

CONTRIBUTIONS to the HISTORY of the GERMINAL VESICLE,
and of the FIRST EMBRYONIC NUCLEUS. By EDOUARD
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(With Plate XIII.)

I HAVE had the honour of presenting to the Academy a summary exposition of the results of my researches on the maturation of the ovule, the fecundation and earliest phenomena in the embryonic development of the rabbit, up to the moment of the appearance of the primitive streak more especially. I have made known those of my researches which establish the disappearance of the germinal vesicle, the formation of the directive bodies (*Richtungsbläschen* of Fritz Müller), and the appearance of the first embryonic nucleus. The recent publication of the researches of Auerbach,¹ Bütschli,² and Strasburger,³ on the formation and division of the nuclei of cells, led me, in the course of my researches on the development of mammalia, to investigate very specially how the first nucleus of the embryo appears and how cells multiply in the embryonic laminae. Long before I became acquainted with these researches, I had observed that the germinal vesicle of the rabbit disappears, independent of fecundation, and that the disappearance of this element is the indication of the complete maturity of the ovum.

At the very moment when I was completing the drawing up of my preliminary communication⁴ on the maturation of the egg, its fecundation and development in the rabbit, there appeared in Germany an important memoir by Oscar Hertwig⁵ on the formation, fecundation, and division of the egg of an echinoderm belonging to the order Echinida, *Toxopneustes lividus*. M. Hertwig and I, endeavouring to solve the same problems, but having chosen for our researches animals belonging to different groups, have arrived at

¹ Leopold Auerbach: 'Organologische Studien,' Breslau, 1874.

² O. Bütschli: "Vorläufige Mittheilung über Untersuchungen betreffend die ersten Entwicklungsvorgänge im Befruchteten Ei von Nematoden und Schnecken," 'Zeitsch. für Wiss. Zoologie,' Bd. xxv. O. Bütschli: "Vorläufige Mittheilung einiger Resultate von Studien über die Conjugation der Infusorien und die Zelltheilung," 'Zeitsch. für Wiss. Zoologie,' Bd. xxv.

³ Eduard Strasburger: 'Ueber Zellbildung und Zelltheilung,' Jena, 1875.

⁴ Edouard van Beneden, "Le maturation de l'œuf, la fécondation et les Premières Phases du Developement Embryonnaire des Mammifères d'après des recherches faites chez le Lapin," (Communication preliminaire), 'Bull. de l'Acad. Roy. de Belgique,' 2e série, t. xl, 1875.

⁵ Oscar Hertwig, "Beiträge zur Kenntniss der Bildung, Befruchtung, und Theilung des Thierischen Eies," 'Morphologisches Jahrbuch von C. Gegenbauer.'

identical conclusions on certain questions of capital importance, and at very different results with regard to other, equally fundamental points.

Among the problems which he and I have solved very differently are, in the first place, the history of the germinal vesicle; and secondly, the question of the formation of the first embryonic nucleus.

My researches on the ovum of the rabbit have proved to me that no morphological part of the germinal vesicle is found in the yolk at the moment of fecundation. The nucleolus united with the substance which constituted the membrane of the vesicle is eliminated to form one of the "directive bodies;" the nucleoplast with the pseudo-nucleoli are thrown off into the perivitelline liquid, to form there the second polar globule. The liquid of the vesicle remains in the yolk, and becomes confounded with the cortical substance of the ovum, which from this moment is no longer distinguishable from the medullary substance. There cannot then be, in the rabbit, any genetic connection between the germinal vesicle or one of its parts, and the embryonic nucleus which appears in the egg after fecundation. I have moreover been able to observe all the phases in the formation of the latter. The first nucleus is developed at the expense of a body formed in the cortical layer of the ovum, which I have called the *peripheral pronucleus*, and of another body which appears in the centre of the yolk, and which I have called the *central pronucleus*. It is probable that the first embryonic nucleus is not formed by the fusion of the two pronuclei:—the peripheral pronucleus, at first smaller than the other, enlarges at the expense of the central. The latter becomes attached to the former, and then its substance becomes absorbed by it, as, I think, by a process of endosmosis.

According to the observations of M. Hertwig on the *Toxopneustes lividus*, things take place in a different way. When the germinal vesicle has quitted the centre of the ovum to place itself under the membrane, and has, so to speak, left the yolk, the germinal spot in its turn forsakes the germinal vesicle, penetrates into the yolk, and becomes what the author calls the nucleus of the ovum (*Eikern*); the germinal vesicle then undergoes retrograde metamorphosis; its membrane becomes dissolved, and the rest is finally absorbed by the yolk.

As to the formation of the first nucleus of the embryo, which he calls *nucleus of the first cleavage-sphere*, or more simply, *cleavage nucleus*, M. Hertwig has ascertained that it is the product of the copulation of two nuclei. In from five to ten minutes after the sperm has been mixed with the eggs,

there appears near the surface of them a small homogeneous body. This element, which appears like a spot or a clear space, is formed by a mass of protoplasm, devoid of granulations. This space enlarges slightly and becomes the centre of a radiated figure, a little sun, from which rays, the length of which gradually increases, spread out in every direction. These rays are nothing but lines along which the yolk-granules are arranged. If the clear space is carefully examined, it is seen to contain a small homogeneous corpuscle. This has nearly the same refractive power as the surrounding protoplasm which makes it a little difficult to see. Sometimes Hertwig has seen a delicate line start from this corpuscle, pass to the periphery of the egg, and become continued into a little filament floating in the perivitelline liquid. The clear spot changes its place; it gradually approaches the centre of the yolk and goes to meet the nucleus of the egg, which also reaches the centre of the vitelline sphere. The two bodies finally meet near the middle of the egg. The homogeneous corpuscle contained in the clear space which comes from the periphery appears to be formed of the same substance as the nucleus of the egg, and is, like that, coloured by carmine. Hertwig calls it a small nucleus—measuring 4μ , while the nucleus of the egg is not less than 13μ . The nucleus of the egg changes its shape, executes amœboid movements, grows, and is soon surrounded by the clear protoplasmic substance which comes from the periphery; finally, it becomes fused with the little nucleus and from the fusion of these two nuclei arises the first cleavage-nucleus. While these last modifications are being accomplished, the radiated figure remains; it even extends, and becomes still more clearly marked; it involves all the yolk. The nucleus of the egg, and the little peripheral nucleus which becomes attached to it, both surrounded by a layer of transparent protoplasm without granulations, occupy the centre of the stellate figure.

Since the clear space near the periphery of the egg appears constantly five or ten minutes after the eggs have been mixed with the spermatic fluid, Hertwig does not hesitate to consider the formation of this space as the result of fecundation. The small homogeneous body which he has found to exist there is the head of a spermatozoon; the filament which starts from it is the tail of the same. The head of the spermatozoon is one of the two nuclei which meet and conjugate; for this reason Hertwig calls it the *spermatic nucleus* (Spermakern). *The first cleavage-nucleus is then the product of the fusion of the nucleus of the egg (Eikern), which is only the original germinal spot, with the spermatic nucleus (Sperma-*

kern) which is the head of a spermatozoon. It is the result of the conjugation of two nuclei.

It is plain that there is in certain respects a remarkable agreement between Hertwig's observations on *Toxopneustes* and my researches on the rabbit. We have both ascertained (1.) that there appears near the surface of the egg a clear space, which I have called, in order not to prejudice in any way its significance, the *peripheral pronucleus*. This peripheral nucleus I have regarded, as at least in part, formed of spermatogenic substance. (2.) This superficial nucleus penetrates into the yolk and goes to meet another transparent body, of which the character and significance are different from those of the peripheral nucleus; and which I have called the *central pronucleus* in contradistinction to the peripheral pronucleus. Hertwig calls it *nucleus of the egg*, in opposition to his *spermatogenic nucleus*.

(3.) We have both ascertained that the nucleus of the first cleavage-sphere, called by me *first embryonic nucleus*, by Hertwig *cleavage-nucleus*, is developed at the expense of the two nuclear elements after they have joined and become united in the centre of the yolk. We have both supposed that the formation of the first embryonic nucleus results from the union of a male element and a female element; and although I have not applied the term conjugation to the fact which essentially characterises the formation of the first nucleus, still the idea was not the less present in my mind, at least in a hypothetical form.

The facts as to which we are in complete disagreement are two in number. (1.) According to Hertwig, the germinal spot does not disappear—it becomes the nucleus of the egg (*Eikern*). According to my observations there exists no genetic bond between the central pronucleus (*nucleus of the egg* of Hertwig) and the germinal vesicle or any of its parts; the central pronucleus which appears after fecundation is an element of new formation. (2.) According to Hertwig, the peripheral nucleus—the *Spermakern*—is the head of a spermatozoon, and the transparent material surrounding it is protoplasm without granulations. In my opinion the clear space which appears in the cortical layer of the egg is a nuclear body (the peripheral pronucleus); the refractive corpuscles which appear on the spot are nucleolar elements. I propose in the following pages to criticise the opinions expressed by Hertwig, and to make known the observations which I have had the opportunity of making on the germinal vesicle of the egg of an echinoderm belonging to the order Asterida; *Asteracanthion rubens*.

I. *Does the germinal spot disappear or does it remain so as to become the central pronucleus (Eikern of Hertwig)?*

Hertwig expresses the opinion that the germinal vesicle disappears in *Toxopneustes lividus*; but he believes that the germinal spot (body or spot of Wagner) remains so as to become his (nucleus of the egg). He has not been able to arrive at a direct proof of this proposition; his opinion rests upon indirect proofs, of which I will give a summary.

(1.) The nucleus of the egg has the same dimensions (13μ) as Wagner's germinal spot; both are corpuscles without any membrane and formed of tolerably firm and homogeneous substance.

(2.) Like the substance of Wagner's spot, the nucleus of the egg is coagulated by osmic acid and coloured more deeply black than the yolk. Both are stained red by carmine. When acted on by acetic acid and chromic acid the two elements undergo a sort of superficial coagulation which produces a finely granular cortical layer, and some spots also granular, in the interior.

(3.) We never observe the nucleus of the egg and the germinal spot at the same moment in the same egg. So long as the germinal spot is seen in the germinal vesicle, when the latter has become superficial and lenticular, it is impossible to discover a nucleus in the yolk. From the time when this nucleus exists the germinal vesicle is destitute of its spot. The two elements are never wanting at the same time.

(4.) Hertwig has never succeeded in tracing any alteration in Wagner's spot, even in those eggs where the germinal vesicle was undergoing retrograde metamorphosis. "Für die Annahme, dass der Keimfleck, wie das Keimbläschen zerfällt, lässt sich daher keine directe Beobachtung anführen." He was equally unable to observe a new formation of the nucleus of the egg.

(5.) The nucleus of the egg at the moment when it appears is situated near the germinal vesicle; the Wagner's spot at the moment of its disappearance is adjacent to the yolk.

Hertwig has not then observed directly the transformation of Wagner's spot into the body which he calls the nucleus of the egg; *he has never seen Wagner's body leave the germinal vesicle in order to penetrate into the yolk.* Hence some doubt must remain as to the identity of these two elements, whatever may be the arguments by which he seeks to establish this conclusion. Hertwig himself acknowledges this fact when he writes: "Bei Abwägung aller dieser Verhältnisse

kann zwar die Möglichkeit dass der Keimfleck sich auflöst und der Eikern neu entsteht, solange nicht der directe Uebergang des ersteren in den letzteren beobachtet ist, nicht ganz von der Hand gewiesen werden."

We may, moreover, make the following remarks on the arguments adduced by Hertwig:

1. Proofs drawn from the physical and microchemical characters of the nucleus of the egg can have only a secondary value; but the characters common to this structure and the spot of Wagner belong to all young nuclei. The only conclusion from these facts is then that the nucleus of the egg is a young nucleus.

2. It does not necessarily follow from our never finding the germinal spot and the nucleus of the egg existing at the same moment that one is a transformation of the other; the disappearance of the nucleolus coincides with the appearance of the nucleus of the egg; but does it follow that the one fact is caused by the other? Nevertheless, the coincidence is surprising; according to my observations on the Mammalia, the formation of the central pronucleus is subsequent to fecundation, and happens, consequently, long after the disappearance of the germinal vesicle.

3. I was much surprised, after reading Hertwig's memoir, to find no mention there of the "directive bodies" (Richtungs-bläschen) which have been seen in many Echinoderms, and which cannot fail to occur also in *Toxopneustes*. It would have been interesting to know how these elements are formed in Echinodermata, since, according to my observations on the Rabbit, one of these bodies is nothing else than the germinal spot thrown off into the perivitelline liquid after its previous fusion with the membrane of the germinal vesicle.

4. I cannot pass over so easily as Hertwig does the differences between the appearance presented by the nucleus and the characters of the germinal spot. For my own part, I do not believe that the nature of the media in which these elements are respectively observed can account for the marked differences which, according to Hertwig's drawings, exist between Wagner's spot and the nucleus of the egg. This opinion is founded upon observations which will be related further on.

5. Hertwig has never seen any modifications produced in the characters of Wagner's body during the retrograde metamorphosis of the germinal vesicle. In this I have been more fortunate, not that I have studied the eggs of *Toxopneustes*, but I made some observations eighteen months ago

on the germinal vesicle in an echinoderm of our coasts, *Asteracanthion rubens*. The result of my researches is that, in this animal, Wagner's spot disappears in the germinal vesicle before the characters of the latter are altered. It cannot be supposed that in the starfish Wagner's spot undergoes progressive modifications which bring about its complete dissolution in the germinal vesicle, and that this element becomes in *Toxopneustes lividus* one of the first constituent parts of the first cleavage nucleus. If M. Hertwig has not seen the germinal spot undergo any modifications, we need not conclude, I think, that such modifications do not occur. His own figures, indeed, will I think show that in *Toxopneustes* Wagner's spot undergoes the same modifications as in the starfish.

Before relating my observations on the disappearance of the germinal vesicle in *Asteracanthion rubens*, I must state shortly in what circumstances, for what reasons, and with what ideas I undertook these researches. When I presented to the Academy my memoir on the composition and significance of the egg, I was convinced that the germinal vesicle does not disappear, but that it divides, after fecundation, so as to produce the nuclei of the two first cleavage-spheres.¹

Up to the time when Johann Müller published his researches on *Entoconcha mirabilis*² no one had any doubts as to the disappearance of the germinal vesicle. Leuckart, in his article "Zeugung," published in Wagner's Handwörterbuch, expressed as follows the conclusion which he felt authorised to draw from a comparison of all the researches made on this subject.³

"Fassen wir alle diese Thatsachen zusammen, dann kann es wirklich kaum noch zweifelhaft bleiben dass das Verschwinden des Keimbläschens einen Vorgang bezeichnet, der mehr der Bildungsgeschichte des Eies, als der Entwicklungsgeschichte des späteren Embryo zugehört. Das Einzige, was der Aufbau eines neuen Thieres voraussetzt, ist die Anwesenheit eines entwickelungsfähigen Materiales."

"If we put all these facts together, there can indeed hardly remain any doubt that the disappearance of the germinal vesicle indicates a process belonging more to the history of the formation of the egg than to the later history of develop-

¹ "Recherches sur la Composition et la Signification de l'œuf basées sur l'Étude de son mode de Formation et des Premiers Phénomènes Embryonnaires," 'Mém. Couronné de l'Acad. Roy. de Belgique,' T. xxxiv.

² Joh. Müller, "Ueber Synapta Digitata und über die Erzeugung von Schnecken in Holothurien," Berlin, 1852, p. 17.

³ "Handwörterbuch der Physiologie von R. Wagner," 'Art. Zeugung von R. Leuckart,' p. 922.

ment. The only essential condition of the construction of a new animal is the presence of a material capable of development."

This was at that time the opinion of most, if not of all naturalists. But so great was the authority of Johann Müller, so complete the confidence which was placed in his observations and conclusions, that the opinion he promulgated on the permanence and division of the germinal vesicle in *Entoconcha* caused all the affirmative statements made in previous works to be called in question.

Shortly afterwards Leydig¹ announced that he had confirmed in the Rotifera the results obtained by Müller in his researches on the development of *Entoconcha*. Mecznikow² observed the division of the germinal vesicle in the Cœcidiomyæ and Aphides. "It is hardly possible," he writes, "to call in question the generality of this fact in insects." Pagenstecher³ made the same observation in the Trichinæ; Leuckart⁴ in the Oxyurides; Keferstein⁵ in *Leptoplana tremellaris*; Gegenbauer⁶ in the Medusæ, Siphonophora (Corynidæ, Calycophoridae, Physophoridae), Pteropoda, Heteropoda, and finally in Sagitta. Häckel⁷ and Kölliker⁸ confirmed as regards the Siphonophora the statements of Gegenbauer. Finally, I myself observed the division of the germinal vesicle in the transparent and easily observed egg of *Distoma Cignoides*.⁹ Resting as much on my personal observations as on those of the eminent naturalists just quoted, I expressed in a hesitating form the opinion already announced in a manner equally general by Leydig,¹⁰ that the disappearance of the germinal vesicle is only apparent, and that the embryonic development begins with the division of

¹ Fr. Leydig, "Ueber den Bau und die Systematische Stellung der Räderthiere," *Zeit. für Wiss. Zoologie*, Bd. vi, p. 102.

² Mecznikow, "Embryologische Studien an Insecten," *Zeitsch. für Wiss. Zool.*, Bd. xvi, p. 484.

³ Pagenstecher, "Die Trichinen," Leipzig, 1865.

⁴ Leuckart, "Die Menschlichen Parasiten," Bd. ii, 2e Lief., p. 322.

⁵ Keferstein, "Beiträge zur Anatomie und Entwicklungsgeschichte einiger Sceplanarien," Göttingen, 1868.

⁶ Gegenbauer, "Beiträge zur Näheren Kenntniss der Siphonophoren," *Zeit. für Wiss. Zool.*, Bd. v. "Zur Lehre vom Generationswechsel bei Medusen und Polypen," p. 24. "Untersuchungen über Pteropoden und Heteropoden," Leipzig, 1855. "Ueber die Entwicklung der Sagitta," Halle, 1856.

⁷ E. Häckel, "Zur Entwicklungsgeschichte der Siphonophoren," Utrecht, 1869.

⁸ Kölliker, "Die Schwimmpolypen von Messina," Leipzig, 1853.

⁹ Edouard van Beneden, *loc cit.*, p. 30.

¹⁰ Leydig, "Lehrbuch der Histologie," p. 10.

this element. I expressed myself in the following words: "En résumé, je considère non comme démontré, mais comme très-probable, que la vésicule germinative se divise au lieu de disparaître" (loc. cit., p. 244).

I supported this proposition by various more or less plausible considerations, and in particular, I pointed out that the disappearance of the vesicle had never been observed, no direct proof of this disappearance having been given.

I should add that the contrary proposition did not, any more than this, rest upon direct observation; no one of the authors who maintained the permanence of the vesicle rested his opinion upon any other considerations than upon a negative fact. *The vesicle is asserted not to disappear because no egg has ever been observed entirely devoid of a nucleus.*

Some observers have been inclined to trace a genetic bond between the nucleus of the impregnated egg and the 'spot' of Wagner. Leydig¹ has expressed this opinion so far as regards the eggs of *Piscicola*; von Baer² with respect to the development of a sea urchin. Bischoff³ derives from the germinal spot of the rabbit, not only the directive bodies but also the nucleus which is found in the egg after impregnation. But Bischoff confesses that it is in his eyes, a pure hypothesis; he moreover, abandoned it shortly afterwards,⁴ and in his later works, has declared it to be untenable. Quite recently, Fol⁵ has found in the ripe egg of the *Medusa* a nucleus, with respect to which he feels some doubt, not knowing whether it is the modified germinal vesicle or the spot of Wagner. Finally, Hertwig has just given new credit to this tottering opinion by the publication of his recent memoir, which cannot fail to produce a great effect.

A short time after the publication of my memoir on the composition and significance of the egg, two publications appeared in Germany, which are of the highest importance with respect to the question now occupying our attention.

Oellacher⁶ established the fact that in the matured ovum of the salmon the germinal vesicle reaches the surface of the germ and opens itself into the space which exists at that

¹ Leydig, "Zur Anatomie von *Piscicola geometrica*," *Zeit. für Wiss. Zool.*, Bd. i.

² K. E. von Baer, "Neue Untersuchungen über die Entwicklung der Thiere," *Froriep's Neue Notizen*, Bd. xxxix, p. 38.

³ Bischoff, "Entwicklungsgeschichte des Kanincheneies," 1842.

⁴ Bischoff, "Entwicklungsgeschichte des Hundeies," 1845.

⁵ Fol, "Die erste Entwicklung des Geryonideeneies," *Jenaische Zeitschrift*, Bd. vii, p. 474.

⁶ Oellacher, "Beiträge zur Geschichte des Keimbläschens im Wirbelthierei," *Archiv. für Mikrosk. Anat.*, Bd. viii.

period between the yolk and the ovular membrane. The opening enlarges, and the membrane of the vesicle becomes gradually detached from its contents. Finally, the latter are evacuated and the membrane spreads itself out on the surface of the germ. Some observation of the same author on "The Germinal Vesicle of the Fowl" furnish a remarkable and complete confirmation of the conclusions at which the illustrious von Baer arrived fifty years ago.

Soon afterwards there appeared the beautiful researches of Kleinenberg "On the Anatomy and Development of the Fresh Water Hydra." Kleinenberg¹ thus describes the mode of disappearance of the germinal vesicle:—"About the time when the production of pseudocells is completed the germinal spot undergoes a retrograde metamorphosis. At first it loses its circular outline and becomes irregular and angular; its substance appears coagulated; then it breaks up into little fragments, and these, unless I am mistaken, finally dissolve. So long as the egg was an amœbiform body, the germinal vesicle was situated at the centre of the yolk; but from the time that the egg begins to become rounded it takes an excentric position, and approaches that pole which is turned towards the surface. It takes its situation near to the surface, and is now covered only with a thin layer of plastic material. Here it also begins to undergo a retrograde metamorphosis which ends in its complete disappearance. Its granular contents become more and more liquefied; a part of these contents escape from the membrane with the result that the latter, which had previously remained uniformly stretched, becomes collapsed so as to form a tube of generally ovoid shape, the wall of which is thickened and folded at certain points. The part of the contents which has remained in the interior breaks up into isolated shining bodies of rounded or angular form, and of very different dimensions; amongst them are scattered some drops of a fatty liquid."

Kleinenberg thinks that these bodies are composed of a fatty material, or at least that they consist of the material which results from the transformation of albuminoid substances, and which we observe in many pathological tissues, where this appearance is a sign of fatty degeneration. According to his view the germinal vesicle disappears by fatty degeneration. On one occasion Kleinenberg believed he observed an actual hole in the germinal vesicle. "If this is a normal phenomenon," says Kleinenberg, "it is possible that the contents of the vesicle escape and mix with the sur-

¹ Kleinenberg, 'Hydra,' Leipzig, 1872, p. 42.

rounding plasma." The question as to what becomes of the membrane has not as yet been resolved; but what is certain, according to Kleinenberg, is that every trace of the germinal vesicle has already disappeared long before the moment when fecundation takes place.

Thanks to the observations of Oellacher and Kleinenberg, the disappearance of the germinal vesicle was directly demonstrated; from that time it was no longer possible to maintain the persistence of the germinal vesicle in all animals, without denying the facts observed. The question then entered into a new phase. Two observers, who must certainly be reckoned amongst the most eminent of the age, had just established the mode in which the germinal vesicle disappears. From that time only two alternatives were further possible; either it must be admitted that the germinal vesicle does not play the same part in all animals; that it disappears in some, and that it persists and undergoes division in others; or else it must be allowed that all the observations made by Müller, Leydig, Gegenbauer, Leuckart, Pagenstecher, Mecznikow, Kölliker, Haeckel, and myself were erroneous, or, at any rate, that the conclusions drawn from the facts observed were but little in conformity with the principles of logic. I believe that the latter of these hypotheses is of the two more probable; the opinion which affirmed the permanence of the germinal vesicle rested, in fact, on negative grounds. It was affirmed that this element does not disappear, because no egg had ever been found which was entirely devoid of all central nucleus; but it does not strictly follow that because no egg had been found entirely deprived of a central nucleus, that therefore the germinal vesicle persists. The doubts which the researches of Oellacher and Kleinenberg had aroused in my mind led me to make fresh researches. In order to make fresh observations on this point it was of importance to choose eggs in which the yolk possessed in the greatest possible degree the properties of transparency and homogeneity, and it was requisite, moreover, that they should be distinguished by the dimensions of the germinal vesicle and the (germinal) spot of Wagner. The ova of the Echinodermata, and in particular those of the *Asteracanthion rubens* realise these conditions in the highest degree. At the end of April, 1874, I betook myself to Ostend with the object of carrying out the artificial fecundation of these ova.

It is not very long ago that we were still in ignorance as to whether the sea starfish are of different sexes. Tiedemann declares that that he has never found the male organs of these

animals.¹ Nothing, however, is easier than to distinguish the ovaries from the testicles. It is true that the sexual organs have the same form, the same position, and the same volume in the two sexes; when they have reached their complete development the five pairs of sexual glands extend along the entire length of the arms, and they cause a marked elevation of the skin of the side of the back, so that one can recognise, even from the external appearance, the individuals in which the sexual products have attained maturity. The most superficial microscopic examination suffices to distinguish the contents of the testicle from those of the ovaries, and one soon learns to recognise the sex, even with the naked eye; the ovaries have a yellowish or very pale brownish tint; the testicles are of a pure milk-white colour. Moreover, the lobules of the ovarian clusters are more rounded and shorter, whilst those of the male sexual gland are elongated and rather of tubular shape.

I will first describe the ovarian ovum, such as it appears when, already free in the cavity of the ovary, it has attained the dimensions of the ripe ovum, but while its germinal vesicle is still lodged in the centre of the yolk. These ova may have either an ellipsoid form, or else they may be pyriform. Their dimensions vary between $\cdot 16$ by $\cdot 13$ and $\cdot 19$ by $\cdot 17$ millimètres. They are composed of a thick and entirely homogeneous envelope, of a finely granular yolk, and of a germinal vesicle which is situated in the neighbourhood of the centre of the yolk.

Membrane.—It is still a question whether there exists around the ovum of the Asteridea a single one or two membranes; nor is anything more certain known as to the nature and signification of these envelopes. If fresh ova which have reached the dimensions of the ripe ovum, and which still have the germinal vesicle central, are examined, we can distinguish a clear zone around the yolk which has a thickness of from $\cdot 003$ to $\cdot 004$ millimètres. This is quite clear, transparent, and homogeneous. It is limited where in contact with the yolk by a sharply defined contour; on the outer aspect, on the contrary, its contour is pale and so slightly marked, that it requires great attention to discern it. The index of refraction of the substance which constitutes this membrane must be very similar to that of water. This substance is very soft; it appears to be a gelatinous, mucilaginous, or albuminoid body; whence the names of "Gallerthülle, Eiweisschicht, and mucilaginous layer" which

¹Tiedemann, 'Anatomie der Röhren-Holothurie, des Pomeranzfarb. Sees-terns,' &c., 1816, p. 42.

have been given to it. More recently Hoffmann¹ has called it the "*vitelline membrane*." To see well the characters of this membrane, examination in water must be avoided. The moment it comes in contact with water it swells considerably; one may study it conveniently by placing the eggs directly in 1 per cent. solution of osmic acid, or in a concentrated solution of picric acid. It is not coloured red by the colouring matter of carmine fluids; it is partially dissolved or contracts strongly by a prolonged stay in absolute alcohol. After remaining for a certain length of time in absolute alcohol it is only a very thin membrane which immediately envelops the vitellus, and which at certain points is in immediate contact with it, whilst at other points it is separated from it, thus forming more or less regular undulations. If one examines the transparent layer in ova which have not attained their complete development, it exhibits a radiated striation which is due to the presence of pores in the form of canaliculi of extreme tenuity; it has been long known that the ova of Holothurians present the same character.

I have never observed in the ova of the sea starfish the canal which J. Müller has discovered in several Holothuria, and which has been thought to fulfil the function of a micropyle. However, in the pearshaped ova the transparent layer is oftentimes a little thinner at the tail end of the pear.

Derbès has found in the ovum of the Echinida, independent of the *mucilaginous zone*, which I have just described, a thin membrane which is immediately applied to the surface of the vitellus, and which he calls a vitelline membrane. Several authors have affirmed since Derbès, the existence of this second membrane, not only in the Echinida, but also in the Holothurida and Asterida. I have not been able to convince myself of the existence of this membrane in the egg of the *Asteracanthion rubens*.

What is the signification of the transparent layer which exists around the ovum in all the Echinodermata? What name must be given to it? It is not possible to answer these questions in the present state of our knowledge on the formation of the egg of these animals. I have reasons for believing that the membrane is not produced by the egg itself, and that its mode of formation is the same as that of the *zona pellucida* of mammals. If my opinion is correct, there would be grounds for designating it by the name of Chorion. As its microscopical characters have some resemblance to

¹ C. K. Hoffman, "Sur l'Anatomic des Astérides," 'Extrait des Archives Néerlandaises,' vol. ix.

those of the zona pellucida of Mammalia, we may, at least provisionally, call it by that name which has the advantage of recalling its physical characters without in any way prejudging its *equivalence* from a morphological point of view.

Vitellus.—The yolk is formed of a clear and transparent fundamental substance (protoplasm) and of feebly refracting vitelline granules held in suspension in the protoplasm. These granules are formed of a substance, the refractive index of which is but little greater than that of the vitelline protoplasm. Hence it results that the transparency of the ovum is scarcely altered by their presence. The absence from the yolk of all vesicular or globular elements and of all highly refractive substances, causes the body of the ovum to be far from the appearance of an emulsion. It is a clear, transparent and finely granular mass. This circumstance renders the eggs eminently favorable to the study of the modifications which the germinal vesicle undergoes in the egg which has reached maturity.

It is possible to distinguish in the yolk of the ova of the sea starfish two layers, or if the expression be preferred, two substances; a cortical layer, the thickness of which is nearly equal to one third of the radius of the yolk, and a medullary mass. The cortical layer is clearer and less granular than the medullary mass; it presents, moreover, a slight radiated striation which appears to me to be wanting in the medullary mass. The limit between the two constituent parts of the vitellus is not marked by a very clear line, the cortical substance of the ovum passes insensibly into the medullary substance. Yet the zone of transition is very narrow. This distinction between the two constituent substances of the ovum of the Asterida has escaped the observation of all those who have studied the sexual products of these Echinoderms. It has not been mentioned up to the present time in any animal of this division, and I am surprised that Hertwig, who has studied the ova of the Toxopneustes with so much care, should not have observed it.

Germinal vesicle.—The germinal vesicle is perfectly spherical; it is lodged in the centre of the medullary mass of the egg. It is marked out by a very sharply defined and deep line. It encloses a transparent and perfectly homogeneous liquid. If we examine the germinal vesicle whilst it is in the yolk, we perceive in this liquid a voluminous and very conspicuous germinal spot, and around it a certain number of much smaller globules which are pseudo-nucleoli. The germinal spot, or spot of Wagner, is of large

size. It is circular, and its contour is quite regular. Sometimes it appears nodulated on its surface; and lastly, at other times it is angular and altogether irregular. It is formed of a highly refractive and very brilliant substance which encloses clear vacuoles, whose number and volume vary in different eggs. The vacuoles have already been noticed by Leydig, in the eggs of *Holothuria tubulosa*: "Der Keimfleck des fertigen Eies ist bedeutend schärfer contourirt als das Keimbläschen, fast fettartig und zeigt ein oder mehrere Cavitäten." Hertwig has observed that in the ova of the *Toxopneustes* Wagner's spot contains sometimes one, sometimes several vacuoles.

In the ova which I am describing, I have not observed amœboid movements performed by the nucleolus. Long ago de la Valette¹ had noted the fact that in the ovum of a *Libellula* the germinal spot might be seen to change its form and place. Mecznikow² has seen, not only in the spot of Wagner of several of the lower animals, but also in the salivary cells of the larva of insects, spontaneous movements of the nucleoli. Balbiani³ has made a similar observation with regard to the ova of spiders. Alexander Brandt⁴ has observed that in the eggs of *Periplaneta orientalis* the germinal spot affects all sorts of forms, and that these changes, which are really active, must be ascribed to the contractility of the substance of the spot or nucleolus. He has remarked that under the influence of heat, these movements become so active, that it is difficult to draw the successive changes of form which are produced.

Auerbach⁵ has recognised changes of form, presenting all the characters of amœboid movements, in the large nuclei of the embryonic cells of the *Muscida* and in the germinal spots of the ovum of the pike; Hertwig has seen the same phenomenon occur in the nuclei of the eggs of the frog and also in the germinal spot of Wagner, in the ovum of *Pterotrachea*. More than four years ago, I observed changes in form, and enlargement and diminution of size, whilst watching Wagner's spot in the germinal cells of the *Polystomum integerrimum*, and I had remarked that the diminution in volume of these nucleoli corresponded with

¹ De la Valette: "Über den Keimfleck und die Deutung der Eitheile," *Archiv. für Mikrosk. Anat.*,² bd. ii, 1866.

² Mecznikow, 'Virchow's Archiv,'² bd. xii.

³ Balbiani (quoted by Auerbach), see Keferstein. 'Jahresbericht,' für 1865.

⁴ Alexander Brandt, "Über retive Formveränderungen des Kernkörperchens," 'Archiv. für Mikrosk. Anat.,' bd. ix.

⁵ L. Auerbach, 'Organologische Studien,' heft i, pp. 167 and 168.

the disappearance of the vacuole which is seen in these at certain moments. I observed, also, that the very numerous nucleoli in the young ova of the frog likewise execute movements which consist in changes of form. I made these observations a little while after having established the alternate disappearance and reappearance of the nucleoli in the nucleus of the *Gregarina gigantea*.¹ Since then I have several times observed the same fact in the *Monocystis lumbricorum*. In the latter species, however, there exists one nucleolus of larger size, which is more voluminous than the rest, and which never disappears, but which changes its form, and in which one sees vacuoles appear and disappear: sometimes the nucleolus encloses only a single very extensive vacuole; a few seconds later it may show a crowd of little ones of all dimensions; at other times there are no longer so many. Other researches and numerous occupations have, however, hindered me from publishing these observations before now. I have not seen changes of form take place under my eyes in the ova of the Starfish; but I have no doubt whatever that the differences shown to exist in the form of the germinal spot must be attributed to the contractility of the substance of the nucleoli. This conclusion results from observations made on mature ova of changes which essentially consist in the reduction of the nucleolus into fragments, a reduction which immediately precedes the disappearance of Wagner's spot.

The pseudo-nucleoli, eight to fifteen in number, are corpuscles of very variable size, composed of a substance which is much less refractive than the nucleolar matter. Sometimes they are disseminated throughout the whole extent of the germinal vesicle; more frequently they are situated in the neighbourhood of the true nucleolus. They have an entirely different composition, and different properties from the latter. It is incorrect, therefore, to say with Hoffmann that there are from one to ten nucleoli in the ovum of the *Asteracanthion rubens*.

Hertwig, in his work, declares himself a partisan of the opinion of Auerbach, who holds that the membrane of the germinal vesicle, and of nuclei in general is produced by the differentiation of a thin layer of protoplasm around a vacuole which is filled with the nuclear substance. Although I do not wish here to go fully into my views on the subject of the

¹ Édouard van Beneden, "Sur une nouvelle espèce de Grégarine, Désigné sous le nom de *Gregarina gigantea*" (On a New Species of *Gregarina*, named the *Gregarina gigantea*), 'Bull. Acad. Roy. de Belg.,' 2nd series, vol. xxviii. See also translation of same in this Journal.

constitution of the cell-nucleus in general, and the germinal vesicle in particular, I think it right to say that I do not in the least coincide in this opinion of Auerbach and Hertwig. A young nucleus is formed by a homogeneous substance which I have called the nuclear essence.¹ When this young nucleus enlarges, the nuclear essence becomes united with a substance which is taken up from the protoplasm of the young cell. The nuclear substance which results from this union forms the body of the nucleus. The membrane of the nucleus, as well as the nucleoli, are the unmodified remains of the primitive young nucleus; they are formed exclusively by the nuclear essence. At the moment when a nucleus is about to divide the nucleoli as well as the nuclear membrane dissolve in the nuclear substance. Hence it results that the contour of the nucleus becomes scarcely distinguishable, and that the nucleoli disappear. All who have studied the multiplication of cells know how little the nucleus is apparent at the moment when the division of the cell is about to take place. It is this fact which has given rise to the theory according to which all cell-multiplication is preceded by the disappearance of the nuclei. The momentary disappearance and reappearance of the nucleoli in the nucleus of the Gregarina were mentioned by me in 1869. I was at that time unable to give any interpretation of these facts; still, they bear witness to the facility with which the nucleolar substance dissolves in the nuclear substance. The observations of which I shall give an account later on, and which show that the spot of Wagner dissolves in the germinal vesicle with the disappearance of the latter element, form an argument which may be turned to account in support of my opinion. Immediately after the dissolution of the nucleoli and of the membrane in the nuclear substance, a separation is effected between the nuclear essence which goes to form the equatorial zone and the nuclear liquid which is driven back to the poles of the nucleus. The latter, after the division of the zone into two nuclear discs which are to become new nuclei, loses itself in the body of the cell.

The vacuoles which appear in so large a number of the nucleoli are, I think, nothing but the result of the momentary union of certain parts of the nucleolar substance with the nuclear liquid.

I believe that this way of looking at the constitution of the nucleus is the only one which can explain the physical and

¹ Édouard van Beneden, "De la Maturation de l'œuf, de la Fécondation et des Premières Phénomènes du Développement Embryonnaire des Mammifères," p. 50.

microchemical characters of the element and of its various parts—to wit, the nuclear membrane, the body of the nucleus, and the nucleoli. It depends entirely on the phenomena which one knows in relation to the vital manifestations, the development and the multiplication of nuclei.

If one breaks the membrane of the egg of a Starfish so as to allow the contents of the egg to escape into weak osmic acid or picric acid, the germinal vesicle presents a peculiarity which I have been able also to make out in the living egg, after having first observed it in the germinal vesicle isolated and treated with these reagents. In the nucleus of the egg there exists a network with large meshes, formed by a very finely granular substance. It is in this reticulum that the pseudo-nucleoli are found; the germinal spot appears to be the centre from which the reticulated filaments start. The characters of this network vary, moreover, in different ova; sometimes one even sees in place of the network a small granular collection formed by the substance of the reticulum and the pseudo-nucleoli. This reticulum I have also found in the rabbit, and I have proposed to designate the substance which constitutes it by the name of *nucleo-plasma*. The first to describe a network similar to that of the Starfish was W. Flemming.¹ He found that in the Anodons and the Unios the transparent liquid of the germinal vesicle is traversed by numerous anastomosing filaments. Kleinenberg has mentioned something similar in the germinal vesicle of the fresh-water Hydra; and lastly Hertwig has observed it in the *Toxopneustes lividus* and in the ova of the mouse. I do not know that anything of a similar nature has been shown to exist in other nuclei of cells. I think, therefore, it will not be without interest to mention here my observations on the constitution of the nucleus of an enormous cell which constitutes by itself alone the whole central part of the body (Leibeshöhle of Kölliker) of the Dicyema. The nucleus, which is more or less regularly ellipsoid in shape, presents a thick membrane, beneath which there exists a very close network composed of a finely granular material, whilst the contents of the nucleus (*nuclear substance*) are perfectly homogeneous and transparent. The body of the nucleus is traversed in certain individuals by a reticulum which makes it resemble a spongy tissue; in other individuals there exists only a bundle of filaments like pseudopodia. I have represented one of these nuclei (figs. 20 and 21). By the application of the picrocarminate after previous treat-

¹ W. Flemming: "Studien in der Entwicklungsgeschichte der Naja-den," "Sitzb. der K. Acad. der Wissensch. zu Wien.," vol. lxxi.

ment with osmic acid, the nuclear substance is stained of a rose colour; the nucleolus and the membrane bright red; the reticular substance does not stain. This is also the case with the nucleo-plasmic meshwork of the germinal vesicle of the rabbit.

But if one opens the ovary of the starfish at the moment of sexual maturity one finds in it not only ova similar to those which I have just now described, and which are essentially distinguished by the fact that although they have attained the dimensions of the ripe ovum they still have their germinal vesicle in the centre of the vitellus; together with these ova one sees others which have not yet attained maturity; others in every respect similar to those of which I have before spoken, but differing from them in the fact that the germinal vesicle has become superficial; and others which show no longer any trace of the vesicle of Purkinje. These latter, however, are ova which are only exceptionally observed; generally speaking, all the ovarian ova still possess their germinal vesicle.

The ova in which the germinal vesicle has reached the surface of the yolk scarcely differ from those above described; they have an ellipsoidal or spheroidal form; their zona pellucida, swollen by the seawater, is very thick, and its surface quite irregular. The yolk invariably presents the same characters; the germinal vesicle has retained its spherical form, and all the sharp definition of its contour. It is difficult to make out whether it is in immediate contact with the zona pellucida, or whether it is separated from that membrane by a thin layer of vitelline protoplasm. Inside the vesicle are seen the nucleus and pseudo-nucleoli in the midst of a little cluster of granules. I have never found the nucleo-plasmic network in the germinal vesicle when it has become superficial, whatever the method to which I have had recourse to convince myself of its presence. From the time when the germinal vesicle has taken its peripheral and superficial position, the nucleo-plasma, together with the pseudo-nucleoli, forms a small nucleo-plasmic mass by the side of the nucleolus.

If the ovarian ova of a completely developed ovary are received into a small vesicle containing sea-water, and if a fragment of testicle which has reached maturity be shaken in it for a moment, a certain number of ova are fecundated, and two or two and a half hours after having performed the artificial fecundation, divided ova are found at the bottom of the vessel. If one has taken care to leave only a small number of ova in the vessel, and to renew the water from

time to time the embryonic development advances rapidly, and at the end of from two to three days the ciliated embryos swim freely in the water. Artificial fecundation was performed for the first time in an Echinoderm by K. E. Von Baer; after him several embryologists have had recourse to the same procedure in order to study the development of Echinida, Asterida, and Holothurida. I will mention only Derbès, Krohn, Busch, J. Müller, A. Agassiz, and Selenka.

If, some few seconds after having performed artificial fecundation, one places a certain number of ova on an object glass, having taken them with a pipette from the bottom of the vessel in which the sexual products have been mixed, one observes that a crowd of spermatozoa have collected together at the surface of the zona pellucida. They move their tails with such force, that they even succeed in making the ova move. If, in order to study the successive phenomena which take place in it, one chooses an ovum which presents a superficially situated germinal vesicle, and if one watches it continuously, one finds that three quarters of an hour or an hour after the fecundation, the germinal vesicle which was so distinct at the time of the commencement of the observation, has completely disappeared. Subsequently one sees the vitellus undergo in succession the phenomena of retraction; distinctive bodies and polar globules appear in the peri-vitelline fluid; then the primary vitelline globe breaks into two parts. The ova, therefore, which are provided with a superficial germinal vesicle, are fit and liable, as well as those which no longer showed any traces of it in the ovary, to be fecundated. In order to be sure whether the disappearance of the germinal vesicle is the consequence of fecundation, or whether it occurs independently of the action of the semen, it is sufficient to turn some ovarian eggs into another vessel, taking care to avoid all mixture with the spermatic fluid. If one observes under the conditions above described ova taken from the bottom of the vessel, one can follow and observe the successive phases of the disappearance of the vesicle, just as when one follows them in fecundated ova. This disappearance then is independent of the action of the spermatozoa. This conclusion might, moreover, be drawn from this fact, that some of the ova lose their germinal vesicle, whilst still in the ovary, and that nevertheless these ova are perfectly fertile.

It was of the highest importance to study the successive phases of the disappearance of the vesicle, in order that we might be able to determine with certainty *how* it

disappeared. Nothing is easier than to make this observation on the ova of the starfish, since the eggs continue to develop on the object-slide, and the changes which they undergo take place under the very eye of the observer. Eight or ten times have I seen the series of modifications which lead to the disappearance of the vesicle of Purkinje evolve themselves under my very eyes. The succession of these phenomena is the same, whether one observes a fecundated ovum or follows them in a non-fecundated one. The whole series of these changes is completed in the same time in either case. I believe, however, that fecundation is often the immediate cause of the disappearance of the germinal vesicle, in this sense, that in a ripe ovum the germinal vesicle disappears from the time that the ovum is placed in the presence of the sperm, whilst that element might have still remained for some time if the ovum had not been fecundated. This appears to me to be shown by the following observation: if we place in two glasses ova from the same ovary, and fecundate those in the one, carefully preventing the sperm from becoming mixed with the contents of the other, an hour after the fecundation all the mature ova of the first glass will have lost their vesicle, whilst most of those in the second still show it perfectly distinct. If amongst the fecundated ova one be chosen which shows a quite superficial germinal vesicle, we may be almost certain to see the germinal vesicle disappear in less than an hour. But it is not so if we select from amongst the non-fecundated ova one which presents similar conditions.

Let us now see the series of modifications that are observed.

1. At first the little granular mass which is situated by the side of the nucleolus, and which is composed of nucleoplasma and pseudo-nucleoli, becomes less and less apparent; soon it becomes impossible to distinguish it; the germinal vesicle now contains only an entirely homogeneous and transparent liquid; with no other granule than the spot of Wagner or nucleolus.

2. The contour of the germinal vesicle becomes paler; the same is the case with the nucleolus, the substance of which appears to become less and less refractile. At the same time the nucleolar vacuoles become united with a single central vacuole, which appears as a clear spot, circumscribed by an irregular ring formed of a highly refractile substance. The nucleolus becomes very irregular, its surface is now knobbed, and the projections are separated from each other by fissures. The nucleolus resembles a small raspberry-like mass.

3. The nucleolus (germinal spot) breaks up abruptly into a large number of fragments which continue to diverge from one another and to spread themselves out into the whole mass of the germinal vesicle. These fragments are of unequal size. There is one of them which is notably of larger size than all the others, and which contains the central vacuole of the old germinal spot. This vacuole is now only surrounded by a thin layer of nucleolar substance, which as looked at appears only as a narrow and irregular ring. The formerly homogeneous contents of the germinal vesicle are now granular, and hold in suspension small bodies of variable form and dimensions which are only the fragments of nucleoli.

4. All the nucleolar fragments increase a little in volume and become less and less refractive. Soon they appear as only little clouds with ill-defined contours, forming spots on the uniformly homogeneous ground of the germinal vesicle. They at last completely disappear from view. The fragment of the nucleolus which encloses the central vacuole is still visible when all the others have already disappeared. Soon afterwards the last traces of this body likewise disappear. The germinal vesicle, which is still perfectly spherical, is now quite clear and transparent. We can no longer see in it any trace of nucleolus, nor any granule of whatever kind. The contour of the vesicle has become less and less marked, as if the substance of the membrane were dissolved at the same time with the nucleus in the nuclear substance. The progressive diminution of refractiveness of the nucleolar substance proceeds side by side with the vanishing of the contour of the germinal vesicle.

5. Some seconds after the last traces of Wagner's spot have disappeared, the membrane of the germinal vesicle becomes torn, or rather a hole is formed in it. This solution of continuity always appears in that part of the vesicle which is turned towards the centre of the ovum. The contents of the vesicle immediately flow out through the hole, forming a clear drop outside the vesicle. This little drop has the appearance of a bud or of a hernia. It enlarges very rapidly. At the same time the membrane of the vesicle withers and becomes wrinkled. The germinal vesicle has now withdrawn itself slightly from the surface and has got nearer the centre of the vitellus. It is enveloped on every side by the vitelline protoplasm. At one moment it appears as if formed of two clear masses adjacent to each other, which on account of their homogeneous appearance encroach upon the granular ground of the vitellus. One of these is formed by that part of

the nuclear substance which is still enclosed in the wrinkled membrane of the germinal vesicle, the other is formed by a drop of nuclear fluid expressed and projecting into the vitellus.

6. The extravasated drop is flattened against the vesicle, in the neighbourhood of the hole by which it made its exit. The nuclear mass then reassumes a more or less rounded form. But in this clear mass one distinguishes an irregular line, which separates the intravesicular from the extravascular portion of the nuclear substance.

This line is produced by the membrane which separates the two parts of the nuclear substance which has become very thin. This line at last completely disappears, proving that the membrane is entirely dissolved in the nuclear substance. There then no longer remains any portion of the germinal vesicle, except a clear spot, whose ill-defined contours become more and more irregular. The spot becomes smaller and smaller, and ends by disappearing completely. It seems as if the clear and homogeneous matter of the germinal vesicle became granular from the periphery to the centre. This appearance is probably the result of the progressive dissolution of the nuclear substance by the vitelline protoplasm.

The successive phenomena which precede the complete disappearance of the germinal vesicle are these: 1. The solution of the nucleoplasmic mass and of the pseudo-nucleoli in the nuclear juice; 2. The breaking up of the germinal spot into fragments, and the progressive solution of these fragments in the nuclear substance; 3. The perforation of the membrane followed by the partial expulsion of the contents of the nucleus; 4. The complete solution of the membrane in the juice of the germinal vesicle; 5, lastly, the solution of the nuclear substance in the vitelline protoplasm.

The modifications which I have shown to exist in the nucleolus, the reduction of the vacuoles into a single vesicle, the changes in the form of that element, and its breaking into fragments cannot be explained unless we admit the contractility of the nucleolar substance. This view is moreover in conformity with the conclusion which one has been able to draw from the amœboid movements which have been seen to be executed by the nucleoli of other cells.

The facts which I have just related have not been observed by M. Hertwig in his *Toxopneustes lividus*. M. Hertwig thinks, on the contrary, without however, being able to affirm it from direct observations, that in that

Echinoderm the germinal spot passes out of the germinal vesicle, and becomes free in the vitellus, and there forms the nucleus of the yolk. But if I may judge from the figures which he gives of the germinal vesicle in process of retrogressive metamorphosis, I am convinced that the spot of Wagner in that Echinoderm undergoes the same fragmentation which I have mentioned in the starfish. I believe that the germinal bodies which M. Hertwig figures in the germinal vesicle (figs. 3, 4, 5, and 6) are nothing but the fragments of the nucleolar substance. It may be remarked that in his work M. Hertwig gives no information on the subject of these granules: he does not describe the phenomena which relate to the progressive metamorphosis of the vesicle; he confines himself to saying: "At the time of maturity of the egg, the germinal vesicle undergoes retrograde metamorphosis, and is driven by contraction of the protoplasm to the surface of the yolk. Its membrane is dissolved, its contents liquefy, and are finally absorbed by the yolk; the germinal spot, however, appears to remain unchanged, to penetrate into the yolk-mass, and to become the permanent nucleus of the ripe fertilizable egg."¹

Of all the observations published up to the present time relating to the history of the germinal vesicle, the only ones which present any analogy with those which I have made on the starfish are those of Kleinenberg, on the fresh-water Hydra.² Kleinenberg, in fact, recognised that in that animal the germinal spot of the mature ovum undergoes a retrogressive metamorphosis; it presents an irregular and angular contour; then it breaks up into little fragments, and these at last dissolve. So far as concerns the spot of Wagner, the description of Kleinenberg is as applicable to the Starfish as to the Hydra. As to the description which he gives of the mode of disappearance of the germinal vesicle, it differs very notably from what I have seen in the Starfish. But at the end of his description Kleinenberg says: "Once I thought I saw an actual hole in the membrane of the germinal vesicle. If this is a normal phenomenon, it would be possible for the contents of the vesicle to escape and mingle with the surrounding plasma." I believe that the formation of the hole which Kleinenberg believed he saw is a normal phenomenon, and that it is by the formation of this hole that the contents of the vesicle are partially eliminated, both in the Hydra and in the Starfish; and it is as a result of the rupture of the membrane that the

¹ O. Hertwig, *loc cit.*, pages 11 and 12.

² Kleinenberg, *Hydra*, page 24.

contents of the germinal vesicle became dissolved in the vitelline protoplasm. This identity of the phenomena which lead to the disappearance of the germinal vesicle in the starfish on the one hand, and the hydra on the other, is so significant that it is needless to insist upon its importance.

The fact of the dissolution of the nucleolus in the nuclear substance is not an isolated fact. I have long ago observed the alternate disappearance and reappearance of nucleoli in the nucleus of the Gregarinida. Strasburger states that the fusion of the nucleoli in the body of the nucleus constantly precedes the division of the nucleus; and I have noticed the same fact in my researches on the division of the cells in the embryonic layers of the rabbit. I believe that what is true of the nucleoli is true also of the nuclear membrane: the substance which constitutes that membrane may be dissolved in the nuclear substance.

When, eighteen months ago, I made the observations of which I have just given an account, I had the intention also of studying the origin of the polar globules and the mode of formation of the first embryonic nucleus. But I was interrupted in my study of the earliest phenomena of development by the opportunity which presented itself one day when I had not any Asterida at my disposal, for investigating the origin of the sexual organs in Zoophytes, in some organisms which came into my hands accidentally. From the time when I entered upon the study of this question in the *Hydractinia*, I thought I saw the possibility of arriving at a positive solution of it. I abandoned for the moment the study of the development of the Starfish; reckoning on being able to resume it when I wished; but since that time I have not again had the opportunity of completing my researches. I have seen the directive bodies (*Richtungsbläschen*) formed under my eyes, and I am in a position to affirm that a fresh nucleus appears in the vitellus before the first segmentation; but I am not in a position to say either how the directive bodies appear, nor how the first nucleus is formed in the embryo.

If I compare the results of my study of the germinal vesicle of the starfish with my observations on the rabbit, I find a complete analogy as to the essential facts; but also differences the importance of which I do not wish to diminish.

In the rabbit as in the starfish the germinal vesicle disappears in so far as it is a morphological element; no part formed by the germinal vesicle exists any longer in the ovum at the moment when the first embryonic nucleus is seen to appear; no genetic bond then exist between the germinal

vesicle or one of its parts and the first nucleus of the embryo.

But whilst in the Starfish the collection of the nucleoplasma, the germinal spot and the membrane of the germinal vesicle are dissolved in the nuclear fluid and secondarily in the protoplasm of the yolk—in the mammalia these elements are thrown off into the perivitelline liquid to form directive bodies, and only the contents of the germinal vesicle remain in the yolk. Since in the starfish directive bodies are eliminated by the yolk, it is probable that in the Echinodermata, as in Mammalia, these bodies are formed by the nucleoplastic substance on the one hand, and by the nucleolar matter, joined to the substance of the membrane, on the other hand. It must be admitted that this is a pure hypothesis. But, however this may be, it follows from my observations that in the starfish as well as in the rabbit, there is no filiation whatever between the germinal spot and the first embryonic nucleus.

II. There is a second point in the observations and views of M. Hertwig, which appears to me irreconcilable with the results of my researches on the rabbit.

Is the clear spot which appears in the cortical layer of the yolk protoplasm without granulations, and is the corpuscle which is found there, and which Hertwig regards as the head of a spermatozoon a cell-nucleus? Or is the clear spot a nuclear body and the corpuscle contained in it a nucleolar element having no morphological connection with a spermatozoon?

The *spermakern* of Hertwig is the head of a spermatozoon enclosed in a clear spot. This is composed of protoplasm without granulations. My peripheral pronucleus, which is certainly homologous with Hertwig's clear spot and with the peripheral nuclei of Auerbach, Bütschli, and Strasburger is, according to the terms employed in my preliminary communication, "a small, round homogeneous body, without granulations; it has, in fact, the appearance of a vacuole. But when treated by osmic acid, the clear substance of the so-called vacuole becomes darker and of a grey colour, while the substance of the yolk is coloured brown." It is only later when the pronucleus is already buried in the yolk, that several very highly refractive corpuscles, which would be taken for so many nucleoli, if seen in an ordinary nucleus, appear in its interior. If then our observations agree so far, that we have both seen the body which appears in the yolk, near the surface of the egg with (according to Hertwig) one, or (according to me) several refractive corpuscles, we differ (a) as regards the moment of appearance of the nucleoli-form

elements, (*b*) as regards the number of the elements. Moreover the interpretation of the observed facts is different. Hertwig regards the refractive corpuscle as a nucleus (*Spermakern*), and this nucleus is, according to him, only the head of a spermatozoon; the clear space in which it is seen is occupied by protoplasm belonging to the yolk. In my opinion the clear spot is the nucleus; the transparent corpuscles which afterwards appear are nucleolar elements, the mode of formation of which I have never been able to observe, but which, in my opinion, based upon the time of their appearance, their number, and even their characters cannot be heads of spermatozoa.

I will make in the first place two observations which are entirely in favour of M. Hertwig. (1.) M. Hertwig has seen the successive phases in the formation of the first cleavage-nucleus produced under his eyes, observing the same egg continue its development on the stage, while my conclusions rest on the comparison of a large number of eggs at different stages of development.

(2.) M. Hertwig's view, with respect to the significance of his spermatic nucleus, rests on a positive fact; he has seen a line start from this body and become continued beyond the yolk into a spermatic filament. For my own part I have not been able to account for the formation of my nucleolar elements, and my opinion as to their significance rests upon negative facts.

But all those who have made observations of the kind of which I am now speaking, will acknowledge how easy it is to deceive oneself about the meaning of a delicate line observed in the yolk of a large egg. It is easy to deceive oneself with respect to the continuity of such a line with a spermatic filament contained in a narrow space between the yolk and the ovular membrane. Hertwig, on the other hand, says himself that he has never seen a spermatozoon bury its head in the yolk, and this head become his spermatic nucleus. Hertwig's direct proof is then still wanting.

As to the opinion which Hertwig has expressed with respect to the nuclear nature of his spermatozoon-head, it appears to me that little can be said for it. I see no reason for calling the little body which he regards as the head of a spermatozoon, a cell-nucleus. The clear spot in which is contained the homogeneous corpuscle seems to me to present rather the character of a nuclear element. Still the name of *cell-nucleus* cannot be given either to the clear spot or to the corpuscle contained in it; for the element looking like a nucleus which is formed near the surface of the yolk does

not become the nucleus of the first cleavage-sphere till it has become united to another element having also the appearance of a cell-nucleus. It is for this reason that I have spoken of the peripheral body as the peripheral pronucleus, and of the element which is formed in the centre of the yolk as the central pronucleus.

If we grant what appears to me hardly to admit of doubt that the small highly refractive bodies of my peripheral pronucleus are homologous with the *spermakern* of Hertwig, and that in the same way as there are nuclei with a single nucleolus and nuclei with many nucleoli, so also there are pronuclei with a single corpuscle, and others with many corpuscles, then it seems to me that Hertwig's view of his spermatoc nucleus being the head of a spermatozoon has little probability. In Mammalia the nucleolar elements of the peripheral pronucleus have certainly not this significance.

(1.) The pronucleus at the moment of its appearance is in the rabbit without any granulations. Now this would be inconceivable on Hertwig's hypothesis; the formation of the clear spot is, according to him the consequence of fecundation; now, fecundation begins with the presence of the spermatozoon. The formation of the spot must then always be consecutive to the penetration of the spermatozoon. The spot must accordingly be formed around the head of the spermatozoon, for the head can never penetrate into a pre-formed spot. We ought then never to see a peripheral pronucleus without at least one nucleolar corpuscle.

(2.) The pronucleus in Mammalia contains several granulations. These granulations have neither the appearance nor the dimensions of the heads of spermatozoa; they are spherical or oval globules, the dimensions of which show much variation; the smallest are almost points, and even the largest have not half the size of the heads of spermatozoa.

(3.) Auerbach, Bütschli, and Strasburger, observed before Hertwig and before myself, in the Nematodes, the Mollusca, and the Ascidians, sometimes one, sometimes several clear elements appear near the surface of the egg. All three describe these bodies as globules which are at first homogeneous, and are, at the moment they appear, quite without granulations. According to the observations of Auerbach, which on this point quite agree with all that I have observed in Mammalia, corpuscles, three to six in number, which Auerbach calls nucleoli, afterwards appear in the homogeneous liquid of these globules. There is not the slightest analogy between these corpuscles and the Nematode spermatozoa.

(4.) Refractive corpuscles, perfectly identical with those which arise in the peripheral pronucleus were seen also in the central pronucleus.

(5.) Corpuscles which perfectly resemble those observed in the peripheral pronucleus are found also in the completely developed nuclei of the cleavage-spheres, and these corpuscles are certainly nucleoli.

The opinion expressed by Hertwig that the transparent body which is formed near the surface of the egg is not a nuclear element, but protoplasm without granulations, appears to me untenable, so far as regards Mammalia, for the following reasons:

(1.) The transparent substance of the peripheral pronucleus does not behave with re-agents like the protoplasm of the yolk, but like the substance which forms the cleavage-spheres. If the pronucleus be treated with a one-per-cent. solution of osmic acid, it is not coloured brown like the protoplasm of the yolk; on the contrary, it appears on the brown ground of the yolk as a clear well defined spot. The pronucleus is faintly coloured pink by picrocarminate applied after osmic acid. By hæmatoxylin it is coloured violet-blue.

(2.) When the peripheral pronucleus has become attached to the central pronucleus, it presents after a time the same appearance as the latter, enlarges, and its outlines become more and more distinct. The nucleolar elements remain for a certain time; finally they disappear and the enlarged peripheral nucleus, not one of the refractive corpuscles, becomes the first embryonic nucleus.

(3.) My view is in harmony with the opinion expressed by Auerbach, Bütschli, and Strasburger, who have all through regarded the transparent bodies formed at the periphery of the egg as nuclear elements.

From all the foregoing considerations I conclude that Hertwig's view respecting the mode of formation, constitution, and morphological significance of the transparent body which appears near the surface of the egg in *Toxopneustes lividus* is very improbable. I regard this body, which is homologous with the peripheral pronucleus of the rabbit, as being a nuclear element, and I regard the corpuscle contained in it which Hertwig calls *spermatic nucleus* not as the head of a spermatozoon, but on the contrary, as a nucleolar element homologous with those which exist in large numbers in the peripheral pronucleus of Mammalia, Nematodes and Ascidiarians.

I have found the number of these nucleolar elements to be very variable in Mammalia. In the rabbit the number

varies from one egg to another. In all the eggs of Cheirop-
tera containing two pronuclei which I have examined, each
of these elements contained a single nucleolar element.¹
These variations in the number of the nucleoli have also been
pointed out by Auerbach in Nematodes, and by Strasburger
in Ascidians.

*A NEW PROCESS for EXAMINING the STRUCTURE of the
BRAIN. With a review of some points in the HISTOLOGY
of the CEREBELLUM.* By H. R. OCTAVIUS SANKEY.
(With Plate XIV.)

THE methods usually adopted in the microscopical exami-
nation of the brain have all proved in my hands more or less
unsatisfactory. I find that when thin sections of hardened
brain are cut and stained, the dye does not sufficiently dif-
ferentiate the various structures so as to render their form
and arrangement obvious, while in teased preparations the
shape of the cells, the connection of their processes, and the
fibres of the brain are just as likely to be torn to pieces as to
be separated from the substance which surrounds them.

The plan which I am about to describe will, I think, be
found to overcome, to a certain degree, several of these de-
fects. The dye which I employ causes the nuclei to appear
black; the cells and their processes are rendered dark purple,
while the rest of the section is of a faint purplish-blue colour,
so that the processes and fibres are rendered by these means
extremely distinct, and may often be readily traced to dis-
tances of a quarter or half an inch, and in some cases even
to greater length.

For the sake of clearness of description I will divide my
process into several stages:—

I. The first stage consists in making slices of brain, which
should be made from the organ as it is obtained from the
post-mortem room, neither hardened nor altered in any way
by reagents. The sections should be cut as thin as practi-
cable, but slices of one eighth of an inch in thickness will
not be found too thick for the subsequent treatment. I find
the following a convenient mode of making such sections.
A large brush is to be fixed to the back of the left ring-finger
by means of two elastic bands; the operator then holding a
piece of brain in the left hand, slices it with a large knife
kept constantly wetted with spirit by means of the brush.

¹ Edouard Van Beneden, 'De la Maturation de l'Œuf,' &c., p. 18.