With the compliments of H J Clark

RECAPITULATION

OF THE

EMBRYOLOGY OF THE TURTLE,

AS GIVEN IN

PROFESSOR AGASSIZ'S "CONTRIBUTIONS TO THE NATURAL HISTORY OF THE UNITED STATES OF NORTH AMERICA."—VOL. II, PART III.

BY

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RECAPITULATION OF THE "EMBRYOLOGY OF THE TURTLE,"

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THE following summary, of the "Embryology of the Turtle," was undertaken at the request of Professor J. D. Dana, one of the editors of this Journal. For certain reasons, given below, it will be seen that neither comment nor criticism upon the work is in place here. The method adopted in writing out this subject, as it stands in the original work, is so far from the aphoristic style that I find it will be next to impossible to make extracts, which will give the pith of the matter, without inserting here a great portion of the whole. On this account I shall be compelled to rewrite, whatever may be presented, in a condensed form, with perhaps here and there a short extract.

Had it not fallen to my lot, as Professor Agassiz's assistant, to write out the embryological portion of these "Contributions" I would not have dared here to take the liberty of reconstructing the fabric of the story of the development of the Turtle. As it is, it will not be possible, for want of room, nor really necessary, to render account of the whole history as originally written, but I will confine myself mostly to what is new in this department of science.

Whenever anything is presented to the scientific world as new and especially controvertive of old and perhaps apparently well established views, every caution is necessary in proving that all liability to error has been guarded against whilst pursuing the investigations.

On this account considerable care has been taken, in the opening part of the "Embryology," to state clearly, and how that the egg and embryo, so called, were kept in as natural a condition as possible. In the study upon the origin and development of the egg, "a young animal was resorted to on account of the greater abundance of the smallest sized eggs and also because the ovary is less opaque than in the adult."

The origin of the egg.—The ovary was cut out entire and floated in serum, so that the eggs might not be distorted by pressure nor by pulling and tearing in order to get them under the microscope. In the first place a comparative view was taken of the whole mass of the eggs, from the youngest to the oldest, with a low magnifying power, and in this manner a rapid survey was made of the respective condition of each egg, and the mind prepared in part, by anticipation as it were, to more readily comprehend the relation of the phase of any one to that of any other or of the whole.

The physiognomy of the egg in its younger stages is so peculiar, with its thick dark outline, brilliant, strongly refractive, homogeneous, yellowish contents, and the lateral nucleus, that it cannot be mistaken for one of the cells, of the corpus graafianum, which press upon it from all sides. "The initial form of an egg is a dark, oily looking, granule-like, spherical body, situated among the interstices* of the cells of the corpus graafianum. As the latter not only, but even their nuclei surpass such an egg in size by several diameters, it is superfluous to debate the question, whether the egg may not be the nucleus of a cell of the generating organ."

At first the egg has no wall about it, but finally by a differentiation of the superficial particles a consistent envelop is elaborated and answers to the name of the *vitelline sac.*

The origin of the Purkinjean vesicle.—About this time, or soon after, the Purkinjean vesicle—germinal vesicle oftentimes called—becomes visible, by a condensation of a portion of the homogeneous yolk against the inner surface of the vitelline sac. The mode of origin of this vesicle is very important to notice, because it bears reference, in a very pointed manner, to the theory of the origin of free cells. Here it is evident that the egg-cell did not originate, as is usually stated of cells, around its nucleus, but that its nucleus is the offspring of the cell which encloses it. The Purkinjean vesicle always remains close to the parieties of the egg, even to the time when it disappears, and in no way points to a falsely claimed relation to fecundation and the origin of the embryo. The wall of this vesicle originates like that of the vitelline sac, about a previously conglomerated mass of particles.

The appearance of numerous vesicular bodies, known as the Wagnerian vesicles, upon the inner surface of the wall of the Purkinjean vesicle, completes the operations, which have here been going on, necessary to the perfecting of the egg-cell, and the rendering of it, although a *cell*, totally different in properties from all other apparently similar cells.

The development of the yolk.—It will be more convenient after this, on account of the complicity of the contents of the egg, to

* "The first blood corpuscles are yolk-cell nuclei which have undergone changes identical with those of the whole 'embryo,' and they alone remain free, circulating in the channels hollowed out in a mass of cells identical with themselves. These are the first cells originating interstitially, but yet, after all, not essentially so, as is the case with the egg; for each blood corpuscle is a segment of an original yolk-cell nucleus, which has gone through the process of self-division; whilst the egg originates just as the primary yolk cell does, by conglomeration of particles, and the formation of a membrane around the parieties of this concretion."

treat of its different constituents separately. We will commence with the yolk first, as that is the fostering substance from which all the other components essentially originate. Previously to the development of distinct yolk cells, the yolk passes through some peculiar granulated phases, the most remarkable of which is the gradual encroachment of granules, commencing at one side of the egg, upon the hitherto homogeneous contents, till they pass across the whole bulk of the vitellus. At one time, during this progressive incursion of the granules, the egg appears as if a half of two different eggs had been stuck together, one-half being homogeneous and the other thickly granulated. After the granular stages, at the time the egg is about one-sixteenth of an inch in diameter, the oily looking granules gradually disappear and at the same time minute hyaline, albuminous, vesicular bodies begin to develop. These again, as is the case with the egg-cell and Purkinjean vesicle, originate without the intervention of a so-called nucleus,* and each one grows for some time without the least sign of a second body within its wall.

"Here then we have essentially, nay in every sense, a cell, a hollow layer of spherical surface derived from the lateral adherence of the superficial particles of a homogeneous globule. It is not a cell formation by the hollowing out of a solid substance, forming at first a very thick wall, which would stretch by the increase of the contents, as it gradually surrounds a larger space, till it thins out to the ordinary crassitude of such envelops. Never, throughout the whole range of cell development in the egg, is there the merest hint of this mode of genesis. From the beginning to the end of the growth of the ectoblast it ever preserves the same thin stratum, apparently of a single layer of corpuscles, and moreover the same tenderness and the same refractory power. Nor can we compare this process to the re-

* "Thus far we have employed, in our descriptions of the egg and its contents' the nomenclature generally in use to designate its different parts, and those of the cell. But this nomenclature, framed to express particular views respecting the mode of formation and the functions of these parts, is completely theoretical in its meaning. It appears desirable, therefore, now that we are about to consider more fully the origin and successive growth of the yolk cells, to discard every technical expression which may imply a theory, and to adopt such only as designate the natural relations of the objects under consideration, especially since the views to which we have arrived cannot be reconciled with the theories which the current nomenclature is intended to express. These parts are therefore designated in the sequel by the following names: *ectoblast* is applied to the outer envelop; *mesoblast* to the so-called nucleus; *entoblast* to the so-called nucleolus; and, when this contains a still smaller body, this is called *entosthoblast*.

In the nomenclature of the egg. similar objections may be raised against the use of germinal or germinative vesicle and dot, as neither of these parts has the slightest reference to the formation of the germ. We shall therefore designate them, henceforth, as some embryologists do, by the names of the Purkinjean and Wagnerian vesicles. Applying our nomenclature to a comparison of the egg with the cell, the *yolk-membrane* is to be considered as an *ectoblast*, the *Purkinjean vesicle* as a *mesoblast*, the *Wagnerian vesicle* as an *entoblast*, and the *Valentinian vesicle* as an *entosthoblast*."

ceived mode of cell origin according to which a wall is condensed around and upon a 'nucleus,' for the mesoblast is often absent in quite large cells; in fact, an egg little more than one-sixteenth of an inch in mean diameter contains numerous cells of considerable size, no one of which contains a mesoblast. Nor can it by any possibility be advocated, that these cells are the contents of other cells, for no others exist; even in a much larger egg, up to full grown ones, this holds good just as undoubtedly, for in such a mass of yolk as larger eggs contain, the mesoblasts and ectoblasts have respectively very peculiar and unmistakable properties, not to be confounded with any other cell contents."

No ectoblast, as these albuminous hyaline vesicles are called, which has attained to a diameter of $\frac{1}{4000}$th of an inch, is without a mesoblast. This latter body, like the Purkinjean vesicle, originates as a conglomeration against the wall of the cell,—the ectoblast,—which directly encloses it, but as soon as it has become well defined in outline it breaks away from its attachment and takes up a position in the centre of the parent cell.

Notwithstanding that the mesoblast soon loses its globular shape, which free cells usually assume, and passes through some rather sharply angular phases, and finally ends with becoming more or less irregularly spheroidal, it appears to have a superficial layer of its mass so consistent as to constitute a wall. Inasmuch as a cell wall has been found, here, in these investigations, to have no definite density and in some cases to be hardly differentiated from the substance which it contains, it has been thought best to extend the definition hitherto used, and characterize it as "a hollow, more or less spherical layer, of indefinite density, tenacity, and refraction, which surrounds the field of some definite though isolated and homogeneous function."

By the time the egg has attained to its ultimate size, within the ovary, the mesoblast has grown so large as to almost completely fill the ectoblast. The size of the mesoblast, at this period, is enormous, far exceeding in this respect the mesoblast of any other free cell known, being about $\frac{1}{730}$th of an inch in diameter.

Perhaps the most remarkable of all the constituents of the egg are the entoblasts of the yolk cells. These bodies appear about the time the egg has reached from one-eighth to one-sixth of an inch in diameter; a single one at first originates in the centre of the mesoblast, and not at the side as we have seen in other cases, but soon others take their places around the first. The marked peculiarity of these bodies is their sharp angularity, from the time of their origin, which increases till they resemble spiculæ; and eventually fill, to surfeiting, the mesoblast. Then, when the egg has reached a diameter of one quarter of an inch, these angular, crystalloid entoblasts begin to lose their corners, and many to disappear altogether, whilst new ones, with rounded angles and more equilateral, develop in place of these.

The Purkinjean vesicle.—We have already described the mode of origin of the Purkinjean vesicle, and mentioned the appearance of the Wagnerian vesicles. These last are developed at first few in number, but subsequently they cover, like drops of dew, the whole inner surface of the wall of the Purkinjean vesicle. Like all cell development heretofore described, the Wagnerian vesicle begins with a faintly visible conglomeration of particles, which eventually obtains a well defined outline. The Valentinian vesicle is remarkable for having very little refractive power, so as to appear like a flat disc in the midst of the parent cell, the Wagnerian vesicle.

By the time the egg has become one quarter of an inch in diameter, the Wagnerian vesicles disappear and give place to an almost total homogeneity of contents in the Purkinjean vesicle. From this time forward the latter grows more and more albuminous, and may be hardened, by boiling the egg, so as to be taken up on the point of a knife. This albuminousness is so marked in the mature ovarian egg that boiling contracts the vesicle by nearly one-half, and the side next the yolk sac becomes collapsed.*

The Zona pellucida.—The zona pellucida, which eventually takes the place of the yolk sac, when the latter becomes resorbed,

* "The clear space, observable in the egg of various animals just previous to segmentation, to which the name of "embryo-cell" has been given, from its supposed intimate connection with the formation of the germ, may be identical with the white area about the Purkinjean vesicle observed in Testudinata. We would take this opportunity to express the opinion, that very probably too much stress has thus far been laid upon the assumption that the Purkinjean vesicle performs a peculiar and exclusive function in reference to the formation of the so-called embryo-cells; and, moreover, that the Purkinjean vesicle is not to be so definitely separated, as regards its essential element, from the immediately juxtaposed substance of similar appearance, but should rather be looked upon as the crowning point of albuminous concentration, to which the opposite side of the egg stands in the reverse extreme of a highly oleaginous nature. A reference to the mode of origin of this vesicle shows this conclusively; for it is developed as a phase of secondary accession in the egg-evolution, and not as the primary basis to a succeeding structure ever after retaining a significance of superior import, and leading, as some would have it, to its becoming in the end the essential element in the genesis of the embryo. This mode of origin alone, we maintain, is sufficient to show that the very foundation upon which its importance is laid cannot be tenable, in this light. The Purkinjean vesicle, therefore, loses all its advocated claims to preponderance over the rest of the egg constituents; to say nothing of the fact that it takes no part in the building up of the blastoderm, excepting that its discharged contents may become absorbed in the endosmotic and exosmotic interchanges of substances between the oily yolk cells, and the albuminous matter in which they float. True enough, the region about this vesicle exhibits a specialized nature; it is there that the embryo first develops certain of its characteristics, previous to its further extension; but it does not follow, that because the Purkinjean vesicle is situated thereabout, it is the basis of this evolution, or in any way causatively connected with it. On the contrary, its presence is itself rather the result of certain tendencies, for instance, the concentration of albumen in that direction; and its disappearance also is the consequence of the consummation of these tendencies."

is formed by a stratification of the inner cells of the Graafian follicle, and therefore is not developed inside of the yolk sac, as has been asserted of it by some authors when treating of its origin in the eggs of other animals. Eventually the zona appears as if made up of columnar cells, and thus remains during the rest of its existence. It may be detected even as late as when the amnios has begun to form.*

The Embryonal Membrane.—The embryonal membrane is a layer, originating by a transformation of the superficial part of the yolk when the egg is hardly visible to the naked eye, and the formation of an excessively transparent cellular tissue, which envelops the whole vitelline mass. In its earliest stages it cannot be compared nor homologized with anything heretofore noticed in the eggs of animals, but when the embryo has begun to develop, it occupies the same relative position as the "*Keimblase*" of Bischoff, or the "*Umhüllungshaut*" of Reichert, and the "*Investing membrane*" of English authors. Yet, insomuch as the "*Investing membrane*" of these authors, according to them, does not develop till the embryo begins to form, it cannot, for a certainty, be homologized with the *embryonal membrane* of the turtle's egg. But it is remarkable that this membrane like the "investing membrane" follows all the curvatures of the dorsal region of the embryo and even becomes a lining to the spinal tube. At the time the turtle is hatched this membrane may be detected as readily as in any of its earlier stages.

Fecundation.—Professor Agassiz's observations are so remarkable, and the facts in regard to fecundation so unprecedented, that I think it worth while to quote here the whole of the section he has written on this subject.

"Ever since I have known that our Turtles lay only once a year, I have been struck with the fact that the ovary nevertheless contains eggs

* "Thompson (article *Ovum* in Cyclop. Anat. p. 78) compares the early yolk-sac of Birds (which he hardly admits as a true *membrana vitelli*, notwithstanding Meckel's researches) to the zona pellucida of Mammals, (the true primary vitelline sac of these animals, interior to the zona, being totally ignored by him; see also p. 50, where he describes the zona as the original yolk-sac and the only one existing in Mammals) and the secondary yolk-sac (the true zona) to the tunica granulosa of viviparous vertebrates. The secondary yolk-sac, he infers, is derived from the cellular lining of the Graafian follicle; but, since at the same time he makes it merely the exterior stratum of a concentric series, the inner of which he insists become the true yellow yolk granules, (the primary yolk-sac, zona pellucida, as he calls it, having disappeared by deliquescence) it looks very much as if he had mistaken the development of the 'membrana investiens' for that of the membrana vitelli. Again he says (p. 78), 'the external edge of the layer of prismatic cells, the length of which is considerably increased, is now surrounded by a narrow pellucid space inclosed by a double line, presenting the appearance as if a small part of the bases of these cells had been fused together in a homogeneous film.' This probably is the true zona pellucida of Birds; he having failed to see the membrana vitelli (already disappeared as he thinks), situated between it and the layer of prismatic cells, from which latter he supposes, but without direct research, that the 'pellucid space' because of its traces of hexagonal markings, is an immediate development."

of very different sizes. I was led by this observation to inquire into the duration of the growth of the ovarian eggs, when I further noticed that these eggs appear in well-marked sets of different sizes, each set being equal in number to the average number of eggs laid by the species under observation. It thus became evident that the eggs require more than one year for their full development. Once upon this track, it appeared practicable to determine how long a period this growth embraces; for, as soon as it could be ascertained how many eggs different species of Turtles lay, there was a standard of comparison obtained for the investigation of the ovaries; and, as I early learned that the species most common about Cambridge exhibit marked differences in that respect, I selected these species for my first studies. Chrysemys picta lays always between five and seven eggs. I have never observed as few as four, and only occasionally eight. Nanemys guttata lays generally two or three; I have only once or twice found four eggs in its nest, and three times in its ovary. There was therefore no chance of making any mistake, when comparing the number of their ovarian eggs with that of the eggs they lay, after I had ascertained that a few weeks before the breeding season there are the same numbers of mature eggs to be found in the ovary as these species usually lay in the spring. I felt still greater confidence in the possibility of coming to precise results, after I had found again and again the very same number of eggs in the oviduct, and noticed that at that time another set of eggs could be readily distinguished, of the same number as the larger eggs left in the ovary. Indeed, the difference between this largest set of ovarian eggs and the smaller ones is so great, even at the time when the eggs about to be laid are still in the oviduct, that they are distinguished at the first glance; for, though they have unquestionably to remain another year in the ovary, they are already nearly as large in diameter as those which have just left it.

"With a knowledge of these facts, it was easy to arrive at a full understanding of the normal periodicity in the growth of the ovarian eggs. It soon became plain, that shortly before the period of laying there were not only two, but as many as four, distinct sets of eggs in every ovary; and that, after the largest set had been laid, a new small set was started from among the innumerable smallest eggs of variable size. It now seemed that a single question remained to be answered. What is the age at which the Turtle discloses for the first time such differences between its eggs? Upon opening large numbers of young Chrysemys picta it was ascertained, that, up to their seventh year, the ovary contains only eggs of very small size, not distinguishable into sets; but that with every succeeding year there appears in that organ a larger and larger set of eggs, each set made up of the usual average number of eggs which this species lays, so that specimens eleven years old, for the first time, contain mature eggs, ready to be laid in the spring.

"Now another question arose, When are the eggs fecundated? Field observations soon taught me that this species copulates before it is eleven years old; I have even seen those that were not over seven years old already performing the act, though I have never seen any in copulation younger than these. Thus it appears that the first copulation coincides with a new development of the eggs, in consequence of which, a certain number of them, equal to that which the species lays, acquire a larger size, and go on growing for four successive years before they are laid, whilst a new set is started every year, at the period of copulation in the spring, enabling this species to lay annually from five to seven eggs, after it has reached its eleventh year.

"The question was then naturally suggested, whether fecundation is the result of the first act of copulation, or of the second, the third, or the last; or whether the first copulation only determines the further growth of a certain number of eggs, which require a series of successive fecundations to undergo their final development. The second alternative appears the more probable when it is remembered that Turtles were observed which did not lay their eggs as usual, though the yolk had undergone all the regular changes through which it passes, up to the time the egg has entered the oviducts. This is another fact which tends to prove that fecundation is a successive act. Though Turtles lay only once every year, soon after the period of copulation in the spring, copulation itself does not take place once merely, every year, as in all the animals known to bring forth young once annually; it is repeated a second time, every year, in the autumn, shortly before the Turtles retire to their winter quarters; and this takes place without apparent connection with any marked change in the growth of the egg at that season. So, in Turtles, fecundation does not appear to be an instantaneous act, resulting from one successful connection of the sexes, as it is with most animals. The facts related above show, on the contrary, that, in Turtles, a repetition of the act, twice every year, for four successive years, is necessary to determine the final development of a new individual, which may be accomplished in other animals by a single copulation.

"It may be suggested, that, by an investigation of the spermatic particles, additional light would be thrown upon these remarkable circumstances. But such investigations present greater difficulties in these animals than could be supposed at first; and notwithstanding the most diligent search, my efforts to trace the spermatic particles through the oviduct, as high up as the ovarian eggs, have been unsuccessful. Turtles do not copulate in confinement; and those which I could catch in coitu in their native haunts have only exhibited spermatic particles in the oviduct. I have, still less, been able to trace the sperm into the egg itself. Indeed, there is no micropyle in the egg of Turtles; and I must confess that I have not yet seen the first fact which could lead me to admit that the spermatic particles penetrate into the egg. I am therefore obliged to abstain from expressing any decided opinion upon the question of the penetration of the spermatic particles into the egg, which has of late attracted so much attention among embryologists. I can only say, that, notwithstanding the high authority upon which it is asserted as a fact that the spermatic particles do pass into the substance of the egg through a definite aperture of its envelope, I am still rather inclined to doubt it.

"The aperture observed in the outer membrane of the egg, which has been called micropyle, has always appeared to me to be the result of the separation of the sac in which the egg is developed, and by no means to pass through the vitelline sac. Without the most careful examination it is not possible to perceive how complicated the sac is, in which the egg is inclosed; and I suspect that a kind of Graafian follicle, which in many animals drops from the ovary with the egg, has frequently been mistaken for a vitelline membrane. I believe, further, that the scar resulting from the separation of that follicle forms the opening called micropyle, and that this opening does not traverse the vitelline membrane. In Turtles the perforated appearance of the yolk sac arises from the presence of the Purkinjean vesicle near the surface of the yolk, and not from the existence of a real hole. After what has been said above of the lateral origin of the Purkinjean vesicle, it is superfluous to insist upon the incorrectness of the view of those who would ascribe its superficial position to the influence of fecundation. It is formed in that position, and preserves it as long as it exists."—pp. 489–492.

Egg Laying.—By careful comparisons, it has been possible to ascertain at what age at least one species begins to lay its eggs and reproduce its kind. Chrysemys (Emys) picta does not lay its eggs before the eleventh year. With other turtles the data were not so complete, but in all probability they may be said to lay their eggs from the eleventh to the fourteenth year, according to the species. They all do this too in the spring, in the month of June, both at the north and south; physical differences not seeming to have the least effect upon this particular function.

The formation of Albumen.—The mode of formation of the albumen is very different from what it is among birds, there being no chalazæ nor spiral arrangement of the layers. The shell and albumen are both deposited at the posterior end of the oviduct, and the albumen is not applied against the surface of the yolk by direct contact of the inner surface of the oviduct, but in a great measure infiltrates through the previously formed shell membrane.

The albumen is composed of innumerable layers of amorphous substance, in which are imbedded a multitude of elongate, oval, coarse, granular bodies pointing in a particular direction in each layer, some along the longer axis of the egg, some across the same, and others in intermediate directions between these two.

The shell membrane.—This arrangement is carried out in a beautiful manner in the shell membrane whose inner layers are composed of the same sort of oval bodies, arranged end to end, and forming a beaded thread. Going outwardly these oval bodies become more and more blended with each other till finally they form a mesh of uniform, even fibres, of excessive thinness and nacreous aspect.

The shell.—The shell is deposited in what appear to be exceedingly tender layers of albuminous substance originating after the manner of the albumen and the shell membrane. The shell is composed of characteristic groups of carbonate of lime, more or less intimately soldered together side by side, each group or nodule consisting of concentric layers of columnar crystals.

The absorption of Albumen.—The albumen is absorbed into the yolk sac in a very peculiar manner; the outer layers are removed first, at a point always above the embryonic disc, so that in the end an inverted conical hole is formed. From this hole the absorption spreads centrifugally till all the albumen is drawn into the yolk sac, or zona pellucida now, and the latter has distended so as to completely fill the shell. At or near the time that the absorption of the albumen commences, the segmentation of the yolk begins; and the embryonic disc is formed, in some species, before the clear hyaline space appears under the embryo.

Self-division of the Mesoblast.—Before we describe the segmentation of the yolk, it is proper to mention another kind of segmentation never before observed in the eggs of any animal. We refer to the self-division of the mesoblast of the yolk cell. This takes place, at least to a certain extent, without the influence of fecundation within a year, but at the same time has been seen only in those eggs which have been expelled from the ovary. Moreover this happens before the so-called segmentation of the yolk mass, and in fact is a forerunner, as if to prepare the way for the latter change.

The mesoblast, which by this time so completely fills the cell that the ectoblast appears like a bright and very narrow halo about it, usually divides at first into two equal portions, rarely into three, each of these into two more, and they each again and again into two more till the ectoblast is filled by an innumerable host of mesoblasts. This process goes on slowly, and lasts as long as the young turtle is within the shell. It is completed first in those ectoblasts which enter into the formation of the embryonic disc, and afterwards in those which are added to the later formed parts of the embryo. After the segmentation of the yolk mass, the ectoblasts become resorbed and the innumerable mesoblasts are left in heaps, which are at first quite distinct, but gradually they blend with each other till finally they form a uniform layer all over the disc and the whole extent of the germinal layer.

The great point gained by these last observations, is to prove that these *self-divided mesoblasts* become the original cells, "*the primitive embryonic cells*," in the composition of the different organs of the body.

The segmentation of the yolk.—The segmentation of the yolk mass has been seen in the eggs of only one species of turtle, *viz*: Glyptemys (Emys) insculpta. This was observed for the first time on the 27th of May, 1854, and afterwards during the two succeeding days, in a series of eggs taken from three different individuals. The process of segmentation is not so regular, and there does not seem to be always, in the beginning, a symmetrical halving of the embryonic area, as has been observed among birds; but in other respects it resembles what takes place within the eggs of the latter animals, and finally results in shaping out the embryonic disc. The most remarkable features to be noticed in the egg at this time, are certain phenomena which tend very strongly to show that segmentation is not confined to the embryonic area, at the upperside of the egg, but that, as has never before been noticed in oviparous allantoidian animals, the whole mass of the yolk becomes segmented.

The whole egg is the embryo.—"Since we have shown in former pages, that the embryonic disc, and its extension, the germinal layer, are formed by the original apposition of yolk-cell mesoblasts minutely subdivided, and that these yolk-cells are all the same through the whole yolk mass from centre to surface, even to the very walls of the superficial Purkinjean vesicle; and, moreover, since it is proved that segmentation obtains beyond the embryonic disc, and very probably all over and throughout the whole yolk, it is evident, that, in the egg of the Testudinata at least, the region around the Purkinjean vesicle cannot be separated from the more exterior or inferior mass which constitutes the greater bulk of the vitelline substance, and that the last cannot be homologous to the contents of the Graafian follicle, which bears no part whatever in the formation of the embryo, but is totally exterior to the Mammalian egg. Again, as will be shown hereafter, that portion of the yolk which is originally excluded from the primary circumscription of the outlines of the embryonic disc cannot be separated from the animal as an appendage, for it very soon afterward becomes an essential part of the 'embryo,' as the latter extends itself in the form of a germinal layer and a vascular area, not only all over the surface of the yolk, but in the case of the area vasculosa, throughout the whole vitelline mass, the latter becoming a great spongy network of blood-vessels, formed by the lateral apposition of the cells composing this large body. This vascular mass is finally drawn into the body, and though gradually disappearing by resorption, remains for nearly six months after birth as one of the essential portions of the organization of the freely moving animal."

The formation of organs.—The first step taken that indicates the formation of organs, is the originating of a furrow around and close to the edge of the embryonic disc. The furrow is the folding of the germinal layer as it begins to turn upward to form the *amnios.* At this time there are two layers all over the surface of the yolk mass; the outer one, the *germinal layer*, is very thin, excepting where it enters into the composition of the embryonic disc; the inner layer, the *subsidiary layer*, is quite thick, and forms the basis of all the organs, except the cerebro-spinal marrow. The germinal layer performs a triple office; in the median line of the embryo, it forms the basis of the "primitive furrow" which is the incipient spinal marrow; exterior to this it constitutes the musculo-cutaneous layer; and more exteriorly still, it folds upwards over the back of the embryo, and becomes the amniotic sac.

The vertebral lamina is formed by the separation of a broad band, about equal in length with the embryonic disc, of loose, unconnected cells, from the upper surface of the subsidiary layer. From this the vertebræ are developed after the usual manner among vertebrates. The cells in the median line of the vertebral lamina become changed very soon, and more compact and united, forming the chorda dorsalis, or the axis of the young vertebræ, which are not as yet apparent. By the development of the chorda dorsalis, the vertebral lamina becomes equally divided into two laminæ lying right and left of the axis of the body. In about twelve days from this time, when four or five vertebræ have appeared, and the spinal marrow is closed over in the anterior of the cerebral region, the subsidiary layer has developed a thick annular ridge on its under side all around the embryo, at a short distance beyond the confines of the original embryonic disc. This ridge eventually becomes a hollow mesh, like a sponge, and then constitutes the *vena terminalis.* Cotemporaneously with the last, another change occurs in the subsidiary layer by which it becomes separated along the median line of the ventral side of the body, from the vertebral laminæ above it, and the heart and dorsal artery are hollowed out in its upper side. Soon after this the omphalo-meseraic arteries are also hollowed out in the upper surface of the same layer from which the heart and dorsal artery originated. In these vessels a granular albuminous fluid surges backward and forward; thus evincing at this early period a beginning of blood circulation in vessels which do not form a complete circle with each other. It is very easily seen that the heart is the impelling power in this case.

The mode of formation and development of the Wolffian bodies is so simple that it is a wonder there has been so much dispute among embryologists about their genesis. They originate as mere thickenings of the subsidiary layer, around and obliquely above each abdominal vein, and leaning over toward the dorsal artery. Being in the same layer with the arteries and veins, the blood vessels of the two systems meet in these organs through very simple channels, running as yet direct from the principal artery to the principal vein. About this time, when the heart has become three-chambered, the vertebræ reach to the root of the tail, and the eyes have become entirely enclosed in complete orbits, the allantois begins to grow. Its formation is as simple as that of the Wolffian bodies, and developed in the same layer with these.

The subsidiary layer approximates its opposite sides and uniting them, arches over a certain space so as to form a sac. At first this sac does not project beyond the body. Soon after or almost synchronously with its formation the allantoidian arteries and veins are developed by simply hollowing out channels direct from the dorsal artery into the allantois, and thence, all in the same layer, into the allantoidian veins which course along the edge of the abdominal opening. Now too the embryo turns upon its axis, and rests always upon its *left side.* Why it rests upon this side and not upon the other, sometimes at least, is simply because this position belongs to its plan of development. A little later the omphalo-meseraic arteries have joined their numerous currents into one single vessel for a short distance from the dorsal artery. The nostrils have begun to develop at this period, and may be recognized as two simple indentations at the end of the head. They have no communication with the mouth at this time.

The vena terminalis merits attention at this time, inasmuch as it is as concentrated in its course as it ever will be. It never has, from the beginning, been a single vessel, as occurs with birds, but is composed of numerous closely set anastomosing currents, running parallel wise to each other. Not long after this time the vena terminalis, to one half of its circuit, sinks a short distance below the surface of the yolk, carrying along with it the afferent vein. The nostrils have grown deeper and broader, and communicate with the mouth, but only through a very shallow furrow, nothing like the deep channel which has been described in Mammals, but just deep enough to show that there is a tendency to follow the type of formation prevalent among higher Vertebrata. In the next step the nostrils become narrowed at the aperture, and the shallow furrow, mentioned above, is closed over, leaving a very narrow fissure to indicate its former position. The degree of communication between the mouth and nostrils in this case, is intermediate between that which obtains among Mammals where the mouth and nostrils are all one cavity during the early periods of development, and that of Fishes, where these two openings are always independent of each other. The shield begins to develop by a budding out laterally of the musculo-cutaneous layer along the sides of the body, and the development of narrow ribs extending to the very edge of the roof-like covering.

The feet, or rather paddles of the lower forms of turtles, the Chelonioidæ, do not remain in a partially undeveloped state, as might be expected from what is observed among other vertebrates, but undergo what may be called an excess of development; the bones of the toes becoming very much elongated, and the web,—which remains soft among some turtles with moderately elongated toes,—is hardened by the development of densely packed scales, so that the whole foot is almost as rigid as the blade of an oar. About two months before the time they were hatched, some young of Chelydra (Chelonura) serpentina, were observed to move the head, feet, tail, lower jaw, tongue, and also the toes separately, and to roll the eyes. In a turtle a little older than these, the omphalo-meseraic vein runs in a direct line through the yolk mass, and joins the exterior boundaries of the vascular area at the lower side of the egg.

By the time the shield has become broadly oval the embryo assumes again an erect position in the egg, and thus remains till it is hatched. The embryo of Chelydra serpentina already shows its predaceous propensities, by snapping at everything which touches it. Just before the young were hatched the edges of the jaws were cut open longitudinally, and disclosed a series of small cavities, into each of which, a branch from the maxillary nerve ran. No doubt these indicate a typical tendency to the formation of teeth. When the young is hatching a large mass of the yolk sac is still hanging outside of the umbilical opening; but within a few hours it is altogether drawn into the body and occupies a large space in the abdominal cavity. The circulation in the yolk sac at this time is in full tide of operation. Externally the allantois withers and falls away, but, within the young turtle, its neck is persistent and becomes the urinary bladder.

The brain at this time does not extend in nearly a straight line, as is the case in the adult, but is still considerably bent downward at its anterior end. The eye presents some hitherto unknown peculiarities at this time; the capsule of the lens is a triple membrane, excessively thin, very tough, glassy, and elastic. It is this membrane which supports the "membrana pupillaris" and not the hyaloid membrane as has been claimed for some of the Mammalia. The "membrana pupillaris is a thick double membrane and does not seem to be resorbing, but on the contrary, would appear to be persistent through life, inasmuch as it was found in a turtle,—Trachemys scabra,—twenty years of age.

Histology.—A great part of the histology relates to the young turtle about the time it was hatched. In the formation of nerve fibres or tubuli, the olfactory nerve furnished the most conclusive proof that these tubuli originate by the soldering end to end of the nervous cells, and the obliteration of the intervening walls at the point of contact. All stages of development were represented here, from cells arranged in a line end to end, with partially absorbed walls, up to those where the only indication of the position of the cells which compose the tubuli, were the mesoblasts (nuclei) arranged in a line at regular intervals. In the bones, the granular ossification presents nothing very remarkable; but, in the ribs, the relation of the outermost fibrous layers to the fibres of the corium of the shield, is very important to notice. The only difference observable between the fibres of the growing and widening edge of the ribs and those of the corium, was that the former were hardened by ossified granules deposited in lines, and the latter were perfectly soft. In all probability the wings of the ribs, which unite eventually and form in some turtles, a solid shield, are developed by the hardening of the component fibres, by calcification. In fact it would be difficult to determine how much of the shield is composed of the fibrous layers of the ribs and how much of calcified fibres of the skin. In the eye, the nervous fibres, which run in lines from the entrance of the optic nerve along the inner surface of the retina to its anterior border, do not spring directly from the optic nerve, as has been usually asserted in regard to the eye of Vertebrata, but are tail-like prolongations from each retinal cell, which lies abutting against the hyaloid membrane.

The whole retina, including the membrana Jacobi, is composed of five apparently distinct layers of cells. When examined closely these layers are seen to be only different varieties of cells. The cells of one layer send tail-like prolongations into the layer above and below, but in no instance have these prolongations been seen united so as to form a continuous string of cells reaching from the outer to the inner surface of the retina. In this respect the retina is lower in degree of development than that of Mammals, where H. Müller and Kölliker have seen the string of fibres in the greatest perfection. The epithelial cells of the stomach and esophagus are endowed with an unheard of arrangement of vibratile cilia. Instead of being scattered over the whole free surface, the cilia are placed in a circle forming a crown to the end of each cell. So that when seen face-wise the interior of the esophageal and stomachial cavity appears as if lined with a net-work of vibratile cilia. The surface of the lungs is covered by a thin layer of very pale round cells, with numerous dark granules intervening. Over the course of the blood vessels these pale cells are almost hidden by densely packed dark granules, arranged in two, three, four, or five rayed star-like heaps.

The blood corpuscles at first are very minute globular bodies, in fact, nothing more nor less than some of the cells of the subsidiary layer, loosened from the sides of the original blood channels. The cells increase in size and grow transparent; then they become oval, and the mesoblast leaves its lateral position and takes a central one, and finally in the last month of incubation they begin to be flattened, and assume the discoid shape proper to the adult state. A short time before the turtle is hatched, the muscles attached to the dorsal arch exhibit very clearly how the fibres originate from the soldering of the ends of the spindle-shaped cells to each other, and the obliteration of the intervening walls. The granular contents of these cells is partially arranged in lines parallel with the sides of the developing fibres. In the muscles of the fore-leg the granules may be recognised as components of the fibrillæ, and as such giving the fibres a transverse striation.