X. *On the Impregnation of the Ovum in the Amphibia.* (*Second Series, Revised.*) *And on the Direct Agency of the Spermatozoon. By* GEORGE NEWPORT, *F.R.S.*, *F.L.S. &c.*

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HAVING shown in a former series of investigations, which has been honoured by a place in the Philosophical Transactions for 1851, that the sole agent of impregnation of the ovum, in all cases of communion of the sexes, is the spermatozoon,—and having then supplied both direct and negative evidence that impregnation is not effected by the *liquor seminis*,—I endeavoured, in a subsequent communication to the Royal Society, in June 1851*, to arrive at some knowledge of the manner in which impregnation is effected, and of the nature of the impregnating influence.

Up to that period, and indeed, until very recently, I had never been able to detect any evidence of the existence of spermatozoa within the envelopes of the fecundated egg, but had constantly found them in great abundance, and easily recognized, in contact with the exterior surface. Experiment also, made by immersion of the egg in coloured fluids, showed that the substance of the envelopes, although permeable by fluids, is uniform in its structure; but no evidence was afforded by it of any natural canal, fissure, or perforation through the envelopes of the egg of the Frog, capable of admitting the spermatozoon to the interior, as has been supposed to exist in the egg of the Mammalia†.

Hence the conclusion which seemed to be fairly led to, was, that some influence was transmitted from the spermatozoon on the surface of the envelopes, through their substance, to the yelk which they inclose,—as the *commencement* of impregnation. But it was especially pointed out in my First Series‡, that "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;" and that, "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;" Further, in my second communication, I remarked,—"I am not yet prepared to assent to the view, that simple contact alone, even of the spermatozoon, is sufficient to complete the changes which result in the formation of the embryo§." Again, in

* Proceedings of Royal Society, vol. vi. p. 82.

† Philosophical Transactions, 1840, p. 533, Plate XXII. figs. 165, 167.

‡ Philosophical Transactions, 1851, p. 242.

§ See "Impregnation of the Ovum (Second Series)," June 19th, 1851, MS. No. 762, p. 49, in the Archives of the Royal Society.

a subsequent part of the same communication, these considerations are further referred to, as being those alone on which the transmission of structural peculiarities seemed likely to be explained*.

Recent observation has now supplied to me a fact which renders these considerations, which heretofore were regarded as hypothetical, much more probable; but, at the same time, it necessitates a revision of the view that an impregnating influence is transmitted from the surface to the interior of the egg; and also some correction of the deductions from experiment by which this view seemed to be supported.

The fact referred to, is, that the spermatozoon of the Frog penetrates bodily into the substance of the thick envelopes of the egg, and comes into direct communication with, at least, the membrane which incloses the yelk in the interior.

It is my duty, therefore, to Science, and to the Royal Society, knowing this to be the case, to revise and to extend my views, and to submit them again to the Society in their amended form; making, in self-correction, the certainty of fact now take the place of negative observation.

In doing this, I shall endeavour to show, that, although still regarding the spermatozoon, for reasons to be adduced, as the organ of a special condition of force, or vitality in the male body,—its influence on the egg in fecundation is direct and immediate, and not operative, as heretofore supposed, merely through the envelopes; but, nevertheless, as I have before shown, that it is exerted only so long as the spermatozoon continues to give evidence of its vitality or force in its power of motion.

Before proceeding to show that the spermatozoon penetrates into the envelopes of the egg it is necessary that I should point out some of the conditions which affect the fulfilment of its fecundatory function, both in regard to the spermatozoon itself and to the ovum. These are, on the one hand, the persistence of vital power in the spermatozoon as influenced by the temperature of the surrounding medium; and on the other, the endosmic property of the gelatinous coverings of the egg, and the susceptibility of the yelk which they inclose, to become impregnated.

1. THE VITALITY OF THE SPERMATOZOON AND OVUM COMPARED.

1. *Of the Spermatozoon.*—It has been elsewhere shown† that when the mature spermatic fluid of the Frog has been more than four hours removed from the living body, and mixed with water, at a temperature of 50° Fahr. or but a few degrees higher, it usually has already ceased to have any power to fecundate the egg; and its efficiency has been found to have been diminished in proportion to the length of time it has been obtained and so mixed. Nearly the whole of the observations I have since made have coincided with these results. Yet there have been two marked instances in which fecundation was effected by fluid, which had been obtained, and

* *Loc. cit.* p. 56.

† Philosophical Transactions, 1851, p. 213.

mixed with a small quantity of water, *twenty-four hours* before it was employed. In the first case, the fluid was obtained when the temperature of the atmosphere was 51° FAHR., and afterwards sunk to 49° FAHR., but had again risen to 51° FAHR. at the time of the experiment. When examined with the microscope, immediately before it was used, the fluid still contained a quantity of very active vibratile spermatozoa, and also an abundance of spermatozoal cells, still in course of development. This fact seemed to afford an explanation of the cause of the efficiency of the fluid for so great a length of time, spermatozoa being liberated from the cells during the whole period. Thus the fact of the prolonged fecundity of this fluid seems to confirm, instead of oppose the conclusion arrived at,—that, the more mature the fluid is before its removal from the body, the more efficacious it is, but only for a given extent of time, since it is then composed almost entirely of very active spermatozoa, with but very few cells of development. SPALLANZANI, as formerly mentioned*, found that the fluid of the fœtid terrestrial Toad (*Bufo calamita*?) at a high temperature of the season, 70° FAHR. to 73° FAHR., at which this species spawns in Italy, had lost its fecundatory influence at the end of six hours†; but that in the temperature of an ice-house, 40° FAHR. it retained its efficacy for twenty-five hours‡. Further, that when the fluid was preserved in the testes of the dead animal, at the temperature of the season mentioned, it became effete in nine or ten hours; but that it continued to be efficacious, when retained in those organs in the temperature of an ice-house for thirty-four hours. Again, he found that the fluid of the green aquatic Frog (*Rana esculenta*), when mixed with a large quantity of water, and placed in an ice-house, the temperature of which he gives at $3\frac{1}{2}$ REAUM. (39·87° FAHR.) retained its fecundatory property for thirty-five hours§. But it must be borne in mind, with regard to these observations, that the species of Toad (*Bufo*), pair later in the season, and at a higher temperature than those of the Frog; and also that the fluid employed by SPALLANZANI in all his experiments, was obtained by vivisection, either from the testes or the vesicles; and no doubt contained, as in my experiment just mentioned, many undeveloped cells; and, consequently, not being fully matured, retained its influence longer than it would have done, under the same circumstances, in its perfect condition.

But PREVOST and DUMAS‖, in some experiments made to ascertain the length of time during which the fluid of the species they experimented on,—the *Rana esculenta*,—retains its fecundatory property, observed that it was efficient at *twenty-four* hours, although removed from the body and preserved during that time, in a temperature that varied from 18 to 22 cents., or from 64′ to 71° FAHR.¶ This result, differing so much, in regard to temperature, from the results formerly arrived at by SPALLANZANI, in regard to the Frog, and from the majority of those since obtained by myself, may, I think, in great part be accounted for in the fact that, like the Toads, the *Rana*

* *Loc. cit.* p. 212. † Dissertations, &c., vol. ii. p. 169. ‡ *Loc. cit.* p. 169. § *Loc. cit.* p. 194.

‖ Annales des Sc. Naturelles, tom. ii. 1824. ¶ *Loc. cit.* p. 140.

esculenta spawns at a higher temperature, and later in the season than the *R. temporaria,* the subject of my observations, and that the fluid employed by PREVOST and DUMAS, was always obtained, as they state, by vivisection, and was expressed from the testes, and necessarily must have been in great part immature, and contained many spermatozoal cells, from which the fecundatory agents were liberated at different periods during the observations, and thus appeared to show that the fluid continues to be efficacious, at a high temperature of the atmosphere, for a much longer period than is really the case. In my own experiments the fluid employed being obtained, without vivisection, by simple compression of the body from the seminal ducts and vesicles, has usually contained only perfectly mature and very active spermatozoa, with but few cells of development. In this condition, as I have stated, it has retained its fecundatory property only during the first three or four hours. Yet, in the exception above mentioned, and also in the second one alluded to, which occurred more recently, the fluid was efficacious at the end of *twenty-four hours* in the first, and *twenty-six hours* in the second instance, the temperature being similar on the two occasions. In both instances the fluid contained a very large quantity of developmental cells, and had been procured from individuals which were not fully prepared for the exercise of their fecundatory function. Further, I may add, that in each of these instances, after the fluid had been supplied to some eggs to test its effect, I found many undeveloped spermatozoal cells adhering to the surface of the eggs, and detected some spermatic bodies in the act of being liberated from them. These facts, then, are confirmatory of the suggestion respecting the results obtained by PREVOST and DUMAS.

The presence of undeveloped cells in the fluid obtained from the living Frog is explicable in two or three ways: first, in that of the animal having but recently been taken from its natural haunts, either early in the season, or at a later period, after a continuance of unusually cold weather and easterly wind, or after the sexes have but recently united; in either of which cases, as also in certain others, in which the individual itself has been late in the development of its reproductive organism, developmental cells are thrown off from the testes together with already liberated spermatozoa, and are usually abundant in the efferential ducts and vesicles. It may thus be seen that in judging of the length of time during which the fluid preserves its fecundatory influence, and consequently, the spermatozoon its vitality, after removal from the body, it is necessary to take into consideration, not only the temperature of the surrounding medium, but also the precise condition of the fluid itself. In perfectly natural impregnation by the Frog there is reason to believe that the fluid contains but very few undeveloped cells, and that it is not employed until it has acquired its full maturity, and that,—as the time of its actual employment is exceedingly brief,—the oviposition of *Rana temporaria* occupying scarcely a single minute, the retention of the impregnating influence and vital force of the spermatozoon may be even for a much shorter space of time than I have mentioned—from three to four

hours. But this time may differ in different species of the *Anoura*, and of the higher vertebrata, and may have some relation to the particular economy of each animal.

The general conclusion which seems to be deducible from a comparison of the observations of SPALLANZANI and of PREVOST and DUMAS, with those by myself, in regard to the tail-less Amphibia, is, that—making allowance for the difference of species and habit of the animals experimented on, and for the degree of maturity of the fluid, which there seems reason to think does not attain its perfect condition until it has passed into the efferential ducts and vesicles—the vitality of the spermatozoon, and the duration of its fecundatory power, are in a ratio *inverse* to that of an increase of temperature in the surrounding medium.

2. EVOLUTION OF VITALITY IN THE SPERMATOZOON AND OVUM.

WAGNER and LEUCKARDT have already pointed out* that the power of motion possessed by the spermatozoon is closely connected with the completion of its structure and composition, and is gradually evolved as the development of this body proceeds. This power of motion I shall presently endeavour to show I regard as the visible exponent of its fecundatory force, or form of vitality, and that a similar power is evolved concurrently with this in the spermatozoon in the contents of the egg, the evolution in both being more or less influenced by temperature, according to the species. These facts are well shown both in the Frog and Toad.

Several pairs of Frogs were collected on the 2nd of March, when the temperature of the season was low, and strong easterly winds prevailed, during which Frogs seldom spawn, except in very sheltered places. As one female had passed some eggs, and believing that the others were equally advanced, I selected a pair, which appeared to be the most mature, for experiment. The fluid from the male was obtained with the greatest ease, in full quantity of a white and somewhat opake colour. When examined with the microscope it was found to consist in chief part of developmental cells, containing each its motionless immature spermatozoon. Besides these cells it also included many others less far advanced, and in which I was unable to distinguish this body. In addition to these there were some also from which the spermatozoa were in the act of being liberated, besides many very active spermatozoa already set free. These constituted nearly one third of the whole mass. It was evident from these circumstances that the fluid was immature, and not fitted for experiment. Five days afterwards, March 9th, I again examined fluid from this male, which had been kept since the previous examination, in a separate vessel, so that there was no mistake in its identity. The mean temperature of the room during the interval was 42°·5 FAHR., and the water in which the Frogs were preserved 40° FAHR., but none of the Frogs had spawned. The fluid was then less dense and of a less white colour, and was composed almost entirely of very active and fully developed spermatozoa, with but very few cells from which these bodies had not

* Article "Semen" in Cyclopædia of Anatomy and Physiology, part xxxvi. (January, 1849) vol. iv. p. 504.

been liberated. There were, however, some of which the contents appeared to be perfectly granular, and these I regarded as spermatozoal cells in the earlier stage of development.

The female with which this male had been paired was killed on the 4th, at the time the experiments were to have been commenced. It was then found that the eggs, like the fluid from the male, were immature. The whole of the eggs had escaped from the ovaries into the cavity of the peritoneum among the viscera, and were crowded together in a mass on each side, at the anterior part of the abdomen, and were without any gelatinous covering. A few only had begun to enter the oviduct on the right side of the body, and acquire their envelopes. In most of these eggs the germinal vesicle had already disappeared.

The fluid and eggs of the common Toad (*Bufo vulgaris*) are developed in precisely the same way as in the Frog. On the 30th of March 1851, I examined the bodies of a male and female Toad for the purpose of experiment. The great efferential ducts and vesicles in the male were then entirely empty, no fluid having yet descended into them. But the testes contained a vast quantity of spermatozoal cells, some of which were distended by the spermatozoon, as if in the act of being liberated. But not a single spermatozoon was free, or showed any sign of motion. The whole were still immature, the caudal portion being as yet short and imperfect.

In the female the ovaries were distended with eggs and occupied the whole of the visceral cavity between the intestines. The eggs were of a sooty black colour, with but a slight trace of the grey surface, and appeared to be nearly mature, but had not yet left the ovary. The germinal vesicle of a large size, circular in its outline, and somewhat flattened, still existed in nearly the whole of them.

It is thus evident that the pairing of the sexes, both of Frogs and Toads, takes place before the semen is fully matured, or the eggs have descended into the oviducts, and that there is a near concurrence in the time of maturity in both.

These facts seem to lead to the conclusion, that when, as in some of the fore-mentioned experiments, the male fluid contains a very large proportion of developmental cells, which occasion its white appearance, it is not fully matured for its function; that the first portions of fluid which descend into the efferential vessels are usually immature; and that it is not absolutely necessary that the spermatozoa should be fully formed before the cells escape from the testes. It seems fair then to infer from these facts that the completion of the development of the spermatozoal bodies takes place in the deferential vessels, or in the vesicles, perhaps in both: further, that the quantity of fluid produced, when too great to be contained in the ducts, may become accumulated in the vesicles, to be furnished at the instant it is required; and that there is a near concurrence in the time of maturity in the fluid in the male, and of the eggs in the female, the former being only slightly in advance, in regard to time, of the latter, at the natural period of their encounter; a condition which perhaps may be necessary to the full and healthy fecundation of the whole brood.

3. OF THE OVUM.

The vitality of the eggs of the Frog appears to be of longer duration than that of the fertilizing agent, the spermatozoon. The egg, as formerly shown*, leaves the ovary quickly after, or at the time when its germinal vesicle disappears; but, as experiment has proved, it is not then entirely fitted for its function. It has yet to be enveloped in a thick covering, which it gains in its transit through the oviduct, before it is susceptible of impregnation. Its condition when escaping from the ovary to the oviduct seems to be analogous to that of the spermatozoon when it leaves the testis to enter the efferential duct. Neither the one nor the other has yet attained its full maturity. When the eggs are collected in the dilated or uterine portions of the oviducts they seem to be in a condition parallel to that of the spermatozoa in the lower portion of the efferential ducts and vesicles. When nearly the whole of the eggs are collected in the uterine cavities their maturity is then almost completed; but a period of retention, even in these structures, seems to be necessary to ensure the fertilization of the whole, as some of them,—as I have found by the persistence of the germinal vesicle for a short time, even after the egg has escaped from the ovary into the cavity of the abdomen, as the undeveloped spermatozoal cell passes from the testis into the efferential duct,—are later in their development than others. Further, it may be remembered†, that so far from the contents of the yelk being in a dormant condition at the time of oviposition, as some inquirers have supposed‡, there are changes still going on within it, and which are perceptible to the eye, in the condition of the white surface, for ten or twelve minutes after oviposition; after which they become less and less marked, and soon entirely cease if the egg be not fecundated.

Thus then from a comparison of the state of the fecundatory agent with the body to be fecundated, we might have expected to have found some close coincidence in the retention of their vitality, and this indeed, in a state of nature, seems to be the fact. Spawning seldom or never takes place by the act of the species until each sex has attained its full maturity of function. The eggs are sometimes voluntarily retained by the female Frog, for several hours or days within the uteri, although arrived at maturity, if the proper evolution of spermatozoa in the male be not completed. I have had evidence of this in the fact, that when the union of the sexes has been of long continuance, the female has sometimes passed a very few ova, and then has continued without further oviposition for many hours, sometimes for a day or two, before the mass was expelled, and the function of the sexes consummated. These first extruded eggs have almost always been found to be unimpregnated.

When the eggs have been deposited, the circumstances which affect their fecundation are precisely similar to those which affect the spermatozoa,—the temperature of the surrounding medium, and the length of time during which they remain im-

* Philosophical Transactions, 1851, p. 180. † *Loc. cit.* p. 185. ‡ Wagner and Leuckardt, *loc. cit.*

mersed in water after expulsion from the body. The length of time during which they are susceptible of impregnation has relation to each of these conditions; and these have now acquired additional interest in the establishment of the fact that the spermatozoon penetrates into the envelopes of the eggs, and that, consequently, the rate of expansion of the envelopes has a further direct relation to its function.

The following experiments will illustrate the foregoing observations:—

It may here be remarked, that although, as in my former paper, the date of the experiment is recorded, it is so only for the convenience of reference, and not with any regard to priority of period at which the experiments were made; the details being given only in illustration of the general statements enunciated.

April 2, 1852. Atmosphere 55° FAHR. The first three of the following experiments were made with the eggs and fluid from a pair of Frogs which had been obtained from their natural haunts only three hours before the female was killed, by division of the spinal cord, for the purpose of the experiments.

No. 1. *Eighty-six eggs* passed from the Frog at *one hour and a half* after death, were supplied with fluid from the male with which this female had been paired,—the fluid having been obtained and mixed with water *one hour and a half* before it was employed.

On the seventh day *fifty-two embryos* had been produced from these eggs, and some of them were then escaping from their envelopes.

No. 2. *Thirty-seven eggs,* obtained from the same female, were supplied with a portion of the same fluid as the above, after it had been mixed with water about *two hours and three-quarters.*

Twenty-eight embryos were produced from these eggs at the same time as the above.

No. 3. *Sixty eight eggs* from the same Frog at *six hours and three-quarters* after death were immersed in the same portion of mixed fluid and water in which the eggs of No. 2 had been fecundated. In this experiment the fluid had been *six hours and three-quarters* mixed with water.

At the end of three days the eggs still had a healthy appearance, but on the seventh day not one had given any evidence of having been impregnated, nor was any embryo ultimately produced. Thus the fluid had lost its fecundatory property, at a temperature of 55° FAHR. between the end of the third and of the seventh hours.

As it was possible that the failure in this experiment was due as much to the eggs as to the fluid employed, it having already been shown that impregnation is sometimes effected with fluid which has been *twenty-four hours* removed from the body,—the following experiments were then made:—

No. 4. *Sixty-nine eggs* obtained from the same female at *one hour and twenty minutes* after death were bathed with fluid procured from another male, and which had been mixed with water *thirty hours* previous. These eggs preserved their regularity of outline for nearly *six hours,* at the end of which time one egg showed traces of

partial impregnation, while the yelks of the others were changing form and becoming irregular. But not a single embryo was produced.

No. 5. *Eighty-one eggs* were obtained from another Frog, a few minutes after it had been killed by division of the spinal cord, and a portion of fluid which had been mixed with water *twenty-four hours and a half*, and kept in a temperature which had sunk from 54° Fahr. to 51° Fahr., but at the time the fluid was employed had again risen to 55° Fahr.—was supplied to them.

This fluid on examination by the microscope was found to contain an abundance of spermatozoa, the whole of which were perfectly motionless, so that I was not able to detect even one which gave any sign of vitality. Yet the eggs in this experiment retained their natural healthy appearance for several hours; but not one was impregnated, nor was even a single embryo produced. On the contrary, after thirteen hours, the yelks of some of them assumed a pyriform shape, while those of others became shrivelled and withered.

No. 6. *Thirty-six eggs* were passed from a Frog, which had been killed about *three-quarters of an hour*, and some fluid which had been obtained and mixed with water *forty-four hours* before was shed over them, and pure water was then added.

The envelopes of these eggs became expanded as under perfectly healthy and favourable conditions; but within two hours of the contact of the eggs with the fluid the yelks of the whole became very irregular, shrivelled, and contracted, and assumed an appearance very similar to that which first results from the application of a strong solution of potass. Not one egg retained its spherical shape, but the whole entirely perished; and it may be needless to add, not an embryo was produced.

These facts then support the statement already made, that only while the spermatozoon continues to give evidence of vitality in its power of motion does it exert any fecundatory influence on the egg. They seem, too, to show that the period during which this vital force is retained by the mature spermatozoon after it is passed from the body of the Frog is perhaps shorter even than what I have stated, and that the limit, in regard to time, depends much on physical influences. In addition to this, they seem to show that after all ocular evidence of power in the spermatozoon is lost, this body is inert, until decomposition has commenced, when its material constituents become injurious and destructive.

The following experiments have more direct reference to the vitality of the egg, and appear to indicate that this is much longer retained than that of the spermatozoon, and is less quickly affected by external causes.

April 2, 1851. Atmosphere 55°.

No. 1. *Thirty-eight eggs* were passed from a Frog killed *twenty-four hours and a half* before, and some fluid which had been obtained and mixed with water about *one hour and a quarter* was then added to them.

Segmentation commenced in some of these eggs in about *four hours and a half*, and on the seventh day *eight embryos* had been produced.

No. 2. *Seventy-three eggs* were taken from a Frog which had been killed about *thirty hours*, and were bathed with fluid which had been *one hour and twenty minutes* mixed with water.

At the end of six hours several of these eggs had become irregular, but *one* had certainly been fecundated, as one *embryo* afterwards came to maturity.

No. 3. *One hundred and sixty eggs* from the Frog which supplied those employed in the experiment No. 2, were bathed with fluid from another male, immediately after it had been obtained, but not a single egg was impregnated.

No. 4. *Thirty-seven eggs* were passed from a Frog which had been killed *forty-four hours* before, the temperature of the atmosphere during this period having risen from 50° FAHR. to 55° FAHR. These eggs were bathed with fluid which had been obtained about *three quarters of an hour*. At the end of six hours I found, to my surprise, that one egg had become segmented, but that the yelks of the others were flattened, shrivelled, and irregular, as if they had been affected by the solution of potass, or by decomposing seminal fluid. On the seventh day the egg which had been segmented had produced *an embryo*.

From these results, then, it appears that while the spermatozoon at a mean temperature of the atmosphere of 55° FAHR. usually loses its vitality, and is inert in less than four hours, and but rarely is efficient at a longer period after immersion in water, the egg retains its reproductive property for a very much longer time, especially if not removed from the body of the dead animal, only losing it at twenty-four hours after the death of the parent, and occasionally retaining it for forty hours.

The experiment No. 4, in which the latter fact is shown, is interesting, from the circumstance that the same results are produced by the living vibratile spermatozoon on the yelks of the dead eggs, as the majority of these were, as that which is occasioned by the application of dead and decomposing spermatozoa to the envelopes of the living one, as shown in No. 6 of the preceding set; and further, that these results so closely resemble in appearance the first effects produced on the yelks of living eggs by the application of strong solution of potass; thereby showing not only that powerful endosmic action takes place rapidly through the coverings of the egg, but also that the influence of the spermatozoon on the yelk, whatever may be its precise nature, is direct and immediate.

4. ENDOSMOSIS OF THE EGG IN RELATION TO ITS VITALITY.

It has before been shown that the endosmic action of the envelopes of the egg, on immersion in water, is closely connected with the act of fecundation; and we have now further proof of this in the fact that the vitality of the egg may be preserved for many hours if the envelopes be not brought into contact with water; so that the insusceptibility of the egg to become impregnated, after a lengthened period of immersion, is due to a diminution of the expansive property of the tissue of the

envelopes, and the distension of the tissue with fluid, rather than to the loss of vitality in the contents of the yelk.

This view has led me to endeavour to ascertain more precisely than heretofore,—*first*, at what length of time, after immersion in water, the egg usually becomes insusceptible of impregnation; and *next*, to what extent the act of fecundation is accelerated by a given increase of temperature, when the experiments are made with portions of fluid from the same male, and with eggs from the same female, at, as nearly as possible, the same time, all the conditions being similar except in regard to the temperature of the surrounding medium.

With this object, I have recently made two sets of observations consisting each of nine experiments. One set was conducted in a room, the temperature of which was raised artificially to 61° FAHR., and the water in which the eggs were immersed to 60° FAHR.; while the other was carried on in an adjoining room where the temperature was only 55° FAHR., and the water employed 53° FAHR. In order to place the whole of the experiments under as similar conditions as possible, it was necessary that the corresponding experiments in each set should not only be made at about the same time, but should also be supplied with fluid in exactly the same state, and the eggs be immersed in like quantities of water, for the time specified in each experiment, before the addition of the fluid. When the time of immersion had elapsed the water was quickly withdrawn, and the fluid poured over the eggs, and fresh water was immediately supplied to them. As much time was unavoidably occupied in passing the eggs into their respective vessels, and noting the period of immersion, it became necessary to commence the experiments with those which had been longest in water, in order that the fluid supplied to the whole should be as nearly as possible in the same condition, in each experiment, at the moment of its application, and be furnished to the eggs in similar quantities. To ensure this, the fluid was mixed, as soon as it was obtained, with fifteen times its quantity of water, and was employed at about *thirty-five minutes* afterwards. The quantity of this mixed fluid poured over the eggs of each experiment was ten minims by measure, excepting only to the first and second experiments of each set, to which twenty minims were added; but even with this advantage, as was seen, with no more favourable change in the result. After the end of the first hour, the eggs which had remained in the lower temperature were removed to the higher, and the two sets of experiments were then placed side by side to watch the time of commencement of segmentation, as indicatory of the more or less accelerated impregnation. The number of embryos subsequently produced showed the ratio in which the eggs had been fecundated after different periods of immersion*. Each set consisted of nine separate experiments, the time of immersion of the eggs being forty-five, thirty-five, twenty-five, fifteen, ten, seven, five, three, and one or two minutes in each.

At the highest temperature, 61° FAHR. atmosphere, and 60° FAHR. water, a tem-

* For the details of the experiments, see MS. paper in the Archives of the Society.

perature which is always most favourable to fecundation, there were no signs that any change had even been commenced in those eggs which had been immersed for a longer period than thirty-five minutes. But it must be borne in mind that the fluid employed had been thirty-five minutes mixed with water, and consequently had begun to lose some of its efficacy. The results of the corresponding experiments at the lower temperature—Atmosphere 55°, Water 53°—were similar, but less marked.

On comparing the two sets of investigations, it was found that no evidence of fecundation occurred in either of them, when the eggs had been immersed during forty-five minutes before the impregnating fluid was supplied to them; and that only the very earliest symptoms of any influence having been communicated to the eggs, after thirty-five minutes' immersion, occurred in those which were submitted to the higher temperature, in the irregular contraction of the yelks; while in each set, both in the higher and lower temperature, some of the eggs, after twenty-five minutes' immersion, showed unequivocal signs of *partial fecundation* in the formation of the *respiratory chamber*, the result of the contraction and depression of the upper portion of the yelk previous to segmentation. But in both sets there were some eggs which, after fifteen minutes' immersion, had become fecundated, and produced embryos. The relative number of embryos produced after immersion for this and shorter spaces of time was certainly greatest in the higher temperature; since, when the total number of eggs employed in the last six experiments in each of the two sets, in which only any embryos were produced, were compared with the number of embryos, it was found that in the higher temperature there were seventy-eight embryos from five hundred and thirty-six eggs; while in the lower temperature, from six hundred and two eggs, there were only seventy-six embryos.

The period at which segmentation occurred in the fecundated eggs of the two sets corresponded also with the above results. In most of the eggs fecundated in the higher temperature segmentation commenced about ten minutes earlier than in those which had been fecundated, and remained during the first hour in the lower temperature.

These results seem to show, that while the eggs are more rapidly affected, they are also more certainly impregnated, and the embryos produced in greater numbers at a higher than at a lower temperature. But it must be borne in mind that the number of eggs fecundated in these experiments can only be regarded as comparative, and as mere approximations to what takes place in a state of nature, the experiments having been made with fluid which had been removed from the body, and considerably diluted with water, more than half an hour before it was employed, and consequently when its efficacy had been considerably diminished.

These facts, then, go to confirm the previous conclusions, that the organic force or vitality of the spermatozoon is of shorter duration than that of the egg; since, if the results of these experiments be compared with those obtained from former ones, it will be seen that the insusceptibility of the egg depends very much more on the endosmic action of its envelopes after immersion in water, than on any loss of vitality:

further, that the non-fecundation of the egg may depend much more on diminished vitality and inefficiency of the spermatozoon, than on the insusceptibility of the egg itself.

5. QUANTITY OF SPERMATOZOA ESSENTIAL TO FECUNDATION.

On a former occasion* I endeavoured to arrive at some conclusion as to the minimum quantity of spermatozoa necessary to effect impregnation, and to draw some comparison between the results obtained by myself and certain of the more remarkable ones by SPALLANZANI†. It then seemed to me, that although the general results obtained were in accordance with SPALLANZANI's, yet that a larger proportion of fluid, and consequently a greater quantity of spermatozoa, was required than was supposed by him. I have, therefore, made further investigation on this subject, and have sought to obtain some knowledge of the quantity really necessary to fecundate the egg. PREVOST and DUMAS formerly made a similar attempt‡, and M. QUATREFAGES has recently done the same§. The results of the observations by PREVOST and DUMAS, QUATREFAGES, and myself, although differing greatly in detail, coincide with the general results obtained by SPALLANZANI,—that only an exceedingly small quantity of seminal influence, and consequently only a very limited number of vibratile spermatozoa is necessary to effect impregnation. They agree, too, in the fact that the number of eggs impregnated is always much fewer than that of the spermatozoa supplied to them; and, consequently, that there is reason to think that fecundation is not the result of a single, isolated spermatozoon, although it has not been ascertained what is the number actually required. The course I have pursued in putting this curious question to the test, has been different from that of either of the distinguished naturalists mentioned. It has not been that of diluting the impregnating fluid in a very large quantity of water, and then applying a minute drop of the water to the Frog's egg, as done by SPALLANZANI;—nor has it been that of reckoning the number of spermatozoa on a given surface of a micrometer plate, and then immersing the plate in water with a quantity of eggs, and afterwards observing how many of them became fecundated,—the course followed by PREVOST and DUMAS;—nor has it been by estimating the number of spermatozoa in a given bulk of fluid, and then ascertaining the number of eggs fecundated by them, as done by QUATREFAGES with those of the *Hermella* and *Teredo*; but it has been by direct application of the spermatozoa on the point of a pin to the surface of a single egg in a glass cell; and then observing beneath the microscope the number of spermatozoa deposited from the point of the pin, applied in a similar way as to the egg, on a plate of glass. Yet only approximative results can be arrived at by this, or by the other modes of proceeding, although it is satisfactory to find that the general results obtained in this way are in agreement with those obtained by the observers mentioned; and further, that the mode adopted has led to some additional results, which seem to be of value.

* Philosophical Transactions, 1851, p. 206.

† Dissertations, &c., vol. ii. p. 189.

‡ Annales des Sc. Naturelles, tom. ii. 1824.

§ Annales des Sc. Nat. 3me série, tom. xiii. p. 129.

6. TESTS OF FECUNDATION.

I have given some account in my former paper* of a space, or *chamber*, which is developed between the vitellary envelope of the egg and the upper surface of the yelk, within the first hour and a half, subsequent to the encounter of the spermatozoon with the egg; and I have referred to it in the details of these experiments, as occurring in some of the eggs which had been immersed during *twenty-five minutes* before the fecundatory agent was supplied to them, and as marking their having been affected by its influence. The formation of this chamber is one of the earliest and most certain indications that the egg has been more or less impregnated. The earliest symptom of impregnation occurs somewhat sooner than the formation of the chamber, or within the first half-hour, and consists in a marked increase in the degree of undulatory heaving and sinking of portions of the surface of the yelk, motions which take place slightly in the unimpregnated egg, but are increased in the impregnated; although, even in that, they are not sufficiently permanent to be easily recognized as proofs of fecundation. The irregular contraction and shrivelled appearance of the yelks, noticed in the preceding observations, may, perhaps, be due in part to this cause. The formation of the chamber is the result of the contraction and depression of the upper surface of the yelk, occasioned, as there is reason to think, by changes which are going on within the substance of the upper hemisphere of the yelk, around the embryo vesicle, or its progeny, and which changes appear to be due to the operation of the spermatozoon; as I have never observed the chamber in any egg which has not been more or less fecundated. The fact of the existence of a chamber, therefore, may always be regarded as a *proof of fecundation*. It is commenced sooner or later according to the temperature of the surrounding medium, and to the more or less complete fecundation of the egg. It is usually perceptible in about *one hour*, or *one hour and a quarter* after the impregnating fluid has been supplied to the egg, and is distinctly marked in less than one hour and a half, when the eggs are preserved in a temperature of from 50° to 55° FAHR., and its size is increased during the first three hours. It is commenced in a slight depression in the centre of the upper surface of the yelk, at a point which corresponds to the entrance to the central canal, and it takes place much sooner in eggs which have been completely fecundated, even at moderate temperatures, than in others which have been more sparingly affected. I have seen it begun in a fully impregnated egg, in a temperature of 52° FAHR., at *fifty-three minutes* after encounter with very mature spermatozoa. When it has attained its greatest superficial extent it appears like a clear watch-glass cavity, filled with a perfectly transparent, limpid fluid above the dark upper surface of the egg. After the third hour it becomes slightly further enlarged in its axis from above downwards by the formation of a shallow, funnel-shaped depression on the surface of the yelk, the centre of which is the minute orifice seen by PREVOST and DUMAS, and BAER, and this is continuous with the canal above alluded to. I have never found this chamber

* Philosophical Transactions, 1851, p. 187.

absent from any egg which has produced an embryo. Its formation is preliminary to the subsequent cleavage of the yelk. It thus affords, within a very short space of time, a certain *test of fecundation*, and as such is exceedingly valuable in all experiments by artificial impregnation.

7. PARTIAL, OR INCOMPLETE FECUNDATION.

But although the formation of the chamber gives certain proof that the egg has been influenced by the spermatozoon, it is only the actual segmentation of the yelk, carried through the first four stages, which shows that its fecundation has been completed; since the egg may be only partially influenced, and consequently may not produce an embryo, although the respiratory chamber may be formed, and the early stages of segmentation of the yelk be commenced. When this is the case the change seldom proceeds beyond the second or third stage of cleavage, and even then there is usually some irregularity in its occurrence. Sometimes only the chamber itself is developed, and the process then stops; at other times the chamber is completed and a little *white spherical body* makes its appearance, and is but *just perceptible within the entrance to the central canal*; but more frequently a white spherical body, not more than one-fourth the size of the original germinal vesicle, appears on the *surface of the yelk* within the chamber; at other times there are *two of these white bodies*, each of which is scarcely more than one-half the size of the single one, and sometimes the bodies are of a dark or grey colour. In other cases the yelk begins to be divided in the margins of the canal, but does not proceed with its change; or it may go on, as just stated, to the several stages. In other cases the yelk divides very unequally. These instances of *partial impregnation* or *fecundation* may occasionally be produced by application of exceedingly minute quantities of seminal influence, as some of the following experiments will show.

8. DEFICIENCY OF FECUNDATORY INFLUENCE.

(*a*.) *Pin-point* application of the fluid.—These experiments were commenced in the season of last year (1851), but have been repeated at different periods during the season of the present year.

March 30, 1851. Atmosphere 56° FAHR.—A *single egg* was passed into each of four glass cells, with a small quantity of water, sufficient only to preserve the eggs moist and promote the expansion of their envelopes. Within two minutes afterwards two of the eggs were each touched once with the point of a common-sized pin, which had been so lightly dipped, into a mixture of one part of fecundatory fluid, obtained about one hour and five minutes before, and four parts of water,—as to cover a portion of its surface equal to about one thirtieth of an inch in extent. The two remaining eggs were touched, the first *once* only, and the second *twice*, with the point of a pin slightly curved, and which thus was made to retain a much greater number of spermatozoa in the fluid which adhered to it than the pin with the straight point.

Each cell was then filled with water, so that the egg which it contained was completely immersed. Within the second minute after the eggs had been touched with the loaded pin, there arose from each, at the point of contact, a microscopic bubble of air, the egg which had been twice touched having two bubbles. These were found by subsequent observations to be due to the circumstance of the eggs having been exposed to the air, for a short time before they were touched, and their surface become slightly dried, so that when the fluid was applied to it a small bubble of air became involved and imprisoned in it, before the eggs were immersed in water. This circumstance and its explanation are mentioned simply to show that the occurrence had no relation to the act of fecundation, as at first I had supposed was probable.

I endeavoured to obtain some idea of the number of spermatozoa deposited from the pin point, during its momentary contact with the egg, in these experiments. But the result seemed to give but little hope that either of the experiments would be successful, or that any fecundation could be effected by the application of so minute a quantity of influence, as it was found that the number of spermatozoa deposited on a plate of glass, beneath the microscope, when the pin point was applied to the glass in the same way as to the egg, did not exceed from six to ten or twelve of these bodies. Yet the results of the experiments were watched. Within the first half-hour the eggs were removed from the temperature of 56° FAHR. to one of 65° FAHR. But at the end of ten hours it was found that only in one instance had any fecundation been effected. The grey central spot on the dark surface of the yelk was still present in three of the eggs, and no respiratory chamber had been formed in either of them. But partial fecundation had been effected in the fourth egg, which had been twice touched with the loaded pin point. In this instance the dark surface of the yelk was depressed, and the respiratory chamber had been formed between it and the envelope, and *two spherical opake white bodies* appeared within it, lying side by side on the middle of the surface of the yelk; but no segmentation of this had taken place, or even had been commenced.

One cause of failure, in these first experiments, appears to have been the diminished efficacy of the fluid, which had been obtained and mixed more than one hour before; as it will presently be seen that even a less proportion of the fluid sometimes is effectual when employed immediately it is procured.

As it was late in the season of last year when these experiments with minimum quantities were commenced, the spawning of the frogs being then nearly at an end, I was fortunate in procuring, on the second of April, a single unspawned pair of frogs from their natural haunts, and thus was enabled to repeat the experiments with the fewest disadvantages. This was done within four hours after the frogs were captured. The fluid in this instance was very mature, was obtained in good abundance, and was mixed with an equal quantity of water, and employed in the experiments a few minutes afterwards.

April 2, 1851. Atmosphere 55°.—*Ten eggs* were passed into a large glass cell, and each egg was touched *once* only with the pin point dipped lightly into the fluid for each egg. This experiment was perfectly successful, as the chamber was formed above the yelk in four or five of these eggs, and at the end of five days three of them had produced embryos. In these instances the pin had been allowed to remain in contact with the egg, and the fluid to drain off it for one or two seconds.

Six eggs were also placed in another cell and each was touched with the loaded pin point when the fluid employed had been obtained about a quarter of an hour. *Two* of these eggs became partially fecundated, as shown in the formation of the respiratory chamber. In each of these the yelk became slightly elongated, a symptom which always precedes the first cleavage; yet they did not undergo further alteration, but with the remaining four were abortive.

Twenty-three eggs were included in a large cell as a further experiment, and each egg was touched once with the pin-point loaded each time, when the fluid employed had been one hour and three quarters mixed with water. *Four* of these also had the chamber formed, and segmentation commenced in them, at the end of four hours and a half; but the majority were only *partially fecundated*, as only one of the twenty-three eggs produced *an embryo*. The fact of this production, however, shows, that an exceedingly small amount of influence, even at a long period after the fecundatory fluid has been removed from the body in which it is generated, is sometimes sufficient to occasion the development of an animated being; and that although a plurality of spermatozoa, supplied to the egg, appears always to be necessary to ensure fecundation, yet that the result seems to depend less upon the abolute given number, or numerical relation of these bodies, to the egg, than on the measure of vitality possessed by those which are brought into actual encounter with it, as a very small number appears to be more efficient when employed immediately after their removal from the body, than a much larger number after a prolonged interval.

(*b.*) *Pin-head* application of fluid.—The preceding experiments having been made with the smallest quantity of fluid, and consequently with the fewest spermatozoa that could be applied directly to the egg, it was desirable to increase the number of these bodies without applying them in excess, and for this purpose the head of a very small pin, the smallest size used by insect-collectors, was employed, instead of the point of a larger sized pin. An endeavour was also made, as in the previous trials, to ascertain the number of spermatozoa which the head of this sized pin, dipped lightly into fluid, was likely to be the means of conveying to the egg. It was then found that the loaded pin-head deposited on a plate of glass, in the minute quantity of fluid which adhered to it, from at least fifty to one hundred and fifty spermatozoa; a number which was not only fully sufficient to fecundate each egg to which the pin-head was applied, but also other eggs which might happen to come into contact with these in the same cell and water in which the experiment was made.

Six eggs, placed in a single cell, were each touched once only with the pin-head,

which had been dipped into fluid only once for the whole, the time occupied in touching them being about five seconds. Each of these eggs became fertilized, and segmentation commenced in two of them in exactly *five hours,* and in the remaining four in from three to four minutes later. The period at which segmentation commenced having been very carefully watched, and the whole of the eggs having been submitted, during the interval, to the same physical conditions of heat, light, quantity of water and degree of aëration, the result, in regard to the difference of time at which they began to change, seemed to be accounted for only on the assumption that it was due to a greater quantity of influence applied to the first two than to the others; and this opinion has since been more fully borne out by other experiments. The fluid at the time it was employed had been thirty-five minutes mixed with water. On the *fifth* day each of these eggs was producing an *embryo.*

Three eggs were placed in separate cells, and each egg was touched once with the pin-head loaded only once for the three, the fluid employed having been *twenty-three* minutes mixed with water. After *four hours and forty minutes* two of these eggs were undergoing segmentation, and on the fifth day both were producing *embryos.* The third egg was not impregnated.

Twenty-two eggs, in a large cell, were each touched once with the pin-head loaded for each egg when the fluid had been *one hour and forty-seven minutes* mixed with water. Some of the eggs in this experiment were touched on the white surface, others on the dark, and others at the side, or about midway between the white and dark surfaces.

In the same cell with these eggs, but at a little distance from them, were placed eighteen other eggs, which were not touched with the pin-head, but were simply allowed to remain in the same cells with the twenty-two eggs experimented on, and the cell was then filled with pure water. At the end of *four hours and fifty minutes* the chamber had been formed, and segmentation had commenced in each of the twenty-two eggs experimented on. But in addition to these there were also *two* of the eighteen g which had not been experimented on undergoing segmentation. These, during the expansion of their envelopes, had come into contact with some of the fecundated eggs, and had also become fecundated by some of the spermatozoa which had been supplied to them. *Five days* afterwards the whole of these *twenty-four eggs* were producing embryos! so that not only had the quantity of spermatozoa employed been fully sufficient to fecundate the eggs actually touched, but also others present in the same water with them.

In another experiment, made before the preceding, but with some of the same brood of eggs and same sample of fluid, I placed five eggs in a cell, and touched each once, very lightly, with the pin's head loaded once only for the whole. This was when the fluid had been only *eighteen minutes* mixed with water. The object of this experiment was to learn whether there is any difference in the result when the eggs are touched at different parts of their surface. Accordingly the trial was made by touch-

ing some eggs on the *dark surface*, others on the under or *white surface*, and others at the sides between the two. At the end of *five hours and seven minutes* the chamber had been formed and segmentation was taking place in each egg, and on the *fifth day* each had produced *an embryo*, thus *appearing to show* that it is of little consequence, with reference to fecundation, as to which part of the egg the fecundatory agent is applied to. But this conclusion is subject to certain conditions, as I shall proceed to explain.

9. PORTION OF THE YELK MOST SUSCEPTIBLE OF FECUNDATION.

On attentively examining the preceding experiments, it seemed difficult to understand how it happened that when similar quantities of the same fluid were applied by the pin-head to different eggs of the same brood, at the same time, and the whole of them placed in exactly similar conditions in regard to heat, light and aëration, that some should become fecundated and others not, if, what *appeared* to be shown in the last detailed experiment,—that the egg can be fecundated equally well by the application of the fecundatory agent to any part of its surface, be strictly correct. There at first seemed to be no other way of accounting for the differences in the results, except on the hypothesis, either that it was due to some insusceptibility in the entire egg itself,—which thus presupposed great imperfection in each brood of eggs, a conclusion at variance with observation,—or that it resulted from the mode in which fecundation was attempted.

During the past season (1852) I have put the question, thus raised to a more rigid test, in order to learn whether the egg really is as susceptible of fecundation in one part of its structure as in another.

In order to obtain some decisive results I repeated my experiments on three separate occasions, with eggs and fluid from three pairs of frogs, at nearly similar temperatures of the atmosphere. On the first occasion, April 5th, the temperature was 54° FAHR., on the second, the following day, it was 55° FAHR., and on the last occasion, April 9th, it was 53° FAHR.

In all the preceding experiments the eggs had been allowed to remain in exactly the same position, at the time of applying the fluid, as that in which they had passed from the body of the frog; so that sometimes the dark, and sometimes the white surface was uppermost, and at other times inclined more or less to either side, and occasionally the two were perfectly horizontal. When the fluid was applied by means of the pin's-head to the part desired, some portion of it necessarily flowed over other parts, if the surface which received it happened to be inclined to one side or the other.

But in my more recent investigations care was taken to place every egg in a perfectly vertical position, before attempting to fecundate it, so that in one set of eggs the central part of the dark surface of the yelks was uppermost, and in another the centre of the white surface. These precautions led to very definite results. In the

first experiment, made on the 5th of April, with fluid which had been mixed with water about *one hour and fifty minutes* before, *twelve eggs* contained in a single cell were each twice or thrice freely touched with the loaded pin's-head over the centre of the *white* surface. *Three* of these eggs became *partially* impregnated, as shown in the formation of the chamber in each, and segmentation commenced in one of them, but *no embryo was produced* from either. *Fourteen eggs* in another cell were touched at the same time, and in the same way as the preceding, with the loaded pin's-head, over the centre of the *black* surface of the yelk. *The whole of these eggs produced healthy and well-formed embryos.*

These experiments were repeated on the following day on the eggs of another frog, with fluid which had been obtained only about *twenty* minutes before it was employed. *Fourteen eggs* were each touched *once* only on the centre of the *white surface*, the pin-head being loaded for each egg. But *not one of these eggs became fecundated*, nor was even one of them partially impregnated. In another cell, at the same time with these, *thirteen eggs* were touched on the centre of the *dark surface*, and *four* of them became partially fecundated, but only *two embryos* were produced. The failure in this experiment certainly appeared to be due to the eggs, rather than to the mode of attempting their fecundation.

At the time of making this experiment I placed two eggs, each in a separate cell, with their white surface uppermost, and then filled the cells, the one with nearly *pure* fluid, and the other with equal parts of fluid and water. Each of these eggs became fecundated, and underwent segmentation, although more slowly than usual, but the development of their embryos was not completed, as it became arrested at a particular period, a circumstance which is presently to be explained. Yet these experiments showed that when the egg is completely immersed in fluid its position at the moment has but little reference to the act of fecundation, provided the fecundatory agents are present in good abundance. A further trial was made on the 9th of April, when *six eggs*, placed in one cell, were each *twice* touched on their *white* surface, the pin's-head being loaded each time it was employed. But neither of these eggs produced an *embryo*. *Six eggs*, placed in another cell, were also *twice* touched, like the preceding, on their *dark* surface. The whole of these eggs became fecundated, but only *five embryos* were sufficiently matured as to leave their envelopes. The fluid employed in these two experiments had been mixed with water about *fifty-five minutes* before it was applied to the eggs, and consequently was becoming slightly deteriorated.

The conclusion which seems to be deducible from a comparison of these experiments is,—that when the egg of the frog is completely immersed in water charged with the fecundatory agents, or to which these are supplied quickly after the immersion of the egg, as in the natural fecundation of the species, the actual position of the egg at the moment of its encounter with the spermatozoon, has but little reference to the act of fecundation, every part of its surface having then an equal chance of

becoming affected. In like manner when the fluid is applied directly to any given part of the surface artificially, when the egg is exposed in an empty cell, and the fluid is allowed to gravitate, before the egg is immersed in water, it is of little consequence what part is first touched with it, since it may spread over the surface to the most susceptible portion; there being in reality one part of the egg which is more susceptible than another. The extremes of susceptibility and insusceptibility appear to be, on the one hand the *centre* of the *dark*, or future upper surface of the yelk, and on the other that of the *white* or under surface*.

That these conclusions, deduced from experiment, are correct, seems to be shown in the fact, formerly pointed out†, that the germinal vesicle originally, and its progeny subsequently, at the time of fecundation, occupies the centre, not of the entire egg itself, but of the upper or dark hemisphere of the yelk; and this possibly may be the structure to be fecundated.

These facts deserve consideration in connexion with that of the first changes in the yelk taking place in this hemisphere; first, in the shrinking of the yelk at this part; the formation of the chamber above it, subsequent to fecundation; the *existence of a canal in its centre,* in the margin of which the cleavage of the yelk commences; and, lastly, in this being the portion of the yelk in which the first lineaments of the embryo become apparent.

In connexion with these remarks, it is but just to mention that a former observer, Dr. Martin Barry, has distinctly referred to the changed germinal vesicle as being the structure in which the future embryo, in the Mammalia, originates‡.

10. EXCESS OF FECUNDATORY INFLUENCE.

At the time of commencing the foregoing experiments with small quantities of fluid, I made others of a directly opposite character with an excess of the same. *Four eggs* were placed each in a separate cell, and within one minute afterwards the cells were filled with a mixture composed of equal parts of seminal fluid and water. This was half an hour after the fluid had been obtained. I had expected, from the vast quantity of spermatozoa present in the fluid, that segmentation of the yelk in these eggs would have taken place very quickly; but to my great surprise, although the eggs were preserved in a temperature of 60° Fahr. no segmentation whatever had commenced in them at the expiration of twelve hours! at which time an abundance of spermatozoa adhered to every part of the surface of their envelopes. But no chamber was formed in either of these eggs, as neither of them had been fecundated.

* During the present year I have most fully confirmed these views by numerous experiments, and have proved to my full satisfaction that the *white surface* of the egg is least, and perhaps not at all, susceptible; while of the *dark surface, that the centre, which is occupied by the canal, and its contents,* is the fertilizable part.—G. N., April 18, 1853.

† Philosophical Transactions, 1851, p. 176.

‡ Ibid. 1840.

This result appeared to be far more inexplicable than any I had previously noticed. A somewhat similar one had occurred once before, but I had attributed that to some imperfection in the eggs employed, an explanation which did not apply in this case, as eggs from the same female had all been fecundated in other experiments.

Four eggs were then passed from another frog, immediately after death, each into a separate cell, which was filled with a mixture of two parts of water and one of seminal fluid, obtained only a few minutes before it was employed. Two of the eggs were allowed to remain in this mixture in their cells, but the others, after a few minutes' immersion, were well-washed, and supplied with pure water, and the whole were then placed in a temperature of 60° FAHR. At the end of four hours I found the surface of the first two eggs covered with an abundance of spermatozoa; and a chamber had been commenced, and the upper surface of the yelk was depressed, in each, thus showing that some degree of fecundation had been effected, but no segmentation had taken place in either of these eggs at the end of *five hours and a half*, although the temperature of the room had been raised during the interval from 60° FAHR. to 67° FAHR. The sixth hour was almost completed before either of these yelks gave any indication of the probability of this change. Segmentation then commenced, but in a very irregular manner. In one egg the division was not in the centre, but at the side of the yelk, and proceeded no further than to about one-half the extent across the surface. In the other the surface of the yelk within the chamber merely became sulcated twice in the same direction. Neither of these eggs proceeded further with their changes, and of course no embryos were produced. The two remaining eggs did not undergo any change whatever, and consequently had not been impregnated.

Although these failures were still attributed to some immaturity of the eggs, I began to suspect that they might be occasioned by the immersion, or *smothering of the eggs* in fluid of too great a density; yet this suspicion appeared to be so hypothetical as scarcely to merit consideration, when it was remembered that on a former occasion when I had covered eggs with a solution of gum* and afterwards applied the impregnating fluid to them, some of them became fecundated. I was well aware that SPALLANZANI had found in his experiments that the greatest number of frogs' eggs became fecundated when the fluid employed was very much diluted with water†; but I did not then know that M. QUATREFAGES had made an observation‡ which completely coincided with the result obtained by myself. This acute naturalist found that when he employed the pure semen of the sea-worms, the *Hermella* and the *Teredo*, not a single egg was fecundated, and that when he mixed it with only a small quantity of water, so as to obtain "un liquide très opalin," that then only a very small number of eggs showed any traces of fecundation. He ascribed these results simply

* Philosophical Transactions, 1851, p. 236.

† Dissertations, vol. ii. p. 190.

‡ Annales des Sc. Naturelles, 3me Série, tom. xiii. p. 129.

to the circumstance of the fluid being too thick; but as we shall presently find, this, perhaps, may admit of a different interpretation.

My first object having been to learn how small a number of spermatozoa is sufficient to fecundate an egg, it may well be supposed that the facts just mentioned were exceedingly puzzling, and they were rendered still more so by the circumstance that a different result occurred with three other eggs from the same frog, employed at the same time as those in the preceding experiments. *Two* of these eggs, placed like the others, each in a separate cell, in a mixture of one part of seminal fluid to six parts of water, *two hours and twenty minutes* after the fluid had been obtained, became fecundated. In each of these the chamber was formed above the yelk, segmentation took place, and proceeded regularly, and each egg subsequently produced an embryo. In the third egg the chamber was formed, but the yelk became irregular in outline, and did not undergo segmentation. It had merely three parallel depressions on its upper surface, after which it became strangely altered in form, and was abortive. The cause of this failure seemed to be the same as in the preceding experiments,—an excess of spermatozoa in encounter with the egg, as subsequent experiments tended to show.

Seven eggs were passed into one large cell, and one egg into each of *two* separate cells, and the cells were then filled with a mixture of fluid and water, which was so dense as to appear turbid and opaline. This appearance was in part due to the fluid containing a large proportion of developmental cells. The result in each instance was very marked. The fluid at the time it was employed had been mixed with water about *one hour and five minutes*, and the temperature at the time the experiment was made was 56° FAHR., but the eggs were soon afterwards removed to a temperature of 62° FAHR. At the end of *five hours* the yelks of two of the eggs had become partially segmented, but the changes were continued only in one. The remaining eggs had been more injuriously affected. Their yelks had become *shrivelled and contracted, and resembled withered apples.* In this experiment it was evident that the effect had been occasioned by an excess of impregnating influence, but in what way it was not then easy to understand. The yelks of these eggs looked very like those of eggs which had been in contact with solution of potass, or of those to which stale and decomposing fluid had been supplied.

But it was possible that this change of form might be due to some injury sustained by the eggs in their removal from the body of the frog, or to some accidental cause in their removal to the cells, before fecundation. In order, therefore, to remove all doubt in this respect, another trial was made with two other eggs, which were so carefully passed into cells as to avoid all suspicion of injury to them, and the cells were then filled with mixed fluid like the preceding. The yelks of each of these eggs also changed their form, and one of them became irregularly contracted and pear-shaped. Of the whole of the eggs employed in these experiments only *one* underwent segmentation, and produced an *embryo.* The others were not fecundated. It was

thus evident that an excess of fecundatory fluid is as unfavourable to the production of the embryo as a deficiency.

11. DEFICIENCY AND EXCESS OF FECUNDATORY INFLUENCE COMPARED.

The results of these experiments with the fecundatory agent in excess require to be further explained, and also to be compared with those obtained with deficiency of the agent, in order that the nature of both may be properly understood. If this be done it will be seen that fecundation of the frog's egg depends very much on the *physical influence of temperature*; and that this has a close relation with the intensity of the force, or the degree of vitality evolved in the spermatozoon, at the time of its application to the egg; and probably also with the influence which the spermatozoon supplies through a greater or less amount of material substance to the body to be fecundated, at the time of its encounter, as in the following experiments made in March 1852.

The experiments by pin-point application of fluid show that only a very small amount of influence is actually required to set up those changes in the yelk which result in the formation of the embryo; and other observations have convinced me that the result is the more certain at a slight increase than at ever so slight a diminution of temperature. The effect of the application of minimum quantities is most certain when the influence has only very recently been obtained, and consequently while still endowed with its greatest degree of vitality. But even at that time a given amount is required, and if such be not supplied the result is incomplete, and partial fecundation only is effected. First then in regard to minimum quantities.

Six eggs were placed each in a separate cell, and three of these were touched *once* only with the pin-head loaded with fluid obtained about twenty-five minutes before, while the other three were each touched once with the fine pin-point loaded in like manner. Yet although there was only an interval of one minute between the application of fluid to the two sets of eggs, two of the former by pin-head application had the chamber developed, and segmentation commenced at the end of *three hours and forty minutes*; while one egg only of the three supplied by pin-point application began to be segmented in *three hours and forty-eight minutes*, although the whole were placed under precisely similar conditions in regard to light, heat, quantity of water and degree of aëration. The third egg of the first set was not impregnated, neither were the two remaining ones of the second set. Thus there was a difference between the two sets of eggs, not only in the extent to which they were fecundated, but also in the *rate of the accomplishment of fecundation* to the extent of *eight minutes*, in about three hours and three-quarters. There was also a perceptible difference of time in the formation of the respiratory chamber of the two, after the first hour, as was remarked by a distinguished physiologist who was present with me when these comparative trials were made. The two eggs of the first set, and the single egg of the second, which underwent segmentation, afterwards produced embryos; the remaining eggs were abortive.

A second comparative trial was made on the 15th of March. *Three eggs* in separate cells were each touched once with the fine pin-point loaded with fluid which had been obtained only twenty minutes before. The temperature at the time of the experiment was 56° FAHR., but the eggs were removed within a few minutes afterwards to a higher temperature, which was gradually increased to 64°·5 FAHR. Segmentation took place in one of these eggs in *three hours and thirty-nine minutes*, and this egg afterwards produced an *embryo*. But the remaining two were not impregnated. Three other eggs in separate cells, were, at the same time as the preceding, each once touched with the loaded pin-head, and two out of the three underwent segmentation at the end of *three hours and thirty-three minutes*. In a further experiment, made at the same time, *sixteen eggs* included in one cell were touched with the loaded pin's-head when the fluid had been obtained about half an hour before. *Four* of these eggs underwent segmentation in *three hours and fifty-three minutes*, and afterwards produced *embryos*, the remaining eggs being unproductive. Another experiment was made with *nineteen eggs* in a single cell, by pin-head application of the fluid, and eleven of these were fecundated and began to be segmented in *three hours and forty-two minutes*, and ultimately produced embryos, the remaining eight being sterile. Some of these failures were probably due to the circumstance that the fecundatory fluid was not applied to the most susceptible part of the egg, yet the general results sufficiently show not only that fecundation is more surely and quickly effected at a moderately high than at a low temperature, but also that it is so, more or less certainly and quickly, in proportion to the amount of influence supplied.

These facts lead us to some further consideration of the results of the application of definite quantities of the fecundatory agent to the egg, as affecting the development of the embryo, and *possibly also as influencing both the evolution of its physical structure and psychical condition*. I have little hesitation in believing that whatever be the precise nature of the influence communicated by the fecundatory agent to the egg, that it is only after full and complete impregnation that perfectly normal and healthy embryos are formed, and ultimately attain to the maturity of the species. And yet we have already seen that when there is great *excess* of the agent, either that no embryo is produced, or that its development is not completed.

A few days after making the last-mentioned experiments I repeated them with another object in view, and then obtained a result which had occurred on previous occasions, but being less distinctly marked had been almost overlooked.

About *twenty eggs* had been placed in separate cells, and fecundation attempted by pin-head application of fluid which had been obtained from a partially exhausted and debilitated male, at the end of the season, and which had been employed from want of a more healthy individual. The fluid, when examined by the microscope, was found to contain very many perfectly motionless spermatozoa, besides a large quantity of cells. The inefficiency of this male was inferred from the circumstance that the

female was prepared to spawn, as was proved by her having passed a few eggs; but these remained unimpregnated, and she still retained the remainder within her for nearly a day, at which time the male was removed for the experiment. The employment of this male was thus accidental, it being the only one I then possessed.

The fluid was applied to the eggs within a quarter of an hour of its being obtained, and the eggs were at once placed in a temperature of 61° FAHR. I remarked that development appeared to go on more slowly than usual, although most of the eggs underwent segmentation, and *fifteen* of them produced embryos, which were placed together in one vessel. A few days afterwards I was surprised to find that two of these embryos were greatly malformed. One had a short falcated tail narrowed at its base, where it was not broader than at its extremity, and the tail of the other was scarcely more than a short narrow stump, so that the embryo had much difficulty of locomotion. A few days later three other of the embryos became distorted, and one of them died much altered in form at the period of absorption of the external branchiæ.

These circumstances are recorded merely as simple occurrences which point to the necessity for further experiment on this curious and important subject, and not as results from which any very positive conclusions can be deduced. Nevertheless, I may add, that I think I have before observed that embryos which have been the result of fecundation with very small quantities of fluid, are usually smaller than others which are produced from full and natural impregnation.

The effect produced by immersion of the egg in pure fluid is as curious as the results above mentioned obtained from minimum quantities, and at first it is less easily to be understood. I can find no more expressive term by which to indicate it than that of *smothering*, and this, perhaps, may be found to be a more literally correct expression of the fact than at first is apparent. It has already been shown that free aëration is most essential to the development of the embryo, and I believe it is equally so, in a minor degree, to fecundation. Thus while the egg may be fecundated in a dense fluid, composed of equal parts of seminal fluid and water, its changes usually take place much more slowly than when the proportion of water is considerably increased; while if it be immersed in pure fluid, and be allowed to remain in that for some hours, it may still be fecundated, and its changes be commenced, but these will not proceed to the full development of the embryo. If the egg when immersed in pure fluid be retained in a moderately low temperature, then the development of the embryo may go on slowly for a time, but with less rapidity than when the egg is freely aërated in pure water. But if, on the other hand, it be retained in seminal fluid, at a high temperature, then it usually happens that although the respiratory chamber be formed, as the result of fecundation, segmentation will proceed slowly, or irregularly, and the embryo, which is begun to be formed, perish. That want of proper aëration is in part the cause of this infertility seems to be shown in the following experiments.

April 5, 1852. Atmosphere 54°.—*Two eggs* were placed in separate cells, which were immediately filled with nearly pure fluid. In about one hour afterwards the respiratory chamber was beginning to be formed in both these eggs, but its progress was slow. One egg was then washed as completely as possible with a powerful jet of water, thrown upon it by a syringe, and the cell was then filled with pure water, while the cell with the other egg was replenished with more of the fecundatory mixture. After the addition of fresh water to the first egg its envelopes began to expand, and it was then seen that the substance of these coverings was *filled with spermatozoa, lying in every direction and so crowded around the envelope which immediately invests the yelk, as to render the whole semi-opake.* The envelopes of the second egg were much more opake than those of the first. Yet the respiratory chamber was developed, after a lengthened interval, in both, but earlier in the egg which had been washed and placed in water than in that which remained in the fluid. At the expiration of twelve hours the second egg was washed and placed in pure water like the first. Both eggs were preserved, from within an hour or two after their first encounter with seminal fluid, for four days in a temperature which ranged from 59° FAHR. to 64° FAHR. At the end of three days and a quarter, or about seventy-eight hours, in this temperature the development of the embryo had advanced so far in the first as to the union of the laminæ dorsales, and the narrowing and elongation of the body, a stage of development at which the function of aëration is becoming more energetic in the evolution of ciliary action. At this stage of formation the development of the embryo became arrested, although the egg was properly supplied with water, and from this period it decayed. The second egg, which had remained twelve hours in the mixed fluid before it was supplied with fresh water, perished much earlier, and before there were any distinct evidences of the formation of an embryo.

On other occasions I have found that the egg does not become fertilized at all, but the yelk contracts irregularly, and resembles the yelk of eggs affected by solution of potass.

These are the usual results of immersion in undiluted fluid, which, contrary to what occurs with minimum quantities, appears to be most prejudicial to the egg, in its pure state, *when the temperature of the surrounding medium is much increased*; so that the cause of failure in these cases *of excess* may reasonably be attributed, in great part, to a smothering of the egg, through impeded aëration; accelerated, perhaps, by some chemical change, through increased temperature, in the remains of the spermatozoa, with which the envelopes of the egg are crowded in their interior.

12. THE MOTION OF THE SPERMATOZOON IN RELATION TO ITS FUNCTION.

The possibility of the fecundatory function being in some way connected with the motion exhibited by the contents of the pollen in plants, and with that of the spermatozoa in animals, has not escaped the consideration of the best observers. Mr. Brown, the most distinguished of botanists, many years ago, made this the

subject of his particular investigation[*]; and M. Brongniart[†], Schleiden[‡], Nägeli[§], Griffith[||], and more recently Suminski[¶] and Henfrey[**] have done the same. But although neither of these able inquirers succeeded by direct experiment in proving that the motion of the particles of plants is essential to the *act* of impregnation, M. Hofmeister[††], and very recently also Mr. Henfrey[‡‡], have noticed facts in regard to that of the spermatozoid filaments discovered by Nägeli and Suminski, in the Cryptogamia, which seem to show that, in plants, it is of great importance to the function of these bodies. The motion of the spermatozoon in animals has equally attracted the attention of zoologists. Prevost and Dumas[§§], as already stated, Siebold, Müller, Wagner, Kölliker, Bischoff, Quatrefages, and especially Wagner and Leuckardt, have studied it attentively; but so intricate is the inquiry concerning its nature and import, that the last two authors dismiss the consideration of the question without arriving at any conclusion, and state that they do not venture to decide[||||]. Heretofore I regarded impregnation as being *commenced* by transmission from the spermatozoon on the surface of the egg, to the contents in the interior, of some influence characterized by motion. But I have regarded this motion as being only the visible indication of a *peculiar force*, or *form of vitality*, in the impregnating agent, the spermatozoon, by which it is destined to arrive at, and is to expend on the object to be fecundated, and the effect of which is to *strengthen*, to *augment*, and possibly also to *modify* the nature of the formative changes, which are going on in the yet unimpregnated egg, *per se*; but which will subside, and soon entirely cease, if not reinforced through the agency of the spermatozoon. Nevertheless, I have not been prepared to assent to the view that simple contact of the spermatozoon, *even with the vitelline membrane*, is sufficient to complete the changes which result in the formation of the embryo (see p. 233). The powerful endosmic action of the envelopes of the ovum, at the time of oviposition, is opposed to this conclusion; since, if simple contact

* A brief account of microscopical observations, made in the months of June, July and August, 1827, on the particles contained in the pollen of plants, and on the general existence of active molecules in organic and inorganic bodies, by Robert Brown, F.R.S., etc., 8vo. July, 1828; also additional remarks on active molecules. (*Id.*) July, 1829.

† Recherches sur la génération et le développement de l'embryon dans les Végétaux Phanérogames (Notes). Annales des Sciences Nat. tom. ix. 1828.

‡ Grundzüge der wissenschaftliche Botanik.

§ Schleiden und Nägeli's Zeitschr. für Wiss.-Botanik; Heft i. 168; Zurich, 1844.

|| Transactions of the Linnean Society of London, vol. xx.

¶ Zur Entwickelungsgeschichte der Farrnkräuter, 4to. Berlin, 1848.

** On the development of the ovule in *Orchis morio*, Transactions of the Linnean Society of London, vol. xxi., and Proceedings, vol. ii. p. 27, April 8, 1849.

†† Untersuchung des Vorganges bei der Befruchtung der Œnothereen, Botanische Zeitung, v. 785, 1847.

‡‡ Annals and Magazine of Natural History, vol. ix. June, 1852; Transactions of Linnean Society, vol. xxi. Part II.; also Proceedings, June 17, 1852, vol. ii.

§§ Annales des Sciences Naturelles, tom. ii. 1824.

|||| Article "Semen," Cyclopædia of Anatomy and Physiology, vol. iv. par. xxxiv. p. 508.

or encounter of the spermatozoon with the ovum were sufficient, it might be expected, as has been well remarked*, that the influence of the spermatozoon of any animal of the same class would be competent to effect the impregnation of any species.

Yet all the phenomena connected with the origin and death of the spermatozoon seem to be in accordance with the view, that its *motion* is essential to its function. Whatever be the relation of this motion to its peculiar faculty, it is evident that motion is intimately *associated with, and dependent on, its material composition, and structural development.* In the Frog, the spermatozoa are usually completed very early in the season, when the animals begin to emerge from their hybernacula, in the beginning or middle of February; but, as we have seen, they only begin to pass into the efferential ducts from the testicle, at the time when the pairing of the sexes is commenced; from which time, to that of spawning, a period of from ten days to a fortnight or three weeks, according to the temperature of the season, they are more fully matured, and acquire greater vibratory power, and are collected in the vesiculæ seminales for expulsion at the instant after oviposition. In the Toad the spermatozoa are developed at a later period of the season, but at a relatively corresponding period in the life of the animal. The male Toad, like that of the Frog, usually emerges from its hiding-place a few days, or a week or two earlier than the female. I have taken the males in the middle and at the end of March, at which time I have not been able to detect any seminal fluid in their vesiculæ seminales; yet they are then exceedingly salacious and disposed to pair, and I have sometimes found the contents of the reproductive organs in a similar state, even after the sexes have been for two or three days in union. The testicles, nevertheless, are then filled with an abundance of spermatozoal cells in the course of development, and also with a great quantity of spermatozoa, each still included in its vesicle of development; but as yet immature, *motionless*, and with only a very short caudal extension from its thick cylindrical body. Besides these there are usually a few spermatozoa, more matured than the rest, *which exhibit movements while still retained within their cells*, which are enlarged, and from which they are soon to be liberated.

Thus motive power in the spermatozoon is coincident with the completion of its structure and composition, and as such, may fairly be regarded as *essential to its function.* In the Frog the motion of the spermatozoon is most intense and persistent at the full period of connubiality. On the other hand, I have constantly noticed in all my experiments on artificial impregnation, that where impregnation has not been effected, all the conditions being favourable to it, or when I have found by trial that the male fluid *has ceased to be efficient*, that then nearly the whole, or perhaps all of the spermatozoa, have been perfectly motionless, and apparently dead. This is also the condition in which I have usually found the few spermatozoa which are retained in the reproductive organs a week or two after pairing, when the male Frog and Toad may be regarded as in the state of aged individuals, the season of reproduction

* Wagner and Leuckardt, *loc. cit.* p. 508.

having passed, and the function being fulfilled. Thus too, I have noticed that, when from accident, but more especially when from reduction of the temperature in the surrounding medium, the season of spawning has been greatly retarded, the impregnating power of the male is much diminished, and perhaps is almost exhausted, through constant shedding of the spermatic fluid, which, I have found, often takes place when the oviposition of the female is delayed, and the individuals are disturbed or interfered with. The female is then forsaken by her partner, and when this occurs it rarely happens that the connubial intercourse of these two individuals is recommenced. When this separation has taken place, there is usually but a small quantity of fluid remaining in the male organs, and even in that, the number of spermatozoa is considerably diminished, and *their power of motion is exceedingly feeble*; while the quantity of molecules and cells is increased. When several days, or a week or two have elapsed, there are not only fewer spermatozoa, but those which remain are much more feeble in action. This is exactly what occurs also in the Toad. On the sixth of June I found that the testes and efferential ducts in a male Toad, which had been kept from pairing during the whole season, were still filled with spermatozoa, together with a very small quantity of liquor seminis with active molecules moving in it; but that, though the spermatozoa were in full abundance, nearly the whole of them were entirely motionless, while the motions of the few which still gave evidences of vitality, were exceedingly feeble, whether the spermatozoa were examined simply in the fluid portion of the semen, or whether they were mixed with water, in which, as is well known, the motions are always at first greatly increased.

A similar reduction in the number of the spermatozoa and diminution of their motive power, appears to exist in animals which have become exhausted through long confinement or want of food; at least, if we may so judge, from a few observations on the Tritons. A *Triton palustris,* which had been captured on the seventh of May, and accidentally confined without food till the sixth of June, was examined immediately after death. The efferential ducts were well-filled with spermatozoa contained in a distinctly perceptible quantity of liquor seminis. When the spermatozoa were examined, without the addition of water, their motions were regular, but apparently very much slower than usual, being uniform and undulating, without that peculiar rapid ciliary action of the spirally twisted tail, which is so constantly referred to as characteristic of the spermatozoa of the Tritons and of some other Amphibia. When water was added, the motions were immediately accelerated, and the tail, which before was merely flexed, and almost longitudinally extended from the body, became folded and entwined around it, and its rapid ciliary movements were commenced. But these gradually subsided within a very few minutes. In another specimen, which had been captured at the same time as the preceding, and confined under similar circumstances, but which, at the time of examination, had been already dead for more than twenty-four hours, scarcely any spermatozoa remained in the testes, or in the efferential ducts. There was a great quantity of cells, with granular nuclei, in

the body of each testis, but scarcely a single spermatozoon. The few spermatozoa which remained were most of them dead, and decomposition appeared to have commenced in them, and there was a large proportion of active molecules. Amongst these were one or two spermatozoa still in action, and the molecules were accumulated around them by the attraction of the current in the fluid, induced by the ciliary action of the tail, and were propelled onwards, frequently in a spiral direction, from the base of the tail to the anterior extremity of the body; thus showing that the motions around the spermatozoon are due to the action of the filamentous tail, flexed upon, and twisted spirally round the body; in accordance with the observation of WAGNER and LEUCKARDT.

But it has been remarked, in opposition to the view, that power of motion in the spermatozoon is essential to its function, that motion is not detected, or but very faintly, in many instances in which the spermatozoa are united into simple and uniform cords, as they often are in the deferential vessels of insects; and that then only a slight waving, or trembling of the mass, the consequence of hygroscopic conditions induced by the fluid around, is, under such circumstances, observed. An aggregation of the spermatozoa into cords takes place in the Tritons as well as in insects. When the semen is expelled by the Triton at the time of conjugation, it is composed almost entirely of white cord-like, flocculent masses of spermatozoa, which, at that moment, exhibit only the faint undulatory motion noticed in them while still within the deferential vessels. Yet, immediately after these masses are ejected from the vessels, and are in contact with water, at the moment of being received into the cloaca of the female, as I shall hereafter have occasion to show,—the motions of the spermatozoa are not only greatly increased, but that peculiar vibratory ciliary action of the tail, which, while the spermatozoa are still within the seminal ducts is very indistinct, and perhaps can scarcely be said to occur—is immediately set up, and the motion of the body of the spermatozoon is changed from the undulatory action before observed, to the jerking or watch-spring movement pointed out by observers, and thought to be the motion peculiar to the spermatozoon of this class of animals.

Thus then, the fact of only a slight wavy motion of the spermatozoon being perceived in some cases, ceases to be of importance, as an argument against the view that motion is closely connected with the function, since it is evident that the true spermatic force is not set up until the spermatozoa have become more or less separated from each other, through their residence in some fluid vehicle. The movements of the spermatozoon in insects are very similar to those of the spermatozoon of the Triton, and the circumstances which affect both are in full accordance with the fact of the existence of, and the necessity for, a distinct liquor seminis, as part of the normal composition of the seminal fluid, in the Mammalia; while this portion of the fluid is not needed, and consequently is almost entirely absent in the Amphibia, Fishes and most Invertebrata. A comparison of the facts in the two divisions of animals seems to point to the true nature and use of the liquor seminis, as being that

of a mere vehicle for the ready transmission of the spermatozoon to the ova, and as allowing greater freedom of motion to these bodies.

It is true, as Wagner and Leuckardt have stated, that but little motion can be observed in the spermatozoa of Insects when they are united into cords; yet this seems to be due rather to their union into these masses, and to have reference to their mode of ejection from the body of the male, as in the Triton, and is known to take place during their collection in the deferential vessels, than to any real absence of a power of motion in them. This is not their condition when brought into contact with the egg at the time of impregnation. When the fluid of the male insect, during pairing, is passed into the spermatheca of the female, where it may be destined to remain for an indefinite time, the spermatozoa compose the chief portion of it, as in the Tritons; but, during their residence in the spermatheca, the spermatozoa are mixed with a fluid, which is supplied by a gland attached to that organ, and which becomes to them a vehicle like the liquor seminis, and allows of their separation and independent motion, and thus appears to answer the purpose of the true liquor seminis in the Mammalia. At the period when the ova are descending from the ovaries, the movements of the spermatozoa become more distinct; and when these bodies are brought into contact with the egg, as it passes the outlet of the spermatheca, they become more isolated, and their movements more intense, as I have seen in the case of the Orthoptera. From these circumstances I am led to believe that some degree of motion will ultimately be observed to mark the perfect condition of the impregnating agent in all animals. The facts already mentioned coincide with this view, and point to the probability that the degree of impregnating force in each individual may perhaps be indicated relatively by the degree or intensity of motion in the spermatozoon, and the duration of this force by the length of time which the spermatozoon continues in motion.

The established fact, that a difference in the structural conformation of a body is the invariable result of a difference in the relations, proportions, and composition of its material constituents, has always afforded reason for presuming that some material influence may be transferred from the substance of the spermatozoon to the contents of the ovum, at the time of impregnation. The function of impregnation appears to be one of definite relations and proportions. Thus we have seen, when only a few spermatozoa were applied to the ovum on the point of a pin, that full impregnation was but rarely effected. In most instances of such limited application, the yelk underwent only partial segmentation, and its changes were then gradually arrested, and no embryo was produced. If, however, the pin point which had been charged with spermatozoa, instead of being applied to the ovum for an instant only, was allowed to remain in contact with the egg for a second or two, and thus by capillary attraction became drained of the spermatozoa which adhered to it, and, as a consequence, thus made to deposit a greater number of these bodies, which were not afterwards attempted to be removed or destroyed, then impregnation was sometimes

completed, and an embryo became developed. But if, instead of the spermatozoa being thus supplied in a minimum quantity from the *point* of a pin, they were supplied in greater quantity from the *head*, then impregnation was almost always effected; and this result was rendered the more certain by allowing the head to be drained of the adhering bodies for a moment or two, as from the point, by which a much larger quantity of the impregnating agents was deposited. In these instances it was rare that impregnation was not effected. These results appear to show, that, whatever may be the precise quantity necessary to effect healthful impregnation, it has some definite relation to the effect to be produced in the contents of the ovum—that a definite quantity of spermatozoa, or spermatic influence is required to fecundate, and that the perfection of fecundation has relation to the degree of impregnating influence. They seem to show, also, that fecundation is not the simple result of the penetration into the egg of *a single isolated spermatozoon*, but probably of some definite number of these bodies, or of a definite amount of influence supplied through their encounter. We are thus led to perceive that the same law of relation between cause and effect,—between a definite amount of influence expended, and definite results,—which has long constituted the basis of our knowledge respecting chemical affinities, and which is now being demonstrated, as that also of the other forces of inorganic nature, may equally pervade and control these material combinations among the organic affinities. Further, the observations, now mentioned, seem to put to rest the last remaining question respecting the independent animality of the spermatic bodies, and to show that these, like the cilia, are mere elementary parts of the adult male organization,—as many physiologists believe*,—as the contents of the ova are of that of the female.

All my experiments on the egg of the Amphibia serve to show, that even with a definite quantity or number of spermatozoa, no impregnation is effected, if, before the spermatozoa are brought into contact with the egg, they have all ceased to exhibit that motion which constitutes their marked characteristic. Thus then, it seems that, independent of the important question as to whether these bodies yield any material substance for combination with the constituents of the egg, through the usual chemical affinities of matter,—the quantity of influence to be supplied to produce the healthful result is definite, and that, whatever be its nature or essence, it is always characterized by a definite degree of *motion* in the spermatozoon. Further, all the observations I have made on the spermatozoa tend to show that their motion is rendered more vivid and intense by an increase of heat, but that, in proportion to such increase, it is so much the sooner exhausted; as, on the other hand, it has long been known that it is diminished by reduction of temperature, but increased in duration. Many of the observations lead to the view, that the power, in a given quantity or number of the spermatozoa, to effect impregnation, is more in proportion to the amount, *quantity, or intensity of the motion* exhibited by these bodies than to their actual

* Kölliker, Siebold, Müller, Wagner, &c.

numbers. Thus the spermatozoa, like the ova, are more efficient at a given temperature, within the first two or three minutes after they are passed from the body, than at a later period. At that time there is not only a greater number of spermatozoa in a given quantity of fluid in a state of activity; but the whole of them exhibit a greater intensity of motion than after they have been for some time mixed with water. In the Toad, which, in this country, as already stated, pairs at a few weeks later in the season than the Frog, and which seems not only to require a higher temperature of the surrounding medium, but also a relatively higher temperature for the development of the ova, the spermatozoa are most active, and most fitted to impregnate at the instant of expulsion; as is shown in the mode of intercourse of the sexes in that animal, and in the fact that, if the fecundatory fluid be not immediately applied to the strings of ova as these are passed, the unsprinkled ova are infertile. The oviposition of the Toad is an exceedingly slow process, and usually lasts, as SPALLANZANI observed, from ten to fifteen hours; in one instance I found it continued during seventeen hours, but it was completed in others in eight or ten. The act of impregnation, therefore, is necessarily also prolonged. But the length of time during which the spermatozoa continue efficient to impregnate after removal from the body, is shorter than in the Frog. SPALLANZANI found that, at a temperature of 81° FAHR., it does not exceed fifteen minutes. I also have noticed that the motions of the spermatozoa cease much earlier than in the spermatozoa of the Frog.

Thus the conclusions to be drawn from the facts of natural impregnation in the Toad, fully agree with those deduced from artificial impregnation in the Frog, and seem to establish the view, that while an increase of temperature is required for the fulfilment of the reproductive function in that animal, and to maintain the efficiency of the spermatozoa, the fecundatory force of the agent is of shorter duration, and corresponds to its more early cessation of motion.

13. PENETRATION BY THE SPERMATOZOON IN EFFECTING FECUNDATION.

The penetration of the spermatozoon into the substance of the egg, or even into its envelopes, has been so much disputed, and so repeatedly denied, that it is only on what may perhaps be regarded as indisputable evidence, that any one ought to reassert it. Up to a very recent period I could not, myself, admit it as probable; because, throughout my investigations, I had not been able to detect any appearance in the fecundated egg or its envelopes, either of the Frog or Newt, which rendered it likely that the spermatozoon penetrates into or through them. Yet the fact of penetration was stated by the older naturalists, not so much, perhaps, from any decided proof of its occurrence, as from a confidence that it was only in this way that the function of the spermatozoon could then be understood. LEEUWENHOEK, and those of his day, not only believed that the spermatozoon penetrates bodily into the substance of the egg, but also that it becomes the future embryo. In later times, M. PREVOST having, with M. DUMAS, seen the spermatozoon within the gelatinous envelope of the

egg of the Frog, as they show, conceived that this body becomes,—not the embryo itself, but the foundation of the nervous system of the embryo. Since then, Dr. MARTIN BARRY announced to the Royal Society that he had *seen* spermatozoa within the substance of the egg of the Rabbit; through the envelopes of which, he has stated, that there is a natural perforation or cleft at the time of fecundation*; a view which has met with much opposition, as no physiologist has hitherto (1852) verified his observation with regard to the existence of a perforation or cleft in the egg-envelopes; or has announced that he has *seen* the spermatozoon *within* the ovum of that animal. Indeed so general has been the opinion that there is no penetration by the spermatozoon, even into the envelopes of the egg, notwithstanding WAGNER's† announcement that he had seen spermatozoa within the envelopes in the eggs of Fishes, that one of the most recent investigators, M. QUATREFAGES, in alluding to MM. PREVOST and DUMAS' views, observes, that "it is useless to allude to the question of penetration by the spermatozoon, as he believes that the only living author of the theory has himself renounced it‡." But very recently it has been announced, in a paper on the *Ascaris Mystax*, by Dr. NELSON, communicated to the Royal Society§, that the spermatozoon in *that* animal *does penetrate* into the substance of the yelk; and a somewhat similar account of that of the Earth-worm was formerly given by Dr. ARTHUR FARRE||. Yet, with all due respect for the observations of these able investigators, I must still have hesitated to admit the fact of any penetration, even into the envelopes of the egg, had I not more recently been convinced of this fact by direct observation, in correction of my former opinions, in so far as relates to the penetration of the spermatozoon into the envelopes of the eggs of the Frog, and its arrival at the vitelline membrane. To them, then, be all honour on this subject, while I subjoin my testimony to a fact which I had heretofore failed to observe.

It was during the month of March last (1852), while making experiments on the Frog's egg by artificial impregnation, before some scientific friends, that certain appearances were noticed beneath the microscope within the expanded jelly of the egg, which led to a suspicion of the probability of penetration by the spermatozoon. Heretofore I had employed, in my observations, either a deep glass cell, in which the egg was completely immersed in water, or the common object-glass of the microscope. In the experiments now referred to, a shallow glass cell was employed, which was capable of containing only a single egg. This, while it admitted light freely around the egg, was too shallow for its complete immersion, and the egg was therefore covered by a drop of water, into which the object-glass of the microscope was passed,

* Philosophical Transactions, Part II. 1840, p. 533, Plate XXII. figs. 164, 165 and 167. Ibid. Part I. 1843, p. 33.

† Elements of Physiology (English Edit. by Dr. WILLIS), note, p. 74, 1841.

‡ Annales des Sciences Nat. 3me Série, tom. xiii. 1850.

§ Proceedings, June 19, 1851, vol. vi. p. 86. Philosophical Transactions, 1852.

|| See Dr. CARPENTER's Principles of Human Physiology, 1st edit. 1842, p. 617.

while viewing the egg, instead of the observation being made through the double medium of air and water. This was the method of examination in all the subsequent experiments.

The appearance which first led me to suspect that spermatozoa do penetrate into the envelope, was produced by an acicular body, which seemed to have its narrowed extremity in near proximity to the vitelline membrane. The direction of the longitudinal axis of this body was in a line with the centre of the yelk. But what appeared to be its larger end was farthest removed from the yelk; and this circumstance seemed to show that the appearance could hardly be due to the presence of a spermatozoon, which, if the observation were correct, must have penetrated in a direction the reverse of that of its usual motion.

It was necessary, therefore, that the suspicion raised by this observation should be settled. I had again and again found, as before shown, that the egg of the frog may be impregnated, under certain conditions, by the direct application of spermatozoa to almost any part of its surface, and this enabled me to put the question of penetration to the test. In the first trial, an egg was placed in a single cell, and immersed in water for *one minute*—the water was then removed, and the egg touched on one point only of its surface with the head of a pin, loaded with the fecundating fluid, which had been obtained and mixed with water about two hours before. I had expected that on watching the egg beneath the microscope, from the instant of contact with the pin's-head and the re-filling of the cell with water, to have been able to detect the spermatozoon during its passage through the envelope. But this I failed to do, in the present instance,—no spermatozoa were detected *in the interior* of the envelope, although many were easily observed on the *surface* at the point to which they had been applied by the pin. Yet, this egg, placed in a temperature of 66° FAHR., underwent segmentation in *three hours* and *thirty-two minutes*, and ultimately produced an embryo. A similar trial was made, at the same time, with an isolated egg after immersion for *two minutes* in water, which was then withdrawn, and the fecundating fluid applied as before. In this instance, several spermatozoa had penetrated for a short distance into the envelope, but had not reached the covering which immediately invests the yelk. No *respiratory chamber* was formed above the yelk in this egg, nor was any embryo afterwards produced. In a third instance, with an egg, which had been immersed for *five minutes*, the experiment was equally unsuccessful. On the 24th of March a further trial was made with an isolated egg, after *one minute's* immersion. The fecundating fluid employed had been obtained only sixteen minutes before it was used, and was applied by the head of a pin, once only, to one point of the egg. In this case, at the expiration of half an hour, I distinctly saw a single spermatozoon sticking by its larger extremity into the vitelline membrane, and a few minutes later there was evidence of the respiratory chamber being about to be formed. This egg underwent segmentation, and afterwards produced a good embryo. In a second egg immersed for *three minutes*, and in a third for *five minutes*, there were no appear-

ances of spermatozoa in the interior of the jelly, although they were in abundance at the point on the surface, to which they had been applied by the pin. Neither of these eggs underwent any change. But in a *fourth trial*, with an egg immersed for *ten minutes*, and to which the spermatozoa were applied in the same way as in the preceding, I saw, at the expiration of fifty minutes, four spermatozoa sticking in the vitellary membrane; soon after which the chamber was commenced, and at a later period segmentation of the yelk took place, and an embryo was produced. This was with fluid which had been obtained nearly an hour before it was employed. In a further trial, with an isolated egg, to which a full quantity of fluid was added, by three or four applications of the loaded pin's-head, after the egg had been *one minute* in the water, I detected several spermatozoa, within half an hour afterwards, sticking like the preceding into the vitelline membrane, and this egg also produced a good embryo.

It was thus evident, that in some cases, spermatozoa certainly penetrated through the envelope; but as there was also one instance, in which the egg had become fecundated, and afterwards produced an embryo, in which I had not seen spermatozoa within the envelopes, it was necessary to pursue the investigation further, to be quite assured of the fact of penetration as connected with the act of fecundation.

On the following day an opportunity occurred to me of examining the egg after impregnation by the natural union of the sexes. I had several pairs of frogs in a basin of water covered by a glass bell jar, when, having my attention directed for a few minutes to some other object, one pair of frogs spawned suddenly, as I knew by the sound of a plunge or splash in the water, which always occurs after the act, at the moment of separation of the sexes. Nearly the whole of the eggs were deposited in a mass, but there were a few which were detached from the rest, and were lying at the bottom of the water. This seemed a good opportunity to examine these eggs, which had been expelled naturally, singly, in search of the spermatozoa within the envelopes. *Fifteen* of these detached eggs were placed, each in separate cells, and carefully examined, during the first hour and a half after their expulsion. In the first eight or nine of these eggs, I was not able to detect even a single spermatozoon within their substance, and I began to look upon the previous observations, made by artificial impregnation, rather as the result of accident. In the tenth egg, however, I most distinctly saw spermatozoa in contact with the vitellary membrane, but in one or two eggs examined after this no spermatozoa were to be seen. Out of the fifteen eggs examined, there were only *two* in which I could detect spermatozoa. As each egg had been carefully marked, and a note made, as to whether or not any spermatic bodies had been detected within it; and as previous investigation had shown, that when any fecundation has been effected the yelk of the fecundated egg becomes depressed, and a chamber begins to be formed, in about one hour, or little more, after the spermatozoon is supplied to the egg, between its upper surface and the investing membrane,—there were ready means of learning, within a short time,

whether any, and which of these eggs had been fecundated. At the expiration of *one hour and twenty minutes,* the temperature being 54° FAHR., the chamber was beginning to be formed in the two eggs in which I had seen spermatozoa sticking in the vitellary membrane, and *these bodies were still readily detected in them,* and continued to be so for two or three hours afterwards. These two eggs ultimately produced embryos. But no change took place in any of the thirteen eggs, in which I could not detect spermatozoa,—no respiratory chamber was formed within, nor was any embryo produced. It was evident, therefore, that these had not been fecundated, and it is probable that the whole of the detached eggs were those which had last been ejected from the oviducts, possibly after the act of fecundation by the male had been completed. Having found spermatozoa in two only of these detached eggs, I then examined several of those from the mass. In each of them I found *numerous spermatozoa sticking around the yelk membrane,* and very many, in the clear space between the membrane and the granular middle portion of the envelope, which had not arrived at the membrane, and in *every instance* the eggs had been fecundated, as the chamber was being formed at the time of examination, about an *hour and a quarter* after the eggs had been deposited, so that the act of penetration by the spermatozoon through the envelopes as far as the vitellary membrane, seemed thus to be clearly established as connected with the act of fecundation. This was the case with all the eggs taken from the upper and middle portion of the mass. But in a very few eggs taken from the sides of the mass, I was not able to detect any spermatozoa, within the envelopes, or found only solitary instances of them. In these cases it was remarkable that no chamber had yet been formed above the yelk, although in some, in which spermatozoa were detected, it commenced at a later period, so that these appeared to confirm the deduction from experiment with reference to quantity of influence, or number of spermatozoa required to effect fecundation; while the penetration of the spermatozoa, as far as the vitellary membrane, and the subsequent development of the chamber above the yelk, appeared in the relation of *cause* and *consequence,* direct or indirect. I could not then observe, in the eggs thus examined, any penetration by the spermatozoa completely *through* the vitellary membrane into the substance of the yelk; although numerous spermatozoa were attached, by their larger ends, to every portion of the membrane, sticking out from it at right angles, with what, at first, appeared to be a knob or knot at the distal end, or rather as if that part of the spermatozoon had been shrivelled up or scorched. This appearance, as I subsequently found, was due to a loop, or distortion of the tail of the spermatozoon, consequent, apparently, on the death of this body. It was this looping of the tail which gave the appearance of a thick end to this part of the spermatic body first detected within the envelopes in a previous observation. All the spermatozoa seen in connexion with the vitellary membrane were perfectly motionless; so that when the looping of the tail has taken place the fecundatory influence of these bodies may be held to have been already exercised and exhausted.

At the expiration of three hours from the commencement of these observations, although the number of spermatozoa which had first been noticed within the coverings of the eggs submitted to examination appeared to have been diminished, there was still an abundance attached to the vitellary membrane. These appeared to afford a good opportunity for endeavouring to ascertain whether any part of the body of the spermatozoon is passed through the membrane into the cavity of the yelk. With this object in view, I placed a glass cell, which contained an egg, on one side, and waited for the rotation, or rather the gravitation, of the yelk within its envelopes. By this change of position of the yelk within its coverings—and which always takes place, if fecundation, or any change in the position of the entire egg, has been effected,—the flattened surface of the yelk, and the chamber above it, were brought beneath that part of the vitellary membrane into which spermatozoa appeared to have sunk deepest,—so that if any of these bodies had passed, or were in the act of passing, or had been partially protruded through the membrane into its cavity, which contained the yelk, it was fair to expect that they might thus have been detected. But not the slightest trace of penetration, even by a single spermatozoon, could then be observed*.

I may here remark, in anticipation of a future communication, that, previous to segmentation, the mass of the yelk does not appear to be invested by any distinct envelope within its so-called vitellary membrane, but the whole seems to be kept together by the natural coherence of the granules or cells of which it is composed; so that when the position of the egg is changed the yelk mass rotates within the vitellary envelope as a consequence of this change, owing probably to the excentric position, within its substance, of the progeny of the germinal vesicle†, contained in the

* Since this paper was communicated to the Society, I have succeeded, through the adoption of a different mode of examination, in detecting spermatozoa *within the vitelline cavity in direct communication with, and penetrating into the yelk.* They were first seen by myself, in company with a friend, on the 25th of March of the present year (1853) within the clear chamber above the yelk, at about forty minutes after fecundation, when the chamber begins to be formed. I have since repeatedly observed them within the chamber, and in some instances still in motion, in which state I have had opportunities of showing them to my friend Professor Ellis of University College, and to two other medical friends, so that the presence of active spermatozoa *within the vitelline cavity* in the fecundated egg of the Frog may now be regarded as indisputable. The details of my investigation I reserve for a future communication, and will merely now add, that the spermatozoa do not reach the yelk of the Frog's egg by *any special orifice or canal* in the envelopes, but actually *pierce the substance of the envelopes at any part with which they may happen to come into contact*; as I have constantly observed while watching their entrance: sometime after they have entered the yelk chamber they become disintegrated, and are resolved into elementary granules. The importance of this fact of actual penetration by the spermatozoon into the yelk is indicated by Wagner and Leuckardt, in their late Article, "Semen" (*loc. cit.* p. 507), in the following remark:—"The truth is, the '*how*' of the fecundation is as far from our knowledge to-day as it was thousands of years ago; this process is still enveloped in what we feel inclined to consider 'its sacred mystery.' *It would be different if we could prove that the spermatozoa really yielded the material foundation for the body of the embryo; that they penetrated into the ovum,* and were developed into the animal (which was the assumption of Leeuwenhœk, Andry, Guatier), or else, that they became metamorphosed into the central parts of the nervous system."—G. N., April 18, 1853.

† Philosophical Transactions, Part I. 1851, p. 176.

middle of the dark hemisphere of the yelk, and which, possibly, may be of less specific gravity than the contents of the light-coloured hemisphere.

The facility with which the yelk can thus be made to rotate, and its flattened surface, and the clear space, or chamber above it,—commenced within one hour after the first encounter of spermatozoa with the egg,—can be brought beneath any portion of the vitellary membrane into which spermatozoa have penetrated,—thus enabled me to observe, within a comparatively short period after fecundation, whether any of those bodies are at that time in the act of passing through it. But although no spermatozoa could then be detected within the vitellary cavity, it must be borne in mind that these observations were made after the commencement of the formation of the clear space, or *respiratory chamber*, the consequence of a shrinking and depression of the surface of the dark hemisphere of the yelk as the *result* of impregnation, so that these negative observations did not afford any *proof* that penetration by the spermatozoon had not previously taken place. Indeed, all circumstances considered, there seems reason to believe that impregnation is effected very quickly after the encounter of the spermatozoon with the egg, as appeared to be shown in my former experiments with solutions of potass*. It is probable that it is *commenced*, as then suggested, *within a few seconds, or at most a very few minutes*, after such encounter; as in one instance of artificial impregnation in some observations made subsequently to those above detailed, I detected a spermatozoon in contact with the vitellary membrane, *within one minute* after the impregnating bodies had been supplied to an egg beneath the microscope. Certainly I believe that it is commenced, and probably completed, within *the first half-hour*; because, after that lapse of time, I have not yet been able to detect any change of place or position of the spermatozoa which have passed into the envelopes of the egg; whether they may have already arrived at, and become partially imbedded in the vitellary membrane, or whether they are still contained in the clear substance of the outer coverings, their force of penetration being exhausted before arrival at the membrane. After the lapse of half an hour, and frequently of a much shorter time, the caudal portion of the body of a spermatozoon which has penetrated into the envelopes becomes looped on itself, and this is a certain indication of the death of the object.

As no spermatozoa were seen, in these observations†, within the vitellary cavity at the early period above stated, it was not to be expected that they were likely to be observed at a later; although the eggs which had been the subject of these investigations, and which had been fecundated naturally, were continued to be watched until segmentation of the yelks commenced. This took place in the two eggs, in which alone, out of the fifteen first examined, spermatozoa were detected,—at the expiration of *five hours and twenty minutes*. But this was much earlier than in the great mass of eggs, of which these were a part; and was due to their having been exposed to a higher temperature, owing to heat radiated from my own body during their

* Philosophical Transactions, 1851.

† See the preceding note.

constant observation beneath the microscope,—rather than to that to which the undisturbed mass of eggs was exposed. The temperature of the atmosphere of the room at the time the eggs were deposited was 54° FAHR., but it gradually sunk to 51°·5 FAHR., and the water in which the eggs were contained to 50° FAHR. at the time when segmentation commenced, at the end of *six hours and thirty-five minutes.* In the most submerged it did not take place until *six hours and forty-five minutes* had elapsed.

In nearly the whole of the eggs of this mass which had been fecundated by the natural union of the sexes, I was struck with the fact that the quantity of spermatozoa which had penetrated into every part of their envelopes was very considerable. Very many of these had arrived at, and were sticking by their larger end into the vitellary membrane, from which they projected like spines from the head of a thistle; while there were many others which had not arrived at this part, their power of penetration being exhausted, and their progress inwards being arrested before they had passed more than half-way through the outer gelatinous coverings. Others, again, had penetrated to scarcely more than their own length into them, while a still greater number were simply in contact with, and adhering to the external surface.

When segmentation took place in these fully impregnated eggs, their yelks seemed to contract more powerfully, and the clear space, or respiratory chamber, formed in each became much larger than in others from the same mass in which but a few spermatozoa were detected; so that the inference deducible from the fact seemed to be, that a *plurality* of spermatozoa is necessary for the full impregnation of the egg, and the production of the robust and healthy embryo—although simple fecundation may result from the influence of only a very few spermatozoa, especially when such influence is aided by a considerable increase in the temperature of the surrounding medium.

Mode of Penetration by the Spermatozoon.—The preceding observations on naturally fecundated eggs were so decisive of the fact of penetration by the spermatozoa into at least the envelopes of the egg, and of the arrival at, and partial imbedment of these bodies in the vitellary membrane, that it seemed desirable to make further observations, by the artificial method, with a view to ascertain more directly the mode and circumstances of their entry; and these it was hoped might be learned through the facility with which the egg may be impregnated by direct application of spermatozoa to almost any part of its surface. Accordingly, on the following day, I placed an egg in a glass cell, beneath the microscope, and quickly afterwards applied to one side of it, by means of a pin-head, a quantity of spermatic fluid, obtained from the male only a few minutes before, and immediately filled the cell with water, and commenced the observation. The fluid was applied four times to the same part of the egg, the pin-head being loaded for each application, in order that a full sufficiency of spermatozoa might be furnished, before the water was added. The temperature of the room at the time of the experiment had been intentionally raised

to 65° FAHR., to afford a greater chance of success; as all my previous experiments had shown that more eggs became fecundated at a moderately elevated, than, under otherwise similar circumstances, at a low temperature. The instant the object-glass (the half-inch of Ross's microscope) was brought into focus, a vast quantity of spermatozoa were seen adhering to the surface of the egg at the part to which the pin's head had been applied. Many of them, attached laterally to the surface, had already ceased to move; others, only partially attached by their larger end, still vibrated the caudal or ciliated extremity rapidly, but did not appear to penetrate; while others, more centripetally attached by their body portion, vibrated the free extremity rapidly, and were seen in the act of gradually penetrating into the substance of the envelopes. I distinctly recognised one of these bodies which had just entered the envelope to a depth equal to about twice its own length, and when first seen had not reached so far as the middle or granulous layer of the envelope. Its motion was then slightly serpentine, and its course from without inwards was in a perfectly centripetal direction, with its thicker or body portion extended forwards, and in a line with the centre of the yelk, its progress inwards, as seen beneath the microscope, being as continued and as direct as that of an arrow. I kept this object in focus for several seconds, and watched it through the granulous layer, but ultimately lost it in the more dense and, as yet, unexpanded portions of the inner layers of the envelope, after it had been distinctly seen by a friend, who was with me at the time of the observation. A few seconds afterwards, as the envelopes became more expanded, very many of these spermatic bodies were seen to have already arrived at, and be in the act of passing through the inner or laminated portion of the envelopes,—the portion which, when the envelopes have acquired their full distension, by the imbibition of water, is seen to be that which immediately covers the vitellary membrane. A few seconds later a great abundance of them were seen in contact with the vitellary membrane itself; and some were even partially imbedded by their thicker extremity in its substance, and some of these showed an appearance as if they were actually penetrating through it. But in no one instance could I then satisfy myself that they did really pass through; since by alternately elevating and depressing the lens, and carefully noticing when the margin of the vitellary membrane was most distinctly defined, the appearances of perforation which some of them showed, seemed to be due to the spermatic bodies being imbedded in the membrane at some inclination to the plane of observation and to that of their direction being not quite centripetal. Yet there were other circumstances which seemed to show a likelihood that some spermatozoa do *actually pass through the membrane*. Thus, within the first few minutes after the impregnating fluid had been supplied to the egg under observation, and at the time when the spermatozoa, which had penetrated its envelopes, were first seen to have arrived at, and begun to enter the laminated portion, or *zona pellucida*, some of them were noticed to pass gradually onwards for a time, and then suddenly to disappear in an instant; as if, having passed into this tissue, they had also escaped

through the vitelline membrane which it covers. This mode of disappearance certainly is that by which the spermatozoa might be supposed to penetrate to the yelk, if in reality they do so*. Thus, presuming the body, or thicker portion of the spermatozoon, to perforate and pass through the vitelline membrane, the more slender or tail portion would follow quickly. But if this occurs with some, then it would be fair to expect that it would happen to the whole which arrive at and become partially imbedded in the membrane; especially to those which have sunk into it to a depth equal to one-half the length of the thicker or body portion; or, further, that the progress of others might be arrested before they had completely passed into the vitelline cavity; and, consequently, that some would occasionally be seen protruding into the interior. But I was not able, in the instance of the observations now detailed, nor in others afterwards made, to prove either of these suggested conditions.

In the observation now referred to, as well as in others since made, there were many spermatozoa which remained distinctly visible for several hours, in the same place, and in almost precisely the same position, sticking into the vitelline membrane, and retaining, at the end of a lengthened period, the same appearance as at first, excepting only that they seemed to have become smaller in diameter and to have their caudal portion more looped. In the present instance, at the high temperature of 65° FAHR. to 66° FAHR., not only were they distinctly seen at the commencement of segmentation of the yelk, which happened at the end of *three hours and twenty-two minutes*, but many of them were present until after the yelk had undergone several of its subsequent divisions. This was the case not only with those which had arrived at the vitelline membrane, but also with others which had never reached it, and had not penetrated further than to about the middle, or granulous portion of the envelopes; as happened with many spermatozoa, both in the eggs which were fecundated naturally, as well as in those which were the subjects of experiment, and were artificially affected. It was those which remained in the substance of the envelopes which usually disappeared earliest, becoming at first gradually fainter, and then more undefined in outline. This change was supposed to be due to a gradual diffluence of the substance of the spermatozoon, through the influence of the water imbibed by the envelopes; but whether this happened as part of the fecundatory process, or whether it was simply the natural process of decay, as other circumstances to be mentioned seemed to intimate, there was no distinct proof. It was remarkable, however, with reference to the *act* of fecundation, that in almost every instance, even of those spermatozoa which never arrived at the vitelline membrane, *the body portion was always directed towards the yelk*, usually peripherally, but sometimes inclined at slight angles to one side or the other; thus showing that it is invariably the body portion which penetrates.

* That this is really the fact, and that the actual penetration by the spermatozoon into the yelk chamber *was observed on this occasion*, is now rendered almost certain by my recent observations stated in the preceding note, p. 271.—G. N., April 18, 1853.

It may be matter of surprise, that, considering the immense quantity of spermatozoa which exist even in a microscopic drop of fluid, and considering also the abundance which come in contact with the surface of the egg, even when but a small quantity of fluid is employed, as in artificial impregnation, a much greater number do not penetrate than are usually observed to do so. There are conditions and circumstances which affect this result. Thus I have found, in repeated observations, that only those spermatozoa which, at the moment of first contact with the egg-envelopes, are in rapid action, and have their body portion directed, either perfectly centripetally towards the yelk, or at angles but slightly inclined to it, do by any possibility enter; while those which happen to be directed horizontally to the surface of the egg at the instant of contact, always adhere to it laterally, and lose their power of motion, but do not penetrate; and the like also is the case with those which become attached by their caudal end, and even with many which adhere by their thicker end, when they come into contact with the egg at very acute angles. Further, I have noticed, that a relatively much greater number of spermatozoa penetrate the envelopes when supplied to the egg immediately after this has been removed from the female into water;—especially when the spermatic fluid also has been recently passed from the male;—and more decidedly so when passed from a male in full season, at which time the movements of the spermatozoa are most energetic. Thus the chances of penetration through the envelopes, and consequently of fecundation of the egg by the spermatozoon, are in direct relation to these circumstances; and inversely to those of an opposite character, being less in proportion to the length of time the egg has been removed from the female, or the fluid from the male; the healthfulness of the parent, and consequent power of motion in the spermatozoon;—the temperature of the season, and the quantity supplied to the egg.

These were the conclusions deduced from the previous observations, and they have been fully borne out by subsequent experiments, some of which have been made in the presence of my friends Professors BELL, BOWMAN, CARPENTER, and Mr. BUSK, Fellows of the Royal Society, and Professor ELLIS, who permit me to mention the circumstance.

Narcotization of the Spermatozoon.—I may now mention some experiments which were made with the view to test the fecundatory influence of recently obtained spermatozoa when narcotized by chloroform. These experiments were suggested by a communication made to me by Mr. BUSK, F.R.S., who, after witnessing my mode of procuring the eggs of the Frog, conceived that a similar result might be attained by narcotizing the gravid animal without killing it, as is necessarily done in my experiment; and on putting this opinion to the test he found that it may be accomplished with ease and success. It then occurred to him to try the effect of exposing the spermatozoa to the vapour of chloroform, by simply covering the spermatic fluid contained in a watch-glass covered with blotting-paper wetted with the liquid,

and afterwards to apply the narcotized bodies to some eggs in the way done by myself.

The facility with which the spermatozoa can be narcotized by the mode now mentioned has enabled me to test the relation of their power of motion to that of their fecundatory property, and although my results differ somewhat from those obtained by Mr. Busk, it may be well, perhaps, to relate them.

MM. Prevost and Dumas found, in addition to their many other excellent results, that spermatozoa are rendered motionless by an electric shock, and that then they do not impregnate the egg. They also found that opium and strychnine have a similar paralysing effect on these bodies. But it has since been suggested that the latter agents act on these bodies only in so far as they affect the chemical composition of their substance, and that the operation of electricity on them also is similar. But it yet remains to be shown whether any chemical change is produced in the substance of the spermatozoon, when simply narcotized by the vapour of chloroform, and not mixed with it in the fluid state; and when, although the power of motion is arrested, the vitality of the body is not destroyed.

Three eggs were placed in separate cells, and the spermatic fluid, immediately it had been obtained, was applied, *once* only to each, by means of the pin's head. These trials with the fluid in its natural state, mixed only with a small quantity of water, were made for the purpose of comparing their results, with those of others to be made with portions of the same fluid after it had been narcotized, and applied to eggs in this state at different periods. *One minute* after the application of the fluid I found an abundance of spermatozoa on the surface of each egg at the part touched, and some spermatozoa had not only already penetrated into the envelopes, but had arrived at, and were in contact with the vitelline membrane, or rather the *zona pellucida*. The respiratory chamber was afterwards formed above the yelk in each of these eggs, and two of these subsequently formed embryos; the third was only partially fecundated.

The spermatic fluid was then exposed to the influence of chloroform, in the way mentioned, about fifteen minutes after it had been obtained; and after seven minutes' exposure to it, and when the majority of the spermatozoa it contained had become narcotized, was employed in experiments. The signs of full narcotization are the entire cessation of all motion in the spermatozoon, which lies with its body extended at length, and not looped on itself. In the latter condition it is usually dead.

Three eggs placed in separate cells were then supplied with the narcotized spermatozoa applied to each egg three times by means of the pin's head. On examining the eggs six minutes afterwards, I was unable to detect even a single spermatozoon within the envelopes of either of them, either in contact with the vitelline membrane or in any part of the substance of their envelopes; although there was a great abundance of perfectly motionless spermatozoa on the surface. No chamber was formed above the yelk in either of them, nor did either of them produce an embryo.

Twenty minutes after narcotization, and during which time not a single spermatozoon had been observed in motion, a portion of them were applied in the same way as above to *three* other *eggs*, but not one of these became fecundated, no chamber being formed within them, and no embryo was produced. Many of the spermatozoa at this time were dead, as was shown by the looping of their bodies, but there was still a quantity which had been simply narcotized and lay extended at length.

One hour after narcotization another portion was applied to *two eggs*, one of which became partially fecundated. The chamber appeared in it above the yelk, the yelk underwent segmentation, and afterwards an embryo was begun to be formed, but its development was not completed, and the egg then perished. The second was not fecundated. The circumstance of this egg having been fertilized at so long a period after the fluid had been narcotized, leads to the conclusion that impregnation had been effected by *revived spermatozoa.*

On the following day I repeated these experiments with very mature fluid immediately after it was obtained, and in which the spermatozoa were exceedingly vigorous. *One minute and a half* after it had been exposed to chloroform, I found that the same effect had been produced on the spermatozoa as that which is produced in them by their admixture with very weak solution of potass; their power of motion was greatly increased, and the whole were in a state of intense action. After a few minutes' longer exposure their motion was perceptibly diminished, and at the end of about ten minutes it had almost entirely ceased, as only a few of them were then observed to move.

Three eggs were supplied, by means of the pin's head, with these narcotized spermatozoa. The respiratory chamber was formed in two of these eggs, and one of them underwent segmentation, and at the end of the fifth day an embryo had begun to be produced, but its development did not proceed, and this egg like the other two perished.

Six eggs were then supplied copiously by means of the loaded pin's head, three times applied to each, nearly the whole of the spermatozoa employed being then motionless, and having already remained so for about *ten minutes*, only an occasional one being observed in feeble action. Two of these eggs became partially impregnated, the usual chamber being formed in them, but neither of them underwent segmentation, nor was any embryo formed either in these or the others.

Three eggs were then supplied as above after the spermatozoa had been motionless during *twenty minutes*, not one being observed with the slightest action at the time of the experiment. No chamber was formed in either of these, but the whole remained unimpregnated.

Three eggs were the subject of further trial, when the spermatozoa employed had been motionless during *half an hour*. At this time one or two spermatozoa were again observed to be in feeble action, but no impregnation was effected.

Thirteen eggs were employed when the spermatozoa had remained motionless

during *one hour*, and when there was reason to believe that the majority of them had perished, but when there were still a very few among them in motion, having either revived, or but recently escaped from spermatozoal cells contained in the fluid. No impregnation took place in either of these instances.

Thus the results of these experiments appear to show that the spermatozoon does not impregnate *when entirely deprived of its power of motion by narcotization*, and disenabled to penetrate into the envelopes of the egg; and, consequently, that its fecundatory power has a close relation with its motion, or force of vitality. In this, then, they coincide with those of the previously detailed experiments, and go with them to show that the *act* of fecundation of the egg of the Frog, and probably also of all the Vertebrata, is the result of a power in the spermatozoon, which, in its operative condition, is characterized *by motion*; and that this power is totally independent of everything approaching to volitional influence, in the impregnating body, but seems to be in direct relation to physical causes.

MM. Prevost and Dumas appear to have thought that the spermatozoa which they observed within the egg-envelopes had entered through means of the infiltration, or imbibition of water by the envelopes; and the gist of their experiments was to show that particles of solid matter *do enter* during such infiltration, or, as it has since been designated, *endosmosis*. But some experiments, elsewhere detailed*, have led me to believe, that only such solid particles as are very much smaller in diameter than the spermatozoon can so enter with the water by endosmosis; while the circumstances now detailed fully prove that the entrance of the spermatozoon is not the result of simple infiltration, but is that of the operation of direct mechanical or physical power in that body. Thus, if it were mainly dependent on endosmosis, the perfectly motionless spermatozoon would enter the tissues and fecundate the egg equally well with the active, but this, as we have seen, is not the case, and this was shown by the physiologists now referred to. On the other hand, the circumstance that the spermatozoon invariably enters the tissues with its thicker or body portion directed forwards, and sometimes even at an angle slightly inclined to the centre of the yelk, and always impelled by an oscillatory or vibratile motion of its caudal portion, seems to show that its power of penetration is not only a necessary condition of its function, but is inherent as such in its organic composition. It may yet be true, nevertheless, that the passing of the spermatozoon through the substance of the envelopes to the vitellary membrane may be indirectly *aided* by the endosmic action of the envelopes. But this aid does not consist of any imbibing property in the tissues. It appears to be simply of a negative character, and to be the result of a decrease of density in the tissues, which, through their imbibition of water, and consequent expansion, are caused to offer less and less resistance to the propulsive force of the spermatozoon. This I believe is the proper explanation of the fact of penetration by this body.

But although penetration be not caused by the endosmic action of the envelopes,

* Philosophical Transactions, 1851, p. 224.

it may yet be thought to be induced by some attractive power in the substance of the yelk itself; and that, therefore, the entrance of the spermatozoon may be as much due to the egg as to any power of motion in the penetrating body. An accident has enabled me to test the validity of this surmise.

I had promised to show the fact of penetration to a friend, but circumstances prevented me from doing so until the season had nearly passed, and the whole of my frogs had spawned. I determined therefore, as a last resource, to endeavour to obtain some fecundatory fluid from a male which had already paired two or three days previously, and to employ it with the only eggs I had then left, which remained in the body of a frog that had been killed *twenty-six hours* before, and which, as former experiments had shown, it was probable had lost their vitality. The fluid required was obtained with ease, but mixed with a large quantity of spermatozoal cells. This was supplied to some eggs from the dead frog, since, although I did not expect that the eggs would be fecundated, I hoped for an opportunity of again witnessing the penetration by the spermatozoon. The spermatozoa in the fluid obtained were very active, and fully efficient, and were supplied in abundance to several eggs in separate cells under the microscope. The envelopes of the eggs expanded as usual, and endosmosis went on in a perfectly natural way, and an abundance of spermatozoa adhered to their surface. At the expiration of from fourteen to twenty minutes I found that several spermatozoa had penetrated the envelopes and were adhering in the usual way to the vitelline membrane. In one egg there were six, in another five, and in a third four, distinctly visible in the plane of observation that could be brought at once within view with the microscope, besides others recognisable on changing the focus. It was thus evident that spermatozoa, even of the previously paired Frog, still retained their *power of penetrating into dead eggs*, as these ultimately proved to be, after careful preservation to the sixth day in a favourable temperature. The spermatozoa were distinctly visible within the envelopes, without change of position, for several hours, but no fecundation was effected by them; no chamber was formed in either of the eggs, no segmentation took place, nor was any embryo produced. These circumstances seem to show that the eggs were already dead, as was supposed, before contact of the spermatozoon; consequently that the entrance of the spermatozoon into the envelope is due to a power inherent in the penetrating body, and not simply to an attraction on the part of the yelk; although from the fact that the spermatozoa usually enter in a centripetal direction, it is probable that some influence may be exerted by the yelk or its vesicle, although penetration is mainly the result of force in the spermatozoon.

The facts now stated of penetration by the spermatozoon seem to lead us better to understand the nature of some experiments with solutions of caustic potass, which are detailed in my former paper. I have repeated these experiments, during the past season, in the presence of several friends, Professors SHARPEY, ELLIS, and BELL, and Messrs. BUSK, TOMES and WATERHOUSE, with results precisely similar to those which

are detailed in that paper; viz. that when diluted spermatic fluid, recently obtained, is applied to a set of eggs, and—as soon afterwards as the experiment can be made, a solution of potass,—of such a strength as is known by previous microscopic observation to have the property of instantly decomposing the spermatic body,—the solution being washed away quickly after its application, by repeated quantities of water,—to prevent its affecting the egg itself—that then—in some instances—even when the interval of time between the application of the spermatic fluid and the subsequent application of the potass does not exceed a few seconds,—impregnation of the egg is effected; as is proved by the formation of the chamber, the segmentation of the yelk, and perhaps the formation of an embryo. Further, that, all circumstances being similar, excepting only that the interval of time between the application of the spermatic fluid, and, subsequently, that of the solution of potass be prolonged,—the production of an embryo is not only more certain to take place, but the number of embryos produced is increased. The now ascertained fact of *almost instantaneous penetration* by the spermatozoon, which, as before shown, sometimes arrives at the vitelline membrane in less than *one minute* after its application to the egg, confirms the principal conclusion deduced from the potass experiments at the time they were made, viz. that impregnation is *commenced at the instant the spermatozoon is in contact with the egg*; while it also seems to afford the true explanation of the nature of those experiments, in which it may now be presumed that some spermatozoa had actually penetrated into the substance of the envelopes before the application of the solution of potass, and thus had already passed out of the reach of its destructive influence, the effect of which on the egg itself was obviated by speedy dilution and ablution with water.

The potass experiments may thus be regarded as confirming by anticipation the results now obtained by direct observation with the microscope, with respect to the rapidity of operation by the spermatozoon; and they seem also to support the view of the essentiality of the motor power of this body to its functional action. A similar view may be taken, as indeed was held at the time, of the nature of the Carmine, the Gum, and the Starch experiments, that the operation of these substances in preventing the fecundation of the egg is *entirely mechanical*, and that they do so simply by offering a mechanical impediment to the spermatozoon, a conclusion which seems to be fully supported in the present inquiry.

14. NATURE OF THE INFLUENCE OF THE SPERMATOZOON.

Having seen in the preceding experiments and observations that fecundation of the egg is effected by the spermatozoon only while this body retains and continues to give evidence of its vitality in its power of motion, and that its vitality either may be destroyed, or its operation be for a time entirely arrested by electricity and by chloroform,—the question naturally arises—in what way, then, is its fecundatory influence to be explained? Is it *simply* by diffluence of the substance of the spermatozoon, and the *chemical fusion* or combination of this with the contents of the egg, after the sper-

matozoon has penetrated through the envelopes and arrived at the vitelline membrane, or the yelk? Or is it by its expenditure on the yelk, of a *force* or power of vitality, which is inherent in the body of the spermatozoon? Or is it by the joint cooperation of both these conditions—the expenditure of a *force* with diffluence of material substance? I have endeavoured to put the first of these questions to the test of experiment;—although, it must be remarked, that the experiments made seem to be open to some objections, which at present cannot be fully answered. Yet knowing what we do of the disposition and great readiness of all matter to enter into new combinations, and affect, or entirely change the condition of the whole body operated upon,—as,—not to mention the effect of the poison of serpents, or the introduction of dead matter into the living, in dissection,—can be shown in the effect, on the yelk of the Frog's egg, of extremely minute quantities of chemical compounds diffused in water,—there has seemed fair reason to think that the question thus started may be examined by experiment. The rapid endosmic action of the envelopes of the egg at the instant of their contact with any aqueous fluid, and the rapidity with which the yelk itself then becomes affected, seemed to favour the idea that if the bodies of the newly obtained spermatozoa could be quickly reduced to a state of diffluence simply by mechanical means, without the addition of any menstruum, saving only a very small quantity of water, and be applied in this state of diffluence to the egg at the instant after it has left the body of the female,—that then an experiment thus made would be a fair test of the question. Some imbibition of the substance of the spermatozoon with the water might thus be expected to take place, and some changes in the egg to follow, if impregnation be the result of simple chemical combination of the substance of the male with that of the female. The presence in the experiment of a portion of water holding the diffluent spermatic substance in suspension, appeared at first sight to be an objection, but this seemed to be met by the fact that water *must* always be present to ensure the natural fecundation of the Frog's egg; and that it not only permeates the envelopes, and is the means of facilitating the entrance of the spermatozoon, but that it passes even to the substance of the yelk itself, as shown in the experiments with large quantities of potass before alluded to, and also in others made by immersion of eggs in water with given proportions of potass in solution. As the details of these latter experiments are contained in a paper which is now in the Archives of the Royal Society*, I need give but little more than the results of these experiments, in the present communication, before proceeding to relate those now referred to with the diffluent spermatozoa.

Immersion of Ova in Solutions of Potass and Soda.—These experiments were commenced in April 1850, before the publication of some which were made with solutions of caustic potass, by M. QUATREFAGES, on the spermatozoa of one of the marine worms, *Hermella*, and read to the Institute on the 24th of June 1850†.

* *MS.*, No. 762, p. 13 to 26; also Proceedings, vol. vi. p. 83.

† Comptes Rendus, June 24, 1850, and Annales des Sciences Naturelles, 3me série, tom. xiii. in Nos. *marked* "February" and "March 1850."

The object which I had in view was to endeavour to learn what proportion of the *carbonates* of *potass* and *soda* may be present in a given quantity of water without destroying the fertility of the egg. The first trials with potass were made with eggs which had already been fecundated, and were undergoing segmentation. It was then found that when fecundated eggs, arrived at the second stage of segmentation of the yelk, were immersed in a solution, composed of *twenty grains* of the *fused carbonate of potass* to one ounce of water, the yelks became shrivelled, and decomposition was commenced within *three minutes* after immersion; thus proving that even when the envelopes of the egg are almost fully expanded, and, consequently, when their endosmic action may be expected to have become greatly diminished, that endosmosis may still be going on with much energy. It is right to mention, however, that in this and the following experiments, the endosmic action may have been, and probably was, increased by the removal of the eggs—which already retained water in their tissues—to the more dense fluid solution; but as the experiments are comparative, the circumstance is not of much importance.

On the contrary, when some eggs were immersed in a solution of *one-fourth of a grain* of the salt to one ounce of water, and the potass in admixture with the water was thus reduced to $\frac{1}{1920}$th part of the whole, development went on more quickly than in other eggs of the same brood, impregnated at the same time, but which were placed in pure water apparently through the potass occasioning a slight increase of temperature.

These results appear to be explicable only on the assumption that the weak potass solution acts as a chemical stimulus,—perhaps both to the egg and to the spermatozoon,—at the time of fecundation, a view which derives some support, in so far as refers to the spermatozoon, from direct observation with the microscope. Thus, when a drop of the *two-grain* solution of potass is applied to spermatozoa on a plate of glass, covered with talc, beneath the microscope, the movements of these bodies are not instantly arrested, as by the stronger solutions, but gradually become slower and slower, until they entirely cease, apparently, in proportion as their substance becomes affected by the potass. The *one-grain* solution very much accelerates the movements of these bodies during the first few seconds; but in a few seconds longer these also become diminished like the preceding. But the effect of the *half-grain* solution, although exciting the spermatic bodies to activity as soon as it comes into contact with them, continues its effect for a much longer time without acting injuriously upon their substance; and it is not until after the lapse of many minutes that the intensity of the motion excited by it is observed to be diminished, and the movement to slacken and afterwards gradually cease.

With regard to the *carbonates of soda*, I may state that the general effect of these on the impregnated and on the unimpregnated egg are very similar to those of potass, but are far less prejudicial, or marked, so that it is unnecessary to detail the experiments.

The general conclusions deducible from these observations, with reference to the nature of the influence of the spermatozoon in impregnation, seemed to be, that as water which holds in suspension or solution any chemical substance, conveys some portion of that substance at all times, with great rapidity, to the immediate vicinity of the yelk itself, which becomes affected by it, and especially so at the time of its first coming into contact with the envelopes of the newly deposited egg,—the substance of the spermatozoon also, if diffused in water, might be so conveyed, in the experiments proposed. In addition to this conclusion, there are others, which, although less directly connected with the object in view, are not less important. Thus it appears that an alkaline fluid, in large quantity, is as injurious to the vitality and fecundatory influence of the spermatozoon as to the fertility of the egg.

Trituration Experiments.—The endeavour in these experiments was to put to the test the question proposed in the preceding observations,—whether, if the spermatozoon be reduced to a state of diffluence in water quickly after it has left the body of the Frog, and before it can reasonably be supposed that any change in the chemical constituents of its body has taken place, the egg can be fecundated through simple imbibition of the substance of the spermatozoon, conveyed to the vicinity of the yelk with the water during endosmosis, at the time when endosmosis of the envelopes of the egg is most energetic?

Although it had been constantly found, both by the authorities before cited, and since by myself, that when the spermatozoon has ceased to move, and is believed to be dead, no impregnation results from the contact of its motionless body with the egg; yet there still appeared to remain some doubt as to what this want of operation is then due; whether it is to be attributed to the organic death and incipient decomposition of the body, or whether to the loss or suspension of some power which is characterized by *motion*, and through which its function is exercised?

The following experiments, with reference to this question, were commenced before I was aware of the fact that the spermatozoon penetrates into the envelopes of the egg; a fact which, when known, seemed further to necessitate the inquiry, as probably tending to show whether impregnation is merely the result of a union of the substance of the spermatozoon with that of the yelk *or its vesicle*; or whether it be not primarily due to the transmission of some dynamic influence, force, or peculiar vitalizing power from the spermatozoon to the egg at the time of, or previous to any fusion with it of its material substance.

The first experiments were made by breaking down the bodies of the spermatozoa mixed with water, and, after filtration, applying the filtered liquid directly to the egg; the following being the method pursued:—

The bottom and sides of a very small glass mortar, and the glass pestle employed with it were ground together with fine sand and water, for the purpose of slightly roughening their surfaces, and of ensuring their contact at every part, and thus to render the crushing of the spermatozoa more certain.

The spermatic fluid first employed was procured from a Frog early in the season, and consequently contained a large quantity of spermatozoal cells from which the spermatic bodies had not been liberated. A difficulty immediately occurred which had not been anticipated. The fluid was divided into two parts, one of which was triturated gently with the pestle and mortar for two minutes, and was then examined with the microscope. It still contained an abundance of active spermatozoa, and many cells. The trituration was continued for three or four minutes longer, without any marked result, as active spermatozoa were still abundant in the specimens of fluid examined. It was then repeated for several minutes with increased rapidity and force, when the fluid suddenly became partially coagulated, and separated into opake white flocculi, composed almost entirely of cells and granules, and a transparent fluid in which there were scarcely any granules. It was then found that nearly the whole of the spermatozoa had been destroyed, as I was not able to detect even one in motion. The thinner portion only of this fluid, when placed on the filter, passed through, and it did so with great slowness and difficulty.

These circumstances are stated to show the mode of proceeding, and the accidents to be guarded against. The conclusions deduced from these trials were, that the spermatic fluid was not fully matured, and probably contained albumen, which, it is stated*, does not exist in mature spermatozoa; also that the substance of the spermatozoa had undergone some chemical decomposition, perhaps from excess of heat evolved in the act of trituration, through too much force being employed; so that the results of these experiments could not be depended on. A further trial was therefore made a few days afterwards. The fluid obtained from a mature Frog was divided into two parts, one of which was reserved for simple artificial impregnation; while the other was triturated slowly and carefully in the mortar with but little more exertion than was necessary to keep the pestle in constant motion for about fifteen minutes; no water being added to the fluid. When placed on the filter it became necessary to add to it about an equal quantity of water to facilitate its filtration. Portions of the fluid which then passed through, holding the substance of the broken-down spermatozoa in solution, were caught in separate glass cells.

Ten eggs were passed into a cell about half filled with the filtered fluid, and the cell was afterwards filled up with water. No other change than a slight enlargement, after the lapse of some hours, occurred in these eggs; no chamber was formed, nor did any segmentation take place in either of them, consequently no embryos were produced.

Fifteen eggs were placed in a cell which contained about half the quantity of filtered fluid employed in the preceding; but no fecundation took place in either of these eggs.

Eleven eggs in a third cell with filtered fluid were equally unfruitful.

Ten eggs in a fourth cell gave similar results.

* Article "Semen," Cyclop. Anatomy and Physiology, vol. iv. p. 506.

One hundred and forty-seven eggs were then placed on the topmost of the filter papers, which had been employed in separating the fluid used in the preceding trials, and on which were the remains of the broken-down spermatozoa, with, probably, some which had not been injured. This was about four hours and a half after the spermatic fluid had been obtained, so that on this ground but little success could be expected. Not a single embryo was produced.

The portion of reserved fluid which had not been triturated was then applied to *four eggs* in separate cells, by means of the pin's head, when each of these eggs became fecundated, and not only underwent segmentation, but each afterwards produced an embryo. As this experiment was made for the purpose of comparison with the preceding, it is clear that the failure in the case of the mass of eggs on the filter paper was not due to the length of time the fluid had been obtained, but to the effect of trituration; and it seems equally clear that the substance of the broken-down spermatozoa dissolved in water and passed through the filter is as inefficient as the *liquor seminis* has heretofore been proved to be*.

These experiments were afterwards repeated, in the presence of Professors SHARPEY and ELLIS, with similar results. Usually there are still some spermatozoa which escape being crushed during trituration, and, being uninjured, remain on the filter paper capable of effecting impregnation when eggs are passed upon it in water, as was the case on the following occasion.

Seventy eggs were first added to some filtered fluid, which resulted from the trituration of spermatic fluid, and had passed through four filter papers, but not one egg became affected, not one embryo was afterwards produced from them; while of *sixty-two eggs* placed on the topmost filter paper in water, amidst the remains of broken-down spermatozoa, with many which were still active and uninjured, more than one-third of the eggs became fertilized, and *twenty-three* afterwards produced *embryos*.

It was thus evident that no impregnation results from an immersion of the egg in fluid which is derived from the broken-down body of the spermatozoon, mixed with water, and separated from the more solid parts by filtration. But as some objections may fairly be offered as to the validity of this experiment, it seemed desirable that trial should be made without having recourse to filtration, and without removing any portion of the destroyed spermatozoon; but, on the contrary, by applying the whole of the triturated fluid immediately to the egg before it is immersed in water; and at the same time to compare the result obtained with that of an experiment by simple artificial impregnation, with a sample of the same spermatic fluid which had not been triturated; the experiments in both cases being made at the same time, and with eggs from the same female, and placed under precisely similar conditions in regard to light, heat, &c. A comparison of the relative number of embryos which might result from these experiments, it was thought, would show, not merely whether

* Philosophical Transactions, 1851.

impregnation can or cannot be effected by the direct application of the diffluent substance of the spermatozoon to the egg, but also whether the influence of the spermatic body is essentially dynamic in its character.

The quantity of spermatic fluid obtained for these experiments, mixed with a small proportion of water, measured about fifteen minims; and to this a further quantity of water was added, making the whole thirty minims. This was divided into three equal portions. The first portion (*a*) was triturated slowly and continuously for fifteen minutes, after which it was slightly turbid, and on examination with the microscope was found to contain a large quantity of granules, each of which did not exceed in size one half of the diameter of a spermatozoon. It also still contained a good abundance of active spermatozoa, although the quantity of these was greatly reduced, and the movements of those which remained were impeded by the quantity of granules. The second portion of fluid (*b*) was triturated with the addition to it of about one grain weight of well-washed sand, and after three or four minutes presented a turbid appearance. At the end of seven minutes, when examined by the microscope, it was found to contain a great abundance of organic granules, intermixed with the sand, and the number of spermatozoa had been greatly reduced; and three or four minutes later nearly the whole of the spermatozoa were destroyed. The following experiments were then made with eggs obtained from the female with which the male that supplied the fluid had been paired. The temperature of the atmosphere at the commencement of the experiments was 57° Fahr., and that of the water employed 55° Fahr. At the end of the experiment, the commencement of segmentation of the yelks, the atmosphere was 61° Fahr., and the water 59° Fahr.

One hundred and seventy-nine eggs were passed into a glass dish, and the triturated fluid (*a*) was immediately poured over them, and the dish quickly filled with water. The yelks of many of the eggs soon became irregular and contracted, and at the end of *four hours and thirty-one minutes* segmentation had commenced in some of them, and ultimately, *sixty-seven embryos* were produced; so that one hundred and twelve eggs were not fecundated.

One hundred and sixty-seven eggs were passed into a second dish, and the fluid (*b*) which had been triturated with sand was immediately added to them, and afterwards the dish filled with water as in the preceding. But *not a single egg* became fecundated, nor was *a single embryo* produced. It must be stated, however, that much of this result was afterwards found to have been due to the admixture of sand with the fluid acting mechanically, as I shall presently show.

Two hundred and thirty-nine eggs were also passed into a dish of the same dimensions as the preceding, and the third portion of fluid (*c*) reserved for simple artificial impregnation, and which had not been triturated, was supplied to them at, as nearly as possible, the same time as the triturated fluid to the preceding. This was at thirty-five minutes after it was obtained. Segmentation commenced in nearly the whole of these eggs in *four hours and fourteen minutes*, and at the end of the fifth day *two*

hundred and six embryos were produced, so that only *thirty-three* eggs failed; thus leading to the inference that the failure in the experiments (*a*) and (*b*) was owing to the spermatozoa being destroyed; and, consequently, that the application of the substance of the body of the spermatozoon to the egg is not alone sufficient to effect fecundation; so that fecundation cannot be regarded as the result of simple chemical combination of the substance of the spermatozoon with that of the egg, but, essentially, may be due to some dynamical influence in that body.

At the time of making these experiments I made also two others with a view to this hypothesis; and for the sake of correct comparison employed eggs from the same female, and placed them in a precisely similar condition with regard to light, heat, and the quantity of water employed. The fecundatory fluid, however, was not from a male in full season, as above, but was what may properly be regarded as *senile*, it being purposely obtained from some frogs which had been kept separate from the females, having already paired and spawned from five to ten days previous. It was slightly translucent, and contained, relatively, but few living spermatozoa, the majority of which were languid in their movements; but there were many which appeared perfectly dead and motionless, and there was a good proportion of spermatozoal cells. In the first experiment *one hundred and sixty-seven eggs* were supplied with a portion of this fluid, and segmentation commenced in some of them in *four hours and forty-two minutes*, or not so soon as in the experiment (*c*) by *twenty-eight minutes*, and afterwards only *forty-three* embryos were produced. In the second trial, made at the same time with *one hundred and seventy-three eggs*, segmentation took place in *four hours and forty-one minutes*, and *one hundred and twenty-six embryos* were produced, forty-seven eggs being unimpregnated. If the difference in the length of time which elapsed between the encounter of the spermatozoon with the egg and the commencement of segmentation in these two experiments, as compared with the preceding one, be not fairly referable to a less degree of vitalizing power in the spermatozoon in the latter, it seems difficult to understand to what other cause it can be assigned; seeing that the eggs employed were from the same female in each experiment, and that all the conditions were similar. It seems, as it were, *inversely*, to show, that the most important condition of the impregnating agent is its possession of some dynamical quality, the degree or intensity of which is expressed in that of its power of motion, and which, possibly, it may transmit from itself to the egg, during the act of fecundation.

These results sufficiently mark the injurious effect produced on the fecundatory fluid by trituration, whether with or without admixture of foreign substances; and while these seem to prove that attrition of the spermatic bodies with particles of solid matter, by lacerating and mechanically destroying them, is fatal to their function, other experiments showed that even their simple admixture with any finely comminuted solid materials greatly interferes with their operation, by the mechanical impediment which such materials oppose to their free motion, and to their penetra-

tion into the envelopes of the eggs, whether such admixture be made with the bodies themselves, or with the water in which the eggs are immersed before they are supplied to it.

The question proposed in these experiments by trituration we may now look upon as to some extent, if not entirely, answered; as it seems that although the spermatozoon be broken down mechanically before any chemical change can be supposed to have taken place in it,—indeed, at the very time it is giving evidence of its vitality,—and its organic substance so broken down be quickly afterwards brought into contact with the egg in water, at the moment when the egg is most susceptible of its influence, yet that no fecundation is then effected by it. It may be urged, it is true, that there was no direct *proof* that the spermatic substance was actually conveyed to the yelk by, or with, the water in these trials; but there was one circumstance which gave fair reason to believe that it really was so conveyed. The yelks of some of the eggs became much contracted, and, as it were, shrivelled, as when affected by potass solution; of the penetration of which to the yelk there is absolute proof in the decomposition of the egg which quickly succeeds to its introduction. It has also been shown in a preceding experiment (p. 241) that the yelk becomes similarly affected when decomposing spermatic fluid is applied to it.

The conclusion then which seems to me to be deducible from these investigations is, that fecundation is not simply the result of a mere fusion or chemical admixture of the substance of the spermatozoon with that of the egg; although such fusion, probably, is necessary to the production of the organized body of the embryo, in determining its structural and psychical peculiarities, and its definite species; and more or less of which may, possibly, help to determine the sex, and the extent to which the structural and psychical peculiarities of the male parent are transmitted to the offspring. That this fusion of some portion of the spermatic substance with the egg does actually take place, either when the spermatozoon has arrived at, and become imbedded in the vitelline membrane, as in the Frog; or, as stated by a recent observer*, to occur in the *Ascaris Mystax*, when it is in immediate contact with the yelk itself, is probable, from the considerations now adduced. These views are countenanced by the now established fact that the spermatozoon of the Frog, and probably also of other Vertebrata, does not fertilize the egg either when it is perfectly motionless,—whether from actual death, or from suspension of vitality by narcotization; nor even, as we now find, though living, while simply in contact with the surface; nor until after it has actually passed into the envelopes, and arrived at the immediate vicinity of the yelk,—facts which seem, I think, inferentially, to show a probability that, *whatever conjugation of materials may be effected, some vitalizing dynamic influence is also expended by the spermatozoon on the contents of the yelk* in the production of its changes,—phenomena which have not been found to take place

* Dr. Nelson, "On the Reproduction of the *Ascaris Mystax*," Proceedings of the Royal Society, vol. vi. p. 86. Philosophical Transactions, 1852.

on the simple application to the egg of the diffluent spermatic substance. In the absence, then, of all proof that simple fusion only of the substance of the spermatozoon with the contents of the egg is the chief condition of fecundation, we have, in the facts now referred to, much reason to regard fecundation as primarily dependent on the existence of a force or power in the spermatozoon, by which this body is enabled to arrive at the object to be fertilized, and which, visibly expressed in that of its power of motion, is lost quickly after it has penetrated into the yelk-membrane or yelk:—this, probably, is the instant of fecundation. May not the spermatozoon, then, be viewed as *the organ of a special form of force in the male body* for the production of these results, in the same way as the nervous structure is regarded as that of the nervous, and the muscular as that of muscular power? We have already seen that its function is exercised more or less readily and perfectly in relation to two conditions: first, that of its full maturity, in which its power of motion is most intense; and next, in relation to the influence of external physical agencies, which cause this power to be evolved through its organic composition, in a greater or less degree, whether the external influence be that of heat, or the operation of a chemical power, as in the instance of potass in the preceding experiments.