparaissent sous forme d'un petit point presque imperceptible; ce point grandit jusqu'à certaines limites; il devient un véritable corpuscule formé d'une substance homogène très-réfringente, puis le corpuscule diminue de volume; il réfracte de moins en moins la lumière, enfin il disparaît."

Claparède ('69) found in the egg of *Lumbricus terrestris* that the nucleolus "ist doppelt, indem er aus zwei einander berührenden ungleich grossen Kügelchen besteht."

1870-79.

Eimer ('71), epithelial cells of the snout of *Talpa*: each nucleolus is surrounded by a clear space ("Hof"), and the outer boundary of this space "war bezeichnet durch zahlreiche kleine Pünktchen. . . . Im optischen Querschnitt stellten diese Körnchen einen Kreis um den hellen Hof des Kernes dar."

Eimer ('72) finds in the earlier stages of the egg of *Lacerta* that all the nucleoli are grouped near the center of the nucleus, while in more advanced ova there are numerous larger peripheral nucleoli, and smaller ones in the other portions of the nucleus; around each of the large peripheral nucleoli are situated concentric rows of smaller ones. Here, as well as in *Cistudo*, *Testudo*, and *Tropidonotus*, the smallest nucleoli are homogeneous, while the larger contain vacuoles. He concludes that "die complicirt gebauten Keimflecke aus einfachen Körnchen" are built up.

Kleinenberg ('72): in the egg of *Hydra* the single spherical nucleolus contains "ein auffallend stark lichtbrechendes Körperchen. . . . Nach kurzer Zeit schwindet es wieder." The nucleolus then becomes irregular in form, breaks into small granules, and he supposes that these latter become dissolved.

Eimer ('73), nervous system of *Beroë*: each nucleus contains one large nucleolus. "Aufmerksamer Beobachtung kann es nicht entgehen, dass jede Epithelzelle von einer Primitivfibrille versorgt wird. . . . Ich kann nur so viel sagen, dass ich dieselbe [Primitivfibrille] stets auf das Centrum des Kerns zugerichtet sah, so dass ich zu der Ansicht hinneige, es werde sich späterhin ihre Endigung im Kernkörperchen feststellen lassen."

Fol ('73) noticed in the egg of *Geryonia fungiformis* one large nucleolus, containing one large, or several smaller vacuoles.

Auerbach ('74). This important paper I have been unable to consult in the original, and quote from citations by R. Hertwig ('76) and Flemming ('82). According to Auerbach the nucleus is originally a vacuole in the protoplasm, around which a layer of the latter becomes differentiated to form a nuclear membrane. In this vacuole a nucleolus appears later, being derived from the protoplasm, either by a separation of particles from the nuclear membrane or is produced out of those protoplasmic particles which had penetrated from the protoplasm into the original vacuole. He distinguishes "enucleolar," "uninucleolar," and "multinucleolar" nuclei, the first being the more primitive state. The nucleolus has the value of an elementary organism: as long as it is homogeneous, it is comparable to a cytode; when a vacuole appears in it, the latter stands in the same relation to the nucleolus as this does to the nucleus, so that that vacuole may be considered the nucleus ("Kern") of the nucleolus. The original single nucleolus can divide into numerous nucleoli, and the latter, by the disappearance of the nuclear membrane, become free, and each develops into a separate cell. Auerbach considers this theory as "eine vorläufige, noch mit Vorbehalt aufzustellende und weiter zu prüfende."

A. Brandt ('74) observed in life (in the blood fluid) slow amoeboid motions of the single nucleolus of the egg of *Blatta*.

Flemming ('74) investigated the egg of *Anodonta*. In young eggs the nucleolus consists of two apposed spheres of equal diameter; in larger eggs one of these spheres is much larger

than the other. "Der kleinere Theil ist stärker lichtbrechend, auch etwas stärker tingirbar, und beim Zerdrücken resistenter als der grosse : beide zeigen sich hierbei als eine homogene, zähe Masse." The smaller has usually one large vacuole ; the larger has several smaller vacuoles. "Bei Anodonta scheinen mir ausserhalb der Fortpflanzungszeit die beiden Theile normal zusammenzuhängen. . . . Kurz vor Eintritt der Befruchtungszeit gewahrt man viele (aber nur reife, grosse) Eier, an deren Kernkörpern eine wirkliche Trennung vorgegangen ist ; aber in der Art, dass der kleinere Bückel stückweise abgesprengt wird."

Haeckel ('74) notes in the nucleolus of some egg cells "ein innerstes Pünktchen, einen Nucleolinus, welchen man Keimpunkt (Punctum germinativum) nennen kann. Indessen haben diese letzteren beiden Theile (Keimfleck und Keimpunkt), wie es scheint, nur eine untergeordnete Bedeutung," only the yolk and the nucleus being of fundamental importance.

Ludwig ('74) gives notes on the number of nucleoli in various germinal vesicles. In the *Cocleenterata* "Das Keimbläschen umschliesst durchgängig einen einzigen Keimfleck, welcher häufig nochmals ein Körnchen beherbergt." There is one germinal spot in *Echinus*, *Amphidectus*, *Solaster*, *Branchiobdella*, and in *Trematodes* and *Rhabdocoeces*.

Van Beneden ('75) remarks in regard to the egg of the rabbit, that there is one nucleolus, and "deux ou trois petits corps arrondis qui j'ai appelés pseudonucléoles." When the nucleus, during the maturation of the egg, has reached the "zone pellucide" of the yolk, "le nucléole s'accôle à la membrane de la vésicule du côté de la surface de l'œuf, là où la vésicule est appliquée contre la membrane. Il s'aplatit contre la membrane et se soude avec elle ; sa substance plastique s'étale en une plaque qui présente d'abord un épaississement médian. Cette lame je l'ai appelée plaque nucléolaire." Shortly afterwards the latter body "grâce probablement à la contractilité inhérente à sa substance, . . . se ramasse en un corps de forme variable, souvent ellipsoïdal, quelquefois lentriculaire ou en forme de calotte, que j'ai appelé le corps nucléolaire." The latter is the first pole body ("corps directeur"), the nucleoplasm plus the pseudonucleolus constituting the second.

Eimer ('75) studied the egg cells of *Silurus* in eye fluid, and found the nucleolus to present amoeboid movements.

Kidd ('75) found slow amoeboid movements of the nucleoli of the epithelial cells from the mouth of the frog. These cells were placed in humor aqueus, and studied on a stage heated to 39° C.

A. Schneider ('75) says: "Les nucléoles ne sont pas un élément constant de la structure des Grégarines; beaucoup d'espèces en sont normalement privées. Dans les genres *Clepsidrina*, *Euspora*, *Gamocystis*, il n'y a jamais qu'un nucléole, permanent, très-volumineux et sphérique. . . . Dans tout ces genres, jamais deux individus ne sont semblables à eux-mêmes au point du nombre, de la grandeur, de la configuration, de l'opacité ou de la transparence de leurs nucléoles."

F. E. Schulze ('75) noticed in life that an equal division of the nucleolus precedes that of the nucleus, in *Amoeba polypodia*.

Auerbach ('76) repeats some of his previous observations ('74) and adds that the nucleoli show a further similarity to the cytoplasm, in that they have a tendency to produce vacuoles.

Balbiani ('76) describes certain structures in the egg of *Stenobothrus*, which may be chromatic filaments, though I may give a brief citation in regard to them in this place. The contents of the nucleus in the fresh state appear "rempli de petites hachures pâles, tantôt parallèles les unes aux autres, tantôt distribuées plus ou moins irrégulièrement dans la cavité nucléaire. . . . À l'aide de l'acide acétique, on s'assure que ces hachures sont déterminées par les corpuscules en forme de bâtonnets étroits . . . chaque bâtonnet paraît formé de petits globules réunis en série." At the time of nuclear division, these "bâtonnets" become less numerous but larger.

Van Beneden in the same year ('76) gives the results of observations on the egg of *Asteracanthion*. There is one large nucleolus, and eight to fifteen small "pseudonucléoles." He did not notice amoeboid motions in these, but found change of form and successive re- and disappearance of the nucleoli in *Rana*, *Polystomum*, *Gregarina*, and *Monocystis*. "Mais je ne doute

pas que les différences constatées dans la forme de la tache germinative ne doivent être attribués à la contractilité de la substance des nucléoles." The vacuoles in the nucleoli are probably "le résultat de l'union momentanée de certaines parties de la substance nucléolaire avec le suc nucléaire." Before its disappearance the nucleolus breaks into fragments, which then dissolve in the "substance nucléaire." In this fragmentation one fragment is always larger than the others, and contains the vacuole of the primitive nucleolus; it persists until all the smaller fragments have disappeared.

Bütschli ('76) found that the nucleolus disappears before the formation of the first pole spindle in *Tylenchus*, *Anguillula*, *Notommata*, *Brachionus*, *Triarthra*, *Aphis*. He mentions that von Siebold, in 1848, first introduced the name "nucleolus" for the micronucleus of the *Infusoria*, and compared it with the nucleoli of metazoan cells. He also cites some of the earlier writers who compared the pole bodies with nucleoli.

O. Hertwig ('76) calls the nucleolus "das wichtigste Formelement des Kerns," and terms its substance "Kernsubstanz" in opposition to the "Kernsaft" (compare his brother's paper of the same year). In the process of maturation he holds that "der Eikern der aus dem Keimbläschen frei gewordene oder ausgewanderte Keimfleck ist." He noticed vacuoles in, but not amoeboid movements of, the germinal spot of *Toxopneustes lividus*; he observed such motions, however, in the germinal spots of *Rana* and *Pterotrachea*.

R. Hertwig ('76) terms the dense substance of the nucleolus "Kernsubstanz." "Entweder leiten sich die vielen Kernkörper direkt aus dem homogenen Zustand des Kernes ab, indem die Aussonderung der Kernsubstanz an verschiedenen Punkten gleichzeitig begonnen hat; oder die zahlreichen Nucleoli sind, . . . durch Theilung aus einem ursprünglich einfachen Nucleolus entstanden." He believes that the "Nucleoli die Träger der Kernfunction sind. . . . Somit müssen wir in allen den Fällen, in denen sich ein oder mehrere Nucleoli im Kerne differenzieren, in diesen die Tätigkeitscentren des Kernes erblicken."

Schwalbe ('76) studied the nuclei of retinal ganglion cells of

the ox, rabbit, and sheep: in the smallest nuclei there is no nucleolus within the nucleus, but there are small peripheral prominences on the inner surface of the nuclear membrane; when a nucleolus is present within the nucleus it is jagged in outline, with fine, thread-like processes. The substance of the nuclear membrane "stimmt in allen Eigenschaften mit der des Kernkörperchens vollständig überein, und ist mit ihr continuirlich." Further, the substance of the peripheral prominences is quite identical with that of the nucleolus, and "Man könnte in dem Falle, wo ein innerer Nucleolus fehlt, geradezu davon reden, dass als Ersatz dafür wandständige Kernkörperchen vorhanden seien." In similar cells of the calf, there are no nucleoli in the smallest nuclei; in larger ones there are from two to four, one or two lying within the nucleus, the others being mere thickenings of its membrane; "beim Wachsen des Kernes ( $12.5\mu$ ) nimmt die Höhe und Zahl dieser Wandverdickungen immer mehr ab, während im Innern ein gut ausgebildeter zackiger oder eckiger Nucleolus von  $2.7$  bis  $3.6\mu$  das gewöhnliche ist." He considers the substance of the nucleoli and of the nuclear membrane to be at first identical, and to be diffused in the "Kernsaft." In the sympathetic ganglion cells of the frog, he noticed, on the heated stage, that the nucleoli exhibited slow changes of form; and in these nuclei he distinguishes "Nucleolarsubstanz, den Kernsaft und die reticuläre Substanz."

O. Hertwig ('77a) found in the egg of *Haemopsis* one large nucleolus, with usually one large vacuole; and also a number of small nucleoli, some of which contain each a small central vacuole. In the production of the pole bodies: "Aus den Theilstücken des Nucleolus und einem Rest des Kernsaftes entsteht ein fasriger, spindelförmiger Kern . . . es muss dahingestellt bleiben, ob der ganze Nucleolus oder nur ein Theil desselben und ob die Nebenkügelchen [Nebennucleolen?] in die Zusammensetzung der Spindel mit eingehen."

v. Kennel ('77) remarks of the ripe egg of *Malacobdella*: "der Kern . . . enthält eine mehr oder minder grosse Anzahl stark lichtbrechender runder Tröpfchen, die sich meist an seiner Peripherie befinden."

Mark (77): the salivary gland cells of *Chionaspis* contain each forty to fifty nucleoli; corresponding cells of *Aspidiotus* have a single large one which may contain from two to seven "nucleoluli." In cells of the oval gland of *Chionaspis* the nucleus contains a true nucleolus, usually without nucleoluli, and also a "Fetttröpfchen," which differs from the former in color and refraction. (The Fig. 32 of the salivary gland cells of *Aspidiotus* shows each nucleus to contain a double nucleolus, containing a larger sphere apposed to, in one case separate from, a smaller colorless sphere.)

A. Brandt (78) gives observations on the germinal vesicles of different forms. In *Aeschna grandis*: "Der vom Keimbläschen umschlossene Keimfleck ist wie dieses, ursprünglich rund, aber in noch viel höherem Grade, und zwar unstreitig bei allen von mir beobachteten Insekten, amöboid beweglich, so dass seine Form meist sehr verschieden erscheint. Nicht selten ist er in einige Theile zerfallen. . . . In einzelnen Keimbläschen lagen ausser dem Keimflecke noch ein oder mehrere Körnchen von verschiedener Grösse;— nur ein Paar Keimflecke wurden aufgefunden, welche anscheinend aus zwei aneinandergedrängten und theils übereinander geschobenen Kugeln bestanden." In *Periplaneta* vacuoles as well as solid "secundäre Keimflecke" occur in the nucleolus. In the egg of *Nemura*, after the action of acetic acid, the vacuoles in the nucleolus increase in size and in each a small granule is to be seen. In *Gryllus*, *Lepisma*, and *Holostomis* the germinal spot is amoeboid: "Die amöboide Beweglichkeit veranlasst nicht selten das Loslösen einzelner Partikel, welche, wie der Keimfleck selbst, amöboid-contractil sind. Die Zahl und Grösse dieser gelegentlich wieder zusammenfliessenden Partikel ist eine äusserst verschiedene"; thus the nucleolus may break into a number of equal-sized pieces, or into a mass of very fine granules. In the egg of *Tegnaria* there is usually a single vacuolated nucleolus, though sometimes there may be present also two "Nebenkeimflecke." In *Distomum* the "Keimfleck . . . ist in sehr hohem Grade mit amöbenartiger Beweglichkeit begabt," and there is a central body in the nucleolus which changes its form periodically. Brandt observes

in regard to the frog's egg : "Der Keimfleck des Froscheies, in den allerjüngsten Eianlagen meist ein zusammenhängendes Gebilde, erscheint bekanntlich später, in eine grössere Anzahl von rundlichen Klümpchen zerfallen — und diese fand ich (bei *Rana esculenta*) amöboid gestaltet"; and adds, against Bütschli ('76), "ist einzuwenden, dass dieser Zerfall des Keimflecks als amöboide Erscheinung keineswegs auf ein Absterben, sondern im Gegentheil auf eine erhöhte Lebensthätigkeit hinweist."

Brock ('78) : the immature ovum of *Anguilla* has one or two large nucleoli ; the number of the latter increases with the size of the egg.

Eimer ('78) notes the great relative and absolute size of the nucleus and nucleolus in ganglion cells, and finds it to be paralleled only in egg cells.

O. Hertwig ('77b, '78a) noticed in the nucleolus of the maturing egg of *Asteracanthion* certain changes, "die darin bestehen, dass die in seinem Innern bisher zahlreich vorhandenen kleinen Vacuolen verschwinden und in seiner Mitte oder mehr der Peripherie genähert eine grössere Vacuole erscheint, die fast ganz von einem kugligen aus Kernsubstanz bestehenden Körper erfüllt wird. . . . Plötzlich verschwinden die in ihm gelegenen Vacuole mit ihrem kugligen Körper unter dem Auge des Beobachters," and in consequence the nucleolus begins to gradually shrink in size, and  $1\frac{1}{2}$  hours afterwards has completely disappeared. The body within the large vacuole of the nucleolus corresponds to the smaller, more deeply staining portion of the original nucleolus, and during the nuclear division reaches out of an opening in the vacuole beyond the surface of the nucleolus, takes on the form of a long, thin rod, and occupies the middle point of the first pole spindle ; while at the same time the remaining portion of the nucleolus gradually breaks into a granular mass, which then disappears. Also in *Sphaerarchinus*, *Ascidia*, some *Coccenterata*, and various *Mollusca*, he noticed a similar differentiation of the nucleolus into two substances, namely, a smaller, deeply staining portion apposed to, or enclosed by, a lighter, larger portion.

O. Hertwig, in still another paper ('78b), investigated the



germinal vesicles of various animals. In *Eucope polystyla* there is one nucleolus in small eggs, several in riper ones: "Es liess sich hier feststellen, dass die zahlreichen Nucleoli durch Ablösung vom ursprünglichen einfachen Keimfleck entstehen."

Klein ('78) studied the stomach cells of the newt, and concludes "that in most cells the so-called nucleoli are local accumulations of the intranuclear network, that they are inconstant in size and number, and that they are only transitory appearances."

Schindler ('78), Malpighian tubules of insects: after a cell has become obliterated by the outflow of its secretion, its nucleus becomes a new cell, and its nucleolus a new nucleus.

Whitman ('78) found in the egg of *Clepsine* one to three nucleoli, each "composed of several highly refractive pieces."

Bergh ('79) found in the egg of *Gonothyraca* (*Campanularia*) a single large nucleolus, which is usually round, but sometimes with irregular outlines caused by slow amoeboid movements (observed in life), these motions being most vigorous later, when the nucleolus begins to divide. It increases in size, and acquires one or two vacuoles. In a later stage, but before the production of the pole bodies, there are a number of irregular nuclear bodies (staining as the original nucleolus), which had been produced by division of the nucleolus; in one case he actually observed the division of the nucleolus, which lasted half an hour, and at the same time the vacuole of the primitive nucleolus seemed to divide into two, so that each daughter-nucleolus received a daughter-vacuole. "Oft macht es den Eindruck, als ob das Volum der secundären Keimflecke zusammengenommen grösser wäre, als das der primären für sich . . . eine active Wanderung der Nucleoli durch den Kernsaft, wie dies Auerbach ['74] bei gewissen Nematoden in den Vorkernen gesehen hat, kommt wahrscheinlich hier nicht vor." The nucleolus also divides in the egg of *Clava*. In the eggs of *Psammocchinus* and *Echinocardium*, the single nucleolus begins to fragment before the chromatic network has disappeared. The *Phallusia* egg contains one large germinal spot, which probably disappears without fragmenting: "ich habe nämlich unter Eiern, die im Keimbläschen einen scharf

begrenzten, durch die Osmium-Carminbehandlung rubinroth gefärbten Keimfleck zeigten, auch solche gefunden, welche statt dessen eine sehr feinkörnige, bisweilen rubinroth, bisweilen weniger intensiv rothgefärbte Masse enthielten, die nicht scharf contourirt war, aber von derselben Grösse wie der Keimfleck. Falls diese Deutung, es schwinde der Keimfleck ohne sich vorher zu theilen, richtig ist, beginnt die Auflösung desselben mit dem Schwinden der Vacuolen in seinem Innern."

Klein maintains his previous views in regard to the nature of the nucleoli in two papers published in the following year ('79a, '79b).

1880.

Van Beneden ('80) studied the egg of the bat, and found one nucleolus (rarely two): "on trouve en outre quelques granules très petits, tous d'égales dimensions, répandus dans le corps de la vésicule (pseudonucléoles)"; the latter have no resemblance to any part of the chromatic filament.

Bütschli ('80) incorporates in his great "Protozoenwerk" the observations of preceding authors. In *Hyalosphenia* there may be as many as six spherical nucleoli; in certain other *Rhizopoda* the "Binnenkörper kann den von der Kernhülle umschlossenen Raum nahezu völlig ausfüllen." In the *Heli-ozoa* the nucleoli are much as in the preceding group. In the *Radiolaria* (for which Bütschli follows some of the observations of R. Hertwig, '79) there is usually a number of rather large nucleoli, frequently containing vacuoles. The nucleus of *Thalassicola* "enthält einen ansehnlichen, strangförmigen und unregelmässig verästelten Nucleolus, dessen Masse nicht ganz homogen, sondern äusserlich feinkörnig ist"; it later breaks into a number of segments. In *Acanthometra* the nucleolus is at first spherical, while later "Aus dem Nucleolus-Pol, welcher der Einstülpungsstelle der Kernmembran zugewendet ist, bildet sich eine helle homogene Masse aus, welche den dunkleren Haupttheil des Nucleolus wie eine Kappe bedeckt oder auch wie eine Vertiefung desselben eingesenkt erscheint. Der Nucleolus erscheint demnach jetzt von zwei verschiedenen Substanzen zusammengesetzt." In many *Flagellata* a nucleo-

lus is absent, in others there is a single one, sometimes with a vacuole; in the *Choanoflagellata* there is always one large, spherical nucleolus, in the *Cystoflagellata* several of various sizes; and in the *Dinoflagellata* there may be several small nucleoli, which are sharply localized from the chromatin, but show the fine reticulation of the latter element. In the *Ciliata* and *Suctorina* there are nucleoli of varying size and number in the macronucleus, but none in the micronucleus.

Chun ('80) finds in the egg of all *Ctenophora* a single large nucleolus, very rarely two.

Engelmann ('80) figures the nucleoli of certain ciliated cells of various invertebrates as each surrounded by a clear space, the outer boundary of which is marked on optical cross-section by a circle of granules.

Flemming ('80) concludes in regard to the nature of the nucleolus: "Dass die Nucleolen überhaupt keinerlei morphologischen Antheil an der Kernvermehrung nehmen"; and "Dass die Dinge, die wir Nucleolen nennen, vielleicht gar keine morphologisch wichtige Theile des Kerns sein mögen, sondern nur Ablagerungen von Substanzen, welche für den Stoffwechsel im Kern verbraucht und wieder neugebildet werden; sie würden damit gewiss physiologisch wichtige Theile des Kerns bleiben, — was ohnehin durch ihr fast allgemeines Vorkommen bewahrt wird, — aber doch keine eigentlich organischen, d. h. morphologisch-wesentlichen Kernbestandtheile."

O. Hertwig ('80) found in the eggs of *Chaetognatha* numerous small nucleoli.

Shäfer ('80), ovum of *Gallus*: there is a single nucleolus, which in young germinal vesicles consists of a homogeneous matrix which stains slightly with haematoxylin, and a number of coarse granules which stain deeply; in larger ova the nucleolus is homogeneous throughout and stains deeply. The threads radiating from the periphery of the nucleolus may be either artefacts or may be regarded as extrusions of the homogeneous substance of the nucleolus. Ovum of *Lepus*: in younger nuclei the nucleolus has the same general structure as in the fowl, though it is more irregular in form. In some larger ova the nucleolus "is represented by a number (a dozen

or so) of globules of varying size which appear to lie loose within the germinal vesicle. An intravesicular network is sometimes present, and serves to unite the granules of the macula. . . . It is possible that the homogeneous matrix above described may represent the remains of such a network, the filaments of which have shrunk up into a mass on contact with the hardening reagent" (picric acid and alcohol).

Trinchese ('80, according to Platner, '86) found in the germinal vesicle of *Amphorina coerulea* a "macchia germinativa laterale o accessoria," and a "macchia germinativa principale," the latter being about seven times the size of the former.

1881.

Balbiani ('81) investigated the salivary gland cells of the *Chironomus* larva. There are here "Deux gros nucléoles irréguliers, larges de 0.03 à 0.04 mm., bosselés à leur surface, et formés d'une substance réfringente granuleuse, creusée d'un plus ou moins grand nombre de vacuoles isolées ou confluentes. Il arrive assez souvent que les deux nucléoles se confondent par une partie plus étroite qui les réunit comme une sorte de pont; d'autres fois enfin, ils se fusionnent plus ou moins intimement en un seul nucléole, dont le diamètre est le double de celui des nucléoles isolés." The ends of the chromatin filament are apposed against the nucleolus; and the latter differs both chemically and morphologically from this "cordon nucléaire."

Giard ('81) observed in the egg of a *Spionid* during life a single central nucleolus. A certain time before completed maturation an "élément cellulaire" appears in the nucleus, which is a little smaller than the latter, and encloses in its center a small "noyau": "D'abord fort éloigné du nucléole, il s'en approche progressivement et vient s'appliquer à sa surface, où il s'aplatit et prend la forme d'une double calotte. En s'appliquant de plus en plus contre le nucléole, il perd son noyau et finit par se réduire à une double membrane qui entoure le nucléole," . . . and finally its substance fuses with that of the nucleolus.

Hubrecht ('81), egg of *Proncomenia*: "in all the different stages of development of the ovum the germinal spot is double:

a larger and a smaller sphere may be distinguished, which, however, are not connected in any way whatever . . . but perfectly free and independent of each other."

Mark ('81) finds that during the maturation of the egg of *Limax campestris* the male as well as the female pronucleus may contain as many as fifty or sixty "pronucleoli," which disappear before the copulation of the two pronuclei. In an undetermined species of *Limax* he "observed in both female and male pronuclei a single nucleolus of much greater size and more deeply stained than the other nucleoli."

Pfitzner ('81) finds that the "Kernsubstanz" is contained in the reticulum and the nucleoli; the latter lie within the meshes of the former, and their rôle is problematical. "Während des weiteren Verlaufes der Karyokinese verschwinden sie, werden anscheinend allmählig aufgezehrt, ohne direkt mit dem Gerüst in Verbindung getreten zu sein."

Retzius ('81, cited by Van Bambeke, '85): the nucleoli are simple local accumulations of the chromatin, derived from the nuclear reticulum.

#### 1882.

Blochmann ('82) observed in the egg of *Neritina* one large nucleolus containing a vacuole. Preceding the pole body production, the nuclear membrane vanishes, and the nucleolus at first retains its original size, then breaks up into several equal-sized fragments. "Dass die Elemente der Kernplatte aus Theilstücken des Nucleolus entstehen, kann bei unserem Objekt keinem Zweifel unterliegen, da ich alle Uebergangszustände vom unversehrten Nucleolus bis zur ausgebildeten Kernplatte beobachtet habe." After the two pole bodies have divided off, the remaining chromosomes in the female pronucleus fuse together to form a deeply staining, spherical body, which resembles the original nucleolus.

Flemming in his classical work ('82) gives the following definition of nucleoli: "Substanzportionen im Kern von besonderer Beschaffenheit gegenüber dem Gerüst und dem Kernsaft, fast immer vom stärkeren Lichtbrechungsvermögen als beide, mit

glatter Fläche in ihrem Umfang abgesetzt, stets von abgerundeter Oberflächenform, meist in den Gerüstbalken suspendirt, in manchen Fällen ausserhalb desselben gelagert." A membrane is absent around all nucleoli. He (erroneously) attributes the discovery of the nucleolus to the botanist Schleiden. Flemming holds it probable that with the possible exception of spermatozoa one or more nucleoli occur in every nucleus, of which it is therefore an important organ (in this conclusion he departs from the views expressed in his previous contribution, '80). "Die Zahl ist bei Thierzellen selten über 8 (mit Ausnahme der Kerne meroblastischer Eier), bei den meisten Arten von Thierzellen durchschnittlich 3-5. . . . Es ist der häufigste Fall, dass einer der Nucleolen an Grösse besonders vorwiegt," this being then the "Hauptnucleolus," the others "Nebennucleoli." In the "Hauptnucleolus" of the egg of *Lepus* two parts are distinguishable, but he leaves it undecided whether "die Unterscheidung von Haupt- und Nebennucleolen eine durchgehende Geltung beanspruchen kann." This investigator notes further: "Die absolute Grösse der Nucleolen steht bei den meisten Zellenarten in annähernder Proportion zur Grösse der Kerne selbst." The nucleolar vacuoles are filled with fluid. In regard to the apparent clear spaces around nucleoli, we read "dass dieses Phänomen nichts anderes ist als ein Reflex, bedingt durch die rundliche Fläche und stärkere Lichtbrechung des Nucleolus." He did not find amoeboid changes of form, but concedes that they may occur. The true nucleolar substance differs from the chromatin. The nucleoli are "spezifische Produkte des Kernstoffwechsels und zugleich auch spezifische Formtheile des Kerns . . . so kann man die Nucleolen ganz wohl Organe des Kerns oder der Zelle nennen." They appear to be "besondere Reproductions- und Ansammlungsstellen des Chromatins. . . . Entweder ist also in den Nucleolen noch ein anderweitiges Substrat vorhanden, in welchem das Chromatin verarbeitet wird und mit dem es in ihren durchlagert liegt, oder . . . die Substanz der Nucleolen mag zwar in sich homogen sein, ist aber dann nicht identisch mit Chromatin resp. Nuclein, sondern eine chemische Modification, Vorstufe oder Doppelverbindung derselben."

Graff ('82) figures in the eggs of *Proporus*, *Plagiostoma*, and *Vorticeros* a single nucleolus containing vacuoles.

Nussbaum ('82) studied the nuclei of gland cells (stomach mucosa of various *Vertebrata*, epidermis glands of *Argulus*). "Es liess sich im Allgemeinen feststellen, dass während des ungestörten Ablaufs der Secretion die mononucleolären Kerne vorherrschten, dass nach längerem Hunger die multinucleolären Kerne an Zahl vermehrt waren. . . . Ein Drüsenzellenpaar der Saugscheibe von *Argulus foliaceus* hatte am 12. Oktober mononucleoläre Kerne; am 18. Oktober zeigten sich viele Kernkörperchen im Kern; nach und nach ging die Granulierung der Zellen, die Strahlung verloren und die Kerne waren platte Ovoide mit mehreren glanzlosen Körperchen darin." From these observations Nussbaum concludes: "So wird man den Kern mit vielen Kernkörperchen als den Ausdruck einer Ruhepause der Kernfunktionen auffassen können, die entweder zum kräftigen Leben oder zum Tode überleitet."

Rauber ('82) figures the nucleoli in the ova of various vertebrates, and distinguishes the following kinds of nuclei, with regard to the mode of distribution of the "chromophile Substanz" (chromatin together with pyrenin): "globuläre," "trabekuläre," "filoide," and "gemischte."

Seeliger ('82) finds that in *Clavelina* the nucleus of the loose mesoderm cell (from which the ovum is derived) becomes the nucleolus of the ovum, and its cytoplasm becomes its nucleus. In the germinal vesicle there is then one large nucleolus, in which nucleolini lie, and also (to judge from his figures) vacuoles.

Vejdovský ('82), egg cells of *Sternaspis scutata*: the young nucleus contains at first one small nucleolus, bounded by a membrane (though the latter structure would appear from his figures to be a clear space enveloping the nucleolus). "Beim fortschreitenden Wachstum des Keimbläschens vergrössert sich auch der Keimfleck, und zwar in der Weise, dass die ihn umgebende Membrane einseitig sich verdickt und schliesslich auf dem runden sich in Pikrokarmine stark färbenden Keimfleck als ein glänzendes, gelbliches Bückelchen erscheint." The nucleolus disappears in the ripe egg.

1883.

Balbiani ('83) renewed his observations on the egg of *Geophilus longicornis*, making several emendations. In very young eggs there are two or numerous nucleoli, in larger eggs only one large one, containing one or several vacuoles. In his previous paper referred to, he assumed that the double tubular structure in these eggs served for the purpose of an intraovular circulation; but in the present paper he offers another explanation: that the double tubular structure later develops into a knotted cord, the distal portion of which then divides into irregular fragments, which become scattered through the yolk; and then each of these fragments, with the exception of one which becomes the "noyau vitellin," differentiates into cytoplasm, nuclear and nucleolar substance, and then represents a cell of the follicular epithelium.

Van Bemmelin ('83) states of the eggs of *Brachiopoda*: "Sie haben meist zwei Kernkörperchen, die enganliegend und stark lichtbrechend sind. Ausser diesen nimmt man oft noch mehrere lichtbrechende Kügelchen in dem gefärbten Inhalte der Eikerne wahr. Von Boraxkarmin werden sowohl diese Körperchen als die Nucleoli stark gefärbt." (Certain of his figures show one of the nucleoli imbedded in another.)

Van Beneden ('83), ovum of *Ascaris megaloccephala*: there is a single "corpuscle germinatif," which contains all the chromatin of the nucleus, and is contained within a special portion of the nucleus termed the "prothyalosome"; from one to three "pseudonucléoles" also occur in the nucleus, but they play no important part in the maturation of the egg.

Fol ('83a), egg of *Ciona intestinalis*: there is here one large, very refractive nucleolus containing a number of vacuoles which he believes are artefacts, since they cannot be found in the living egg, though their appearance after the action of reagents would show that the substance of the nucleolus is chemically not homogeneous. The nucleolus consists of a more refractive cortical substance, and of a less refractive, clearer medullary portion; in the latter, the vacuoles are produced. Fol maintains that the follicle cells arise by budding from the



egg nucleus: "Ce nucléole a une tendance bien évidente à se placer dans le voisinage immédiat des noyaux folliculaires en voie de formation. Le fait n'est pas constant, mais il est trop fréquent"; he did not actually observe that the nucleolus gives off a part of its substance to the follicle cell, but supposes this to be the case.

Fol, in a second paper ('83b) of the same year, finds that in *Ciona* during the "production endogène" of the follicular cells a segment (diverticulum) of the egg nucleus breaks off, while the (then peripherally situated) nucleolus gives a part of its substance into this diverticulum, and the nucleolus then wanders back to another portion of the nucleus. "Chez *Ascidia mamillata*, le bourgeonnement de l'enveloppe a lieu simultanément en une foule de points, et il est tout ou moins admissible que la substance de la tache germinative dispersée à la formation de ces bourgeons."

Gruber ('83) describes in *Actinosphaerium* the growth of a supposed nucleolus and its division during mitosis into two equatorial plates; though his figures would show that he mistook true chromatin masses for a nucleolus.

Jensen ('83) studied the ovum of *Cucumaria*; there are from fifteen to thirty nucleoli flattened against the nuclear membrane, and containing vacuoles. As shown by treatment with acetic or picrosulphuric acid, the outer layer of the nucleolus seems to be a continuation of the nuclear membrane, so that the inner, less refractive portion of the nucleolus appears to be situated in a depression of the outer surface of the nuclear membrane.

La Valette St. George ('83, quoted after Platner, '86) found in the egg of an *Isopod* one nucleolus which is at first homogeneous, later granular, and which may enclose a vacuole and show amoeboid movements. In other cases there are either several smaller vacuoles or one or two larger ones.

Leydig ('83), from comparative studies, concludes that the nucleoli "sind Theile des Kernnetzes," and that each of them is enclosed in a small, clear cavity of the nucleus. "Die Nucleoli können als eine Vielzahl von Körnchen erscheinen, die unter sich gleichwerthig sind. . . . Nicht selten lässt sich bei genauem Zusehen in der Menge kleiner und unter sich gleicher

Kernkörper ein grösserer Nucleolus . . . auffinden (Epithel des Eierstocks von *Aglia tau*). . . Wahrhaft riesige Kernkörper kommen zu Stande, wenn viele Nucleoli zu einem einzigen Körper zusammenfliessen. . . Prüfen wir Herkommen und Beschaffenheit der Kernkörper näher, so ist bezüglich der kleineren Nucleoli leicht festzustellen, dass sie aus Verdichtungen oder Knotenpunkten des Kernfadennetzes den Ursprung nehmen. Daher schon im frischen Zustande solche Kernkörperchen einen zackigen Saum haben, auch durch Spitzen und Striche sich verbinden, die bis zum Rande des Kernes gehen. Aber selbst die grösseren Nucleoli . . . erweisen sich als Umbildungen von Partien der Kernfäden." In the ganglion cells of the brain of *Limax* and *Arion* the nucleoli are jagged in outline, with long fibers. In the cells of the salivary gland of *Nepa* they are three or four in number, bent and elongated in form. Those of the corresponding gland in *Naucoris* have often the shape of a half ring, or may be lobular or band shaped, with cross striation. In the salivary gland of *Chironomus plumosus* there is usually a single nucleolus, spherical, lobular or tubular, its radiating cavity filled with a homogeneous, refractive substance; its wall contains vacuoles, "und starke Linsen lassen deutlich werden, dass der ganze Kernkörper eben wieder die Struktur eines Schwammgebildes besitzt." Besides the nucleoli there are in these cells several looped or contorted bodies, one of which is always in connection with the nucleolus, and all of which evince a cross striation, the nature of which is as follows: "Mit Tauchlinsen unterscheiden wir abwechselnd je eine dunkle und helle Querlinie und sehen die erstere, welche leicht gekerbt ist, zusammengesetzt aus einzelnen kleinen Stückchen, vergleichbar den Elementen einer Muskelscheibe. Die feinen Abtheilungslinien der den Querstrich bildenden Stückchen erstrecken sich ferner durch die helle Zwischenzone, so dass dadurch auch eine Art von zärtesten Längslinien zum Ausdruck kommen kann." He believes that these cross-striated structures "durch Umbildung des den Kern durchziehenden Maschenwerkes entstanden sind." In young larvae these structures are not seen immediately in life, but "nach und nach, während das Thier noch lebt, tauchen die querstreifigen

Bildungen auf. . . . Man darf wohl annehmen, dass die fraglichen Gebilde, bevor sie dem Auge sichtbar werden, schon dagewesen sind und nur erst jetzt sich abheben, weil die Lichtbrechungsverhältnisse sich geändert haben." Similar cross-striated bodies were noticed in the cells of the Malpighian vessels of *Chironomus*. In the ovarian egg of *Libella* Leydig found one nucleolus, which consisted of a mass of granules grouped around a central cavity, these granules being connected together by fine threads; "der lebende Nucleolus zeigt ferner langsam ablaufende Gestaltsveränderungen, wobei sich nach und nach einzelne Klümpchen mehr oder weniger absondern."

Ogata ('83) investigated the pancreas cells of man, which had been treated with various poisons and with the induction current, then fixed in aqueous solution of corrosive sublimate, and with osmic acid. One to more than eight nucleoli may be present: "Die einen färben sich wie die Kernmembrane tief mit Haematoxylin. . . . Die anderen oder vielmehr das andere, denn es ist in der Regel nur eins, färbt sich nicht mit Haematoxylin, sondern mit Eosin. . . . Manchmal hat es einen ganz feinen blauen Saum, als habe es selbst wieder eine Membran. Es ist viel größer als die anderen Kernkörperchen, und das Feld, in dem es liegt, ist durch eine stärkere Linie von dem übrigen Kern getrennt. . . . Man wird es am unbefangenen wegen seiner Färbung als Plasmosoma von den übrigen die Kernfärbung annehmenden Karyosomen des Kerns unterscheiden." Sometimes several smaller plasmosomata are also present. Close to the nucleus is a body he terms "Nebenkern," which stains as the plasmosoma, but is much larger, and is apposed to the surface of the nucleus like a hat; its substance is homogeneous, refractive, enclosing small cavities in which minute spherules occur, the latter having a resemblance to zymogen granules. The "Nebenkern" is produced by a plasmosoma which has wandered out of the nucleus, and there becomes the nucleus of a new cell. (This process is called "Zellneuerung.")

Pfützner ('83) found in the resting nuclei of the ectodermal cells of *Hydra* usually one central, spherical nucleolus. Its substance is not identical with the chromatin in the resting stage of the nucleus, but becomes metamorphosed into the latter

substance during the following mitosis ; wherefore he suggests the term "Prochromatin" for nucleolar substance. In the prophase of the mitosis only one nucleolus is present in the nucleus, while in the "Rückkehr der Tochterkerne zum Ruhestadium waren dagegen stets mehrere vorhanden. In einem gewissen Stadium, wo die Nucleolenbildung beginnt, ist eine ganze Anzahl vorhanden ; jemehr sich der Tochterkern dem Ruhestadium nähert, desto mehr vermindert sich die Zahl unter gleichzeitiger bedeutender Grössenzunahme der übriggebliebenen, bis für das ausgesprochenste Ruhestadium das Vorhandensein eines einzigen grossen central gelegenen Nucleolus geradezu typisch wird," and this he concludes to be a process of fusion. The nucleolus plays only a passive rôle in mitosis, "nämlich die eines aufgespeicherten Nahrungsmaterials zur Neubildung von Chromatin."

Rein ('83) studied the eggs of *Lepus* and *Cavia*. In each there is one large nucleolus which disappears during maturation and is succeeded by several smaller ones, which have the same consistency as the first, and at the time of their first appearance occupy a central position in the nucleus. "So weit ich den Vorgang am Säugethiere verfolgen konnte, machte mir derselbe eher den Eindruck eines successiven Zerfalles des ursprünglichen Keimflecks in immer kleinere Stückchen, welche schliesslich in der Substanz des Keimbläschens verschwinden."

Roule's ('83) conclusions are, in the main, confirmatory of Fol's ('83) observations in regard to endogenous cell formation. In the egg of *Ciona* there is one large and two or three smaller nucleoli, the latter being "formés pendant l'évolution des cellules endotheliales en cellules ovulaires." In eggs a little larger these "nucléoles adventifs" become more numerous (five to six), and certain of them show a limiting membrane. Later still some of these adventive nucleoles are found in the yolk, where each becomes surrounded by a clear zone ; these he considers at this stage to be the nuclei of endogenetically formed cells (follicular cells), the clear zone around each representing its cytoplasm.

Schauinsland ('83) noticed in the egg of *Distomum* a single large nucleolus.

A. Schneider ('83) studied *Klossia*, one of the *Coccidia*. One or several nucleoli are present, "formant un ensemble souvent très complexe que j'appellerai le *corps nucléolaire*." Sometimes the largest nucleolus is enveloped on one side by a number of secondary, much smaller ones ("nucléolites"), which are portions loosened from the inner substance of the large nucleolus, from which they break out through a "canal micropylaire" (such a canal was not observed in life, and on only a single fixed preparation; cf. his Fig. 7). "Corrélativement à la multiplication du corps nucléolaire, le nucléole principal diminue de volume. . . . Touts les petits nucléoles qu'on observe dans le corps nucléolaire me paraissent descendre aussi sûrement du nucléole primitif ou ancêtre que les jeunes d'une espèce de leurs parents. Les nucléolites, une fois produits, grossissent et, d'homogènes qu'ils étaient d'abord, peuvent offrir à leur tour la différenciation d'une couche corticale et d'une zone centrale et faire office de producteurs nouveaux . . . j'ai de bonnes raisons de penser qu'à ce moment tous les nucléolites produits sont de taille sensiblement égale et qu'ils paraissent tous homogènes. . . . Je n'ai pas vu ce que deviennent ces fragments du nucléole, quelque soin que j'aie mis à scruter leur destinées. Je suppose que l'enveloppe du noyau se rompt, que les nucléolites mis en liberté gagnent par des mouvements propres la zone superficielle de la masse granuleuse pour s'y diviser activement. . . . Si ma hypothèse était fondée, le corps nucléolaire mis en liberté dans le plasma du kyste représenterait en réalité les débris de la fortune d'un noyau; ce serait le noyau lui-même, segmenté, morcelé, et le nom employé, celui de nucléoles, serait complètement impropre."

Weismann ('83), ova of *Hydromedusae*: in all the genera studied there is always a single large nucleolus, which sometimes contains one or several vacuoles.

1884.

Ayers ('84) germinal vesicle of *Oecanthus niveus*: in smaller eggs a single nucleolus, in larger ones several; these nucleoli he considers as "nodules of nuclear filaments."

Carnoy ('84) distinguishes three kinds of nucleoli: (1) "nucléoles nucléiniens," which are parts of the chromatin network; (2) "nucléoles-noyaux," which contain all the elements of a normal nucleus (namely, a membrane, chromatic filament, and nucleolar substance), while the substance in the remainder of the nucleus is allied to cytoplasm; such nucleoli occur in *Gregarines*, large *Radiolaria* and *Rhizopoda*, *Spirogyra*, the asci of lichens, testicle cells of *Littobius*, and eggs of *Pleurobrachia*, *Ascidia*, and *Nephtlys*; (3) "nucléoles plasmatiques," which contain no chromatin, but consist of a plastin network in which an albuminous enchylema is imbedded.

Frommann ('84) studied fresh ganglion cells from the anterior horn of the medulla of the ox; their nucleolus shows "eine Zusammensetzung aus feinen und derberen Körnchen und aus sehr kurzen Fäden, mitunter auch einen netzförmigen Bau mit theils ganz engen, theils etwas weiteren Maschen." In the ganglion cells (of the ganglion Gasseri) of the rat, the nucleolus is usually homogeneous, as are those of the sympathetic ganglion cells of *Bufo*.

R. Hertwig ('84), *Actinosphaerium*: in the resting nucleus there is one central nucleolus which consists of deeply staining nuclein and faintly staining paranuclein. The nucleolus is rarely spherical; when so, it consists mainly of nuclein, except for a small portion of paranuclein superimposed on the margin. In other cases the larger nuclein portion is of a curved dumb-bell shape, and "gleichzeitig bildet das Paranuclein ein schwach gekrümmtes Stäbchen, dessen Krümmung zur Krümmung der Nucleinmasse senkrecht gestellt ist." The connecting portion of the dumb-bell may disappear, "so dass sich zwei Nucleoli bilden, welche von einander durch ein queres Stäbchen Paranuclein getrennt werden. . . . Hiermit beginnen die plurinucleolären Kerne, wie sie für gewöhnlich bei *Actinosphaerium* beobachtet werden." In most nuclei there lies a mass of from six to twenty nucleoli, which are smaller as they become more numerous: "Hier ist es sehr schwer festzustellen, was aus dem Paranuclein geworden ist, und . . . bin ich zu dem Resultat gekommen, dass es als ein Korn im Centrum des Haufens von Kernkörperchen ist, dass es mit einem Fortsatz an jedes

derselben herantritt und alle somit unter einander zu einer Rosette vereinigt. . . . Die staubförmigen Nucleoli sind ursprünglich vorhanden, erst allmählich vereinigen sie sich zu grösseren Stücken, bis endlich nur ein einziger Nucleolus und Paranucleolus gegeben ist; dann tritt die Theilung ein." In the resting nucleus all the chromatin is contained in the larger nucleolus.

Jijima ('84) found that there are one or several nucleoli in the ripe eggs of Triclad *Turbellaria*, but none in younger germinal vesicles.

Korschelt ('84), following Balbiani ('81) and Leydig ('83), investigated the interesting structures in the cells of the salivary gland of *Chironomus*. The form and number of the nucleoli is mainly such as was described by Balbiani, "meist aber sind sie ausgehöhlt und von der Form einer mit sehr dickem Boden versehenen Schale. . . . Die Convexität der Schale richtet sich immer nach der zunächst gelegenen Aussenfläche des Kernes. . . . Der Kernkörper besteht aus einer feinkörnigen Masse, in welcher Vacuolen auftreten. . . . Von den Vacuolen fliessen oft einander benachbarte zu einer grösseren zusammen." The cross-striated structures described by Balbiani are not to be seen in the fresh nucleus, but, as noted by Leydig, first appear after the nucleus has remained under the microscope for some time; thus they may be possibly products of coagulation. "Dass sie sich, wie dies Balbiani zeichnet, mit ihren fransenartig gebildeten Enden an die sog. Kernmembranen anheften oder dass (nach Leydig) Anheftungsfäden von ihrer Oberfläche zur Umgrenzung des Kernes hingehen, habe ich allerdings nie bemerken können. . . . Ich muss nach meinen Befunden . . . sagen, dass die "Querstreifung" der Bänder auf einer Faltung ihrer Oberfläche beruht und dass eine Zusammensetzung aus verschiedenartigen Schichten nicht vorhanden ist." Further, Korschelt did not observe the enveloping membrane of these structures, described by Balbiani, though he corroborates the observation of this author that the end of the band gradually fuses into the mass of the nucleolus. From experiments on starving larvae, he concludes: "Es scheint demnach das eigentliche Chromatin nicht die ganze Masse der

Bänder auszumachen, sondern nur einen Bestandtheil derselben zu bilden, der bei mangelhafter Ernährung der Gewebe zuerst schwindet."

Lang ('84) remarks of the egg cells of Polyclad *Turbellaria*: "Das Kernkörperchen oder der Keimfleck ist stets als ein kugliger, relativ sehr grosser, intensiv gefärbter Körper zu unterscheiden."

Vejdovský ('84) noticed a single nucleolus in the eggs of *Oligochacta*.

Wielowiejski ('84) studied the egg cells of various *Arthropoda*. In the *Araneina* and *Acarina* the larger nucleolus contains a single large or several smaller vacuoles, though no pulsating or amoeboid movements were noticed (in opposition to the observations of Balbiani). In *Drassus* and *Lycosa* there is a small mass of granules in place of a germinal spot; in *Oniscus*, a single large nucleolus; in *Astacus*, numerous peripheral ones; and in *Musca*, a large, irregularly spherical one. (He notes that the germinal vesicle differs from all other nuclei in that its contents do not stain at all, or only faintly, with acetic acid methylen-green solution.)

Will ('84) studied in life the eggs of *Bufo* and *Rana*. Larger and smaller nucleoli may be distinguished; the latter increase somewhat in size, but never attain the dimensions of the preceding. Those nucleoli, then, which lie close to the nuclear membrane cause small protuberances ("Knospfen") of this membrane, each such bud next breaks off from the nucleus, and, still enclosing a nucleolus within itself, wanders towards the periphery of the cell, and there becomes a "Dotterkern," the disintegration of which furnishes the yolk granules.

1885.

Van Bambeke ('85) reviews the opinions of the following writers in regard to the nature of nucleoli: Flemming ('82), Strasburger, Pfitzner ('81), Retzius ('81), Leydig ('83), Balbiani ('81), Korschelt ('84), R. Hertwig ('84), Van Beneden ('83), Frommann ('84), Carnoy ('84), Brass, Wielowiejski ('84), and Rabl ('84). Nucleoli are rarely absent, and hence they must be regarded as an essential element of the nucleus. "Le



mode d'origine des nucléoles généralement admis explique le rapport de ces éléments avec la charpente nucléaire. . . . Flemming est dans le vrai en disant que si les nucléoles sont généralement suspendus au *reticulum*, ils ne sont pas en continuité de substance avec ce dernier, mais constituent des éléments spéciaux. . . . Nous croyons devoir rapprocher du nucléole principal la formation récemment désignée par Ed. Van Beneden, sous le nom de *corpuscule germinatif*, et plusieurs de celles appelées par Carnoy *nucléoles-noyaux*." The nucleoli are probably reservoirs for masses of chromatin.

Van Beneden and Julin ('85) found, in contradiction to Roule, that in the ovum there is only a single large "corpuscule germinatif" in *Clavelina*, and neither smaller nucleoli nor any migration of nucleoli into the cytoplasm.

Bütschli ('85), *Ceratium tripos*: most of the nucleoli of the individuals examined contained no nucleoli; only occasionally are one or two present, and then these evince a honey-combed ("wabige") structure. In many *Flagellata* there is no trace of a nucleolus.

Carnoy ('85) amplifies his observations of the preceding year, in which he had distinguished the following four types of nucleoli: (1) "nucléoles nucléiniens"; (2) "nucléoles plasmatiques"; (3) "nucléoles mixtes" ("qui sont constitués par la réunion des deux espèces précédentes en un corps unique"); (4) "nucléoles-noyaux." Types 1, 3, and 4 are closely related, and all are sharply demarcated from type 2. The "nucléoles plasmatiques" are plasmatic, albuminoid accumulations, and not chromatin material in reserve (in opposition to the views of Heuser, Guignard, and Pfitzner): "Nous préférons dire qu'ils concourent avec les autres éléments plasmatiques du noyau à l'élaboration du fuseau, dont les filaments constituants sont formés d'une substance, ou de diverses substances, présentant beaucoup d'analogie avec la plastine." The "nucléole nucléinien" may be composed of amorphous masses or of a skein of chromatin (the latter is the case in the testicle cells of *Chilopoda*, ova of *Pleurobrachia* and *Cymbulia*): "Le nucléole central de beaucoup de cellules ganglionnaires est de nature nucléinienne et présente souvent

la même constitution floïde"; and similar nucleoli occur in the *Protista* and in various cells of the *Arthropoda*. The "nucléole-noyau" of the eggs of *Cymbulia* and *Lithobius* has a fine external membrane and a convoluted chromatin filament. In the amitotic division of the capsular ovarian cells of *Gryllotalpa* the nucleolus (formed of a central portion of chromatin and a peripheral layer of plastin) divides first so that each daughter-nucleus receives one nucleolus. But in the amitosis of the intestinal cells of *Aphrophora* the "nucléoles plasmatiques" do not divide; and in the testicle cells ("métrocytes") of *Scolopendra* there is also a "nucléole plasmatique," and at the commencement of the mitosis the "nucléole se liquéfie pour enrichir le caryoplasma," and is not to be found later. The amitotic division of the fat cells of *Geotrupes* is introduced by a division of the "nucléole-noyau."

Frenzel ('85) studied the cells of the mid-gut in insects at various stages of development. *Bombyx dispar*, larva: one large nucleolus containing a vacuole, in which lies a small spherical "Nucleollolus." *Tachina*, larva: here is one large nucleolus, "mit kurzen zackigen Ausläufern. In seinem Innern umschliesst er fast stets wenigstens einen, in der Regel aber mehrere, etwa 6 bis 12, kugelige oder matt aussehende Gebilde, welche nicht gerade den Eindruck von festeren Körpern, sondern vielmehr von Vakuolen machen." In cylinder and gland cells of various insect larvae the nucleus is filled with a homogeneous fluid, "in welche sowohl echte Nucleolen, wie auch nucleolenartige Körper ('Keimflecken' oder 'Nucleolide') einerseits und andererseits zahlreiche verschieden angeordnete sehr klein aber stets gleich grosse Körnchen eingelagert sind, die hier 'Kerngranula' oder '-granulationen' heissen mögen."

Leydig ('85) noticed in ganglion cells of *Astacus* a large, spherical, granular nucleolus, in which is a large cavity; this nucleolar cavity stands in communication with that of the nucleus itself. We read further: "Die Körper im Kern, die man Nucleoli nennt, sind Bildungen verschiedener Art"; some arise out of the nodal points of the nuclear network, others out of the "Kernplasma."

Rabl ('85) studied mitoses in cells of the larva of *Salamandra*, and found that in the prophases of mitosis the nucleoli gradually vanish and take part in the production of the chromatin threads. In the unripe germinal vesicle of *Proteus*, on the inner surface of its membrane, "sieht man in unregelmässigen Abständen von einander kugelige, stark glänzende, wie Oeltropfen aussehende Körperchen," which he assumes are neither nucleoli nor masses of true chromatin.

Will ('85) studied the ovogenesis of *Notonecta* and *Nepa*. The young "Ooblast" contains one nucleolus bounded by a membrane and surrounded by smaller "Chromatinballen"; subsequently the latter bodies fuse together and form a closed ring around the nucleolus. The nuclear division of the oöblast is an amitotic one, and is preceded by a division of its nucleolus; in each daughter-nucleus, then, the divided half of the primitive nucleolus breaks up into fragments, which become distributed through the nuclear sap, and the daughter-nucleus produces a new nucleolus without the aid of these particles. When the ovum proper is ripe, the nucleolus finally disappears.

1886.

Van Bambeke ('86) found that in the germinal vesicles of *Arachnida*, *Isopoda*, *Hymenoptera*, and *Meconema*, the nucleoli and the chromatin do not stain with methylen green (corroborating Wielowiejski) though they stain with carmine and haematoxylin; "Rien ne s'oppose, me semble-t-il, à ce que l'on considère le corpuscule germinatif comme étant équivalent à l'ensemble de la charpente chromatique des noyaux ordinaires [somatiques]." He concludes that there is no proof of the identity of the true nucleoli of the somatic cells with the germinal spots of egg cells. Two stages in the formation of the nucleolus may be distinguished in the ova of various Arachnids (*Lycosa*, *Amaurobius*, *Argyronecta*, *Tegenaria*, *Attus*, *Theridium*, *Epeira*, *Zilla*, *Phalangium*): (1) there is a single large nucleolus (sometimes accompanied by smaller accessory ones), in which at first a few vacuoles arise, which later fuse to produce a single voluminous vacuole; and (2) the nucleolus becomes replaced by a mass of fine granules. In the ovarial egg of *Amaurobius*

*ferox* the nucleolus consists of (1) a peripheral, less deeply staining portion; and (2) of a more deeply staining and more highly refractive central portion, in which one large and several smaller vacuoles lie: "Chose remarquable dans la vacuole centrale se voyait, à l'état frais, un granule foncé, doué d'un mouvement très vif"; in this germinal vesicle a small, finely granular nucleolus is also present. Amoeboid movements of the germinal spot of *Periplaneta* were noticed. In the egg of *Zilla* there are from one to three homogeneous, spherical nucleoli, as also a large "tache principale"; the latter is composed of two or three different substances, somewhat as in *Amaurobius*.

Carnoy ('86), egg of *Spiroptera strumosa*: there is one large, central "nucléole nucléinien," sometimes also one or two small "nucléoles plasmatiques"; the former nucleolus is the only part of the nucleus which stains deeply with methyl green; it is bounded by a fine membrane, and contains eight "bâtonnets" (chromosomes), so that it is comparable to a "nucléole-noyau." *Nematode* from the stomach of *Scyllium canicula*: in the "œufs très jeunes . . . le filament nucléinien y est assez puissant, il paraît continu. . . . Nous n'avons pu voir s'il se scindait d'abord en tronçons; nous croyons plutôt qu'il se localise par le retrait de ses anses, pour constituer un nucléole nucléinien pelotonné. Ainsi naît la tache de Wagner. Elle est toujours simple; elle se colore peu par le vert de méthyle"; no "nucléoles plasmatiques" are present in this nucleus. In the egg of *Filaroides mustclarum* one or two "nucléoles plasmatiques" occur; but in that of *Ascaris lumbricoides* such nucleoli are usually absent, and the chromatic filament extends through the whole nucleus. In *Ascaris* sp. (from the dog) there is one "nucléole plasmatique" in young eggs.

Heathcote ('86) noticed in the egg of *Julus* one nucleolus with vacuoles; it disappears before the production of the pole bodies.

Knappe ('86), ovarian ova of *Bufo*: The nucleoli show amoeboid movements in life, and these movements probably lead to the dissolution of the nucleoli, by causing the latter to first break into fragments, these fragments afterwards dissolving in the nuclear sap.

Pfützner ('86a) distinguishes in the nucleus: "Das Achromatin, eine geformte färbbare Substanz, das Chromatin (mit der Unterart der Nucleolensubstanz, des Prochromatins) und eine geformte nicht färbbare Substanz, das Parachromatin." In a second paper ('86b) he studied *Opalina*: here are several nucleoli flattened against the nuclear membrane; "bei der Kinese verschwinden sie allmählich, aber später als bei anderen Objekten bisweilen sind sie noch bis zur Metakinese vorhanden." Though they are occasionally found at the poles of the spindle they take no part in the formation of the chromatin elements, and in the daughter-nuclei reappear at a distance from the latter elements. For denoting the substance of the nucleoli he substitutes for his earlier term "Prochromatin" the term "Pseudochromatin," since "das Chromatin und die Nucleolensubstanz wohl nichts Anderes mit einander gemeinsam haben, als die untergeordnete Eigenschaft, sich bei den meisten Färbemethoden gleicherweise stark zu färben."

Platner ('86) investigated the ovogenesis of *Arion* and *Helix*. In *Arion* there appears first in the "primitives Ei" a small, completely spherical nucleolus, to which he limits the name "Nucleolus"; "weiterhin enthält das Keimbläschen den eigentlichen Keimfleck. Dieses ist zu Beginn seines Auftretens meist rundlich mit hervorspringenden Erhabenheiten, als sei er durch Contraction eines Knäuels entstanden. Zuweilen erscheint er auch mehr ringförmig oder ganz unregelmässig. Immer aber verdichtet er sich bald zu einem völlig runden homogenen Element, welches Kernfarbstoffe begierig aufnimmt und den Nucleolus bedeutend an Ausdehnung übertrifft." (His figures show the two to be in close contact.) A number of clear vacuoles begin to appear in the "Keimfleck": "Sie sind rund und von verschiedener Grösse . . . und scheinen nur dazu zu dienen, weitere Veränderungen einzuleiten. Sie verschwinden nämlich alsbald wieder, und in dem stetig an Grösse zunehmenden Keimfleck scheidet sich mit wachsender Deutlichkeit eine heller gefärbte und eine dunklere Partie. Letztere, dem "corpuscle germinative" van Benedens entsprechend, ist von geringer Ausdehnung, rundlich oder länglich oval und liegt excentrisch in der von runden Contouren

begrenzten hellen Substanz, die demnach auf dem Querschnitte halbmondförmig erscheint. Sie dürfte dem von van Beneden als "prothyalosome" bezeichneten Gebilde entsprechen. Es sei mir daher gestattet, sie Hyalosoma zu benennen. In völlig entwickelten Eiern ist dieses Element nahezu völlig farblos und erscheint aus feinen Körnchen zusammengesetzt. Die gefärbte Partie des Keimflecks tritt dadurch um so schärfer hervor, man kann sie im Anschluss an van Beneden Keimkörperchen nennen." The nucleolus of the ripe egg "liegt excentrisch und besteht wieder aus dem runden zart granulirten Hyalosoma, sowie in dem peripher in demselben gelagerten Keimkörperchen, welches sich stark färbt und keine weitere Differenzierung erkennen lässt. Dem hellen Hyalosoma meist dicht anliegend findet sich der intensiv sich färbende Nucleolus oder der kleinere Keimfleck." Platner considers that by the last division of the ovocyte the "Nebenkernel" disappears and becomes a constituent of the nucleus. "Bei Ausbildung der Furchungsspindel konnte ich mit Sicherheit constatiren, dass die Spindelfasern aus der unfärbbaren Substanz des Eikerns hervorgingen. Diese ist bei sich entwickelnden Eiern im Keimfleck enthalten, in welchem sie sich bald als Hyalosoma differenzirt." In *Helix* the "primitive Eier . . . entbehren des schönen grossen Nucleolus. . . . Daher enthält ihre definitive Form auch nur einen Keimfleck, welcher weiterhin dieselben Veränderungen zeigte wie bei *Arion*." It may be noted in conclusion that in the spermatogonium of *Arion* the nucleolus appears in the nucleus at the same time that the "Nebenkernel" appears in the cytoplasm.

Schauinsland ('86) found one or two large nucleoli in the egg of *Bothrioccephalus rugosus*.

Stuhlmann ('86) investigated the early stages of the ovum in a large number of species, more particularly of the *Arthropoda*. *Carabus memorialis*: there are numerous "chromatin" granules in the young eggs, which increase in number and size; later a granular nucleolus appears: "Es ist schwer zu entscheiden, ob der Haufe von chromatischen Körnern zu einem grossen Ballen zusammenschmilzt, oder ob sich einer, wohl das ursprünglich central gelegene, zum Nucleolus ausbildet oder endlich ob

letzterer eine ganz neue Bildung ist. . . . Wenn aber schon Dotter ausgeschieden ist, hat der Nucleolus fast stets eine Form, die aufs Täuschendste einer Eichel gleicht. . . . Wir sehen an dem Nucleolus einen helleren, völlig homogenen Theil und einen dunkler gefärbten, welcher fein granulirt ist und wie mit einer Menge von winzigen Vacuolen durchsetzt erscheint. Dieser dunklere Theil umgreift wie die Cupola einer Eichel den helleren Theil. Um die Formähnlichkeit ganz zu vollenden, sitzen häufig auf der Kuppe der homogenen Hälfte noch einige dunkle Körnchen. . . . Auf einem Aequatorialschnitt sieht man nun, dass der dunklere Theil eine Zone um den helleren Theil bildet." This enormous nucleolus measures  $67\mu$ ; it disappears when the nucleus wanders to the periphery of the egg. *Carabus auratus* and *Pterostichus elatus*: one spherical nucleolus, containing a few small vacuoles, and its size increases with that of the nucleus; later it assumes a peripheral position, "und in seiner Nähe treten mehr oder weniger kleine Chromatinkugeln auf, während der Nucleolus selbst kleiner zu werden scheint"; the nucleolus disappears, then the small "Kugeln" unite to form a larger spherule, and finally the latter also vanishes. In the egg of *Dytiscus marginalis* there are no true nucleoli, only irregular masses of chromatin. Egg of *Silpha*: one granular nucleolus, which increases in size up to a certain point, and later, when vacuoles arise in it, a number of small spherules become apparent outside of the nucleolus: "Ob dieselben aus dem Nucleolus stammen oder ob sie als Paranucleolen des Kerngerüstes aufzufassen sind, weiss ich nicht," though he does not think that they are products of the nucleolus; the nucleolus, as well as a portion of the nucleus, disappears later. *Necrophorus vespillo*: several non-homogeneous germinal spots, later a single nucleolus, which finally vanishes. Eggs of *Geotrupes* and *Cetonia*: several small, spherical or elongated nucleoli, which occupy a central position in the nucleus, and increase in number and size; "Dieselben liegen in concentrischer Anordnung um einen homogenen Kern, der etwas dunkler als die Kerngrundsubstanz gefärbt ist." *Lina populi*: at first there is one large and one small nucleolus; in this stage "sind im ganzen Keimbläschen

mit Ausnahme der Randzone ganz feine klare Bläschen vertheilt, welche ich jedoch als Kunstprodukte ansehen möchte"; later there lies in one part of the nucleus a group of minute nucleoli; then a portion of the nucleus breaks off and wanders into the cytoplasm, while the remaining portion of the nucleus retains one small nucleolus; and lastly, when the nucleus becomes amoeboid in shape, it contains one large vacuolated nucleolus, "sowie mehrere kleinere chromatische Körper." *Lycus aurora*: at first there is no nucleolus, later a large and a small one (both granular); when the nucleus wanders to the periphery of the egg it retains one of the nucleoli, which subsequently disappears at the same time as the nucleus does. *Periplaneta orientalis*: at first there is no nucleolus, "derselbe bildet sich erst allmählich heraus. . . . Wir sehen ausser dem etwas körnigen Nucleolus eine Anzahl kleinerer stark färbbarer Kügelchen, die wohl als Bestandtheile des Kerngerüsts, als Paranucleolen aufzufassen sind." *Gryllotalpa vulgaris*: in the immature egg "ein eigentlicher Keimfleck ist nicht vorhanden; vielmehr liegen in der Kerngrundsubstanz zerstreut Chromatinpartikel von  $4\mu$  Durchmesser bis zu unmessbarer Feinheit"; when the nucleus has assumed a peripheral position a large nucleolus is produced in it, "wohl durch Verschmelzung mehrerer kleinerer." *Locusta viridissima*: in maturer ova a large but lightly staining nucleolus, "von dem aus ein Kernnetz seinen Ursprung nimmt." *Pieris brassicae*: one large, homogeneous germinal spot, which later acquires vacuoles and divides into three parts. *Sphinx ligustris*: in the immature germinal vesicle lies a large, excentric nucleolus, containing vacuoles; "ausser letzterem finden sich noch einige wenige Paranucleolen"; at the time when the nuclear fragments break off, the nucleolus becomes paler and then vanishes. *Zygaena filipendulae*: at first no nucleolus is present, later there is a larger one with vacuoles, as well as a smaller one, "der sich wohl von dem grossen abgelöst zu haben scheint"; subsequently both disappear. *Musca vomitoria*: there is at first in the germinal vesicle a single, large, excentric nucleolus, but later appear in it "eine Anzahl von Paranucleolen und ein Nucleolus, . . . von welchen letzterer aus einem Häufchen von



kleinen, gefärbten Kügelchen besteht." In the egg of *Anabolia* there is one large nucleolus, but in those of *Vespa germanica* and *V. media* apparently no true nucleoli are present. There is a large granular nucleolus in the larger germinal vesicles of *Bombus terrestris*. *Trogus lutorius*: there is one large, irregularly shaped nucleolus and two smaller ones; all these finally disappear, and their place is taken by smaller granules. *Bauchus fulvipes*: at first no nucleolus is present, later one or three large nucleoli appear, but all of them vanish subsequently. In the egg nucleus of *Pimpla* sp. only a number of small granules are to be found, and at a later period still smaller granules. *Anomalon circumflexum*: in the youngest germinal vesicles no nucleolus is to be found, in older ones there is a single large one; this has nothing to do in the formation of the "Dotterkerne," and disappears when the nucleus does. There is one spherical germinal spot in *Ophion ventricosum*, but not in *O. luteum*. *Ephialtes liturater*: in the smaller nuclei a considerable number of "chromatic" bodies occur, while in the older ones there is a single large nucleolus. *Ambyteles castigator*: one large nucleolus, in older ova also several smaller ones. *Epeira diademata*: here is one large spherical nucleolus, which later becomes jagged in outline and evinces vacuoles, which may unite to produce a single larger vacuole: "In seltenen Fällen kann man einen Zerfall des Nucleolus in mehrere kleinere sehen, was jedoch wohl eine pathologische Erscheinung sein dürfte." *Glomeris marginata*: one large, spherical or angular nucleolus, and later also a smaller one: "Höchst wahrscheinlich stammt dieser von dem grossen Nucleolus ab"; the smaller nucleolus disappears subsequently. In the egg of *Peripatus edwardsii* one nucleolus forms itself gradually, and vacuoles begin to appear in it. In *Amaroecium rubicundum* a single large nucleolus is present; while in *Clavelina lepadiformis* the nucleolus is probably formed out of the central chromatin masses. From these numerous observations Stuhlmann draws the conclusion: "Aus Allem schien mir hervorzugehen, dass das Schwinden des Nucleolus nicht zum Wesen der Eireifung gehört, besonders weil ich ihn bisweilen (so bei *Silpha*) so lange verfolgen konnte, als noch ein Rest des Keimbläschens im Ei sichtbar war."

Vigelius ('86) finds in the egg of *Bugula* one large nucleolus, containing vacuoles.

Will ('86) studied the maturation of the egg of *Colymbetes*. "Dem Kernkörperchen oder Nucleolus . . . kann nach meinen Untersuchungen keinerlei morphologische Bedeutung zukommen. Was wir Kernkörperchen nennen, ist nach meiner Auffassung nichts als ein besonders grosses Stück Chromatin-substanz. So können wir es verstehen, dass bald eines, bald mehreres, bald gar keine vorhanden sind."

1887.

Boveri ('87): in the ovum of *Ascaris megalocéphala bivalens* there are no true nucleoli when the tetrads are formed. In the variety *univalens* there is usually one "achromatisches kugeliges Körperchen. Von dem "Prothyalosoma," das an den van Beneden'schen Eiern den Keimfleck [Vierergruppe] umgiebt und welches im weiteren Verlauf bei ihm eine so grosse Rolle spielt, habe ich weder auf diesem Stadium, noch später die geringste Spur wahrgenommen."

Eisig ('87) remarks in regard to the egg of *Capitellids*: "Der ursprünglich rundliche, jederzeit durch Dichtigkeit und hohes Tinctionsvermögen auffallende Keimfleck erleidet im Laufe seines Wachstums offenbar Theilungen; denn man findet ihn in späteren Stadien mit ein oder zwei verschiedengradig abgesehnürten Kuppen besetzt; ausserdem trifft man schon frühe mehrere Pseudonucleoli, welche offenbar Produkte des Hauptnucleolus darstellen, in dem Keimbläschen zerstreut." He notes, further, that in the maturing ovum the nucleolus does not increase in size in equal proportion to the size of the nucleus. (To judge from his figures, the nucleoli are not homogeneous.)

Fraipont ('87) found in the germinal vesicle of *Polygordius* several nucleoli of unequal size.

Henking ('87) studied the eggs of *Phalangids*. In the ovarian egg a sickle-shaped body lies at one pole of the nucleolus: "es scheint, als wenn in ihm und dem Keimfleck die Chromatin-substanz des Keimbläschens sich concentrirt hätte." In the nearly ripe egg there is one large nucleolus, which is not homo-

geneous, and a number of smaller globules, these latter staining as the former, and some of them containing vacuoles: "sie stellen einerseits eine Zusammenballung der bisher ganz unregelmässigen, im Keimbläschen vertheilten Chromatinsubstanz dar, rühren andererseits aber wohl vom Keimfleck her." These bodies have all disappeared in the ripe egg.

Hubrecht ('87) noticed only a single nucleolus in the egg of *Cerebratulus* sp.; as to the egg of *Pelagonemertes*, he figures one nucleus containing one large and several smaller nucleoli, and another nucleus with only numerous small nucleoli.

Kosinski ('87, '93, mentioned by Lavdowsky, '94): within the nucleolus of cancerous cells there is sometimes a vacuole, and within the latter a small body which Kosinski considers may correspond to Carnoy's "nucléoles-noyaux"; such nucleoli have the faculty of division, and of wandering through the nuclear membrane into the cytoplasm.

Lukjanow ('87a), stomach epithelium of *Amphibians*: in the cytoplasm of the cylinder epithelium are structures of various form ("Nebenkerne"), which stain in general like the nucleoli. In some of the nuclei of the deep layer of gland cells each nucleolus is joined with a karyosome.

Lukjanow ('87b) distinguishes three kinds of nucleoli in muscle cells of *Vertebrates*: (1) "Plasmosomen"; (2) "Karyosomen"; (3) "Kernkörperchen von gemischtem Charakter." The first stains deeply red (eosin), the second blue-violet (haematoxylin), while the third stains a mixed color with these two stains (when used together). He remarks also: "dass in manchen Kernen die Kernkörperchen gänzlich fehlen, in anderen entweder nur eine Kategorie derselben, oder mehrere zugleich vertreten sind. . . . Zuweilen liegt das Kernkörperchen sogar ganz ausserhalb des Kernes."

Nussbaum ('87) found in smaller eggs of *Hydra* a single large nucleolus, while in larger ova several are present. "In frischem Zustande sieht man in den allerersten Stadien neben den Keimflecken noch eine blasse Kugel, die im Gegensatz zu den Nucleolen des Keimbläschens keine Farbstoffe in sich aufnimmt."

O. Schultze ('87) studied the maturation of the egg in *Rana* and *Triton*. In the unripe germinal vesicle there are larger

nucleoli near the nuclear membrane, and smaller ones at the center of the nucleus: "Dass sie sich durch Theilung vermehren, kann keinem Zweifel unterliegen, denn nicht nur sind dieselben in ganz jungen Eiern grösser und weniger zahlreich, . . . sondern die grösseren Keimkörperchen weisen durch Einschnürung und Zerklüftung auf eine Vermehrung durch Theilung hin." He does not consider that such daughter-nucleoli are again capable of division, but that the process is rather a "Lösungsphänomen." All the nucleoli are homogeneous, but vacuoles are produced in them by  $\frac{1}{2}$  per cent normal salt solution. In larger ova a considerable number of nucleoli lie peripherally, and there is also a central group of them; and, still later, the peripheral nucleoli commence to stain less intensely, and the greater number are centrally situated. The nucleus of the maturing egg consists of "Membran, Kernsaft und Keimkörperchen," a chromatin network being absent; and the microsomes of the chromosomes are formed from the smallest, most centrally placed nucleoli.

1888.

Böhm ('88) found in the egg cell of a 5 cm. long *Ammocoetes* of *Petromyzon* a homogeneous nucleolus, "an dem sich sehr oft eine kleine Vacuole zeigt, welche mit einer feinen Strasse bis an die Oberfläche des Fleckes [nucleolus] reicht." At the animal pole of the nucleus lies a disc-shaped mass ("Deckel"): "räthselhaft ist die Bedeutung des Deckels." (Compare the extranuclear structure found by Lukjanow, '88.)

Boveri ('88): in the female pronucleus is neither a prothyalosoma nor a hyalosoma, such as were described by Van Beneden ('83); the hyalosoma is probably "ein durch Schrumpfung entstandenes Artefakt." Just before copulation "zeigen sich die ersten Spuren achromatischer Kernkörperchen als ganz kleine Körnchen, die . . . stets . . . in nächster Nachbarschaft der chromatischen Elemente sich finden, . . . so dass die Vermutung nahe gelegt wird, dass sie sich aus diesen absondern."

Fiedler ('88) studied the egg development of *Spongilla*: one large homogeneous nucleolus is present in the germinal vesicle. In the nuclear division (which is intermediate between the

mitotic and the amitotic) "der gesammte sonstige — übrigens spärliche — Chromatininhalt des Kernes vereinigt sich . . . mit dem Kernkörperchen zu einem kugeligen Gebilde, und erst dieses zerfällt dann durch allmähliche Zerschnürung in zwei kleinere, unter sich gleich grosse Kernkörperchen, welche an die beiden Pole des Kernbläschens rücken."

Graff ('88) found in the egg of *Spinther* either a mass of granules or a single nucleolus; the nucleolus may be either granular or contain a large vacuole.

R. Hertwig ('88): in nuclei occur "chromatische" nucleoli, and "das unter gewöhnlichen Verhältnissen nicht färbende Paranuclein, welches zumeist rundliche Körper, die Paranucleoli, bildet. Die Paranucleoli können entweder die einzigen Kernkörperchen im Kern sein (gewöhnliche Gewebszellen, reifes Ei und Furchungszellen) oder sie finden sich neben den chromatichen Nucleoli, unter Umständen auch als Einschlüsse derselben (Keimbläschen der unreifen Eier, Kerne von Actinosphaerien und anderen Protozoen) vor. . . . Zweifelhaft wird es dagegen gelassen, ob auch der Substanz des achromatischen Gerüsts . . . nicht . . . vielleicht auch Paranuclein [ist], welches sich durch seine Anordnung von den Paranucleoli unterscheidet." The centrosomes are probably derived from the paranucleoli, and the paranuclein is "die befruchtende Substanz" (these views have subsequently ('96) been retracted).

Kultschitzky ('88) found in the youngest eggs of *Ascaris marginata* one "Kernkörperchen," which afterwards "in zwei Stückchen zerfällt, deren eines sich intensiv mit Karmin färbt und alle Eigenschaften des Chromatins bewahrt, das andere sich in die blasser gefärbte gewöhnliche Kernkörperchen verwandelt"; the latter he terms the true "Kernkörperchen," which from this stage on gradually decreases in size, and finally disappears.

Leydig ('88) gives the results of numerous comparative investigations on germinal vesicles; most of these observations were made on the living egg, fixing reagents having been little employed. *Nephele vulgaris*: here there is one nucleolus, which sometimes has a long process, "in dessen Nähe kleine rundliche Ballen von gleicher Art, wie er selber ist, liegen, so

dass man die Entstehung der letzteren durch Abschnürung von dem Fortsatz sich denken darf." *Argulus foliaceus*: in young eggs there is one large nucleolus with clear spaces in it, showing that the nucleolus "aus Theilen besteht, die allmählich von einander weichen, so dass man alsdann in anderen Thieren anstatt eines Keimflecks eine ganze Anzahl kleinerer vor sich hat"; these nucleoli are often jagged in contour; by treatment with chromo-acetic acid "bekommen die Keimflecke eine Querzeichnung, so dass sie wie aus Querstücken zusammengesetzt erscheinen." *Tetragnatha*: one large nucleolus with dark contours, and several smaller pale, granular ones, which gradually disappear during the maturation of the egg. *Lycosa*: "Ein einziger, grösserer Keimfleck zeigt sich . . . und dieser bietet das Bild eines Knäuels dar." *Theridium*: the large "Hauptkeimfleck hat die Beschaffenheit eines stattlichen, aus scharf geränderten kleinen Körpern zusammengesetzten Ballens. Von ihm nun weg zieht sich ein Strang solcher Körperchen oder Theilstücke über die Grenze des Keimbläschens hinaus in den Dotter hinein. In einzelnen Eiern, deren grosser Keimfleck das Bild gewundener und geknäuelter Fäden giebt, können die kleinen Theilstücke zusammenhängend oder in bereits abgelösten Gruppen abermals in den Dotter sich erstrecken. Ja ich glaube an dem lebenden Ei verfolgt zu haben, wie Theile der geknäuelten Fäden sich zu einzelnen Ballen zusammenschoben und in den Dotter vordrangen"; there are present also one or several pale "Nebenkeimflecke." *Phalangium*: the young ovum has one large nucleolus containing vacuoles; "Wiederholt habe ich beobachtet, dass ein solcher Keimfleck — das lebende Ei mit Mundspeichel befeuchtet — unter dem Mikroskop allmählich verblasste und zuletzt für das Auge völlig verschwand." *Lithobius*: there may be one granular nucleolus, or numerous nucleoli, each with a granular core: "Wieder eine andere Form ist die, dass die amöbenartigen Gebilde in ihrem Innern einen hellen, kernartigen Fleck mit centralem Pünktchen zeigen und am Rande feinstrahlig sind"; in other germinal vesicles there may be present numerous small nucleoli, either irregularly grouped or arranged in "kurze, goldrollenähnliche Säulchen . . . ; ein andermal stösst man auf

längere fadige Aufreihungen, deren Stränge zu Schlingen gebogen oder geknickt sind." In these ova two kinds of nucleoli may occur, namely, numbers of the small ones just described, and a large one with dark contours, which has a central vacuolar, granular portion, and is peripherally homogeneous; but nucleoli also occur which are intermediate between these two kinds. *Geophilus electricus*: here are numerous small, pale nucleoli and a large one, which has a finely granulated core, and an outer homogeneous layer, the latter portion consisting of concentric layers; further, he noticed the infundibular structure first found by Balbiani on the outer surface of the nucleus, though he remarks that it is especially apparent in eggs in which post-mortem changes have commenced (!), and concludes: "Wir haben es sonach bezüglich des Trichters mit einer Ausbuchtung jenes Hohlraumes oder Lichtung zu thun, welche von der Höhlung um das Keimbläschen herum in den Dotter dringt." The basis of this infundibulum empties into a space around the nucleus, and not into the nucleus itself (as opposed to Balbiani's observations); Leydig also thinks that particles of finely divided nucleoli penetrate separately out of pores which are present in the nuclear membrane, and that these particles, arrived in the cytoplasm, fuse together to form a large "Ballen." *Stenobothrus*: in the ova of the proximal portion of the egg tube there are either numerous small nucleoli or a dense mass of very fine granules; in riper germinal vesicles they are much larger and resemble somewhat the nucleoli in the salivary glands of *Chironomus*; masses of nucleolar substance wander out of the nucleus into the cytoplasm. In *Pemphigus bursarius* there is one compound nucleolus, with fine radiating processes; and in *Meloë violaceus* there are numerous nucleoli, each of which has the structure of the single one of the preceding species. *Gasterosteus aculeatus*: in the month of May there are numerous germinal spots, sometimes densely grouped, sometimes arranged in rows; the gradual thickening of the nuclear membrane takes place at the cost of nucleolar substance. *Triton taeniatus*: the germinal vesicle at the end of October contains numerous nucleoli of unequal size, many of which are arranged in columns; the peripheral ones probably

wander into the cytoplasm. *Salamandra maculosa*, larvae : the "Urei" has a single large nucleolus. *Bufo cinereus*, larvae of several months : concludes "dass die Keimflecke, wenn noch winzig klein, aus den Knotenpunkten des Spongioplasmas entstanden sind, und nachdem sie eine gewisse Grösse erreicht, die Form und Sonderung einer Amöbe besitzen. Dieselben stellen sich jetzt dar wie hüllenlose, kleine Zellen, an denen wir einen homogenen körnigen Körper, der feinzackig oder selbst in feine Strahlen ausgezogen ist, unterscheiden und im Innern einen lichten, kernähnlichen Fleck, in dem sich noch ein Körperchen abzeichnet"; numbers of such nucleoli may later fuse together, "unter Vermittelung ihrer Zackenspitzen." *Rana esculenta*: in the smallest ova there is only a single large nucleolus, with a vacuolar central portion and peripheral radiating strands ; in larger eggs there are a number of smaller nucleoli, each of which has the same structure as the primitive one ; Leydig believes that nucleoli wander out of the nucleus, since he found a granular mass on the outer surface of the latter. The ova of *Sus scrofa*, *Myoxus nitela*, and *Talpa europea* contain each a single nucleolus.

Lukjanow ('88) investigated the stomach mucosa of *Salamandra*. There are several, usually club-shaped nucleoli ("Nucleoli claviformes"), the smaller, often funnel-shaped, end of which is in contact with the nuclear membrane. He concludes "dass die kolbenähnliche Form des Nucleolus . . . auf eine Vorbereitung zur Inhaltsentleerung hinweist. Der Kolben entleert seinen Inhalt etwa ebenso, wie die Becherzelle ihren Schleim entleert" ; and he supports this conclusion with the observation that a mass is often found on the outer surface of the nuclear membrane which stains like the nucleolus.

Nagel ('88) studied the human egg. The "Primordial-Ei" has a single nucleolus ; those which contain no nucleoli he believes do not develop further. In the ripe egg amoeboid motions were noticed in life (studied in liquor folliculi).

Sanfelice ('88) terms the nucleolus of the spermatoblast "nucleus," and the nucleus, "cell." What he calls the nucleus then divides karyokinetically (but that this process is a division of the nucleolus may be deduced from his figures 60 and 62).



Scharff ('88) studied the maturation of the eggs of various *Teleosts*. In the smallest ova examined (.011 mm.) there are numerous peripheral nucleoli, and a few which are central in position. In larger eggs (.03 mm.) "the nucleoli show an inclination to gather still more towards the periphery of the nucleus . . . one or more of the nucleoli become larger than the others, and in their interior refractive specks are visible which have sometimes been described as endonucleoli." In still larger ova (.08 mm.) "in some cases the big nucleoli disappear almost completely, leaving an unstained part around them." In *Conger* he "noticed a small nucleolus being constricted off from a larger one." He figures outside of the germinal vesicle of *Gadus* certain granules, and these he considers are emigrated nucleoli which are destined to become dissolved there, though he holds it possible that "some find their way to the surface of the egg to form the nuclei of the follicular epithelium"; in eggs which have attained the dimensions of .132 mm. the nucleoli become very irregular in shape. In the *Trigla* egg of .13 mm. the surface of the nucleolus is raised into small protuberances, most of which contain a nucleolus; these protuberances later break off and become the yolk spherules (in corroboration of Will, '84).

Schewiakoff ('88), *Euglypha*: the nucleolus gradually disappears in the prophase of mitosis.

Steinhaus ('88), intestinal cells of *Salamandra*: karyosomes and plasmosomes are distinguished within the nucleoli, and are usually combined in pairs with one another. Plurinucleolar nucleoli are formed by continued divisions of a single nucleolus, "et les nouveaux nucléoles s'éloignent l'un de l'autre, probablement à l'aide de mouvements amoeboides ou d'autres qui leur sont propres." Plasmosomes when extruded into the cytoplasm increase greatly in size, though this increase is due to mere imbibition of some substance; each such extruded nucleolus, combining with a karyosome, develops into a new nucleus.

Vejdovský ('88) studied the maturation of the egg of *Rhynchelmis*. The embryonal genital cells contain no true nucleoli. The nucleolus does not stain when it first appears (in very young stages). Subsequently it is always excentric in position,

perfectly spherical, and consists of a central, homogeneous, deeply staining portion, and an outer unstaining envelope (judging from his Fig. 5, Tab. 3, I would consider this supposed envelope to be a vacuole in which the nucleolus lies). In the more advanced ovum this envelope has disappeared, and the nucleolus has increased in size, but is no longer homogeneous, since it contains a number of deeply staining granules. When "das Kernkörperchen die oben angedeutete Grösse [.013 mm.] erlangt hat, beginnt es sich einzuschnüren, was gewiss auf dessen Theilung hinweist"; he believes that this division is rapid, "dass es aber thatsächlich so geschieht, beweist die Thatsache, dass in den reiferen Eiern in der Regel zwei Kernkörperchen vorhanden sind. Das neu entstandene Kernkörperchen liegt anfänglich in der Nähe des älteren und ist etwas kleiner als dieses; später entfernt es sich mehr oder weniger und wächst zu der Grösse des ersteren heran." In the ripe egg two nucleoli are present, or there may be three or four, the latter two having been divided off from the former; each of these consists of an inner chromatic portion and an achromatic envelope; the latter is porous, and "man kann voraussetzen, dass durch die Poren die flüssige Nahrung in das Innere des Kernkörperchens eindringt." When this envelope has vanished, each nucleolus is formed of (1) a hyaline, homogeneous fluid, in which (2) a delicate network arises, the nodal points of which are represented by the previous granules of the nucleolus; "kurz und gut, die Kernkörperchen unserer Eier sind chromatische Kernfäden. . . . Die intensive Färbung sowohl der Knötchen als des Fadenwerkes erleichtert die Verfolgung des metamorphosirten Kernkörperchens, welches jetzt ganz und gar den Kernen des späteren Blastomeren gleichkommt." (The descriptions do not enable one to determine whether all the nucleoli become thus metamorphosed.)

Waldeyer in his "Referat" ('88) agrees with Klein "dass die Nucleolen nur stark verdickte Knotenpunkte des Netzwerkes der Gerüstfäden [chromatin], also mit den letzteren identisch seien. . . . Die Bedeutung aller dieser Dinge für das Zellenleben ist noch fast vollkommen dunkel."

1880.

Bergh ('89), *Urostyla*: the fragments of the macronucleus contain true nucleoli, while the micronuclei do not.

Brass ('89) states: "Für gewöhnlich erscheint jedes Kernkörperchen rund, sehr häufig kugelförmig; es besteht entweder aus einer gleichartigen Masse oder es sind in derselben einige hellglänzende Körnchen ausgeschieden, oder aber es finden sich in ihm dichtere, weniger glänzende Körnchen. . . . Im Umkreis der Kernkörperchen ist vielfach ein heller Hof, der von feinen Körnchen kugelschalenartig umgeben wird. Der Hof wird als Kernkörperchenhof beschrieben; er ist in sehr vielen Fällen sichtbar zu machen."

Davidoff ('89) observed in the egg of *Distaplia* a single large, spherical nucleolus, consisting of a homogeneous mass in which a few granules are imbedded. These nucleoli increase in size as follows: "Sie werden grössere Partien des Reticulums in sich aufnehmen, sich mehr und mehr verdichten und demgemäss sich immer deutlicher und deutlicher färben." Subsequently, but antecedent to the production of the pole spindle, the nucleolus contracts, and its contour becomes irregular, often with regular branched processes: "Vielleicht, ja sogar wahrscheinlich, werden sie dadurch hervorgerufen, dass der Nucleolus Flüssigkeit ausscheidet"; and the central portion of the nucleolus becomes lighter in color. Next, first the lighter portion, then the whole nucleolus, becomes filled with fine granules ("Chromosomen"). Then these chromosomes collect and form in the center of the nucleolus a compact, granular body, in the middle of which is one especially large chromosome, and the whole is surrounded by a membrane. And finally, other chromosomes, not concerned in the formation of the central granular body, form a reticulum around it. Davidoff concludes "dass aus dem Nucleolus ein Kern mit Kernnetz, mit einem Nucleolus und Nucleolus hervorgegangen ist. Wir können diesen Kern weder als Keimbläschen, noch als Nucleolus bezeichnen. Es ist eben ein neues Gebilde, dass wir einstweilen mit dem Namen Polkern belegen wollen"; out of this "Polkern" the first pole spindle is formed.

Fol ('89), ovarian egg of *Dentalium*; the nucleolus is at first absent, and single. In larger nuclei there are two apposed nucleoli (which disappear when the nuclear membrane has vanished). "Le nucléole présente d'abord deux parties distinctes, dont l'une, plus volumineuse et moins foncée, entoure l'autre un peu comme un bonnet posé sur la tête. La partie foncée est sphérique; elle retient l'hématoxyline ou le carmin alunique avec une nuance rougeâtre ou vineuse. Sa texture est compacte. L'autre partie est formée des corpuscules plus clairs [vacuoles] et d'un réseau plus foncé; elle prend les colorants que nous venons de nommer avec une teinte violacée tirant sur le bleu. . . . Lorsque l'ovule approche de l'époque où la vésicule germinative va se dissoudre, les deux nucléoles, au lieu de s'emboîter, sont simplement accolés, et le nucléole clair s'est accru beaucoup plus que l'autre."

Hermann ('89a) investigated the spermatogenesis of the mouse. The "Spermatoblastkern" (nucleus of a v. Ebner's cell) possesses one nucleolus, which is made up of two parts, "einen von Safranin sehr intensiv gefärbten, und einen ungefärbt bleibenden Bestandtheil. Letzterer tritt stets in Form einer einfachen Kugel auf, die chromatische Substanz aber besteht entweder aus zwei kleinen, leuchtend roth tingirten, an zwei gegenüberstehenden Polen der farblosen Kugel liegenden Kügelchen, oder das chromatische Element stellt eine einzige, in diesem Falle grössere Kugel dar, die dem ungefärbten Bestandtheile des Nucleolus sich innig anschmiegt. Im ersteren Falle erscheint dann das ganze Kernkörperchen als ein annähernd spindelförmiges Element, im anderen als eine Doppelkugel, und ist in beiden Fällen die Längsaxe des Nucleolus stets in dem grössten Durchmesser des Zellkerns eingestellt." The nucleoli of the spermatogonia are sometimes biscuit-shaped. Those of the spermatids are at first multiple in number, but later they unite to form a biscuit-shaped one. Still later, by the formation of the spermatozoon out of the spermatid, the two parts of this nucleolus wander apart, "dabei aber noch durch eine chromatische Brücke mit einander in Verbindung stehen." He observed in the follicle cells of the testicle of *Salamandra* "neben kleinen Nucleolen einen

grösseren, . . . der vollkommen die gleichen Strukturverhältnisse zeigt, wie sie oben von dem Kernkörperchen der Spermastoblastkerne der Maus beschrieben wurden und wie dies für den Frosch von Sanfelice angegeben wird."

Hermann ('89b), testicles of immature white mice : the nuclei of the follicle cells contain compound nucleoli, similar to those of the cells of v. Benda of *Salamandra*.

Korschelt ('89) made observations on the germinal spots of *Epeira*, *Dolomedes*, *Phalangium*, *Spinther*, and *Ciona*. In *Epeira* the nucleolus is at first a compact mass of granules "von stark lichtbrechenden Körnchen umlagert. . . . Ich will damit nicht sagen, dass eine direkte Aufnahme von [nutritiven] Körnchen stattfände, welche letzteren sich dann unmittelbar zum Keimfleck formirten, sondern möchte vielmehr glauben, dass die Substanz in flüssiger Gestalt aufgenommen und erst im Kern wieder geformt wird"; in later stages small vacuoles are frequently present in the nucleolus. In *Dolomedes* the nucleolus is at first homogeneous, it later contains vacuoles, and finally becomes simply a membrane surrounding a cavity. In *Spinther* there is a single large nucleolus with a vacuole. Korschelt draws the general conclusion : "Ich muss es nach meinen Erfahrungen, . . . als zweifellos hinstellen, dass eine Auflösung der Nucleolarsubstanz stattfindet. Die Erklärung dieser Erscheinung fand ich darin, dass die Nucleolarsubstanz in und vielleicht ausserhalb des Kerns zur Verwendung gebracht werden sollte."

Lukjanow ('89) describes the nucleoli ("plasmosomata") of the germinal vesicle and cleavage nuclei ; they disappear during mitosis.

Platner ('89a), Malpighian tubule cells of *Dytiscus*, fixation in Kleinenberg's fluid : there are one or several nucleoli, of irregular form, and around each one usually "ein hellerer Hof, welcher aussen von einer Anzahl grösserer unregelmässiger Chromatinbrocken eingefasst wird." The division of the nucleoli introduces the amitosis of the nucleus : "Der anfangs mehr runde Nucleolus zeigt eine Abplattung zur Scheibe, welche der umgebende Hof mitmacht. Zugleich tritt in der Richtung seiner kürzern Durchmesser eine Streifung an dem-

selben auf, als wenn er aus einer Anzahl nebeneinander liegender schmaler Elemente zusammengesetzt wäre. Weiterhin tritt eine Spaltung in der Richtung des längsten Durchmessers auf. . . . Die auf diese Weise entstehenden Tochterplatten zeigen an den einander zugewandten Seiten spitze Hervorragungen, an den abgekehrten Flächen dagegen mehr abgerundete Erhabenheiten. Beide besitzen wieder eine längsgestreifte Struktur, als seien sie aus parallelen Stäbchen zusammengefügt. . . . Den auseinanderweichenden Tochterplatten passt sich der helle, umgebende Hof an, der also in der Richtung dieser Bewegung sich verlängert."

Platner ('89b) contends, in opposition to the views of Ogata ('83) and others, that in the pancreas cells the nucleoli do not wander out of the nucleus.

Platner's ('89c) observations on the egg of *Aulastomum* shall be mentioned in the course of our observations on the egg of *Piscicola*. In accord with O. Schultze ('87) he finds in amphibian ova that the contents of the nucleus are composed only of "Kernsaft und Keimkörperchen," a portion of the latter forming the nuclear filament, the rest being extruded from the nucleus; the true chromatin loops were not seen by him.

Weismann and Ischikawa ('89) find in the ovarial winter ova of *Leptodora* one large nucleolus (rarely is a smaller one apposed to it), containing a large vacuole; it wanders out of the nucleus and becomes the "Nebenkern, Paranucleus," which ultimately disappears, and corresponds to the nucleus alone of the paracopulation cell of the other Daphnids. In nearly ripe ova of *Bythotrephes* "findet man . . . innerhalb des Keimbläschens und dem Nucleolus desselben ganz nahe einen scheibenförmigen Körper, der sich wie der Nucleolus färbt. Etwas später, wenn das Keimbläschen bereits an die Oberfläche des Eies gestiegen ist, liegt dieser Körper ausserhalb des Keimbläschens und ist in einen Protoplasmahof eingebettet"; then it rapidly disappears.

Wheeler ('89), ovarial follicle cells of *Blatta*: there is a "nucleolus of unusual structure. The latter consists of an irregular mass, not stainable in carmine or methyl green, and is regarded as plastin by Carnoy. . . . The mass of plastin encloses

a smaller mass of chromatin, or at least of a substance which does not differ in its reactions from the chromatin of the coiled filaments in the same nuclei." This nucleolus divides first in mitosis.

1890.

Auerbach ('90) distinguished two kinds of chromatin substance: "erythrophile," *i.e.*, substances staining with eosin, fuchsine, aurantia, carmine, picrocarmine; and "kyanophile," substances staining with methyl green, aniline blue, haematoxylin. The nuclear reticulum is not the fundamental portion of the nucleus, but the nucleoli are its important elements. He finds "dass in einer Grundsubstanz, die im frischen Zustande homophon, im gehärteten . . . höchstens feinkörnig erscheint, grössere, scharf begrenzte, isolirte, stärker lichtbrechende und stärker färbbare Körperchen, Nucleoli, von wechselnder, aber für die verschiedenen Zellarten und Thierspecies typischer Anzahl eingebettet sind"; thus in the *Batrachia* most of the nuclei contain numerous nucleoli, and when they are particularly abundant the greater number are peripheral in position. There are two kinds of "Kernkörperchen," those which stain blue (or green) and those which stain red (or yellow); both kinds occur in most nuclei. In the giant nuclei of the gland cells from the skin of *Urodelea* are found (1) numerous small cyanophilic nucleoli, and (2) from one to fifteen (usually two to five) much larger, erythrophilic nucleoli, which sometimes contain vacuoles. Embryonal nuclei contain only cyanophilic nucleoli, while in maturer nuclei erythrophilic nuclei become differentiated from the former. Thus in the blood corpuscles of frog larvae there is at first only one large nucleolus, which later differentiates into an inner erythrophilic and an outer cyanophilic portion. The peripheral layer next breaks up and divides into small cyanophilic nucleoli, while the central portion remains as a large erythrophilic nucleolus. Subsequently the smaller cyanophilic nucleoli ("Nebenkügelchen") may fuse together so as to produce six or eight larger cyanophilic nucleoli, each of which attains the size of the original "Stamm-Nucleolus"; at the conclusion of the larval period of the frog, the latter

nucleolus entirely disappears, becoming dissolved in the nuclear sap. "Die erythrophile Kernsubstanz ist übrigens dem Protoplasma des Zelleibes offenbar ähnlicher als die kyanophile."

Bürger ('90) made observations on the maturing ovum of various *Nemerteans*. *Carinella*: there is one large, spherical nucleolus. In the ripe egg of *Cerebratulus marginatus* "in der Regel kann man zwei umfangreiche Keimflecke konstatiren, welche aus einer schwärzlich-grünen körnigen Substanz zusammengesetzt sind, aber einen membranartig scharfen Kontour besitzen. Die beiden Keimflecke sind nicht von gleicher Grösse." In the immature germinal vesicle of *Drepanophorus*: "Dem wenig tingirten Binnenraum des Kernes durchflieht ein zartes Netzwerk feiner Fäserchen; peripher sind gröbere dunklere Körnchen angeordnet"; the ripe ovum of this Nemertean contains one finely granular, central nucleolus, in which are found "kuglige, noch intensiver gefärbte Körperchen." *Prosadenopus janthinus*: constituting the inner portion of the wall of the genital ducts are seen numerous cells, "welche ganz wie in der Entwicklung im frühen Stadium stehen gebliebene Geschlechtsprodukte aussehen," and each of these cells has one large nucleolus; while in the ripe egg the "Keimbläschen ist ausgezeichnet durch eine Menge kugliger Bläschen von über 5 $\mu$  Durchmesser mit scharf kontourirter und stark gefärbter Peripherie."

Eimer ('90, cited by Mann, '92), recalls his previous observations ('73, '78) in regard to the termination of nerve fibrils in the nucleolus; he mentions further that such radiating fibers are also to be found in the nucleolus of the egg cell, such fibers serving at first as paths for nourishment, and later becoming nerve fibrils.

Henking ('90), spermatogenesis of *Pyrrhocoris*: the single peripheral nucleolus of the first spermatocyte gradually becomes smaller in the prophase of division, and it is considered probable "dass er späterhin eine Einschnürung erfährt."

O. Hertwig ('90), *Ascaris megaloccephala*: in the spermatocytes of the growth zone the nucleolus is usually flattened against the periphery of the nucleus, or it may be irregularly elongated, or in addition to it a "Nebennucleolus" may be also



present ; from these differences in form he concludes that the nucleolus may be capable of amoeboid movements. Subsequently it wanders towards the center of the nucleus, becomes larger and more spherical. When the chromatin has assumed the characteristic radial distribution, before the first maturation division, the nucleolus passes again towards the periphery, and there becomes gradually smaller, partly by fragmentation, and so gradually disappears.

Holl ('90) found one spherical nucleolus in ova of the newly hatched chick : "Da das Kernkörperchen so auffallend verschieden vom Kernnetze und Kernsaft e hinsichtlich des Verhaltens zur Farbe sich zeigt, so muss es wohl aus einem anderen Stoffe bestehen als jene. Auch bei *Salamandra*, *Rana*, und *Lacerta* fand ich das Kernkörperchen immer sich verschieden halten von den anderen Theilen des Kernes." The nucleolus is always situated excentrically at the upper pole of the nucleus. Towards the end of the spirem stage the nucleolus lies on the periphery of the chromatin, with which it stands in no close connection ; it is no longer present in ova of  $491\mu$  diameter.

Kastschenko ('90) investigated the maturation of the ova of *Pristiurus*, *Scyllium*, and *Torpedo* : there are numerous nucleoli, which attain a diameter of  $16\mu$ , and all disappear at the spirem stage (in the prophase of the first pole spindle). Each nucleolus contains a large unstaining globule (but in his Fig. 1, in several of the nucleoli, all of which had been stained with borax carmine, this globule is colored blue, while the peripheral portion of the nucleoli is red).

Masius ('90) : in the ovum of *Asplanchna* the nucleolus forms the greater part of the nucleus. In *Lacinularia* it is at first as in the preceding genus, but at a later stage several much smaller nucleoli are found.

Mellissinos and Nicolaides ('90), pancreas cells of *Canis* : The "Nebenkern" is a plasmosome which has wandered out of the nucleus ; this migration is caused by an injection of pilocarpin into the living gland.

Sheldon ('90) found one germinal spot in *Peripatus capensis*, which disappears when the nucleus reaches the periphery of the egg.

Smirnow ('90), sympathetic ganglion cells of *Rana* and *Bufo*: a "Kernkörperchenkreis" is figured around the nucleoli of some of the cells.

1891.

Brauer ('91) studied the maturation of the ovum of *Hydra*. As a rule in the smaller eggs there is a single large nucleolus which occupies an excentric position within the nucleus; in larger ova numerous small nucleoli arise, which gradually become grouped near the large one. "Die Anzahl [der kleinen] wechselt, was zum Theil darin seinen Grund zu haben scheint, dass der grosse — selten sind zwei grosse vorhanden — wahrscheinlich durch Aufnahme kleinerer wächst . . . zum Theil aber auch darin, dass in verschiedenen Keimbläschen die Masse der Nucleolen eine verschieden grosse ist, was mit der Ernährung zusammenhängen möchte. . . . Sehr oft lag in der Nähe des grossen Nucleolus eine etwa halb so grosse blasse Kugel . . . möglich wäre es, dass diese sich vom grossen Nucleolus abgespalten hat, und den achromatischen Theil derselben vorstellt." Just before the formation of the first pole spindle the large nucleolus breaks into fragments, which, together with the smaller nucleoli, wander towards the periphery of the nucleus: "Ein Theil scheint im Keimbläschen selbst aufgelöst zu werden, ein Theil tritt unverändert nach dem Schwinden der Membran in das Eiprotoplasma über." Brauer contends that the nucleoli have no morphological significance in the maturation of the egg.

Cuénot ('91), ovarian egg of *Synapta inhacrens*: "la tache germinative primitive bourgeonne une quantité de petits nucléoles secondaires, qui errent dans le protoplasma clair de la vésicule germinative; presque toujours la tache a un aspect mamelonné par suite de la formation de ces nucléoles."

Davenport ('91) figures in the germinal vesicle of *Plumatella* a double nucleolus.

Macallum ('91), following Ogata ('83), distinguishes two kinds of nucleoli, namely, plasmosomata and karysomata. He finds the "Nebenkerne" of Nussbaum to be abnormal structures. In the pancreas cells of *Amphibia* an extrusion of plasmosomata occurs, but it is not a normal process, and the extruded portion

does not become a "Nebenkernel" (in opposition to the views of Ogata). In the eggs of *Rana* and *Necturus* the chromatin is "principally collected in the form of nucleoli at the periphery," but it is also contained in certain threads in the nucleus. He concludes from the study of the reactions of the substances to the indigo-carmin stain: "the peripheral nucleoli generate a substance, therefore, which diffuses gradually through the nucleus, then into the cell protoplasm, the point in time of the latter occurrence corresponding with the formation of the yolk spherules. The mode of origin is through a process of deposition from the nucleus of a substance allied to chromatin in the cytoplasm. . . . I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm."

C. Schneider ('91) concludes that the true spherical nucleoli "ebenso wie die Klumpen [of the chromatic network] aus [achromatischen] Gerüst und Chromatin bestehen und die Unterschiede beider nur morphologischer Natur sind." In the testicle cells of *Astacus* the nucleoli are spherical, with "eine deutliche Membran an und durch welche genau wie bei der Kernmembran [achromatische] Gerüstfäden treten. . . . Der ganze Unterschied zwischen Nucleolus und Klumpen besteht also hier darin, dass um ersteren die Fasern zu einer Membran sich zusammenlegen . . . was man ringförmig am Rande des Nucleolus wahrnimmt, ist sicher nicht die optische Wiedergabe einer Membran, sondern durch das Brechungsvermögen der Wandung des Nucleolus veranlasst." The nucleolus in eggs of *Echinodermata* is homogeneous only in the final stages of its formation. Nucleoli are only metamorphosed portions of the true chromatin, and represent reserve masses of this substance: "die Zusammenballung kann nur eine Befreiung der chromatischen Substanz von ihrer Arbeitsleistung bedeuten."

Wolters ('91) studied the sporulation of *Monocystis*: in the youngest individuals there is one nucleolus, which "in seinem Innern sich stärker tingierende chromatische Kugeln führt." In larger individuals the nucleolus consists of eight spheres, "Diese Kugeln führen in ihrem Innern wieder Stäbchen und Körner." Just before the conjugation of two individuals this compound

nucleolus breaks into a number of nucleoli of various sizes. After the copulation and encysting the nucleoli fuse together and gradually disappear (but I am unable to determine from his description whether the substance of the chromosomes is derived from the nucleoli). Shortly after the nuclei themselves copulate, the nucleoli reappear in them. In *Clepsidrina blattarum* there is a single primitive nucleolus, formed as in the preceding species; later there are numerous smaller nucleoli, which have probably arisen by division from the primitive nucleolus.

1892.

Bannwarth ('92) figures a division of the nucleolus in leucocytes from the spleen of the cat.

Born ('92) finds that in the *Amphibian* egg, in opposition to the observations of O. Schultze ('87), the chromatic "Fadenknäuel" has no origin in the nucleoli, but is directly derived from the chromatin network of the "Urei."

Brauer ('92) made observations on the maturation and fecundation of the egg of *Branchipus*. Each germinal vesicle from the "Wachstumszone" of the ovary has one large, slightly staining nucleolus, and near it a much smaller, deeply staining one. Each "Nährzelle," however, contains numerous nucleoli, and its nuclear sap also stains deeply. When the chromosomes are being produced, the larger nucleolus of the egg cell gradually ceases to stain, and it finally disappears. In the male pronucleus small nucleoli are present.

Frenzel ('92) noticed in *Carcinus moenas* and in a species of *Amphipod*, in the ferment cells and "Fettzellen" of the hepatopancreas, amitotic division of the nucleus, but no division of the nucleolus; "sondern dass vielmehr an geeigneter Stelle des Tochterkernes noch vor der Abschnürung desselben ein ganz neuer Nucleolus entstehe, der alle Charaktere des ersten besitzt"; in this nuclear division one of the daughter-nuclei retains the whole original nucleolus. In similar cells of *Idotea tricuspidata* he found the nuclear division to be as in the preceding species (but his Figures 8b, 10, and especially 11, would seem to represent stages of division of the nucleolus).

Häcker ('92a) studied the early development of *Acquorea forskalea*: in the ripe egg there is one spherical or kidney-shaped nucleolus, containing vacuoles. At the time of the first pole body mitosis the nucleolus does not accompany the nucleus, but remains behind in the cell, at the place previously occupied by the nucleus; and from this time on he applies to it the name "Metanucleolus." It is to be observed in one of the cleavage cells until about the 32-cell stage. "Zur Zeit wenn sich dann in der schwärmenden Blastula die Zellen des hinteren Poles . . . zu differenzieren beginnen, kann man in einzelnen von ihnen neben dem chromatischen Fadennäuel kleine nucleolenähnliche Körper beobachten, welche den nicht differenzierten Blastula-Elementen fehlen. Es wäre denkbar, dass man es hier mit den Abkömmlingen des Metanucleolus zu thun hat, ich vermag aber weder hierrüber, noch über das weitere Schicksal dieser Gebilde etwas bestimmtes zu sagen." Häcker assumes that what Metschnikoff ('86) supposed to be the "Sperm-nucleus" in *Nitrocoma* was in reality a Metanucleolus; and also that the "Paracopulationszelle," described by Weismann and Ishikawa ('89) in the winter egg of *Daphnia*, to have been also a nucleolus.

Häcker in a second paper ('92b) studied the maturation of the ovum of *Canthocamptus*. In the smallest eggs the nucleolus is large and contains vacuoles. Later it becomes differentiated into a lighter central portion and a denser peripheral part containing small vacuoles. At this stage the nucleolus presents a concavity facing the chromatic spirem. Then "aus dem Kernkörper tritt unter plötzlicher Verkleinerung desselben eine Masse aus, welche vermuthlich dem grossen, bis dahin in den meisten Kernkörpern kugligen Einschluss entspricht." The nucleolus apparently disappears when the first pole spindle is perfected.

Heidenhain ('92), cells of *Salamandra*: the nucleoli lie enclosed within the chromatin and linin network; he was unable to decide whether each nucleolus has a particular chromatin envelope. The nucleolus has no processes, and "nur die ihm auflagernde, von ihm selber stofflich differente Schicht ist mit dem Chromatin- und Lininfadengerüst kontinuierlich verbunden. . . . Mir ist es wenig wahrscheinlich, dass die Substanz

der Nucleolen etwas dem Chromatin ähnliches sei. Zwar sind sie durch einige Chromatinfarbstoffe stark färbbar, wie z. B. durch Safranin, allein auf eine andere Gruppe derartiger Farbstoffe reagieren sie nicht, hierher gehört das Methylgrün."

O. Hertwig ('92) in his recent text-book materially changes some of the views expressed in his previous papers. The true nucleoli consist of "Paranuclein" (Pyrenin), and he uses the term "Nuclein" for chromatin. "Nuclein und Paranuclein betrachte ich als die wesentlichen Substanzen des Kerns. . . . Beide scheinen mir in irgend welchen Beziehungen zu einander zu stehen." Further, he distinguishes "Keimflecke" from "echte Nucleolen." "Je nach dem Alter oder der Entwicklungsstufe einer Zelle kann der ruhende Kern . . . in der Zahl, Grösse und Beschaffenheit seiner 'Nucleolen' erhebliche Veränderungen erleiden."

Kostanecki ('92a) is preliminary to his '92b.

Kostanecki ('92b) studied mitoses "in sämtlichen embryonalen Zellen" of *Lepus*, *Cavia*, and *Equus*, with especial regard to the central spindle; I quote this paper here, since the "Centralspindelkörperchen" may have some relation to nucleoli. "Im Bereich dieser Centralspindel sieht man in diesem Stadium [Dyaster] in der Nähe der beiderseitigen Tochterfiguren der Chromosomen kleine Körperchen auftreten, die ich als "Centralspindelkörperchen" bezeichnet habe. Grösse und Zahl dieser Körperchen zeigen ganz beträchtliche Schwankungen. . . . Meist fand ich nun jederseits vier, fünf oder sechs grössere Körperchen, . . . daneben aber immer noch eine grössere Anzahl kleinerer Körnchen. Diese Körnchen sowohl als auch die grösseren Körnchen standen in inniger Beziehung zu den Fäden der Centralspindel." These granules then wander from both sides towards the equator of the spindle, "so dass sie . . . eine äquatoriale Körnchenplatte bilden. . . . Sobald die Einschnürung des Zelleibes bis zur Centralspindel vorgeschritten ist, werden die mehr peripher gelegenen Centralspindelfasern gerade im Aequator da, wo die Centralspindelkörperchen liegen, durchschnitten, und man sieht die Körperchen zugleich mit den verkürzten und undeutlich werdenden Fasern sich wiederum polwärts begeben." At each pole, then, these granules become

so densely grouped that often only one or two large granules appear to be present. "Mit der völligen Durchschnürung der Zellen wird schliesslich . . . der Zwischenkörper in zwei Theile getrennt, von denen jeder einer Tochterzelle angehört." Similar in the main points is also this process in the *Chick*, *Frog*, *Axolotl*, *Triton*, and *Salamandra*: "Wenn wir uns nun fragen, ob diese Vorgänge bei tierischen Zellen mit Recht mit den Vorgängen der Zellplattenbildung bei den pflanzlichen Zellen homologisiert wurden, so kann ich diese Frage nur zum Teil bejahen"; for two processes take place together, "nämlich eine äquatoriale Differenzierung der Centralspindelfasern zum Zweck ihrer Halbierung und eine eigentliche Zellplattenbildung, aus der die Zellscheidewand hervorgeht. Von diesen beiden Prozessen ist der eine, nämlich eine eigentliche Zellplattenbildung zum Zweck der Scheidewandbildung, bei tierischen Zellen gar nicht vertreten, wodurch der zweite desto deutlicher und unverhüllter zu Tage tritt." (Kostanecki mentions the following observations of previous authors on the occurrence of such a granular aequatorial plate in animals: Van Beneden, germs of *Dicyemida*; Balbiani, epithelial cells of an *Orthopterous* larva; Fol, eggs of echinoderms and *Cymbulia*; Flemming, eggs of echinoderms; Bütschli, egg of *Nephelis*; Mark, egg of *Limax*; van Gehuchten, egg of *Ascaris megalocephala*; Pre-nant, testicle cells of *Scolopendra* and *Lithobius*; Henking, similar cells of *Pyrrhocoris*; numerous observations of Carnoy; Van Beneden, ectoderm of vertebrate embryos; Strasburger, cartilage cells of vertebrates; Mayzel, corneal epithelium of *Fringilla*; Schleicher, cartilage cells of *Batrachia*; Carnoy, *Triton*; Bütschli, embryonal blood corpuscles of chick; Schottländer, inflamed epithelium of the cornea of the frog.)

Kraepelin ('92, cited by Braem, '97) noticed in the Bryozoan egg a division of the nucleolus.

Lönnerberg ('92) studied the nucleoli of various ova and somatic cells. In the liver cells of *Mytilus* there are two "Nebennucleoli" and one "Hauptnucleolus." In the cells of the intestinal epithelium of *Tellina* a granule is sometimes found on the outer surface of the nucleus, which resembles a small nucleolus, and stains in the same manner. *Doris*, egg: "eine stärker

sich färbende Kugel (meist auch eine oder mehrere kleine Vacuolen) in einer grösseren hineingesenkt war und so den Nucleolus darstellte." *Mytilus*: "In den Einucleolen von *Mytilus* liegt oft eine (oder bisweilen zwei) grosse, blasse Kugeln in der Mitte oder ein wenig excentrisch, aber von der stärker sich tingirenden Substanz vollständig umschlossen; es ist schwer zu unterscheiden, ob es sich hier nur um Vacuolen handelt. . . . Bei *Aeolidia papillosa* [Ei] . . . zwei, ein wenig abgeplattete Kugeln die in einanden teilweise eingesenkt sind. Diese Kugeln sind aber hier beinahe gleich gross und die blasse ist in der gefärbten eingesenkt, bei *Unio* [nach Flemming] umgekehrt. . . . In den jungen Keimzellen fand ich nur einen einfachen Nucleolus, und dieser färbte sich stark." In the liver cells of *Doris proxima* there are two nucleoli: "Der eine von diesen ist ganz kugelförmig und stark lichtbrechend, glänzend; dieser, der sich auch intensiv tingiert, muss als eigentlicher Nucleolus aufgefasst werden. Der andere ist blasser und grösser, seine Gestalt ist bald rundlich, bald länglich, bohnenförmig also mehr unregelmässig; diesen möchte ich als Nebennucleolus bezeichnen"; the two stain differently; "Die Lage der beiden Kernkörperchen ist auch wechselnd, indem sie bald ganz neben einander liegen oder sogar der Nucleolus im Nebennucleolus hineingesenkt, bald völlig getrennt sind. . . . Der Nebennucleolus, der immer scharf begrenzt ist, enthält oft eine kleine Vacuole. Ein Paar Mal traf ich in demselben Kern zwei Nebennucleoli." The latter are homogeneous, with an outer clearer layer, while the "Hauptnucleolus" is granular. Lönnberg found similar nucleoli also in the liver cells of *Polycera* and *Aeolidia*. Liver cells of the "*Krebs*" (*Astacus*?): "Meist sieht man . . . einen blassen Körper, der sich schwach wie der Nebennucleolus bei den Nudibranchiaten färbt, und daneben einen oder mehrere kleine Körperchen, die sich intensiv tingieren und sich wie Nucleolen verhalten; . . . bald liegt ein stark gefärbtes Kügelchen an einem Pole des Nebennucleolus, bald eins an jedem Pole desselben und in wieder anderen Fällen schmiegen sich drei Nucleolkörperchen dem Nebennucleolus an. Bisweilen treten Nebennucleolen in zwei- oder dreifacher Zahl auf." Lönnberg concludes



that the "Nebennucleoli" may play a part in the acquisition of nourishment or may hold reserve nourishment.

Marshall ('92) studied the sporulation of *Gregarina blattarum* v. Sieb. In the youngest individuals there is one large nucleolus. In larger ones there are one large and two or three smaller nucleoli, or four or five smaller ones of equal size; these now increase in size, accompanying the growth of the nucleus. He believes that the smaller nucleoli which are subsequently produced, arise in only one (as a rule) of the four or five original nucleoli: "Im Innern dieses Formationsnucleolus erscheinen dann klare, runde Ballen von verschiedener Grösse, welche keine bestimmte Grösse haben. Sie sind in wechselnder Zahl vorhanden und etwas heller als die übrige Masse des Nucleolus. Bei vielen Formationsnucleoli . . . waren alle Stadien der Entwicklung zu finden; kleine und grössere Ballen im Innern, und einige, die schon halb nach aussen getreten waren." After leaving the "Formationsnucleolus" they stain like the latter, and become either irregularly or spirally grouped together. "Die Vermehrung dauert bis zum Beginn der Cystenbildung fort. . . . Am Anfang der Encystierung enthält jeder Kern etwa 25-40 deutlich erkennbare Nucleoli, welche bald in dieser, bald in jener Art angeordnet sind. In beiden Fällen liegt der jetzt unregelmässig gestaltete Formationsnucleolus der von ihm ausgegangenen Gruppe gegenüber"; the latter is smaller than heretofore, "doch zeigt er noch Ballen im Innern." The smaller nucleoli increase in number, but now by repeated divisions of their own; the small granules resulting from these divisions are termed "Chromatinkörner": "Jedes Chromatinkorn bildet nun eine Hülle um sich, nachdem es sich vorher mit einer Schicht Plasma umgeben hat. Auf diese Weise vollzieht sich die Bildung der jungen Sporen. . . . Kurze Zeit, nachdem die Spore gebildet ist, nimmt dieses Chromatin-Korn die Gestalt einer 8 an und teilt sich in zwei Hälften, die beide an die entgegengesetzten Seiten der Spore treten." Later each of these divides into two, and each of the resulting four then divides into two, so that eight is the result; then one such "Chromatin-Korn" is allotted to each "Keim" (young Gregarine) and represents its nucleus.

Rückert ('92) studied the maturation of the eggs of *Scyllium*, *Pristiurus*, and *Torpedo*. In young germinal vesicles there are a few small nucleoli, most of them peripheral in position. In larger ova they have increased in number and size, and become grouped in a cluster at that part of the nucleus which is nearest the animal pole of the egg; this cluster may occupy one-fourth of the whole space of the nucleus. Later, but still antecedent to the formation of the pole spindles, the nucleoli decrease in size and commence to stain very faintly. Rückert considers the nucleolus of an egg cell as strictly comparable to that of any somatic cell. From the fact that the nucleoli are largest, and color most intensely, at the same time that the chromosomes do, and simultaneously with the latter become gradually invisible later, he concludes: "dass es die Stoffwechselforgänge der Chromosomen sind, zu welchen die Nucleolen in direkter Beziehung stehen, sei es nun, dass sie notwendige Stoffe an die letzteren abgeben (vielleicht das Chromatin, wie schon Flemming vermutete), oder dass sie Stoffe von ihnen aufnehmen, oder endlich dass beides zugleich der Fall ist. . . . Später freilich, wenn die Chromosomen merklich an Substanz verlieren, wird man eher geneigt sein, die betreffenden Nucleolen als Träger von Zerfallsprodukten der Chromosomen anzusehen." He also observed that the number of the nucleoli varies in different germinal vesicles of the same age, that a number may coalesce to form a larger one, and that a few wander out into the cytoplasm, where they become paler and finally vanish.

Wirén ('92) found that the smallest germinal vesicles of *Chaetoderma* contain no nucleoli; in nuclei of about  $15\mu$  diameter a nucleolus appears for the first time, and consists of a dense mass of granules, which stain differently from the other nuclear granules. More than one nucleolus is never to be found.

1893.

Van Bambeke ('93) found one to five homogeneous nucleoli in the germinal vesicles of *Scorpaena scrofa*, and notes that in older eggs they do not stain as deeply with carmine as in younger ones.

Böhmig ('93), *Rhodope veranii*: the single nucleolus in older eggs contains one or several vacuoles.

Brauer ('93) investigated the spermatogenesis of *Ascaris megalcephala*: there is one homogeneous nucleolus in the spermatogonium, which becomes smaller in the spermatocyte and often evinces a large vacuole. The nucleolus is smaller than the centrosome (which is at this stage enclosed in the nucleus), and stains differently from the latter.

Brooks ('93), *Salpa*: the single large nucleolus of the ovarian ovum "is suspended near the center of the nucleus by a network of fine threads."

Fick ('93) studied the maturation of the egg of the *Axolotl*. In the germinal vesicle lies a group of nucleoli, which vary in size from  $3\mu$  to  $16\mu$ ; some contain a single vacuole, and some stain more deeply than others. The greater number of them disappear at the time of the longitudinal division of the chromosomes, though a few may remain in the yolk for a certain time. "Bei den Nucleolen des Keimbläschens liegt es sehr nahe mit Strasburger und Pfitzner daran zu denken, dass sie vielleicht eine Art Reservestoffbehälter darstellen"; further, he holds that the nucleoli "in einer allerdings noch nicht aufgeklärten Beziehung zu den Veränderungen des Chromatins stehen, da sie bei der Ausbildung der Chromosomen für die erste Spindel vollständig verschwinden."

Frenzel ('93a) studied the nucleoli of various *Gregarines*. In *Gregarina statirae* the single nucleolus, which he terms "Morulit," appears "eigenthümlich glänzend mit einem schwach gelblichen Schimmer und dabei an der Oberfläche rau und warzig-runzelig. . . . In seinen Reaktionen verhält er sich an allen Orten ähnlich wie Nuklein." In *G. bergi* a single Morulit is present. In the embryo of *Pyxinia crystalligera* there is a single Morulit; in older individuals the nucleus "enthält mehrere helle, klare, glatträndige und lebhaft glänzende Nucleoli . . . die oft noch einen Flüssigkeitsraum im Innern bergen." In *Gregarina portuni*, *Callyntrochlamys*, and *Aggregata portunidarum* there are several nucleoli in the nucleus.

Frenzel ('93b) hepatopancreas cells of *Astacus*: in the fat and ferment cells a single nucleolus is present; in the amitosis

of the nuclei he concludes that the nucleolus divides ("nukleoläre Kernhalbierung"), since the nucleoli of the daughter-cells are of equal size.

Häcker ('93a) divides the maturation stages of the ovarian eggs of *Moina*, *Cyclops*, and *Sida*, into two periods, "von denen der erste gekennzeichnet ist durch die Anwesenheit eines einzigen 'Nucleolus' und durch die leichte Färbbarkeit des Fadenspirems (chromatische Stufe), der zweite durch die Anwesenheit mehrerer 'Nucleolen' und die Abneigung der chromatischen Substanz, die Mehrzahl der Färbungsmittel anzunehmen (achromatische Stufe)." In the first period ("Wachstumsphase") there is one excentric, deeply staining nucleolus ("Hauptnucleolus"), which possesses a "Hüllmembran"; in the second period, in addition to the "Hauptnucleolus" there are also one or two "Nebennucleoli" of greater size than the former, but staining less deeply, and somewhat irregular in form. Both kinds of nucleoli contain vacuoles. The "Nebennucleolus . . . stellt sich vielfach als hohles Gebilde von ellipsoidischer Gestalt dar, dessen einem Pole der Hauptnucleolus kappenförmig aufsitzt." Only the outer shell of this nucleolus stains deeply. Subsequently the "Hauptnucleolus" grows gradually smaller and finally disappears; and at the same time the "Nebennucleolus" increases in size and becomes irregularly lobular in shape, and finally breaks into pieces. The nucleolar relations in *Moina* are as in *Cyclops* (just described). In *Sida* only a "Hauptnucleolus" is present, and this contains a large central and several smaller peripheral vacuoles. Häcker distinguishes the following types of ova with regard to their nucleolar structure: (1) *Lamellibranchiate type*, with one "Hauptnucleolus" and one or two "Nebennucleoli," the latter larger and less chromatic than the former, but both frequently in close connection (*Naja*, *Anodonta*, *Cyclops brevicornis*); (2) *Echinoderm type*, with one large "Hauptnucleolus," which increases in size, and only towards the close of the "Keimbläschenstadium" do a few smaller nucleoli appear (*Toxopneustes*, *Sida crystallina*, primiparous *Cyclops strenuus* and *C. signatus*); (3) *Vertebrate type*, with several nucleoli varying in size, number, and form (*Rana* and

numerous other *Vertebrates*, *Sagitta*, *Moina*, *Cyclops brevicornis*, multiparous *C. strenuus*). "Aus der obigen Zusammenstellung . . . geht . . . hervor, dass das unter der Bezeichnung 'Nucleolus' oder 'Keimfleck' im Eikern auftretende Gebilde hauptsächlich in zweierlei Gestalt auftritt: entweder stellt dasselbe einen in der Einzahl vorhandenen, stetig seine Grösse verändernden, formbeständigen Körper dar, oder aber finden sich als 'Nebennucleolen' Bläschen oder Tröpfchen von wechselnder Zahl, Grösse und Gestalt vor." The "Hauptnucleolus" remains in the nucleus until just before the formation of the first pole spindle; after that it either diminishes rapidly in size, or it passes out of the nucleus into the cytoplasm, where it remains for a time as a "Metanucleolus"; the "Hauptnucleolus" is phylogenetically derived from a "Nebennucleolus," and has developed into "einen membranumhüllten, formbeständigen und stetig durch Diomose wachsenden Organ." "Es dürfte vielleicht zunächst die Thatsache heranzuziehen sein, dass ein Auftreten von 'Nebennucleolen' von wechselnder Zahl, Form und Grösse und von analoger chemischer Reaktion auch in den ruhenden Furchungskernen der betreffenden Thierformen festzustellen ist, und dass diese 'Nebennucleolen' hier nicht mit einem als Hauptnucleolus anzusprechenden Körper vergesellschaftet sind. Nebennucleolen treten folglich auch da im ruhenden Kerne auf, wo kein Zellenwachsthum stattfindet." Häcker considers that the "Nebennucleoli" are not drops of a nutritive fluid, but "Abspaltungsprodukte oder Sekretstoffe der chromatischen Substanz. Diese Auffassung findet vor allem in der Thatsache eine Stütze, dass die Nebennucleolen, z. B. bei *Moina* und *Cyclops strenuus* (mehrgebärend), im Lauf der Wachsthumphase stetig an Grösse und Massigkeit zunehmen und dass sie das Maximum ihrer Entwicklung erst in dem Moment erreichen, wenn bereits die Vierergruppen zur Ausbildung gelangt sind, wenn also von einem Wachsthum der chromatischen Substanz kaum mehr die Rede sein kann."

Häcker ('93b): a preliminary contribution to the following paper.

Häcker ('93c) found in the germinal vesicle of *Echinus microtuberculatus*, in addition to the "Hauptnucleolus," a few small

globules which stain in the same manner as, and are probably homologous to, the "Nebennucleoli" of other animals; the "Hauptnucleolus" increases in size by the absorption of these latter. "Der Hauptnucleolus des Echiniden-Keimbläschens ist . . . ein pulsirendes Organulum, in welchem periodisch eine grosse Hauptvacuole sich durch Zusammenfluss kleinerer Vacuolen bildet, um dann wieder langsam abzunehmen. . . . Was die Dauer der Perioden anbelangt, so wurden solche von vier bis zu solchen von acht Stunden beobachtet"; the central vacuole at the time of its maximum size passes from the center to the periphery of the nucleolus: "Die Centralvacuole tritt also in Beziehung zur äussersten Wandschicht des Hauptnucleolus, anscheinend um ihren Inhalt mit . . . den Kernsaft in Kommunikation zu bringen." Accordingly, the "Hauptnucleolus" may be "als ein osmotisches System betrachtet werden, in welchem die feste Substanz (Rindensubstanz) nach zwei Seiten hin, einerseits mit dem Kernsaft, anderseits mit den Vacuolen, in diosmirender Verbindung steht. Sobald jedoch ein Körper nach zwei Seiten diosmirt, so ist eine Anhäufung in demselben nur durch das Eingehen einer neuen Verbindung möglich [Pfeffer (91)]. Es folgt schon hieraus, dass die aus dem Kernsaft aufgenommene Flüssigkeit in der Nucleolarsubstanz nicht nur eine Verdichtung, sondern auch eine weitere chemische Umsetzung erfahren muss." The fluid vacuoles of the "Hauptnucleolus" represent an excretion which in *Echinus* is periodic, while in the *Copepoda* "im Laufe der Eireife wächst unter Mitwirkung der Rindenvacuole die Centralvacuole langsam heran, nimmt allmählich eine excentrische Lage an und entleert sodann kurz vor der Bildung der Richtungskörper ihren Inhalt nach aussen." He compares the vacuole of the "Hauptnucleolus" to the pulsating vacuole of the *Infusoria*: "so würden die Centralvacuole des Hauptnucleolus mit der eigentlichen pulsirenden Vacuole des Protozoenkörpers, die Rindenvacuolen des Hauptnucleolus mit den Bildungsvacuolen zu vergleichen sein." From a study of the pole-body mitoses he concludes: "dass der Hauptnucleolus während der Auflösung der Keimbläschenwandung zunächst noch in seiner ursprünglichen Grösse erhalten bleibt und sich

von dem Kernplasma langsam zu trennen beginnt." He could not exactly determine how long the nucleolus remains in the egg after that, but considers the fact important, "dass der Hauptnucleolus zur Zeit der Umbildung der Keimbläschen-substanz ohne bemerkbare Volumverminderung fort besteht"; it is at this time (after the disappearance of the nuclear membrane), to use his terminology, a "Metanucleolus."

Henneguy ('93) studied principally the genesis and occurrence of the yolk nuclei, "corps vitellin de Balbiani," in the ovarian egg of various vertebrates. This body is absent in the ova of the *Rabbit*, *Bitch*, *Mole*, *Rhinolophus*, *Cow*, *Antelope*, *Baboon*; and in *Lizards*, *Galcus*, *Raja*, and *Scyllium*. In the rat it consists of a peripheral clearer portion and a central denser core; it stains with eosin, safranin, and haematoxylin, but not with methyl green or gentian violet. In the bat it encloses a spherical corpuscle. It is also present in the chicken. Though absent in *Bufo* and *Triton*, it is found in *Rana temporaria*, where it is much the same as in birds, enclosing a more deeply staining portion. In the trout the corpuscle of Balbiani is as in the rat, but larger (twenty). *Sygnathus*: the very young egg contains a nucleus "renfermant un réseau chromatique bien développé" (his Fig. 20 would show three small nucleoli enclosed in this "réseau"); in older eggs the nuclear membrane is lined by a large number of nucleoli, and "le centre du noyau est occupé par une petite masse finement granuleuse et teintée en rose par la safranine, tandis que le reste du contenu demeure incolore. Le protoplasma ovulaire est également faiblement coloré et renferme un corpuscule arrondi, réfringent comme les taches germinatives et retenant la safranine avec la même intensité que ces dernières"; this corpuscle at the time of its first appearance is flattened against the outer surface of the nuclear membrane. Subsequently this intravitelline body becomes elliptical in form, with its long axis parallel to the surface of the egg: "il est de plus au contact immédiat par son bord externe avec une amas arrondi, constitué par une substance fondamentale d'apparence homogène, mais remplie de granulations très colorées. A un stade plus avancé tout le corps réfringent s'est transformé en un amas let qu'on l'observe dans

la plupart des ovules des Poissons"; in this manner it develops into a Balbianian corpuscle, and later breaks into small granules. In no eggs of any of the species studied are more than one of these corpuscles present; and it always arises during the maturation period of the ovum, before fecundation. "C'est très probablement une partie de la tache germinative, ou une tache germinative entière, qui sort la vésicule [germinative] pour pénétrer dans le vitellus. . . . C'est un organe ancestral qui, avec les éléments nucléolaires de la vésicule germinative, correspond au macronucleus des Infusoires, le micronucleus étant représenté par le réseau chromatique, prenant seul part aux phénomènes de fécondation."

Heuscher ('93) noticed in the ovum of *Proneomenia* either one nucleolus, or two of different size which were usually separated from each other.

Holl ('93) studied the maturation of the ovum of the mouse. "Die Fäden [Chromatin] zeigen eine innige Verbindung mit dem Kernkörperchen derart, als wäre dasselbe ein Centrum, von welchem die Fäden des Netzwerkes auslaufen." The nucleolus is not homogeneous, but contains granules ("Schroen'sche Körner") to the number of twenty; these gradually become stained during the growth of the nucleus, until the whole nucleus becomes evenly stained. "Im weiteren Verlaufe der Entwicklung treten die Schroen'schen Körner aus dem Kernkörperchen heraus und gelangen als chromatische Ballen in das Kernnetz, wo sie sich mit den Fäden desselben verbinden. . . . Endlich wird das Kernkörperchen von seinem Inhalte ganz frei; es bleibt nur die Kernkörperchenmembrane übrig, und im Kernraume liegen zerstreut eine grössere Anzahl der chromatischen Ballen. Dieselben sind anfangs klein und schwach gefärbt, wachsen auf  $2\mu$  heran und färben sich immer besser. . . . Die chromatischen Ballen wandern aus dem Kerne aus, und das übrige [Fadennetzwerk] rückt als 'Kernrest' ganz an die Oberfläche der Eizelle. Die chromatischen Ballen liegen in 6 Gruppen von je 4 neben einander, und jeder Ballen wandelt sich in eine dicke, kurze Schleife um," *i.e.*, a chromosome of the "Richtungsspindel."

Jordan ('93) studied the development of the ovum of the



newt. He thinks "that certain deutogenic substances are formed in the [germinal] vesicle, perhaps through the agency of the nucleoli, and are then sent forth to share in the building up of the cell," *i.e.*, of the yolk particles. "The nucleoli in the young egg appear arranged along the chromatin threads, and possibly originate from the thread substance." Later they lose this connection, grow larger, and assume a peripheral position within the nucleus. There is apparently no division of the nucleoli; they "attain their maximum size shortly before their centripetal movement." Having arrived at the periphery of the nucleus, the nucleoli commence to stain less deeply, their contours become uneven, and they then wander back to the center of the nucleus, where they disintegrate. He does not agree with O. Schultze ('87) that the nucleolar particles build up the chromosomes.

Kaiser ('93) found in the egg of *Echinorhynchus bipennis* one large, spherical, peripherally situated nucleolus. It disappears before the pole spindle is produced.

Lustig and Galeotti ('93), mentioned by Lardowsky ('94), consider that the centrosome does not proceed from the nucleolus.

Mertens ('93), ovum of *Homo*: two or three nucleoli are present, consisting of a central clearer and a peripheral darker portion; it is probable that several smaller ones may fuse together to form a larger one; they are at first in intimate connection with the chromatin filaments, but later lose this connection and gradually cease to stain with safranin. The Balbianian corpuscle is an extruded nucleolus: "c'est alors aussi que nous nous étendrons quelque peu sur l'expulsion des parties chromatiques du noyau, expulsion qui paraît affecter les mêmes caractères chez les oiseaux et les mammifères"; eliminated nucleoli ("grains chromatiques") as well as attraction spheres have been described as Balbianian corpuscles. Ovum of *Pica*: in young ovules there is one nucleolus which arises as follows: at one point in the nucleus the reticulum concentrates itself, and here a certain number of the filaments fuse together, thus producing the nucleolus. The chromatin is at first irregularly arranged in the nucleolus, but "finit par être également dense dans toutes les parties de la tache germinative," and subse-

quently accumulates on its surface. "Le nucléole devenu indépendant [from the chromatin reticulum] est expulsé : les chromosomes s'écartent pour lui livrer passage. Il n'est pas rare d'en rencontrer qui, arrivés à la périphérie, sont coiffés par un filament nucléinien. . . . Le filament se rompt bientôt et le nucléole est libre." The presence of a vacuole in the nucleolus is explained by the assumption that the chromatin wanders to the periphery of the nucleolus, thereby leaving a clear space at the center of the latter. (Safranin the only stain employed.)

Minchin ('93) states that the single nucleolus of *Gregarina irregularis* "consists of a darkly stained ground substance containing an immense number of clear vacuoles of all sizes. One of the vacuoles is much larger than the others, and being excentrically placed, constitutes the clear spot seen in the thick sections." The nucleolus of *G. holothuriae* has a similar structure.

Pizon ('93), ova of *Botryllida*: a single large nucleolus containing several vacuoles.

Repiachoff ('93) figures a large vacuole in the single nucleolus of the ovarian cells of a pelagic, acoelic *Rhabdocoele* (species undetermined).

From Rhumbler's contribution ('93) to the morphology of the nucleoli, or "Binnenkörper," the following extracts are important: "Mir scheint es . . . noch keineswegs sicher, ob die Nucleolen der Gewebszellen und die Nucleolen der Keimzellen bezw. vieler Protozoen (vielleicht ausgenommen die Ciliaten und Suctorien) analoge Gebilde sind; obgleich auch das Gegentheil wegen des ähnlichen Verhaltens der beiderlei Nucleolenarten während der Mitose sehr zweifelhaft bleiben muss." In *Saccamina sphaerica* there are from 1 to 300 nucleoli: "ähnlich wechselnd wie ihre Zahl ist ihre Grösse, ihr Lichtbrechungsvermögen und ihre Gestalt." The largest of them "zeigen meist eine, durch stärkeres Lichtbrechungsvermögen ausgezeichnete Innenmasse, in welche kleinere, noch stärker brechende und oft von der Kugelgestalt abweichende unregelmässige Körperchen eingelagert sind, und eine dunklere, weniger lichtbrechende Aussenmasse, die in gleichmässiger Dicke wie

eine feste Membran um die Innenmasse herum gelagert ist"; this latter portion also stains more intensely with eosin. Rhumbler concludes that the "Binnenkörper . . . durch Zusammenfliessen anfänglich leicht flüssiger, dann zähflüssiger und schliesslich erstarrender Massen entstanden sind. Ich nehme an, dass die Binnenkörpersubstanz an allen oder auch nur an bestimmten Stellen (das Letztere da, wo eine fixirte Nucleolenzahl Regel ist) des Kernplasmas zuerst in Gestalt kleinster, erstarrender Tröpfchen abgeschieden wird, die auf verschiedenen Stadien ihrer Erstarrung an einander treffen," this deduction being based in part on an observation of A. Schneider ('75). He explains why the nucleoli are not evenly distributed in the nucleus, on the ground "dass die einzelnen Tröpfchen jedenfalls nicht an allen Stellen des Kernraumes zu genau derselben Zeit entstehen." The nucleoli probably represent "Reservestoffe," which are consumed in the later growth of the nucleus, and since in *Saccamina* they decrease in size as the amount of the chromatin increases, it is probable "dass die Nucleolensubstanz [die sehr verschieden sein kann] in irgend welcher Beziehung zum Chromatin steht." Further, he holds that the nucleolar substance is produced in the nucleus, "und dann erst erzeugt wird, wenn sie in kleinen Tröpfchen auftritt." But it is not yet possible to decide whether the nucleoli of the *Metazoa* also arise in this manner, and hence the use of the general term "Binnenkörper" instead of the more specific one "Nucleolus." That amoeboid movements of nucleoli have been noticed is not contradictory to his theory, since changes of form would be caused by the processes of fusion, or these motions might denote "Auflösungsvorgänge": "Die Auflösung der Binnenkörper muss nach unserer Annahme von zwei, im Kernsaft enthaltenen, sich gegen die Binnenkörper konträr verhaltenden Substanzen, auf eine Ueberschreitung des angestrebten Mischungsoptimums von Seiten der lösenden Substanz zurückgeführt werden. . . . Der Verschmelzungsvorgang ist schon von mehreren Forschern erschlossen oder vermuthet worden — neu dürfte nur die Annahme einer allmählichen oder auch rascheren Erstarrung der ursprünglich flüssigen Binnenkörpersubstanz sein." Rhumbler concludes that the

“Binnenkörper” are not organs, since they show no fixed organic structure, but represent accumulations of various substances. There is more nucleolar substance, “Reservestoff,” accumulated in the nucleus before mitosis than is necessary for it, so that after a mitosis some always remains to serve for the production of daughter-nucleoli (this being an explanation for the reappearance of nucleoli after mitosis).

Stauffacher ('93), maturation of the egg of *Cyclas*: the “Urei” contains a single large nucleolus; later one or two “Nebennucleoli” also appear in the nucleus. When the ovum has so increased in size that it adheres to the wall of the ovary only by a narrow thread of cytoplasm, two nucleoli are present, which are of unequal size but are in close contact with each other; in one case the nucleolus was trilobular. After borax-carmin staining, the smaller one appeared more refractive and deeply stained than the larger. Subsequently the two became separated, and both vanished before the formation of the first pole spindle.

Strasburger's paper ('93) presents a general discussion of certain problems of mitosis in animals and plants; his remarks on the aequatorial plate are apropos here. He believes that the “Körnchen” found by Kostanecki ('92) in the equator of the central spindle are similar to, and comparable with, structures found by himself in the mitoses of plants, and are masses of nucleolar substance (these bodies being termed “Centralspindelkörperchen” by Kostanecki, “Zwischenkörper” by Flemming, and “Zwischenkügelchen” by O. Hertwig, '92). “Vergegenwärtige ich mir nun das, was ich seinerzeit bei der Bildung pflanzlicher Zellplatten beobachtet habe [*Histol. Beitr.*, vol. i, p. 161], nämlich das Fortschreiten jener tingirbaren Substanz, die ihrem Auftreten und ihren Tinctionen nach nur als Nucleolarsubstanz gelten konnte, zwischen den Verbindungsfäden bis zum Aequator, so muss in mir die Vorstellung erwachen, dass es sich in der von v. Kostanecki geschilderten Erscheinung um einen entsprechenden Vorgang handle. . . . Mit den durchschnittenen [achromatischen] Verbindungsfäden . . . wanderte dann auch die Substanz der halbirtten Zwischenkörper nach den Zellkernen zurück, ähnlich,

wie wir das für die unverbrauchte Nucleolarsubstanz bei Pflanzen angeben konnten. . . . Bei Pflanzen treten die Elemente der Zellplatten als Anschwellungen der Verbindungsfäden im Aequator der Zelle auf. Diese Anschwellungen bilden sich dort erst, wenn jene tingirbare Substanz . . . den Aequator erreicht. Diese Substanz wird in gelöster Form zwischen den Verbindungsfäden dorthin befördert. Aus den verschmolzenen Elementen der Zellplatte geht die Scheidewand hervor. . . . Man könnte denken, dass in tierischen Zellen ein mittlerer Teil der 'Zwischenkörper' in eine lösliche Substanz sich verwandle und so die Halbierung der Zwischenkörper und damit auch der Verbindungsfäden bewerkstelligte."

Ver Eecke ('93), pancreas cells of *Rana* and *Canis*: he distinguishes one "nucléole nucléinien" and several "nucléoles éosinophiles," or plasmosomes, the latter being the larger. When the cell enters on its functional activity "le plasmosome unique devient plus volumineux; il n'est pas rare d'en trouver plusieurs dans un seul noyau; ils se rapprochent de la membrane nucléaire, la soulèvent, la perforent et se placent en définitive a côté du noyau pour former un noyau accessoire. D'ordinaire le plasmosome dans sa migration est accompagné de petits karyosomes qui lui forment parfois une véritable couronne"; the mother-nucleus subsequently degenerates. Against the opinion of Platner (that the supposed migration of the nucleoli is artificially produced) "il suffit de faire remarquer que la migration ne s'observe pas ou très rarement à l'état de repos pour ne se manifester dans tout son éclat qu'au début de l'activité sécrétoire." In the cytoplasm the nucleolus and its attendant karyosomes gradually change into a nucleus.

Wasielevsky ('93) found the "Urgeschlechtszelle" of *Ascaras* with one or two nucleoli. While in the resting state of the nucleus only one nucleolus is present, two are regularly seen in the spirem stage, and these he believes have originated by division of the primitive one. He noticed no difference in size or stain between these nucleoli and the centrosomes, and hence concludes that the latter are identical with, or have some genetic relation to, the former.

1894.

Blochmann ('94) gives a preliminary account of the results of the observations of Keuten ('95).

Born ('94) investigated the maturation of the ovum of *Triton*. In the "Urei" are one or several large, spherical nucleoli. In the second stadium of the maturation (production and degeneration of a "Chromatinfadenknäuel") there are at first ten nucleoli, then they become more numerous, increase in size, and lie close to the nuclear membrane. In the third stadium (eggs of from  $200\mu$  to  $350\mu$  in diameter) the nucleoli increase still more in size. In the fourth stadium (eggs measuring from  $350\mu$  to  $800\mu$ , first appearance of yolk in the cytoplasm) most of the nucleoli lie in the peripheral "Karyohyaloplasma," only a few pale ones being in the center of the nucleus (this part of the nucleus he terms "Centralkörper"). At the commencement of this stage the nucleoli increase, at its conclusion decrease, in number, and "während der ganzen Periode steigt die Zahl der verkleinerten und abgeblassten Nucleolen im Centralkörper," only a few of these pale ones being situated at the periphery of the nucleus. Thus while at the beginning of this period the nucleoli attain their maximum size, at its end most of them wander towards the center of the germinal vesicle, become smaller, and lose their staining power. Fifth stadium (the nucleus passes to the periphery of the egg): the nucleoli decrease still further in size, and continue to wander to the center of the nucleus; some of the larger ones contain vacuoles, and for the first time appear granular; the smaller, lightly staining nucleoli are division products of the larger ones. At the commencement of the sixth stadium (formation of the first pole spindle) all the nucleoli lie in irregular rows around the "Centralkörper," stain quite intensely, and are regularly vacuolated; the few in the midst of the "Centralkörper" are smaller and stain more faintly; when the nucleus has decreased still further in size, all the nucleoli vanish at once. Born concludes as follows: "Eine sichere Herleitung der peripheren Nucleolen von den Nucleolen des Ureies, bin ich freilich nicht im Stande zu geben. . . . Die Nucleolen stehen in Beziehung

zum individuellen Zelleben, nicht zur Fortpflanzung; denn beim Beginn der Mitose verschwinden sie, um nach Beendigung derselben — im Ruhestadium des Kerns — wieder aufzutreten." He notes that their peripheral position is "Eine Lage, die für eine Wirkung auf den Zelleib die denkbar günstige ist."

Brauer ('94), *Actinosphaerium*: in the cyst of the second order ("Ruhecyste") there is a nuclear reticulum consisting of chromatin granules imbedded in a linin network, and usually numerous nucleoli of irregular form, arranged either in rows or circles. Probably the nucleoli take no part in the formation of the chromosomes, and are equivalent to those of metazoan cells; they disappear in the prophase of mitosis.

Bunting ('94) found in the eggs of *Hydractinia* and *Podocoryne* a single large nucleolus, containing one central vacuole of large size.

Flemming's ('94) "Referat" includes some of the more recent papers on nucleoli.

Foot ('94), egg of *Allolobophora*: during the first maturation division the nucleoli are distributed in the cytoplasm. Each pronucleus contains from one to seven nucleoli: "the nucleoli persist during the cleavage spindle, but how much later I am unable at present to state."

Hodge ('94), nerve cells of *Rana* stimulated by the electric current: amoeboid movements of the nucleolus were noticed; "it was possible to make out granules in the nucleolus which moved slowly about and in several instances were seen to be extruded into the nucleus"; and in cells which had not been stimulated, but simply fixed in osmic acid and stained with safranin, "the granules were stained brighter red than the body of the nucleolus, and several were found partially extruded."

Lavdowsky ('94) studied nuclei from the epidermis of the fins of *Amphibian* larvae, as well as various tissues of plants. The nucleolus consists of: (1) an outer, thick "Pyrenin-Chromatinschale"; (2) an enclosed vacuole; and in the latter (3) the "Nucleololus" ("das noch in Entwicklung begriffene Centrosoma"). The animal nucleolus varies from a spherical to an angular or star shape. In the resting nucleus the chro-

matin and pyrenin shells are the largest, since "die Bestandtheile noch nicht für die Karyokinese verbraucht sind." The centrosomes "sind wahrscheinlich Teile von Kernkörperchen und wandern zur Zeit der Karyokinese von den Kernelementen aus" (these centrosomes are spherical or oval, homogeneous and compact, and stain very slightly). He concludes "dass die Kernkörper nicht zu jeder Zeit des Zellenlebens persistieren, dass ihr Verschwinden während der Karyokinese keinem Zweifel unterliegt und dass dies in innigem Zusammenhang mit dem Erscheinen des Centrosoma steht." The nucleoli divide amitotically (not seen in life, however) into very small pieces, which "scheinen in das Gerüstnetz eingeschaltet und verwandeln sich in den Vorbereitungsstadien der Karyokinese in Chromatinfäden"; other "Kernkörper" pass out of the nucleus, at the points where its membrane is broken. The nucleoli are not sufficient for supplying the whole mass of chromatin necessary for the mitosis; "es muss also eine andere Quelle der Chromatinentwicklung da sein und hauptsächlich im Eidotter und in den pflanzlichen Samen muss man die Quelle aufsuchen. . . . Durch nichts unterscheiden sich die Chromosomen von den zerteilten Dotterkörnchen und den getheilten Nucleolen. Alle diese Gebilde . . . können somit als 'Kariosomen' betrachtet werden."

Metzner ('94), cells in the testicle of *Salamandra*: he concludes "dass die Nucleolen in keinem Stadium der Mitose fehlen, obwohl sie von sehr verschiedener Grösse sind." In resting nuclei the smaller nucleoli stain entirely with gentian violet (after Flemming's triple stain), the larger ones with safranin except for a blue-stained peripheral zone. Smaller nucleoli are budded off from the surface of the larger ones, and the "Leitkörper" (granules which serve to attach the chromosomes to the spindle fibers) resemble such buds in stain and size; "es ist mir vorerst nicht möglich zu entscheiden, ob diese Leitkörper von dem Nucleolus stammen, doch ist es wahrscheinlich, dass gerade an ihm sich die ersten, den Kern- und Zelltheilungsprocess einleitenden Vorgänge abspielen. Denn an den Zellen mit ziemlich gleichmässiger Vertheilung der Chromatingranula und geringer Anzahl der Nucleolen kann



man immer schon den Vorgang der Ausstossung kleinster Kügelchen beobachten." In mitosis the nucleoli wander into the cytoplasm, where the larger of them disappear, while the smaller persist; "dass aber diese Nucleolen in den Tochterkern einwandern, ist nicht sehr wahrscheinlich, denn es liegen in den Tochterzellkernen nur die gelösten Leitkörper. . . . Vielleicht persistiren nur einige von ihnen in der jungen Zelle und zwar als Nucleolen. . . . Eine 'Vermischung von Nucleinsubstanz' kann ich . . . an meinen Präparaten . . . für Chromatingranulastrange nicht annehmen, denn die augenscheinlich von den Nucleolen stammenden Leitkörperchen adhären nur den Segmenten und erfüllen ihre . . . Function als Anheftungspunkte der Spindelfibrillen; sie lösen sich intakt in den Tochterknäueln wieder ab. Dass sie noch andere Functionen ausüben (als Nucleolen) ist wahrscheinlich, doch nicht ganz sicher. . . . An den Nucleolen treten die ersten Erscheinungen der Zelltheilung auf. Sie lassen aus sich eine Menge kleiner Kügelchen hervorgehen, die z. Th. aus dem Kerne in das Protoplasma wandern, z. Th. aber als Leitkörperchen über den Kern sich vertheilen und so wohl den Anstoss geben zur Strangbildung der Chromatingranula. Dem Nucleolus fiele also für die Fortpflanzung der Zelle eine wichtige Function zu."

Murbach ('94) considers it probable that the "Kapselkeim" of the nettle capsules of hydroids is derived from one of the two nucleoli of the parent cell, in accordance with his view that the capsule is of nuclear origin.

Purcell ('94) describes the nucleolus of the retinula cells of *Acantholophus* as structurally "wabig."

Reinke ('94) found in the cells of the spleen of the mouse one oval or elongate nucleolus; during the prophase of mitosis this divides into three or four smaller ones, while at the end of mitosis each daughter-nucleus has a single nucleolus.

Rückert ('94) studied the maturation of the ovum in three species of *Copepoda*. *Cyclops strenuus* (his species he assumes is not identical with the "*C. strenuus*" of Häcker): in the "Wachsthumszone" of the ovary there is one large, sometimes also two smaller nucleoli, which stain with haematoxylin as does the chromatin, and together represent the "Hauptnucleolus"

of Häcker. The single "Nebennucleolus" appears a little later, and is regular in its occurrence, both in females with egg sacks ("mehrgebärend," after Häcker) and in those without egg sacks ("erstgebärend," according to Häcker); Häcker found the "Nebennucleolus" only in the ova of the former category of females. It is paler and much larger than the several "Hauptnucleoli," and has a more central position within the germinal vesicle, while the latter are usually peripheral. When the "Hauptnucleoli" have disappeared the "Nebennucleolus" increases in size and thereby at first assumes a mulberry shape, or is produced into long processes (though at the start it was spherical). "Während er anfänglich ein kompaktes Gefüge besitzt, lockert er sich später auf. Schon frühzeitig sieht man in seinem Innern einen lichterem Raum, und später entrollt er sich zu knäuelartig gewundenen Zügen, die ein sehr wechselndes Ansehen bieten, sehr häufig bilden sie eine einzige, ziemlich einfach verschlungene Figur, ein Achtertour, ein S u. a., neben der jedoch noch ein oder ein paar kleinere kugelige Stücke im Keimbläschen liegen können. . . . Er ist . . . nicht einheitlich gebaut und homogen, wie ihn Häcker abbildet, sondern zusammengesetzt aus rundlichen Anschwellungen, die in einer Reihe hinter einander liegen, stellenweise getrennt durch schwach gefärbte, schmälere Zwischenstücke. Man könnte daher das Ganze als eine Kette von Kugeln bezeichnen. . . . In etwas späteren Stadien verlieren diese Bildungen an Färbbarkeit, erscheinen aber zunächst immer in sehr wechselnder Form. Man trifft entweder einen mehr kompakten Substanzhaufen oder meistens eine Anzahl durch das Keimbläschen zerstreuter Stücke. . . . Häufig sieht man ein vielfach verschlungenes, sehr unregelmässig angeordnetes Fadensystem. . . . Es ist schwierig zu entscheiden, ob die beschriebenen, sehr wechselvollen Bilder der Ausdruck nur für verschiedene Funktionszustände des Nucleolus sind, oder für einzelne, zum schliesslichen Zerfall führende Entwicklungsstufen"; they disappear before the true maturation processes commence. In *Heterocope robusta* and *Diaptomus gracilis* there is a single large, vacuolated nucleolus; it disappears when the chromatin has arranged itself into "Vierergruppen."

Schaudinn ('94) finds in *Amoeba crystalligera* a large nucleolus, with "wabiger Struktur"; in the mitosis it divides into two equal parts.

Watasé ('94), in the course of his theoretical deductions as to the structure of the cell, concludes in regard to the nucleolus: "The nucleolus is not a permanent body in the nucleus. It may exist at one stage of the cell, and may disappear at the next. The micro-chemical reaction of the nucleolus is entirely different from that of the chromosome. It appears probable that three or more different bodies are included under the name of nucleolus. Indeed, one sees no reason why the inside of the nuclear membrane may not be used as a depository for some solid products of cell metabolism. . . . And thus some of the bodies included under the generic name of *nucleolus* may belong to the group of metaplasm."

H. V. Wilson ('94), *Tedanione foetida*: the youngest germinal vesicle contains a single, centrally placed nucleolus. Later there are two nucleoli, "which are invariably placed on opposite sides of the nucleus and adhere to the inner surface of the nuclear membrane. In eggs which have reached the adult size it is the rule to find either one nucleolus peripherally placed, . . . or the nucleus contains no nucleolus at all. It sometimes happens that an egg of full size is found with two nucleoli, but this is rare. From this evidence it would seem that the two nucleoli present in the developing egg are lost, one after the other, at the time when the egg reaches its full size. As to how the first of the two is lost, I have no evidence, but the second nucleolus may often be seen lying just outside of the nucleus in the yolk, . . . showing that it has been extruded from the nucleus." What Fiedler ('88) described as polar bodies in *Spongilla* are probably extruded nucleoli. In the egg of *Hircinia acuta* the nucleolar changes are as in *Tedanione*.

1895.

Balbiani ('95), reviewed by v. Erlanger in *Zool. Centralbl.*, 1895, macronucleus of *Spirochona*: the nucleolus of the authors arises in a vacuole of the chromatin, and is formed by the separation of microsomes which fuse together to form one

or two nucleoli. The nucleolus then wanders through the chromatin to take position in the center of the achromatic substance ; it combines the qualities of a true nucleolus with those of a centrosome. There is thus no fundamental difference between a nucleolus and a centrosome ; when it remains in the nucleus it has the value of the former, when in the cytoplasm it has the significance of a centrosome.

Böhmig ('95) noticed in the ovarian eggs of *Haplodiscus* that the nucleolus is at first small and homogeneous, while later it becomes larger, and one or more vacuoles appear in it.

Bremer ('95a), blood cells of *Testudo* and *Chelydra* : there is normally one paranuclear corpuscle to a cell ; "seiner Natur nach ist das Paranuclearkörperchen ein vom Innern des Kernes in das Diskoplasma [Cytoplasma] ausgewanderter Nucleolus oder vielleicht ein Nucleolusfragment, umgeben von einer dem Kerne entnommenen Hüllsubstanz. . . . Seine Grösse, die Schwierigkeit der Färbung und seine Lage sprechen für den nucleolären Charakter." In a second paper ('95b) he identifies this corpuscle with a centrosome, and states : "Hertwigs Vermuthung, dass ein Zusammenhang des Centrosoms mit dem Nucleolus existire, wird durch meine Beobachtungen wahrscheinlicher gemacht."

In Bürger's monograph ('95) of the *Nemertean*s the following statement in regard to the structure of the germinal vesicle is of interest : "Im Keimbläschen findet man ausser den intensiv färbbaren Körperchen, den Nucleolen, von denen meist zwei, ein grösseres und ein kleineres, vorhanden sind, ein Netzwerk feiner Fäden, in welche sehr feine Kügelchen aufgehängt sind."

Coe ('95), ova of *Cerebratulus lacteus* : "as the ovum increases in size its nucleus develops into the germinal vesicle which has many germinal spots, of which one or two are much larger than the others." In the mature ovum the nucleus "often contains a highly refractive germinal spot one-third as large as the vesicle itself."

Cunningham ('95), ovarian eggs of fishes : in the youngest ova there is a single large nucleolus, in older ova a number of peripheral ones ; the latter are produced in part by a division of the primitive nucleolus, in part by an increase in size of

“minute nucleolar granules” which were present in early stages. In contradiction to the view of Scharff ('88) he finds that no nucleoli wander out of the nucleus to form yolk globules.

Delage ('95) opposes the view that the nucleoli and the centrosomes are genetically related (as against the theory of Julin ('93b) and Wasielevsky).

Galeotti ('95), embryonal cells of *Triton* and *Spelerpes* (fixation in Hermann's fluid with chloride of palladium substituted for chloride of platinum; stained for five minutes in sat. sol. acid fuchsine in aniline water at 60° C., then stained in 1½% sol. methylen green in equal parts of water and alcohol for three or four minutes): “Auf diese Weise erhält man roth gefärbt die Körnchen des Cytoplasma und alle Elemente des Kerns mit Ausnahme des Nucleolus . . . ; gelblichgrün erscheint der protoplasmatische Grund der Zelle und lebhaft grün die basophilen Granulationen.” In the pancreas cells of *Spelerpes* the green-stained nucleolus passes out of the nucleus and persists as “Nebenkern,” which in the cytoplasm seems to increase by continued division; and from this he concludes “dass der Nucleolus ein endonucleares Arbeitsprodukt des Kernes ist, bestimmt aus der Kernmembran auszutreten und im Cytoplasma so umgeändert zu werden, dass er in Secretionsprodukte umgewandelt wird.”

Häcker ('95) first describes the nucleolar relations in the eggs of *Canthocamptus*, and then gives expression to general views, based on his numerous previous observations, in regard to the nature of nucleoli. *Canthocamptus staphylinus*: in the smallest eggs there is one large nucleolus, which increases in size, but not to same relative extent as does the nucleus itself; subsequently vacuoles arise in it, one of which becomes a “Hauptvacuole”; smaller “Kernkörper” appear first when the chromatin elements commence to thicken; “wenn endlich die Kernsubstanz auf das Minimum ihres Volumens zusammengedrängt ist, so fehlt in der Regel jede Spur von nucleolärer Substanz.” Then follows his general conclusions in regard to the physiology and structure of the nucleoli: “Die Nucleolen sind nach meiner Ansicht im allgemeinen als nicht strukturirte Gebilde aufzufassen. . . . Sie stellen als solche . . . ein Abspaltungsprodukt,

welches während der vegetativen Thätigkeit der Zelle und des Kerns in oder an den chromatischen Elementen zur Abscheidung gelangt und zu Beginn der Mitose aus dem Kernraum entfernt wird. Wie bei allen organischen Wachstums- und Umbildungsprocessen, so würden . . . Sekret-Substanzen zur Abspaltung kommen, welche in Form eines Hauptnucleolus oder mehrerer Nebennucleolen auftreten. . . . Die Gründe, welche theils für die Auffassung der Nucleolen als nicht organisirter Stoffwechselprodukte sprechen, theils speciell darauf hinweisen, dass es im Kern entstandene und dem Kern verlassende secretartige Stoffe sind," are the following: (1) "Die bedeutende Entfaltung der nucleolären Substanz in den Kernen solcher Zellen, für welche eine intensive vegetative Thätigkeit angenommen werden muss (Keim-Mutterzellen, Drüsenzellen, Ganglienzellen, Wimperzellen), würde zum mindesten dafür sprechen, dass die Nucleolarsubstanz ein Stoffwechselprodukt darstellt, dessen Erzeugung in einem gewissen Abhängigkeitsverhältniss zur Intensität der vegetativen Leistungen von Kern und Zelle steht." He cites numerous cases to show that all germ cells with little yolk and with usually adequal cleavage have a large "Hauptnucleolus" (sponges, *Hydromedusae*, *Siphonophora*, *Acalephae*, *Ctenophora*, *Echinodermata*, *Copepoda*, *Tomopteris*); while all large ova with a considerable amount of yolk and with discoidal or superficial cleavage have numerous "Nebennucleoli" (most *Insecta*, many *Crustacea*, lower *Vertebrata*). He explains the time of the appearance of the "Nebennucleoli" in the egg of *Canthocamptus* in this way: "Zur Erklärung dieser Erscheinung ist anzunehmen, dass irgend welche die ganze Eizelle betreffenden Veränderungen physiologischer Natur, die um diese Zeit eintreten, die weitere Apposition der neu sich bildenden Nucleolarsubstanz an den Hauptnucleolus verhindern und das Auftreten mehrerer Verdichtungscentren hervorrufen, welche häufig nicht mehr das Färbungsvermögen des ursprünglichen Hauptnucleolus erlangen . . . vom rein morphologischen Standpunkt aus darf man aber wohl mit diesen in den Endstadien der Eibildung auftretenden Bildern jeden intermediären Keimbläschentypus vergleichen, welcher sich im Lamellibranchiaten-Ei vorfindet."

He notes that "die Bildung nucleolärer Substanz auch unabhängig vom Zellwachsthum in erheblichem Masse stattfinden kann. Bekanntlich treten nämlich auch in den zur Copulation sich anschickenden Geschlechtskernen Nucleolen auf, welche nicht selten beträchtliche Dimensionen annehmen, und dasselbe gilt für die Kerne der früheren Furchungsstadien. Hier ist von einem Zellwachsthum nicht die Rede." Accordingly, he concludes: (1) "dass die Menge der nucleolären Substanz in einem direkten Verhältniss steht zur Intensität der Wechselbeziehungen zwischen Kern und Zelle"; (2) "hier möchte ich nur wiederholen, dass ich aus den verschiedenen Bildern eine Entstehung der Substanz der Nucleolen an oder in den Chromatinschleifen und die Möglichkeit einer Verschmelzung derselben ableiten und mich so entschieden gegen die Auffassung aussprechen möchte, dass die Kernkörper aus dem Zellplasma in den Kern hereingelangen und hier in die Bildung des Chromatins eingehen, sowie im allgemeinen dagegen, dass die kleinen durch Theilung der grösseren entstehen"; (3) he brings a few observations to show "dass der Kern die nucleoläre Substanz an das Zellplasma abgibt, dass es sich also hier wohl kaum um Stoffe handelt, welche als Nährmaterial dem Chromatin zugeführt werden, sondern um solche, die während der Veränderungen des letzteren zur Abspaltung und dann zur Ausscheidung aus dem Kerne kommen. . . . Ich denke . . . , dass sie [die vorhergehenden Erörterungen] in ihrer Gesamtheit sehr wohl eine Stütze für die Kernsekret-Theorie bilden können." Finally, Häcker gives his own explanation of the maturation stages of *Triton*, based on the description of Born ('94), and, comparing the changes here with those observed by himself in the maturation of *Canthocamptus*, generalizes the two as follows: 1. Stadium (growth of the germinal vesicle), "Abscheidung einer dunkel tingirbaren Nucleolarsubstanz"; 2. Stadium, "Verdichtung der chromatischen Substanz und Concentrierung in die Kernmitte. Beginn der Auflösungsvorgänge. Der neu sich bildende Nachschub an nucleolärer Substanz erlangt . . . nicht mehr das ursprüngliche Färbungsvermögen"; 3. Stadium, "Grössenreduktion des Keimbläschens: Die chromatische Figur liegt unmittelbar im Zellplasma."

Held ('95) finds that in the ganglion cells of vertebrates, when stained with erythrosin followed by methylen blue, the nucleolus stains blue and the "Nebennucleoli" violet.

Herrick ('95) found that the nucleolus of *Homarus* contains one large and several smaller vacuoles; the gravitation of the nucleolus in the caryolymph, *i.e.*, its movement to the lower side of the nucleus, may be post-mortem phenomena (at least I learned as much from Dr. Herrick during a brief conversation).

Keuten ('95) investigated the nuclear division of *Euglena viridis*. In the nucleus there is an elongate body, the "Nucleolo-Centrosoma," which stains more intensely than any other portion of the nucleus. At the commencement of mitosis it elongates, "und während die Segmente [Chromosomen] bisher eine annähernd senkrechte Richtung zur Oberfläche des Nucleolo-Centrosomas eingenommen hatten, bilden sie jetzt einen spitzen Winkel mit demselben," and gradually come to lie parallel to it. At this time the middle piece of the "Nucleolo-Centrosoma" stains more lightly than its ends, so that these latter parts are sharply demarcated from it (with the stain of Heidenhain, namely, Bordeaux R. followed by haematoxylin). "In der folgenden Phase rücken die parallel zum Nucleolo-Centrosoma gelagerten Chromosomen von beiden Polen her nach dem Äquator zu, so dass die Enden des Nucleolo-Centrosomes nunmehr frei in die Kernhöhle hineinragen, während die Chromosomen als breite äquatoriale Zone das Mittelstück des Nucleolo-Centrosomes umgeben." Next, the nucleus assumes the form of a rotation ellipse, in the short axis of which the "Nucleolo-Centrosoma" lies. After the longitudinal splitting of the chromosomes, from three to five vacuoles appear in each end of the "Nucleolo-Centrosoma"; then the latter structure elongates and breaks into two parts, while at the same time the long axis of the nucleus gradually changes so as to coincide with the long axis of the "Nucleolo-Centrosoma," and part of the chromosomes become grouped around the one end, the remainder around the other end, of the "Nucleolo-Centrosoma." Keuten believes his "Nucleolo-Centrosoma" to be comparable to the nucleolus of *Amoeba crystalligera* (Schaudinn), to the "Centralspindel" in *Diatomea* (Lauterborn), and to the centro-



some plus central spindle of *Ascaris megaloccephala*; it is probably an important mechanical factor in the mitosis.

Korschelt ('95) finds that in the amitosis of the intestinal cells of *Ophryotrocha puerilis* the "Kernkörper" divides into two. Ovarial and cleavage stages of the same annelid: the "Kernkörper" in the cleavage cells arises as "eine Anhäufung von Chromatin, die sich zu einer Kugel abrundet. In ihr taucht bald eine polygonale Felderung als Ausdruck einer schon ganz früh beginnenden wabigen Struktur des Kernkörpers auf." The "Kernkörper" increases in size rapidly, attaining its maximum size and staining intensity when the chromatin filament for the next mitosis becomes well marked. From this time on "beginnt sein allmählicher Verfall"; it stains less intensely, owing to the walls of its meshes becoming thinner; the regularity of the latter becomes lost, and granules appear within and between them, while at the same time the "Kernplasma" ["Kernsaft"] stains more deeply: "Während vorher das Kernplasma hell und der Nucleolus dunkel gefärbt erschien hebt sich jetzt umgekehrt der helle Kernkörper von dem dunklen Kernplasma ab. . . . Immerhin halte ich es für möglich und sogar für wahrscheinlich, dass zu dieser Zeit ein Austausch zwischen dem Kernsaft und der geformten Substanz des Kernes stattfindet, bei welchem vielleicht ein Theil des vorher im Kernkörper niedergelegten Chromatins dem Kernfaden beigefügt wird." Similar nucleolar changes take place in the male and female pronuclei, antecedent to the stage of the first cleavage spindle; in the male pronucleus "man sieht . . . bei dem aus dem Kopf des Samenfadens sich herausbildenden Spermakern im Gerüstwerk den Nucleolus auftauchen." The younger germinal vesicles contain one deeply staining, homogeneous "Kernkörper"; later vacuoles arise in it, so that it eventually evinces an alveolar structure; the time when the nucleolus disappears is quite variable, thus it may sometimes remain when the chromatin filament is perfected: "Dieser kann übrigens auch noch vorhanden sein, wenn die vier Kernschleifen bereits gebildet sind. Das letztere Verhalten möchte man entschieden so denken, dass die Substanz des Kernkörpers von keinerlei Bedeutung für die Ausbildung der chromatischen

Substanz ist. Das oben eingehend besprochene Verhalten der Embryonalkerne liess dagegen eine ganz andere Auffassung zu, obwohl es auch bei diesen allerdings abnormer Weise vorkommt, dass neben den bereits gebildeten Chromosomen (sogar in der angelegten Spindel) der Kernkörper noch vorhanden ist. . . . Was die erwähnten Verschiedenheiten des Verhaltens der Nucleolen in dem Ei- und Embryonalzellen betrifft, so liessen sich diese vielleicht durch die recht verschiedenartige Ausbildung und Funktion der Kerne in den beiderlei Zellen erklären."

Lauterborn ('95a), nuclear division of *Ceratium hirundinella*: from one to four oval nucleoli are present and are frequently apposed to the nuclear membrane. One nucleolus is still present in the spirem stage (the mitosis advances no further than this); but he was unable to decide whether this nucleolus divides into two.

Lauterborn ('95b), *Multicilia*: each nucleus contains a relatively large nucleolus, which frequently shows a "netzgewabige" structure.

Macallum ('95) concludes that less iron is contained in the nucleolus than in the chromatin, as is shown by its lighter stain with haematoxylin. Nucleoli "are always attached to the chromatin network, and sometimes there appears about them a membrane derived from, and continuous with, the fibrils with which they are connected." In a nucleus of a gland cell from the kidney or liver of *Necturus* "which is passing into the mitotic phase, the nucleolar body disappears, apparently by solution into the chromatin threads, for in the nucleus of a renal cell, in which the meridional disposition of the chromatin filaments obtained preparatory to the formation of the loops, I saw, attached to one of the filaments and partly embraced by its substance, what appeared to be the remains of such a body." The nucleoli of the amphibian ovum are derived from the chromatin of the nuclear reticulum. In support of his previous observations ('91) he adds, "that the iron in the cytoplasm of the ovum makes its appearance only after the solution of the peripheral nucleoli commences." In plant cells (*Erythronium*) there are at least three kinds of nucleoli: the first stain intensely with eosin; the second are composed of chromatin;

and the third kind, which occur in the embryo sac, "are not present in the mitotic nucleus, but in the retrogressive stage [metaphase] they appear on the course of the filaments as spherical elements enclosing one or more refracting corpuscles and containing but a small amount of iron, which, however, in later stages . . . is more abundant. These nucleoli are eventually formed chiefly of chromatin, and in stained preparations appear to contain nearly all the chromatin of the nucleus. When mitosis again commences the filament forms at their expense, the increase in size of the filament keeping pace, apparently, with the decrease in the quantity of chromatin which the nucleoli contain. Finally, before their disappearance, when they contain but a minimal quantity of iron, they take the eosin stain deeply. All these forms of nucleoli take up safranin from solutions as readily as do the chromatin elements in the same nuclei, and they hold the stain as tenaciously when they are washed with alcohol. They are in this respect different from the eosinophilous nucleoli in the animal cell, which appear to be unrepresented in the vegetable cell." In *Spirogyra* and *Corallorhiza* "the greater portion of the chromatin in each nucleus forms a single large spherical element unconnected with the chromatin network." He corroborates Leydig's view of the structure of the chromatin loops in the nuclei of the salivary glands of *Chironomus*; the nucleolus is often vacuolar and amoeboid, and may be transversed several times by the chromatin loop; "the presence of granules and vacuoles . . . appears to indicate that it is physically active, which cannot be postulated of the vast majority of the nucleoli of Vertebrate cells." In *Euglena* the nucleolus stains deeply with eosin (except after fixation in picric acid), but does not stain with safranin; it is "intermediate in composition between the nucleolus of higher animal cells and the chromatin of the nuclear reticulum."

Mead ('95), egg of *Chaetopterus*: "in the second cleavage, as in the first, the nucleoli are dropped out into the cytoplasm in the equatorial plane."

Montgomery ('95) described the various arrangements of the nucleoli ("Chromatinmassen") in the ova of *Stichostemma*

*eilhardi*. "Was diese Chromatinmassen chemisch darstellen, ist mir völlig unklar: vielleicht sind sie als von dem Dotter aufgenommene Nährsubstanzen zu betrachten, oder vielleicht stellen sie Konglomerate mehrerer Kernsubstanzen dar." (In my present paper I have no corrections to make to these previous observations, but add only fuller descriptions of the genesis of these nucleoli.)

Moore ('95), spermatogenesis of *Selachii*: the resting nuclei of the first spermatogenetic period contain each a single large nucleolus, which disappears in the following mitosis. In the subsequent resting stage the nucleolus reappears, and also there appears a smaller "secondary nucleolus" surrounded by a vacuole. The larger one then "takes a position, generally, but not always, in line with the long axis of the archoplasm. . . . These two peculiar forms of nucleoli are always to be found after the transition from the first into the second spermatogenetic period, and throughout all the generations of the latter."

Pflücke ('95), ganglion cells of *Invertebrata*: "Ob . . . die zum Nucleolus tretenden Lininfäserchen mit der Substanz desselben verschmelzen oder jener dem Vereinigungspunkt der Gerüstbälkchen nur aufgelagert ist, muss ich unentschieden lassen. Die Nucleolen erhalten sich hierin komplicirter als die Chromatinkörnchen, und die Möglichkeit, dass der intensiv färbbaren Substanz des Kernkörperchens ein eigenes stützendes Liningergüst zu Grunde liegt, ist nicht ausgeschlossen." Nucleolar vacuoles are normal structures, and are especially abundant in the cells of gasteropods; he followed in life the process of the detachment of smaller vacuoles from a larger one, as well as the process of fusion of two vacuoles. In *Helix* "kommen neben drei bis fünf grösseren Hauptnucleolen mit einem oder mehreren Hohlräumen im Inneren, sehr zahlreiche ganz zerstreut liegende kleinere Nebennucleolen bis zur Grösse eines Chromatinkornes herab vor, denen Vacuolen ganz fehlen und die sich von Chromatinkörnchen nur durch die Färbung unterscheiden." He also observed (cells of gasteropods) the "Kernkörperchenkreis" first described by Eimer, and found that the circle of granules around the nucleolus was connected

with it by linin fibers; but he was unable to decide whether these granules are thickenings of linin fibers, or whether they correspond to "Nucleolen bzw. Nebennucleolen . . . , welche sich vielleicht vom Mutterkörper getrennt haben und durch Wirkung centraler Lebensherde in jener typischen, regelmässigen Stellung verharren."

vom Rath ('95a) studied the maturation of the ovum of *Euchaeta marina*: on Pl. VII he figures a number of various sized, all rather large nucleoli, in the germinal vesicle, at the stage when the chromosomes are longitudinally cleft.

vom Rath ('95b) finds that the secretion of the gland cells of the head in *Anilocra* stains exactly like the nucleoli, and concludes that both substances are probably chemically related. He briefly mentions (footnote, p. 5) having seen double nucleoli in liver cells of molluscs and *Amphibia*; these dumbbell-shaped nucleoli may be either regarded as states of fusion or of division. In liver cells of *Astacus* the nucleolus consists of "zwei verschieden tingirten einander dicht anliegenden Kugeln einer dunklen und einer blassen." There is no relation between centrosomes and nucleolar substance.

Rhumler ('95) studied the nucleolar relations of *Cyphoderia*. From three to nine "Binnenkörper" lie within the nucleus, the largest nuclei having the smallest number; so that accompanying the increase in size of the nucleus, a gradual fusion of the "Binnenkörper" takes place, though without an appreciable increase in the total volume of their substance.

Sacharoff ('95) concludes that since the eosinophilic granules of the blood have the same appearance as the nucleoli, "und weil diese Kernkörperchen bei dem Herausfallen der Kerne auch herausfallen müssen, um dann unweigerlich von Leukocyten verschlungen zu werden, so ist mit grösster Wahrscheinlichkeit anzunehmen, dass bei Säugern die eosinophilen Granulationen auf dem Wege der Phagocytose von aus Hämatoblasten herausgefallenen Kernkörperchen entstehen." In birds the nuclei do not fall out of the erythrocytes, but the eosinophilic corpuscles are nucleoli which have wandered out of the nucleus; these nucleoli are rod shaped. (Only medical literature is cited in this paper.)

Sala ('95), ovum of *Ascaris*: in the first maturation mitosis the single nucleolus breaks into small pieces of various size, which gradually become scattered throughout the nucleus; then they become smaller and spherical, and come to lie directly under the nuclear membrane. These fragments may possibly stand in a genetic connection with the corpuscles which are subsequently found at each pole of the spindle. And since the latter corpuscles may stand in some connection to a centrosome, "es ist . . . nicht unmöglich, dass eine enge Beziehung besteht zwischen der Auflösung des Nucleolus und dem Auftreten des Centrosoma."

Schloter ('95), gland and liver cells of *Salamandra*: in the nuclei may be distinguished, besides the chromatin and paralinin, red-staining spherical corpuscles, the larger of which are regarded as plasmosomes.

Sobotta ('95), ovum of *Mus*: in contradiction to the view of Holl, the chromosomes are not derived from the nucleoli only, but from the whole chromatic substance of the nucleus.

van der Stricht ('95) observed in the larger ovarial eggs of *Amphioxus* that each contains a large nucleolus with an excentric vacuole; it disappears at the time of formation of the pole spindle.

Vejdovský ('95a) found large, homogeneous nucleoli in the yolk cells of *Prorhynchus hygrophilus*, "die nicht die gewöhnliche kugelige Gestalt bewahren, sondern immer in Theilung begriffen sind. Man findet meist doppelte Kernkörperchen, deren Hälften durch eine ziemlich tiefe Furche von einander getrennt sind und die eine centrale Höhlung erkennen lassen. Nebstdem findet man in Drei- selbst Viertheilung begriffene Kernkörperchen. . . . Ich glaube . . . , dass man es hier mit einer Hypertrophie der normalen Kernkörperchen zu thun hat, welche schliesslich zur Degeneration der Kerne führt"; these nucleoli occupy more than two-thirds of the space within the nucleus. In the ovum the nucleolus is much smaller, and shows a division into two parts (Fig. 89), but here these two parts are not of equal size.

Vejdovský ('95b) found in the egg of *Bothrioplana* a spherical nucleolus, "mit einem Nucleolinus."

Waldeyer ('95), cited by Flemming ('96), regards the nucleoli as morphologically distinct from the chromatin reticulum.

Wheeler ('95) observed in *Myzostoma glabrum* that the nucleolus is large and vacuolated, and after the reduction mitosis, "remains in the cytoplasm as an inert mass, gradually melting away, but not disappearing until about the eight-cell stage, when it may often be found in the largest blastomere."

Wilcox ('95) holds that in the spermatocytes of *Cicada* the nucleoli stand in genetic connection with the centrosomes, and adds, "It is probable that different structures have been called nucleoli by different authors."

1896.

Auerbach ('96) studied the spermatogenesis of *Paludina*: the nucleus of the spermatogonium contains a number of large, more or less spherical bodies ("Karyosomen"); each nucleolus (of the resting spermatogonium), after simultaneous staining with acid fuchsine and methylen green, shows a central red portion and a blue peripheral shell. "Es besteht also eine Zeit lang der Nucleolus aus einer erythrophilen Centralmasse und einer kyanophilen Rinde." In the subsequent nuclear division of these cells the nucleoli disappear. "Fest steht nur, dass in dem Netzstadium die Nucleoli als solche verschwinden, und dass ihre Rindensubstanz auf die angegebene Art zu einem Teile des intranukleären Netzwerkes wird, der anfangs noch unterscheidbar ist, dann aber durch Auseinanderücken der Knotenpunkte sich in dem übrigen Fadennetze verliert." In the spirem stage there are one or two small, spherical, red-staining bodies in the nucleus; he was unable to determine whether these stand in any genetic relation to the nucleoli, which had previously vanished. In the spermioblast (which changes directly into the hair-shaped spermatozöon) a small, red-staining body lies within the nucleus, but subsequently disappears; Auerbach supposes that it wanders out of the nucleus and fuses with the "Nebenkern."

Doflein ('96), maturation of the egg of *Tubularia larynx*: the single large nucleolus is suspended by achromatic fibers in

a clear, structureless space within the nucleus ; at first homogeneous, it later contains from one to five unstaining "Körperchen," which he thinks are not vacuoles, on account of their refractibility. In the amitotic division of those nuclei which degenerate and eventually become absorbed by a definitive egg cell, division of the nucleolus precedes that of the nucleus.

Floderus ('96) studied the maturation and embryonal development of various *Tunicata*. A "Hauptnucleolus" and "Nebennucleoli" are present. The former is homogeneous in only very young cells, and later differentiates into two different substances : (1) a refractive, larger portion, which encloses (2) a less-refractive, paler portion. He considers the small vacuoles of the nucleoli to be "Kunstprodukte," though the large one is normal. "Nicht selten findet man in dieser grossen, allem Anscheine nach mit Flüssigkeit erfüllten Höhlung eine Anzahl fester, lichtbrechender Körnchen, vielleicht Coagulationsprodukte, die wahrscheinlich bei der Fixierung entstanden sind." As a rule there is one, but sometimes two "Nebennucleoli" in most though not all eggs ; these rarely attain half the diameter of the "Hauptnucleolus," and appear in the germinal vesicle shortly before the yolk granules arise in the cytoplasm ; they are similar to, but paler than, the refractive portion of the large nucleolus. The "Nebennucleoli" are absent in *Clavelina* ; they probably arise by gemmation from the "Hauptnucleolus," and he figures to this effect a lobular "Hauptnucleolus." In the cytoplasm of the ova of *Stylopsis* and *Ciona* (but not *Clavelina* and *Corella*) certain spherical "intravitelline Körper" occur, usually one to a cell, and frequently close to the nuclear membrane ; in size and staining reactions these are similar to the "Nebennucleoli," and, following Roule, "sehe ich mich genöthigt, anzunehmen, dass sie von Nebennucleolen herrühren, die aus dem Kern des Eies in den Dotter hinausgewandert sind," thereby supposing that they press out through a preliminarily produced pore in the nuclear membrane, and that the larger intravitelline bodies are probably fused masses of smaller ones. In accord with Hennequy ('93) and Roule he considers the intravitelline bodies not as "Dotterkerne" nor astrospheres, but as atavistic or rudimentary organs, which



together with the nucleoli correspond to the macronucleus of the *Infusoria*.

Gerould ('96), ovarian eggs of *Caudina*: in the youngest ova there are numerous peripheral nucleoli; these increase in size as the nucleus grows, and subsequently each contains a vacuole, but they are always close to the nuclear membrane.

Greenwood ('96), macronucleus of *Carchesium polypinum*: the nucleoli ("protomacrosomes," in distinction to the "protomicrosomes," or chromatin granules) are numerous and vacuolated, and stain like true metazoan nucleoli. They vary in size and form, and are probably amoeboid, though this point could not be determined in the living nucleus, which is first rendered visible by reagents. The vacuoles are fluid accumulations, and arise first in the center of the nucleolus. "No vacuoles surround the macrosomes of *Carchesium* at any time, nor do they ever show general increase of fluidity or swelling such as might accompany the penetration through them of some secretion from without; . . . the deposition of vacuolar fluid is centrifugal; . . . thus the macrosome may become a bladder-like or honey-combed structure, its residual solid (?) forming a well-defined membrane-like investment for fluid contents."

Henneguy ('96) distinguishes true and false nucleoli (the latter being "nœuds du réseau," in the sense of O. Hertwig, '92). He reviews the observations upon nucleoli made by several previous authors.

R. Hertwig ('96), unfecundated ova of echinoderms poisoned with strychnine: the nucleoli vanish within the nucleus as the chromosomes appear. "Meine eigenen Untersuchungen lassen es mir ausgeschlossen erscheinen, dass im Ei der Seeigel Nucleolen und Centrosomen irgend etwas mit einander zu thun haben. . . . Dagegen ergeben sich unzweifelhafte Beziehungen der Nucleoli zur Entwicklung der Chromosomen. . . . Dieses Wechselverhältniss ist nun nicht so zu verstehen, als wäre das gesammte Material der Chromosomen in den Nucleoli enthalten. Dagegen spricht die geringe Masse der Nucleolar-Substanz und ihr verschiedenes Verhalten den üblichen Chromatin-Färbungsmitteln gegenüber. . . . Die Nucleolen können somit den Chromosomen ein zur endgültigen Fertigstellung nothwendiges

Ergänzungsmaterial liefern." "Chromatin-Nucleoli" are such as contain the whole chromatin of the nucleus (*Actinosphaerium*, *Spirogyra*, salivary glands of *Culex*); "solche Kerne zeigen dann ein achromatisches Gerüst und in demselben einen grossen chromatischen Körper, im übrigen Nichts, was man den Nucleoli oder den Chromosomen der Gewebszellen vergleichen könnte. . . . Derartige Nucleoli wären dann nicht, wie mein Bruder annimmt, und auch ich früher geglaubt habe, von den echten Nucleoli als etwas wesentlich Verschiedenes zu unterscheiden; sie würden Nucleoli sein, die ausser der specifischen Nucleolensubstanz noch das Chromatin des Kernes enthalten. . . . Bei der Umwandlung zur Spindel lösen sich Chromatinkörnchen vom Nucleolus ab und treten auf das Kernnetz über, ein Substrat hinterlassend, das man wohl den echten Nucleolen vergleichen muss. Später wird auch dieses aufgelöst."

Korschelt ('96), employing a modification of the Ehrlich-Biondi stain, finds in the spinning glands of caterpillars that the macrosomes stain green and hence consist of chromatin, while the microsomes stain red and so must be regarded as nucleoli (cf. '97).

List ('96) made comparative studies on various nucleoli, principally with a view to their chemical constituents, by applying a new staining method, whereby Berlin blue is produced in the fixed tissues. "Wir sind zu dem Resultate gekommen, dass die Nucleolarsubstanzen nach ihrem chemischen Verhalten 3 verschiedene Gebilde darstellen, von denen jedes wahrscheinlich wieder eine eigene complicirte chemische Zusammensetzung besitzt. Nach der bisherigen Bezeichnungswiese sind zu unterscheiden: Hauptnucleolus, Nebennucleolus und Nucleolus schlechtweg"; the substance of all the nucleoli differs from that of the nuclein (chromatin) proper. "Wir haben gesehen, dass (bei *Mytilus* und *Pristiurus*) die Umsetzung des Ferrocyankaliums durch Salzsäure, wodurch Ferrocyanwasserstoffsäure und hieraus durch den Sauerstoff der Luft Berlinerblau entstand, allein genügte, um die Nebennucleolen zu färben. . . . Wenn wir die Reagentien concentrirter anwenden, . . . so tritt in jeder Zelle die Substanz des Nucleolus in Gestalt eines oder

mehrerer blauer Kügelchen hervor. . . . Nach ihrem chemischen Verhalten stehen also Nebennucleolus und Nucleolus einander näher als Haupt- und Nebennucleolen"; he concludes that the "Nucleolus" and the "Nebennucleolus . . . mindestens verschiedene Modificationsstufen des Paranucleins . . . darstellen."

*Mytilus* egg: what Lönnerberg supposed to be vacuoles within the nucleoli, List holds are "Nebennucleoli," and these alone evince the characteristic Berlin-blue reaction; by afterwards staining the preparation with carmine, "die Masse des Hauptnucleolus, das Nuclein, hatte sich scharf roth gefärbt, die Nebennucleolarsubstanz, das Paranuclein, rein blau." Even in eggs where no yolk was as yet present, both these substances could be demonstrated. The "Hauptnucleolus" represents the greater part of the nucleolus, and is usually single; in it may lie one spherical "Nebennucleolus," or the latter may cover, cap-like, one pole of the former; sometimes "Nebennucleoli" occur in the nuclear cavity, separated from the "Hauptnucleolus"; occasionally there are true vacuoles within the latter. *Pholas* egg: (treatment with iron chloride, nitric acid, then "Ferrocyankaliumlösung") the "Nebennucleolus" is much larger than the "Hauptnucleolus," except in very small ova, where they may be equal in size. The last-named nucleolus may enclose a large, excentric vacuole, or in place of this, a "Nebennucleolus"; in the chromatin network of the nucleus there are small nodules of paranuclein, and sometimes a free "Nebennucleolus." "In älteren Eiern überwiegt bei Weitem der Nebennucleolus den Hauptnucleolus an Masse"; the latter is either apposed to one end of the former, or there may be a large "Nebennucleolus" with a small "Hauptnucleolus" at each end of it. *Pristiurus* egg: in the youngest germinal vesicles the minute nucleoli all lie at the nuclear periphery, the larger ones being central; in larger ova all the nucleoli are placed at the periphery of the nucleus. *Sphaerichinus* egg: the supposed (Häcker, '93b) vacuole of the "Nebennucleolus" is in reality the "Hauptnucleolus": "Jedoch weichen meine Resultate von denen Häcker's darin principiell ab, dass eben festgestellt werden konnte, dass das, was H. Hauptnucleolus nennt, wie ein Nebennucleolus reagirt, und die Vacuole

wie ein Hauptnucleolus." With the three staining methods employed (all used on material fixed with corrosive sublimate), only the "Nebennucleolus" is plainly stained, "nicht aber der Nucleolus schlechthin, wie er in jeder Zelle vorkommt." By treatment for half an hour with a drop of .5 % iron chloride solution, then stained by the Berlin-blue reaction, in each somatic cell the nucleolar substance appears in the form of bluish-green spherules. "Im Mollusken- wie im Vertebraten- gewebe hatte jede Zelle einen rundlichen Nucleolus; in secernirenden Zellen, z. B. Darmzellen, traten 2 oder 3 auf, oder Grössenunterschiede, wie z. B. der Nucleolus in der Leberzelle von *Mytilus* durch seine Grösse auffällt."

Michel ('96), ova of *Nephtlys* and *Spiophanes*: each double nucleolus consists of (1) a darker, more granular, portion, which in *Spiophanes* contains either a small granule or a vacuole (he is undecided which it is), and in *Nephtlys* is vacuolated; and (2) of a clearer, refractive, unstaining portion. In *Nephtlys* there are usually two double nucleoli, "la substance colorable recouvrant plus ou moins complètement la masse claire comme d'une calotte"; but other states were also found: "trois nucléoles doubles, une sphère claire entre deux parties sombres presque à l'opposé; inversement, une partie sombre et deux sphères claires presque opposées, nucléoles plus composés avec plusieurs sphères claires et même comme spumeux, sphérules claires libres en plus de celles des nucléoles doubles jusqu'à une douzaine. . . . Les masses claires, avec leur aspect, leur forme sphérique et leur déformation temporaire par la pression, leur variation de taille suivant les conditions osmotiques, l'épaississement de leur paroi par réduction de volume, apparaissent comme des vésicules à contenu liquide spécial," while the colorable portions are composed of pyrenin, and hence are true nucleoli (the pyrenin proved "par l'absence de gonflement par l'eau et par le gonflement par les acides, par l'insolubilité dans le sulfate de cuivre ou le ferrocyanure de potassium. . . . l'aspect des vésicules et leur disposition dans les nucléoles ou à l'état libre. . . . portent à croire à des vacuoles à contenu spécial formées dans le nucléole et finalement éliminées").

Morgan ('96) studied Echinoderm eggs placed in artificial

media : immature ova of *Sphaerechinus*, placed in sea water to which 1.5 gr. NaCl had been added, show artefacts in the nucleolus : "Each [body] consists of an outer darker shell, which is filled with a clear fluid, and the center of each sphere is occupied by a small black granule"; several of these structures are usually found on each section through the nucleolus. (For previous descriptions of somewhat similar productions, cf. Ransom ('67), Leydig ('88), and O. Schultze ('87). The upper of the two figures numbered "24" in Morgan's plate should be "23," since it refers to the nucleolus.)

Rohde ('96), ganglion cells of *Doris* and *Pleurobranchus* : the nucleoli wander out of the nucleus and finally into the neuroglia, and there acquiring an envelope (derived from the neuroglia) form new cells. [Judging from his figures, however, these supposed nucleoli would seem to be myelin drops.]

Wagner ('96a), spermatogenesis of *Arachnids* : "Bei der ersten Spermatocytentheilung theilt sich der Nucleolus entweder in der Ebene der Aequatorialplatte mit den Chromosomen zusammen, oder ausserhalb derselben neben einem der Spindelpole. Im letzteren Falle tritt er nach dem Verschwinden der Kernhülle . . . aus dem Kerne heraus."

Wheeler ('96) gives no description of the nucleoli in the text, but he figures several stages of the development in eggs of *Myzostoma* (Figs. 9, 10-15, *M. cirriferum*; Figs. 23, 52-54, 56, *M. glabrum*). In *M. cirriferum* (Figs. 12-15) is figured, in addition to the single large nucleolus, also one smaller nucleolus.

E. B. Wilson ('96) states of the true nucleoli or plasmosomes : "There is strong evidence that the true nucleoli are relatively passive bodies that represent accumulations of reserve-substance or by-products, and play no direct part in the nuclear activity." In germinal vesicles he assumes that the "principal nucleolus" is chemically different from the nucleoli of somatic cells; but that the "accessory nucleoli" of the former correspond to the nucleoli of the latter. He concludes that "we can hardly doubt the conclusion of Häcker, that the nucleoli of the germ-cells are accumulations of by-products of the nuclear action, derived from the chromatin either by direct transformation of its substance, or as chemical cleavage-products or secretions."

1897.

Toyama, cited by R. Hertwig ('96), holds that the nucleoli become centrosomes in the spermatogenesis of *Bombyx*.

Van Bambeke ('97a), ovocyte of *Pholcus*: there is usually a single large nucleolus, rarely also accessory ones; the nucleolus is vacuolated, "les vacuoles . . . faisant fréquemment saillie à sa [tache germinative] surface; dans certains vacuoles, on découvre des granules safraninophiles." At a later stage the nucleolus retains much the same appearance, "mais fréquemment le contour net, safraninophile, qui la délimitait, a disparu en tout ou en partie, et l'on remarque parfois une solution de continuité au niveau de laquelle le contenu de la tache s'épanche dans le reste du contenu nucléaire. Cette sorte d'évacuation ne doit pas être confondue avec la rupture de vacuoles nucléolaires, laquelle peut s'observer à tous les stades." ('97b, the same, with figures.)

Bouin ('97), giant spermatogonia of *Cavia*: the accessory part ("corps juxtanucléolaire") of the double nucleolus stains red in safranine and blue in haematoxylin (in opposition to Hermann), though less deeply than the spherical portion of the nucleolus, and is sometimes hemispherical in form. This part is single, and appears to consist of a mass of very fine granules. In degenerating cells, "les uns nous montrent deux nucléoles flanqués chacun d'une ou de plusieurs petites masses hémisphériques, réfringentes et teintées en rose pâle; lors des mouvements intranucléaires, les corps juxtanucléolaires contractent des rapports plus intimes avec les vrais nucléoles, deviennent plus réfringents et moins colorables, s'accolent à leur substance, se divisent à leur suite, et les accompagnent dans leurs migrations. Après plusieurs divisions répétées, ces noyaux contiennent un certain nombre de nucléoles, cinq ou six généralement." In the process of formation of the cells of Sertolli the nucleoli fuse successively with one another.

Braem ('97), *Plumatella*: in the egg of .013 mm. diameter the nucleolus contains one to four vacuoles: "Sie sind allem Anschein nach Flüssigkeitsbläschen, welche im Nucleolus auftreten und auf dem Höhepunkt ihrer Entwicklung an die

Peripherie rücken, um da ihren Inhalt nach aussen zu entleeren." The nucleolus becomes ovoid, and its substance paler at its smaller end; the vacuoles are usually, but not always, at the paler end. "Zuweilen ist der Gegensatz der beiden Nucleolus-Hälften lediglich in der verschiedenen Färbbarkeit derselben ausgesprochen. In anderen Fällen wird er durch eine Einschnürung bezeichnet, die den Nucleolus in einen grösseren, dunkeln und einen kleineren, hellen Abschnitt zerlegt. . . . Die Einschnürung kann nun zu einer völligen Abschnürung führen, so dass der Nucleolus doppelt erscheint und von zwei neben einander liegenden Kugeln gebildet wird, oder bei gegenseitiger Entfernung der Theilstücke in zwei räumlich getrennte Nucleoli zerfällt. . . . Selten ist der Nucleolus dreitheilig . . . , wo das mittelste Stück dunkler ist als die beiden seitlichen. . . . Dies lässt vermuthen, dass der Keimfleck im Stande ist, unabhängig vom Wachsthum des Eies seine Gestalt zu verändern, und dass die Zweitheiligkeit auf der Bildung eines pseudopodienartigen Fortsatzes beruht, der sich bald mehr, bald weniger deutlich vom Hauptkörper abgliedert und auch hinsichtlich seiner Substanz bald mehr, bald weniger von demselben verschieden ist."

De Bruyne ('97), double cells of the ovarian follicle of *Nepa*, *Periplaneta*, *Meconema*, and *Aeschna*: in the amitotic division of the nucleus the nucleolus divides first. (Since the cytoplasm does not divide, each such cell finally receives two nuclei.)

Carnoy and Lebrun ('97a) (an abstract of this paper may also be found in the *American Naturalist* for July, 1897). This contribution deals particularly with the relations of the nucleoli in the growth period of the ovum of *Salamandra* and *Pleurodeles*. In the youngest nuclei observed there is a nuclein filament, but no nucleoli; the first nucleoli arise as buds from the filament, and these are termed "nucléoles primitifs." Then the nuclear filament becomes changed into an amorphous magma, composed of irregular granules, and the latter then subsequently disappear, so that all trace of the original filament becomes lost. All further changes within the nucleus are of nucleolar character. From the "nucléoles primitifs" are derived the "nucléoles secondaires" which "sont dûs à des

associations de granules provenant de la désagrégation de l'élément nucléinien"; and then follow the "nucléoles tertiaires," which differ from the nucleoli of the preceding two generations in that they do not come from degenerating granules of preceding generations, but are detached from them in the form of spherules. Each nucleolus of each generation arises, increases in size, becomes more complex in structure, and then passes through a polymorphic "figure de résolution"; the form of these figures varies according to the particular generation, and also according to particular ova. The greater part of the "figure de résolution" then disappears, except a few granules which serve as the starting point for the next generation; that portion of the substance which disappears serves as nourishment for the egg. So all the generations of the secondary and tertiary nucleoli arise "à l'aide des produits de la résolution antérieure." After each "résolution" new nucleoli arise, and the number of these generations is large, continuing through a length of three years. The number of primary nucleoli is usually from two to six; of secondary, from 400 to 500; of tertiary, from 500 to 1000; the number varying in different ova. Fusions of nucleoli are of normal occurrence: "cette attraction des masses nucléiniens rappelle à l'esprit ce qui se passe au sein de l'œuf entre les noyaux de conjugaison." In the radiation exerted by each nucleolus upon the surrounding caryoplasm "nous voyons . . . la confirmation d'une thèse soutenue dans la 'Cytodiérèse,' à savoir: que c'est sous l'influence du noyau que se forment les asters de division." The chromatin filament does not reappear, but there is a "grand nombre de générations nucléolaires et filamenteuses qui naissent et disparaissent tour à tour, l'une après l'autre, jusqu'à l'époque des globules polaires." The authors necessarily regard all the previous observations on the amphibian ovum as erroneous. General conclusions for all kinds of cells, based in part on previous observations: there may be distinguished "nucléoles plasmatiques," "nucléoles nucléiniens," and "nucléoles mixtes" ("qui sont rare"). Plasmatic nucleoli consist of at least two substances, "une plastine et une globuline digestible." All nucleoli, "lorsque leur formation est achevée . . . représentent



la totalité de l'élément filamenteux d'un noyau ordinaire ; . . . dans bien de cas — aujourd'hui nous pourrions peut-être dire dans tous — on constate dans ces corps la présence d'un véritable appareil filamenteux, tortillé sur lui-même, comme dans un noyau ordinaire, et présentant les mêmes propriétés que dans ces derniers. C'est que l'on voit surtout dans les nucléoles-noyaux, c'est-à-dire dans les nucléoles nucléiniens uniques, qui ont absorbé tout l'élément filamenteux primitif." All nucleoli develop from the chromatin filament ; and chromosomes are derived from "nucléoles noyaux." The chemistry of nucleoli is also considered.

Carnoy and Lebrun (197b), fecundation of the ovum of *Ascaris megalocéphala*: the centrosomes are "nucléoles plasmatiques ou achromatiques" which have left the nucleus at the commencement of mitosis ; one is derived from the male, the other from the female, nucleus. They totally disappear after mitosis, and neither reënter the nuclei nor divide to produce the centrosomes of the subsequent division.

Cunningham (197) : "There are indications in the ova of the turbot that the substance of the nucleoli is absorbed into the central fibrils to form the chromosomes of the polar mitoses, but the actual formation of these chromosomes was not followed."

v. Erlanger (197), a brief mention of certain recent views upon the nucleolus : "Als echte Nucleolen waren allein solche Körper zu bezeichnen, welche sich durch ihr Verhalten gegen Chemikalien . . . scharf von dem Chromatin unterscheiden. . . . Vorderhand bleibt also die Bedeutung der echten Nucleolen rätselhaft, falls man diese Gebilde nicht mit Häcker als eine Sekretion des Kernes beurteilen will." They bear no relation to centrosomes.

Fauvel (197), ovogenesis of *Ampharete* : ovarian ova of 30 $\mu$  diameter, and at this stage only, contain two nucleoli. "On rencontre toutes les modifications : nucléole simple, nucléole étranglé par le milieu, deux nucléoles accolés, et enfin deux nucléoles bien nettement séparés. . . . Nous n'en avons jamais rencontré deux dans l'œuf mûr, ni dans l'œuf non détaché de l'ovaire." The nearly mature ovum contains one

large nucleolus, with a large vacuole ; he believes that subsequent to the two-nucleolus stage one of the nucleoli is extruded from the nucleus. Two nucleoli were observed also in the ova of *Amphicteis*, *Sanytha*, and *Melinna*.

Flemming ('97) recurs to the controversy between Korschelt ('96) and Meves ('97), and agrees with Meves that the macroosomes are nucleoli, and the microsomes chromatin granules. He also mentions the following observation on the ovum of *Ascidia canina* : here there is one "Nucleolus" and one much smaller "Kernkörper" ; "beobachtet man ihn [Kernkörper] am lebend entnommenen Ei, so findet man ihn so gut wie stets in Molekulärbewegung, und zwar oft in recht grossen Exkursionen."

Häcker ('97a) ('96 is a preliminary communication), cleavage stages of *Cyclops brevicornis*. This paper deals particularly with the "intrasphärale," "extranucleäre," or "Aussen-Körnchen (Ektosomen)" found in certain of the astrospheres of the cleaving ovum. These ectosomes are small spherical bodies of various size, which stain like the nucleoli, but somewhat more intensely. In the resting stage of the cell there are several nucleoli in the nucleus, and no ectosomes outside of it ; when the nucleus enters on the aster stage, the nucleoli have disappeared and ectosomes are present in one of the astrospheres, at first at the base of, subsequently on the whole periphery of, the latter ; towards the close of the metakinesis there appear in the place of the ectosomes larger clumps of red-staining substance. He concludes : "So glaube ich es denn mit Sicherheit aussprechen zu dürfen, dass diese gröberen Brocken auch genetisch mit den Körnchen [Ektosomen] zusammenhängen, sei es, dass sie direkte Umwandlungsprodukte derselben, sei es, dass sie Neubildungen sind, welche dem nämlichen Process ihre Entstehung verdanken, aber in Folge der während der Theilung eintretenden Zustandsänderungen der Zelle eine etwas andere Beschaffenheit, einen anderen Aggregatzustand angenommen haben. Wie ich gleich hier hinzufügen möchte, verschwindet die Erscheinung, sowohl im Zweizellenstadium als in den späteren Stadien, während der eigentlichen Ruhepause vollständig, indem vermuthlich jene Massen einer Resorbtion

oder chemischen Umwandlung anheimfallen." In only one astrosphere of only one cell in each of the following cleavage generations this process is repeated, and the line of these particular cells ("Körnchen-Zellen") constitutes the line of development of the sexual cells; but the ectosomes are present in these particular cells only during mitosis, and in the resting stages are absent, while nucleoli occur in the nuclei; this process was observed from the first through the ninth cleavage stages. He concludes that in each generation there is a production *de novo* and a subsequent solution ("Auflösung") of the ectosomes. The first appearance of the latter coincides in point of time approximately with the disappearance of the nucleolar substance in the nucleus; from this and certain other factors he concludes: "So . . . würde also der Annahme kaum etwas im Wege stehen, dass die zu Beginn der Mitose noch vorhandenen oder neugebildeten Nucleolen aus dem Kernraum in der Richtung der *einen* Spähre auswandern und sich hier in die Aussenkörnchen umwandeln. . . . Für die Kerne der Körnchenzellen ist dann allerdings, in Gegensatz zu den übrigen Embryonal-Elementen, eine besonders reichliche Produktion der Nucleolarsubstanz und demnach eine besonders intensive vegetative Thätigkeit [*cf.* '95] anzunehmen." The explanation for the arrangement of the ectosomes in only one of the astrospheres he finds in the assumption "dass die beiden Centrosomen einen verschiedenen (vielleicht einen verschieden 'kräftigen') Einfluss auf das umgebende Plasma, beziehungsweise auf die beweglichen Inhaltkörper desselben ausüben."

Häcker ('97b) finds that in germ cells of both animals and plants there is to be noted "das Auftreten eines einzigen, vacuolenhaltigen, dunkel tingierbaren "Hauptnucleolus" in den jüngeren Stadien, das Hinzutreten von blässeren adventiven oder "Neben-Nucleolen" in einer früheren oder späteren Phase." Nucleolar substance arises during one or several stages of nuclear activity as a by-product of metabolism, possibly also as chromatin substance which has become structureless and chemically changed; and, finally when the nucleus begins to divide, is removed out of the latter. He confirms Wheeler's ('96) observations on the ovum of *Myzostoma*, that the nucleolus

wanders out of the nucleus into the cytoplasm, where it slowly decreases in size.

Hermann ('97) figures (Fig. 20) a spermatogonium nucleus of *Scyllium* containing a single and a double nucleolus ; the latter consists of two apposed spheres, which differ chemically and dimensionally.

Korschelt ('97) maintains his previous opinion ('96) of the chromatin nature of the macrosomes of the nuclei in the spinning glands of caterpillars, in answer to the criticism of Meves ('97) (reviewed immediately below). Korschelt employed the Ehrlich-Biondi stain with increased strength of the methyl green, and thereby obtained a coloration of the macrosomes and microsomes the very opposite of that procured by Meves. "Ob man überhaupt achromatische, chromatische Substanz und Nucleolen in allen Kernen so scharf auseinanderhalten kann, wie dies vielfach geschieht, ist mir höchst zweifelhaft. Wenn man in verschiedenen Zuständen der Kerne Nucleolen auftreten und wieder verschwinden sieht, wird man annehmen müssen, dass sie sich aus den sogenannten achromatischen oder chromatischen Substanzen des Kerns, vielleicht aus beiden herausbilden. So können sich möglicher Weise auch die von mir als Makrosomen bezeichneten Theile in Nucleolen umbilden und das von Meves angegebene Auftreten von Vacuolen in ihnen würde damit seine Erklärung finden."

Meves ('97) contends that the microsomes in the spinning glands of caterpillars, which Korschelt regarded ('96) as lanthanin granules, are chromatin ; and what Korschelt regarded as chromatin granules (*i.e.*, the macrosomes) are nucleoli. Meves employed the usual formula of the Ehrlich-Biondi stain (Heidenhain's receipt), and finding that the macrosomes thereby become stained red, concludes from this reaction their chromatin nature.

Stauffacher ('97) finds in the aster stage of the mitosis of one of the pronephral cells of *Cyclas*, that the nucleolus still persists intact in apposition to the spindle fibers.

Wheeler ('97), maturation of the ovum of *Myzostoma* : this object, previously described by the author ('95), is here more fully described with the addition of figures. A remarkable mode of formation of nucleoli in the pronuclei is described ;

each "chromosome" consists of "two granules, at first of the same size [which] grow very unequally, so that one is often considerably larger than the other. Hereupon some, but not all, of these granules break down to form irregular strings of minute karyomicrosomes which are distributed along the fibers of the achromatic reticulum. . . . The large chromatin granules which do not break down become the nucleoli of the pronuclei. I am unable to state positively that in each Diplococcus-shaped chromosome one of the granules breaks down to form a chain of minute karyosomes while the other persists as a nucleolus, but I am very strongly inclined to believe that this is the case." These nucleoli are cast out into the cytoplasm when the first cleavage spindle is formed, and there rapidly dissolve. Wheeler accepts "Häcker's view of the secretory nature of the nucleolus, at least so far as the germinal vesicle is concerned."

Bancroft ('98), germinal vesicle of *Distaplia*: the nucleolus "does not form the stellate body found in the old ova, as Davidoff maintained, but is found within this body, which is itself the remains of the germinal vesicle. The nucleolus at this stage is quite complex, consisting of a homogeneous cortex, an excentric finely granular medulla, and within the latter several very highly refractive bodies, the largest of which may have a granular appearance. During the greater part of the growing period these refractive bodies are the only substance in the germinal vesicle that takes the chromatin stain with a methyl green and acid fuchsine combination."

1898.

Kostanecki ('98) confirms the observations of Wheeler ('95, '97) in regard to the casting out of the nucleolus into the cytoplasm, in the maturation of the ovum of *Myzostoma*.

#### B. BOTANICAL LITERATURE.

Schleiden ('38) is the discoverer of the nucleolus in plants, but he gives it no name: "einen kleinen, sich scharf abgrenzenden Körper, der, nach dem Schatten zu urtheilen, einen

dicken Ring oder ein dickwandiges hohles Kügelchen darzustellen scheint"; while in other cases it may be a simple spot, or may be wholly absent. "Aus meinen Beobachtungen an allen Pflanzen, die eine vollständige Verfolgung des ganzen Bildungsprocesses erlaubten, geht hervor, dass dieser kleine Körper selbst früher sich bildet, als der Cytoblast [Nucleus]."

Macfarlane ('81) examined various plant cells, in all of which he found one or several bodies ("nucleolo-nuclei") within the nucleolus. The nucleolus of *Spirogyra* has a distinct membrane, which disappears at the period of the nucleolar division; the karyokinesis results in the formation of a "nuclear barrel," at each end of which is a mass of nucleoplasm, these two masses being connected by fibers with the nucleolus which lies between them. The nucleolus then divides, preceded by a division of the nucleolo-nucleus, so that each daughter-nucleolus receives a daughter nucleolo-nucleus, and the daughter-nucleoli then wander apart to the nearest masses of nucleoplasm, "as they retreat from each other they drive the polar masses before them, thereby elongating the nuclear barrel. . . . The nucleoli at length advance to the polar masses and bury themselves in the nucleoplasm of these." From these and numerous other observations, Macfarlane concludes: "that the nucleolus, or more probably the nucleolo-nucleus, is the center of germinal activity, and that as we pass outwards to the periphery of the cell, this reproductive activity becomes less and less. In no other way, to my mind, can the number of nucleoli and nucleolo-nuclei at different ages in the cells of any plant be explained."

Strasburger ('82a) gives reviews of previous observations on the chemical constituency of nucleoli.

Strasburger ('82b) studied nuclear division in various plant cells (*Fritillaria*, *Lilium*, *Hemerocallis*, *Tradescantia*, *Galanthus*, *Dicotyledons*). "Pollenmutterzelle" of *Fritillaria*: between the nucleus and its membrane collects a homogeneous, refractive, lens-shaped mass of substance; "sie geht nicht unmittelbar aus den Kernkörperchen hervor, die ja schon auf vorausgehenden Stadien verschwunden waren, vielmehr repräsentirt sie, allem Anschein nach, ein Secret"; this body he terms "Secretkörperchen." At first it stains deeply with methylen green; but

subsequently it ceases to stain, vacuoles arise in it, and it decreases in size, until at the time of the spindle formation it disappears. "Sie [Secretkörperchen] treten erst auf, nachdem das Kernkörperchen oder die Kernkörperchen in dem Fadenknäuel des Kerns Aufnahme gefunden. Ihre Entwicklungsgeschichte unterscheidet sich auch von derjenigen echter Nucleolen, denn sie treten nicht im Verlauf der Fadenwindungen auf, vielmehr ausserhalb derselben, stets an der Wand der Zelle. Ausgeschlossen ist ja nicht, dass in der so ausgesonderten Substanz die Substanz früher Kernkörperchen vertreten sei, aber erweisen lässt sich dies nicht." So he concludes that before the mitosis of the spores and "Pollenmutterzellen" a certain change occurs in the nucleoplasma, in connection with the formation of the "Secretkörperchen." The nucleoli of many plant cells contain vacuoles. In the embryo sac of *Galanthus* a division of the large nucleolus takes place, which division is probably passive, caused merely by the tension of the cytoplasm. Gradations are to be found between the nucleoplasmic-microsomic substance and the substance of the nucleoli: "ob die Nucleolen-Substanz trotzdem nur eine Modification der Mikrosomen-Substanz sei und aus dieser hervorgehe, will ich dahingestellt bleiben lassen. Wahrscheinlich ist mir aber das letztere, wenn ich bedenke, dass bei Eintritt in die Theilungsvorgänge selbst die stark modificirte Nucleolen-Substanz in das Kerngerüst findet und sich in demselben nicht anders als wie die Mikrosomen-Substanz verhält. Man könnte die Nucleolen-Substanz vielleicht als einen Reservestoff des Zellkerns auffassen, als eine momentan ausser Aktion gesetzte Substanz."

Tangl (82) studied the nuclear division of three species of plants. *Hemerocallis fulva*, flower buds: the "Pollenmutterzelle" contains at first three or five nucleoli, which are homogeneous. "Mit fortschreitender Entwicklung der Mutterzellen verringert sich die Anzahl der Nucleolen," until only one is to be found; this one is always peripheral in position, never in contact with the central "Körnermasse." Later, vacuoles appear in the nucleolus (he believes these to be the results of reagents), and while it still stains with carmine it no longer does with acidified

methylen green. In mitosis, when the nucleus is uninucleolar, the substance of this nucleolus becomes dissolved in the nucleus; when multinucleolar, however, one of the nucleoli may pass out into the cytoplasm. *Hesperus*, "Pollenmutterzelle": here there is one nucleolus, which stains with methylen green, as does the chromatic filament, and disappears in mitosis. *Pisium*, same cells: here there is one hat-shaped nucleolus, which stands in no connection with the "Fadenknäuel"; "Sehr eigenthümlich ist das Verhalten des Nucleolus in den die Kerntheilung vorbereitenden Stadien. Anfänglich besteht derselbe aus homogener, stark lichtbrechender Substanz. Später sind am Nucleolus eine dichte äussere und eine innere, bedeutend schwächer lichtbrechende Schichte unterscheidbar. Endlich findet man Stadien, auf denen neben dem noch unveränderten Fadenknäuel ein sehr schwach lichtbrechender Körper gefunden wird, dessen Umriss vollkommen demjenigen des ursprünglichen Nucleolus entsprechen"; finally even this disappears.

Zacharias ('82) studied the epidermis cells of *Phajus*, and concludes that the nucleoli (one or two in number) consist of plastin. They do not dissolve in distilled water; swell with .1% nitric acid; do not stain with methylen green; and dissolve in weak "KalilaugeLösung."

Heuser ('84) studied the mitoses in the embryo sac of *Fritillaria imperialis*. In the resting nucleus there are from five to nine nucleoli: "Dieselben sind intensiv gefärbt und stehen in deutlich wahrnehmbaren Zusammenhang mit dem Nucleo-Hyaloplasma." In the prophase of the mitosis they lose their staining power and apply themselves to the chromatin threads. He considers them, with Strasburger, "als Reserve-Behälter der Kernsubstanz" (using the term "Kernsubstanz" as equivalent to "Chromatin"); their ground substance consists of plastin, permeated with chromatin. In *Fritillaria*, as well as in *Galanthus* and *Leucojum*, "fließt das gesammte Kernkörperchen in die Kernelemente über, während in anderen Fällen ein Ueberschuss an Plastin als 'Secretkörper' ausgeschieden werden mag."

Strasburger ('84), nuclei in the embryo sac of *Fritillaria*: in the spirem stage the large nucleoli disappear, "wobei sich um



dieselben der Kernsaft wieder zu färben beginnt." He concludes that the nucleoli are not immediately taken up into the chromatic thread, but dissolve in the caryolymph; "auch ist hiermit wohl sicher der Nachweis gegeben, dass sie nicht identisch mit den Mikrosomen sein können." The nucleoli arise in the meshes of the chromatin network. Strasburger agrees with Flemming that they represent a substance distinct from the chromatin and nuclear sap, but does not consider it to be a living substance, but rather a reserve stuff.

Guignard ('85) investigated nuclear division in several species of plants. *Lilium*, young embryo sac: the nucleus usually contains a single nucleolus, which is very large, finely granular in structure, and situated excentrically between the strands of the chromatin network; with the double stain, methylen green and fuchsine, it stains red, while the chromatin stains green. At the time of the longitudinal division of the chromatin filament, the nucleolus commences to stain less intensely, vacuoles arise in it, and it finally fragments into small pieces which subsequently disappear; the fine granules appearing in the nuclear sap at this time are not derivatives of the nucleolus, but originate from the cytoplasm when the nuclear membrane vanishes. "Dans le *Lilium* . . . rien ne fournit la preuve d'un apport direct effectué dans la formation du fuseau par le nucléole, dont la substance se dissout dans le suc nucléolaire, pour s'incorporer et se mélanger, . . . aux autres éléments figurés qui contiennent la chromatine." In each daughter-nucleolus there are several nucleoli of unequal size; these disappear also in the subsequent mitosis. *Clematis*, embryo sac: the nucleoli in karyokinesis gradually decrease in size, and it seems "comme si la plus grande partie de leur substance était absorbée par les segments [chromatiques]." *Northoscordum*: here there are several large nucleoli which disappear when the spindle is produced, their substance being possibly incorporated in the chromosomes. In the metaphasic spirem they reappear in contact with the chromatin: "leur aspect général fait supposer qu'ils naissent là où on les aperçoit dans les jeunes noyaux . . . il est à croire que les nucléoles tirent une partie de leur substance, tout ou moins, du filament nucléaire auparavant

homogène. Ils se nourrissent ensuite dans le suc nucléaire. . . . Les nucléoles peuvent être considérés comme une substance de réserve que se sépare à un moment donné de la charpente nucléaire pour être reprise par elle ultérieurement"; he assumes that Strasburger's "corpuscule du sécrétion" is a true nucleolus. "Dans le *Lilium* et dans l'autres plantes, les noyaux filles n'offrent pas de nucléole avant d'entrer en division; en outre, leur aspect général au début du phénomène est bien différent de celui du noyau mère. . . . Le fait qu'ils se séparent du filament dès que le noyau . . . arrive à l'état de repos, pour être repris par lui aux premiers stades de la division, permet de les considérer, avec M. Strasburger, comme une sorte de réserve."

Macfarlane ('85) studied nuclear division in *Chara fragilis* (fixation with osmic acid): the nucleus of the apical cell contains one nucleolus, in which lies an "endonucleolus" (a term here substituted for his earlier term "nucleolo-nucleus"). At the commencement of all cell divisions this part of the nucleolus first divides, then the nucleolus, last of all the nucleus. After this division of the apical cell a nodal and internodal cell are produced, and the former "continues to divide regularly, forming cells each with one nucleus and nucleolus. In the internodal complete cell division is henceforward absolutely arrested: but the earlier steps are taken; for while the nodal cell has divided into three or four, the nucleolus of the internodal has divided and redivided, so that four nucleoli are present in the nucleus of it. The internodal cell then increases rapidly in length, the four nucleoli meanwhile continuing to proliferate, so that in internodal cells, such as in the third removed from the apex, we soon get a large nucleus with many little dark nucleoli. The nucleus then divides in the simple manner figured by Johow, so that in the fourth internodal cell there may be two nuclei, each with many nucleoli, in the fifth, three or four nuclei, and so on, so that the internodal cells soon become multinuclear, and their nuclei multinucleolar." The cortical nodal cells do not divide further, but "their nucleoli follow the example of that of the internode . . . the consequence being that the cortical nodal, and soon after the cortical

internodal cells, become multinucleolar"; the nodal leaf cells proceed in the same way. From these observations Macfarlane concludes: "in every active embryonic cell one nucleolus only is present in the resting state"; in some cases a fluid globule is present in the nucleolus, and this probably represents a "degradation of the endonucleolus." "The nucleolus, or more probably the nucleolo-nucleus, is the center of germinal activity, and that as we pass outwards to the periphery of the cell, this reproductive activity becomes less and less. . . . The result is that in all plants thus examined, after cell division has ceased, continued division of the cell contents from the endonucleolus outwards goes on. . . . I venture, therefore, to regard it as a general principle that after cell formation has ceased, the cell contents (especially the endonucleolus and nucleolus) persist in their activity for a shorter or longer period; . . . the most exalted type of cell is one with abundant protoplasm containing a single nucleus, nucleolus, and endonucleolus; . . . a cell with vacuolated protoplasm, one nucleus and two to four nucleoli is less exalted, while the multinuclear state is the most degraded form of cell."

Zacharias ('85) gives critical reviews of numerous preceding papers on nucleoli, besides observations of his own on various cells of plants. *Galanthus nivalis*, cells of the inner layer of the "Fruchtknotenwand": the single nucleolus is about  $\frac{3}{10}$  the size of the nucleus; examined in water it is homogeneous; after the action of absolute alcohol it appears to be composed of granules of various indices of refraction. Bast cells of *Cucurbita pepo*: the nucleoli, when stained with "Blutlaugensalz-Eisenchlorid," become very intensely colored, while the remaining nuclear substance stains only faintly. In the cells of *Spirogyra* and of the asci of Lichens he finds that there are no "nucléoles-noyaux," such as Carnoy described. "Alle Autoren stimmen gegenwärtig darin überein, dass die Nucleolen bei der Kerntheilung verschwinden." In opposition to Strasburger he contends that during the mitosis the dissolved nucleolar substance might as probably enter into the formation of the spindle fibers as of the chromosomes. In *Chara* (observed living) each nucleus contains one large nucleolus, with vacuoles: "Naht

die Kerntheilung heran, so verliert der Nucleolus an Deutlichkeit, er erfährt langsame Gestaltsveränderungen, die schliesslich einen amöboiden Charakter annehmen," and the nucleolus gradually disappears (this process lasting a half hour); "1½ Stunde später wurden in jedem Tochterkern vier kleine Nucleolen bemerkt, nach 3½ Stunden waren nur noch je zwei Nucleolen vorhanden und nach weiteren 1½ Stunden nur noch je einer. . . . Bei der Verschmelzung bilden die Nucleolen zunächst einen bisquitförmigen Körper, der sich dann später kugelig abrundet. Die Deutlichkeit der Nucleolen nimmt während des Vorganges der Verschmelzung stark ab, um später wieder zu steigen." Contrary to Strasburger and Tangl, he believes that no "Paranucleolen" wander out of the nucleus, but that where such have been observed, it has been due to the method of fixation. He notes that while the egg cells always contain nucleoli they are frequently absent in the male cells. "In alternden Zellen sind Gestaltsveränderungen, Kleinerwerden und Schwinden des Nucleolus beobachtet worden. . . . Mir scheint es nicht begründet zu sein, den Nucleolus als eine Ablagerung von Reservestoffen zu betrachten. Weshalb sollte er nicht ein Organ der Zelle sein, wie es Flemming annimmt? . . ." Against Strasburger's views "ist zu erwidern, dass wir über das active oder passive Verhalten der Nucleolen im ruhenden Zustande oder dem der Theilung überhaupt gar nichts wissen, und das Bestehen einer Organisation für die Nucleolen ebenso gut angenommen werden kann wie für irgend einen anderen Theil der Zelle."

Meunier ('86), *Spirogyra*: the single large nucleolus has a limiting membrane, and in the fresh state contains no vacuoles, vacuoles only appearing in the dying cell, and then are probably introduced drops of water. It stains with methylen green more intensely than any other structures of the nucleus, and also stains with acid picrocarmine, alkaline carmine, and haematoxylin; "ainsi . . . on constate que les matières colorantes, réputées spécifiques de la nucléine, limitent uniquement leur action efficace et significative au corps réfringent et apparemment réticulé du nucléole." After the action of nitric acid of from 2% to 4% a reticulation is found in the nucleolus; a 10%

or 12% solution of the same acid dissolves this reticulation and only preserves the clear, non-refractive stroma; 2% or 4% hydrochloric acid solution shows the reticulation of the nucleolus to be "un boyau continu et pelotonné. . . . Le filament chromatique du nucléole ne se digère pas dans la liquer digestive [suc gastrique]. . . . Nous ne craignons pas d'affirmer que le nucléole des *Spirogyra* reproduit fidèlement, dans ses traits essentiels, la structure des noyaux les plus parfaits. Il a une membrane propre, probablement une partie protoplasmatique, quoique fort réduite; il renferme toute la nucléine du noyau, et celle-ci est exclusivement confinée dans un étui de plastine, qu'elle remplit plus ou moins complètement. . . . Quoi qu'il en soit, nucléole par position, noyau par nature, on ne peut lui refuser le nom de nucléole-noyau, dans le sens attaché à ce mot par J. B. Carnoy."

Schwarz ('87) studied the microchemistry of plant cells. He distinguishes the following substances in the nucleus: "chromatin," "pyrenin" (nucleolar substance), amphipyrenin" (substance of the cell membrane), "linin" (achromatic fibrils), and "paralinin" (nuclear sap). The pyrenin and amphipyrenin "stimmen in fast allen Reactionen überein, sie unterscheiden sich jedoch durch ihre Tingirbarkeit, indem das Pyrenin der Kernkörperchen Farbstoffe fast immer sehr leicht aufnimmt und festhält, während das Amphipyrenin nur wenig oder gar nicht tingirt wird. . . . In den weitaus meisten Fällen liegt das Maximum des Nucleolusvolumens vor der Zone, in welcher der Kern sein Maximum erreicht, und in vielen Fällen tritt gerade dann die bedeutendste Verkleinerung des Nucleolusvolumens ein, wenn der Kern sein Volumen am stärksten vergrößert. Es scheint mir demnach wahrscheinlich, dass ein Theil der Kernkörperchensubstanz direkt bei der Neubildung der übrigen Kernsubstanz verbraucht wird."

Went ('87), mitosis in various cells of plants. *Lcucorum*, embryo sac: at the commencement of the prophase there are two large nucleoli, which lie between the fibers of the chromatin network; later they become apposed to these fibers, and he notes how "die Masse des Nucleolus langsam in die des Kernfadens übergeht. . . . Im Wandbelege des Embryosackes von

*Helleborus viridis* scheinen die Nucleolen auch im Kernfaden aufgenommen zu werden"; and there is apparently the same process in *Fritillaria imperialis*. "Bei den Kernen im Wandbelege des Embryosackes von *Narcissus pseudonarcissus* findet die Aufnahme des Nucleolus ungefähr wie bei *Galanthus* statt; er wird also von allen Seiten vom Kernfaden umwunden; allmählich windet dieser sich wieder los. Oft ist dann der Nucleolus schon ganz aufgenommen, zuweilen aber werden noch Theile davon vom Kernfaden fortgeschleppt und bleiben dann wohl einmal sichtbar bis zum Anfang der Metaphase. Wenn man Präparate mit diamantfuchsin-jodgrün tingirt hat, sieht man, dass die Farbe des Kernfadens vor der Aufnahme des Nucleolus blaugrün ist, während dieser letztere roth gefärbt ist; nach der Aufnahme des Nucleolus und während der ganzen Meta- und Anaphase ist die Farbe des Fadens deutlich violett geworden, was naturgemäss verursacht ist durch die Aufnahme des Nucleolus"; also during the mitosis of similar cells in *Hyacinthus* and *Tulipa* nucleolar substance is taken up by the nuclear filament. "Ich glaube aus den hier mitgetheilten Thatsachen wohl den Schluss ziehen zu dürfen, dass in vielen Fällen wenigstens der Nucleolus beim Anfang der Kerntheilung im Kernfaden aufgenommen wird. . . . Am wahrscheinlichsten ist es wohl, dass, wo der Nucleolus vor der Theilung im Kernfaden aufgenommen wird, er sich nach der Theilung auch wieder daraus bildet."

Strasburger (88) studied nuclear division in *Spirogyra polytactniata*. In the resting nucleus there is usually one large nucleolus, which disappears immediately before the formation of the nuclear filaments, and by dissolving in the nuclear sap causes the latter to stain more intensely: "Als wahrscheinlich stellte ich [84] es aber hin, dass die im Kernsaft gelöste Nucleolussubstanz den Kernfäden als Nahrung diene. . . . Auf Grund meiner neueren Erfahrungen erscheint es mir überhaupt unwahrscheinlich, dass die Nucleolarsubstanz, auch nach ihrer Auflösung im Kernsaft, den Kernfäden als Nahrung dienen sollte." In each daughter-nucleus several nucleoli arise, and these have the same number, position, and size in the two nuclei; later the several nucleoli of each daughter-nucleus unite to form

a single large nucleolus, and during this process the nuclear sap gradually loses its staining power. He shows that when the nucleolar substance is dissolved in the nuclear sap, and after the cell division, a portion of this substance plays a part in the production of the cellulose walls of the daughter-cells ; but he holds that not all of it is thus consumed, but that the nucleoli have probably some other, as yet unknown, function.

Mann (91) introduces a new method of differential nuclear staining : when plant tissues are stained for ten minutes in saturated solution of heliocin in 50% alcohol, and then from ten to fifteen minutes in a saturated aqueous solution of aniline blue, the nucleolus is red, the rest of the nucleus and the cell blue.

Macfarlane (92) constructs the following hypothesis, based on previous observations of his own and of Mann : "We would consider, then, that the nucleolus is the special chromatic and cell center ; that it sends out fine radiating processes — the intranuclear network — which partially fuse externally to constitute the nuclear membrane, the interspaces of the network being occupied by nucleoplasm concerned in metabolic change ; that radiating continuations of the chromatic substance pass out beyond the nuclear membrane and form a network in the protoplasm, while we would suggest for future proof or disproof that they further may be continued through wall pores to form an intercellular chromatic connection. . . . We would thus view a plant as a group of connected hermaphrodite cells, . . . bound together by a fine chromatic ramification, in the center of which in each cell is the nucleolus."

Mann (92) studied the cells of the embryo sac of *Myosurus minimus*. At the commencement of the conjugation of the two nuclei resulting in the formation of the primary endosperm nucleus, each nucleus contains "a large deeply stained nucleolus enclosed by a very faintly stained nucleolar membrane," and in each nucleus are also one or two smaller globules, which "seem to originate thus : when the nuclei about to conjugate have come in contact, one or two small nucleoli arise by the unequal division of the primary nucleolus. . . . These secondary nucleoli seem to have at first the power of division, but gradually they lose this power and their property of becoming

deeply stained, and change into globular colloid-looking masses with a central more deeply stained spot. I propose to call these bodies paranucleoli, because of their origin they may always be found in the micropylar nucleus and occasionally also in the antipodal nucleus." When these nuclei begin to conjugate, the large nucleoli of both fuse to form the single nucleolus of the primary endosperm nucleus; at the same time a new structure makes its appearance, in close contact with the nuclear membrane of the primary endosperm nucleus: "This body . . . corresponds, I believe, to the nucleolar membrane of the antipodal nucleus"; it is at first granular, later homogeneous. Still other, smaller spherical bodies later appear in the nucleus, which may have some connection with the paranucleoli. Finer structure of the nucleolus: in the nucleolar membrane "a number of very minute dark radially placed pores or striae can be observed, and . . . these striae are continued into very delicate cilia-like fibrils radiating out from the nucleolar membrane into the nuclear hyaloplasm. . . . The nucleolus is differentiated into an outer zone and an inner zone. The outer zone is less deeply stained, and on careful examination is found to be made up of a circle of peripheral endonucleoli, which are slightly elongated radially. The inner zone of the nucleolus is very darkly stained, and shows a number of large and irregularly disposed endonucleoli." The structure of the nucleolus may be somewhat different in other stages of its development, thus it may be composed of "(1) A thin unstained nucleolar membrane; (2) a great number of peripheral endonucleoli; (3) a deeply stained, apparently structureless, layer; (4) a corona of minute, slightly elongated, endonucleoli surrounding (5) a large central endonucleolus. . . . In a resting cell, . . . the center of the nucleolus is occupied by a large endonucleolus, which sends out minute fibrils through the nucleolar substance. . . . I believe the endonucleolar fibrils probably to pass through the finer pores in the nuclear membrane"; and Mann conjectures that "the endonucleolar filaments constitute the linin element of the chromosomes." Functions of the nucleolus: it is "concerned in the assimilation of food-material." He holds "the nuclear chromatin to be less highly elaborated



and less assimilative albuminoid material than the nucleolar chromatin. On the assumption just stated, we could explain also why we find . . . at the time of maturation portions of nucleolar matter detaching themselves from the main nucleolus to undergo a peculiar gelatinous change. The gelatinous change would correspond to a conversion of the assimilative material into achromatic elements, an explanation which would also explain the disappearance of nucleoli during the division of a cell. . . . I believe the hypothesis that the nuclear chromatin-segments and perhaps the nucleoli are organs for the conversion of assimilated material into material directly available for the achromatic elements of the cell to be not quite erroneous." In the mechanism of cell conjugation: "The endonucleolar fibers running through the body-plasm of the two sexual cells . . . are brought into contact with one another whenever the pseudopodial processes of the two cells have met. As soon as an union of fibrils has taken place, each fibril will commence to contract similarly to a muscular fibril," which results in drawing the two nuclei, afterwards also the two nucleoli, together; thus the endonucleolus is the "tropic center" of the cell.

Rosen ('92a) studied the differential staining of the nuclear elements in plants. Flowers of *Scilla*: in the nuclei of the "Bündelparenchym" are numerous large nucleoli, which differ in form and size; the one or two larger ones, "Eunucleoli," are each surrounded by a clear space, but none is present around the smaller "Pseudonucleolen." With the double stain, Altmann's acid fuchsine and methylen blue, the Eunucleoli stain red and the Pseudonucleoli blue, or *vice versa*. Similar cells of *Hyacinthus*: by the application of the double stain, aqueous solutions of fuchsine and methylen blue respectively, the Eunucleoli stain red, the Pseudonucleoli blue; but when these stains are applied in the reverse order, the nucleoli stain reversely. He considers, following Auerbach ('90), that the Eunucleolus is erythrophilic, the Pseudonucleoli kyanophilic, the latter staining as does the chromatin network. "Meine Pseudonucleolen aber sind eben offenbar weiter nichts, als besonders selbständig ausgebildete Bestandtheile des chromatischen Kerngerüstes und sind wie dieses und sein Produkt, der

Kernfaden, kyanophil"; these disappear before the mitosis, while the Eunucleoli remain until about the end of the spirem stage. Vacuoles arise only in the Eunucleoli.

Rosen in a second paper ('92b) presents further observations upon nucleoli. *Myxomycetes*: the spore nucleus contains one large nucleolus. *Fuligo septa*, plasmodium: one large, cyanophilic nucleolus, which he terms "Mittelkörperchen," since in the atypical mitosis this body lies in the middle of the pole plate, and disappears at the end of the nuclear division. *Synchytrium*: one large nucleolus with several vacuoles; in the first mitosis the division of this nucleolus precedes that of the nucleus, but during subsequent divisions the nucleoli vanish. In *Cystopus* there is no nucleolus.

Schottländer ('92), cells of cryptogams: the nucleus consists of a blue-staining substance (network), and a red-staining (nuclear membrane, nucleoli). Egg cell of *Gymnogramme chrysophylla*: here are one or several large nucleoli, each surrounded by a vacuole; in the ripe egg the nucleoli are filled with small globules. Egg cell of *Chara*: the nucleoli contain vacuoles, which later become so large in the largest nucleoli that they become polygonally flattened against one another, and their thin walls then present the appearance of a network within the nucleolus.

Demoor ('93), mitosis of *Tradescantia*: the nucleoli gradually disappear during the prophase.

Gjurasin ('93) investigated the nuclear division of *Peziza*. In the nucleus is one large, excentric nucleolus, which stains red with Flemming's triple stain, while in it as many as six granules may occur, and these stain violet. In the mitosis these granules disappear, but otherwise the nucleolus does not change at first, but occupies its original position within the cell, though now in the cytoplasm; eventually it disappears gradually. In each daughter-nucleus a new nucleolus arises, which apparently has no genetic connection with the mother-nucleolus (now vanished). "Ich bin der Ansicht, dass . . . das Kernkörperchen nicht eine Art von Reservestoff darstellt, sondern ein spezifisches Organ des Zellkernes ist."

Karsten ('93), nuclear division of *Psilotum*: in the resting nucleus are two or three nucleoli, which are homogeneous, oval

or spherical, and after haematoxylin-eosin, stain a rose color, while the chromatin is blue. At the time of the appearance of the chromosomes, "treten die Nucleolen aus den sich zusammenordnenden Plasma und lassen sich hier in Form scharf umschriebener, homogener, roth gefärbter Kügelchen nachweisen." Usually two nucleoli wander out, at least never more than two were found outside of the nucleus. These two come to lie at opposite poles of the nucleus, occupying the positions of centrosomes; and when the longitudinal splitting of the chromosomes takes place, each of the nucleoli also divides into two. Karsten believes these nucleoli are identical with the centrosomes of Guignard; but he does not explain what becomes of the third nucleolus during the division.

Lauterborn ('93), quoted by Karsten ('93), diatoms: there is a centrosome lying in a concavity of the nucleus; he noticed, further, "beim Beginn der Theilung aber zwischen Kern und Centrosom noch ein anderes Gebilde —, welches im späteren Verlauf der Karyokinese eine sehr bedeutsame Rolle spielt, nämlich die Anlage der Centralspindel"; this body must be derived either from the nucleus or the centrosome (I mention it here since it may in the future be found to have some connection with a nucleolus).

Moll ('93) studied karyokinesis in *Spirogyra*. There are one or two nucleoli, which stain more intensely with gentian violet than any other portion of the nucleus. They may be vacuolar in structure, or contain a skein of chromatin; they appear homogeneous only when too deeply stained. The skein structure (the skein itself staining as chromatin) is found in resting nuclei, as well as in the prophase of mitosis, and at the same time vacuoles may be present. He assumes that the thread in the nucleolus contains all the chromatin of the resting nucleus, and "that by the nucleolus the chromatin substance for the segments [chromosomes] is furnished"; this chromatin leaves the nucleolus in mitosis, and "it seems as if the chromatic substance were squeezed from the nucleolus by an aperture." After the chromatin skein has left the nucleolus, the latter disappears.

(Strasburger's paper, '93, was reviewed under the head of zoölogical literature.)

Wager ('93), nuclear division in *Hymenomyces agaricus*: each nucleus of a basidium contains one large nucleolus, besides the nuclear network. The two nuclei of the basidium fuse together and form one nucleus, in which afterwards the two nucleoli later fuse to form one nucleolus. This latter is often vesicular in structure. In the mitosis it lies close to the nuclear membrane, it gradually loses its staining intensity, decreases in size, and finally disappears; at the same time the cytoplasm in its neighborhood stains more deeply. But sometimes it persists until the diaster stage. "From the fact that the chromosomes begin to stain red at the time of the disappearance of the nucleoli, it would further appear that the former can take up nucleolar substance from the nuclear sap, and as fast as the nucleoli disappear the chromatic elements become more deeply stained red." In *A. stercorarius*, in the daughter-nucleus, "the chromatin mass appears to be transformed at once into the nucleolus," and only later a chromatin network appears. "I would suggest that the nuclear threads take up the dissolved nucleolar substance at some period during the division, and carry it over into the daughter-nuclei, to be given up again later as the nucleoli of the latter. . . . But a certain quantity of the dissolved nucleolar substance probably escapes into the cytoplasm when the nuclear membrane disappears, and this would be taken up at a later stage into the daughter-nuclei, as is shown by the increase in size of the nucleoli, and by the decrease in the capacity of the protoplasm for taking up stains."

Zacharias ('93) finds in plants that the nucleolus and cytoplasm are erythrophilic, the nuclein (chromatin) network is cyanophilic.

Belajeff ('94), "Pollenmutterzellen" of *Larix*: after the disappearance of the nuclear membrane in mitosis the nucleolus becomes gradually smaller and then disappears; several nucleoli reappear within each of the daughter-nuclei. "Es ist zu bemerken, dass nach der Auflösung der Nucleolen der Mutterzelle im Zellplasma eine gewisse Anzahl grober Körnchen erscheint, welche mit Safranin färbbar sind. Mit dem Beginn der Nucleolenbildung in den Töchterzellen verschwinden die Körnchen vollkommen. . . . Ich erklärte mir die Ergebnisse

meiner Beobachtungen derart, als lösten sich die Nucleolen, nach vorausgegangener Auflösung der Kernmembran, unter der Einwirkung der in die Kernhöhle aus dem Zellplasma gedrungener Substanzen, gänzlich auf, um später durch den Einfluss des Kernsaftes, der die ganze Zelle durchdrungen, wieder hergestellt zu werden, indem der Kernsaft die Nucleolensubstanz im Zellplasma so zu sagen gerinnen macht. Nach der Bildung der Töchterkerne, welche ihren Kernsaft aus dem Zellplasma absorbiren, werden die Körnchen abermals vom Zellplasma aufgelöst, um zum zweitenmal im Inneren der jungen Kerne (Töchterkerne) in der Gestalt von Nucleolen zu erscheinen." In *Fritillaria* and *Lilium* also the nucleolus is dissolved after the disappearance of the nuclear membrane.

Humphrey ('94) studied the "Pollen-" and "Sporenmutterzellen" of *Convallaria*, *Ceratozamia*, *Osmunda*, and *Psilotum*, and cells from the apex of the root of *Vicia* and *Hyacinthus*. The nucleolar substance is usually not to be found in the cytoplasm during mitosis. The nucleoli are "keine individuellen Bestandtheile, sondern unbestimmte Massen von Nucleolarsubstanz, und ihr Vorkommen im Cytoplasma hat keine weitere Bedeutung als zu zeigen, dass eine Communication zwischen Kernhöhle und Cytoplasma bisweilen, wenn auch nicht immer, sich herstellen kann und dass entweder die Nucleolen in einigen Fällen aus der Kernhöhle, bevor sie von den karyokinetischen Kräften angegriffen werden, austreten können, oder dass die Menge der Nucleolarsubstanz in einem Kerne grösser sein kann, als diese Kräfte zu lösen oder zu verbreiten vermögen. . . . Die 'Vacuolen' der Nucleolen scheinen mir das natürliche Resultat der nachherigen Trennung der flüssigeren von den festeren Theilen der Nucleolarsubstanz zu sein. . . . Wenn also Zimmermann ['93] den Satz aufstellt 'Omnis nucleolus e nucleolo,' so kommt er zu einer Verallgemeinerung, die nicht zulässig und derjenigen 'Omnis nucleus e nucleo' nicht gleichwerthig ist." In every nucleus of the "Pollensäcke" of *Ceratozamia* there is a large, peripherally placed paranucleolus (Strasburger): "In extremen Fällen kann die Anhäufung von Substanz eine so grosse sein, dass die Kernmembran hier bedeutend hinausgestossen wird. . . . Auf der Fuchsin-

Jodgrün tingirten Schnitten werden die Paranucleolen weder reinroth wie die Nucleolen, noch blaugrün wie die chromatische Substanz gefärbt, vielmehr nehmen sie eine Zwischennuance, welche mehr der des Chromatins als der der Nucleolen ähnelt, an"; he believes these paranucleoli to be artefacts. In contradiction to Karsten ('93) he found no body in *Psilotum* comparable to a Nucleo-Centrosoma.

Zacharias ('94) concludes, from numerous observations on cells of plants that as the size of the nucleus increases (or decreases) with the size of the cell, so also that of the nucleolus increases (or decreases) with the size of the nucleus.

In Rosen's ('95) contribution a large number of new facts are recorded, which may be briefly mentioned. The kyanophilic nucleoli of Auerbach "sind eben keine Nucleolen und bedürfen als wenig constante Theile des Chromatingerüsts überhaupt keines besonderen Namens." *Hyacinthus*: in meristem nuclei all the nucleoli except the smallest lie in special clear spaces, and though fibrils are rarely found in connection with them, "gleichwohl muss das Kernkörperchen in seiner scheinbar schwebenden Lage wohlbefestigt sein, da es . . . stets seine Lage im Centrum seines Hofes bewahrt." The large nucleoli of the "Gefässzellen" become vacuolar as they increase in size. In mitosis of root cells the nucleoli become gradually dissolved within the nucleus in some species, in others they are extruded into the cytoplasm; in the latter cases "erfolgte die Zerklüftung und Auflösung des Nucleolus viel langsamer, sodass bei dem Schwinden der Kernmembran noch bedeutende Nucleolarreste vorhanden waren." The nucleoli reappear in the dispirem stage before the daughter-nuclei have produced membranes, and the new nucleoli stain from the commencement intensely; from which the general conclusions are drawn: in the prophase the diminishing nucleolar substance penetrates, perhaps as a micellar solution, into the cytoplasm, and this process may cease before the nuclear membrane has disappeared. In some cases larger particles of nucleolar substance may penetrate into the cytoplasm, but only after the nuclear membrane has disappeared, and these particles become subsequently dissolved in the cytoplasm; in either case "das lösende Agens muss wohl

der Kernsaft sein, vielleicht unter Mitwirkung eines nur während der Prophasen gebildeten Enzyms. Während der Anaphasen wandert die Nucleolarlösung als solche in den Raum der Tochterkerne ein, und hier wird die Nucleolarmasse wieder fest. Bei der Hyacinthe — und anderen Objekten — erfolgt die Rekonstituierung der Nucleolen auch ausserhalb der Dispiremfigur. Die derart im Cytoplasma entstandenen Nucleolen wandern, wie ich glauben möchte, in die Tochterkerne ein, ehe sich diese mit einer Kernmembran umhüllen; wenn letzteres geschehen ist, so findet man anscheinend niemals mehr Nucleolen im Cytoplasma, die, wenn überhaupt, auch wohl nur nach nochmaliger Auflösung in den Kernraum gelangen könnten. Nicht ganz unmöglich scheint es mir, dass die Nucleolen, die man an fixirten Präparaten . . . im Cytoplasma auffindet, doch durch die coagulirende Wirkung des Fixierungsmittels entstanden sind. Ich glaube aber, dass dies von keiner grossen Bedeutung ist, denn an den Stellen, wo wir extranucleäre Nucleolen vorfinden, muss dann die Masse der Kernkörperchen als Lösung angesammelt gewesen sein." Also in the mitosis of root cells of *Aspidistra*, are nucleolar fragments seen in the achromatic spindle. Root cells of *Phaseolus*: in the resting stage there is a single nucleolus; in the mitotic prophase it becomes first lobular, then lengthened in the direction of the spindle, while at the same time it is undergoing a slow dissolution; "wenn die Spindel gebildet und die Kernwandung verschwunden ist, sieht man fast stets inmitten der zur Kernplatte angeordneten Chromosomen einen mehr oder minder ansehnlichen Nucleolarrest, welcher in derselben Richtung wie die Chromosomen und die Spindelfaden gestreckt ist. Dieser Nucleolarrest wird nun in der Mitte eingeschnürt, sodass er Hantelform erhält; die beiden Hälften reissen schliesslich von einander und gelangen an die Spindelpole. In anderen Kernen wird der Nucleolarrest einseitig aus der Kernplatte herausgedrängt oder auch doppelt getheilt; endlich findet sich meist an einem oder an beiden Spindelpolen ein Restchen des Nucleolus; seltener liegt ein solches neben der Spindel. Die Auflösung ist nun meist bald beendet"; and only exceptionally is there a minute nucleolar remnant in the cytoplasm at the end of

mitosis. "Unzweifelhaft sind auch bei *Phaseolus multiflorus* die Nucleolen der Tochterkerne Neubildungen. Wenn auch die Nucleolarsubstanz möglicherweise bei der Karyolyse erhalten bleibt und sich in den Tochterkernen nur wieder auf Neue sammelt, so besteht doch keine von Generation zu Generation sich fort spinnende Continuität in den Nucleolen als solchen und von einem 'omnis nucleolus e nucleolo' [Zimmermann] kann keine Rede sein." Root cells of *Vicia faba*: the nucleolar mass diminishes as the cell degenerates; "dieselbe stellt das erste Zeichen der Kerndegeneration . . . dar und ist, wie sonst, mit einer Zertheilung des Nucleolus verbunden," while a large nucleolus surrounded by a clear space is an embryonic condition. In the mitosis of these cells no nucleolar fragments pass into the cytoplasm, and in each daughter-nucleus two nucleoli arise which subsequently fuse into one. In opposition to Lavdowsky ('94), he contends that the centrosomes have no genetic connection with nucleoli, and that the nucleolar substance does not serve as nourishment for the chromosomes; "nichtsdestoweniger wäre es voreilig zu behaupten, dass von der Substanz der Nucleolen nichts in die Fadensegmente gelangen könne. . . . Die Violettfärbung der Segmente in den späteren Phasen der Karyokinese . . . könnte auf eine Einlagerung erythrophiler Nucleolarsubstanz in den kyanophilen Kernfäden schliessen lassen." In buds of *Psilotum triquetrum* the nucleoli are excentric, while in most plants they have a central position. In the mitosis nucleolar fragments are extruded into the cytoplasm (in agreement with Zimmermann, in opposition to Karsten and Humphrey), and none of the extruded masses can be regarded as centrosomes (against the view of Karsten). Three nucleoli usually arise in each daughter-nucleus: "Sie entstehen nahe der Peripherie des jungen Kerns, oft in Contact mit dem Cytoplasma, bevor die Tochterkerne sich mit einer Membran umschliessen und verschmelzen später nicht miteinander." In the mitosis of sporangia the nucleoli are usually "aus den karyokinetischen Figuren ausgestossen"; and the "Secretkörperchen" of Strasburger is a true extruded nucleolus.

Strasburger ('95, cited by Lauterborn, *Zool. Centralbl.*, 1896)



concludes that the nucleolar substance, dissolved in the nuclear sap, may be used in the production of the spindle fibers.

Koernicke ('96), study of mitosis on *Triticum*: in the development of the embryo sac when the two pole nuclei fuse together, the two nucleoli also join to form one. In the mitosis of the pollen the nucleolus always disappears before the formation of the spindle, but it could not be determined whether it takes any part in the formation of the latter.

Lauterborn ('96), nuclei of diatoms: there are several nucleoli present; in the spirem stage of division they commence to gradually disappear; "es scheint mir ziemlich sicher, dass ihre Substanz mit derjenigen der Chromatinkörnchen und des Liningerüstes zur Bildung der Knauelfaden verbraucht wird." It is important to note that the central spindle arises outside of the nucleus, before the nucleoli begin to disappear, so that there can be no genetic connection between the two.

Poirault and Raciborski ('96), binucleated ("conjugate") *Uredineae* during the production of the ascidiospore generation: in the mitosis the nucleolus becomes extruded into the cytoplasm, almost always in the equatorial plane. "Bei manchen Arten bleiben sie sehr lange erhalten so z. B. bei *Peridermium Pini acicola*, wo neben den längst ruhenden, mit neuen Nukleolen versehenen Kernen noch in den Plasma, die alten Kernkörperchen der Elternkerne herumirren. Mit den Centrosomen haben somit diese extranukleolären, vakuolirten Nukleolen nichts zu thun."

Zimmermann ('96), a general critical summary upon the vegetable nucleolus, with consideration of a part of the previous literature. Nucleoli are almost always present in the cells of the higher plants, and are of wide occurrence also in the lower forms; double staining serves to differentiate them from the chromatin. There are usually from one to three to a nucleus, but in the embryo sac of *Lilium martagon* there are from twenty to thirty. In *Chara* the older nuclei show the nucleolar substance in the form of very numerous, irregular fragments. The distinction of "Hauptnucleolus" and "Nebennucleolus" is not tenable, since the latter may be possibly chromatin globules. "Mit dem Chromatingerüst scheinen die Nukleolen

innerhalb der ruhenden Kerne in keinem Falle in direkter Verbindung zu stehen." The space frequently observed around the nucleolus is probably not an artefact. Its substance is probably homogeneous; "als die alleinigen mit Sicherheit nachgewiesenen Einschlüsse derselben können Vakuolen angeführt werden. . . . Diese Vakuolen sind dem gewöhnlichen Einschluss in Kanadabalsam häufig ganz oder teilweise mit Luft erfüllt oder stellen luftleere Räume dar. Sie erscheinen dann bei höherer Einstellung schwarz, bei niederer etwas rötlich, und es dürften wohl die namentlich in der die Kerne beiläufig behandelnden Litteratur vorliegenden Angaben über stark lichtbrechende Einschlüsse der Nucleolen zum Teil auf derartige Bilder zurückzuführen sein" (c.g., the "endonucleoli" described by Mann). During mitosis nucleolar bodies are often found in the cytoplasm, and such are probably extruded nucleolar fragments; "immerhin muss aber die allgemeine Gültigkeit des früher von mir als möglich hingestellten Satzes *omnis nucleolus e nucleolo* nach den neueren Untersuchungen als nicht sehr wahrscheinlich angesehen werden." In the Pollenmutterzellen of *Lilium martagnon* the nucleoli "zerfallen . . . in sehr zahlreiche kleine Kugeln, die . . . im Asterstadium ungefähr gleichmässig über den gesamten Zellinhalt zerstreut sind." He made similar observations also on *Hyanthis candicans*, *Fritillaria imperialis*, young sporangia of *Equisetum* and *Psilotum*, cells of the root apex of *Vicia*, and stem apex of *Phascolus* and *Psilotum*. There is also an extrusion of nucleolar substance in *Chara*, but it is doubtful whether this process occurs in other low forms. This extruded substance may in some cases, but perhaps not as a rule, return into the daughter-nuclei. That in mitosis the nucleolar substance may be incorporated into the chromosomes, "sei noch erwähnt, dass ich neuerdings an den Kernteilungsfiguren des Embryosack-Wandbelags von *Lilium martagnon* nach der Fixierung mit Chromsäure und Platinchlorid und Färbung mit Fuchsin und Jodgrün in den Endstadien des Spirems beobachten konnte, dass einzelne rote Kugeln, die ausserdem auch in grosser Zahl in der Umgebung der betreffenden Kerne zu beobachten waren, den violettgefärbten Chromosomen teils

seitlich ansassen, teils auch ganz von denselben aufgenommen waren, so dass sie . . . kleine Auftreibungen an denselben bildeten." It is doubtful whether the nucleoli have any genetic connection with either the centrosome or the nuclear membrane. In the synapsis (Moore, '95) of the nucleus the nucleolus becomes flattened against the nuclear membrane in most *Angiospermia*, having thus on section a sickle shape ("Sichelstadium"); and the coincidence of this form of the nucleolus with the synaptic stage "macht es jedenfalls sehr wahrscheinlich, dass die im Sichelstadium eintretenden Metamorphosen den Nucleolus eine gewisse Bedeutung besitzen."

Dębski ('97), *Chara*: the space surrounding the large nucleolus is caused by shrinkage of the latter, due to the fixing fluids, and is not present in life. In the nucleolus are numerous vacuoles which may become confluent. Within the cytoplasm occur extranuclear nucleoli, which stain like the others. In the mitotic prophase the nucleolus usually divides into two, and the latter either gradually diminish in size and finally disappear or else they persist for a while after the disappearance of the nuclear membrane. Then the extranuclear nucleoli collect at the poles of the spindle and "bewegen sich während der Metakinese von beiden Seiten her gegen den Ort der späteren Zellplattenbildung und verschmelzen dabei nicht selten während des Diasterstadiums miteinander zu unregelmässigen Kugeln, Klumpen und Fäden . . . die nucleolenartigen Körper sind später, nach der Bildung der Zellplatte und der Membran, nicht mehr dort zu sehen; es finden sich alsdann nur noch wenige durch das ganze Plasma der Zelle zerstreut, oder sie fehlen, besonders in den älteren Zellen gänzlich. Einige, wahrscheinlich solche, welche während des Diasters nicht in die Zellplattenebene gerückt sind, finden sich während des Dispirems in der Nähe der Tochterkerne ein; später sind sie zwischen den Fäden des Kerngerüsts zu sehen; in späteren Stadien findet man an ihrer Stelle einige kleine Nucleolen, deren Zahl immer mehr beschränkt wird, so dass sich schliesslich gewöhnlich in jedem Kern ein einziger grosser Nucleolus befindet."

Fairchild ('97), *Basidiobolus*: "Das Verschwinden des

Kernkörperchens . . . spricht entschieden für Strasburgers Annahme, dass es zur Bildung der Spindelfasern benutzt werde."

Harper ('97), ascus of *Erysiphe*: the nucleolus and the centrosphere stain in the same way, and "die achromatischen Fasern, aus welchen diese intranucleären Strahlenkegel gebildet werden, entstehen wahrscheinlich grösstentheils auf Kosten der Kernkörperchenssubstanz, die zu dieser Zeit regelmässig verschwindet."

Huie ('97), cells of *Drosera*: the nucleoli ("nucleolar chromosomes") are spherical and usually central; "endonucleoli" are enclosed spaces, not granules. During the process of food assimilation by the nucleus the nucleolus becomes smaller, and its vacuoles less apparent.

Lidfors (97) gives a thorough review of the "Sichelstadium" (Strasburger's "Sekretkörperchen") of the nucleolus in plant cells, as also the results of observations of his own on the embryo sac. *Tulipa*: at first there are several small nucleoli within the nuclear cavity, which later by their fusion produce a large one which becomes flattened against the nuclear membrane (the process is essentially the same in *Fritillaria*, *Anthericum*, and *Lilium*). *Gagea*: the nucleolar changes are as in the preceding forms, except that when the nucleolus reaches the periphery it remains spherical; this is also the case in *Ornithogalum*. *Oenothera*: in the youngest cells there is one central nucleolus; subsequently this flattens against the nuclear membrane, but finally wanders back to the center and becomes spherical. He concludes that in the angiosperms the sickle stage of the nucleolus is a normal phenomenon, as is also its excentric position. In male and female germ cells these metamorphoses occur at corresponding stages, namely, when the reduction of the chromatin takes place; "indessen bleiben vorläufig alle Speculationen über die Bedeutung des Sichelstadiums von problematischen Werth. . . ."

Mottier ('97), cells of *Podophyllum* and *Lilium*: in mitosis, at the time of disappearance of the nuclear membrane, the nucleolus breaks into fragments of various size. "Bei der Anlage der vielpoligen Spindel nun treten im Cytoplasma

kleinere, dem Nucleolus ähnlich tingirte Körper auf. . . . Es unterliegt keinem Zweifel, dass dieses die zerfallene Kernkörperchensubstanz darstellt . . . nachdem der Tochterkern mit einer Wandung versehen wurde und in ihm Kernkörperchen zum Vorschein kamen, sind oft noch extranucleäre Nucleolen in dem Cytoplasma zu sehen. Dasselbe gilt für die zweite Theilung. Ob die in dem Tochterkern zum Vorschein kommenden Kernkörperchen aus den im Cytoplasma liegenden Körperchen entstehen, lässt sich nicht feststellen. Hingegen wäre hier hervorzuheben, dass die im Kern wieder entstehenden Kernkörperchen stets in Contact mit den Kernfäden sich befinden . . . meine Ansicht geht aber dahin, dass in den Kernkörperchen ein Kraftvorrath gegeben ist, welcher der Zelle nach Bedarf zur Verfügung steht."

Pennington ('97), cells of *Spirogyra* treated with .1478% palladium chloride: "The nucleolus showed a dark bounding layer of double contour. . . . The dark layer is undoubtedly a true membrane dividing the nucleolus from the nucleus."

Strasburger ('97) reiterates his view ('95) that in plant mitoses the achromatic spindle is formed from nucleolar substance, and that also the "Zellplatte" and "Centralspindelkörperchen" of animal cells must be of nucleolar origin.

Swingle ('97), algae (*Sphaclariaceae*): the vacuolization of the nucleoli occurs simultaneously with the separation of the two centrosomes, and probably at the same time that the differentiation of the chromosomes occurs. Though "die schnelle und vollständige Auflösung der übrigen Substanz des stark vacuolisirten Kernkörperchens findet statt, wenn die Spindelfasern an den Polen einzutreten beginnen," there yet seems to be no direct proof that these fibers have their origin in nucleolar substance. "Könnte er [Nucleolus] nicht eher einen speciellen Vorrath organischer Nahrung zur Erhaltung des Kinoplasmas während der Karyokinese vorstellen?"

### C. SYNONYMS OF THE TERM NUCLEOLUS.

Since there are quite a large number of synonyms of the nucleolus, they may for convenience' sake be classified together

at this place. Certain of the following terms, however, apply not to the true nucleoli but to the *Caryosomata*.

*German writers.*—Nucleolus (Valentin) Keimfleck, Keimkern, macula germinativa (Wagner); Kernkörper (chen) (Schwann, Valentin); Keimkörper (chen); Wagner'scher Fleck; Binnenkörper (Rhumbler); Hauptnucleolus, Nebennucleolus (Flemming); Metanucleolus (Häcker); Plasmosoma (Ogata); Formationsnucleolus (Marshall); Kernfleck, Nucleolide, Morulit (Frenzel); Nucleolo-Centrosoma (Keuten); Mittelkörperchen, Eunucleolus (Rosen); Nucleolkörperchen (Lönningberg); Stammnucleolus, Nebenkügelchen (Auerbach); Hauptkeimfleck, Nebenkeimfleck (Leydig); Chromatin-Nucleolus, Paranucleolus (R. Hertwig).

*English and American writers.*—Wagnerian vesicle, entoblast (Agassiz); pronucleolus (Mark); nucleole, germinal spot, germinal dot, principal nucleolus, accessory nucleolus, protomacrosome (Greenwood).

*French writers.*—Nucléole, tache germinative; pseudonucléole (Van Beneden); tache de Wagner, nucléole plasmatique, n. mixte, n. nucléinien, nucléole-noyau (Carnoy); nucléole adventif (Roule); corps nucléolaire, nucléolite (A. Schneider); nucléole primitif et secondaire (Carnoy and Lebrun); corpuscule germinatif (Van Beneden).

*Italian writers.*—Macchia germinativa, macchia germinativa principale, m. g. laterale, m. g. accessoria.

*Synonyms of the nucleolus.*—Nucleololus, Nucleollolus (Frenzel); Schrön'scher Korn, Valentinian vesicle, entosthoblast (Agassiz); Centrosoma (Lavdowsky); nucleolo-nucleus, endonucleolus (Macfarlane); Nucleolinus, Keimpunkt, punctum germinativum (Haeckel).

### III. OBSERVATIONS.

#### A. METHODS OF STUDY.

The following observations have been made upon material collected, fixed, stained, and sectioned by myself, with the exception of the preparations of the ova of *Rodalia*, which were kindly loaned to me by Dr. E. G. Conklin. In no case were observations made upon the living tissue; however, but

little could be gained from a study of the living cells, in regard to the minute structures with which we are chiefly engaged. With only few exceptions (*Rodalia* and the two gregarines examined) no cells were studied which had not been preserved with at least three fixing reagents, and in some cases at least half a dozen different fixatives were used. The preserving reagents employed were the following: saturated solutions of corrosive sublimate in distilled water (this being the only fluid used hot), sat. sol. of the same in 50% or 35% alcohol, Flemming's stronger fluid (chromo-aceto-osmic acid), Hermann's fluid (platinum chloride, acetic acid, osmic acid), sat. sol. of picric acid in 50% alcohol, Perenyi's fluid (chromo-nitric acid), 2% aqueous sol. of chromic acid, absolute alcohol, picro-nitro-osmic acid. Those reagents which gave the best general results were the fluids of Flemming and Hermann, and the alcoholic solution of corrosive sublimate; though the particular reagent demanded depends both upon the object of study, as well as upon the method of staining which is to follow. It is hardly necessary to state that a structure found after the use of a given fluid, but not apparent on material treated in a different manner, was either regarded as an artefact, or doubts were expressed as to its naturalness; that is, only when a structure was found to present itself to the eye in more or less the same manner, after various methods of preservation had been employed, have I regarded it as a natural appearance and not as a result of the fixatives used. Thin serial sections were cut of objects imbedded in paraffin, in the usual way. All staining done was upon the sections on the slide, and the stains employed were as follows: Ehrlich's or Delafield's haematoxylin followed by eosin (sat. sol. in distilled water), nigrosine (a sat. sol. in water diluted by six vols. water), sat. sol. of acid fuchsine in 50% alcohol, the triple stain of Ehrlich-Biondi-Heidenhain (as prepared by Grüber, Leipzig), Flemming's triple stain (safranin, gentian violet, and orange G.), Lyons blue (sat. sol. in 50% alcohol), gentian violet (sat. aqueous sol.), methylen blue (sat. aq. sol.), brasilin (sat. sols. in water and in 35% alcohol), Mayer's acid carmine, cochineal (sat. sol. in 70% alcohol); while Grenacher's borax carmine and alum carmine, Heidenhain's iron haematoxy-

lin, indigo-borax carmine (Norris and Shakespere), and certain others were tried, but proved unsatisfactory. With the exception of the three triple stains mentioned, the others were used in various combinations as double stains; worthy of recommendation are (with especial regard to the differentiation of the nucleolus) Delafield's, or better, Ehrlich's haematoxylin followed by eosin; acid carmine followed by nigrosine; methylen blue followed by brasilin. Other combinations were also used, but it is not necessary to mention these here, nor to speak of the duration of the staining baths, since in the explanation of the figures these data are given for each case separately.

For the study of the finer structural details, the  $\frac{1}{12}$ th homogeneous immersion lens of Zeiss was used, in combination with oculars 2 and 4. I would emphasize the fact that the drawings from the preparations were made gradually, as I proceeded in the study of each particular cell, and were not postponed until the end of the particular investigation, so that almost all were made before I had arrived at any views upon the nature of the nucleolus; and I have pursued this method in order to eliminate from the figures as much as possible of the subjective element. In other words, I have made as close copies as possible of the preparations, drawing every cell or structure presenting some appearance with which I had not as yet become acquainted, or rather the significance of which I had not learned, and then from the figures so made I have endeavored to learn the nature of the phenomena there presented, at the same time recurring to the preparations themselves. This method of study is the one employed by many investigators, though it can scarcely be termed the one most in vogue. The colors of the original figures have on the whole been most excellently reproduced by the lithographs of Werner and Winter.

## B. PROTOZOA.

### 1. *Gregarine* from *Lineus gesserensis* (O. F. Müll).

(Plate 21, Figs. 1-19.)

(*Description of the animal.* — The largest individuals are just visible to the naked eye, and are of a whitish color. No synzigia were observed among the thirty individuals exam-



ined. Form : elongate, slightly larger at one end than the other, the thinner end sometimes flattened, slightly curved or sickle-shaped ; the greatest diameter is found in the region of the nucleus, which is situated nearer to the larger than to the smaller end ; both ends of the animal are rounded. In one individual (Fig. 2) the surface of the body was slightly furrowed in a spiral direction. Nucleus large, with a very thick membrane, and seldom oval, usually irregular in outline. In a single case (Fig. 1) two nuclei were present in one gregarine (the youngest individual seen), the two nuclei were of unequal size, though each contained a single nucleolus. Kölliker ('49) has described a gregarine with two nuclei ; I am unacquainted with any other cases. Sporocysts were not observed ; but in one case the cytoplasm was quite densely filled with minute spherical and oval bodies, which stained lightly with eosin, and in each occurred a small granule (this staining with haematoxylin) ; in the same individual a normal nucleus was also present (Fig. 4). These small bodies cannot be other than spores, even though they occur in the endoplasm of a gregarine in which a nucleus occurred at the same time ; this observation stands in no accord with what has thus far been described of the sporulation among gregarines, and I am thoroughly at a loss to explain the phenomenon. These gregarines occurred only in the posterior intestine of *Lincus*, but were not present in all the individuals of this nemertean sectioned. The absence of synzgia, the transverse furrows of the body, and the oval-shaped spores would relegate this form to the neighborhood of the genus *Gonospora* of Schneider.)

In the smallest nuclei found (the size of the nucleus stands in some degree in proportion to that of the animal) only one nucleolus was present (Figs. 3 and 5) ; in all the larger nuclei their number varied from two to four, though since four nucleoli were found in only two cases, two or three nucleoli may be regarded as the usual number in the larger individuals. As an inspection of Figs. 3-19 shows, the comparative size of the nucleoli within the same nucleus is very variable, and the nucleoli of one nucleus are always of unequal size. When only two nucleoli occur, one is about one-half or three-quarters the

size of the other; but when three nucleoli are present, either (1) one is particularly large, and the other two small; or (2) two are large, and the third is much smaller than either; or (3) all three are large, the smallest being about one-half the size of the largest. In the two cases of nuclei with four nucleoli apiece, in the one there were two larger and two smaller nucleoli, in the other one large and three small ones.

The nucleoli vary from a spherical to an oval shape. In the smallest usually no vacuoles (*n. Vac.*) are to be seen, but such vacuoles are always to be found in the larger nucleoli. In the largest there is usually a large excentric vacuole, while small ones may or may not be present in other portions of the nucleolus. In nucleoli of medium size it is most usual to find a number of small vacuoles. These vacuoles have already been noticed in numerous other gregarines, but I would call especial attention to a remarkable polarity of the nucleolus with regard to their position. In all those nucleoli in which vacuoles occurred, with the exception of not more than five or six, the single large vacuole, or the group of smaller ones, was situated at that pole of the nucleolus nearest the nuclear membrane (Figs. 7-9, 16, 17-19). There are almost no exceptions to this phenomenon in the smaller nucleoli, those, namely, in which only a single small vacuole or a few small ones are present. Accordingly, it would seem to be the rule that the vacuoles first appear in that portion of the nucleolus which approaches nearest to the nuclear membrane. The number and size of these vacuoles increase with the size of the nucleolus; or, as is more usually the case, as the nucleolus increases in size they gradually fuse together to form a single large vacuole, which may occupy the greater part of the nucleolus (Fig. 15). Thus the vacuoles first arise at one point in the nucleolus, so that here one can speak of a polarity of the nucleolus; but as the vacuoles increase in number and commence to fuse together the fluid substance of them begins to diffuse more widely throughout the nucleolus, so that evidences of this primitive polarity gradually become obliterated.

The ground substance of the nucleoli is very finely granular, and stains deeply red with eosin, and brownish red with the

Ehrlich-Biondi stain. The vacuoles are filled with a structureless fluid, which stains but lightly. But in four nuclei, the sections of which were stained in aqueous solution of methylen blue followed by brasilin, a differential stain of the ground substance was acquired: that pole of the nucleolus which contained vacuoles was stained a bluish green (methylen blue), the opposite pole, where no vacuoles could be seen, being of a light pinkish color (brasilin), the vacuoles themselves appearing as clear unstained spaces (Figs. 17-19). In one nucleus, in which two minute nucleoli were present, the one without, the other with, a single small vacuole, both nucleoli stained a bluish green throughout (Fig. 18). Further, in an unstained nucleus fixed with Flemming's fluid a somewhat similar differentiation was visible in the two larger nucleoli (neither of which contained vacuoles), the pole of each nucleolus nearest the nuclear membrane being of a deeper color than the opposite pole (Fig. 11). This differentiation produced by staining would show that the ground substance of the smallest nucleoli is homogeneous, but that in the larger ones a chemical change takes place in it, whereby that portion of the substance opposite the pole where the vacuoles first appear differentiates itself chemically from that portion of the ground substance lying at the latter pole. Unfortunately I had too little material to carry further the study of this differentiation.

In the nucleus is a faintly staining nuclear sap, in which irregular granules of various size are massed together especially near the center of the nucleus; they do not come into contact with the nucleoli, usually leaving a clear space around each of the nucleoli (Figs. 7, 8, 11, 14, 17-19). These do not stain with haematoxylin or with methylen green, but stain red with eosin and brownish red with the Ehrlich-Biondi mixture, in their staining differing little from the substance of the nucleoli. With the methylen-blue-brasilin stain mentioned above they stain pink, a little more deeply than does the inner pole of each of the larger nucleoli (Figs. 17-19). Whether they represent physiologically chromatin, or whether they are masses of (perhaps nutritive) substance taken into the nucleus from the cytoplasm, which might be chemically and genetically

akin to part of the substance of the nucleoli, I am unable to decide. I am also unable to determine from the preparations at hand whether the nucleoli themselves are partially composed of chromatin; but the usual diagnostic stains for chromatin do not show the presence of this substance within the nucleus.<sup>1</sup>

To revert again to the polarity of the nucleoli. The fact that the vacuoles first arise in that portion of the nucleolus nearest the nuclear membrane would seem to prove that the substance of these vacuoles is extranuclear in origin, or else is secreted in the peripheral portion of the nucleus. But since it would be obscure how the peripheral portion of the nucleus should secrete a substance, and the central portion should not, I incline to the former explanation, namely, that the substance of the vacuoles is first produced in the cytoplasm, and then this substance penetrating through the nuclear membrane, it, or a part of it, arrives at that pole of the nucleolus nearest the nuclear membrane, and then is taken into the nucleolus at this pole. The size of the vacuoles stands in a more or less direct ratio to the size of the nucleolus itself; at the same time the ground substance of the nucleolus also increases in amount, though apparently not as rapidly as the amount of the vacuolar fluid.

2. *Gregarine from Carinella annulata.*

(Plate 21, Figs. 20-35.)

(*Description of the animal.*—Monocystid gregarines occurring in the body cavity of this nemertean. No synzigia observed. Form: elongate, though not attenuate, the end in which the nucleus lies being broader and terminally more obtuse than the opposite end (Figs. 20 and 21). The longitudinal axis is never perfectly straight, and the cuticula shows no transverse furrows. The single nucleus is usually spherical or oval, rarely lobular in outline. In the entosarc of many individuals occur numerous minute, refractive granules. Neither cysts nor spores having been observed, I was unable to determine the genus of

<sup>1</sup> However, the chromatin here might exist in the state in which it is found in the growth period of ovocytes, namely, commingled with plastin.

this form. Only two individuals of *Carinella* were examined (both from Bergen, Norway); in the one all the gregarines were large, in the other of a smaller size.)

The nucleoli are nearly always more numerous than in the preceding species of gregarine, the number varying from four to about twenty-six, in those stages found (Figs. 22-35). In the larger nuclei they are usually more numerous than in the smaller ones, but exceptions to this rule are quite frequent. In the same nucleus some are nearly or quite spherical, others very irregularly lobular in outline. Their size within a given nucleus is also very variable, though as a rule they are unequal in their dimensions. In the larger nuclei the nucleoli are larger (or at least some of them are) than in the smaller nuclei. In a given nucleus there may be either (1) from two to four larger nucleoli and a number of smaller ones; or (2) a single large nucleolus and several much smaller ones. In the smaller nuclei the nucleoli are more equal in size than in the larger ones. The largest nucleoli in a nucleus are as a rule of oval or spherical form, with regular contour (an exception is seen in Fig. 26); the irregularly lobular nucleoli (Figs. 23, 25, 27, 28, 33) are usually of medium or small size. There is no apparent regularity with regard to their distribution in the nucleus. None of the nucleoli appear to have limiting membranes.

All these gregarines were fixed with alcoholic solution of corrosive sublimate. With the double stain, haematoxylin and eosin, the larger nucleoli were stained with a deep blackish red, the smaller ones either of the same color or a clearer red; all became stained so intensely by this method that the vacuoles in them were greatly obscured (Figs. 27 and 28).

The Ehrlich-Biondi method produces a yellowish brown or reddish stain of the nucleoli, differences of stain being observable in the different nucleoli of the same nucleus (Figs. 26, 31-35). This staining method brings out very clearly the vacuoles in the homogeneous (?) ground substance of the nucleolus; the structureless substance of these vacuoles stains less intensely than the enveloping substance. Vacuoles are absent in the smallest nucleoli, as well as in those of irregular form; in the larger ones they are almost invariably

present, though variable in size and number. They do not regularly arise at one particular part of the nucleolus, as we found to be the case in the preceding species. Further, there is rarely in this species a single large excentric vacuole ; but as the figures show, usually a number are present, either arranged in a circular row near the periphery, or in a row around a larger central vacuole, or grouped together at one point in the nucleolus. There can be no doubt that the larger vacuoles are produced by the fusion of smaller ones, since two or three smaller ones are frequently found in close contact with each other.

The double stain, haematoxylin and alum carmine, gives different results from the preceding stains, in that by it not only the different nucleoli within a nucleus become colored differently, but also in some cases different stains of the different portions of the same nucleolus are attained (Figs. 22-25). It is only the larger nucleoli, those with regular contours, which become differentially stained in this manner. In such a large nucleolus a portion of its substance stains a deep blue (haematoxylin), another portion or portions purplish or reddish (alum carmine) ; the part stained blue is usually central in position, and encircling it is a zone of red-stained substance. In one case (Fig. 22) the two opposite poles of the nucleolus were reddish, the intermediate part being a deep blue. The medium-sized, irregular nucleoli always stain blue throughout, the smaller ones usually red, but sometimes blue. This stain, accordingly, shows that in this gregarine some of the larger nucleoli are composed of two different substances similarly as we had found two substances in the preceding species, though there by using the methylen-blue-brasilin stain.

With all three staining methods employed, a mass of irregular granules is present in each nucleus, which stain less intensely than the nucleoli. In the smallest nuclei (Figs. 22-25) these granules are more or less regularly distributed through the nucleus, but in the larger ones (Figs. 28, 31-35) they compose a dense mass around the nucleoli or around the largest nucleolus, while the peripheral portion of the nucleus remains nearly free of them. Delicate, faintly stained fibers transverse

this peripheral part of the nucleus, which may be radially disposed or else form a loose network. The size of the granules, their abundance and staining intensity vary in different nuclei of the same size, and there is no sharp distinction between the smallest nucleoli and the largest of these granules. In this species, as in the preceding, I was unable to detect any substance which stained like chromatin.

I have been unable to determine the origin and ultimate fate of these nucleoli, owing to lack of material ; but a few justifiable conclusions may be drawn from the facts at hand. Thus the number and size of the nucleoli stand, as a rule, in a direct ratio to the size of the nucleus. Further, those irregularly lobular nucleoli described above probably represent amoeboid changes of the nucleolus, such as have been seen in life by previous investigators, though it is strange that these nucleoli differ from all others in consisting of a single substance and in containing no vacuoles. Lastly, the number and size of the vacuoles increase, as a rule, with the size of the nucleus.

It is worthy of mention that usually there are a larger number of very small nucleoli in the larger nuclei than there are in the smaller nuclei, although the largest nucleoli of the former are much larger than the largest nucleoli of the latter nuclei. We must conclude, then, that though the size of the nucleoli increases as a rule with that of the nucleus, new nucleoli are also being formed as the nucleus grows larger. Now some of these new small nucleoli found in the largest nuclei have undoubtedly been produced by division from some of the larger ones : thus I have frequently observed irregular (amoeboid) nucleoli with oval prolongations, or with small nucleoli closely apposed to their surfaces, and it probably is correct to conclude that such small nucleoli are in process of division from the larger ones (Figs. 23, 25, 27, 28, 33). Whether all the small nucleoli of the larger nuclei have had such a formation is difficult to determine, since in some of the largest nuclei most of the smallest nucleoli may be peripheral in position, close to the nuclear membrane, and far removed from the larger nucleoli, so that it might seem that the substance of these was extranuclear in origin. The

mass of irregular granules within the nucleus appears to stand in some relation to the growth of the nucleoli, at least there is a relatively greater amount of this substance in the larger nuclei; it envelops the largest nucleoli and imbibes the same stains, though more faintly, with which the nucleoli become stained. Now as the gregarine grows, at the same time both nucleus and the total mass of nucleolar substance increase in size; but the nucleus cannot grow without the addition of a substance or substances to it, which have been derived from without. Accordingly, I suppose that the substance of these granules has an extranuclear origin, a substance, *i.e.*, which, having penetrated the nucleus from the cytoplasm, undergoes a chemical change in the nucleus and there becomes precipitated in the form of granules, for no such substance occurs in granular form in the cytoplasm. The growth of the nucleoli might then be explained on the assumption of the intussusception of this substance by the nucleoli. This explanation is offered merely as a hypothesis, since I cannot prove its correctness with the limited material at my disposal. Since no chromatin was demonstrable in these nuclei, it remains for future workers to show whether the chromatin is in these stages commingled with the nucleolar substance, or whether it is represented by one of the two substances of which some of the nucleoli are composed; and if so, whether all, or whether only a certain number, of the nucleoli are thus partially constituted of chromatin.<sup>1</sup>

### C. METAZOA.

#### a. Egg Cells.

##### 1. *Montagua pilata* (Verr.).

(Plate 22, Figs. 57-63, 65-87.)

In the germinal vesicles of this mollusc two kinds of nucleolar structures occur: the true nucleolus, which is of large size and almost invariably single; and certain secondary structures,

<sup>1</sup> For observations of other authors on nucleoli in *Gregarinida*, *cf.* the reviews of Minchin ('93), Van Beneden ('69), Marshall ('92), Frenzel ('93), Koelliker ('49), A. Schneider ('75, '83), Wolters ('91), Carnoy ('84).



which appear at only a certain stage of the cell. The true nucleolus may be considered first, then these other structures, or "pseudonucleoli."

There is always one true nucleolus to each nucleus, and in only two cases out of hundreds of ova examined have I seen two nucleoli (Figs. 57 and 61). The position of the nucleolus within the nucleus is in most cases excentric, seldom central, and never apposed to the nuclear membrane; it apparently lies free in the caryolymph, and is not supported by the chromatin threads. In the youngest, most immature germinal vesicles (I have not studied it in the ovogonia) it is apparently wholly homogeneous, dense, not noticeably refractive, and usually spherical (Figs. 57-61); sometimes, however, it shows an oval or more elongate form, and in the latter case its long axis usually coincides with that of the nucleus (Fig. 58); it is never irregular in outline.

The nucleolus always colors differently from the chromatin, when treated with double stains, as follows:

STAIN.	NUCLEOLUS.	CHROMATIN.
Ehrlich-Biondi . . .	maroon . . .	green.
Haematoxylin, eosin . . .	orange red . . .	blue.
Acid carmine, nigrosine . . .	blue . . .	red.
Haematoxylin, fuchsine . . .	purple . . .	blue.
Flemming's stain . . .	yellow . . .	violet.

With the increase in size of the nucleus the nucleolus enlarges, and in such a way that the size of the latter usually preserves its proportion to that of the former; but as the figures show, this proportion is quite frequently not preserved. What may be termed the *first stage* of this nucleolar growth consists merely in an increase in the amount of the homogeneous substance, and between the largest homogeneous nucleoli (Fig. 65) and the smallest (Fig. 57) there is no difference except one of size.

The *second period* of nucleolar growth is introduced when vacuoles commence to appear in the substance of the nucleolus (Fig. 62). Since my observations show that these nucleolar vacuoles are derived from small fluid globules which first appear

in the nuclear sap, these globules may best be treated first. In the nuclear sap, at a certain stage in the growth period of the germinal vesicle, small globules of varying size occur; there are usually one or two of them in a given nucleus, but sometimes they are quite numerous (*Nut. Gl.* in Figs. 62, 63, 69-71, 73, 75, 81). When I first noticed these structures I conjectured that they might represent centrosomes such as have been found within nuclei at stages previous to mitosis (by Brauer in the spermatocytes of *Ascaris*); but further investigation shows that they have no kind of relation to centrosomes, since they vary in number and size, and further they readily imbibe stains, which centrosomes do not. They have a close resemblance to the smallest yolk granules found in the cytoplasm in point of form, size, and manner of staining. However, sometimes one or two of these bodies may be found in the nucleus when there is no evidence of yolk in the cytoplasm. Accordingly, they would seem to consist of a substance very similar to the young yolk at the time of its first formation. And since they may arise in the nucleus before yolk spherules appear in the cytoplasm they are probably not always taken up by the nucleus from the cytoplasm in the form of globules, but acquire this spherical form first in the nucleus. In other words, we may consider that the nucleus assimilates from the cytoplasm a thin fluid, similar to, if not identical with, that from which the yolk spherules themselves are ultimately formed, and that in the nucleus this substance becomes deposited in the form of globules, perhaps after having undergone a chemical change within the nucleus. Further, this substance must be regarded as having a nutritive value, on account of its similarity to the substance of the yolk, which certainly is nutritive in function. In the more mature, larger germinal vesicles (Fig. 78) large yolk globules are usually found, and are wholly similar to those in the cytoplasm in these stages; as can be easily determined, their position within the nucleus is not due to removal by the knife in sectioning, so that as the nucleus becomes larger it regularly takes up large yolk globules from the cytoplasm, and from these probably derives the greater part of the nourishment necessary for its rapid growth. We

may conclude, then, that when the nucleus is comparatively small, and when no yolk or only small yolk globules are present in the cytoplasm, the nucleus derives a nutritive substance from the cytoplasm, which is closely similar to that composing the youngest yolk globules ; but when the nucleus has grown large, and the cytoplasm is packed with large yolk globules, it has the power to take up these larger globules also.<sup>1</sup>

To return, then, to the second stage of nucleolar differentiation. This stage does not commence when the nucleolus has attained a certain size, but may commence in some nucleoli earlier than in others; and again it is not marked by a particular stage of development of the yolk in the cytoplasm. The fluid vacuoles probably stand in a genetic relation to the small nutritive globules found in the nucleus, which have been just described. That is, these globules of the nucleus penetrate into the nucleolus and then constitute the fluid vacuoles of the latter structure. I have reached this conclusion after observing that the vacuoles of the nucleolus and the small nutritive globules within the nucleus always stain in exactly the same way. This assumption is further strengthened by the fact that, when the nutritive globules lie in the nuclear sap at some distance from the nucleolus, they have invariably a spherical form ; but in those numerous cases where they may be seen apposed to the outer surface of the nucleolus they become flattened against the surface of the latter, as if the nucleolus were (figuratively speaking) a loadstone which attracts them to itself (Figs. 63, 69, 75). If this origin of the vacuoles of the nucleolus were not the true one it would be difficult to explain their mode of genesis, since there appears to be no other substance within the nucleolus from which they could be derived, and there is no reason for supposing that the

<sup>1</sup>The intensity in the staining of the yolk globules increases with their size, and the largest stain much more deeply than does the nucleolus. During all the earlier growth stages the nuclear membrane is retained, and it is seldom, and then only slightly, irregular in outline ; therefore the yolk cannot be taken up by the mechanical aid of amoeboid processes of the nucleus, but its substance must osmotically penetrate the nuclear membrane. And as I mentioned above, it does not seem probable that the yolk globules retain their shape while penetrating this membrane, but diffuse through it in the form of an irregular fluid mass, and then in the nucleus this fluid becomes re-formed into globules.

substance of these vacuoles is a differentiation of the nucleolar ground substance. We may assume, then, that this explanation of the genesis of the nucleolar vacuoles is the correct one, and now proceed to explain the changes in the nucleolus during the successive development of its vacuoles. If we take the size of the nucleolus as a general criterion (though it is not an infallible one, since there are considerable individual differences in different nucleoli (*cf.* Figs. 62, 65, 80)) of the stage of the nucleolus, the process of assimilation of the nutritive globules from the nucleus by the nucleolus seems to be in general as follows: first, one or two globules are taken into the nucleolus, and later when others (apparently a varying number) are also taken up into it, we reach a stage when the nucleolus contains a number of fluid vacuoles (the assimilated nutritive globules) (Figs. 64 and 70). Then these vacuoles commence to fuse together (Figs. 63, 66, 72), finally by their fusion giving rise to one large vacuole, which fills about three-quarters of the space of the nucleolus, and always lies excentrically within the nucleolus (Figs. 68, 69, 73, 77, 79). The nucleolus has now attained its greatest dimension and is either perfectly spherical, or more usually ovoid in shape. Its large excentric vacuole is encircled by a peripheral layer of the primitive homogeneous ground substance of the nucleolus, which has undergone no structural or chemical change. This layer of ground substance becomes necessarily thinner as the vacuole becomes larger, *i.e.*, as the pressure from within becomes greater. But since the large vacuole lies peripherally, the peripheral substance of the nucleolus remains thickened at that point opposite the vacuole, and this thickened portion of the nucleolar wall has most frequently the form of a concavo-convex lens (or on a cross-section, of a half moon), the concave side of which borders upon the vacuole. This thickened part, as the remaining portion of the peripheral layer of the nucleolus at this stage, is in every respect identical with the ground substance of the nucleolus in earlier stages, before vacuoles had made their appearance in it; and the total amount of the substance of the peripheral layer seems to be equal to the amount of the homogeneous substance of the nucleolus at the end of the preceding

stage. Accordingly, in this second period of the nucleolar growth there appears to be no increase in the amount of the true nucleolar substance, but merely an increase in the amount of the vacuolar substance. The thickened portion of the peripheral layer of the nucleolus is at first biconvex, but as the large vacuole grows larger the pressure of the latter causes it to gradually assume a concavo-convex form (Figs. 84-86). Thus the shape of the large vacuole is at first concavo-convex, and later spherical or oval. This thickened portion of the outer layer of the nucleolus is usually homogeneous in structure, as is the remainder of the true nucleolar substance which envelops the vacuole ; but sometimes small vacuoles may occur within it also (Fig. 71).

Two poles may be distinguished in the nucleolus at this second stage of its differentiation : (1) the pole at which the large vacuole lies ; and (2) the pole at which the thickened mass of the true peripheral substance is situated. From the study of a large number of nuclei at this period I find that in about 75% of them the second of these poles is directed towards the nuclear membrane, the first pole towards the center of the nucleus ; at this stage, as in the preceding, the nucleolus lies usually excentrically within the nucleus.

The later differentiation of the nucleolus consists, accordingly, in the accumulation in it of fluid vacuoles (their substance identical with that of the nutritive globules of the nucleus), but the true nucleolar substance undergoes no change whatever, as far as can be determined from differential staining. There is no chemical union of the vacuolar with the true nucleolar substance, but the fluid vacuoles simply push aside this substance, so that, after these numerous smaller vacuoles have united to form a single large vacuole, the true nucleolar substance remains unchanged as a peripheral layer around this vacuole. The substance of the vacuoles becomes colored with the same stains, though always more lightly, as does the true nucleolar substance, so that we find in this stage a more deeply staining envelope of substance around a less deeply stained portion. This difference of staining between these two parts of the nucleolus is best shown by employing haematoxylin

and eosin (Figs. 68 and 69). With the Ehrlich-Biondi method this difference is not quite so clearly demonstrable. The latter stain is peculiar and differs from all other stains used by me for these cells, in that it very often gives to the smaller vacuoles of the nucleolus the appearance of black, refractive granules ; but a careful focusing of these supposed granules shows them without doubt to be vacuoles, their apparent solidarity being probably due to the refraction of light by the enveloping nucleolar substance.

The chief result derived from the foregoing observations is that the nucleolus takes up some or all of those nutritive globules which lie in the caryolymph, and whose substance had been probably derived from the cytoplasm. Some of these globules then become collected within the nucleolus, representing its fluid vacuoles ; and these globules, increasing in number at the same time, gradually fuse together and thus give rise to a single large excentric vacuole, which is enveloped by the unchanged true nucleolar substance. Since the substance of these small globules is probably nutritive in function, the nucleolus in thus collecting some or all of them would appear to act as a reservoir for nutritive substance, or as a reservoir for that portion of the nutritive substance accumulated in the nucleus, for which the nucleus may have no use. Of course it is not *a priori* impossible that these globules may represent waste products of a nutritive substance, so that the nucleolus might here fulfill the office of an excretory organ. But the function of these nucleoli can only be decided when the behavior of the nucleolus during the pole-body mitosis is known ; I had no ova showing pole-spindle formations.

Finally, the true nucleolus appears not to be bounded by a special membrane ; after staining with acid carmine and nigrosine the nuclear substance appears bluish green and a red membrane seems to envelop it (Fig. 80), but this appearance is probably due to the refraction of light, since nothing of the kind can be found after the use of other staining methods.

We now come to speak of what I have called the "pseudo-nucleoli," but merely in order to distinguish them from the true nucleolus, and without wishing to express by the use of

this term any particular significance of these bodies. In eight individuals of *Montagua* which were sectioned, and which were of slightly different sizes, though the various growth stages of the ova were more or less the same in all, in only four were pseudonucleoli to be seen, and in only one of these four were they quite abundant, occurring in about 30% of the larger germinal vesicles. There are never more than from one to three in a nucleus. They are usually irregularly spherical and sometimes even angular in form (*Ps. n.* in Figs. 72-77, 79). The largest attained about three-quarters the size of the true nucleolus (of the same nucleus), though this size was attained by few, since they are, as a rule, but little larger than the nutritive globules which are observed in the caryolymph. Each pseudonucleolus consists of a denser, more deeply staining layer surrounding a less dense, more faintly staining core. The denser outer layer is homogeneous, somewhat refractive, and stains in the same manner as the ground substance of the true nucleolus. In smaller pseudonucleoli this outer portion appears on cross-section as a deeply staining ring, with regular outlines, but in the larger ones small, irregular prominences may often be seen on its inner surface. The peripheral layer or ring, further, shows a double contour, but I am unable to determine whether it is bounded by an outer membrane. It increases slightly in thickness with the growth of the pseudonucleolus, and in one case (Fig. 77) it was noticeably thickened at one pole, which gave to it somewhat the appearance of the *tout ensemble* of a true nucleolus. This peripheral layer surrounds a homogeneous, non-refractive, probably fluid mass, which either stains not at all or else only faintly; when it stains, it is either in the manner of the caryolymph or of the vacuoles of the true nucleolus. I have never noticed that the nutritive globules of the nuclear sap were apposed to these pseudonucleoli. What their origin is, and what their relation to the true nucleolus, I do not know. They are never found in contact with a true nucleolus and so are probably not buds from one. It is curious that they were frequent in the ova of only one mollusc, and in the same stages of the eggs of three other individuals were present in only a few cells, and in four other individuals were

present in none of the ova, though here the same stages of the ova were present as in the first individual. When they occur it is only in the larger germinal vesicles. They are apparently structures *sui generis*, and I have only the suggestion to offer, that they might be characteristic of a particular generation of egg cells, as their absence in the ova of some of the individuals of the mollusc would render probable (compare the observations of Häcker, '93a, where nucleolar differences were found in the ova of primiparous and multiparous individuals of *Cyclops strenuus*).

In Fig. 70 is a remarkable case depicted, namely, two small nuclei lying within a larger germinal vesicle, the former having apparently been assimilated by the latter.

### 2. *Doto*.

(Plate 22, Figs. 64, 68, 69.)

The nucleolar differentiation of these ova is essentially as in *Montagua*, so that no detailed description of the process need be given here. But in the five individuals of *Doto* which were sectioned, no traces of pseudonucleoli were seen, and the nutritive globules within the nuclear sap are usually smaller and much more numerous than in *Montagua*. The yolk globules also have different shapes in these two genera.<sup>1</sup>

### 3. *Amphiporus glutinosus* (Verr.)

(Plate 24, Figs. 140-158.)

(For descriptions of the connective-tissue elements of the nemerteans, from which the genital products are derived, *cf.* my previous paper '96.)

In the nuclei of the connective elements, by a differentiation of which the ova are produced (without any intervening

<sup>1</sup> For the observations of other authors on molluscan germinal vesicles, *cf.* the reviews of the papers of Wagner ('35, '39), Flemming ('74), O. Hertwig ('78b), Lönnberg ('92), Balbiani ('65b), Platner ('86), Leydig ('55a, '50), Stauffacher ('93, '97), Stepanoff ('65), Lovén ('49), Mark ('81), List ('96), Blochmann ('82), Trinchese ('80), Heuscher ('93), Hubrecht ('81), Carnoy ('84, '85), Wirén ('92), Fol ('89), Lacaze-Duthiers ('57), Quatrefages ('49).



mitosis), I could find no nucleoli ; but one or two small minute nucleoli might nevertheless be present within these nuclei, but escape detection, owing to their small size and to the comparatively great amount of chromatin. These nuclei are usually elongated and irregular in form (Figs. 144 and 145, *C. T. N.*).

The smallest germinal vesicles, which are recognizable as such by slightly larger dimensions and more regular, spherical shape, show likewise no recognizable nucleoli.

In what may be termed the *first nucleolar stage*, the nuclei have grown still larger, and in them are to be seen from one to about twelve small nucleoli. These are all peripheral in position, being flattened against the inner surface of the nuclear membrane, which results in their not being spherical, but more or less flattened, lens-shaped, or hemispherical (Figs. 140 and 141).

*Second nucleolar stage.* — The peripheral nucleoli commence to wander towards the center of the nucleus, at the same time growing larger and increasing in number (Figs. 142–145, 152). This process goes on until a considerable number of quite large nucleoli are present, none of which are any longer in contact with the nuclear membrane. As a rule they are not evenly distributed throughout the nucleus, but groups of them occur at different points in the nucleus (Figs. 153, 146–150). This period of differentiation, then, consists in the grouping of most or all of the nucleoli at or near the center of the nucleus, accompanied by their increase in size. There is no ground for supposing that at this stage they fragment into smaller nucleoli ; but very frequently groups of two or three nucleoli may be seen in close contact with one another, and these would represent states of fusion rather than of division, since they are found to be flattened at the point of contact, and not attenuated. Thus the increase in the size of the nucleoli would be due, in part at least, to fusion of contiguous ones. While some of the nucleoli have left the periphery of the nucleus, others are at the same time forming there, which in their turn eventually reach the center, so that a continual process of formation of nucleoli, and wandering of those already formed towards the center, takes place at this stage.

*Third nucleolar stage.* — The nucleoli increase in number, but gradually become smaller and wander towards the periphery of the nucleus (Figs. 154 and 155), until they all lie close to the inner surface of the nuclear membrane. In this stage they attain their maximum staining intensity, as is well seen after the use of Heidenhain's iron haematoxylin, by which they become colored a greenish blue (Fig. 157), while in the previous stages they are brownish yellow, unstained by the haematoxylin.

*Fourth nucleolar stage.* — Vacuoles of varying size arise in the nucleoli, and become somewhat irregular (instead of spherical) in outline (Figs. 156 and 158). In numerous nuclei it may be noticed that all the nucleoli lie close to the nuclear membrane, except a single one, which is placed nearer the center and differs from the others in not staining with haematoxylin, though it usually contains vacuoles; it may be a nucleolus which has not developed as fast as the others have (Fig. 156).

All nucleoli in the third and fourth stages are very uniform in size, and smaller and much more numerous than in the second; since there are no facts which permit us to conclude that new nucleoli are being formed in the last two stages we must consider that in them a division of the nucleoli must take place, and this would explain their increase in number and concomitant decrease in size. The fourth stage would seem to be characterized by the commencement of a degeneration of the nucleoli, if the presence of vacuoles and the irregularity of form may be taken as a criterion of degeneration. Neither in this species nor in the other nemerteans examined have I seen stages showing the formation of the pole spindle, so that I cannot describe the ultimate fate of the nucleoli. But the observations of those who have studied these divisions seem to show that they all disappear before the pole spindles are produced; and accordingly the phenomena characteristic of our fourth nucleolar period might represent the commencement of these degenerative processes.

The method of formation of the yolk may next be considered, since the yolk stands in a certain relation to the genesis of the

nucleoli. The cytoplasm, when the yolk first arises in it, stains with haematoxylin (with the double stain of this and eosin); this blue stain of the cytoplasm I have noticed to be characteristic for the cytoplasm of many immature ova, while the cytoplasm of somatic cells usually stains with eosin. The yolk first appears in the form of large yolk balls (Figs. 144 and 145, *Yk. Bl.*), as they may be termed; the number of these balls varies in cells of the same size, as well as in those of different dimensions, and they appear to be produced successively in a cell, until at the end of the third nucleolar stage they all have disappeared, having given place to the mature yolk spherules. They arise in the cytoplasm at no fixed point, though usually at some distance from the nucleus; it is hardly necessary to state that they stand in no genetic relation to the nucleus, either in this or in the other nemerteans studied. The yolk balls are at first dense and homogeneous, and stain intensely with eosin; the size that they may attain while still homogeneous is very variable. Subsequently they become vacuolated, even sometimes granular, and different portions of the same ball may stain differently, which shows that both a chemical and a physical change takes place in their substance. Finally, they fragment into unequal sized granules, which stain less deeply, and then these latter split up further, until the ultimate yolk spherules (*Yk. Gl.*) are produced. In the largest ovarial eggs all the yolk balls have disappeared (they linger longest at the periphery of the cell), the cytoplasm being densely filled with the yolk spherules. In some cases yolk balls lie in the cavity of the gonad (Fig. 155), and these are probably derived from degenerated ova.

The following facts show, I think, that the nucleoli stand in a genetic connection with the yolk substance. The nucleoli stain in the same way and have in other respects the same appearance as the smaller fragments of the yolk balls and as the mature yolk spherules (Figs. 144-146). Fragments of yolk balls occur frequently in close contact with the outer surface of the nuclear membrane. Now since the nucleoli first appear in contact with the inner surface of this membrane, the conclusion is plausible that the nucleoli represent portions of

a yolk substance, either of the yolk-ball fragments or a substance equivalent to that out of which the latter are differentiated, and this substance, then penetrating osmotically the nuclear membrane, becomes deposited or precipitated in the nucleus in the form of spherical globules, which are the nucleoli. From this yolk substance taken into the nucleus the chromatin, linin, and nuclear sap might derive the nourishment necessary for their growth, and those nucleoli which remain through the fourth nucleolar stage might represent either a reserve supply of this nourishment, or chemically changed portions of it, from which all nutritive substances have been extracted; the latter view would seem substantiated by the fact that the nucleoli stain somewhat differently in the third and fourth stages.

The nuclear membrane is present during all these stages. The nucleus is always regular in outline, usually oval, except during the third stage, when it may become slightly irregular, though it never becomes noticeably lobose or amoeboid.

In the first nucleolar stage (Figs. 140 and 141) the chromatin appears as a network of delicate fibers, which stain with haematoxylin. Towards the end of the second stage (Figs. 146-150) it assumes the form of irregular masses, and the fibers become less numerous. In the largest ovarial nuclei (Figs. 154 and 157) it is finely distributed throughout the nucleus in the form of minute microsomes; traces of fibers may be found only at the periphery of the nucleus, though I have not determined whether these are fibers now for the first time forming, as is the case in the other nemertean. The nucleoli are never suspended by the chromatin fibers.

This species is characterized by the formation of a membranous structure in the cytoplasm, during the second and third nucleolar stages, which is present in none of the other nemertean. This is a membrane within the cytoplasm, separated from the nucleus, as well as from the cell membrane by cytoplasm; it lies close to the nucleus (Figs. 146 and 155, *lv. Mb.*). It is thicker than the nuclear membrane, though not so dense, and differs in no wise structurally from the cytoplasm, except in its greater density, the cytoplasmic granules in it lying closer together (these granules appear to be

the nodal points of a "Wabenwerk" in the sense of Bütschli). This intracellular membrane is not open at any point, and a longitudinal section of it shows it to be not spherical but oval in outline, the apices of the oval being furthest removed from the nucleus. It is present only in the second stage of the nucleolus, and between it and the nucleus no yolk balls occur. I have never seen such a structure in any other egg cells except in the ova of *Gryllus abbreviatus*; a similar structure was found by van Bambeke ('83, eggs of *Leuciscus, Lota*), Schäfer ('80, egg of *Lepus*), and Gerould ('96, *Caudina* egg).

4. *Tetrastemma catenulatum* (Verr.) Montg.

(Plate 23, Figs. 103-133; Plate 24, Figs. 137-139.)

The formation of the yolk may be spoken of first, then the nucleoli proper, and afterwards certain large nuclear structures which may or may not represent nucleoli of another kind.

The yolk first appears in the form of one or two yolk balls (*Yk. Bl.*, Figs. 107, 108, 112, 114-116) in the cytoplasm; the larger ones are regularly oval as a rule, and the smaller ones spherical. A number of these yolk balls are produced successively in each cell, and by their fragmentation the ultimate yoke spherules (*Yk. Gl.*) are evolved. Each such ball is at first smaller than the nucleus of the cell in which it occurs, but gradually increases in size, though the maximum size which it may attain is not a fixed quantity, but is quite variable. As it increases in size it also gradually becomes more deeply stained, attaining its most intense staining when it has attained the limit of size. The substance of these balls is dense, finely granular, not brittle, somewhat refractive; in the youngest stages of their formation they often appear nearly homogeneous. About the time a ball has reached its maximum size it commences to change both structurally and chemically, vacuoles appear in it, it begins to stain less intensely, and becomes irregular in outline. Thus it becomes either coarsely granular, or else unstaining vacuoles appear scattered through it, and with eosin stains no longer a deep red, but a light red or even yellowish. Next it breaks into a number of pieces, whereby

the primitive yolk ball may break either into two fragments (which are usually unequal in dimensions), each of which then fragments further, or it breaks at once into a considerable number of larger granules. The final stage in this process of division shows the daughter yoke balls fragmenting to form the ultimate yolk spherules (Fig. 118); the latter stain an orange red with eosin, are homogeneous in appearance, and usually oval or spherical in form, seldom irregular. Two main stages may accordingly be distinguished in the formation of the yolk: (1) the formation of a large, regularly shaped yolk ball; and (2) the successive fragmentation of this ball, accompanied by a gradually lessening affinity for stains, resulting in the evolution of the mature, small yolk spherules, the cytoplasm of the ripe egg being thickly filled with the latter. It is usually the case that the yolk ball attains its greatest size at the end of the first stage. In cells of medium size all the various stages of yolk formation may be found, which shows that the yolk balls are being successively produced and are successively fragmenting; quite a number of these balls need to be produced in order to furnish the large quantity of yolk globules of the mature egg. The time when the yolk balls first appear, the size they reach, and the manner in which they segment, seem to vary much in individual cells.

I have not been able to determine the manner of the first differentiation of the yolk substance in the cytoplasm. Two possible explanations suggest themselves: (1) either a certain portion or constituent of the cytoplasm changes into yolk substance; or (2) the yolk balls may represent a nutritive substance accumulated in the cytoplasm, which may have been derived from the blood or from some neighboring tissue, if not directly from the posterior intestine. But it is without doubt that this substance is not of nuclear origin, for the yolk balls at their first appearance are not in contact with the nucleus, but usually at some distance from it; and also during the earlier stages of the yolk formation the nucleus is irregular in outline, with short, blunt processes, which would show that it is taking up substances from the cytoplasm, rather than excreting substances.

The cycle of the formation of the nucleoli may here also be divided into three stages, which do not quite correspond to the four of *Amphiporus gelatinosus*.

*First nucleolar stage.*— In the smallest germinal vesicles found one or two relatively very large nucleoli were present, one of them often in the center of the nucleus, the other more excentric or even against the nuclear membrane (Figs. 103, 114, 115). The nucleoli in these smallest nuclei are as large or nearly as large as in any of the following stages. In germinal vesicles of slightly greater dimensions three or four nucleoli may be present, and some of these may have increased a little in size; the amount of nucleolar substance at this stage is often so great as to occupy a fifth of the nucleus. They now increase in number, until at the close of this period we find a considerable number of mostly large nucleoli quite evenly distributed through the nucleus (Figs. 104–106, 109, 110, 116), but often they are at one of its poles more numerous than at other points. This stage would seem to correspond to the first and second of *Amphiporus glutinosus*.

*Second nucleolar stage.*— The nucleoli continue to increase in number but now decrease in size and commence to pass to the periphery of the nucleus, until at the end of this period they all lie close to the nuclear membrane, are regular in outline, and adequal in size (Figs. 107, 119, 122, 124–126, 130, 131). At the beginning of this stage numbers of nucleoli may be found arranged in chain-like rows, as is to be seen in Fig. 111. This would correspond to the third stage of *Amphiporus*.

*Third nucleolar stage.*— Nearly all the nucleoli are close to the nuclear membrane, often flattened against it (Figs. 117, 120, 127, 129, 137, 138). They show signs of degeneration; thus they stain less intensely, are irregular in outline, and have a vacuolar or granular structure. In the largest germinal vesicles their number has apparently decreased and small non-coherent masses of granules may be seen, which are probably degenerated nucleoli. Sometimes a nucleus may be found in this stage in which almost all of the nucleoli contain each one large, excentric, lightly stained globule or vacuole (Fig. 117).

*Staining of the nucleoli.*— The natural color would appear

to be a light yellow. In a preparation stained with haematoxylin and eosin, though not very thoroughly colored with the latter stain, the large nucleoli of the first nucleolar stage were of a light-yellow color, apparently stained only slightly with the eosin; those of the end of the second stage were mostly stained red, and those of the third stage were stained red, except those which had broken into granules, these latter being stained very little. In another preparation, in which the eosin had acted for one or two minutes longer than in the preceding preparation, the nucleoli in the first stage were stained orange, those of the second stage red, and those of the third stage very slightly or not at all stained. Accordingly, they stain more lightly at the commencement of the first and at the end of the third stage than during the second stage; these differences of stain are probably due to chemical differences in the nucleoli at different stages.

The chief differences between the nucleoli of this species and those of *Amphiporus glutinosus* are as follows: in the former there is no stage which exactly corresponds to the first stage of the latter, where we found a number of small peripheral nucleoli; in *T. catenulatum* there are at first one or two large nucleoli which are not always peripheral in position. The nucleoli in the third stage of *T. catenulatum* are more irregular in form and dimensions and stain less intensely than those of the fourth stage of *Amphiporus*. But the most important difference between the two species is to be found in the fact that in *T. catenulatum* new nucleoli continue to be produced even in the third stage. Thus there are at the periphery of the nucleus, between the larger degenerating nucleoli which had their origin during the first stage, also much smaller, newly formed nucleoli arising while the former are disappearing. Such younger nucleoli may be seen at the close of the third stage, when the nuclei are largest and chromatin filaments appear in them, arranged in contact with the chromatin threads or near to them (Figs. 127, 137, 138). These smallest nucleoli of the third stage always stain intensely red with eosin, while the much larger ones of the first and second stages stain more of an orange color with this stain. This difference of staining in these two kinds of nucleoli might be explained thus:



As we had concluded for the preceding species, so also in the present and in the species of nemerteans yet to be described, the nucleoli are in all probability accumulations within the nucleus of a substance taken up from the cytoplasm, this substance being related to that which constitutes the yolk balls. In the least mature germinal vesicles of *T. catenulatum* we found one or two very large, lightly staining nucleoli; these stain in the same way and show the same structure and degree of refraction as do the daughter yolk balls (Figs. 107 and 116). Further, I have noticed in the cytoplasm small yellowish spherules (yolk-ball fragments) which are in every way similar to the smaller nucleoli, and quite frequently I have observed one or two of them pressed so close against the outer surface of the nuclear membrane as to cause a depression of the latter (Figs. 112 and 118). In other words, it would seem that the substance of some of the yolk-ball fragments is taken into the nucleus and in the latter is re-formed into nucleoli. As long as yolk balls or their fragments are found within the cytoplasm lightly stained nucleoli of approximately the same dimensions as these may be seen in the nucleus. I have never seen a pore in the nuclear membrane through which a yolk-ball fragment could penetrate, though this membrane sometimes appears to be thinner at the point of contact with a yolk-ball fragment than at other points in its circumference. But in the third stage, when all yolk balls and their fragments have disappeared and the whole cytoplasm is thickly filled with their derivatives, the mature yolk spherules, large, faintly staining nucleoli, are no longer present in the nucleus, but the smallest nucleoli present at this time resemble in form, size, and stain, the yolk globules. Therefore we must conclude that the young, small nucleoli which first appear about the end of the third nucleolar stage represent mature yolk spherules, or at least that the substance of the two is equivalent. While the nucleoli of the first generation (formed in the first stage) are commencing to degenerate, new nucleoli of a second generation begin to arise in the nucleus, and the latter, which may serve as nourishment for the chromatin threads, differ from the former genetically, in that they are not assimilated portions of yolk-ball fragments, but

assimilated yolk spherules. Thus, as we find in the cytoplasm first yolk balls, then their fragments, and finally the mature yolk spherules, so in the nucleus the first generation of nucleoli are assimilated yolk balls and their fragments, while the small ones of the second generation are derived from the only yolk elements then present in the cytoplasm, namely, yolk spherules. The nucleoli of the first generation also differ from those of the second, at the time of the first appearance of both, in their manner of staining; so that they would seem to differ chemically from each other.

*Nuclear structures of problematical significance.*—In only one out of the three individuals of this worm studied were the following remarkable structures to be observed, though the fixation method of both of the other individuals was exactly the same. These bodies first appear in ova of the second nucleolar stage, but here show always the same typical structure, so that I can say nothing as to the manner of their first formation. In preparations stained with haematoxylin and eosin they are colored by the former stain a little more deeply than the nuclear chromosomes, so that they stand out sharply in the nuclear substance (*N. Bd.*, Figs. 122–139). The smaller ones, *i.e.*, those of the younger germinal vesicles (Figs. 122–126), are finely granular, though whether they each consist of a mass of fine granules or of homogeneous ground substance in which granules are distributed, I cannot determine. In the larger nuclei they often appeared wholly homogeneous (Fig. 132). In shape they are usually nearly spherical, with a sharp outline, which may or may not represent a limiting membrane; the larger ones are often more irregular in form (Figs. 132, 133, 139). In the smaller nuclei they are as a rule, but not always, smaller than in the larger ones; in the smallest nuclei in which I have found them there is only one of these bodies to a nucleus; while in the larger nuclei they are not only larger, but also there may be from one to four of them in each nucleus. In only one small nucleus were three of them present (Fig. 128). In two cases, both larger nuclei, I found division stages of these bodies: in the one case (Fig. 131) the body was ovoid in outline, with a shallow constriction at right angles to its

longitudinal axis, at about its middle; in the other case (Fig. 129) the body was plainly biscuit-shaped, with a well-marked medial constriction: these would probably represent respectively successive stages of division.

The various stages found would show the metamorphoses of these structures to be as follows: in the medium-sized nuclei, those in which they first appear, there is only one to a nucleus. This one increases in size up to a variable point, when it begins to divide, producing two daughter-bodies, which are not always of equal size. One or both of these bodies may now divide again, resulting in the formation of (respectively) three or four bodies. Since, however, the four bodies sometimes found in the larger nuclei are often quite unequal in size, we must assume: (1) either that the divisions have been very unequal, and each daughter-body had divided; or (2) that after the first division, which may or may not have resulted in unequal daughter-bodies, only one of the latter divides further, and it divides once, and one of its products divides once. It is to be noted that the number, the size, and the time of the division of these bodies stand in no regular relation to the size of the nucleus. Thus in one small nucleus (Fig. 128) three were already present, so that here two divisions must have taken place; while in some much larger nuclei (Figs. 130 and 133) a single, much larger one was present, which showed no signs of division. In the larger nuclei these bodies are often quite irregular in form; may this increasing irregularity portend an on-coming dissolution or other degeneration? They were found, as remarked above, in the ova of only one of the three individuals of this species examined, though in all three individuals the stages of egg development were very much alike; in the single individual in which they occurred they were not present in all the larger eggs. Their whole appearance and consistency show that they are not artefacts (the fixation was with hot aqueous corrosive sublimate), and they have no resemblance to any parasitic organisms, as *e.g.*, *Protozoa*, with which I am acquainted. Nor can they be centrosomes nor true nucleoli, and stand in no apparent relation to the nucleoli. In a single case I found two nucleoli enclosed by one of these bodies;

but in no other cases were these structures in contact with nucleoli. They are also never in contact with the nuclear membrane. Male pronuclei they cannot be, since the fecundation takes place in later stages than those which I have had opportunity to observe. I must conclude, though with reserve, that they are either parasitic *Protozoa*, or, more probably perhaps, structures which characterize ova of a certain generation. (Compare my remarks on the "pseudonucleoli" of *Montagua*. The structure figured by Henneguy ('93), in the immature germinal vesicles of *Sygnathus* may have some connection with these bodies.)

*Chromatin.*—The chromatin in the youngest germinal vesicles (Figs. 103–105, 112–114) is distributed throughout the nuclear sap in the form of minute microsomes. In the second and sometimes the first nucleolar stage such microsomes can often not be detected, but the whole nuclear substance, with the exception of the nucleoli, appears homogeneous and stains with eosin a yellowish red (Fig. 115). This peculiar coloration might be accounted for on the ground that in these stages there is a diffusion of nucleolar substance throughout the nucleus. Towards the conclusion of the second and the commencement of the third nucleolar stage, the minute chromatin microsomes again become evident (Figs. 118 and 130). At the end of the third stage a few chromatin threads begin to arise in the nucleus (Fig. 127), and these stain slightly with haematoxylin in the same manner as the microsomes do; they appear to arise separately and at different points in the nucleus, and are at first short, but gradually increase in length. As noted above, the small nucleoli of the second generation are often apposed to these threads, and sometimes lie in the meshes of them.

*Nucleus.*—In the first and second nucleolar stages the nucleus has often short, lobular processes, which may be amoeboid in life (Figs. 109, 112, 114, 116, 125); these changes in the form of the nucleus no doubt stand in a direct relation to the assimilation of yolk substance from the cytoplasm. Towards the end of the third stage the nucleus becomes regular in outline, with no traces of amoeboid processes; at this stage also

the nuclear membrane has attained its greatest thickness. The thinness of this membrane in previous stages would allow the penetration of nutritive substances into the nucleus from the cytoplasm. The small nuclei from which the germinal vesicles are directly derived, without any intervening mitoses, are irregular in shape, and no nucleoli are to be seen in them (Figs. 108 and 112, *C. T. N.*).

5. *Tetrastemma elegans* (Verr.).

(Plate 28, Figs. 282-299.)

Having only two mature individuals of this worm for study, I am unable to give as thorough a description of the nuclear metamorphoses of the egg as was possible for the other nemerteans; one preparation was fixed with Hermann's fluid, the other with aqueous solution of corrosive sublimate, but the latter had been too deeply stained (haematoxylin, eosin) to allow the study of certain details, as *e.g.*, the cytoplasmic changes leading to the formation of the yolk. Yolk balls were observed in only a few ova, and are much less numerous than in *T. catenulatum*; it is possible that the development of the yolk in the present species may be as in *Zygonemertes*, that is, the mature yolk spherules may as a rule be directly formed without the interpolation of a yolk-ball stage.

*First nucleolar stage.* — The youngest germinal vesicle, recognizable as such, showed a large nucleolus close to the nuclear membrane (Fig. 282); I have seen no smaller nuclei than this one, but would conclude by analogy from the facts in the other metanemerteans that also here all the nucleoli have an extra-nuclear origin. In slightly larger nuclei (Figs. 283-287) there are from one to three nucleoli, whose size varies considerably with regard to that of the nucleus, as well as to the size of one another. In such cases (Fig. 283) where only two nucleoli are present, one near the center of the nucleus, the other close to the nuclear membrane, the former is probably the older and has left the periphery for the center of the nucleus, while the other is younger and is still in process of formation. These first-formed nucleoli are usually rather large in proportion to

the size of the nucleus, seldom small. It is the rule that in one, sometimes in all the nucleoli, a large unstaining globule is present, which has the appearance of a vacuole (Figs. 284–287, 298); no nucleolus has more than one such globule. Quite often there is only a single large vacuole-containing nucleolus in a nucleus; or there may be from one to six nucleoli, only one of which contains a vacuole, and then the latter is usually the largest; or again, there may be two or three large nucleoli, nearly equal in point of size, each of which contains a vacuole (of course numerous intermediate stages may be found). There is certainly a successive production of nucleoli, but it is difficult to decide whether some of these after leaving the periphery of the nucleus fuse together, or whether some divide into smaller nucleoli. Now it seems probable that those nucleoli which are formed first are usually unequal in size, both in the same nucleus and in different nuclei, as a comparison of the figures shows. And though a gradual fusion of the nucleoli might play some part in the youngest germinal vesicles, nevertheless it would seem more probable that we have to do in these early stages with divisions of the nucleoli, especially since in the following stage they are much more numerous, as well as smaller. Fig. 287, in which three apposed nucleoli are to be seen, may thus represent a division of a single nucleolus. It is not unlikely that the unstaining globule within a nucleolus might aid, if it is not the direct mechanical cause of, such division. This first nucleolar stage is then characterized by the successive formation of a few comparatively large nucleoli at the periphery of the nucleus, and the migration of these towards the center; the presence of large vacuoles within some of the nucleoli is also a criterion of this period.

*Second nucleolar stage.* — We find a group of numerous nucleoli near the center of the nucleus, which are frequently more numerous than in our Fig. 292. At this stage they attain their smallest dimensions, and are approximately equal in size; they are completely homogeneous and contain no vacuoles. The total number of the nucleoli is apparently greater at this stage than at any other.

*Third nucleolar stage.* — This is characterized by an increase

in the size of the nucleoli, a decrease in their number, and the gradual migration of them towards the periphery of the nucleus. At the beginning of this period (Figs. 290, 291, 293, 294), the nucleoli are quite evenly distributed throughout the nucleus; at its close they are mainly peripheral in position, near the nuclear membrane (Fig. 297). The increase in the size of the nucleoli is due, in some part at least, to the coalescence of every two or three neighboring ones, and such juxtaposed groups of two or three nucleoli may be often found (Fig. 294). None of the nucleoli contain vacuoles.

*Fourth nucleolar stage.* — Now we find unstaining globules or vacuoles reappearing in the nucleoli, and there may be either a single large one to each nucleolus, or a number of smaller ones; the large one is probably formed by the coalescence of smaller ones. Almost all the nucleoli are in contact with the nuclear membrane, often flattened against it (Fig. 299). They have become larger than in any preceding stage, and less numerous, but are now quite unequal in size. This stage may mark the commencing degeneration of the nucleoli, though I have observed no evidences of a commencing fragmentation.

At the beginning of the first stage the nuclear sap never stains; but at the end of this period, when the nucleoli have become more numerous, it stains very noticeably with eosin (Fig. 286), which would point to a solution of nucleolar substance in the nuclear sap.

#### 6. *Zygonemertes virescens* (Verr.) Montg.

(Plate 27, Figs. 236–248.)

*Yolk.* — In only two cases out of the numerous egg cells examined (three individuals of this worm were sectioned) have I seen yolk balls, so that the formation of yolk balls must be regarded as abnormal, if not pathological; in this species the yolk arises as minute yolk spherules in the cytoplasm (Fig. 246), without (except in the cases noted) a yolk-ball stage being passed through. These minute globules stain at first very faintly, and when they first appear are isolated from one another. There is no given point in the cytoplasm where they

are first produced, but a varying number are formed simultaneously and at different parts of the cell; it is usually, though not always, the case that they first arise at the periphery of the cell at some distance from the nucleus. The mature yolk globules are slightly larger than these and stain somewhat more intensely, which shows that they gradually become denser as they increase in size; in the largest ova these spherules are so abundant that the true cytoplasm is quite obscured (Fig. 247).

*First nucleolar stage.* — In the smallest nuclei found there is a peripheral group of several nucleoli lying close to the nuclear membrane, which are spherical in form (Figs. 236–238).

*Second nucleolar stage.* — The nucleoli have increased in number, and, departing from their original peripheral position, now occupy the center of the nucleus (Figs. 239 and 240). So small are they, and so densely grouped may they become, that at first sight one might be led to suppose that each group of numerous nucleoli was a single nucleolus. In those cases where the nucleus is oval or elongated in form, instead of spherical (the usual case), in the place of a single cluster two are commonly present, or else the single mass or cluster of nucleoli is elongate in shape, its outline being more or less parallel to the contour of the nucleus. The nucleoli in this stage are always more numerous and usually also smaller than those of the previous period; their increase in number might thus be brought about, in part at least, by divisions of the earlier nucleoli.

*Third nucleolar stage.* — The nucleus now is much larger, and the nucleoli begin to wander apart towards the periphery of the nucleus (Figs. 241, 243, 246, 247). I have observed all stages between nuclei containing centrally grouped, small nucleoli and those in which they have come to lie close to the nuclear membrane. In this stage, as in the preceding one, the nucleoli are perfectly homogeneous without vacuoles, and spherical in form. In a few nuclei, however, they appear greatly vacuolated, but these cases are so rare that they must be considered abnormal. At the end of this period they attain their greatest dimensions, though they thereby become some-



what unequal in size. In this stage, accordingly, they increase in size (perhaps by the fusion of contiguous ones (Fig. 242), and decrease in number, whereas in the preceding one the reverse process took place.

*Fourth nucleolar stage.* — Almost all the nucleoli are flattened against the nuclear membrane (Figs. 245 and 248), and they commence to show a vacuolated structure; these apparent vacuoles, which are unstaining globules, when stained by the Ehrlich-Biondi method, whereby only the ground substance of the nucleolus is colored, appear as refractive granules (Fig. 248). At the conclusion of this period the nucleoli become irregular in shape, granular in appearance, stain less deeply, and each finally breaks up into a mass of granules. In this manner they decrease both in number and size.

During the third and fourth stages, while the nucleoli are undergoing the metamorphoses described, a small number of newly formed ones appear in the nucleus, which are of later formation than the others (Figs. 245, 247, *n.* 2). These may serve as nourishment for the chromatic filaments, as in *Tetrastemma catenulatum*; but in the present species I have not observed any distribution of them along these filaments, and further they are numerically scarcer than in *Tetrastemma*.

No yolk is present in the cytoplasm in the first and second nucleolar stages. This fact is easily proved by the use of the Ehrlich-Biondi stain, by which the cytoplasm is stained green, and the yolk substance, when present, a brownish maroon color. Yolk first appears in the third nucleolar stage, and at the commencement of the following stage the whole cytoplasm is nearly filled with it. Further, the nucleoli stain differently from the yolk globules by the use of the stain mentioned. These facts show that the origin of the nucleolar substance is not to be found in the yolk substance proper, but in a cytoplasmic substance from which the latter may later be evolved. That the substance of the nucleoli is extranuclear in origin is shown by the fact that the nucleoli at their first appearance lie in contact with the nuclear membrane (Figs. 236–238), and only later do they take a central position. Though I have seen no nuclei smaller than those figured, which could without doubt be

classed as germinal vesicles, yet it seems so probable that the substance out of which the nucleoli are formed is extranuclear, that I would conclude, *a priori*, that no nucleoli are present in stages of the germinal vesicle much earlier than those which have been here described. Those small nucleoli of a second generation, which are first produced in the third and fourth nucleolar stages, may represent yolk globules assimilated by the nucleus, since in these stages the cytoplasm is filled with such globules.

On the other hand, the yolk cannot be considered as having its origin in nucleoli which have wandered out of the nucleus, since in none of these stages are nucleoli found in the cytoplasm. And if such were the case, one certainly should be able to observe the large nucleoli of the third nucleolar stage in the cell substance, for it is at this period that the yolk first appears. I conclude that the yolk globules have their origin in some substance contained in the cytoplasm, and that the nucleolar substance also has its origin in some cytoplasmic substance. But whether the primitive nutritive substance of the yolk globules and that from which the nucleolar substance is derived are identical, is of course open to question; however, judging from the similarity in appearance, we might conclude that the primitive cytoplasmic substance was the same in both cases, and especially if we consider, which seems plausible, the nucleoli to represent the nutritive substance of the nucleus, as the yolk globules certainly represent that of the cell body.

In the first nucleolar stage the nuclear membrane is usually very thin, but always perceptible; in the later stages it becomes thicker. The nucleus is never noticeably irregular or amoeboid in outline. Might this be explained by the absence of yolk balls in the cytoplasm?

In the second and at the beginning of the third nucleolar stages, the central mass of nucleoli is usually surrounded by a clear space, in which space few or no chromatin microsomes occur, though it may be transversed by a few achromatic fibers (Figs. 239 and 240). This space was found in most of the egg cells of this stage in the three individuals sectioned, though it

may have been produced by the action of the preserving fluid (hot aqueous solution of corrosive sublimate).

7. *Stichostemma eilhardi* (Montg.).

(Plate 27, Figs. 213-235.)

The yolk changes may first be delineated, then those of the nucleoli. In my paper on this fresh-water form ('95), I have described the ovogenesis to some extent, and here shall follow it more in detail.

*Yolk.*—The yolk first appears in the cytoplasm in the form of small, more or less spherical masses (Fig. 213, *Yk. Bl.*), which at first stain like the cytoplasm; but these youngest recognizable yolk balls consist of a substance in which the fine granules (or nodal points of an alveolar structure) are much more densely grouped than in the surrounding cytoplasm. Thus the young yolk ball may be distinguished from the cytoplasm proper by its greater density. A number of these yolk balls appear to arise simultaneously, though in these earliest, as well as in the later stages of yolk formation, a successive production and metamorphosis of yolk balls take place, since in all but the earliest stages of their development yolk balls occur in the cytoplasm in various stages of formation. There is no rule as to the part in the cell at which these balls are destined to arise, for they may be found anywhere between the nucleus and the periphery of the cell; the fact that they first arise just as frequently at some distance from the nucleus as in its immediate neighborhood shows that they have no nuclear origin. An anabolic and a katabolic series of changes of each yolk ball can be distinguished, and these series of metamorphoses may be described in succession and termed respectively the prophasis and metaphasis of the yolk balls.

*Prophasis* (*Yk. Bl.* in Figs. 217, 218, and the median ones of Fig. 215).—The progressive or anabolic changes of the yolk balls consist in (1) their absorbing protoplasmic stains with great intensity, so that they stand in marked contrast to the cytoplasm; and (2) in their becoming quite homogeneous in structure, this homogeneity probably explainable by supposing

that a dense condensation of the fine granules of which they are composed takes place. They continue to increase in size, and gradually stain deeper as they do so, until they attain about the dimensions given in Fig. 217; but I am unable to determine whether they all reach exactly these dimensions before the metaphasic changes commence. At the conclusion of this period of their development they are large bodies, regularly spherical or oval in outline, and apparently without a limiting membrane; especially characteristic is their homogeneity and their intense staining.

*Metaphasis.* — These katabolic metamorphoses are introduced when a few unstaining globules arise in the substance of the yolk balls. These globules increase in number and size until the yolk ball assumes a vacuolated appearance (Figs. 215, 217, 228). At the same time its ground substance loses its staining power and no longer stains homogeneously. At the commencement of these changes the yolk ball may even increase somewhat in size, since the substance of the globules is added to it. These changes continue until the yolk ball either breaks up into the mature yolk globules (*Yk. Gl.*, Fig. 235), or first breaks into a varying number of larger pieces, and then each of the latter divides into yolk globules. The yolk globules are usually nearly spherical in shape, and though by no means equal in size are always larger than those of the other nemerteans examined.

During the prophaseis each yolk ball is enveloped by a clear, structureless zone of cytoplasm; but this surrounding zone is usually not noticeable around the larger yolk-ball fragments, and never around the mature yolk globules.

As to the cause of the fragmentation of the yolk balls, I can find no sure explanation from the facts at hand. However, the appearance of the colorless fluid globules within their substance must have an important connection with these katabolic changes, since they characterize the commencement of this period of change. It would seem likely that these colorless globules represent a fluid constituent of the cytoplasm which has actively or passively been taken into the yolk ball, — perhaps from the clear cytoplasmic zone enveloping each yolk ball, —

since the yolk balls increase in size at the beginning of the metaphasis, though there appears to be no increase in their own ground substance. These unstaining globules are at first localized at different points in the yolk ball ; and it would seem probable that their substance later mixes itself with the ground substance of the yolk ball, since this supposition would account for the lessening intensity of the stain of the yolk ball during the metaphases. It would appear less probable that these globules are metabolic products of the true substance of the ball ; however, we have too few facts to enable us to determine which of these is the correct view.

Certain curious structures found in the cytoplasm of an immature worm fixed with Lang's fluid (aqueous corrosive sublimate solution, NaCl, acetic acid) may be mentioned here. In the cytoplasm of a number of ova, in none of which any yolk was present, I found a few small, ring-shaped bodies, which stained with haematoxylin much more intensely than the surrounding cytoplasm (*Yk. Bl.?* Fig. 233). Each consisted of a ring composed of a dense, homogeneous substance, the inner surface of the ring being smooth, but the outer irregularly jagged, this whole ring (as it appears on a section) enclosing an unstaining vacuole or globule. In reality these bodies are spheres, but my description applies to sections of them. These structures vary considerably in size, and sometimes are not spherical but oval, the larger ones usually staining more deeply than the smaller ones. I found them only in some of the ova of this one individual, and nothing of the kind was to be seen in the ova of about twenty other individuals sectioned, which had been variously preserved in picric, osmic, and chromic acids, in simple aqueous solution of corrosive sublimate, and in the fluids of Hermann and Flemming. Accordingly, they must be regarded as artefacts, produced by the action of the acetic acid, which I have long since found to be a very unreliable fixative for the cytoplasmic elements of the nemerteans. It is most probable that they are young yolk balls, to which the acetic acid has given an abnormal appearance. Or might they represent multiple asters, such as have been recently described by Mead in *Chaetopterus?*

*Germinal vesicles, nucleoli.*—In this genus the earliest egg stages are more favorable for study than in the other metanemertans. In the connective-tissue nuclei from which the germinal vesicles are directly derived (with no intervening cell generations) no nucleoli are present, though this conclusion was possible only after much careful observation. These small nuclei (Figs. 213, 217, 218, 220, 228, *C. T. N.*) are characterized by a relatively thick membrane and by chromatin which is usually granular in distribution, but which may sometimes occur in the form of granular fibers. These chromatin masses might at first sight be confounded with nucleoli, but their small size and irregular contours show that they are true chromatin granules. Further, when these nuclei are stained by the Ehrlich-Biondi method, these fibers and granules always stain with methylen green (chromatin reaction) and not a single one stains with fuchsine (which invariably stains any true nucleoli). Accordingly, what could not be finally proved for the other metanemertans, though all observations pointed to its being the case there, could be definitely settled for *Stichostemma*, namely, that these connective-tissue cells contain no nucleoli; in other words, nucleoli first arise in the definite germinal vesicles.

Before proceeding to the description of the egg cells it may be noted that not all the undifferentiated connective-tissue cells within the gonad become germinal vesicles. I have previously ('95) shown that the young gonad is a cell syncytium in which numerous nuclei are unevenly scattered through a mass of cytoplasm, but cell boundaries cannot be seen (Figs. 217 and 218). A few of these nuclei increase in size and eventually become germinal vesicles, and the latter reach maturity not simultaneously but in succession, so that no gonad contains more than one large ovum at a given time. The numerous other nuclei which do not become thus differentiated degenerate, and their substance is eventually absorbed by the gradually increasing mass of cytoplasm of one of the growing egg cells. These regressive processes are as follows (Fig. 218, *C. T. N.*): the nuclei increase a little in size, but become much clearer in appearance, *i.e.*, the relative amount of their chromatin appears to decrease;

next the cell membrane gradually disappears; then the chromatin granules no longer become colored by any of the stains employed, but become refractive and yellowish. All the chromatin granules do not lose their affinity for stains simultaneously, but two or three of them may often remain stained as before, while the remaining granules of the same nucleus may have entirely lost their stain. At this period in the nuclear degeneration we find small masses of these unstaining, yellowish granules in the cytoplasm, each mass still preserving the form of a nucleus. Later these individual granules wander apart, or those of several nuclei may partially fuse together to produce a larger mass; these larger masses of granules are always enveloped by a clear zone of cytoplasm, sometimes of considerable extent, so that they appear to be situated in vacuoles of the cytoplasm. The degeneration stages of these nuclei are most frequent in the cytoplasm, before yolk balls begin to arise in it; as the latter appear, the remnants of the degenerated nuclei gradually vanish, so that when the cell is filled with the yolk balls all vestiges of these nuclei have vanished. We must suppose that they become assimilated by, or dissolved in, the cytoplasm. These formations, the katabolic changes of degenerating nuclei, can in no way be confounded with stages of yolk development, since the small size, yellowish color, and refrangibility of these granular masses serve to distinguish them sharply from any stage of the yolk balls, even though both are often found in the immediate vicinity of each other.

The nuclei which are destined to become germinal vesicles increase in size to some extent before nucleoli appear in them; they now differ from the connective-tissue nuclei, apart from their greater dimensions, in having a relatively greater amount of chromatin and in being regularly spherical or oval in form. The first nucleoli to arise always lie in close contact with the inner surface of the nuclear membrane (Figs. 214, 216, 219, 220, 224, 225). They usually appear in the form of a thin disc-shaped mass on the inner surface of the membrane, but there is considerable irregularity in the form of this mass, which may be angular or nearly spherical in outline. At the com-

mencement of this *first nucleolar stage* the nucleolar substance appears at only one point in the periphery of the nucleus, and always in the shape of an irregular mass.

*Second nucleolar stage.*— This period is characterized by the formation of other nucleolar masses at various points in the periphery of the nucleus, the successive detachment of all of these from their connection with the nuclear membrane, and their migration towards the center of the nucleus. The commencement of this process is to be seen in very young nuclei, where but a single peripheral nucleolar mass is present; from the inner side of this mass small particles become divided off (Figs. 219, 224, 225), then each of these particles assumes a more or less spherical shape and wanders to the center of the nucleus; this process continues until the whole mass of nucleolar substance has reached the center in the form of separate particles (Figs. 217, 218, 223, 227). The peripheral nucleolar mass usually stains less intensely than the portions which have already reached the center of the nucleus. While the first-formed peripheral nucleolar mass is thus gradually wandering to the center, other masses are successively forming at the periphery of the nucleus, and their detached portions successively passing to the center. When a considerable number of these nucleoli have reached the center of the nucleus they naturally come into mutual contact, and then a process of fusion sets in, which results in the coalescence of neighboring groups of nucleoli, so that a smaller number of larger ones are formed. Sometimes this fusion may proceed to such an extent that one single, enormous nucleolus results (Fig. 226), but usually several large nucleoli are the result, these being unequal in size. The irregularity both in the dimensions and the forms of the nucleoli is particularly characteristic for this stage; thus the individual nucleoli often have elongated processes and angles, and this irregularity is frequently so excessive that the nucleoli within the nucleus appear like smears of ink upon a page (Figs. 226, 227, 230). I think that this irregularity in form may be explained by the assumption that at this stage the substance of the nucleoli is viscid in its consistency, while in the following one, where the spherical form is the rule, its nature must



be more freely fluid. Further, at this period we usually find vacuoles within some of the nucleoli of each germinal vesicle (Figs. 217, 218, 226, 229-231); sometimes no vacuoles are present in any of the nucleoli of a nucleus, but it is the rule that at least one of them, and that usually the largest, contains one or several vacuoles. Sometimes four or five of the nucleoli, which may be very unequal in size, may each have vacuoles. Occasionally a nucleolus contains only one vacuole, and in the latter there may be one or several small solid bodies, which stain like the ground substance of the nucleolus, and may be termed nucleololi; one of the latter may be fused with the inner surface of the nucleolar ground substance (Figs. 217, 218, 230, 231). These nucleololi vary in number and size, and are absent in the greater number of the vacuoles; so no particular significance should be attached to them, since they are probably nothing more than portions of the ground substance of the nucleolus which have become detached from the surrounding substance and have come to lie within the vacuole. During this period the nuclear membrane is thinner than at any other stage, and the nucleus is very noticeably amoeboid in form, the amoeboid processes being much more pronounced than in any of the other nemerteans examined; these processes in reality represent changes in the form of the nucleus, and are not artefacts, since they are seen equally well after preservation in the most diverse fixing fluids (Figs. 226, 227, 230, 232, 233). The nuclear membrane is always particularly thin around these nuclear processes, but, as far as I could make out, never becomes broken.

*Third nucleolar stage.*—The large nucleoli which were present at the end of the preceding stage now commence to fragment into smaller nucleoli, which are more or less equal in size, and then the latter wander towards the periphery of the nucleus; at the conclusion of this period, which must take place in a very short time, since I found only a few germinal vesicles exhibiting it, there are a large number of rather small nucleoli close to the nuclear membrane (Fig. 234). At this time the nucleoli attain their maximum staining intensity; the nucleus usually shows no traces of an amoeboid form, and its membrane has increased in thickness. None of the nucleoli

contain vacuoles ; and in every respect the nucleolar changes during this stage are the very reverse of the preceding.

*Fourth nucleolar stage.* — This is characterized by the gradual degeneration and disappearance of the nuclei (Fig. 235). Small vacuoles arise in them, and these increase numerically, while at the same time the nucleolar substance stains less intensely. Fusion of neighboring nucleoli is very frequent at this time, or perhaps a little time before the nucleoli lose their staining power ; accordingly, in the largest germinal vesicles it is the rule to find a small number of large nucleoli. The nucleoli are not evenly distributed along the periphery of the nucleus, and are often flattened against the nuclear membrane. This nucleolar stage is found only in the largest ovarian eggs, where the nucleus is perfectly regular in outline, without amoeboid processes, and its membrane has attained its greatest thickness.

Since this species is a protandric hermaphrodite, in which male and female sexual products ripen successively in each gonad, I found it at first difficult to determine whether a young nucleus in a given gonad corresponded to a male or to a female cell. But after comparing briefly the spermatogenesis of the other metanemerteans mentioned in this paper, and finding in them that no nucleus in any stage of spermatogenesis was larger than any of the smallest germinal vesicles here figured, I concluded that also in *Stichostemma* no male nuclei can attain the dimensions of even the smallest nuclei of our second nucleolar stage, and hence that all these nuclei were correctly concluded to be germinal vesicles, and not nuclei of spermatogenetic stages.

We notice in the succession of the nucleolar stages described the rhythmic sequence in regard to (1) the position of the nucleoli, (2) their states of fusion and division, and (3) the absence and presence of vacuoles in them ; these successive changes may be expressed as follows :

Stage.	NUCLEOLI.		
	Position.	Vacuoles.	Fusion, division.
First . . .	peripheral . . .	absent . . . . .	fusion ?
Second . . .	central . . . . .	present . . . . .	division, then fusion.
Third . . . .	peripheral . . . .	absent . . . . .	division.
Fourth . . . .	peripheral . . . .	present . . . . .	fusion, then division.

There is without doubt in this genus, as in the other metanemerteans, an extranuclear origin of the nucleolar substance. This is proved (1) by the absence of nucleoli in the nuclei from which the germinal vesicles are derived; (2) by the nucleoli first appearing close to the nuclear membrane. And since yolk globules do not arise in the cytoplasm until nearly the close of the second nucleolar period, when most of the nucleoli are near the center of the nucleus, to the yolk substance cannot be attributed a nucleolar derivation, and other reasons, such as the fact that the yolk balls usually appear at some distance from the nucleus, would contradict such an assumption. The nucleolar substance is apparently formed from an unstaining fluid constituent of the cytoplasm, which after it is taken into the nucleus undergoes a chemical change, since it stains there and is deposited in the form of nucleoli. In the second nucleolar stage, when the formation of nucleoli is at its height, the nuclear sap stains more deeply than at any other period (Figs. 224-227, 233), so that it is probable that at this time nucleolar substance is finely distributed throughout the nuclear sap, as well as in the form of nucleoli. (This staining of the nuclear sap is especially well seen on material fixed with Flemming's fluid and stained with alum carmine.)

In the third and fourth nucleolar stages a few yolk globules are often found in a number of germinal vesicles (Figs. 234 and 235, *Yk. Gl.*); these have probably been taken up by the nucleus from the cytoplasm.

*Chromatin.* — In the nuclei of the first stage, the chromatin is always demonstrable in the form of coarse granules (Figs. 214, 216, 219). In the beginning of the second it may usually be found in the form of a reticulation (Figs. 218, 229, 233), but at the end of this stage it is not demonstrable (Fig. 227). In the third and fourth stages it reappears, but now in the form of fine microsomes (Fig. 235); and at the conclusion of the fourth stage short chromatic filaments begin to arise, similar to those described for *Tetrastemma catenulatum*.

8. *Lincus gesserensis* (O. F. M.).

(Plate 24, Figs. 159-177.)

*Yolk.* — The yolk first arises in the cytoplasm in the form of irregular yolk balls, which are much smaller than in the other nemerteans examined (*Yk. Bl.*, Figs. 159, 160, 177); these increase in number and size, the largest sometimes containing vacuoles. In the largest ovarial ova seen (though I had only immature individuals of this species) yolk balls are no longer present, but in their place smaller yolk globules, which in all probability represent fragments of the earlier balls. The yolk usually makes its first appearance in a zone of the cytoplasm, midway between the nucleus and the cell membrane, which is characterized from the rest by a less dense structure (Fig. 177). The extreme peripheral portion of the cytoplasm retains its density longest, as is also the case in the other species. The cytoplasm of the connective-tissue cells (Fig. 159), from which the egg cells take their origin, stains very faintly, while that of the young egg is dense and stains deeply.

*Nucleoli.* — Only three worms out of eighteen sectioned contained ovogenetic stages, and since in these individuals only the earlier stages of this development were found, I am able to describe only the younger stages of nucleolar formation. The egg cell of this heteronemertean contains a single nucleolus; apparent exceptions will be considered later.

In the smallest nuclei (Fig. 159) of the cell syncytium of the gonads no nucleoli are to be seen; we find nucleoli for the first time in cells whose nuclei are a little larger and whose cytoplasm commences to stain more intensely. These are the earliest stages of the oocytes.

Now in these youngest germinal vesicles (Figs. 159, 161, 164, 166) the nucleolus is very frequently peripheral in position, close to the inner surface of the nuclear membrane; while in the later stages (certain mitotic stages excluded) it is almost invariably never in contact with the nuclear membrane. Further, yolk balls first appear in the cytoplasm when the nucleus contains a nucleolus. These facts, being considered together with the fact that nucleoli are absent in the nuclei of the con-

nective-tissue cells, lead to the conclusion that the nucleolus first appears in the young germinal vesicle, and more particularly, that the substance of the nucleolus is extranuclear in origin, and stands in a genetic relation to the substance of the young yolk balls. The substance of both is homogeneous and stains identically; by fixation in Hermann's fluid, followed by the triple stain of Flemming, the nucleolus and the yolk balls stain a brownish yellow (Fig. 160); by fixation in corrosive sublimate and staining in haematoxylin and eosin both structures are colored a yellowish red (Fig. 177). Still more conclusive is the following observation: while the greater number of the yolk balls may lie at some distance from the nucleus, one or several are very frequently in close contact with the outer surface of the latter, and yolk balls may even be found which are halfway through the nuclear membrane, or which have completely transversed it and lie within the nucleus (Fig. 160). Thus the nucleolus would seem to owe its origin to the substance of yolk balls which have been taken into the nucleus. The very marked increase in the size of the nucleus and the nucleolus is probably caused by a continued process of yolk-ball assimilation on the part of the nucleus. This may be observed in numerous cases where small globules of yolk-ball substance lie within the nucleus, some at its periphery or close to the nuclear membrane, others flattened against the nucleolus (Figs. 160 and 177). By the use of the haematoxylin-eosin stain the nucleolar substance usually stains a little more intensely than the substance of the yolk balls (Fig. 177); this would show that this substance, after being taken up by the nucleus, undergoes a chemical change within the latter. Those yolk balls which are not assimilated by the nucleus remain in the cytoplasm and give rise to the yolk globules, as has been described. Thus the nucleolus probably has an extranuclear origin and represents a portion of the yolk-ball substance taken into the nucleus; its rapid increase in size is due to the addition to it of other similarly assimilated globules of substance.

In the largest germinal vesicles seen (though these were not mature) the nucleolus is usually spherical in form, seldom oval, and homogeneous in structure, except that it sometimes

contains a single large, unstaining globule, which appears as a vacuole (Figs. 162, 175-177); or there may be from one to three minute globules in it, which, when seen in their entirety, present the optical appearance (due perhaps to refraction) of black granules, which might be mistaken for solid bodies. The nucleolus has no limiting membrane. The largest are relatively enormous and stain more intensely with eosin than the smaller ones. There is no clear zone in the nucleus around the nucleolus.

In *Lincus* the study of the metamorphoses of the nucleolus is complicated by the occurrence of nuclei in various mitotic stages. Karyokinetic figures were absent in the ovarial stages of the other nemerteans examined, so that in those species the connective-tissue nuclei and the egg nuclei both stand in the same cell generation, and the germinal vesicle may either be regarded as equivalent to an ovogonium or to a true ovocyte of the first order. In those species no cell generation separates the connective-tissue nucleus and the germinal vesicle, but the latter is merely evolved from the former by a gradual process of differentiation. But in *Lincus* the germinal vesicle is separated from the connective-tissue nucleus by at least one and probably by two or three generations (if the differences in the size of the cells offer a sure criterion). Here, accordingly, the indifferent connective-tissue cell represents an ovogonium, and perhaps another generation of ovogonia may intervene before the germinal vesicle, the ovocyte of the first order, is produced. Of the two individuals on which these nuclear studies were made, I found mitotic stages in only one individual, while none were to be seen in the other individual, though here these nuclei had reached nearly the same degree of development. I have studied the mitosis merely with regard to the behavior of the nucleolus. The most abundant stages were those of the spirem and dispirem, asters and dyasters being much less frequent (Figs. 163, 166, 169, 170-172); the time duration of the latter stages may be less than that of the former. In by far the greater number of the spirem stages one nucleolus was present; it is probably present in each nucleus of this stage, but sometimes may escape observation by being

covered by the chromatic filament or by lying in a part of the nucleus outside of the plane of the section. In this stage, further, two nucleoli are never present; accordingly, in the spirem there is neither a disappearance nor a division of the nucleolus. In the dispirem stage each daughter-nucleus contains one nucleolus (Fig. 171), the two nucleoli being, however, often unequal in size. I found very few aster stages, and these were either so unfavorably placed for study or the chromosomes so densely entangled that I could not determine whether a nucleolus is present in this stage and whether a division of it takes place at this time. The facts determined are (1) that no division of the nucleolus occurs in the typical spirem stage, since here only one nucleolus is present; and (2) that each nucleus of the daughter-spirem has one nucleolus. But I cannot show whether a division of the nucleolus occurs in the time between these two stages or whether the original nucleolus passes over into one of the daughter-nuclei, while in the other one a new nucleolus is produced. In these various mitotic stages the nucleolus usually lies at the periphery of the nucleus, and it is most frequently the case that it is not in contact with the chromatin filament; it preserves its former shape and staining intensity, and apparently does not decrease in size during the mitosis. To be sure, in the karyokinetic stages under consideration it usually appears small in proportion to the size of the particular nucleus, but then it is usually the case in most mitoses, and probably so here, that before the disappearance of the nuclear membrane the volume of the nucleus greatly increases.<sup>1</sup>

Two nucleoli, never quite equal in size, are frequently found in certain small nuclei, which the distribution of the chromatin would show to be in a stage at the commencement of the prophase of the mitosis or at the conclusion of the metaphase (Figs. 163, 164-167, 170, 172). As the figures show, all these nuclei which contain two nucleoli are more or less of the same size. Nuclei which are a little smaller than these, as well as those which are larger, invariably contain a single nucleolus.

<sup>1</sup> The chromatin filament has considerable thickness and is apparently a continuous thread; it is looped around the inner surface of the nuclear membrane.

It is probable that the two nucleoli of such nuclei have not arisen by division from a single nucleolus, but are nucleoli which have been developed at different points in the nucleus and which are destined to fuse together later and form a single one. This assumption was based upon the observation of nuclei where two nucleoli lie at opposite poles of a nucleus (Fig. 166) and each is apposed to the nuclear membrane, or where only one occupies such a peripheral position, the other being in the center of the nucleus (Fig. 164). In one figure (Fig. 165) we see a nucleus in which the two nucleoli lie near the center, close together, which might denote the beginning of such a fusion. On a little reflection this explanation of the presence of two nucleoli will appear quite allowable. In the more usual mode of development a larger nucleolus is formed at the periphery of the nucleus, wanders towards its center, and then much smaller masses of nucleolar substance are similarly formed and later fuse with the large nucleolus; while in the cases under consideration two nucleoli of nearly equal size are produced, either simultaneously or in succession, and these afterwards fuse together. These two nucleoli of nearly equal size cannot be division products of a single primitive nucleolus, since two nucleoli are never found in the larger germinal vesicles.

The nuclear sap of the smaller germinal vesicles does not stain at all; in the larger ones (Figs. 168, 173-175, 177) it does, and the explanation for this staining may be given by the assumption that there is a dissolution of nucleolar substance throughout the whole nucleus, *i.e.*, of that substance of the assimilated yolk balls which does not enter into the formation of the nucleoli. During the mitotic stages no constituents of the nucleus stain except the nucleolus and the chromatin filament, but these do not stain in the same manner.

At first sight the heteronemertean *Lineus* seems to differ markedly from all the metanemerteans here examined, in that it contains a single, enormous germinal spot. But in *Lineus*, though a single large nucleolus is first formed, it nevertheless grows by the addition to it of much smaller nucleolar globules (*Nut. Gl.*, Figs. 168, 174, 177) which have the same method of



formation and fuse with the former. Were these secondary nucleolar globules in *Lineus* as large as the first-formed nucleolus, and were they all to remain separate from one another, the nucleolar metamorphosis in this genus would correspond to that of the metanemerteans; accordingly, the difference in the nucleolar production is not very important. (For the nucleolar relations in the other nemerteans, cf. my reviews of the papers of v. Kennel, Hubrecht, Coe and Bürger.<sup>1</sup>)

9. *Siphonophore (Rotalia?)*.

(Plate 26, Figs. 204-212.)

(Dr. Conklin kindly loaned me the preparations on which his earlier studies were based ('91); these were preserved in alcohol and stained with haematoxylin.)

There were no very young stages of the ovogenesis in this specimen; I have studied the ova in the egg pouches and in the gonophores, each gonophore containing a single large ovum (as shown by Conklin and Brooks), while in the egg pouches a number of smaller ova may be present.

A single large nucleolus is contained in each germinal vesicle. This is not only large in relation to the size of the nucleus, but is also absolutely probably one of the largest nucleoli ever described in animal cells (Fig. 212). It is always excentric in position, though seldom close to the nuclear membrane. In those younger stages where the nucleus is still near the center of the egg (Fig. 205, and the dorsal cell of Fig. 211)

<sup>1</sup> The only other observations of the yolk development in the nemerteans are those of Bürger ('90) on *Drepanophorus*. Near the young germinal vesicle lies in the cytoplasm a homogeneous, deeply staining body, of smaller size than the nucleus, which Bürger assumes may correspond to a yolk nucleus. This body disappears, "und es sammeln sich nämlich, dem Keimbläschen anliegend, in jenem [Plasmahügel] kuglige oder längliche, tröpfchenähnliche Gebilde an, erst spärlich ein einziges, zwei und mehrere, später aber mit dem immer noch fortschreitenden Wachstum des Keimbläschens sich zahlreich vermehrend in grösster Menge. Sie sind durchaus homogen, von mattem Glanze und äusserst tinktionsfähig. . . . Erst nach der Entwicklung des Keimbläschens geht die des Deutoplasmas vor sich und zwar nun auf Kosten der glänzenden Dotterballen, welche aufgebraucht werden und so im reifen Ei verschwinden." In the ripe egg the cytoplasm is granular and stains lightly.

the nucleolus is usually nearer the center of the nucleus than in those more mature stages where the nucleus lies near the periphery of the cell. But in the more mature stages the nucleolus may lie at the animal pole, or the vegetal pole, or at one side of the nucleus, so that no coincidence between the position of the nucleolus and the age of the nucleus can be determined. Thus the nucleolus stands, *e.g.*, in no relation to the animal pole of the more mature nucleus, that pole where amoeboid processes are produced (Figs. 204 and 209). The ground substance of the nucleolus is dense and homogeneous, and stains quite deeply; the nucleoli of the smaller germinal vesicles stain, as a rule, less intensely. In the ground substance of all the nucleoli more or less numerous fluid globules occur, which stain very faintly or not at all, and their presence gives a vacuolated appearance to the nucleolus; those within the same nucleolus are of unequal size, and among them two or three usually occur which far exceed the others in size. Occasionally there is one large central vacuole (Fig. 206), but as a rule the larger ones are peripheral, and may produce prominences of the surface of the otherwise perfectly smooth and spherical nucleolus (Figs. 209 and 212). In one large vacuole (Fig. 212) a finely granular mass was found, though this may have been an artefact. Since in the smaller nucleoli these vacuoles are less numerous and smaller in size, it would seem probable that in stages antecedent to those found by me the nucleolus may be wholly devoid of such vacuoles. The nucleolus has no enveloping membrane, for what at first view appears to be such a structure careful study shows to be merely the result of refraction.

In addition to the single large nucleolus described, there are in the most mature nuclei also from about one to five minute nuclei (Fig. 209). These vary somewhat in size, are perfectly spherical and homogeneous, without vacuoles, and stain more deeply than the larger one. Sometimes they are found in close contact with the nuclear filaments (*cf.* the nucleoli of the second generation in *Tetrastemma catenulatum* and the observations of Rückert ('92) on the germinal vesicles of *Selachii*). These probably have no genetic relation to the large nucleolus, since

they never lie in contact with the latter and are frequently situated at some distance from it. Were they buds from the large one, one would expect to find in them vacuoles such as occur in the large nucleolus, but they never contain vacuoles. In one nucleus (Fig. 207) I saw a disc-shaped mass apposed to the inner surface of the nuclear membrane, which stained more intensely than the chromatin. Such a peripheral mass may be regarded as a substance taken up from the cytoplasm by the nucleus, which, after passing through the nuclear membrane, undergoes a chemical change to such an extent that it stains with haematoxylin. The minute nucleoli may stand in a genetic connection with such a mass of substance, that is, be portions of a substance assimilated by the nucleus and afterwards scattered through the latter. They might serve as nourishment for the chromatin threads with which they are often in contact.

In seven nuclei out of about one hundred or more examined the large nucleoli differed much from the ordinary type described above. In one egg pouch there was a smaller ovum apposed to the animal pole of a larger one (Fig. 211); a normal nucleolus was present in the nucleus of the smaller one. But in the larger ovum two nuclei were present, in close contact with one another, though separated by a membrane (coalesced nuclear membrane). It is in each of these latter nuclei that an abnormal nucleolus is present. Each of these nucleoli is finely granular, without enclosed vacuoles, and stains faintly with haematoxylin; the one is regular in outline, but the other is jagged at one pole, and a ring-shaped portion of its substance stains more deeply than the remaining portion. In another ovum I also found two nuclei, in each of which was a nucleolus similar to those just described. In still another ovum two nuclei were found in contact with each other, the nucleolus of one of which was similar to those here described, but the nucleolus of the other nucleus was intermediate in structure between these and the ordinary type of nucleoli (Fig. 210). In only one case was such an abnormal nucleolus present within an ovum contained in a gonophore (Fig. 208); in the other six cases the abnormal nucleoli were in ova of egg pouches.

Now what do these lightly staining, granular nucleoli represent? In all except the seven cases here mentioned the nucleolus was always of the deeply staining, vacuolar type, irrespective of its occurrence in ova of egg pouches and of gonophores. The abnormal nucleoli, with one exception, were found in the largest ova of the egg pouches. Types intermediate between the two are represented in Fig. 210. Conklin and Brook's observations, which I can corroborate, show that a number of ova are produced in an egg pouch, but that only one of these passes into a gonophore, and there develops into the ripe ovum, while the others remain behind in the egg pouch and do not reach maturity, but degenerate. I would hold that the abnormal nucleoli described by me are degenerating nucleoli of degenerating ova. All the facts seem to favor such an explanation.

The cytoplasm of the youngest egg cells appears finely granular (it may be an alveolar meshwork). In the largest it was coarsely vacuolar, especially near the center of the cell; I find no evidence of yolk. Conklin and Brooks evidently mistook the vacuoles of the cytoplasm for yolk globules.

No chromatin threads were apparent in the smallest germinal vesicles (Figs. 204-206), but only a fine granulation in the nuclear sap; chromatin threads make their appearance gradually in the larger ova (Figs. 207, 209, 211) and stain more intensely as they increase in number and size. Each thread often has the form of a chain of transversely placed discs; or sometimes it would seem to consist of a large number of short fibrils, placed at right angles to a common longitudinal axis, as is the structure of the chromosomes of the Selachian egg. These threads usually make their first appearance in the neighborhood of the nucleolus, from which they sometimes radiate outwards; only in the largest nuclei are they more generally distributed throughout the nucleus. This fact might show a physiological relation between these two structures. But there is in all probability no genetic connection between the two; rather, the chromatin threads are built up of the minute microsomes found in the nuclear sap of the smaller ova. But the formation of the chromatin threads must be determined by the investigator who has more abundant material at his disposal,

and material which has been more advantageously fixed and stained.<sup>1</sup>

10. *Polydora*.

(Plate 28, Figs. 249-281.)

The egg cells of this form, as those of most *Polychaeta*, are derived from the peritoneal cells of the body cavity, the latter cells building pseudoepithelia around the intestine, as well as occurring free in the body cavity. Those in the pseudoepithelia (Fig. 249) are more or less flattened, disc-shaped, while the free cells (Figs. 250-254) are oval in shape, with more regular outlines. Their cytoplasm is not dense, and one or several large vacuoles are frequently found at the periphery of the cell; a delicate cell membrane is present. The cytoplasm of these sexually indifferent cells does not stain with haematoxylin. The nucleus is small, irregular in outline, and contains a few chromatin granules; very frequently the greater part of this substance lies close to the nuclear membrane. I have never found more than one minute nucleolus, and this is almost always close to, or in actual contact with, the nuclear membrane (Figs. 251, 252, 254); in many nuclei I failed to find nucleoli, though in these cases they may have been obscured by the chromatin. I found one division stage of a nucleus (Fig. 249); there were two daughter-nuclei of the same size and form lying close together; the nucleolus of each was somewhat elongate in form (in all others of these cells examined it is spherical), which might show that the nucleoli had been produced by the division of a single one in the mother-nucleus. In many of the smaller free peritoneal cells a peculiar body often occurs (*N. P.* Fig. 253). This is always smaller than the nucleus, more or less spherical, often homogeneous in appearance, and it may stain either deeply red with eosin or faintly with haematoxylin, or in other cases it may not stain at all, but appear as a light yellowish, refractive mass. From the comparative study of a large number of cells containing these bodies it may be determined that they are degenerated nuclei or portions of nuclei. Thus in Fig. 250, which

<sup>1</sup> For other observations on nucleoli of *Siphonophora*, cf., besides the paper by Conklin and Brooks, the review of O. Hertwig ('78b).

probably represents the commencement of such a degeneration, there lies close to the nucleus what seems to be a much smaller nucleus, or a portion of one; and I have found all intermediate stages between such a body, which is granular and stains with haematoxylin, and the body reproduced in Fig. 253, which appears nearly homogeneous and stains with eosin. These bodies then seem to be degenerated or cast-off portions of nuclei. We might conclude also that the cells in which these structures are found, are themselves fated not to develop into egg cells, even if they are not degenerating; for no such bodies are to be seen in the cytoplasm of the true egg cells.

These peritoneal cells have the morphological value of ovogonia. Those which are destined to become ova seem to become detached from the pseudoepithelial connection, but in such a way that they do not become detached singly, but portions, each of which is composed of a number of cells, become loosened from the epithelium. Thus the earliest ovogenetic stages are to be found in strings of cells arranged radially around a common longitudinal axis, each such string of cells situated free in the body cavity (Fig. 270 represents a portion of such a string). At the one end of such a cellular string lie, densely grouped, the numerous mitoses of the ovogonic stages, while the remaining portion of the string is usually composed of young ova, *sensu strictiori*. I have never found mitoses in cells which lie singly in the body cavity.

The first change noticeable in the ovogonium leading to the formation of the ovum consists in (1) the increase in the size of it and of its nucleus, and (2) in its cytoplasm gradually staining with haematoxylin. This deep blue staining of the cytoplasm, accompanied by its increasing density and the loss of the vacuoles in it, continues from now on until yolk granules begin to arise in it, when the cytoplasm commences to stain faintly with eosin and loses its dense structure. At the conclusion of the ovogonium rest stage the nucleolus has increased a little in size, accompanying the growth of the nucleus.

The next stage is a mitosis. Whether there is more than one mitotic generation separating the ovogonium from the ovum I have not been able to determine; the slight differences

in the size of the mitoses hardly afford a satisfactory criterion for deciding this point (Figs. 255-261). All the typical stages of the prophase and metaphase are to be found, though only in the arrangement of the chromatin, for I have been unable to find either centrosomes or achromatic spindle. After careful study of a large number of these dividing nuclei I find the nucleolus to persist in the nucleus throughout the mitosis. Further, it appears to retain its original size throughout this process, without any diminution in volume. Thus the nucleolus seems to be retained without change in the spirem and aster stages of the prophase. In the dyaster stage (Fig. 258) each pole of the nucleus usually contains a nucleolus, so that the nucleus contains two nucleoli; and when the nuclear division is completed, *i.e.*, when in one and the same cell two nuclei occur in close contact with each other, in the aster as well as in the spirem of the metaphasis, each daughter-nucleus has its own nucleolus (Fig. 257). Now the ovogonium contains only one nucleolus, so that we must assume either (1) that a division of the nucleolus has taken place during the mitosis, or (2) that to one of the daughter-nuclei is allotted the whole original nucleolus, while in the other nucleus a new one is produced. I have not seen any dividing nucleoli in these mitoses, their small size being a great obstacle to their study. But I should judge that such a division occurs, for these reasons: (1) the nucleus of one or of both the daughter-nuclei has sometimes a somewhat elongate form (Fig. 257); and (2) in later stages of the ovum proper I have found dividing nucleoli, and these cases would show that if such divisions take place in stages subsequent to the mitosis they might also occur during the mitosis. The two cases of division of the nucleolus found are here figured (Figs. 264 and 265), and in each of the elongate nuclei is a dumbbell-shaped nucleolus lying in the longitudinal axis of the nucleus; in these figures the two halves of each nucleolus appear unequal in dimensions, but this is so because neither of these nucleoli happened to lie wholly in the plane of the section. I have found numerous other cases of elongated nuclei, each with an elongate nucleolus without any median constriction (Fig. 270). These facts would show that a division

of the nucleolus may take place during the mitosis, and probably does so.

After the completion of the mitosis just described, each daughter-nucleus, which now has the value of a germinal vesicle, first passes through the spirem stage of the metaphasis and then enters upon the stage of synapsis, namely, the nucleolus has a more or less central position, and all the chromatin of the nucleus becomes grouped immediately around it (Figs. 264-266, 270, 271, 278), the peripheral part of the nucleus being transversed by only a few fine, unstaining strands of substance (linin?). All intermediate grades between this and the preceding stage of the nucleus may be found. This is not an artificial appearance caused by the use of a particular preservative, since it is equally demonstrable on preparations fixed with aqueous or alcoholic corrosive sublimate, sublimate with acetic acid, Flemming's fluid, and alcoholic solution of picric acid; only after the use of Perenyi's fluid is this arrangement of the chromatin not found, but this fluid seems to be rather a poor one for most cytological study. It cannot be an artefact, since this appearance is found only in ova of a certain size but not in those which are larger; thus it cannot be produced by the resistance offered by the cell membrane to the penetration of the fixatives, since this membrane is much thicker in the larger ova. This central arrangement of the chromatin then represents a definite stage of the germinal vesicle concomitant with the first appearance of yolk globules in the cytoplasm.<sup>1</sup> So at this point we may briefly describe the yolk development and then return to the changes of the nucleolus.

The yolk first arises in the cell during the stage just described, that is, immediately after the conclusion of the spirem stage of the metaphasis. It appears in the form of small globules (*Yk. Gl.*, Figs. 262-264, 266, 270, 271), most of which are arranged close to the outer surface of the nuclear membrane, the first globules rarely arising at a distance from the nucleus. At this period they stain less deeply than later. The yolk

<sup>1</sup> This stage of synapsis (Moore) appears to be characteristic of the anaphase of the last spermatogonic and spermatocytic division in all the higher animals, and no doubt can any longer be expressed of its representing an artefact.



rapidly increases in amount, spreading from the region of the nucleus (which is central) to the cell periphery. In the largest ovarian ova the cytoplasm is densely filled with larger and smaller yolk globules; the larger ones appear homogeneous when stained with eosin (Fig. 269), but the Ehrlich-Biondi stain shows them to be composite masses of small globules.

The nucleolus rapidly increases in size, at a somewhat greater proportionate rate than the nucleus itself. It is now large enough for its structure to be clearly made out: it consists of a homogeneous ground substance, which seems to stain more deeply with eosin as it grows larger; a limiting membrane is clearly demonstrable in the largest nucleoli (Figs. 271-277, 279-281) after staining by the Ehrlich-Biondi method or after fixation with Flemming's fluid, though it does not differ chemically or in structure from the ground substance and is only a thin layer of the latter in which vacuoles never occur. At the close of the metaphasis of the mitosis small vacuoles make their first appearance in the ground substance of the nucleolus (Figs. 263 and 270). There are only a few of them at the start, but their number rapidly increases as the nucleolus grows larger, until there are large numbers of them in its center (Figs. 268 and 269). They are always more numerous at the center than at the periphery of the nucleolus, and usually first appear at the former point. On preparations stained with eosin the small vacuoles appear either as clear spaces or as black granules, according to the focusing of the microscope; after the use of the Ehrlich-Biondi stain they become a light grayish color (note the contrast, — that in the eggs of *Doto* and *Montagua* the nucleoli appear as black granules only after treatment with the latter stain); after fixation in the fluid of Flemming the substance of these vacuoles is of a lighter color than the ground substance. This vacuolar substance is homogeneous, and is probably of a thin, fluid nature. With the growth of the nucleolus the number of the vacuoles becomes very great, though their size does not seem to increase. In the nucleoli of the largest germinal vesicles examined the vacuoles no longer retain their original spherical form, but become mutually confluent to some degree, not in such a manner as to pro-

duce one or a few large vacuoles, but as to produce an irregular canicular network of vacuolar substance in the nucleolus (Figs. 272-277, 279-281). This process often goes so far that in the largest nucleoli the deeply staining ground substance may appear in the form of a skein of threads, or merely of scattered granules surrounded by vacuolar substance. Especially on preparations stained by the Ehrlich-Biondi method is the skein-like arrangement of the ground substance well marked. I have no doubt that it was the observation of similar nucleoli in like stages which led Carnoy to the assumption of a "nucléole-noyau," that is, a nucleolus with a limiting membrane, and containing a wound thread of chromatin; it is probable that Carnoy mistook the reticulum of the true ground substance of the nucleolus for chromatin, and considered what is really vacuolar substance to be the original ground substance; only studies on the genesis of a nucleolus can explain its various components.

In the largest ova found in the body cavity the nucleolus reaches its maximum size (Figs. 279-281). It contains a greater amount of vacuolar than of ground substance, and instead of being regularly oval, as it was before, is often quite irregular in form, and very frequently apposed to the nuclear membrane (a position not noticed in any of the preceding stages). Whether this irregularity of form denotes the commencement of a degeneration of the nucleolus I cannot say, since I had no preparations of the stages of reduction.

Two nucleoli were found in only two germinal vesicles (Figs. 262 and 266), and in a spirem stage of an ovogonium three small nucleoli were present in one nucleus (Fig. 261). In the hundreds of other resting nuclei examined a single nucleolus was invariably present. These exceptional cases must, therefore, be considered abnormal, and not typical for certain stages of the nucleus.

In the larger germinal vesicles there is a peculiar body in contact with the nucleolus, which remains to be described. This body (*ux.*, Figs. 272, 274-277, 279, 281) is homogeneous, somewhat refractive, and lies either in close contact with the surface of the nucleolus, projecting beyond the periphery of the

latter, or (and this is the rule for the largest, irregular nucleoli) it is imbedded in the peripheral portion of the nucleolus ; in the former position it is concavo-convex, in the latter, biconvex in outline, always being thickest in its median diameter. With the Ehrlich-Biondi staining method it almost invariably colors yellowish, and in only one or two cases did it stain somewhat similarly to the ground substance of the nucleolus ; after fixation in Flemming's fluid, and staining with safranin, gentian violet, and orange G., it always appeared yellowish, while the ground substance remained wholly unstained. The largest nucleoli, *i.e.*, those of the largest germinal vesicles, have always at least one of these bodies in contact with their surface, but quite frequently two may be found on opposite sides of the nucleolus, and in one case I found three (Fig. 277). Those of different nucleoli vary slightly in their dimensions, but my observations give no clue as to their origin. All that can be said of their growth is that in the smaller nucleoli they lie upon the surface of the latter, while they are sunk into the peripheral portion of the larger nucleoli. It differs both chemically and structurally from the ground substance of the nucleolus, and from the vacuolar substance ; and it would seem to be derived from some part of the nucleus outside of the nucleolus, since it at first lies upon the surface of the nucleolus. This body may be comparable to the "Nebennucleolus" described by Flemming in the egg of *Anodonta* ; but I have found no structure in any of the other ova here examined which is identical with it.

Yolk globules are assimilated by the nucleus from the cytoplasm, though without the production of amoeboid processes. Such assimilated globules are usually of small size, but sometimes large, compound ones are taken into the nucleus (Figs. 267-269, 272, 274, 280); they occur most frequently singly or in small masses close to the inner surface of the nuclear membrane (Figs. 274 and 280) in almost all of the larger germinal vesicles, and in a few cases some globules may be found near the center of the nucleus. Careful observation shows that the yolk globules really occur within the nucleus, and are not artificially removed there by the knife in sectioning. Usually these

stain in the same manner as those contained in the cytoplasm. But occasionally from one to three of the larger globules (Fig. 267) in the nucleus stain much more intensely than the others, though intermediate degrees of staining are to be found between these largest, most deeply colored ones and the smaller, less deeply stained ones; so that there can be no doubt of the genetic relation of the two kinds. By staining with eosin these largest yolk globules in the nucleus stain almost or quite as deeply as the nucleolus itself, so that at first I mistook them for nucleoli; but that they are chemically metamorphosed yolk globules and not nucleoli is shown, even leaving aside the fact that all intermediate forms may be found between them and the less deeply staining globules of the cytoplasm, by the fact that vacuoles are never found within them. By the Ehrlich-Biondi staining method no color differentiation was to be obtained for the larger and smaller yolk globules of the nucleus. But nevertheless I would think that these large yolk globules (or accumulations of such globules) which have been taken into the nucleus from the cytoplasm and there have undergone some degree of chemical change, possibly stand in genetic connection with that body which is apposed to the nucleolus in the larger germinal vesicles, and which has been described in the preceding paragraph.

*Chromatin.* — We found the chromatin in the primitive peritoneal cells and in the youngest ovogonia to be arranged in the form of granules (Figs. 250–254). In the following mitoses it is arranged in the form of a spirem, then of chromosomes, and again of a spirem (Figs. 255–261). Just after the conclusion of the spirem stage (of the metaphasis) it comes to lie in a more or less dense mass around the nucleolus, this mass appearing to be composed of a reticulum of short fibers (Figs. 263–266, 270, 271, 278). In all these stages the chromatin is marked by its deep blue staining with haematoxylin. After the last stage described it gradually departs from the close vicinity of the nucleolus and becomes evenly distributed throughout the nucleus. But when it has thus become diffused it does not stain with haematoxylin as before, but appears in the form of a very large number of minute microsomes, which

appear not to stain at all, and of a few delicate fibers, which stain a lilac color (Figs. 267-269). As the germinal vesicle increases in size these chromatin fibers gradually become thicker and more numerous, commence to stain more deeply with haematoxylin, and gradually connect together to build a chromatin reticulum; the minute, unstained microsomes still occur between these fibers. Finally, in the largest nuclei at my command, and ones which had been fixed with the fluid of Flemming and stained by the triple stain of this cytologist, we find, in addition to the abundant unstained microsomes, short, rod-like masses of chromatin, which stain deeply with gentian violet, and each appears to be formed of a row of granules or thickened discs (Fig. 280). Whether the minute microsomes are true chromatin or are lanthanin (oedematin) granules is open to question; the latter assumption might be the correct one. We notice two remarkable phenomena in the chromatin changes just depicted: (1) the grouping of the chromatin in the center of the nucleus, around the nucleolus, at the completion of the mitotic stages; and (2) immediately subsequent to the preceding, the lilac stain of the chromatin after haematoxylin. Now, concomitant with the former of these two phenomena, the yolk makes its first appearance in the cytoplasm, and as we have shown above, usually in the close vicinity of the nucleus. It would be quite erroneous to conclude that the yolk globules are in any way produced by the chromatin, as *e.g.*, by a migration of chromatin particles out of the nucleus; for in this stage all the chromatin is removed from the periphery of the nucleus. On the other hand, however, I would suggest the hypothesis that the reason for the chromatin being removed from the periphery of the nucleus is because at this period the peripheral portion of the latter is chiefly concerned in the assimilation of yolk substance from the cytoplasm. In support of this assumption the fact may be recalled that in the following stage the chromatin fibers are stained a lilac color, as if they were stained with eosin, as well as haematoxylin, and not as before, simply with the former stain; this would show that during this period there is an addition of a cytoplasmic substance to the chromatin fibers, perhaps allied to the substance of the yolk globules, and

this substance would superinduce the lilac staining of the chromatin threads. This addition of a probably nutritive substance would seem necessary, in order that the amount of the chromatin continue to increase as the nucleus itself grows larger. Subsequently all that nutritive substance attached to the chromatin threads would seem to become metamorphosed into chromatin, since in the largest germinal vesicles these threads again stain a deep blue. And as a matter of fact, the quantity of the chromatin must increase with the growth of the ovum, since it can easily be demonstrated that in the larger nuclei there is an absolutely greater amount of this chromatin present than in the nuclei of the primitive peritoneal cells.<sup>1</sup>

11. *Piscicola rapax* (Verr.) (= *Pontobdella rapax* Verr., which Dr. Percy J. Moore assures me is a true *Piscicola*).

(Plate 29, Figs. 300-316.)

(The ovary is a tubular, contorted sack; from its inner surface numerous smaller, likewise tubular (round on cross-section), acini project into its cavity, each acinus containing numerous ovogenetic stages, the least mature of which lie at its proximal end, the most mature at its distal. These several acini are not continued as far as the external opening of the ovary, but their distal ends apparently open into a large ovarian cavity, and the ova drop into this cavity before they can arrive at the external genital opening. Each single acinus of this leech may be compared to either of the two whole ovaries of *Ascaris*.)

The youngest ovarian stages are small ovogonia in stages of mitotic division (Fig. 300). In them no nucleoli were to be seen; a minute nucleolus might be present in each of these nuclei and be obscured by the dense mass of chromatin. In all stages subsequent to these a single nucleolus is present in the nucleus (now a germinal vesicle) until the pole spindle is formed; in the smaller nuclei the nucleolus is usually oval, in the larger ones spherical. The growth of the nucleolus keeps

<sup>1</sup> For the researches of other authors on germinal spots of polychaetous annelids, cf. the reviews of the papers of Korschelt ('89, '95), Graff ('88), Giard ('81), Vejdovský ('82), Eisig ('87), Fraipont ('87), Mead ('95), Fauvel ('97), Michel ('96), and Carnoy ('84).

pace proportionally to that of the nucleus (Figs. 301-304). Then vacuoles arise in the nucleolus, these being neither very numerous nor very minute (Figs. 304-310, 312-316). The time when these vacuoles first arise is very variable, though in the majority of cases they do not appear before the nuclear sap begins to stain red. The size of the nucleolus does not always stand in the same proportion to that of the nucleus. Its ground substance is dense, stains deeply with eosin, and no limiting membrane is present; but by the use of the double stain Lyons blue and acid carmine, whereby the nucleolus stains blue and the chromatin red, a deep red line appears to surround the nucleolus: I cannot determine whether this line is a nucleolar membrane or a layer of chromatin, or whether it is merely an appearance caused by the refraction of the nucleolus.

When the nucleolus first appears it is usually situated at that pole of the nucleus opposite the chromatin mass and is not in contact with the nuclear membrane (Fig. 301). In nuclei of intermediate size, before the nuclear sap commences to stain with eosin, it is most frequently in contact with the nuclear membrane (Figs. 302-304); but in the largest germinal vesicles it is never in contact with this membrane, though often lying excentrically in the nucleus.

As soon as the germinal vesicle has nearly, or quite, attained its maximum dimensions (quite frequently, however, in those of still smaller size) two very noticeable changes take place in it: (1) the chromatin assumes a different form and stains differently (these chromatin changes shall be delineated later); and (2) the nuclear sap, which had heretofore remained colorless or was merely of a light lilac shade (by the double stain haematoxylin and eosin), now becomes a yellowish-red color, so that the nuclei in this stage may be easily distinguished from those of preceding ones (Figs. 304, 305, 307-310, 316). Simultaneously two changes occur in the nucleolus: (1) it stains no longer a deep red with eosin, as before, but a yellowish red, and appears more refractive; and (2) the several vacuoles within it gradually fuse together and so produce a larger one, which has usually a central position. The fluid, structureless

substance of the vacuole stains more faintly than the ground substance of the nucleolus, and has much the same color shade as the nuclear sap. In certain germinal vesicles, which appear to be of a somewhat later stage of development, numerous small globules (*n.D.*, Figs. 306 and 310) are scattered through the nuclear sap; they stain with eosin a little more deeply than the last-named nuclear portion, vary in number and size, and have no regular distribution. In one case (Fig. 316), which stood in a stage immediately antecedent to the pole spindle formation, where there was a centrosome at either end of the nucleus in the cytoplasm (the nuclear membrane had not yet disappeared), such globules were present in the nucleus; so that we may infer that these globules are one of the latest formations in the germinal vesicle before the pole spindle is formed. I have not found any stages between the stage just described and the perfectly formed spindle (Fig. 311). About fifty or sixty ova were examined in the stage of the first pole spindle, and in all of them the nucleolus had completely disappeared, and no trace of it could be found either in the nucleus or in the cytoplasm.

What has been the manner of this disappearance of the nucleolus? Its total disappearance must occur within a relatively short time, since otherwise one would expect to find stages showing this process. The observations which I was able to make would demonstrate at least the mode of the commencement of the vanishing of the nucleolus. We have seen that when the germinal vesicle has attained its greatest size or, in some cases, a little before its maximum size is reached, its nuclear sap stains red; therefore some substance must be suspended in the caryolymph at this period which was not contained in it before. Now such a substance must have been derived either from other elements of the nucleus or from the cytoplasm. It has probably not been derived from the cytoplasm, since the nuclear membrane at this stage has its maximum thickness and hence could not be easily penetrable; and also there is no appearance of any similar substance in the cytoplasm, since no yolk globules or other nutritive elements seem to be present, but the whole cytoplasm (at least the



nodules of its meshes) stains a lilac-blue color. And since it is wholly improbable that it should be derived from the chromatin we must conclude that it takes its origin from the nucleolus. In other words, a substance emanates from the nucleolus and dissolves in the nuclear sap, and this process must be regarded as the commencement of the dissolution of the nucleolus. In support of this conclusion is the fact that in many germinal vesicles the nuclear sap stains most intensely in the neighborhood of the nucleolus (Fig. 309). Further, the minute red-staining globules which later occur in the nuclear sap must also be nucleolar in point of formation, *i.e.*, be either a substance given off in globular form from the nucleolus, or be accumulations (perhaps chemically changed by the action of the nuclear sap) of that nucleolar substance which has already diffused through the nucleus. Of importance in this connection is the fact that these globules are often found in contact with the nucleolus (Figs. 306 and 316). In all preceding stages the nucleolus is regularly oval or spherical in outline, but in the largest germinal vesicles not only may the size of its contained vacuole be increased to such an extent that the original ground substance forms only a thin shell around it (Figs. 308, 312, 314), but also its outline becomes frequently irregular (Fig. 313); and in one case I found it broken at one pole, so that its large vacuole communicated with the cavity of the nucleus (Fig. 315). A morphological change in the shape of the nucleolus which seems to take place with great regularity consists in the indentation of the nucleolar wall at that point where it is thinnest (Figs. 308, 314, 316). It would seem that the pressure from without, *i.e.*, the pressure of the nuclear sap, being greater than the pressure of the fluid within the vacuole, would cause the nucleolar wall to be pressed in at that point where it is thinnest. The fact remains that the nucleolus persists in the nucleus until a very short time before the production of the pole spindle, and when the latter is formed no trace of it can longer be found in any part of the nucleus or cell. And since there is no reason for supposing that it is extruded from the cell we must assume that it dissolves within it. The red-stained substance and small globules

within the nucleus would show that dissolution commences in the nucleus; and we must assume that when the nuclear membrane has disappeared the cytoplasmic substances which then come into contact with the nucleolus would cause its rapid and total dissolution. It may be remarked that in the region of the fully formed spindle (Fig. 311) no trace of the red-stained nuclear sap is longer to be seen; accordingly this sap with its contained nucleolar substance must either have been distributed through the cytoplasm or have been chemically changed by that portion of the latter which immediately surrounds the spindle.

In the ovary no ova are to be found which have advanced beyond the production of the first pole body, so that the formation of the second pole body must occur after the egg has been discharged from the ovary; I had no material at hand to enable me to determine the relation of the nucleolar substance in the female pronucleus.

Of considerable morphological interest are the metamorphoses of the chromatin in the various ovarian stages. In those small ovogonic mitoses (Fig. 300) from which the true egg cells (first ovocytes) are derived aster and dyaster stages are to be found; with the lens used for this study (the homogeneous immersion  $\frac{1}{12}$  of Zeiss) I could not determine the form of the chromosomes. As the ovum increases in size the dense chromatin mass of the aster gradually loosens, until up to the time when the nuclear sap commences to stain red (Figs. 301-304) the chromatin is arranged in the form of rather numerous granules, which are situated mostly close to the nuclear membrane. Thus far the chromatin has stained intensely blue, with the double stain haematoxylin and eosin; but when the nuclear sap begins to stain with eosin a marked change takes place in the character and arrangement of the chromatin; it now stains a lilac color, often more reddish than bluish, and has no longer a peripheral position, but becomes arranged in the form of threads, sometimes in the form of a small number of loops, the two ends of each loop being joined together (Figs. 304, 305, 307, 309). In some of the larger germinal vesicles absolutely no trace of chromatin can be found (Fig. 316). In the equator

of the first pole spindle (Fig. 311) lie twelve small chromosomes, which stain an intense blue black with haematoxylin and have an oval or slightly elongate form. It remains for investigators working with more abundant material and with stronger microscopical lenses, to penetrate more deeply into these phenomena of the chromatin changes, but it would seem that the chromosomes of the first pole spindle have the value of either tetrads or dyads. The lilac or even reddish stain of the chromatin at a particular period would seem at first sight to be due to the assimilation by the chromatin of that nucleolar substance diffused in the nuclear sap; but even as probably it might be due to the mere penetration of this substance between the individual microsomes of each chromatin thread, without any chemical change of the chromatin substance (Fig. 309). The red-staining globules in the nuclear sap, which I have assumed to be of nucleolar derivation, cannot be considered as metamorphosed portions of chromatin substance, since they vary so considerably in size and number; this point needs to be emphasized, since in some of the larger germinal vesicles no trace of chromatin is to be seen, and it might be thought by some one that these globules, which occur in such nuclei, represented the supposedly absent chromatin. (Platner, '89c, had, in *Aulastomum* seen only nucleolar fragments and overlooked the true chromosomes.) Where in the largest germinal vesicles, before the formation of the pole spindle, the chromatin appears to be absent in the nucleus, we must assume that it is merely obscured by the large amount of diffused nucleolar substance.

In the first pole spindle (Fig. 311), after treatment with Flemming's fluid or with corrosive sublimate, the mantle fibers have a remarkable thickness and appear even thicker than in Fig. 311; they stain a reddish-lilac color with the haematoxylin and eosin stain, not a lilac blue, as do the rays of the asters and the cytoplasm; I could not determine whether they extend quite to the centrosomes. I am also unable to decide whether each chromosome lies upon a single spindle fiber which extends from centrosome to centrosome, or whether its ends are connected with separate fibers. The centrosomes are rather large,

refractive granules, and stain with cosin ; they were present in one egg, close to, and opposite, the two poles of the nucleus, before the nuclear membrane had disappeared (Fig. 316), so that they may be extranuclear in origin. The radiations of the asters are very clear, especially after fixation in Flemming's fluid, and may be traced nearly to the cell membrane. Immediately around each centrosome a central portion of the aster is differentiated, namely, an attraction sphere (in the terminology of van Beneden), and this differs from the remaining portion in staining less intensely, and appears to be quite sharply bounded from it. In this attraction-sphere the cytoplasmic granules are smaller and more densely grouped, so that at first sight it might appear to consist of a homogeneous "archoplasm," but careful study shows that in it the cytoplasmic microsomes are arranged in radial rows around the centrosome, and each of these rows appears to be continuous with a ray of the outer aster. Or, to express it differently, the microsomic rays of the sphere extend to the centrosome, but this terminal part of each ray differs from the remaining distal portion in that its microsomes are smaller and closer together. Thus in *Piscicola* the finer structure of the attraction-sphere seems to have much resemblance to that of *Ascaris*, as described by Kostanecki and Siedlecki (*Arch. mikr. Anat.*, 48, 1896).

It remains to describe the mode of arrangement of the ova within each ovarial acinus. The proximal, small end of the latter is filled with small ovogonia (the youngest stages), and from mutual contact these are polygonal in form (Fig. 300). As we proceed towards the distal end of the acinus (Fig. 301) the ova not only become gradually larger, but have a different arrangement, in such a manner that they become epithelially grouped along the wall of the acinus, each cell having a pyramidal shape, with its apical end directed towards the central cavity of the acinus. A little more distally in the acinus (Figs. 302 and 303), the ova become not only larger, but fewer of them are to be found on a given transverse section of the acinus ; the individual ova have more of an oval shape and become separated from one another. Now when we proceed still further towards the distal end of the acinus (Fig. 304) we find a single

ovum commencing to outstrip the others in point of size, *i.e.*, in rapidity of growth, until we reach a point where this fortunate cell nearly fills the whole cavity of the acinus, driving the neighboring ova aside. Those cells which come into contact with such a rapidly growing ovum, as well as those in more proximal portions of the acinus which did not chance to lie close to the wall of the acinus, do not develop further, but disintegrate, and various stages of such disintegration may be seen in the cavity of the acinus, such as irregular cells with a nucleus, those which have lost their nuclei, and finally refractive cytoplasmic masses which stain deeply with eosin (the cytoplasm of the developing ova stains with haematoxylin). Perhaps such degenerated masses of cellular substance are destined to be assimilated by their more fortunate brethren. Often a number of such degenerating ova are to be seen grouped at one pole of a large ovum, and these cases present a certain similarity to cleavage stages, the large ovum resembling a macromere, the others micromeres. It is not difficult to find an explanation for the disintegration of certain of the ova, for only those close to the wall of the acinus can procure nourishment in amount sufficient for their growth, since this nourishment must be derived through the wall of the acinus from the body cavity (there being no yolk in the ova); and the peripherally situated ova must obtain all the nourishment thus furnished, so that those in the center of the acinus simply die for want of food. Further, a particular ovum of those placed peripherally, if it procures a greater amount of nourishment than its neighbors do, because, *e.g.*, of being in contact with a greater surface of the wall of the acinus, grows faster than the others and, pushing them aside, eventually gets full control of the whole amount of nourishment, so that a slight advantage at the start would increase in value in a geometrical ratio. Here, accordingly, we have a beautiful example of that process termed by Roux "der Kampf der Theile ums Dasein," that cell becoming a mature ovum which has succeeded in obtaining the greatest amount of nourishment. It is also interesting to note the position of the nucleus within the growing ovum; in all the younger stages of the egg it is placed in that part of the cell

which is nearest to the wall of the acinus, *i.e.*, nearest to the source of the food supply ; only then does it come to occupy a central position within the cell, when the latter has attained its maximum size and the thickness of the cell membrane shows that the cell is assimilating little or no nourishment from without.<sup>1</sup>

*b. Somatic Cells.*

12. *Ganglion Cells of Doto.*

(Plate 21, Figs. 36-49.)

(I have studied those nerve cells which occur in the cerebral, pleural, and pedal ganglia. Three kinds of these cells may be readily distinguished and described in succession : (1) colossal cells, which are found only in the cerebral ganglion; (2) cells of medium size; and (3) small cells.)

*Colossal ganglion cells* (Figs. 43-49). — The number of the nucleoli in the nuclei of these cells varies from about six to thirteen; they are also variable in regard to the position which they occupy in the nucleus, and though always excentrically placed they never lie in contact with the nuclear membrane. Sometimes all the nucleoli in a given nucleus are of approximately equal size, but as frequently one or two are several times larger than any of the others. Where such larger nucleoli occur along with a number of smaller ones, the former are usually vacuolar in structure ; sometimes nearly all the nucleoli contain vacuoles, in other cases none of them are vacuolar. Quite often the nucleoli in a nucleus show slight differences in their staining intensity, and one of them may stain quite differently from the rest (Figs. 44 and 46). None of the nucleoli have limiting membranes. No cases of nucleolar division were found, unless those cases where two nucleoli lie near to one another may represent the completion of such a division.

*Ganglion cells of medium size* (Figs. 37-42). — In these the nucleoli vary in number from one to four, two or three being the rule. Those of the same nucleus frequently show differences in size and form, as well as slight staining differences. In only one

<sup>1</sup> For the observations of other writers on germinal spots in *Hirudinea*, cf. O. Hertwig ('76), Leydig ('49), Whitman ('78), and Platner ('89c).

case (Fig. 41) I found three nucleoli of approximately equal dimensions and homogeneous ; usually they vary somewhat in size and contain vacuoles. The shape of the nucleoli is either spherical or oval, or it may be irregular ; certain ones stain scarcely at all, and appear granular : these might represent cases of degeneration.

*Smallest ganglion cells* (Fig. 36). — Here a single nucleolus is the rule, though two may occasionally be found. They are spherical or oval, and vary considerably in size. Vacuoles do not seem to occur in them, though they might well escape observation from the small dimensions of the nucleoli, which often renders it difficult to distinguish the nucleoli from the larger chromatin granules.

In all these ganglion cells the chromatin appears in the form of small granules, but on a preparation fixed with Hermann's fluid and stained with Lyons blue (Fig. 45) it appeared as a network ; in this preparation the granules seemed to be united by fine fibers, which stained less intensely than the granules. But even here the connecting threads might consist rather of linin than of chromatin, since the solution of Lyons blue employed by me stained all the nuclear substances except the nuclear sap (paralinin). Such fibers often appear to radiate outwards from the surface of the nucleoli, as if the latter were suspended by them. The nucleoli always stain differently from the chromatin.

There is, as a rule, a relatively small amount of nucleolar substance in the cells of the second and third types in comparison with most of the other nuclei which I have examined ; but the nuclei of those of the first type, on the contrary, usually contain a relatively large amount of this substance, for not only may one or two of the nucleoli in a nucleus be quite large, but also a considerable number of nucleoli are frequently present.

13. *Ganglion Cells of Montagua pilata* (Verr.).

(Plate 22, Figs. 90-97.)

(The same types of cells may be roughly distinguished as in *Doto*.)

*Colossal ganglion cells* (Figs. 90-92, 94-97).—In the nuclei of these there are never more than from one to three nucleoli, which neither contain vacuoles nor become noticeably irregular in size, as is the case in *Doto*. Most frequently only a single nucleolus is present. It is the rule that they are oval and not spherical, though in some cases they may appear perfectly spherical; perhaps the great majority of them are oval and seem to be spherical only when they do not chance to be longitudinally sectioned. Their substance is perfectly homogeneous, without a limiting membrane. When two or three occur in the same nucleus they are usually of approximately equal dimensions (Figs. 94 and 95). Further, it would seem to be the rule that when one nucleolus is present in a nucleus it is larger than any one of the two or three which may be found in other nuclei; but, nevertheless, the relative amount of nucleolar substance seems to vary in different nuclei.

*Ganglion cells of medium size* (Fig. 93).—Here one or two nucleoli are present in each nucleus, and these are of homogeneous appearance.

*Smallest ganglion cells*.—The nucleoli are similar to those of the corresponding cells of *Doto*.

On a preparation preserved in Flemming's fluid I find many of the nucleoli present a different structure from those fixed with corrosive sublimate or Kleinenberg's fluid. Thus many of them do not appear homogeneous, but finely granular and refractive (Figs. 96 and 97). On the surface of such nucleoli occur small, refractive, yellowish globules, which appear black or yellow, according to the focus of the microscope; some of them are very small. These never occur within the nucleolus, but only on its periphery. They may easily be distinguished from the chromatin granules by their rounded form and high degree of refrangibility, as well as by their deeper yellow color (this preparation had been stained with haematoxylin and eosin, but the nuclei had not become stained, probably owing to too long a fixation in the fluid of Flemming). Numerous other nuclei on the same sections showed none of these globules, and none were to be seen on preparations which had been differently preserved. Accordingly, I consider them to be artefacts,



caused by (1) the direct action of the fluid of Flemming, or more probably (2) they might be post-mortem exudations of the nucleoli, which might well be produced before the slowly penetrating fixative had reached to the cells in question. At any rate, they cannot be regarded as normal structures. Do they represent the "Kernkörperchenkreis" of Eimer?

The chromatin, as in *Doto*, occurs in the form of granules, which are connected by fine fibers. After fixation with Kleinenberg's fluid a clear space encloses each nucleolus (Figs. 93 and 94); but this space is not to be found after fixation in other fluids.

As in *Doto*, the nuclei of the colossal ganglion cells contain a relatively greater amount of nucleolar substance than do those of the second and third types. But in the former genus there are in the colossal cells from about six to thirteen nucleoli, and these vary noticeably in size and structure, while in *Montagua* there are only from one to three, which are always homogeneous and usually quite equal in dimensions. Why should there be this marked difference in the form and number of the nucleoli?<sup>1</sup>

14. *Ganglion Cells of Piscicola rapax* (Verr.).

(Plate 23, Figs. 134-136.)

In the ganglia of the brain occur cells of different dimensions. Each nucleus contains most usually a single small spherical nucleolus; seldom are there two present, and in these cases they are unequal in size. None of the nucleoli contain vacuoles. They are excentric in position, but are never in contact with the nuclear membrane. These nucleoli are small in proportion to the size of the nucleus.

15. *Muscle Cells of Lineus gesserensis* (O. F. M.).

(Plate 21, Figs. 51-56.)

(The nuclei of the circular muscular layer of the body wall were studied. Those of *Cerebratulus lacteus* Verr. are essen-

<sup>1</sup> For other observations on nucleoli in ganglion cells of molluscs, cf. the reviews of the papers of Pflücke ('95), Leydig ('83), and Rohde ('96).

tially similar to those of *Lineus*; in the metanemerteans they are too small for satisfactory study.)

These nuclei are very variable in shape, all extremes being found between ovoid or oval and elongate rod-like forms. But they are rarely angular. I have remarked in a previous contribution that the nuclei of the muscle cells are more variable in form than those of the cells of any other tissue in the nemerteans, and now I would offer the following explanation for this variability: when the muscle fiber (a single, smooth fiber with attached nucleus constitutes a muscle cell) contracts, this contraction must produce likewise a contraction (shortening) of the nucleus; but when the fiber expands the form of the nucleus must become more elongate, corresponding to the elastic extension of the fiber, for the fiber cannot contract without causing a shortening of its nucleus, since the latter is closely adherent to it.

One very small nucleolus is usually to be seen in each nucleus (Figs. 51-54, 56); sometimes it does not appear to be present (Fig. 55), but whether in these cases it is absent or only escapes observation by reason of its minute size, it is difficult to decide; in the greater number of nuclei it may be seen by careful focussing of the microscope. It most usually lies very close to the center of the mass of nucleoplasm, so that if the nucleus be larger at one pole than at the other it is situated in the larger end, while in elongate nuclei, of nearly equal diameter throughout, it usually lies at an early equal distance from both ends of the nucleus. The nucleolus may be said, as a general rule, to occupy the center of the nuclear substance, and is not often markedly excentric; in none of the other cells examined in the course of these investigations did the nucleoli show a similar tendency to occupy the center of the nucleus. The nucleolus always stains differently from the chromatin.

The relative amount of chromatin varies in different nuclei. It is always found, after the action of various fixatives, to occur in the form of small granules, which are connected by delicate irregular fibers, which stain exactly as the granules do. The nuclear sap stains faintly with haematoxylin (this has not been shown in the figures). The nucleolus is either in contact

with chromatin granules or with fibers of chromatin, which pass between it and the nuclear membrane; there is never a clear space around the nucleolus, but it seems to be held in position by the chromatin.

16. *Muscle Cells of Piscicola rapax* (Verr.).

(Plate 29, Figs. 325-337.)

(The nuclei of the longitudinal muscle layer of the body wall were studied. For the examination of the different stages of these nuclei worms of different sizes must be studied; I examined the nuclei of leeches of about 6 mm. in length, where the cells and their nuclei are smallest, as well as of larger and fully mature individuals, where these cells and their nuclei attain their maximum dimensions.)

In the smallest nuclei (Fig. 325) a single nucleolus is invariably present and lies centrally; it is of medium size, more or less oval in outline, and contains a varying number of small vacuoles. In larger nuclei it becomes larger and more elongate in form, lying in the longitudinal axis of the nucleus (Figs. 328 and 331); at the end of this stage its greatest dimensions are reached. Next commences a process of fragmentation of this original nucleolus into a number of smaller nucleoli, which are of different sizes. There appears to be little uniformity in the mode of this nucleolar division (Figs. 327, 329, 332, 333): the nucleolus may become dumbbell shaped and then divide into two larger pieces; or when much elongated it usually breaks simultaneously into a number of consecutive portions; or buds of nucleolar substance may be divided off from its surface. This segmentation is not strictly dependent upon the size of the nucleus, nor upon the size or form of the nucleolus. The fragmentation continues, the larger daughter-nucleoli also dividing, until in the largest nuclei (those of the mature worm) as many as twelve small nucleoli may be present, which are irregularly distributed through the nucleus (Figs. 335-337). In all these stages at least some of the nucleoli contain vacuoles, though they have not been reproduced in all the corresponding figures.

All the nucleoli of the largest nuclei are thus produced by a

series of divisions from the single original one. This division usually commences, then, when the form of the nucleus changes from the original oval to a more elongate shape. It seems probable that this elongation of the nucleus may directly cause the division of the nucleolus, since the long axis of the latter coincides with that of the nucleus; and were the nucleolus in any way fixed in position in the nucleus, the nuclear elongation would draw out the nucleolus and cause it to break into fragments. But the division of the daughter-nucleoli does not always take place in the direction of the long axis of the nucleus, so that some other factor might be at work to produce this division.

The chromatin is arranged in the form of a reticulation (Fig. 326). The nuclei of the younger cells are usually regular in outline, but those of the larger ones become very irregular; this irregularity of the contours of the nuclei is more marked by fixation with corrosive sublimate than with Flemming's fluid, so that it might be regarded as an artefact caused, *e.g.*, by the obstacle offered to the rapid penetration of the preserving fluid by the dense outer (fibrillar) layer of the cytoplasm in the largest muscle cells.

#### 17. *Blood Corpuscles of Doto.*

(Plate 22, Figs. 98-101; Plate 23, Fig. 102.)

(These cells are usually to be found abundantly in the cavity of the cirratida and of the sheaths of the tentacles, though their number varies greatly in different cirratida. They lie in the meshes of the loose network of mesenchym cells, either singly or grouped together into bundles. I have been unable to find them in other parts of the body. These cells appear to be free mesenchym cells, with perhaps the function of blood corpuscles.)

There is always a single large nucleolus, which is usually very large in proportion to the size of the nucleus. It varies in form from a perfect sphere to an elongate oval. The nucleolar substance is usually homogeneous, but in some cases it is granular (Figs. 99-102) and then it stains faintly as if it were

undergoing a degeneration. Quite frequently a small spherical granule lies in the center of the nucleolus and this always stains more intensely than the surrounding substance (Figs. 99-102). In only about half a dozen cases, out of hundreds of cells examined, did I find attached to the surface of the nucleolus one or two much smaller bodies, which also stained less intensely (Figs. 100 and 101). Can it be that in certain cases the nucleolus becomes differentiated into a "Hauptnucleolus" and a "Nebennucleolus," in which case these small bodies would represent the "Nebennucleoli"? In certain of the cirratida of a young individual the nucleoli of the greater number of nuclei were situated at that pole of the nucleus directed towards the median axis of the cirratidum, *i.e.*, those in the nuclei on the right side of the cirratidum were in the left-hand poles of the nuclei, and those in the nuclei of the left side of the cirratidum (as seen on sections) were placed at the right-hand poles of the nuclei. I did not observe this regular position of the nucleoli in the cirratida of the other individuals sectioned and hence would conclude that it was not a normal phenomenon, but an osmotic consequent of the fixing reagent.<sup>1</sup> The size of the nucleolus preserves approximately the same ratio to that of the nucleus.

The nucleus is either spherical or oval in outline. The apparent arrangement of the chromatin varies according to the fixative employed. After picro-nitro-osmic acid (Fig. 102) it appears granular; after Hermann's fluid (Figs. 99-101), in the form of delicate fibers which radiate from the nucleolus to the nuclear membrane; after alcoholic solution of corrosive sublimate (Fig. 98) we find a few fibers radiating from the surface of the nucleolus, but the greater amount of the chromatin appears in the form of granular masses, which lie mainly near

<sup>1</sup> In a previous paper ('96) I figured for the nuclei of those mesenchym cells which surround the distal end of the ventral nerve cords of *Cerebratulus lacteus*, the nuclei with their chromatic masses pressed against that side of the nuclear membrane which was situated nearest to the central point of the section. At that time, I regarded this excentric position of the chromatin as a normal but peculiar phenomenon; but now, on comparison with the cells of *Doto*, I am convinced that it is an artefact produced by the osmotic action of the fixing reagent.

the periphery of the cell, so that the nucleolus is surrounded by a clear space. These nuclei thus offer a suggestive object lesson, to teach how careful one must be in the determination of the form of delicate cellular structures by the study of preserved material.

Cells which are isolated have a spherical form ; those grouped together are polygonal, owing to their mutual pressure (Figs. 99 and 101). A cell membrane is present. The cytoplasm is for the most part finely granular ; portions of it, however, are always more dense and stain more deeply than the former portion ; there are great individual differences in different cells (Figs. 100 and 102). Often the cytoplasm is more or less vacuolar or a clear space may partially surround the nucleus and a similar space be present between the cytoplasm and the cell membrane, this space being transversed by a few radiating fibers. Such spaces are best shown after the action of the fluid of Hermann ; they are seldom to be seen after fixation in micro-nitro-osmic acid ; but whether a coarse alveolar layer of cytoplasm at the periphery of the cell be normal or be an artefact, there are certainly marked differences in the structure of the cytoplasm in neighboring cells, and these differences might be regarded as the morphological changes corresponding to functional phases in the cells. Cases of degenerating cells are numerous, and may be recognized by their faint staining properties and by their granular appearance.

#### 18. *Giant Cells of Doto.*

(Plate 30.)

(These enormous cells, which are the largest cells in the body, not excepting the ova, lie at the anterior part of the body just behind the head region and are closely apposed to the folds of the nidamental gland. They do not produce a closed mantle on the outer surface of this gland, but either are isolated or occur in small groups of from two to four cells each. In each individual their number appears to be about thirty or forty. These cells do not seem to have any open communication with the neighboring tissues, and I cannot

conclude from their structure what their function is; perhaps they have a function similar to that of lymph glands. Such cells are absent in *Montagua*.)

The form of these is a more or less polyhedral one, caused by the pressure of the surrounding organs (Fig. 339). The nucleus is relatively and absolutely very large and is very variable in form, sometimes irregularly oval, sometimes with obtuse or pointed processes, or again concavo-convex, that side being convex which lies near the nuclear membrane (on a transverse section such a nucleus appears sickle shaped). The chromatin is arranged in the form of rather coarse granules (Figs. 339 and 342), which after fixation in Hermann's fluid (Fig. 340) appear to be the nodal points of a reticulum.

The nuclei (Figs. 338-346) are numerous, vary in number from about six to about forty, and are irregular in size. Their shape is usually oval, seldom perfectly spherical, though quite frequently, as the figures show, they may be more or less elongate or even very irregular in form. Vacuoles are frequently present in them. The nucleoli stain as do all true nucleoli, but different degrees of staining density may be observed in the nucleoli of the same nucleus (Figs. 338, 342, 346). In two cases, one of which is here figured (Fig. 342), a dense ring of chromatin was found around a nucleolus, but such cases, judging from their infrequency, must be regarded as very abnormal, if not attributable to the action of the fixing fluid. Divisions of the more elongate nucleoli certainly take place. Thus I have observed dumbbell-shaped nucleoli in three cases (Figs. 343, 345, 346), and Fig. 340 probably represents a stage just after a division, where two smaller nucleoli have apparently been divided off from a larger one, one end of the latter being drawn out to a point. Thus it might seem that the large number of nucleoli are produced by divisions of a smaller number of larger nucleoli. The variability in the size, form, and number of these nucleoli recalls those of the subcuticular gland cells of *Piscicola* (*cf. infra*); but in these cells of *Doto* I have been unable to make out different morphological phases.

The cytoplasm of these cells is also remarkably differentiated (Fig. 339). In a given cell certain portions of the cytoplasm

may be dense and stain deeply ; other portions are less dense in structure, with a corresponding less intensity of stain ; and still other portions of the cell substance appear structureless and do not stain at all. The cytoplasm in at least a portion of the peripheral area of the cell is always dense and deeply staining ; rarely is the cytoplasm in the whole cell of this dense structure. With low powers of magnification (*e.g.*, Zeiss Obj. C, oc. 2 or 4) there may appear to be either several cavities in the cytoplasm or a single large one at one side of the nucleus. These differentiations of the cytoplasm (which fixation in corrosive sublimate or in Hermann's fluid bring out always in the same manner) probably denote certain metabolic states of the cytoplasm, but it would be difficult to determine from the structure alone to what physiological processes these states might correspond. There is no definite secretion produced by the cytoplasm, *i.e.*, no secretion with a definite form. As has been noted, a wholly or nearly wholly clear space often occurs in the cytoplasm at one side of the nucleus ; such a space usually lies at that margin of the nucleus situated closest to the center of the cell, and the nucleus may often surround it to some extent. Where the nucleus comes into contact with this space its membrane is thinnest and its outline irregular, and quite frequently this margin of the nucleus is produced into long, irregular, amoeboid processes, which extend into the space in question and pass around it. These appearances would show that the nucleus stands in a certain functional relation to the metabolic changes of the cytoplasm, not improbably that it assimilates certain substances produced in the latter.

To return to the nucleoli, I cannot find any genetic connection between these structures and the cytoplasm. They are usually grouped near the center of the nucleus, and though often quite peripheral in position, never come into contact with the nuclear membrane, nor are they found in the amoeboid processes of the nucleus. It will be necessary to study very young individuals of this mollusc in order to determine the mode of nucleolar development.

The cell (Fig. 339) is developed by a delicate membrane, which seems to be interrupted at no point on the surface of the



cell. The cell has thus no external openings and no ducts or fibers which penetrate into the enveloping tissues.

19. *Subcutical Gland Cells of Piscicola rapax* (Verr.).

(Plate 25; Plate 26, Figs. 198-203.)

(These cells lie for the most part in the body cavity, between the body muscular wall and the intestine. Two modifications of them may be distinguished: (1) those at the ends of the body, near the suckers and in the wall of the latter, which are comparatively small, and the relatively short cell ducts of which open at all points of the surface of the body at the ends of the body and on the inner surface of the suckers; these seem to resemble the second modification in all respects except size; (2) the larger type of these gland cells, which I have studied exclusively, are massed together in that portion of the body cavity which extends from the region a little anterior to the brain, nearly to the posterior end of the body, the greater number of them being in contact with the inner surface of the body wall.)

In order to find all the functional stages of these cells one must study preparations of worms of various dimensions, since all the stages cannot be found in a single individual; I made consecutive series of sections of seven different individuals, the smallest being about 6 mm. in length, and the largest being fully matured. The remarkable cycles of the nuclear and cell stages, to be described below, were equally well discernible with all three of the fixatives employed, namely, Flemming's fluid and alcoholic and aqueous solutions of corrosive sublimate; various double stains were used.

These cells, when they reach their fullest dimensions, are so enormous that they may be readily seen with the naked eye. Their single ducts all open on the surface of the body, between the epithelial cells, a little anterior to the region of the sexual pore; their openings are at this point equally numerous on the dorsal, lateral, and ventral sides of the worm. The most posterior gland cells of the body send their ducts a distance of four-fifths the total length of the body before they open on the

surface of the latter, these ducts transversing a large number of body segments (in certain of the enchytraeid *Oligochaeta* there have been described subcutical gland cells whose ducts pass through a number of segments, but I believe that they are not of the same relative length as those of *Piscicola*). Each cell has its own duct, the latter being morphologically merely a process of the cell (Figs. 178, 181, 202); and as these individual ducts run in bundles parallel to one another, on their way to the surface of the body, they become closely apposed to one another, but there are apparently no open communications between the several ducts, nor do they unite to form larger, compound ducts. The ducts of those gland cells which are situated behind the sexual pore necessarily have an anterior direction, while those which are situated near to the head end of the animal send their ducts posteriorly. The duct departs from the cell more or less at right angles from its distal end, *i. e.*, that end which is usually directed towards the central axis of the worm. Since the greater number of these cells become filled with secretion only when the worm is sexually mature, and since they all open on the surface of the body near the sexual pore, they have probably the same function as the clitellar glands of the *Oligochaeta*; after these observations had been completed I found that Bourne ('84) had described such gland cells in *Pontobdella* as "clitellar glands," but he made no observations on their finer structure.

In studying the cycle of the structural changes of these cells two main morphological periods may be distinguished: (1) the *prophasis*, from the immature cell to the cell filled with secretion; and (2) the *metaphasis*, from the time when the cell begins to discharge its secretion until it becomes re-formed into a functionally immature cell again. I have no means of determining whether a given cell becomes filled with secretion only once a year (as, *e.g.*, at the period of sexual maturity) or whether it may secrete several times in succession during the sexual period. At any rate, all appearances lead me to conclude that it secretes periodically, most probably once during each period of sexual maturity. I have found no evidences that it secretes only once and then dies to become absorbed by the other tissues of the body; in other words, there were no

evidences of cell degeneration or of a formation of new cells, so that we must conclude that each of these cells continues to functionate periodically during the whole time of the existence of the leech.

We may now describe in succession the prophase and the metaphase of the structural changes.

*Prophasis* (Figs. 178–196). — In the smallest cells found in the youngest leech examined no trace of secretion is present (Figs. 178–180). In these the nucleus is usually central in position, with a delicate chromatin network, and with a single, most frequently oval, nucleolus, in which one or a few small vacuoles are commonly present. Around the nucleus, and filling the cell duct, is a somewhat dense cytoplasm, which becomes more vacuolar at the periphery of the cell. The chief cytoplasmic changes from now on are as follows (I have not figured these changes, since they may be briefly characterized): that portion of the cytoplasm close to the nucleus gradually becomes more dense and begins to stain differently from the rest, and then becomes quite homogeneous; most frequently there is a layer of this homogeneous substance between the nucleus and the cell duct, only that portion of the cytoplasm at the proximal end of the cell, as well as a thin layer around the homogeneous substance, retaining its primitive appearance. Next, this homogeneous mass gradually breaks up into the numerous secretion corpuscles (Fig. 181, *Secr.*), the shape of the latter being ovoid after fixation in corrosive sublimate, but more spherical after the action of Flemming's fluid. These secretion corpuscles stain at first just like the homogeneous substance, but gradually commence to stain otherwise, and in the functionally mature cell stain differently from the primitive cytoplasm, as well as from the homogeneous substance from which they were derived. The whole cell thus gradually becomes filled with these small corpuscles, until finally no trace of the original cytoplasm is to be seen, except a few faintly staining fibers. The cytoplasm which fills the duct undergoes the same morphological changes as that of the cell body just described, so that the first secretion corpuscles in it are the derivatives of its own substance; the cytoplasm of the

duct and of the distal portion of the cell are as a rule the first portions to become differentiated into the secretion. At the end of the prophase the cell has attained its maximum size, and the duct its greatest diameter, both containing hundreds of the mature secretion corpuscles lying in an unstained, structureless fluid. The duct in all stages is always larger at its proximal than at the distal end, though it narrows very gradually.

But the most interesting morphological changes are those of the nucleus. While the secretion is being produced in the cytoplasm the nucleus increases rapidly in size, and at the same time becomes very irregular in form, until in the nearly physiologically mature cell it attains enormous dimensions and sends out through the substance of the cell long branching processes, which anastomose with one another and some of which reach even to the cell membrane (Figs. 178-196). Korschelt has described (89) branched nuclei in the spinning glands of certain insect larvae, which are somewhat similar to the nuclei here delineated. The nucleus attains its greatest dimensions and its most marked degree of ramification when there is the greatest amount of the homogeneous substance in the cell, *i.e.*, just before this substance becomes metamorphosed into the secretion corpuscles. At this stage we find the greater portion of the nucleus situated at the proximal part of the cell, and from that point it sends out irregular branches which envelop the mass of homogeneous substance, and which penetrate into it. At this period, further, no two nuclei are alike in form, so that it would be in vain to attempt to figure all the shapes which they may assume. The nuclear membrane becomes very thin, often scarcely perceptible, around the branched processes. I know of no other nuclei which are more interesting in point of size and variability of form than these; and it would well repay accurate investigation in the endeavor to decide in what way they may influence or modify the cytoplasmic changes which are simultaneously taking place, for they obviously have a close physiological connection with the formation of the cellular secretion. Since the nucleus undergoes a rapid process of growth in these stages, we are obliged to assume that it is taking up substances from the cell body; but

it probably does not assimilate the mature secretion corpuscles, since when the latter are produced, as we shall see, the nucleus commences to retract in size and to withdraw its processes. As the nucleus increases in size its chromatin reticulum becomes looser, as if it were elastically stretched by the expansion in volume of the nucleus; the chromatin is continued into the ramifying processes of the nucleus.

The nucleolar changes during the prophase are as follows : in the immature cell there is invariably a single rather large nucleolus, which occupies a more or less central position in the nucleus (Figs. 178-181, 184) ; it may be either oval or spindle shaped, and most frequently contains one or several small vacuoles. Its substance appears homogeneous after treatment with corrosive sublimate, granular after the action of the fluid of Flemming, and has no limiting membrane ; in all its stages within the nucleus it stains very intensely, though always differently from the chromatin. Now as the nucleus increases in volume so also does the nucleolus, though at first at a relatively more rapid rate than does the former ; and in growing larger it gradually becomes more elongated, rod shaped, and at this stage is most frequently in contact with the nuclear membrane (Fig. 182). When it has taken up this peripheral position its period of most rapid growth commences, so that at this stage there is a proportionately greater amount of nucleolar substance in the nucleus than at any other period in its history. When it is apposed to the nuclear membrane it has at first more or less the form of a rod (often of a slightly curved rod), but as its substance commences to increase in volume this rod shape gradually becomes changed and the nucleolus becomes bent inwards (towards the center of the nucleus), frequently in the form of a V, an S, or a W, though there is marked variability in regard to the form it may assume, since no two nucleoli can be found at this stage which have exactly the same form (Fig. 189). It is about this time that the nucleolus attains its greatest staining density. Then this large and irregularly shaped nucleolus leaves the nuclear membrane and begins to fragment into pieces, which are very irregular in shape and variable in number and size ; the nucleolus may show thereby

a number of constrictions, or buds of nucleolar substance may project from its surface; it may first break into two larger pieces, and then these may fragment further, or it may at once break into a number of pieces which are irregular in their dimensions (Figs. 185–188, 190, 191, 193). These fragments gradually wander apart from one another, the nucleus now being larger and already somewhat irregular in shape; and at the same time each of the primitive nucleolar fragments divides into smaller pieces of unequal size, until when the nucleus has attained its greatest dimensions and most pronounced degree of ramification it contains a very large number of irregular nucleoli, which are unequal in their dimensions (Figs. 194–196). The figures given of this last stage show only sections of nuclei, and since as many as five or six sections may be made of one of these colossal nuclei (my sections were between 3 and  $5\mu$  in thickness), not one of these figures shows more than a portion of the total number of nucleoli in these largest nuclei; in some of the latter nuclei I compute the number of the nucleolar fragments to be at least three hundred. But the total mass of nucleolar substance in these largest nuclei is certainly considerably greater than the mass of the primitive nucleolus at the time of its greatest size; accordingly, though the division products of the primitive nucleolus might constitute the greater part of the nucleolar substance in the largest nuclei, they do not constitute all of it. Therefore there must be a formation of new nucleolar substance after the primitive nucleolus has divided, *i.e.*, a production of nucleolar substance not derived from the primitive nucleolus; I cannot determine the manner of formation of this new nucleolar substance, but would suggest that either new nucleoli are formed, or that the fragments of the primitive nucleolus increase in size by the addition of new nucleolar substance to them. The greater number of nucleoli in the largest nuclei are collected in or near the thicker portion of the nucleus and few or none lie in the branched processes; they are at this time seldom in contact with the nuclear membrane. Only a few of them contain vacuoles, and those which do may be regarded as derivatives of the primitive nucleolus, the vacuoles of the latter still being preserved in its daughter-nucleoli.

A nucleolar change now occurs which I have never seen paralleled, and to my knowledge no similar morphological change has ever been described. At the time when the homogeneous substance of the cell is commencing to differentiate into the secretion corpuscles, the nucleus begins to withdraw its branched processes and to decrease in size; while so doing it discharges its nucleoli into the cell body (Figs. 197-199). There can be no doubt of the genuineness of this process, since I have examined at least two hundred nuclei at this stage, which showed all intermediate stages between nuclei which had discharged only a few nucleoli and those which had discharged all except a single one of their nucleoli into the cell. The study of these nuclei gives the impression that successive contractions of the nucleus take place, whereby at first all the more peripheral nucleoli, and later those which are more central in position, become successively extruded, for in the cell two or three more or less parallel rows of nucleoli may be found, or more properly speaking, concentric circles of nucleoli (Figs. 197 and 198). In some cases I have observed nucleoli which were halfway through the nuclear membrane, but by far the greater number of the nucleoli are found either within or without the nucleus, and this would prove that the contractions of the nucleus are sudden in their action. I think that it is the sudden contractions of the nucleus which alone cause the expulsion of the nucleoli, for as the nucleus diminishes in volume its chromatin network may be seen gradually to become closer and denser, and the pressure within the nucleus becoming greater than the pressure without it, the nucleoli, not being fixed in position, are forced out into the cell body where there is comparatively little pressure, since the secretion corpuscles are not densely grouped, but lie scattered through a thin and structureless fluid substance. The nucleoli, when they have arrived in the cell body, are not found in equal number at all points around the nucleus; accordingly they are probably not discharged from all sides of the nucleolus in equal number, but only there where the nuclear membrane is thinnest (it is probably thinnest at those points whither the nuclear processes had withdrawn themselves). But though the nuclear membrane appears to be thinner at some

points than at others, there are no visible pores in it, so that the nucleolar substance must be squeezed through the nuclear membrane itself. When one takes a sponge filled with water and presses it in the hand the water is forced out of it in the form of jets or columns, which are radial to the surface of the sponge; exactly similar seems to be the method of the discharge of the nucleoli in the case at issue, except that the nucleus is itself actively contracting. Thus we find the greater number of the nucleoli which lie in the cell body close to the surface of the nucleus to be irregularly columnar or rod like in shape (Fig. 198) and radially grouped around the nucleus. Those which lie nearer the periphery of the cell, however, and which had probably been discharged by a previous contraction of the nucleus, are more irregular in form, and their axes have a less regular position with regard to that of the nucleus. Further, those ends of the rod-like nucleoli in the cell which are directed towards the surface of the nucleus are usually more attenuated than the opposite ends, *i.e.*, a nucleolus lying in the cell close to the nucleus has often the form of a pyramid the apex of which is directed towards the surface of the nucleus, and this form we would expect to result in the squeezing of a more or less viscid substance, like that of the nucleoli, through the nuclear membrane. I give only two figures showing the stage of the discharge of the nucleoli from the nucleus, simply in order to save time in the drawing of the numerous nucleoli; but my preparations show very clearly all the stages of this process: one has only to examine sections of the mature leech to find them in abundance. The extrusion of the nucleoli continues until only about twenty, then a dozen, then four or five, and finally only a single nucleolus (Fig. 199) remains in the nucleus; corresponding to these successive states of the discharge of the nucleoli we find cells in which only a few nucleoli, and then those in which the greater number of the nucleoli, lie in the cell body. One nucleolus always remains in the nucleus, though this one appears to differ in no wise from those which are discharged. Those nucleoli which lie in the cell body (Figs. 197-199) differ from those in the nucleus in their lesser density, greater size, and different reactions to certain stains (we shall



return to the chemical change later); in other words, the substance of those nucleoli which have come to be situated in the cell body undergoes a physical and perhaps a chemical change in this portion of the cell, and their expansion in volume might be accounted for on the ground of there being a smaller degree of pressure in the cell body than there is in the nucleus.

It will be noticed that the prophase and the metaphase of the cell body and of the nucleus do not exactly coincide in point of time, the metaphase of the nucleus commencing earlier than that of the cell body. Thus the nucleus attains its greatest dimensions and most diverse ramification at the time when the cell body contains the greatest amount of homogeneous substance, and the nucleus enters on its metaphase (diminution in volume, retraction of processes, expulsion of nucleoli) when the secretion corpuscles are only commencing to arise in the cell body. At the beginning of the metaphase of the cell body (when the latter is filled with the secretion corpuscles and commences to excrete them) the nucleus has already assumed a nearly spherical or oval form, has greatly decreased in size, and has discharged most of its nucleoli into the cell, *i.e.*, the nucleus has advanced already some distance in the path of the metaphasis.

The metaphase of the cell body (Figs. 198-203) commences when the cell is filled with the secretion corpuscles, all traces of the previous homogeneous substance being absent, and begins to discharge them through its duct. During this process the cell gradually decreases in size, and the primitive cytoplasm again comes into view, at first in the form of delicate fibers. When the cell has shrunk to about one-third of its former size (the diameter of the duct does not decrease quite so rapidly, since it may be still full of secretion corpuscles after they have all disappeared from the cell body) the nucleus has simultaneously decreased in size, but with greater proportionate rapidity than the cell body, and so at the close of the metaphase (Fig. 202) the nucleus reaches its smallest relative size. The latter contains at this stage invariably a single nucleolus, of spherical or oval form, very regular in outline, and exactly similar to the nucleolus at the commencement of the anaphase except that

it does not appear to contain vacuoles. The nucleus itself is somewhat elongate and irregular in outline, and, owing to its maximum degree of contraction (a characteristic of the end of the metaphase), its chromatin builds a dense network within it. A study of the cell body at this stage allows us to follow the morphological changes undergone by those nucleoli which had been discharged by the nucleus (Figs. 198-203). The cytoplasm gradually assumes a reticulate or a somewhat granular structure, and finally a most regular vacuolar or alveolar structure. As the cell body decreases in size the discharged nucleoli lying in it gradually stain less deeply, they lose their rod-like form, and no longer remain isolated, but all the nucleolar substance in the cytoplasm gradually becomes confluent, and becomes arranged in the form of a coarse, irregular network of substance distributed in the cytoplasm, and readily distinguishable from the latter by its different staining properties (Figs. 201-203). By a hasty inspection this network of nucleolar substance might appear to represent branches of the nucleus, but a careful study shows that at this period of its growth the nucleus has no branches. As the cell continues to become smaller the amount of nucleolar substance in the cytoplasm gradually becomes less and less, first the network at the periphery of the cell disappearing, then that in the vicinity of the nucleus, until at the conclusion of the metaphase no nucleolar substance is any longer to be seen in the cytoplasm. I am unable to determine whether it is finally discharged through the cell membrane or whether it becomes metamorphosed into cytoplasm; it certainly is not excreted through the cell duct, since no nuclear substance occurs in the latter, and at this stage the duct is no longer an open tube, but all the secretion corpuscles having been expelled from it, it is again filled with cytoplasm. The suggestion may be made that at least a portion of this nucleolar substance remains in the cytoplasm, so that in the succeeding prophase the nucleolus within the nucleus might find the material necessary for its growth in the nucleolar substance suspended in the cytoplasm; thus there might be, in the history of the nucleolar substance, periods of its expedition into the cytoplasm alternating with those when it is again taken

into the nucleus. And that in the prophase the single nucleolus of the nucleus derives the material necessary for its further growth from the cell substance, seems highly probable when we recall the fact that at the time of its most rapid growth it is usually apposed to the nuclear membrane, which would denote that it is taking up a substance which penetrates that membrane from the side of the cell body.

We have alluded to certain chemical changes which occur in the nucleolar substance when discharged from the nucleus during the metaphase of the latter. These staining differentiations and the coloration of the cytoplasm as observed on five different preparations are as follows (the first preparation was fixed with Flemming's fluid, the others with corrosive sublimate).

*First preparation* (Ehrlich's haematoxylin, two hours ; eosin, ten minutes) : cytoplasm pale lilac ; nucleoli in the nucleus, and when first discharged from it, reddish or rusty brown ; nucleolar substance at the end of the metaphase lighter in color.

*Second preparation* (gentian violet in aqueous solution, twenty-five minutes ; eosin, four and one-half minutes) : cytoplasm very faintly stained ; nucleoli in the nucleus deep violet, those in the cytoplasm yellowish red.

*Third preparation* (Ehrlich's haematoxylin, one hour ; eosin, five minutes) : cytoplasm pale pink ; nucleoli in the nucleus, and when first discharged from it, purple ; nucleolar substance in the cytoplasm at the end of the metaphase pure blue.

*Fourth preparation* (Ehrlich's haematoxylin, forty minutes ; eosin, five minutes) : nucleolar substance within and without the nucleus yellowish red ; cytoplasm of a paler red.

*Fifth preparation* (Mayer's acid carmine, twenty minutes ; Lyons blue, five minutes) : cytoplasm unstained ; nucleoli in the nucleus, and, when first discharged, bluish green ; nucleolar substance at the end of the metaphase reddish purple in the cytoplasm. These methods of double staining show that the nucleolar substance, when discharged from the nucleus, undergoes some chemical change in the cytoplasm ; and they serve to distinguish, further, this substance from the true cytoplasm.

I have no material of *Piscicola* after the breeding season, and accordingly could not follow the changes of these gland cells in their metamorphosis from the end of the metaphase to the commencement of the prophase. But these two end stages do not differ much from one another, since the cell at the former stage differs from that of the latter merely in that its nucleus is smaller and more irregular in shape.

It is not difficult to determine the sequence of the stages described ; only in the smallest individuals do all the stages of the prophase occur, and only in the largest those of the metaphase.

20. *Mesenchym Cells of Cerebratulus lacteus* (Verr.).

(Plate 29, Figs. 315a, 316a-324.)

(I have described these cells in a previous contribution ('96), and so shall treat of them in this place mainly with regard to their nucleoli.)

The smallest nuclei (Figs. 316a and 317) are densely filled with chromatin, and nucleoli appear to be absent ; the nuclear sap also stains with haematoxylin, so that these nuclei may be easily recognized by their deep stain and sometimes nearly homogeneous appearance. I have made a careful examination for nucleoli on preparations stained by the Ehrlich-Biondi method, as well as with haematoxylin and eosin, and am certain that nucleoli are either wholly absent or, if present, must be very minute in point of size. Such, then, is the structure of the smallest nuclei, namely, those found in the body cavity, and those of the smallest cells of the pseudoepithelia lining the body cavity.

The non-continuous pseudoepithelia of the body cavity are layers of differentiated mesenchym cells, which differ from the primitive cells in their greater dimensions and more oval or spherical outlines (the undifferentiated cells are bipolar or multipolar, with long branching processes). In these larger cells we find for the first time a spherical, deeply staining nucleolus. Now the size of the latter stands in a pretty constant ratio to that of the nucleus. Further, in the smallest

nuclei which contain nucleoli, from one to three of the latter occur, and one or all of these are frequently found in close contact with the nuclear membrane (Figs. 315a, 318, 320), while in the largest nuclei observed only a single nucleolus is present, and this one is relatively large and is always at or near the center of the nucleus, never at its periphery (Figs. 319, 322, 323). In connection with the problem of the origin of this nucleolus we recall those small granules contained in the cytoplasm, which I have ('96) termed nutritive particles. These particles (*Nut. Gl.*) stain with eosin quite as intensely as the nucleolus, and in the smallest cells are either wholly absent or present in only small number; but in the larger cells they are usually much more abundant, or when not more numerous they are of greater size, and are often quite densely grouped around the nucleus. It would seem probable that the nucleolar substance is derived from these supposed nutritive particles. Thus when the nucleoli first appear they are most frequently in contact with the nuclear membrane; and this shows that they are formed at the periphery of the nucleus, and only later come to occupy a central position within it. And since the nutritive particles are usually very numerous in the immediate vicinity of the nucleus, we may conclude that the nucleoli are formed from substance of these nutritive particles, which has been taken up by the nucleus. In the smallest nuclei alone do more than one nucleolus appear, so that the nutritive substance would seem to be taken into the nucleus from several points on its periphery, and then subsequently these several assimilated portions of nutritive substance may fuse together and so produce a single large nucleolus. Accordingly, the substance of the nucleolus would in this way appear to have an extra-nuclear origin. That these nutritive particles are being successively absorbed by the nucleus is shown by the fact that the increase in the size of the nucleus and of the nucleolus go hand in hand. On the other side, these nutritive bodies in the cytoplasm cannot be considered to be of nucleolar origin, since they usually make their first appearance in the cell body before a nucleolus arises in the nucleus; and if they did have a nucleolar origin, *i.e.*, if they were excreted portions of the nucleolus,

we should expect to find the largest nucleoli in the smallest cells and the smallest ones in the largest cells. Further, the nucleolar substance cannot be regarded as a secretion of the nucleus itself, since this would leave unexplained the peripheral position which it at first occupies in the nucleus. Thus the mode of origin of the nucleolus in these cells would seem to be similar to that of the nucleoli in the ova of the nemerteans. A final point may be noted: the nucleolus accepts the same stains, though more intensely, than do the nutritive particles in the cytoplasm; accordingly, the substance of those bodies which have been absorbed by the nucleus, and then by their fusion in the nucleus produce the nucleolus, must have undergone either a slight chemical or physical change within the nucleus.

The largest mesenchym cells of the pseudoepithelia probably represent the youngest stages of the ova, though in the single individual of this species at my disposal no gonads were present, so that I can bring no proof positive that this is the mode of origin of the egg cells. In *Carinella* it is from similar cells that the genital products are derived, as I have previously shown ('96). Coe ('95) described certain of the more mature egg stages.

In my earlier paper on these cells (*l.c.*) I termed all the nuclear divisions of these cells "amitotic." But renewed study of these elements shows that only the divisions of those cells are amitotic (Figs. 316a and 317), from which the free mesenchym cells are produced. Whereas, in the nuclear divisions of the cells of the pseudoepithelia from which the masses of larger cells are derived I now find evidences of regularity in the distribution of the chromatin, so that probably these divisions are mitotic. However, in these small nuclear divisions it is almost impossible to decide whether we have to do with mitoses or with amitoses without the use of better lenses than those which were at my disposal.

#### 21. *Ganglion Cells of Nemerteans.*

I may here briefly mention the relations of the nucleoli in these cells, and for other details refer to a previous contribution of mine ('97).

*Lineus gessserensis*.—Cells of the first type : one or two small nucleoli. Cells of the second type : one nucleolus. Cells of the third type : a single nucleolus, or two of unequal size.

*Cerebratulus lacteus*.—Cells of the first type : as in the preceding species. Cells of the second type : one or two nucleoli. Cells of the third type : one or two nucleoli, which in one case stained differently. Cells of the fourth type : usually one peripheral nucleolus ; rarely are two present, and then they are unequal in dimensions.

In all these cells the nucleolus is comparatively small, homogeneous, and no evidences of nucleolar division were seen.

#### IV. GENERAL COMPARISONS AND CONCLUSIONS.

Here I shall summarize merely the results of my observations on the nucleolus, and compare them with the conclusions of other investigators. Numerous other morphological points have been brought up, however, in the preceding pages, such as yolk development, differentiation of ova, nuclear divisions, distribution of the chromatin elements in the germinal vesicle at different stages in the growth period, changes in the structure of cytoplasm, etc.

##### I. *Chemistry of the Nucleolus.*

I have made no special chemical study of these structures, except what may be learned from their reactions to stains. In the gregarines no substance could be demonstrated which chemically corresponds to the chromatin of the metazoan cell ;<sup>1</sup> but the following table represents the mode of staining of true nucleoli in the somatic and germ cells of the *Metazoa* :

STAIN.	NUCLEOLUS.	CHROMATIN.
Del. or Ehrl. haematoxylin, eosin . . . . .	red . . . . .	blue.
Ehrlich-Biondi stain . . . . .	maroon or red . . . . .	green.
Acid carmine, nigrosine . . . . .	blue or greenish . . . . .	red.
Del. haematoxylin, cochineal . . . . .	pink or red . . . . .	blue.
Safranin, gentian violet, orange . . . . .	yellow . . . . .	blue.

Schwarz ('87) distinguishes in plant cells pyrenin, the substance of the true nucleoli, from the other nuclear substances

<sup>1</sup> That is, not to chromatin in the form of pure nucleic acid.

and finds that it has a closer chemical affinity to the substance of the nuclear membrane (amphipyrenin) than to any other substance. Judging merely from the reactions of these two substances to stains I would agree in this point with Schwarz. Zacharias ('82) shows also for plant cells that the nucleolar substance is *sui generis* and is allied to plastin. O. Hertwig ('92) terms the nucleolar substance "Paranuclein" and observes: "Nuclein und Paranuclein betrachte ich als die wesentlichen Substanzen des Kerns. . . . Beide scheinen mir in irgend welchen Beziehungen zu einander zu stehen." But it is important to note that the true nucleolar substance probably has no chemical relation to the true chromatin (nuclein). Thus karyosomes should not be considered as a particular group of nucleoli, since they are not nucleoli at all, but nodal points of the chromatin reticulum. The substance of every true metazoan nucleolus apparently differs chemically from the chromatin, linin, paralinin, and oedematin (lanthanin); and accordingly "pyrenin" is a term preferable to "paranuclein," though "pyrenin" may include divers substances.

There are also chemical differences between the nucleoli proper ("Hauptnucleoli") and the paranucleoli ("Nebennucleoli"), which occur together in many ova and in a few somatic cells; the substance of the paranucleoli stains more lightly than that of the nucleoli proper. List ('96) distinguishes three kinds of true nucleoli, from a chemical standpoint: (1) the nucleolus of somatic cells; (2) the nucleolus proper of germinal vesicles; and (3) the paranucleolus of germinal vesicles; and he considers the substance of the paranucleolus of the germ cell to be closer related chemically to the nucleolus of somatic cells than either of them is to the nucleolus proper of ova. List promises a more complete paper on this subject. The so-called "nucleoli," which react like chromatin, are of course not true nucleoli, but either *karyosomes* (thickened nodal points of the chromatin reticulum) or *chromatin nucleoli* (independent lumps or spheres of chromatin). It is my intention to devote a special paper to the consideration of the latter structures. Other papers on the chemistry: Macallum ('95), Michel ('96), Carnoy and Lebrun ('97).



2. *Number of Nucleoli.*

As Flemming ('82) has stated, the number of nucleoli is small in most cells, not more than from one to five. But in certain stages of some cells there may be several hundred (ova of *Reptilia*, *Amphibia*, *Selachii*, nemerteans, subcuticular gland cells of *Piscicola*). Even in those cases just mentioned, where the number of the nucleoli is very large, the immature cell contains only one or a few nucleoli, so that the large number is attained only when the nucleus has increased in size, cf. the observations of Auerbach ('74a). Among somatic cells a large number of nucleoli is much more infrequent than among egg cells. At a given stage of a given cell of any one species of metazoan the number of nucleoli is pretty constant, and there is less variability in the number among those cells where the typical number of nucleoli is a small one than in those where a large number is present. In cells where the usual number of nucleoli is one or two, as in those of the nidamental gland of *Montagua*, three may quite frequently be found, but no cells are found in which not a single nucleolus occurs; in other words, there is in most cases some degree of variability in the number of the nucleoli, and the amount of this variability stands in a more or less direct ratio to the number of the nucleoli, but it is numerically progressive as a rule, tending to produce more than the normal number, and in no cases where cells normally contain nucleoli do we find a regressive numerical variation leading to the total disappearance of nucleoli. In certain few cells no nucleoli are present, and this is the case in more cells than Flemming ('82) was disposed to admit, since not only are specialized cells like mammalian blood corpuscles without them, but they are also absent in certain connective-tissue elements of nemerteans, and in certain other cells of a low degree of vitality.

Auerbach ('90) formulated the law that the number of nucleoli is more or less constant for all the cells of a given species. But this conclusion is certainly erroneous, since in *Doto* there is one nucleolus found in the blood corpuscles and in the ovum, from one to five in the ganglion cells, from one to three in the cells of the nidamental gland, and in the giant cells as many as

forty ; and in *Piscicola*, usually one in the ovum and the ganglion cells, about twelve in the mature muscle cells, and three hundred or four hundred in the subcuticular gland cells. From the data at hand we accordingly conclude that the number of nucleoli is not constant for the species. (On the number of nucleoli at different stages in amphibian ova, cf. Carnoy and Lebrun, '97a).

In order to determine whether the number of nucleoli in egg cells were fixed for, or in any way determined by, the particular groups of *Metazoa*, I have compiled the following tables (pp. 501–505) for the larger groups, these tables representing the data of previous investigators and of my own observations. In them four classes of germinal vesicles are distinguished according to differences in the number and kind of the nucleoli ; this classification is only for convenience' sake, only arbitrarily chosen, and is probably not a natural one. On the left hand is given the name of the genus or group ; the asterisk corresponding to each form indicates by its position in a particular vertical column the nucleolar relations of the ovum of the form specified ; and next to the asterisk is placed the name of the authority. In some cases two investigators may have reached different conclusions in regard to the nucleolar relations, so that for these cases two asterisks were employed.

One must be extremely cautious in any attempt to draw conclusions from these data, not only because the data are so meager, but also because where data have been culled from so many different observers some of the facts may ultimately prove to have been erroneous. Thus many of these ova may have been examined at only one point in their development, and in others paranucleoli may have been entirely overlooked, or may have been confused with true nucleoli. But taking this mass of observations as it stands, the following general conclusions may be drawn: we find that a large number of nucleoli is not always characteristic of ova with a considerable amount of deutoplasmic substances, for a single nucleolus is typical for the birds and for many of the *Arthropoda*. Further, the number of the nucleoli does not seem to be dependent upon the amount of yolk, nor upon the mode of cleavage,

FORM.	A SINGLE NUCLEOLUS.	MORE THAN ONE NUCLEOLUS, ALL ALIKE.	NUCLEOLUS AND PARANUCLEOLUS.	
<i>Coelenterata.</i>				
<ul style="list-style-type: none"> <li>{ Esperella</li> <li>{ Spongilla</li> <li>{ Tedamione</li> <li>{ Hircinia</li> <li>{ <i>Hydrozoa</i></li> <li>{ Aequorea</li> <li>{ Hydractinia</li> <li>{ Podocoryne</li> <li>{ Geryonia</li> <li>{ Tubularia</li> <li>{ Eucope</li> <li>{ Aeginopsis</li> <li>{ Hydra</li> <li>{ Nausithoë</li> <li>{ Pelagia</li> <li>{ Physophora</li> <li>{ Rodalia</li> <li>{ <i>Ctenophora</i></li> </ul>	<ul style="list-style-type: none"> <li>* (H. V. Wilson)</li> <li>* (Fiedler)</li> <li>* (Weismann)</li> <li>* (Häcker)</li> <li>* } (Bunting)</li> <li>* }</li> <li>* (Fol)</li> <li>* (Doflein)</li> <li>* (Chun)</li> </ul>	<ul style="list-style-type: none"> <li>* } (H. V. Wilson)</li> <li>* }</li> <li>* (O. Hertwig)</li> </ul>	<ul style="list-style-type: none"> <li>* (O. Hertwig)</li> <li>* (Brauer)</li> <li>* }</li> <li>* }</li> <li>* }</li> <li>* (mihi)</li> </ul>	
	<i>Plathelminthes.</i>			
	<ul style="list-style-type: none"> <li>Bothriocephalus</li> <li>Distomum</li> <li>{ <i>Polycladidea</i></li> <li>{ <i>Tricladidea</i></li> <li>{ Rhabdocoele</li> <li>{ Prorhynchus</li> <li>{ Bothrioplana</li> <li>{ Haplodiscus</li> </ul>	<ul style="list-style-type: none"> <li>* (Schauinsland)</li> <li>* (Lang)</li> <li>* (Repiachoff)</li> <li>* }</li> <li>* }</li> <li>* (Böhmig)</li> </ul>	<ul style="list-style-type: none"> <li>* (Schauinsland)</li> <li>* (Jijima)</li> </ul>	
	<i>Nemertini.</i>			
	<ul style="list-style-type: none"> <li>Carinella</li> <li>{ Cerebratulus</li> <li>{ Lineus</li> <li>{ Malacobdella</li> <li>{ Drepanophorus</li> <li>{ Tetrastemma</li> <li>{ Amphiporus</li> <li>{ Stichostemma</li> <li>{ Zygonemertes</li> <li>{ Proneurotes</li> <li>{ Prosadenoporus</li> <li>{ Pelagonemertes</li> </ul>	<ul style="list-style-type: none"> <li>* (Bürger)</li> <li>* (Hubrecht)</li> <li>* (mihi)</li> <li>* (Bürger)</li> </ul>	<ul style="list-style-type: none"> <li>* (v. Kennel)</li> <li>* }</li> <li>* }</li> <li>* }</li> <li>* }</li> <li>* (Bürger)</li> </ul>	<ul style="list-style-type: none"> <li>* (Bürger, Coe)</li> <li>* (Hubrecht)</li> </ul>

FORM.	A SINGLE NUCLEOLUS.	MORE THAN ONE NUCLEOLUS, ALL ALIKE.	NUCLEOLUS AND PARANUCLEOLUS.
<i>Annelida.</i>			
{ Nereis			* (E. B. Wilson)
Spinther	* } (Korschelt)		
Ophryotrocha	* }		
Sternaspis	* (Vejdovský)		
Polydora			* (mihi)
Spio			* (Giard)
Capitellids			* (Eisig)
Polygordius			* (Fraipont)
{ <i>Oligochaeta</i>	* } (Vejdovský)		
Rhynchelmis	* }		
Lumbricus			* (Claparède)
{ Nephelis	* (Leydig)		
Branchiobdella	* }		
Haemopis	* }		* (O. Hertwig)
Piscicola	* (mihi)		
Clepsine		* (Whitman)	
Piscicola		* (Leydig)	
<i>Arthropoda.</i>			
{ Homarus	* (Herrick)		
Porcellio	* (St. George)		
Oniscus	* (Wielowiejski)		
Heterocoepo	* }		
Diaptomus	* }		
Argulus		* (Leydig)	
Astacus		* (Wielowiejski)	
Cyclops			* }
Sida			* }
Canthocamptus			* }
Moina			* }
Euchaeta			* (vom Rath)
{ Epeira	* (Korschelt)		
Dolomedes	* (Korschelt)		
Phalangium	* (Korschelt,		
	Leydig)		* (Henking)
Lycosa	* (Leydig)		
Theridium	* (v. Wittich)		* (Leydig)
Tetragnatha			* (Leydig)
<i>Arancina</i>			* }
<i>Acarina</i>			* }
Zilla			* (Van Bambeke)

FORM.	A SINGLE NUCLEOLUS.	MORE THAN ONE NUCLEOLUS, ALL Alike.	NUCLEOLUS AND PARANUCLEOLUS.
{ Julius	* (Stuhlmann)		
{ Geophilus		* (Balbiani)	* (Leydig)
{ Glomeris			* (Stuhlmann)
{ Lithobius			* (Leydig)
{ Peripatus	* (Stuhlmann)		
{ Blatta	* (Brandt)		
{ Nepa	* } (Will)		
{ Notonecta	* }		
{ Carabus nemoralis	* }		
{ Gryllotalpa	* }		
{ Pieris	* }		
{ Anabolia	* }		
{ Bombus	* } (Stuhlmann)		
{ Anomalia	* }		
{ Ophion	* }		
{ Ephialtes	* }		
{ Pemphigus	* (Leydig)		
{ Musca	* (Wielowiejski)		* (Stuhlmann)
{ Necrophorus		* }	
{ Geotrupes		* }	
{ Banchus		* } (Stuhlmann)	
{ Pimpla		* }	
{ Stenobothrus		* }	
{ Meloe		* } (Leydig)	
{ Libella			* (St. George)
{ Melolontha			* (Wagner)
{ Lina			* }
{ Lycus			* }
{ Sphinx			* } (Stuhlmann)
{ Ambyteles			* }
<i>Echinodermata.</i>			
{ Psammechinus	* } (Bergh)		
{ Echinocardium	* }		
{ Echinus			* }
{ Toxopneustes			* } (Hacker)
{ Asteracanthion			* }
{ Sphaerechinus			* } (O. Hertwig)
{ Amphidetus	* }		
{ Solaster	* }		
<i>Mollusca.</i>			
{ Chaetoderma	* (Wirén)		
{ Proneomenia			* (Hubrecht)

FORM.	A SINGLE NUCLEOLUS.	MORE THAN ONE NUCLEOLUS, ALL ALIKE.	NUCLEOLUS AND PARANUCLEOLUS.
{ Neritina	* (Blochmann)		
Doto	* (mihi)		
Montagua			* (mihi)
Tellina			* } (O. Hertwig)
Helix	* (Platner)		* }
{ Limax			* (Mark)
Arion			* (Platner)
Doris			* }
Aeolidia			* } (Lönnberg)
Amphorina			* (Trinchese)
Paludina			(Leydig)
{ Anodonta			* (Flemming)
Unio			* (Hessling)
{ Mytilus			* (Lönnberg)
Pholas			* (List)
{ Cyclas			* (Stepanoff)
<i>Tunicata.</i>			
Distaplia	* (Davidoff)		
Phallusia	* (Bergh)		
Botryllus	* (Pizon)		
Clavelina	* (Seeliger)		
Ciona			* }
Styelopsis			* } (Floderus)
Ascidia			* (O. Hertwig)
<i>Vertebrata.</i>			
Amphioxus	* (Van d. Stricht)		
Petromyzon	* (Böhm)		
{ Scyllium		* }	
{ Torpedo		* } (Rückert)	
{ Pristiurus		* }	
{ Scorpaena		* (Van Bambeke)	
{ Conger		* }	
{ Gadus		* } (Scharff)	
{ Trigla		* }	
{ Gasterosteus		* (Ransom)	
{ Anguilla		* (Brock)	
{ Cyprinus		* (Eimer)	
{ Rana		* (Born)	
{ Amblystoma		* (Fick)	
{ Triton		* (Leydig)	
{ Emys		* (mihi)	
{ Lacerta		* (Eimer)	
" Turtle "		* (Agassiz)	

FORM.	A SINGLE NUCLEOLUS.	MORE THAN ONE NUCLEOLUS, ALL ALIKE.	NUCLEOLUS AND PARANUCLEOLUS.
{ Gallus	* (Holl)		
{ Fringilla	* (v. Wittich)		
{ Columba	* (mihi)		
{ Felis	* (St. George)		
{ Cavia	* (Rein)		
{ Mus	* (Holl)		
{ Vespertilio	* (v. Beneden)		
{ Sus	* } (Leydig)		
{ Myoxus	* }		
{ Talpa	* }		
{ Ovis			
{ Lepus			* (St. George)
{ Homo	* (Nagel)		* (Flemming)

nor yet upon the mode of deposition of the egg (*i.e.*, whether it is pelagic, hatched in a cocoon, or nourished in an uterus). These facts hardly warrant an attempt to explain the factors limiting the number of nucleoli, and perhaps such explanations should rather be expected from experimental workers than from purely structural observers. On examining the metazoan groups in detail we find in certain of them a degree of uniformity in regard to the number of nucleoli. Thus the only vertebrate ova with two kinds of nucleoli are those of *Lepus* and *Ovis*. A single nucleolus is the rule for *Amphioxus*, *Petromyzon*, the birds, and most of the mammals; the *Reptilia*, *Amphibia*, *Teleostii*, and the *Selachii* have numerous nucleoli. In the *Tunicata* there is either a single nucleolus or a nucleolus and paranucleoli; this is also the rule for the *Echinodermata*, *Mollusca*, and *Annelida*. In the *Arthropoda* there is considerable diversity in regard to the number and differentiation of the nucleoli. In the nemerteans we find most usually either a single nucleolus or a large number of small ones. In the *Plathelminthes* one or two is the rule; this is also most frequently the case for the coelenterates, but in some of the latter paranucleoli have been described.

### 3. *Position of the Nucleolus in the Nucleus.*

Where a single nucleolus is present it almost always lies excentrically, though not against the nuclear membrane. Those cases where it regularly occupies the center of the nucleus must be regarded as exceptional ; thus I am unable to agree with Macfarlane that the nucleolus is either the morphological or the tropic center of the cell. At the time of its origin, and often at the time of mitosis, the nucleolus may be in contact with the nuclear membrane. Where a number of nucleoli are present they may be scattered irregularly through the nucleus, or grouped at one point in it, or be concentrically arranged ; their position is often dependent upon the stage of the development of the nucleus. Thus in the metanemertans examined by me they lie at the periphery in the smallest germinal vesicles, then wander towards its center, and finally migrate to the periphery again.<sup>1</sup>

The nucleoli lie in the nuclear sap, as a rule not in any close connection with the chromatin reticulum. But in those cases where the nucleolus may be unusually large it appears to be suspended by the fibers of this reticulum, but not in such a way that the fibers penetrate into its substance, but become simply wound around its surface ; thus it appears that when the nucleolus increases in size it forces apart the fibers of the nuclear network in such a way that the latter gradually produce a latticework on its surface. In this way the nucleoli may be more or less held in position in the nucleus, but Herrick's observations on the gravitation of the nucleolus show that it is not firmly held by the chromatin fibers. The nucleolus is, as it were, a ball lodged in the branches of a tree, its movements hindered by the intervening branches, but nevertheless not immovable. Various views on the mode of suspension of the nucleolus : Pflücke ('95), Heidenhain ('92), Rosen ('95), Jensen ('83), Zimmermann ('96). Note also its peculiar position in *Synapta* (Leydig, '52).

<sup>1</sup> For the opinions of other authors, cf. the reviews of the papers of Pflücke ('95), Heidenhain ('92), Rosen ('95), Jensen ('83), Leydig ('52), Zimmermann ('96), Schneider ('91).



4. *General Morphological Structure of the Nucleolus.*

The ground substance of the nucleolus is more or less dense, but not brittle, and either homogeneous or finely granular, rarely coarsely granular. It may be either fluid or viscid in consistency.

In the greater number of cases it has no limiting membrane. Such a membrane was found by me only in the germinal spot of *Polydora*, and here it appeared to be merely a denser portion of the ground substance. When any small nucleolus is viewed in its totality a membrane appears to surround it, but this phenomenon is due to the refraction of light from its convex surface, and many observers have been misled by this appearance into supposing that a membrane is present. Others in describing those states of nucleoli in which a large vacuole is present have erroneously described the peripheral layer of true ground substance as a nucleolar membrane; it is necessary to distinguish between such a peripheral layer, which consists of true ground substance, and a nucleolar membrane proper, which is a differentiation of the ground substance. Some authors, *e.g.*, Lavdowsky ('94), have described a membrane of chromatin enveloping the nucleolus, and I have found that those of the giant cells of *Doto* may sometimes be surrounded by a mass of chromatin. But this apposition of a mass of chromatin in *Doto* is certainly an artefact, though it would seem probable that the nucleolus in some cases has an envelope of chromatin forming a distinct capsule separated from the chromatin network of the nucleus. I am able, however, to corroborate the observations of Macfarlane ('81) and Pennington ('97), that the nucleolus in *Spirogyra* has a true membrane.<sup>1</sup>

A very unusual structure of the nucleolus is that afforded by the salivary gland cells of *Chironomus* as described by Balbiani ('81), Leydig ('83), Korschelt ('84), and Macallum ('95). C. Schneider ('91) supposes the nucleoli, as well as the rest of the nuclear substance, to consist of "Gerüst" (linin?) and chro-

<sup>1</sup> The following writers have described nucleolar membranes: Macallum ('95), Carnoy and Lebrun ('97a), Will ('85), Holl ('93), Roule ('83), Bürger ('90), Ogata ('83), Vejdvský ('82), Meunier ('86), Carnoy ('86), Mann ('92).

matin, and considers the nucleoli to be only isolated masses of chromatin surrounded by linin sheaths; these observations have not been corroborated by any other writers and would seem to be due to faulty methods of fixation.

In opposition to Meunier ('86), and in agreement with most investigators, I must conclude that vacuoles are normal structures in nucleoli, since they may be seen after the most diverse methods of fixation, and their size and number are not only to some extent limited for the particular cell, but are also different at different periods in the metamorphoses of the nucleus. It is the rule that the youngest nucleoli are homogeneous, and that vacuoles first arise when they have increased in size. Their size and number vary at different phases in the development of the nucleolus. Very frequently a number of smaller ones appear, and then these subsequently fuse together and produce a larger one. The nucleoli of egg cells are characterized as a rule by more numerous or larger vacuoles than those of somatic cells, and in many somatic cells these vacuoles appear to be wholly absent. The vacuolar substance appears in some cases not to be a derivative of the ground substance of the nucleolus, but to be derived from without the nucleolus (ova of *Doto* and *Montagua*). Perhaps this vacuolar substance always has an extranuclear origin, since in many cases a germinal spot grows larger merely by an increase in its volume, while the ground substance seems neither to increase nor diminish.

The alveolar structure of nuclei as described by Purcell ('94), Schaudinn ('94), Korschelt ('95), and Lauterborn ('95b) is probably referable to the regular distribution of equal-sized vacuoles in the nucleolus.

A "Kernkörperchenkreis," a shell of minute granules arranged concentrically around the nucleolus, has been described by Eimer ('71, '72), Auerbach ('74a, who considered it to be the result of opposing repulsive forces of the nucleolus and nuclear membrane), Brass ('89), Pflücke ('95), Platner ('89a), Smirnow ('90), Engelmann ('80), Carnoy and Lebrun ('97a). A more or less similar phenomenon has been described by me for ganglion cells of *Doto*. Such a nucleolar circlet must be considered, in most cases at least, an artefact. But in this cate-

gory should not be classed small masses of nucleolar substance grouped around a larger one, these being normal phenomena during the growth period of nucleoli.

Reticulations within the nucleolar substance have been described by some few authors. Thus Carnoy ('85, '97a), Meunier ('86), and Moll ('93) described nucleoli containing a skein of chromatin, but Zacharias and Strasburger ('88) did not find anything resembling these supposed skeins described for *Spirogyra*. Leydig ('88) states that the germinal spot of *Lycosa* "bietet das Bild eines Knäuels dar." Fromann ('84) described the nucleolar substance as consisting of granules connected by fibers, Bütschli ('80) found the nucleoli of *Dinoflagellata* to contain a fine reticulum, and Davidoff ('89) states that the germinal spot of *Distaplia* takes up portions of the nuclear reticulum into itself (but cf. Bancroft, '98, and Schäfer, '80). The only structure which was found by me to resemble a skein was present in the later stages of the germinal spot of *Polydora*; but in this object, owing to the gradual confluence of the vacuoles, which thus produce anastomosing channels of vacuolar substance in the ground substance of the nucleolus, it is the true ground substance which represents a skein-like appearance. It is very probable that Carnoy and his followers have mistaken the vacuolar substance for the ground substance, and have considered the true ground substance to be chromatin; I am forced to conclude that in all probability there are no skeins of chromatin lying in any metazoan nucleolus, since I have never found any evidence of chromatin in it in any metazoan cell. But it is not improbable that in the nuclei of gregarines chromatin may be massed in some or all of the nucleoli.

Nucleolini, granules within the nucleolus, have frequently been observed. A single nucleolinus to a nucleolus has been described by Vejdovský ('95b), Morgan ('96), Agassiz ('57), Kleinenberg ('72), Leydig ('88), Macfarlane ('85), Lavdowsky ('92), A. Brandt ('78), Van Bambeke ('86), Kosinski ('87, '93); several nucleolini to a nucleolus, by Bürger ('90), Rhumbler ('93), Holl ('93), Wolters ('91), Schrön ('65), Scharff ('88), See-liger ('82), Gjurasin ('93), Haeckel ('74), Mann ('92), Van Bam-

beke ('97b), Mark ('77), Bancroft ('98). Compare also the following: Huie ('97), Van Bambeke ('97), Kosinski ('87, '93), Mark ('77), Zimmermann ('96), Hodge ('94): I have found these bodies occurring in varying number, though most frequently absent, in the nucleoli of various cells, and they appeared to be merely loosened portions of the ground substance which had come to lie within a vacuole. Macfarlane and his pupil Mann have described nucleolini under the names "endonucleolus" and "nucleolo-nucleus" as occurring singly and with great constancy in certain plant cells, though Zacharias ('85) studied Macfarlane's object (*Chara*) and makes no mention of any of these structures. Macfarlane ascribes the utmost importance to his "endonucleolus," regarding it as the tropic center of the cell and as an important mechanical agent during nuclear division. Mann has not only described a most complex structure of the nucleolus, such as no other observer has yet seen, but also has found fine fibrils radiating out from it, which he supposes to penetrate through the nuclear cavity. From my own observations, and in agreement with the majority of observers, I can attach no particular morphological significance to the nucleolinus; it appears to be only a detached portion of the nucleolar ground substance, to be in most cases absent, and when present to vary greatly in regard to size, position, and number. It is undoubtedly the case that many structures which have been described as nucleolini are in reality minute vacuoles, which from their refrangibility appear to be granules; such is the case with the minute vacuoles of *Polydora* and *Montagua* when studied after the action of certain stains, and has been shown for other objects by Zimmermann and Huie, Lavdowsky found in the nucleolus a central vacuole, and in the latter a small granule, which he supposed to be "das noch in Entwicklung begriffene Centrosoma," destined to finally pass out of the nucleolus; he was unable to determine how it does wander out of the nucleolus and become the centrosome, so that his suggestion has merely the value of a hypothesis. Van Bambeke describes the nucleolinus of the germinal spot of *Amaurobius* as "doué d'un mouvement très vif"; this interesting phenomenon certainly deserves investigation, though

it is not impossible that the supposed nucleolinus was in reality a microorganism inclosed in the vacuole of the nucleolus. (Cf. also Flemming's observation on the egg of *Ascidia*, '97.) Supposed nerve fibrils in the nucleolus have been described by Eimer ('73, '90).

##### 5. *Polarity of the Nucleolus.*

In the gregarine (*Gonospora?*) from the intestine of *Lincus gesserensis* it is the rule that the vacuoles make their first appearance at that pole of the nucleolus which is nearest to the nuclear membrane. In the germinal spot of *Montagua* the opposite position of the large excentric vacuole is the rule, though the percentage of cases in which the vacuole has a particular position with regard to the nuclear membrane is less than in the gregarine. On the contrary, in the germinal spots of *Piscicola* and *Rodalia* there is no regularity in regard to the position of the vacuoles, and in that of *Polydora* the vacuoles are, at the time of their first appearance, usually central in position. In the germinal spots of many other *Metazoa*, where a single large vacuole is present it more usually lies excentrically than centrally, though its position appears to be independent of the proximity of the nuclear membrane; so that in these cases we can speak of a certain polarity in regard to the position of the vacuole within the nucleolus, and not of a polarity of the axis of the nucleolus in regard to the position of the nuclear membrane. But in the two gregarines examined by me the substance of the nucleolus, or of some of the nucleoli, is differentiated at two poles of the nucleolus, so that the portion of the ground substance at one end stains differently from that of the other end of the nucleolus; this state apparently does not occur in the nucleoli of metazoan cells. It remains to be solved whether in the gregarines the chromatin or its physiological equivalent is localized at some particular point or pole of the nucleolus, *i.e.*, whether or not such nucleoli should be compared to the nucleoli of the *Metazoa*.

6. *Amoeboid Movements, Divisions, and Fusions of Nucleoli.*

Amoeboid movements have been seen in life in metazoan cells by the following observers (germinal vesicles): A. Brandt ('74, *Blatta*), Eimer ('75, *Silurus*), O. Hertwig ('76, *Rana*, *Pterotrachea*), La Valette St. George ('66, *Libella*; '83, *Isopoda*), Bergh ('79, *Gonothryaca*), Van Beneden ('69, '76, *Polystomum*, *Rana*), Balbiani ('64, several genera of spiders), Leydig ('83, *Libella*), A. Brandt ('78, numerous *Insecta*, *Distomum*), Van Bambeke ('86, *Blatta*), Knappe ('86, *Bufo*), Auerbach ('74a, *Telcostii*). In somatic cells: Schwalbe ('76, sympathetic ganglion cells of *Rana*), Kidd ('75, epithelial cells from the mouth of *Rana*), Hodge ('94, nerve cells of *Rana*), Auerbach ('74b, salivary gland cells of *Musca*). In *Protozoa*: Van Beneden ('69, '76, *Gregarina*, *Monocystis*). In plants, Zacharias ('85) has observed amoeboid movements in the nucleoli of *Chara* (an observation overlooked by Zimmermann, who states that such movements have not been seen in plants).

These observations would show that amoeboid movements are probably natural phenomena of certain nucleoli, but one should not be too positive of the naturalness of these phenomena, since some of the observations were made upon the heated stage, and in all of them the object was probably more or less compressed and placed in artificial conditions. But they are in all probability frequently normal phenomena, since, as we shall see, divisions and fusions of nucleoli are certainly normal and of wide occurrence, and the latter can only be classed as forms of amoeboid motion. The question arises, Are these movements wholly passive, caused by movements in the other parts of the nucleus, or should they be considered an inherent function of the nucleolus? The latter alternative would seem the more probable, since no movements of the other nuclear elements are known in the resting cell. Van Beneden ('69) has described rhythmic expansion and contraction of the volume of nucleoli in gregarines. But all these movements of nucleoli should not be regarded as automatic motions of the nucleolus in the sense that an *Amoeba* forms and retracts processes; but rather with Rhumbler ('93) they should be regarded as "Auf-

lösungsvorgänge," due to chemical changes in its substance. Cf. the movements described by Flemming ('97) for the ovum of *Ascidia*.

The nucleolus has in some cases a viscid consistency (as described by me for *Stichostemma*) and then may be irregular in form; in other cases it is more fluid, and this is probably the case when it has regularly a spherical shape, *i.e.*, the globular form characteristic of drops of a thin liquid. Its more or less fluid consistency allows changes of form, division into particles, and fusions of neighboring nucleoli.

The division of a nucleolus into two or more parts is a normal and regular phenomenon in many cells, though all nucleoli do not show this property. Two kinds of nucleolar division may be distinguished: (1) that mode by which the nucleolus becomes elongated and then breaks into two or more parts, whereby the daughter-nucleoli are usually capable of further division; and (2) that mode by which the nucleolus fragments nearly simultaneously into a number of small granules. From my own observations the former mode is evinced by the nucleoli of the muscle and giant gland cells of *Piscicola*, the giant cells of *Doto*, and the germinal spots at certain stages in the ovogenesis of the metanemertean. This mode of division cannot be regarded as a phenomenon of nucleolar degeneration, since the nucleolus and its products may often continue to increase in size during the process of division. But the second mode, that by which the nucleolus breaks into a large number of granules, since it is particularly characteristic of the nucleolus in nuclear division, may be regarded as a process of degeneration; the case of divisions during nuclear division shall be considered later. A strange mode of nucleolar division has been described by A. Schneider ('83). According to his observations on *Klossia*, the smaller nucleoli are portions of the inner substance of the larger nucleoli and wander out of each larger one by passing through the pore ("canal micropylaire") of the cortical substance of the latter; this intranucleolar origin of the smaller nucleoli is still open to question, since it was not observed in life, and since the canal micropylaire was observed in only one nucleolus. Marshall ('92) has described

a somewhat similar method of formation of the smaller nucleoli of *Gregarina blattarum*. Now I found in the nucleus of the gregarine from *Lineus* numerous nucleoli of different dimensions, and often irregular in their outlines; and this irregularity in form would point not only to amoeboid movements of the nucleoli, but also to nucleolar divisions, since in the largest nuclei we find a large number of small nucleoli. All appearances showed that these smaller nucleoli are division products of the larger ones; but it seems that they simply bud off from the surface of the latter, and are not preformed in their interior. In other words, Schneider and Marshall are probably correct in concluding that the smaller nucleoli are disassociated portions of the larger ones; but they may perhaps be mistaken in assuming that they are preformed in the interior of the latter, since these investigators may have mistaken vacuoles for intranucleolar nucleoli. (Other observations on nucleolar divisions in resting cells: Hermann, '89; Vejdovský, '95a; Bütschli, '80; R. Hertwig, '76; Kultschitzky, '88; Bergh, '79; Bannwarth, '92; Stuhlmann, '86; A. Brandt, '78; Scharff, '88; Eisig, '87; Cunningham, '95; Kosinski, '87, '93; Carnoy and Lebrun, '97a; Steinhaus, '88; Cuénot, '91; Metzner, '94.)

Fusions of nucleoli are not as widely known as divisions, but there are some facts which would show that the former processes are by no means unusual in their occurrence. Such fusions have been described for cells of plants by Zacharias ('85), Mann ('92), and Wager ('93); for animal cells by Rhumbler ('93, '95), Brauer ('91), Leydig ('50), Pfitzner ('83), and Rückert ('92). I have found fusions of the nucleoli to be characteristic phenomena of certain stages in the maturation of the germinal vesicles of nemerteans, an extreme case being furnished by *Stichostemma*, where sometimes all the nucleoli may fuse together at the center of the nucleus, and so produce a single large one. The nucleolus at the time of its origin may be said to be undergoing a process of fusion, since it is produced by the coalescence of numerous smaller portions of nucleolar substance. There is nothing problematical in regard to the fusion of nucleoli, since it is a physical property for bodies of like nature (when fluid) to fuse together when they come into contact, though this



process is to some extent dependent upon the nature of the medium in which they are suspended (*cf.* Rhumbler, '93). (*Cf.* also Hermann, '89b; Bouin, '97; Mertens, '93; Debski, '97; Carnoy and Lebrun '97a; Koernicke, '96.)

### 7. *Paranucleoli and Pseudonucleoli, Double Nucleoli, etc.*

The term paranucleolus is here adopted as equivalent to Flemming's "Nebennucleolus," and I shall use simply the name "nucleolus," or "nucleolus proper," instead of "Hauptnucleolus." E. B. Wilson's terms, "principal nucleolus" and "accessory nucleolus," are somewhat inconvenient on account of their length, and may be misleading, since the "principal nucleolus" is often smaller than the "accessory nucleolus." "Paranucleolus," as used here, is not employed in the same sense as by Stuhlmann ('86), since he expresses by this term portions of the nuclear reticulum; in my paper the term "nucleolus" has not been used for any part of the chromatin elements of the nucleus.

In many egg cells, especially those of the *Mollusca*, *Annelida*, *Tunicata*, and *Echinodermata*, two kinds of nucleoli occur according to the writers on these objects, which differ from one another chemically and in some cases also structurally; these are the nucleolus proper and the paranucleolus. Of these it is the nucleolus proper which seems to be morphologically comparable to the nucleoli of somatic cells, however the two may differ chemically. The paranucleolus may be either larger or smaller than the nucleolus, and appears usually to be distinguishable from the latter by staining less deeply with the specific nucleolar stains. In the spermatoblast of the mouse these two kinds of nucleoli have been found by Hermann ('89); and in somatic cells by Lönnberg ('92, liver cells of *Doris*, *Polycera*, *Aeolidia*, and *Astacus*); perhaps the smaller of the two nucleoli found by me in the blood corpuscles of *Doto* might represent a paranucleolus. In plant cells apparently only one kind of nucleolus is present, this being comparable morphologically to the nucleolus proper of the germ cells and to the nucleoli of the somatic cells of *Metazoa*. Thus paranucleoli are quite

frequent in many egg cells, infrequent in somatic cells of the *Metazoa*, and apparently never present in plant cells. In each such egg cell there may be either one nucleolus proper and from one to several paranucleoli (this being the most usual case), or there may be a single paranucleolus and a few nucleoli proper. In the ova of three forms examined by me there were two kinds of nucleoli present, namely, in *Montagua*, *Polydora*, and *Rodalia*. In my descriptions I have employed the term "pseudonucleolus" for these secondary nucleoli, since in this form they have a different structure from that of the nucleolus proper, but nevertheless stain in the same way, so it is difficult in this case to decide whether they correspond to paranucleoli, and hence I have used the indifferent name "pseudonucleoli" for them. In *Polydora* we found from one to three paranucleoli in the larger germinal vesicles, and these are always apposed to the nucleolus. Then the smaller, deeply staining bodies in the maturer stages of the ovum of *Rodalia* may be comparable to paranucleoli. Whether the remarkable structures of the germinal vesicles of *Tetrastemma catenulatum* are paranucleoli, I am wholly unable to decide. This problem of different types of true nucleoli in the same nucleus is one of the most difficult in the study of nucleolar structures, so that it is necessary to discuss it more in detail.

A. Schneider ('83), Brauer ('91), and Floderus ('96) consider the paranucleoli to be derivatives of the nucleolus proper, more especially to be buds from its surface. Häcker ('93a) considers them to be secretions of the chromatin. Flemming ('82) doubts whether "die Unterscheidung von Haupt- und Nebennucleolen eine durchgehende Geltung beanspruchen kann"; he finds that in *Anodonta* the two are at first in contact, but that later they become separated. Giard ('81) finds in the ovum of a Spionid one nucleolus, and later there appears in the nucleus a much smaller body, which fuses with the former. Lönnberg ('92) thinks that the paranucleoli may serve for the acquisition of nourishment, or may contain reserve nourishment. List ('96) considers that the paranucleoli and the nucleoli of the somatic cells are more closely allied to one another than to the nucleolus proper of the ova, and that the former two "mindestens

verschiedene Modificationsstufen des Paranucleins . . . darstellen." Hessling ('54) found that in the ovum of *Unio* the smaller paranucleolus is divided off from the larger nucleolus proper. Häcker, in his last paper on the subject ('95), considers that the paranucleoli are of later formation than the nucleolus proper.

Now in many of those cases where a paranucleolus and a nucleolus have been described lying in contact with one another it is very probable that the vacuolar portion of the vacuole has been described as a paranucleolus. I have no doubt that many of the earlier observers, who studied the nucleolus mainly in the living egg, have been thus misled, since only sections of nucleoli can show the true nature of the nucleolus. Thus Lönnerberg, in speaking of "helle Kugeln" in the germinal spot of *Mytilus*, says: "Es ist schwer zu entscheiden, ob es sich hier nur um Vacuolen handelt"; and any one studying the unsectioned nucleolus of *Montagna* would be misled into supposing that here two nucleoli of different consistency are apposed to one another. Accordingly, we must be very careful in treating as facts some of the observations of the earlier workers, which were made upon unstained and unsectioned material.

But there are undoubtedly many cases in which two kinds of nucleoli do occur,<sup>1</sup> and this is especially so in germinal vesicles. The nucleolus and the paranucleolus may be in contact with one another, may be always separated, may at first be in contact and later become separated, or finally may be at first separated and later come into mutual contact. Are these paranucleoli derived from the nucleolus proper, or have they a distinct origin? In the ovum of *Polydora* the paranucleoli appear towards the close of the maturation period, and then are always in contact with the outer surface of the nucleolus proper.

<sup>1</sup> Cf. the reviews of the following papers: Floderus ('96), Hermann ('89a, b, '97), Vojdovsky ('95a), Flemming ('74, '82), Häcker ('93a), Kultschitzky ('88), Lukjanow ('87b), Brauer ('91, '92), Nussbaum ('87), Rein ('83), Henking ('87), Van Beneden ('80), Leydig ('55a, '50), Stauffacher ('93), Stepanoff ('65), Giard ('81), Mark ('77, '81), Lönnerberg ('92), Stuhlmann ('86), List ('96), Van Bemmelin ('83), Platner ('86), Claparède ('69), Hessling ('54), Rückert ('94), Bouin ('97), Vom Rath ('95b), Moore ('95), Weismann and Ishikawa ('89), Fol ('89), Lacaze-Duthiers ('57), Fauvel ('97), Held ('95), Michel ('96), Steinhaus ('83), Metzner ('94), Braem ('97), Siebold ('39), Reinhard ('82), Kraepelin ('92), Davenport ('91).

In the ova of *Montagua* and *Rodalia* they are never in contact with the nucleolus. In none of these three cases observed by me does there seem to be any genetic connection between the paranucleoli and the nucleoli proper. And in other cases, where the two are separated (this separation is the most usual state), no genetic connection between the two has been described; and even in that smaller number of cases where they are in contact with each other at some period of their development, no positive proof of their genetic relation has been offered. Therefore we might conclude, though with reserve, that in the greater number, if not all, cases the paranucleoli are not derivatives of the nucleolus, but are products *sui generis*. It is the rule that the nucleolus proper appears in the nucleus before the paranucleoli arise, the latter usually arising first towards the close of the growth stages. Accordingly, though I cannot corroborate Häcker's ('95) conclusions as to the origin of the nucleolar substance, I am inclined to agree with him that portions of nucleolar substance are successively deposited in the nucleus, and that those portions which are deposited last, after the nucleus has undergone important physiological and chemical changes, would differ from the portion first produced (that of the nucleolus proper), and so would represent the paranucleolus. And there are certain facts from my own observations which would support this view. In the earlier stages of the maturation of the ovum of *Tetrastemma* and *Zygonemertes* there are a large number of nucleoli produced successively at the periphery of the nucleus; these then wander successively to the center of the nucleus, and then from that point again to the periphery. Now in this last stage, when the nuclear filaments are commencing to arise, we find, usually in contact with the latter, much smaller, more deeply stained nucleoli, and these I have termed "nucleoli of the second generation." We have found, accordingly, that after the nucleus has passed through very marked physiological changes (increase in size, redistribution of chromatin), another kind of nucleoli appears, which may or may not be morphologically compared to the paranucleoli of other ova. These nucleoli of the second generation have neither a genetic nor a physiological

relation to those of the first generation ; and their difference from the latter is probably due to the fact that they have been produced at a time when very different physiological conditions exist in the nucleus.

It is not my intention in this contribution to deal in any detail with those cases where double nucleoli occur in a cell, or those where two chemically and morphologically different kinds of "nucleoli" occur in the same nucleus ; to these cases it is my intention to devote a special study. But preliminarily, from those observations which I have made on this subject, the following conclusions are in order. In a nucleus there sometimes occurs a double nucleolus, the component parts of which may each represent a true nucleolus ; or such a double nucleolus may consist of a true nucleolus apposed to a chromatin-nucleolus (according to my unpublished observations on the spermatocytes of the beetle *Harpalus*). Further, and this is frequently the case in resting spermatocytes of the first order, the nucleus may contain a true nucleolus separated from a chromatin-nucleolus ; and in *Pentatoma*, the account of the spermatogenesis of which will be shortly published by me, the unique process occurs of the chromatin-nucleolus being a metamorphosed chromosome (one of the fourteen chromosomes of the last spermatogonic division becoming the chromatin-nucleolus of the first spermatocyte) ! This peculiar structure of *Pentatoma* divides with the true chromosomes in the first reduction division. In another case where I have been able to follow all the developmental stages of a chromatin-nucleolus, namely, in cells of the hypodermis of the larva of *Carpocapsa*, I found it to originate from one of the granules of the nuclear reticulum, — a particular one of these granules (karyosomes) gradually increasing in size until it attains large dimensions ; during its growth period it is usually attached to one of the true nucleoli of the cell. What is of importance in these two cases (*Pentatoma* and *Carpocapsa*) is the distinction emphasized between the true nucleolus and a karyosome or chromatin-nucleolus : the latter always standing in genetic connection with the true chromatin, while the former, so far as my observations go, is never derived from this substance. These observations are not

wholly out of place in the present paper on the true nucleolus, since they are necessary to prove that the true nucleolus is in all cases never derived from the chromatin ; where " nucleoli " have been described as arising from the chromatin elements of the nucleus, such structures cannot correctly be included under the term " nucleolus," when the latter is used in the proper sense.

#### 8. *Relation between Nucleoli and Centrosomes.*

The greater number of cytologists agree that there is no genetic relations between these two structures ; and my observations on the egg of *Piscicola* as well as more recent studies on other objects corroborate this view. But some few have been led to contrary conclusions by observing the fact that in mitosis the nucleolus often disappears about the time that the centrosome becomes apparent. Thus Karsten ('93) assumes that the nucleoli wander out of the nucleus into the cytoplasm, and there become the centrosomes of the spindle ; this observation has been refuted by Humphrey ('94). Also Wasielevsky ('93) believes that the centrosomes of the egg of *Ascaris* stand in some connection to the nucleoli, but this stands in direct opposition to the conclusions of all other workers on this object, except those of Carnoy and Lebrun ('97b), and the supposition of Sala ('95). Then Lavdowsky ('94) concludes that the nucleolinus is the centrosome in the process of formation, but he failed to observe the steps by which this body develops into a centrosome. Further, Julin ('93b) is said by Delage ('95) to have assumed a genetic relation between the centrosome and the nucleolus. Other supporters of the nucleolar origin of the centrosome : Balbiani ('95), Wilcox ('95), Bremer ('95b), F. Toyama ('94). I believe that these are the only investigators who have assumed this genetic relation. We may conclude, from the greater number of observations at hand, that there is probably no connection between these structures in the metazoan cell. But it is difficult to decide the homologies of the body found by Keuten ('95) in the nucleus of *Ceratium*, and termed by him nucleolo-centrosoma ; he considers it as equivalent to the central

spindle and centrosome of *Ascaris*, but might it not be compared to the nucleolus alone, or to the nucleolus plus centrosomes of the metazoan cell? However, the significance of most protozoan "nucleoli" is very problematical. (Cf. the later observations of Lauterborn, '95a.)

### 9. *Ontogenetic Origin of the Nucleolus.*

Very few observations have been made to determine the mode of origin of the nucleolus, though there are numerous hypotheses intended to explain it. We may leave aside, for the time being, its mode of reappearance in the daughter-nuclei after nuclear division, since a special section will be devoted to that subject.

In order to determine the mode of origin of the nucleolus in resting stages of nuclei, I have studied those cells in which at first no nucleolus is present, but which after a certain period of growth acquire one. Objects well adapted for such investigation are the ova of the nemerteans and the mesenchym cells of *Cerebratulus*. For details of these processes the reader is referred to the observations.

In the ova of the nemerteans the nucleoli at the time of their first appearance are always in close contact with the nuclear membrane; this is also the case for the mesenchym cells of *Cerebratulus*, and probably for the paranucleoli of the ova of *Rodalia*. In all these cells the nucleoli only then leave the periphery of the nucleus and wander towards its center, after the nucleus has increased more or less in size. There is only one explanation for the peripheral position of the nucleoli at the time of their first appearance, namely, that their substance is extranuclear in origin. This process of formation has already been discussed in detail for the several cells, and it is not necessary to repeat here all the detailed observations on which the main deduction is based. If the nucleolar substance were a secretion of the nucleus, as Häcker ('95) assumes, how would this assumption explain the strictly peripheral position of the nucleoli when they first arise? For on Häcker's hypothesis we should expect the supposed nucleolar secretions to be de-

posited evenly throughout the nucleus, and not only at the periphery. And his deductions are based in great part, as those of most other investigators, on the study of maturation mitoses, and he had not observed their first mode of origin, namely, their origin in nuclei which are not in the prophases of mitosis, but are only gradually becoming differentiated from somatic cells. I have found no evidences in any cell that the nucleoli stand in any genetic relation to the chromatin elements of the nucleus; and while the chromatin may derive substances from the nucleoli, I am unacquainted with any observations which show that the nucleoli derive any part of their substance from the chromatin. In all the cases observed by me, the nucleus appears to assimilate a substance or substances from the cytoplasm, and after this substance has entered the nucleus it apparently undergoes there a chemical change, and becomes deposited on the inner surface of the nuclear membrane in the form of masses of varying dimensions, which may be either globular or irregular in shape, according as they are fluid or viscid in consistency. In the case of the ova of the nemerteans the substance taken up into the nucleus, and which there becomes deposited in the form of nucleoli, is sometimes exactly similar to the substance of the yolk-balls which lie in the cytoplasm; in other cases it is probably similar to those metabolically changed portions or inclusions of the cytoplasm, out of which the yolk-balls are later differentiated. In *Lincus*, indeed, the yolk-balls may often be found halfway through the nuclear membrane, and their appearance is exactly similar to that of the nucleoli. In the mesenchym cells of *Cerebratulus* the substance of the nucleoli appears to be identical with that of the numerous nutritive granules which are dispersed in the cytoplasm; the latter globules arise in the cytoplasm before the nucleolus appears in the nucleus, and as soon as they become numerous in the neighborhood of the nucleus, peripheral nucleoli begin to appear in the latter. In the subcutical gland cells of *Piscicola* the nucleolus, at the time of its most rapid growth, is apposed to the nuclear membrane; but when this period of volume-increase has ceased, it is never found in this position. Further, the paranucleoli of *Rodalia* appear first in contact with the



nuclear membrane. Schwalbe ('76) found in the nuclei of various vertebrate embryos that when the nucleoli first arise they appear as thickenings of the inner surface of the nuclear membrane.

From these observations I conclude, accordingly, that the nucleolar substance, in many if not all cells, has an extranuclear origin ; and that, though it may undergo a chemical change after entering the nucleus, it can be regarded neither as a secretion nor as an excretion of the latter. In making this conclusion I can corroborate the views of only one investigator, namely, Korschelt ('89), though he changed this opinion in a later paper ('97). He concluded that the nucleolar substance stands in some connection with the nutritive processes of the cell, and that the nucleus probably derives it from the cytoplasm.

Other views on the origin of the nucleolus (those of Häcker have already been mentioned): Auerbach ('74a, '76) first supposed the nucleolus to be cytoplasmic in origin ; more recently ('90) he appears to champion its nuclear origin. Rhumbler ('93) assumes that the "Binnenkörper" of *Protozoa* are products of the nucleus, but he does not attempt to decide whether those of the *Metazoa* have a similar origin. Strasburger ('82b) also postulates a nuclear origin for the nucleolus, and assumes that its substance is allied to chromatin. Jordan ('93) holds that the nucleoli probably arise from the chromatin threads. Flemming ('82) considers them to be "spezifische Produkte des Kernstoffwechsels." Schwalbe ('76) supposes the nucleolar substance to be at first identical with that of the nuclear membrane, since he found it to arise as thickenings of the latter. C. Schneider ('91) supposes it to be a metamorphosed portion of the chromatin. Leydig ('83) concludes that the nucleoli are portions of the chromatin reticulum. Guignard ('85) assumes that they are derivatives of the chromatin filaments. Watasé ('94) considers them to be metabolic products of the cell, but he gives no detailed observations in regard to their mode of formation. Mertens ('93) and Retzius ('81) consider them to arise by concentration of the chromatin reticulum.

10. *Discharge of Nucleolar Substance from Resting Nuclei.*

Will ('84) holds that the larger nucleoli of the amphibian germinal vesicle pass out into the cytoplasm, and there become the yolk-nuclei; and Scharff ('88) corroborates this view for the ova of *Trigla*, though it is opposed by Cunningham ('95). Macallum ('91) concludes that in amphibian ova the peripheral nucleoli generate a substance which diffuses first in the nucleus and from there into the cytoplasm, and that this substance combines with the cytoplasm to form the yolk substance; Jordan ('93) expresses a somewhat similar view in regard to the yolk formation of the newt. Henneguy ('93) assumes that the corpuscle of Balbiani in the ova of *Vertebrata* "est très probablement une partie de la tache germinative, ou une tache germinative entière, qui sort de la vésicule [germinative] pour pénétrer dans le vitellus," and Mertens ('93) holds a similar view. And for egg cells of *Tunicata*, Floderus ('96) confirms Roule's ('84) observations, that the "intravitelline Körper" are paranucleoli which have wandered into the cell body. Cf. also Bremer ('95a, b).

Leydig ('88) finds that in ova of *Geophilus*, *Stenobothrus*, *Rana*, and *Triton* particles of nucleolar substance penetrate into the cytoplasm. Lukjanow ('88) concludes that in the case of the cells of the stomach mucosa of *Salamandra*, the nucleolus discharges a portion of its substance from the nucleus. Humphrey ('94), from observations on plant cells, maintains that in some cases portions of nucleolar substance may pass into the cytoplasm.

Fol ('83a, b) concludes that the follicle cells of the ascidian egg arise as buds from the surface of the germinal vesicle, and that each of these buds contains a particle of nucleolar substance; these conclusions are affirmed by Roule ('83). Scharff ('88) supposes that the follicle cells of the ovum of *Gadus* are derived from nucleoli which have left the germinal vesicle, such nucleoli becoming the nuclei of the new cells. (Ogata '83) studied human pancreas cells and finds that a nucleolus wanders out of the nucleus, becomes a "Nebenkern," and the latter finally changes into the nucleus of a new cell, a conclusion which is opposed by Platner ('89b).

I have found a wandering of nucleolar substance out of resting nuclei in one very beautiful and unique case, namely, in the subcuticular gland cells of *Piscicola*; at one stage in its cycle of development the nucleus commences to contract in volume, and in so doing discharges all except a single one of its nucleoli into the cytoplasm. This and certain of the observations cited from other investigators show that a discharge of nucleolar substance from the resting nucleus takes place in some cells. But the more recent observations of Morgan, Floderus, and others on Tunicate development render it very probable that Fol and Roule were mistaken in assuming that the nucleoli which pass out of the germinal vesicle become the constituents of follicle cells. There is still some question, also, as to whether the nucleolar substance in the cytoplasm takes any part in the formation of the yolk substance. Other pertinent observations: Mertens ('93), Bremer ('95a, b), Kosinski ('87, '93), Galeotti ('95), Melissinos and Nicolaidis ('90), Auerbach ('74), Ver Ecke ('93), Steinhaus ('88), Rohde ('96).

### II. Behavior of Nucleoli during Nuclear Division.

It is in cases of nuclear division that the nucleolus has received the most attention from morphologists. The behavior of the nucleolus in mitosis and amitosis may be treated separately.

1. *Amitosis*. — In this mode of nuclear division it is frequently the case for the nucleolus to divide first, so that each of the daughter-nuclei receives a half, or approximately a half (for the division of the nucleolus is not always into two equal parts), of the parent-nucleolus. In support of this deduction the following observations may be mentioned: Schaudinn ('94, *Amoeba crystalligera*); F. E. Shulze ('75, *A. polypodia*); Will ('85, ova of *Nepa*, *Notonecta*); Doflein ('96, degenerating ova of *Tubularia*); Carnoy ('85, ova of *Grylotalpa*, *Lithobius*, *Geotrupes*); Korschelt ('95, intestinal cells of *Ophryotrocha*); my observations on the peritoneal cells of *Polydora*; Hoyer ('90, intestinal epithelium of *Rhabdonema*); Frenzel ('93b, hepatopancreas cells of *Astacus*); Platner ('89a, Malpighian tubes of *Dytiscus*; Wheeler

('89, follicle cells of *Blatta*); de Bruyne ('97, follicle cells of *Nepa*, *Periplaneta*, *Meconema*, *Aeschna*). E. B. Wilson ('96), in speaking of amitosis, states: "In many cases, however, no preliminary fission of the nucleolus occurs; and Remak's scheme must therefore be regarded as one of the rarest forms of cell division." But the list of cases which I have given shows that such cases of nucleolar division are frequent in amitosis, so that I conclude that a fission of the nucleolus, if not exactly typical for this mode of nuclear division, is nevertheless well represented and occurs here much more frequently than in mitosis. Dr. E. G. Conklin has demonstrated to me preparations of nucleolar division in follicle cells of *Gryllus*, which he has kindly allowed me to mention here.

2. *Mitosis*.—In karyokinesis the nucleolus may either not disappear, or, and this is the most usual case, it disappears before the spindle is formed. These two modes may be considered in turn.

(a) *The nucleolus does not disappear*.—In some few cases the nucleolus wanders out into the cytoplasm after the disappearance of the nuclear membrane and may remain there for some time without undergoing any change. Such cases have been described by Häcker ('92a, egg of *Aequorea*), Wheeler ('95, that of *Myzostoma*), H. V. Wilson ('94, ova of *Tedamione* and *Hircinia*), Tangl ('82, flower buds of *Hemerocallis*), Gjurasin ('93, *Peziza*), and Karsten ('93, sporangia of *Psilotum*). In all these cases the nucleolus ultimately disappears in the cytoplasm, though in *Aequorea* it may be observed still in the cell body of one of the blastomeres at the thirty-two cell stage, and the daughter-nuclei produce their own nucleoli. (Similar are the observations of Mead, '95; Häcker, '96, '97; Rosen, '95; Zimmermann, '96; Metzner, '94; Foot '94; Poirault and Raciborski, '96.)

In the other cases where the nucleolus does not disappear it remains within the nucleus. In some of these cases it appears to divide into two or more parts; in other cases it may be that one of the daughter-nuclei receives the whole parent-nucleolus, while in the other one a new nucleolus is produced. There are a few observations which show that it sometimes divides; thus Strasburger ('82b, embryo sac of *Galanthus*) and Rosen ('92b,

*Synchytrium*); Reinke studied the mitosis of the spleen cells of the mouse, and found that the single parent-nucleolus divides into three or four pieces, while at the end of the mitosis each daughter-nucleus contains a single nucleolus. In the mitoses of the ovogonia of *Lineus* and *Polydora* my own observations show that the nucleolus persists in the nucleus, and each daughter-nucleus contains one nucleolus, so that it is very probable that in these cases the parent-nucleolus divides into two, and each daughter-nucleus thereby receives a half of it; but these mitoses were so small that I was unable to decide this point definitely. Rosen ('95) finds nucleolar division in root cells of *Phascolus*; J. Wagner ('96a) describes a similar division of a "nucleolus" in spermatocytes of Arachnids, though this case, like that described by Henking ('90), probably represents a chromatin nucleolus. This persistence of the nucleolus in the nucleus during mitosis must be considered atypical.

(b) *The nucleolus disappears during mitosis.*—This is the most usual mode of behavior of the nucleolus during mitosis. The nucleolus either gradually diminishes in size, and so finally vanishes, or else it first fragments into a number of smaller pieces, and then these disappear. The only cell which I had for the study of this phenomenon was the ovum of *Piscicola* during the formation of the first pole spindle. When this spindle is complete no trace of nucleolar substance is to be seen anywhere in the cell. In stages immediately antecedent to that of the spindle, numerous minute granules, as well as a smaller number of larger globules, are dispersed through the nuclear sap; all these stain with eosin, and I regard them as particles of nucleolar substance which had become separated from the nucleolus. Thus a dissolution of the nucleolar substance commences before the nuclear membrane has disappeared, and after this membrane has vanished it is probable that all the nucleolar substance must be dissolved by the action of the cytoplasm, or at least become dispersed through the latter, so that no remnant of it is to be found in the region of the spindle or of the chromosomes. During the process of dissolution of the nucleolar substance in the nuclear sap the chromatin elements stain red (with eosin), and this fact may be

explained either by the assumption that the nucleolar substance unites chemically with the chromatin, or that it simply penetrates into the meshes of the latter; since no nucleolar substance appears to be united with any of the twelve chromosomes we may conclude that it does not unite chemically with the chromatin, and therefore the chromosomes probably do not serve to carry it over into the daughter-nuclei. We may now briefly review the results of other observers on the mode of disappearance of the nucleolus during mitosis.

It is not necessary to discuss the earlier view of O. Hertwig, which he has since discarded, that "der Eikern der aus dem Keimbläschen frei gewordene oder ausgewanderte Keimfleck ist," nor yet the view of Kölliker. Kleinenberg ('72) believes that the germinal spot of *Hydra* dissolves during mitosis; Brauer ('91) finds that it breaks into fragments, of which a part seems to be dissolved in the cytoplasm, "ein Theil tritt unverändert nach dem Schwinden der Membran in das Eiprotoplasma über." Fick ('93, germinal spot of *Amblystoma*) finds that the nucleoli disappear at the time of the longitudinal splitting of the chromosomes; and Böhm ('88) reaches the same conclusion for *Petromyzon*. Davidoff ('89, ovum of *Distaplia*) concludes "dass aus dem Nucleolus ein Kern mit Kernnetz, mit einem Nucleolus und Nucleolinus hervorgegangen ist"; and Vejdovský ('88, *Rhynchelmis*), Blochmann ('82, *Neritina*), and Marshall ('92, *Gregarina*) conclude that the nucleoli become chromosomes. In the egg of *Ascaris* the nucleoli gradually disappear, according to most observers. Strasburger ('82b) first contended that the nucleolar substance is taken up into the nuclear filaments; later ('88) he writes: "Auf Grund meiner neueren Erfahrungen erscheint es mir überhaupt unwahrscheinlich, dass die Nucleolarsubstanz, auch nach ihrer Auflösung im Kernsaft, den Kernfäden als Nahrung dienen sollte," and he considers that after it is dissolved in the nuclear sap a portion of it forms the cell membranes of the daughter-cells (*cf.* also his paper of '93). Rein ('83, ova of *Lepus* and *Cavia*) finds that the nucleolus breaks into small fragments, which finally disappear in the substance of the nucleus. Pfitzner ('83, ectoderm cells of *Hydra*) terms the nucleolar substance "prochromatin," since

he finds that in mitosis it changes into chromatin. Rabl ('85, larval cells of amphibians) and O. Schultze ('87, ova of *Rana* and *Triton*) contend that the nucleolar substance takes some part in the formation of the nuclear filaments; but Born ('94) subsequently found that these filaments stand in no connection with the nucleolar substance. Holl ('93, ovum of *Mus*) finds that the central granules of the nucleoli wander out of them and so become the chromosomes. Van Beneden ('75, ovum of *Lepus*) originally supposed that the nucleolus becomes the first pole body. Kastschenko ('90, ova of *Selachii*) finds that all the nucleoli disappear in the spirem stage, while Rückert ('92) finds that a few of them pass into the cytoplasm. Stuhlmann ('86, ova of *Insecta*) finds that the nucleoli gradually disappear during the maturation of the egg; and similar conclusions were reached by Stauffacher ('93, *Cyclas*), Rhumbler ('95, *Cyphoderia*), Sheldon ('90, *Peripatus*), Heathcote ('86, *Julus*), Van der Stricht ('95, *Amphioxus*), Brauer ('92, *Branchipus*), and Vejdovský ('82, *Sternaspis*). Auerbach ('96, spermatogonium of *Paludina*) holds that the nucleolar substance becomes incorporated with the chromatin elements. Meunier ('86) and Moll ('93) for *Spirogyra*, and Carnoy ('85) for other cells also, hold that the chromosomes are derivatives of the chromatin skein of the nucleolus. Heuser ('84, mitoses of various plant cells) contends that the nucleoli become gradually apposed to the nuclear filaments, and that their substance unites with these elements, though in some cases a superfluous portion of the nucleolar substance may be discharged from the nucleus. Korschelt ('95, ovum of *Ophryotrocha*) finds that the nucleolus gradually disappears by dissolving in the nuclear sap, and believes that a part of this substance may be introduced into the nuclear filaments. Zacharias ('85) somewhat prematurely concludes that the nucleoli always disappear in mitosis. Tangl ('82) finds that in *Hemerocallis*, in uninucleolar nuclei, the nucleolus dissolves in the nucleus, but in those which are multinucleolar one may pass out into the cytoplasm; in *Hesperus* and *Cisium* they gradually disappear. Humphrey ('94, plant cells) holds that "die Nucleolen in einigen Fällen aus der Kernhöhle, bevor sie von den karyokinetischen Kräften angegriffen werden, austreten können."

Wager ('93, *Agaricus*) describes the nucleoli as becoming dissolved in the caryolymph, and then, this dissolved substance penetrating the chromatin elements, the latter serve to carry it over into the daughter-nuclei. Went ('87, plant cells) holds "dass in vielen Fällen wenigstens der Nucleolus beim Anfang der Kerntheilung im Kernfaden aufgenommen wird," and that "er sich nach der Theilung auch wieder daraus bildet." Rückert ('94, egg of *Cyclops*) finds that the nucleoli gradually break into fragments and the latter disappear. But there is not space here to mention all the views of students of mitosis.

There are only a few observations which would show that in mitosis the chromosomes are derived from the nucleoli (Davidoff, Vejdovský, Blochmann, Marshall, Sobotta, '95, Macallum, '95, Carnoy, '97a, R. Hertwig, '96, not corroborated by Brauer, '94), and these cases stand in such marked contradiction to the observations of other morphologists that a reinvestigation of them is very necessary.<sup>1</sup> Then we have the observations of Carnoy, Meunier, and Moll, which would show that the chromosomes are derived from a part of the nucleolus; but the existence of a "nucléole-noyau," *i.e.*, of a nucleus within a nucleus, as assumed by Carnoy and his followers, in any metazoan cell, seems to be very problematical. On the other hand, most observers agree that the nucleoli disappear more or less gradually during mitosis, and that the chromosomes are not derived from them. Now we have reached the crucial question: What is the mode of transference of the nucleolar substance to the daughter-nuclei? In answer to this, some observers hold that this substance may be distributed in the cytoplasm and taken up therefrom into the daughter-nuclei; others, that it combines with the chromatin elements and is transferred with these; still others maintain a position intermediate between these two.<sup>2</sup> But when we find so much variance in the conclusions of competent investigators only one deduction is allowable, namely, that the mode of transportation

<sup>1</sup> On the relation of nucleoli to chromosomes, *cf.* also Cunningham ('97), Sobotta ('95), Macallum ('95), Platner ('89c), Carnoy ('97a), R. Hertwig ('96), Van Beneden ('83), Zimmermann ('96), Lauterborn ('96), Boveri ('88), Wheeler ('97).

<sup>2</sup> *Cf.* also Belajeff ('94), Mottier ('97), and Rosen ('95).



of the nucleolar substance is probably different in different objects.

We have found above that in the simplest though secondary nuclear divisions, the amitotic, the nucleolar substance of the parent-cell is transported into the daughter-nuclei by the mechanically simplest process, namely, by a direct division of the parent-nucleolus ; this is very frequently the case in amitosis, though it does not always occur. But in most mitotic divisions the nucleolus first disappears, *i.e.*, there would seem to be an indirect mode of transference of its substance corresponding to the indirect mode of transference of the chromatin and linin elements. Now all mitotic divisions do not proceed on exactly the same plan, for we find differences in regard to the presence of a central spindle, in regard to the number of the chromosomes, etc. Accordingly, one would expect also different modes of transference of the nucleolar substances. Thus in some cases, as Wager ('93) suggests, the chromosomes may serve as mechanical vehicles for the transportation of this substance. In many other cases it is very probable that this substance, after the disappearance of the nuclear membrane, becomes dispersed in the cytoplasm ; and then each of the daughter-nuclei may either take up this substance from the cytoplasm again, or may produce its own nucleolus from a new substance, owing to the primitive nucleolar substance having been assimilated by, or even discharged from, the cytoplasm. There are observations in support of each of these three modes of re-formation of nucleoli in the daughter-nuclei. But since when the nuclear membrane disappears the cytoplasm probably comes into contact with the substance of the nucleoli, it is most probable that it would produce either a physical or a chemical change in the latter, and hence the second and third modes would appear the more probable. Accordingly, I agree with Humphrey ('94) that there is no substantial basis for Zimmermann's ('93) conclusion "omnis nucleolus e nucleolo," or more strictly speaking, that the nucleolus in most cases is not derived from a previously existing one. But the third mode of diffusion of the nucleolar substance is in reality not a transference of this substance at all, since it probably becomes lost in the cytoplasm ; and hence,

though the mode of disappearance of this substance may be more or less dependent upon the mode of mitosis, the substance of the parent-nucleolus may be in many cases not transferred to the daughter-nuclei, but the latter (perhaps as a rule) may produce their own nucleoli *de novo*.

Strasburger ('93, '97) assumes that the small granules found by Kostanecki ('92) in the equatorial plate of the central spindle may be nucleolar particles, and accordingly that the nucleolar substance may be in this way very evenly distributed to the daughter-nuclei; but it is not as yet clearly shown that these granules are derivatives of the nucleolus (*cf.* also Debski, '97, Sala, '95, Pfitzner, '86b, and Rosen, '95).

Zacharias ('85), Carnoy ('85), and Platner ('86) have concluded that in some cases the achromatic spindle fibers are derived from the nucleolus; similar views are held by Strasburger ('95, '97), Harper ('97), and Fairchild ('97), but most facts would show this view untenable.

Rhumler ('93) assumes that a greater amount of nucleolar substance is accumulated in the nucleus before mitosis than is necessary for its growth, and this superfluous amount would serve for the formation of the nucleoli in the daughter-nuclei.

## 12. *The Function of the Nucleolus.*

The attempt to deduce the physiological economy of a structure from a mere study of its morphological relations is always difficult, and this is especially the case with regard to the nucleoli.

Balbiani ('64) found contractile and discharging vacuoles in the germinal spot of *Phalangium*, and notes that they differ from the contractile vacuoles of the *Rhizopoda* in that they are not formed again at the same point. Häcker ('93c) regarded the nucleolus of the ovum of *Echinus* as an excretory organ, since he found its large vacuole to be contractile; he compared it directly to the contractile vacuole of *Infusoria*. Balbiani ('65b) also observed contractile vacuoles in the germinal spots of *Helix*, *Vortex*, and *Prostomum*, and in these the vacuole discharges through a small orifice in the cortical substance of the

nucleolus. Böhm described ('88) the vacuole of the germinal spot of *Petromyzon* as connected by a fine duct with the surface of the nucleolus. Lukjanow ('88) found in the stomach cells of the salamander that the nucleolus is apposed to the nuclear membrane, through which it discharges an excretion. Compare also Van Bambeke ('97a) and Michel ('96). These observations would show that the nucleolus in some cases contains a contractile vacuole, and that the fluid substance of the latter is periodically discharged from it (*cf.* Hodge '94, Van Bambeke, '97, and Michel, '96).

Flemming ('82) considers the nucleoli to be nuclear organs, and regards them either as containers or reserve supplies of chromatin, or as "eine chemische Modification, Vorstufe oder Doppelverbindung" of the latter substance; this view is also held by Van Bambeke ('85). Zacharias ('85) also thinks that they are organs, but does not agree with Flemming that they are reserve masses of chromatin; Gjurasin ('93) corroborates the views of Zacharias. Strasburger originally contended ('84) that they represent reserve material, a view shared by many later observers; more recently ('88) he shows that the nucleolar substance may play some part in the formation of the cell membrane, but considers that they may also have some other, as yet unknown, function. Korschelt ('89) concludes that they are formed as depositions of nutritive substances, and that their substance "in und vielleicht ausserhalb des Kernes zur Verwendung gebracht werden sollte." Rhumbler ('93) assumes that the nucleoli ("Binnenkörper") of the *Protozoa* represent "Reservestoffe" deposited in the nucleus and consumed in the growth of the latter, standing in some connection with the chromatin; they are not organs, but secretions of the nucleus. Häcker ('95) concludes that they are not nuclear organs, but secretions of the nucleus formed in or from the chromatin elements and destined to be discharged from the nucleus during mitosis; he observes that the nucleolar substance "ein Stoffwechselsprodukt darstellt, dessen Erzeugung in einem gewissen Abhängigkeitsverhältniss zur Intensität der vegetativen Leistungen von Kern und Zelle steht," and that its amount stands in a direct ratio "zur Intensität der Wechselbeziehungen

zwischen Kern und Zelle"; he opposes the view "dass die Kernkörper aus dem Zellplasma in den Kern hineingelangen und hier in die Bildung des Chromatins eingehen." Leydig ('85) holds that certain of the nucleoli are differentiations of the chromatin reticulum, others of the "Kernplasma." Watasé ('94) considers that they may be metabolic products of the cell. Auerbach ('90) holds them to be the fundamental constituents of the nucleus, which is a retrogression to the earlier views of O. and R. Hertwig. Born ('94) states: "Die Nucleolen stehen in Beziehung zum individuellen Zelleben, nicht zur Fortpflanzung." Lavdowsky ('94) considers them to be reserve masses of chromatin. Macfarlane ('81, '85) regards them as the tropic centers of the cell, and as the most important mechanical agents in cell division. Julin ('96b) believes they conduct the vegetal processes of the cell. Mottier ('97) considers the nucleolus "ein Kraftvorrath, welcher der Zelle nach Bedarf zur Verfügung steht"; and Swingle ('97), as a reserve fund of nourishment for the kinoplasm in mitosis. Metzner ('94) considers them to be of importance in the processes of mitosis (compare his observations). Henneguy ('93) regards the nucleolus and Balbianian corpuscles as corresponding with the macronucleus of the *Infusoria* (cf. Julin, '93b). These, then, are the most important views on the nature of the nucleolus.<sup>1</sup>

From my own observations the nucleolar substance would seem to be extranuclear in origin, and not a secretion or excretion of the nucleus. To be sure it may, and probably does, undergo chemical changes within the nucleus, but it is derived in the first place from the cytoplasm. I regard the nucleoli as

<sup>1</sup> The following list includes, I believe, all who have written on the function of the nucleolus: Korschelt ('89), Häcker ('93a, '95, '97b), O. Hertwig ('77a, '92), Rhumbler ('93), R. Hertwig ('76, '96), Fick ('93), Lukjanow ('88), Brauer ('91), Nussbaum ('82), Strasburger ('82b, '84, '88, '95, '97), Jordan ('93), Flemming ('80, '82), Van Beneden ('75), Wasielevsky ('93), A. Schneider ('83), Henneguy ('93), Rückert ('92, '94), C. Schneider ('91), Born ('94), R. Wagner ('36, '37), Auerbach ('74a), Kölliker ('43), Lönnberg ('92), Klein ('78), Macallum ('91, '95), Stuhlmann ('86), O. Brandt ('78), Schwarz ('87), Giugnard ('85), Macfarlane ('81, '85, '92), Zacharias ('85), Watasé ('94), Humphrey ('94), Gjurasin ('93), Mann ('92), Julin ('93b), E. B. Wilson ('96), Van Bambeke ('85), Mottier ('97), Swingle ('97), Rosen ('95), Metzner ('94), Wheeler ('97), Carnoy ('84, '86, '97a, '97b).

consisting of a substance, or different substances, taken into the nucleus from the cell body. It seems probable, further, that these substances stand in some relation to the nutritive processes of the nucleus, and in a relation to the growth of the latter. Thus those nuclei which are characterized by an especially large amount of nucleolar substance are growing nuclei, *i.e.*, those of egg cells in the maturation period, those of the subcuticular gland cells of *Piscicola*, the mesenchym cells of *Cerebratulus*. In the gland cells of *Piscicola* the volume of the nucleolar substance rapidly increases in amount during the phase of growth of the nucleus, but diminishes when the latter decreases in volume. Somatic cells, on the contrary, at least those which are undergoing no dimensional changes, contain a relatively small amount of this substance. It is doubtful whether Häcker ('95) is quite correct in assuming that the amount of the nucleolar substance stands in a direct proportion to the intensity of the functional changes which take place between the nucleus and the cytoplasm; at least there are but few criteria to enable one to compute the degree of such an intensity. Thus one would suppose that in nerve cells there was a close and intimate correlation between nucleus and cell body, but the nucleoli of the ganglion cells of the nemerteans and *Piscicola* are very small. Häcker's deduction might be modified as follows: where there is a close physiological *rapport*, in regard to processes of nutrition, between the nucleus and the cell body a relatively large amount of nucleolar substance occurs in the former.

Accordingly, we find a relatively large amount of nucleolar substance in growing nuclei, and hence conclude that this substance stands in some connection with the processes of nutrition, is itself either nutritive in function or represents that portion of substances assimilated by the nucleus from which all nourishment has been extracted, and in this case it would be a waste product. A third possibility is that the nucleoli may represent accumulations of nutritive substance retained in the nucleus as a reserve supply; but this does not seem to be very probable, for by this assumption it would be difficult to explain the uniformity in the size of the nucleoli in a given species of cell.

It would be premature to attempt to decide the exact manner in which the nucleolar substance is concerned in the metabolism of the cell. But the facts at least show that it has an extranuclear origin, and is especially abundant in growing nuclei, which shows that it stands in intimate connection with the phenomena of nutrition of the nucleus.

Vacuoles are characteristic for certain stages in the development of many nucleoli, especially those of germinal vesicles. For the nucleoli of the ova of *Montagua* and *Doto*, I showed that the vacuolar substance is at first present in the form of small globules in the nuclear sap, that these become applied against the surface of the nucleolus, and, finally penetrating into the latter, represent within it the vacuoles. I was unable to decide the mode of derivation of the vacuoles for the other nucleoli studied. So in some cases this vacuolar substance would appear not to be a derivative of the ground substance of the nucleolus, but to be derived from without the latter. Thus such nucleoli may be considered as diosmosing structures. The manner of growth of nucleoli is apparently by a process of apposition of smaller particles of nucleolar substance to their surfaces, and the addition of vacuolar substance to them differs from this only in that the vacuolar substance is intussuscepted. This vacuolar substance may be also a product of the nutritive processes of the nucleus.

It is a difficult question to determine whether the nucleolus at some stage of its development should not be considered a nuclear organ. In most nuclei it has a regular shape, in others it may be oval; in many cases the nucleolus has no regular shape, and in the salivary gland cells of *Chironomus* (according to Balbiani) it is convoluted. From the facts at hand we may conclude that the shape of the nucleolus is pretty constant for the particular species of cell. Now, taking constancy in form as a criterion of an organ, one might conclude that the nucleoli are organs. But, on the other hand, the most frequent form of the nucleolus, namely, the spherical, might simply be due to its thin fluid consistency, and when it is more viscid in consistency its shape would be more irregular. Thus Rhumbler ('93) concludes that the irregular nucleoli of

*Foraminifera* "durch Zusammenfliessen anfänglich leicht flüssiger, dann zähflüssiger und schliesslich erstarrender Massen entstanden sind." It may be asked: Why does the nucleolus persist through the whole resting state of the nucleus if it be not an organ? It may be simply stored in the nucleus until at the time of mitosis, when the nuclear membrane disappears, it has an opportunity to leave the nucleus. The only observations which would prove that the nucleolar substance may functionate as an independent organ are those according to which the nucleolus contains a contractile vacuole, and thus rhythmically contract and expand; in these cases the nucleolus might be regarded as a pulsating excretory organ of the nucleus. The hypothesis might be suggested that though the nucleolus probably consists of substances which stand in some relation to the nutritive processes of the nucleus, and so at the time of its first formation may be a functionless, inert mass of substance, yet it may at later periods in the history of the resting nucleus acquire some active function and thus gradually come to acquire the value of a nuclear organ; this hypothesis is put forward merely as a tentative one. According to this view the nucleolus might be considered as an organ which serves to accumulate in itself the waste products of the nucleus, thus serving as a reservoir for such substances; or it might be considered as an organ of excretion, to discharge waste products out of the nucleus: in either case the nucleolus would seem to stand in direct connection with the nutritive substances and forces of the nucleus.

### 13. *Comparison of the Nucleoli in Plants, Protozoa, and Metazoa.*

I have made no morphological studies on the nucleoli of plant cells, but would judge from the results of botanical investigators that they are probably strictly comparable to the nucleoli of the metazoan cells.

Rhumbler (93) doubts whether the nucleoli of the *Metazoa* and the "Binnenkörper" of the *Protozoa* are homologous structures; and, indeed, there are certain nucleolar structures

in *Protozoa* which are unique, such as the nucleolo-centrosome of Keuten ('95). Henneguy considers that the corpuscle of Balbiani, together with the nucleolar elements of the metazoan cell, corresponds to the macronucleus of the *Infusoria*; in connection with this view may be mentioned the observations of Bütschli ('80), according to which only the macronuclei of the *Ciliata* contain nucleoli. Henneguy's hypothesis is very ingenious, and opens an interesting field for investigation, but it is difficult to determine whether it corresponds to the facts at hand, or whether it does not.<sup>1</sup> Some of the nucleoli of *Protozoa* are comparable to those of *Metazoa*, but it is doubtful whether all of them are.<sup>2</sup> Thus it may be the case in some of the gregarines that the chromatin (or its physiological equivalent) is localized in some or all of the nucleoli, and such structures could not be compared with the nucleoli of the metazoan cell.

As to the metazoan nucleoli, there is the question whether the nucleoli of egg cells and of somatic cells should be considered homologous. In my opinion this may be answered in the affirmative, since the nucleoli of both kinds of cells appear to be depositions of substances which are concerned in the nutritive processes of the nucleus. In making this conclusion I limit myself to the true nucleoli and do not consider those structures which have been erroneously termed nucleoli, but which in reality are portions of the chromatin reticulum of the nucleus. Numerous writers have considered the thickened nodal points of the nuclear network to be nucleoli, and here may be mentioned Leydig, Klein, Waldeyer, and others. The "cyanophilic" nucleoli of Auerbach ('90), the "pseudonucleoli" of Rosen ('92a), the "nucléoles nucléiniens" of Carnoy ('85), and the "Karyosomata" of Ogata ('83), Lukjanow ('87b), and Macallum ('91) are undoubtedly not nucleoli but portions of the nuclear reticulum. While the "erythrophilic" nucleoli of Auerbach, the "Eunucleoli" of Rosen, the "nucléoles

<sup>1</sup> On the genetic relation of nucleoli to Balbianian corpuscles (true yolk-nuclei), a relation which seems to me very doubtful, cf. Mertens ('93), Galeotti ('95), Melissinos and Nicolaides ('90), Weismann and Ishikawa ('89), Ver Ecke ('93), Steinhaus ('88), Henneguy ('93), Julin ('93b).

<sup>2</sup> For the central masses of chromatin found in many protozoan nuclei, Doflein ('98) proposes the term "chromatosphere."



plasmatiques" of Carnoy, and the "Plasmosomata" of the other observers correspond to true nucleoli in the sense in which this term should be used. The existence of Carnoy's "nucléoles mixtes" and "nucléoles-noyaux" in cells of *Metazoa* appears to be doubtful. List ('96) considers that the paranucleoli of the egg cells and the nucleoli of the somatic cells are homologous, but that the nucleolus proper of the ova is different from both; but the chemical differences which he finds between these kinds of nucleoli do not prove that they are morphologically distinct structures.

#### APPENDIX TO THE LITERATURE REVIEWS.

Siebold ('39) noticed "in den Eiern von *Plumatella campanulata* Lam. . . . ein deutliches Keimbläschen mit gedoppeltem Keimfleck."

Koelliker ('43) concludes: "Es bestände . . . das Ei aus einer primitiven Zelle, dem Keimbläschen, die sich um einen Kern, den Keimfleck, gebildet, und um die sich nachher Körner und eine secundäre Zelle, die Dotterhaut, gelegt hätte."

Auerbach ('74a) was the first to emphasize and prove clearly that the number of nucleoli is usually quite large, and that they are frequently irregular in form (before this time it was generally assumed that the usual number of nucleoli was one or two). The nucleus is filled with "Grundsubstanz" (the "Zellsaft" of Kölliker) and "Zwischenkörnchen"; the latter are distinguishable from the nucleoli by their smaller size and different refraction. He explains the clear zone around the nucleolus and the "Kernkörperchenkreis" of Eimer by the action of a repulsive force on the part of the nucleolus and of the nuclear membrane. He distinguishes several successive stages of the nucleus with regard to the number of the nucleoli: *enucleolar* nuclei, at an early embryonal stage; *paucinucleolar* nuclei, with one or two nucleoli; *plurinucleolar* nuclei, with two to four; and *multinucleolar*, with more than four. "Die Zahl der Kernkörperchen in einem Kerne beträgt 1-16, und in extremen Fällen selbst noch viel mehr, bis über 190. Und zwar ist nur eine kleine Minderheit aller Kerne durch den Gehalt von nur

einem oder zwei Nucleoli ausgezeichnet." He gives a large series of data on the number and size of nucleoli in embryonal and adult cells of vertebrates and *Musca*. The enucleolar condition is characteristic for embryonal cells; later a nucleolus makes its appearance in the center of the nucleus, though its substance is probably derived from the cytoplasm; new nucleoli are formed by successive divisions of the first one. In *Teleostii* the nuclei have fewer nucleoli than those of *Amphibia*, and those of *Reptilia* fewer than those of *Mammalia*; from which is concluded that the number increases in advancing phylogeny as in the ontogeny. "Je schneller und absolut bedeutender das Wachsthum der Zellen ist, desto mehr scheint auch die Tendenz zur Vervielfältigung der Kernkörperchen obzuwalten." The nuclei of the stomach mucosa of *Rana* are multinucleolar in summer and autumn, while after hibernation they contain only one to four nucleoli, which may be due to a process of fusion. The substance of nucleoli is similar to that of the cytoplasm in structure, capability of movements and of producing vacuoles; just as the nucleus is first formed as a vacuole in the cytoplasm, so in the substance of a nucleolus (which is cytoplasmic in origin) a vacuole is formed which has the same relation to the nucleolus as the nucleus has to the cell; "bei dieser Betrachtungsweise erscheint demnach der Zellkern als ein hohler Brutraum, bestimmt, eine junge Zellenbrut in sich zu entwickeln, die Nucleoli aber als wahrhaft endogen entstandene Tochterzellen." In higher animals all nucleoli do not become daughter-cells, but fulfill some new function; "und so werden wir auch die ursprüngliche Bedeutung der Nucleoli als Fortpflanzungszellen nicht für ganz unmöglich halten dürfen, wenn wir auch auf der anderen Seite nicht zweifeln können, dass sie in den meisten Kernen der höheren Organismen ganz andere Aufgaben zu erfüllen haben müssen."

Auerbach ('74b) studied in life the fecundation and cleavage of *Strongylus* and *Ascaris*. A short time after the appearance of the two copulation nuclei in the ovum, arise in each from one to five nucleoli; "wenn eine Mehrzahl sich einfindet, so kommen sie nicht alle gleichzeitig, sondern eines nach dem anderen, in Intervallen von einer halben bis zu einigen Minuten

zum Vorschein, und zwar in unregelmässigen, oft beträchtlichen Entfernungen von einander." When the nuclei wander towards one another the nucleoli move about, "indem sie innerhalb des Kernraums allerlei gerade, zickzackförmige, bogenförmige, Bahnen durchlaufen, mit einer vergleichsweise erheblichen Geschwindigkeit, so dass zuweilen in weniger als einer Minute Strecken von der Länge des Kern-Durchmessers zurückgelegt werden"; during these movements the nucleoli remain perfectly spherical. When the copulation nuclei are apposed the nucleoli in them suddenly disappear, and the mode of this disappearance was determined in one case, though it is exceedingly rapid; "das Kügelchen wurde allmählich blasser und etwas grösser und fuhr dann plötzlich auseinander, ein Wölkchen bildend, welches einen Augenblick darauf nicht mehr zu sehen war." The nucleoli reappear in the resting nuclei, and in the successive generations up to the eight-cell stage have the same cycle of changes, except that in each generation they are somewhat larger than in the preceding. These nucleoli are formed independently of one another. By the re-formation of the nuclear vacuole a number of cytoplasmic granules pass into the cavity of the nucleus, and there fuse to form the nucleoli.

Reinhard ('82, cited by Braem, '97) describes in the egg of *Plumatella* different stages of the nucleoli, which may be single, double, or even trilobular.

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EXPLANATION OF PLATES XXI-XXX.

All the figures have been drawn with the aid of the camera lucida, and represent sections of the structures delineated. Those parts of them which are colored represent as accurately as possible the stained preparations from which they were copied; in most of the figures only certain portions are colored, the other details being filled in with the pencil. In order to show the correct proportionate size of the various cells and nuclei the greater number of the figures have been made at a magnification afforded by the homogeneous immersion lens  $\frac{1}{2}$  of Zeiss, with the ocular 4, and unless otherwise specified this may be understood to have been the magnification employed. The following abbreviations have been used in the figures:

<i>C.</i>	cell.	<i>N. P.</i>	metamorphosed portion of nucleus.
<i>C. D.</i>	cell duct.	<i>N. Sap.</i>	nuclear sap.
<i>Cen.</i>	centrosome.	<i>Nut. Gl.</i>	nutritive globule.
<i>Chr.</i>	chromatin.	<i>n.</i>	nucleolus.
<i>Chr. F.</i>	chromatin filament.	<i>n. 2.</i>	nucleolus of the second generation.
<i>Chrom.</i>	chromosome.	<i>n. D.</i>	derivatives of the nucleolar substance.
<i>C. Mb.</i>	cell membrane.	<i>n. Gr.</i>	granules of degenerated nucleoli.
<i>C. Sp.</i>	centrosphere.	<i>n. Mb.</i>	nucleolar membrane.
<i>C. T. N.</i>	nucleus of connective tissue.	<i>nn.</i>	nucleolinus.
<i>C. T. S.</i>	connective tissue sheath of the ovarian acinus.	<i>n. Sub.</i>	nucleolar ground substance.
<i>Cut.</i>	cuticula.	<i>n. Vac.</i>	nucleolar vacuole.
<i>Cy. Pl.</i>	cytoplasm.	<i>nx.</i>	nucleolar body of unknown origin.
<i>d. C.</i>	degenerated cells (or cell substance).	<i>Ps. n.</i>	pseudonucleolus.
<i>End. Pl.</i>	endoplasm.	<i>Secr.</i>	secretion corpuscles.
<i>Gon. Mb.</i>	gonadal membrane.	<i>Sp.</i>	spores.
<i>Ir. Mb.</i>	intravitelline membrane.	<i>Sp. F.</i>	spindle fibers.
<i>N.</i>	nucleus.	<i>Vac.</i>	vacuole.
<i>N. Bd.</i>	problematical nuclear body.	<i>Yk. Bl.</i>	yolk ball.
<i>N. Fib.</i>	nuclear fibers.	<i>Yk. Gl.</i>	yolk globule.
<i>N. Gr.</i>	nuclear granules.		
<i>N. Mb.</i>	nuclear membrane.		





## EXPLANATION OF PLATE XXI.

*Figs. 1-19: Gregarines from Lincolns gesserensis.*

FIG. 1. Smallest individual found (hom. immers., oc. 2. Hermann's fluid; Del. haematoxylin, eosin).

FIG. 2. Outline of the largest individual. Obj. C., oc. 2.

FIG. 3. Nucleus (corros. sublimate; Del. haematoxylin, eosin).

FIG. 4. Portion of a longitudinal section, though an individual in which spores were present (as in 3).

FIG. 5. The smaller of the two nuclei of Fig. 1.

FIG. 6. The same gregarine drawn in Fig. 1, but with obj. C., oc. 2 to show its relative size to the one of Fig. 2.

FIGS. 7-9. Nuclei (alcohol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).

FIG. 10. Nucleus (Flemming's fluid; Del. haematoxylin, eosin).

FIG. 11. Idem (Flemming's fluid; Ehrlich-Biondi stain, 23½ hrs.).

FIGS. 12-16. Nuclei (Flemming's fluid; Del. haematoxylin, eosin).

FIGS. 17-19. Idem (sublimate with 2% acetic acid; aq. sol. methylen blue, 30 min.; aq. sol. brasilin, 2¼ hrs.).

*Figs. 20-35: Gregarines from Carinella annulata (fixation with alcohol. sol. sublimate).*

FIGS. 20 and 21. Outlines of two individuals. Obj. C., oc. 2.

FIGS. 22-25. Nuclei (Del. haematoxylin, 15 min., alum carmine, 6 hrs.).

FIG. 26. Nucleus (Ehrlich-Biondi stain, 3 hrs.).

FIGS. 27 and 28. Nuclei (Del. haematoxylin, eosin).

FIG. 29. Nucleus, only the outlines of the nucleoli drawn.

FIGS. 30-35. Nuclei (as in 26).

*Figs. 36-49: Nuclei of ganglion cells from the brain of Doto (Fig. 36, of the smallest type of cell; Figs. 37-42, of medium-sized cells; Figs. 43-49, of the colossal cells).*

FIG. 36 (Hermann's fluid, 1¼ hrs.; Lyons blue, 15 min.).

FIGS. 37 and 38 (Hermann's fluid, 1¼ hrs.; Ehrl. haematoxylin, 1½ hrs., eosin, 7 min.).

FIGS. 39 and 40 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).

FIGS. 41 and 42 (as in 37).

FIG. 43 (as in 39).

FIG. 44 (Hermann's fluid, 1¼ hrs.; safranin, 92 hrs., gentian violet, 1½ hrs., orange G., 2 min.).

FIG. 45 (as in 36).

FIGS. 46 and 47. Two sections of one nucleus (as in 37).

FIG. 48 (as in 37).

FIG. 49 (as in 39).

FIG. 50. Immature germinal vesicle of *Emys* (picric acid; Del. haematoxylin).

*Figs. 51-56: Nuclei from the muscle cells of the circular musculature of  
Lineus gesserensis.*

FIG. 51 (alcohol. sol. sublimate ; Ehrlich-Biondi stain,  $3\frac{1}{4}$  hrs.).

FIG. 52 (aq. sol. sublimate ; cochineal, 1 hr., Del. haematoxylin, 20 min.).

FIGS. 53 and 54 (aq. sol. sublimate with 2% acetic acid ; Ehrl. haematoxylin,  
eosin).

FIG. 55 (Hermann's fluid, 30 min. ; Ehrl. haematoxylin, 3 hrs. ; eosin, 5 min.).

FIG. 56 (as in 54).







## EXPLANATION OF PLATE XXII.

*Figs. 57-63, 65-87: Germinal vesicles of Montagua pilata; Figs. 64, 88, 89: germinal vesicles of Doto.*

FIGS. 57-59 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIG. 60 (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIGS. 61-63 (aq. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

FIG. 64 (alcohol. sol. sublimate; Ehrlich-Biondi stain,  $3\frac{1}{4}$  hrs.).

FIGS. 65-69 (as in 60).

FIG. 70 (as in 61).

FIG. 71 (as in 57).

FIGS. 72-75 (as in 61).

FIG. 76. Outlines of pseudonucleoli from various ova of one individual (aq. sol. sublimate).

FIG. 77 (as in 61).

FIGS. 78-80 (alcohol. sol. sublimate; Mayer's acid carmine, 15 min.; nigrosine, 10 min.).

FIGS. 81-87 (Flemming's fluid; Del. haematoxylin, eosin).

FIGS. 88 and 89 (as in 64).

*Figs. 90-97: Nuclei of ganglion cells from the brain of Montagua pilata (Fig. 93, from a cell of medium size; the others from the colossal cells).*

FIGS. 90 and 91 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 92-94 (picrosulphuric acid; Del. haematoxylin, 25 min.; eosin, 5 min.).

FIG. 95 (as in 90).

FIGS. 96 and 97 (Flemming's fluid; Del. haematoxylin, eosin).

*Figs. 98-101: Blood corpuscles of Doto.*

FIG. 98 (alcohol. sol. sublimate; Ehrlich-Biondi stain,  $3\frac{1}{4}$  hrs.).

FIGS. 99-101 (Hermann's fluid,  $1\frac{1}{4}$  hrs.; safranin, 92 hrs.; gentian violet,  $1\frac{1}{2}$  hrs.; orange G., 2 min.).









## EXPLANATION OF PLATE XXIII.

FIG. 102. Blood corpuscle of *Doto* (picro-nitro-osmic acid, 35 min.; Del. haematoxylin, 30 min.; eosin, 5 min.).

*Figs. 103-133: Egg development of Tetrastemma catenulatum.*

FIGS. 103-106. Germinal vesicles (aq. sol. sublimate; Ranvier's picrocarmine; Del. haematoxylin).

FIG. 107. Portion of a young gonad (as in 103).

FIG. 108. Immature ovum (as in 103).

FIG. 109. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

FIGS. 110 and 111. Germinal vesicles (aq. sol. sublimate; Del. haematoxylin, 15 min.; eosin, 5 min.).

FIG. 112. Germinal vesicle with portion of the surrounding cytoplasm (as in 110).

FIG. 113. Germinal vesicle (as in 110).

FIG. 114. Idem, with portion of the surrounding cytoplasm (as in 110).

FIGS. 115 and 116. Outlines of young ova (as in 110).

FIGS. 117-119. Germinal vesicles (as in 110).

FIG. 120. Outline of germinal vesicle, the natural color of the nucleoli shown (aq. sol. sublimate).

FIG. 121. Tangential section of the inner surface of the nuclear membrane, the dotted line representing the greatest diameter of the nucleus (as in 109).

FIGS. 122-133. Germinal vesicles (as in 109).

*Figs. 134-136: Outlines of the nuclei of ganglion cells of Piscicola.*

FIGS. 134 and 135 (alcohol. sol. sublimate).

FIG. 136 (Flemming's fluid, 1 hr.).







## EXPLANATION OF PLATE XXIV.

FIGS. 137-139. Germinal vesicles of *Tetrastemma catenulatum*, the nucleoli omitted in Fig. 139 (aq. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

*Figs. 140-158: Egg development of Amphiporus glutinosus.*

FIGS. 140-143. Germinal vesicles (aq. sol. sublimate; Del. haematoxylin, 20 min.; eosin, 5 min.).

FIGS. 144-146. Germinal vesicles with surrounding cytoplasm (as in 143).

FIGS. 147-150. Germinal vesicles (as in 143).

FIG. 151. An abnormally large yolk ball (aq. sol. sublimate; aq. sol. dahlia, 15 min.; eosin, 5 min.).

FIGS. 152-154. Germinal vesicles (aq. sol. sublimate; haematoxylin, 45 min.; ferro-ammonio-sulphate, 45 min.; haematoxylin, 45 min.).

FIG. 155. Ovum and a part of the gonadal cavity in which yolk balls lie, only a portion of the cytoplasm drawn (as in 152).

FIGS. 156-158. Germinal vesicles (as in 154).

*Figs. 159-177: Egg development of Lineus gesserensis.*

FIG. 159. Nuclei from which the germinal vesicles are derived, from the cytoplasm of the gonad (Hermann's fluid; safranin, 70 hrs.; gentian violet, 1 hr.; orange G., 2 min.).

FIG. 160. Ovum (as in 159).

FIGS. 161 and 162. Germinal vesicles (as in 159).

FIG. 163. Group of neighboring nuclei from a gonad, showing mitotic stages (aq. sol. sublimate; Del. haematoxylin, 20 min.; eosin, 5 min.).

FIGS. 164-172. Nuclei from gonads (as in 163).

FIGS. 173-176. Germinal vesicles (as in 163).

FIG. 177. The largest ovum found, only a part of the cytoplasm drawn (as in 163).









## EXPLANATION OF PLATE XXV.

*All the figures refer to the large subcuticular gland cells of Piscicola rapax*  
*Figs. 178-196 show stages of the prophase, and Fig. 167 the commencement*  
*of the metaphase of the nucleus.*

FIG. 178. Outline of an immature cell, only a portion of its duct drawn (aq. sol. sublimate).

FIGS. 179-181. Immature cells (aq. sol. sublimate; Mayer's acid carmine, 20 min.; nigrosine, 25 min.).

FIG. 182. Immature nucleus (alcohol. sol. sublimate).

FIG. 183. Idem (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 184-189. Nuclei (alcohol. sol. sublimate).

FIGS. 190-194. Stages of the ramification of the nucleus (Flemming's fluid).

FIGS. 195 and 196. Nuclei at the end of the prophase (alcohol. sol. sublimate).

FIG. 197. Nucleus at the commencement of the metaphase, discharging its nucleoli (Flemming's fluid).



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## EXPLANATION OF PLATE XXVI.

*Figs. 198-203: Large subcuticular gland cells of Piscicola, in stages of the metaphase.*

FIGS. 198 and 199. Nuclei discharging their nucleoli, only outlines drawn (Flemming's fluid).

FIGS. 200-203. Subsequent stages of the metaphase (as in 198).

*Figs. 204-212: Egg development of a siphonophore (Rotalia(?)); all fixed in alcohol and stained with Del. haematoxylin.*

FIG. 204. Ovum from gonophore, chromatin unstained. Obj. A, oc. 4.

FIG. 205. Ovum from egg pouch. Obj. C, oc. 4.

FIG. 206. Germinal vesicle from egg pouch. Obj. C, oc. 4.

FIGS. 207-209. Germinal vesicles from gonophores. Obj. C, oc. 4.

FIG. 210. Ovum from egg pouch. Obj. C, oc. 4.

FIG. 211. A large and a small ovum from an egg pouch. Obj. C, oc. 4.

FIG. 212. Nucleolus from a large ovum of a gonophore.

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## EXPLANATION OF PLATE XXVII.

*Figs. 213-235: Egg development of Stichostemma eilhardi.*

FIG. 213. Portion of the cell syncytium of an immature gonad (aq. sol. sublimate; aq. sol. methylen blue, 5 min.; brasilin, 20 min.).

FIG. 214. Germinal vesicle (as in 213).

FIG. 215. Yolk balls (as in 213).

FIG. 216. Germinal vesicle (as in 213).

FIGS. 217 and 218. Portions of cell syncytia of gonads (as in 213).

FIG. 219. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 15 min.; borax carmine, 20 hrs.).

FIG. 220. Portion of the cell syncytium of a gonad (aq. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 221-223. Germinal vesicles (as in 220).

FIGS. 224-227. Idem (Flemming's fluid; alum carmine, 24 hrs.).

FIG. 228. Portion of a gonadal syncytium (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 22 hrs.).

FIGS. 229 and 230. Germinal vesicles (as in 228).

FIG. 231. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 45 hrs.).

FIG. 232. Idem (aq. sol. sublimate; picrocarmine, 22 hrs.).

FIG. 233. Ovum, only a portion of the cytoplasm drawn (Lang's fluid; alum carmine; Del. haematoxylin, 15 min.).

FIG. 234. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 16 hrs.).

FIG. 235. Germinal vesicle and portion of the cytoplasm (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 24 hrs.).

*Figs. 236-248: Egg development of Zygonemertes virescens.*

FIGS. 236-241. Germinal vesicles (aq. sol. sublimate; Del. haematoxylin, 20 min.; eosin, 5 min.).

FIGS. 242 and 243. Idem (aq. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 244 and 245. Idem (alcohol. sol. sublimate; picrocarmine; Del. haematoxylin, 20 min.; eosin, 5 min.).

FIG. 246. Portion of an ovum (as in 242).

FIGS. 247 and 248. Germinal vesicles (as in 242).

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## EXPLANATION OF PLATE XXVIII.

*Figs. 249-281: Egg development of Polydora.*

FIG. 249. Nuclear division in a peritoneal cell (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIGS. 250-254. Free cells of the body cavity (as in 249).

FIG. 255. Nuclear mitosis (as in 249).

FIGS. 256-259. Mitoses of genital cells (as in 249).

FIG. 260. Nuclear mitosis (as in 249).

FIGS. 261-266. Immature ova (as in 249).

FIGS. 267 and 268. Germinal vesicles (as in 249).

FIG. 269. Ovum, only a portion of the cytoplasm drawn (as in 249).

FIGS. 270 and 271. Ova (aq. sol. sublimate with 5% acetic acid; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 272-275. Germinal vesicles (as in 270).

FIGS. 276 and 277. Idem (Perenyi's fluid, 1 hr.; Ehrlich-Biondi stain, 2½ hrs.).

FIG. 278. Ovum, only a portion of the cytoplasm drawn (Flemming's fluid; safranin, 70 hrs.; gentian violet, 2¼ hrs.; orange G, 2 min.).

FIGS. 279-281. Germinal vesicles (as in 278).

*Figs. 282-299: Egg development of Tetrastemma elegans.*

FIGS. 282-291. Germinal vesicles (alcohol. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

FIG. 292. Ova (Hermann's fluid; Del. haematoxylin, 45 min.; eosin, 5 min.).

FIGS. 293-299. Germinal vesicles (as in 292).









## EXPLANATION OF PLATE XXIX.

*Figs. 300-316: Egg development of Piscicola rapax.*

FIGS. 300-304. Transverse sections of ovarian acini (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIGS. 305 and 306. Germinal vesicles (as in 300).

FIG. 307. Ovum (as in 300).

FIG. 308. Germinal vesicle (as in 300).

FIGS. 309 and 310. Ova (as in 300).

FIG. 311. First pole spindle in the ovum; only one attraction sphere is drawn, and that only partially, the dotted line showing how far its rays extend into the cytoplasm (as in 300).

FIGS. 312 and 313. Germinal vesicles (alcohol. sol. sublimate; Mayer's acid carmine, 20 min.; Lyons blue, 5 min.).

FIG. 314. Germinal vesicle; the dotted line shows the extension of the indented surface of the nucleolus, the unstained small oval space being the external opening into it (alcohol. sol. sublimate; fuchsine, 10 min.).

FIG. 315. Germinal vesicle (Flemming's fluid; Ehrl. haematoxylin, 2 hrs.; eosin, 10 min.).

FIG. 316. Ovum with attraction spheres at opposite ends of the nucleus; the rays of only one attraction sphere drawn (alcohol. sol. sublimate; Ehrl. haematoxylin, 40 min.; eosin, 5½ min.).

*Figs. 315a-324: Mesenchym cells of Cerebratulus lacteus (fixation with alcohol. sol. sublimate).*

FIG. 315a. Nucleus (Ehrlich-Biondi stain, 2 hrs.).

FIGS. 316a and 317. Nuclear division in free cells (Ehrl. haematoxylin, 2 hrs.; eosin, 5 min.).

FIGS. 318 and 319. Nuclei (as in 317).

FIGS. 320-324. Whole cells (as in 317).

*Figs. 325-337: Nuclei of the muscle cells of the longitudinal musculature of Piscicola rapax.*

FIG. 325 (aq. sol. sublimate).

FIG. 326 (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIG. 327 (Flemming's fluid, 1 hr.).

FIG. 328 (aq. sol. sublimate).

FIG. 329 (as in 327).

FIGS. 330 and 331 (as in 328).

FIG. 332 (as in 327).

FIG. 333 (Flemming's fluid, 1 hr.).

FIGS. 334 and 335 (as in 326).

FIGS. 336 and 337 (as in 333).

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## EXPLANATION OF PLATE XXX.

*All figures refer to the giant cells of Doto.*

- FIG. 338. Nucleus (alcohol. sol. sublimate; Ehrlich-Biondi stain,  $3\frac{1}{4}$  hrs.).  
FIG. 339. Cell (as in 338; hom. immers., oc. 2).  
FIGS. 340 and 341. Nuclei (Hermann's fluid,  $1\frac{1}{4}$  hrs.; safranin, 92 hrs.; gentian violet,  $1\frac{1}{2}$  hrs.; orange G., 2 min.).  
FIGS. 342 and 343. Two sections of a single nucleus (as in 338).  
FIG. 344. Nucleus (Hermann's fluid; Ehrl. haematoxylin,  $1\frac{1}{2}$  hrs.; eosin, 7 min.).  
FIG. 345. Dividing nucleolus (as in 344).  
FIG. 346. Nucleus (as in 344).

















