The Development of the Vascular System in the Human Ovum Prior to the Establishment of the Heart.

by

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General Introduction.

Our knowledge of the earliest stages of blood-vascular development in the human suffers from a dearth of suitable material. Early human ova are not frequently available for examination; many are pathological; some, although of value for other purposes, are not sufficiently well preserved to furnish observations on angiogenesis; direct microscopic observation of the tissues while undergoing development cannot be carried out as is possible, say in the chick embryo. Our knowledge of the process must be based on descriptions of separate specimens representing different stages. Individual specimens, therefore, are worthy of careful record.

I have the opportunity of recording the blood-vascular development in two ova of the presomite stage, viz. the Teacher-Bryce ovum No.2 and the McIntyre ovum. For permission to describe the stage of development in the former I wish to express my great indebtedness to Professor Teacher. These two ova have recently been described by Professors Bryce and Teacher and the reader is referred to their papers for the anatomical detail. The development of the system of which this paper treats has been considered in broad principle by Professor Bryce but only in so far as it completes the anatomical description. Herein it is proposed to set down in some detail the blood-vascular picture as it appears in these

specimens. This will be followed by a brief description of the blood-vascular development as recorded for a number of early ova. The third section of this thesis will be devoted to a consideration of the data thus accumulated.

(A)

TEACHER-BRYCE OVUM NO. 2.

The ovum was found by Professor Teacher at autopsy. The fixation and histological details are excellent. The following summary of measurements and anatomical notes indicate the stage of development reached:-

External dimensions of chorionic vesicle (roughly)

	4 x 4.5 x 3.5 mm.
Cavity of Chorion	2.8 x 2.6 x 2.25 mm.
Blastoderm.	0.2 x 0.1 to 0.15 mm.
Yolk-sac	0.05 to 0.2 x 0.396 mm.
Amniotic cavity	0.09 to 0.1 x 0.16 mm.

The yolk-sac, greater in size than the amnion, is conical in shape and is prolonged in a tapering process to form a second attachment to the chorion. (fig.1 Pl.I). The primitive knot and primitive groove have appeared. An allantoic diverticulum is not recognised. The villi are well developed and show simple but not extensive branching. The sections are 6.25 microns thick.

Professor Bryce in his memoir refers to the part of the chorionic vesicle at the point of entrance as the "vegetative pole" and the area opposite on the decidua basalis and in the vicinity of the body-stalk as the "embryonic pole". These terms will be employed similarly here. (1).

1. Teacher uses "Closing Pole" and following Graf Spee "Implantation Pole".

CHORION.

Vascular development is recognised in the mesoderm of the chorion in the form of angioblastic strands lying in channels or spaces. Before, however, considering these in detail, I shall refer to the general arrangement and structure of the chorionic mesoderm. This latter layer has on its inner surface and in direct contact with it the granular reticular coagulated contents of the blastocyst which have been described by Professor Bryce. This material which had settled towards the embryonic pole lies on the inner surface of a very loosely arranged tissue and makes it difficult to define the inner limit of the mesoderm and, therefore, also to determine the relationship of the angioblastic strands and the spaces in which they lie to the inner aspect of this layer.

The nuclei of the chorionic mesoderm are of varied form, spherical, ovoid or rod-shaped and the size varies within limited range. This tissue cannot be resolved into individual cells although usually there is a condensation of protoplasm around the nuclei. Most often this takes an elongated form with tapering extremities and with the long axis corresponding to that of the contained nucleus. A distinct cell envelope is absent. The rod-shaped nuclei are most numerous at the inner aspect of the chorionic mesoderm, the rounded variety at the outer margin.

While the connecting stalk has a practically complete layer of mesothelium, the mesoderm at the embryonic pole and frequently at some little distance from the stalk shows small

patches of what resembles a mesothelial covering. One area more particularly like mesothelium, (fig.3 Pl.I),is considered by Bryce in his memoir but is rejected as being mesothelium by him. These small patches are not continuous with the mesothelium of the connecting stalk. Where they exist there is a zone of fine reticulated structure devoid of nuclei separating them from the under-lying mesoderm. Where this mesothelium-like layer is present the angioblastic strands and spaces can be more clearly located. They are found most often in the inner region of the mesoderm close to the above mentioned reticulated zone.

Rarely one sees a space near the trophoblastic covering but never in direct relationship to it. The spaces are never continued into the mesoderm of the villi although,if the chorionic mesoderm where it runs out to form the stroma of a villus contains an angioblastic space, such space may be deflected outwards slightly.

The spaces form a complicated branching network disposed parallel to the trophoblast layer. (Text fig.1). The system is not continuous throughout. Some channels disappear when traced through several sections, while a few appear to open into the lumen of the chorionic vesicle. The walls of the spaces cannot be said to have an endothelial lining. Now and then a rod-shaped nucleus is seen in the wall but this is no more frequent than in the mesoderm elsewhere. More often the nuclei in the wall of a channel are ovoid and without any more

definite condensation of protoplasm around them than exists in relation to the mesoderm nuclei in general. Often no nuclei are seen in one wall of the space for a considerable distance and this occurs mostly on the side towards the lumen of the vesicle. Again, at times a space appears in part of its course without any nuclei in the immediate vicinity of its walls and the walls themselves are ragged and irregular. One is left with the definite impression that these are spaces which have opened up in the mesoderm and that there is very little, if any, special condensation of the mesoderm cells around them. The spaces form a striking feature of the mesoderm at the embryonic pole where they are of greatest calibre. Passing round the blastocyst towards the vegetative pole they are seen to diminish in size until near the operculum where the mesoderm layer is thin they are infrequent and inconspicuous. At the area on the equator of the blastocyst where the prolongation of the yolk-sac is attached, they are specially large interrupting the gradual transition from pole to pole.

Where patches of mesothelium-like cells exist no connection of these with the spaces could be made out.

Angioblastic strands in the chorion can be recognised only in relation to the spaces or channels described above (fig.4 Pl.I). In its typical form a strand consists of an elongated syncytial mass running in the lumen of a space. The protoplasm is small in amount and drawn out into a thin thread between nuclei where

these are far apart. The protoplasm stains more deeply with eosin than that around the ordinary mesoderm nucleus. In the neighbourhood of the base of the stalk the protoplasm in places may swell out to contain four, five or six nuclei which are oval, rounded or kidney-shaped and are a little more regular in size than those of the mesoderm. The protoplasm has usually a clean cut edge but now and then the surface appears to have very minute thread-like processes passing from it. The nuclei where the strand is a slender one are elongated and sometimes show a curved axis. In individual sections a strand may appear to be entirely free in the lumen but often a narrow attachment to the wall is present, (fig.4 Pl.I). This attachment may be broader and at times the strand forms one side of the wall of the space. Where a strand of some length appears it may run obliquely from one side of the space to the other with attachment at either end.

These syncytial strands never open to disclose a lumen, and it is not the rule to find a double row of nuclei for any distance such as would suggest the early appearance of canalisation, although a double row of nuclei is occasionally seen.

BODY-STALK.

Angiogenesis in the base of the stalk takes the same form as in the chorion. Here the mesoderm forms a considerable mass but is broken up by the spaces which contain the angioblastic strands. The spaces are irregular in shape and taper off in

several directions in the plane of the chorion into the more regular channels seen in that membrane. The contained strands are here less slender. Sometimes quite a mass of protoplasm with numerous nuclei is present and this, at intervals, shows a broad attachment to the wall of the space. Again, the mass may appear broken up into small portions containing 1, 2 or 3 nuclei only. The spaces in the stalk come into close relationship with the amnion-duct but close to the amnion itself, that is nearer the embryonic end of the stalk, another method of blood-vascular development is found. Collections of cells situated in the mesoderm of the stalk have all the appearances of early blood cells. The two largest of these can be traced through four and five sections respectively. The remainder are smaller and consist of four to six cells. The cells stand out from the surrounding mesoderm by virtue of their nuclei being more rounded and more regular in size than those of the mesoderm in general, also, each nucleus has a narrow rim of deeply stained protoplasm making the individual cells spherical. Although the protoplasm is not frankly haemoglobin-coloured, the deep staining is highly suggestive. The spaces in which these cell masses lie differ from those already described in that they have the lumen almost completely filled by the contained cells. Further, there are cells arranged around the wall in appearance similar to those in the lumen of the space except that here and there the nucleus is elongated and is disposed along the wall. In

one section (fig.5 Pl.II) the collection of cells appears as if it had been formed by a sinking in from the surface in the direction indicated by the arrow. The space in which the cells lie might thus be regarded as continuous with the mesothelium. Traced from this section, the space with its contained cells is found to run obliquely to the middle of the stalk in a direction away from the chorion. This arrangement and the appearance of the cells gives quite a different picture to that of the spaces at the base of the stalk and in the chorion.

YOLK-SAC.

The yolk-sac is sectioned almost at right angles to its long axis. For the most part both endoderm and mesoderm are composed of a single layer and in both of them localised thickenings are seen sparsely scattered throughout. In the mesoderm this takes the form of a duplication of the single layer with a suspicion of radial arrangement of nuclei. Otherwise they differ no way in appearance from the rest of the layer in which they lie. There is nothing which enables one to label these collections "angioblastic".

In one instance situated in the mid-lateral wall a small mass lies between the mesoderm and endoderm (fig.6 Pl.III). This mass, which is present through seven sections, at one end in two sections shows quite definite continuity with mesoderm (fig. 7 Pl. III). Elsewhere it lies free between the two layers. It is clearly a mass of mesoderm which has projected in towards

the endoderm and has then made its way along between the two layers. There is no haemoglobin colouration but the protoplasm stains fairly deeply and the nuclei are more spherical than the adjacent mesoderm and endoderm nuclei. The nuclei are not arranged in any definite manner and the mass cannot be resolved into individual cells. There is scanty evidence to show that this is the precursor of a blood-island but it is the nearest approach to such seen in the yolk-sac. In the narrow prolongation of the yolk-sac to the chorion no evidence of angioblastic tissue is recognisable. As already stated, spaces and strands are larger and more numerous in the mesoderm of the chorion where this attachment of the yolk-sac is placed. The endoderm cells of this yolk-sac stalk terminate in the wall of a specially large space containing angioblastic strands. This space with a strand is continued into the slender mesodermic process which replaces the yolk-sac prolongation and terminates in the cavity of the blastocyst. The appearances of this particular area have been fully described in Bryce's memoir. No further description, therefore, is necessary here, but reference will be made to this peculiar arrangement later.

VILLI.

In the mesoderm of the villi there is no evidence of commencing vessel formation. Minutes spaces or clefts are present without any special condensation of protoplasm or nuclei in their vicinity and without protoplasmic strands in their lumen. (fig.8 Pl.III). Many of the spaces were traced in the direction

of the chorion and in no case was communication with a space containing angioblastic tissue in the chorionic mesoderm established; nor do the spaces form a continuous network within themselves in a villus. It is thought that these spaces in the villi represent the fluid parts of the mesodermic tissue and also that they may have become slightly exaggerated in the course of preparation of the specimen as shrinkage of the mesoderm from its trophoblastic covering is not at all common in the villi while it is seen in places in the wall of the chorionic vesicle.

Although vascular development is present in the mesoderm of the body-stalk, no evidence of its commencement is found in the prolongation of this on to the amnion.

There is no evidence of the commencement of the process in the tissues of the embryonic shield.

SUMMARY.

Chorion: The mesoderm contains a network of angioblastic strands and spaces. This network is most plentiful around the base of the stalk. The spaces are not continuously connected.

Body-Stalk: In the base of the stalk a network similar to that in the chorion exists but the spaces and strands are larger. Towards the embryo spaces unlike the above contain collections of cells having the appearances of primitive blood cells. This is the most advanced stage of vascular development found in the embryo.

Yolk-sac: A few groups of cells of origin from mesoderm might be

taken to represent the earliest stage of blood-island formation. This interpretation is open to question.

Embryonic Shield and Villi: No angioblastic formation is recognised.

MCINTYRE OVUM.

This ovum was found by the writer in the course of his work as Pathologist at the Royal Samaritan Hospital for Women, Glasgow, in a uterus after supravaginal hysterectomy. The uterus was partially opened until the blastocyst projected, was washed in running water and then placed in Kaiserling's formalin and salt solution. The specimen was immersed in the latter fixative less than an hour after operation. The shrinkage of the uterine wall was sufficient to cause complete separation of the ovum which came away free when the specimen was next handled.

Measurements.

External dimensions of chorionic vesicle including villi (after fixation) 14 x 13 x 8 mm.

Anterior extremity of yolk-sac to posterior extremity of amnion 1.75 mm.

Blastoderm (including primitive streak) length 1.37 mm.
greatest breadth 0.5 mm.

Primitive streak 0.32 mm.

Yolk-sac (approximately) 1.44 x 1 mm. x 1 mm.

Amnion - length 1.28mm..

Sections 10 microns thick are numbered from the head end of the embryo to the base of the stalk and pass through the embryonic axis exactly transversely.

In this embryo (fig.2 Pl.I) the primitive streak is fully

developed.

There is a notochordal plate and a neurenteric canal and the fore-gut has commenced to close in. The yolk-sac is relatively large and is greater in size than the amnion. The villi show plentiful branching. The specimen is of special value as it represents an important stage in vascular development.

YOLK-SAC.

The yolk-sac as yet cannot be said to possess any vessels. Blood-islands are numerous and consist of multi-nucleated masses of haemoglobin-coloured protoplasm projecting from the surface of the vesicle. Nowhere is an endothelial lined lumen with free individual blood cells found.

As seen in transverse section the endoderm in the middle line on the ventral aspect of the sac consists of a single layer of cells of a low columnar type with centrally situated oval nuclei. Traced dorsally to the embryonic area the cells gradually become cuboidal or spheroidal with spherical nuclei. For a short distance before reaching the embryonic shield the cells are flattened out in the line of the yolk-sac wall. This area is the thinnest part and here there is a longitudinal in-folding of the wall. The mesoderm cells have a less regular arrangement and are less constant in size, shape, and in shape of nuclei. The intensity of nuclear staining also varies. In the middle line ventrally the mesoderm forms a thicker layer than elsewhere. Towards the embryonic area it is represented by a single layer. There is a tendency for the mesoderm cells

to clump together and the nuclei in these groups of cells have a radial arrangement towards the surface. The blood-islands are situated principally in the lateral wall midway between the embryonic area and the ventral aspect. (Text figs.2 & 3). This corresponds to an area just ventral to the thinnest part of the wall. The cephalic end of the yolk-sac for a short distance is totally devoid of blood-islands but these are seen to appear before the anterior end of the blastoderm is reached. On the left side of the yolk-sac the blood-islands are larger and more numerous than on the right. Again, on both sides they are more plentiful opposite the head end of the embryo and in the area of yolk-sac wall bridging across the tail fold. Between these two areas they are scanty in number on the left side and still less numerous on the right. Away from the mid-lateral wall towards the blastoderm margin or the ventral aspect blood-islands are rare and when present are small and in an early stage of formation.

The blood-islands show a variety of forms, and these may be divided for purposes of description into four types which are taken to represent different stages in development.

Stage 1. This form which is regarded as the earliest evidence of blood-island formation, consists of a hemispherical mass with the central nuclei becoming spherical. (fig.9 Pl.IV). The mass projects slightly from the surface of the yolk-sac. The protoplasm possesses no haemoglobin colouring. This form is taken to represent a stage following on the grouping of mesoderm

cells already described. There is no proof that it is the precursor of a blood-island except that the next stage is similar to it with the addition of haemoglobin colouring of protoplasm in the centre of the mass.

Stage 2. In this stage (fig.10 Pl.IV) the central nuclei are mostly spherical, have a bold outline and stain less deeply with haemalum than the adjacent mesoderm nuclei. In the periphery of the mass showing haemoglobin colouring one or more deeply stained crescentic nuclei may be seen. These are not constantly present, but when present appear to belong to the central mass rather than to the surrounding protoplasm.

Stage 3. Increase in size of the blood-islands results in definite projection from the surface and the mass originally hemispherical becomes almost spherical in section. The bulk of it is composed of protoplasm showing haemoglobin colouring and numerous faintly stained spherical nuclei of regular size (fig.11 Pl.IV). This mass cannot be resolved into individual cells. Surrounding it there is a narrow zone of protoplasm uncoloured by haemoglobin with nuclei in a single layer. These nuclei are well spaced and irregular in shape. The protoplasm of this surrounding zone cannot be sharply demarcated from the central mass.

Stage 4. This still more advanced picture (fig.12.Pl. IV) is seen only in a few of the largest blood-islands in the posterior extremity of the yolk-sac at the tail fold and, therefore, near the commencement of the body-stalk. The central mass

now shows the presence of clefts in the protoplasm dividing it up into irregular masses closely packed together. Some of these masses have attachment to the surface zone. In one or two instances what appeared to be individual cells were seen, but examined alongside neighbouring sections these were decided to be merely narrow terminations of a mass cut transversely so as to show only one nucleus. A greater number of the nuclei of the outer covering now assume a flattened shape.

The blood-islands vary considerably in size and the size is not related to the intensity of haemoglobin colouring of the contained protoplasm. Some of the smallest, having only four or five central nuclei, show the protoplasm as intensely coloured as the largest. The blood-islands vary in shape as viewed on the flat and have an irregular arrangement. Attempts to make a transparency reconstruction of them from the sections were only partially successful so irregular is the distribution. It was possible, however, to appreciate that they do not form a continuously connected network although connection between neighbouring blood-islands is established. At the head end of the vascular zone the blood-islands appear to have their greatest measurement in the axis of the embryo whereas at the tail end there is a definite tendency for them to be disposed at right angles to the embryonic axis and here also they are more elongated. In addition they are not so sharply limited by their outer zone with its elongated nuclei. The blood-island posteriorly, as a result

of this difference, furnishes a different picture in the sections. The projection outwards from the surface of the yolk-sac as the result of its presence is not so striking. It appears to produce a thickening of the wall for a distance rather than a localised outward projection as seen at the cephalic end.

Although there is often difficulty in resolving mesoderm and even endoderm into individual cells there is no difficulty in differentiating mesoderm and endoderm. This enables one to state that the vascular tissue present is more closely connected to mesoderm than to endoderm.

BODY-STALK.

In section No.115 the yolk-sac merges into the funnel-shaped diverticulum which becomes the allantoic duct. This is taken to represent the upper limit of the body-stalk. In section No.190 the stalk merges with the chorion. Its length, therefore, is 0.75 mm. The tail-fold of the embryo reaches as low as section No.150; the amnion with its lower limit in section No. 175 passes off from the dorsal aspect of the stalk (Text fig.2). The allantoic duct definitely established in section No.122 ceases in section No.166. The stalk in transverse section may be taken as roughly circular in outline. The allantoic duct is situated almost in the centre and furnishes a useful landmark in describing the relationship of the vascular structures present (fig.18 Pl.VII).The stalk increases gradually in diameter towards the chorion until it breaks up unevenly into

strands which turn outwards to join the chorion very obliquely. This gives a very complicated picture in the sections through the lower end of the stalk.

The mesoderm of the stalk consists of a finely reticulated protoplasm which stains faintly with the basic stain employed. The nuclei are vesicular, are lightly stained with haemalum, are of fairly regular size and are slightly ovoid or short rod-shaped. This tissue cannot be resolved into individual cells nor is there any sharp condensation of protoplasm around the nuclei. A mesothelial layer is present. (fig.13 Pl.V). It is most complete at the level of the middle of the stalk where it consists of a thin film of protoplasm which has taken up the eosin stain and a single layer of round nuclei, smaller than and more deeply stained than the ordinary mesoderm nuclei. The nuclei are irregularly spaced; sometimes wide intervals exist between two nuclei. This covering is incomplete at both extremities of the stalk.

The presence of two large vessels (the umbilical arteries) containing free cells and of approximately equal size forms the most striking feature of the stalk. (Text fig.4). These are situated one on either side of the allantoic duct at the embryonic extremity. Both have a wide lumen. They commence in section No.121 and as they pass towards the chorion, they increase in size and gradually come to lie anterior to the allantoic duct. In section No.156 they connect across in front of this duct by a narrow open channel; in section No.159 they connect by a solid strand; in section No.168 they unite to form a single large vessel

which in section No.180 again splits up into two main branches running to the chorion.

The vessel walls consist of a condensation of nuclei and protoplasm. The nuclei are of similar shape to the ordinary mesoderm nuclei and lie one, two or three deep in the wall of the vessel. This contrasts with the surrounding mesoderm which is specially scanty in nuclei in the ventral half of the stalk through which the vessels run. The condensation of protoplasm corresponds to the nuclear zone and takes up the eosin stain. Elongated nuclei which might be regarded as belonging to endothelium are present only at wide intervals. The lumen contains free nucleated blood cells. These are rounded or polygonal and have a relatively large amount of protoplasm which shows unmistakeable haemoglobin colour. The nuclei are very regularly spherical and are situated centrally. They vary greatly in intensity of staining. Some stain so faintly that the presence of a nucleus is made out only with difficulty. In others the nucleus stains very deeply. Mitotic figures are seen and the presence of two nuclei, one sometimes larger than the other, in a single cell is not uncommon. No syncytial masses are seen in the lumina of the vessels but, now and then blood cells lie closely applied to the wall. In the latter, however, direct protoplasmic continuity does not exist.

Occasionally a V-shaped depression or cleft in the wall of the vessel passes outwards towards a small mass of angioblastic tissue. Such masses are not specially common in association with

the vessels at present being described. They resemble the cells within the vessels and are sharply demarcated from the surrounding unaltered mesoderm.

Sometimes the vessels appear to communicate with the mesothelium by a cleft or depression in the vessel wall and another cleft on the surface of the stalk passing towards one another, the interval being bridged across by a solid strand. This is not frequently noted nor is it a conspicuous feature. Complete open communication is not found.

Traced towards the embryo the two vessels end blindly and there is no connection established with the angioblastic tissue of the yolk-sac. The possibility of connection by solid strands was also ruled out by careful examination of this area. In the stalk above the termination of the vessels there is angioblastic tissue but this is less in amount than at any other level of the stalk.

Although in the mid-line the yolk-sac is replaced by its posterior diverticulum in section No.115, the lateral walls are continued downwards to bridge across the tail fold of the embryo to section No.131. The amnion is now interposed between the yolk-sac and the body-stalk (Text fig.2). The possibility of angioblastic connection between the stalk and this part of the yolk-sac wall rich in blood-islands by way of the amnion wall was excluded. Several small isolated masses of angioblastic tissue were encountered in the amnion mesoderm here,but only close to the stalk,and might be regarded as belonging to the latter.

Another picture of vascular development is found in the dorsal part of the stalk and would appear to be unconnected with that already described. At the junction of the embryonic and middle thirds of the stalk behind the allantoic duct and frequently situated near the angle between the body-stalk and the amnion on either side, angioblastic masses are seen. Some of these have an appearance rather like the blood-islands of the yolk-sac. They lie just under the mesothelium and have a few crescentic nuclei disposed around them with the central mass showing haemoglobin coloured protoplasm. They form, however, no projection on the surface. Passing towards the chorion this gives place to larger irregular masses of the same structure, only differing in shape, running directly dorsal to the allantoic duct. About the middle of the body-stalk these become smaller in size, are diffusely distributed and sometimes show connection by clefts with a network of spaces which has appeared at this level. The walls of the spaces have the same appearance as that of the vessels already described except that condensation of nuclei is less marked, endothelium-like nuclei are less frequently seen and the protoplasm in the wall stains less deeply with eosin. The spaces connect up with one another behind the allantoic duct in an irregular manner. In transverse section the lumen is often stellate. Numerous V-shaped depressions pass outwards to end in solid processes which sometimes run to isolated angioblastic masses. The majority, however, are unconnected with such. Two clefts passing outwards from a space may converge and almost

isolate a mass as it were in the lumen. This mass has the same structure as the wall of the space elsewhere. There is no haemoglobin colouring of the protoplasm nor other indication that it will be any more closely concerned in the production of endothelium or blood cells than the other parts of the wall.

The main channels of this network are represented in the diagram as they exist in the upper two thirds of the stalk (Text fig.5). Nearer to the chorion they become smaller in size, more numerous and more complicated in arrangement. They can be traced to the mesoderm of the chorion. At this end of the body-stalk they cannot be traced as continuously connected. Some of them establish connection by solid processes with the walls of vascular channels in the chorion which contain undoubted blood cells. Although this network of spaces contains no blood cells, it is certainly the commencement of a capillary network, the future (vitelline) umbilical veins.

Throughout the examination of this area particular attention was directed to the relationship of the angioblastic tissue with the mesothelium. Mention of this has already been made in connection with the umbilical arteries. In one instance what is almost certainly a connection between a well formed angioblastic mass and a funnel-shaped depression of the mesothelium was found (fig.13 Pl.V). This mass, which can be traced through 12 sections, in one part of its course may almost be regarded as a vessel. At two points it appears to have connection with the mesothelium, while at another level

it connects by a solid strand with the network of spaces behind the allantoic duct.

CHORION.

Again, it is necessary in the first place to make reference to the structure of the mesoderm before describing the vascular picture. In the vicinity of the base of the body-stalk the mesoderm has a loose arrangement. It cannot be definitely resolved into individual cells although here, unlike the mesoderm of the stalk, there is evidence of some concentration of protoplasm often in the form of a spindle around the nuclei. The nuclei vary in shape from short ovoid to rod-form with blunt extremities. They lie principally with their long axes parallel to the chorion. One frequently finds the mesoderm marked off into two layers by a very loose arrangement of the tissues in its middle. Under such circumstances, the mesoderm lining consists of one sheet clothing the trophoblast and another lining the blastocyst cavity. The latter contains the largest chorionic vessels seen and would appear to be formed as a result of these large vessels leaving the chorion to pass into the stalk. Away from the attachment of the stalk the mesoderm of the chorion is a thinner, more compact layer, and gives indication of the presence of wavy fibrillae. The nuclei are relatively less numerous and approach nearer in appearance to the adult fibrous tissue nucleus. The inner surface of the mesoderm near the body-stalk has, in areas, fine irregular protoplasmic strands streaming off into the cavity of the blastocyst. A mesothelium as such is

not recognisable.

Vessels and solid angioblastic strands are present in the chorion but only in an area limited to the vicinity of the base of the body-stalk. In this area they are quite numerous.

The earliest stage of vessel formation recognisable consists of a thin, solid strand of protoplasm staining rather deeply with eosin and partially but not completely marked off from its surroundings. It is never situated in a space as described for the angioblastic strands in the chorion of the T.B.2. ovum. Three or four rod-shaped nuclei are arranged in single row in the protoplasm. The next stage is the appearance of a nucleated haemoglobin coloured cell in the strand. This cell is sharply demarcated and is distinctly a free cell. It lies in a space provided for it in the protoplasm of the strand sometimes at its middle, sometimes at one extremity. This cell may be as great in thickness as the strand which contains it and the protoplasm of the strand where it passes on either side to enclose it, is thinned out and may be readily overlooked by the observer.

More commonly, the strand, before the appearance of frank haemoglobin colouring in the cell, contains a double or treble row of elongated nuclei (fig.14 Pl.V). Next, several haemoglobin coloured cells appear in a row and are situated in the middle of the strand (figs.15 & 16 Pl.VI). These are sharply marked off from their surroundings and from one another, and the ends of the cells where in contact are flattened so that the cells are frequently square in shape, the other two sides being flattened

against the walls of the space in which they lie. The appearances are such as to suggest almost that the cells are under compression.

These strands may be isolated or may establish protoplasmic connection with others of any stage in development. Intermediate stages are seen right up to the largest vessels present. Some of the latter have a lumen almost equal in size to that of the vessels in the stalk. Their walls consist of a condensation of protoplasm with numerous rod-shaped nuclei disposed parallel to the lumen. The structure of the vessel wall, apart from its richness in eosin staining and the regular arrangement of the nuclei has no special feature to distinguish it from the surrounding mesoderm. There is not as yet a lining to be compared with adult endothelium. In these larger vessels the lumen contains free nucleated blood corpuscles, sometimes of irregular shape but the majority spherical. They differ in no way in appearance from those in the vessels of the body-stalk. The angioblastic tissue present tends to run parallel to the chorionic membrane and takes part in the formation of a network. The more mature vessels in the vicinity of the attachment of the stalk communicate together in a complicated manner. The earliest representations of vessels may connect up over limited areas either by solid strands alone or in part by open channels. Angioblastic strands are seen isolated and these sometimes already possess cells in which haemoglobin staining of the protoplasm is beyond doubt.

No angioblastic tissue is found directly in contact with the chorionic epithelium; only a few of the earliest strands are seen near the epithelial layer. As already stated the vascular development is specially prominent on the inner (cavity) aspect of the chorionic mesoderm. This, along with the short distance in which a vessel with a wide lumen may be replaced by a solid strand form two striking features of vascular development in this area.

Special attention was directed to the vascular connection between the body-stalk and the chorion. Tracing the rudiments of the umbilical arteries from their common trunk at the lower extremity of text figure 4, section No.172, this single vessel divides into two. In section No.180, the two branches passing away from one another become continuous with the network of vessels in the chorion. The arterio-vascular system in the body-stalk and chorion thus communicate by open channel. The venous plexus of spaces at the junction of the body-stalk and the chorion establishes connection with solid angioblastic strands which are specially numerous in the vicinity of its termination and by way of some of these with a few of the smallest vessels in the chorion. A connection by open channel was not made out. As far as could be gathered by tracing vessels and angioblastic strands, no connection exists either directly in the body-stalk or indirectly in the chorion between the two sets of vessels in the stalk, viz. the two arteries on the one hand and the venous plexus in the dorsal part of the stalk on the other.

VILLI.

The villi are well formed, are of considerable length and show intricate branching. The mesoderm of the villi differs from that of the chorionic membrane in that there is no concentration of protoplasm around the nuclei. The protoplasm which is of finely granular structure with a very fine network of fibrillae stains faintly with the basic stain. It, therefore, resembles the mesoderm of the body-stalk in its staining reaction. The nuclei are situated wide apart and are not equidistant from one another but are sometimes grouped together in collections of three or four. The blood-vascular development in the villi has reached a critical stage.

There are,in the first instance,in the villi in the vicinity of the base of the stalk,angioblastic strands consisting of a single row of nuclei usually, but sometimes of a double row, surrounded by protoplasm which stains deeply with eosin. These therefore, stand out sharply in contrast to the general mesoderm of the villus which is faintly stained with haemalum (fig.17 Pl.VII). A detailed description is unnecessary as they have the same appearance as strands of similar dimensions in the chorion. In several the origin of haemoglobin cell from angioblastic strand is seen. In the vast majority of instances these strands run in the middle of the mesoderm in the axis of the villus. Exceptionally a short strand may run obliquely until it reaches near to the epithelial covering but never comes in contact with it. There is no space separating the strands from the mesoderm.

Such angioblastic strands as have now been described exist as already stated in a very restricted area at the base of the body-stalk. Elsewhere the villi show no evidence of commencing blood vessel formation. Even in the area where these strands are present the great majority of villi as yet show no indication of the commencement of the process.

The strands may lie in the base of the villus or half way between the two extremities. In the few instances where a strand is seen near the distal extremity it stops a considerable way short of the tip of the villus. One angioblastic strand in the base of a villus was traced by direct continuity back into the chorion and eventually into what was sufficiently well developed to be termed a vessel. Numerous strands at all levels in villi traced backwards are found to terminate short of the chorion, and, therefore, have no connection with the vascular system therein. In fact, in examining a villus, one may encounter two angioblastic strands at different levels unconnected with one another or with the chorion.

To complete the description of the developmental picture of this system in the villi, it is necessary to give a detailed account of the appearances in two villi opposite the base of the body-stalk, the only two villi encountered which showed the presence of free cells with a haemoglobin reaction or what may be termed vessels.

(a) Situated in the chorionic mesoderm on the body-stalk side of one of the larger villi (0.63 mm. long) there is a good

example of a vessel with a well formed lumen containing plentiful free nucleated red cells. Opposite the base of the villus which has an overall diameter of 0.15 mm. this vessel divides into two, one branch, the smaller, does not enter the villus but runs through three sections steadily becoming smaller until in the next and last section in which it can be identified, it is represented by a solid strand. The larger branch preserving its lumen and contained free cells passes into the base of the villus but only for a short distance. In the villus at a point on a level with the epithelial covering of the chorion the lumen is lost and the vessel is now represented in section by three or four haemoglobin coloured cells in the mesoderm of the villus. This picture is maintained through four sections and then the cells showing haemoglobin colouration become more numerous, seven or eight appearing in the sections and establishment of a lumen gradually takes place. This lumen occasionally passes out in the form of a cleft to reach a haemoglobin coloured cell or cells still in protoplasmic connection with the general mesoderm of the villus. This arrangement holds through twelve sections. The villus now divides into several branches and in one of these, the one nearest to the chorion, the vessel is continued as a strand showing condensation of nuclei in deeply staining protoplasm. This strand ceases some distance short of the termination of the branch in which it lies. The fact that this villus has been cut in section at about an angle of 45^{o} to its long axis made it specially easy to trace this early vessel

throughout its course and simplified a rough reconstruction which was made.

(b) Another villus with a narrow elongated origin from the chorion almost immediately divides into two main branches which, at some distance from the chorion, break up into smaller branches. Some of these can be traced a distance of 1 mm. from the chorion. In one of the two main branches a young vessel with lumen established and containing nucleated red cells is seen. Followed into the chorion this vessel is continuous with a solid angioblastic strand which terminates opposite the base of the villus without establishing connection with any vessel of the chorion. Traced distally the vessel in the villus terminates abruptly at no great distance from the chorion. Alongside of this vessel but separate from it and having no identifiable connection with it, two angioblastic strands in their earliest form are seen. These run parallel to the vessel. Again, distal to the vessel and unconnected with it a similar strand is present.

EMBRYO.

The pericardial coelom is present in the form of a U-shaped space (Text fig. 6). The limbs of the 'U' extend backwards in the mesoderm near the lateral border of the embryo. Here the mesoderm consists of a double layer of cells and the cavities are formed by separation of this double layer. When formed, therefore, the walls of the cavities dorsally and ventrally consist of mesoderm with a single row of nuclei (Text fig. 7). Here and there the roof and floor come into apposition with one another

for a short distance dividing the lumen into two. On the right side the canal extends back to section No.107, on the left to section No.102.

At the anterior extremity of the embryonic area they unite with one another to complete the formation of the "U". This portion of the "U" is bent slightly downwards conforming to the shape of the embryo. If the sections are examined from the head end backwards this union in front appears first in section No.42 which is also the first section in which the cavity of the amnion is apparent.

The entire embryo was carefully examined, especially the floor of the pericardial cavity, and nowhere were any changes found which could be regarded as early evidence of blood or vascular development. Nothing which could represent the commencing formation of the endothelial heart tubes was recognised.

SUMMARY.

Yolk-sac: Blood-islands are numerous and are situated in the mid-lateral walls. They are greater in number at the extremities of the yolk-sac than at its middle, and greater in number on the left side than on the right. No vessels are present.

Body-Stalk: Two large vessels (umbilical arteries) are present and dorsal to them a venous plexus. Neither of these are connected with the blood-islands of the yolk-sac, but both communicate with the vessels of the chorion, the former directly by means of open channels, the latter through the medium of solid strands. The two systems do not communicate with one another. Small solid

masses of angioblastic tissue are present in addition to the above.

Chorion: All stages of vessel formation are seen in the vicinity of the attachment of the body-stalk. Away from this area no indication of vascular development is found.

Villi: Vascular development at a very early stage is evident only in the villi in the neighbourhood of the base of the body-stalk. Angioblastic tissue is found isolated in the mesoderm of villi.

Embryo: There is no angioblastic tissue recognisable. The pericardial cavity has the form of a "U" shaped tube.

(B.)

BRIEF NOTES OF THE VASCULAR DEVELOPMENT IN EARLY OVA.

For purposes of comparison with the two ova recorded, brief notes of the vascular development in other twentysix early human ova are given. For this I have taken Prof. Bryce's selected list and maintain the same order which is an approximation to a sequence in respect of differentiation. I have, however, included three other ova of interest from the point of view of vascular development. These are Meyer's, Triepel's and Ingalls' 1920 ova. The source of each specimen is indicated, and, where available, a measurement of the embryo and of the blastocyst is given. Meyer regards his ovum as older than v. Mollendorff's (Op.) but younger than the Strahl-Beneke ovum. I have included it here between the T.B. 2. ovum and Strahl-Beneke's. Very short notes of the T.B. 2 and McIntyre ova are also inserted here to make the list complete.

J. W. ~~T.A.~~ Miller (1913).

Curettage. Embryonic Rudiment solid. Blastocyst, 0.44 mm. There is no extra-embryonic coelom. There are no villi.

There is no indication of the commencement of vascular development.

Teacher-Bryce No. 1 (1908).

Abortion. Embryo about .15 mm. Blastocyst .77 x .63 x .52 mm. There is no extra-embryonic coelom. There are no villi.

There is no indication of the commencement of vascular development. In this I agree with Bremer who has also studied the specimen.

v. Mollendorff (Sch.) (1921).

Abortion. Embryonic Knot. .17 mm. Blastocyst .26 mm. There is no extra-embryonic coelom. There are no villi.

There is no indication of the commencement of vascular development.

Linzenmeier (1914).

Vaginal Hysterectomy. Embryonic anlage. .21 mm. Blastocyst .75 x .615 x .525. The extra-embryonic coelom is present.

Villi have formed and in these as in the mesoderm in general there is no trace of the commencement of vessel formation. The body-stalk has not yet assumed its characteristic appearance, the embryo lying in a collection of mesoderm on the wall of the blastocyst. The anlage of the allantois is described as lined with epithelium coloured like red-blood corpuscles. It is of interest to note that the cells of the allantoic duct in the McIntyre embryo have a somewhat similar appearance; so much so that this structure might readily be mistaken for angioblastic tissue. No further note of vascular development is made.

Peters (1899).

Suicide autopsy. Embryo .19 mm. Blastocyst 1.6 x .8 x .9 mm. The extra-embryonic coelom is present. The villi have a mesodermic core. A body-stalk can hardly be said to exist.

In the model made by Keibel from drawings by Selenka, the external surface of the yolk-sac appeared uneven, but it could not be decided if these represented the anlagen of vessels.

Jung (1908).

Curettage. Embryonic anlage about .25 mm. Blastocyst 2.5 x 2.2 mm.

All trace of vessels is absent from the chorion and villi. In the body-stalk there are present collections of cells with a lumen in their middle. The lumina have no contents resembling blood corpuscles. He hesitates to decide whether or not these are the first vessel anlagen. Vessels are not recognisable in the embryonic mass.

Schlagenhaufer and Verocay (1916).

Suicide autopsy. Embryonic shield .24 mm. Blastocyst 2 x 1.6 x 1 mm.

A well-defined body-stalk is present. In the mesoderm of the yolk-sac are thickenings which are regarded as the anlagen of blood-islands. The commencement of vascular development is not noted in the body-stalk, chorion or villi.

Fetzer (1910).

Curettage. Embryonic shield .23 mm. Blastocyst 1.6 x .9 mm.

The villi are vessel free. There is no mention of vascular development in the chorion. No vessel anlagen are present in the body-stalk or in the amnion wall. The mesoderm of the yolk-sac shows numerous projections but there is nothing found which could be called a vessel anlage. A process passes out from the yolk-sac to end free and without attachment to the chorionic wall.

v. Mollendorff (Op.) (1921).

Hysterectomy. Chorionic vesicle 2.25 x 2 x 2.5 mm.

In one instance in the embryonic shield a collection of mesoderm cells is found near the entoderm. Similar cell groups are found in the mesoderm of the yolk-sac. He does not decide definitely that these are concerned in the formation of blood or of vessels. In the chorion he finds here and there channels lined with flattened cells. These are pronounced to be vessels. Their cavities do not yet connect up to form a continuous system. There is apparently no vascular development in the body-stalk or in the villi.

Herzog (1909).

Autopsy. Embryonic shield .154 mm. Blastocyst 2.3 x .8 x 1.2 mm. The villi consist of projections without dichotomous branching.

At the junction of the yolk-sac and body-stalk mesoderm

there are found some solid and some open circular masses of mesoderm cells. These formations are taken to represent the earliest "anlage" of the yolk-sac blood vessels. Bremer regards Herzog's interpretations of these cellular rings as incorrect. The mesoderm of the chorion and of the villi do not yet show any trace of blood vessels. There is no mention of blood vessel development elsewhere on the yolk-sac or in body-stalk or in the embryo itself.

Teacher-Bryce No. 2 (1924-25).

Autopsy (acute rheumatism). Blastoderm .2 mm. Blastocyst cavity 2.8 x 2.6 x 2.25 mm.

Angioblastic strands are found in spaces in the chorionic mesoderm. These are most plentiful around the base of the body-stalk and are not continuously connected. The body-stalk contains similar but larger strands and spaces at its base. Nearer the embryo several spaces contain collections of cells having the appearances of primitive blood cells. What is probably the commencement of blood-island formation is found in the wall of the yolk-sac. No angioblastic formation is recognised in the embryonic shield or in the villi.

Meyer (P.M. 1923) (1924).

Curettage. Embryonic shield .41 mm., Chorionic cavity 2.6 x 2.1 x 2.72 mm.

No indication of vascular development is found in the mesoderm of the chorion or in that of the villi. The yolk-sac

possesses blood-islands which do not communicate with one another and are furthest developed over the ventral aspect. A blood-island consists of a collection of cells between the yolk-sac entoderm and the mesoderm coat with a narrow connection to the latter. He finds in the body-stalk dorsal to the allantoic duct a collection of cells which he regards as the first anlage of the Umbilical Artery. There is no mention of vascular development in the embryonic shield.

The yolk-sac possesses a long process which passes across the blastocyst cavity to terminate in a vesicle in the chorionic mesoderm at the implantation pole. The yolk-sac prolongation contains no blood-islands. The vesicle he regards as lined on the outer (trophoblast) side by mesoderm, on the inner (towards the chorionic cavity) by entoderm. This is covered by mesoderm which separates it from the lumen of the chorionic cavity and between the two layers he figures a blood vessel anlage (fig.12, p.59). One is unable to recognise the entoderm lining of the vesicle from the figure, otherwise, apart from the absence of strands in the lumen, the vesicle has the appearance of the spaces in the mesoderm at the attachment of the yolk-sac duct in the Teacher-Bryce ovum No.2. Blood-islands, he points out, are found nowhere away from entoderm and he takes this as indicative that the blood-islands have some relation to entoderm.

Strahl-Beneke (1910).

Curettage. Embryonic shield .75 mm. Blastocyst 3.8 x 2.2 x 1.2 mm,

In this ovum on the under surface of the yolk-sac there are thickenings of mesoderm containing cell masses which are regarded as the forerunners of vessels but these have not yet acquired the definite characters of vessels. Sharply defined spaces in the mesodermic envelope of the embryonic body and specially marked on the yolk-sac side are referred to as "like capillary vessels". These spaces do not form a closed tube system and are empty except in one place where free cells are present. These cells, however, show no haemoglobin colouring of their protoplasm. Beneke thinks he can recognise similar spaces in the mesoderm of the Teacher-Bryce ovum No. 1 in the photograph (Plate 11) reproduced by the authors. In the mesoderm of the chorion of the Strahl Beneke ovum, spaces similar to these described in the embryonic sections are present and are referred to as having the appearance of empty endothelial tubes. The authors' figure 1, however, shows mesoderm of the chorion which in the description of the figure is stated to be vessel-free. The villi have a mesodermic core in which there are no vessels. This ovum has a prolongation of the yolk-sac which runs to the chorionic wall. There is no note of vascular development in this structure, nor does there appear to be any particular development of spaces in the mesoderm of the chorion where this structure reaches it.

Graf. von Spee (v.H.) (1896).

Abortion. Embryonic shield .37 mm. Blastocyst 4 mm.

Spaces in the chorionic mesoderm and in the body-stalk have no visible content; neither blood nor endothelial cells are found. The yolk-sac wall has irregular protuberances of the mesoderm. Corresponding to these are blood-islands situated between mesoderm and entoderm. The youngest stage of blood-island lies nearest to the embryonic disc. The formation of blood-islands is noted as extending nearer to the embryonic disc than in his older embryo "Gle".

Evans, in Keibel and Mall's "Manual of Human Embryology" states that some of the vascular anlagen of the yolk-sac of this embryo show evident differentiation into endothelium and blood cells. Also, that in the body-stalk and chorion there are, as Graf Spee has described, "highly characteristic strands of spindle cells". This, he thinks suggestive of endothelium although there is no blood cell formation.

Lewis (Minot) (1912).

In the yolk-sac wall among the mesodermic cells are vessels with a true endothelial lining and containing nucleated blood corpuscles. Sometimes a corpuscle is closely applied to the endothelium as if arising from it. The vessels of the yolk-sac do not pass into the body-stalk which contains numerous vessels. There are many spaces in the chorion which are regarded as vessels. "Frequently these contain strands of darkly staining

cells suggesting collapsed endothelium". There are no vessels in the embryo proper. It is not definitely stated that the vessels in the body-stalk are not in continuity with the spaces in the chorion but this may be inferred.

Streeter (Mateer) (1920).

Hysterectomy. Embryonic Plate 1 mm. Blastocyst 6.1 x 5.6 x 2.5 mm.

Evidence of blood vessel formation is present in all parts of the chorion. All stages from simple multinucleated protoplasmic strands to completed endothelial tubes are seen. In the villi the stage of development is similar to that in the chorion except that the vessels appear to be more numerous, although a great many villi, as a rule the smaller ones, show no sign of vessel formation. Embryonic blood cells are recognised. In the body-stalk vascularisation has occurred to the same degree as in the chorion, and, as in the latter vessels are mostly empty. Blood vessel formation is also recognised over the greater part of the parietal mesoderm covering the amnion. Angiogenesis in the yolk-sac is limited to the caudo-ventral half, and is represented by clumps of cells up to completely formed endothelial tubes. In this process of vessel formation in the yolk-sac relatively few complete cells (blood corpuscles) result, and none of these as yet show evidence of the presence of haemoglobin. The process does not appear to have commenced in the embryo proper. The continuity or other-

wise of the vessel-forming tissues in the different areas is not definitely stated.

Debeyre (1912).

Hysterectomy. Embryonic shield .85 mm. Blastocyst 5.6 x 2.1 mm. There is no intra-embryonic coelom.

The yolk-sac possesses blood-islands over the distal pole. These vary from a solid group of cells arranged concentrically to irregular festoons containing cells compactly arranged. Apparently the cells show no haemoglobin colouring. The islands do not form a network. In the body-stalk blood-islands are present. Two of these are specially large and one of them describes a third of a circle spirally round the allantois. In the embryo there is a cellular collection which Debeyre thinks may be the first cardiac formation. In the villi occasionally a lumen is seen but without endothelial lining. He states that if these are vessels they represent a very early stage of development. Vessels are present in the chorion. The question of continuity of vessel forming tissue in the different areas is not discussed.

Thompson and Brash (1923).

Curettage. Embryonic shield including caudal fold .9 mm. Chorionic vesicle 10 x 7.5 x 4 mm.

There is no intra-embryonic coelom. Blood-islands are found on the ventral aspect of the yolk-sac and appear to be more advanced in development in the cranial half. All

stages from small clumps of cells to completely formed vessels containing developing blood cells are encountered. The description and figure would suggest that the blood cells arise from entoderm while the endothelium arises in the vicinity of mesoderm, although the writers do not arrive at any definite conclusion with regard to this. The doubtful presence of angioblastic tissue in the body-stalk is noted, otherwise there is no further evidence of vascular development in this specimen.

Rossenbeck (Peh.I)(1923).

Hysterectomy. Embryonic shield 1.4 mm.

In this specimen the presence of the anlage of the aorta is suggested but not definitely affirmed. The mesoderm of the amnion contains endothelial lined spaces not continuously connected. Two strands run from the amnion to the chorionic mesoderm, one traversing the body-stalk. Both end in connection with vessel anlagen in the chorion. The picture of angiogenesis in the yolk-sac is stated to be the same as in Streeter's (Mateer ovum). In the body-stalk at its caudal end, two endothelial lined lumina - vessel anlagen - appear. These traced caudally end in an unpaired vessel anlage which lies near the ventral surface of the stalk and which finally divides and passes into numerous but not continuously connected vessel anlagen in the chorionic mesoderm. Vascular development in the villi is not mentioned.

Strahl. (1916).

No history. Embryonic Anlage .7 mm. Chorionic vesicle with villi about 10 mm. dia.

There is no mention of vascular development in the embryonic shield or chorion. Vessel anlagen are not recognised in the body-stalk or villi. Vessel anlagen are present as thickenings in the mesoderm of the yolk-sac wall. Cells, free in the lumen of the yolk-sac, are regarded as nucleated red-blood corpuscles.

Grosser. (1913).

Abortion(after operation). Embryonic shield including primitive streak .67 mm. Chorionic cavity 6 to 8 mm. across.

Vessels are absent from the embryonic area. The yolk-sac shows blood-islands on the distal pole. Blood corpuscles are recognised by their intensive staining and the blood-islands possess an endothelial lining to their lumina. The blood-islands are already connected together in some parts. In the body-stalk and chorion there are empty cleft-like spaces resembling vessels and lined by fairly irregular endothelium. At a single place in the body-stalk there is a true blood-island. Vascular-isation of the villi is not mentioned. A mesoderm strand passing from the yolk sac right across the cavity of the blastocyst to the chorion shows the presence of entodermal cysts. In the wall of one of the larger cysts blood-islands are present.

Ingalls (1918).

Abortion. Blastoderm 2 mm. Ovum external measurements. 9.1 x 8.2 x 6 mm.

In the embryo there are no vessels or blood cells. Spaces present "might stand in some relation to the future pericardial coelom". Over the fundus of the yolk-sac there is extensive formation of blood cells and blood vessels. The body-stalk contains numerous vessels filled with nucleated red cells. There are a few "funnel" ingrowths of mesothelium, but connection of these with "unlined" spaces and angioblast cords, which are also present, is not specially evident. Vessels and solid strands are present in the chorion and villi and are most frequent near the attachment of the body-stalk. The question of continuity of vascular tissue in the body-stalk, chorion and villi is not entered into, but it is definitely stated that the vessels in the stalk are not in connection with the vascular tissue of the yolk-sac.

Frassi (1907-08).

Vaginal Hysterectomy. Embryonic shield 1.17 mm. Blastocyst cavity 9.4 x 3.2 mm.

There is apparently no vascular development in the embryonic area. The yolk-sac posses early blood and blood vessel anlagen which are situated between mesoderm and endoderm (fig.16, 1908). As far as I can make out from fig.16 (1908) the cells

in the centres of the blood-islands are individual cells and the limiting cells approach more nearly to endothelium than is the case in the McIntyre ovum. Blood vessels are present in the body-stalk and according to Grosser one of these contains blood cells the remainder being empty. Vessel anlagen in the chorionic mesoderm are only recognised with certainty near the insertion of the body-stalk and two of these at the base of the body-stalk (fig.17. 1908) show the presence of free cells. Vessel anlagen are not found in the villi. Fig. 15 (1908) shows a small cyst on the wall of the yolk-sac in association with "the anlage of a blood vessel with blood". The epithelium of this cyst is similar to the coelomic epithelium where it passes over the blood vessel anlagen.

Jung, referring to this ovum in the description of his own ovum implies that all trace of vessels is absent from the chorion.

MCINTYRE (1924-25).

Hysterectomy. Blastoderm (including primitive streak) 1.37 mm. Chorionic vesicle external dimensions including villi (after fixation) 14 x 13 x 8 mm.

Blood-islands are seen in the lateral walls of the yolk-sac. They are more numerous on the left than on the right side. They do not form a continuously connected network. None of these structures has reached a stage in development which would permit one to call it a "vessel".

The body-stalk possesses two large vessels regarded as the umbilical arteries and dorsal to these a venous plexus of spaces. Neither of these systems is in connection with the blood-islands of the yolk-sac nor do they appear to communicate with one another, but both can be traced into the chorion. In addition to the above elements, small solid angioblastic masses, not unlike the blood-islands of the yolk-sac, are found scattered throughout.

All stages of vessel formation are seen in the chorionic mesoderm in an area around the base of the body-stalk and are restricted to this area. The commencement of vascularisation of the villi is noted only in the villi belonging to the same area. In only two villi could a vessel be said to be present. Angioblastic tissue is found isolated in the mesoderm of the villi.

In the embryo, no evidence of the commencement of formation of the heart or of vessels was found. The pericardial coelom has the form of a "U" shaped tube.

Eternod (Vulliet) (1894).

Abortion. Embryo 1.3 mm. Blastocyst cavity 6 x 4.8 x 3.6 mm.

There is in this embryo a horse-shoe shaped heart of symmetrical outline giving rise to two primitive aortae (with 3 or 4 aortic arches) which are continued into two umbilical arteries in the body-stalk and presumably through these into vessels in the chorion. The villi are commencing to become vascularised. On the venous side, veins run from the chorion to

unite in the body-stalk to form the future umbilical vein; this divides into two, the branches running forward in the mesoderm of the yolk-sac on either side to the primitive heart. Circulation is thus established, Posteriorly a venous loop is formed around the allantoic canal. There is evidence of vessel formation in the amnion wall and some of these elements are already canalised. In addition to the veins mentioned above, the yolk-sac possesses blood-islands and vessels in which the lumen is already established.

Graf. v. Spee (Gle.) (1889).

Abortion. Embryonic shield 1.54 mm. Internal diemnsions of chorion 7.5 x 8 mm.

No vessel formation nor commencement of development of the heart is found in this embryo. A space is present in the embryonic mesoderm. Blood anlagen are present exclusively in the wall of the yolk-sac. The mesoderm of the body-stalk and yolk-sac is rich in spaces having a smooth lining of low cells like embryonic endothelium. In the yolk-sac, cell strands lying between mesoderm and entoderm are more closely connected with the mesoderm.

Evans, in "Keibel and Mall" reproduces a drawing of a section in which the above mentioned space in the embryonic mesoderm is shown and he regards it as the pericardial cavity. In the same section the anlage of the cardiac endothelium is seen. Vascular anlagen are recognised in the embryonic area and these

ca be traced into the vessels in the body-stalk - the anlagen of the umbilical arteries. He also recognises the presence of vessels in the chorion, but does not state if these are in continuity with the vascular elements in the body-stalk.

Triepel (1917).

Abortion. Embryonic shield 1.6 mm.

In the embryonic shield projections between the mesoderm and entoderm are taken to represent fine vessels. In these no trace of blood cells is found. The endothelial heart tube is not recognisable. The mesoderm of the yolk-sac possesses blood cells and vessel anlagen in great numbers. Near the anterior end of the yolk-sac extra large blood-islands are found. He finds that both blood cells and vessels arise from the mesoderm but considers that in a few places cell processes of endoderm take part in the vessel formation. He concludes however that the vessel forming potentiality of the mesoderm is greater than that of the endoderm. In the middle of the yolk-sac a few quite separate "Erythrocytes" are found. These are nucleated cells.

The body-stalk has at its middle short representations of vessels which can be followed only through one or two sections. The villi show the presence of channels regarded as the anlagen of vessels but these contain no blood cells. In the mesoderm of the amnion near the body-stalk are a few isolated blood cells.

Ingalls (1920).

Curettage. Embryo - greatest length 1.38 mm. Ovum 7.5 x 10.5 x 12 mm.

In this embryo the pericardial coelom is present, a heart plexus has formed and the dorsal aortae and the rudiments of two aortic arches are evident. In the yolk-sac, vessels are abundant especially laterally and posteriorly but all are not canalised. In the body-stalk are two umbilical arteries and a venous plexus. The chorion contains numerous vessels many with widely open lumina. Formed elements are present, however, only at the base of the body-stalk. The villi have abundant slender anastomosing channels or cords and many detached strands.

One umbilical artery establishes connection with the vascular elements in the yolk-sac wall. The venous plexus of the body-stalk is not in connection with the yolk-sac vessels. "Slender and circuitous" connection between the plexus in the body-stalk and the chorionic vessels is established by small, thick-walled vessels regarded as ingrowths from the chorion to meet the independently formed vessels of the stalk. Vascular structures in the villi are in continuity with the deeper vessels of the chorion. Circulation in the sense of a pulsating heart is not yet established.

(C)

DISCUSSION.

YOLK-SAC.

Reviewing the specimens considered we find the following steps in vascular development in the yolk-sac.

The earliest ovum in which vascular development is mentioned is that of Schlagenhaufer and Verocay who find the anlagen of blood-islands present. The first definite statement that blood-islands are present is found in Meyer's description of his ovum. Vessels (with endothelium and blood corpuscles) are first encountered in the Lewis (Minot) embryo. Vessels having a definite course over the yolk-sac are found only in Eternod's ovum.

Of the ova earlier in the list than T.B.2. only those of Schlagenhaufer and Verocay and Herzog show indication of vascular development, and in the latter, this is seen not in the yolk-sac proper but at its junction with the body-stalk. Following on T.B.2. blood-islands are present in all the specimens. The thickenings or grouping of cells in the mesoderm of T.B.2. present also in Fetzer and v. Mollendorff's Op. are almost certainly blood-islands in the process of formation. With reference to the collection of mesoderm cells in T.B.2. which lies between mesoderm and endoderm, it is interesting to note that v. Mollendorff in his ovum "Op." describes a similar solitary collection which he also regards as of mesodermal origin.

The McIntyre ovum appears after several (Lewis, Grosser, Ingalls, (1918) and Frassi) in which vessels are clearly formed.

From the description given it is obvious that the term "vessel" cannot be correctly applied to the most mature form of blood-island which I have described. This doubtless represents a variation within normal limits. Another example of this variation is found in Strahl's ovum which although belonging to an earlier group than the McIntyre, yet is placed after the Lewis ovum in which vessels are present. In it vessel anlagen are described merely as thickenings in the mesoderm of the yolk-sac wall.

Regarding the distribution of the Blood-Islands. In all specimens where definite mention of the distribution of the blood-islands is made, only one, (Ingalls 1920) corresponds at all closely to the arrangement in the McIntyre embryo. In the remainder, with two exceptions, the blood-islands are present or furthest developed on the ventral or distal pole. In the Thompson and Brash ovum, although the blood-islands are situated on the ventral pole, they are more advanced in development at the cranial extremity. Triepel, in his ovum, finds the largest blood-islands at the anterior extremity. The restriction of the blood-islands to the lateral walls in the McIntyre ovum has permitted me to figure them graphically (Text figs. 2 & 3) as they appear in side view of the yolk-sac. I have no explanation to offer for this departure from the arrangement usually described.

The Source of Cells forming Blood-Islands. In the majority of cases the blood-islands are described as being situated in the mesoderm layer or as producing thickenings or protuberances of

that layer (Schlagenhaufer and Verocay, Strahl-Beneke, Lewis, Strahl, and Triepel). In a few cases they are said to lie between mesoderm and endoderm. (Meyer, Graf Spee v. H, and Graf Spee "Gle"). In Meyer's and in Graf Spee's "Gle", although situated between the two layers, connection to mesoderm is noted. Thompson and Brash, in describing their specimen, hint at the possibility of blood-cell origin from endoderm and endothelium from mesoderm, although they come to no definite conclusion on the matter. Triepel finds in a few places in his ovum that processes from endoderm take part in vessel formation. In the original description of all the human ova considered, with the exceptions mentioned, no statement that blood-islands are connected to or arise from endoderm is encountered.

These facts at first sight might appear to decide the question under consideration, but one has to contend with the possibility of the early migration of endoderm cells into the mesoderm layer, there to give rise to the formation of blood-islands. If we accept the thickenings of the mesoderm present in Fetzer, v. Mollendorff (Op.) and T.B.2. as blood-islands in process of formation then, as proof of endodermal origin, we might expect to find in those cell masses or in parts of them, in addition to mere alteration in arrangement, some slight departure from the appearance of the mesoderm layer in which they lie. If, for instance, we imagine a yolk-cell as the nucleus of one of these cell groups, in well preserved specimens one would expect to recognise at least a different staining reaction of the

protoplasm. I have not encountered any statement that such an appearance is present in any specimen in this series, nor is it found in the T.B.2. ovum which represents a stage at which it would most likely be found if such were the arrangement.

Mann, in "Quain's Anatomy" accepts the mesodermic origin of the yolk-sac vessels. In "Keibel and Mall" Evans also practically accepts a mesodermic origin for the vascular anlagen but with the reservation that they may actually have arisen from the entoderm. Minot, in the same work holds that the angioblast is formed from cells which separate from the yolk or from the layer of yolk-cells. The most recent work bearing on this problem is that of Florence Sabin who has been able to observe in the living chick the actual differentiation of mesoderm to angioblast. This work would appear to furnish conclusive proof that in the embryo of the chick at least the blood vessels arise not only in but from mesoderm.

The more recent suggestion of origin of endothelium and blood cells from different germ layers is found in the work of Wang. In ferret embryos he finds the blood cells are formed first and in connection with the endoderm. Endothelium arises from the mesoderm layer, and growing round and engulfing the blood cells, takes them into the circulation. The Thompson and Brash ovum, it has already been noted, is the only one in the human series in which a similar appearance is described. The vascular elements in the yolk-sac wall of the ferret embryos produce projections on the endoderm side, another appearance not encountered in the human.

The evidence regarding the source of origin of the blood-islands in the human may still be regarded as inconclusive but the weight of evidence is decidedly in favour of origin from mesoderm.

The Differentiation to Blood Vascular Tissue. Accepting that the blood-islands in the wall of the yolk-sac take their origin from mesoderm, at what stage does differentiation of mesoderm to blood-island cease? From the descriptions of many of the older specimens, even when vessels are present, it is apparent that early blood-islands also are found. Definite statements regarding the continuity or otherewise of these are not always encountered, but it would appear that the differentiation is progressive and that these early representations are not the result of growth from the more mature elements. The arrangement in the wall of the yolk-sac in the McIntyre ovum at any rate supports this view. The stage at which this differentiation ceases is, I think, a stage beyond that embraced by the material examined or reviewed here.

Origin of Corpuscles, Plasma and Endothelium. Turning now to the separate elements of the yolk-sac vessels, are we to regard the blood corpuscles and endothelium as both arising from the blood-islands? If we regard the blood-island as consisting of the whole thickness of the mesoderm layer where a blood-island exists, then both elements arise from the blood-island. If, on the other hand, we regard only the central part of the total mass (different in appearance to the adjacent

mesoderm) as the blood-island, then it is possible that the blood-island is responsible for the production of blood corpuscles, while from the surrounding mesoderm endothelium takes its origin. The former is the view generally held (Bremer, Sabin) but here again there is little evidence one way or the other in the descriptions of early human ova. Jordan, in the yolk-sac of a 13 mm. human embryo, finds the latter method of differentiation present. In the McIntyre embryo the appearances generally suggest, first, a differentiation into angioblastic tissue which is responsible for production of both blood corpuscles and endothelium. (fig.10 Pl.IV). The most mature form of blood-island in the McIntyre ovum represents the stage at which plasma first appears. This arises as a result of splitting up of the central mass into smaller nucleated masses which no doubt by further division will produce individual cells - the blood corpuscles. Streeter finds "considerable conversion into clear plasma". Sabin in the living chick finds that whole masses are destroyed in the process of liquefaction to form plasma. A comparison of direct observation of the process with the picture representing an isolated stage is impossible, but it may be said that in the material I have examined, there is no evidence of destruction of nuclei in the process of plasma formation. Whole cells cannot be destroyed as individual cells do not exist, or at least cannot be recognised if they do. The formation of plasma would appear to occur pari passu with the splitting up of the protoplasmic mass into individual cells. It is not suggested

that there is an immediate production of single free cells by this process of cleft formation, but rather that for a time masses of nucleated protoplasm remain attached to the outer boundary of the blood-island (the future endothelium). They are already haemoglobin coloured and will produce blood corpuscles. They, therefore, represent blood-islands in the sense in which the term is employed by Sabin, and their appearance corresponds to her description of such.

The Blood-Islands are not continuously connected. It will be remembered that in the McIntyre ovum the blood-islands in the yolk-sac are not continuously connected. Meyer states that in his ovum the yolk-sac blood-islands do not communicate with one another. Debeyre states that they do not form a network. Grosser finds the blood-islands already connected together in some parts. In the other ova where blood-islands are present if not definitely stated, in the majority of cases the description implies that they are not continuously connected. If, as Minot suggests, the angioblast appears as a reticulate grouping of cells between the mesoderm and endoderm and maintains its complete independence throughout life, then the yolk-sac blood-islands would be connected up together at all stages and progressive differentiation of mesoderm could not occur. The evidence in human material is entirely against this view. The blood-islands are at first isolated from one another, intercommunication being established later. There is indication that this linking up of the blood-islands has commenced in the

McIntyre ovum.

I have not encountered any funnel-shaped arrangement of the mesoderm of the yolk-sac as described by Bremer in Jung's ovum. The projection of mesoderm cells noted in T.B.2. is solid.

In two cases blood cells are found free in the cavity of the yolk-sac. Strahl in his ovum finds nucleated red-blood corpuscles free in the yolk-sac lumen. Triepel describes erythrocytes (nucleated cells) as present in the middle of the yolk-sac. In this connection it is interesting to note that a few cells, in appearance very similar to the nucleated red-blood corpuscles in the stalk, were found in the yolk-sac cavity of the McIntyre ovum. They were situated at the angle of junction of the yolk-sac wall and blastoderm on the left side. After careful consideration it was decided that these were endoderm cells heavily laden with yolk which produced a staining reaction of the protoplasm simulating haemoglobin colouring.

Minot states that "in man, if red blood-islands occur at all, they must break up very early". That haemoglobin deposit occurs in the blood-islands of the yolk-sac before these break up is clear from the appearances in the McIntyre embryo. This, however, may be a very transitory stage as Minot suggests.

No endodermal "blisters", as described by Sabin, were encountered in either specimen examined. What were taken to

be "blisters" in the mesoderm of the amnion receive mention shortly.

AMNION.

The earliest ovum in which vessel formation is noted is that of Streeter where the process is recognised over the greater part of the amniotic mesoderm. Rossenbeck finds endothelium - lined spaces present. Eternod describes early vessel formation, some of the elements of which are canalised. Triepel notes a few groups of blood cells near the body-stalk. The early vascular tissue present in the McIntyre ovum is so near to the body-stalk that I prefer to regard it as part of the process in that area. In this ovum ring-like structures or "blisters" in the mesoderm of the amnion are seen projecting outwards in some of the sections (fig.18 PlVII) but these were not regarded as concerned in vascular development. I am unable to offer any opinion with regard to their significance.

It is rather remarkable that the Streeter ovum - the earliest in the series in which vascular development is recognised - should show comparatively extensive development. More so is this the case when we meet with no mention of the commencement of the process in many older embryos.

BODY-STALK.

Mention of vascular development is encountered at the earliest stage at which a body-stalk exists, viz. in Jung's ovum.

The cellular collections, with lumina described, he does not, however, definitely decide to be vessel anlagen. The next reference is found in Herzog's specimen where the anlagen of the yolk-sac vessels are located at the junction of that structure and the body-stalk. The next in order is the earlier of the two ova under consideration in this paper, and in it two separate regions of the stalk are involved in the process. The angioblastic strands and spaces situated at the base of the stalk indicate essentially the same process as is going on in the chorionic mesoderm. Consideration of this area is deferred until the vessel formation in the chorion is discussed. The question as to whether this process extends ~~upwards~~ into the stalk to represent the ingrowths from the chorion described by Ingalls (1920) will also require consideration. In the body-stalk proper the early commencement of vessel formation in a different manner has been indicated. The drawing reproduced (fig.5 Pl.II) is not so convincing as the actual specimen. It is reproduced mainly to show, as indicated by the arrow, the possible interpretation that the mass has sunk in from the surface, a point of interest in connection with Bremer's theory of origin of the vessels.

The figure, however, demonstrates the difference between the mass and the surrounding mesoderm. There is no doubt that this represents the early formation of blood and vessel, and from its position it probably represents one or other umbilical artery.

In Debeyre's embryo the next step in development is found. In the body-stalk blood-islands are described. Two of these are particularly large and elongated and might well be taken to represent the first recognisable differentiation into umbilical arteries. In Rossenbeck's specimen we meet with the first open channels. Two endothelium-lined spaces present are described as having a course strikingly like that of the two vessels of the McIntyre ovum. The combination of two regular vessel channels with nucleated red cells free in their lumina is first encountered in the latter specimen.

With minor variations vascular development in this region advances steadily in the specimens considered. Two exceptions may be noted, viz. Strahl's and Triepel's. In the former no blood vessel anlagen are present, in the latter, a somewhat more advanced stage of development than is described might be expected.

Returning now to the McIntyre ovum, in the body-stalk we find two umbilical arteries, a venous plexus and isolated angioblastic masses. These last named in some cases resemble the blood-islands of the yolk-sac. Blood-islands in the body-stalk are described by Debeyre and Ingalls (1920). From their descriptions it appears that in the former the blood-islands cannot stand in relation to vessels as vessel lumina are not yet established, while in the latter the blood-islands are situated in the vessels. In my specimen the angioblastic tissue is not located in the vessel channels. No blood-islands, in the sense

in which the term is employed by Sabin, or as described by Ingalls are found. Nucleated blood corpuscles are numerous in the umbilical arteries, in fact as far as I can make out, are more numerous than in any of the specimens reviewed, but no syncytial or cell masses are attached to the vessel walls. My interpretation of this is that the angioblastic masses, as they mature and produce blood cells, are incorporated in the vessel lumen. This inclusion of these masses of cells must coincide with the freeing of the cells, otherwise their attachment to the vessel wall would have been encountered. In the venous plexus of spaces no free cells are present, but communication partly open and partly solid between the spaces and the angioblastic masses is very common. Such connection with the umbilical arteries exists, but in comparison, is infrequent. Speculating on this I should offer as an explanation that in the umbilical arteries blood cells are now being produced partly by the existing free blood cells, whereas, the venous plexus having none to start with is entitled to a more generous supply of blood cell forming tissues. In other words the umbilical arteries represent a later stage in development than the venous plexus.

In describing the walls of the vessels in the body-stalk it was stated that their structure does not entitle one to apply the term "endothelium-lined" to these channels. The inner lining has to undergo further differentiation before it can be so described. It is quite obvious, however, that the lining of these channels will form endothelium. If this is so, then in the

plexus of spaces, we have tissue which is undergoing differentiation into endothelium in the absence as yet of blood cell contents. Here it may be noted that Stockard finds in Teleost embryos that the "endothelium is in all cases utterly incapable of giving rise to any type of blood cell". When spaces become lined by endothelium blood cell reproduction stops. "The red-blood corpuscles are always produced so as to be delivered into the vessels....." This description in some respects fits the arrangement described in the body-stalk of the McIntyre embryo. Sabin, however, maintains that it is proved for the chick that endothelium can form erythroblasts.

Finally, with regard to the body-stalk in the McIntyre embryo, the condensation of protoplasm and nuclei which forms the walls of the umbilical arteries is a relatively thick layer. This layer is of a greater thickness, one might suppose, than is necessary for the production of a simple endothelial lining. Is it possible that we have here, already, evidence of the commencement of formation of the extraendothelial structures of the walls? I think one is almost justified in concluding that such is the case.

The commencement of vascular development can be recognised in the body-stalk and yolk-sac at about the same stage. It is usually stated that vascular development can first be recognised in the yolk-sac, but in the human, the evidence points rather to a simultaneous commencement of the process in these two regions.

CHORION.

Among the ova earlier than the T.B.2., in one only, (v. Mollendorff's Op.) is there mention of vascular development. v. Mollendorff describes channels in the chorion which are accepted as vessels, and these are lined with flattened cells. In the T.B.2. ovum we meet with the angioblastic strands and spaces which have been described. Vessels are present in the chorion of Debeyre's and Ingalls'(1918) specimens and numerous large vessels with plentiful free nucleated red cells have appeared in the McIntyre ovum.

Recent work on angiogenesis has been directed principally to solving the problem of development of intra-embryonic vessels. In the literature, therefore, one finds the descriptions of vessel development in the chorion less satisfactory than for the regions already considered. A noticeable feature is that a gradual sequence in development is not so readily made out.

The appearances in the T.B.2. ovum are of particular interest and are reproduced at all closely in only one specimen, viz. Lewis (Minot). In the v. Mollendorff (Op.), Strahl-Beneke, Graf v. Spee (v.H.), Grosser, and Ingalls (1918) ova, spaces only are found in the chorionic mesoderm. In the Lewis (Minot) embryo, however, spaces with contained strands, the latter being regarded as collapsed endothelium, are described. Bremer describes spaces and strands in several ova he examined but these are found more particularly in the body-stalk. The v. Mollendorff (Op.), Strahl-Beneke, Graf v. Spee and Lewis specimens all represent

a stage in development very near to that of T.B.2, while Grosser's and Ingalls' ova are slightly older. It would seem almost as if the appearances in the T.B.2. ovum which have been detailed in the early part of this paper were present only for a very short period. There is nothing comparable to these spaces found in the McIntyre ovum or, as far as one may judge from the descriptions, in other ova representing a similar or a later stage in development.

The spaces or channels in the chorionic mesoderm of T.B.2 possess no lining which might suggest that they are, or, in themselves are likely to form, vessels. Although it cannot be denied that some of these spaces communicate with the extra-embryonic coelom, such communications are not numerous in comparison with the many spaces present. The chorionic mesoderm has a loose open arrangement of its tissues and its inner limit is indistinct. No matter what type of spaces or cavities were distributed generally throughout this zone, it would be surprising if a few of these did not communicate with the extra-embryonic coelom as mere accidental occurrences. On the other hand, the spaces, whether they communicate with the coelom or not, may have been produced by the identical process which brought about the formation of the coelom. The extra-embryonic coelom in this ovum is established and the arrangement of the spaces makes it unlikely that they will go to produce its further development, so that they are not a part of the process of coelom formation. The fact that most of the spaces do not communicate with it merely indicates

that they are not formed by tubular extensions of the coelomic cavity into the chorionic mesoderm. It should be noted also that they never contain any of the granular coagulum present in the coelomic cavity.

A point which is worthy of consideration is the possibility that these spaces have been merely potential during life and that they have become actual only in the course of preparation of the specimen. Against this we have the absence of distortion of the tissues in their vicinity and the presence of many with a wide and sharply defined lumen. In the preparation of the specimen slight exaggeration of the spaces, present as actual channels in the living state, may have occurred.

The solid protoplasmic strands in these channels are regarded as angioblastic tissue; they are destined to form endothelium and, with establishment of a lumen, blood corpuscles as seen in the earlier forms in the chorion of the McIntyre ovum. There is no evidence to support the possible view that the spaces represent vascular channels and that the strands will go to form blood cells only. This can be excluded by consideration of the picture of vascular development present in the chorion of the McIntyre and other ova.

I venture to suggest in explanation of these channels that they are present to facilitate the rapid diffusion of material for the nutrition of the tissues of the ovum. As might be expected, they are found in direct relation to the angioblastic tissue which at this stage requires special provision for its rapid, even

precocious development. They persist only for a very short period because when the extra-embryonic coelom is fully formed and the vesicle has become larger, the chorionic mesoderm is relatively a much thinner layer and has a greatly increased area. This improvement in facilities for diffusion having been establised, the spaces disappear, the vascular elements lie in direct contact with their surroundings and the chorionic mesoderm as a whole becomes a more condensed layer. The McIntyre ovum may be taken to represent this stage completed.

This might be made clearer if we again consider the process of formation of the extra-embryonic coelom. Starting with the early mesoderm filling the chorionic cavity as in the T.B. No.1 ovum, the coelomic cavity is probably formed by an increase, and running together in the centre, of the fluid matrix of the tissues. The cavity being more or less established, differentiation into angioblastic tissue occurs in strands and around these the fluid parts of the mesoderm run to facilitate their nutrition temporarily as an aid to their recognised rapid development. Whether it is that the angioblastic strands determine the appearance of the spaces, or that the converse holds, it is impossible to say. The ultimate arrangement as described for the T.B.2. ovum would be the same in either case.

In this ovum, although an occasional space communicates with the cavity of the vesicle, no connection of angioblastic strands with the inner surface of the mesoderm layer was encountered. The strands and spaces, already mentioned, at the base of the body-

stalk differ in no respect but size from those elsewhere in the chorion. The area where they lie, it is impossible to allocate with any certainty to either body-stalk or chorion, but as the appearances are essentially the same as in the chorion I prefer to regard it for descriptive purposes as chorionic. Away from the chorion there is no indication of downgrowth of angioblastic tissue into the chorionic mesoderm. The possibility of migration of endoderm cells from the yolk-sac to the chorionic mesoderm, there to provide the origin for angioblastic formations, has never been suggested. We must conclude, therefore, that in the chorionic membrane angioblastic tissue arises *in situ* by differentiation of the mesodermic elements.

With reference to the McIntyre embryo, blood-islands as described in the yolk-sac are not encountered in the chorion. In the chorion the vessel and blood forming tissue is present in more elongated form. Although wide vascular channels containing nucleated red cells are found, these can always be traced a considerable distance in the wall of the chorion. The earlier types in their narrow elongated form give the impression that there is considerable effort to produce rapidly, channels with a lining destined to form endothelium. In the yolk-sac on the other hand, the effort would appear to be directed more to the forming of blood cells. Even in the chorion, nevertheless, as soon as a channel exists, the presence of primitive blood-cells can be made out.

The collected evidence points to the commencement of vascularisation of the chorion as occurring at about the same time

as it commences in the yolk-sac and body-stalk. This would correspond to a stage in development represented by an ovum slightly younger than the T.B.2 (when the extra-embryonic coelom is established but not fully so). In practically all the ova older than T.B.2, vascularisation of the chorion is noted in some form or other.

VILLI.

Descriptions of the process of vascularisation of the villi are even less satisfactory than those of the chorion. In the list of ova surveyed, the process receives notice first in Streeter's ovum, in which solid strands and endothelial tubes are present. Debeyre refers to doubtful early vessel formation. In Ingalls'(1918) ovum and in the majority of those older than his, vessels can be recognised. It is apparent that vascularisation of the villi commences at a later stage in development than is the case in the yolk-sac, body-stalk and chorion.

In the McIntyre embryo there is no difference in the process of vascularisation as seen in the chorionic membrane and in the villi except in degree. In the T.B.2. there is no proof that the (empty) spaces present in the mesoderm of the villi are concerned in vascular development. It is possible that these spaces might later take on the characters of those in the chorionic mesoderm and contain similar angioblastic strands. If such a stage exists for the villi, it has not been described. The presence of angioblastic tissue isolated in villi, as seen in the McIntyre ovum, and

as described by Ingalls (1920), entitles one to assume that here again such masses result from differentiation of the mesodermic elements in situ.

PERICARDIUM AND HEART.

Of the ova earlier than Eternod's in three only does mention of the presence of vascular development in the embryo appear. v. Mollendorff (Op.) finds one group of cells of doubtful significance; Debeyre thinks a cellular collection present may represent the first cardiac formation; Rossenbeck suggests the possible presence of the anlage of the aorta.

The failure to find, after a careful search, any appearances in the McIntyre ovum indicative of the commencement of the aortic or heart rudiments makes it more than probable that the findings in these three specimens cannot be interpreted as suggested by those who have described them. The description of the Frassi ovum also definitely more advanced in development than the three ova mentioned would bear out this contention. The sudden transition to the presence of a heart and an established circulation in Eternod's ovum seems almost too sudden to be accepted as normal. In Graf Spee's "Gle" Evans finds the cardiac endothelium commencing, and Ingalls' (1920) embryo has a heart plexus dorsal aortae and aortic arches. From the latter at least it is a very short step to an established circulation. The size of the ovum or of the embryo we now also know cannot be taken by itself as an indication of the stage of development. It must be conceded, however, that, as describ-

ed, the cardio-vascular development of Eternod's ovum has probably been a little precocious. For comparison, it is interesting here to note that Sabin finds in the chick, that in the embryo the angioblast can be first seen to differentiate from mesoderm at the stage of 5 somites and that the first beats of the heart occur at the stage of 10 somites.

With regard to the formation of the pericardium, Ingalls in his 1918 ovum regards tubular ingrowths (two on either side) from the extra-embryonic coelom as standing in some relation to the future pericardial cavity. If this is the method of origin there is certainly no trace of any communication between the "U" shaped cavity in the McIntyre embryo (which conforms to the description given by Robinson for mammals in 1903) and the extra-embryonic coelom. The material and the literature I have examined throws no further light on the earliest phase of pericardial formation in the human.

YOLK-SAC PROLONGATIONS AND MESODERM STRANDS IN THE CHORIONIC VESICLE.

The yolk-sac connection to the chorion in the T.B.2. ovum is so well established and so accurately described by Bryce that particular attention is directed to this type of structure in relation to vascular development. In Fetzer's ovum a process from the yolk-sac ends free in the chorionic vesicle, and has no particular relation to vessel formation in the yolk-sac. It is rather curious that the following three ova showing yolk-sac prolongations extending to the chorion should appear in direct

sequence in the list. These are the T.B. No.2, Meyer's and Strahl-Beneke's. In two of these, viz. T.B. 2 and Meyer's, the strand from the yolk-sac terminates in relation to a space or spaces in the chorionic mesoderm which are in some way related to vascular development. In the former angioblastic strands are more plentiful there than at any other part of the chorion with the exception of the base of the body-stalk, while, in the latter a blood-island is present, although these are absent elsewhere from the chorion. The similarity in appearance in some respects at this area, in these two specimens has already been indicated. In the T.B.2 ovum, the yolk-sac prolongation, after reaching and running in the chorionic mesoderm a short distance, is replaced by an elongated protoplasmic mass which ends free in the chorionic cavity. Into this projection an angioblastic strand runs and is converted into a tubular structure which might be regarded as a vessel. I am inclined to regard this projection into the cavity of the chorion as consisting of mesoderm only, and the conformation of the angioblastic strand which it contains as an accidental occurrence. When one bears in mind that these angioblastic strands throughout the chorion have no regular disposition but show curves and twisting of their axes it is not improbable that an accidental tubular formation for a short distance might be found. It is necessary to state, however, that no instance of tubular form was noted elsewhere in the chorionic mesoderm. My interpretation of the appearances in this area of the T.B.2 ovum are against any involvement of endoderm cells in vascular or blood

formation in this region. This is contrary to Meyer's findings in his ovum. In the Strahl-Beneke ovum the yolk-sac prolongation to the chorion possesses no early vascular elements, and although spaces are described in the chorionic mesoderm, these do not appear to be specially prominent at the attachment of the structure.

Rossenbeck describes two mesodermic strands running from the amnion to the chorion (one through the body-stalk) and these end in connection with vessel anlagen in the chorion. Grosser's ovum possesses a prolongation of the yolk-sac to the chorion and in the wall of one of the entodermal cysts, which this structure contains, blood-islands are present.

The information we have available with regard to these connections between the embryonic mass and the chorionic mesoderm does not permit one to offer even the merest conjecture with regard to their significance in relation to vascular development. I am prepared to state, however, that after carful examination of one specimen in which such a communication is present, I can find no evidence to indicate that the yolk-sac endoderm has any relation to the angioblastic tissue present. In any ovum described in the future, which contains a structure resembling the above, particular attention should be directed to the relationship of such structure to the vascular development,as it is quite apparent there is some connection the significance of which may be of great importance.

CONTINUITY OF VASCULAR TISSUES.

The question of the continuity of the vascular elements has already been discussed with regard to the yolk-sac and may now be considered for the other regions of the ovum, first individually and later collectively. In the body-stalk while still at the stage of angioblastic masses or blood-islands, the umbilical arteries are being defined. This stage is represented by Debeyre's ovum where two elongated blood-islands might be regarded in this light. Plexus formation in the case of these arteries must represent a very transitory phase, as in Rossenbeck's ovum, while still referred to as "anlagen they have a course corresponding closely to that of the umbilical arteries in the McIntyre ovum. In the case of the veins, the plexiform phase would appear to be of much longer duration as it is still present in Ingalls' (1920) ovum, and to a lesser extent in Eternod's,

As regards the chorion, definite statements are rarely encountered. Rossenbeck, however, finds the chorionic vessel anlagen not continuously connected. This is in agreement with the appearances in the T.B.2 ovum, and also in the McIntyre ovum. At this stage it is interesting to note that in a number of these early ova the area in the vicinity of the body-stalk shows a more advanced vascular development than elsewhere in the chorion. This is found in the T.B.2 ovum and also in the McIntyre ovum. Ingalls (1918) finds vessels by far most frequent near the attachment of the body-stalk. Frassi recognises vessel anlagen with certainty only near the body-stalk. In the Ingalls' (1920) specimen, where vessels

are abundant, formed elements are found only at the base of the body-stalk. As it is already clear that in the human the information we have is entirely against a progressive growth of vascular tissue outwards from the body-stalk, this can be regarded only as an early indication of differentiation of placenta from chorion. It is probable that at no stage of development is the chorion equally well vascularised throughout. Similarly, the villi at different points of attachment to the chorion, from the beginning, are vascularised in varying degree. Two ova indicate an independent origin for the vessels in the villi. These are the McIntyre ovum and Ingalls' (1920) ovum.

From human material, therefore, the evidence collected would indicate that in the different areas the vessels have their origin not in one angioblast but by progressive differentiation at many points into angioblastic tissue.

Turning now to a consideration of the continuity of the vascular tissues in the different regions, we again find in many of the specimens considered absence of definite statements on this question. In an ovum, for instance, where vascular elements are recognisable in both the yolk-sac and the body-stalk one is frequentlydisappointed to find absence of a definite statement that these are or are not connected up with one another.

In the T.B.2. ovum, if the interpretation of the appearances is correct, then one may assume that there is no extension of vascular tissue from the yolk-sac to the body-stalk. Similarly one may conclude that there is at this stage no extension of angioblastic tissue from the body-stalk to the chorion. This ovum,

however, suggests the possibility of extension of the angioblastic spaces and strands from the chorion into the body-stalk to communicate with vessels formed independently in the latter structure. This has already been suggested by Ingalls from observations on his 1920 specimen, and the appearances in the T.B.2. ovum lend some support to his view. Possibly it is not so much an extension of vascular tissue into the body-stalk as a taking up of the chorionic mesoderm and its contained vascular elements to assist in formation of this extremity of the stalk.

Here one might again refer to the distribution of the angioblastic strands and spaces in the T.B.2. ovum. These are well distributed throughout the chorionic mesoderm and although smaller and less numerous in the vicinity of the vegetative pole, are nevertheless present in that area. In the McIntyre ovum no vessel or vessel-forming tissue is found in the chorion except around the attachment of the body-stalk. What has become of the angioblastic strands near the vegetative pole of the T.B.2. ovum? These strands must either have reverted to mesodermic tissue or the mesoderm in which they were situated has, in the course of enlargement of the chorionic vesicle to the size it has attained in the McIntyre ovum, gradually moved round to the vicinity of the body-stalk.

Continuity of vascular tissue in the body-stalk and chorion is established in Rossenbeck's ovum. Vascular communication between the yolk-sac and body-stalk is found in Ingalls' (1920) ovum where open communication with the right umbilical artery

exists. The McIntyre ovum is especially interesting in this respect that it presents a stage at which the umbilical arteries are well formed but do not communicate with the blood-islands of the yolk-sac, nor does it appear that they communicate with the venous plexus in the body-stalk.

In Ingalls'(1920) and the McIntyre Ova continuity of angioblastic strands or vessels in the chorion and in the villi in some cases is established.

From consideration of human ova we may conclude that the vessels arise independently in the different regions of the embryo, and that at an early stage the body-stalk vessels are linked up with those of the chorion before vascular communication is established between the yolk-sac and the body-stalk or between the chorion and the villi. The only apparent exception to this might be found in the body-stalk as already explained. There is absolutely no evidence that the vessels in the body-stalk result from extension of the yolk-sac vessels as has been suggested.

The question of establishment of vascular communication with the embryo proper does not come within the scope of this thesis but, it is clear from the descriptions of the various ova that, in the human, the vascular tissues in the areas here considered are connected up before communication with the embryonic area proper is established.

Much has been written about the origin of the intra-embryonic vessels because a proof of their independent origin in the embryo settles the question of the possibility of origin of

vessels *in loco*. A very complete review of the literature and experimental work in this connection is given by McClure in his presidential address to the American Association of Anatomists, 1921. He concludes that the angioblast theory of His, in which it is maintained that the vascular tissue in the embryo is an ingrowth from the yolk-sac,no longer holds, and, "that the general principle of a local origin of intra-embryonic endothelium has been completely confirmed by experiment". Some additional evidence from human material that the "general principle" holds also for the extra-embryonic vascular development is supplied in this paper.

REGARDING BREMER'S THEORIES.

This thesis would not be complete without further reference to the work of Bremer and his fascinating and ingenious theories of vascular development in the human ovum. So attractive did these appear at first sight and so convincing the arguments advanced that I made several drawings as evidence in support of them before considering the question in detail. These drawings (fig.5 Pl.I,fig.13 Pl.V) I have reproduced. After a broader consideration of the matter,I have concluded that the evidence in the two ova I have personally examined does not favour full acceptance of the theories advanced by Bremer.

After examining many human embryos Bremer concludes that "the earliest blood vessels arise separately in the yolk-sac and in the body-stalk, by multiple anlages. The anlages in the body-stalk (and perhaps also in the yolk-sac cf. Jung's figure 17) are

funnel-shaped ingrowths of the surface mesothelium,... By partial fusion of the walls of an ingrowth a portion of the coelum, still bordered by mesothelium, may be cut off as a separate cavity, lying deep within the substance of the body-stalk. The endothelium seems to arise either (a) by delamination from the walls of such a detached portion of the coelom, or (b) by direct extension, in the form of an angioblast cord, from the mesothelial ingrowth..... Extension within the limit of the areas covered by the mesothelium is achieved by confluence of the detached portions of the coelom, or union of the cords; the result is a net comprising the various vascular units. Extension into the chorion, where the mesothelial layer is absent in the early stages, appears to be by direct centrifugal growth of the angioblast cords, without the addition of new elements from the surrounding mesenchyma....."

After delamination of the endothelium we have spaces regarded as isolated portions of the coelom referred to by Bremer as "unlined spaces" containing strands which will go to form endothelium. This conforms in some respects to the appearances in the T.B.2. ovum. Spaces and strands are present abundantly in the chorion and at the base of the body-stalk, but do not fulfil Bremer's description in that neither forms a continuous network, and therefore, cannot have arisen by direct extension from the body-stalk. No connection of spaces or of strands with the mesothelium of the body-stalk could be made out. Spaces in the chorionic mesoderm may communicate with the extra-embryonic coelom but no mesothelium is present. The angioblastic mass shown

in (fig.5 Pl.II) lying at a higher level in the body-stalk might be regarded in the one section reproduced as having arisen by sinking in of the surface. It is left to the reader to form his own opinion. Even if this mass were in continuity with the surface, it is unconnected with the spaces and strands at the base of the body-stalk. These spaces with their contained strands I have already suggested belong to the chorion rather than to the body-stalk, and a theory regarding their method of formation and their significance has been propounded.

In the case of the McIntyre ovum, the conditions present are against Bremer's contention that "no surely isolated endothelial cords have been found in the chorion". In the body-stalk of this specimen, however, the appearances are in some cases very suggestive of a communication between the surface and the developing vessels through the medium of solid strands. One of these is reproduced in (fig.13 Pl.V).. We may, therefore, conclude that the mesothelium of the body-stalk may play some role in vascular development in that part of the ovum, but that extension of this vascular tissue into the chorion and villi does not occur. The latter postulation is dependent on assuming that the spaces and strands in the chorionic mesoderm are the same as those described by Bremer. The appearances correspond sufficiently closely to warrant this.

REGARDING ABORTION.

Of the 28 ova here considered, 8 are abortion specimens. I had hoped that this investigation might disclose some anomalies

of vascular development which in some way could be associated with the production of abortion. These ova have all been published by their authors as representing the normal, and we have seen that vascular differentiation progresses in a fairly definite sequence alongside general development. In the present state of our knowledge of the normal vascular development in the earliest stages in the human, the hope of finding errors in development of this system and assigning these as a cause of abortion was perhaps premature. This, however, is a point, which, I think, is worth consideration in the investigation of specimens of early abortion which occur without apparent cause.

GENERAL REMARKS.

In reading through the literature, some difficulty is experienced from the wide range of terms employed to denote vascular elements and their different forms. For instance, in reference to the yolk-sac, does "the first anlagen of blood vessels" represent a less mature or a more mature stage than "blood-islands"? I have been content to employ some of the existing terms, but I think a simplification of the terminology merits the consideration of embryologists. Throughout I have restricted the term "vessel" to those instances where there is a lumen containing free blood cells.

In some of the ova it is quite obvious that the vascular development has only received passing mention and, no doubt, if all

the ova here considered were examined by one and the same individual, the record would show many differences. In some cases notably Streeter's, Debeyre's and Ingalls' (1920) ova the vascular development receives special notice.

The personal factor may to some extent come into play in recording the picture of the vascular system in isolated specimens of early human embryos. Existing theories familiar to the observer may influence his interpretation of the sections. The method of recording in this case adopted by one whose work did not previously necessitate a knowledge of the literature was as follows. In the first instance a complete examination of the material was carried out, the findings recorded and illustrations prepared. This explains the absence of reference to the literature in the descriptive part. An investigation into the literature was then made and subsequently the material was again examined and a few additional drawings prepared. Some alterations in the original description were necessary, but where doubt arose, the first interpretation was allowed to stand. The very favourable plane of section in the case of the McIntyre ovum was of the greatest help in investigating the continuity of vascular tissues.

The identification of the very earliest representation of angioblastic tissue can be submitted only as an expression of opinion.

I am responsible for all the drawings with the exception of the three which Professor Bryce has kindly permitted me to reproduce from his memoir and the microphotograph of the body-stalk

(fig.18 Pl.VII) which Professor Teacher was good enough to prepare for me. In many cases a slight exaggeration or distortion would have brought out more clearly descriptive points in the text. This I have most carefully avoided.

I am indebted to Professors Bryce and Teacher and to Dr. Norman MacLaren for many helpful suggestions. To these I wish here to make acknowledgement and to offer my thanks.

CONCLUSIONS.

From a consideration of the material here recorded and from a review of the literature the following conclusions are set forth. Some of these may not be fully justified by the evidence available, and are, therefore, to some extent opinions rather than conclusions.

1. The blood-vascular system in the earliest stages of development in human ova arises in the extra-embryonic areas by progressive differentiation of mesoderm at multiple points in situ. Its commencement may be recognised while yet absent in the embryonic area proper.

2. The theory of vascularisation of the chorion and villi by centrifugal growth of angioblastic tissue from the yolk-sac or body-stalk is disproved for the human ovum.

3. Differentiation to angioblastic tissue occurs at about the same time in the yolk-sac, body-stalk and chorion while it appears at a slightly later stage in the villi. The first differentiation to angioblastic tissue occurs at a stage just earlier in development than that represented by the T.B. 2 ovum.

4. The vascular elements in the separate areas become connected together and the different areas establish connection with one another in the following order: 1. Body-stalk and chorion. 2. Chorion and villi. 3. Body-stalk and yolk-sac. 4. Yolk-sac body-stalk and embryo.

5. The pericardial coelom is formed before the formation of the heart rudiment, or vessels can be recognised as having commenced in the embryo.

6. The blood-islands of the yolk-sac in the McIntyre ovum are found principally along the lateral walls and are more numerous at the extremities of these areas. They are also more numerous on the left side than on the right side. This distribution differs from that usually recorded.

7. Red blood-islands can be recognised in the yolk-sac in human ova.

8. From examination of the McIntyre ovum the impression was formed that, in the wall of the yolk-sac the main effort is directed to the rapid production of blood cells, whereas, in the body-stalk, chorion and villi, channel formation is of equal importance to blood cell production.

9. Prolongations of the yolk-sac across the blastocyst cavity to the chorionic wall have some bearing, not properly understood, on vascular development in the chorionic membrane. It does not appear, however, that the endoderm elements which these may contain have any part in the origin of the vessels in the chorion.

10. The mesodermic layer of the amnion wall appears to play

practically no part in vessel formation during the period of development here considered.

11. Vessel formation in the body-stalk outplaces that in the other regions so that the first channels, which can be identified as vessels having a recognisable course are those destined to be the umbilical arteries.

12. The arterial and venous systems in the body-stalk do not communicate with one another or, if they are ever in communication in that structure they become isolated from one another at a very early stage.

13. The blood cell forming tissues in the body-stalk communicate with the vessel channels but do not project into the vessel lumina.

14. The mesothelium of the body-stalk may play some part in vessel formation.

15. Extension of vessel growth does not take place from the body-stalk into the chorion but there is some evidence that the reverse process may occur in connection with the venous channels in the body-stalk.

16. In the earliest stage at which angioblastic tissue is found in the chorionic mesoderm, it appears in the form of nucleated protoplasmic strands which run in spaces. These strands are destined to form vessels and blood cells while the spaces are regarded as a temporary provision for the ready diffusion of substances for the nutrition of the ovum and, more particularly, of the angioblastic tissues which the spaces con-

tain.

17. Such spaces may be produced by a process similar to that which results in the formation of the extra-embryonic coelom but they are not parts of that cavity.

18. At the stage when angioblastic tissue appears in the chorionic mesoderm the latter possesses no mesothelium, and, therefore, mesothelium in this region has no role in vessel formation.

19. At a very early stage of development the distribution of the vascular tissues in the chorion indicates a differentiation of the area around the body-stalk towards placenta.

20. No blood-islands attached to the vessel walls, as described by Sabin in the chick, were encountered in the material examined. In some blood-islands in the yolk-sac of the McIntyre ovum nucleated protoplasmic masses attached to the periphery and projecting in towards the centres of the blood-islands are described. These can be reconciled with Sabin's descriptions of blood-islands.

(D).

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(E)

DESCRIPTION OF PLATES.

A scale indicating the magnification is supplied with each drawing taken from the sections.

Plate I.

Fig. 1. Teacher-Bryce ovum No.2. Diagram of the rudiment placed within an ideal section of the chorionic vesicle. ch.ep., chorionic epithelium; ch.mes., chorionic mesoderm; c.st., connecting stalk; a.m., amnion-embryonic vesicle; yk. sac., yolk-sac; st., stalk of yolk-sac. (By permission of Professor T.H. Bryce).

Fig. 2. Drawing of model by Dr. Norman McLaren of the McIntyre Embryo seen from above. The amnion has been opened to show the blastoderm. A bristle occupies the amnion duct. The yolk-sac is seen projecting forwards in front of the blastoderm; it has been opened up from the side. (By permission of Professor T.H. Bryce).

Fig. 3. Teacher-Bryce ovum No. 2. Wall of chorionic vesicle showing an inner mesothelium-like layer (Mth.), a reticulate zone (Rz), Mesoderm (Mes.), and the trophoblast (Tr.).

Fig. 4. Teacher-Bryce ovum No.2. Wall of chorionic vesicle including the base of a villus. The mesoderm contains spaces, in one of which a large angioblastic strand appears in cross section (A). C., cavity of chorionic vesicle; V., villus; Tr. trophoblast.

Plate II.

Fig. 5. Teacher-Bryce ovum No.2. Drawing from a section through the body-stalk to show a space containing early blood cells (Bc)

situated near the embryonic extremity of the stalk. The mesothelial covering on the surface (M) is apparent and its possible relationship to the space is indicated by the arrow. S., surface of body-stalk.

Plate III.

Fig. 6. Teacher-Bryce ovum No. 2.

Yolk-sac wall showing a small mass (blood-island?) between endoderm and mesoderm. M., Mesoderm; E., Endoderm.

Fig. 7. The same mass as in Fig. 6 two sections removed, showing protoplasmic connection with the mesoderm layer. M.,Mesoderm; E., Endoderm.

Fig. 8. Teacher-Bryce ovum No.2. A high power drawing of the mesoderm of a villus to show its structure and the spaces referred to in the text.

Plate IV.

Figs. 9 to 12. McIntyre ovum. Examples of the four stages of Blood-island formation in the wall of the yolk-sac as described in the text. M., Mesoderm; E., Endoderm.

Plate V.

Fig. 13. McIntyre ovum. The field embraces the dorsal part of the body-stalk on one side. The amnion wall (Aw.) is seen passing off from the stalk. Near the angle between the amnion wall and the body-stalk a depression of the mesothelium (Mth.) runs towards an angioblastic mass (AM.), which at this particular level may almost be regarded as a vessel. A., cavity of Amnion.

Fig. 14. McIntyre ovum. Wall of chorionic vesicle showing condensation of protoplasm and nuclei to form the earliest

representation of an angioblastic strand (As.) Tr., trophoblast; M., Mesoderm of chorion.

Plate VI.

Fig. 15. McIntyre ovum. Chorionic mesoderm showing the presence of an angioblastic strand in which haemoglobin coloured cells have appeared.

Fig. 16. McIntyre ovum. A further stage in development of the chorionic vessel is shown. Contained blood cells are seen and a lumen is present for some distance.

Plate VII.

Fig. 17. McIntyre ovum. The mesoderm of a villus showing an angioblastic strand. No haemoglobin coloured cells have formed.

Fig. 18. McIntyre ovum. Microphotograph by Prof. J. H. Teacher of the body-stalk. The prolongation of the amnion also appears and is seen passing off from the dorsal aspect. The allantoic duct can be recognised about the centre of the stalk. On either side of it an umbilical artery is present; behind it on one side a venous space is seen and on the other side an angioblastic mass (the dark elongated area). In the mesoderm of the amnion wall the "blisters" referred to in the text are shown. X120. Section No. 154. See Text fig. 2.

Text Figures.

Text Plate I.

Text. Fig. I. A flat reconstruction (20 sections) of the angioblastic strands and spaces in an area of the chorionic mesoderm at the embryonic pole directly opposite the operculum of the

Teacher-Bryce ovum No. 2. The reconstruction is equivalent to a view at right angles to the microscopic sections or to a silhouette view through the chorionic membrane.

Text Figs. 2 and 3. McIntyre ovum. The drawing represents a sagittal section of the embryo, amnion and body-stalk with superimposed a lateral elevation of the yolk-sac. The blood-islands of the yolk-sac have been plotted in to scale from individual drawings of the sections. The numbers of the sections are indicated. It was not considered necessary to correct the reversal of the view as taken from the sections.

Text Plate II.

Text Fig. 4. McIntyre ovum. Front elevation of the Body-Stalk showing the two arteries present and a lateral view of the left vessel assembled from drawings of individual sections.

Text Plate III.

Text Fig. 5. McIntyre ovum. The plexus of spaces in the dorsal region of the stalk in the same view as text fig. 4.

Text Fig. 6. McIntyre ovum. The pericardial coelom viewed antero-posteriorly as in fig. 2, plate 1. Graph reconstruction from individual sections.

Text Fig. 7. McIntyre ovum. Section No. 65. See text figs. 2 and 5. The pericardial coelom is seen on either side. (By permission of Professor T. H. Bryce).

— Plate I —

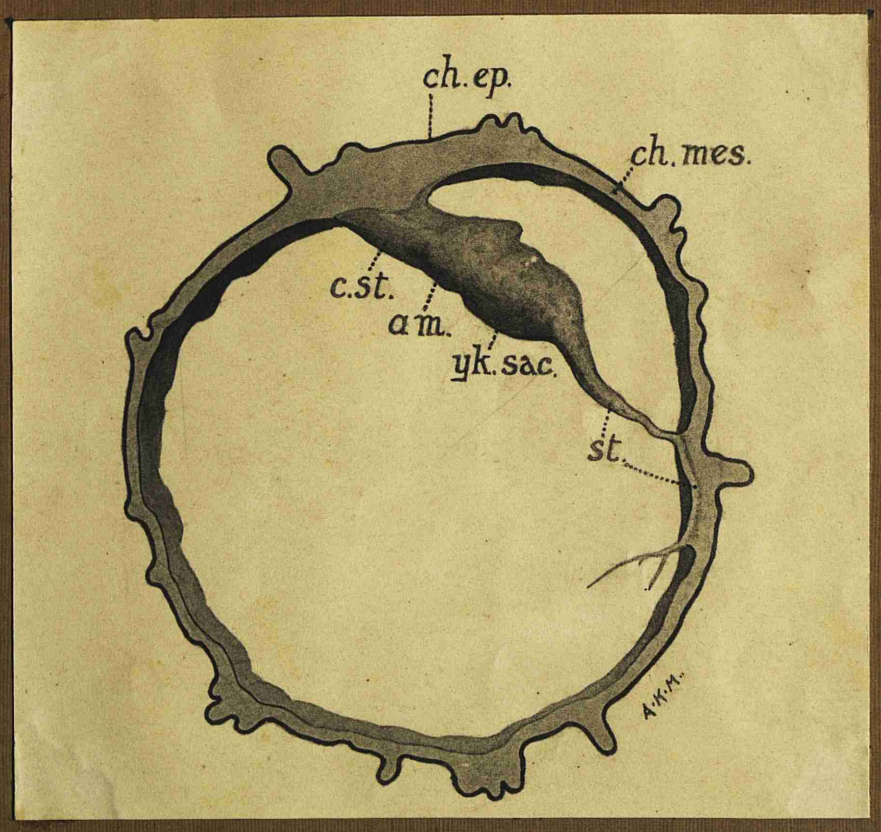
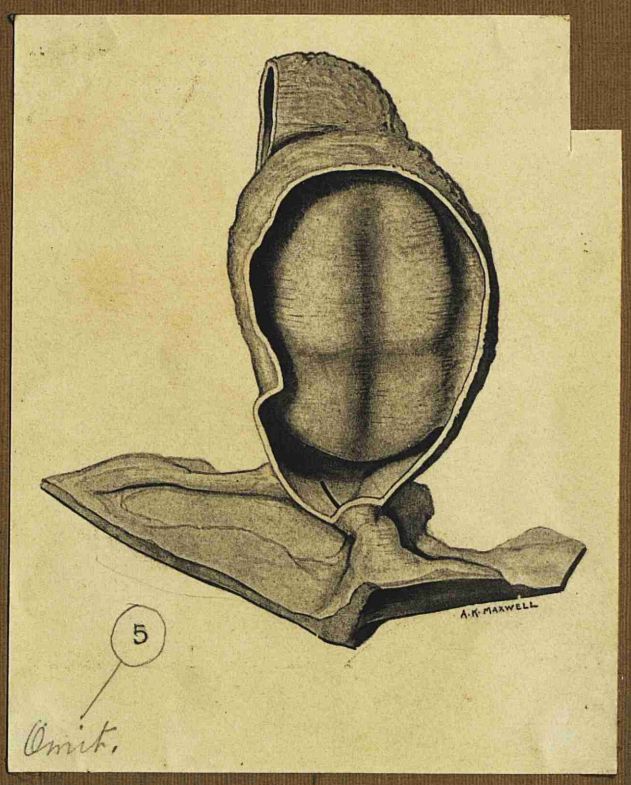


Fig. I.



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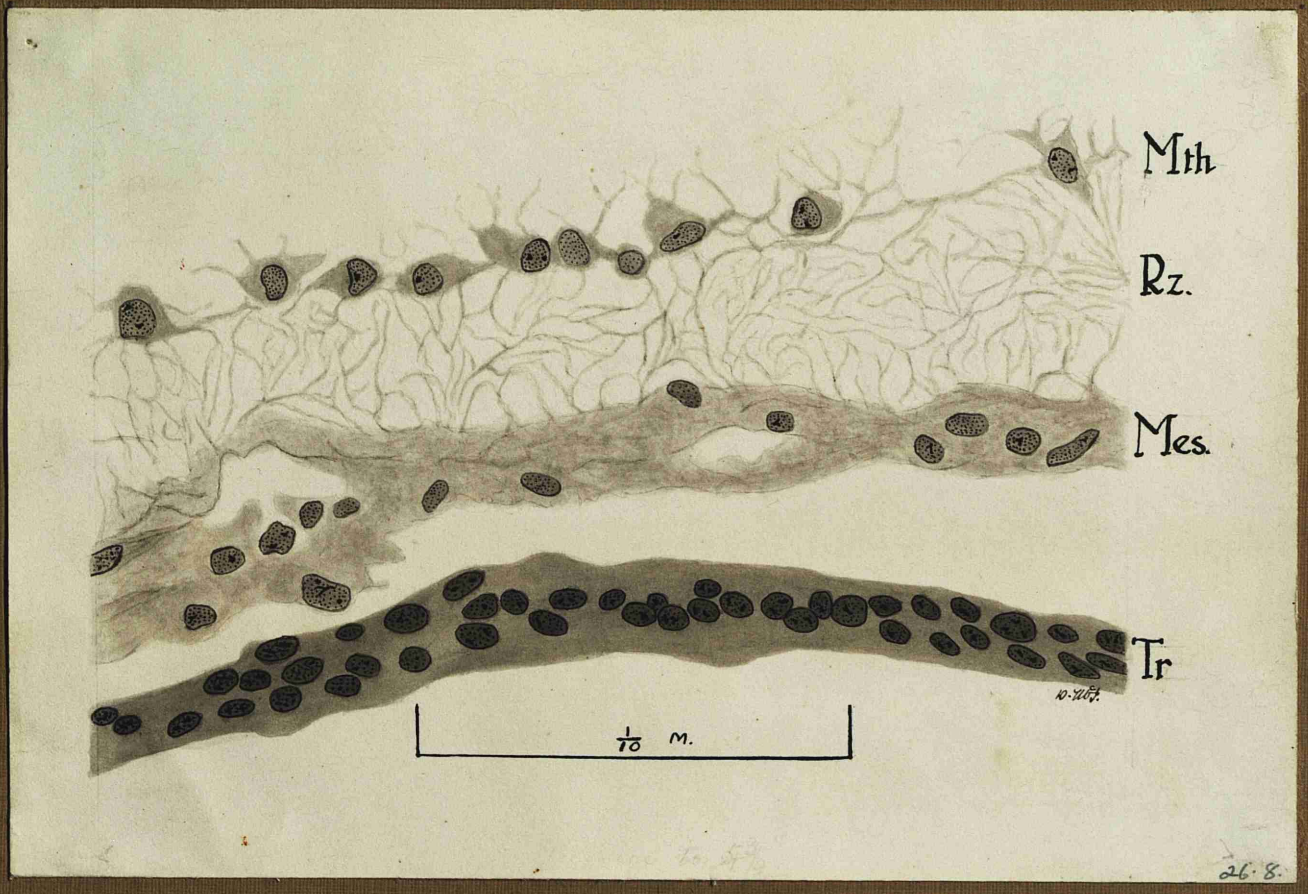

Fig. III.

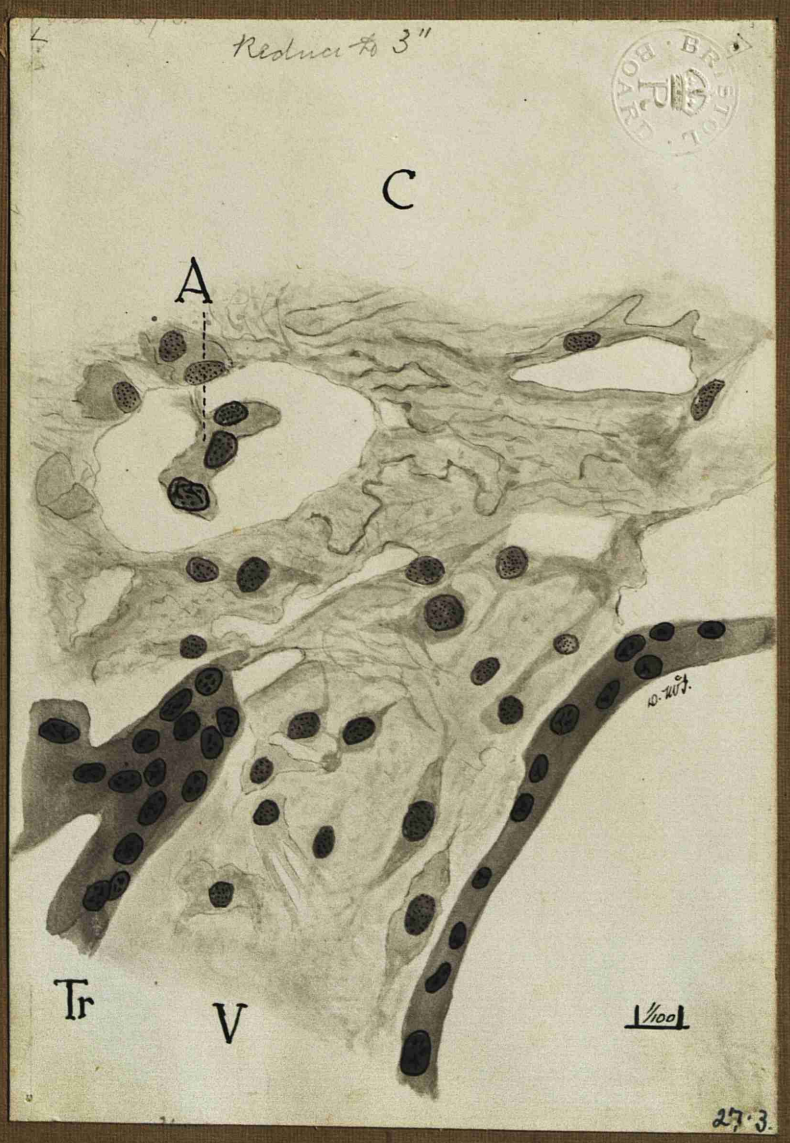

Fig. IV.

Plate II

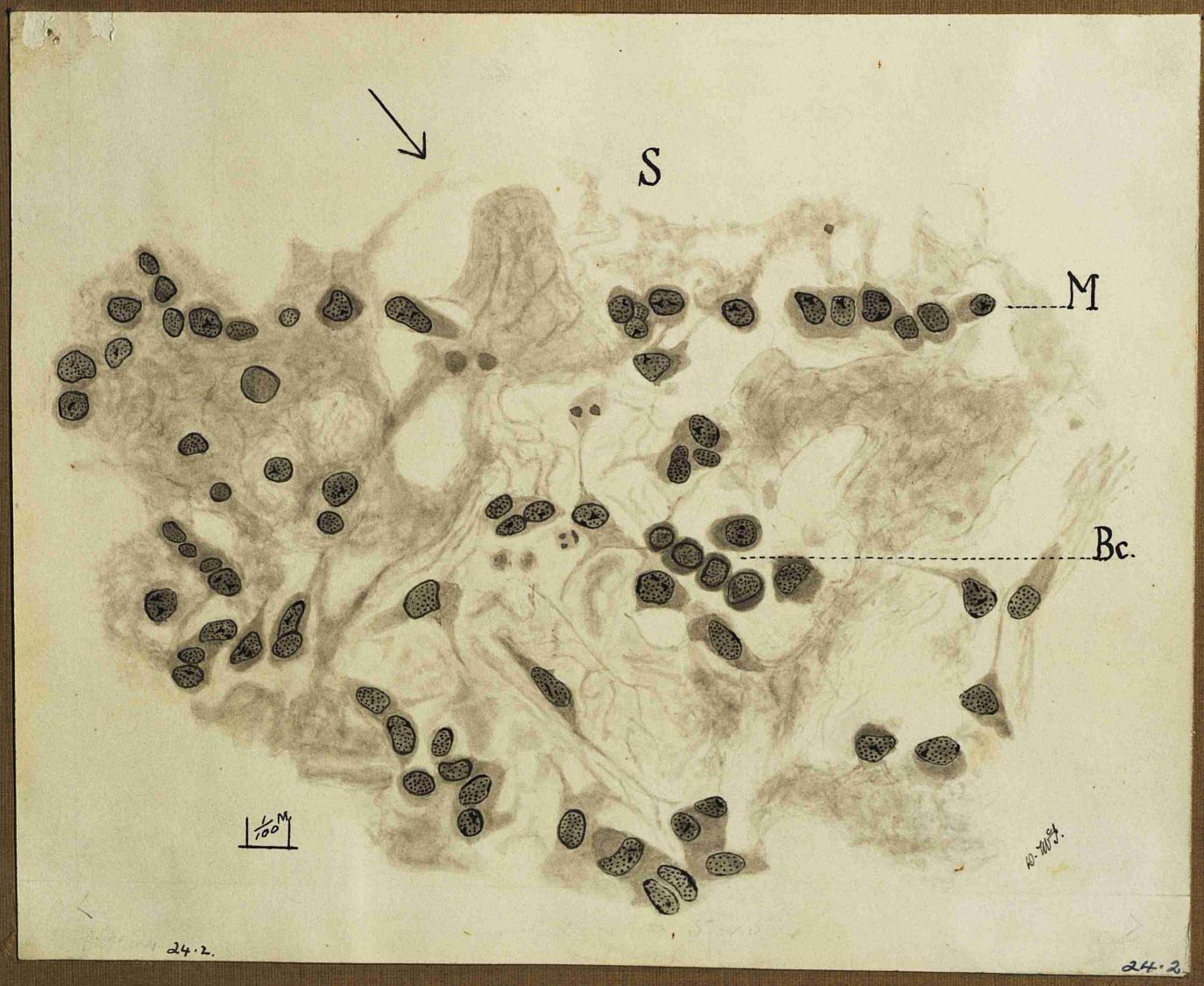

Fig. V.