IX. *On the Impregnation of the Ovum in the Amphibia.* (*First Series.*)

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THE communication which I have now the honour to present to the Royal Society is a portion of a series of investigations on the Development of the Embryo on which I have been for some years engaged, and which was commenced in a paper on the Development of the Myriapoda, that was honoured with a place in the Philosophical Transactions for 1841. I now propose to give the results of my observations on the Amphibia, reserving to a future early occasion the continuation of those on the Invertebrata commenced in the paper alluded to.

The Amphibia, of all the vertebrated animals, afford to us the readiest means of investigating the difficult subject of Impregnation by actual experiment, and it is only, perhaps, by combining experiment with careful observations on the physical conditions that affect the development of the germ, and comparing these with the facts of the natural history and instincts of the species, that we may hope, ultimately, to obtain some further insight into this one of Nature's most hidden secrets.

I shall endeavour, therefore, in this communication, to show the condition of the ovum in the Amphibia through its earliest changes, and also before and immediately after impregnation, and to detail experiments made with a view to learn by what means its fecundation is effected;—and in a future communication I propose to trace the development of the embryo from the time of fecundation to that of its liberation from the ovum, in the two chief divisions of the class,—the tailless and the tailed Amphibia. The subjects thus naturally form two series—Impregnation and Development.

IMPREGNATION OF THE OVUM.

The history of what we can now prove to be the agent of impregnation, the spermatozoon, deserves to be especially noticed. Although great attention has been paid by physiologists during the last thirty years to almost every point of inquiry connected with the production and physical composition of the seminal fluid of animals, and its relation to the fecundation of the ovum, we have remained to the present time without any acknowledged proof either of the part which the different constituents of this fluid take in impregnation, or of the mode in which it effects impregnation. This perhaps is little to be wondered at when we remember how many years elapsed before the great discovery of Ham and Leewenhoek of the existence of moving bodies in the fluid, as part of its normal composition, was admitted. It is now one hundred and eighty-three years since Leewenhoek communicated the important discovery

of these bodies to the Royal Society*, and regarded them as in some way essential to the fecundation of the ova; although his conjecture that they became the future embryos was erroneous. Eighty-five years afterwards, Dr. PARSONS, Foreign Secretary of the Royal Society, believed it to be "extreme nonsense to imagine that the insignificant animals called *spermatic animals* can contribute anything towards propagation," &c.†, and it was not until the publication of the observations of LEDERMÜLLER‡, a few years after that, that the production of spermatozoa, as part of the fluid, began to be admitted. Dr. HILL, in the notes to his English translation of SWAMMERDAM's Biblia Naturæ§, two years later, mentioned them as abundant in the Frog at the season of pairing, but that it was then the fashion to doubt even their existence. Yet NEEDHAM, ten years after this, while acknowledging that these bodies are found in the fluid of all animals, adopting the views of BUFFON and DAUBENTON, stated that they do not exist until after the fluid is removed from the vessels, and decomposition has commenced‖. And later still, even in our own time, their existence has been denied in the most positive manner by Sir EVERARD HOME¶. SPALLANZANI, however, was so well acquainted with them, as found in the Frog and Toad, that he has recorded his great surprise at not observing them in the latter on two occasions**. BONNET†† and GLEICHEN‡‡, also, well knew them to abound in the males of animals of distinct species at the season of impregnation, but discovered that they are usually absent in hybrids, a fact that has since been confirmed by PREVOST and DUMAS§§. These two observers, regarding the spermatic bodies, with LEEWENHOEK, as essential elements of the semen, believed that they actually penetrate bodily into the ovum, and become by metamorphosis part of the future embryo. Still more recently it has been stated by Dr. BARRY‖‖ to this Society that he has actually seen the spermatozoon within the ovum, a statement which my own observations do not enable me to confirm.

Before any satisfactory conclusion could be arrived at respecting the importance of the spermatic bodies in impregnation, it was necessary to ascertain their nature, to trace their mode of development and production, to establish the periods of their occurrence in different classes of animals, and to learn something of their chemical

* Philosophical Transactions, 1667, vol. xii. p. 1040.

† Philosophical Observations on the Analogy between the Propagation of Animals and that of Vegetables. 8vo. 1752 (note), p. 44.

‡ Physikalische Beobachtungen der Samenthierchen. Nuremb. 1756. And also, "Beyträge zu denen Beobachtungen deerer Saamenthiergen und Kleiste Aale gehörig. 12mo. Frankfurt und Leipzig, 1759."

§ Book of Nature, folio, part 2 (note), p. 105, 1758.

‖ Notes des Nouvelles Recherches sur les Découvertes Microscopiques de l'Abbé SPALLANZANI par M. NEEDHAM. Lond. 1769, tom. i. p. 196.

¶ Lectures on Comparative Anatomy, 4to, vol. v. pp. 332 and 337. 1828.

** Dissertations relative to the Natural History of Animals and Vegetables. Lond. 1789, p. 151.

†† "Contemplations de la Nature;" and "Œuvres d'Histoire Naturelle," 4to, tom. iii. p. 454, &c. 1779.

‡‡ Abhandlung über der Samen- und Infusionsthierchen. Nuremb. 1788.

§§ Annales des Sciences Naturelles (Prem. Série), tom. i. p. 182, 1824.

‖‖ Proceedings of the Royal Society, vol. iv. p. 432. Phil. Trans. part 1, 1843, p. 33.

composition, as well also as of the fluid portion of the semen in which they move. Most of these inquiries have been well followed out by WAGNER, SIEBOLD, MULLER, and more especially KÖLLIKER, and more recently by WAGNER and LEUCKARDT, from whose labours we have now some positive information which enables us to deduce a fair conclusion respecting their function, although a direct proof of its correctness is still to be supplied. Most observers now believe with KÖLLIKER that the spermatozoa (still so called) are not independent living organisms, but are merely elementary constituent parts of the male body, an opinion in which my own investigations lead me fully to coincide. This opinion, indeed, is not entirely new, as a like view was held by some observers at the beginning of the last century, when it was still questioned whether the spermatozoa are normal constituents of the semen. Dr. DRAKE*, in his "New System of Anatomy," while acknowledging that he had seen the seminal animalcules, and combating on the one hand the theory of LEEWENHOEK respecting them, and on the other the view that had previously been held with regard to the ovum, doubted their separate organization, and suggested that they "may be nothing more than some large particles of mixed fluid, whose motions and different figure the microscope discovers to our eyes," &c. G. TREVIRANUS† more recently held a similar opinion, that they are not independent animals, but are analogous in their structure and properties to particles in the pollen of plants, and that their motion is of the kind discovered by ROBERT BROWN in vegetables. KÖLLIKER‡, however, first distinctly referred them to a class of known organic constituents of the living body, the vibratile cilia, a view which had previously been discussed and inclined to by MÜLLER§.

But however much our knowledge has become settled in regard to the nature of the spermatic bodies themselves, and their mode of development, their relation to the fluid portion of the semen in which they are contained is still a matter of doubt. H. GOODSIR‖ regards certain albuminous flakes in the fluid portions of the semen of Crustacea as the debris of dissolved cells, and as the source of nourishment and development of the spermatozoa; while a more recent observer, Dr. KIRKES¶, regards the spermatozoa as the elaborators of the fluid, and the conveyers of it to the ovum at the time of impregnation. This latter supposition was originally advocated by WAGNER, VALENTINE and BISCHOFF. But two of these observers have recently changed their views**, and now regard the fluid portion as only of secondary importance in impregnation, and the spermatic bodies as of essential. This view, as WAGNER states††, is founded chiefly on the fact that in some of the invertebrata the whole mass

* New System of Anatomy, by JAMES DRAKE, M.D., F.R.S. vol. i. p. 352, 1707.

† TIEDEMANN, Zeitschrift, vol. v. part 2, 1835.

‡ Beiträge zur Kenntniss der Geschlechtsverhältnisse und der Samen-flussigkeit wirbelloser Thiere. Berlin, 1841.

§ Elements of Physiology (Eng. ed.), part 6, 1841, p. 1478.

‖ Anatomical and Pathological Researches, 1844, p. 40. ¶ Handbook of Physiology, 1848, p. 610.

** BISCHOFF in MÜLLER's Archiv, 1847. WAGNER in Article "Semen," Cyclopædia of Anatomy and Physiology, part xxxvi. January 1849. †† *Loc. cit.* p. 507.

of the semen appears to be constituted almost or entirely of spermatozoa, while scarcely any *liquor seminis* can be detected;—and further, on the great improbability, perhaps impossibility, of the liquor seminis of those animals which expel their ova into water before impregnation being brought into contact with the ovum. But the same author justly remarks, that "even up to the present day this hypothesis of the influence of the liquor seminis has not met with any direct refutation." To this I may add, that however strong the presumption may be in favour of the agency of the spermatozoa in those instances in which a liquor seminis has not been observed, it affords no sufficient reason for disbelieving that the spermatozoa are not resolved into fluid at the moment of fecundation; or that in those animals in which the liquor seminis occurs in abundance it is not that which impregnates the ovum.

The question then, so far as *proof* is concerned, both of the direct agency of the spermatozoa, and of the non-efficiency of the liquor seminis in impregnation, remains open, as well also as that which involves the knowledge as to how impregnation is effected.

It is to these questions that this communication which I have now the honour of laying before the Royal Society is chiefly directed. I propose *first* to show the time and mode of disappearance of the germinal vesicle, and the condition of the ovum in the Frog and Newt, immediately before and after impregnation, and to endeavour to supply proof from actual experiments that the spermatozoa alone, in all cases of communion of the sexes, are the sole agents in impregnating the ovum; and further, that impregnation *cannot* be effected by the *liquor seminis*; and next to examine in what way the agency of the spermatozoa is influenced, impeded, or exerted.

1. CHANGES IN THE OVUM WITHIN THE BODY.

The ovum of the Amphibia has so frequently been the subject of examination by the best observers that a further detailed account of its development may at first appear to be useless, after what we already know of its changes through the labours of Swammerdam, Leewenhoek, Roesel, Spallanzani, Prevost and Dumas, Rusconi, Baer, Reichert, Vogt, Bell and others. But apart from the fact already mentioned, that the ovum of the Amphibia affords us the best means of actual experiment on impregnation, there are questions which relate to its earlier conditions on which the observers named are not agreed, but which are of importance with regard to the physiology of reproduction in the whole of the vertebrata.

I shall state, therefore, what I have myself observed with regard to these questions from the time when the ovarian ovum is approaching to maturity to that of its expulsion from the body, before entering on the subject of its impregnation.

As our means of comparing and testing the accuracy of all observations in natural history, and of experimental results in physiology, depend mainly on the correct identification of the objects examined, I may here state at once that the objects of the following details have been the Frog, *Rana temporaria*, and the Toad, *Bufo vul-*

garis, among the *Anoura*; and the *Triton palustris*, *Lissotriton punctatus* and *L. palmipes*, among the Water Newts, the *Urodela*. Neither SPALLANZANI, PREVOST and DUMAS, nor RUSCONI, to whose observations I shall have frequent occasion to refer, mention the species they have examined. SPALLANZANI has given only the popular names of his animals, but RUSCONI has given a figure of his, which appears to have been *Rana esculenta*, and as the description he has given of the ovum of this species agrees with the description given by PREVOST and DUMAS, it is probable that the species they employed was the same.

The Ovarium Ovum.—When the frog, *Rana temporaria*, is examined in the autumn, after it has ceased to feed, and is preparing to retire to its winter quarters, the ova within it have already attained to more than two-thirds of their ultimate dimensions before leaving the ovaries, and have begun to distend the abdomen. They remain in this state through the greater part of the winter, while the frog is hybernating, as Professor BELL remarks*, in the mud at the bottom of ponds and stagnant waters. But as the spring approaches, and the animal is aroused from its lethargy by increased temperature, the ova then rapidly acquire their full development. A female frog, taken at the end of September, with her body enlarged with ova, was confined in water in a cold room, undisturbed through the winter, excepting only at intervals of examination. The weather being mild, and the temperature of the room during October and November being sometimes at or but little below 50° FAHR., the frog remained active, and came frequently to the surface to respire. In December the temperature sunk to below 40° FAHR., when the frog became lethargic, and scarcely changed its place at the bottom of the water during nearly a fortnight, while the temperature was almost stationary, and ranged only from 35° FAHR. to 37° FAHR. On the 10th of January, when it had again risen to 40° FAHR., and that of the water to 37°·5 FAHR., the animal was still submerged and motionless; but on sudden exposure to the light of a candle it crept languidly, and almost imperceptibly, to a distance of about two inches, and again became quiet. On the 26th of January the temperature of the room had risen gradually and continuously to 52° FAHR., and that of the water to 48° FAHR., but the frog remained submerged and perfectly quiet with its eyes partially closed. Although it was not cognizant of any object by sight, the irritability of its body was now much increased, as it moved instantly when touched ever so lightly, but quickly relapsed into its previous state of rest. On the morning of the 1st of February, the temperature being then 48° FAHR., it was still submerged and motionless, but in the evening, when the warmth was increased to 52° FAHR., I found it with its nostrils only above the surface of the water, and evidently beginning to respire freely, but its eyes were completely closed. When the light of a candle was suddenly cast upon it, the eyes were slowly opened, but its body remained immoveable. On the following day, February 2, it was evident that its hybernation had been brought to a close, as it was then active, with its head out of the water, and its eyes widely open,

* History of British Reptiles, 1832, p. 89.

and perfectly alive to external objects, as when the hand was slowly approached it withdrew beneath the water. The temperature at this time was 53° Fahr. During this period of hybernation the frog became slightly emaciated, and its abdomen, instead of being more enlarged, was somewhat diminished in bulk,—a good proof that during the inactivity of the respiratory and circulatory functions, the secretory also are lessened, and the development of the ova is arrested. But although no food was supplied to the frog at this time, and none probably is taken by the creature in its natural haunts, as at the time it comes forth but few of the objects on which it feeds are abroad, its body soon became enlarged, showing that the ova were then rapidly attaining their full development. Between the 2nd and 22nd of February the temperature of the room was occasionally as low as 42° Fahr., yet the creature remained active beneath the water, without relapsing into its previous state of hybernation. It only continued longer beneath the surface without rising to respire. At this period, having found that some frogs in their natural haunts had already come forth, I removed the subject of these observations also from the water to a damp locality, and on the following day found it greatly changed in appearance. While confined in the water it was of a dull dirty brown colour, but some hours after its removal it cast its tegument, and changed to a bright yellow, with the usual brown markings, and had increased in size, both in its body and limbs.

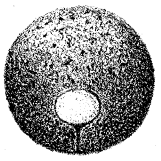
The conclusion to which these circumstances seemed to lead was, that quickly after the frog leaves its hybernaculum, it casts its tegument as the insect escapes from its puparium, and acquires new vigour, while the ova are attaining their full growth. The Toad undergoes a similar change. About a fortnight later in the season than the Frog, I have seen many toads in a shallow ditch of slow moving water in the act of casting their dark brown tegument, and acquiring one of a greenish yellow.

On examining several frogs taken from their natural haunts, I found them in, as nearly as posible, the same state of development with regard to the ova, judging from external appearance, as the specimen I had watched through the winter. A few had just paired, but the majority were still single. On opening the bodies of the latter, I found the ovaries greatly enlarged, and the ova apparently ripe, but still contained in the ovisacs.

It is at this period, therefore, immediately after hybernation, and before the ova have left the ovaries, that the condition of the ovum is a matter of great interest with reference to the structure and contents of the germinal vesicle, the period at which the vesicle is changed or disappears, and the circumstances under which the ovum escapes from the ovary, and is received into the oviduct.

The Germinal Vesicle.—The fate of the germinal vesicle in the matured ovum is still a matter of doubt. Previous to the embryological researches of Dr. Martin Barry, it was usually believed that the vesicle entirely disappears before or at the time of fecundation of the ovum. But this view was combated by the author named, who, quoting the opinions of previous inquirers, contended that the germinal vesicle in

Fig. 6



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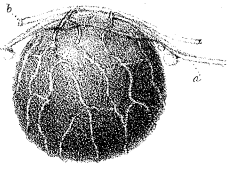
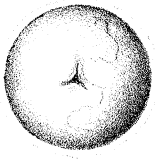


Fig. 7.

Fig 11.



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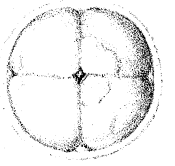
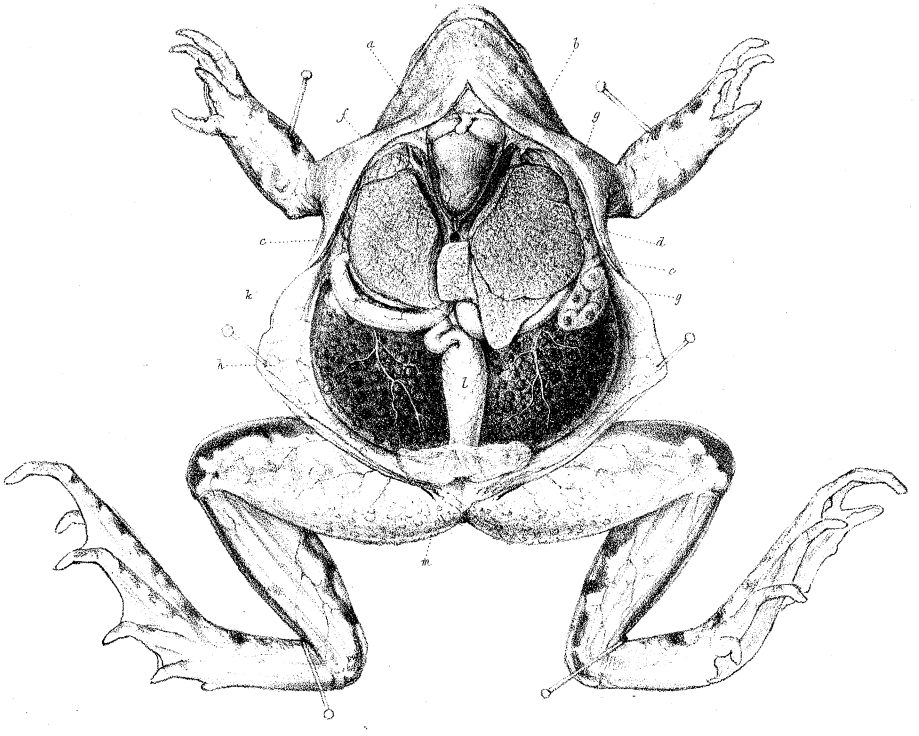


Fig 1.



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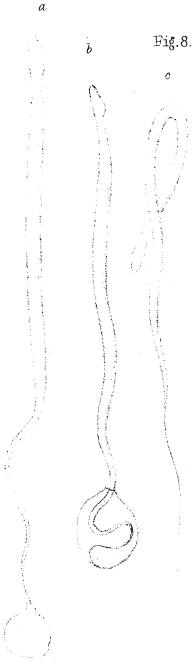


Fig. 10.

Fig. 9.

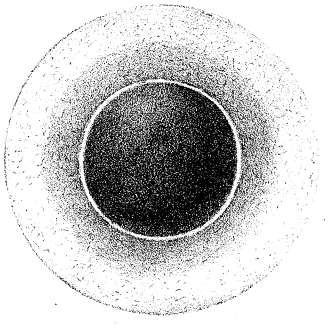


Fig. 3.

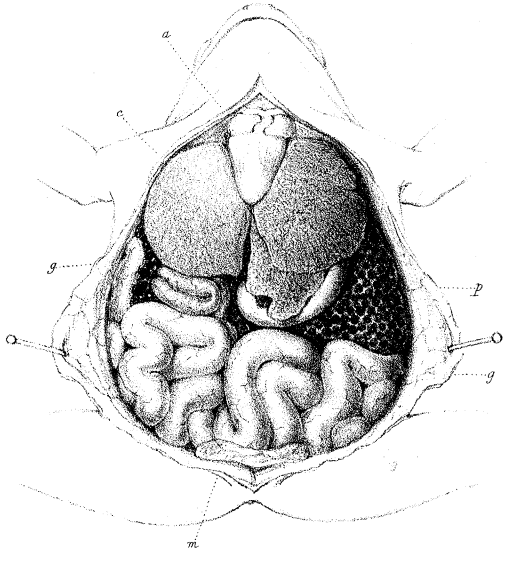
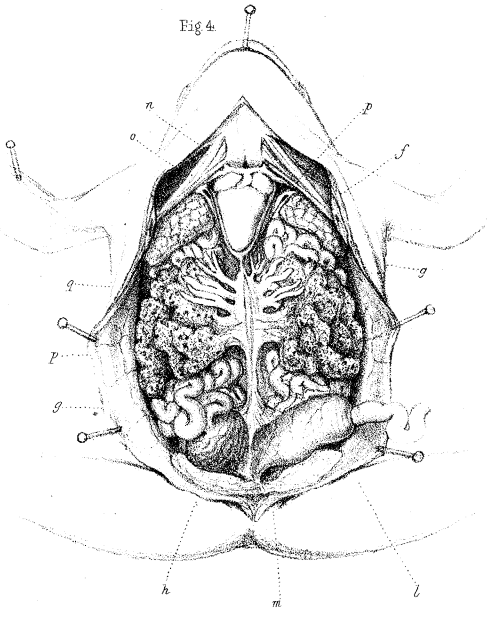


Fig. 4.



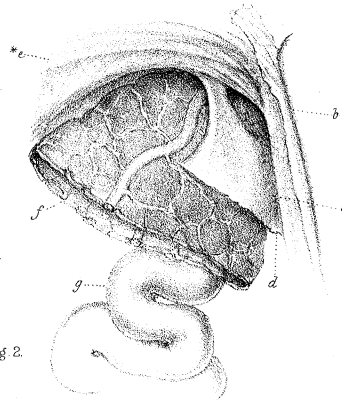


Fig. 2.

Mammalia does not disappear, as believed by PURKINJE*, in Birds and Amphibia, by bursting during the generative act, and pouring its contents into the germinal layer of the fecundated ovum. Neither, as supposed by BAER†, by being urged forwards and burst between the vitellus and its membrane before fecundation. Nor, as stated by RATHKE‡, with reference to the Crustacea, by disappearing while still within the ovary. Or, as supposed by WHARTON JONES§, in Birds and Amphibia, by approaching the surface of the yelk, and by the giving way of the coats of the vesicle, and the effusion of its contents on the surrounding surface of the yelk. Or, further, as believed by BISCHOFF‖, by disappearing at the time of exit of the ovum from the ovary. On the contrary, Dr. BARRY has stated that the germinal vesicle returns to the centre of the ovum, and the germinal spot to the centre of the vesicle, before the ovum leaves the ovary, and that these do not become dissolved, but only are changed in character by a process of cell development within them, which ends in the production of two cells in the centre of the yelk, which are the foundation of the body of the future embryo.

Perhaps what I am about to mention may assist us to reconcile or correct the views of this embryologist, as well as those of the authorities he has quoted. It is well known that if the ovum of the Frog is examined before the yelk has attained to one-half of its ultimate dimensions, the germinal vesicle is distinctly visible as a large circular body near the centre of the yelk, apparently granular in its interior, and more opake than the yelk itself. In the centre of the vesicle its nucleus, the germinal spot, is then equally distinct. If the ovum is examined when it has nearly acquired its full dimensions, the vesicle is still found to exist, but, as compared with the size of the entire ovum, is relatively smaller than at earlier periods, and is recognized with more difficulty, owing chiefly to the yelk cells having both increased in number around it, and also acquired a darker colour. It is equally well known that all appearance of the vesicle is lost in the Amphibia, as in Birds, before the ovum is prepared for fecundation; but as to the way in which it disappears, observers are not agreed, or even as to the time.

Structure of the Germinal Vesicle.—Having collected a number of frogs that had recently left their hybernacula, and had not yet paired, I placed some in spirit for examination. On dissecting them afterwards, I found that in some the ova had nearly reached maturity, but had not left the ovary (Plate XIV. fig. 2 *p*). The yelks being rendered firm by the spirit, I was able, by gentle pressure, to break open some of these beneath the microscope, without diffluence of the contents. The aggregated yelk cells were then seen to consist of two kinds; the one dark-coloured, which form

* Symbolæ ad Ovi Avium Historiam ante Incubationem, 1825; and Article "Ei," Encyclop. Wörterbuch, Band x. p. 112, 1834.

† "Lettre sur la Formation de l'Œuf," in Breschet. Repertorium, 1829.

‡ Untersuchungen ueber die Bildung und Entwickelung des Fluss Krebses, fol. Leipzig, 1829.

§ Philosophical Transactions, 1837, part 2. ‖ R. WAGNER's Lehrbuch, &c., 1839.

the upper portion of the yelk, and which diminish in intensity of colour from brown or black at the circumference to a leaden or grey in the centre; the other, which forms the inferior portion, consisting of cells that become lighter-coloured as they approach the surface. The whole form a mass of nucleated cells of nearly uniform size, closely aggregated together. In the interior of the yelk, amidst the dark-coloured cells, and *much more near the surface of the dark* than that of the white portion of the ovum, I was surprised to find the germinal vesicle still entire (fig. 6), and of a somewhat oval, lenticular form, although irregularly compressed by the contraction of the whole egg in the spirit. It was of a dense white colour, and opake, from the action of the spirit, and was in striking contrast to the dark cells of the yelk, which adhered to its surface, and amidst which it lay imbedded like the kernel in a peach or apricot. On the surface of the black portion of the yelk was a minute orifice, already noticed by Prevost and Dumas*, the outlet of a canal that passes through this portion of the yelk to the germinal vesicle in its interior (fig. 6). This I believe to be the result of the yelk cells having only imperfectly closed around the germinal vesicle. It is however of some consequence in the future development of the ovum, as it is in this canal that the cleavage of the yelk is commenced. The vitelline membrane was already formed, but I could not discover any orifice or perforation in it, either corresponding to the canal in the yelk, or to any other part of the surface. It is not possible to mistake the germinal vesicle for any portion of the lighter substance of the yelk,—first, from the fact that the vesicle at this period is of an intense, opake, white colour, very different from that of the yelk substance,—next, from its being completely isolated from the lighter, and imbedded in the dark substance,—and lastly, from its being still invested with a distinct envelope. On removing the vesicle, and examining it separately, first, as taken from the yelk, without crushing it, and next by gentle compression, and with the highest powers of the microscope, the interior was seen to be filled with secondary cells. Each of these, formed by a distinct envelope, *appeared* to contain other, or tertiary cells, and strongly reminded me of the developmental cells of the spermatozoa in the male organs, since these again seemed to contain granules, or quaternary cells. In the midst of the secondary cells I was able to distinguish, in the centre of the germinal vesicle, in some specimens, one or two cells of larger size than the rest, and which I regarded as the remains of the germinal spot, or central nucleus. In those ova which, from their size and general appearance, seemed to be the most mature, the peripheral series of cells within the germinal vesicle were of smaller diameter than those nearer to the centre, as if the earlier developed secondary cells had disappeared and liberated their contents. The cells of the dark portion of the yelk that adhered to the germinal vesicle were also nucleated, but were of much smaller size than the peripheral cells of this body. I must remark, however, that saving the fact noticed, of having seen in some vesicles one or two cells near the centre of larger size than the rest, I have not been

* *Loc. cit.* tom. ii. p. 104.

able to distinguish any separate nucleus, or germinal spot, which seems, at this time, to have disappeared as a distinct body. The germinal vesicle thus near its maturity is a mother-cell that contains a multitude of daughter or secondary cells, each, apparently, including its own progeny.

It is thus certain that the germinal vesicle exists in the ovum of the Frog until after the period of hybernation. But in individuals that have been several days abroad, and in which the ova are so far matured as greatly to distend the body, and be nearly ready to leave the ovisacs, I have not always been able to detect the vesicle. The yelk has then a greater proportion of white substance in its interior, intermingled with the dark, and this, I suspect, is the result of the disappearance of the vesicle.

In the ovum of the *Toad*, when as nearly mature as that of the Frog before leaving the ovisacs, I have been unable to detect any trace of the germinal vesicle. The dark portion of the yelk is then quite distinct from the light, and forms a cortical stratum of intensely black pigment, scarcely more than one-sixth of the diameter of the yelk in thickness. The light portion is of a yellowish white, and from which the germinal vesicle, when present, is not readily distinguished. But at a little earlier period, although only a few days before the pairing of the Toad, I have found the vesicle fully as large and as distinct as before its disappearance in the egg of the Frog, but situated more distant from the centre, and nearer to the black, the future dorsal surface of the egg.

In the great Water Newt, *Triton palustris*, the yelk of the matured ovarian ovum is of a dull pea-green colour, which in spirit is changed to a greenish yellow. On breaking open the yelk of this species, after it has been hardened in spirit, I have always found the germinal vesicle of the same opake, intense white colour, of precisely the same structure, and situated in the same part of the yelk as in the Frog. It has also a canal passing from the middle of the surface of the darker-coloured portion of the yelk, through its substance to the vesicle, as in the Frog.

In the lesser Newts, *Lissotriton punctatus* and *L. palmipes*, the yelk is of a brown or liver colour on the future dorsal surface, with a white central spot, and of a white or pale straw colour on the ventral. In ripe ovarian ova of these species also I have found the germinal vesicle of the same white colour, and having the same structure as in the larger Triton and the Frog.

In each of these instances, not merely when the ovum is immature, but even when nearly ready to leave the ovisac, the germinal vesicle is situated in the interior of the yelk (fig. 6), and not immediately at the surface, and thus far the fact is in accordance with the observation of Dr. Barry, that in the Mammalia the germinal vesicle is in the interior of the ovarian ovum, and does not disappear on the surface. But, nevertheless, it is not in the *centre* of the yelk, its place, as I have stated, is *excentric*.

Time of disappearance of the Vesicle.—It is well known that in frogs and toads the ovum is never impregnated until after it has left the body, and consequently not until long after it has left the ovary, and the germinal vesicle has entirely disappeared.

This disappearance does not, I think, take place at the instant the ovum is about to leave the ovisac, but a short time before; as I have found the ova of the Frog in one instance more matured than in that already described, still contained in the ovisacs, and yet most of them without any remains of the vesicle which I could identify as such, and with the yelks containing a larger proportion of white substance, as in the Toad. In a very few, however, the vesicle was still present, and exhibited the structure I have described. Certainly, then, the vesicle is not burst at the moment the egg escapes from the ovisac. There seems to be a short period of time between the disappearance of the vesicle and the full maturity of the ovum, during which the yelk itself undergoes some further change, and acquires the appearance noticed in the matured eggs of the Toad, and in the most advanced of those of the Frog. No trace of the germinal vesicle can be detected in any ova that have left the ovary and are contained in the cavity of the abdomen, before entering the oviducts. I have examined many ova, both of the Frog and Newt, from the abdominal cavity, but in every instance the vesicle has entirely disappeared. In some specimens, which seemed to have been in the act of escaping from the ovary when the animal was killed by immersion in spirit, I have found in the place occupied by the vesicle an aggregation of white nucleated cells, which, examined by the microscope, exhibited a close resemblance to those seen in the interior of the vesicle. In the midst of these there has occasionally been one or two of larger size than the rest, and which I have imagined to be the remains of the germinal spot, and possibly the origin of the future embryo vesicle of the impregnated ovum, an opinion, however, which I have not had the means of verifying, and I must further state that I have failed to recognize these larger cells in ova that were free in the cavity of the abdomen. Each of the three species of Newt, as well as the Frog, have presented similar appearances in the germinal vesicle and ovum under similar circumstances.

Thus it is quite certain that the germinal vesicle disappears in the Amphibia before the ovum enters the oviduct. I believe it does so in the interior of the yelk, not in the centre, but nearer to the dorsal than to the future ventral or white surface; and not, as has been supposed, on the dorsal or dark surface, between the vitellus and the vitelline membrane. This view is supported by the fact, that that portion of the yelk which incloses the vesicle in an advanced stage of the ovum in the Frog is of a more or less intense black colour, while the vesicle is perfectly white; and that at a further advanced stage, after the vesicle has disappeared, and its place is occupied by a collection of white cells, the dark portion of the yelk still preserves its intense black colour, except at the point that corresponds to the central canal, which then has a leaden hue. Prevost and Dumas*, and also Rusconi†, have mentioned that there is a yellow spot at a corresponding part of the dark surface of the egg of the species they have examined after impregnation, *Rana esculenta* ?, but these appearances must

* Annales des Sciences Naturelles, tom. ii. p. 104.

† Développement de la Grenouille Commune, 4to. Milan, 1826. p. 9.

not be mistaken for the germinal vesicle arrived at the surface. There is a similar spot, and that too of an elevated form, on the egg of each of the *Lissotritons*. But independent of the fact that the germinal vesicle has entirely disappeared from the interior of the egg before it escapes from the ovary, this spot is shown not to be the vesicle, both in the fact that in the egg of *Rana temporaria* the dark portion of the yelk is unchanged, while in each case the spot is perforated, and leads into the canal that passed originally to the vesicle. I regard the spot as simply a protrusion outwards of the edges of the canal while closing, after the vesicle has disappeared. I shall presently show that a similar white spot is formed on the under surface of the egg of the Frog soon after deposition, and which might equally well be mistaken for the germinal vesicle.

Mode of disappearance of the Vesicle.—The mode in which the vesicle disappears may be inferred from the facts of its structure. Being filled with a progeny of cells which we may regard as of different periods of growth, and these again containing others, it is fair to conclude that this process of cell formation is that by which the parent vesicle is ultimately destroyed. At the time when the germinal vesicle has nearly attained its full size, the peripheral cells are smaller than those nearer to its centre, while the yelk cells that surround the vesicle are still smaller than either, and are of a dark colour. When, therefore, the vesicle has acquired its full size, by the simple vegetative endogenous growth of the contained cells, we may fairly presume that the death of the parent mother-cell, or germinal vesicle, takes place as the result of their enlargement, by the diffluence of its investing membrane; and the enclosed daughter cells, thus gradually set free in the midst of those of the yelk, as in the ideal (fig. 7), form one mass with the latter, and the moment of the actual disappearance of the vesicle thus escapes direct observation, its previous existence being indicated only by the unbroken outline of the investing membrane.

These views lead me to agree with Wagner and Barry in regard to the structure and mode of growth of the germinal vesicle, but not as to that by which it disappears. Dr. Barry indeed believes that the vesicle only becomes changed by its mode of development, and does not cease to exist. But most certainly it does disappear in the Amphibia, and, as I believe, through the growth of the young cells in its interior. I cannot therefore agree with Dr. Barry that the changes in the vesicle end in the production of two cells in the centre of the yelk, that give immediate origin to the embryo; but rather believe that it is from one of the central cells of the germinal vesicle that the future *embryo vesicle* takes its origin, while the remainder of the liberated cells are distributed with this through the substance of the yelk, when the segmentation of this body takes place. This opinion is in accordance with that of Vogt*, who found that in the ova of *Alytes obstetricans* the germinal spots increase in number, and that a few hours after fecundation small vesicles, similar to these spots, are scattered through the yelk. I have myself found similar vesicles in the fecundated egg

* Untersuchungen über die Entwicklungsgeschichte der Geburtshelfer-Kræte (*Alytes obstetricans*), 4to, 1842.

of the Frog about three hours after impregnation (fig. 10 *a*), but have not traced them to their origin. With regard to the disappearance of the spot in the germinal vesicle, the facts observed in the ova of the Frog agree with those noticed by KÖLLIKER* in the ova of intestinal worms, that all appearance of the spot is lost before that of the vesicle. This circumstance, however, may be owing either to the spot having given origin to cells in the vesicle which quickly attain to similar dimensions, and from which it is not otherwise distinguished; or to its becoming entirely obscured by their multiplication. Although no observations have been made on the origin of the embryo vesicle that appears in the yelk after the disappearance of the germinal, I am still inclined to regard this as being in some way derived from the lost germinal spot, notwithstanding that KÖLLIKER found a certain period of time elapse between the disappearance of the vesicle and that at which he was able to recognize this body.

Transit of the Ovum.—Thus, then, when the ovum escapes from the ovisac and ovary into the cavity of the abdomen, the germinal vesicle and spot have entirely disappeared, and it consists only of the yelk enclosed in an exceedingly delicate, structureless, vitelline membrane. This is its condition in the Frog, Toad and Newts. It is then extremely delicate and easily lacerated. The mode in which the ovum passes into the oviduct has been the subject of much inquiry. I am quite certain, as SWAMMERDAM long ago showed, that the ova, when mature, pass from the ovaries into the cavity of the abdomen, and from thence into the oviducts, in the Frogs, Toads and Newts, quite independent of any intercourse with the male, as I have myself recently had an opportunity of proving. The frog I have already mentioned (p. 173.) as having watched through its season of hybernation, was kept apart from all others until the 2nd of April, at which time she had not deposited any ova. But from the altered form of her body it was evident that the ova had passed, or were at that time in the act of passing, into the oviducts. I then placed her in a vessel with others, some of which were males, but not one of these joined with her. Nevertheless, on the 6th of April she cast her ova, without having paired, and died on the following day. On examination after death, I found that the whole of the ova had left the ovaries, all of which, excepting only two still free in the cavity of the abdomen, had passed through the oviducts without any intercourse with the male. These ova of course were sterile, but it was worthy of note that their envelopes did not expand to so great an extent as those of the eggs of paired individuals.

Passage into the Oviduct.—In what way the ova pass from the cavity of the abdomen into the mouth of the duct has never been satisfactorily explained. SWAMMERDAM examined the question with much care†, but was unable to form any decided opinion respecting it, as he correctly states that the mouths of the ducts are at a distance from the ovaries, and are not free to grasp the ova like the fimbriated extremities of the Fallopian tubes in Mammalia, but are confined in the peritonæum, which is continuous with that which passes over the pericardium and heart. PREVOST and DUMAS

* MÜLLER's Archiv, 1843.

† Book of Nature, part 2, pp. 108, 109.

have since stated* that the ova after leaving the ovaries are seized by the tubes ("sont saisis par des trompes"), but they do not show in what way this seizure is effected. They have omitted to describe the structure of the parts concerned in the act, and have not mentioned the way in which the ova are conveyed to the tubes from the ovaries, or whether the mouths of the tubes approach the ovaries, as in Mammalia.

The entrance to the tubes in the Frog, as SWAMMERDAM has correctly shown, is in the peritonæum (Pl. XIV. fig. 1 *b*) at each side of the heart (*a*), and I have found it in nearly the same place in the Newts. The apex of the pericardium in the Frog is attached to the cartilage of the sternum by two layers of peritonæum, which together form the mediastinum, and enclose between them the trunk of the median abdominal vein, a branch of the vena cava. Tracing one of these layers of peritonæum upwards and over the pericardium, we find in it an orifice (fig. 1. and 2 *b*), at the part where it is reflected on itself to form the lateral portion of the suspensory ligament (*e*) of the liver (*c*). This orifice (*b*) is elongated, oval and funnel-shaped, and, when dilated, forms a kind of pouch at the anterior boundary of the space or cavity between the liver (*c*) and the heart (*a*), and laterally it is in free communication beneath the suspensory ligament with the common cavity of the abdomen. The oviduct (*g*) commences in this dilated orifice as a narrow tube with thick muscular parietes and with a thick mucous lining. It passes at first upwards and forwards, confined to the peritonæum, and then outwards above the base of the lung (*f*), gradually increasing in its dimensions. Immediately after it has passed the lung it becomes more enlarged, and as it passes backwards to the side of the spine forms many convolutions, which end in the dilated oviduct (fig. 1. and 4 *h*) or common receptacle for the eggs ready to be deposited.

The commencement of the oviduct in the Newts is very similar to that of the Frog. In the *Triton palustris* it differs only in the entrance being larger, and situated more to the side of the body, and above the lung, to the base of which, as well as to the peritonæal investment of the heart, it is confined, as in the Frog; but it has a more free communication laterally with the common cavity of the abdomen than in that animal.

The ova escape from the ovaries into the cavity of the abdomen among the viscera both in the Frogs and Newts, and are carried forwards to the spaces between the liver and heart on each side to the dilated mouth of the oviduct. They certainly are not seized by the tubes as they escape from the ovaries, as they are constantly found free in the abdominal cavity, while the mouths of the tubes being confined in the peritonæum, and having no appendages, cannot be extended to reach them. Their transfer seems to be effected in the Frog in part by the action of the abdominal muscles forcing them onwards in the spaces between the viscera, aided perhaps by the peristaltic action of the stomach and intestines; and their entrance into the tubes, when arrived in the vicinity, seems to be induced by an ingurgitory or suction action at the mouth, occasioned by the alternating and pulsatory motion of the heart, with which the tube is

* *Loc. cit.* tom. ii. p. 105.

connected by means of the peritonæum. The tube itself is formed of strong longitudinal and transverse fibres, which are continued into the peritonæum, and the former especially into the suspensory ligament, the free external margin of which bounds the outer side of the orifice. The transverse fibres are strongly marked at the commencement of the orifice, where there is a slight pouch; so that when the eggs are entering, these fibres doubtless prevent their return and transfer them onwards. This, I believe, is the way in which the eggs enter the oviducts. It is quite certain from the anatomy of the parts that they cannot be grasped by the oviducts until they are conveyed to them. I have not actually witnessed the passing of the eggs from the abdomen into the ducts in the Frog, but I have seen the eggs moved onwards in the smaller Newt, *Lissotriton palmipes*. Having deprived a female of this species of sensation and power of motion by division of the spinal cord through the medulla oblongata, I proceeded to open the abdomen to obtain ova from the oviducts for experiments on artificial impregnation. I then found that a number of ova were free in the abdominal cavity, and that some had very recently entered the ducts, while others were in the immediate vicinity of the mouths. The heart was still pulsating vigorously and with great regularity, and I then saw that at each pulsatory action the ova passed slowly forward between the liver and lung, towards the mouth of the oviduct, which still contained two or three ova that appeared to have entered at the moment of the operation. I did not witness the actual entrance of an ovum, but saw that the action of the heart certainly had the effect of inducing the advance of it to the mouth of the tube, and quite sufficient to lead me to regard this as one of the chief means of its entrance into the duct.

It is not until the ovum has become clothed in the oviduct with its gelatinous envelope that it is susceptible of impregnation. This remark applies equally to the Frogs, Toads and Newts. The ova of the Frog and Newt at large in the abdominal cavity are always entirely without this envelope, and consist simply of the yelk mass enclosed in an extremely delicate vitelline membrane. They are so easily injured that it is only with great difficulty that they can be removed from the abdomen for examination unbroken. Those of the Newt, when taken up ever so carefully by means of a hair pencil, often burst the membrane simply by their own weight. But immediately after they have entered the oviduct and begin to acquire their envelopes, the yelk appears to undergo some change, as it becomes much firmer and is less easily injured. Shortly after the egg has entered the duct, it gains the first layer of an investment, which, from the great similarity it bears to the gelatinous layer gained by the ovum of the Rabbit in the Fallopian tube, and regarded by its discoverer in that animal, Mr. Wharton Jones*, as the origin of the chorion, I am disposed, with him, to look upon as the analogue of that layer. This covering adheres very closely to the vitelline membrane, and is scarcely to be distinguished from it, except at certain periods of change. It is acquired before the egg has arrived at the first convolutions of the ovi-

* Phil. Trans. part 2, 1837.

duct. During the remainder of its passage the egg gains two other distinct layers of similar investment, which, together, we afterwards recognize as the gelatinous envelope of the Frog and Toad, and the capsule of the Newts. These envelopes are not merely simple means of protection to the egg during the production of the embryo, as has been supposed, but, as I shall presently show, are essential to it at the period of fecundation, and without which the egg is not susceptible of impregnation. The layer of envelope which I regard as the foundation of the *chorion*, is a dense, but very transparent thin covering, in immediate contact with the vitelline membrane, and is formed of cells so closely aggregated together as to have coalesced into a fibrous structure. The two layers external to this are also formed of cells, which, with their nuclei, are distinctly visible in the envelope of *Triton palustris*, in which they alternate in regular series. Although these layers, which constitute the jelly in the egg of the Frog, become detached in that of the Newts quickly after oviposition, and, expanding as in the Frog, they leave the egg at liberty in a chamber in their interior, they are nevertheless essential to the impregnation of the ovum, which takes place before or at the time of leaving the body, as in Frogs and Toads. Rusconi* removed the envelopes of the egg of frogs, and found that the embryo still became developed, and thence concluded that these coverings serve only mechanical purposes during the changes; but it will presently be seen that they have a more important function at a much earlier period. During the time they are in course of formation around the egg the yelk undergoes some further change. The light portion becomes of a whiter, and the dark portion of a deeper colour. Internally the cells vary more in size, the lighter-coloured being the largest. I have not succeeded in recognizing any embryo or central vesicle up to this period.

2. CHANGES AFTER SPAWNING AND IMPREGNATION.

First period of development.—It has been long known that a division or cleavage of the yelk of the Frog's egg is one of the earliest and apparently invariable results of fecundation. The primary division was first seen by Swammerdam, and was figured† and mentioned, but was not understood by him. Spallanzani long afterwards recognized it in the egg of the Toad (*Alytes obstetricans*‡), which he says becomes about a day after fecundation marked "with two furrows which meet to form an angle,"—that the furrows afterwards become deeper, and that "two small tumours arise on each side of the furrows,"—changes which have since been more accurately and completely described by Vogt§. Spallanzani also says that the egg of the common Toad is "marked with four furrows which intersect each other at right angles nearly like the husk of a chestnut half-opened," but he seems to have thought this was the usual condition of the ovum. To Prevost and Dumas||, however, we owe the important

* Développement de la Grenouille commune. Milan, 1826.

† *Loc. cit.* tab. xlviii. figs. 5, 8. ‡ *Loc. cit.* vol. ii. p. 159.

§ Untersuchungen über die Entwicklungsgeschichte der Geburtshelfer-Kræte (*Alytes obstetricans*), 1842.

‖ Annales des Sc. Nat. tom. ii. p. 110, 1824.

discovery of the cleavage of the yelk as a process of the fecundated egg; to Rusconi*, Baer† and others, its full exemplification in the Amphibia; and to Barry‡ and Bischoff§ its detection and elucidation in the Mammalia.

The agent immediately concerned in these changes is believed to be the embryo vesicle and its progeny, produced after the disappearance of the germinal vesicle. But it is yet uncertain what is the origin of the embryo vesicle, or whether it exists in the unfecundated ovum. As cleavage of the yelk certainly is not the result of the disappearance of the germinal vesicle, which disappears from all ova of the Amphibia, whether they are afterwards impregnated or not, I was desirous, at the commencement of my experiments on impregnation, to learn *from direct observation* whether the unfecundated ovum ever passes through any stage of cleavage; since the ascertainment of the fact in the negative would be an important test in the experiments I was about to make. For this purpose it was necessary to collect many pairs of frogs at the proper season, and when from symptoms which are soon recognized, it was found they were about to cast their ova, to wait patiently, perhaps for many hours, for the result, in order that the exact condition of the ovum, impregnated naturally, should be first ascertained. Swammerdam long ago remarked that the spawning of the frog takes place very rapidly "by a single effort||." It is often completed, as I have found in the English species, in a few seconds, and usually in less than a minute, during which the male impregnates them, so that if the animals are not closely watched the opportunity of observing the earliest appearances of the ovum is lost. Having noted the condition of the impregnated ova of several pairs of frogs within the first few minutes after spawning, I found those of different individuals vary much with respect to the white or inferior surface, and exhibit appearances that may readily be mistaken for the breaking up of a vesicle on the surface. This appearance is due to a more or less complete state of maturity of the eggs of different broods, and according as their spawning has been retarded or hastened. The peculiarities are the most marked in the least matured, the white surface of the egg being the last completed part, and forming the base of the egg in the ovisac (fig. 5). Having noticed the appearances of the eggs when impregnated naturally, I was enabled to compare them with others impregnated artificially, and these with some of the same brood not impregnated.

Immediately after the frog has spawned the ova form a close rounded mass, which at first is scarcely so large as a walnut. They then seem to consist almost entirely of dark-coloured yelks with thin gelatinous envelopes. The form of the egg is then somewhat oval, with the white portion a little more conical than the dark and differing slightly in different ova. In some there is a dark spot in the centre of the white, that looks like a depression or cavity, or perhaps a vesicle. I am not certain

* *Loc. cit.*

† Müller's Archiv, 1834.

‡ Philosophical Transactions, 1839, 1840.

§ Entwickelungsgeschichte des Kaninchen-eies, 1842.

|| *Loc. cit.* part 2. p 111.

that this appearance is in reality a vesicle, and therefore am content to describe it as a spot, although it conveys the idea of being a vesicle. In some ova there are two, four or six of these spots imbedded each in a small portion of white substance. When only a single spot exists, the white surface of the egg for some space around it is more defined than afterwards, and exhibits faint indications of a crucial division of the yelk on this surface immediately around the spot. This is the condition of a few ova immediately after spawning; but the majority have then advanced farther in their changes, and show four or six rounded dark-coloured spots at a little distance in the place of the single central one. When four spots occur they are usually arranged in a quadrangle, and are less than their own diameter apart. They convey the idea of being derived from the central one; but I have never seen any division of this, and if such division takes place, I think it must occur before or at the very moment the ova are expelled. In a further advanced stage of the ovum the four dark spots have become larger, and are each imbedded in a distinct portion of the white surface.

One minute after deposition the spots are more widely separated, and are then each encircled by a separate patch of white substance. *Two minutes* after spawning six dark spots have made their appearance, one of which is situated nearly in the centre, and the remaining five are so arranged around this that the white patches in which they were imbedded seem to have coalesced. In *three minutes* the spots are further enlarged, and appear joined by a dark line of colour extending from each, so that the whole form, as it were, a knotted ring that includes a patch of the white surface of the yelk with one of the dark spots near the centre. In *four minutes* the ring around the included white substance is more distinct, and the white surface of the egg has increased in extent. In a further advanced stage at this period the white portion included in the ring exhibits the appearance of a white, very opake patch, the dark spot in the centre having disappeared. Around this opake white patch is the dark-coloured knotted ring, now become more uniform, and resembling a ragged chink or slight circular furrow or division in the white surface. The centre of this hemisphere of the egg thus comes to be occupied by a white patch instead of the dark spot. At *five minutes* this central white patch,—which, as before stated, and from what afterwards occurs, may readily be mistaken, on casual inspection, for the germinal vesicle, altered in its appearance and arrived at the surface,—were it not that we now know that this has long before entirely disappeared,—becomes more defined, and the dark circle around it is more uniform and distinct. At *ten minutes* the central patch is a little reduced in size, and the circle that incloses it begins to take the appearance of a diffused halo. At *fifteen minutes* the central white patch is more reduced, and the halo is spread wider, while the whole of this hemisphere of the egg has acquired a whiter appearance, and become more distinct from the dark colour of the sides and future dorsal hemisphere. At *twenty minutes* the central patch has become still smaller and rounder, and the dark halo much broader.

At this period an interesting circumstance occurs which may hereafter be found

to have some reference to changes in the interior of the yelk, possibly to some rapid evolution of the so-called central or embryo vesicle in the locality originally occupied by the germinal vesicle and spot, which, as we have seen, is nearest to the dark surface —it is the partial rotation of the entire yelk. Up to about this period the ova remain undisturbed in the water in a mass as they are expelled, and lie indiscriminately, some with the dark and some with the white portion of the yelk uppermost, or horizontal. But during the time that has passed since the ova have been in contact with water, the envelopes have imbibed fluid and expanded until these investments of the yelk have acquired a thickness equal to about two-thirds of the diameter of the yelk itself. The yelks that have remained to this time with their white surface uppermost now change their position spontaneously by a partial rotation of the whole mass of each on its axis, within the vitelline membrane, until the dark surface of the whole is placed uppermost. Whether this change of position is merely the result of an expansion of the vitelline membrane at this period, when the ovum is rapidly ceasing to be susceptible of impregnation, as I shall presently show is the case after this lapse of time in the water, or whether it be also connected, as we may fairly believe, with changes going on in the interior of the yelk, I am not prepared to decide. It is important, however, to note that the change takes place at about the time at which I have found a great abundance of bright clear rounded vesicles distributed throughout the yelk, but chiefly in the place originally occupied by the germinal vesicle. In some of these vesicles, which I regard as the progeny of the germinal vesicle, I have seen irregular-shaped nuclei that appeared to be formed of a multitude of nucleoli. These vesicles convey to me the same idea as those seen by Bischoff in mammalian ova, excepting only that in the egg of the Frog they contain compound nuclei.

At *thirty minutes* the central patch on the white surface of the egg has almost disappeared, and the halo around it is still more diffused. At *forty-five minutes* it has entirely disappeared in most specimens, and its place is occupied by a broad dark area which includes the boundary of the previous halo, and which appears to be occasioned by a slight depression in the centre of this surface of the yelk. *One hour* after spawning this depression is somewhat deeper. The white surface has become still more defined, and the dark has acquired a more intensely black colour. The egg remains in this state without further perceptible change during the succeeding *second* and *third hour*, excepting only that the depression in the white surface becomes a little deeper, but it has almost disappeared at the end of the *fourth* hour, when *segmentation* or cleavage of the yelk is about to take place. But this is not invariably the case. When it does remain it is always of an oval form, and the primary cleavage of the yelk, as it proceeds on either side from above downwards, meets in its centre and invariably passes through it transversely to its long diameter. These are the first perceptible changes in ova that are impregnated by the natural union of the sexes, and when spawning has not been retarded. But in some broods of eggs that have been retained longer than usual in the oviducts, the whole of these changes have

already taken place, in so far as regards those of the yelk, the white surface of which then exhibits an uniform appearance.

Changes immediately before segmentation or cleavage of the yelk.—Segmentation usually commences in from four to five hours. At about *one hour and a half* after spawning, the peripheral layer of cells on the middle of the dark or uppermost portion of the yelk of the impregnated ovum becomes separated from the inner surface of the vitelline membrane, and this separation goes on until a broad free space is left between this envelope and the superior layer of yelk-cells. This space, which we may designate the *respiratory chamber*, is at first but a small area above the middle of the dark surface of the yelk, and is commenced above the central canal. It seems to be occasioned by a recedence towards the interior, or a shrinking, at this period, of the yelk-cells of the dark hemisphere of the egg, commencing in the centre of this part and extending gradually, but in a less degree, to the circumference. This recedence goes on until the space left between the vitelline membrane and the yelk is equal to about one-sixth of the diameter of the whole mass, when the space appears to be occupied by a very transparent fluid, interposed between the now depressed surface of the yelk and the vitelline membrane. In the centre of the black surface is the minute orifice noticed by Prevost and Dumas*, and Baër†, which leads into the central canal that communicated with the germinal vesicle in the ovarian ovum. It is in the margins of this canal that segmentation is commenced. While the space or chamber between the black portion of the yelk and the vitelline membrane is being formed, and from fifteen to thirty minutes before there is any sign of cleavage, the yelk becomes extended horizontally in a direction transverse to that in which the first cleft afterwards takes place, and assumes a transitory obtuse oval form, which it retains until the yelk begins to divide. The division, as correctly shown by Baër‡, commences in the extension in opposite directions of at first a faint indentation in the margin of the central canal, which quickly becomes deeper, and is carried across the surface of the yelk, and gradually more and more deepening and widening as it proceeds, is carried round the sides, and meeting in the middle of the depression on the under surface, or of the remains of the white patch when this has not already disappeared, is completed by passing through the middle of the yelk; which is thus divided into two portions. This first division occupies from twenty to thirty minutes before it is finished, and it is not until then that a second fissure is commenced. I have not had any opportunity of proving whether this division is the direct result of subdivision of the central vesicle, and the attraction of the yelk-cells in equal proportions around each division of that body, as believed by Kölliker§, and, as it seems fair to infer, is the case; but in addition to the observation by Prof. Sharpey‖, that the contraction of the entire yelk at the commencement of these changes, and the movements he has observed among its granules as they proceed, are in favour of this opinion—I may remark, that the extension of the yelk of the Frog's

* *Loc. cit.* tom. ii. p. 104, 1824.
† Müller's Archiv, 1834.
‡ *Loc. cit.*
§ Müller's Archiv, 1843.
‖ Quain's Anat., Fifth Edition, 1848.

egg in a direction transverse to the first cleavage, may also be advanced as conformable to the same view. It is further supported by the fact which I have seen in the egg both of the Frog and Newts, that before the second or crucial cleavage is commenced the yelk becomes contracted, so that the first cleft for a time is almost imperceptible while it is extended in the transverse direction, or line of axis of the first division, after which the second or crucial cleft is commenced. To this I may add another fact which appears to be equally significant, and which occurs in the unimpregnated eggs both of the Frog and Newts, but more especially in the latter. Although no recedence of the yelk from the vitelline membrane takes place in the unimpregnated egg of the Frog, it becomes, nevertheless, slightly oval after the first few hours, but returns to its original shape some time afterwards. But the unimpregnated egg* of the Newts is not only separated from the vitelline membrane, but also is depressed, and has a distinct pit in the centre of its upper surface, and also assumes an obtuse oval form, both which it retains, when the egg is preserved in water, until decomposition has commenced.

Changes in the impregnated and unimpregnated Ovum compared.—Having traced the egg impregnated by natural union of the sexes through its first period of development, I was able to compare the phases it exhibits with those of the artificially impregnated, and these with the appearances in unimpregnated eggs of the same brood, placed under precisely similar circumstances with reference to light, heat, air, water, and locality. The ova experimented on were all procured from the same female, and the seminal fluid from the male with which she was paired, and at the time the female was about to spawn.

Spallanzani obtained unimpregnated eggs of the Frog for his experiments by opening the body of the female and removing them from the distended oviducts. This mode is exposed to the objection, that in the removal of the ova they are liable to be brought into contact with the blood of the animal from the cut vessels, and that the ova thus obtained may not be the most mature, and fitted for experiment. It seemed desirable, therefore, to obtain them by another mode,—the total withdrawal of the influence of sensation, and power of tension in the muscles by division of the spinal cord through the medulla oblongata. The attempt was made with a female frog that had been paired for several days, and, from appearances, would have deposited her ova naturally in the course of a few hours. The spinal cord was divided as quickly as possible with a strong pair of scissors immediately behind the brain, and this organ was also destroyed, so that all consciousness was annihilated. The attempt was successful. The muscles deprived of voluntary power instantly became relaxed and allowed of the ova being passed, by gentle compression of the body, through the natural passage without contact with the blood of the animal, in greater or smaller number at pleasure, and thus afforded easy means of experiment.

I may once for all state that it was in this way that the ova were always obtained in the following investigations.

* Or, possibly, partially impregnated.

Recourse was had to a similar expedient to procure the seminal fluid from the male. SPALLANZANI had obtained the fluid, by vivisection, from the seminal vesicles themselves. But there seemed more objection to the adoption of this mode with the male than the female, the certainty of much of the fluid being lost, independent of the severity of the operation. Indeed SPALLANZANI states, that he was never able to procure more than from two to three grains from a single individual. I therefore availed myself of a habit in the male frog, and which SPALLANZANI had previously noticed and taken similar advantage of in the Newt, to obtain the fluid in greater quantities than by the mode constantly adopted by that physiologist. When the male frog, like the Newt, is taken in the hand, or slightly compressed, at the season of pairing, a quantity of fluid is immediately passed. This consists chiefly of seminal fluid mixed with water expelled from the effect of the compression, or during the efforts to escape, as water is passed by other animals at the moment of capture. It abounds with spermatozoa in their most active state, and thus is fitted for experiment. It required therefore only to secure the limbs of the animal and compress it slightly, to obtain the fluid without severe injury. This ready mode was adopted on all occasions when the fluid was required, and the precaution taken always to examine a portion with the microscope, to be assured of its nature before employing it. Spermatozoa have never, during the season of pairing, been absent from it. At the end of the season they have been less abundant, and spermatozoal cells in greater proportion than at an earlier period. But in these cases I had reason to think that the chief part of the fluid consisted of water. It is probable that this was the case in the two instances of apparent absence of spermatozoa in the Toad, mentioned by SPALLANZANI*, and that the fluid did really contain spermatozoa, although few in number, and consequently easily overlooked, and that the ova were impregnated by these, and not by the fluid portion of the semen, as he appears to have supposed.

On comparing the white surface of the yelk of the unimpregnated with that of the impregnated egg, whether the egg had been fecundated naturally or artificially, I was not able to detect any difference during the first twelve minutes. The changes went on in both, and appeared to be almost identical in each. But after the time specified no further progress was perceptible in the unimpregnated ovum, which continued to exhibit the same appearance for several hours. But the white surface of the impregnated egg became more and more changed, up to the time of cleavage of the yelk, when it was almost an uniform surface.

These observations were afterwards repeated with similar results, and the conclusion to which they led was, that changes take place in the yelk from the period when the germinal vesicle disappears and the ovum leaves the ovary to the moment of its expulsion from the body, and which changes may proceed for some time afterwards quite independent of impregnation; and that these have some reference to the evolution of the central or embryo vesicle: possibly also that they do not cease imme-

* Dissertations relative to the Natural History of Animals and Vegetables, 1789, vol. ii. p. 151.

diately, but subside gradually when the stimulus imparted by impregnation is not supplied.

The experiments made to ascertain whether the unimpregnated ovum passes through any stage of cleavage, consisted of four sets, placed in four vessels of equal size, containing each about two ounces of water. Ova were passed from the same female as quickly as possible at the same time into each of these vessels. To one of these marked A, a considerable quantity of a mixture of seminal fluid, one part to three parts of water, was immediately added; to a second, B, only a single drop of this mixture; while the third and fourth, C and D, contained only water with the unimpregnated ova, and the four vessels were then placed in every other respect under precisely similar conditions.

A few minutes after the impregnating fluid had been added to A, I examined some of the ova beneath the microscope, and found a vast abundance of spermatozoa adhering to every part of the surface of their gelatinous envelopes. On other ova from the set B, there were also many spermatozoa attached, but in much smaller number than on the ova of set A.

In *five hours and fifteen minutes*, the temperature of the room during the interval having ranged only from 53° FAHR. to 54° FAHR., segmentation had commenced vigorously, and was strongly marked in the whole of set A. But it had not commenced in set B. It did not occur in these until *five hours and twenty-two minutes*, when it began in these also. Thus there were *seven minutes'* difference in the commencement of the changes in these two sets of ova, a circumstance which led to the belief that this difference might have some reference to the relative quantities of the impregnating fluid employed,—an opinion which I had long before been led to by observations on the impregnation of the common Earwig, *Forficula*, in which it had appeared to me that deficiency in the quantity of the impregnating fluid is unfavourable to fecundation.

No segmentation or cleavage of the yelk took place in the sets of ova marked C and D, which, except in becoming a little oval, as already mentioned of unimpregnated eggs, remained, in so far as the appearance of the yelk surface was concerned, in the same state as at a few minutes after spawning, and they continued in exactly the same condition at the end of twenty-two hours. This I have since found constantly to be the case with unimpregnated ova, whether they happen to be exposed to a high or low temperature of the surrounding medium. The yelk of the impregnated egg gradually acquires a more intense black colour, which strikingly contrasts with the dull colour of the unimpregnated.

At the end of *six days* the majority of the ova in A and B were producing embryos, while those in C and D were fast decomposing.

These trials afforded the positive test I required from direct observation, as a fixed point in the experiments about to be commenced,—that segmentation certainly does not take place in the unimpregnated ovum.

3. SUSCEPTIBILITY OF THE OVUM.

I was not aware, at the time of commencing my experiments in March 1849, nor indeed until very recently, of the extent to which the original investigations of SPALLANZANI, and of PREVOST and DUMAS had long ago been carried*, and it has only been since my experiments were completed, and during the preparation of this paper for presentation to the Royal Society, I have learned by careful reference to their first memoirs, that they have anticipated me in part of this inquiry—that of endeavouring to separate the spermatozoa by filtration from the more fluid portion of semen, and testing the effect of these two constituents in artificial impregnation. To them therefore be all honour for the result; although even they, as they honourably mention, had themselves been anticipated in this by SPALLANZANI, and that too with similar success. The extraordinary results obtained by SPALLANZANI† in artificial impregnation, and the imperfect knowledge which we possess of the nature of the means by which it is effected, has induced me to endeavour to repeat and vary his experiment, and to conceive others, which, so far as I am aware, have not yet been attempted. I have been the more urged to this from the circumstance mentioned by SPALLANZANI, and already alluded to (p. 189), the occasional supposed absence of spermatozoa from fluid that is capable of fecundating; and also from a belief formed long ago with regard to the Articulata, that the spermatozoa, nevertheless, certainly are the efficient agents in impregnation, although full proof of the fact has been wanted. I have been desirous therefore of learning how far this belief can bear the test of direct experiment, or the fact be capable of demonstration by artificial means in the Amphibia. As, however, the experiments I have myself made vary from those of the authors mentioned,—have not been influenced by the result they had previously arrived at,—have been somewhat more extended, and, as I believe, will now tend to place the fact of the direct agency of the spermatozoa in impregnating the ovum beyond doubt,—it has seemed desirable still to give them in detail, as assisting to establish an important point of knowledge by facilitating a comparison of the results of independent investigations.

Duration of susceptibility.—The length of time during which the ovum, after it has been passed, remains susceptible of fecundation, is affected by several circumstances. I had reason to believe at the commencement of my experiment that this time is very short. SPALLANZANI found that when the egg of the Toad was expelled into water it was not susceptible of fecundation after a lapse of fifteen minutes‡. This was at a raised temperature of the atmosphere, 81°·5 FAHR. On the other hand, he also found that, at this temperature, ova retained fourteen hours within the body of the female after death, and of course not in contact with air or water, might still be fecundated; and that when preserved in an ice-house fecundation might be effected at two days after the death of the parent§. But PREVOST and DUMAS arrived at the conclusion‖

* Annales des Sciences Naturelles, tom. i. et ii. 1824.

† Dissertations, &c. vol. ii.

‡ *Loc. cit.* vol. ii. p. 178.

§ *Loc. cit.* vol. ii. pp. 176 and 177.

‖ An. des Sc. Nat. vol. ii. p. 135.

that the time is much more extended in the Frog;—that fecundation may take place when the temperature ranges from 53° FAHR. to 59° FAHR. at the expiration of one hour after immersion of the eggs;—that some eggs are fecundated at two, and a very few even at the end of three hours, after which no fecundation takes place. The results obtained by myself both on the Frog and Toad have been most in accordance with those by SPALLANZANI. This is the more worthy of notice from the circumstance that a slight difference between his and mine is readily accounted for by a corresponding slight difference of temperature, which SPALLANZANI, and PREVOST and DUMAS, have remarked, and since them also Mr. BELL*, has great influence on the changes of the eggs and young. The temperature at which my test experiments were made, was a little lower even than that at which PREVOST and DUMAS made theirs; and yet I was not able to find any ova susceptible of fecundation after they had remained from thirty to forty minutes in water. On careful examination of PREVOST and DUMAS' experiments, I think the difference may perhaps be due to a circumstance which seems equally to affect some of SPALLANZANI's results, namely, the mode in which the impregnating fluid employed was obtained. These authors state that the fluid they employed was expressed from the testicles of the frogs, so that from what we now know of the mode of origin of the spermatozoa, this fluid in all probability contained a large proportion of developmental cells that included spermatozoa not fully matured, but which might become liberated in the water at a longer or shorter period. Or, possibly, the fluid added to ova that had been long in the water, had been very recently obtained; in which case the vigorous spermatozoa might effect the impregnation of ova that had become almost insusceptible through the imbibition of water by their envelopes. I am led to this view by the fact that the jelly-like envelope of the Frog's egg begins to imbibe and expand the instant it is brought into contact with fluid; and from having ascertained that there is a close relation between the degree of expansion and imbibition of this envelope and the susceptibility of the ovum to become impregnated, and that these conditions are also greatly affected by temperature. The act of expansion of the envelope is an act of endosmose, and possibly this is one of the means by which the impregnating agent is made to exert its influence on the yelk. The yelk is not a passive recipient during the endosmic action of its coverings, but seems to participate in that action, as I have seen portions of its surface heave and contract within the vitelline membrane during the first hour the egg has remained in water. It may thence be inferred, that if the impregnating stimulus be not supplied quickly, the fitness of the ovum to become impregnated is diminished in proportion as its envelopes are expanded. If then it be proved that the spermatozoon is the agent in impregnation, but, so far as can be discovered, does not penetrate bodily into the ovum or its envelopes, and yet, as may be shown, must always come into contact with their surface, the more rapidly and to the greater extent this expansion takes place, and removes the efficient body from that which it is in some way destined to affect, the

* British Reptiles, p. 92.

less will be the chance of its impregnating the ovum, and the less will the ovum become susceptible of impregnation even by the most healthy and vibratile spermatozoa.

The extent and rate of expansion of the envelope of the Frog's egg, during the first half-hour it remains in water, very nearly coincide with the diminution of the fitness of the ovum to become fecundated. This is shown by observing the rate of expansion of the envelope during the first fifteen minutes of submersion, and then testing the fitness of the ovum, by experiment, during a similar period.

At the *moment* when the ovum is expelled from the body, the envelope is merely a thin gelatinous layer, its entire diameter being equal only to about one-sixth of the diameter of the yelk. After it has been *one minute* in water, and begun to imbibe and expand, it is then equal to about one-fourth of the diameter of the yelk. At the end of *two minutes* it is enlarged to one-third, and in *three minutes* to one-half the diameter of this body. In *four minutes* it exceeds three-fifths, and in *six minutes* two-thirds, and it continues to imbibe fluid and expand at the same rate, until, at from *ten* to *fifteen* minutes, it very nearly equals in thickness the whole diameter of the yelk; and at *half an hour* (fig. 9) it is one-fourth greater than this. Prevost and Dumas* noticed the expansion of the envelope during the first *six hours*, but entirely overlooked the rate of expansion during the most important period, the first hour, and noticed only the general fact that the diameter of the envelope, at the end of the first hour and a half, was as 5 to 2·5 at the time of spawning, and that it had nearly acquired its full size at the end of three hours. My own observations agree with this latter statement. The expansion of the envelope is greatly retarded at the end of the third or fourth hour, until after cleavage of the yelk has taken place, when it again proceeds, but much more slowly than at first. If then we bear in mind the rate of expansion of the envelope during the first half-hour, the following experiments will give some idea of the degree of susceptibility of the ovum to become impregnated during that period.

Set E. April 6, 1850.—The temperature of the room, at the commencement of this set of experiments, being 60° Fahr., ova were obtained from a female frog and seminal fluid from a male, by the mode already mentioned; the latter being mixed with an equal quantity of water.

I may here remark, that the ova in each of this set of experiments were placed in nearly similar quantities of water, and that as it had been shown in the experiments A, B, C and D (p. 190), that segmentation of the yelk proves the ovum to have been impregnated, although, as we shall hereafter find, not always sufficiently so as to produce the embryo, I adopted this as a fair test of the susceptibility of the ovum. I may here also mention, that although the date of making the several experiments detailed in this paper is recorded, it has been necessary, for reasons that will be obvious, to disregard the order of time at which the several sets were

* *Loc. cit.*, vol. ii. p. 108.

made, and to detail them when considering the subject to which they more especially refer.

No. 1. P.M. $2^h\ 52^m$.—*Eighty-three ova* were passed into water, and the impregnating fluid added to them at the expiration of *one minute*. This was *forty-three minutes* after the fluid had been obtained and mixed with water; but on examination with the microscope at this period, the spermatozoa contained in it were still very active. This long period was determined on with a view to the more effectually testing the susceptibility of the ovum, as it will be shown that the spermatozoa are less and less efficient in proportion to the length of time they have been mixed with water. Segmentation commenced in a very large proportion of the ova at the end of *three hours and fifty-five* minutes. On the 14th of April, the *eighth day*, *thirty-two* embryos had been formed.

No. 2. P.M. $2^h\ 51^m$.—*Ninety-two ova* were passed into water, and at the expiration of *two minutes* impregnating fluid was added to the same, *forty-three minutes* after it had been obtained. Segmentation commenced in these also at *three hours and fifty-five minutes*, and took place in almost every ovum. On the *eighth day* there were *forty-five* embryos.

No. 3. P.M. $2^h\ 24^m$.—*One hundred and twenty-seven ova* were immersed in water for *three minutes*, and then exposed and bathed with impregnating fluid during *twenty seconds*, water being immediately afterwards added to them. No segmentation had taken place at the end of *four hours and three minutes*, but it took place in many of the ova at a later period, the exact time having escaped my notice. The fluid employed had been mixed with water only *seventeen minutes*. On the *eighth day* there were *thirty-three* embryos.

No. 4. P.M. $2^h\ 15^m$.—*Eighty-one ova* were exposed to the air on a dry surface for *three minutes* without having been in contact with water, and were then bathed with impregnating fluid during *five seconds* and water immediately afterwards added to them. The fluid in this experiment had been obtained only *eight minutes*. Segmentation commenced in several ova at *four hours and five minutes*, and on the eighth day there were *fifty-three* embryos.

No. 5. P.M. $2^h\ 25^m$.—*One hundred and thirty-six ova* were passed into water for *five minutes*, and were then exposed and bathed with impregnating fluid for *several seconds*, and water immediately afterwards added to them. The fluid had been obtained *twenty minutes*. Segmentation occurred in one ovum at *four hours and eight minutes*, and in others quickly after. On the eighth day only *ten embryos* had been formed.

No. 6. P.M. $2^h\ 17^m$.—*One hundred and thirty-nine ova* were exposed to the air, on a dry surface, for *five minutes*, and were then touched freely with fluid during *five seconds*, applied with a hair-pencil, and water was then quickly added to them. The fluid employed had been obtained *twelve minutes*. Segmentation took place in *four hours and eleven minutes*. On the eighth day there were *thirty-seven* embryos.

No. 7. P.M. $2^h\ 21^m$.—*Two hundred and five ova* were retained in water for *fifteen*

minutes, were then exposed, well-bathed with impregnating fluid, and water immediately added to them. The fluid employed had been obtained *twenty-six minutes*. Segmentation commenced in *four hours and fourteen minutes*, but was more general in *four hours and seventeen minutes*. On the eighth day there were *forty-five* embryos.

No. 8. P.M. $2^h\ 24^m$.—*About one hundred ova* were submerged for *half an hour*, and impregnating fluid obtained *forty-four minutes* before was then supplied to them, but not more than *six or eight ova* became segmented, and only two embryos were formed.

The following summary will more immediately indicate the results :—

TABLE I.—Set E.

Experiment.	Ova.	Time.	Medium.	Fluid obtained.	Segmentation.	Embryos.	Per-centage.
		″		″	′ ″		
No. 1	83	1	Water.	43	3 55	32	·38
No. 2	92	2	Water.	43	3 55	45	·49
No. 3	127	3	Water.	17	4 3	33	·26
No. 4	81	3	Air.	8	4 5	53	·65·5
No. 5	136	5	Water.	20	4 8	10	·07
No. 6	139	5	Air.	12	4 11	37	·26
No. 7	205	15	Water.	26	4 14	45	·22
No. 8	100	30	Water.	44	.....	2	·02

Thus then at a temperature of 60° FAHR. the susceptibility of the ovum to become impregnated is greatest at the time it is passed into water, and for two or three minutes afterwards, and segmentation then takes place more quickly, even when the seminal fluid has been for nearly three quarters of an hour mixed with water, than after longer immersion. The fitness of the ovum to become impregnated is gradually diminished, and segmentation takes place more tardily, according to the length of time which the ovum has remained in water, as is seen by comparing the results of Nos. 1 and 2 with 7 and 8. On the other hand, while the desiccating effect of exposure to air more arrests the fecundation of the ovum and the occurrence of segmentation of the yelk than a continuance for a corresponding length of time in water, it seems to be less prejudicial to the fecundity of the ovum than immersion in that fluid, as appears to be shown by comparison of Nos. 4 and 6 with 3 and 5, the difference in the number of ova produced being too great to lead us to attribute this to difference in the length of time the impregnating fluid had been obtained.

In the foregoing set of experiments, the quantity of impregnating fluid supplied to the ova was but little attended to, it being added very freely in each case. In the following set I was desirous of knowing what difference would result from the fluid being applied more sparingly, or but for a very short space of time. SPALLANZANI had made experiments with a similar view, but his appeared to be open to some objections, as he had not noted some important circumstances which greatly affect the result, as the temperature of the medium, the length of time the fluid employed had been obtained, &c. In the experiments now made, these circumstances were

attended to, and I noticed a curious fact which I first remarked in experiments in 1849. It is what I may designate *partial impregnation*, and is indicated by a portion only of the yelk becoming segmented. This frequently happens with ova that have been brought into contact with only very small quantities of seminal fluid, and but for short spaces of time, as in some of the following experiments. These ova, so far as I have observed, never produce embryos. Segmentation is arrested in some at the very commencement (Plate XIV. fig. 11), in others it goes on to the second or crucial fissure, and in a very few cases may proceed somewhat further (fig. 12), but is never completed to granulation of the yelk. This, I think, is a fact which deserves some consideration with reference to the formation of the embryo.

Set F. March 22, 1850.—Temperature of the room at the time of the experiment was 48°·5 FAHR., and that of the water employed 46°·5 FAHR. As the proof of impregnation is the segmentation of the yelk, and as my object now was to observe the effect of small quantities of impregnating fluid applied only for very short periods of time at a low temperature of the surrounding medium, it is not of consequence that this set was not watched to the full development of the embryo. To show the degree of susceptibility of the ovum under the combined influence of these circumstances, it was sufficient to attempt the impregnation at a low temperature, and after the lapse of an interval to remove the ova to a room of the same, or nearly the same temperature as in the set E. This was done at the end of one *hour and a half*.

No. 1. A.M. $12^h\ 12^m$.—*Fifty-one ova*, passed on a dry surface, were each touched lightly and quickly, *once* only, with a small hair-pencil dipped in impregnating fluid mixed with water, at *eleven minutes* after it was obtained, and water was then added to them. At the expiration of one hour and a half they were removed to a room of the temperature of 59° FAHR. Segmentation did not occur until the expiration of *six hours* and *a half*, and at the end of eight days only *four* embryos had appeared.

No. 2. A.M. $12^h\ 20^m$.—*Forty-two ova* were immersed in water for *five minutes*, and then exposed, and touched for an instant only as above, and again placed in water. The impregnating fluid had now been obtained *nineteen minutes*. Segmentation commenced in some of these at *six hours and three quarters*, but nearly all of them were only *partially impregnated*, and not a single specimen produced an embryo.

No. 3. A.M. $12^h\ 19^m$.—*Fifty-eight ova* immersed in water for *five* minutes, were exposed, touched for an instant as above, and again immersed for *one minute*, after which, they were well rubbed in the water with a clean pencil, and fresh water then supplied to them. The fluid employed had been obtained *twenty-three* minutes.

Not a single egg gave any signs of having been impregnated, either perfectly or partially, nor did a single ovum produce an embryo.

No. 4. A.M. $12^h\ 23^m$.—*Forty-two ova* were passed into a solution of *carmine* (the pigment employed by water-colour painters) for *five minutes*, and were then washed with water, touched for an instant, as above, with impregnating fluid at *twenty-seven minutes* after it was obtained, and water then supplied to them. Not a single egg

became fully impregnated or afterwards produced an embryo. In two or three ova there were slight indications of partial impregnation.

No. 5. P.M. 12^h 37^m.—*Sixty-five ova* having remained *fifteen minutes in water*, were exposed, and thoroughly bathed with impregnating fluid applied with a hair-pencil, *fifty-one* minutes after it had been obtained, and water was then added to them. Segmentation did not take place in these ova.

No. 6. P.M. 12^h 38^m.—*Seventy-seven ova*, passed into a *solution of carmine* for *fifteen minutes*, were exposed, and thoroughly bathed with impregnating fluid as in No. 5, and water was then added; but not a single egg gave any sign of impregnation.

Influence of Temperature.—From these experiments, it seemed evident that the susceptibility of the ovum to become impregnated is diminished in proportion to the degree of expansion of its envelope and its imbibition of fluid, conditions which are greatly affected by the temperature of the medium in which the ovum is placed during the first hour; and there seems reason to suppose that this diminution may be due to the extent to which the envelope becomes influenced by temperature, rather than to any insusceptibility at that time in the yelk itself. The following experiments, made a few hours after the above, tend to support this view.

Set G. March 22, 1850. Atmosphere 48° FAHR. Water 47°.

No. 1. P.M. 4^h 30^m.—*Fifty-eight ova* were passed from the female from which the ova used in the preceding experiments were obtained, four hours and a half after division of the spinal cord; and seminal fluid mixed with water, and obtained *fifteen minutes* before, was *immediately* added to the water in which they were immersed. The ova were removed at the end of twenty-five minutes to a room in which the temperature was then 62° FAHR. Segmentation took place in almost every ovum a few minutes *within* the *sixth hour*, at which time the temperature of the air was 64° FAHR., and that of the water 62°. At the *eighth* day, *fifty-three* out of fifty-eight ova had produced embryos.

No. 2. P.M. 5^h.—*Sixty-three ova* from the same female were well bathed with impregnating fluid, and water was then added to them. The fluid in this case had been obtained three quarters of an hour. Segmentation took place in the majority of these at the end of *five hours and a half*.

No. 3. March 23. P.M. 12^h 45^m.—*One hundred and twenty-four ova* from the same female, twenty-four hours after section of the spinal cord, were passed into water, and impregnating fluid soon after it was obtained supplied to them. The ova were placed in a temperature of about 60°, and nearly the whole produced embryos.

These facts proved that the ova employed were still fitted to become impregnated when the fluid was supplied to them in sufficient abundance, and for a sufficient length of time, and within the period during which the envelope continues to expand and imbibe most rapidly. This condition is always promoted by an early removal from a low to a comparatively high temperature during the period of expansion, as in *Set* G, in which segmentation took place in from *five hours and a half to six hours*, and when

the removal from low to high temperature was within the first half hour; while it did not occur, in the only experiment in which it happened in *Set* F, No. 1, until the end of *six hours and a-half*, when the removal from a similar low to a like high temperature was not made until one hour and a half after impregnation.

The influence of temperature is thus as marked in its effects on the impregnation of the ovum as it can be proved to be on the future development of the embryo. Impregnation is accelerated, and also is more certain in its occurrence in a high than in a low temperature. In the latter it becomes retarded and is less determined. This applies equally to the susceptibility of the ovum, and to the fitness of the impregnating fluid to effect impregnation. But in proportion as this fitness is exalted by increase of temperature, so is the duration of the capability to receive in the one, and the efficiency to communicate in the other diminished. SPALLANZANI found that the ova of toads placed in an ice-house could be impregnated at the end of forty-one hours*. PREVOST and DUMAS† also mention that they were successful, and that too to a great extent, with ova that had been twenty-four hours *in water*, the temperature during the period ranging from 18° Cent. (64°·4 FAHR.) to 22° Cent. (71°·6 FAHR.), and with some eggs that had not been immersed even at thirty-six hours‡, the temperature being then from 12° Cent. to 15° Cent. (53°·6 to 59° FAHR.). The results obtained by myself have been much less successful. Out of one hundred and forty ova obtained from a female frog, killed twenty-four hours before and preserved at or below the temperature of 55°·5 FAHR., at which the experiment was made, only a very few became partially segmented, but not one produced an embryo; although an abundance of impregnating fluid, abounding with spermatozoa, and obtained only a few minutes before it was employed, had been supplied to them. It is evident therefore that this failure was due chiefly to the ova, and not to inefficiency of the impregnating fluid. On the other hand, I have been equally unsuccessful with ova from a frog that had been killed only two hours and a half when the impregnating fluid employed had been more than four hours and a half mixed with water. In this case the failure appeared to have been due chiefly to the spermatozoa, nearly the whole of which, on inspection by the microscope, were found to be motionless and appeared to have lost their vitality. At the same time it must be mentioned that the female from which the ova employed in No. 3 of the last set of experiments were obtained still existed, in so far as the vitality of the muscular system was concerned, and therefore can hardly be mentioned in comparison with MM. PREVOST and DUMAS' observation. But while the numerical results obtained by myself have been less favourable than those of SPALLANZANI or the physiologists now mentioned, the general facts, so far as they are open to comparison, are in full accordance with them. The difference in the details of our respective observations appears to have been due in chief part to the influence of temperature at the time of the impregnation of the ova, or within the first two or three hours after the impregnating fluid has been supplied. Thus, if the temperature

* Dissertations, &c., vol. ii. p. 177. † *Loc. cit.*, vol. ii. p. 140. ‡ *Id.*, p. 134.

has been gradually rising at the time of impregnation, the fecundation of the ovum, as I have stated, has more certainly taken place than when the temperature was subsiding, the condition of the ova and of the impregnating fluid employed being equally fit in each case. SPALLANZANI has shown that in his experiments ova did not become impregnated after they had remained fifteen minutes in water. In the experiments by myself I could rarely obtain fecundation after thirty minutes' immersion. The difference of time between these results may fairly be attributed to difference in the temperature at which the experiments were made, and in great measure to the influence of this on the endosmosis and expansion of the envelopes. But it was possible that some other agent might be concerned in these results, and that light, as well as heat and immersion in water, might greatly influence them. To put this to the test, and to learn whether the difference depends entirely or chiefly on the amount of temperature, I have made two sets of experiments at precisely the same time, performed in the same way, with ova from the same female and impregnating fluid from the same male, the only difference being that within a very few minutes after the impregnating fluid was supplied, one set was removed to a higher and slightly rising temperature, from which all light was excluded; while the other was allowed to remain freely exposed to light, but in a room of ten or twelve degrees lower temperature, and which was becoming still further reduced.

The influence of light and heat on the development of the embryo has already been referred to by SPALLANZANI, PREVOST and DUMAS, RUSCONI, Dr. W. EDWARDS, and Mr. BELL. RUSCONI expressly states that light has no influence on the development of the germ*, but his observations, as well as those before made by SPALLANZANI, show that heat has a very marked influence, and this has been fully confirmed by Dr. W. EDWARDS, and Professor BELL. Very recently also the subject has been referred to by Mr. HIGGINBOTTOM†, and I have great pleasure in stating that my own observations on the influence of heat, and the little effect of light on the development of the tadpole, are in accordance with the observations made by him. But the object I have had most in view has been, as above stated, to mark the effect of heat, without light, on the changes of the ovum, more especially during the period of fecundation, the first three or four hours after the egg is laid; and onwards to the termination of what I shall hereafter propose to consider, when describing the development of the embryo,—as the end of the *third period*—the closure of the *laminæ dorsales* and the establishment of ciliary aëration on the surface of the body.

Set H, March 20, 1850. Atmosphere 59° FAHR. Water 57° FAHR.

No. 1. P.M. 1^h 14^m.—*Eighteen ova*, as they passed from the body of a frog, were touched lightly *once* with a hair-pencil that had been dipped in impregnating fluid obtained two minutes before, and mixed with about three parts of water. After these ova had remained ten minutes in water, this was removed and fresh supplied.

These ova assumed the ovoid form at the expiration of three hours and thirty-six

* *Loc. cit.*, p. 20. † Proceedings of the Royal Society, May 16, 1850; and Phil. Trans. Part II., 1850.

minutes, and segmentation commenced at *three hours and fifty-six minutes*, and was general in two minutes longer, at which time the temperature of the dark cupboard in which the ova of this set of experiments were placed had been raised to 64° FAHR., and that of the water they were contained in to 60° FAHR.

In four days and a half, thirteen of these ova had produced embryos that were then at the end of the *third period* of development, and on the eighth day the whole of them had advanced to the period when they leave the ovum and attach themselves to the exterior of the envelopes,—the end of the *fourth period* of development. The mean temperature of the locality in which these ova and the embryos produced from them were placed, was about 60° FAHR. for the entire period of eight days.

No. 2. P.M. 1^h 55^m.—*Forty-eight ova* were touched *once*, as they passed from the body of the frog, with a hair-pencil that had been dipped in a small quantity of residual fluid retained with spermatozoa on a filter, in separating these from the fluid portion of frog's semen, obtained and mixed with water forty minutes previous.

The temperature of the cupboard having been raised as in No. 1, segmentation commenced in *three hours* and *fifty-five minutes*, but was more general in *four hours*. Many of these ova were only partially impregnated, and of consequence did not produce embryos. Others passed through their changes, as in No. 1, and in nearly similar periods of time. On the eighth day *ten embryos* had been produced.

No. 3. P.M. 2^h 3^m.—*Fifty-seven ova* were well bathed as they passed from the frog with the fluid portion of semen that had passed through two filter papers and been separated from most of the spermatozoa, and which when examined with the microscope was found to contain only a very few of these bodies.

No segmentation had taken place in any of these ova at the end of *four hours and five minutes*, but several had become ovoid. At *four hours and thirty-seven minutes* segmentation had taken place in *one ovum*, and this alone produced an embryo.

Set I, March 20, 1850. Atmosphere 48° FAHR. Water 47° FAHR.—This set was the counterpart of the preceding, *Set* H.

No. 1. P.M. 1^h 15^m.—*Nineteen ova* were treated in exactly the same way as in No. 1 H, and at the expiration of ten minutes were removed to fresh water, and placed where they were most exposed to light.

No segmentation occurred in any of these ova until the expiration of *seven hours and forty-five minutes*. The temperature of the room had then sunk to 47° FAHR., and that of the water with the ova to 46° FAHR. All the changes in these ova were so exceedingly slow, that at the end of the eighteenth hour, the temperature during the interval becoming slightly further reduced, the segmentation of the yelk had not advanced further than to the formation of the first equatorial and secondary median furrows. On the fifth day the development of the germ had not proceeded further than to the commencement of the formation of the *area germinativa*, the end of the *second period*, the mean temperature during the interval having been 45°·49 FAHR.; while the ova in No. 1 H had reached the end of the third period, the mean tem-

perature in which they had been retained being 59°·5 FAHR. On the *eighth day*, when, as already shown, the *thirteen embryos* of No. 1 H had left the egg, only *three* had been developed in this set of observations, No. 1 I, and these had reached only to the commencement of the formation of the laminæ dorsales, the mean temperature of the room during the entire period being then advanced to 45°·72 FAHR.; and the completion of their third phase of development did not take place until the *tenth or eleventh day*.

Thus while *three embryos* only were produced in this experiment, No. 1 I, during exposure to light and at a mean temperature now raised to 47°·38 FAHR., *thirteen* in No. 1 H were developed in about one-half the space of time from a similar number of eggs removed from the light, and at a mean temperature of 59°·5 FAHR.; so that we seem here to have good reason to believe that a low temperature of the medium not only retards the development of the embryo, even when exposed to light, but injuriously affects the fecundation of the ovum.

The result of the next experiment coincides with the above.

No. 2. P.M. $1^h\ 56^m$.—*Fifty-one ova* were touched in the same way with spermatozoa from the filter paper, as in No. 2 H, and were retained in the same temperature as the preceding.

A few of the yelks became ovoid in about *six hours*, but segmentation did not commence until *seven hours and two minutes*, and then only in a very few. *Two embryos* only were produced from this set.

No. 3. P.M. $2^h\ 5^m$.—*Fifty-five ova* were bathed with filtered fluid in exactly the same way as in No. 3 H; but not a single ovum became segmented. Not one produced an embryo.

Thus while segmentation took place in No. 1 H, in three hours and fifty-six minutes, when the temperature was rising from 59° FAHR. to 64° FAHR., it did not occur in No. 1 I, until seven hours and forty-five minutes, when the temperature during the interval was sinking from 48° FAHR. to 47° FAHR. This sufficiently marks the great influence of temperature during the earliest periods of change in the ovum; and this injurious effect of reduction of temperature at that period is further shown in the relative number of embryos in these comparative experiments. That the injurious effect of reduced temperature at the time of impregnation is mainly the cause of this result, and not the diminution of temperature after the period of impregnation, seems to be shown in the circumstance, that while at the end of the eighteenth hour the ova in the set H had already passed through all the stages of segmentation, and the surface of the yelk had become granulated, and the blastoderma had begun to be formed even although the temperature in that case subsided a little after segmentation had commenced,—from 59° to 57°,—the corresponding set of ova, No. 1 I, had advanced only to the octuple division of the yelk. A similar difference in the rate of development we have seen takes place in the growth of the embryo. At the end of three days the embryos of set H were advanced to the stage at which the laminæ

dorsales are proceeding rapidly to meet, and form the median dorsal sulcus of the growing body. But the corresponding ova in set I had not been carried further than to the earliest perceptible indications of the *area germinativa*.

These facts sufficiently prove the great influence of temperature on the development of the embryo in its earliest stages, as the comparative numerical results do also its effects on the impregnation of the ovum. Out of eighteen ova placed in the higher and increasing temperature, thirteen produced embryos at nearly similar stages of growth; while of nineteen ova maintained in a low and diminishing temperature only eight became segmented, and but three of them arrived at the tadpole state.

A somewhat similar but more marked result took place with the ova of No. 2 in the two sets of experiments. The impregnating means employed in these trials had already been forty minutes mixed with water on the filter. Out of forty-eight ova employed in set H, twenty-five became segmented, and ten of these produced young. But in set I fifty-one ova gave birth to only two embryos.

In the third experiment of each set the difference is as strongly marked. As the filtered fluid employed in both was the same, and the very few spermatozoa contained in it were by the same means brought into contact with the ova in each, it might have been expected that each would have produced embryos. But while the production of a single tadpole in the one case, at a high temperature, may be looked upon as leading to the inference that these bodies are the efficient agents in impregnation, the entire absence of all appearance of impregnation in the ova of the other set, to which the same fluid had been equally applied, seems to point to the cause of failure in this case as depending on the prejudicial effect of a low temperature of the surrounding medium on their agency.

The temperature of the surrounding medium ought, therefore, always to be borne in mind when we are attempting to deduce conclusions from experiment on impregnation and development. The presence of light appears to be only of secondary consideration as compared with heat; since in set H, from which light was carefully excluded, not only did impregnation take place more certainly and rapidly than in set I which were exposed to light, but the embryos also were produced in greater number, and acquired maturity in less than one-half the space of time than in the latter; the only difference of circumstance between the two sets being degree of temperature.

Influence of Aëration.—Next in importance to heat is a free *aëration* of the ovum. This is of less consequence with reference to impregnation than to the subsequent production of the embryo. In every set of experiments there are always some ova more advanced than others. These are ova which have been nearest to the surface of the water, and which, consequently, have been more completely aërated as well as exposed to a slightly higher temperature than others at a greater depth. It is from this cause chiefly that the results of experiments on artificial impregnation, and even of observations on naturally impregnated ova, are always less complete and

successful than what takes place with regard to the ova in the natural haunts of the species. The ova in a state of nature are usually deposited in well-aërated places, clear, slow-moving water, or shallow and but slightly turbid water. It is almost impossible to afford to ova that are the subjects of experiment, either in broad flat dishes, or in glass vessels in one's study, the amount of aëration required to ensure complete success. By too frequently changing the water in the vessels the embryos often become injured; while if the water be not changed, development is arrested, and decomposition commences, and the experiment entirely fails. Even when these difficulties are obviated by a gentle withdrawal of the water, and a renewal of it with equal care, the perfect stillness of the fluid in the interval of our observations does not allow of that extent of aëration to the embryo which it gains in a perfectly natural state, either in slow-moving waters, where I have usually found the eggs deposited, or in pools of still water, the surface of which is agitated by currents of air, and affected by diurnal changes of temperature.

Thus then we may conclude that the procreative force of the germ, and of the impregnating fluid, is augmented by increase of heat, but the duration of the force is lessened. It becomes less and less energetic in proportion as the temperature is diminished, but the period during which it is capable of being exerted is extended. In each of these conditions aëration is of essential consequence, and becomes more and more necessary in proportion to the increase of heat.

4. THE AGENCY OF THE SPERMATOZOA IN IMPREGNATION.

It is evident from the last-mentioned experiments, that however great may be the influence of temperature in accelerating or retarding impregnation and development, and however much the operation of this influence may be interfered with by want of proper aëration of the ovum, the impregnating force does not equally pertain to all parts of the seminal fluid, but is to be found in some only of its constituents. Experiments made before those now detailed,—and to which I had been led by a conviction that the opinion formerly entertained, that impregnation is effected through means of the fluid portion only of the semen, was not in accordance with facts I had very long been acquainted with in the Articulata,—convinced me that the spermatozoa themselves, and not the other constituents of the semen, are the efficient agents of impregnation. Leewenhoek, and, as I have since found, Prevost and Dumas, not only believed this to be the fact, but also held the opinion that the spermatozoa penetrate bodily into the ovum; and this view has been more recently insisted on by Dr. Martin Barry, with the additional belief that a perforation or fissure exists in the envelopes of the ovum, through which the spermatozoon enters. On careful examination of the envelopes of the ovum of the Frog, I have not been able to detect any fissure or orifice. The question of the agency of the spermatozoa, nevertheless, appears to be capable of solution, however difficult it may be to ascertain the mode and particular nature of such agency. The separation, as far as possible, of

the spermatozoa, by filtration from the fluid in which they move, and testing the ova with these and the fluid separately, afford good proof of the agency of these bodies; while immersion of the ova in coloured fluids, at the moment of their passage from the body of the frog, seems equally fitted to ascertain the believed existence of a fissure or perforation through the envelopes during their expansion. The experiment of filtration was originally performed by PREVOST and DUMAS with well-marked results, and it has since been repeated by the first of these observers* by a different mode, —endosmose through the operation of galvanic currents. The mode pursued by myself was that originally adopted by these observers:—careful mechanical filtration, by simply passing the fluid portion of diluted semen through folds of filtering-paper. The paper I have employed, and which alone was fitted for the purpose, was the best Swedish filtering-paper employed by chemists in their most delicate analyses. A large proportion of the spermatozoa were always retained, even on a single filter, although a few usually passed through; but this, as the results show, was not in reality a disadvantage, when a few only were present in the filtered portion. When three or four folds of filtering-paper were employed, the whole of the spermatozoa were removed.

Filtration of Seminal Fluid.—Fluid obtained from a male frog, immediately after removal from the female, was mixed with about twice its quantity of water and placed on the filter. Portions of this fluid as they passed through were repeatedly examined with the microscope. Some of these filtered specimens contained a very few spermatozoa, usually not more than three or four in the drop examined, but sufficient occasionally, as the results proved, to effect impregnation.

Filtration Experiments.—Set K. March 14, 1849. Atmosphere 55°·5 FAHR. Water 55° FAHR.

No. 1. A *single drop* of the *filtered fluid* was added to one ounce of water, in which *forty-six ova* were immersed. Not a single egg became segmented or produced an embryo.

No. 2. A *single drop* of the diluted fluid, *not filtered*, but two hours after it had been obtained, was added to one ounce of water with *ninety ova*. Not a single egg was segmented or produced an embryo.

No. 3. *Two drops* of *filtered fluid* were added to one ounce of water with *sixty ova*, but not one egg became impregnated.

No. 4. *Three drops* of *filtered fluid* were added to one ounce of water with *one hundred and five ova*. *Two* of these were partially impregnated, as shown by their becoming imperfectly segmented (Plate XIV. fig. 11 and 12), but neither of them produced an embryo.

No. 5. *Three drops* of diluted fluid, *not filtered*, but two hours after being mixed with water, were added to one ounce of water with *seventy-six ova*. Several of these became segmented, but more tardily than in the following experiment, No. 7. At the end of seventeen days *fifteen* embryos had been produced from these ova.

* Journal de l'Institut, 1840, No. 362, p. 908.

No. 6. *Thirty drops* of *filtered fluid* were added to one ounce of water with *two hundred and ten ova.* At the expiration of five hours two ova had become segmented, and two embryos were afterwards produced.

No. 7. *Thirty drops* of diluted fluid, *not filtered,* were added to water with *two hundred and fifty ova.* At *four hours and forty-two minutes* segmentation had commenced in two or three of these, and in *five hours* had occurred in almost every ovum. Nearly the whole of these produced embryos.

No. 8. About *thirty drops* of the same diluted fluid, *not filtered,* were added to water that contained about *two hundred ova,* passed from the body of a frog killed twenty hours before. A few of these ova became imperfectly segmented, but not one produced an embryo.

From these first experiments with filtered fluid, it seemed that the portion of semen which passes through the filter has not the power of impregnating, unless there are spermatozoa present in it; while similar quantities of diluted semen that has not been filtered, are efficient and impregnate, as in Nos. 4, 5, 6 and 7. Further, it is shown, from No. 8, if we may judge from one experiment, that ova which have remained in the body of a frog twenty hours after actual death and cessation of the organic functions, and in a temperature of 55° FAHR., may be affected by the stimulus of the impregnating fluid, but not sufficiently so perhaps as to result in fruitful impregnation.

During the time these ova were under observation, in March 1849, an opportunity occurred of observing the effect of reduced temperature on the rate of development of the embryo when its formation has been somewhat advanced. On the seventh, eighth and ninth days after impregnation of the ovum, and when the temperature had already been considerably reduced, the season became severe, and in order to test the effects of cold, the eggs were removed to the open air and exposed to a keen wind. The temperature of the atmosphere was then 38° FAHR. During the night of the tenth day, the water in which the ova were contained was frozen to a mass of ice. Yet many of these ova, as above shown, produced embryos. SPALLANZANI had already remarked, that the eggs of the Frog may be inclosed in ice, and yet afterwards produce embryos, if the envelope does not become frozen*.

The experiments made to ascertain whether cleavage of the yelk may be taken as a test of impregnation (p. 190), seemed also to show that, *within certain limits,* a large or small quantity of seminal fluid has some influence on the more or less early occurrence of this phenomenon. It occurred to me, therefore, that in making the experiments now given, two questions might be examined: one, as to whether the extremely minute quantities of seminal fluid disseminated in water, as mentioned by SPALLANZANI, are as efficient to produce the embryo at the low temperature of the season at which the Frog spawns in this country, as in the warmer region of Italy; and the other, whether the presumed efficiency of such minute quantities depended on the presence of the spermatozoa; and it seemed possible to put these

* Dissertations, &c., vol. ii. p. 49.

questions to the test in one set of experiments. But, in attempting to do so, it was a matter of importance, first, to obtain some approximative knowledge of the actual quantity of spermatic fluid employed by SPALLANZANI, in his more remarkable experiments, to enable me to compare the results of my observations with those of his. SPALLANZANI states, that by his mode of obtaining the seminal fluid, by vivisection, he could only procure from "two to three grains*" from each individual. Three *grains* weight of fluid are equal, by measure, to nearly three *minims* of our medicinal standard. But it may be presumed that in so delicate an operation as that of removing the fluid from the vesiculæ seminales, from which SPALLANZANI states he usually obtained it, he could rarely be very precise in his determination of the quantity. I assume, therefore, for the sake of comparison, that the quantity he speaks of as "a *grain*" was about equal to a *minim* of our medicinal measure. Three grains of fluid (? minims), he says, were mixed with a *pint and a half* of water, and one *drop* of this mixture (the seminal fluid in which was equal to $\frac{1}{3840}$th part of the whole at 16 oz. per pint) applied directly to an ovum on the point of a needle, was "frequently" sufficient to render it fruitful†. The *drop* spoken of in this experiment was a much less quantity than the grain or minim; indeed, SPALLANZANI states that it did not exceed the "fiftieth of a line" (? fifth) in diameter. The quantity of fluid obtained by myself from a frog was usually about *six minims*, and this I mixed with twice its quantity of water, thus making eighteen minims. One *drop* of this mixed fluid measured, as I found, one-third of a minim, and consequently contained one-third part of seminal fluid, or one-ninth of a minim of the seminal fluid. Yet the result of the employment of one drop of this mixed fluid added to one ounce of water, in which the proportion of seminal fluid was then made to be $\frac{1}{4320}$th part of the whole, did not lead to the same result as the experiment by SPALLANZANI. The difference arose, perhaps, from the operation of two or more causes:—first, the much lower temperature of the atmosphere at the time of making my experiment, than at that of SPALLANZANI's; next, the diminished efficiency of the seminal fluid, owing to the length of time (two hours) it had been removed from the body. Both these circumstances, but especially the first, as already shown, operate unfavourably in experiments on impregnation, more eggs being fertilized at a high temperature, when the changes go on rapidly, and especially when the seminal fluid has been most recently obtained, than under the opposite conditions. The general results of my experiments, however, may be regarded as quite confirmatory of SPALLANZANI's more remarkable one, as they prove, like that, that only an exceedingly small proportion of seminal fluid is necessary to fertilize the ovum.

* *Loc. cit.*, vol. ii. p. 189.

† *Loc. cit.*, vol. ii. p. 192. There is some confusion of statement in the translation of SPALLANZANI's work, now referred to, respecting the quantities mentioned by the author, as "pint" is the word used in some passages and "pound" in others (p. 191), apparently synonymously, while the latter is further spoken of as "twelve ounces."—G. N.

But however satisfactory these experiments were with regard to that fact, they still left the question of the immediate agency of the spermatozoa in impregnation in doubt. I therefore repeated them with greater precision, and with that object only in view, and took especial care to obtain as perfect a filtration and separation of the spermatozoa from the fluid as possible. The results were far more interesting and instructive than I had anticipated, as I found by repeated examination by the microscope that the filtered fluid was almost completely deprived of spermatozoa, one or two only being occasionally detected in it, with a very few nuclei and spermatozoal cells. One circumstance that tended to increase the value of this set of experiments, and to prove the influence of the spermatozoa, was, that the ova employed were not fully matured, and hence I had less expectation of a favourable result. The quantity of seminal fluid obtained was larger than usual, and this was mixed with twice its quantity of water. This mixed fluid was divided, as before, into two portions, one of which was filtered, and the other not. The experiments were commenced in the early part of the day, and the temperature of the atmosphere of the room, and that of the water employed, was nearly the same, 51° FAHR., and the whole of the experiments were placed as nearly as possible under similar circumstances.

Set L. March 18, 1849. Atmosphere 51° FAHR. Water 51° FAHR.

No. 1. *Ten minims* of the mixed fluid were added to two ounces of water, into which *one hundred and fifty ova* were immediately passed. One hour afterwards I found a great abundance of spermatozoa adhering to the surface of the envelopes. Segmentation of the yelk commenced in *five hours and forty minutes* in a few ova; and took place in others at a later period. A few only of these ova produced embryos.

No. 2. *Ten minims* of the filtered portion of the mixed fluid were added to two ounces of water, and about *one hundred and fifty ova* were passed into it. When these ova were examined at the expiration of an hour, not a single spermatozoon was detected on any of them; and when repeatedly examined at the end of five and six hours, not one showed any signs of cleavage. This change did not take place in any of them even at a later period, and *not one produced an embryo.*

As the ova in these two experiments were the first that passed from the body of the Frog, it was fair to regard them as being the most matured; segmentation ought, therefore, if it occurred at all, to have taken place at an earlier period in these than in others afterwards obtained from the same female.

No. 3. *The filter paper* employed in separating the fluid used in the last experiment, No. 2, and which retained the separated spermatozoa in a minute quantity of fluid that had not passed through, was placed in two ounces of water, and *one hundred and thirty ova* from the same female were shed upon it. When some of these ova were examined at the end of an hour and a half, spermatozoa in vast abundance were found adhering to every part of their surface, but the whole were then motionless, and apparently dead and partially coiled on themselves (Plate XIV. fig. 8 *c*). In

some of these the body appeared to be slightly enlarged. Segmentation commenced in very many of these ova in *five hours and ten minutes*, and was almost universal in them a few minutes later. *Nearly the whole of these ova produced embryos*, there being only nine out of the one hundred and thirty that were abortive. I have sometimes found a much larger proportional number of unproductive ova in some broods in the natural haunts of the Frog.

There was one exceedingly interesting fact in this experiment,—it was that the smaller and apparently less matured ova were as fully impregnated as the larger and more perfect. The whole set of observations was the more interesting from this circumstance. These ova were smaller than usual and had not the white surface so complete, but were very like the ova in which I have described the changes in the light-coloured surface (p. 185). When first placed in water with the filter paper and spermatozoa, the surface of the yelks became more contracted and irregular, within the vitelline membrane, than in other ova I have employed. Hence I had much doubt, at first, whether any satisfactory evidence would be obtained from this set. But the contrary has been the case, as it is evident that some ova may be impregnated at a little earlier period than usual; and that when a great abundance of spermatozoa is supplied, ova less matured than others may be equally well impregnated. The greater efficiency of a larger as compared with a smaller number of spermatozoa, with reference to the earlier or later segmentation of the yelk, has already been shown; and the difference is very marked in the first and third of this set of experiments, both with reference to the occurrence of segmentation and to the relative fecundity of the ova. On the other hand, the experiment No. 2 proved that the *liquor seminis is not* the fecundating portion of the seminal fluid.

Circumstances having prevented me from making known the result of these experiments at the time they were obtained, I determined, during the past spring, to repeat and vary them, to obtain, if possible, still more conclusive proofs that the spermatozoa alone effect impregnation. Accordingly, in March and April of the present year (1850) I repeated them, with the following precautions:—first, that the frogs in each case had been some days paired, and at the time of the experiment were nearly ready to spawn; next, that the seminal fluid used was obtained from the male paired with the female from which the eggs were taken; further, that the condition of the specimen of fluid used was correctly ascertained; and lastly, that the ova were placed in flat dishes, under precisely similar circumstances, with similar quantities of water, repeatedly changed. Two sets of experiments were made at the same time.

Set M. April 4, 1850. Atmosphere 60° Fahr.

The seminal fluid employed, mixed with an equal quantity of water, was placed on a *single*, and caught on a double filter paper, and the clear fluid that passed was then examined with the microscope. The fluid that had traversed the three filters was almost completely deprived of spermatozoa; as, after many very careful examinations, both

of drops as they passed the last filter, and of portions from the capsule it was caught in, there was only one instance of the presence of spermatozoa. In this I saw *two*, but perfectly motionless. The fluid that passed through the *first* or top filter, I had reason to suspect was contaminated by a small quantity having travelled over the sides of the filter. I knew also, that a few spermatozoa always penetrate through a single filter, or rather perhaps are carried through by the fluid. Accordingly, I detected several spermatozoa in this portion. The fluid that remained in the interior of this filter abounded with very active ones. It was not employed until fifty-three minutes after it had been procured from the Frog.

No. 1. *Seventy ova* were passed on a dry surface, and a portion of the fluid which had traversed three filters was poured carefully over them, and water was then added.

In *four hours and twenty-five minutes* I found *one ovum* partially impregnated, and the yelks of others were somewhat shrunk, and a little irregular, an appearance frequently seen in ova that have not been impregnated. Not a single ovum, however, was fruitful.

No. 2. *One hundred and twenty-seven ova* were shed into water on the second of the three filters, which still retained a portion of the fluid from the first, and in which I had found a few spermatozoa. At *four hours and fifty minutes* several of the ova had begun to be divided, and out of this set of eggs *sixteen embryos* were produced.

No. 3. *One hundred and sixty-three ova* were passed into water on the topmost of the three filters, and *four hours and fifty minutes afterwards* a very large proportion of them had become segmented, as was expected, although from the circumstance of the seminal fluid having been more than one hour mixed with water before it was employed, the number was not so great as usual. But it was not entirely due to this cause. Many of the ova had been injured before impregnation, and these became irregular and pear-shaped. At the end of twelve days *forty-nine* well-developed embryos had been formed.

Set N. April 4, 1850.

Four filters were now used instead of three, the seminal fluid being obtained and mixed as before. The fluid which had passed through the whole of these filters, contained not a trace of spermatozoa or of the nuclei of cells. That which had passed through two only still gave an occasional perfectly motionless spermatozoon, but not a single one in motion; the fluid from the uppermost or first filter, as in *Set* M, still swarmed with myriads of active spermatozoa. It was thus proved that the filtration was complete.

No. 1. *One hundred and thirty-one ova* were passed on a dry surface, and a portion of the fluid which had traversed the four filters was poured over them, and water was then added. But not a single ovum gave any sign of having been impregnated, not one became segmented, nor was a single embryo produced. The ova became slightly irregular, shrunken, and depressed at parts of the yelk, a condition which, as I had

noticed this in other ova, I was inclined to attribute to some normal change in the yelk itself, perhaps of an imbibing or endosmic character.

No. 2. *One hundred and ninety-seven ova* were shed into water mixed with the remaining portion of fluid which had passed through the four filters, but not a single egg gave even a trace of segmentation. Many of the yelks had the same peculiarly irregular outline as in No. 1. These two experiments I regarded as a satisfactory proof that it is not the fluid portion of the semen which impregnates.

No. 3. *Two hundred and four ova* were passed into water upon the third filter, already immersed in it, and the fluid on which showed an occasional spermatozoon. I was unable to detect any impregnated ova in this experiment, but, as the result showed, a few had been affected, as *four embryos* were produced.

No. 4. *Three hundred and seventy-one ova* were passed into water upon the first or topmost of the four filters, and which had already been placed in the water. At *four hours and thirty minutes,* almost every yelk had become segmented. The change had occurred some length of time before this, as the second or crucial segmentation was commenced. This experiment seemed to be a most direct and conclusive proof of the agency of the spermatozoa. At the end of four days almost the whole of the eggs were producing embryos, many of which were advanced to the *fourth period of development. One hundred and twenty-seven* became fully formed and vigorous, besides nearly as many more which did not complete their changes, from an accidental cause.

Before these concluding experiments were made, I had already, in March last, repeated the preceding; but, as the filtration was less perfect, have thought it unnecessary to give them in detail; they agreed however in the results. The whole have confirmed in the fullest manner the experiments first made, and have proved, as I trust, satisfactorily that the spermatozoa alone are those parts of the semen which effect the impregnation of the ovum. Having repeated the filtration in five separate sets of experiments, on different occasions, and with exactly the same general results, I can no longer entertain any doubt of the direct agency of the spermatozoa. The conclusion, I think, is rendered certain by facts now shown, which escaped the notice of Spallanzani, and of Prevost and Dumas. Segmentation of the yelk takes place earlier when impregnation is effected by a large, than when occasioned by a very small number of spermatozoa, the temperature of the surrounding medium, and all other circumstances, being alike in the two cases, as in the experiments *Set* A as compared with *Set* B (p. 190). This fact is supported by another, equally significant. When only a very small number of spermatozoa exist in the fluid, then the remarkable result of partial impregnation often takes place, and the ova are unproductive. On the other hand, when spermatozoa are supplied in full abundance to the ova, not only does segmentation of the yelks take place more rapidly, but also more extensively, and almost every ovum produces an embryo.

With regard to the liquor seminis, it seems equally decisive that this portion of

the seminal fluid *does not* effect impregnation. WAGNER and LEUCKARDT* have justly remarked, that it is almost impossible that the *liquor seminis* can have any action on ova which are expelled into water before the semen is ejected by the male, as in the case of frogs and fishes; and it is worthy of note, that in the experiments now detailed not a single ovum was either *completely* or *partially* impregnated when immersed in water mixed only with the liquor seminis, obtained through filtration; nor even when the ova were carefully bathed, as they passed from the body of the Frog, and before they had been brought into contact with water, with the filtered fluid from which the spermatozoa had been completely separated, as in *Set* N, No. 1, or even when the fluid still contained a very few dead and perfectly motionless spermatozoa. Yet this fluid can hardly be regarded as entirely without use, although it now appears to be of very secondary consequence. When the ova were placed in water with which only the *liquor seminis* had been mixed, the yelks became contracted exactly as is often the case in the unimpregnated ovum. Whatever may be the nature of this fluid, it does not appear to be essential to the conveyance of the structural peculiarities of the male parent to the offspring. These appear to be communicated by the spermatozoa alone, as not only did the ova that were impregnated by spermatozoa from the filter paper, as in *Set* N, No. 4, become segmented quickly, but the embryos produced from them came forth with all the usual characters of tadpoles, and have passed, or are now passing (June 20) through their stages of growth as perfectly and as quickly as others which have been produced in the natural haunts of the species through the mutual concurrence of both sexes. Thus the liquor seminis does not even hasten the course of development of the young. Neither does it accelerate that of fecundation, either through direct imbibition or from becoming a solvent to the bodies of the spermatozoa; as we have seen that segmentation of the yelk takes place most quickly in proportion to the number of spermatozoa, within certain limits, in contact with the ovum. And such also is the case in a state of nature.

These facts appear to give that *direct negative* and refutation to the hypothesis of the immediate agency of the liquor seminis in impregnation which WAGNER and LEUCKARDT† remark it has not hitherto met with; and they lead to the supposition that one of the chief uses of the fluid is merely that of a vehicle through which the spermatozoa are more readily brought into contact with the ova. Possibly it may bear that relation to the spermatozoa in the viviparous vertebrata, in which it chiefly occurs, which the fluid medium into which the ova of Amphibia and Fishes are expelled, bears to the spermatozoa in those classes. This view may derive some support from the fact that the liquor seminis has recently been shown by chemical analysis to consist chiefly of a thin solution of mucus, with small quantities of chloride of sodium and phosphates and sulphates of the alkalies‡.

* Cyclopædia of Anatomy and Physiology, vol. iv. "Semen," part xxxiv. January 1849, p. 507.

† *Loc. cit.*, p. 507. ‡ *Id.*

5. NATURE OF THE AGENCY OF THE SPERMATOZOA.

Having obtained full evidence by direct experiment that impregnation is effected through means of the spermatozoa, we have now to inquire as to the manner in which it is induced by them, and as to the nature of the agency they exert. Spermatozoa have been found adhering to the surface of the impregnated ovum by Barry, Bischoff, Pouchet and others, in the Mammalia; as they were long ago seen by Prevost and Dumas on the ovum of the Amphibia; and since by Siebold, Kölliker, myself and others in the Invertebrata. They have been constantly present in those experiments on artificial impregnation which I have now detailed, in which the yelks became segmented after the egg had been in contact with seminal fluid, or with the filter paper used to remove them from the liquor sanguinis; but they have not been detected on ova which did not undergo the cleavage of the yelk, or which had been immersed only in fluid separated during filtration. Whenever present in fluid in which ova have become impregnated, the spermatozoa have always been found in motion, until after they have become attached to the surface of the ovum, when their motion has soon ceased. In the *Lissotritons*, in the few experiments I have made on this division of the Amphibia, I have seen the motion continue for a long time after they have been in contact with ova. This leads to the supposition that a vibratile condition or power of motion is in some way essential to their power to effect impregnation; notwithstanding that, as Wagner and Leuckardt have remarked*, no movements have as yet been perceived in the spermatozoa of the *Isopoda* and *Amphipoda*. If this vibratile condition be essential to their function, then the length of time which it is continued may be of importance.

Duration of Motive Power in Spermatozoa.—Spallanzani found that water mixed with but a small quantity of seminal fluid of the Frog retained the property of impregnating ova longer than pure semen†. When inclosed in a glass tube, the semen of the Toad was not impaired at the end of six hours, but was useless at the end of nine‡; while a small quantity from the Frog, mixed with water and preserved at a temperature of about 40° Fahr.§, was still efficient at the end of thirty-five hours. Results obtained by myself have fallen short of this extended period, even when the influence of temperature has been attended to. The difference of result may to some extent be accounted for in the condition of the fluid, and in the way in which it has been obtained. Spallanzani says he obtained it both from the vesiculæ seminales and the testes after opening the body. It is probable therefore that a large part of what he procured had not arrived at maturity, and instead of consisting almost entirely of active spermatozoa, as when it is obtained by compression of the body, the

* *Loc. cit.*, p. 503. † Dissertations, vol. ii. p. 193. ‡ *Id.* p. 168.

§ It may be well here to mention, that for the purpose of more easily comparing the observations recorded by Spallanzani, and Prevost and Dumas, with the results obtained by myself, I have, throughout this paper, reduced the data given by them to the scale of Fahrenheit, employed by myself; the scale used by Spallanzani being that of Reaumur, and by Prevost and Dumas the Centigrade.

mode adopted in my experiments, it included a large proportion of developmental cells from which the spermatozoa escaped at longer or shorter periods after the fluid had been mixed with water, or had been retained for some time out of the body. I have very rarely found the seminal fluid of *Rana temporaria* obtained in the way stated, and at a temperature of about 50° FAHR., retain any impregnating influence for more than four or five hours. Thus, after mixing it with an equal proportion of water, very many of the spermatozoa have soon become motionless, and in less than two hours a moderate proportion only have continued active. At three hours there have been still fewer moving; while at four hours the great majority of them have exhibited most unequivocal signs of lost vitality, being either extended at length or coiled on themselves (Plate XIV. fig. 8 *c*), as they usually appear when motionless, and adherent to the surface of ova. If any, at this length of time, have been still moving in the fluid, they have been few in number, and their motions exceedingly feeble. Occasionally I have detected others, at this lapse of time, in the act of escaping from the cells (fig. 8 *a* and *b*), and these have always been the most energetic in their movements immediately after their liberation. Further, I have noticed that in those specimens of fluid which have contained most developmental cells, the spermatozoa have been longest in a state of activity.

The following have been the results of observations on spermatozoa attached to the surface of ova, or contained in the water in which ova were immersed. At *three-quarters of an hour* after mixing *recently obtained* seminal fluid with the water and ova, vibratile spermatozoa have continued to be exceedingly abundant and in a state of great activity. *At one hour and a quarter* there were still an abundance in motion, but many were now perfectly motionless, and apparently dead. At *one hour and a half* I was not able to detect any movement in even a single spermatozoon out of a vast abundance which adhered to the surface of the gelatinous coverings of the ova, although I sought for this very carefully. Neither could I detect even the slightest indication of the spermatozoa having penetrated into these coverings, either near the surface or in the vicinity of the thicker envelope, which I regard as the *chorion*, and which immediately covers the *vitelline membrane*. After a lapse of some time all the appearance of spermatozoa on the exterior of the envelopes ceased. The longest period, after contact with spermatic fluid in water, at which I have hitherto been able to recognize these bodies on the surface of the frog's egg has been *six hours and one or two minutes*, and about half an hour after segmentation of the yelk had commenced. This was on ova impregnated artificially, on the 14th of March, at a temperature of the atmosphere of the room of 54°·5 FAHR. and 53°·5 of the water employed. A few motionless spermatozoa were then still found on the surface, but most of them appeared to be becoming disintegrated. The surface of the egg-envelope was then covered at places with numerous small granules, possibly the remains of spermatozoa which had disappeared.

A somewhat similar result has ensued when spermatozoa have been *two hours* mixed

with water before ova were passed into it. At *five minutes* after immersion of these ova, I have found large quantities of spermatozoa already adhering to the surface of their then expanding envelopes. But many of them have already been coiled on themselves, and were perfectly motionless. The water still contained very many disseminated through every part of it, but most of them, with few exceptions, have appeared to be rigid, and to have become enlarged in diameter, but not increased in length. This change has appeared to be due to the hygroscopic nature of these bodies, as formerly pointed out by Siebold*. Possibly this nature may have some reference to impregnation. Repeated observations lead me to believe that, in whatever way the spermatozoa are concerned in impregnation, they do not penetrate bodily into the ovum, but merely adhere to the surface.

Prevost and Dumas concluded† from their investigations, that the spermatozoa of the Triton and Frog do penetrate into the envelope of the egg; and they state that they had fecundated ova taken from the ducts of the Triton, and after the lapse of three hours, having first carefully washed them to remove all that were merely adhering to the surface, have made sections of the envelopes of the egg, and, with the aid of the microscope, have found living spermatozoa still struggling within. Their words are—"Une grande quantité d'animalcules encore mouvans, et qui semblaient se débattre dans cette espèce de gelée où ils se trouvaient emprisonnés. On en voyait partout même au contact des membranes de l'œuf." Further, that they had repeated this experiment on the ova of the Frog, and found the envelope penetrated in like manner with spermatozoa, still moving, but not changing place. I regret much that my investigations do not enable me to confirm these observations, which seem to me to be due to the circumstance of these physiologists having regarded the objects on the surface as being in the interior. I have many times sought for spermatozoa within the substance of the egg-envelope of the Frog, at different periods between that of first contact with impregnating fluid and the time when cleavage of the yelk has commenced, and have constantly found them on the surface, but have never, even in a single instance, observed any appearance of them in the substance of the envelope, nor anything which induced me to suspect that they penetrate bodily into it. They have been present on the surface, and adherent to it, even from within a few seconds after contact, to so late as the sixth hour, but have usually been motionless; and most of them have had the caudal portion folded back on the body. I was led to make these observations on the egg of the Frog,—before I was aware of MM. Prevost and Dumas' views,—from the circumstance of Dr. Martin Barry having mentioned‡ that an orifice or fissure exists in the thick investing membrane of the ovum of the Rabbit, through which, at the time of impregnation, he believed the spermatozoon to enter. All the observations I have been able to make on the ovum of the Frog, both microscopically and experimentally, are opposed to the belief in the existence of any perfo-

* Müller's Archiv, 1836.

† *Loc. cit.*, vol. ii. p. 133.

‡ Philosophical Transactions, 1840, p. 535.

ration either before or at the time of impregnation. With regard to the ovum of the Tritons, I have recently made the following observations, since becoming acquainted with the views of the authors named.

A female *Lissotriton punctatus*, obtained on the 17th of May, produced several ova on the morning of the 19th. Cleavage of the yelk (which, I may remark, was entirely overlooked by Rusconi* in his account of the Newts) commenced in two of these ova at the expiration of eight hours, the temperature during the period having ranged from 56° Fahr. to 62° Fahr. On examining these ova very carefully about an hour before the cleavage commenced, there were what I regarded as portions of the bodies of spermatozoa on the surface, but certainly no traces of any in the interior. While engaged in this examination the same female produced another egg, which she inclosed as usual in a folded leaf. On this specimen, examined at the end of half an hour, I could not detect any spermatozoa on the surface, which led me to imagine that it had not been impregnated, a supposition which ultimately proved to be correct, as no segmentation of the yelk took place in it. Some time after this, the temperature of the atmosphere being 62° Fahr., I saw the same newt enclosing another egg in a leaf. This I immediately removed for examination, and thinking that this, like the previously deposited egg, had not been impregnated, no traces of spermatozoa being found on its surface, I placed it for about a minute in a small capsule filled with water, into which a quantity of fluid had just been expressed from a male that had been kept separate from the female. The fluid on examination was found to be composed almost entirely of very active spermatozoa. The egg was examined three minutes after immersion, and scarcely five minutes after it had been laid, and multitudes of spermatozoa were then seen adhering to its surface. Most of them were still vibrating rapidly, while others were motionless. But although I was able to distinguish every part of these bodies, I could not detect any in the act of penetrating, or which had already penetrated into the substance of the envelope, and most certainly not one was imbedded in the interior. Neither were there any in contact with the yelk-membrane, or in the yelk-chamber of the envelope.

The egg of the Newt is peculiarly fitted for an examination of this kind, from the fact of the existence of this yelk-chamber, or space in the interior of the envelope. This is formed by the gelatinous covering which the egg gains in the oviduct imbibing fluid by endosmose and becoming expanded immediately it comes into contact with water, when the inner layer separates from the vitellary membrane, with which it has been in contact within the duct; and as the outer layers more and more expand, the yelk, covered only by the vitellary membrane, is left free in a large cavity in the interior, surrounded by a thin fluid. The spermatozoa of the Newts, as is well known, are of large size, and are easily recognized; so that in the event of their having penetrated the egg-covering before it leaves the duct, or at the moment of its expansion, or after the chamber has been formed in it, they can hardly escape observation.

* Amours des Salamandres Aquatiques, 4to. Milan, 1821.

I preserved this egg confined to one spot in a minute glass capsule, and in exactly the same position beneath the microscope, for forty-eight hours. During the first three quarters of an hour many of the spermatozoa on its surface exhibited as vivid motions as at first, but still adhered to the same parts, and had not, so far as I could perceive, changed their posture or their place in the least, or had penetrated in the slightest degree into the envelope. At the end of twelve hours I found that the yelk had undergone the usual process of cleavage, which, at that time, had already been advanced to the stage of coarse granulation of the surface, a fact which proved most distinctly that this egg had been impregnated. Spermatozoa were still distinctly recognized over the whole surface of the envelope, but their motions had now ceased. On the following morning, May 20th, the changes were found to have proceeded uninterruptedly, as the yelk was then finely granulated over its whole surface. At a little later period, the *end of twenty-four hours*, a few spermatozoa were still adhering to the surface of the envelope, but the whole were perfectly motionless, and many had evidently disappeared. Still, not one could be detected in the substance or in the chamber of the envelope. At *twenty-eight hours* the granulation of the yelk was nearly completed, and its surface was becoming smooth, and there were still a very few motionless spermatozoa on the exterior of the envelope. At the end of *forty-eight hours*, May 21st, I was still able to recognize the bodies of several spermatozoa which had not yet disappeared, but which had become very indistinct, as if in a state of diffluence. At the end of *sixty hours* I could no longer detect any trace of them. The egg that was the subject of these observations proceeded regularly in its changes, and ultimately produced the embryo; all the stages of which I have traced and delineated for future communication to the Royal Society.

Subsequently to these observations I saw ova passed by another female, *Lissotriton palmipes*, and on submitting these to the same close examination as the above, within five minutes after their production, I again found what I regarded as the remains of spermatozoa on the surface of the egg-covering, but not a trace of any in the interior or in the vicinity of the yelk.

Endosmosis of the envelopes during impregnation.—But although the facts now mentioned are so opposed to the view that the spermatozoa penetrate bodily into the ovum, it is due to the distinguished observers to whom I have referred to enter somewhat more fully into the questions which their observations involve; and while I am free to admit the possibility of mistake or oversight on my part, to mention the details of some experiments made expressly with the view to ascertain whether the envelopes of the ovum of the Frog are permeable in any part to solid particles of matter; or whether there exists any orifice in them by which such particles can enter. It is well known that during the transit of the ovum through the oviduct the vitellary membrane becomes invested with a thick gelatinous covering, the first thick layer of which may be regarded as the rudimentary *chorion*, and perhaps may be analogous in its function, in some respects, as it seems to be in its place and mode of origin, to

the albuminous investment of the ovum of the Rabbit. I cannot, with Rusconi*, regard this envelope of the Frog's egg as being merely a mechanical protection during the process of development. It is formed of cells with distinct nuclei, and from what I shall presently mention, seems to be essential to the ovum at the commencement of the changes, and to be intimately connected with the act of impregnation. Rusconi deprived the ova of the green aquatic frog, ? *Rana esculenta*, of their gelatinous envelopes at a period subsequent to impregnation, and found that they passed through their changes as well as when covered by them; and he thence concluded that the envelope is of no use further than to protect the egg from the injury it might receive through mechanical disturbance, "des petits chocs qui pourroient nuire à son developpement†." Certainly it affords this protection to the germ, but to conclude that this is its sole office appears to be somewhat premature. I have found that it is almost impossible to remove this envelope from the Frog's egg at the moment of deposition, or even during the first few minutes after submersion, and before it has become expanded by imbibition of fluid; although it may be removed without much difficulty from the egg of the Newt, the yelk of which, in the vitelline membrane, lies free and unattached in its interior. But some time after the expansion has taken place I have myself found that the frog's egg may be deprived of a large portion of this covering, and yet produce an embryo equally well as if it had remained protected. On the other hand, one most important function of this investment seems to be indicated in the following facts. Spallanzani found that ova of the Frog deprived of their envelopes before contact with the male influence, were not impregnated; and further, that ova taken directly from the ovaria, are not susceptible of impregnation‡.

A remarkable fact which I noticed, at a time when I was not fully aware of its interest and importance, enables me to confirm this observation. I captured a pair of frogs, the female of which, a short time after they were in my possession, had a large hernia formed by a protrusion of part of the great oviduct through an accidental wound in the posterior part of the right side of the body, and in consequence of which she was unable to deposit her ova. This wound had been received before the union of the sexes, but the hernia was formed afterwards, during the passing of the ova from the ovaria. The result of this was, that when some of the ova had passed into that part of the duct which protruded through the wound, the sac formed by it was constricted, and became so enlarged by the expansion of the egg-envelopes, that the remainder of the ova were prevented from entering it. On opening the abdomen of the frog after death, I found that a very large proportion of the ova which had left the ovarium on that side of the body, were lying in the cavity of the peritonæum, among the viscera, being entirely prevented from entering the duct, which was filled throughout its whole extent, to its very orifice, with eggs which had already entered, and were prevented from passing on. On the left side of the body the ova had

* *Loc. cit.*, p. 8. † *Loc. cit.*, p. 9. ‡ Dissertations, &c., ii. 152, 3.

also quitted the ovarium, but the whole on that side had passed into the oviduct in the usual way. The eggs found in the cavity of the body consisted only of yelk-masses in their vitelline membranes. I immediately placed some of these eggs in water, with seminal fluid obtained from the male with which this female had been paired; but not a single egg became impregnated, or gave afterwards any sign of formation of the embryo. I had some hesitation in regarding this experiment as quite conclusive,—that impregnation cannot take place before the egg has gained its gelatinous envelope, and consequently while it is still within, or has but just escaped from the ovary,—from the possibility that these eggs might have been for some time in the cavity of the body, and that some change might have been induced in them through long detention. In so far, however, as that this was in accordance with SPALLANZANI's experiment, it seemed to point to the nature and importance of the covering which the egg gains in the oviduct.

Since my attention has been more particularly directed to this point of investigation, I have repeated the experiment on the ova of the Newt, *Lissotriton palmipes*, with precisely similar results. I opened the body of a female with great care (after dividing the spinal cord through the medulla oblongata), for the purpose of obtaining ova from the oviducts, for artificial impregnation, and immediately saw that a number of ova were free in the cavity of the abdomen, and were in the course of being transferred to the entrance of the tubes, as stated in the first part of this paper. These ova, like those which had recently escaped from the ovarium in the Frog, were without any other covering than their vitelline membranes; most certainly I was unable to detect any other, and they were so delicate that it was with difficulty they were removed into water to which fluid from the male had been added. But although uninjured in the removal, and in every way carefully treated, not one gave any sign of cleavage of the yelk, which, as I have before stated, I have constantly found take place in the impregnated ova of newts as well as of frogs, although the fact of its occurrence was overlooked by RUSCONI; not one egg afterwards produced the embryo. Thus then it seems fair to conclude that the egg in the *Amphibia* is not fitted for impregnation until after it has entered the oviduct and acquired its gelatinous covering.

I have already shown that there is a remarkable coincidence between the rate of expansion of the gelatinous covering, immediately after the egg is placed in water, and the susceptibility of the egg to become impregnated; and that in proportion as the covering becomes enlarged and distended by imbibition of water, the susceptibility of the egg becomes diminished; until at the end of about half an hour it is almost completely lost, at which time the rate of expansion of the envelope is also greatly lessened, and the envelope itself has attained to more than two-thirds its future diameter. NOW SPALLANZANI found that the susceptibility of the ovum, when immersed in water, had ceased at the end of fifteen minutes, at which time the envelope is considerably enlarged. PREVOST and DUMAS also observed that the expansion of

the envelope is greatest during the first three hours, and rightly regarded the occurrence as connected with impregnation, and with this opinion made experiments to test their views. They placed ova taken from the oviducts in ink, and found the envelopes blackened with the imbibed fluid*; but they remark—"bientôt cette imbibition s'est arrêtée à cause de la reaction chimique de l'encre qui coagulait la matière muqueuse." Afterwards they employed the blood of the Frog mixed with water, and found the envelope deeply reddened when immersed in it; and thence concluded that the envelope in its normal condition admits of the entrance into it of solid particles of matter held in suspension in the fluid. But each of these experiments appears to be open to a different explanation. It is probable that the chemical action of the ink, by altering the condition of the envelope, allowed of the admission into it of solid particles only in proportion to the change in the tissue; and that the colour given to the envelope by frog's blood was due to particles of colouring matter which adhered to the surface, rather than to the admission of these into the substance of the tissue. This conclusion is founded on the following trials.

The imbibition of water by the covering of the egg being so distinctly marked, I had intended, like the authors mentioned, to endeavour to ascertain whether coloured water could be as readily absorbed as pure water. Through accident, however, I omitted to put this question to the test until late in the season of last year, and after the whole of my frogs had spawned. But having placed several ova in rectified spirit for future examination, at the moment of passing them from the body of a frog, I determined to test the result of the immersion of these in coloured fluid, although well aware of the correct objection that would be made, that the experiment must of necessity be inconclusive. I put some of these into a solution of carmine in water, and watched the result. The envelope, which, while the egg was in spirit, was white, opaque, and adhered closely around the yelk membrane, began to imbibe and expand the instant it was placed in the solution; and at the expiration of an hour there seemed reason to believe that the trial had succeeded. The envelope was much enlarged, and the fluid had penetrated into it, carrying with it some colouring matter; as on carefully washing the surface repeatedly in clean water, to remove the deposit on the exterior, the substance of the interior was seen to be coloured, and it was evident that the colouring matter had penetrated as far as the deepest or thick layer of the envelope. This result appeared to favour the view that the spermatozoon enters the ovum. When the ova were again examined at the end of the third day's immersion, they were of a deep red; the deepest stratum of colour being then between the vitelline membrane and the thick or innermost layer of the envelope. The entire egg presented the appearance of a globule or bead of red glass, with a dark red centre, surrounded by a lighter-coloured halo. The eggs were then thoroughly washed and placed in clean water. At the end of six hours part of the colouring matter had again been removed from the interior, and the eggs were of a less deep hue, and the

* *Loc. cit.*, ii. p. 132.

water they had been immersed in was coloured. I then again removed them to clear water. At the end of sixteen hours they had parted with more colour, but were still red, more especially between the inner portion of the envelope and the vitelline membrane, and the water had again acquired a red hue, thus showing that both endosmose and exosmose must have taken place.

As it might fairly be objected that these ova, changed by immersion in spirit, were unfitted for experiment, I made trial with others which had not been impregnated, and being infertile, had remained in water many days without giving signs of decay. When these were placed in the carmine solution, their envelopes became as deeply and thoroughly imbued with colouring matter throughout their whole substance as in the former; and when placed in clear water they parted as readily with a portion of it, so that it was evident that whenever the density of the fluid in which these dead and infertile ova were immersed, was altered, a change by endosmose or exosmose immediately took place in the fluid retained mechanically in their tissues. To this cause, perhaps, may be ascribed the colouring of the ova in PREVOST and DUMAS's first experiment with ink, while other experiments, which I shall mention, made on living and impregnated ova, lead me to regard the colour in the experiment with frog's blood as merely the result of adhesion of colouring matter to the surface.

The immediate objects I had now in view were, to learn whether impregnation is effected by any direct and palpable infiltration of seminal matter through the envelopes of the ovum;—whether the admixture of other matters with the seminal fluid will prevent or arrest impregnation;—and whether the spermatozoa collected on a filter paper, and then placed with this in a fluid of great density, are as efficient as in clear water.

With these views, I prepared a very dense solution of carmine pigment in water, and added parts of this to small quantities of water with ova, either before the seminal fluid was mixed with the water, or immediately afterwards, and I expected the results to show whether any solid particles, held in suspension in the fluid, passed through the envelopes. The previous trials had shown that solid particles do pass through the *dead* tissue, but it was doubtful whether the like result would occur in the living.

Carmine Experiments.—Set O. March 13, 1850. Atmosphere 53° FAHR.

No. 1. *Eleven unimpregnated ova* were passed into water mixed with carmine.

The envelopes became as fully expanded, and imbibed fluid as freely as in the impregnated ova, and acquired a red tint; but much of the colour was due to the deposition of granules of matter on the surface, while I was unable to detect any similar granules within their texture. On the contrary, on removing part of the surface of the envelopes, the interior, although slightly reddened, exhibited an uniform appearance.

No. 2. *Thirty ova* were passed into water that had been mixed with seminal fluid, and immediately *afterwards* a solution of carmine was also added.

Twenty-six of these ova became impregnated and produced embryos; thus showing that impregnation takes place very quickly, and is not prevented by the addition of a dense colouring fluid, added *after contact* with the impregnating fluid.

No. 3. *Forty-one ova* were passed into a solution of carmine in water which had been mixed with seminal fluid immediately *before* the passing of the ova.

Thirty-three of these ova also produced embryos. It was evident, therefore, that when seminal fluid is freely mixed with a dense medium that holds solid particles of matter in suspension, the spermatozoa are not necessarily prevented from effecting impregnation of the ova. Thus the ova of the Frog, although usually deposited in slow-running or clear still water, may be deposited even in slightly turbid water without impediment to the natural process of impregnation, as the water and spermatozoa may be brought into contact with the ova at the same instant.

No. 4. *Thirty ova* were passed into water mixed with fluid that had been almost completely deprived of spermatozoa by filtration.

Only *one ovum* exhibited any signs of impregnation, but not a single embryo was produced.

No. 5. About *two hundred and twelve ova* were passed into a dense solution of carmine and water in which the filter paper with spermatozoa, separated from the fluid employed in No. 4, had already been placed, and the water and ova were then freely agitated together.

The result of this experiment was very marked. Only a few of these ova became segmented, and the change proceeded much slower in them than in the ova of experiments Nos. 2 and 3. At the end of twelve days only *five embryos* had been produced. Thus a dense solution of carmine, applied to the spermatozoa *before* they are brought into contact with ova, may have the effect of preventing impregnation, apparently by operating as a mechanical impediment. These ova, excepting only a few removed for the following experiment, No. 6, which were taken from the mass as stated, were allowed to remain in the carmine for twenty-four hours before they were placed in clear water.

No. 6. *Forty ova* taken from the last experiment were removed to clear water at the end of one hour and a quarter, having first been thoroughly washed. The result was as decided as in No. 5. *Only two embryos* were formed; so that there was further reason to believe that impregnation takes place very quickly, and that the result in No. 5 was not entirely due to long continuance in the solution, but to some impediment at the time of contact.

No. 7. A thick solution of carmine was mixed with seminal fluid and water, and *three minutes afterwards* a mass of ova were passed into it.

This experiment was similar to No. 3, excepting only that the solution of carmine was much more dense, and the ova were not passed until three minutes after the fluids had been mixed. There was a marked difference in the result. Only a few of these eggs became segmented, and only *eight* out of a large mass produced embryos.

But there were several eggs that appeared to have been *partially impregnated*, the whole of which were abortive. *Partial impregnation*, as before stated, is shown in a very imperfect cleavage of the yelk, sometimes on one surface only, and sometimes complete as regards one half of the yelk, but imperfect or irregular in the other.

No. 8. *Forty-two ova* from the last experiment, No. 7, were removed from the carmine at the end of *thirty seconds*, and were immediately well washed to get rid of the adhering spermatozoa and granules of colouring matter. Not one of these ova produced an embryo, but several had become *partially impregnated*.

These experiments were made in the middle of March, when the season was unusually cold, and the mean lowest temperature of the room during twelve days was only 43° FAHR., and the mean highest 47° FAHR.; at a higher temperature the results, I have little doubt, would have been more favourable. The eggs had been impregnated however when the temperature, during the first twelve hours, was 55° FAHR., so that the question respecting the infiltration of solid matter with the water absorbed by the envelopes of the eggs was not affected.

About *four hundred and twenty* eggs were employed in this set of experiments; yet I could not detect any granules of the colouring matter of carmine held in suspension, and of dimensions equal to those of the spermatozoa, which had passed into the tissue of the envelopes of the eggs, although they had become tinged by the colouring matter in combination with the water. Abundance of granules of colouring matter of most minute size, and not more than one-third the diameter of the spermatozoa of the Frog seen beside them, adhered to the surface of the envelopes, and it was to these chiefly that the red colour of the whole was due. Every part of the envelope exhibited the same uniform appearance, the granules being pretty equally distributed over the surface, and the suffusion of colour was uniform in the interior. These facts appeared to be conclusive with reference to the question of the presumed existence of a fissure or perforation through the coverings of the egg of the Frog before, or at the moment of fecundation, as is supposed to exist in the ovum of the Rabbit. I have not been able to detect any appearance of orifice or fissure in the egg of the Frog-envelopes, and the course of which, if such really exists, would no doubt be indicated by some deposition of the colouring matter of the carmine, to a greater or less extent, in its tract. The result of these experiments was thus most unfavourable to the belief that the spermatozoa penetrate bodily through the membranes of the ovum; and to that of the supposed existence of a special opening in these membranes for their admission.

I ought now, however, to mention one experiment that seemed to favour the opinion that the spermatozoon enters the ovum. I had taken several ova, together with the oviducts into which they had passed, from the body of a *Lissotriton palmipes*, and others from that of *Triton palustris*, for the purpose of artificial impregnation. Some of these I pressed from the oviducts into a very clear solution of carmine, taken from a solution which had remained undisturbed for nearly a fortnight, so that the granules

in suspension had subsided, and only the colouring matter actually combined with the water gave it its red hue. At the end of half an hour I removed the ova from the solution to clear water for examination, and then found that the interior of the envelope was coloured by the water which had entered, but that the greater portion of the colouring matter had been arrested and separated at its entrance and adhered to the surface. One ovum of *Lissotriton palmipes*, however, to my great surprise, had a little dense mass of colour deposited at one point only of the dark surface of the ovum, not merely within the envelope or its chamber, but actually beneath the vitelline membrane, between it and the yelk, as was distinctly proved by turning the egg on one side and viewing it in profile. Not one of the other eggs, placed in the solution, either of the *Triton* or *Lissotriton*, showed any appearance like this; so that while I am debarred from expressing a decided opinion that the spermatozoon does not enter the ovum, I can only regard the appearance mentioned as entirely accidental, and not as a normal occurrence; but as resulting, perhaps, from some minute puncture or other accident during the removal of the eggs from the body or the oviduct.

But in order, if possible, to remove another source of doubt, it seemed necessary to make some trial with the colouring material employed by Prevost and Dumas in their experiments; and some further examination of that used in my own; and to ascertain whether any solid particles or granules of matter, held in suspension in ink or in carmine, and equal in size to the spermatozoa of the Frog or the Newt, can be passed through the filter, or can be separated from the fluid portion by filtration, like the spermatozoa, when precisely the same mode is followed, and the same means and same description and number of filter-papers are employed, as in the filtration of the seminal fluid. The solution of these questions it was evident must tend to confirm or to unsettle the previous conclusions. I first tried *ink*, and used a part of the identical filtering-paper employed to separate the spermatozoa. The ink passed quickly and freely through three filters without losing any of its intense black colour, and carried with it only a very few extremely minute granules, much smaller in size than the spermatozoa of the Frog; so that it seemed fair to conclude that the colour imbibed by the ova from *ink*, in MM. Prevost and Dumas' experiment, was due to the admission of the chemically combined colours of the fluid, and not to an admission into the texture of the egg-envelopes of solid particles held merely in suspension in the fluid. Consequently this experiment seemed to negative the supposition that, from the fact of the interior of the egg-covering becoming blackened, solid particles of matter, equal in size to the spermatozoa, must have penetrated into the envelope during its expansion; and there seemed less reason to believe that the spermatozoa,—bodies very much larger than the ink-granules,—could enter it. *Carmine* was then tried. A solution of this colour could scarcely be made to pass through even a single filter. This seemed to be due chiefly to the fact that the greater proportion of the colouring matter of the carmine used (the water colour pigment of artists) was combined with gum and an earthy base, and consequently most of the colour was in

suspension rather than in chemical combination. When placed on a single filter, the solution passed through with extreme difficulty and slowness. When a microscopic drop of the fluid so passed was examined with a power of three hundred diameters, it was found to contain a large quantity of granules suspended in clear fluid. When made to pass, but with still greater difficulty, through a second filter, it still contained a quantity of minute granules, but each *less than one-half the diameter* of the spermatozoon. It is *possible*, therefore, that some extremely minute granules may penetrate into the texture of the envelope, formed as it is of aggregations of cells; but it seems to be very improbable that any of the larger-sized objects, such as the spermatozoa, can enter: and it is even much more improbable, that if the chief colour of the ova in my experiments was due, as I believe, to granules of carmine on the surface, and not in the interior of the ova, that in MM. PREVOST and DUMAS' experiments with frog's blood, the ova should have become reddened by the admission of particles of this into their interior, since it need scarcely be mentioned that the colour of the blood is due only to the particles suspended in it; and MM. PREVOST and DUMAS remark, that they were not able to detect any blood-globules on the surface. To what else, then, than to these, or to their broken-down particles, could the reddened colour of the ova in their experiments be due?

The conclusion, then, to which I am led by these experiments is, that although the envelopes of the egg imbibe coloured fluid, they do so less easily than when the fluid is not coloured, unless it is in chemical combination; and although atoms of solid matter, very much smaller than the spermatozoa, may possibly be carried by infiltration into the texture of the egg-envelope by the act of endosmose during its expansion, it appears to be extremely unlikely that the large bodies of the spermatozoa are so carried in; an improbability which is raised almost to a certainty by the fact that the spermatozoa are not seen attached to the egg with a centripetal direction of the axis of their bodies, but are constantly applied laterally to, or are entangled amongst the loose tissue of the surface, extended at length or partially folded on themselves.

6. AGENCY OF SPERMATOZOA AS AFFECTED BY CHEMICAL MEDIA.

The experiments with carmine having led to an unexpected result in the impediment which this medium offers to the impregnation of the ovum when immersed in it before contact with the spermatozoa, I was desirous of ascertaining what effect would be produced on the ovum by the destruction of the spermatozoa by chemical means, immediately after they had been applied to it. Mr. GULLIVER* long ago showed that the spermatozoa of different animals are variously affected by different chemical tests; and Dr. FRERICHS†, more recently, has found that a solution of caustic potass has the property of entirely dissolving and destroying them. This material, therefore, seemed to be peculiarly fitted for the object in view. But before any experiment, in

* Proceedings of the Zoological Society, part 10. p. 101. July 26, 1842.

† In Cyclopædia of Anatomy and Physiology, Article "Semen," p. 506, January 1849.

which this was employed, could be relied on, it was necessary to confirm the facts ascertained by chemical investigation, by observing the mode of action of solutions of caustic potass, and other chemical agents on the spermatozoa, by means of the microscope. As my observations on the effect of chemical agents on the spermatozoa have been confined for the present to those of the Frog, I shall state the results of these observations with the microscope before mentioning the experiments.

All the observations were made immediately after the spermatozoa employed had been obtained, by the course already mentioned, and not by vivisection from the vesiculæ seminales or the testes, sources which are objectionable from the facts shown in Dr. Frerichs' analyses, that a large quantity of albumen is always found in the immature cells in the testes, with which the spermatozoa, obtained from that source, are constantly mixed, while there is no trace of albumen in the mature spermatozoa.

1. *Solution of Caustic Potass.*—The solution employed was in the proportion of twenty grains of caustic potass (*Potassa fusa*) to one ounce of water. This was the solution employed on most occasions in the following experiments, and which quickly and entirely dissolves the spermatozoa. When a drop of semen, in which the spermatozoa are active and abundant, covered by a pellicle of talc on a plate of glass, is attentively examined, while a very small quantity of the potass solution is applied to the edge of the talc, the act of dissolution is easily witnessed. As the solution spreads beneath the talc the spermatozoa first brought into contact with it are instantly destroyed, while the motions of those at a distance become slower and slower, until, when the fluid has nearly approached, they entirely cease. The instant the fluid comes into contact with the spermatozoa, they roll up on a sudden into a spiral form, the change commencing at the apex of the caudal extremity, and each becomes a rounded mass, which quickly dissolves and disappears in the homogeneous fluid. The action of the potass in this destruction of the spermatozoa, as seen by the microscope, is very similar to, in appearance, and strongly reminds one of the action of fire on the barbs of a feather, which become frizzled in an instant, leaving only a scoria that soon disappears.

2. *Nitrate of Potass.*—This, as in the preceding case, was in solution in the proportion of twenty grains to an ounce of water. It destroys the spermatozoa much less quickly than the caustic potass. When applied, as above, to the edge of the talc, the spermatozoa first become on a sudden motionless, and are in general elongated, and afterwards are very slowly dissolved.

3. *Diluted Acetic Acid.*—When this is applied to the spermatozoa in the same way as the solution of potass, it quickly destroys all signs of vitality. The movements immediately become slower and very soon entirely cease, and the spermatozoa are extended at full length, and are but rarely folded on themselves, as they usually are in natural death. I could not satisfy myself that the acid has any other effect on them chemically than that of contracting and rendering them smaller. It did not appear to dissolve them. Mr. Gulliver* has mentioned that the spermatozoa of the

* *Loc. cit.*

snake (*Natrix torquata*) are not affected by acetic acid, but he makes no reference to its action on those of the Frog.

4. *Gum-Arabic.*—A thick solution of gum appears to act on the spermatozoa mechanically only, and almost immediately deprives them of motion by the obstruction it opposes to them. When a minute drop of spermatic fluid is placed in the midst of one of gum solution, and covered with talc, those spermatozoa which have become mixed with the gum cease to move instantly, and remain with the tail and body coiled in various directions; while others at the edges and in the midst of the fluid, where they are less mixed with gum, still move feebly for a few seconds, but become motionless as the gum collects around them.

These circumstances will better enable us to understand the following experiments, the object of which was to endeavour to learn how far the influence of the spermatozoa, and the *act* of impregnation of the ovum partake of a chemical or of a mechanical nature; and also will help to determine the length of period of contact requisite for impregnation.

The following experiments bear on these inquiries:—

Potass experiments.—*Set* P. March 25, 1850. Atmosphere 48° Fahr. Water 46° Fahr.

No. 1. P.M. $1^h\ 40^m$.—*Fifty-four ova* were passed from the Frog on a dry surface, and were instantly bathed with recently-obtained impregnating fluid mixed with water; and at the lapse of *fifteen seconds* were washed by means of a hair-pencil loaded with the solution of caustic potass before mentioned; after which the eggs were again washed freely with water.

No. 2. P.M. $1^h\ 45^m$.—*Thirty-nine ova* were treated in precisely the same way, except that the interval between the application of impregnating fluid and the solution of potass was only *five seconds.*

The ova were removed, after the first day, to a room in which the average temperature was about 60° Fahr., and at the end of the *eighth day twenty-one embryos*, advanced to near the end of the *fourth period* of development, had been produced in No. 1, and *two embryos*, at a similar stage, in No. 2. This, at first thought, appeared to be an extraordinary fact, seeing that the solution of potass so quickly decomposes the spermatozoa, and even renders the ovum sterile, as was afterwards found; but, on examination of the details, the experiments admit of explanation:—*first*, the seminal fluid was employed immediately it was obtained, and *before* the application of the potass, which was not used, in the first case, until after a lapse of *fifteen seconds*, and in the second case, of *five seconds*; next, that in both instances the solution of potass was, as quickly as possible after its application, diluted and removed by repeated washing of the ova with water. Nevertheless, these experiments prove that the ovum becomes impregnated very quickly after the application of spermatozoa, and, in these cases, even within the short interval of *fifteen seconds* in the one, and *five seconds* in the other, the difference in the number of embryos produced in the two apparently indicating the extent in each of the deleterious effect of the solution.

No. 3. P.M. 1^h 50^m.—*Forty-seven ova* were passed on a dry surface, and spermatic fluid was instantly applied to them, and within *ten seconds* afterwards the ova were washed with the solution of potass, which was allowed to remain, and water was then added. At the end of the *eighth day* not a single embryo had been produced. The difference between this experiment and the above was the non-removal of the potass, and the more free bathing of the ova.

No. 4. P.M. 1^h 53^m.—*Forty-five ova* were passed on a dry surface, and were *first* bathed with solution of potass, and then with seminal fluid in water, and afterwards they were removed with No. 1 and 2, to higher temperature.

At the expiration of the *eighth* day *three embryos* had been formed. This result at first appeared to be more difficult of explanation than the former. But, when the circumstances are considered, it seems to admit of quite as easy an interpretation. The bathing of the ova freely with seminal fluid mixed with water, *five seconds after* the application of the solution of potass, diluted this solution too much to allow of its effect on the whole of the spermatozoa applied to the ova, while this very dilution enabled the impregnating influence of these bodies to take effect in some of the ova. The fact, however, leads to an inference of some importance with reference to the action of the potass on the envelopes of the ovum, and seems to show that this action is less immediate on the envelopes than on the spermatozoa.

No. 5. *Fifty-nine ova* were bathed with seminal fluid and water, and *five seconds afterwards* with a solution of nitrate of potass (in the proportion of twenty grains of the salt to one ounce of water), and water was then added to them.

No. 6. *Seventy-two ova* were treated in precisely the same way.

Not one embryo was produced in either of these experiments.

No. 7. *Forty-four ova* were washed with diluted *acetic acid* immediately after they had been shed from the female, and *five seconds afterwards*, seminal fluid with water was added to them.

No. 8. *Seventy-six ova* were washed with diluted acid, and treated in every way as in No. 7.

Not a single embryo was produced in either of these trials.

The action of acetic acid is almost instantaneous on the envelope of the ovum, which it quickly contracts, and renders slightly opaque.

Besides those media which act *chemically* on the spermatozoa and the ova, I made trial, in this and the two following sets of experiments, with a solution of gum-arabic, the effect of which appears to be entirely *mechanical*; and as the results are curious and seemingly important with reference to the nature of the agency of the spermatozoa in impregnation, I defer the mentioning of them until I have to show the effect of media which operate mechanically on the ovum or the spermatozoon in impregnation.

The result of the preceding experiments was so remarkable, that it seemed necessary that they should be repeated with greater precision, with reference to exact periods

of time, than can always be done when alone and unassisted. It was evident that a proper understanding of the nature of the act of impregnation, if ever this becomes known, will be led to chiefly by attention to the *periods of time* in which it is effected. I obtained therefore the assistance of a friend, to note the spaces of time which elapsed in each stage of the following experiments, so that these might be performed with the quickest dispatch, and the attention of the experimenter be not withdrawn from each until it was completed.

Set Q. March 30, 1850. Atmosphere 49° FAHR.

The seminal fluid employed in this set of experiments was not obtained from the usual source. From some cause or other it could not be so procured. I therefore killed a male frog, by dividing the spinal cord in the *medulla oblongata*, and pressed out the fluid from the testes, which were gorged with spermatozoa, as found by examination with the microscope. From the presence of a great number of spermatozoal cells, and from the water with which I mixed the fluid becoming slightly turbid and albuminous, it was seen that it was not fully matured, a circumstance to be borne in mind with reference to the experiments, which were commenced some minutes after the fluid had been thus obtained. It was doubtful also whether the eggs were quite mature.

It is necessary further to mention, that in these experiments two solutions of potass, with different proportions of the salt, were employed; one having twenty grains in one ounce of water, and which, to avoid repetitions, I shall designate "*strong solution*;" and the other having only eight grains of the salt in one ounce of the fluid, and which I shall refer to as the "*weak solution*."

No. 1. P.M. 4^h 45^m.—*Forty-five ova*, passed on a dry surface, were bathed with seminal fluid and water, and *five seconds afterwards* with the *weak solution* of potass, and were then washed, and placed in clean water. The whole time occupied in the experiment did not exceed *thirty-five seconds*.

On the following morning, segmentation was found to have taken place in *twelve ova*. This as well as the following sets of ova were then removed to a higher temperature, in which they were allowed to remain; but *no embryos* were produced.

No. 2. P.M. 4^h 58^m.—*Fifty-three ova* were treated in exactly the same way, but with the *strong solution* of potass; the *interval* between the application of the impregnating fluid and the solution being only *two seconds*, and the whole time occupied *forty-five seconds*.

Segmentation took place in *three or four ova*, but not completely. Several ova also were altered in form; but *not a single embryo* was produced.

No. 3. P.M. 4^h 52^m.—*One hundred and twenty-two ova*, passed on a dry surface, were immediately well bathed with seminal fluid and water, and *two seconds afterwards* with *weak solution* of potass, which was *allowed to remain with the ova*, and water was then added. The whole time occupied was *forty-five seconds*.

Segmentation took place in a great number of these ova, certainly from *fifty to*

sixty, and there were nine also which became shrivelled and decayed. Notwithstanding the great proportion of ova segmented, *not one produced an embryo.*

No. 4. P.M. 5^h.—*Sixty-six ova*, passed on a dry surface, were bathed as in No. 2, with impregnating fluid, and *one second afterwards* were washed quickly with *strong solution* of potass, and water was then added; the period occupied being *sixty-two seconds.*

Only *two ova* became segmented, while, excepting only eight or nine ova, the whole of the remainder were shrivelled, irregular, or compressed in form, and distinctly spoiled, apparently by the action of the potass, thus showing that endosmose and exosmose through the envelopes had taken place.

No. 5. P.M. 4^h 55^m.—*Fifty-four ova* were passed, and washed with the *weak solution* of potass, and *afterwards*, at an interval of *two seconds*, with impregnating fluid and water, and water was then added and allowed to remain. The whole time occupied was *fifty seconds.*

One ovum only became segmented; but several others had slightly altered their form to the obtuse oval, as if about to become divided: *no embryo* was produced.

No. 6. P.M. 5^h 2^m.—*Seventy-nine ova* were washed with *strong solution* of potass, and one *second* afterwards with impregnating fluid, and water was then added.

Not one ovum became segmented, nor did even one yelk retain its proper shape. The whole were irregular and spoiled. *Five ova* had the envelope clouded and opaque, and the surface of others was translucent with refracted light, like crystallized carbonate of lime. In one egg only had there been any attempt at segmentation. This experiment, like No. 4, seemed to show that the act of expansion of the chorion is an act of endosmose.

No. 7. P.M. 5^h 15^m.—*Nitrate of Potass. Seventy-seven ova* were passed on a dry surface, and were well bathed with impregnating fluid in water, and *one second* afterwards with a *weak* solution of nitrate of potass, and water was then added to them; the whole time occupied being *thirty seconds.*

Segmentation took place in *forty-three* of these ova, and the whole retained their natural form and size. The effect of the nitrate of potass, as compared with the caustic solution, was thus very marked, as showing that the momentary application of the nitrate does not prevent or arrest impregnation in weak solution (eight grains to one ounce of water), even when applied *after* the impregnating influence. The experiment was also interesting in another respect. It proved that the ova were susceptible of being impregnated, and that the fluid from the testes was efficient to induce the first evidences of impregnation. But none of these ova, or of the ova in the preceding experiments of this set, produced embryos. Subsequent observations will show that this failure was not due to the nitrate of potass, but perhaps was attributable to the conjoint causes of low temperature at the time of impregnation, and of some imperfection both in the seminal fluid and the ova.

No. 8. P.M. 5^h 9^m.—*Seventy-six ova* were washed with diluted *acetic acid* as in *Set* P,

No. 7, and *one second* afterwards with impregnating fluid, and water was then added to them. The time occupied was *forty-five seconds.*

The result was more decided than in the experiment referred to. The envelopes of the ova immediately became clouded, and no segmentation took place in any of the yelks, some of which became shrivelled and changed in form.

The result of the preceding experiments being doubtful as to the cause of the non-production of embryos, especially with reference to the first three, and the seventh experiments, in which many ova became segmented, I obtained some additional pairs of frogs from their native haunts, and within twenty-four hours afterwards, before they had in any way become debilitated by confinement, repeated the experiments at a higher temperature.

Set R. April 3, 1850. Atmosphere 60° FAHR.

No. 1. P.M. 3^h 5^m.—*Eighty-two ova,* passed on a dry surface, were touched for *an instant only* with a pencil dipped in impregnating fluid and water, and *one second afterwards* were washed with *strong* solution of potass, and then with water, and water was then added. The whole time occupied was only *fifteen seconds.*

No. 2. *Twenty-five ova* were treated in precisely the same way with the same solution (which also was employed in the following experiments); the interval being *two seconds,* and the whole time *twenty seconds.*

Segmentation took place, but *only very partially,* in about *twelve ova* of the first, but completely in *one only* of the second experiment. The whole of the remaining ova were shrivelled and decayed; their envelopes exhibiting the same clouded and refractive property noticed in the last set. At the end of five days *two embryos* had been produced in the first experiment, and *one* in the second. It was thus far confirmatory of the experiments with potass in *Set* P, that if this salt be applied to the envelope *several seconds after* the application of the impregnating fluid, and be again *quickly removed or diluted* with water, impregnation may already have taken place, and the action of the caustic will not in that case affect the production of the embryo; especially if the experiment be made when the temperature of the surrounding medium is becoming increased. But if the solution be applied *before* the application of the seminal fluid, then the spermatozoa will in most cases be decomposed, and no impregnation follow. In either case, however, the undiluted solution acts also on the ovum itself within a very short period, and destroys or renders it sterile. This was further proved in the succeeding experiments.

No. 3. P.M. 3^h 10^m.—*Fifty-eight ova* were passed on *a moistened surface,* and were immediately afterwards washed with the solution, and at the expiration of *one second* were bathed with seminal fluid and water; the time occupied being only *fifteen seconds,* as in No. 1.

No. 4. *Sixty-nine ova* were treated in exactly the same way, the interval being *one second*; and the whole time occupied only *twelve.*

Partial segmentation had taken place in *one ovum* of No. 3; but the whole of the

remaining ova, both in No. 3 and 4, were destroyed. Many of the yelks had begun to change form within the first hour, and the envelopes exhibited the same refractive appearance as in the previous experiments.

Anticipating from the former experiments what probably might be the ultimate result in these, I now determined to put beyond the possibility of doubt, both the fitness of the seminal fluid employed to effect impregnation and the healthiness of the ova, and their susceptibility to become impregnated; and to show from these facts that a non-production of the embryo *in this set of experiments* must be due to the action of the potass solution, and not to any unfitness in the spermatozoa or the ova. Accordingly,—

No. 5. P.M. 3^h 17^m.—*Sixty-two ova*, from the same female employed in the preceding experiments, were bathed with a portion of the seminal fluid and water which had been employed in No. 1 and 2, and were then placed side by side with these, in a separate dish.

At the expiration of *four hours and thirteen minutes*, the temperature being 60° FAHR., from *thirty to forty* of these ova had become segmented. Some of the ova had been injured mechanically, but nearly the whole that had not been injured were impregnated. On the *seventh day* there were *twenty-three embryos*, *thirteen* of which had already left the egg-envelopes; others were somewhat less advanced, thus proving the fitness of the seminal fluid to impregnate, and the ova to produce. The number of embryos too was fully as great as could have been expected, seeing that many of the ova had been slightly injured, and that the seminal fluid had already been *one hour and twenty-six minutes* mixed with water.

The result of this experiment was borne out by the following.

No. 6. P.M. 3^h 31^m.—*Nitrate of Potass. Seventy-four ova* were well bathed with impregnating fluid and water on a previously dry surface, and *one second* afterwards with a *strong solution* of nitrate of potass (twenty grains to one ounce of water), and water was then added to them; the whole time of the experiment being *twenty seconds.*

No. 7. *Fifty-nine ova* were treated in exactly the same way, save that the interval between the application of the impregnating fluid and the solution of potass was *three seconds*, and the whole period *twenty-five seconds.*

Segmentation commenced in each of these sets in *four hours and fourteen minutes*, when from *twelve* to *fifteen* ova were undergoing this change in No. 6, and *thirteen* in No. 7. At a later hour there were many more in each experiment only very partially segmented, and which proved to be unproductive. At the end of the *seventh day* there were *twelve embryos* in No. 6 advanced to the same stage as in the *simply artificial impregnation* No. 5, and *ten embryos* in No. 7, but at a little less early stage of growth, a circumstance which I attributed at the time to imperfect aëration.

No. 8. P.M. 3^h 37^m.—*Seventy-nine ova* were bathed with the same solution of nitrate of potass as above, and *three seconds afterwards* with impregnating fluid in water; the whole time occupied being *twenty seconds.*

Segmentation took place at a little later period in this than in the preceding experiments. It commenced at *four hours and twenty minutes,* when *twenty-five ova* were undergoing the change. This was a full proportion of impregnation as compared with No. 5, seeing that the impregnating fluid had already been mixed with water one hour and forty-six minutes. *Twenty-five embryos* were the result of this experiment.

The results thus support the explanation already given, with reference to the effect produced on the envelopes of the ovum being less immediate than on the spermatozoa; since, in this case, twice as many ova became segmented, and ultimately produced embryos, as in those experiments in which *the solution* was applied *after* the seminal fluid, and while endosmosis of the egg was most rapid, and when the solution remained undiluted.

The general results of this set of experiments, compared with those of the last *Set*, Q, appear also to show that the non-production of embryos in the whole of that set,—after segmentation had taken place in several of the experiments, as in Nos. 1, 2, and especially No. 3, with solutions of the caustic potass;—and still further, No. 7 with the *nitrate*,—may fairly be attributed to some defect in the seminal fluid or in the ova; since, if such were not the cause, and the failure had been due either to the chemical effect of the media on the ova, or to the moderate temperature of the atmosphere (49° FAHR.) at the time of experiment,—segmentation of the yelk would hardly have taken place. This supposition appears to be the more likely, when we recollect that in the *Set* Q, and *in that set only*, the impregnating fluid was obtained from the testes, compressed and broken down in water,—that the eggs were of doubtful maturity,—and that this was *the only set of experiments in which no embryos were ultimately produced*; although, I may now mention, that greater care was taken to ensure a favourable result than in most of these investigations,—the ova being removed at the end of twenty hours to an average temperature of 60° FAHR.,—were retained in flat shallow dishes,—and had the water changed daily. Both sets, however, Q and R, seem to prove that the *act* of impregnation, as evidenced in the fact of the yelk becoming segmented, must take place, or be commenced very rapidly; and, apparently, *almost at the instant of contact of the spermatozoon with the coverings of the ovum*; as seems to be shown in the fact, that segmentation took place in many of the ova when the space of time between the application of the spermatozoon, and that of the solution,—which previous observation (p. 225) showed was sufficient to decompose it immediately,—was scarcely more than *one* or *two seconds*. Thus in Q 4, and R 1, it must have commenced in the interval of *one second*, even when the strong solution was used; and in Q 2 and R 2 with the same solution in *two seconds*. When the weaker solution was used, a greater number of ova became affected in similar spaces of time, as in Q 2 and 3. These experiments seem to show that the *act* of impregnation had already been *commenced* before the application of the solution; as, in the experiments which are the converse of those now mentioned, in regard to the time when the spermatozoa and the solution were applied, a different result ensued. Thus when the *solution* was applied to the ovum *first* as in Q 6 and R 3 and 4, and *one*

second afterwards the impregnating fluid with spermatozoa, no impregnation, or but a very partial one, was effected. The ova in these three experiments amounted to *two hundred and six*, and yet only *one ovum* became very partially affected. A like result ensued even when the weaker solution was employed at an interval of *two seconds*, as in Q 5, when out of *fifty-four ova* segmentation occurred but in *one*.

When the interval between the application of the impregnating fluid, in the first instance, and that of the solution subsequently, was extended to *five seconds*, then a greater proportion of ova became segmented, as in Q 1, with the weak solution, when out of *forty-five* ova *twelve* became changed.

These were the results when the experiments were made at different temperatures, as at 49° FAHR. with the *Set* Q, and 60° FAHR. with the *Set* R. They cannot, therefore, be attributable to inertness of the fecundating agent, or of the object to be fecundated, occasioned by an unfavourable temperature of the surrounding medium. The fact of the occurrence of segmentation in some ova, but not in the majority of the ova of different experiments, as in Q 1, 2, 3 and 4, seems further to show that the influence of the momentary application of the potash solution was produced chiefly, and in the first instance, on the spermatozoa, or impregnating bodies, and not so immediately on the ova; since if the ova had been first, or most affected, none of them, probably, would have become impregnated.

Further, I may perhaps be allowed to remark, that the arrest of impregnation was due mainly to the nature of the chemical agent employed; and the extent of interference with the fecundatory process was in proportion to the more or less immediate action of this agent on the spermatozoon. Thus we have seen that but few ova were impregnated when the solutions of caustic potass were employed; but when the nitrate of potass was used as in Q 7, *forty-three* out of *seventy-seven ova* were segmented; while in R 6, 7 and 8, in which the total number of ova was *two hundred and twelve*, there were *fifty-three* segmented, and these produced *forty-seven embryos*.

The object of these sets of experiments, therefore,—that of endeavouring to ascertain within what period of time after the contact of the spermatozoon with the ovum its fecundatory function is exerted,—appears to have been somewhat fulfilled;—in so far as that in these experiments on the *Amphibia* the *commencement of the act of impregnation appears to have been almost instantaneous*. Yet there seems reason to believe that momentary contact of the impregnating body, even in the ovum of these animals, is not in itself sufficient to complete the fecundation, although it may tend to induce that condition of the yelk, segmentation, which we now are assured is always indicatory of its having been influenced by the fecundatory agent. If momentary contact were sufficient for the completion of the function, then partial impregnation, which so frequently takes place when spermatozoa are few in number, or in contact only for very brief periods, could hardly happen; while every ovum in which the process of cleavage is begun ought to pass through all its changes to the production of the embryo, circumstances being favourable to its development. But this we have

seen in the foregoing experiments is not the case. On the contrary, *duration* of at least some *seconds* of contact, varying no doubt in different tribes of animals, and, apparently also, *quantity* of spermatozoa, seem to be essential to fruitful and healthy impregnation, as appears to be shown in the *filtration experiments*, *Set* L 3, as compared with L 1 (p. 207). Possibly momentary contact may suffice to occasion segmentation, but certainly with *duration* of contact the ovum is fecundated.

This leads us further to inquire whether any endosmosis of the material substance of the spermatozoon is imbibed by the ovum during any period of impregnation, before or during segmentation of the yelk?—and whether those media which do not act chemically on the spermatozoon or the ovum can arrest the agency of the former? BISCHOFF has already shown that spermatozoa are in contact with the ovum in some Mammalia, the Rabbit* and Dog†, from quickly after the entrance of the ovum into the Fallopian tube until segmentation is nearly completed, and the yelk has acquired a tuberculated or mulberry-like surface. In the ovum of the Frog I have shown that the spermatozoa are in like manner seen on the envelopes from immediately after immersion in impregnating fluid until segmentation has commenced. In the Newt we have seen that *when impregnation is effected artificially*, they may be recognized on the surface for a much longer period,—from the time of contact with fluid, until the surface of the yelk has reacquired its original smoothness, a period, in my observations, of from thirty-six to forty-eight hours. The persistence of these bodies to a period after the first evident changes in the yelk have commenced, seems to favour a supposition that their function is not completed in momentary contact. Although we are at present unable to trace their influence beyond what is now stated, I think it can be shown that their function can be arrested by media which affect them mechanically, when submitted to such media at the moment of contact with the ovum.

7. AGENCY OF THE SPERMATOZOA AS AFFECTED BY MECHANICAL MEDIA.

The object of the next experiments was to learn whether the interposition of a dense fluid medium, which does not act chemically on the spermatozoa, would be as effectual in preventing the influence of these bodies on the ovum as in the *carmine experiments*, the effect of which seemed to be mechanical.

As the experiments were made at different periods, it will be seen, that although on two of these occasions the temperature of the atmosphere differed, the general results were similar.

Gum and Starch Experiments.—Set S. March 25, 1850. Atmosphere 48°.

(*a.*) *Gum.* No. 1. P.M. $2^h\ 22^m$.—*Seventy-six ova* were passed on a *dry surface* and were immediately bathed with a thick solution of gum-arabic, and *fifteen seconds afterwards* with seminal fluid and water, and fresh water was then added to them. The whole time of the experiment was *sixty seconds.*

* Entwickelungsgeschichte des Kaninchen-eies. 4to. 1842, tab. 2, 3 and 4, fig. 17 to 28.

† Entwickelungsgeschichte des Hunde-eies. 4to. 1845, tab. 1 and 2, figs. 10 to 16.

No. 2. *Fifty-eight ova* were treated in exactly the same way, the interval being about *fifteen seconds*, and the whole time *sixty*.

The seminal fluid employed was obtained from two males, the fluid used to No. 2 being from a male which had paired four days before. Out of the whole number of eggs in the two sets, amounting to one hundred and thirty-four, not one produced an embryo.

No. 3. March 30, 1850. P.M. 5^h 5^m. Atmosphere 49° FAHR.

One hundred and eight ova were passed on a *moist* surface, and were immediately bathed with a thick solution of gum as above, and one *second afterwards* with seminal fluid in water; the whole time occupied being *sixty seconds*.

Segmentation took place in *two*, or at most only *three* of these ova, and even in them very imperfectly, and much slower than in the corresponding ova of the set to which they belonged, *Set* Q (p. 228–9), in which the fluid employed was obtained from the testes of the Frog, and regarded as immature.

No. 4. P.M. 5^h 18^m.—*Fifty-eight ova* passed on a *moistened* surface were immediately bathed with solution of gum, and one *second afterwards* with seminal fluid from the same male as No. 3. The whole time occupied was *forty-five seconds*.

The result of these two experiments, as compared with others of the set to which they belonged, *Set* Q, was exceedingly curious. In the experiments with the nitrate of potass as in Q 7, segmentation was carried to some extent, and the divisions of the yelk were multiplied; while only *four ova* out of the fifty-eight, in this with gum, gave any evidence of segmentation, and the process was not advanced further, either in this or in the preceding experiment, No. 3, than to the completion of the primary division of the yelk into two hemispheres. Thus not only was the process entirely prevented in the great majority of the ova, but it was also very much retarded in those in which it did take place, and this simply, as it appeared, by the mechanical hindrance of the gum. Could it be that the effect was produced on the endosmic action of the yelk? These trials certainly appeared to show that the obstruction was a mechanical one. I need scarcely remark, that *no embryo* was produced in either of these experiments.

No. 5. April 3, 1850. P.M. 3^h 25^m. Atmosphere 60° FAHR.

Sixty-one ova were passed on a *moistened* surface and were immediately bathed *with impregnating fluid*, and *two seconds afterwards* with a thick solution of gum-arabic, and water was then added; the whole time occupied being only *twenty seconds*.

This experiment, when compared either with the four preceding ones, made at a temperature of the atmosphere eleven degrees lower, or with that which follows, No. 6, seems to point to the exact nature of the operation of the gum. At *four hours* and *five minutes* from *fifteen* to *twenty* ova had become segmented, and others were in the act of becoming so. At *seven hours and a half* more than one-half of the whole number had changed, and were perfectly healthy. Thus, in this case, in which the gum was applied *after* the seminal fluid, impregnation occurred *earlier* than in corresponding experiments of the same set, R 6, 7 and 8, with nitrate of potass, when it happened in from *four hours*, and *fourteen* to *twenty minutes*. It was even as

rapid as in the artificial impregnation, R 5 (p. 231), in which it took place in *four hours and thirteen minutes.* On the eighth day *twelve embryos* had been produced.

These facts seemed to show, precisely as in experiments with solutions of potass, that impregnation is commenced very quickly; and further, that it was not arrested by the gum when applied only *two seconds* after the spermatozoa, but that the change proceeds almost as uninterruptedly as in a perfectly natural impregnation, since the number of embryos was almost as great as in No. 5 R, seeing that the fluid employed had been obtained and mixed with water one hour and thirty-four minutes.

No. 6. P.M. 3^h 22^m.—*Seventy ova*, passed on a moistened surface, were bathed with a thick solution of gum, and *two seconds afterwards* with some of the impregnating fluid employed in the last experiment, and water was then added; the whole time occupied, as above, being only *twenty seconds.*

This experiment was the converse of the preceding. At *four hours and twenty-eight minutes* only one egg out of the whole had become segmented; but others gave signs of being about to change, and some hours later a few had done so, but there were not at most more than *ten.* At the end of the *seventh* day *two embryos* had been produced.

Thus while a comparison of these two experiments seems to show that the gum acts simply as a mechanical obstruction to the process of fecundation, this experiment, No. 6, when compared with Nos. 1 to 4, made at eleven degrees lower temperature, shows also the influence of a higher degree of temperature in accelerating fecundation.

The two following experiments were made with a view to test the efficiency of the fluid and ova employed, now at one hour and fifty minutes after the fluid had been obtained: when examined at this time with the microscope, there were still an abundance of active spermatozoa.

No. 7. P.M. 3^h 41^m.—*Eighty-five ova* were accordingly placed in water with some of the impregnating fluid and allowed to remain to test its efficacy.

At *four hours and twenty-four minutes* nearly the whole of the ova had become segmented, and on the seventh day *forty-two embryos* had been produced.

No. 8. P.M. 5^h 50^m.—*One hundred and thirty-two ova* were now passed into the remainder of the impregnating fluid, which had at this time been *four hours* mixed with water.

When examined at the end of five hours and ten minutes, not a single specimen had become impregnated. This was proved by the result, that at the end of seven days *not a single embryo* had been formed. The fluid had thus lost its fecundating property at the end of four hours in a temperature of 60° FAHR.

No. 9. April 6, 1850. Atmosphere 60° FAHR.

P.M. 1^h 50^m.—One more experiment was now made with the gum solution, for the purpose of comparing it with the following experiments with starch.

One hundred and twenty-two ova were passed on a dry surface and covered with a thick solution of gum, and *three seconds afterwards* with impregnating fluid that had been mixed with water only *thirty minutes.* The time occupied was not noted, but fresh water was added to the ova at the end of fourteen minutes.

Segmentation took place only, in two or three ova, at *four hours and twenty-four minutes*, and at the end of ten days only *three embryos* had been produced.

(*b.*) *Starch.* No. 10. P.M. 1^h 40^m.—*One hundred and fifty-eight ova* were passed into a solution of starch in water, and at the end of *ten seconds* one-half of the whole quantity of seminal fluid, obtained from a single frog, and previously mixed with water, was added to them.

After the ova had remained in the solution and been gently agitated during twenty minutes, they were carefully washed and removed to clear water.

At *four hours and twenty-six minutes* only a very few of these ova, not more than eight or ten, had become segmented, notwithstanding the large quantity of recent impregnating fluid that had been added to them. At the end of ten days only *five embryos* had been produced.

It was remarkable that very few of the ova in this experiment cohered together, as the frog's ova almost invariably do when placed in fluid. On the contrary, most of them remained separate and isolated, although their envelopes had imbibed water and expanded to their usual extent in a similar space of time.

No. 11. P.M. 1^h 47^m.—*Seventy-one ova* were passed on a perfectly dry surface, and immediately afterwards were covered, by means of a hair-pencil, with a thick solution of starch, and at the expiration of *ten seconds* with impregnating fluid, and water was quickly added.

The water was changed at the end of fifteen minutes. At *four hours and twenty-five minutes*, only two or three ova had become segmented. At the end of ten days *three embryos* had been produced.

The concluding experiment with starch was the counterpart of No. 5, with *gum*.

No. 12. P.M. 1^h 44^m.—*One hundred and nineteen ova* were passed into water, with which one-eighth part only of the seminal fluid, obtained from the Frog, had already been mixed. *Two seconds* afterwards a solution of starch was added to these ova, and at the end of sixteen minutes they were removed to clear water.

In *four hours and twenty-six minutes* segmentation had commenced in many of these ova. The exact number I omitted to ascertain. But the change had taken place in a much shorter space of time, and was more general, although scarcely one-fourth part of the quantity of seminal fluid that had been employed in No. 10 was used in this case. Nevertheless, in ten days *twelve embryos* had been formed.

This experiment, therefore, was quite confirmatory of the conclusions drawn from its counterpart, No. 5, with *gum*, and Nos. 10 and 11 as fully bore out those deduced from Nos. 1 to 5, with the same; while the entire set seem to be in full accordance with the already arrived at conclusion, that fecundation is commenced almost immediately the fecundating body is in contact with the ovum. Thus, then, with regard to the nature of impregnation, we seem to have obtained sufficient proof that the *act* is effected through the agency of the spermatozoon, and not through that of the *liquor seminis*, as was formerly supposed.

TABLE II.—Experiments with Media that act *chemically* or *me-*

No. of experiments.	Date and hour of experiment.	Temperature at the time of the experiment. Atmosphere.	Water.	Mean daily temperature after the experiment.	Segmentation comenced in hours.	No. of eggs Employed.	Segmented.	Embryos at End of third stage.	In days and hours.	Nature of the impregnating fluid.	Time, procured and mixed with water.
1850.											
Set P. 1.	March 25. P.M. 1 40	48	46	61	.....	54	.....	20	$4^d\ 12^h$	Semen and water.	10^m
2.	P.M. 1 45	48	46	61	.....	39	.....	2	$4^d\ 12^h$	Semen and water.	15^m
3.	P.M. 1 50	48	46	61	.....	47	.....	Not one.	Not one.	Semen and water.	20^m
4.	P.M. 1 53	48	46	61	.....	45	.....	3	5^d	Semen and water.	23^m
5.	P.M. 2 21	48	46	61	.....	59	.....	Not one.	.....	Semen and water.	51^m
6.	P.M. 2 26	48	46	61	.....	72	.....	Not one.	.....	Semen and water.	56^m
7.	P.M. 2 16	48	46	61	.....	44	.....	Not one.	.....	Semen and water.	46^m
8.	P.M.	48	46	61	.....	76	.....	Not one.	.....	Semen and water.	10^m
Set S. 1.	P.M. 2 22	48	46	61	.....	76	.....	Not one.	.....	Semen and water.	52^m
2.	P.M.	48	46	61	.....	58	.....	Not one.	.....	Semen add water.	12^m
Set Q. 1.	30. P.M. 4 45	49	48	61·5	.....	45	12	Not one.	.....	Semen from testes.	10^m
2.	P.M. 4 58	49	48	61·5	.....	53	4	Not one.	.....	Semen from testes.	23^m
3.	P.M. 4 52	49	48	61·5	.....	122	50 to 60	Not one.	.....	Semen from testes.	17^m
4.	P.M. 5	49	48	61·5	.....	66	2	Not one.	.....	Semen from testes.	25^m
5.	P.M. 4 55	49	48	61·5	.....	54	1	Not one.	.....	Semen from testes.	20^m
6.	P.M. 5 2	49	48	61·5	.....	79	.....	Not one.	.....	Semen from testes.	27^m
7.	P.M. 5 15	49	48	61·5	.....	77	43	Not one.	.....	Semen from testes.	40^m
8.	P.M. 5 9	49	48	61·5	.....	76	.....	Not one.	.....	Semen from testes.	34^m
Set S. 3.	P.M. 5 5	49	48	...	18 to 20^h at temp. 49 to $48°$	108	3	Not one.	.....	Semen from testes.	30^m
4.	P.M. 5 18	49	48	...	18 to 20^h	58	4	Not one.	.....	Semen from testes.	43^m
Set R. 1.	April 3. P.M. 3 5	60	...	...	4^h	82	12 partial	2	6 to 7^d	Semen and water.	$1^h\ 12^m$
2.	.....	60	...	...	.....	25	1	1	6 to 7^d	Semen and water.	.....
3.	P.M. 3 10	60	...	...	$4\frac{1}{4}^h$	58	1 partially	Not one.	.....	Semen and water.	$1^h\ 17^m$
4.	.....	60	...	...	.....	69	.....	Not one.	.....	Semen and water.	1^h
5.	P.M. 3 17	60	...	...	$4^h\ 13^m$	62	30 to 40	23	5^d	Semen and water.	$1^h\ 24^m$
6.	P.M. 3 31	60	...	...	$4^h\ 14^m$	74	12 to 15	12	5^d	Semen and water.	$1^h\ 38^m$
7.	.....	60	...	...	$4^h\ 14^m$	59	13	10	5^d	Semen and water.	.....
8.	P.M. 3 37	60	...	...	$4^h\ 20^m$	79	25	25	5^d	Semen and water.	$1^h\ 46^m$
Set S. 5.	P.M. 3 25	60	...	...	$4^h\ 5^m$	61	33	11	5^d	Semen and water.	$1^h\ 34^m$
6.	P.M. 3 22	60	...	...	$4^h\ 30^m$	70	10	2	7^d	Semen and water.	$1^h\ 31^m$
7.	P.M. 3 41	60	...	...	$4^h\ 24^m$	85	74	42	6^d	Semen and water.	$1^h\ 50^m$
8.	P.M. 5 50	60	...	...	.....	132	Not one.	.....	.....	.....	4 *hours*
Set S. 9.	6. P.M. 1 50	60	...	...	$4^h\ 24^m$	122	3	3	7^d	Semen and water.	30^m
10.	P.M. 1 40	60	...	...	$4^h\ 20^m$	158	8 or 10	5	7^d	Semen and water.	20^m
11.	P.M. 1 47	60	...	...	$4^h\ 25^m$	71	3	3	7^d	Semen and water.	27^m
12.	P.M. 1 44	60	...	...	$4^h\ 18^m$	119	.....	12	$5\frac{1}{2}^d$	Semen and water.	24^m
					Total number	2634	369	176	in Sets P, Q, R, & S.		

RECAPITULATION AND CONCLUSIONS.

It may now be well to recapitulate briefly some of the facts and views derived from the foregoing observations and experiments. *First*, then, the *germinal vesicle* disappears in the Amphibia before impregnation; and before, or at the time of the bursting of the ovisac, and extrusion of the egg from the ovary into the cavity of the

chanically on the Spermatozoon or the Ovum in Impregnation.

Material employed.			Period of experiment.			Remarks.
Operating mechanically.	Operating chemically.	Quantity per ounce of water.	Interval *before* fluid.	Interval *after* fluid.	Whole period.	
.....	*Solut. caust. pot.*	℈j to ʒj.	.....	15 sec.	1m	No. 1 to 8 of this set removed after fifty minutes to temperature 61° Fahr. These ova were passed on a dry surface, and fluid applied freely with a pencil, and afterwards the potass washed off freely with water.
.....	Solut. caust. pot.	℈j to ʒj.	.....	5 sec.	40 sec.	
.....	Solut. caust. pot.	℈j to ʒj.	.....	10 sec.	30 sec.	Potass allowed to remain.
.....	Solut. caust. pot.	℈j to ʒj.	5 sec.	.....	30 sec.	Ditto, well bathed with fluid *after* potass.
.....	*Solut. nitr. pot.*	℈j to ʒj.	.....	6 sec.	30 sec.	Well bathed with solut. nitr. potass and water then added, the solution allowed to remain.
.....	Solut. nitr. pot.	.....	.....	5 sec.	30 sec.	
.....	*Acetic acid.*	ʒj to ʒj.	5 sec.	.....	20 sec.	Impregnating fluid applied quickly and profusely, and water then added.
.....	Acetic acid.	ʒj to ʒj.	5 sec.	.....	20 sec.	
Solut. *gum-arab.*	.....	.....	15 sec.	.....	1m	Water was added quickly after the gum, but not sufficient to remove it; one or two ova segmented.
Solut. gum-arab.	.....	.....	15 sec.	.....	1m	
.....	*Solut. caust. pot.*	*gr. viij to* ʒj.	.....	5 sec.	35 sec.	No. 1 to 8 of this set also removed at end of nineteen hours to high temperature, 62° Fahr., No. 1 and 2 being thoroughly bathed with seminal fluid.
.....	Solut. caust. pot.	℈j *to* ʒj.	.....	2 sec.	45 sec.	
.....	Solut. caust. pot.	Weak.	.....	2 sec.	45 sec.	Water added and solut. of potass not washed off.
.....	Solut. caust. pot.	Strong.	.....	1 sec.	62 sec.	Nearly the whole at twenty hours spoiled.
.....	Solut. caust. pot.	Weak.	2 sec.	.....	50 sec.	
.....	Solut. caust. pot.	Strong.	1 sec.	.....	45 sec.	All spoiled in a few hours.
.....	*Solut. nitr. pot.*	gr. viij to ʒj.	.....	1 sec.	30 sec.	Ova passed on a moist surface and *well bathed* with fluid.
.....	*Acetic acid dilut.*	.....	.....	1 sec.	45 sec.	
Solut. *gum-arab.*	.....	.....	1 sec.	.....	1m	This was a thick solution of gum and applied with difficulty, but freely and quickly; the eggs were then well bathed with fluid and allowed to remain in a low, and slightly diminishing temperature.
Solut. gum-arab.	.....	.....	1 sec.	.....	45 sec.	
.....	*Solut. caust. pot.*	Strong.	.....	1 sec.	15 sec.	Ova touched lightly for an instant only with the fecundating fluid, and water afterwards added.
.....	Solut. caust. pot.	Strong.	.....	2 sec.	20 sec.	
.....	Solut. caust. pot.	Strong.	1 sec.	.....	15 sec.	Time of applying the potass was from two to three seconds; the eggs were spoiling within twenty minutes.
.....	Solut. caust. pot.	Strong.	1 sec.	.....	12 sec.	
.....	.....	.....	.....	.....	.....	Made with *fecundating fluid* without solution of potass to test the above.
.....	*Solut. nitr. pot.*	Strong.	.....	1 sec.	20 sec.	These ova were passed on a dry surface and were then thoroughly bathed with seminal fluid and water.
.....	Solut. nitr. pot.	Strong.	.....	3 sec.	25 sec.	
.....	Solut. nitr. pot.	Strong.	3 sec.	.....	20 sec.	The fluid was applied *after* the solution.
Solut. gum-arab.	.....	.....	.....	2 sec.	20 sec.	Impregnation must thus have occurred very quickly.
Solut. gum-arab.	.....	.....	2 sec.	.....	20 sec.	
.....	.....	.....	.....	.....	.....	Made to test the fecundatory fluid at *two hours* after mixture with water.
.....	.....	.....	.....	.....	.....	Not one egg segmented or one embryo found.
Solut. gum-arab.	.....	.....	3 sec.	.....	.....	Gum applied thickly and the ova then bathed with fecundating fluid.
Solut. of starch.	.....	.....	10 sec.	.....	.....	The ova *passed into* the starch, and a large quantity of fluid then added.
Solut. of starch.	.....	.....	10 sec.	.....	.....	Ova passed on a dry surface, starch applied with a pencil.
Solut. of starch.	.....	.....	.....	2 sec.	16m	Changed to fresh water after sixteen minutes.

abdomen. It does not return to the centre of the yelk, nor escape to the surface, but is lost much nearer to the latter than to the former position; and its disappearance is the result of the endogenous development of cells in its interior. The egg is cast loose into the abdomen, and then consists only of the yelk mass in its vitelline membrane, and it is transferred to the mouth of the oviduct by the joint action of

the abdominal muscles and the motions of the viscera, and not necessarily through the aid of the male during copulation. *Second*, changes are going on in the constituents of the egg, both before and after oviposition as well in the unimpregnated as in the impregnated condition; but they soon cease in the former, and do not proceed to the cleaving or segmentation of the yelk. *Third*, that the egg is not susceptible of impregnation until after it has acquired the envelopes which it gains in the oviduct. *Fourth*, that endosmosis of the entire egg takes place through these envelopes, and is most rapid during the few minutes the egg is most susceptible of impregnation. Further, that this endosmosis is augmented and hastened by an increase, and is lessened and retarded by a diminution of temperature; and that the susceptibility of the egg to become impregnated, and produce, is in exactly the same condition with regard to heat; whether the egg be exposed to, or whether it be excluded from light. *Fifth*, that only extremely minute granules of solid matter can by any possibility pass into the tissue of the envelopes during endosmosis; and that there is no evidence whatever of the existence of a fissure or orifice, in the envelopes of the egg of the Amphibia, at the time of, or before impregnation, capable of admitting the spermatozoon to the interior of the yelk-membrane or its contents. *Sixth*, that it is the spermatozoon alone which effects impregnation; and that this does not take place until the spermatozoon is brought into immediate contact with the external envelopes of the ovum. *Seventh*, that the *liquor seminis*, when entirely separated from spermatozoa, certainly does not effect impregnation. *Eighth*, that although direct contact of the spermatozoa with the ovum is indispensable to effect impregnation, I have never been able to detect any traces of these bodies in contact with the yelk-membrane, or even within the substance of the external envelope. *Ninth*, that impregnation is *commenced* the instant the spermatozoa are brought into contact with the egg, but a certain *duration of contact* is essential to its completion. *Tenth*, that impregnation is not effected when the whole or the majority of the spermatozoa in contact with the envelopes have previously become motionless and, apparently, have lost vitality, as they are found to have done after the lapse of a longer or shorter period. *Eleventh*, that although an exceedingly minute quantity of spermatozoa suffice to impregnate the ovum, the phenomenon of impregnation takes place more tardily, even with duration of contact when the number is extremely limited, than when it is in full abundance, without excess; while when the quantity is deficient, or the duration of contact too limited, then the phenomenon is incomplete, and partial impregnation only is effected. *Twelfth, partial impregnation* is shown in imperfect segmentation of the yelk; and is due chiefly to the spermatozoa being insufficient in quantity, or in duration of contact, or inefficient through diminished vitality; and it may also result from diminished susceptibility in the ovum. *Thirteenth*, partial impregnation of the ovum is of frequent occurrence, as I found in my first experiments with fluid that had passed through filtering-paper, but which still contained a very few spermatozoa, either motionless or exceedingly feeble; and further, partial impregnation is of

much the most frequent occurrence when the ova are placed in dense fluid before contact with the spermatozoa, as in the experiments with carmine. *Lastly*, when the ova are only partially impregnated they are usually, and perhaps always unproductive.

These facts lead us to inquire, whether impregnation takes place through any catalytic influence of the spermatozoa as suggested by Bischoff*, while in a state of activity, and at the instant they are brought into contact with the ovum, or whether impregnation results from a diffluence of the spermatozoa thus brought into contact with the surface, the substance into which they may be dissolved being carried by endosmosis with the water imbibed through the tissues; or whether it is the result of the conjoint influence of both these conditions;—the first action induced being instantaneous and catalytic, and possibly dependent on the persistence of organic vitality in the spermatozoa, while the completion of the impregnation may depend on the imbibition of some material influence or substance derived from the impregnating body;—a view which the gradual disappearance of the bodies of the spermatozoa from the surface of the ovum, both in the Frog and Newt, seems to favour; as we have already seen that endosmosis is an active and important function of the envelopes of the ovum at the very period when impregnation is effected.

All the experiments now detailed seem to show that in those vertebrata which expel their ova into water before impregnation, as in the tail-less *Amphibia*, and in which—from the nature of the medium into which the ova are passed—we may infer that the function takes place most quickly, impregnation is commenced at the very instant of contact of the spermatozoon with the ovum, and even may be completed within very short spaces of time—but *duration* of at least some seconds of actual contact,—even in these animals' ova, is essential to the perfection of the function;—but this period, we may fairly conclude, may differ in different classes of animals, and possibly may have some relation to the greater or less facility with which the spermatozoa are brought into contact with the ova.

When the experiments last detailed are compared,—the effects produced by the application of media which influence the spermatozoon and the ovum chemically,—with those of which the effect is merely mechanical, we seem to have made some advance towards a future knowledge of the nature of the impregnating power. Although we are as yet entirely without proof that any material influence or substance is actually transmitted from the spermatozoon on the surface of the ovum to the yelk in the interior, we have evidence that fluids are imbibed by the ovum by endosmosis through its tissues; and although not a trace of the spermatozoon is detected in the interior of the ovum, we have seen that it remains for a long time on the surface, and gradually disappears, apparently by diffluence; so that it may be fair to conclude, that the agency of this body is material in its operation. On the other hand, the effect which we find is produced on the yelk by the direct and even momentary contact of the

* Müller's Archives, 1847.

spermatozoon with the envelopes of the ovum, seems closely to resemble that of the so-called catalytic power of certain known bodies, in so far as that contact, during only very short spaces of time, with the surface of the ovum, appears to be sufficient to induce certain changes in the interior. These changes, too, as known of catalysis, are carried only to a certain extent when the exciting agents,—in this instance the spermatozoa,—are feeble in action or but very few in number; and then, as we have seen, the yelk may become only more or less partially segmented; or the changes in it, having proceeded to a certain extent, may then become arrested, apparently from deficiency of the originally exciting cause. Then, again, we find that although segmentation of the yelk may take place, embryos are not produced unless there has been some continuance or duration of contact of the impregnating with the impregnated body; and that the number produced seems to have reference to the duration and to the full sufficiency of the exciting cause. But neither what we at present know of the so-called catalytic power or of endosmosis, appears alone to be sufficient to account for the whole of the phenomena of impregnation. Simple contact of the spermatozoon does not appear to be sufficient to determine the transmission of more or less of the material structural characters of the male parent to the offspring; while diffluence and endosmosis of the substance of the spermatozoon can hardly be imagined to occur in a brief second or two of time sufficiently to effect the full impregnation of the yelk, and induce its invariable consequence, segmentation. Possibly, we may hereafter find that the first changes induced by contact of the impregnating body are completed by its diffluence, and by the material constituents into which it is dissolved, being transferred to the yelk by endosmosis.

Description of the Plate.

PLATE XIV.

Fig. 1. The female Frog, *Rana temporaria*, dissected to show the situation of the entrance to the oviducts (*a*) at each side of the heart (*b*). The liver (*c*) is drawn back and removed a little from its natural position to show the spaces (*d*) along which the ova pass from the cavity of the abdomen to the mouths of the oviducts (*g*), to be received into the dilated or uterine portion of the ducts (*h*). (*i*.) The stomach. (*k*.) Intestine. (*l*.) Colon and rectum. (*m*.) The bladder.

Fig. 2. A portion of the commencement of the oviduct magnified, partially concealed by the root of the lung.

a. The entrance to the duct between the heart and liver. (*e*.) The suspensory ligament of the liver. (*f*.) The base of the lung around which the oviduct (*g*) passes.

Fig. 3. The female Frog, exhibiting the viscera *in situ* before the ova have left the ovaries (*p*) and with the oviducts (*g*) enlarged with secretion, for the for-

mation of the envelopes of the ova as they pass through to the uterine or dilated portions of the ducts (*h*).

Fig. 4. The female Frog after oviposition, with the organs of digestion and the liver removed to show the condition of the ovaries (*p*) with their fatty appendages (*q*), the hyoid and thyroid muscles (*n*) (*o*) (*p*), the lungs (*f*) and the contracted state of the oviducts (*g*), and their uterine enlargement (*h*) with the rectum (*l*), and the bladder (*m*) then beginning again to be enlarged.

Fig. 5. Structure of the ovarian ovum.

a. The ovum while still attached to the inner surface of the ovary and projecting into the cavity, exhibiting the dark surface within the ovisac, which is traversed by minute vessels (*b*).

Fig. 6. Vertical section of the ovum, showing the situation of the germinal vesicle and the canal in the yelk, which corresponds to the centre of the dark surface of the yelk.

Fig. 7. The presumed mode of disappearance of the vesicle.

Fig. 8. Spermatozoa of the Frog. *a* and *b* escaping from the vesicle of development, *c*, as seen on the egg after contact.

Fig. 9. An ovum with spermatozoa half an hour after impregnation.

Fig. 10. A small portion of surface of the yelk at the commencement of segmentation, highly magnified. (*a*.) Yelk-cells at the same period. (*b*.) The smaller cells of the last, more highly magnified.

Figs. 11 and 12. Examples of partial impregnation at twenty-eight hours after contact with the spermatozoa.