à Penard ou à Schaudinn le soin de choisir un nouveau vocable approprié.

Nancy, 17. Mai 1897.

2. Rectification.

Je viens de publier dans le Bulletin de la Société Zoologique de France, tome XXII. page. 98 1897 une note intitulée:

Observations sur Divers Céphalopodes; Troisième note; Céphalopodes du Musée Polytechnique de Moscou. — Cette dernière ligne doit étre ainsi modifiée: Céphalopodes du Musée Zoologique de l'Université Impériale de Moscou.

Cette rectification est faite sur la demande du Musée intéressé et afin d'éviter une confusion.

Dr. L. Joubin
Prof. de l'Université de Rennes.

3. The Follicle Cells in Salpa.

By Maynard M. Metcalf, Baltimore.

eingeg. 30. Mai 1897.

Through the courtesy of Professor Brooks of the Johns Hopkins University, I have been enabled to examine a number of finely preserved embryos of several species of *Salpa*, and I desire after briefly referring to certain points in recent papers upon *Salpa* embryology to describe such of the results of my study as bear upon the nature and role of the follicle cells. —

Since the publication, fifteen years ago of Salensky's careful studies [see references at end of paper], interest in the development of this genus has centered more around the follicle cells and their role than around any other point. Salensky showed that the young embryo was composed, in part of a few true blastomeres derived from the fertilized egg, but more largely of a great mass of cells derived by proliferation from the follicle. He claimed that the true blastomeres early disappear, serving probably to nourish the inwandering follicle cells, from which the adult organism is derived. That is, according to Salensky, the fertilized ovum serves merely as food for its unfertilized sisters (the follicle cells), which are the really important elements.

He says — page 362 — »Aus den vorgeführten Stadien läßt sich der Schluß ziehen, daß die Blastomeren fortwährend an Zahl abnehmen, bis sie endlich ganz verschwinden. Diese Erscheinung kann auf

zweierlei Weise erklärt werden. Entweder gehen die Blastomeren unter allmählicher Verkleinerung zu Grunde — sie könnten als Nährmaterial für die Bildungszellen dienen —, oder sie verändern unter fortwährender Theilung Form und Bau und vermischen sich so mit den Gonoblasten, daß sie endlich von den letzteren nicht zu unterscheiden sind. Diese Frage durch direkte Beobachtung zu entscheiden, ist sehr schwer, und bei dem Material, das mir zu Gebote stand, war das unmöglich. Ich will deshalb hier nur Thatsachen vorführen, welche für und gegen diese beiden Voraussetzungen sprechen können.

Erstens will ich bemerken, daß Form und Bau der Blastomeren so characteristisch ist, daß sie mit den Gonoblasten schwer zu verwechseln sind. Selbst bei den kleinen Blastomeren, wie wir in Fig. 2 sehen, kann man nach dem Kern jedes Blastomer, wenn es auch nur von Gonoblastengröße ist, ganz gut von den Gonoblasten unterscheiden. Der Blastomerenkern ist rund, opak, färbt sich mit Carmin besser als der eines Gonoblasten, welch letzterer eine ovale Form besitzt und ein kleines punctförmiges Kernkörperchen beherbergt.

Zweitens will ich darauf aufmerksam machen, daß man in dem zuletzt betrachteten Stadium Blastomerenkerne antrifft, welche noch ihre Größe behalten, aber deren Begrenzung nicht so scharf ist, wie es in den Blastomeren der früheren Stadien der Fall ist. Sie verlieren also ihre scharfen Contouren, was schon darauf hinweist, daß diese Kerne in der That solchen Veränderungen unterliegen, welche ihr Absterben sehr wahrscheinlich machen. Endlich gegen die Verwandlung der Blastomeren in gonoblastenähnliche Zellen spricht auch der Umstand, daß man nie Übergangsformen antrifft, was doch der Fall sein müßte, wenn eine solche Verwandlung in der That existierte. Auf Grund aller dieser Thatsachen bin ich zur Überzeugung gelangt, daß Blastomeren in der That allmählich schwinden, um die Hauptrolle bei der Entwicklung den Gonoblasten zu überlassen«.

Todaro, in 1881, had described a peculiar fragmentation of the blastomeres by which each broke up into numerous small nucleated cells. Salensky, referring to this point, describes the phenomena as follows, — page 99 — »Das Protoplasma derselben (of the blastomeres), welches in allen früheren Stadien feinkörnig, beinahe homogen war, zerfällt jetzt in kleine mannigfaltig gestaltete Parcellen, die theils um den Kern, theils in der Peripherie der Zellen gelagert sind. Als ich zum ersten Mal diesen eigenthümlichen Zerfall des Protoplasma beobachtete, glaubte ich es mit dem Product der Einwirkung der Conservations- oder Färbeflüssigkeit zu thun zu haben. Derselbe kommt aber so beständig in gewissen Stadien der Entwicklung, namentlich nach dem ersten Furchungsstadium, vor und erscheint von der Art der Con-

servierung so unabhängig, daß ich bald zur Überzeugung gelangte, daß diese Veränderungen des Protoplasma normale Entwicklungsvorgänge darstellen«. He speaks in another place, page 125, of »kleinen polyedrischen Protoplasmastückchen, in welchen ich trotz aller Mühe selbst an sehr schön gefärbten Praeparaten keinen Kern zu unterscheiden im Stande war. Ich muß deshalb die Zellennatur dieser Protoplasmastückchen vollständig in Abrede stellen«. From these quotations it is seen that he denies the cellular nature of the bodies within the blastomeres, but offers no explanation of their true nature.

More recent papers by Brooks, Heider and Korotneff have dealt with the relation between blastomeres and follicle. —

Brooks, in 1893, confirmed Salensky's description of the complex character of the young embryo, pointing out with the greatest clearness that the follicle cells multiply very rapidly by amitotic division, the resulting cells pushing in among the blastomeres which for a long time remain few in number.

He further showed that, as Salensky described, the migrated follicle cells give rise to rudiments of the organs. He, however, took issue with Salensky as to the ultimale fate of blastomeres and follicle, claiming and clearly showing that in the later stages the follicle cells composing the rudiments of the organs are replaced by true blastomeres which give rise to the adult. He says page 27 »Stated in a word, the most remarkable peculiarity of the *Salpa* embryo is this. It is blocked out in follicle cells which form layers and undergo foldings and other changes which result in an outline or model of all the general features in the organization of the embryo. While this process is going on the development of the blastomeres is retarded, so that they are carried into their final position in the embryo while still in a very rudimentary condition. Finally when they have reached the places they are to occupy, they undergo rapid multiplication and growth, and build up the tissues of the body directly while the scaffolding of follicle cells is torn down and used up as food for the true embryonic cells«.

Brooks' Figs. 1 and 2 Plate XLII, also Fig. 12 Plate IX, as well as his descriptions, demonstrate that the peculiar granular bodies seen within the blastomeres at certain stages of development are not an indication of the fragmentation or degeneration of the blastomeres, but are nuclei of follicle cells that have been ingested and are undergoing digestion. This statement I have fully confirmed, as described a few pages beyond. The amitotic division of the migrating follicle cells confer the belief that they are on the road to degeneration, and in the centre of the embryo there are found masses of such disintegrating cells.

Heider's account of the embryology of *Salpa fusiformis*, published in 1895, differs in certain points from preceeding accounts.

He interprets the granular bodies in the protoplasm of the blastomeres as ingested follicle cells and figures them as containing nuclei (Plate I, Figs. 4, 10^a, 10^b), and largely from this observation argues, as Brooks had shown, that the follicle cells serve as food for the blastomeres.

Heider places emphasis upon the unequal cleavage of the *Salpa* ovum, claiming, contrary to Salensky and Brooks, that, except in the early stages, the micromeres can not be distinguished from the follicle cells, and that organ rudiments which are apparently formed from follicle cells are really composed of small blastomeres.

The insufficient reference in Heider's paper to Brooks' Monograph may perhaps be explained by the fact that Heider's paper was practically complete before Brooks' work was published.

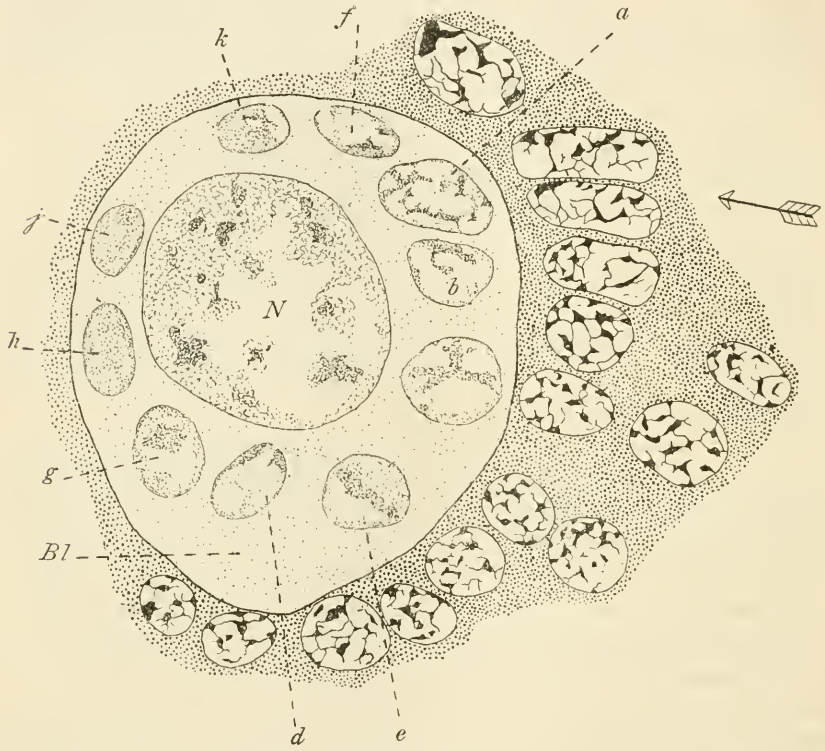
Korotneff's several papers are the most recent dealing with this subject. This author denies Heider's contention that the smaller blastomeres are difficult to distinguish from follicle cells, figuring and describing them as distinctly different, even in advanced embryos. On this point, then, Salensky, Brooks and Korotneff agree in opposition to Heider.

As to the nature of the granular bodies in the protoplasm of the blastomeres Korotneff says [Korotneff III. page 342]: »In meiner Schrift über die Embryologie von *S. democratica* habe ich mich gegen die Vermuthung von Heider, wonach diese Ablagerungen keine Dotterpartikelchen, sondern von den Blastomeren verzehrte Follikelzellen seien, ausgesprochen. Jetzt kann ich meine Meinung bekräftigen und ganz positiv behaupten, daß in den als Dotterplättchen bezeichneten Gebilden niemals eine Spur von Kernen zu sehen ist«, which is certainly true, at least for *S. hexagona* and *S. pinnata*, since these granular bodies are not ingested cells, but ingested follicle nuclei, as Brooks had shown, and as is evident in the material I have worked upon. The needle-like bodies figured by Korotneff in the protoplasm of the blastomeres of *S. cordiformis-zonaria* I have not seen described before. They appear from his figures to be peculiarly arranged chromatin particles within the ingested follicle nuclei. (Compare Korotneff III. Plate B, Fig. 14.)

Korotneff fully confirms Brooks' description (without however mentioning Brooks) of the disintegration of the follicle cells in the central region of the embryo, speaking of a retrogressive metamorphosis of the Kalymmocytes, »welche ganz blaß werden, sich schlecht färben und zuletzt nur noch in Spuren zu erkennen sind. Kurz und

gut, die Kalymmocyten gehen ganz zu Grunde, und ihre Bruchstücke dienen gewiß den Histogenen [Blastomeres] als Nährmaterial« [by osmosis]. (Korotneff III. page 335).

Korotneff denies Brooks' statement that the organs are blocked out in follicle cells which later are replaced by blastomeres, saying that the organ rudiments are from the first composed of blastomeres. If he is not contending over definitions, his statements on this point



Portion of a section of a young embryo of *Salpa hexagona* showing one blastomere and fifteen migrated follicle cells. *Bl*, blastomere; *N*, nucleus of blastomere; *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *j*, *k*, nuclei of follicle cells ingested by the blastomere.
Leitz $^1/_{12}$ objective, no. 8. compensating ocular.

are difficult to understand, for his figures show with the greatest clearness just the condition of affairs Brooks has described [Compare Korotneff IV. Taf. XVIII. Fig. 5 (rudiment of cloaca composed of follicle cells alone), Fig. 6. 7 and 8 (rudimentary walls of amnionic cavity composed wholly of follicle cells), Fig. 9 (pharynx rudiments composed chiefly of follicle cells) etc.].

I wish now to call particular attention to my own observations

upon the nature of the disputed bodies within the protoplasm of the blastomeres of *Salpa*.

After having examined several hundred blastomeres all showing the intra-protoplasmic bodies under discussion, I have selected a single blastomere to figure and describe, not because there are not many others showing a similar condition, but because this seems sufficient to establish the point. The blastomere figured is one of five appearing in a section of an embryo of *S. hexagona* at that stage of development when the follicular epithelium of one half of the surface of the embryo is most rapidly proliferating, about the stage shown in Brooks' Fig. 2 Plate XI. The arrow indicates the direction of movement of the follicle cells as they wander into the center of the embryo, where, as described by Brooks, many of them degenerate. The figure is carefully drawn with a Seitz $^1/_{12}$ immersion objective and a number 8 compensating ocular.

The large blastomere, *B l*, has a very large nucleus, *N*, and evenly granular protoplasm which does not stain deeply with hematoxylin, borax-carmine, or saffranin. Outside the blastomere is a mass of more coarsely granular and deeply staining protoplasm in which no cell walls can be discerned, but in which appear many nuclei all exactly resembling the nuclei of the follicular epithelium. These have a definite chromatic reticulum with rather small nodal swellings and no nucleolus.

Within the protoplasm of the blastomere as shown in this one section are seven bodies similar in size to the follicle nuclei just described, but quite different in appearance. I believe them to be ingested follicle nuclei. They do not stain so deeply as the nuclei outside, though they are much darker than the protoplasm of the blastomeres in which they lie. We do not find in them the clear cut chromatin reticulum with sharp contours such as we see in the follicle nuclei, but in certain of them we do find what appears to be such a chromatic reticulum degenerating, because undergoing digestion. Observe especially the nucleus *a*. The reticulum is evident and I think no one can doubt that the body is really a nucleus. Compared with follicular nuclei the reticulum is seen to stain less deeply and the fibrils and nodal masses do not have sharp contours. The whole appearance indicates the beginning of desintegration. I believe this nucleus to have been ingested but a short time previous to the killing of the embryo. At *b* and *c* are nuclei which have gone further in the process of degeneration, the chromatin threads being more diffuse. At *d*, *e*, *f* and *g* we see a further stage in the same process and at *h*, *j* and *k* we observe within the ingested nuclei an almost evenly granular mass of

disintegrating chromosomes. In other blastomeres and in another section of this same blastomere one can observe the last step in the degeneration, a mere mass of debris no longer delimited by a nuclear membrane from the surrounding protoplasm of the blastomere.

Notice that the less degenerated of these ingested nuclei lie on the side nearer the periphery of the embryo. The inwandering follicle cells, as they push toward the center of the embryo, penetrate the blastomeres that lie in their path. Apparently the most recently ingested nuclei, entering from the peripheral side, crowd the partly digested ones toward the inner side of the blastomere, giving the appearance figured. Not every section of a blastomere shows such diagrammatic arrangement, but this condition is noticeably frequent. The section figured was chosen because of the diagrammatic way in which it shows this point, and because of the clearly nuclear nature of the body *a*.

As before mentioned, Brooks has given exactly this interpretation of the bodies within the blastomeres and this confirmatory note would be uncalled for except for Heider's and Korotneff's more recent papers giving a different interpretation. It is possible that in *S. runcinata-fusiformis* (the species Heider studied) not only the follicle nuclei, but also their cytoplasm may be ingested by the blastomeres, but I am more inclined to believe Heider was mistaken when he figured these bodies as nucleated cells. His figures are not drawn with careful attention to detail, so it is hard to judge from them. In *S. pinnata* and *S. hexagona* no cell walls can be made out in the mass of migrated follicle cells. It is therefore by no means probable that the cytoplasm of the follicle cells could be seen if it were ingested with the nuclei. This is especially true in view of the digestive action upon these bodies within the blastomeres.

Korotneff's and Salensky's statements that these bodies contain no trace of a nucleus within them is, of course, true if they be themselves nuclei.

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— III. Zur Embryologie von *Salpa cordiformis-zonaria* und *musculosa-punctata*: Mitth. a. d. Zool. Stat. zu Neapel. 12. Bd. p. 331—352.

— IV. Zur Embryologie von *Salpa runcinata-fusiformis:* Zeit. f. wiss. Zool. 62. Bd. p. 395—414.

The Biological Laboratory of the Woman's College of Baltimore.

May, 13th 1897.

4. Ein neuer Fall von Brutpflege bei Holothurien.

Von Prof. Hubert Ludwig in Bonn.

(Vorläufige Mittheilung.)

eingeg. 31. Mai 1897.

Es sind bisher sieben brutpflegende Seewalzen bekannt, die alle entweder zu den Dendrochiroten oder zu den Synaptiden gehören. Unter jenen benutzt *Phyllophorus urna* Grube die Leibeshöhle als Brutraum, während bei *Cucumaria crocea* (Lesson) und *Psolus ephippifer* W. Thomson die Eier auf dem Rücken des Mutterthieres, dagegen bei *Cucumaria laevigata* (Verrill) und *Cucumaria glacialis* Ljungman in besonderen ventralen Bruttaschen (Hauteinstülpungen) zur Entwicklung gelangen. Mit Ausnahme des mittelmeerischen *Phyllophorus urna* sind alle diese Dendrochiroten arctische (*Cuc. glacialis*) oder antarctische Formen (*Cuc. crocea*, *Cuc. laevigata*, *Psol. ephippifer*). Bei den zwei brutpflegenden Synaptiden *Synapta vivipara* (Oerstedt) und *Chiridota rotifera* (Pourtalès), die beide dem westatlantischen Meeresgebiete angehören, dient ähnlich wie bei *Phyllophorus urna* die Leibeshöhle als Brutraum. Von *Synapta vivipara* haben wir neuerdings durch Clark [1] Näheres über die Entwicklung und Brutpflege erfahren, nachdem ich schon vorher das von mir in der Leibeshöhle dieser Art gefundene Gastrulastadium kurz erwähnt hatte [2]. Über *Chiridota rotifera* besitzen wir nur die fragmentarischen Beobachtungen, die ich 1881 veröffentlichte [3].

Daß es aber auch eine antarctische *Chiridota* mit ausgeprägter

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[1] Clark, The viviparous Synapta of the West Indies. Zool. Anz. 1896. p. 398, und Notes on the Life History of *Synapta vivipara* Oerstedt; Journal of the Institute of Jamaica, Kingston (Jamaica) Vol. II. Part 3. 1896. p. 278—282.

[2] Ludwig, Die von Chierchia auf der Fahrt der kgl. ital. Corvette »Vettor Pisani« gesammelten Holothurien. Zool. Jahrbb. II. 1886. p. 28—29. Clark citiert diese Publication nicht, behauptet hingegen irrthümlich, daß ich schon 1881 ein Exemplar der *Synapta vivipara* beschrieben hätte. Meine Arbeit aus dem Jahre 1881 (Archives de Biologie II. p. 41) bezieht sich nicht auf *Synapta vivipara*, sondern auf *Chiridota rotifera*.

[3] S. die vorige Anmerkung.