VIII. *Researches on the Impregnation of the Ovum in the Amphibia; and on the Early Stages of Development of the Embryo.* (*Third Series.*) *By the late* George Newport, *F.R.S., F.L.S. &c. Selected and arranged from the Author's MSS.**, *by* George Viner Ellis, *Professor of Anatomy in University College, London. Communicated by Sir* John Forbes, *M.D., F.R.S.*

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In a note dated April 18th, 1853, which was added to my last paper on the "Impregnation of the Ovum †," whilst it was printing, I recorded the fact that, "through the adoption of a different mode of examination" from that which I had previously employed, I had seen the spermatozoon pass through the gelatinous covering and the vitelline membrane of the egg into the vitelline chamber and the yelk. This fact of the penetration of the spermatozoon into the yelk is of such importance as to make it necessary for me to state, with precision, all the circumstances connected with it, and to detail the exact course I have pursued. These particulars were not given when I announced the fact, because they would have exceeded the limits of a supplementary note; but as the omission of them has exposed my statement to considerable doubt on the part of one of the most distinguished and truthful of physiologists (Professor Bischoff ‡), it is the more necessary that I now detail precisely my mode of proceeding §. I think it will then appear, that the difficulties which have hitherto prevented a solution of the question of the penetration of the egg of the Frog by the spermatozoon are in chief part, if not entirely overcome, and that this egg, far from being the least fitted, is perhaps one of those best adapted for arriving

[* A great part of this paper was written by Mr. Newport, with the intention of presenting it to this Society; and the rest has been compiled from his *Note Books*. None of the observations were made later than the Spring of 1853.—G.V.E.]

† Philosophical Transactions for 1853, p. 271.

‡ In a paper purporting to refute the opinions of Dr. Keber and Dr. Nelson concerning the penetration of the spermatozoon into the ovum, "Widerlegung, &c. &c., von Dr. Th. L. W. Bischoff, mit einem Zusatz von Dr. Rud. Leuckart."

[§ Since the above sentence was written Professor Bischoff has investigated, this spring (1854), the impregnation of the egg of the Frog, and has confirmed the statement of Mr. Newport respecting the penetration of the spermatozoon. See his pamphlet headed "Bestätigung des von Dr. Newport bei den Batrachien und Dr. Barry bei den Kaninchen behaupteten Eindringens der Spermatozoiden in das Ei." In this publication he asserts that Mr. Newport is the discoverer of the phenomenon of the *penetration of the spermatozoon into the egg by its own movement*.—G.V.E.]

at the settlement of the dispute about penetration, which has so long engaged the attention of physiologists.

Mode of proceeding.

I have elsewhere mentioned that I employed a glass cell to contain the egg whilst it was examined*, with the view of keeping it in one position, and preventing the movement derived from accidental causes: it is made of a section of a piece of barometer tube, from one-eighth to one-fourth of an inch deep and three lines in diameter in the clear, which is cemented on a plate of glass of convenient size. This piece of apparatus, which I name a *tube-cell*, is of a size sufficient to contain only a single egg after its covering is fully expanded. For the purpose of making an observation, the egg is to be placed in the centre of the cell, immediately after removal from the body of the frog, and before it has come into contact with any fluid; by this proceeding the gelatinous envelopes adhere so firmly to the glass as to render the egg almost or quite immoveable, when the jelly expands on the subsequent addition of water. In order that the proper focal distance of high magnifying powers may be obtained, I commonly use a cell which allows the object-glass to be immersed in the fluid.

As this cell admits light on every side, it is well adapted for viewing the penetration of the spermatozoon into the egg envelopes when the microscope is placed vertically, and a strong transmitted light is employed. It is convenient also for viewing the egg laterally with a simple plano-convex lens of low power, with the view of observing the formation of the chamber above the yelk, or watching the cleavage of the yelk; but the experience of some years has proved that the cell is not suited for the lateral examination of the egg with the compound microscope, owing to the thickness and the convexity of its wall. True, the passing of the spermatozoon into the external envelope, and even *into* the vitelline membrane, can be readily observed when the tube cell is employed in the upright position of the microscope, but I have been unable to follow satisfactorily the course of the spermatozoon completely *through* the yelk membrane in that position of the object, in consequence of the dark colour and opacity of the egg.

To ascertain the fact of the impregnation of the ovum by penetration, it was then necessary to invent some means by which the egg could be examined laterally with the compound microscope. The great difficulty to be overcome was the tendency of the dark surface of the fecundated ovum to maintain a vertical position, with the consequent rotation or rather gravitation of the whole mass of the yelk, whenever there was any change in the position of the cell. I contrived for this purpose a cell or box larger than the one before described, which may be designated a *cistern box*; and with it I could note all the changes that took place whilst the egg was quite undisturbed. This box resembles the animalcule cage of Mr. TULLY†, since it is

* Philosophical Transactions for 1853, p. 267.

† A Practical Treatise on the Microscope, by JOHN QUEKETT, p. 130, 2nd edit.

constructed of brass with glass at the top and bottom, and it is inserted into a brass plate by which it may be fixed to the stage of the microscope: the front glass can be approximated to or removed from the hinder one by means of the sliding of the piece of brass in which it is cemented, though this motion never allows the glasses to touch. The glass employed is thin, especially the front one; and a piece is cut out of the anterior one (about one-third of the whole) to allow of water being added or removed by a siphon without disturbing the box or its contents. Its diameter is eight-tenths to nine-tenths of an inch, and its least depth is about one-sixth of an inch, or about sufficient for the lodgment of a single row of the frog's eggs, before they are expanded. During the use of this box the microscope is placed horizontally; and a camera is attached to the eyepiece to allow of the immediate delineation of the changes seen.

The magnifying power used has been commonly the half-inch object-glass (Ross) with No. 2 eyepiece, but when any doubt has arisen the quarter-inch object-glass has been taken; and the illumination has been derived from the apparatus called Gillet's condenser.

If the following points are attended to, the entrance of the spermatic body may be readily seen. The eggs are to be passed uninjured from the frog*, and are to be attached immediately to the inner surface of the glass plate in the moveable front of the cistern box; the front of the box is to be replaced and the objects brought into focus, and then the box is to be filled with spermatozoic water. As soon as the fluid touches the eggs, these imbibe it and expand, but they remain firmly attached to the glass. In order that the success of the experiment may be ensured, equal parts of the sperm and water may be used, and within a few minutes after the former has been obtained; this will cause a large number of spermatozoa to enter the egg; but as the mixture is too opake for the satisfactory observation of the phenomena, I remove it at the end of two or three minutes by means of a siphon, and supply its place with pure water. By this time many of the sperm bodies have begun to enter the ovum, and their transit to the vitelline chamber is facilitated by the endosmose of the water.

When these circumstances have been attended to, spermatozoa may be sometimes seen at the *zona pellucida* of the envelope within the first minute, though only those that encounter the egg at right angles; but in from four to five minutes many may be visible, according to the number contained in the water. After the lapse of some time, varying with the temperature, the formation of the *chamber* may be noticed over the centre of the dark surface of the yelk: it is usually at this stage, and for a short time afterwards, that the spermatozoa are first detected in, or passing into, the vitelline chamber.

Penetration of the Spermatozoon into the Yelk.

The fact of the penetration was first observed on the 25th of March, 1853, not

* Philosophical Transactions for 1851, p. 188.

only into the chamber, but also into the substance of the yelk; and as soon as I was satisfied of it, the following precise observations were made.

First set of observations.—An observation was begun at 11^h 28^m A.M. with the temperature 60° FAHR. of the room, though possibly it was three or four degrees higher to the eggs, from the radiation of the lamp.

Within five minutes an abundance of spermatozoa could be observed sticking in the vitelline membrane.

At twelve minutes the number was greatly increased, and extended around all the circumference of the membrane within focus: some were still in motion, and passing slowly on through the gelatinous envelope with their characteristic serpentine movement.

At seventeen minutes the number of the spermatozoa sticking in the vitelline membrane appeared to be lessened.

At twenty-two minutes the yelk had changed its position, the dark part being uppermost; and I could distinctly see some spermatozoa sunk in the vitelline membrane and shining through it, as in former observations, but I could not yet detect any within the cavity of the yelk: some of the bodies, both those in the yelk membrane and those in the jelly, were perishing, as the curling up of the tails showed.

In thirty minutes the yelk had begun to separate from its envelope (forming the *chamber*), and in the small space thus forming at the middle of its upper surface, I saw two spermatozoa in motion.

At thirty-seven minutes the space was increased and more spermatozoa were in it.

At 1^h 4^m the bodies were still moving, though the greater number were folded up both in the chamber and on the yelk, but some had their tails projecting out of the yelk membrane; the chamber had attained to half of its future dimensions.

At 1^h 22^m all motion had ceased, and there was a heaving of the yelk. From this period till segmentation began, the spermatozoa in the chamber became gradually fainter, till they appeared to change into fine elementary granules, and then disappeared; but some of those that entered the dark surface of the yelk remained for more than twenty minutes after its first cleavage, whilst others that were sticking in the vitelline membrane were perceptible for many hours.

In the glass box were three other eggs, in all of which the same facts could be noted. The segmentation of the yelk began at the end of three hours and thirty minutes at the temperature stated. I may mention, that in each egg two spherical bodies, to be presently described, were present, and that these exist in all perfectly or imperfectly fecundated eggs.

The observations were repeated on the evening of the same day on a set of four eggs; and were repeated on the three following days with precisely similar results.

Second set of observations.—I now proposed to vary the conditions of the eggs by having a temperature of the room of 64° FAHR., there being the corresponding

increase from the lamp, and by using pure sperm that had been obtained an hour before.

At 12^h 50^m a single egg was immersed in undiluted male fluid.

At twenty-two minutes after immersion I saw spermatozoa passing through the vitelline membrane at all points of its surface, whilst some had passed through it. The yelk was beginning to separate from its membrane, and its surface had an undulatory motion. As the chamber enlarged, several spermatozoa became evident within it in motion.

At 1^h 45^m several of the spermatozoa were fast disappearing, as if breaking up into granules, but those outside the membrane did not disappear so rapidly as those within the chamber.

Some other eggs, four in number, were experimented on with some of the same fluid used for the egg referred to above, and in this case the fluid had been removed from the male for two hours and a quarter; the fluid remained on the eggs half an hour, and its place was then supplied by pure water. Temperature 64° Fahr. as before.

At thirty minutes, that is to say, as soon as the water was added, the chamber was in three eggs largely developed, as much as it would have been in an hour in eggs only moderately supplied with spermatozoa, though in the fourth egg the degree was not quite so great. From eight to twelve spermatozoa were detected in each chamber, and some motionless in the fluid; others on the vitelline membrane had their serpentine movements; and others were projecting from the membrane motionless, as if their force had been expended before they could effect an entrance.

This last experiment seems to favour my view, that the changes in the yelk are hastened by excess of the fecundating fluid.

From the facts stated above, and before detailed in my former papers, the conclusion seems to be arrived at, that the fructifying of the egg depends on the force or power residing in the sperm body to make its way through the thick coverings of the yelk; and that, this being the case, an explanation will be afforded of the failure of the fluid to occasion fecundation when those bodies are deficient in number or well-being, or are deprived of the power of moving: and at the same time the penetrating power may afford a clue to the inability of filtering paper, even when it is twice or thrice folded, to stop their progress through it.

The action of the spermatozoon is influenced by the temperature of the air, and by mechanical impediments to its passage into the egg.

With respect to temperature, I have frequently referred to its influence in expediting or retarding the development of the embryo, and the following general statement may be given in support of it. A given number of eggs, at a mean temperature of 61° Fahr., will advance in *four* days as far as a corresponding set, at a mean temperature of only 47° Fahr., will reach in fourteen days. Further, the embryos exposed to the low temperature mentioned above die and decompose, whilst

in a running stream, and in a natural state, they would come to maturity, and this difference appears to be owing to the more perfect aëration of the water in the natural than in the artificial development.

There is a certain condition of the envelopes of the egg, of not very unfrequent occurrence, which affects the impregnation. In this condition the envelope is semi-opake and thickened, and the alteration is induced I suspect by too long retention of the ova in the oviduct during a period of excitement: this pathological state seems interesting, as it may have its representative in the ova of other animals, and may be operative in like manner in them.

On the 15th of March, 1853, I employed in an experiment a pair of frogs that had been in constant union since their capture eight days before, and on passing the eggs in the usual way I noticed (what I had before observed) that the envelope was more opake or clouded, thicker and more irregular than usually. On fecundating the eggs with recent healthy sperm I found the changes were slower in their occurrence, and the number of embryos formed was much smaller than in experiments on eggs with healthy envelopes. Thus out of fifteen eggs in separate cells, not more than a third were fecundated; and in these, at a temperature of 60° FAHR., the chamber could not be perceived with a lens till after the lapse of an hour and ten minutes, instead of less than an hour; and segmentation of the yelk did not begin for three hours and fifty-five minutes at a temperature of 60° to 65° FAHR., instead of at about three hours and twenty minutes.

The results were still more marked in two other sets of eggs, fifty in each, where a smaller quantity of spermatozoa was employed, and more time occupied in making the experiments; for in the one set, only three or four eggs were segmented with but one embryo; and in the other set, a very few underwent segmentation, and but three embryos were afterwards produced.

These and other experiments show that the unusual condition of the egg affected the process of fecundation, and that to this cause the failure in the production of embryos is to be attributed. And as the act of fecundation is accomplished through the motor power or force of the spermatozoon, by which that body is enabled to pass through the coverings of the healthy egg, it appears that, when there is any deficiency in the usual power, arising from an unhealthy condition of the fertilizing body, or an increase in the resistance in the yelk coverings, the spermatozoon is unable to pass through the membranes into the yelk, and the egg remains unfertilized.

Of the spherical bodies which appear on the Yelk after fecundation.

I have already alluded* to the presence of certain spherical bodies, which appear within the clear chamber of the egg of the Frog, and on the surface of the yelk subsequent to fecundation. Like bodies have been noticed, especially in the Gasteropodous Mollusca, among the Invertebrata, and have been even seen in some of the

* Philosophical Transactions, 1851.

Mammalia, but their evolution has not hitherto been traced, nor their signification been understood*.

I may now detail the results of an experiment carefully made for the purpose of ascertaining somewhat of their origin and destination; this one will serve as a type of all.

On the 27th of March 1853, and at a temperature of 61° FAHR., three eggs were put in a cell and impregnated in the usual way.

* A single spherical body, projected from the surface of the yelk and maintained for a period at a distance from it on a pedicle, was first noticed in *Lymnæus stagnalis* by CARUS in 1824 (Von der äusseren Lebensbedingungen der weiss- und kaltblütigen Thiere, p. 33. t. i. fig. 4), before the investigations of BAER and PURKINJE had directed attention to the structure of the ovum. But this pediculated body was not further regarded by CARUS than as the imagined axis of rotation of the yelk. A similar body, but followed also by a second, was also seen in *Lymnæus* by DUMORTIER in 1837 (Mém. de l'Acad. de Bruxelles, tom. x. p. 136; also Annales des Sciences Naturelles, 2me série, tom. viii. 1837, p. 136), and this was immediately supposed by him to be the analogue of the Purkinjean vesicle, and with its fellow, was believed to give origin to the head and foot of the animal. A single body was also noticed in *Lymnæus* by POUCHET (Sur le développement de l'Embryon des Lymnées, Comptes Rendus, July 1838, pp. 86, 87), and by him likewise was thought to be the Purkinjean vesicle set free from the yelk. But SARS observed two vesicles in *Tritonia, Eolis* and *Doris* (WIEGMANN's Archiv, 1837), and VAN BENEDEN found two in *Limax agrestis* (MÜLLER's Archiv, 1841, p. 180) and *Aplysia depilans* (Annales des Sc. Nat. tom. xv. p. 126. pl. 1. figs. 4, 7, 9, 1841), and remarked that in both species these bodies come from the yelk before segmentation is commenced, and that their presence indicates that the body of the *Limax* will be formed on that side of the yelk at which they appear. Nevertheless VAN BENEDEN, observing that they are suspended for a time in the transparent fluid which surrounds the yelk from which they proceed, believed that they become dissolved in this fluid and do not take any direct part in the formation of the embryo; and although he appears to have regarded them as the representatives of the Purkinjean vesicle, he seems to have doubted whether this body plays the important part which has been attributed to it in the higher animals. At the same time he called attention to the fact of the constancy of these vesicles in *Lymnæus, Limax* and *Aplysia* as meriting consideration. Yet KARSCH, who afterwards saw the vesicles in *Lymnæus* (WIEGMANN's Arch. xii. 1846, p. 255), thought their presence an abnormal condition. The late Dr. J. REID, however, saw them in *Doris bilamellata* (Annals and Mag. of Nat. Hist. June 1846), and F. MÜLLER in the eggs of *Limapontia* (WIEGMANN's Arch. 1848, p. 1); and although VOGT failed to notice them in *Actæon*, he appears to have seen them in *Limax*, and his remarks (Ann. des Sc. Nat. 3me série, tom. vi. 1846) as to their disappearing in the albumen, coincide with those of VAN BENEDEN.

In the *Nudibranchiata*, NORDMANN appears to have observed at least one of the vesicles in *Tergipes Edwardsii*, although he refers to it as a little vesicle "of air" separated from the yelk (Ann. des Sc. Nat. 3me série, tom. v. 1846, p. 143).

In the *Acephalous Lamellibranchiata* one vesicle at least has been noticed in several genera. It has been remarked by QUATREFAGES in *Teredo navalis* (Ann. des Sc. Nat. 1848), by LOVÉN in *Modiolaria marmorata, Cardium pygmæum, Patella pellucida,* and *Solen pellucida* (in Kongl. Vetenskaps-Akademiens Förhandl. Decemb. 1848, and MÜLLER's Archiv, 1848, p. 531), and more recently by KEBER in *Unio* and *Anodonta* (De Spermatozoorum introitu in Ovula, Königsberg, 4to, 1853). In *Modiolaria* and *Unio* the vesicle is removed from the yelk on a pedicle, as in Gasteropoda, and this KEBER has miscalled "a *Micropyle*;" while in *Cardium, Patella, Solen* and *Teredo* the vesicles rest on the surface of the yelk. LOVÉN, who mentions that the germinal vesicle itself disappears in these mollusks before the eggs quit the ovary, has well remarked that the body seen on the surface of the yelk in the fecundated egg before segmentation, cannot therefore be the Purkinjean vesicle itself as supposed by DUMORTIER, and he ingeniously inquires whether it may not be its nucleus.

In the *Annelida*, as in *Mollusca*, one, and in some instances two, vesicles have been seen. KÖLLIKER observed

At thirty-seven minutes the chamber had begun to form, and several moving spermatozoa were in it. The spherical bodies made their appearance on the surface of the yelk; and when first noticed they were closely applied to it, and were surrounded by a small irregular heap of granules. Some of the spermatozoa remained in motion $1^h\ 22^m$, and I have subsequently seen them alive in the chamber for a longer period.

At $1^h\ 30^m$ the two bodies differed somewhat in character, and one was before the other: the anterior or *granulous* one appeared to have a distinct membrane, and to

one in the eggs of *Exogena Œrstedii*, which he at first looked upon as the embryo cell (in Einige Worte zur Entwickelungsgeschichte von *Eunice*. Nachworte von A. Kölliker in Zurich, Nauenberg, 1846, note). Quatrefages also has noticed one in *Hermella* (Ann. des Sciences Nat. 3 série, tom. x. 1848, p. 177. pl. 3. fig. xv. xviii.), and more recently Van Beneden has seen and figured one in the eggs of a Cestoid worm, *Echineibothrium variabile* (Mém. de l'Acad. de Bruxelles, tom. xxv. p. 68, pl. 3. fig. 15. 1850). Thus the existence in many Invertebrata of at least one detached spherical body on the surface of the fecundated yelk, before segmentation, seems to point to the conclusion that these bodies have some important signification with reference to the future embryo. Quatrefages, who has recently directed attention to the fact of their occurrence, properly remarks, that their presence at this stage of development of the egg is much more general than it has hitherto been supposed.

These bodies have been seen equally in the Vertebrata as in the Invertebrata. In the Vertebrata they appear to have been first noticed, but entirely misunderstood, by Dr. Martin Barry in the Rabbit. Although repeatedly seen and figured by him (Philosophical Transactions, 1840, p. 538, Plates XXIV. XXV. and XXVI., figs. 185, 186, 187 and 193, 200, 206, 209, 212), imagining that the germinal vesicle does not disappear before, but only changes its form, becomes enlarged, and takes the place of the yelk, subsequent to fecundation, he conceived that these spherical bodies were the remains of the yelk, or, as he terms it, "*substance*" (p. 538), which he supposed had undergone dissolution and disappeared. Professor Bischoff however, having noticed these bodies in the egg of the Rabbit (Müller's Archiv, 1841, p. 14. tab. 1. fig. 6), directed particular attention to them (Entwickelungsgeschichte des Kaninchen-Eies, 4to. 1842, tab. 2. fig. 17 *b*, figs. 19, 20), and believed them to play an important part in the segmentation of the yelk. Subsequently to this, these spherical bodies were also noticed by Bischoff in the egg of the Dog (Entwickelungsgeschichte des Hunde-Eies, 4to. 1845, tab. 1. figs. 11, 12, 13, 14); and afterwards Pouchet, who had noticed a single vesicle, as before stated, in the Mollusca, gave also *one only* in the egg of the Rabbit, which he has equally regarded as a descendant of the germinal vesicle (Théorie positive de l'Ovulation spontanée et de la Fécondation des Mammifères, Paris, 8vo. 1847, pl. 15. fig. 9). More recently Professor Bischoff has noticed, as in the Rabbit and Dog, two of these spherical bodies in the egg of the Guinea Pig (Entwickelungsgeschichte des Meerschweinchens, 4to. 1852, tab. 1. figs. 5, 6), and I have also found two in the egg of the Frog (Philosophical Transactions, 1853, p. 247). It is thus evident that the appearance of certain spherical bodies on the surface of the yelk, between it and the zona pellucida subsequent to fecundation, and after the yelk has become contracted and ceases to fill the entire zone, and before any subdivision of the yelk is commenced, is a general and perhaps universal occurrence in the fecundated egg both of vertebrated and invertebrated animals, but of the signification of these bodies very different opinions are entertained.

Dr. Bischoff, in his most recent work on the Guinea Pig, states that he formerly believed these bodies to play an important part in the segmentation of the yelk, and that the view he then held has been adopted by Lovén; while F. Müller conceives that these vesicles determine the direction of the segmentation of the yelk. Rathke, on the other hand, regards these vesicles but as accidental occurrences, and of no organic or developmental importance (Erichson's Archives, 1848, p. 187), a view in which he is supported by Desor, in some observations on *Nemertes* (Müller's Archiv, 1848, p. 511), and to which Professor Bischoff himself states he is now somewhat inclined, although he properly remarks that the regularity of the appearance of these vesicles at a definite stage of development of the egg is not favourable to such an opinion (*loc. cit.* p. 19).

be granulated throughout; the other, or *nucleated* body, was clear, and had a distinct granular nucleus on one side. The changes proceeded more rapidly in one of the eggs, but the *bodies* were the same in all.

At 1^h 45^m the two bodies were passing in a straight line from the centre to the circumference of the chamber. The spermatic particles were fading away and becoming granulous.

At two hours the nucleated one began to change; its nucleus dividing into two granular masses, each of these having a bright spot in its centre. The same change took place in the corresponding body in each egg. Towards the end of the third hour the two masses subdivided, so that four granular masses were present at the centre of the nucleated cell; and in each a bright central spot became very distinct.

The anterior or granular spherical body was still present, and had passed outwards, followed by the nucleated one, to about midway between the central canal and the margin of the chamber above the yelk: the same transference took place in all.

Shortly after the completion of the third hour, it was noticed that the rate of moving towards the margin of the chamber became more rapid, and that the bodies had reached the margin in a direct line with the centre of the yelk surface, one being still at a short distance before the other. When, on viewing the egg laterally, the line of transit corresponds to the line of sight, the change of place is not readily perceived, except the bodies go out of focus; but if their course is at right angles to the line of vision, then the movement is most marked and interesting. When the granular or foremost vesicle has arrived at the margin of the chamber, this space becomes further enlarged by a depression in the centre, marking the commencement of the cleavage of the yelk: granular remnants of the spermatozoa were still recognizable in the chamber.

At 3^h 10^m the four granular masses in the interior of the nucleated body still cohered together, and seemed to be again subdividing. At this time the little heap of granules, which originally escaped with the spherical bodies from the central canal and remained at its margins, gradually enlarges into a number of small transparent vesicles, in which at first no nuclei are visible: in some of the eggs six or eight of these are to be recognized. In consequence of the transparency of these cells, they are not seen unless the eggs are examined in the way recommended; but during the observation, some attain a third of the size of the spherical bodies. These vesicles usually remain in the vicinity of the central canal from which they came.

About 3^h 17^m the first division of the yelk is indicated by two clefts which appear at the margins of the canal, and sinking into the substance of the yelk, gradually extend outwards. As the cleft is produced, I have constantly observed the spherical bodies sink into it, and become for a time more or less lost to view; but they do not pass into the substance of the yelk, for they are readily seen in the triangular furrow between the halves, either by means of a reflector above the egg, or when the cleavage lies in the line of vision by the usual mode. When the

cleavage of the egg is at right angles to the line of view the spherical bodies suddenly disappear, and are seen again only when the crucial or second cleft is about to begin.

I may state, that in the eggs under observation, as well as in others examined both before and since, I found, with but very rare exceptions, that both spherical bodies pass together to the same side of the yelk in a line with the entrance of the canal, and one at a little distance behind the other; and that after the completion of the cleavage of the yelk at right angles to the line of sight, the bodies make their appearance again at the spot where they disappeared, and never at any other part of the yelk.

At the end of the fourth hour the first cleft was finished, and the yelk mass contracting at right angles to the cleft, and extending in its direction, the two bodies were again brought into view almost at the very spot at which they disappeared. The same was observed in each egg. This transit, which may be regarded as an indisputable fact, will set aside the opinion that the bodies enter the yelk, and become the foundation of the nuclei of the different parts into which the yelk splits.

It may here be mentioned, with reference to these bodies, that they occasionally are more or less completely detached for a time from the yelk mass during the formation of the chamber. In some instances too, they *appeared* to be absent, as they were not in the usual place, but I found them afterwards adhering temporarily to the inner surface of the vitelline membrane at the point with which they were in approximation at the time of fecundation; in this position they remained till after the first segmentation of the yelk was completed, and then being detached, apparently by the movement of the yelk within its envelope, occupied their usual place in the first furrow. Eggs, with the exceptional condition above described, have always produced embryos; so that whilst we doubt whether the spherical bodies are necessarily connected with the yelk-changes, we see some analogy with the corresponding bodies observed in several of the mollusca, in which they are removed from the yelk during the time it is undergoing the commotion of its first great divisions. In the ovum of the Rabbit (BARRY) and in that of the Dog (BISCHOFF) they are suspended in the fluid, quite free of the yelk, during the first divisions; they are also on one side of the yelk, usually in the cleft between the primary divisions of the mass; and they continue to be seen at the same place, as in the Amphibia, until about the fourth or fifth subdivision, when they are suddenly lost.

At 4^h 15^m, when the second crucial division of the yelk was nearly completed, I found that the granulated or anterior spherical body floated in the fluid in the first formed furrow, and was as yet unchanged in appearance, saving only that its contents seemed to be divided into two masses; whilst the nucleated or posterior body had already undergone some change, since there appeared in its place a group of four distinct cells: these cells I am disposed to believe, in the absence of proof not yet obtained, were the progeny of the nucleated body that had disappeared.

After this stage the tracing of the vesicles was more difficult. The granulated or anterior body, still unchanged, is seen at the same place when the yelk undergoes its

equatorial or third great subdivision: this cleavage begins on the side of the yelk opposite to that at which the granular body is seen.

After the completion of this equatorial division, the granular-like nucleated body is suddenly lost, and I have been unable to trace it further. But I am not inclined to believe that these bodies are simply dissolved in the fluid surrounding the yelk in the Invertebrata, according to the supposition of VAN BENEDEN, RATHKE, QUATREFAGES and others; supported as this view has been by Professor BISCHOFF*, who is inclined to think that such may be their ending in the Vertebrata. On the contrary, the regularity of their appearance at a given period, their presence at a particular part of the yelk, their special course on the surface, and their disappearance at a given stage of the yelk-changes, furnish a presumption of their greater importance.

VAN BENEDEN† has already pointed out, notwithstanding his above-mentioned opinion, that it is on that side of the yelk of the *Limax* at which these bodies appear, that the body of the animal is afterwards formed. Also DUMORTIER‡ had previously believed that the spherical bodies gave origin to the head and foot of the future animal. Although I am unable to specify the exact part which these bodies take in the formation of the embryo, all my observations on the eggs of the Frog and Toad have proved to me that they are usually, and perhaps invariably, at that part of the yelk at which the head of the embryo is afterwards formed. Their isolation from the yelk for a time, during which it is in a very disturbed state, and their constant localization, with their subsequent disappearance in that part at which the head is afterwards to be developed, seem favourable to the conclusion that their function is as definite as their presence is certain.

It has been supposed by F. MÜLLER§ that the spherical bodies determined in the Mollusca the line of the first cleavage of the yelk; and with this idea he named them the *direction* vesicles. But my observations on the development of the embryo lead me to believe, that, though the transit of these bodies is usually in the same line as the first cleft, the direction of the fissure is not determined by them, but is owing to some other cause.

With the view of tracing the origin of these bodies, the egg of the Frog and Toad was examined from a period before the disappearance of the germinal vesicle to the time at which they appear on the yelk after fecundation; and for this purpose the eggs were hardened in rectified spirit and then dissected||. * * * * *
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* Entwickelungsgeschichte des Meerschweinchens, 1852, p. 19.

† Ann. des Sci. Nat., tom. xv. p. 126, and plate 1, 1841; and MÜLLER's Archiv for 1841, p. 181.

‡ Mém. de l'Acad. de Bruxelles, tom. x. p. 136.

§ WIEGMANN's Archiv for 1848, p. 1.

|| [The results obtainable from this inquiry are not apparently contained in the MS. note books. It is however well known to the writer that Mr. NEWPORT intended, had his life been spared, to employ those means in learning the *destination* of the spherical bodies; and the circumstance is here mentioned because the mode of investigation seems most judicious, and likely to be useful to any one engaged in prosecuting the inquiry.—G.V.E.]

On the production of the Body and Head of the Embryo from definite portions of the Yelk.

Repeated observations have demonstrated that in one part of the yelk the head, and in another the body and tail of the forthcoming being begin. This appointment of the parts is further indicated, very early, by conditions in the yelk cleavage—conditions so constant that I am able to predicate soon after the completion of the horizontal cleft, or at the beginning of the fourth change, where the head, and where the tail of the future embryo will be placed. In support of this statement the changes in the egg undergoing segmentation must be referred to, but they will be traced, here, only to the point indicatory of the diagnosis to be made.

To ascertain the correctness of the following statement it will be necessary to put the impregnated eggs, dark surface uppermost, in *tube*-cells, and to mark on these where the future head and body should be, according to the deduction from changes in the segmenting yelk.

First change.—In eggs kept at a temperature of 60° FAHR., the first change begins at about the end of four hours, and consists in a cleft that runs vertically round the egg. This cleft may be called *axial* from its position, as it will afterwards appear, to the body of the future embryo. It begins in the centre of the dark surface in a slight depression, so that in some instances the canal may be almost detected: then suddenly a sulcus appears on each side of the depression, and quickly extends outwards around the yelk, though it is deepest and most strongly marked above. In the egg of the Frog as in that of the Newt, the halves are not always of equal size in this stage, but this disproportion is obviated in the second division.

Second change.—Another cleft now surrounds the egg crossing the first at right angles, and this may be named *crucial.* An interval of about an hour elapses before its appearance, and its commencement is not seen at the same instant on each side of the axial line, but is perceived first at one side. After this, as after the axial cleft, the pieces into which it divides the yelk are unequal in size; but these variations have in general but little influence on the result obtained by the segmentation.

In some ova there is such an unusual displacement of the segments as almost to prevent the identification of the parts in the subsequent changes, the lines of the axial and crucial clefts being rendered very irregular by unequal increase of the sectional pieces on opposite sides of the first cleft. This irregularity I am inclined to think arises from unequal division of the yelk in the production of the first cleft; and to it I attribute the partial horizontal movement of the yelk itself, which may be sometimes recognized.

But when the yelk has divided with perfect equality both axially and crucially, the pieces on one side of the crucial line (in front say) always become larger than those on the other side (behind); and during this increase in size the anterior divisions project backwards over the posterior so as to cause a bending of the crucial sulcus, the convexity being forwards.

Third change.—In this a third cleft is formed horizontally around the egg, nearly midway between the upper and under surfaces: this may be called from its position, the *equatorial* cleft.

Fourth change.—About two hours after the completion of the crucial cleft, a new series of changes is set up in the egg. The clefts no longer include the whole circumference of the egg, but are confined to the splitting of the larger into smaller pieces after a binary plan: and this process does not begin at once over the whole surface, but appears first in a given spot, and then pursues a definite course; thus each of the two pieces seen from above on one side (behind) of the crucial cleft become subdivided, producing four segments on one side of that line, whilst there are only two on the other. When this subdivision is nearly completed, and not till then, a corresponding change takes place in the two segments on the other side (in front) of the sulcus.

When this stage of the segmentation of the yelk is arrived at, the position of the body and head of the coming being can be determined with certainty, so that it is not necessary to follow further the changes during segmentation. If the cell be marked opposite the first commencing post-crucial subdivisions, and then set aside for the formation of the embryo, the trunk and tail of the developing being will be found to originate in this first-subdividing part behind the crucial sulcus, and the head to be produced in the part on the other side, or in front of the sulcus, in which the secondary segmentation last appears.

On the correspondence of the primary cleft of the Yelk with the axis of the future Embryo.

I have been long aware that the axis of the embryo was in the line of the first cleft of the yelk, but my endeavour to show this was not always satisfactory, in consequence of the difficulty of making the egg keep a given position, whilst it was free to move; but since I have employed the tube-cell I have obtained the desired evidence with great ease. The results of the following observations will support my statement.

Obs. 1.—I took an egg that had just divided for the first time, and placed it in a glass cell only sufficiently large to contain it when the jelly was fully expanded, and filled the cell with water. The dorsal surface turned uppermost, as usual, consequently I had under my eye the whole surface, and could watch the changes with the microscope. I marked the plate of glass supporting the cell with a line parallel to the primary cleft of the yelk, and indicated the position of the ends of the sulcus by other marks. The whole was placed in a temperature of 60° FAHR.

At the time of the closing-in of the dorsal laminæ, I found the correspondence between the axis of the embryo and the line of the first cleft to be exact. As I knew however that some movement would be excited, I made a drawing of the appearances. In twelve more hours the dorsal sulcus was nearly closed, and the embryo had passed to the left of the line marked on the glass, viz. to about an angle of 30°. A second drawing was made to remove doubts.

On the day after, the embryo had moved still further round. Finally, the embryo was perfected, like those of the same set that were left in mass in water.

Obs. 2.—Nine eggs were put into separate cells on March 11th, and when segmentation began, the line of the first cleft was carefully marked on the glass in the manner before explained. One of the eggs was abortive.

March 13. The dorsal sulcus was forming in three of the embryos exactly in the line of the cleft.

March 14. In each of the eight instances the axis of the body is more or less precisely in the line of the sulcus: thus in five it was in the exact line, in one about five degrees to the left, in another about three degrees to the left, and in the remaining one rather more to the left of the given line.

Obs. 3.—Five eggs were placed in separate cells, and marked, as above directed, and then removed to a high temperature.

March 26. The results obtained from these were confirmatory of the facts before stated, as the variation between the line and the axis of the beings was only slight; the axis of two being directed to the right, one to the left, and one perfectly coinciding.

Although these observations have shown that the axis of the body of the future embryo corresponds primarily with the first cleft in the yelk, they point out that, at times, the axis deviates to the left or right of that line. The cause of this deviation I may attribute to a change in the position of the yelk, for on March 29, 1853, I observed that the entire yelk occasionally shifts its position during the progress of segmentation, in consequence probably of unsymmetrical division, in accordance with the explanation before given at page 240.

On the power of the Spermatozoon to influence in artificial impregnation the direction of the first cleft of the Yelk.

In connexion with the influence of the spermatozoon on the egg, I determined to try whether the artificial application of that body to different parts of the egg's surface could affect the position of the first cleft of the yelk.

Obs. 1.—Several eggs were placed, March 29, in separate tube-cells, with each turned on its side so that both the dark and the white surface were exposed. Very recent spermatic fluid was then applied, by means of a pin's head, to the lower part of the dark surface, and the cell was carefully marked close to the spot, to show where the egg was touched.

The eggs rotated in the usual way, so as to bring upwards the dark surface, and at the end of one hour and a half only two eggs were fecundated. In these the spherical vesicles had the usual position and appearance, and were directed outwards across the centre of the flat surface of the yelk to that side of the egg to which the spermatic fluid had been applied. After the formation of the primary cleft, the cells were marked and set aside for the production of the embryo.

April 3. Each egg has formed an embryo, and in each instance with the head to the side of the egg touched.

Obs. 2.—Four eggs were placed in separate cells as before, and only two became fruitful.

In one the primary cleft was in the precise line of the spot touched, although the egg subsequently diverged to the left; and the head corresponded to the part fecundated. In the other egg the cleft was about ten degrees to the left of the part impregnated, and the head was also turned to the part touched with the fluid.

Obs. 3.—Four other eggs were taken, but two of them were sterile; and in the development of one the head deviated remarkably from the usual position.

The first cleft in one (*a*) was about six degrees to the right; and in the other (*b*) about five degrees to the left of the point touched. Both formed embryos: in one (*a*) the head was at the end of the cleft nearest the point touched, but in the other (*b*) at the end furthest from the same point. The peculiarity in this last experiment I cannot explain; possibly there might be some want of precision in conducting it.

Similar experiments were repeated four other times, and the results showed that the first cleft of the yelk is in a line with the point of the egg artificially impregnated, and that the head of the young frog is turned towards the same point. But in this set, as in the other, the nascent being in the course of its development deviated to the right or the left of a line through the centre of the spot fecundated.

In another set of experiments the spermatic fluid was applied to an unknown part of the egg.

On taking my last female frog (April 5, 1853) I found the white part so dark in colour (not a very unusual change), that the usual black surface could not be well distinguished. Accordingly the eggs were put into separate cells, and the fluid was applied and the cell marked in the usual way, without a knowledge of what was the spot touched.

Experiment 1.—Six eggs were used: all were fecundated, and segmented in about three hours and a half in an atmosphere varying from 61° to 64° Fahr. The following is the result:—

The axis of the embryo was in one in a line with the part touched, in four diverging slightly to the left, and in one to the right of that line. The head was in four nearest to the spot fecundated, and in two furthest from it.

Experiment 2 with five eggs: all of these were fruitful. The axis coincided exactly with the impregnated point in three, and diverged slightly to the left in two eggs. The head was nearest the same point in four, and furthest from it in one embryo.

Experiment 3.—Five eggs were employed, and being fertilized, gave the following result:—

The axis coincided with the given point in one, and diverged more to the right or left in the other instances. The head was nearest the side to which the spermatic fluid was applied in four, and furthest from it in one.

From these and other experiments it results, that, when part of the black surface of the egg was certainly touched with the spermatic fluid, the head of the future embryo was turned towards it in ten eggs, and removed from it in only one; and that the axis of the embryo nearly coincided at first with the line of the point touched, though it afterwards deviated to the left or right of that line (most to the left), the distance not exceeding fifteen degrees.

When however the part of the egg to which the spermatic fluid was applied was not known, the axis kept much the same position as before, but the head was far distant from the impregnated spot in four out of sixteen instances.