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I.—On the Development of the Smaller Blood-vessels in the Human Embryo.

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PLATES LXXXVII., LXXXVIII., LXXXIX., AND XC.

THE researches upon the formation and development of the smaller blood-vessels in the human embryo, described in the following pages, were made some years ago simultaneously and on the same material with those upon the origin and development of the blood-corpuscles in man, published in the February number, 1874, of this Journal. The coloured blood-corpuscles, as set forth in that article, were found to originate within certain primary follicles in the wall of the umbilical vesicle which, in this instance, was attached

EXPLANATION OF PLATES LXXXVII., LXXXVIII., LXXXIX., AND XC.

FIG. 1.—Fragment of the chorion of the small human ovum with the abnormal embryo, showing the manner in which the villi arise by small roots, and also the process of multiplication of nuclei by gemmation. Magnified 465 diam.

FIG. 2.—Various forms of the villi. Magnified about 6 diam.

FIG. 3.—Formation of embryonic blood-vessels by the coalescence of cells, arising from the nuclei by budding. Magnified 465 diam.

FIGS. 4 and 5.—Embryonic blood-vessels in the course of development. Magnified 465 diam.

FIG. 6.—Fragment of a villus from the chorion of a human embryo, about 16 millimètres in length; *a*, portion denuded of its epithelium, showing its fibrous structure with two capillary vessels in the process of formation; *b*, portion covered by the epithelium, which, out of focus in the middle, is only seen at the margin. Magnified 265 diam.

FIG. 7.—Deeper layer of epithelial nuclei of the same villus, showing their multiplication by gemmation. Magnified 465 diam.

FIG. 8.—Formation of blood-vessels by the fusion of spindle-shaped bodies in the pia mater of the spinal marrow of a human embryo about twelve weeks old. Magnified 465 diam.

FIG. 9.—Blood-vessels of the pia mater from another embryo of the same age. The granular fibrillous bundles, of which the larger vessels were originally composed, are still distinguished. Magnified 465 diam.

FIG. 10.—Artery from the pia mater of the spinal marrow of a human embryo about nine to ten weeks old, showing three coats of the vessel. The interior is filled with blood-corpuscles, the greater portion of which became discoloured by the action of the chromic acid solution, in which it had been laying; the rest, comprising the younger ones, remained unaffected. Magnified 465 diam.

FIG. 11.—Vein from the same preparation. Among the blood-corpuscles a

to an imperfectly developed embryo of an ovum of very small dimensions. The embryo, arrested in its growth, consisted only of a mass of embryonic cells, nuclei, fibrils, &c.

It was in the outer membrane of this small ovum, which, like the umbilical vesicle, appeared to be normal in its development, that I observed the primary formation of the smaller blood-vessels, as well as a certain process of multiplication of nuclei, which, as far as I know, had up to that time not been observed in the tissues of vertebrated animals.

From the examinations made on the chorion of this ovum, together with others of older embryos in various stages of development, it appeared that the formation of the smaller blood-vessels occurred in two different modes. The one, belonging to the earlier period of development, was observed to consist in a coalescence of certain cells, while by the other, at a somewhat later period, the formation of the blood-vessels was effected by the fusion of certain spindle-shaped bodies.

A short time ago, however, long after these observations were committed to the paper, a human ovum, still smaller than the one already mentioned, was put into my hands, only a few hours after its abortion. This specimen was the smallest I had ever seen, for the embryo which it contained scarcely measured 6 mm. in length; it was perfectly normal and fresh, and still buried in the membrana decidua, from which I had to remove it.

While the examination of the umbilical vesicle of this embryo corroborated my former statements regarding the origin of the coloured blood-corpuscles, as we shall see hereafter, that of the

number of mother-blood corpuscles are seen, and also some free blood-crystals. Magnified 465 diam.

FIG. 12.—Blood-vessel from the pia mater of the spinal marrow of a human foetus 5½ months old; *a*, nuclei belonging to the fibrous coat; *b*, nuclei belonging to the muscular coat; *c*, the same, bearing vesicles; *d*, cells of the epithelium in its formation. Magnified 465 diam.

FIG. 13.—Small human ovum; *a*, membrana decidua vera; *b*, decidua reflexa, separated from the ovum and pinned down. The light spot upon it represents the embryo seen through the membrane. Natural size.

FIG. 14.—The embryo of this ovum. Natural size.

FIG. 15.—The same enlarged; *a*, the elongated coccyx, making a spinal turn.

FIG. 16.—The same, showing the left side. The spinal turn of the inferior portion of the spinal column has been straightened, in order to show its proper shape; also enlarged.

FIG. 17.—The same embryo (enlarged), after having remained about eighteen hours in a weak solution of chromic acid. Its various organs described in the text are here exhibited; the umbilical vesicle has, for the sake of completeness, been represented entire, though at this time a piece of it had already been removed for examination.

FIG. 18.—Represents the structure of the umbilical vesicle of this embryo, as described in the text. Magnified 465 diam.

FIG. 19.—Embryonic blood-vessels from the chorion of the same specimen, containing embryonic blood-corpuscles in the form of granular nuclei. Magnified 465 diam.

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chorion, however, showed the formation of the smaller blood-vessels not to take place, as I had observed in the former cases, by the coalescence of certain round cells, but by the fusion of spindle-shaped bodies. From this apparent discrepancy, observed in the formative process of the smaller blood-vessels of the chorion of these two different ova, it must, however, not be inferred that my former observations were incorrect, but we must rather seek for an explanation elsewhere. For this reason, too, I shall in the following pages confine myself to an accurate statement of the phenomena I observed, from which the reader may draw his own deductions.

In returning now to the first ovum, containing the imperfect embryo, we shall commence our statements with a description of the structure of its wall. This consisted of two membranes, an outer and stronger one, the so-called chorion, giving origin to the villi, and an inner one, consisting of an exceedingly delicate loose fibrous tissue. The outer surface of the chorion, including its villi, was covered by a comparatively thick layer of primary epithelial cells or nuclei, imbedded in a granular matrix. In removing this layer, it was found that its base, the membrane itself (Fig. 1), consisted of an amorphous finely granular substance, in which, however, a considerable number of fine granular fibrillæ were crossing each other in various directions. These represented the elementary form of the later fibrous or connective tissue. A considerable number of certain forms of nuclei and also cells were imbedded in the membrane. The most interesting of these elements were a considerable number of nuclei, distinguished by bearing on their surfaces concave depressions, corresponding to the segment of a sphere, either empty or occupied by double-contoured vesicles or cells (Figs. 1 and 3). They remind us of those mother-blood corpuscles, described in the paper before mentioned, having, as will be remembered, similar depressions on their surfaces, which, however, differed in this respect, as they were produced by the separation of a consistent, though soft body (the embryo blood-corpuscle), in the case of the nuclei in question, they were caused by the separation of a small vesicle, arising from the substance of the nucleus; in other words: in the one case, the product was an embryo blood-corpuscle, while in the other it was a vesicle or cell, destined, as we shall see, to take part in the formation of the primary embryonic blood-vessels, or to develop, perhaps, into a nucleus itself. The process of multiplication of the nuclei and cell, therefore, instead of consisting in a division of the old, we find here to take place by a separation of the young brood in the form of a vesicle or bud, being in reality a process of *budding* or *gemmation*. The whole process takes place as follows: on the surface of the nucleus, and partially imbedded in its substance, a small globular body appears, which, gradually enlarging, is developed into a clear double-contoured cell. This may either

remain in its place and take part in the building up of the small blood-vessels, or be thrown off from its mother-body and be developed into a nucleus itself. In the latter case its contents become more opaque and small granules appear within them.

The concave depression which the young nucleus leaves behind on the surface of the mother-nucleus never disappears entirely, no matter how much the latter may change its original form by the production of others. For this reason the productive force of the mother-body can always be inferred from the number of depressions. Several vesicles or young nuclei may arise simultaneously from the old one (Fig. 3); accordingly, I observed in the chorion of the ovum in question a number of them, bearing three to four vesicles, and, judging by the number of depressions, one nucleus may even produce five to six of its kind. Along with the production of new vesicles the substance of the old nucleus seems to be rendered more dense, its double contour and the granules it contains become less distinct, and the whole body assumes at last a shining, greenish tint. This is especially the case in specimens which have been laying for a few days in a weak solution of chromic acid; in these, the vesicle, at its first appearance, is distinguished by a reddish tint. In consequence of the concave depressions, produced by the formation of the vesicles, and in proportion to the diameter of the latter, the mother-nuclei appear in many fold, mostly unsymmetrical forms (Figs. 3, 4, and 5), and it is difficult to find an object of suitable comparison. In many cases the vesicle is developed, as in the formation of blood-vessels, into a large, clear, double-contoured cell. The greater now the diameter of the cell, the greater is also the depression observed on the nucleus, and it is for this reason that, after the production of a number of cells, very little of the substance of the nucleus is left. Equally as varying as the form of these nuclei were their diameters; while, namely, those of the smaller ones only reach to about $\frac{1}{100}$ mm., those of the larger attain a size of about $\frac{3}{100}$ mm. in length, by $\frac{1}{100}$ mm. in breadth. The latter are found in connection with the formation of the first embryonic blood-vessels. The more the entire embryo advances in its development, the more also the size and fertility of these mother-nuclei are reduced.

Long before I discovered these mother-nuclei, bearing buds in the form of cells, in the membranes of the ovum, I had met with them in the nervous tissues as well as in the pia mater of those human embryos on which I had worked. But as they appeared here in a smaller form, and with smaller vesicles or corresponding depressions, I failed to recognize their true nature, and considered them passingly as old nuclei, deformed by degeneration. Nevertheless, however, did I observe in their immediate neighbourhood, their offsprings in the form of small, round, and pale bodies, which,

as I could nowhere discover evidences of a process of multiplication by a direct division of the nuclei, almost inclined me to think more seriously of a primary formation of nuclei from the plasma itself. It was not until I met in the epithelium, covering the villi of the chorion of a human embryo of about 16 mm. in length, with nuclei, provided with vesicles or their corresponding depressions, that my attention was called to the process of multiplication by budding or gemmation. But further proofs in corroboration of this idea were still wanting, until chance threw the small human ovum under consideration into my hands, in the membranes of which I observed the whole process in full operation.

Besides these mother-nuclei, a considerable number of others, of a more or less oval form and bearing no vesicles, were imbedded in the membrane; they were distinguished by a double contour and a fine granular aspect, containing a number of fine granules. A small number of nucleated cells of a granulous appearance were further observed here. The concave depressions, which many of these bodies showed upon the surface, inclined me to look upon them as mother-cells.

The villi, arising from the chorion of the ovum, were tuberos bodies, connected to each other in a manner similar to that of the branches of a tree, by shorter or longer stems. (Fig. 2.) To the larger bodies, smaller pear-shaped ones were attached by means of fine pedicles. The entire tree-like body thus formed seemed to be connected by a fine stem to the membranous chorion. But by a closer examination, and after the removal of the epithelium, I found that this stem arose in reality from the membrane, by a number of fine branches or roots. (Fig. 1.) The tissue of the villi resembled that of the chorion itself; it consisted of very fine granular fibrillæ, with a number of more or less oval nuclei imbedded in it. The epithelium covering the membranous portion of the chorion, and extending over the villi, consisted in this small ovum of a granular matrix with oval nuclei, a considerable number of which were provided with the above-described vesicles and concave depressions. This fact proves that it is a production of the ovum itself.

The inner membrane of this ovum consisted of a loose, spider-web-like connective tissue of fine, wavy fibrillæ, containing a number of free nuclei and nucleated cells. Some of these latter were open, and apparently in the act of setting free their nuclei, showing that here the process of multiplication took place by the endogenous mode. Besides these, however, a number of small nuclei-bearing vesicles were also observed. No trace of epithelium could be discovered on the inner surface of this membrane.

Let us now direct our attention to the formation of the primary blood-vessels, such as I observed it to occur, not only in the chorion of the small ovum above described, but also in that of some-

what older ova, as well as in the pia mater of the spinal marrow of a very small embryo. As has been remarked before, some of the vesicles or cells, springing from the mother-nuclei, were probably developed into ordinary nuclei, pertaining to the fibrous tissue of the membrane; a portion of these might even assume the function of mother-nuclei and give rise to other vesicles. The rest, however, especially the larger ones, were observed to take part in the formation of the embryonic vessels of the membrane. This seems to be effected by a number of these cells, arising from their respective nuclei at different points, and meeting each other in such a manner as to form a row. (Figs. 3, 4, and 5.) To form this row, however, they were not placed, as might be expected, in regular order, cell by cell; on the contrary, the nuclei, from which they were seen to arise, were distributed throughout the membrane without any special arrangement or order; neither was their size and form more regular. The formation of a vessel, of course, is brought about by the coalescence of cells arising from neighbouring nuclei, and opposite to each other. The tube of the vessel is not necessarily formed by one single row of cells; for when, by the meeting of two cells unequal in diameter, a vacancy is left, this will be filled up by a third cell, arising from the nucleus of one of the former. Rarely less than two cells were seen to arise from one nucleus, but more frequently five to six. Often, large irregularly-formed cells were met with in the course of a vessel, the nucleus of which was marked on its margin by a number of crescent-shaped indentations. In these cases, the large cell was formed by the absorption of the contiguous walls of a number of small ones, which originally had arisen from one and the same nucleus, of which the crescent-shaped indentations were indicating the place of their origin. (Fig. 4.) After the tube of the vessel is fully formed by the absorption of those portions of the cell-walls contiguous to each other, the nucleus assumes, as it seems, by a gradual rounding of its indentated margin, a round or oval form. Very frequently, however, by the production of a number of cells, it is deprived of so much of its substance, that finally nothing of it remains but a long indentated body, which by the rounding of its angles assumes an oblong form. This form is often met with in the walls of young vessels, long after the cellular formation of the blood-vessels has ceased to occur. In some instances, especially with the smaller vessels, we meet with two cells arising from two opposite points of one and the same nucleus, the latter occupying the whole diameter of the row (Figs. 3 and 4), thus seeming to form an obstacle to the opening of the vessel. This, however, is overcome, I think, by the growth of the cells, at the expense of the substance of the nucleus, of which at the end of the process only little will be left. Thus the absorption of the entire nucleus will sometimes be effected. In the nervous tissues

of the human embryo, I observed the nuclei, belonging to the primitive fibrils of the axis cylinder, disappearing during the process of development by fusing with the latter. In the small ovum under discussion, the formation of the blood-vessels, as described above, was observed to occur throughout the entire chorion to the roots of the villi, but had as yet not extended into the latter.

In examining the chorion of an embryo of about 16 mm. in length, we still find a considerable number of mother-nuclei with vesicles, budding from their surfaces, imbedded in the tissue. The structure of the membrane is on the whole further developed, for the fibres of the connective tissue are more definite in their form, and the cells of the epithelium placed nearer to each other. The stems of the tuberous villi, arising from the surface of the membrane, have gained in thickness, also their epithelium, which now consists of two layers of cells. (Figs. 6 and 7.) The fine fibres of the connective tissue of the villi themselves are fully developed and have lost their granular aspect. But the smaller blood-vessels, although more numerous, and having penetrated into the villi, still consist of cells, only partially fused with each other. (Fig. 6.)

Among the blood-vessels of the pia mater of the spinal marrow of embryos of about the same period as the preceding, some, especially the larger ones, were found to contain a considerable number of coloured blood-corpuscles, showing that the circulation of the blood through them had already been established; while others, especially the capillaries, still showed their original formation by the cellular structure of their walls, and the entire absence of blood-corpuscles in their interior.

The muscular structure of the heart at this period consists of embryonic muscular fibrillæ, each of them representing a row of simple granules. The walls of the larger blood-vessels, such as the aorta, umbilical vessels, &c., consisted of a granular, amorphous, already partially fibrous matrix, in which a considerable number of oval or even round nuclei were imbedded. On their inner surface a number of clear cells, containing a coarsely granular nucleus, devoid of an enclosing membrane, were met with. These cells, being present in various other tissues of the embryo, probably stand in some relationship to the multiplication of the nuclei.

Judging from the development of the heart and the larger blood-vessels of this period, it would seem that the circulation of the blood were already complete; that this is not the case is proved by the almost entire want of fully formed capillaries in a number of organs which I examined. For this reason I am inclined to believe that it occurs only through anastomosing branches of the smaller arteries and veins. Further, the fact that the smaller vessels of the chorion, and particularly those of its villi, still mostly consist of cells, the contiguous walls of which are as yet not fused,

proves sufficiently that even here the circulation is not fully established, and that accordingly the embryo does as yet not receive its nutriment from the mother directly through the circulation of its own blood.

From these observations on the formation of the primary embryonic blood-vessels we may infer that the larger vessels are very probably formed, in the same manner and simultaneously with other parts and organs of the embryo, from the embryonic cells of the *area germinativa*, while those smaller ones, imbedded in membranes, are formed by the coalescence of larger and smaller cells, as above described.

Continuing our examination of the blood-vessels of the pia mater of the spinal marrow, which membrane in regard to its delicate structure and transparency is better adapted than other tissues for this purpose,—at a later period, in embryos, about eight to nine weeks old, we find that the embryo has entered another stage of development, in which these vessels are no more formed by the coalescence of cells. The pia mater here is still represented by an amorphous, granular membrane, in which, however, a thin layer of delicate connective-tissue fibres has already been developed, and containing besides a considerable number of oval or round nuclei. The latter consist of small granules, surrounded by a thin membrane. Among them, however, a number of mother-nuclei, with small buds arising from their surface, are also observed, showing that multiplication of these nuclei still occurs by the process of budding or gemmation. The blood-vessels within the pia mater show different stages of development.

The formation of those vessels described in the preceding pages was brought about, as we have seen, by the coalescence and subsequent fusion of cells, and so were the greater number of those within the tissue of the pia mater of embryos from nine to ten weeks old, through which, as they were filled with blood-corpuscles, the blood had been evidently freely circulating. A smaller portion of these, however, still in process of development, were observed to be formed by another process. Where, in the former instance, the formation of the tube of the vessel was accomplished by the coalescence of cells, it is now by the fusion of elementary fibrils. The process of gemmation, though still occurring, as far as the multiplication of nuclei is concerned, has ceased for the formation of vessels; the *fibrillous* formative process of the blood-vessels has now commenced. This consists in the formation of granular fibrillæ, lying parallel to each other, and becoming in the course of their development fused into the form of a tube.

Postponing at present a more minute demonstration of this process, let us return to the already formed blood-vessels of the pia mater of the last-mentioned embryos. The greater part of the

vessels, imbedded in this membrane, embracing, as it seemed, a considerable portion of the larger and smaller capillaries, consisted of tubes of about $\frac{3}{100}$ to $\frac{1}{100}$ mm., the wall of which represented a clear, very transparent membrane. In this, a number of oval and irregular oblong nuclei were observed. The irregular form of many of these bodies lead me to regard them as the remains of those mother-nuclei from which, in the chorion of the smallest embryos, those cells, forming the primary embryonic blood-vessels, were seen to arise. The larger vessels were filled with blood-corpuscles, which, by the action of a weak chromic acid solution, in which the specimens had been preserved, had lost their colouring matter, and thus appeared now in the form of clear double-bordered cells. The smaller ones, on the contrary, contained only a few single corpuscles, which appeared to have been forcibly pressed into their interior. This circumstance points to the probability, already mentioned in connection with the development of the coloured blood-corpuscles in man, that these bodies penetrate with every contraction of the heart only gradually into the newly formed but still closed blood-vessels. Among the blood-corpuscles, contained within larger vessels, a number of mother-corpuscles, bearing one embryo-corpuscle, were always observed.

In examining the larger vessels, imbedded in the pia mater, of this period, viz. the small arteries and veins, their structure was already found considerably more complicated. Their walls, namely, consisted no more of a homogeneous membrane with large nuclei, but of two distinct layers. In place of the large nuclei, often irregular in form, a larger number of smaller ones, of an oval or roundish form, and imbedded between the two membranes, are now observed. These I presume are the descendants of a small number of mother-nuclei with small vesicles budding from their surfaces, always found in company of the smaller nuclei, which they seem to have preceded. The inner membrane probably represented the original tube of the vessel, while the outer one, distinguished by fine, longitudinal, fibrillous lines, represented its later fibrous coat. In the arteries, the fibrous character of the latter was more strongly marked than in the veins. In the larger arteries and veins of the pia mater these two layers, forming the wall of the vessel, and distinguished by their regular, sharply-defined double contour, were found to be separated from each other by a third layer of irregular thickness. (Figs. 10 and 11). The latter most probably represented the muscular coat of the vessel in progress of formation. In those cases where it was found, the nuclei were imbedded in it, and a number of them were observed to assume a position in which their long axis comes to lay at right angles with the axis of the vessel. No muscular fibres, however, could as yet be distinguished.

The greater number of the vessels just described were filled

with coloured blood-corpuscles, of which the greater portion had lost their colouring matter by the action of the chromic acid solution, and appeared in the form of clear double-bordered cells, while the rest, comprising the younger corpuscles, had remained unchanged; in many vessels they were crowded in such a degree that the former, by mutual pressure, had assumed a hexagonal form. A number of mother-blood corpuscles were always found among them; they had also become discoloured, while their embryo-corpuscles had retained their colouring material. In many of the larger vessels, besides the blood-corpuscles, a number of coloured molecules and also small blood-crystals were found. Sometimes even blood-corpuscles were met with, in the substance of which small crystals were found to be imbedded. Many vessels were completely coloured by the hematin escaped from the blood-corpuscles.

The blood-vessels were probably found, as already mentioned, by the coalescence of cells. But besides these, I observed in the same pia mater, a number of capillary vessels in progress of formation, the fibrillous composition of which attracted my attention. The result of a series of close examinations, which I subsequently made on the smaller vessels of the pia mater of this embryo, as well as of a number of others of different periods of development, convinced me that these vessels were formed in a different manner from the preceding, to which I have already alluded. This consists in the formation of granular fibrillæ, laying parallel to each other and becoming eventually fused into the form of a tube. (Figs. 8 and 9.) In connection with the development of the coloured blood-corpuscles, I have already pointed to the different modes of origin and development during the earlier and later periods of embryonic life. In the development of the embryonic blood-vessels, according to these observations an analogous change in the mode of formation seems to take place. The exact time at which the first or *cellular* process of formation is superseded by the second, or *fibrillous*, I am at present unable to state.

In an embryo of 16 mm. in length, I found all the vessels of the liver still to consist of granular fibrillæ, while those of the chorion and its villi, as well as those of the pia mater, were formed by the *cellular* process, above described. It appears, therefore, that the formative process of the vessels is not always the same in different organs. In the pia mater, as far as I am able to judge, the fibrillous formative process seems to have already commenced about the eighth or ninth week, and to be in full operation some weeks later. We will now describe its course, as I observed it in the pia mater of human embryos of about twelve weeks, where, at this period, the small arteries and veins, as well as the capillaries, prove to be quite numerous.

The primary elements of the vessels to be formed represent gra-

nular, spindle-shaped bodies, with an oval nucleus in their centre ; they are formed by the granules of the plasma, arranging themselves into rows at the opposite poles of the nucleus. The latter, therefore, forms the basis of development. After one row has thus been formed by the mutual arrangement and attachment of these granules, and attained a certain length, a second one, starting also from the nucleus, begins to be formed in the same manner ; after this, a third and fourth, until their aggregate breadth equals that of the nucleus. But, as with the beginning formation of the succeeding rows of granules, or fibrils, those first formed continue to elongate by the attraction and appropriation of new granules, a spindle-shaped body is the result. In the formation of a vessel by a greater or smaller number of these bodies, they join—already during their own formation—each other laterally in such a manner that a part of the length of one overlaps another ; they finally are fused with each other into the form of a tube.

In consequence of the various directions in which these spindle-formed bodies, or the bundles formed by them, are developed, they frequently cross or meet each other. When this occurs, it is usually found that the point of the smaller body or bundle adapts itself to some extent to the lateral border of the larger. As the result of a number of such connections, or points of fusion, the meshes of the capillaries are formed. (Fig. 8.) In this manner, capillaries, still in progress of development, are frequently observed to be fused with larger vessels, through which blood-corpuscles have already been circulating. The small arteries and veins are formed by the fusion of a number of bundles, composed of the spindle-shaped bodies. The contiguous borders of the latter can still be recognized on the vessels of the embryo of twelve weeks. (Fig. 9.) Frequently one of the larger vessels is observed to be formed by the union of two smaller ones, the latter still being in process of development themselves. In the pia mater of human embryos of this age, the fusion of the spindle-formed primary elements into bundles, and of these into larger or smaller vessels, is observed to take place in an irregular manner, and also, as already mentioned, in various directions. It is owing to this circumstance that up to this period the form of the meshes, and even that of the vessels still in process of development, is quite indefinite. Equally irregular are the numerous nuclei, contained in the walls of the arteries and veins, in size as well as distribution.

The tube of the vessel being thus formed by a parallel apposition and subsequent fusion of those fibrillous spindle-shaped bodies, is opened and distended by the blood-corpuscles penetrating into it from the neighbouring more fully developed vessels ; thus we meet in the newly-formed capillaries only a few blood-corpuscles, which have evidently been forced into them from larger neighbouring

vessels, as they are seen to distend the calibre of the vessel only to a certain distance. (Fig. 8.) The further development of the vessels is moreover promoted by the nutrient matters contained in the liquor sanguinis. While a number of blood-vessels are thus gradually approaching their final development, and in consequence the circulation of the blood is further extended, others are still formed by the same process.

At what period the formation of new vessels in the human embryo ceases to take place I have not determined, as my examinations concerning this subject were extended only to the vessels of the foetus, about $5\frac{1}{2}$ months old, in the pia mater of the spinal marrow of which new capillary vessels were still seen in the process of formation.

The further development of the newly-formed capillary vessels consists, as already mentioned, of the fusion of the granular fibrils of which they still consist, into a structureless membrane. In the small arteries and veins, formed by the *fibrillous* process, the granular fibrils are developed into permanent, smooth fibrillæ of connective tissue, while the diameter of the vessel increases by the continuous formation of new fibrils from the plasma of the blood circulating within them. The multiplication of the nuclei takes place by gemmation.

In the pia mater of the embryo of about nine weeks we found, as will be remembered, the largest vessels already composed of three distinct layers, though in the middle and inner one no particular structure could as yet be recognized; the outer layer alone was composed of delicate fibres of fibrous tissue. In the succeeding periods also, it is especially this layer which is most distinctly developed. The first traces of the formation of the muscular layer consist in the appearance of a larger number of oval nuclei, the long axis of which lays at right angles with that of the vessel. The inner layer, however, which in the beginning was equal in thickness to that of the outer one, is gradually rendered more indistinct, perhaps by the increasing development of the latter.

In the foetus of $5\frac{1}{2}$ months, finally, the outer coat of the larger vessels of the pia mater has attained sufficient consistency to permit its separation from the loose areolar tissue by which these vessels are surrounded, thus affording an opportunity for a closer examination of their respective coats. In the walls of such vessels of about $\frac{1}{100}$ mm. diameter, and freed from the loose fibrous tissue surrounding them, only two layers, distinct from each other, are recognized (Fig. 12). The outer of these, representing the fibrous coat of the vessel, consists of delicate wave-like fibres of connective tissue, holding numerous, more or less oval nuclei, their long axis being parallel to that of the vessel. They are distinguished by a fine double contour, and filled with small granules. The inner layer, which appears

somewhat darker than the outer, represents the muscular coat, already tolerably advanced in development; it also contains a considerable number of nuclei, with their long axes, however, at right angles to the course of the vessel. A number of these nuclei, though somewhat larger and of an oblong form, show, like those of the outer coat, a fine double contour, and are filled with granules. The rest, however, represent mother-nuclei, being distinguished by a greenish lustre. A number of these bear those characteristic small vesicles or buds, which show that the process of their multiplication is still in active operation: the remaining ones appear in the form of irregular spindle-shaped bodies, with crescent-shaped notches, the traces of their former activity, in their contours. The muscular coat itself appears as a fine granulous substance. Besides those nuclei just described, a number of others of an oval form are observed on the inner surface of the vessels; these probably represent the epithelium of the vessel in the process of formation.

I shall next proceed to give an account of the results obtained from the examinations made on the very small ovum, alluded to in the beginning of this article.

This was the smallest specimen of the human ovum I had ever seen. (Fig. 13.) Being removed from the membrana decidua, which had come away entire, it was found to be perfectly fresh and normal. Slightly oval in shape, it only measured 13 by 11 mm. in diameter. The oval form, however, may have been produced by a stretching of the membrana decidua, which was pinned down upon a cork during the operation of its removal. Its surface was almost entirely covered by villi. The embryo it contained (Fig. 14), measuring only 6 mm. in length, was bent upon itself, the inferior part of the spinal column making a spiral turn to the left side of the body. The rudiments of the vertebræ when examined through a lens (Fig. 15), manifested themselves in the form of opaque spots. The superior portion of the body was divided into three segments, the upper and larger one of which represented the future cranium, the two others visceral arches; there was no trace of the formation of the eye or ear to be seen. Directly below the visceral arches, and occupying the middle portion of the body, there were three round prominences, which, as I suppose, represented the rudiments of the heart. (Figs. 15 and 17.) They were of a reddish colour, and the larger of them was, by a subsequent examination, found to be hollow. Below these prominences the pedicle of the umbilical vesicle was seen to arise from the rudimentary alimentary canal; and still lower down, within the curve of the spinal column, also the umbilical vessels. The extremities manifested themselves only in the form of four small delicate buds; the upper ones laterally and behind the above-mentioned prominences, and the lower ones within the curve of the inferior portion of the spinal column. That

portion of the vertebral column situated beyond the rudiments of the lower extremities and representing principally the coccyx, formed an appendix of such a length that it might, without any impropriety, and in support of Darwin's theory, well be looked upon as a tail. (Fig. 16.) The embryo itself was attached to the walls of the egg by a delicate membrane, which, after arising gradually from the abdominal surface, and including, but *not* closely surrounding, the umbilical vessels, was soon blended with the walls of the egg. (Figs. 16 and 17.) The rudimentary heart, alimentary canal, and umbilical vesicle, were not embraced by this membrane, but, laying at its right side, were projecting into the general cavity.

Referring, for a better understanding of the general form of the embryo, the reader to the drawing accompanying this article, which I carefully took from the object in its fresh condition, while immersed in water, I shall now state and consider the results of the microscopical examination, regarding the structure of the umbilical vesicle and chorion of this interesting specimen of human embryo, as they really formed the principal object of the investigation. To do so, however, I must somewhat deviate from the object, in order to recall to our mind the structure of the umbilical vesicle, in which I discovered the origin of the coloured blood-corpuscles, described in my treatise on that subject.* It will be remembered that the embryo to which it belonged had been arrested in its growth, being represented only by an accumulation of embryonic cells. Further, that the wall of this vesicle was found to be a system of primary follicles and canals, the interior of which was lined by large hexagonal cells, in which the blood-corpuscles originated in the form of pale double-contoured nuclei. The interior of the follicles and canals were occupied by considerable accumulations of fully-developed coloured blood-corpuscles.

The examination of the umbilical vesicle of the small normal embryo, described above, became therefore a matter of interest, as its results would either disprove or corroborate the correctness of the conclusions which I had drawn from the observations made on the former specimen. They proved to have been correct, for in examining a portion of the wall in its fresh unchanged condition, the individual follicles with their intervening canals could be distinctly recognized. As in the former case, the follicles consisted of the same large hexagonal cells (Fig. 18), which also contained, besides a number of small nuclei, one or two larger ones. But there were no fully-developed coloured blood-corpuscles to be seen either within the follicles or the canals. In place of these, however, a considerable number of free small round nuclei, containing a number of granules, were observed. The same kind

* 'M. M. J.,' February number, 1874.

of nuclei I found within the cavity of the rudimentary heart and also within the newly-formed blood-vessels of the chorion. (Fig. 19.)

In examining the chorion I found it to consist of an amorphous, granular membrane, covered by an epithelium, consisting of a decidedly granular matrix, in which a considerable number of small nuclei were imbedded; the villi consisted of the same elements. Within the structureless membrane of the chorion I observed, to my astonishment, a considerable number of the smaller blood-vessels and capillaries (Fig. 19), some of them far enough developed to allow the circulation of the blood, others still in process of development. They, however, extended not into the villi. As their structure showed, they had been formed by the fusion of spindle-shaped bodies. A number of those small granular nuclei, above mentioned, were met with in their interior. A considerable number of oval nuclei were distributed throughout the membrane, occupying the meshes of the capillaries. Some of these latter nuclei I discovered in the act of multiplication, not by the process of budding, however, as in the chorion of the abnormal embryo, described in the first pages of this article, but, on the contrary, by that of direct division.

In reviewing now the different facts, regarding both the mode of origin of the blood-corpuscles within the follicles of the umbilical vesicle, as well as the development of the earliest embryonic blood-vessels in the chorion, elicited by the above examinations, and comparing them with those observed in the same parts of that abnormal embryo, some discrepancies existing between them will become apparent, and it would almost seem as if my former observations had been incorrect. But this is not the case, and for this reason we will give an explanation of the probable cause of these discrepancies, consisting firstly in the presence of granular nuclei within the follicles of the umbilical vesicle and in the blood-vessels of the chorion of the normal embryo, instead of fully-developed coloured blood-corpuscles, as were met with in those organs of the abnormal, undeveloped embryo; and secondly, in the mode of development of the embryonic blood-vessels.

In comparing the diameters of the two ova, we shall see at once, that while the former abnormal one measured $2\frac{1}{2}$ ctm. in diameter, the size of the latter normal one only amounted to about half of this, that is, 13 mm. in length to 11 in breadth. It becomes evident, therefore, that the abnormal one, containing the undeveloped embryo, must have been considerably older than the latter. The structure of the umbilical vesicles of the two ova being found to be the same, we must presume that the fully-developed blood-corpuscles found within the follicles of the umbilical vesicle of the older ovum, had, at an earlier time, likewise been represented by granular nuclei as in the other case, and by a gradual continuous develop-

ment only attained the more perfect form in which they were discovered. In consequence, however, of the embryo being arrested in its development at a very early period, no heart or blood-vessels were formed to communicate with the system of canals of the umbilical vesicle for the establishment of the provisional circulation. The primitive blood-corpuscles, in the form of round nuclei therefore, being thus prevented from escaping from the canals, accumulated there, as well as in the interior of the follicles, where they were eventually developed into perfect coloured blood-corpuscles. The development of the umbilical vesicle with its follicles, and the coloured blood-corpuscles found within the walls of the latter, of course, must be regarded as an abnormal phenomenon of nature, almost in the same light as those isolated parts of embryos, as fragments of jaws with teeth, &c., found sometimes in the interior of ovarian cysts.

As regards the presence of round granular nuclei within the follicles and canals of the umbilical vesicle, as well as within the rudimentary heart and the primary vessels of the chorion of the small *normal* embryo, it seems to indicate that at an early period, when the blood commences to circulate through the vessels of the provisional circulating apparatus, it carries small round granular nuclei, which are either gradually metamorphosed into coloured corpuscles, or replaced by such originating directly in the cells of the follicles. The latter seems to be the more probable, as a number of pale, smooth nuclei were observed in some of the cells.

The formation of the primary blood-vessels in the chorion of this embryo was observed to be effected, as mentioned before, by the fusion of spindle-shaped cells. In the chorion of that abnormal ovum, however, the blood-vessels, as my former observations showed, were formed by the fusion of certain round cells, arising in the form of buds from the nuclei, distributed throughout the membrane. Now, it might be presumed that, as the development of the embryo in this case was abnormal, the mode of formation of the blood-vessels would probably be the same; but remembering that I observed this same process still taking place in the chorion and the pia mater of much older embryos, of from 16 to 18 mm. in length, this argument loses its force. With these facts before us, we can only presume that the blood-vessels of the provisional circulatory apparatus, during the earliest period of development of the human embryo, are formed by the fusion of spindle-shaped cells, while somewhat later the formation of the permanent vessels is effected by the fusion of smaller or larger cells or vesicles arising by the process of gemination from the nuclei, as described in the first pages of this article. At a still later period this process, too, ceases in its turn, and the vessels are formed, as we have seen, by the fusion of granular spindle-shaped bodies.

Of the morphological development of the embryo of the small normal ovum, I have little to say; as it was not my particular subject of study in this case. I rather directed my attention first to the examination of the umbilical vesicle in its fresh condition. This examination, together with the removal of the ovum from the membrana decidua and the execution of the sketches, occupying all the time which I had at my disposal until twilight, I was obliged to put the specimen in a weak solution of chromic acid for preservation. In resuming my examinations on the next day, the body of the embryo had so much lost in transparency by the action of the chromic acid, that I was obliged to use compression in order to render it sufficiently transparent for microscopical observation. The compression of course would obliterate the form of any rudimentary internal organ. The whole embryo seemed to consist only of a multitude of small embryonic nuclei imbedded, or held together by a homogeneous plasma. With the fact of the developed blood-vessels in the chorion, however, before us, there can remain no doubt as to the existence of such within the body of the embryo; though I believe, as I have said before, that the larger vessels, as aorta, &c., being developed simultaneously with other internal organs, consist at this period, like the latter, only of embryonic nuclei imbedded in the plasma, until a differentiation of the various tissues commences.

Those three prominences on the anterior side of the body of the embryo, representing, as I supposed, the rudimentary heart, were, as before mentioned, of a decidedly reddish tint, which was fading towards the body of the embryo. The question arises here, whether this tint was the natural colour of the tissues, or whether it was due to the blood, which had been circulating within the tissue. As has been stated, my examination of the walls of the umbilical vesicle showed no coloured elements whatever; still, as the specimen was examined in water, we might venture to suppose that those small round nuclei, which undoubtedly represented the coloured corpuscles in their earliest condition, had already possessed the characteristic colouring material, and lost it again by the action of the water. This supposition would explain the phenomenon, as in the absence of any muscular fibre formed, the reddish tint could hardly be due to the embryonic tissue itself, without the presence of the colouring element of the blood.

During the whole course of my researches made on embryonic tissues, I have endeavoured to discover some general law concerning the different modes of multiplication of nuclei during embryonic life; that is, whether one or the other process of multiplication would regularly appear only during a certain period of the development of the embryo. This seems to be the case, however, only to a limited extent. During the earliest periods of embryonic

life, the multiplication of nuclei seems to be solely effected by the process of fission, or direct division. Having observed in the chorion of the small normal embryo, above-discussed, a number of nuclei in the act of multiplying only by this mode and no other, I suppose that in the human embryo this process of multiplication of cells and nuclei, beginning with the cleavage of the yolk of the egg, prevails only throughout the first weeks of embryonic life, after which it is superseded by that of gemmation or budding. I base this supposition upon the fact that, with the exception of the cells of cartilage, I have throughout all succeeding periods of development of the human embryo, never been able again to discover a cell or nucleus dividing by simple fission. In that abnormal specimen of human ovum, probably one or two weeks older than the other, I observed, as already stated, the multiplication of nuclei in the chorion as well as in the embryo stunted in its growth, only taking place by budding or gemmation, and the same process I observed in the tissues of other embryos up to the age of $6\frac{1}{2}$ months. During a certain period, however, the multiplication of nuclei seems to be effected also by the endogenous mode. In various tissues of human embryos, of about from 16 to 20 mm. in length, as, for instance, the brain, spinal marrow, skin, &c., I met with a number of double-contoured cells, which were filled by small nuclei of a greenish lustre. On some of them the double contour had disappeared, showing the obliteration of the cell-wall for the purpose of liberating the nuclei. Another kind of clear cells, containing a coarse granular nucleus, which in many instances is seen dividing into a number of fragments, is met with in various tissues of the embryo; these cells stand very probably also in some relation with the multiplication of nuclei.