XIV. *Observations on the Ovum of Osseous Fishes.*

By W. H. Ransom, *M.D.* *Communicated by Dr.* Sharpey, *Sec. R.S.*

Received June 21,—Read June 21, 1866.

In November 1854 I had the honour of presenting to the Royal Society a short paper, containing the principal results of observations made on the ova of two species of *Gasterosteus*, and I then expressed an intention of furnishing a more detailed record of them at a future time. Since that date my experiments have been continued and extended, and I now purpose so to recount them, that physiologists interested in this department may be able to judge of the value of the results, and to repeat the observations.

It is intended in the following pages to consider, first, the unimpregnated ovum, with reference to its structure, its physical and chemical properties in the mature state, and during its development; and afterwards the impregnated egg, in reference to the mode in which fecundation is effected, the phenomena which follow it, and their modifying and essential conditions.

The Unimpregnated Ovum.

The Stickleback (Gasterosteus leiurus and G. pungitius).

1. *Ripe deposited ova.*—The 3-spined and 10-spined sticklebacks may be taken together, as there are no important differences in the structure of their eggs. These observations, however, were for the most part made on the former, which are more easily obtained in numbers, and where the latter were employed it will be so stated.

The freshly deposited ova are held together by a colourless, transparent, viscid, mucoid matrix; they are few in number compared with those of many other fishes, and of large size, measuring about $\frac{1}{17}''$ on the average. To the naked eye they have a pale amber tint, and are semitransparent, spherical in form, but irregular, from mutual pressure (Plate XV. fig. 1). Each consists of an outer covering, the well-known dotted membrane or chorion, which I shall speak of as yelk-sac, understanding that term to mean that covering of the yelk which, being formed in the ovary, is placed next in contact with the yelk, but takes no part in cleavage; and a yelk-ball, divisible into formative and food-yelk, the former forming a complete cortical layer of granular matter, the latter, the chief mass, containing oil in large drops.

a. *The viscid layer* is a secretion from the oviduct. It resists for some time the action of water and prevents its imbibition in unimpregnated eggs, so that they remain flaccid after at least $2\frac{1}{2}$ hours immersion. It is then no longer distinguishable as a viscid layer, but it makes the eggs cohere firmly together, as if it had the property of setting in water.

This substance has an alkaline reaction on red litmus-paper, but does not change turmeric. A very weak solution of potash destroys its viscidity, and permits water to enter and distend the egg. A weak solution of acetic acid destroys its viscidity, and renders it opaque and flocculent. Boiling and nitric acid do not coagulate it.

b. *The yelk-sac* is a rather thick membrane, measuring $\frac{1}{525}''$. It surrounds the yelk-ball, and is, on its inner surface, in immediate contact with it, so long as no water has been imbibed. It is characterized by a tolerably regular fine dotting, the dots being arranged in lines which cross each other, so that lozenge-shaped spaces are left between them. At the folded margin corresponding radial lines appear, themselves resolvable into dots, as if the membrane consisted of concentrically arranged laminæ, each dotted so as to correspond with the next layer. The further details of this structure will be given in the section devoted to the development of the ovarian ova.

One part of the surface of these eggs is distinguished from the rest by a scattered group of stalked, cup-shaped processes, or buttons, which covers about one-fourth of the surface, and marks the germinal pole of the unimpregnated egg. The form of these buttons varies a great deal, and the size is by no means constant, but for the most part they a little exceed in length the thickness of the yelk-sac, and the form, when not changed mechanically, is shown in Plate XV. fig. 2. Further details of their structure are given in the section on development of ovarian ova.

In the centre of this group of buttons is the micropyle. It may be seen in various modes. I first noticed it while crushing an unimpregnated egg in June 1854, but its characters are best studied by removing the germinal segment, and examining it separately after washing away the contents. Sections may also be made by imbedding ova in strong warm jelly, and slicing the mass when cold, and in this way, some may be obtained which cut the micropyle vertically. It consists of a wide-mouthed, funnel-shaped pit, directed towards the centre of the egg, near its apex becoming more acute, and terminating in a short narrow tube with almost parallel sides (Plate XV. fig. 3). The inner end of this tube is apparently open, and however viewed, whether from within or without, whether in sections or in whole eggs, looks like a clear, pale-blue, oval or circular aperture, and measures $\frac{1}{2350}''$ (Plate XV. fig. 4). The fine dottings of the yelk-sac cease abruptly at the margins of this opening. Powers of $\times 100$ to $\times 200$ are well suited for the examination of these sections, but higher ones may be used without difficulty. To examine the relation of the micropyle to the egg as a whole, or to the yelk-ball, powers of $\times 50$ to $\times 100$ are more convenient.

Unimpregnated ova yield to moderate pressure without rupture, and when the germinal pole presents, the micropyle may be seen either full face, or at various inclinations; and if the position be suitable, the terminal opening is still visible as a clear bluish spot, although the whole of the yelk-ball is below it. When the germinal pole is in profile under suitable pressure, the funnel is seen projecting into that portion of the yelk-ball which corresponds to this pole (Plate XV. fig. 5), and which I have called the discus proligerus. In unimpregnated ova this relation is not quite so distinct as in those which

have been recently impregnated. If eggs are examined in a similar manner about four or five minutes after having been fertilized, the funnel is seen very clearly, half withdrawn from a corresponding pit in the centre of the discus proligerus (Plate XV. fig. 6). The ordinary mode of examining the surface of these eggs under a lens is not convenient for observing the micropyle, on account of the strong reflection from the viscid layer, which cannot be got rid of by drying or wiping.

The yelk-sac resists the action of water for a very long time, in so far as it is not decomposed or materially changed in its optical properties by it, and indeed it differs markedly from the contents of the egg by its greater stability. It is, however, changed by imbibition of water, from a soft easily lacerable membrane to a firm elastic one, each small section of which returns to its normal form very quickly after pressure. It is not much altered by spirit of wine; it is rendered clearer, slightly swollen, and its markings are made less distinct by dilute acetic acid and by dilute solutions of potash.

c. *The yelk-ball.*—A delicate, colourless, translucent, homogeneous membrane, which I call provisionally the inner sac, covers the whole surface of the yelk-ball within the yelk-sac. It is not so easily shown in the eggs which have not imbibed water, but with care may be seen to escape with its contents, by causing a sudden rupture with a large opening in the yelk-sac. By examining recently impregnated eggs, which, being elastic, allow the contents to escape with a jerk under suitable pressure, it may be better seen, as it escapes, thrown into distinct folds, contracting or collapsing as its contents pass out into the water around (Plate XV. fig. 7). I have reason to think that it is hardened by the action of water on its outer surface, its membranous characters being more distinct in eggs which have been some time impregnated. When free in water it shows a double contour at the folds or wrinkles only; for where it still contains in its pouches yelk-substance, the inner surface line is not well defined. Under pressure it seems capable of almost indefinite extension before it ruptures. It thus presents more the characters of a firmer layer or crust upon the surface of the thick fluid yelk-ball, than of a separable membrane. Perhaps the best view of it is got while it is yet within the yelk-sac, after partial escape of the yelk (Plate XV. fig. 8). I formerly spoke of this membrane as elastic, but I have now some doubt of this, as its tendency to shrink and collapse after rupture may be due merely to the escape of its fluid contents.

Dilute acetic acid and dilute solution of sal-ammoniac do not dissolve it; dilute hydrocyanic acid does.

The formative yelk is that portion of the yelk-ball which is afterwards directly transformed into the germ. It exists in the unimpregnated egg as a superficial layer completely surrounding the food-yelk, and is closely connected near the germinal pole with the soft, ill-defined internal surface of the inner sac, which, as it ultimately takes part in the cleavage, may to that extent be considered a part of the formative yelk. At the germinal pole it forms a thicker layer or disk, extending over about one-fourth to one-third of the surface of the yelk-ball, marked at its centre by a pit which receives the

micropyle, and at its margins passing imperceptibly into the thinner layer of similar material which extends over the rest of the yelk (Plate XV. fig. 5, and Diagram A).

Although this layer is found in the eggs of both species of *Gasterosteus* with identical structure and properties, it is best observed in those of *G. pungitius*.

It may be traced over the whole surface by examining the eggs under gentle pressure, either rolling over the same egg, or using at the same time a number of eggs in different positions, or by treating an egg with a weak solution of acetic acid, which so hardens the cortical layer that it cracks under pressure, and is then very distinct, even at the ventral pole, where it is thinnest. To show the thicker portion of it at the germinal pole, or discus proligerus, it may be viewed either full face under gentle pressure short of rupture, when it is seen as an opaque, yellowish halo around the micropyle; or in profile, when it is seen as in Plate XV. figs. 5, 6 & 8.

A power of $\times 50$ enables one familiar with the object to trace this layer very well, but to make out its constituent parts $\times 100$ is required, and then it is better to examine both before and during rupture, under suitably graduated pressure. By such means it may be made out to contain a number of droplets, sometimes irregular in form from mutual pressure, but usually round, and varying much in size from a diameter equal to the thickness of the yelk-sac down to an immeasurable granule, in which case the characters of a drop are lost. They have a yellowish colour, are placed near the surface of the egg next to the inner sac, and the larger ones are much more numerous in the germinal than in the ventral segment. They are imbedded in a mass of fine, yellowish, granular matter, which in part at least consists of very minute similar droplets, but principally of a substance having somewhat different reactions.

These elements are held together by a homogeneous matrix, which in the discus proligerus makes the mass semi-solid, and under some conditions may be seen drawn into threads, as if very viscid.

In consequence of the presence of the granular elements of the formative yelk in the cortical layer of the unimpregnated eggs, they are more opaque to the naked eye than impregnated ones.

Attached to the basal surface of the discus proligerus, in contact with the clear food-yelk, there is a small collection of dark oil-granules, distinct from the larger drops which float in the food-yelk.

The yellow droplets are characterized by their reactions with water, and indeed they are so unstable that I could find no neutral medium in which to examine them when the egg is ruptured. When seen *in situ* in unimpregnated, unruptured eggs, into which no water has been imbibed, in consequence of the defensive action of the viscid layer, their aspect is perfectly homogeneous and highly refractive, although less so than oil. On rupturing an egg in water, they exhibit vacuolation very rapidly, and undergo a very varied series of changes, during which they become pale and disappear, often presenting appearances like cells, with clear, lilac-tinted, vesicular nuclei in a mass of a deeper yellow colour, either granular or homogeneous. Similar changes occur, but less rapidly,

if eggs be ruptured with no other fluids than the adhering maternal secretions, such as the serum, or the alkaline viscid layer; and care being taken to note the position of the escaping currents, it seemed as if the acid food-yelks had a similar effect.

These changes, so common in protoplasmic matter, wherever met with, give rise to the suggestion that they may be due to the separation of two immiscible fluids, by the contact of a third, from a previous state of feeble combination. The lilac or faint blue tint of the vacuoles may be an optical effect of contrast, as I produced a similar appearance by shaking together water and yellow fat, but, as the depth of the lilac tint is not in the ratio of the depth of the yellow, I am not certain on this point. That I am justified in calling them droplets, is shown by the fact that they sometimes fuse together.

The granular basis, which, besides the yellow droplets in a granular condition, forms the formative yelk, is exhibited best by the action of water, which, while it causes the latter to disappear gradually while vacuolating, makes the former at once become darker and more distinct, and does not cause its ultimate disappearance.

The homogeneous matrix of the cortical layer contains also a peculiar form of albumen, which water precipitates in fine molecules, and which I shall have to speak of as albumen *b*.

The yellow droplets disappear also with vacuolation in a solution of sal-ammoniac, although less rapidly than in water. A weak solution of acetic acid acts on them somewhat similarly. A weak solution of potash rapidly dissolves them, and those of them which are in the granular condition are similarly acted on by these reagents.

The larger part of the granular substance of the cortical layer, rendered darker by the action of water, is not dissolved by sal-ammoniac or by dilute acetic acid. These agents cause a precipitate to appear, of dark granules of a coarse kind, and make the whole layer solid, by coagulation. A weak solution of potash also leaves a granular solid layer after dissolving the yellow droplets.

That portion of the granular matrix which is dissolved by sal-ammoniac, is finer than the substance precipitated by it, and if after this reaction a solution of acetic acid be added, a further precipitate of very fine granules is formed, which is due to the presence of albumen *b*. The finer granular deposit caused by the contact of water is easily dissolved by the solution of sal-ammoniac, not by acetic acid.

Thus it is possible to distinguish in the formative yelk (1) part of the substance of the inner sac, (2) the matter of the yellow droplets and the granules of the same material, (3) the granules darkened by the action of water and not dissolved by the alkaline chlorides, (4) the smaller oil-drops—all existing as separable substances already formed. In solution or otherwise not optically separable, (1) the albumen *b*, (2) the matter so largely precipitated by the sal-ammoniac.

No trace of a germinal vesicle or of its contents could be found after the most careful and repeated searching.

The food-yelk forms the chief mass of the egg. It is a thick fluid drop, covered by the formative yelk, colourless, transparent, and without visible contained particles, ex-

cept the oil-drops. The surface of the drop within the inner sac I had some reason to think a little less dense than the centre, as it ran rather more freely, but all parts flowed from a rupture like a very thick syrup.

The oil is collected into a group of large and small drops and granules, which moves freely through the fluid food-yelk, but not through its centre, to get to the uppermost segment when the egg is rolled; in so doing the drops often separate to unite again. Sometimes, especially in impregnated ova, they adhere to the germinal mass, and then cause it always to float uppermost.

The food-yelk is acid in reaction to blue litmus, to an extent which more than suffices to neutralize the alkalinity of the viscid layer, but its taste is astringent rather than acid. It is coagulated firmly by boiling, by nitric acid, or by spirit of wine. Water causes a fine molecular precipitate in it, soluble in alkaline chlorides and acetates. Dilute acetic acid precipitates freely very fine dark molecules in rapid vibratory movement, after the action of the chlorides, or previously, and then dilute nitric acid causes a still further molecular deposit of a darker aspect.

I tested for cellulose in the different parts of the egg without finding any.

2. *The ovarian ovum.*—The development of ova in ovario I have only attempted to trace in its later stages, *i. e.* after the first germs of the egg in a distinctly recognizable form have been laid. This has been done, mainly, with a view of throwing light upon the mode of growth of the parts of the ovum, and upon the ultimate fate of the germinal vesicle and its contents.

a. *The ovaries* appear completely formed, and containing their characteristic elements in very young fry, certainly in those not more than a month old and about $\frac{1}{2}''$ long. They are, however, better studied in the adult, in which they exist as complex folds of vascular connective tissue, not separable from the peritoneum, attached on either side of the bodies of the vertebræ, and projecting as leaflets, in which lie the ova contained in ovisacs which are lined with epithelial cells, but have no demonstrable basement membrane. There is no connexion, beyond that of simple contact, between the outer surface of the yelk-sac and the ovisac; certainly no peduncle. As the position of the micropyle can be easily and certainly determined in early ovarian ova by the buttons which surround it, the absence of such attachment connected with it is not difficult to prove, by watching eggs escape from the ovisacs under graduated pressure.

The ovisacs are supplied with blood-vessels running in one, two, or more directions, so that they are not pedunculated. Younger and more advanced eggs are met with together in all parts of the ovary without any definite arrangement.

The ovaries are enclosed in a sac, which is attached to their bases on either side, and anteriorly, but continuous with the sexual aperture behind the vent, and must be looked upon as the oviduct. Into its cavity the ova escape when ripe, and remain there for a short time before they are deposited; its walls are muscular, and its inner surface secretes a viscid substance which defends the eggs when deposited from the too rapid action of water, and which serves as a suitable medium for the spermatozooids to move in.

b. *Nearly ripe ova* will be conveniently described here before the earlier ovarian ova; and at the same time I shall attempt to trace the fate of the germinal vesicle and its contents. As the germinal spots have some resemblance to the yellow droplets of the formative yelk, and as both are so very unstable that they undergo visible changes in all ordinary media, a large number of observations had to be made with a view of determining their characteristic reactions.

Nearly ripe ovarian ova, having a diameter of $\frac{1}{23}''$ to $\frac{1}{18}''$, have the oil collected but imperfectly, and are not quite so yellow and clear-looking as ripe ova. The germinal vesicle can be seen *in situ* very well, by placing an egg without the previous contact of water on the slide, and using some pressure, the micropyle either presenting or in profile: the latter is the better mode. The vesicle is then constantly found excentrically placed in the egg, imbedded in the centre of the semi-solid discus proligerus, so that the apex of the micropyle comes nearly into contact with the centre of its surface directed to the germinal pole. At this period it appears lenticular when viewed in profile, and so closely connected with the substance of the discus proligerus, that on rupture of the egg by pressure, the vesicle carries with it, in escaping, a portion of the granular matter in which it lies.

In an egg which measured $\frac{1}{20}''$ the germinal vesicle had a diameter of $\frac{1}{104}''$. Viewed with a power of $\times 50$ the germinal spots are just visible, the other contents of the vesicle not at all, while it is *in situ*. To see the spots with higher powers, it is better to puncture the yelk-sac before applying pressure, so that the contents may escape with less violence. If an egg be ruptured by pressure in water, the germinal vesicle often escapes detection altogether; but pressure short of rupture, without water, shows the vesicle very well, and if it be then increased so as to produce rupture, the vesicle may be easily traced as it passes from its natural position to the aperture in the yelk-sac, where, however, it often breaks and is lost. In all these modes the contents of the vesicle are apt to be displaced, and are so variable in aspect, that no doubt can remain of their being changed somewhat, either by the media they are examined in, or by mechanical violence. When by pressure and rupture without water the vesicle escaped without being destroyed, it was much distorted at the opening in the yelk-sac, but as it lay in the fluid of the egg it appeared round. Examined with a power of $\times 200$ the germinal spots were numerous, loosely aggregated, rounded irregularly, of considerable refractive power, and without any distinct vacuolation in their substance, which had a homogeneous aspect and was almost solid looking (Plate XV. fig. 9). They measured on the average $\frac{1}{1960}''$. Besides the germinal spots, the vesicle was nearly filled with a very delicate molecular matrix. When to this slide water was added, the vesicle imbibed it, and became distended at first unequally, the molecular matrix was displaced, the molecules seemed to darken (although this might be an effect of the greater contrast of refractive index merely), and among them delicate vacuoles appeared (Plate XV. figs. 10 & 11). The wall of the vesicle resisted the action of water and showed considerable tenacity, admitting of free manipulation. It was not dissolved by a solution of sal-ammoniac, or by weak acetic acid, which shrivelled it.

When an egg is ruptured by pressure in water, and the germinal vesicle is seen as it escapes, it shows the contents more changed, and it is very apt to vanish quickly in the water, as if from some injury received during its expulsion. However, sometimes it may be examined in this mode, if no time be lost, and then the germinal spots are very irregular. At a rough estimate the number of spots is about 100 at this period. They are slowly dissolved by a solution of sal-ammoniac, the molecular matrix more quickly so. Dilute acetic acid, added after the action of the sal-ammoniac, causes a copious, very fine dark granular precipitate within the vesicle.

In one instance I found eggs measuring $\frac{1}{17}''$, the average size of ripe free ova, which contained the germinal vesicle with contents precisely as above described. In this case the oil was more concentrated, the eggs clearer-looking to the naked eye; and as a further proof of their being nearly ripe, the oviduct was furnished with a store of the viscid material ready to cover the ova as they burst the ovisacs. Not all the eggs, however, of the same batch have exactly the same dimensions, and still less have those of different individual parents when ripe and free. In one, at least, of these ovarian ova I ascertained that the germinal vesicle had disappeared.

In the nearly ripe ovarian ova, from about $\frac{1}{18}''$ to $\frac{1}{23}''$, in which the germinal vesicle is visible, there is a cortical layer of formative yelk, a thicker layer of it, or discus proligerus, at the germinal pole, essentially identical in structure and properties with the same parts in the deposited eggs. There are some slight differences however, the most distinctive being, that larger droplets, apparently identical with the matter forming the yellow droplets, but a little paler in colour, occupy a deeper plane in the cortex, chiefly of the germinal segment. These undergo similar changes of vacuolation, and have identical reactions with the yellow droplets. This vacuolation presented at the same time in various parts of the escaped formative yelk both lilac-tinted and colourless vacuoles, shown, by their inverting an image seen through them when beyond focus, to have less refractive power than the surrounding medium, and by their gradual growth and fusion, to be in reality drops of a limpid fluid. Between these limpid drops there appeared various kinds of granules and semisolid-looking refractive, yellow, crescent-shaped masses, partly or wholly surrounding the vacuoles, thus giving rise to appearances like young cells. Sometimes a number of minute vacuoles formed within a large homogeneous-looking yellow droplet, and thus a pseudo-granular corpuscle resulted. But all these appearances were fleeting, and the variations infinite, depending in some degree on the nature of the medium which had been used. Thus water caused the vacuolation most rapidly; the viscid secretion of the oviduct and the food-yelk, I have before said, excite it, although slowly; the same may be said of the scanty succus, or the blood or serum contained in the tissues of the ovary, or of weak solutions of glycerine or sal-ammoniac; this latter, by causing a precipitate in the matter of the formative yelk, complicates still further the forms which result from these changes. There is seen at the under surface of the discus proligerus, and forming a part of it, the little heap of fine dark oil-granules, distinct from the store of oil which floats in the food-yelk. The inner sac is distinctly to be made out in these ova.

In still more advanced eggs, *i. e.* those of full size, with concentrated, grouped oil-drops, although still within their ovisacs, no trace of germinal vesicle could be found on repeated and careful examination; so that its disappearance precedes the escape of the eggs into the oviduct. The most careful and prolonged examination of the substance of the discus proligerus, more particularly of that part of it which had so recently contained the germinal vesicle, failed to show any trace of the germinal spots, or any other change in the structure or reactions of the matter of the discus proligerus, after the germinal vesicle had vanished, which could be looked on as due to its disappearance. In face of this negative result, however, it may be mentioned that in cases where I watched the germinal vesicle escape through a rupture in the yelk-sac into the surrounding fluid, whether water was present or not, it often happened that the vesicle was ruptured and its contents escaped; and when this did occur, I frequently could not see the spots among the surrounding materials if, perchance, they were lost sight of for a few minutes. This was doubtless due, in part, to the fact that all the objects in the field were in a state of constant change; still, the changes which occur in the germinal spots are such as might distinguish them from the only visible elements of the formative yelk which can be compared with them, viz. the yellow droplets; for these latter vacuolate, fade, and disappear in water, while the former vacuolate and become darker in outline and do not disappear, at least for a very long time, in water. Perhaps the saline or other constituents of the yelk-ball may have a solvent action on the germinal vesicle or spots; but this point, which might be submitted to experiment, I had not an opportunity of determining.

The ripe or nearly ripe ovarian ova have their ovisacs decidedly thinner at that part which covers the germinal segment. These eggs, placed in water, speedily imbibe it, and become faintly opalescent. Vacuolation soon appears in the matter of the cortical layer, beginning at the germinal segment, and in ten minutes the yellow drops disappear, and a slight interval appears between the yelk-sac and the outer surface of the yelk-ball—a true breathing-chamber. In one or two cases, where some rupture or injury had taken place, a partial concentration of the formative yelk also took place, but this was exceptional.

c. *Earlier ovarian ova.*—An adult female fish, taken from the natural haunts in the month of June, after she has deposited her first batch of eggs, and in which a second is ripening, may be used conveniently to examine the developing ovarian ova in all their earlier stages. Three principal groups may then be made out with the naked eye; 1st, large, nearly ripe, semitransparent, yellowish ova, the oil grouped more or less; 2nd, medium-sized creamy tinted opaque ones, with oil scattered; 3rd, smallest, colourless or whitish, semitransparent ones without oil-drops. The two latter groups alone remain to be described, and I shall examine them chiefly with reference to their mode of growth.

There is no advantage gained in the study of the earliest distinguishable ova by taking young fry; for adults in the autumn, winter, or early spring contain the smallest visible ova as easily observable as in young fish of $\frac{1}{2}''$ in length, and not a month old. I may

mention here, incidentally, that young fry, taken from the streams in November, measure about 1″ to $1\frac{1}{4}$″, and their ova, which belong to Groups 2 and 3, reach about $\frac{1}{75}$″. Young fry in June of $\frac{1}{2}$″ in length, contain ova not much exceeding $\frac{1}{200}$″, but some fry are met with in this month about 1″ in length, and in all respects as far advanced as are some of the later hatches met with in early spring, and which do not seem to have grown at all during the winter. In adult females in November, the largest eggs are about $\frac{1}{33}$″.

3rd Group.—The smallest certainly recognizable ova measured $\frac{1}{800}$″ (excluding now a single observation in which I met with what appeared to be still earlier ova without distinguishable yelk of any kind around the germinal vesicles), Plate XV. fig. 12. They are spherical, have a distinct ovisac lined with cells, a central, comparatively large germinal vesicle, a yelk of one kind only, which is solid, yellowish, refractive, homogeneous, semitransparent, and is not covered by a distinctly separable yelk-sac, but has a smooth defined border, probably indicating its first trace (Plate XV. fig. 13). As the eggs grow larger, the first change noted is a faintly granular aspect of the yelk, and, with certain methods of examination, an appearance as if a clear substance occupied the centre around the germinal vesicle.

The yelk-sac is separable in eggs measuring about $\frac{1}{200}$″, and may be seen in the fluids on the slide as a homogeneous-looking, collapsed sac. Eggs a little larger are less translucent, the yelk is more granular, the free yelk-sac is seen to be furnished with buttons, and has the dotted structure; these eggs measured $\frac{1}{140}$″, the germinal vesicle measuring $\frac{1}{322}$″. Later on, the cortical layer is seen to have the yellow droplets.

2nd Group.—The oil begins to appear in eggs about $\frac{1}{80}$″ in diameter, at first as scattered small granules, and the whole egg is then more opaque, not only from the presence of the oil, but partly from its larger size, partly from the more markedly granular structure of the cortical layer (Plate XV. fig. 14). These eggs pass into the above described first group, with gradual increase of the oil and grouping of the large drops.

In both of these groups the germinal vesicle is central and globular; nor could I succeed at any time in making out how it became transferred to its excentric position, and received its lenticular form. I saw also no discus proligerus, although in all the eggs of the second group a food-yelk exists and escapes on rupture, apart from the formative yelk or cortex, and probably also in some of the later stages of the third group.

To examine the relations of the parts of the eggs in both these groups, water is not a good medium, as it changes them too rapidly by imbibition; but its action on the ovisac is noteworthy. It distends the contained cells rapidly, and passes through the yelk-sac so as to act on the yelk without forming a breathing-chamber. A solution of chloride of sodium, 1 per cent., is a very good medium to be used,—it exhibits well the cells lining the ovisac, and causes very little distension; but a 1 per cent. solution of glycerine is perhaps better for taking measurements in, as it neither distends nor shrivels for some time, while it leaves the whole field very clear. A solution of the acetate of potash of the same strength is also a good medium. All these, after some minutes, permit one

to see in eggs of about $\frac{1}{100}''$, a clear halo around the germinal vesicle, bounded by a defined, but irregular granular outline. At present I hesitate to express any decided opinion as to whether this indicates a first separation of the yelk into two kinds.

A solution of chloride of sodium of 2 per cent. contracts or shrivels the tissue of the ovary, and makes the eggs, when they are above the very smallest size, opaque, by precipitating in them the matter of the cortex.

Solutions of acetate of potash of 5 per cent. or 2 per cent. cause the egg, with its yelk-sac, to shrink within the ovisac, which dilates; make the larger eggs of the third group opaque by precipitating the formative yelk, and leave the smallest homogeneous eggs clear, do not permit the clear halo to be seen, and make the yelk-sac paler.

Solution of glycerine, $2\frac{1}{2}$ per cent. is a very good medium, it leaves the field clear, all the objects well defined, but shrivels the egg a little in the ovisac.

Strong glycerine is quite unsuitable, it changes the appearance and form of the yelk-mass, and obscures the germinal vesicle at times completely; nor can the natural appearances be restored to specimens preserved in it by adding water. The ovarian stroma is also obscured in it.

The yelk requires to be examined in various media in order to make out its structural elements, and its separation into food and formative yelk.

Water, although in using it great care is required, on account of the rapidity with which it changes everything, is very useful. When used abundantly it causes a fine granular precipitate in the substance of the yelk, in the very smallest ova met with, due probably to the presence of albumen *b*, but does not cause visible vacuolation in the yelk. It also permits, in the larger eggs of group 3, and the smaller of group 2, the clear halo around the germinal vesicle to be seen; at the same time it causes a granular deposit in the cortical layer, and then gives rise to vacuolation; and if the eggs are at that stage that yellow droplets have appeared, they grow pale and disappear.

A 1 per cent. solution of acetate of potash slowly causes a precipitate in the cortex of eggs above the very smallest; very slightly also a turbidity of the smallest egg.

A solution of $1\frac{1}{4}$ per cent. of chloride of sodium, which does not alter the blood-disks of the same fish, also causes a dark precipitate in the cortex of eggs which have a distinction of yelks.

A 1 per cent. solution of glycerine is the most neutral agent, as far as regards the yelk-substance, but after some time the yelk of all ages becomes slightly granular in it.

In trying to determine at what stage of development the granular elements of the cortical layer appeared, it was necessary first to find a fluid medium which did not determine a precipitate. The maternal fluids may be used, but do not enable one to obtain a clear field.

5 and $2\frac{1}{2}$ per cent. solutions of acetate of potash precipitate the larger of these eggs strongly, the smaller less so, the smallest not at all, and their yelks escape in a solid form. The yellow droplets change very slowly in these solutions.

Hence it is safe to say, that the substance which is first seen around the germinal

vesicle is neither food-yelk nor formative yelk, but differs in structure and reactions from both in a very marked manner, although it contains probably a little albumen *b**. It may be called primitive yelk. That water and other fluids cause no visible vacuolation in its substance may possibly be due to the fact, that if it were delicate it might be obscured by the changes which are produced at the same time in the cells of the ovisac, and which at times are very confusiug.

The primitive yelk is very firm, and often escapes on cutting up a fragment of an ovary in 1 per cent. solution of chloride of sodium, or in a solution of glycerine 2 per cent., as a solid-looking, somewhat angular body, with its contained germinal vesicle (Plate XV. fig. 15). In water also it is solid, though much paler, when escaped, than in the other media. Although it is not like the perfect formative yelk in structure or properties, it may possibly be continued in a modified form, and exist in some proportion in the ripe ovum, as its reactions are rather negative than positive, and it might therefore easily escape detection.

To the primitive yelk, as the eggs grow, are added the other elements; first, of the formative yelk, and afterwards of the food-yelk, as the above-mentioned reactions prove. The precise time of the appearance of the food-yelk was not made out with certainty; it is probably some time before the oil-drops make their appearance, and possibly the clear halo around the germinal vesicle is the first optical expression of it. What its precise relation to the germinal vesicle and formative yelk may be at first, I could not determine. The solidity of the primitive yelk reminds one forcibly of the early condition of the yelk in the ovum of Birds.

Whether any inner sac exists in ova of the groups 3 and 2, I cannot say. I could only find it in group 1, *i. e.* nearly ripe ova; and one observation seemed to indicate that in group 3, at least, it is not present; for these eggs, when examined in saliva, show the yelk-sac distended, together with the ovisac as one membrane, and then the surface of the yelk is granular and irregular, not smoothly defined as it would be, were an inner sac present.

At no time did I observe any contractions of the protoplasm of immature ova: perhaps I did not use the requisite media; but the solid state in which it exists at first makes it difficult to conceive how such could occur.

The germinal vesicle and its contents also require that various reagents, of different degrees of concentration, shall be employed in their examination.

The first difficulty is to get to understand the natural aspect of such variable objects, and to appreciate duly the influence of the media used.

By cutting up a large piece of an ovary without any fluid, and selecting a small fragment for examination, the smaller eggs and the germinal vesicles may be studied, and the latter seen both *in ovo*, and free in the field; but the field is turbid, and the refractive index of the medium, which is a mixture of escaped yelk and serum, is too much like

* See page 451 for a description of this variety of albumen, which is probably a constant constituent of the yelk of vertebrata.

that of the objects to be examined, for good definition. The germinal vesicle appears to be filled with a homogeneous and colourless colloid material. It is true, however, that a finely molecular structure in it might in this way escape notice. The germinal spots are imbedded in this colloid matrix, on its surface only, so as to be in contact with the inner surface of the wall of the vesicle; unless, as often happens from mechanical causes and from imbibition, they have been displaced. They have a round form, homogeneous aspect, and a refractive power but little greater than that of the yelk-fluid (Plate XV. fig. 16).

A more convenient mode of seeing the natural condition of the germinal spots, is to use a very minute quantity of water in cutting up the fragment, and to examine rapidly before time is allowed for changes to take place. Then, the spots are seen round and homogeneous-looking, when within their vesicles in larger ova, water not having reached them by imbibition. But in the vesicles of the smallest eggs, or in those which lie free in the field, the spots when first seen are variously tailed and vacuolate, parietally placed in the vesicle, lenticular when seen edgeways, their outlines much darker, and harder, perhaps, in part, an effect of contrast. The colloid matrix is usually seen delicately shaded by a fine molecular deposit, but the conditions of the formation of this molecular deposit I could not feel quite sure of, except that water favours it.

More abundant and prolonged action of water is apt to displace the germinal spots, by distending the vesicle, but this it does irregularly, so as to make it appear in some measure a result of mechanical injury (Plate XV. fig. 17). The colloid matrix, after a time, becomes more granular, and this change may even obscure the germinal spots, when a weak solution of chloride of sodium dissolves the fine granules, without impairing the consistence of the colloid matrix. This was well seen in one instance, at a rupture in the wall of a large escaped vesicle, in which also the extreme toughness and strength of the vesicular wall was manifest. The action of water, however, on the spots and on the colloid matrix is not the same on free uninjured vesicles, as it is on those still within the egg, especially the larger ones; in which I found that the results were in great measure due to the influence exerted by the saline or other constituents of the yelk, which were carried into the vesicle by osmose. Thus, in larger eggs of group 3 and smaller ones of group 2, when long acted on by water, the germinal spots of contained vesicles are seen to get pale and disappear; at the same time the ovisac and yelk-sac show evidences of abundant endosmose, and there is also some granular deposit in the cortical layer of the yelk, but not such as will account for the obscuration of the spots, as the position of the germinal vesicle is well seen, marked by a clear area (Plate XV. figs. 13 & 14). Still later on, the contents of the germinal vesicle are seen as distinct granules in rapid tremor. These facts strongly suggest the notion that the germinal spots are soluble in some of the constituents of the yelk, and we may thus explain their disappearance in ripe ova.

It should be here mentioned that free germinal vesicles, being uninjured, remain in water for hours, without much visible distention or displacement of their contents, or disappearance of the germinal spots; and the same may be said of those contained in very

small eggs; and if the fragment has been prepared in an abundance of water at first, the free vesicles may show no molecular deposit in the colloid matrix after seven hours (Plate XV. fig. 18).

The germinal spots, after this prolonged action of water on the free vesicles, are not soluble in a 10 per cent. solution of acetate of potash, but the spots in the unbroken larger ova are; thus water must chemically change the substance of the spots or their surface, as before its action $2\frac{1}{2}$ per cent. of the same salt dissolved them.

A 1 per cent. solution of glycerine is an excellent medium for showing the germinal spots; they remain for ten minutes in it without showing changes of form or vacuolation even in the free vesicles. It does not precipitate the colloid matrix.

A $1\frac{1}{4}$ per cent. solution of chloride of sodium, which does not change blood-corpuscles, added to a fragment of ovary, prepared in the maternal fluids, made the colloid matrix which was not previously granular look brighter, and changed the germinal spots from round homogeneous-looking bodies to variously tailed and vacuolated forms (Plate XV. fig. 19). On then gradually increasing the strength of the solution to 5 per cent., it was observed in a ruptured free vesicle that the germinal spots, as they lay adhering to the colloid matrix near, and partly within the rupture, gradually became paler, coalesced, and fused into a large pale drop, with vacuolation in and around it. The stages of this change are seen in Plate XVI. fig. 20. Precisely similar changes were seen to occur in the spots of germinal vesicles while yet contained in the eggs. It seems probable, then, that we must look on the germinal spots as drops of a thick fluid, or at least not as solid bodies.

A solution of only $2\frac{1}{2}$ per cent. of chloride of sodium which crenates the blood-disks, similarly caused fusion of the germinal spots. In a solution of 1 per cent. only, the spots vacuolate and become tailed very slowly, and after an hour I found them again round—suggesting the possibility that they may have a power of changing their form analogous to that possessed by the protoplasm of white blood-corpuscles. This solution causes the red blood-disks of the same fish to become paler: it does not ultimately dissolve the spots, but like water changes them, so that they are no longer soluble in even a 10 per cent. solution of chloride of sodium.

The solutions of acetate of potash act very much like those of chloride of sodium.

A weak acetic acid solution does not dissolve the wall of the germinal vesicle or further distend it. It precipitates the colloid matrix, leaving the spots dark-bordered and distinct.

The yelk-sac also merits a minute investigation.

The precise period at which it is formed is difficult or impossible to determine. In the smallest eggs seen, those of $\frac{1}{800}''$, it is not separable, but is probably indicated by the smooth hard outline which the yelk shows on its surface, when a $2\frac{1}{2}$ per cent. solution of glycerine or of chloride of sodium is used, which contracts the yelk-ball with the yelk-sac, and leaves a space between it and the ovisac. A little later it is seen indicated by folds on the surface of the yelk, the result of the shrivelling which the solution

causes; and in eggs about $\frac{1}{200}''$ in diameter it may be separated, but I failed to show any structure in it, probably from not succeeding in mounting it for examination with the highest powers. It is known by its characteristic foldings and refractive index.

The best way to get out the yelk-sac is to cut up a fragment of the ovary with very fine scissors in all directions, after having macerated it well in water; then by removing the larger pieces several yelk-sacs are seen free from their ovisacs.

The yelk-sacs of eggs of about $\frac{1}{140}''$ have certainly a fine dotted structure, and are furnished with buttons and a micropyle. The buttons can be seen on still smaller eggs, in which I found no dotted structure. As the eggs grow the yelk-sac gets thicker, and its markings more distinct: at first it is flaccid at all times; but in eggs of the 2nd group, and larger, the segments, after imbibition of water, during which they seem to increase in thickness, become elastic, so that each segment springs into its shape again like a segment of an india-rubber ball. When the fine dottings can be observed, they have the same characters in all the stages of growth.

The dots have a similar aspect on both inner and outer surfaces of the sac, are arranged in tolerably regular diagonal, curved lines, alternating, so that they enclose lozenge-shaped spaces. With powers up to $\times 500$ they appear round, and even with the highest used, $\times 2600$ and $\times 3000$, they are but obscurely hexagonal. They are seen blackest when a plane rather deeper than the true surface is in focus, and then appear round. With very careful adjustments, and the true surface in focus, they have a polygonal or hexagonal, not very sharp outline, and seem like pits; the elevated ridges between which look like a very fine, rather irregular reticulation. At a folded edge they produce an appearance of radial striation, the striæ resolvable into dots, due to the laminated structure of the sac. This is best seen at a cut edge, especially after longer maceration. The cut edge reminds one of the edge of cut lace or perforated zinc, but whether this is due to an actual falling out of the matter which caused the appearance of dots, or is an illusion, I cannot say. The dots act, in focusing, like a substance of low refractive power, and I incline to the view that the appearance described is illusory. In the smallest-sized eggs of which I measured the dots, they were $\frac{1}{24000}''$ apart; these eggs were about $\frac{1}{100}''$ in diameter. In nearly ripe ova, on the point of quitting the ovisacs, they were $\frac{1}{11000}''$ apart, measured from centre to centre, each dot being about $\frac{1}{30000}''$ in diameter; these results were the mean of several measurements with a power of $\times 1000$. The dots are the same distance from each other on the inside as on the outside of the sac, and the radial lines are the same distance apart. All the measurements were made in the eggs of the 3-spined species.

The outer surface of the sac suffers sometimes, after long maceration in water, a peculiar change in its consistence, so that on its rupture by very strong pressure, the surface-layer yields like a soft, almost viscid substance, seen as a colourless, structureless film, stretched across the rent, with a power of $\times 250$ (Plate XVI. fig. 21). But with a power of $\times 3000$ it has an exquisitely delicate structure, like net, very regular and perfect, and

evidently identical with the dotted structure. It forms thin layers, of which several may be counted. The dottings are dark with light interspaces, or light with dark interspaces, according to the focus (Plate XV. figs. 22 & 23).

Besides these minute regular dots, in larger eggs of group 2 there is a darker kind of dot, which I will call the stellar dot. It is irregularly scattered over the inner surface only, and can only then be seen from the outside, when a lower objective than $\frac{1}{8}''$ is used, which penetrates sufficiently. They are larger and much blacker than the regular small dots, of a stellar form, are wider apart (on an average $\frac{1}{3000}''$), but vary much in this respect. Examined and measured with a power of $\times 930$, each has a diameter of about $\frac{1}{8000}''$. They are in sharpest focus and blackest when a plane rather deeper than the true inner surface is in focus, and with that surface in focus they look like stellar-shaped pits. On focusing they act like bodies of low refractive power. At the cut edge they may be seen to pass radially about two-thirds into the substance of the yelk-sac, gradually coming to a point and ceasing. They do not look like spaces at the cut edge, as do the fine regular dots.

The buttons may be well examined in unripe ova, especially those nearly ripe. They are attached to the outer surface of the yelk-sac by a bright, highly refractive point, from which radiate along their under surfaces to the periphery, little folds of the substance, which is clear, homogeneous, soft, and easily distorted, by contact, in consequence of its adhesiveness.

I counted the number of buttons on five small eggs of group 3, and on the average found 80 to each. The average number on each yelk-sac of nearly ripe ova, or group 1, is 207, a result of five countings. In ripe deposited ova I could not prepare the yelk-sac so as to count them, on account of the readiness with which they became detached, by adhering to external objects; but there is no reason to think the number increases after the stage of group 1. I think it probable that they are organs of adhesion, and serve to fix the egg.

d. *The method of staining tissues*, so strongly recommended by Dr. Beale, was tried with reference to its importance as a test of germinal matter, a term which I assume to be synonymous, or nearly so, with protoplasm.

If a fragment of ovary be digested in the carmine solution* for half an hour, and then washed with the acetic acid glycerine, it will be found to be irregularly and unequally dyed, this irregularity affecting the ovarian stroma, the yelk-sac, and the yelk. The tissue of the ovary is softened to an almost viscid consistence; whether an effect of the ammonia or of the acetic acid I did not stop to ascertain.

After twenty-four hours' digestion in the carmine solution the stroma is deeply dyed, but less so than the yelk of the youngest ova. Those parts of the ovarian tissue which are thickest, such as vessels, have the deepest tinge; the films of connective tissue show no colour, perhaps on account of their extreme tenuity and translucency.

In the fragment which had been digested for half an hour, the minutest eggs showed

* This was prepared according to Dr. Beale's formula.

the deepest tinge in their yelk-mass, especially those most exposed to the fluid. Those larger eggs which had a food-yelk showed less colour; what stain had taken place was limited to the formative yelk, but many of these yelks were not dyed at all; the food-yelk flowed out from a rupture as a colourless fluid, or changed chemically into a mass of clear, starch-like corpuscles. The whole primitive yelk, when dyed, was deformed, rendered opaque, vacuolating, and granular, a physical condition favourable for reflecting its colour, but unfavourable for exhibiting its true structure or characters. The germinal vesicle and spots were obscured or quite undistinguishable.

To test the action of this dye-fluid on the germinal vesicle and contents, I prepared a piece of ovary in a 1 per cent. solution of chloride of sodium, as a neutral solution; then bringing into view a free germinal vesicle, I gradually added the carmine solution; slowly the vesicle swelled out, the spots became pale and vanished, the vesicular wall seeming to shrivel up.

That this was due to the ammonia was shown afterwards by repeating the experiment, using a dilute solution of ammonia (about $1\frac{1}{2}$ per cent. of Liquor Ammoniæ); the germinal spots vanished as in the dye-solution, and the vesicular wall also, but an hour later.

Thus it may be said, I think, that whatever value we may attach to this process of dyeing tissues, we must not neglect the consideration of the changes which may be produced in these sensitive states of matter by the menstrua employed. If the strong, tough, and very distinctly solid wall of the vesicle may vanish, if the highly refractive, striking-looking spots may be rapidly dissolved by BEALE'S carmine solution, who can tell what changes it may produce in the almost equally unstable and sensitive substances which constitute the growing parts of tissues, and probably even the functionally active parts? It certainly seems necessary to supplement this method by others capable of determining the appearances presented in perfectly indifferent media.

The yelk-sac took the dye freely; considering its thinness and translucency perhaps as much so as the formative yelk; the dye was deepest in the thickest sacs. When an egg was crushed by strong pressure, so as to reduce the layer of yelk-substance to the same thickness as the yelk-sac, the colour was seen to be quite as deep in the buttons on this latter as in the formative yelk, but of a somewhat more yellowish red.

The dyeing is independent of any acid reaction of the substance dyed, as macerated yelk-sacs, which had become alkaline from decomposition, took the dye freely. This independence of the acid reaction is also further seen by the fact that the acid food-yelk does not take the colour at all.

The fine structure of the yelk-sac is rendered less distinct by this method, partly in consequence of the action of the glycerine, partly from the action of the acetic acid. Ammonia does not impair the distinctness of this structure, although it makes the sac very clear.

It will perhaps not be entirely out of place to introduce here the following

Observations on the staining of the eggs of the Pike.

Ripe ova put into water tinged with common alkaline magenta dye, after ninety-six hours show a rose colour in the yelk-sac, and a deeper tinge in the granular contents of the yelk, in those eggs only, which had a ruptured and collapsed inner sac, with alkaline contents. But in those with clear acid food-yelk and unruptured inner sac, there was no dye seen beyond the yelk-sac; while in those with partly diffused formative yelk, and partly emptied inner sac, the dye extended only to the diffuse granular matter. Thus the inner sac resists the passage of the solution, and the deeper parts of the egg are not stained until materially altered.

Fertile ova, ninety-six hours after impregnation, placed for twenty-four hours in a similar solution, continued to develope as in water; the yelk-sac alone took the dye, the embryonic tissues resisted it completely.

Unimpregnated eggs, put fresh from the parent fish into the same solution, soon become stained in the yelk-sac, and if then they be broken, the contents escape free from colour.

A weak ammoniacal solution of carmine acts just like that of magenta, and when tried on ovarian ova, I could not succeed in staining the yelk-matter of either kind by cutting them in halves, and leaving them in it, although the yelk-sac was easily dyed in the same time. The yelk-sac must, I suppose, be looked upon as "formed material," yet it takes the carmine dye even more quickly and almost, if not quite as deeply, as does the formative yelk. The food-yelk, with its portion of inner sac, must, I imagine, be looked upon as "germinal matter;" at least it is a protoplasm and contractile, yet it cannot be made to take the dye.

The substance of the primitive yelk after a time takes the dye strongly, and then, compared with the more delicate translucent tissue in which it lies, is a very prominent object; but it is important not to forget the effect of thickness and physical condition in influencing the apparent colour of objects.

The substance of the formative yelk appears only then to take the stain when it is no longer defended by that of the inner sac; so that it is changed in form and structure before it can be dyed.

I am therefore not disposed to consider staining a satisfactory test of germinal matter, for some "formed material" takes the colour quicker and some "germinal matter" is destroyed, while other is much changed by the dye fluid; and some cannot be stained if the food-yelk and its cortex be considered one of its varieties. So far as my own observations permit me to form an opinion on the constant characters of protoplasm, I should at present say that the tendency to vacuolate is the most trustworthy test; in other words, protoplasm is in such unstable equilibrium that its proximate elements easily separate by contact with most aqueous solutions.

e. *Remarks on the mode of growth of the yelk-sac and on the germinal vesicle.—The former* I am, notwithstanding its highly complex structure, disposed to consider as a cell-membrane. Whatever may be said as to the mode of its earliest formation, it cannot

be conceived to grow by apposition of layers added from the inside or outside, although its laminated structure might at first be supposed to afford some support to this view; for the increase in number, as the growth proceeds, of the buttons placed on the outer surface, and their early appearance, make it impossible to understand growth by either of these modes. It is, I think, certain that it grows interstitially, and the suggestion arises that the larger stellar dots may in some way be connected with this increase.

The fact that young ovarian ova, of $\frac{1}{100}''$ in diameter, when the dots are first measured show them to average about 24,000 to an inch linear, and ripe eggs five or six times their diameter have 11,000 dots to an inch, thus but little more than doubling their size or distance apart, proves that during growth the number of these structural elements of the yelk-sac must increase, as well as their size. This may be taken as an additional proof of interstitial growth.

There is no evidence of the conversion of the substance of the outer layer of the protoplasm, *i. e.* the cortex of the yelk, into yelk-sac, in the sense in which that gradual conversion is believed by Dr. BEALE to take place in cartilage; at least after arrival at that stage, and it is a very early one, at which the yelk-sac is separable: as then it always shows its inner and outer surfaces equally sharp, hard, and distinct.

There are no facts known to me to point out whether the pabulum for the growth of this membrane is derived directly from the currents passing inwards, or from the material elaborated in the egg and passing out of it, or from both sources indifferently.

The extreme delicacy of the film which covers the yelk at first, makes it impossible to say positively whether it appertains rather to the layer of cells lining the ovisac, or whether it is more closely adherent to the yelk; but I incline to the latter view.

The germinal vesicle, which, both from the facts here recorded, and from the analogy of the eggs of invertebrata, appears to be formed before the primitive yelk, may be supposed, from its disappearing when the egg is ripe, before fecundation, or the action of external agents, to preside over the origin and growth of the egg. However, the position in which it is last seen with respect to the micropyle, as strongly indicates that its remnants have some important relation to the act of fecundation.

BALBIANI* has recently, in a paper which received a prize from the French Academy, stated that the germ (with him the equivalent of the formative yelk) may be traced to a preexisting nucleated cell spontaneously arising on the surface of the food-yelk, which cell by endogenous development of cells at the expense of the "primordial protoplasm" (food-yelk) forms the future germ on its surface. He expressly extends this view to osseous fishes, although he repeats the error made by COSTE, that the germ (formative yelk) only appears after fecundation.

This description of the mode of origin and growth of the parts of the ovum, I feel justified in stating is not in accordance with observed facts.

* "Sur la Constitution du Germe dans l'œuf animal avant la fécondation," Comptes Rendus, 1864, t. lviii.

The Trout (Salmo fario).

In ripe ova expressed from the fish I found the micropyle, December 1854, by carefully turning the egg over before water was applied; then by drying the surface somewhat, it was visible with the naked eye, but more easily with a lens (Plate XVI. fig. 24). At first it corresponds to the centre of the discus proligerus or germinal pole, but after the egg has been in water a few minutes, even when not fertilized, water enters, and the formative yelk which is at first as in *Gasterosteus*, a complete cortical layer, concentrates, and is collected into a nipple-shaped heap at the germinal pole; and from having attached to it some oil, it always floats uppermost, the yelk-ball being free to move in the now distended yelk-sac; so that the correspondence between the micropyle and the centre of the discus proligerus ceases. The terminal opening measures $\frac{1}{6300}''$ across, and the funnel or pit at its mouth $\frac{1}{96}''$.

The formative yelk, the discus proligerus with a deep central pit to receive the micropyle, the clear food-yelk, and the group of oil-drops, are all essentially the same as in *Gasterosteus*.

These eggs, pressed from the parent into water, stick to the dish for a time, but if first left exposed to the air for a little while, do not. This was not explained. They formed a breathing-chamber by imbibition, but no active protoplasmic contractions were seen.

The Salmon (Salmo salar).

These ova were examined in January 1855. They have a micropyle precisely similar to that met with in the egg of the trout, and the general structure of the egg is the same. The yelk-sac is very well suited for examining the dotted structure, especially after prolonged immersion in water, in which it retains its structure for four months at least; the details of its structure are essentially the same as in *Gasterosteus*.

These eggs, like those of the trout, imbibe water when not impregnated. If uninjured, they remain in water without apparent change, at least forty-three days. If injured, the inner sac often ruptures, and then the yelk coagulates, by the action of the water. If kept for the same time in damp moss, they decompose, become fœtid and alkaline, and then, if crushed in water, do not coagulate, the salt of ammonia produced keeping the albumen *b* in a state of solution. The inner sac thus seems, when intact, to resist the passage of osmotic currents through it.

I tried to test this in the following manner:—

Eight clear ova which had been kept without change in water for twenty days were well shaken in an empty bottle for a minute or two; then distilled water was added, and all became opaque at once, showing no longer a breathing-chamber, the inner sac being ruptured. The water was found to be acid, and contained an organic substance, which, when incinerated, left an alkaline ash containing chlorides and phosphates.

The chemical reactions of the yelk.—In the three species of Salmonidæ which I have examined, the yelk reacts similarly, but it differs somewhat from that of most other osseous fishes, in having a larger proportion of a peculiar variety of albumen precipitable

by water. This substance, which I have spoken of as albumen *b* provisionally, I have thought worthy of a somewhat more detailed examination, because it appears to be present in some proportion in the yelk of all the osseous fishes which I have been able to procure, and it, or a closely allied substance, is a constituent of the yelk of frogs and birds.

Its ready precipitation by water suggests the notion that the inner sac may possibly be formed by a gradual hardening of the surface of the yelk, through a chemical action of the surrounding medium, which in the ovarian ova would be an exudation from the blood. Certainly the inner sac was noticed to become firmer and more distinct in eggs which had been long exposed to the action of water. Whether this property of albumen *b* has any part in the formation of cell-walls is an interesting speculation, but one to which these observations give no direct support. Be that as it may, a substance easily precipitable by water is, I believe, very widely met with in animal protoplasms, and the firmer limiting surfaces, which in the protoplasmic balls of rapidly growing structures are the only representatives of true cell-walls, may owe their formation to its precipitation.

a. *Fluid albumen b.*—The food-yelk of the salmon is a thick fluid albumen, entirely precipitable by water in excess, if free ova be used; if ripe ova *in ovario* be used, a small proportion of ordinary albumen remains dissolved in the supernatant water, and may be coagulated by boiling, and nitric acid; but this is derived from the vascular tissue of the ovary.

The characters of this albumen, when in solution, may be studied in the entire egg, or in crushed eggs treated with water, in too small a proportion to precipitate all the albumen.

It then is coagulated by boiling, nitric acid, hydrochloric acid, alcohol, and ether. Dilute acetic acid coagulates it, and stronger acetic acid redissolves the coagulum, after which water will not precipitate it, nor will carbonate of potash added to alkalinity. In the alkaline solution mineral acids do not cause a precipitate unless heat is applied, nor does boiling without a mineral acid coagulate it. The acetic acid solution is, however, precipitated by yellow prussiate of potash.

b. *The solid albumen b.*—In the solid state this albumen, obtained by precipitating it with water, is white, finely pulverulent, composed of immeasurably fine molecules; while moist, it gives an acid reaction, after the most prolonged washings, short of decomposition; part of it always passes through the filter on account of its fineness and the absence of dense flocculi.

A strong solution of *chloride of ammonium* dissolves it, and the solution is not reprecipitated by alcohol unless boiled, and only imperfectly coagulated by boiling alone. It is precipitated, on the addition of strong acetic acid, and also by water in excess, when the precipitate is soluble in phosphoric, nitric, hydrochloric, sulphuric, tartaric, and acetic acids, also in potash and ammonia. The nitric acid solution is not coagulated by boiling, and the potash solution only then coagulated on boiling when nitric acid is added hot.

A strong solution of *acetate of potash* acts much as does the above reagent. The solution may be boiled without much coagulation, unless acetic acid be added, when flocculi form.

Chlorides of sodium and *potassium* dissolve it, and the solution is reprecipitated by water; it then may be again dissolved by acetic acid, phosphoric acid, hydrochloric acid, and nitric acid, the acid solutions not being coagulated by boiling unless nitric acid be added while hot, and not being precipitated by water in excess. This solubility of the second precipitate by water, in nitric acid, is the more remarkable, as the first solution of the albumen *b* in chloride of potassium is precipitated by nitric acid.

Ammonia dissolves it, the solution is not precipitated by water in excess, and only very imperfectly coagulated by prolonged boiling, after which acetic acid causes a copious precipitate.

Weak solutions of *potash* and its *carbonate* do not dissolve it; they partially coagulate a clear egg.

The common *phosphate of soda* dissolves it, and if precipitated again by excess of water, and redissolved by phosphoric acid, the acid solution may be boiled without coagulating, unless ammonia be added while hot, or may be diluted freely with water, without clouding. If acetic acid be used instead of phosphoric, the solution may be boiled without coagulation, unless potash be added.

The *phosphate of ammonia* acts like the above; the precipitate from it by water may be dissolved in phosphoric acid, and the solution, which is faintly clouded by prussiate of potash, and more so by nitric acid, is not at all so by chloride of mercury or by boiling, unless ammonia in excess be added hot.

Weak *acetic acid* does not dissolve it; stronger does, and the solution is not precipitated by water used freely; the acid solution clouds a little on boiling.

Weak *phosphoric acid* dissolves it; the solution is not reprecipitated by water or by boiling.

A concentrated, acid-reacting, aqueous, solution of the *salts* and watery extractive of the egg dissolves it, the solution being precipitable by water in excess.

While a strong solution of *sugar*, in which the eggs float, causes the albumen *b* to precipitate if an egg be broken in it, a weaker solution of *glycerine* does not.

These somewhat complicated reactions do not precisely accord with any modification of albumen with which I am familiar. Perhaps it has the closest affinity with MYOSIN, recently discovered by KÜHNE* in the juice of muscle. Besides its precipitation by water and easy solubility in most alkaline salts, the most characteristic reactions are, that under certain conditions its acid solutions do not coagulate on boiling, even when the acid is the nitric, and that its solution in some neutral salts is not precipitable by alcohol. The ichthuline of VALENCIENNES and FREMY† may be the same substance, but I cannot feel sure.

The natural salts of the yelk probably hold this albumen in solution, at least it has been shown that they can do so; whether the acid of the yelk contributes thereto I could not make out.

* Untersuchungen über das Protoplasma und die Contractilität, 1864.

† "Recherches sur la Composition des œufs dans la série des Animaux," Journal de Pharmacie et de Chimie, t. xxv. and t. xxvi.

c. *The acid reaction.*—I sought in vain to isolate the acid of the yelk. In precipitating the albumen *b* by water, the supernatant fluid becomes acid, and the precipitate itself is so, until it commences to decompose.

The acid, whatever it may be, is insoluble in alcohol, soluble in water, and forms a non-crystallizable soluble salt with baryta, which remains in solution in the alkaline reacting fluid, after the phosphates and sulphates have fallen, and is not precipitated from it by carbonic acid, but is by ammonia; when dried and burned, it chars, and leaves an alkaline ash. It is, I believe, a compound into which phosphoric acid and an organic substance enter as constituents.

A substance much resembling leucin was found among the alcoholic extractives of the yelk.

The Grayling (Thymallus vulgaris).

The ripe ova resemble those of trout and salmon in all essential particulars.

The Pike (Esox lucius).

The ripe ova have a general structure, essentially the same as that of *Gasterosteus*; the inner sac is particularly easy to demonstrate, and may be separated, teazed with a needle, and mounted. The yelk-sac is covered externally by a thin layer quite structureless under a power of $\times 400$, the equivalent of the "eikapsel" in Perch. It is visible only in moderated light, at least with the lower powers, and then requires, in order to distinguish it from a diffraction effect, that note be taken of the flow of fluids, and the position of solid particles on its surface.

It is this layer which causes freshly expressed eggs to adhere to each other and to the dish when in water. They do not thus adhere in air. Yelk-sacs long digested in a weak ammoniacal solution of carmine become rather friable, but the homogeneous outer layer retains its plasticity, and on rupture by pressure may be seen stretched across the gaping fissures of the yelk-sac, tinged faintly with the dye, but quite structureless under a power of $\times 400$, and careful illumination.

The micropyle may be sought for in the same way as in trout eggs. The aperture at the apex is easily seen under a power of $\times 200$ in full face. When the pit is viewed in $\frac{3}{4}$ face with oblique light, it seems to have a trumpet-shaped tube, standing erect from the bottom of it (Plate XVI. fig. 25). This appearance is due to the fact that in a strong illumination the clear, colourless, outer layer is quite invisible, and the trumpet-shaped tube appearing to stand erect and unsupported, is the thick wall of the canal, which penetrates the outer layer, where it dips into the pit of the micropyle.

The Ruffe (Acerina vulgaris).

The general structure of the ripe egg is the same as in *Gasterosteus*. The oil, however, forms but one large drop, the inner sac seems thicker, and the yelk-sac has an outer layer or "eikapsel." Eggs expressed from the parent may be manipulated with care if no water be added, as although very soft they do not adhere strongly; but if

water be present they imbibe it rapidly, and then become so adhesive, that every attempt to roll them and examine the surface tears off the outer layer of the yelk-sac, or "eikapsel." Before water is added they have a convoluted surface, such as a lax membrane presents, which is marked by irregular impressions of the cells which line the ovisac. When water is added it rapidly distends and effaces the convolutions, but I could not see any regular hexagonal facets such as have been described. A beautifully regular fine dotting, however, is seen arranged so as to enclose lozenge-shaped spaces.

Any attempt to move the egg while in its adhesive stage, exhibits the wonderful extensibility of the outer layer, the shreds of which are drawn out so as to appear homogeneous. This adhesiveness is lost, however, after twenty-four hours' immersion in water. The fine dottings may be best examined by placing eggs which have been thus macerated, in a solution of chloride of sodium, and after cutting them in halves, washing the yelk-sac and its capsule in a 1 per cent. solution of chromate of potash, so as to preserve it free from adhesiveness, and to remove the coagulated albumen *b*. In this way the outer layer appears to be continuous with, and of similar structure to the yelk-sac proper, from which it can in no way be separated as a distinct membrane. It seems probable, that the outer layer serves to fix or anchor the spawn upon the weeds or other bodies on which the female deposits them.

The micropyle is very similar to that seen in the pike. It is, however, not easy to find in water while the eggs are adhesive, but weak solutions of the alkaline chromates, or of chromic acid with chloride of sodium, destroy the adhesiveness of the outer layer, without otherwise changing the aspect of the egg, and thus it may be manipulated, and the micropyle easily found.

The Perch (Perca fluviatilis).

The eggs may be expressed from a ripe female, cohering, so as to form a long flat band. folded in zigzag. This band is a collapsed tube, a network of eggs with irregular meshes, altogether not unlike a netted bead purse.

The unimpregnated egg rapidly absorbs water so as to distend the yelk-sac and its outer layer or "eikapsel," and to form a water-chamber, while the formative yelk concentrates as in pike and salmon. The structure of the egg as a whole, is the same as in the ruffe, and the oil is in one large drop.

The yelk-sac, under which term is included the outer layer or "eikapsel" of MÜLLER*, merits a very careful examination, but I must give only a brief description of such observations as I have been able to make upon it.

Like the outer layer of the yelk-sac in the ruffe, that of the perch is probably an organ of adhesion, but in this case the eggs adhere to each other before extrusion from the parent fish, and are not adhesive after they are expelled. The time they lie in the oviduct, free from the ovisacs, and during which they are definitely arranged to form the tube, is very short, probably about twelve hours only, as I found a female at 10 P.M.

* "Ueber zahlreiche Poren-canäle in der Eikapsel der Fische," MÜLLER's Archiv, 1854, p. 186.

yielding on pressure but a very few free, not yet cohering eggs, mixed with some still within their ovisacs, and at 10 A.M. the next day, on pressure her spawn came out in the usual way, forming a flat zigzag band.

The outer layer, or "eikapsel," has a consistence much like that of fresh fibrine, is much thicker than the dotted yelk-sac, and is characterized by a radial striation. The striæ look like tubes, have a distinct double contour for each wall (Plate XVI. fig. 28), but are filled with a vacuolating material, and do not seem to convey anything, either fluid or solid, into or out of the egg. They are vertically set in a clear matrix, and terminate on the outer surface by expanded ends or mouths, arranged in a regular alternating order (Plate XVI. fig. 26). The surface of the outer layer is thrown into delicate folds, which radiate from the ends of the "tubes," and the aspect of these when viewed full face is seen in Plate XVI. fig. 31. I could not make out after very careful search, hexagonal outlines such as MÜLLER has figured, having the ends of the "tubes" placed in their centres. The appearance of a vertical section is shown in Plate XVI. fig. 27, which represents the point of junction of two eggs. At the free surface the profile view is crenated, the clear matrix forming lenticular elevations between the depressed expanded ends of the "tubes." The outer layer is separable only by tearing from the yelk-sac, and does not leave a clean surface. The "tubes" at their inner termination divide into branches like roots, and are in some way intimately adherent to the outside of the thick dotted yelk-sac (Plate XVI. figs. 29 & 30). They have no expanded funnel-shaped mouths at this inner termination, such as MÜLLER has described. Here and there in the substance of the outer layer, very delicate connecting branches pass from one "tube" to the other. The clear matrix is delicately shaded, as if faintly granular on its outer surface, which under high powers is seen laminated concentrically, and is sufficiently elastic to turn inside out, when a minute segment is cut off it. Its central substance is so translucent as often to escape detection in these segments. The appearance described by MÜLLER, of oil-granules passing along the "tubes," may possibly have been due to vacuolation in them. Be that as it may, I saw appearances capable of being so construed, in the tubes of segments which had been cut off the surface of the outer sac without touching the yelk-sac, so that it is certain nothing passed from the inside of the egg along them.

In various ways I tried to make out whether any absorption of fluids took place along them, but always with a negative result; for these experiments I used weak ammoniacal solution of carmine, solution of prussiate of potash, and then a salt of iron, and performed artificial impregnation in these fluids, that they might be present at the moment of the greatest inward current. The cleavage went on, the yelk-sac was dyed throughout, the clear matrix more so than the tubes, the germinal mass not at all after five hours. In short I satisfied myself that these tubes either do not at all serve for imbibition, or in a much smaller degree than the clear matrix, which has marked powers of absorption, swelling up so as very much to increase its thickness after long action of water and in various solutions. I also tried the unimpregnated egg with like results, after forty-

eight hours in carmine. Neither can they serve for admitting the spermatozooids, both because they are, if examined in ripe ovarian ova, full of a semisolid vacuolating matter; and because the micropyle exists as in other osseous fishes, and in the same position, marking the germinal pole. It is interesting to note that the eggs in the mass of spawn are all so placed that the micropyle looks directly towards the inside of the collapsed tube which forms the band. One effect of this arrangement must be to prevent its occlusion by contact.

In perch spawn, taken from the river, the micropyle is easily seen, after letting out the young embryo with a needle; a deposit of fine mud being usually deposited in the furrows around it, thus rendering it visible to the naked eye.

The dotted sac has a structure in all essential particulars like that of *Gasterosteus* (Plate XVI. figs. 32 & 33).

The river Bullhead (Cottus Gobio).

The eggs are held together by a viscid secretion of the oviduct, the yelk-sac is furnished with a micropyle surrounded by button-shaped processes, just as is seen in the allied genus of *Gasterosteus*.

The Gudgeon (Cyprinus Gobio).

The egg has a micropyle at its germinal pole, consisting of a conical pit perforated at its apex. The dotted yelk-sac is villous on its outer surface. The villi are soft, tenacious, easily deformed by pressure. The unimpregnated eggs imbibe water, form a breathing-chamber, and the formative yelk concentrates without exhibiting any active contraction.

The Minnow (Leuciscus phoxinus).

The eggs have a micropyle similarly placed, with a raised margin around the mouth of the funnel. In water they act just like those of the gudgeon, except that some very slow changes of the form of the yelk-ball occur.

The Chub (Leuciscus cephalus).

The egg has a similar micropyle, the margin of the funnel is crenated, and its sides are furrowed, it reacts in water like that of the gudgeon.

The Impregnated Ovum.

The Stickleback.

I purpose first to describe the changes which follow fecundation, up to the time at which the yelk commences to contract, then to relate the experiments made to show

how impregnation is effected, and afterwards to consider the conditions of the protoplasmic movements.

1. *Earlier sequences of impregnation.*

To trace the changes which follow the action of the spermatozooids upon the egg in their earliest stages, it is necessary to fecundate artificially upon the stage of the microscope. For this purpose I used POWELL and LEALAND'S animalcule-cage, a glass ring being fastened upon its lower plate, to convert it into a cell, and a portion of the thin glass cover being cut away so as to permit the fertilizing agent to be applied at its edge. The male and female fishes about to be used may be conveniently manipulated, if the spinal cord be first divided just behind the gill-covers, after which they live very well in water for forty-eight hours or more. The semen is not easily pressed from the male, and hence in these experiments it is convenient to have a number of them ready, and to open the abdominal cavity and use a fragment of testis; this, if quite ripe, will impregnate the eggs in the cell if pushed just under the cut edge of the glass cover, so as to be not too much exposed to the action of water, which soon arrests the movements of the spermatozooids. It will not do to put the male fish into water after the abdomen has been opened, but if it be kept moist the spermatozooids live some time.

a. *Formation of the breathing-chamber.*—The earliest change which occurs after an egg is fecundated, is the formation of a space between the yelk-sac and the outer surface of the yelk. This space is the breathing-chamber of NEWPORT; it commences first close to the micropyle and gradually extends over the rest of the yelk-ball*, being complete in from three to five minutes after the spermatozooids have been applied to the edge of the glass cover, in successful experiments. It begins by a withdrawal of the funnel of the micropyle from the pit in the discus proligerus, so that as water enters the funnel is gradually shortened, and at length may be almost effaced. This withdrawal was seen to begin in about fifteen seconds after the first spermatozooid was seen to enter; but ordinarily it is visible about one minute after the testis has been applied to the eggs. After the breathing-chamber has been once formed, it for some minutes longer increases in size, by expansion of the yelk-sac, so as to efface the indents on the surface of the egg, increase its size, and render it globular, tense, and elastic, remarkably resisting, and difficult to injure, the very reverse of its state before it had imbibed water.

The formation of the breathing-chamber is, I think, not entirely due to the entrance of water, but in part to a contraction of the substance of the yelk, which commonly produces a flattening of one surface. In the eggs of *Gasterosteus* it seems certain that the water mainly enters to fill the breathing-chamber through the micropyle, and under ordinary circumstances, not by imbibition through the yelk-sac. It is somewhat difficult to conceive how the passage of such a minute body as a spermatozooid through the tube of the micropyle, closed as it seems to be only by the viscid secretion of the oviduct,

* This is only ascertained to be the fact in *Gasterosteus*. In most other osseous fishes water enters freely through the yelk-sac, and the breathing-chamber may probably commence simultaneously at all parts of the surface. In frogs I believe that I have witnessed its commencement, as in *Gasterosteus*, first near the micropyle.

can determine the prompt entry of the surrounding medium, unless it is assumed that the inward current is assisted by a contraction of that part of the protoplasm with which the spermatozooid comes into contact*. I tried in vain to observe the entry with the stream into the egg, through the micropyle, of minute particles of carmine while the breathing-chamber was forming. A solution of caramel somewhat acid decomposes the viscid layer, and stains the yelk-sac, but cannot be seen to colour the fluid in the breathing-chamber; and the same result was obtained by using a salt of iron tested afterwards with prussiate of potash. It is, however, difficult to tell the colour of the contents of the breathing-chamber seen through a dyed yelk-sac. A watery solution of the extract of safflower gave a similar result, and after twenty minutes, on rupture all the contents of the egg were colourless; the inner sac was especially noted to be colourless. It would seem that the viscid layer does not entirely prevent the absorption of watery solutions of pigments into the substance of the yelk-sac. This does not, however, invalidate the conclusion as to the mode in which water enters to form the breathing-chamber. That it is the inner sac which presents the obstacle to the imbibition of aqueous solutions of colouring-matter into the yelk, is shown by the fact that eggs which have lain for forty-eight hours in the dead body of the parent, and have become slightly decomposed, permit the tint of the caramel, and of iron when tested by prussiate of potash, to appear in the substance of the yelk, in such of them only as have the inner sac ruptured, a change which often occurs in dead eggs, and will be again referred to.

b. *Concentration of the formative yelk.*—Very soon after the funnel of the micropyle begins to shorten, the formative yelk commences to undergo the series of changes which eventually terminate in the formation of the germinal disk.

In one instance, where the spermatozooid was seen to enter, the yellow droplets were distinctly paler in $1\frac{1}{4}$ minute, and an obscure puckering was visible at the same time on the surface of the discus proligerus, which, after the completion of the act of fecundation, I would call the germinal disk. Gradually all the granular and other elements of the cortical layer or formative yelk move away from the ventral segment, and concentrate into a disk at the germinal pole, where it then covers a somewhat smaller area than that previously occupied by the discus proligerus, but is thicker. The egg becomes clearer in consequence, partly through the removal from the surface of the clear food-yelk of a granular opaque layer, partly from the distension and increased translucency of the yelk-sac. At the same time the structural elements of the cortical layer undergo certain changes, which show that some slight action of water takes place through the substance of the inner sac. The yellow droplets grow paler, and disappear without distinct vacuolation, commencing to pale first at the germinal pole. Although, in a short interval of time after these changes begin, all, or nearly all the granules of the cortex are transferred to the germinal pole, and the yellow droplets either carried with them or in some way rendered invisible, I failed to see any distinct movement of them streaming towards the germinal pole.

* Such contractions have been shown by Newport to take place in the eggs of frogs.

In one instance, seven minutes after impregnation, the germinal disk was seen not yet fully concentrated, and as it presented full face I watched in vain for several minutes some of the granules at a short distance from its outer margin, to see their progress towards it. This attempt was often repeated with a like result. But as from the first moment there are slight contractions of the protoplasm, minute displacements of such granules, if observed, would not be conclusive evidence of a streaming movement. That such streaming does, however, take place is, I think, certain from the ultimate position in which the granular matter of the formative yelk is found, and I have frequently seen the granules of the cortex arranged in lines radially placed around the periphery of the then concentrating germinal disk. The germinal disk is visibly increased in bulk three minutes after fecundation, but I have no doubt that it begins to concentrate much sooner; it continues to increase until all but a very few scattered remnants of the formative yelk are collected, and it is complete some time before cleavage, for which it is the necessary preliminary.

That the disappearance of the yellow droplets from the cortical layer is due to the action of the water which has entered into the breathing-chamber, is shown by the fact that not only does it begin near the micropyle where the water enters, but it proceeds more slowly in eggs which are too scantily supplied with water.

As the concentration of the formative yelk goes on, and the discus germinativus increases preparatory to cleavage, the accumulation of minute oil-granules distinct from the large reserve oil-drops at the under surface of the discus increases.

When at various stages after impregnation the egg was ruptured, and the germinal disk in process of formation examined, which was done in various media, it was found to contain no additional structural elements beyond those in the discus proligerus before fecundation; but the yellow droplets were very few in number, or absent altogether, unless in cases where the egg had been treated with too little water, in which cases they were numerous. The same vacuolation and pseudo cell-formation were seen as were met with in the matter of the formative yelk, but no true vesicles or cells. The mass is essentially granular with a clear matrix in very small proportion, and is somewhat more solid than before impregnation.

The inner sac during these first stages appears to get thicker and firmer, at least it is more easy to observe; it is adherent to the germinal disk, over the outer surface of which it passes, and of which it probably constitutes the clear matrix.

c. *Mode of effecting impregnation.*—To ascertain the function of the micropyle, the following observations were made.

I first sought to close it by gentle pressure, while allowing the spermatozooids to have free access to all other parts of the surface of the egg. This was done with the animalcule-cage, prepared as before described (p. 457), the depth of the cell being somewhat less than the diameter of the egg. In this way a power of $\times 100$ may be used, which enables one to follow the spermatozooids distinctly.

Experiment 1.—An egg was so compressed that its micropyle which presented was

seen to be closed; a fragment of testis from a ripe male was applied to the edge of the glass cover, so that the spermatozooids came at once into contact with the viscid layer. They were then watched incessantly, for about eighteen minutes, and seen vividly moving in contact with all other parts of the yelk-sac, except near the micropyle within the area pressed upon by the glass cover. My attention was then withdrawn; seventeen minutes later they were languidly moving in the same parts, twenty minutes later they were nearly all still; the pressure was continued till two hours twenty-five minutes after the testis had been applied, when no signs of impregnation appeared, and the next day the egg was addled.

Experiment 2.—Two ova were then impregnated in a similar manner, for control, without pressure. The breathing-chamber was distinct in each in three minutes and a half, and vivid contractions of the yelk began in eleven minutes.

Experiment 3.—An egg was strongly pressed, in such a way that the micropyle being in profile, was not closed; the spermatozooids were seen in active motion quite near to the aperture, and the evidence of impregnation was discoverable before removing the pressure in the changes which the cortical layer underwent, although no breathing-chamber could be seen until the pressure was removed. This egg went on to cleavage, although it was later than normal, and the cleavage masses were irregular. This experiment was intended to show that pressure alone, if it does not close the micropyle, does not prevent impregnation.

Experiment 4.—I put seven eggs into a larger but otherwise similar cell, and applied pressure so that the cover flattened an area of each egg the diameter of which was equal to half that of the egg. To these eggs I carefully applied the testis from a vigorous male on two occasions: and the spermatozooids were seen actively moving during the twenty-five minutes I watched, but I could find no indications of impregnation having occurred. I then removed the pressure and applied a fresh piece of the testis, and in three minutes five of the seven eggs showed a breathing-chamber. Of the two failures, one at least had its micropyle so placed that it might be closed by pressure against another egg (Plate XVI. fig. 34), and the other had it looking downwards in such a position on the inclined stage of the microscope, that the current would tend to carry the spermatozooids away from it.

Experiment 5.—I put four eggs of *Gasterosteus pungitius* (which are clearer and rather better for this inquiry than those of the three-spined species) into the cell without pressure and fertilized them. I watched closely one egg, which was placed with the micropyle in full face, so that the aperture at its apex was well seen. Spermatozooids were seen approaching and entering the funnel, and one was watched till it disappeared, apparently in the direction of the interior of the egg, just at the moment when it seemed to occupy the aperture at the apex of the micropyle. Immediately after, the depth of the funnel began to diminish, and a breathing chamber commenced to form; two or three more spermatozooids were less distinctly seen playing about in the apex of the funnel as it was shortening: one of these appeared to become still before it vanished, apparently

inwards. The breathing-chamber was complete in five minutes, and the funnel of the micropyle was effaced in fourteen minutes. During the first shortening of the funnel it seemed as if the aperture at its apex also became smaller, but this appearance may have been deceptive. This experiment was repeated, the spermatozooids seen moving in the apex of the micropyle, and in half a minute a breathing-chamber began to form.

Experiment 6.—I then impregnated similarly five eggs, using no pressure, and noted that one egg, which was so placed that the mouth of the micropyle was directed towards the stream carrying the spermatozooids, was the first to show indications of being impregnated. I saw in this case the spermatozooid enter the mouth of the funnel, but could not in this position of the egg trace it any further. This egg showed the breathing-chamber $1\frac{1}{2}$ minute after the testis was applied; in two minutes more all the eggs showed a breathing-chamber, and in every case those eggs which had their micropyles directed from the current were the latest to give evidence of being fertilized.

Experiment 7.—I placed four more eggs in the cage, and applied a fragment of ripe testis to the edge of the cover, using no pressure; one egg was so placed that the micropyle could be viewed full face, and the aperture at its apex was brought into focus; this egg was in the second row, so that the current being diverted by the upper row and flowing quickly in consequence of the inclination of this stage, carried the spermatozooids wide of it. I watched carefully and painfully for seven minutes; no spermatozooid approached the micropyle, and no trace of a breathing-chamber appeared. I applied a fresh fragment of testis and watched closely for nine minutes longer, still no spermatozooids were seen near the funnel, and no change was seen in the egg, although other parts of the egg were in contact with active spermatozooids. I then put another piece of testis to the edge of the cover, and turned the cell the other way upwards, so that gravity tended to bring the seminal particles back to the egg, which was constantly and carefully watched. In two minutes I saw an active spermatozooid enter the apex of the funnel and disappear as if inwards: a quarter of a minute more had not elapsed before the clear bright circle, which marks the aperture, became indistinct from shortening of the funnel: during the next two minutes I saw three more spermatozooids enter the apex, and vanish apparently inwards: $1\frac{1}{2}$ minute after the appearance of the first spermatozooid in the funnel, the yellow droplets became paler: the breathing-chamber was complete $3\frac{1}{2}$ minutes later, and the usual vivid contractions of the yelk appeared in fifteen minutes. The eggs, two in number, which were in the front row were impregnated by the first application of the testis. Thus for eighteen minutes, active, moving spermatozooids were seen in contact with the yelk-sac, but not in the micropyle, and no sign of impregnation appeared; yet in a quarter of a minute after one was seen to enter, the indications of perfected impregnation began and went on in the usual way. The fourth egg in this experiment was not impregnated; it lay in the back row, its micropyle closely pressed against one of the eggs in the front row, so that the access of spermatozioods was rendered difficult. These results leave no room to doubt that the function of

the micropyle is what its position and structure suggest, viz. to admit the spermatozooids to the surface of the yelk.

It may be here mentioned, although it adds little to the strength of the evidence adduced, that ripe eggs yet within the ovisacs cannot be impregnated. All attempts to see the spermatozooids in the breathing-chamber failed; nor is this to be wondered at, as the funnel of the micropyle dips so deeply into the pit in the granular opaque discus proligerus, that it is impossible to see its apex clearly until it is withdrawn to some extent; thus the first moments of the entry of the spermatozooids are lost, and their extreme minuteness and delicacy, as compared with the egg, add to the difficulty of the observation. It was observed during these experiments that the spermatozooids continued to move freely for twenty minutes or more in the viscid layer, but became still very soon if they had first to float a very short distance through water. The surer plan therefore is to apply the testis while only a little moisture covers the egg, and afterwards to fill the cell with water.

d. *Relation of these sequences to the surrounding medium and to the Spermatozooids.*—An accident occurred during these observations which shows how well the spermatozooids continue to move in the viscid secretion of the oviduct. Eggs yet within the parent fish were unintentionally fertilized by applying forceps which had just before held a piece of testis to the sexual orifice of the female.

I was thus led to make an experiment with a view of ascertaining what share water had in inducing the changes which follow impregnation.

I fertilized the ova yet in the oviduct of a three-spined female, by applying to the sexual aperture a fragment of testis from a ripe male. In ten minutes some of these eggs, pressed out and examined without water, were found to have a concentrated discus germinativus, and the yellow droplets had disappeared from the cortical layer; the breathing-chamber was not, however, distinct, partly in consequence of the strong refraction of light, partly from its small size; but on adding water it was at once apparent, so promptly, indeed, that it must have been present before. The fish was then covered with oiled silk and put aside, and eggs pressed from her twenty-eight minutes after impregnation were found contracting. Forty-eight minutes after they were still contracting, and then water was added, under observation, to see if it increased the activity of the movements, but such result was not observed. Two hours and a quarter after, more eggs, pressed from the fish, were just about to cleave. Water being then added, to make the object more distinct, the funnel of the micropyle was seen dipping into the deep pit of the discus germinativus, thus proving how imperfectly the yelk-sac had distended.

Five of these eggs which had not touched water were put into pure nut-oil twenty minutes after impregnation; five minutes later they were seen contracting, and at two hours ten minutes after impregnation four out of the five were cleaving. Thus concentration of the formative yelk, formation of a small breathing-chamber, and even cleavage may occur without the presence of water, if maternal fluids are present. But I ought

to add, that the contractions seemed to be scarcely so vivid as in eggs normally fecundated.

With the view of testing whether any changes were due to the mechanical action alone of the spermatozooids, I tried to cause the micropyle to be forced by minute animalculæ, but could not succeed in any instance, in consequence perhaps of the animalculæ being all somewhat larger than the spermatozooids. No better result followed similar attempts with the spermatozooids of *Lissotriton punctatus* and of *Unio tumidus*.

If eggs be exposed to water without being fertilized, the viscid layer prevents its action to a great extent, for they may be left in it two or three hours without losing their flaccidity; after a still longer time they imbibe a little, even before the viscid layer has lost its characteristic properties; in so doing they become rounded and more tense, the yellow droplets become paler, but do not vacuolate: an imperfect concentration of the formative yelk occurs. If the unimpregnated eggs be submitted to a stream of water of considerable strength, and for some time, by which means a part of the viscid layer is removed, although no effect is seen at once, yet in an hour a good breathing-chamber appears, and the formative yelk is concentrated.

Some ill-understood changes take place in the eggs after the death of the parent, which diminish the readiness with which they may be impregnated. I kept a dead ripe female moist for $19\frac{3}{4}$ hours, and found that only five, out of ten of her eggs, could be fertilized, although all the ten seemed alike; four hours later, only two out of seven could be fecundated. The testis of a male was used successfully after it had been dead twenty-one hours.

If a dead female be kept moist forty-eight hours in summer weather, the eggs inside her become a little decomposed, and then a breathing-chamber soon forms when they are put into water; the formative yelk concentrating, the yellow droplets vanishing at the same time.

Thus it appears that although in *Gasterosteus* the formation of a breathing-chamber and the concentration of the formative yelk, under normal conditions, only occur after fecundation, yet they are only an indirect consequence of the action of the spermatozooids, which act by favouring the entrance of the surrounding medium into the cavity of the egg.

2. *Later sequences of impregnation.*

a. *The yelk contractions* are the most striking of the phenomena which follow the entrance of the spermatozooids into the egg. They may be watched with a $\frac{1}{2}''$ lens, or better, with the compound microscope, using a power of $\times 50$ or $\times 100$, and may be spoken of as rhythmic yelk contractions. From the first moment of entry of the spermatozooids, slow contractions of the yelk begin, and assist in the formation of the breathing-chamber, causing first a flattening of the surface of the yelk near the germinal pole, and afterwards slight changes of outline due to travelling waves at other parts of the surface, but not before the breathing-chamber has reached that part. Gradually more vivid contractions commence, at various times after fecundation, according to the

temperature. In warm weather they have been noted in six minutes, in cooler weather in fifteen or twenty minutes after impregnation. They cause a flattening of one side of the yelk-ball, to see which it is often necessary to roll the egg over (Plate XVI. figs. 35 & 35′). The flat surface gradually becomes a sulcus, giving a reniform outline to the yelk (Plate XVI. fig. 36). It then extends all round, giving rise to a dumbbell shape (Plate XVI. fig. 37). This sulcus, which may be termed equatorial, travels with considerable but variable rapidity towards the germinal pole, producing as it passes on, the flask form (Plate XVI. fig. 38). The sulcus is lost by passing forwards to the germinal pole, not by relaxation. It is seen for a brief space affecting the thickness of the germinal disk only, to which it gives a nipple-like form, while the food-yelk is round (Plate XVI. fig. 39). When effaced, the whole yelk-ball is globular and at rest, the germinal disk being no longer prominent (Plate XVI. fig. 40). This series of forms recurs with more or less of regularity, and with some variations both of time and form, about fifteen or twenty times; each series being the result of a travelling wave. About five waves pass in ten minutes. Sometimes a wave commences as usual near the equator, and then for a short space passes towards the ventral pole; but it soon returns, and passing forwards towards the germinal pole, is then lost; occasionally other irregularities occur, such as two or even three waves travelling at the same time, a new one having commenced before the previous one had ceased (Plate XVI. figs. 41 & 42). This is more often the case in warm weather.

The concentration of the discus germinativus is somewhat greater as each wave comes to that pole, although some diffusion occurs again, always as the round form is reproduced (Plates XVI. & XVII. figs. 35 to 49 inclusive). The contractions continue, although gradually declining in vigour, up to the period at which cleavage begins, after which I could not trace them beyond the area of the germinal disk. (See Plate XVII. figs. 44 to 49 inclusive, which are drawings made at short intervals, until the commencement of cleavage; and show some singular forms of the germinal surface of the food-yelk, which are difficult to understand, as contractions of its substance. The figures show also the constantly recurring elevation and depression of the germinal disk caused by the travelling waves.) The periphery of the germinal disk is perpetually varying, being now sharply defined, now shaded off and diffused, but it always has a circular outline.

Coincidently with these contractions, oscillation of the whole yelk-ball takes place. At first this is so slow that it requires the use of a cobweb micrometer. As the contractile waves increase in vigour and rapidity, the oscillations quicken.

During the early feeble contractions, the micropyle, except in cases where from deficient supply of water the funnel is not quite withdrawn from the pit in the germinal disk, has its position changed relatively to the germinal pole of the yelk, by a slight imperceptible swing of the latter.

With the *vivid* contractions begin visible oscillations of the yelk-ball, so that its germinal pole swings through about 60°, usually in a plane, which cuts the micropyle, and which may be vertical, horizontal, or inclined, but is not a true plane, as the germinal pole describes an ellipse.

Somewhat similar movements in the eggs of the pike have been spoken of as rotations, but in those of *Gasterostei* there is no rotation on the polar axis; as I ascertained by carefully watching fixed points, on each side of a spider thread, placed so as to correspond to the polar axis. However, it is not intended to deny the occurrence of occasional slight rotations on any axis of the egg, during the various and irregular contractions which occur. The yelk rests on the lower part of the yelk-sac, being of greater specific gravity than the water in the breathing-chamber, and the oscillation takes place from the point of rest.

The oscillations are caused by the contractions of the yelk. Not only do they commence with the beginning contractions, and become pronounced as the latter become vivid, but each commencing wave is shortly followed by an oscillation. In one observation six to and fro oscillations were counted in thirteen minutes, each corresponding to a travelling wave; this was during the second quarter-hour after impregnation. During the next half-hour, the contractions being less vivid, thirteen to and fro oscillations, proportionally more limited in extent and rapidity, were counted, each corresponding to a wave. Gradually, as the contractions became feeble, and limited to the germinal surface of the food-yelk, the oscillations ceased, and the germinal pole became stationary, about 35° from the micropyle. In short, the contractions measure the oscillations, so that irregular contractions cause irregular oscillations. In one instance, where two waves began on the left-hand side of the yelk-ball, so that the first had not ceased before the second had begun, there were two oscillations to the right, and none to the left.

I could not always with certainty connect the direction of each oscillation with the position of each commencing wave, yet, as a rule, the germinal pole swung to the right if a sulcus appeared on the left side of the yelk-mass, and *vice versâ*, provided the sulcus was in the germinal hemisphere, or near the equator; but when the first depression of the surface was in the ventral hemisphere, the oscillation carried the germinal pole to the same side as the sulcus. The result was, however, often modified by the direction of the wave, as well as by the rapidity at which it travelled.

The oscillations were influenced occasionally by oil-drops of larger size than usual, adhering to the germinal disk, and making it float uppermost; then, the oscillations were in a vertical plane, or nearly so. The yelk-ball is of nearly equal specific gravity throughout, as it retains any position it may be placed in, the oil-drops usually floating so freely in the outer portion of the food-yelk, that they move up to the top during an oscillation or any other movement.

It is therefore a fair inference that the oscillations depend on the contractions, which by altering the form of a globular mass of nearly equal density throughout, and partly floated, displace its centre of gravity, and determine the movement to restore the equilibrium. The onward movement of the wave would further modify the result.

The unimpregnated eggs, in a ripe female which had been dead forty-eight hours and kept moist, were a little decomposed, and in many instances had the inner sac ruptured, so that the whole or a portion of the food-yelk had escaped into the cavity of the

yelk-sac, and the inner sac had shrunk to a variable amount. Over the partly emptied inner sac, where it still contained some food-yelk, a wave of contraction was sometimes seen to pass slowly, but distinctly and repeatedly. The observation was made in several eggs.

Thus the contractions of the yelk in *Gasterosteus* are independent of impregnation, although ordinarily they are only seen in fecundated eggs, and they may continue long after all vital processes might be supposed to have ceased, and while all around, and in contact with the contracting matter, is decomposing.

b. *The cleavage*, which is limited to the germinal disk, begins usually about two hours after fecundation, although sometimes as early as $1^h\ 25^m$, or as late as 4^h, varying chiefly with the temperature.

The formative yelk, having been concentrated as described, varies considerably, as to its form, at the moment when it is about to cleave. It may be flat and somewhat diffused, with its periphery well defined, or not, it may be prominent and conical, or hemispherical; in short those modications of its form which result from the then fading remnants of the waves of contraction, are still going on at the moment when a fresh set of contractions begin, viz. those which result in cell-formation. Plate XVII. fig. 49 shows its usual aspect immediately before cleavage, and the moment when the first cleft is beginning is shown in Plate XVII. fig. 50.

Adhering to the under surface of the germinal disk is the group of minute oil-granules, which are more numerous at this stage than at an earlier one; and as there is a constant consumption of the stock of oil in the group of larger drops, during the development of the germinal mass, it appears probable that at the surface of contact between the two kinds of yelk, a digestion of oil, so to speak, goes on; the process having for one of its constant phenomena a subdivision of the oil into minute granules. The remarkable appearances which attend the vacuolation of the matter of the formative yelk have often, irrespective of the evident absorption of oil into its substance during development, led me to infer that it was a compound, containing some fatty substance, easily separable from its associated matter. As to its structural elements, the germinal disk differs in this stage in no other respect from its earlier condition; but when crushed and examined under higher powers, a few yellow droplets are seen in it, and it is more solid than it was.

The cleavage begins in a faint well-defined line, which, seen in profile, appears as a notch, dividing the germinal disk into two equal halves (Plate XVII. figs. 50 & 52). This deepens and gradually separates the germinal disk into two conical elevations (Plate XVII. figs. 51 & 53). Even during the cleavage, constantly recurring, very slight waves of contraction go on, change the form of the cleavage masses, and cause the periphery of the germinal disk to vary. The two first cleavage masses, after the stage of greatest separation (Plate XVII. fig. 51), approach each other and appear as if about to fuse; this would seem due to the yelk contractions; it is, however, common, if not constant, just before the next cleft begins (Plate XVII. fig. 54).

The inner sac is thrown into folds at the margin of the cleft during its formation,

reminding one of the "Faltenkranz" described by REICHERT*, and M. SCHULTZE†, in the frog's egg (Plate XVII. figs. 55 & 56).

The cleavage masses at no time can be seen to contain any nucleus, vesicular or solid, nor could I find any in the germinal disk prior to cleavage. After twenty hours the germinal mass consists of a cup-shaped group of cell-like corpuscles, the result of repeated segmentation, seated upon the germinal pole of the yelk (Plate XVII. fig. 57), without any differentiation of parts. It is closely connected with the inner sac, which may be seen at its outer boundary forming radially arranged folds. The mass is solid, and its elements cohere with some tenacity, but on rupture in water the cell-like corpuscles in part separate, and as they float away undergo vacuolation.

The surface of contact of the germinal mass with the food-yelk is difficult to study. It seems to be merely the corpuscles resulting from segmentation in contact with the fluid food-yelk.

It is not without interest to note, in passing, how frequently, from slight causes, among which pressure seems to be the most important, an asymmetrical cleavage occurs; and the possibility of artificially inducing the formation of monsters is thus suggested (Plate XVII. figs. 56 & 58). One egg, in which the irregular cleavage had been seen, showed on the eighth day a well developing embryo, about to burst the yelk-sac, but no visible deformity.

3. *Conditions of the Yelk Contractions and of Cleavage.*

a. *Poisons.*—Experiments were made on impregnated ova of the two species of *Gasterosteus*, to ascertain how far the contractions of the yelk, and the cleavage were influenced by poisonous substances.

Hydrocyanic acid, when very dilute, produced no visible effect upon the rhythmic contractions of the yelk, but caused a little delay in the commencement of cleavage. When used a little stronger it slowly produced rupture of the inner sac, without previously seeming to influence the rhythmic movements; and it delayed still more the cleavage in those eggs which had not ruptured the inner sac. When used still stronger it arrested the rhythmic contractions, but at the same moment caused bursting of the inner sac and opacity of the yelk.

Atropia.—A supersaturated aqueous solution had no apparent effect on the yelk contractions or on cleavage.

Aconite.—The spirituous extract, mixed with water, did not influence the yelk contractions, but retarded the cleavage. The next day the yelks had undergone chemical change.

Strychnia.—The aqueous solution had no apparent effect.

b. *Galvanism.*—The following observations were made to ascertain the influence exerted by the galvanic current upon the yelk contractions, and on cleavage. In my

* Archiv für Anatomie, Physiologie, &c., 1861 and 1863.

† Observationes Nonnullæ de Ovorum Ranarum Segmentatione, 1863.

previous paper it was said to have no effect on the yelk contractions. I used then a single cell containing $\frac{1}{2}''$ of gold wire, and the same of zinc, excited by a solution of chloride of sodium. A like negative result was obtained, with reference to the cleavage, while using a cell containing carbon and zinc, each 2 square inches, excited by common salt.

Later experiments in May 1864 with more powerful currents and better appliances have reversed the result.

I used an induction apparatus made by STOEHRER, which admits of nice graduation, and has as much power as is usually required for medical purposes.

A cell was prepared, the idea for which had been suggested by KÜHNE*, of sufficient depth to permit the eggs to be covered, without pressure, and the conductors were so suspended that the movements of the stage of the microscope were unimpeded. A contact breaker was introduced into the circuit, so that it could be closed for any required time without touching the stage or removing the eye from the microscope. A very weak secondary current was employed, using only $\frac{1}{2}''$ of metallic contact, and pushing the coil as far off the core as possible; the shocks were then barely felt by the moistened fingers. The primary current was found to be less suitable for these experiments, being less perfectly graduated.

An egg, fifteen minutes after impregnation, was placed between the poles in the cell, and watched for a minute or two with a power of $\times 75$. The yelk was seen to be languidly changing its form, by flattening one segment; no travelling waves were present (Plate XVII. fig. 59). On then making contact for about $\frac{1}{4}$ minute, there appeared, after a very brief interval, which I could not accurately measure, a deep notch surrounded by radial foldings of the inner sac in that part of the yelk-ball nearest to the platinode (Plate XVII. fig. 60). The tardiness of this reaction made it impossible to note the relative effect of making and breaking contact.

After another similar application of the current, another notch formed near the first, and directly after, the inner sac burst at a point distant from the notch, and the food-yelk escaped (Plate XVII. fig. 61).

Other applications of the current were followed by indentation of the yelk, on the side opposite to the first formed one, and the inner sac burst near the new indents; the escaping food-yelk showed signs of chemical change, being very granular from electrolysis (Plate XVII. fig. 62).

These excited contractions were followed by oscillations.

At the rupture in the inner sac the torn edge is well seen, as it retreats during the shrinking of the yelk-ball; it is often folded and ragged, sometimes drawn into threads.

The circuit being again closed as before, the inner sac shrunk into a lobular mass, which contained the greater part of the formative yelk, and a little food-yelk, which was seen to escape more rapidly under the influence of the current. Electrolysis was more marked in the yelk of both kinds (Plate XVIII. fig. 63). After this stage repeated

* Untersuchungen über das Protoplasma und die Contractilität, 1864, p. 147.

applications of the current were made, but they were only followed by slow shrinking of the remaining pouches of inner sac, by which the food-yelk and even the oil were squeezed out, and the whole mass at length became darkly granular (Plate XVIII. fig. 64).

I afterwards ascertained that one application of the current, sufficiently strong to cause a deep indent, was generally followed after a time by rupture of the inner sac, and all the further changes above described. Repeated applications of the current hastened them however.

It was somewhat difficult to adjust the strength of the current, so as to excite well-marked contractions, and yet not cause rupture; however, by using the smallest amount of metallic contact requisite to put the machine in motion, by pushing the coil quite off the core, and by closing the circuit for only two to five seconds, I obtained trustworthy results.

With these, which may be called zero-currents, I excited peristaltic waves, distinguishable from the normal ones by their greater depth, abruptness, rapidity of formation and of progress, by the varying directions in which they travelled, and the positions at which they originated. An excited contraction may begin near to either electrode, or distant from both; the sulcus may be in the direction of the current as in Plate XVIII. fig. 65, or at right angles to it, as in Plate XVIII. fig. 66. Such waves may be equatorial or meridional, as the same figures show. The zero-currents cause no observable electrolysis, and are slower in exciting the contractions than the stronger currents are.

The position of the rupture in the inner sac varied much; it had no constant relation to the electrodes, or to the poles of the yelk-ball. Sometimes it took place near to the indent, especially if the current was strong; sometimes at the part of the yelk-ball most remote from the contraction, and then it was preceded by a protrusion and distension of the inner sac, which exhibited a marvellous extensibility.

Ova which had arrived at that stage, when, being about to cleave, their natural contractions had nearly ceased, contracted in a similar manner, but required perhaps somewhat stronger shocks, and the interval which elapsed between the application of the galvanism and the commencement of the contraction was rather longer. Ova in the second stage of cleavage, when the normal contractions had ceased, were markedly contractile on the application of moderately strong currents. Unimpregnated ova, when submitted to a moderately strong current, soon imbibe water, form a breathing-chamber and contract; and then rupture of the inner sac and electrolytic changes are very apt to occur near the electrodes.

The excited contractions, although, like the normal ones, they began almost constantly upon some part of the outer surface of the food-yelk, extended afterwards to that surface which lies in contact with the germinal disk.

Neither the germinal disk, nor the separate cleavage masses could be made to exhibit any contractile movements by galvanic irritation, although certain changes of their form

appeared, which were due to the contractions of the food-yelk with its covering. Electrolytic changes, however, appeared very readily in the substance of the cleavage masses, which became regularly crenate at the margin, as if composed of small globular cells (Plate XVIII. fig. 70). The current used in this observation decomposed water.

Ova nine minutes after impregnation, before visibly moving contractile waves had commenced, contracted to the zero-currents.

The cortical layer of the food-yelk being carefully examined at 40^m and at $2^h\ 30^m$ after impregnation, with a power of $\times 195$, it was found that a few scattered granules and yellow droplets always remained, which had not been collected into the germinal disk; and these, on rupturing the inner sac by a strong current, were observed to retreat with it from the broken part, and to undergo vacuolation at the same time. In this way, better than by any other, the intimate connexion which exists between the inner sac and the remnants of the formative yelk where it is spread over the food-yelk, is shown.

c. *Heat.*—Some observations were made to ascertain how far abstraction of heat diminished the susceptibility of the contractile material of the yelk to galvanic stimulus.

Control experiment.—Ova were fertilized and kept, some in cells, others in capsules, at the temperature of the room, 58° F. After thirty-five minutes they were actively contracting and rotating in the usual way; they completed the first cleft after $2\frac{1}{2}$ hours, and the second after five hours. The germinal mass was in the fine mulberry stage of cleavage after twenty-four hours, and then no contractions of the yelk were seen, but zero galvanic currents excited them distinctly in the yelk, without causing any movements in the germinal mass.

Experiment *a*.—Some of the same ova fifteen minutes after impregnation were placed in a chamber cooled to 45° or 48° F.: forty-five minutes after, they were contracting normally but languidly, and responded to the galvanic current apparently as well as did the ova in the control experiment.

Experiment *a'*.—Some of the same ova, two hours after impregnation, were put into a chamber cooled to 40° F. The first cleft was not completed until $3^h\ 45^m$ after impregnation; so that cold, even when it does not act until late in the stages which precede cleavage, retards its progress.

Experiment *b*.—Some of the ova which had been cooled to 45°–48° F. were, one hour after impregnation, further cooled, so that the thermometer on the cell stood at 32° F., the water not being frozen. The yelk-ball became round and still, but zero-currents of galvanism somewhat slowly excited very distinct contractions. In this experiment, however, the cell was rapidly receiving heat from the stage of the microscope, as I had then no means of maintaining it at a constant temperature.

Experiment *c*.—Some of the stock of control ova were, one hour after impregnation, cooled so that the water was nearly all frozen, as well as some of the eggs, which were then allowed to thaw. Those which had been frozen were decidedly opake, and various

degrees of opalescence were seen in most of the eggs. Examined by a power of × 35, the opaque ova had their inner sacs ruptured and shrunken in degrees, varying with the opacity, the discus germinativus being lobular and darkly granular. Those ova which were faintly opalescent only, exhibited but slight shrinking of the inner sac, which had evidently healed soon after rupture; the site of this was marked by a deep pit surrounded by radial folds. Gradually, under the influence of the warmth of the room, the slight contractions natural to this stage returned. Zero-galvanic currents produced strongly marked contractions in these eggs. Those which were only thus partially ruptured, cleft for the first time about five hours after impregnation, but the masses were not symmetrically arranged, so that perhaps in this way also monsters may be formed (Plate XVIII. fig. 71).

Cold, then, delays the changes which follow impregnation, but does not, within those limits which fall short of mechanical rupture and complete derangement of the structure of the egg, destroy irritability. Observations are wanted, however, with eggs cooled down to the state described in experiment *b*, upon an insulated stage, kept at the required temperature, while the galvanic current is applied.

Observations were then made on the effects of elevated temperatures, upon the movements of the yelk, and on the cleavage.

Control experiment.—Ova impregnated and kept at the temperature of the room (58° F.), were 30^m after, contracting and rotating slightly, 45^m after, vigorously, $2^h\ 30^m$ after, were not cleft, but $6^h\ 30^m$ after, were cleft into eight masses.

Experiment *d*.—Ova ten minutes after impregnation, being warmed on the stage of the microscope to about 73° F., at first did not seem to be influenced; but after ten minutes' continuance of the warmth, they were seen to be moving more rapidly than those of the control experiment. The temperature was then raised to about 80° F., which, after some minutes, produced a state of almost complete rest, with the yelk-ball globular, the discus germinativus nipple-shaded, and the oil-drops displaced. At the same time the control ova were vigorously contracting. Being then removed, and left at the temperature of the room, they completed the first cleft at $1^h\ 50^m$ after impregnation, and by $6^h\ 30^m$ after, were cleft into the coarse mulberry stage, and much in advance of the control ova; a fact, the more remarkable, because the contractions had been arrested for a time by the highest temperature used. In this experiment the thermometer was laid upon the cell, but probably indicated a temperature somewhat higher than that reached by the eggs.

Experiment *e*.—Ova ten minutes after impregnation, put into a chamber, warmed to 83° F., and kept there for twenty minutes, were found actively contracting. In this case I had reason to think that they did not reach the temperature of the chamber, which was then heated to 102° F., and after some minutes, when the control ova were actively contracting, these eggs became relaxed and still, so that their globular yelk-balls filled the yelk-sac, and effaced the breathing-chamber, and the oil-drops were displaced and scattered. On cooling the eggs again slowly to 58° F., the contractions reappeared in

five minutes, but were languid. Cleavage began six hours after impregnation, the masses being arranged irregularly and without symmetry.

Another mode is thus suggested by which monsters may be formed. Some of these ova were left in the chamber at 102° F. for 50 minutes. They became opalescent, their inner sacs ruptured and shrunk up.

Experiment *f*.—Ova one hour after impregnation were warmed in a cell, upon a metal plate, the thermometer resting upon which stood at 103°–104° F. In $2\frac{1}{2}$ minutes they began to be opalescent, and in four minutes they were opake; the yelk-ball was round and filled the yelk-sac, and the inner sac was not ruptured. The opaque yelk not being coagulated, diffused in water, on cutting an egg through. In this mode of applying the heat, the eggs approached more nearly the temperature indicated by the thermometer.

Experiment *g*.—Ova $1\frac{1}{2}$ hour after impregnation, put for 5 minutes in a chamber at 90° F., had their yelk-balls globular, relaxed, so as to fill the yelk-sac, and their oil scattered and displaced towards the periphery; but were not at all opaque. Being then replaced in the chamber, warmed to 103° F., they soon became faintly opalescent to the naked eye, and when examined with a power of $\times 75$ the yelk-balls were found to fill the yelk-sac; even the germinal mass was diffused and the oil scattered in small drops, but no coagulation was visible. These eggs were gradually cooled to 58° F., and soon contracted so as to form a breathing-chamber; no contractile waves appeared, but the germinal disk concentrated. They were again put, but only for a few minutes, into the chamber heated to 109° F., when again the yelk-balls became globular, and effaced the breathing-chamber; again, as they cooled, they contracted so as to cause its reappearance. Once more they were put into the chamber at 110° F., when a faint increase of opacity was visible, and being removed to the metal plate, at 103° F. they all shortly became opaque. In this experiment, I have no doubt that the thermometer in the chamber indicated a temperature considerably higher than that reached by the eggs.

Although the difficulties which stand in the way of warming the yelks to a given temperature, and maintaining them there, were not satisfactorily overcome in these experiments, it is, I think, fair to infer that a moderately elevated temperature quickens the yelk contractions, and hastens the commencement of cleavage. It is probable that 75° F. is somewhere about the upward limit of this temperature.

A higher temperature, which begins probably about 80° F., arrests the contractions, and relaxes the yelk-ball, which on cooling recovers itself, unless the heat has been carried too far. This limit was not made out with certainty, but is probably about 100° F. Imperfect coagulation of some of the contents of the yelk, or at least a granular precipitation, occurs at about 103° F.

d. *Oxygen and carbonic acid.*—The question which I have attempted to answer is this; Is oxygen in the surrounding medium a necessary condition of yelk-contraction and of cleavage?

Control experiment 1.—Ova five minutes after impregnation were placed, in a large

capsule, in tap-water and lightly covered. At thirty minutes and at forty minutes after impregnation, the contractions were observed to be normal; at 1^h 10^m they were almost limited to the neighbourhood of the germinal disk, and its surface directed towards the food-yelk; at two hours cleavage began; at 2^h 40^m progressing, the "Faltenkranz" distinct; at 3^h 30^m the second cleft commencing; at five hours complete; at eight hours there were thirty-two cleavage masses; at twenty-four hours the germinal mass was composed of minute cell-like corpuscles.

Control experiment 2.—About six ova were impregnated, and kept in a lightly covered cell in tap-water. At thirty-five minutes after impregnation they were contracting normally, at 2^h 15^m were about to cleave, at 3^h 30^m the first cleft was completed, and the second about to begin; at twenty hours after, the germinal mass was a cluster of corpuscles having the general aspect of cells.

Experiment 1.—Into a similar cell, five minutes after impregnation, an equal number of ova were put in ordinary aërated distilled water. The cover was sealed with hot wax and lard, an operation which lasted about two minutes. The eggs were then compared with their control ova, at thirty minutes, at forty minutes, at 1^h 10^m, at two hours, at 2^h 40^m, at 3^h 30^m, at five hours, at eight hours, and at twenty-four hours after impregnation, and were on each occasion found to be progressing equally with them. Accidentally one or two of these eggs were injured, so that the inner sac broke, and partially emptied itself, but cleavage went on in an irregular manner, although with a rapidity equal to that observed in uninjured ova.

Experiment 2.—An equal number of ova, twenty-five minutes after impregnation, were put into a similar cell, in distilled water which had been well boiled, and the cover was sealed as before. At forty minutes, at one hour, at two hours, at 3^h 30^m, and at twenty hours after impregnation, they were found to be progressing, equally well with their control eggs.

Experiment 3.—The same as the above, using water in the cell which had been saturated with hydrogen after having been boiled. At forty-five minutes, at one hour, at 1^h 55^m, at 2^h 15^m, at 3^h 30^m, and at twenty hours after impregnation, they were also found to be progressing, like the eggs in the control experiment.

Experiment 4.—The same as the last, using distilled water impregnated with oxygen. At forty minutes, at 1^h 10^m, at two hours, at 2^h 40^m, at five hours, at eight hours, and at twenty-four hours after impregnation the ova were examined, and found to be quite like their control ova.

Experiment 5.—The same as the above, using water which had been moderately charged with carbonic acid, after having been well boiled. Forty minutes after impregnation, immediately after the cell had been sealed, the yelks were seen in the dumbbell form, and under observation the sulcus was effaced without travelling on, the yelk-ball becoming round, even the germinal mass ceasing to be prominent. For some minutes there was no visible movement, but afterwards, by imperceptible degrees the germinal mass was slowly reprotruded from the surface in a nipple-shaped and rather

irregular form, while the food-yelk underwent no change of form (Plate XVIII. fig. 72). None of these eggs passed on to cleavage, and all ultimately had ruptured inner sacs, shrunken into a dark granular mass.

Experiment 6.—Forty minutes after impregnation the last experiment was repeated, with a stronger but otherwise similar carbonic-acid water. The eggs were examined directly, and the yelk, which had been actively contracting, suddenly ceased to move; the sulci were effaced without travelling on, and under observation the yelk-balls became globular, in ten minutes the germinal disk being level with the surface. About fifteen minutes later the germinal mass again projected above the surface of the yelk-ball, and the further fate of these eggs was the same as in those of experiment 5.

Experiment 7 was the same as the last, using eggs $3^h\ 35^m$ after impregnation, at which time the first cleft was fully formed. They were not examined until four minutes after the action of the carbonic acid; the germinal disk was then withdrawn into the yelk-ball in a singular manner (Plate XVIII. fig. 73). In ten minutes more it was projecting again, but irregular in form; afterwards, by still slower steps, it was again flattened, but not drawn into the yelk-ball. While this went on the cleavage masses underwent a gradual fusion, which commenced as early as thirty minutes after closing the cell; and at length, $5^h\ 30^m$ after impregnation, the yelk-ball was globular and at rest (Plate XVIII. fig. 74). By degrees the diffused germinal mass became darker and more granular, and eight hours after impregnation many of the eggs had ruptured inner sacs, and the contents were changed to a darkly granular mass, which consisted chiefly of the decomposed formative yelk-substance, with some oil-drops and granules (Plate XVIII. fig. 75). All the changes of figure observed after the yelk has first effaced its sulcus, are imperceptible in progress.

Experiment 8 was the same as the last, using eggs $3^h\ 45^m$ after impregnation, the germinal disk being then cleft into four masses. The eggs were examined at once, in order to trace the steps of the first retraction of the germinal disk into the yelk, this took place under observation until the yelk-ball exhibited no prominence on its surface. (See Plate XVIII. fig. 79, which shows the complete retraction, and figures 77, 78, which show the intermediate stages: fig. 76 is given as a normal standard.) I could not ascertain that any changes of form took place in the mass of the food-yelk, and the explanation of the withdrawal of the germinal mass into the food-yelk is at present not made out. During the next ten minutes, by invisible movements, the germinal disk became again prominent, the cleavage masses being irregular in form and wanting in symmetry. Much more slowly after this, sinking of the germinal disk began, and gradual fusion of the cleavage masses; so that five hours after impregnation, or $1^h\ 15^m$ after the action of the carbonic acid, some of the eggs had still prominent and irregular germinal disks, but all showed more or less fusion of the cleavage masses, and by $6\frac{1}{2}$ hours after, the fusion was complete. It was not, however, till eight hours after, that the germinal mass was quite reduced to the state shown in Plate XVIII. fig. 74. Later still, in many, but not in all the eggs, rupture of the inner sac and dark granular precipitation occurred.

The slow reprotrusion of the germinal disk and commencing fusion of the masses are shown in Plate XVIII. figs. 80, 81 & 82, and the ultimate flattening and fusion of the cleavage masses in Plate XVIII. figs. 74 & 83.

In order to compare the developing embryos with the developing early germs in their relations to oxygen in the surrounding medium, particularly with reference to the relative need for oxygen shown by striated muscle and protoplasm during their contraction, I made the following additional experiments.

Experiment 9.—One young stickleback, three days hatched, was put into a similar cell in tap-water and sealed; 1^h 30^m after, it was quite strong, and at 4^h 30^m after, it could still swim about, although rather less vigorously.

Experiment 10.—Two such young fishes were sealed in a similar cell, in some of the boiled distilled water before used. One of them was accidentally injured, it ceased to swim in a few minutes; the gills became still first, and the heart, although it did not contract more than thirty minutes, last. The uninjured fish swam about for thirty minutes; by forty-five minutes it had turned on its back, by 1^h 30^m the gills and fins were still; the circulation ceased in two hours, but the heart continued for 2^h 30^m.

Experiment 11.—The last experiment was repeated, using but one fish. In ten minutes the gills and fins ceased to move, and the trunk to be sustained in its position, while in 1^h 10^m the heart was motionless, but the tissues were not opaque.

Experiment 12 was the same as the last, using boiled water saturated with hydrogen, and taking care to choose a vigorous fish. In ten minutes the gills and fins ceased to move, the fish turned on its back, and after fifty-five minutes the heart alone was moving.

Experiment 13 was the same as the above. In ten minutes the fish turned on its back, in twenty minutes the gills and fins ceased to move, in fifty-five minutes all movements had ceased, and the tissues were opalescent.

Experiment 14 was an attempt to find chemical evidence of the presence of carbonic acid in the water in which eggs which had passed through the earlier stages of cleavage had been immersed; but although the observation was repeated no result was obtained, and the details are therefore omitted.

Although the preceding experiments were made upon such a small mass of material, and the methods of excluding oxygen from the surrounding medium were so imperfect, it may be inferred, without much risk of error, that the proportional demand for oxygen, of equal masses of organic matter, undergoing the changes of growth and development, is much less in the early germ than in the free embryo. Indeed the rhythmic protoplasmic contractions and the cleavage were, to all appearance, quite unchecked by water deprived of most of its oxygen. The movements of striated muscle, on the other hand, were shown to be very soon checked in water similarly deprived of its oxygen.

That the excess of oxygen does not hasten the cleavage, or promote or excite the protoplasmic contractions, appears also a legitimate inference.

That carbonic acid acts as a potent poison is clearly shown, and that it relaxes the con-

tractions promptly. Also that it causes afterwards an irregular deformed projection of the germinal disk, soon followed by a state of complete permanent relaxation, and fusion of the cleavage masses into one formless mass. Ultimately it causes rupture of the inner sac and chemical change in the yelk.

The Trout.

In this fish the impregnated ova do not exhibit any visible contractions of the yelk, although a breathing-chamber forms; the formative yelk concentrates at the germinal pole, and its elements undergo changes like those in *Gasterosteus*. Cleavage did not take place in my experiments made in January 1855 until the next day. Prior to the commencement of cleavage no distinction is visible between the impregnated and non-impregnated eggs. I was struck with the great length of time which these unimpregnated eggs remained clear and unchanged in water: if the inner sac be not injured, it is at least twenty-three days; while ova kept in moist air for the same period decompose, are fœtid, alkaline, and coagulate when put into water less than a fresh egg broken in water.

The Ruffe.

Impregnated eggs of the ruffe exhibit slow changes of form of the yelk-ball without distinct oscillations. Unimpregnated eggs in water form a breathing-chamber, and show similar slight changes of form of the yelk. In four minutes the formative yelk gradually concentrates.

The Perch.

Impregnated ova of perch undergo changes of form like those of the ruffe, and the same may be said of unfecundated ova put into water.

The Pike.

1. *The sequences of impregnation.*

These ova are better adapted for the examination of some points than are those of *Gasterosteus*, and their study has helped me to correct some errors which I fell into at first.

Impregnated eggs show a commencing breathing-chamber and slight changes in the droplets of the cortical layer after about a minute; unimpregnated eggs are similarly affected by water only. After twenty minutes the breathing-chamber was complete, and the formative yelk concentrated into a well-defined discus germinativus in the fertile eggs, and the barren ones appeared in all respects the same. After three hours and twenty minutes contractions began with a slow flattening of one side of the yelk-ball and a slight oscillation. After three hours and forty minutes cleavage began, and up to this time no distinction was visible between the impregnated and the unimpregnated eggs. Four hours after deposition in water, in these latter the discus proligerus was not lobulated.

The contractions and oscillations resemble those which occur in the egg of the stickleback, but they are more frequently irregular; thus the sulcus may be represented by a circular pit, it may be a furrow parallel to the equator in either hemisphere, and may travel towards either pole; or more rarely, it is a meridional one; frequently there are two or three present travelling over the surface at the same time.

At first the yelk-ball retains any position which it may have within the yelk-sac, so that its polar axis may be vertical, inclined, or horizontal, and these positions modify the direction and extent of the oscillation. Usually, about the time of the commencement of cleavage, the oil-drops tend in part to adhere to the germinal disk, and then the polar axis is always vertical. In this position it is easy to see that the germinal pole in oscillating describes a very wide ellipse, and there is at the same time a partial rotation on the polar axis; but the oscillations vary as much as the contractions. This general description applies equally to the impregnated and unimpregnated eggs.

After seven hours, in the impregnated eggs the germinal disk was cleft into sixteen regular segments.

At the same time the unimpregnated ones showed a remarkable and very interesting lobulation of the concentrated formative yelk, a sort of irregular asymmetrical cleavage. This lobulation or pseudo-cleavage continued to increase, and to become more and more irregular. After twenty-five hours it was noted that portions of the discus proligerus were pinched off and appeared either as projecting buds, or as detached masses in the breathing-chamber. (This tendency of the formative yelk material to pinch off portions of its substance, may explain the so-called "Richtungsbläschen.") Sometimes the whole mass of the concentrated formative yelk is pinched off, and lies free in the breathing-chamber, leaving a scar at the germinal pole of the yelk-ball, indicated by the collection of smaller oil-granules which do not separate with it, and by the ragged and radially puckered edges of the torn inner sac around. It is somewhat singular that, as a rule, from this scar no food-yelk escapes. Some hours later nearly all the unimpregnated eggs exhibited the separation of the discus proligerus, but no food-yelk had escaped. After fifty-seven hours some of them had their inner sacs ruptured, and a part of the fluid food-yelk had escaped into the breathing-chamber, but the diminished yelk-ball went on contracting as before. After seventy-four hours, decomposition having made considerable progress, the contractions were visible but feeble; after eighty-three hours they required the greatest care to see them at all. In other instances I found these movements continue for 105 hours, provided some acid clear food-yelk still remained in the inner sac.

In an unimpregnated egg twenty-five hours after it had been pressed from the parent, the inner sac escaped, on rupture, in a very distinct and firm state; its surface was marked by fine dots, having much the appearance of impressions of the inner surface of the yelk-sac. I examined this surface carefully for cilia, or ciliary motion, which have been considered a possible cause of the oscillation. I used a power of $\times 250$ without finding any.

The formative yelk in these eggs breaks up ultimately into a formless, dark, granular mass.

The fecundated eggs continued to contract and oscillate vividly during the progress of the cleavage, and often, where the sulcus was strongly marked, the surface of the inner sac was beautifully wrinkled, like a ripple upon water. Fifty hours after fecundation the contractions were more active than in the unfertilized eggs, which then were seen to have vibriones upon them, appearing to be liable to decomposition earlier than fertile eggs, and to suffer in consequence some diminution of their contractility. The fertilized eggs, after fifty hours, have a germinal mass composed of polygonal cell-like corpuscles, on the surface of which I failed, with a power of $\times 250$, to detect any trace of ciliary action.

To ascertain, if possible, in what part of the yelk-ball the contractile property resided, I ruptured, by pressure, one which had been fecundated fifty hours, the contractions being at the time vigorous.

An irregular shred of the inner sac was retained within the crushed yelk-sac, and a pouch of the former, filled with food-yelk, projected from the rupture in the latter. Very active contractions were seen $1\frac{1}{2}$ hour afterwards in both of these, causing a to-and-fro movement, during which the pouch was alternately protruded and retracted from the opening in the yelk-sac; and the shred of inner sac, which had partial attachments, moved right and left, each time, seeming to alter its form somewhat: the rapidity of these motions was much greater than that seen in unbroken eggs. I counted on the shred three contractions, each causing a right and left motion in one minute, and a point on this shred passed through $\frac{1}{440}''$ in ten seconds. For six hours longer contractions continued in this ruptured egg.

The torn shred seen moving within the broken yelk-sac could scarcely have been other than a portion of inner sac, and it had precisely the same appearance, examined with $\times 250$. At this stage of development of the germ there is no differentiation of parts, and no contractile property, and on its mass of cell-like corpuscles, as already stated, no cilia or ciliary movement could be found.

It was found impossible to repeat this observation exactly, at least so far as regarded the happy accidental position of the torn shred of inner sac, although a number of attempts were made. However, I often found escaped and projecting pouches of the inner sac containing food-yelk, over the surface of which contractile waves passed, with varying degrees of rapidity; and in one instance, while endeavouring to stain an unimpregnated egg with an extremely weak ammoniacal solution of carmine, these waves were seen, an hour after it had been crushed, moving with considerable velocity. The pouch of the inner sac was in this instance so large, as to contain the greater part of the food-yelk of the egg. The contractile waves had a certain rhythm, but not a very regular one; two or more waves sometimes coexisted, and as they travelled along they proceeded in the same or opposite directions, and then if two met, a very deep angular sulcus resulted, which showed a tendency to recur at the same spot. One of

these sulci, which passed nearly halfway across the field of the microscope, equal in this case to about $\frac{1}{50}''$, was effaced in forty seconds. Three hours later these contractions were still going on, but nine hours after rupture of the egg they had ceased. During their continuance there was a gradual emptying of the fluid food-yelk and shrinking of the inner sac.

So long as in impregnated ova any portion of the food-yelk remains uncovered by the gradually advancing germinal mass, its surface is constantly moving, and the waves of contraction are seen to pass beyond the margin of the germ, and under it, to the contained food-yelk. Seventy-four hours after fecundation there remains but a small area at the ventral pole uncovered by the advancing germ. But long after the whole of the yelk is covered by the germ, at least as late as the ninth day after impregnation, slow rhythmic contractions of the contained food-yelk are visible, producing alternate depressions of the lateral poles of the abdominal region, and consequent oscillations of the embryo, which have been described by REICHERT*.

On the tenth day some of these eggs were hatched. I could not see any contractions in the food-yelk of free embryos, but the search was not very carefully made.

In the pike, as in the stickleback, eggs which have been allowed to remain in the dead parent for a certain time cannot be fertilized, even when they have undergone no discoverable change of structure. In the pike, after seven hours, the capacity of being impregnated was lost; but then a physical change had occurred, for water no longer passed through the yelk-sac to form a breathing-chamber.

On the whole, I think it may be said that the inner sac is essentially connected with the exercise of this contractile property. It is difficult to ascribe contractility, at least of this rhythmic kind, to a substance so fluid as the food-yelk, in which minute monads can swim about freely, and in which, when escaped or escaping, I never saw the slightest evidence of contractility. On the other hand, except the single observation above related, of a retained shred of inner sac in a crushed egg, I have no satisfactory evidence that the inner sac alone is capable of contracting; and that instance may possibly have been fallacious, for the closely connected pouch of the inner sac filled with food-yelk, might at each of its contractions have pulled at and moved the shred. The persistence of the movements of the food-yelk contained in the embryo, when one bears in mind that the inner sac is folded in during cleavage, and might therefore fairly be expected to be used up in the gradual extension of the germ over the yelk, would seem to give support to the notion held by REICHERT†, that the substance of the food-yelk is the contractile matter. But it may be replied that the inner sac may possibly be retained on the surface of the contained food-yelk in the abdomen of the embryo, and to this view I incline. But while it appears probable that the contractile property resides in the inner sac, I am disposed to think that the presence on its inner surface of some of its acid yelk is an essential condition of its action.

* "Der Nahrungsdotter des Hechteies,—eine kontraktile Substanz," MÜLLER's Archiv, 1857, p. 46.

† MÜLLER's Archiv, *loc. cit.*

The substance of the formative yelk, at least where it is collected into a discus proligerus or germinativus, appears to possess the same contractility in a less degree (it may be that its solidity is a hindrance to its manifestation). Certainly, as the contractile waves pass forward to become lost at the germinal pole, the nipple-shaped form which the disk assumes (Plate XVI. fig. 39) is best explained on this assumption; and if, as I conceive, the substance of the inner sac is thicker at the germinal pole, so as to embrace the whole of the discus, the production of that shape is easy to be understood.

But the formative yelk possesses also another different contractile property, by which, when acted on by water or the maternal fluids, it tends to subdivide itself into smaller masses. This property it seems to be the function of the male element to regulate.

2. *Conditions of the yelk contractions and of cleavage.*

Pike ova being obtainable in greater numbers than those of the stickleback, and being in some other respects better adapted for experiment, the inquiry into the modifying and essential conditions of protoplasmic movements was continued with them.

a. *Poisons.—Morphia.* A solution of the acetate of morphia of 2 grs. to 60 grs. of water, which had a slight excess of acetic acid, was added in small proportion to the water of a cell containing several ova, vividly contracting, thirty-six hours after impregnation. In less than a minute they ceased to move, the yelk-ball became round, no rupture of the inner sac took place. On repeating this experiment, after adding carbonate of potash in slight excess to the solution of acetate of morphia, the movements again seemed to cease, the yelk-ball became round in less than a minute. But a source of fallacy always exists in these observations, viz. that normally the yelk tends to become round and remain at rest for a variable but brief space of time after each wave has passed. Half an hour later contractions were visible.

Acetic acid.—The above result being somewhat doubtful, a few drops of a solution of one drop of strong acetic acid, in sixty of water, were added to a cell containing several actively contracting eggs. At once an arrest of the movements took place, but the yelk-ball, instead of becoming round, which is the position of relaxation, remained for some minutes marked by the sulcus, which at the moment existed; but afterwards the movements began again, the sulci were remarkably deep and irregular, and travelled very slowly. A little more of the acetic acid solution being added no effect appeared at first, but in two hours, three eggs out of five had become opaque by coagulation of the food-yelk, the two ova which remained clear being motionless and globular.

Acetate of potash.—A weak, faintly alkaline, solution of acetate of potash was then added to a cell containing some eggs freely contracting. Soon the yelk-ball became round, the formative yelk changed in structure, became firmer, more opaque, and projected from its surface little rounded masses. After two hours the food-yelk was still slowly contracting. Thus a solution of a strength which chemically changes somewhat the formative yelk-matter, does not arrest the contractions, although it hinders them.

Tincture of opium.—On adding two or three drops of tincture of opium to a cell containing ova, vividly contracting, the movements seemed to cease for a time, but the sulcus remained. In about three minutes the movements reappeared and continued for half an hour, after which more tincture of opium was added, and still the contractions were found going on with moderate vigour an hour later.

Spirit of wine.—A few drops of spirit of wine added to a cell containing freely contracting ova, either did not affect the contractions and oscillations at all, or slightly quickened them.

Tincture of cantharides acts much the same as spirit of wine does; after twelve hours eggs treated with it were developing normally.

Solution of potash.—A very minute drop of Liquor Potassæ, L. P., was mixed with the water of a cell containing several freely contracting ova. The inner sac at once burst, its contents escaped into the cavity of the egg, and it was soon crumpled into a distinctly membranous bag, still marked by the ripples due to the previous contractions. No further contractile waves appeared, nor did it dissolve. After a very few minutes the yelk-sac burst, the solution having been too strong.

Strychnia.—A neutral solution of acetate of strychnia, 2 grs. to 480 grs. of water, was added, in small amount, to a cell containing vigorously contracting ova. No visible change appeared in the movements, and after twelve hours the eggs were normally developing. More strychnia was then added, but no result followed.

Aconite.—A solution of 5 grs. of extract of aconite in 60 minims of spirit of wine was added, by drops, to a cell containing freely moving ova, but no effect was observed, except perhaps a slight acceleration of the movements.

Hydrocyanic acid.—A few drops of dilute hydrocyanic acid (2 per cent.) added to the water in which ova were actively contracting, produced no effect in a quarter of an hour, during which time I watched.

In each of these experiments there were some unimpregnated eggs present, easily recognized by their irregular, often detached, proligerous disks. In no case was there any difference observable between them and the fertilized ones as to the action of the poisonous agents.

Hydrochlorate of morphia.—Two drops of a strong spirituous solution of hydrochlorate of morphia were added to some unimpregnated ova, which had been deposited nine hours, and were rapidly contracting and oscillating. No effect following, four drops more were added without result; again four drops were added, and during a quarter of an hour no result was observed; twelve hours later they were all opaque.

Chromic acid.—Eight days after impregnation, a healthy developing embryo was let out of the yelk-sac into a weak solution of chloride of sodium and chromic acid, and the contractions at the lateral poles, mentioned by REICHERT, were seen to go on apparently unchecked.

Ammonia.—Contractions of the yelk continue in eggs placed in a very weak ammoniacal solution of carmine; and even when the egg is ruptured, and the inner sac

escapes into this solution, they go on, provided the inner sac is not too much injured. A 2 per cent. solution is too strong, it causes prompt rupture of the inner sac, but does not dissolve it.

Ether.—Unimpregnated ova, thirty hours after deposition, while freely contracting, put into a watch glass, with only enough water to moisten them, and exposed to the vapour of ether under a small bell-glass, exhibited no diminution of movements, although slight opacity of the eggs was produced. Similar eggs treated with an aqueous solution of ether, became opaque, and exactly in the same ratio the movements became slower, and ultimately ceased. The opacity preceded any visible diminution of the movement.

Chloroform.—Some chloroform in vapour was applied 144 hours after impregnation, at which time the heart is seen beating, the muscles of the trunk acting, and the yelk still contracting at the lateral abdominal poles. The first effect was to excite writhing movements in all the embryos, but in five minutes the muscles of the trunk and the heart had ceased to move, and the contractions of the yelk were arrested, the sulcus remaining uneffaced. Five minutes later the embryos began to recover, the yelk-contractions moved on slowly, and after adding fresh water the heart began to beat feebly; but the trunk could not be seen to move in any of the embryos for half an hour in spite of free addition of water. After $2\frac{1}{2}$ hours the trunk moved freely, the heart beat regularly, and the yelk contracted vigorously.

As the general result of these observations, and those of a similar kind made on the ova of the *Gasterosteus*, it may be stated that the rhythmic contractility of the yelk is not materially influenced by any of the poisons used, which did not act chemically, with the exception of chloroform and of carbonic acid. It is true that acetate of morphia appeared in some experiments to arrest the movements, but the results were not confirmed by the later observations recorded with other solutions of morphia, and never were free from certain fallacies which have been mentioned. Whether alcohol or cantharides quicken these movements or not must also remain in some doubt, as the results obtained were not sufficiently marked to justify a positive assertion, and some fallacy might exist due to the currents which occur during the mixture of spirit with water.

b. *Galvanism.*—The effects of the application of galvanic currents to these eggs are like those already mentioned, as seen in the ova of *Gasterosteus*.

c. *Heat.*—Eggs nine hours after fecundation, when they are cleaving and actively contracting, warmed on the stage of the microscope to about 80° F., became still, or nearly so, and the oil-drops were a little displaced. At the temperature of the room (58° F.) they did not soon recover, but ultimately they cleft, although irregularly. Eggs at the same stage, gently warmed to about 70° F., moved much more quickly; on being cooled to about 40° F. the contractions ceased entirely; warmed again, the vivid movements returned, cooled again, they ceased: left at 60° F. until the next day, the impregnated ova were seen cleaving, the barren ones contracting, their proligerous disks being detached. In these experiments the temperature stated is only approximative, as a ther-

mometer on the stage of the microscope does not duly register the temperature of the eggs in the cell.

d. *Oxygen.*—The experiments made with the impregnated eggs of the stickleback having failed to show in a satisfactory manner to what extent the contractions of the yelk and the cleavage are dependent upon the presence of oxygen in the surrounding medium, I attempted to ascertain this by employing a larger number of ova of the pike, which, as they exhibit the contractions of the protoplasm irrespective of impregnation, make it easier to separate experimentally the conditions of the cleavage from those of the yelk contractions; and as they are free from any appreciable amount of maternal secretion, are more suitable for prosecuting the inquiry whether any and what product of oxidation passes into the surrounding medium, during the movements of the protoplasm, or the development of the germ.

In these experiments I divided the spinal cord of the parent fishes, without injuring the bodies of the vertebræ, just behind the edge of the gill-covers, and then wrapped them in a cloth which was kept wet, by which means they continued to live and breathe for some hours, and could be easily manipulated.

Having previously prepared a curved glass tube of about $2\frac{1}{2}''$ in length, tapered at one end so as to leave an aperture of about $\frac{1}{10}''$ to $\frac{1}{8}''$ in diameter, to which was fixed an elastic thread for securing it when in position, I filled the tube when warm with pure olive-oil and closed both ends with hot tallow, excluding air-bubbles. By this arrangement I was able to pass ova direct from the body of the parent into any fluid, without previous contact with air, or any air containing liquid.

Being unable to obtain water absolutely freed from dissolved oxygen, I prepared several small beakers of distilled water in which were fragments of broken glass, by prolonged boiling, until the bumping became so violent that they were in great danger of being broken (indeed two or three were thus broken), and then poured pure olive-oil upon the water whilst boiling to a depth of $\frac{3}{4}''$ to $1''$, and left them to cool. In this way I hoped to have water sufficiently freed from dissolved oxygen, for the purposes required, and in a state in which it could not support respiration.

Thus prepared, I attempted a first series of experiments on April 5th, 1866, to ascertain whether the rhythmic contractions of the yelk persisted in water deprived, as above described, of its dissolved oxygen; and at the same time whether the slower protoplasmic contractions which cleave the germinal disk persisted under similar conditions. As, however, it was ascertained that some dissolved oxygen still existed in the water, I sought to obviate this difficulty by repeating the experiments with varying proportions of water to ova: because if oxygen be used up during these movements they must cease sooner when the proportion of ova to water is greater.

I used distilled water in order that it might be afterwards tested for any product such as carbonic acid.

Control experiment 1.—For the sake of having a standard of comparison, I put into a vessel of ordinary distilled water some unimpregnated eggs in the proportion of one

part of ova to sixteen of water without covering the surface with oil. After twenty hours the rhythmic contractions of the yelk were vigorous, the formative yelk was lobular, and often detached in part or wholly. After fifty-three hours they were contracting freely, several inner sacs ruptured. After seventy-two hours most of the inner sacs were burst, and the yelks were more or less opake, and no contractions were seen in those few in which the inner sac was not ruptured. The water was slightly opalescent. 100 hours after, the water did not precipitate baryta-water, the broken eggs reddened blue litmus. Several of these eggs, from the deeper layers in the vessel, had not properly imbibed water; indeed the very lowest, fifty-three hours after, had the appearance of eggs freshly expressed from the fish, being still adhesive; they could not then be made to absorb water freely as fresh eggs do.

Control experiment 1′.—I fecundated ova successfully in ordinary distilled water, not covered with oil, by dropping in a fragment of ripe testis. Many of them cleft, but the water soon became turbid; and fifty-three hours after, many of them had opaque germinal masses; seventy-two hours after, all were opaque, decomposition being evidently favoured by the presence of the fragment of testis.

Control experiment 2.—I fertilized some spawn in the usual way in tap-water contained in a dish, and changed the water daily. Not all of these ova were impregnated, but the fecundated and non-fecundated were seen rhythmically contracting with equal vigour twenty-five hours after, when the former were in the mulberry stage of cleavage. At fifty-seven hours, at seventy-five hours, and 100 hours after, the same contractions were seen in all the unimpregnated eggs, of which the inner sac yet contained some yelk, and in the impregnated ones in that part of the food-yelk still uncovered by the advancing germinal mass. The unimpregnated ova were all still the next day. The fertile eggs were hatched from the 16th to the 18th of April, that is, after eleven to thirteen days.

Experiment *a*.—The fish being secured on a raised shelf, and the beakers on supports near, I passed the tube filled with oil into the sexual aperture, and held it in position by means of the elastic threads. Then by gentle pressure upon the abdomen the ova were made to pass into the tube until they had displaced the oil, the lower end being closed by a drop of oil. Unimpregnated ova were then passed through the tube, into one of the beakers, in the proportion of about one part of ova to ten of water, so that the tube dipping below the layer of oil, the eggs were scarcely, if at all, greased. After twenty-seven hours their yelks were contracting and oscillating freely; the discus proligerus was concentrated and had become irregularly lobular, and often detached in fragments, or as a whole; the detached masses being granular and opaque. The food-yelk was clear. The upper layers of the eggs in the beaker, however, alone exhibited these movements distinctly, the lower ones not having imbibed enough water to duly distend them; hence they were not clear on the general surface, and had not a well-concentrated discus proligerus. At this time the contractions of the eggs in the upper layer were as strong as in control experiment 1. After forty-nine hours the lower layers of the eggs,

still imperfectly distended with water, contracted slowly, resembling eggs three or four minutes after impregnation while the breathing-chamber is forming. The eggs of the uppermost layers contracted vigorously, and quite as much so as those of the control experiment 1. Nearly all had their proligerous disks entirely separated and broken into masses. The intermediate layers of ova in the beaker exhibited the formative yelk concentrated, but not at all lobulated; from which it would seem that the tendency of the discus proligerus to lobulate, and to be pinched off in portions or as a whole, like the rhythmic contractions, depends on a free supply of water as one of its conditions. The same may be said of the concentration of the granules of the formative yelk, and I think also of the tendency of the inner sac to rupture. Seventy-two hours after, the contractions were as vigorous as ever in the clear yelk-balls of the upper layers of eggs; the discus proligerus was entirely detached in all. 100 hours after, contractions had ceased, the water had a very faintly milky aspect, and the eggs were opaque. Three days later the water drawn off was not fœtid, it reacted faintly acid, and gave a slight precipitate with baryta-water.

Experiment *b*.—The last experiment was repeated, with one part of unimpregnated ova to about three parts of water. The contractions were observed twenty-seven, forty-nine, and seventy-two hours afterwards, and found to be as vigorous as those in experiment *a*, or in the control experiment 1. They were seen only in the eggs of the surface layers, which alone had imbibed water freely. 100 hours after, and three days later still, they resembled experiment *a*. The fact that water was not absorbed freely, except by the upper layers of these eggs, deprived the experiment of a part of its value, as the proportion of actively contracting eggs to the water had no definite relation to the numbers used, and could not be determined.

Experiment *c*.—The experiment was repeated, using equal parts of unimpregnated eggs and of water. At twenty-seven, fifty, and seventy-six hours after, the rhythmic contractions of the yelk were as vivid as in experiment *a* or in control experiment 1; but the discus proligerus was less lobular, and not so often detached. 104 hours after, all the eggs were opaque.

In this experiment mechanical disturbance was employed at first, to ensure a nearly equal action of water on all the eggs, but they were not quite fully distended, although nearly all the water was taken up; from which we may conclude that normally the eggs imbibe about their own bulk of water.

Experiment *d*.—The same as the above, using two parts of unimpregnated ova to one of water. Very few eggs of this experiment imbibed water, and those did so imperfectly. The rhythmic contractions were feeble, the formative yelk scarcely lobulated at all. The lowest layers did not visibly absorb any water, efface their indentations, or concentrate the formative yelk in the slightest degree. At 104 hours after, they were opaque.

Experiments were then attempted to be made in a similar manner with impregnated eggs, but being unable to insert a tube into the male fish, I passed a piece of ripe testis

quickly through the oil on to the eggs, directly after they had been introduced into the beaker in the way before described.

Experiment *a'*.—I used one part ova to ten parts of water, but the attempt to fecundate failed, perhaps from the action of the oil upon the testis.

Experiments *b'*, *c'*, and *d'*, made with varying proportions of ova to water, also failed, apparently from a similar cause. The results only served to confirm those obtained from the experiments upon unimpregnated eggs, as to the long duration of the rhythmic contractions.

I then sought to ascertain the comparative duration of these movements in aërated distilled water, with a layer of oil on its surface, employing varying proportions of eggs to water; but as in all these experiments the proportion of eggs which, being defended by the superincumbent layers, did not freely imbibe water was large and variable, the results obtained lost much of their value. I will mention, however, that the supernatant water, after 100 hours, did not precipitate baryta-water more freely than that from the experiment *a*, and had the same very faintly acid reaction.

These results not being conclusive, partly because of the difficulty of properly watching with the higher powers contractions going on in the eggs contained in beakers, partly on account of the eggs not being all equally acted on by water, partly in consequence of the failure to fecundate through a layer of oil, I obtained a further supply of ripe male and female pike, and on April 12th and 13th made a second series of observations, using glass cells having a depth of $\frac{1}{10}''$, and a cubic capacity of about $\cdot 05''$. In these experiments I sought to ascertain, by varying the proportions of eggs to water, air being excluded, whether the duration of the rhythmic contractions of the yelk, or their vivacity and the activity of the cleavage, were inversely as the number of eggs.

Control experiment 3.—For purposes of comparison, unimpregnated ova were passed from the female fish into ordinary distilled water in a wide beaker, so that they formed only one layer on the bottom. Four hours after, they were contracting freely, and the discus proligerus was concentrated, but smooth on its surface. Twelve hours after, it was in some lobulated, and in many detached, wholly or in part. Twenty-five hours after, they were vigorously contracting and oscillating; in nearly all the discus proligerus was detached, and in many of these its substance was fused, so as to run like a stream of lava: in a majority the inner sac was ruptured and more or less emptied. Thirty-five hours after, in all the discus was detached and diffused, but where the inner sac was not ruptured, or ruptured and only partly emptied, the yelk was actively contracting and rotating. Forty-eight hours after, the water, which had not been changed, was faintly milky, of a neutral reaction, and did not precipitate baryta-water: filtered and concentrated, it was alkaline, was precipitated by baryta-water, and the flocculent precipitate was only in part dissolved by hydrochloric acid: dried and strongly heated, it charred, and gave off fumes having the smell of burning hair, and it left an alkaline ash which did not effervesce with dilute hydrochloric acid. In this case there must have been a transudation of some organic substance, which probably was limited to the eggs with ruptured inner sacs.

Control experiment 3′.—The last experiment was repeated with eggs which had been impregnated in the usual way. Ordinary distilled water was used, care being taken to wash away all adhering seminal fluid. Twelve hours after, they were contracting freely, the germinal disk was cleft into a coarse mulberry mass. Twenty-six hours after, the contractions were rapid, the germinal disk a fine mulberry mass: a few eggs were opaque. Thirty-five hours after, they were contracting freely, and the germinal mass covered nearly half the yelk. Forty-eight hours after, they were contracting as before, and the germinal mass covered nearly three-fourths of the yelk. The water, which had not been changed, was faintly milky, neutral in its reaction, did not precipitate baryta-water: on concentration it remained neutral, and contained faint traces of a phosphate. In this instance also the presence of several opaque eggs with ruptured inner sacs makes the examination of the water for products of respiration unsatisfactory.

Control experiment 4.—I fertilized in the usual way a number of eggs in a large dish in tap-water, which was changed daily; and as there were some eggs in which the impregnation had failed to take place, they could be compared with the control experiment 3, to ascertain whether distilled water modified the contractions and development of the germ. During the first forty-eight hours no difference was observed when they were compared with control experiments 3 and 3′.

Experiment *e*.—I placed thirty-five unimpregnated eggs, fresh from the pike, in one of the above-described cells, taking care that all were well bathed with distilled water, and I sealed the cover quickly with hot wax. Four hours after, they were contracting freely, but with a smooth discus proligerus. Twelve hours after, contracting as before, they showed less lobulation of the discus proligerus and rarer separation of it than did the eggs in control experiment 3. Twenty-six hours after, they were moving freely, but less so than those in control experiment 3. Unfortunately, however, the luting had got loose, and an air-bubble had entered. Thirty hours after, the contractions had ceased; and the yelk-ball was round, except in those eggs near the air-bubble, which were still contracting well. Fifty-three hours after, all were motionless.

Experiment *f* was the same as the above, with only eighteen eggs in the cell. A small air-bubble got in, but the yelks ceased to contract in thirty-five hours. Those nearest to the air-bubble continued to move the longest, and became still ultimately by rupture of the inner sac, which explains their early cessation.

Experiment *g* was the same as the above, with but nine eggs in the cell. At twenty-six hours after, greater vivacity of the yelk-contractions and more lobulation, and separation of the discus proligerus were observed than in the eggs of experiment *e* at the same time. After thirty-five hours they were still contracting. After fifty-three hours they had ceased to move. Into this cell also a very minute air-bubble found its way.

Experiment *h* was the same as the above, but with thirty eggs in the cell, tallow being used instead of wax to seal the cover. Twelve hours after, they rotated less freely than the control ova to which they belonged. Twenty-three hours after, they had all

ceased to move, except two or three which lay near a very minute air-bubble. Twenty-nine hours after, all were still, the yelk-ball was round, the discus proligerus was not detached but flat and diffused.

Experiment *j* was the same as the above, with only seven eggs in the cell. Twelve hours after, the eggs were contracting freely, and resembled the control eggs more than those in cell *h*. In twenty-three hours the movements were languid, but distinct. After twenty-nine hours the contractions were barely visible. In this cell there was no air-bubble.

Experiment *e'* was the same as experimant *e*, with forty-eight impregnated eggs in the cell. After four hours the germinal mass was cleft into two or four. After twelve hours it was cleft into eight or sixteen masses, the control experiment 3' being at the same time in the coarse mulberry stage, containing hundreds of cleavage masses. The germinal mass was flat and rather diffused, and the outlines of the separate cleavage masses indistinct, as if they were about to coalesce. The eggs looked granular, notably the germinal mass. The yelk-ball went on contracting, however, although not so strongly as in experiment *e*. After twenty-three hours the cleavage had not progressed, the yelk-ball continued to contract in a few eggs with still further impaired vigour; but in most eggs it was round and still. After twenty-six hours all were motionless, and the yelk-ball so relaxed as nearly to fill the yelk-sac. These appearances reminded me of those which resulted from the action of carbonic-acid water on the eggs of the stickleback. Into this cell a little air entered.

Experiment *f'* was the same as the last, with seventeen eggs only in the cell. After twelve hours they were in a coarse mulberry stage of cleavage, being, however, less advanced than the control ova, but more than those of experiment *e'*; the yelks were contracting freely. Twenty-three hours after, they were in a finer mulberry stage, and were well contracting. Thirty-five hours after, the germinal mass had become diffused and darkly granular, but the contractions of the yelk continued. Fifty-three hours after, all were motionless. There was an air-bubble in the cell.

Experiment *g'* was the same as the last, with only ten eggs in the cell: air-bubbles entered again. Twenty-three hours after, the cleavage was further advanced than in experiment *f'*, but not so far as in the control experiment 3; the yelks contracted freely, however. Thirty-five hours after, they had dark, granular, germinal masses and contracted slowly. After fifty-three hours all were still.

Experiment *h'* was the same as the last, with thirty-eight eggs in the cell, and tallow employed instead of wax as a luting. After twelve hours they were cleft in eight, the cleavage masses were dark, granular, and almost fused together; the contractions, however, were distinct. After twenty-three hours the cleavage had not progressed, the contractions were languid. After twenty-nine hours the cleavage masses were quite fused, the contractions had ceased, the yelk-ball was round, relaxed, and at rest. In this cell there were no air-bubbles.

Experiment *j'*.—The same as the above, with only seven eggs. After twelve hours

they were cleft into the mulberry stage, but somewhat coarser than their control ova: the contractions were vivid. After twenty-three hours the germinal mass was in the finer mulberry stage. After twenty-nine hours no further progress was made in the cleavage, but the contractions continued.

Concurrently with these suffocative experiments, as they may be termed, I tried to ascertain whether any, and what respiratory products could be detected in the surrounding medium, and I considered separately impregnated and unimpregnated ova, with the view of comparing the rhythmic contraction with the movements of cleavage.

Experiment *k*.—I placed unimpregnated ova, fresh from the fish, in a tube with two parts of distilled water, agitating to ensure that all were well exposed to the water. The contractions continued for forty-eight hours, although latterly with less vigour than in the control eggs. The water, which was then clear, and neutral when filtered, gave no precipitate with baryta-water, was not coagulated by boiling or by alcohol, but was by protonitrate of mercury.

Experiment *l* was the same as the above, with one part of eggs to five of distilled water; the results after forty-eight hours were the same.

Experiment *k'* was similar to the last, but made with impregnated eggs in the ratio of one to four of water, the semen being washed away as quick as possible. Development was arrested before the germinal mass had extended over one-third of the yelk. After fifty hours all were still. The water, which was not quite bright even when filtered, was neutral or faintly acid in its reaction, was not precipitated by baryta-water, was coagulated by heat and nitric acid and by alcohol, more freely precipitated by protonitrate of mercury. Probably in this experiment an egg may have been ruptured while introducing it into the tube.

Experiment *l'* was the same as the above, with one part of ova to eight of water. Fifty hours after, all were still. The water when filtered was clear, the reactions were the same as those in experiment *k'*, except that it was not coagulated by heat and nitric acid, or by alcohol; so that the albumen must have been accidental in *k'*.

Experiment *m*.—As the number of ova used in experiment *k* was small, I repeated it in a flask, in which about half an ounce of spawn was put into two ounces of distilled water. Agitation was kept up for a time to ensure that all the eggs were duly exposed to the water. After twenty-eight hours they were contracting freely. No ruptured eggs were seen among them. The water was bright; it was filtered and evaporated; the concentrated liquid was faintly alkaline, the dry residue resembled dry serum, contained an organic colloid, not albumen, with alkaline phosphates, chlorides and sulphates; but no evidence of carbonic acid was found. The organic matter was not further examined.

Experiment *m'* was the same as the above with fertilized ova. After thirty hours, while all the movements were active and cleavage was progressing, there being but few opaque ova in the flask, the water was filtered quite bright, and evaporated. The residue had the same reactions as had that of experiment *m*.

In order to permit a comparison of the consumption of oxygen in protoplasmic move-

ments with that which takes place in cell-development and multiplication, and in the muscular movements of the embryo, a third series of observations was made on more advanced developing ova of the pike, and on recently hatched embryos. In this series I employed the same cells, and using the same bulk of organic matter of the same kind, in different grades of development, I observed how the duration of the different activities varied.

Experiment *n*.—Two healthy developing ova were put into a cell in water, and sealed with hot tallow, seventy-six hours after impregnation, when the yelk-contractions were very vigorous, and no muscular movements of the trunk or contractions of the heart could be seen. Seven hours after, but little progress in the development was seen; the yelk-contractions were vigorous. Eighteen hours after, no further development, and no yelk-contractions were seen: the embryonic tissues were not opaque. Forty-eight hours after, the tissues were somewhat opaque, and the germs of the vertebræ were partially fused together.

Experiment *o* was similar to the above, using two eggs 101 hours after impregnation, when the yelk-contractions were vigorous, but no beating of the heart or movements of the trunk were seen. Seven and a half hours after, the contractions were visible, but somewhat reduced in vigour; a barely recognizable progress in the development had occurred. Eighteen hours after, no further progress had been made in the development of the organs, and all motion had ceased. The ova were much behind the control eggs, and the tissues were still clear.

Experiment *p* was similar to the above, using two healthy eggs 127 hours after impregnation, when the yelk-contractions were distinct. The germ of the heart was visible but was not seen to move, the trunk struggled rarely and fitfully. After $13\frac{1}{2}$ hours all movement had ceased.

Experiment *q* was similar to the above, using two eggs 150 hours after impregnation, when the yelk-contractions were vigorous, the heart was beating regularly, and the trunk frequently moving; a few circulating corpuscles were seen. After $6\frac{1}{2}$ hours the heart was still, the trunk was not seen to struggle, nor could I by using the micrometer thread detect any yelk-contractions.

Experiment *r* was similar to the above, using two healthy eggs 174 hours after impregnation, when the heart was acting vigorously, the stream of blood was seen entering the auricular opening, the trunk often moved, and the yelk contractions were well seen. After $7\frac{3}{4}$ hours the movement had ceased in one egg, in the heart, trunk, and yelk. In the other the heart was moving slowly, and feeble trunk-movements were seen, but no yelk-contractions. In this egg the heart was irregularly and slowly acting after twelve hours. After eighteen hours all was still. The discrepancy which this result shows as compared with experiment *q*, is explained by the fact that there were several deaths in the dishes from which the eggs were taken, and some parasitic growth on the yelk-sacs, which I neglected to wash off in the case of experiment *q*, but carefully attended to in experiment *r*, in which instance I also selected the eggs under a lens.

Experiment *s* was similar to the above, using two free embryos hatched twenty-four hours previously. The beating of the heart was regular, 112 per minute, the blood was circulating vigorously, no yelk-contractions being seen, and as yet no movement of the gill-covers. After three hours the heart's action was reduced to ninety per minute, the movements of the trunk seemed unimpaired. After five hours the heart beat only fifty a minute, the blood-corpuscles tended to block up the channels near the auricular aperture. After seven hours the heart's action was irregular, and failed at times for forty seconds, afterwards beating once a second; the blood formed a red coagulum near the heart; the trunk did not move. Nine and a half hours after, the heart was beating feebly at long intervals, although the trunk was rigid, and recurved as it often is in dead fry, and the tissues were opalescent. In eighteen hours all movement had ceased.

Experiment *t* was similar to the above, using two free embryos, two days hatched (I may say here that the young fry burst the yelk-sac at various stages of development, so that the number of days they have been hatched is no safe measure of the stage of development). At this time they were somewhat further advanced than those in experiment *s*, but no action of the gills was seen. After $4\frac{3}{4}$ hours they became still as regards the trunk, and the heart beat rarely and feebly.

Experiment *v*.—As a supplement to these experiments, and as a measure of the value of my tests for carbonic acid, I sought for respiratory products in the water in which free embryos had been suffocated.

Sixteen young pike, about one week old (when they are seen to move the gill-covers and to vibrate the pectoral fins), were put into 800 grains of distilled water, and the beaker, which was full, was covered by a glass plate. They soon showed indications of distress, moving the gill-covers much more than their fellows in the aquarium did. $5\frac{3}{4}$ hours after, most of them were motionless at the bottom; a few were attached to an air-bubble which had got in, and these were able to swim. Six and a quarter hours after they were all unable to move. The water was filtered and evaporated, the concentrated fluid was neutral, and gave an indistinct cloud with baryta-water.

Experiment *w*.—In order to have a physiological test of the extent to which, by prolonged boiling, I had exhausted the oxygen of the distilled water in preparing the beakers for the first series of experiments *a*, *b*, *c*, *d*, I put into one of the same beakers twelve young pike, about six or seven days old, passing them through the oil on the surface, wrapped in moist bibulous paper unrolled afterwards by a needle. In one hour all were quiet but one, although their hearts were acting feebly. In one hour and forty-five minutes they were dead. The beaker contained 700 grains of water.

Experiment *x*.—Ova passed into pure olive-oil formed no breathing-chamber, and did not undergo any change resembling those which occur in water.

Experiment *y*.—Ova passed into spirit of turpentine resembled the above, but became more opaque.

These two experiments were made in reference to NELSON'S observations on the ova of *Ascaris mystax*.

Without giving a detailed analysis of this group of experiments, I will briefly state the inferences which they appear to justify.

The contractions of the yelk, and to a somewhat less degree, the cleavage, are remarkable for the small amount of oxygen which they demand for their maintenance. This was shown indeed in the experiments upon the eggs of the stickleback (pages 472 to 475), which seemed almost to justify the view that oxygen in the surrounding medium is not an essential condition of protoplasmic contraction, until the more extended observations on the pike ova enabled me to arrive at a more correct conclusion.

The experiments *a*, *b*, *c*, also establish the fact, that the rhythmic contractions demand but little oxygen for their support, as they persisted for seventy-two to seventy-six hours in water deprived of oxygen, as far as it is possible to do, by boiling in air.

The persistence of yelk-contractions in eggs which are already in part decomposing, is probably another illustration of this general rule.

That cleavage also demands but little oxygen appeared from the experiments 1, 2, 3, on the ova of the stickleback, page 473, for it progressed in limited areas, and in water partly deprived of oxygen apparently as rapidly as it did in open vessels.

One is almost led to infer, on comparing the results of the experiments upon the pike ova with those of the stickleback, that the former require proportionally more oxygen than do those of the latter during cleavage. Nor does there appear to be any difficulty in believing that variations in this respect exist among different species of animals.

That some oxygen in the surrounding medium is, however, a necessary condition of these protoplasmic movements appears from a careful consideration of the whole of the observations here related, although some of the results are such as to require explanation, and no one of the experiments taken alone is entirely free from possible objections. I will briefly explain some of the apparently opposing results.

In the control experiment 1, the yelk-contractions ceased in less than seventy-two hours, therefore earlier than they did in the ova of experiments *a*, *b*, *c*; this was due to the early setting in of decomposition in the control experiment 1, and consequent rupture and shrinking of the inner sac, phenomena which are favoured by the presence of oxygen in the water if it be not from time to time renewed, and were shown to be delayed in the boiled water covered with oil.

But in the ova of the control experiment 2, in which the water was changed daily, the yelk-contractions persisted for more than 100 hours, or about thirty hours longer than they did in the experiments *a*, *b*, *c*.

The series of suffocation experiments *e* to *j*, and *e'* to *j'* inclusive, also support the inference that oxygen is an essential condition; for in all of them the yelk-contractions ceased long before they did in the eggs of the control experiment, and in all they persisted longest in those eggs which lay nearest to the accidentally admitted air-bubbles.

It is true that a constant inverse relation was not observed between the numbers of the eggs in the cell and the duration of the yelk-contractions, but this was explained

by the varying amounts of air in bubbles which entered the cells, and by the accidental bursting of the inner sacs in many eggs, a mode of terminating the contractions not due to the exhaustion of oxygen, and which is most apt to occur when the proportion of water is greatest, provided it be not changed. Nevertheless, in experiment *h*, with thirty eggs in the cell, and imperfect exclusion of air, the contraction ceased in twenty-three hours in eggs distant from the air-bubbles, while in experiment *j*, with only seven eggs in the cell, and complete exclusion of air, they continued for twenty-nine hours.

The experiments with impregnated eggs gave more definite results still. For in experiment *e'*, with forty-eight eggs in the cell, the yelk-contractions ceased in twenty-six hours, leaving the yelk-ball globular and relaxed, while in experiment *g'*, with only ten eggs in the cell, they persisted for more than thirty-five hours.

The pseudo-cleavage, or contractions of the concentrated formative yelk in unimpregnated eggs, and probably also the concentration of the formative yelk, seem to demand the presence of oxygen as well as a due supply of water: for in experiment *e*, after twelve hours it was less advanced than in the ova of control experiment 3. It also was shown to cease long before the yelk-contractions, and may be supposed to consume more oxygen, although there are other explanations which may be offered of this fact, especially the tendency of the matter of the discus proligerus to undergo chemical change and disintegration.

Cleavage may be said to demand more oxygen than do the yelk-contractions, as in experiments *e'* to *j'* it always ceased long before, and was more promptly checked by increasing the number of eggs in the cell. It is also more quickly arrested than pseudo-cleavage, and would seem therefore to need oxygen more.

Indirectly, bursting of the inner sac and consequent cessation of the yelk-contractions depends upon access of oxygen, which acts by favouring decomposition when the water is not changed.

Although some of the changes seen in the eggs in the suffocation experiments may be attributed to the poisonous action of some product, and not alone to the absence of oxygen, yet the general inference, that oxygen is consumed, is not thereby weakened; and it is a significant and interesting fact, that the cleavage masses in suffocated eggs undergo a species of fusion, which much resembles one of the effects of the action of carbonic acid on them.

It remains, however, a weak point, that I failed to obtain chemical evidence as to the product of oxidation, which might be expected to be in very small amount, as the consumption of oxygen was so minute.

Despite the difficulties of deciding what interchanges take place between the substance of the yelk and the surrounding medium during the functional activity of the former, the fact came out with sufficient clearness, that some non-albuminous organic matter, and some salts, passed into the water.

Experiments *n* to *t* inclusive, show that cell-multiplication and differentiation, in the

developing embryo, more urgently demand oxygen than do the earlier stages of cleavage, and *à fortiori*, than the yelk-contractions.

Thus the progress of development and of growth of the embryo ceased as early as seven hours after the cell was closed; and so rapidly had the oxygen been consumed, that even the yelk-contraction ceased in eighteen hours, although the proportion of ova to water was small: while the earlier cleavage, under less favourable conditions, had continued for twenty hours at least, and had not so exhausted the oxygen as to stop the yelk-contractions for thirty hours or more.

The higher the stage of development at which the embryos used for experiment had arrived, the sooner did they so exhaust the oxygen as to arrest the yelk-contractions.

In young embryos in which no striated muscle was seen, the trunk movements persisted as long as the yelk-contractions, and the heart, which is but a mass of protoplasmic balls, did the same, but the striated muscles in the trunk of more advanced embryos lost their contractility more rapidly when oxygen was withheld. The heart, even in hatched embryos, continued to contract longer than any other structure, perhaps, because it continually helped to renew the medium around it.

Concluding Remarks.

The observations detailed in this communication seem to me to be confirmatory of the view that the egg of osseous fishes is a cell, and to be looked on as a structural unit, the prototype of those units which, variously aggregated and modified, form the mass of the higher organisms. The minute, simply constructed, early ovarian ovum would, I doubt not, be accepted as such by most observers; and the larger ripe egg can scarcely be held to differ essentially if its mode of growth and development be considered.

The complex structure of the yelk-sac cannot be urged with much force against this opinion, as analogous structure has been met with in other parts, which are admittedly cell-walls or their descendants; for example, the striations in the matrix of some cartilages, and in the surface-layer of intestinal epithelium, and the so-called pore-canals in the cuticular tissues of many lower animals. It is certainly somewhat difficult to conceive what is the true position of the egg if not that of a cell.

Assuming that the opinion now advocated is sound, the question as to the mode of growth of cell-walls receives some additional light from the evidence here brought forward, to prove that the yelk-sac grows interstitially, and not by accretion upon either surface, or by gradual transformation of the surface of the yelk-ball.

To the yelk-ball, however, as the essential cell, the greatest share of interest attaches, and it, like the cell contents, alone is capable of undergoing multiplication. A convenient definition of a cell-wall might therefore be,—the first separable covering of the protoplasmic mass, which does not take part in multiplication by fission.

The cell-wall must be considered as a living substance, at least so long as it continues to grow interstitially, although it is probable that a time occurs in the life-history of most cells, which possess such walls, when they cease to grow, when they render

only physical services to the organism, and then they are generally marked by singular stability.

The inner sac is to be looked on as the homologue of the primordial utricle, and its thicker portion with the granules of the discus proligerus would then correspond to the granular mass around the nucleus in the plant-cell.

The food-yelk is held to be the equivalent of the fluid cell-contents, and the germinal vesicle and spots hold the position of the nucleus and nucleoli.

Contractility, which there is some reason to think is a property common to all cell-contents or protoplasm, in the egg, as in Tradescantia, appears to have its seat in the surface-layer. It may be spoken of as of two kinds, Rhythmic and Fissile.

The former is met with, at least in the egg, as in very many other cells, in a portion only of its life-history, and varies very much as to the vividness of its manifestations in different organisms. Its essential conditions do not appear to differ from those which govern all other known vital actions, and its normal excitors are the same as those of higher motor structures, but it seems to be less liable to be influenced by most poisons than are the vital properties of higher tissues. It is not influenced in any manner by the spermatozooids. From the contractile matter of striated muscle it differs in one important particular, viz. that while the former is permeated by an alkaline fluid it is bathed with an acid. No explanation of its rhythmic character has yet been found, and its uses in the economy are also unknown. In the ova of osseous fishes, and in those of Batrachia, its existence has been ascertained, but usually its manifestations are slow and indistinct. That the rhythmic contractions have no essential relation to growth in the ovum of osseous fishes, is shown by the fact that they do not begin until the egg has reached its full size. I venture to suggest that they may be connected in some way with the conversion of a lower form of organic matter into a higher, such as occurs when food-yelk is transformed into formative yelk. It seems probable that the exceptional vividness of the contractions noted in some fishes, as the pike and the stickleback, is connected with the rapidity of the changes which take place in the egg. These eggs hatch in a shorter time than do those in which the contractions are slow and indistinct. Were it not for their orderly recurrence, one might be tempted to refer them to the same category as those motions which occur during the admixture of certain fluids, as of spirit and water.

The fissile contractility is also independent of the action of a male element, although so far influenced by fecundation as to owe persistence and regular progress to it. Its essential and modifying conditions are otherwise like those of the rhythmic contractility, but its normal excitors, if we except heat, are but little known. It requires for its maintenance that a portion of the lower form of protoplasm united with oil shall be continually converted into the higher. Its results are growth and development.

To show the extent and importance of the question as to the nature and properties of protoplasm, I will draw a brief parallel. The first germ of an animal, as the egg; the first stages of organic matter about to be formed into tissue in the body, as the white

blood-corpuscle; the lowest known existing organisms in the animal or vegetable kingdom, as the *Amœbæ*; the earliest ascertained traces of organic beings in the geological record, as the *Eozoon Canadense*,—are all essentially masses of protoplasm, and some of them have been shown to possess some important properties in common, as the researches of KÜHNE* and M. SCHULZE† have shown.

This cursory glance beyond the limited area which I have been hitherto examining, gives some support to a view, on other grounds probable, that the rhythmic contractions of the lower forms of protoplasm precede and lead up to the fissile movements which result in cell multiplication in the higher forms of protoplasm. Witness the amœboid stages of some monads before they encyst and multiply by fission, as described by CIENKOWSKI‡.

One is thus easily led to form the general conception that matter, in passing from the inorganic to the organic world, first takes on a homogeneous thick fluid form, the denser surface of which is endowed with a rhythmic contractility; that it then is gradually converted into a higher form, which is granular, and contains fat, which loses its power of rhythmic contraction, and acquires that of dividing into separate masses by fission.

EXPLANATION OF THE PLATES.

PLATE XV.

DIAGRAM A.

a. Dotted yelk-sac.
b. Buttons.
c. Micropyle.
d. Inner sac indicated by the dark line (it should be in contact with the yelk-sac).
e, *e*. Cortical layer, or matrix of the formative yelk, continuous with the inner surface of inner sac indicated by the fine shading.
f, *f*. Yellow droplets and granules of the formative yelk imbedded in the cortical layer, and forming at its thicker portion the discus proligerus.
g. Germinal vesicle with contents (introduced to show its position when last seen).
h. Group of large store oil-drops.
j. Food-yelk.
k. Smaller oil-granules of the formative yelk.

Figures 1 to 23 inclusive refer to eggs of *Gasterosteus*.

Fig. 1. The unimpregnated egg, indented by pressure, viewed with a low power:—*a*, the micropyle (proportionally too large in the figure); *b*, the buttons; *c*, oil-drops; *d*, the discus proligerus.

* *Loc. cit.* † Das Protoplasma, 1863.

‡ "Beiträge zur Kentniss der Monaden," Archiv für Mikroskopische Anatomie, 1865.

Fig. 2. The buttons, when not deformed by mechanical violence. × 200.

Fig. 3. Section of yelk-sac near the apex of the micropyle:—*a*, cut edge; *b*, folded edge; *c*, apex of the micropyle.

Fig. 4. The micropyle, front view, the apex in focus. ×200 (the fine dotting is too coarse).

Fig. 5. An unimpregnated egg under pressure, the micropyle (*b*) in profile projecting into the discus proligerus (*a*).

Fig. 6. An egg, five minutes after impregnation, showing the funnel of the micropyle (*b*), and the pit in the discus proligerus (*a*).

Fig. 7. Escaped and partially emptied inner sac:—*a*, food-yelk; *b*, formative yelk changed by the action of water.

Fig. 8. An egg crushed forty-five minutes after impregnation:—*a*, contents escaping at the rupture in the yelk-sac; *b*, collapsing inner sac thrown into folds; *c*, germinal disk.

Fig. 9. An escaped germinal vesicle in the fluids of the egg:—*a*, the germinal spots displaced; *b*, the colloid matrix changed and faintly granular; *c*, escaped yellow droplets; *d*, free oil-globules. ×200.

Fig. 10. The same vesicle acted on by water:—*a*, the germinal spots vacuolating, with dark hard outlines and irregular forms; *b*, the colloid content or matrix of the spots, more darkly granular and vacuolating. ×200.

Fig. 11. A portion of the same vesicle more highly magnified:—*a*, a germinal spot vacuolating. ×400.

Fig. 12. Youngest ova seen; no primitive yelk around the germinal vesicles.

Fig. 13. *a*, an ovum of the smallest size seen furnished with primitive yelk; *b*, an ovum of somewhat larger size changed by imbibition of water, so that the germinal spots have vanished; *c*, escaped germinal vesicle without contents; *d*, free granular matter from larger eggs; *e*, stroma of ovary.

Fig. 14. An ovum of group 2, showing granularity of the surface of the yelk, oil-drops distributed, and germinal spots vanished from prolonged action of water through the substance of the egg.

Fig. 15. An ovum of group 3, escaped from its yelk-sac, exhibiting the subangular form indicative of its solidity:—*a*, clearer zone around the germinal vesicle; *b*, granular aspect of the superficial part of the primitive yelk after the action of water.

Fig. 16. Germinal vesicle with its colloid matrix sustaining the germinal spots in their natural positions. Seen in the fluids of the ovary. (The drawing is faulty in showing the spots as if those nearest the observer were central.)

Fig. 17. A germinal vesicle which has imbibed water unequally, the colloid matrix retaining the germinal spots in their natural peripheral position.

Fig. 18. A free germinal vesicle uninjured mechanically, but acted on by water for seven hours, the position of the spots and the vesicular wall not changed.

The colloid matrix not granular, the germinal spots variously tailed, dark-bordered and vacuolating.

Fig. 19. Germinal spots, more highly magnified, seen in a $1\frac{1}{4}$ per cent. solution of chloride of sodium, variously tailed and vacuolating.

Fig. 20 (Plate XVI.). Germinal spots in various stages of fusion and solution, in a 5 per cent. solution of chloride of sodium.

Fig. 21 (Plate XVI.). A macerated yelk-sac:—*a*, the changed homogeneous-looking extensile outer layer; *b*, the dotted yelk-sac not changed. ×250 (drawn with a camera).

Fig. 22. Appearance presented by the extensile outer layer under a magnifying power of ×3000 diameters; the dots dark (diagrammatic).

Fig. 23. Aspect of the same when the focus is so arranged that the dots are light. ×3000 (diagrammatic).

PLATE XVI.

Fig. 24. Egg of the Trout, showing the cup of the micropyle under a $\frac{1}{2}''$ lens.

Fig. 25. The micropyle of the egg of the Pike seen in $\frac{3}{4}$-face with reflected and transmitted light:—*a*, the trumpet-shaped tube. ×190.

The figures 26 to 33 inclusive refer to the ova of Perch.

Fig. 26. A diagram explanatory of the arrangement of the outer ends of the "tubes" in the outer layer of the yelk-sac of the ovum of the Perch:—*a*, the funnel-shaped mouths; *b*, the "tubes;" *c*, radiating curved furrows and folds of the surface.

Fig. 27. Shows the arrangement of the "tubes" in a vertical section where two eggs meet. In the figure the "tubes" are stretched and broken, and their spiral twist destroyed. ×190.

Fig. 28. Small fragments of the "tubes" more highly magnified to show the double contour of the walls. ×500.

Fig. 29. The inner branched ends of the "tubes:"—*a*, the outer laminated surface of the dotted yelk-sac; *b*, a detached "tube."

Fig. 30. The inner branched ends of the "tubes" seen from the inner surface of the yelk-sac through its substance, showing their relation to the finer dottings.

Fig. 31. The outer ends of the tubes seen full face, showing their regular arrangement in the clear matrix, and the aspect of the funnel-shaped ends. ×190.

Fig. 32. The dotted yelk-sac, showing its laminated structure at a fissure. ×500. (The drawing exaggerates somewhat.)

Fig. 33. The dotted yelk-sac, showing a cut edge. ×500 (also somewhat exaggerated).

The figures 34 to 83 inclusive refer to the eggs of *Gasterosteus*.

Fig. 34. Two ova of *Gasterosteus pungitius*, pressed together so as to seem to close the micropyle of one of them. ×100.

Fig. 35. An impregnated ovum of *G. leiurus*, just before the commencement of vivid contractions, showing the flat surface.

Fig. 35′. The same egg rolled a little.

Fig. 36. The first stage of active contraction producing the reniform figure.

Fig. 37. The dumb-bell figure.

Fig. 38. The flask-shaped figure.

Fig. 39. The wave having passed forwards, has left the food-yelk globular, but still influences the germinal disk, which is nipple-shaped.

Fig. 40. The whole yelk-ball globular and at rest. The germinal disk diffused somewhat.

Figs. 41 & 42. Abnormal forms of the yelk, caused by two or more waves present at the same time.

PLATE XVII.

Figs. 43 & 44. Slower changes of form of the germinal disk and neighbouring part of the food-yelk.

Figs. 45 to 49 inclusive. Further successive slow changes in the form of the germinal disk and of the germinal surface of the food-yelk, shortly before the commencement of cleavage.

Figs. 50 & 51. Show the commencement and progress of the first cleft, and the separation of the masses.

Figs. 52 & 53. Also show the gradual deepening of the first cleft.

Fig. 54. The cleavage masses closely pressed together as seen before the second cleft begins.

Fig. 55. Shows the "Faltenkranz" of REICHERT, or folds of the inner sac during the progress of the first cleft.

Fig. 56. Shows similar folds in one cleft of an egg which is cleaving irregularly.

Fig. 57. An egg twenty hours after impregnation. The germinal mass is composed of cell-like corpuscles; *b*, a portion of the germinal mass more highly magnified.

Fig. 58. An egg cleaving asymmetrically.

Fig. 59. The yelk-ball before galvanism was used (given as a standard of comparison):—*a*, the micropyle; *b*, the germinal disk.

Fig. 60. Contraction, the effect of one application of the galvanic current.
P. The Platinode. Z. The Zincode.

Fig. 61. Another contraction, the effect of a second application of the current, with rupture of the inner sac and escape of the food-yelk.

Fig. 62. Further contraction, with rupture of the inner sac on the opposite side, from a third application of the current. The escaping yelk granular.

PLATE XVIII.

Fig. 63. The inner sac contracted by repeated applications of the current. Electrolytic changes visible.

Fig. 64. Final result after repeated application of the galvanic current.

Figs. 65 & 66. Contractions, the effect of zero-galvanic currents, too weak to cause rupture of the inner sac.

Figs. 67 & 68. Contractions excited by weak galvanic currents in eggs which had been partially frozen.

Fig. 70. Electrolytic effect of the galvanic current upon the cleavage masses.

Fig. 71. Irregular cleavage in an egg of which the inner sac had been ruptured by partial freezing.

Fig. 72. Second stage of the action of a weak solution of carbonic acid upon an egg before cleavage. The yelk-ball globular; the germinal disk very prominent and conical (drawn seventy minutes after impregnation, and thirty-five minutes after the action of the carbonic acid).

Fig. 73. First stage, four minutes after the action of carbonic acid upon an egg, after the first cleft is complete. The yelk-ball globular, the germinal disk retracted.

Fig. 74. Last stage of the action of carbonic acid on an egg in the same grade of development, not ruptured or chemically changed. The food-yelk globular and still, the cleavage masses fused and diffused. (The breathing-chamber is too large.)

Fig. 75. Final stage of the same eggs when the inner sac had ruptured and chemical change taken place.

Fig. 76. Normal aspect of the germinal disk cleft in four masses (as standard of comparison).

Figs. 77 to 79 inclusive. Stages of the recession of the germinal disk into the yelk, which took place during the first few minutes after the action of the carbonic acid.

Figs. 80, 81 & 82. Stages of the gradual reprotrusion of the germinal disk, with commencing fusion of the cleavage masses.

Fig. 83. State of complete fusion of the cleavage masses, three hours after the action of the carbonic acid. (The yelk-ball should more nearly fill the yelk-sac.)

Postscript.

Partly from an unwillingness to enter upon any discussion as to priority, and partly not to add to the length of this paper, I avoided historical references entirely. But as my silence might seem to indicate an acquiescence in claims which are unjust to other observers, I have since thought it better to append a short history of the observations relating to the micropyle in Fishes.

K. E. Von Baer (Untersuchungen über die Entwickelungsgeschichte der Fische, 1835) saw in the egg of *Cyprinus Blicca* (white Bream) a clear circle in the centre of the germ (discus proligerus), which when viewed full face had a dark halo around it and resembled an area pellucida, but when seen in profile was recognized as a funnel-shaped depression in the outer membrane. He says further, "I can only conceive the formation of this funnel taking place by the passage outwards of the germinal vesicle, through the centre of the germ, as I have seen occur in the egg of the frog."

It may be said therefore that Baer observed the funnel of the micropyle, and correctly described its position relative to the parts of the egg, but did not see the aperture through the yelk-sac or properly interpret the structure.

M. Doyére communicated a paper to the Soc. Philomathique de Paris, Dec. 15th, 1849 (see l'Institut, vol. xviii. p. 12, 1850), in which he described the micropyle in *Syngnathus Ophidium*, clearly indicated its relation to the discus proligerus, measured the aperture, and without doubt appreciated its significance in the act of impregnation, without, however, affording any experimental evidence of its uses.

My paper, read November 23, 1854, and published in the Proceedings of the Royal Society, was next in order of time.

C. Bruch (Zeitschrift für Wissensch. Zool. B. vii. 1855) announced his discovery of the micropyle in the eggs of trout and salmon in a letter to Professor Siebold, December 28, 1854. He did not make out its relation to the proligerous disk, and failed in his attempts to prove that it served for the entrance of spermatozooids. He, however, rightly interpreted the structure, and claimed to have first established on a firm basis the existence of a micropyle in vertebrata.

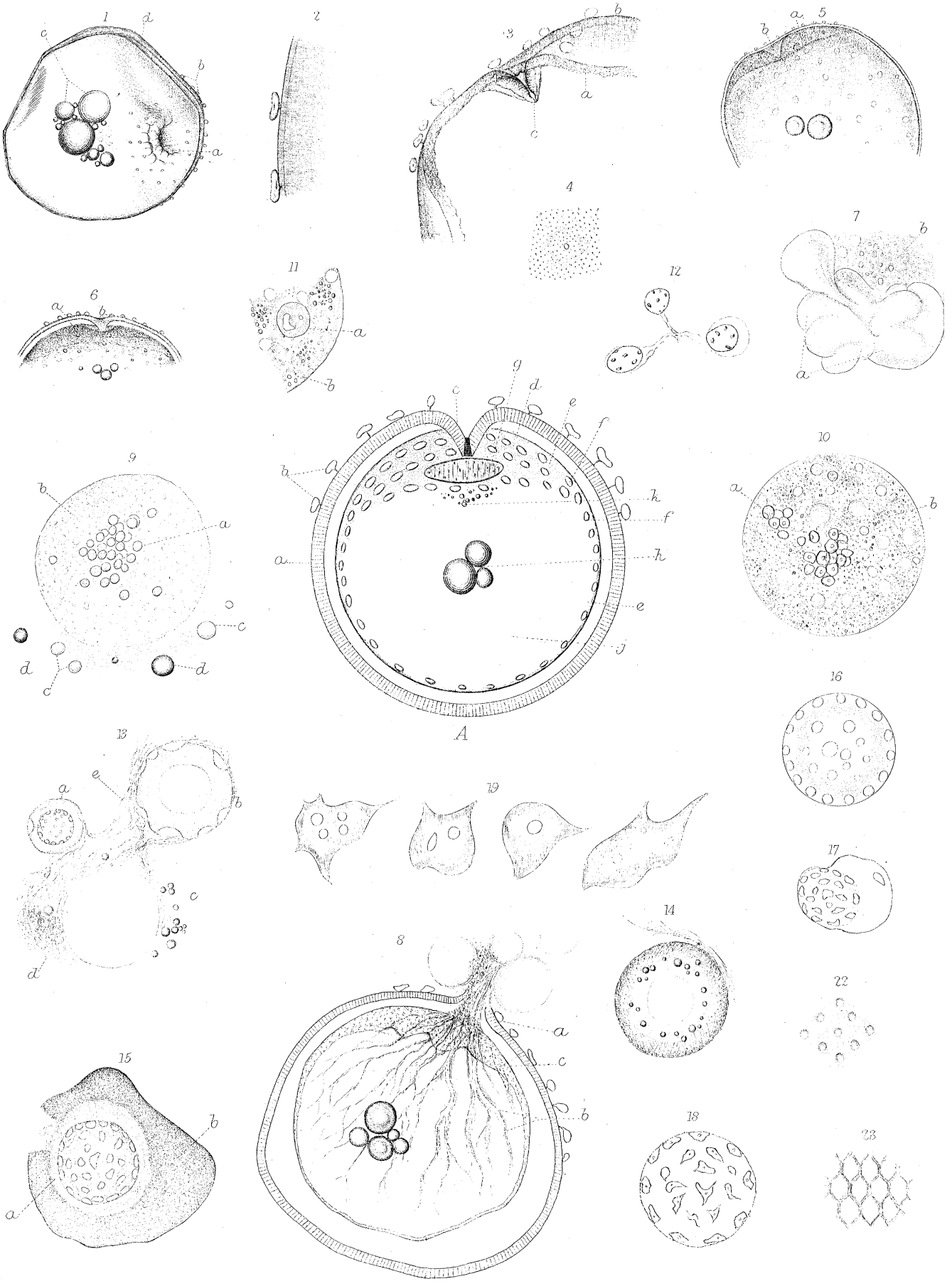
My observations on the eggs of trout and salmon were made in the same season, December 1854 and January 1855, and the results were communicated in the latter month to Dr. Allan Thompson (Cyclopædia of Anatomy and Physiology, vol. v. p. 104).

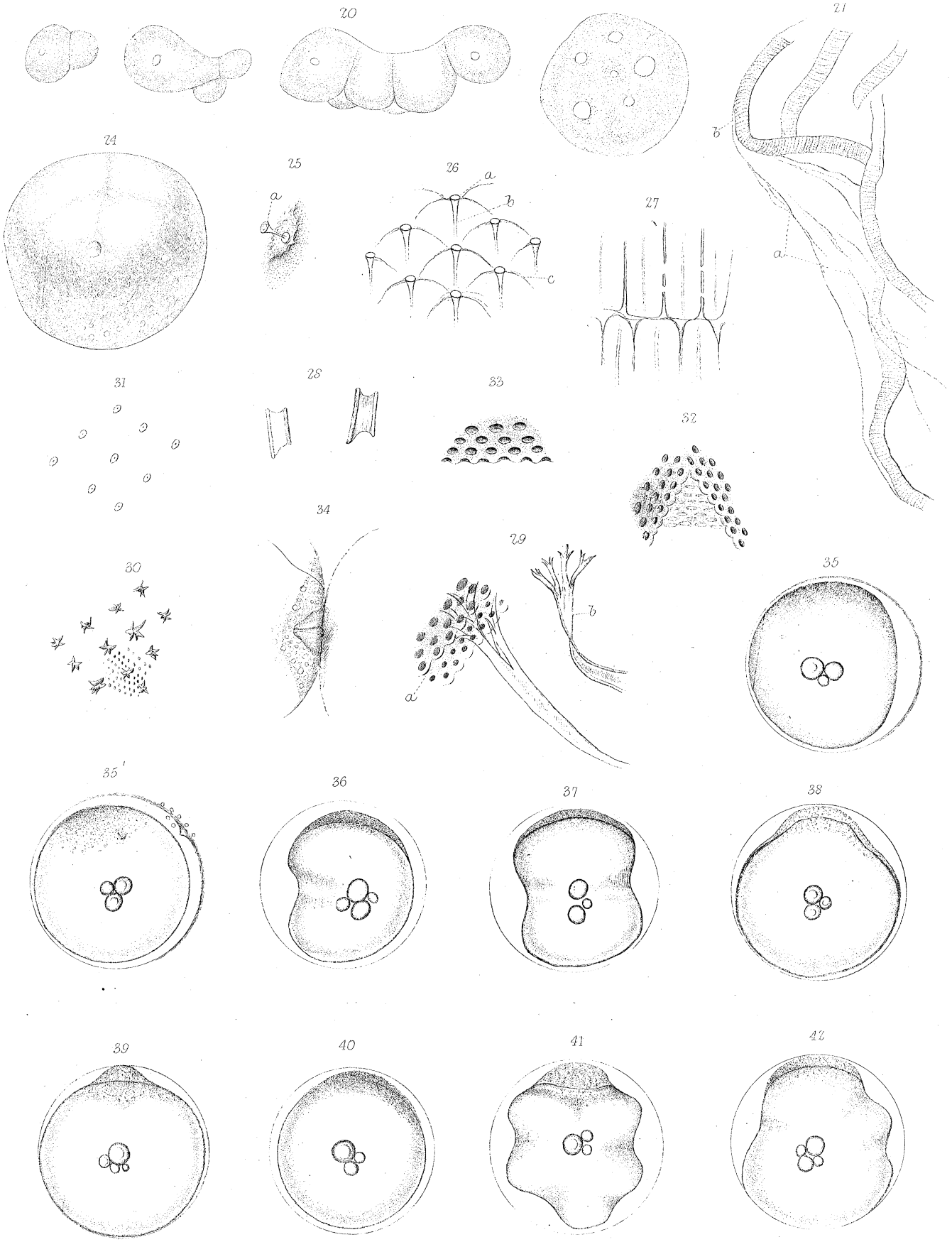
Reichert (Müller's Archiv für Anat. Physiologie, &c. 1856, p. 83) described the micropyle in the egg of the pike and of several other fishes, failing, however, to find it in perch. His first observation was made immediately before Professor Bruch's paper came to his hand. He did not describe the relation of the micropyle to the other parts of the egg, or give any proofs that it served to give entrance to the spermatozooids.

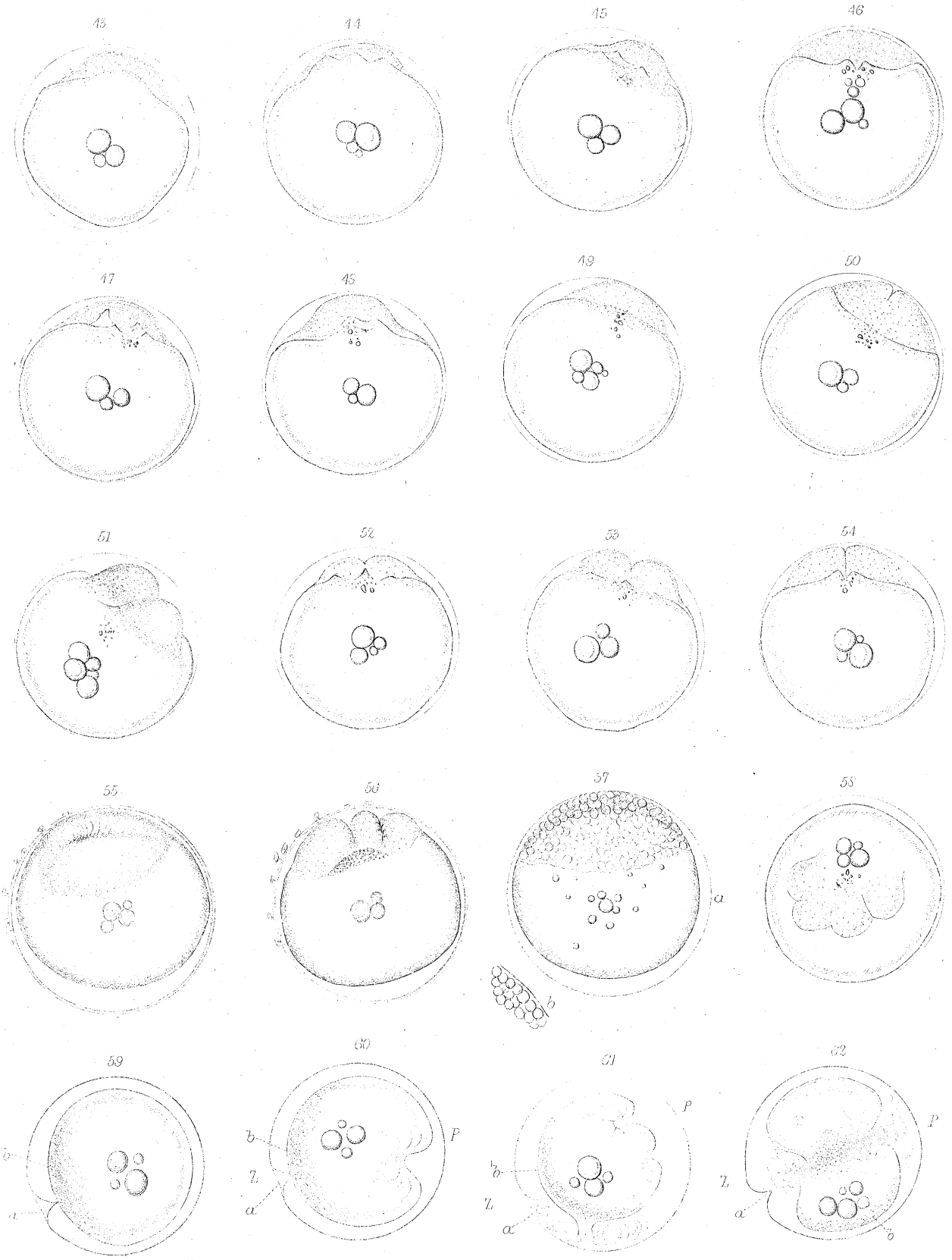
The credit of priority rests mainly with Doyére. His observation was unnoticed for some time, and my experiments were made and published without any knowledge of his discovery or of the earlier one by Von Baer.

Bruch worked on this subject also without a knowledge of what had been done before, and Reichert with only a partial knowledge.

February 23, 1867.

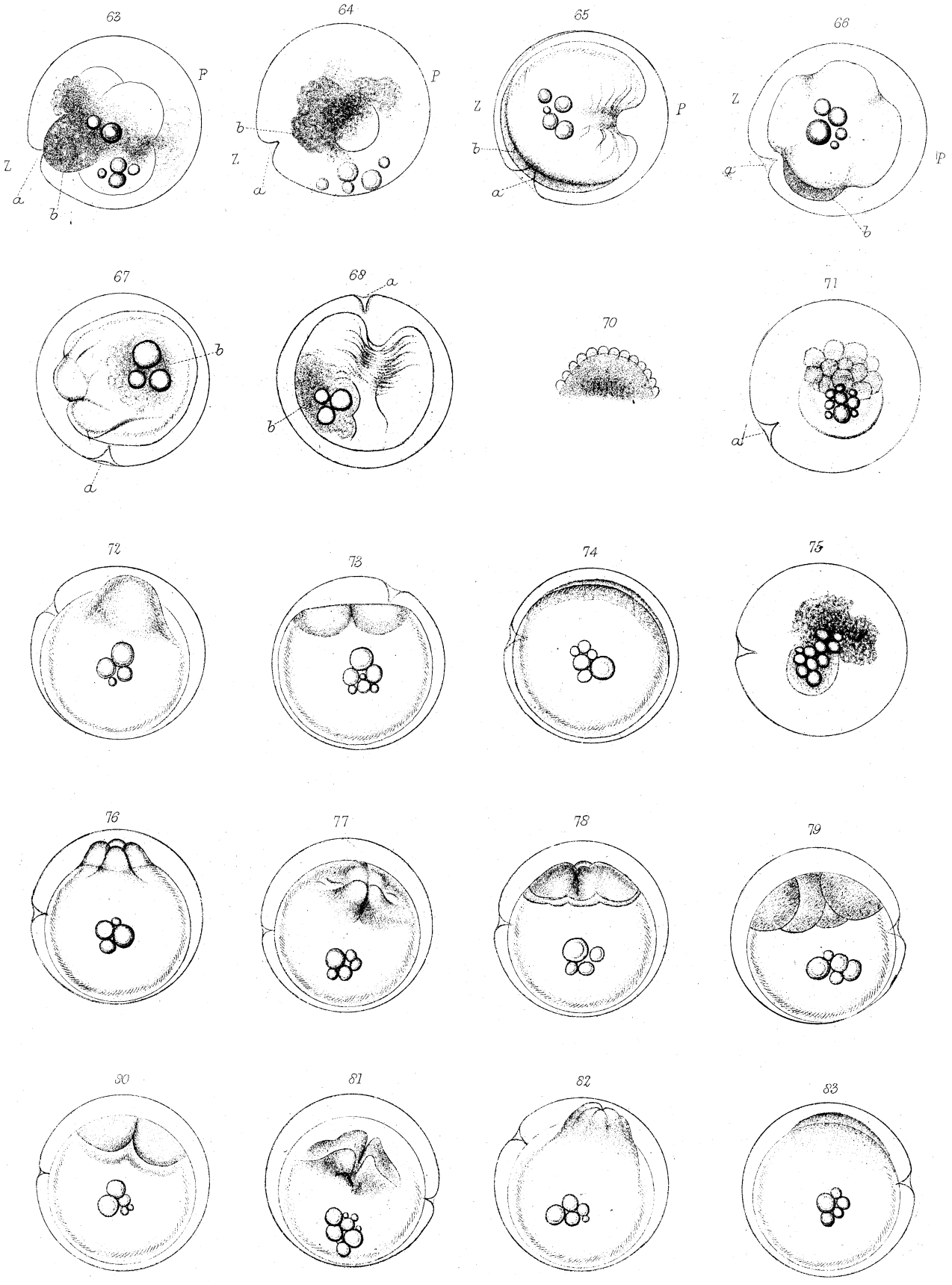

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
22
23
A


20
21
b
a
24
25
a
26
a
b
c
27
31
28
33
32
34
30
29
b
a
35
35′
36
37
38
39
40
41
42



W.H.Rowsom del.

W.West Lith.



W.H. Ransom del

W. West Lith