SOME OBSERVATIONS ON SPANNER'S CONCEPTION OF THE PLACENTAL VASCULAR ARRANGEMENT AND THE MATERNAL BLOOD CIRCULATION THROUGH IT.

by

A. El SHAHED (El Shahed)

M.B., Ch.B., L.M., D.G.O.

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SUBJECT MATTER OF THESIS.

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INTRODUCTION.

While the development of the vascular system in other organs is regulated by functional influences and hereditary factors; in the case of the placenta the site of implantation in the uterus and the characteristics of the maternal circulation also play an important role.

The maternal and foetal blood circulatory paths within the placenta are regarded from the haemodynamic point of view as most complicated because the placental structure is looked upon as being most complex. Many contradicting views are held in that respect and many theories are formulated regarding the placental structure and maternal blood circulation through it. It is clear that in order to understand these questions the placental connections with the uterus and foetus must be studied both with the naked eye and microscope.

The human placenta according to Grosser is described as a placenta haemochorialis, thereby meaning that the foetal chorion is in direct relationship with the maternal blood.

This fact underlies many risks that the pregnant, parturient, and puerperal woman undergoes and it follows/

follows that a sound knowledge of the vascular conditions in the pregnant uterus underlies the pathology of reproduction. For instance: is the mechanism of antepartum and postpartum haemorrhage really understood? or the factors that are associated with embolus complicating child birth known? - advancement in obstetrics depends to some extent on the complete knowledge of this subject.

In 1935 Rudolf Spanner of Kiel brought forward a new conception formulating a new idea not only of the structure of the placenta but of the circulation of the maternal blood through that organ and back to the uterine veins. His work depended on injection experiments on the pregnant uterus and placenta. In the present scope of this work Spanner's work on the subject has been studied and experiments done to compare with his results.

REVIEW OF LITERATURE.

PART I.

THE PLACENTAL VASCULAR ARRANGEMENT.

THE ANATOMICAL ARRANGEMENT OF THE UMBILICAL VESSELS IN THE CHORIAL PLATE OF THE PLACENTA.

Bacsich and Smout (1937) have studied the anatomical architecture of the foetal vessels within the placenta, directing their attention mainly to the umbilical arteries. In their opinion ordinary dissection does not give a clear conception of the arrangement of foetal vessels. Also X-Ray photography after the vessels have been injected with an opaque material such as barium in gelatine as has been used by Fraser, does not show any anastomoses of blood vessels or their stereoscopic arrangement. On the other hand, corrosion technique provides specimens which may be seen and handled and accordingly is obviously superior.

In their study of corrosion preparations they considered three principles in regard to the arterial system.

(1) The pattern of the vessels.

(2) The area of placenta supplied by each artery.

(3) Any connection between the vessels and their branches.

I. The umbilical arteries enter the placenta either marginally or centrally. Then they break up into/

into several branches which run towards the margin of that organ, giving off further branches along their course. All the large arterial branches being in the same horizontal plane and lying immediately below the amnion as was first pointed out by Spanner.

According to the pattern of the arteries Schordania (1929) divided the placenta into two main types:

(A) A disperse type - (Fig.1.) in which the two arteries divide dichotomously, and rapidly diminish in calibre, resembling in their spider-like arrangement the spokes of a wheel.

(B) The magistral type - (Fig.2.) in which the two arteries extend almost as far as the margin of the placenta before their calibre diminishes.

Some of the placentae are definitely of a border-line type.

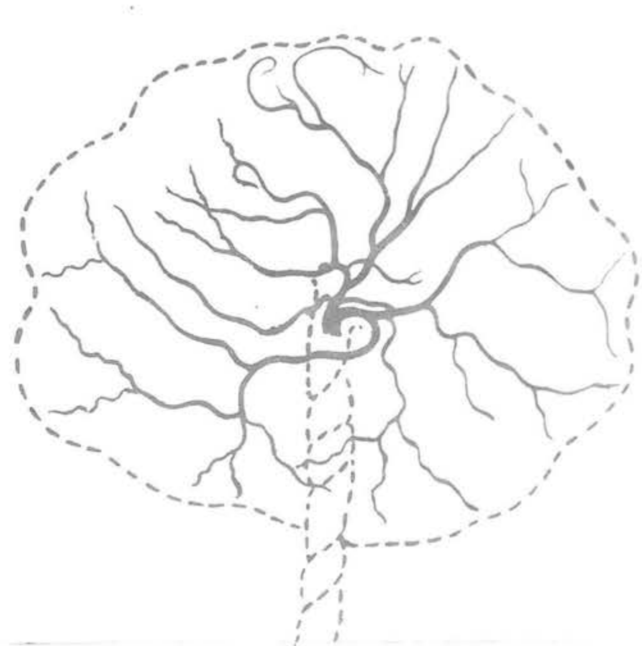

Fig 1 - Disperse Type.

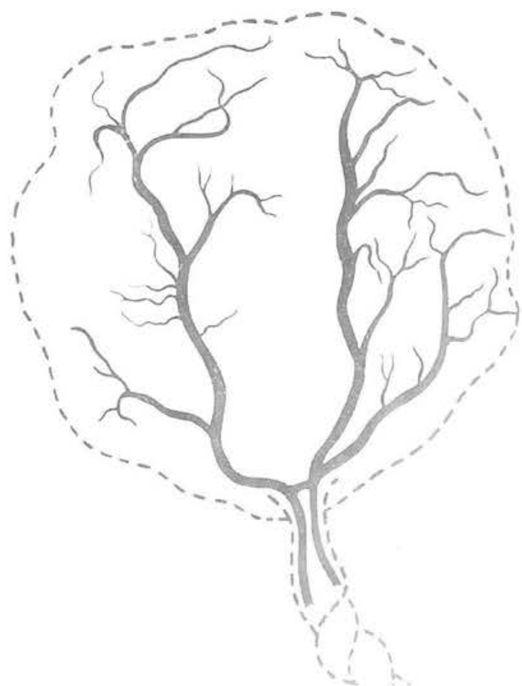

Fig 2 - Magistral Type.

II. The areas supplied by each of the arteries show great variation. In over half the cases there is a symmetrical distribution i.e. equal areas of the placenta are supplied by each umbilical artery. In one third of the cases the distribution is asymmetrical, e.g. one third would be supplied by one artery and two thirds by the other.

The fact noted by Bacsich and Smout was whatever the relation of the areas supplied by the two arteries, these vessels were always of equal calibre in the cord.

III. Bacsich and Smout point to the constant existence of a communication between the two arteries either/

either immediately before or immediately after their entrance into the placenta. This transverse communicating branch may sometimes be as wide as the main branches, while at other times it is considerably narrower. This communicating branch usually arises before the arteries branch, sometimes one or two small branches come from one or other of the umbilical arteries before they unite by the transverse branch (Fig.3.) while still in others, branches arise from the transverse branch (Fig.4).

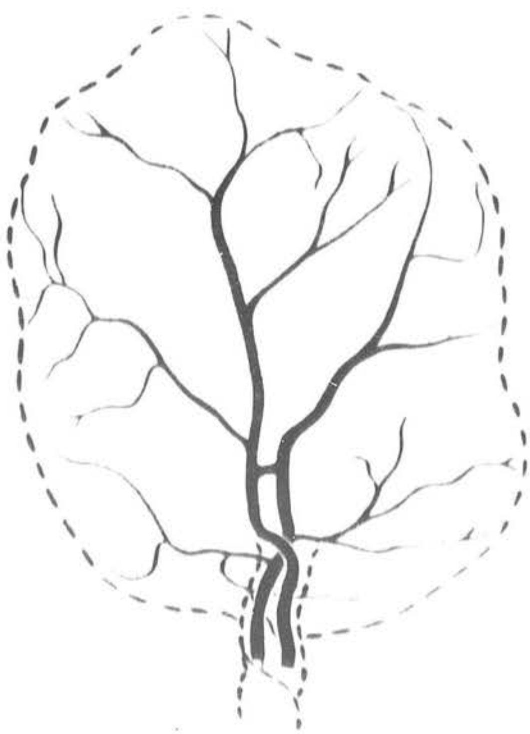

Fig 3. Diagram showing the two umbilical arteries each giving off a small branch then uniting through a transverse communication soon after their entry into the placenta.

Rarely the actual transverse branch is missing but in these cases the umbilical arteries are fused at the entrance of placenta and then separate later. (Fig. 5). Bacsich and Smout state that there are no

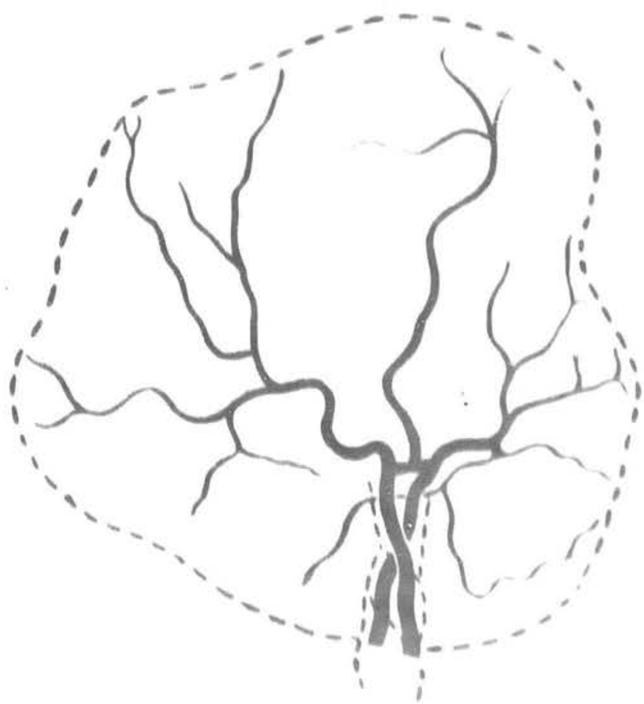

Fig.4 Placental diagram showing branches arising from the transverse communication between the two umbilical arteries.

signs of any peripheral anastomosis and they believe that the two arteries do not communicate except by the transverse branch. i.e. All the arterial branches distal to the transverse communicating branch are end/

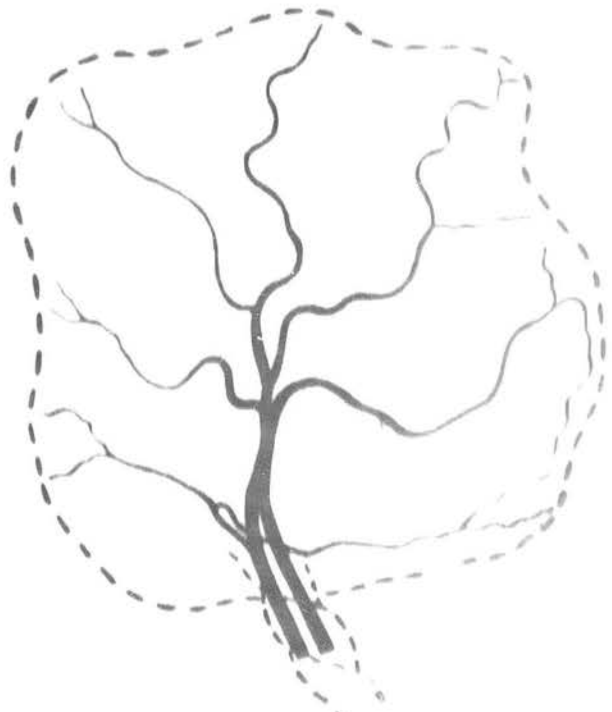

Fig.5. Diagram showing the two umbilical arteries fused together at the entrance of the placenta and the transverse communicating branch to be missing.

end arteries thus explaining the anatomical fact of the occurrence of white infarcts. Hyrtl (1870), Holl (1881), and Fraser (1923) on the other hand believe in the existence of numerous anastomoses in the peripheral branches of the umbilical arteries.

The umbilical vein accompanies the arteries, but it is wider in calibre and more deeply situated.

It will be noted that Burg (1929) was the first writer/

writer that has mentioned the presence of the transverse communicating branch. But he did not seem to attribute much importance to it. Bacsich and Smout, however, believe it plays a most important role in regulating the circulation of the placental organ. Its function, they believe, is comparable to the function of the circle of Willis which is usually considered to equalise the pressure and establish an equal blood supply to all parts of the brain as stated by Cunningham and Buchanan. They support their view by stating that the pregnant uterus carrying out rhythmic contractions would, during the contraction wave, cause a rising in blood pressure in the correponding part of the intervillous space and cotyledons of placenta, and that the presence of a pressure-equalising or "buffer" system is well justified. That arrangement may also be compared with the arterial arcades of the intestines, where the continuous peristaltic movement is comparable to the contractions of the uterus.

The presence of the transverse communication between the two umbilical arteries also explains why these vessels are always equal in calibre,irrespective of the size of territory supplied by them and therefore the transverse branch plays an active role in the distribution of the foetal blood in the placenta.

THE FOETAL VESSELS WITHIN THE PLACENTAL COTYLEDONS.

The chorio decidual space is divided by means of placental septa into smaller blood spaces - the intervillous spaces. Into these blood spaces are immersed the foetal villi containing the foetal vessels. The manner in which these villous stems divide was always held to be irregular and most complex like the branching of a tree. Studying corrosion preparations, however, Spanner (1935) maintains that the villous branches run from chorion to basalis perpendicularly to both surfaces, they then loop again chorialwards running parallel but in the opposite direction to the main vessels in the villous trunk. In other words the shape of the villous tree may be compared to the shape of an inverted drooping ash or like an old fashioned chandelier.

Spanner states that in these corrosion specimens he noticed that the small and middle sized veins were beaded along their course. Microscopic study of longitudinal sections of these veins and towards their endothelial lining, showed the existence of plain muscle fibres forming sphincters round the vessel lumen. These sphincters, Spanner believes, regulate the blood outlet from foetal villous capillaries. Namely when they contract they cause great/

great slowing reaching almost to stagnation of blood in the foetal capillaries; thereby favouring food and gaseous exchange between mother and child.

In the middle sized villous veins, besides those sphincters, Spanner holds that microscopic study showed the existence of muscle fibres running longitudinally along the long axis of the vein wall making them comparable therefore to veins in liver and intestine.

Since these villous capillaries containing the longitudinal muscle tissue in their walls run from chorion to decidua basalis, some actually dipping into decidua acting as anchoring villi, they will therefore cement the placenta, more or less, to uterine wall. This longitudinal muscular system in villous veins would in like manner serve also to keep open the intervillous spaces (i.v.s.).

Graaf Spee first demonstrated how these villi anchor in the basal decidua.

Ruge states that those portions of villous vessels dipping into the decidua basalis turn up again and flow into the intervillous space.

To differentiate between utero-placental arteries and those portions of villous vessels embedded in decidua it will be found that the villous vessels are very small having a trophoblastic layer outside their walls/

walls. Also the injection of villous vessels with a dye such as cinnabar would demonstrate the course of these vessels and how they run to be embedded in the decidua.

THE UTERO-PLACENTAL ARTERIES.

Bumm (1890) and Klein (1905) studied the number, position, and direction of the utero-placental arteries. Bumm maintains that the utero-placental arteries to reach the intervillous space run in the septa between the cotyledons and very seldom do they open on the basal plate of cotyledon beside the veins. This he explains by stating that at an early date the villi grow in the direction of the venous openings and away from the arteries, the blood stream naturally drifting them in this direction. They stretch the venous sinuses, and by pressure necrosis destroy the decidua compacta between the veins until only the septa carrying the arteries are left.

Bumm also states that the utero-placental arteries retain their same calibre width throughout their course. These arteries are more spiral and wider than the utero-placental veins. Spanner believes that Bumm studied only the decidual portions of these vessels and therefore his work is not complete in that respect. He points out the difficulty encountered in demonstrating the utero-placental arteries and veins by gelatin injections owing to the thickness of the uterine wall and owing to the fact that often the placenta is torn off the uterine wall with the result that these injected utero-placental/

utero-placental vessels will be torn off too. Corrosion preparations in his opinion, therefore, afford the best method of demonstrating the utero-placental vessels. Using this method Spanner was able to demonstrate the communication between the utero-placental arteries and the intervillous spaces, also the irregular distribution of these arteries throughout the basal surface of the placenta. The entrance of these arteries into the intervillous spaces was at right angles to the basal plate and their calibre was not evenly maintained throughout their course as held by Bumm; but was wider as they enter the intervillous spaces. Spanner was able to count 488 such coiled utero-placental arteries each having a diameter varying from 400 to 600 μ and he divides these vessels into two types:

Firstly, those supplying the uterine muscle wall; these divide and subdivide till they become capillaries.

Secondly, those destined to run into intervillous spaces and these are to be looked upon as end arteries.

THE PLACENTAL SEPTA.

According to Spanner (1935) the placental septa have not been credited with much study. They arise from the basal decidual plate, run perpendicularly or slightly obliquely across the chorio-decidual space stopping short one third of the distance away from the chorion. In other words, they are only found in the basal two thirds of the chorio-decidual space. This he was able to study by means of microscopic examination of the placenta in situ after fixation in formalin. Also by means of injecting a white gelatin mass into the foetal placental vessels when the masses of villi will take up a whitish appearance while the septa would stand out as dark sharp dividing lines between those whitish cotyledons.

Spanner maintains that no foetal vessels traverse the septa going through it from one cotyledon to another. If these foetal vessels ever dip into a septum then they always emerge again on same surface from which they entered.

Although Bumm (1890) holds the view that the utero-placental arteries run mainly in the septa to enter the intervillous spaces; Spanner believes that in the septa there are seldom any maternal vessels.

Since no foetal vessels pass through the septa from one intervillous space to the other; each cotyledon,/

cotyledon, therefore, is an independent unit in as far as its foetal vascular inlet and outlet is concerned.

Spanner was able to show by injecting the maternal vessels with carmine gelatine that in the basal two thirds of the placenta the injection mass was divided up by the presence of septa whereas in the subchorial third the injection mass ran confluently due to the absence of septa. Also by double injection of contrast mass, namely of Indian ink into the umbilical vessels and of white gelatine mass into the intervillous space through the basal membrane, the white mass could be seen filling up the intervillous space, its lateral flow being limited by the placental septa thus marking the boundary of the cotyledon. Now if injection of gelatin mass is continued, there comes a point where it overflows the tops of the septa to flow into neighbouring intervillous spaces, gradually making its way towards the placental margin.

This drove Spanner to conclude the following:

(i) There is no connection between neighbouring cotyledons in their basal two thirds owing to the presence of septa.

(ii) Above the septal level, cotyledons communicate with one another in the subchorial space.

(iii) From this subchorial space the stream of injected mass flows towards the placental margin in the direction of the blood stream.

(iv)/

(iv) Each cotyledon, besides forming an independent unit as regards its foetal vessels, also forms a maternal unit owing to the presence of septa preventing any communication between the basal portions of neighbouring cotyledons - placental cotyledons communicating with each other only subchorially.

(v) The maternal blood stream in one cotyledon has no influence on the blood stream of neighbouring cotyledons as such an influence would be prevented by the presence of placental septa.

THE VENOUS OUTLET OF THE PLACENTA.

(1) THE UTERO-PLACENTAL VEINS OF THE MARGINAL ZONE.

Waldeyer (1890) was the first to notice that in the uterine wall underlying the marginal zone - i.e. that area about 2-3 c.m. in width along the free margin of the placenta - there existed a venous network which he called "the border veins draining the marginal zone" of the placenta. This venous network was especially developed under the margin of the placenta but was poorly developed under its central zone.

Spanner (1935) amplified that by demonstrating fairly big oval openings or outlet communications between the floor of the marginal cotyledons and those utero-placental veins under the placental margin. These openings he counted to be one hundred in number and of varying diameters 1 - 9 m.m. with an average of 2.4 m.m. And, in his opinion, they constituted the blood outlet from the marginal zone to the uterine veins. He also states that there were never any communications between the intervillous spaces in the central placental zone and those veins underlying it. Outside the placental area the venous network was very poorly developed. The network of veins extends from the basalis through the whole thickness of the uterine wall till it almost reaches the peritoneal coat/

coat of the uterus where it merges into the uterine and ovarian veins.

It will be noted that the above view of Spanner thus differs from that of Stieve (1935) who believes that all intervillous spaces drain through their basal plate into the venous network in the uterine wall from an early date of placental development.

The oval openings between the marginal cotyledons and the venous network are crenated and irregular owing to the attachment of villous stems and the latter serve to keep those communications open. Differentiation between the utero-placental arteries and the utero-placental venous network under the basalis, according to Spanner, can be made because although the structure of vessel walls in either is more or less the same yet the differentiating point is that the utero-placental arteries run perpendicularly to the basal plate to flow into the intervillous space; whereas the veins run longitudinally parallel to the basal membrane and are never perpendicular. These points were pointed out by Spanner by means of double injection and corrosion technique. A point to be kept in mind is that the endothelial lining of the utero-placental arteries runs all the way up to the termination of the artery, i.e. to the point where it opens into an intervillous/

intervillous space, when the endothelial lining ends and trophoblast lines the space. Some trophoblast may, however, line the mouths of these arteries so that Nietabuch described these arterial mouths as having a fibrous endothelium inside which there is a thin trophoblastic lining. At the point where the utero-placental arteries pass into the intervillous space they suddenly lose their muscular coat.

(2) THE MARGINAL SINUS.

Also called the circular sinus. This is a venous channel that runs along the margin of the placenta in the chorio-decidual angle and contains no villi. Budde (1907) was first to notice the presence of the marginal sinus in the placenta but he thought that its presence was not a constant feature.

Spanner (1935) on the other hand admits the difficulty of finding the marginal sinus in fresh placentae after they are born because the bleeding that takes place from the placenta at its delivery causes the inevitable collapse of that sinus. He, however, maintains that the marginal sinus is of constant occurrence and is of fundamental importance in the placental circulation. By removing the upper chorial wall the communications of the marginal sinus with the intervillous spaces at the periphery or margin of the placenta could be seen. These take different forms, either port-hole-like openings or the medial partition of the sinus may be lacking so that it merges into the intervillous space without a border. Spanner interprets this medial wall as the altered septa of the marginal cotyledons which unlike the other septa, reach as far as the chorionic membrane.

The/

The marginal sinus is divided by a partition wall but this division wall is incomplete in the sense that it does not extend throughout the whole circumference of the sinus.

The port-hole-like communications between the marginal cotyledons and the marginal sinus are 70 in number and have a diameter varying from 1.5 - 5 c.m. while the trophoblastic element in their walls keeps them open. The utero-placental venous network is well developed under the periphery of the placenta to drain the marginal sinus and the marginal zone, whereas under the central placental zone they are not so well developed and have no communication with the overlying central placental cotyledons. The calibre of the marginal sinus varies along its course, thus measuring 2 c.m. by 3 c.m. in places, but may be smaller, with a diameter of as little as 0.5 c.m. in places. According to Spanner, the marginal sinus is found from the third month of pregnancy onwards i.e. at the time when the placenta is formed. By celloidin injection into the uterine veins and subsequent corrosion, he was able to see firstly the filling of the utero-placental venous network, then the filling of the marginal sinus and zone, and lastly the subchorial space in a retrograde manner. The basal portions of the intervillous space, however, showed no/

no such filling, thus making Spanner come to the conclusion that there were no direct communications between the intervillous space and the uterine veins through the basal plate; a view contrary to that held by Stieve (1935).

Double celloidin injection technique, injecting white celloidin into the uterine veins and red celloidin into the uterine arteries and injecting both systems at the same time and under the same pressure, shows the filling of the basal portions of the intervillous space with red celloidin whereas the white celloidin fills the marginal sinus and zone and then later flows to fill the subchorial space. If the injection of white mass was delayed, then there occurs a flow of red celloidin into the subchorial space and thence to the marginal zone and sinus. Becher's experiments on the haemochorial placenta of tamandua showed similar results.

THE SHAPE OF THE INTERVILLOUS SPACE.

The shape and form of the intervillous space depends on the structure of the foetal villi and their density, as some parts of the intervillous space are more dense in villi than others. However, the intervillous space could be divided generally into three parts:-

Firstly, the basal two thirds; this is characterised by its great density in villi.

Secondly, the subchorial third of the intervillous space; this is situated immediately subjacent to the chorionic membrane and is traversed by villous trunks, but is itself free of villi. It measures 0.5 c.m. or more in depth, but it is not shown in sections of separated placentae owing to the blood draining out immediately the placenta is born through the large openings in the marginal sinus. Its existence was shown by injection experiments and its connections with the marginal sinus were demonstrated by Spanner (1935).

Thirdly, the placental caverns, these exist in the basal two thirds of the intervillous space scattered here and there among the villi and are due to the relative scantiness of villi in those areas. Carmine gelatine injection into the utero-placental arteries would demonstrate the presence/

presence of these caverns.

Stieve (1935) demonstrated the presence of vasavasorum that serve to nourish the villous stems and demonstrated their anastomosis with the villous vessels. Although Stieve (1935), Fraser (1923), Hyrtl (1870), and Holl (1881) believe in the existence of anastomoses between the terminal villous branches, yet Spanner (1935), Bacsich, and Smout (1937) believe that the terminal villous vessels are end arteries. By studying micro-corrosion specimens of the villous tree, mounted in canada balsam and examined under the binocular microscope, Spanner claims to have been able to see the terminal villous vessels ending in the form of closed loops i.e. arteries coming back as veins.

By injecting the foetal vessels with indian ink and the intervillous space with white gelatin, and cutting sections transversely at various levels parallel to the basal plate, with a half millimeter divided square cover-slip put on for microscopic examination; Spanner saw that those sections cut through the basal part but near the chorial portion of the placenta exhibited more or less uniformity in villous distribution throughout the field with an average of thirty villi per $\frac{1}{2}$ m.m. square. On the other hand those sections cut through the deep basal part/

part of the cotyledon showed big wide spaces or caverns between the villi with channels leading from them to the subchorial space. Why does not the villous tree divide in the intervillous space in such a manner as to fill up these caverns? Could it be that the villi first filled the whole intervillous space and then atrophied in some parts with subsequent cavernous formation? The answer to these questions is not yet known. Fritscheck (1927) believes that as the placenta is born, its basal membrane gets damaged with subsequent air entry into the intervillous space and formation of these caverns. Spanner does not believe in that hypothesis and argues that if air was to enter through the basal plate then it would diffuse regularly throughout the intervillous space and would not confine itself to any areas in particular. He also states that these caverns, in a large number of cases, are not in direct contact with the basal membrane but high up in the intervillous space and therefore cannot be explainable by this theory.

After the birth of the placenta the blood drains out of the subchorial blood-space and therefore the chorial placental surface sinks down with subsequent depressions on that surface which could be easily seen on naked eye examination of the placenta.
To demonstrate this subchorial blood space, Spanner injected/

injected a mixture of beef fat and lard through the chorial plate of the placenta close to the insertion of the umbilical cord, the placenta having been previously washed with Ringer's solution. It was then fixed in 20% formalin, passed through ascending alcohols, then chloroform to dissolve the fat, then finally kept in formalin again or alcohol - sections of such placentae show a compact basal portion and a relatively empty subchorial chamber where all cotyledons intercommunicate, opening into the common chamber. Carmine - gelatine - injected uteri would also demonstrate this subchorial blood lake.

REVIEW OF LITERATURE.

PART II

THEORIES OF THE CIRCULATION OF THE MATERNAL BLOOD THROUGH THE PLACENTA.

I. THE MATERNAL BLOOD FLOW THROUGH THE INTERVILLOUS SPACE IS DEPENDENT ON THE FORCE OF THE MOTHER'S HEART BEAT:

It will at once be noted that the circulation through the placenta is more difficult to follow than in organs elsewhere in the body owing to the complexity of its structure. Spanner points out, that in the case of the placenta, the blood passes through its intervillous spaces meeting the resistance of those villi growing in these spaces while having on the one side the utero-placental arteries as afferent and on the other side the subchorial blood lake as efferent channels. This essentially differs from organs in the rest of the body where blood flows through capillaries.

Shröder maintains that the circulation through the placenta is dependent on two factors.

Firstly, the uterine tonicity. This serves to exert intermittent contractions on the placenta which lies between the uterine muscular wall and the incompressible mass of the amniotic sac with its contained liquor and foetus.

Secondly, pulsations of the villous vessels which, as they contract and relax, create a stream of flow in the intervillous space. Spanner, however, does not think that the intermittent uterine contractions or villous pulsations could possibly play/

play the prime role in driving the maternal blood through the intervillous space. He points out that the uterine arteries through their origin from the hypogastrics are near the aorta; also the ovarian arteries arise directly from the abdominal aorta; and since both these sets of arteries develop enormously during pregnancy assuming large sizes; they therefore make the uterus during pregnancy nearer than ever to the abdominal aorta for its blood supply.

These main arterial trunks divide in the uterus into two main types. Those supplying the uterine muscle wall; which divide and subdivide till they become capillaries. Secondly those destined to run into the intervillous space which do not divide but run directly into the intervillous space as the coiled utero-placental arteries. The blood stream through the utero-placental arteries is directly derived from the great maternal arteries. In other words the utero-placental arteries serve as intermediaries between the big arteries and the intervillous space. According to Spanner they number some 488 with a diameter of 152μ but just before they get into the intervillous space they come to measure 400 - 600μ . That change in calibre, according to Spanner, serves to damp down and regulate the blood pressure/

pressure from the aorta to the intervillous space as for example in hypertensive mothers or in cases of emotional excitement. Also the intermittent contractions of the uterine muscle may affect the circulation in one of the following ways.

One, by altering the inflow of blood;

Two, by pressure on the subchorial blood lake in the placenta itself and;

Three, by altering the pressure in the venous outflow.

The venous return flow through the placenta, according to Spanner is via;-

Firstly, the marginal sinus with a diameter of 2 c.m. or more, comparable to the marginal villous space but without villi and having about 70 venous outlets with a diameter varying from 2-8 m.m. communicating with the utero-placental venous network.

Secondly, the marginal zone of the placenta; occupying an area of 2.5 c.m. along the placental free margin. There are about 100 venous communications between the floor of the intervillous spaces of the marginal zone and the utero-placental venous network; they are smaller than those of the marginal sinus; with a diameter of 2 - 4 m.m.

Following the haemodynamic law Spanner believes that the blood flow would take the shortest route through the placenta from the afferent utero-placental arteries through the basal plate, to the efferent utero-placental/

utero-placental venous network at the placental margin. At the same time the placental septa regulating the blood flow through the chorio-decidual space not allowing the quick passage through of blood from the arterial to the venous side. These septa extend two thirds of the way up from the basal plate to the chorionic membrane, i.e. stopping a little distance short of the chorion. Thus the maternal blood after flowing up through the adjoining cotyledons in distinct streams merges in the subchorial space to form the lake of blood which drains to the margins of the placenta into the circular sinus and thence to the uterine veins. The number of the septa corresponds to the number of cotyledons between which they lie. The number of cotyledons varies from 14 - 30 in individual placentae. Each cotyledon, therefore, is a unit which may be likened to a box (Fig. 6) with holes in its bottom representing the utero-placental arteries; and the boundaries of which do not reach the chorion so that the maternal blood from all cotyledons mingles in the subchorial blood lake.

The height of the intervillous blood spaces diminishes as they get towards the placental margin. Therefore, septa between marginal cotyledons are not as high as those in the central placental zone. The septa which form the very lateral boundary to the marginal/

marginal cotyledons and separate them from the marginal sinus - also looked upon as the medial wall of the marginal sinus - reach as far as the chorionic membrane but exhibit the port-hole-like openings or may be lacking in places to allow of drainage into the sinus.

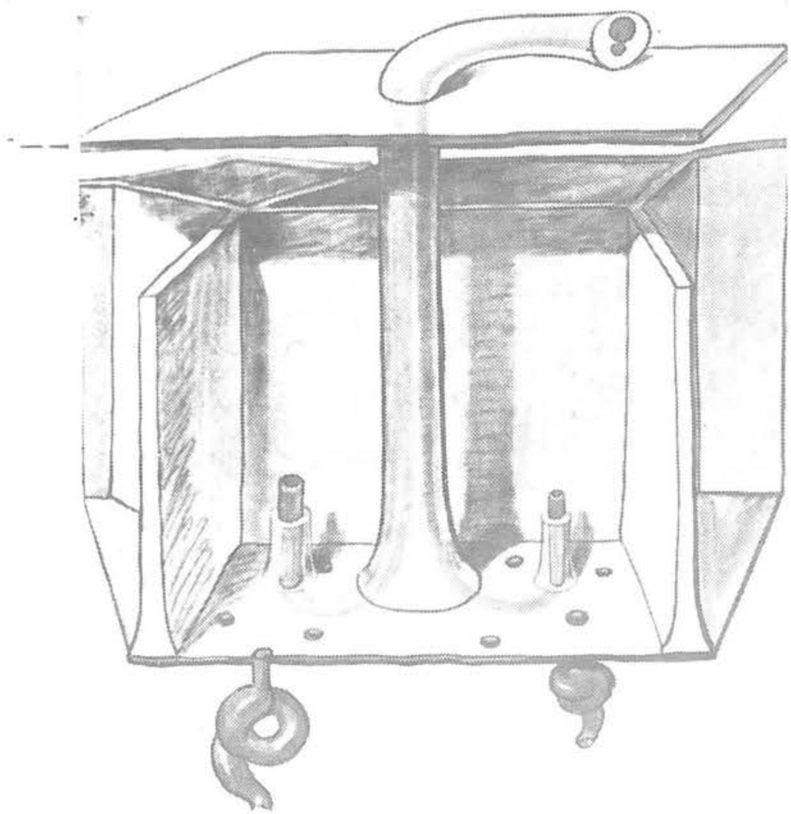

Fig. 6. A box representing an I.V.S. the boundaries of which do not reach the top and the floor exhibits holes representing the openings of the coiled utero-placental arteries.

It will be noted that Stieve (1935) holds the view that the central septa reach to the chorion but exhibit holes in them to allow of an intercotyledon blood flow.

Bumm/

Bumm (1890) believes that each cotyledon acts as an independent unit having its own maternal blood inflow and outflow through arteries in the septa and veins on the basal plate.

Hinselmann accepts Bumm's teaching of two streams, an inflow and an outflow in each intervillous space, but he also states that normally stagnation almost reaching coagulation of blood may take place in the intervillous space. Spanner, however, states that stagnation cannot occur because the experimental injection of carmine gelatine shows that it flows easily into the intervillous space gradually filling it till it reaches the subchorial space and thence flowing smoothly in an outward direction towards the placental margin. Therefore, should there be any resistance to the flow of the blood stream through the placenta it is to be sought for in the basal two thirds of the intervillous space where septa exist and where villi are relatively dense. Spanner estimated the distance between the villi in the basal part of the intervillous space as about 40μ and with an average of 115 villi per square millimeter. Hartmann states that the minimal distance between villi is 50μ . This distance between villi is comparatively large and therefore there could not be much resistance offered to the flow of blood as held by Hinselmann.

The following table represents Spanner's conception/

conception of the big venous drainage of the placenta with relatively very low resistance to the blood flow in the intervillous space:-

Efferent venous openings 175 with 3.4 m.m. calibre.

Measurements in I.V.S.

Macroscopic	Few villi	Subchorial 1/3
40μ between villi	115 vill/1 sq.m.m.	Basal 2/3

Afferent 500 utero placental arteries 152μ diameter

Stieve thinks it unlikely that the force of the maternal blood pressure would drive the maternal blood through the placenta because he believes that firstly there is a great slowing in the rate of the maternal blood flow before it gets to the intervillous space. Secondly, the complexity of the villous structure would form a mechanical obstacle to the maternal flow. Spanner does not agree with Stieve's point of view because he thinks that although the basal third of the intervillous space offers some resistance to the maternal blood flow, yet that resistance is too small as evidenced by the taking of blood pressure in the marginal sinus which was found to be only a little less than that in the uterine or ovarian arteries. Spanner emphasises the observation/

observation that there was a definite but slight difference in the two blood pressures thus taken. This was enough to allow the direction of the blood flow to take place from the basalis towards the chorion. He believes therefore that since the blood flows from basal to chorial portions of the intervillous space due to relatively low pressure in the latter, it obviously cannot flow back against pressure from chorion to basalis. This observation leads Spanner to the belief that there is one stream of maternal blood flow through the placenta and one stream only and that is from the basalis towards the chorion thus contradicting Bumm's contention of an inflow and an outflow of maternal blood through the basal plate of each cotyledon. Bumm believed that the utero-placental arteries run up in the septa thus attaining various heights within the placenta, then they terminate by opening into the intervillous space. Blood thence circulates amongst the villi and drains out through the basalis via the utero-placental veins. Bumm, therefore, believed that each cotyledon forms an independent unit with regard to its blood circulation and that the direction of maternal blood flow follows the simple physical laws of difference in height level - namely inflow through the relatively high utero-placental arteries and outflow through the veins in the floor of the intervillous space. (Fig. 7).

Besides/

Besides this Bumm recognised also another stream of maternal blood flow in the intervillous space, namely, from the utero-placental arteries in the septa directed chorialwards and hence connected with other cotyledons and flowing out towards the margin of the

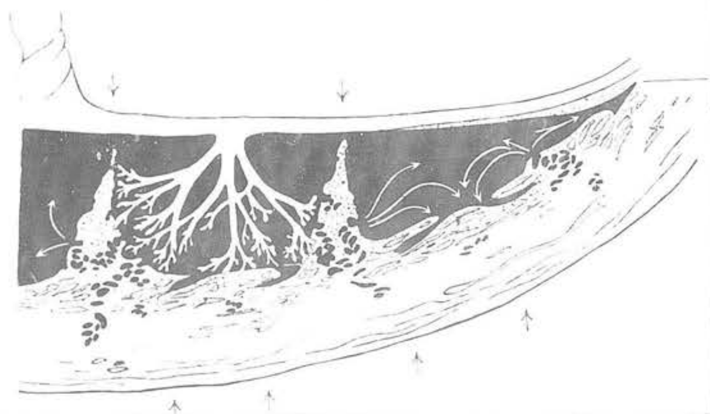

Fig. 7. Diagramatic representation of Bumm's contention of the existence of two streams of maternal blood flow (arrows). The intermittent uterine contractions acting on both the chorial and the basal placental surfaces are also represented by arrows.

placenta. Bumm thought that the first stream of maternal flow depends on the maternal blood pressure, the pulsations in the villi, and also on the physical laws of difference in height level, whereas the second stream of flow chorialwards is maintained by intermittent uterine contractions.

Kermauner (1924) does not recognise the presence of septa in the placenta and believes that what are taken/

taken to be cotyledons do not exist in intra uterine life. His theory of the maternal blood flow through the placenta is dependent on the force of the intermittent uterine contractions. When these contractions occur, according to him, they drive the maternal blood out of the placenta in a manner similar to squeezing water out of a sponge.

Wieloch (1923) believes that the utero-placental arteries in the placental septa are not so high relatively to the veins in the floor of the intervillous space as to play the main role in the stream of maternal blood flow through the intervillous space. Thus he does not accept Bumm's (1890) theory but believes that the maternal blood pressure is the driving force of maternal blood through the placental cotyledons.

Budde (1907) was the first to describe the marginal or circular sinus but he does not believe in its constant existence.

Winkler describes his belief about the maternal circulation through the placenta as the latter being highly irregular and varying in different parts of the placenta - namely, maternal blood flows in in certain parts of the placenta, and flows out in other parts, while it stagnates in certain other areas of the placenta.

Mikulicz-Radecki (1929) using the injection technique/

technique found that the injection mass accumulated in the basal part of the placenta round the mouths of the utero-placental arteries. He interpreted this accumulation as being due to a great resistance offered by the presence of villi round the mouths of these arteries thus hindering any maternal blood flow from basalis chorialwards. He, therefore, does not accept the theory of the existence of this latter stream. Spanner attributes this accumulation of injection mass in Mikulicz-Radecki's experiment to faulty injection technique. This is because with a uterus that has been kept in warm saline immediately on removal at operation and injection carried out properly, Spanner never saw such an accumulation of injection mass round those sites.

Dyroff (1929) recommends the keeping of the uterus in warm saline immediately on removal at operation and the carrying out of injection while it is in warm saline as otherwise if left dry and cold then unnatural conditions will be simulated when injection is being carried out.

Franken (1929) recognises the division of the placenta into two parts, namely a basal portion rich in villi and a chorial portion which is relatively poor in villi. He also believes in the presence of two maternal blood streams, namely, one from the basal plate chorialwards and another from the chorial membrane/

membrane basalwards. Spanner argues against the theory of two streams by asking how we could have two such streams when we have a more or less equal distribution of utero-placental arteries throughout the floor of each cotyledon - namely 20 such arteries per cotyledon? He also states that if we were to accept Franken's double stream theory - each being in an opposite direction to the other - then there should occur stagnation subchorially to allow of the other stream to form. But there is no proof of such stagnation taking place; on the contrary, should there be any stagnation, then the contractions of the uterus would empty the so called stagnating blood from these spaces and would not permit of such an occurrence; there could be therefore only one direction of flow of maternal blood stream through the placenta.

II. THE INFLUENCE OF INTERMITTENT UTERINE CONTRACTIONS ON THE CIRCULATION IN THE INTERVILLOUS SPACE.

Grosser (1929) believes that the slow blood flow in the human placenta through the intervillous space is a phylogenetic progression phenomenon in contradistinction to what pertains in lower animals. This slow flow reaching almost to stagnation allows of better exchange of gaseous and nutrient substances between the maternal and foetal blood. This slowing down of blood flow is particularly noticeable in between the intermittent uterine contractions when the blood nearly reaches a state of stagnation thus allowing easier and better exchange to take place. Grosser believes that in the basal portion of the intervillous space gaseous exchange takes place whereas in the chorial portion protein exchange takes place. Spanner dismisses this phylogenetic theory of blood stagnation because the placenta, as organs elsewhere in the body, have afferent and efferent blood vessels to allow of the oxygen intake and the carrying away of waste products. Stagnation therefore would hamper this normal physiological process; and would therefore be detrimental to the foetus.

Kermauner (1924), Grosser (1929), and Franken (1929) believe that the maternal circulation through the/

the placenta is dependent on the intermittent uterine contractions and not on the driving force of the maternal blood pressure. Spanner, however, holds that the uterine contractions only play a secondary role in promoting the maternal circulation through the placenta for the following reasons:

Firstly, these uterine contractions never reach such an extent as to empty the placenta of the blood it contains - admittedly however, they occur and they may render the placenta partially ischaemic.

Secondly, Mikulicz-Radecki (1929) by means of posterior pituitary injections was able to produce long lasting uterine contractions with resultant stagnation of blood in the placenta and slowing of the foetal heart rate due to lack of oxygen, therefore, Spanner believes that uterine contractions, if marked, would hinder rather than help to promote the circulation with deleterious effects on the foetus.

Thirdly, if these uterine contractions were to be the factor in promoting the maternal circulation through the placenta then they would have to be frequent and rhythmical from the start of pregnancy. Wagner (1929) has shown, however, that the earlier the pregnancy the less frequent are these uterine contractions which may even be separated by as long an interval as two hours between two successive contractions.

Pankow/

Pankow (1929) and Halban (1929) state that during pregnancy especially in the first month the uterine contractions may not exist at all and it would be hard to believe that such an important function of oxygen supply to the foetus would be dependent on such an unsure mechanism. They, therefore, do not accept this stagnation theory of uterine contractions and although Pankow believes in the existence of uterine contractions yet, according to him, these are so small that they cannot be recorded by means of Crodel's Hystergometer. They come to the conclusion, therefore, that uterine contractions may only help in the emptying of the intervillous space.

Dyroff (1929) is of the same opinion as Pankow because he was able to observe only partial contractions of the uterus after it has been removed and kept in physiological solution at body temperature.

Spanner thinks it possible that uterine contractions may influence the emptying of the intervillous space only if the amplitude of contraction is not too great and not too long sustained as otherwise blood stagnation would occur.

Dubireuil and Rivière believe in the intermittent uterine contractions as a factor in maintaining maternal circulation through the placenta; but they also/

also believe in the possible existence of placental contractions which would be an equally important factor. The possibility of placental contractions occurred to them because they were able to demonstrate isolated plain muscle fibres in the subchorionic connective tissue and in the larger villous stems. These muscle fibres had the same characteristics as plain muscle tissue found anywhere else in the body as for instance in the blood-vessel walls. Small villous stems, however, did not possess any such muscle tissue. They believe, therefore, that this fibro-muscular system plays the main role in emptying the intervillous spaces into the uterine veins; and that the uterine contractions only play a secondary role.

Dubireuil believes that this muscular plexus may also play a part in promoting the flow of the foetal blood stream through the placental villi.

It will be noted that if Dubireuil's and Rivière's conception is true then they have confirmed Jizuka's work (1915) in having first demonstrated spindle plain muscle fibres arranged in stripes between the amniotic and chorionic layers in the central placental zone.

Happe (1907) was unable to demonstrate the presence of this layer of plain muscle tissue at all.

Spanner/

Spanner tried to demonstrate the presence of these plain muscle fibres in the subchorionic connective tissue as follows:-

The Chorionic surface of the placenta is put in 33% alcohol for 24 hours. This allows the easy stripping off of the amnion from the chorionic membrane. If this chorionic membrane is now stained with iron and haematoxylin or eosin and methylene blue with the Mölendorf technique the muscular layer could easily be demonstrated microscopically, as consisting of two main types of fibres:-

(i) Short muscle cells with multiple filaments like a brush at either pole of the cell. These filaments may remain free or interlace with those of neighbouring cells. The nucleus carries a constriction belt near its centre and with a vacuole surrounding it. The body of the muscle cell contains the sarkosome. With iron and haematoxylin stain these muscle fibres show as thick dark threads whereas with the eosin and methylene blue they take up an intense red colour quite distinct from the blue mesenchymal collagen tissue that lies between these muscle fibres. It is these muscle fibres with their multiple filaments extending over a wide area in the subchorionic connective tissue layer that cause the chorion to exhibit the power of contractility.

(ii) Spindle cells lying singly or in bundles. When they/

they are single neighbouring cell poles may touch each other or may actually unite end to end. The nucleus is longish, indented on one side and having a vacuole surrounding it. Where cells unite end to end, then microscopically they would appear as a big long cytoplasmic mass with two or more nuclei according to the number of cells thus united.

Those muscle fibres in large villous stems run obliquely or transversely to the long axis of the villus.

Having been able to demonstrate the fibro-muscular network in the placenta Spanner accepts Dubireuil's and Rivière's contention that it plays a part in emptying the intervillous space and he states that one therefore would be dealing with two factors in the intervillous space, namely:-

Firstly, a passive tension; evidenced by lower blood pressure subchorially than at the basal portion of the cotyledon, thus allowing stream of blood flow chorialwards.

Secondly, an active tension; acting through the muscular network in the subamniotic connective tissue and large villous stems.

Both these factors act together thus allowing the filling of the subchorial blood space and regulating the blood flow in the same manner as do blood vessels elsewhere in the body.

Spanner/

Spanner claims to have been able to see a wave of contraction over the chorionic surface of the placenta at times, thus exhibiting the active power of contractility in the placenta. Spanner, therefore, draws the conclusion that:-

(i) It is only the force of the maternal blood stream which is responsible for the maternal blood inflow and outflow through the placenta.

(ii) The intermittent uterine contractions and placental contractions may, however, play an accessory part in regulating the maternal blood circulation through the placenta. These accessory factors only come into play when there is lowered pressure in the placenta; because as they come into play they cause a holding up of the circulation in the intervillous spaces.

III THE INFLUENCE OF VILLOUS PULSATIONS ON THE MATERNAL BLOOD CIRCULATION IN THE INTERVILLOUS SPACE.

Hofbauer (1905), Mikulicz (1929) and Wagner(1929) believe that the pulsations of the chorionic villi which are at the rate of 140 per minute create volume changes in the intervillous spaces and thereby allow the mixing of the maternal blood and the maintenance of its circulation in these spaces.

Hofbauer was the first to demonstrate villous pulsation movement in 1905 using a hand syringe and injecting fluid through the umbilical vessels of the placenta and he described it as a swaying movement of the villi.

Mikulicz-Radecki tried to demonstrate this movement of villi under as near physiological conditions as occur in vivo. Thus he experimentally imitated the foetal circulation, which normally has a blood pressure of between 60 - 70 m.m. Hg, by connecting both the femoral artery and the jugular vein of a dog to the umbilical vessels of the placenta, and thereby maintaining the circulation through the placenta. On cutting a window through the placental surface he observed villous movement with each pulse wave and he described two types of movements. Namely, the single villi exhibited a swaying movement; while groups of villi exhibited a pushing movement - pushing each other with each pulsation.

Spanner/

Spanner carried out similar experiments injecting placentae under physiological conditions and under normal pressure. He was able to observe the filling up of the villi with every quantity of fluid that was injected, also the rising up and sinking down of the placental organ with each injection pressure. On cutting a window either through the basal or chorionic surface of the placenta, the villi could then be seen moving. Spanner, therefore, believes that this villous movement only begins to occur after the window is cut through the placental surface and that in vivo these villi do not move or sway. According to him these villi only stretch and fill up under the pressure of injected fluid and what has been described as an active swaying villous movement is in reality only a passive movement of distention under pressure. Spanner thinks that what Wagner and Mikulicz described as swaying villous movement in their experiments may be attributable to the cutting of a window on the placental surface which would make the villi come out through that window and swim in the saline current in which the placental organ is immersed.

Spanner's experiment was carried out as follows: the placenta was kept in Ringer's solution at body temperature. The umbilical vessels were dissected out in the cord; then a canula was inserted into each vessel. The vein canula carried a forceps to regulate outflow/

outflow. The arterial canulae were attached to a rubber tube at the other end of which there was a Y-shaped junction piece joining it at the one limb to a water manometer to record the pressure used for injection. This should vary between 60 - 80 c.m. of water. The other limb of the junction tube is attached to a burette containing the fluid to be injected into the placenta. This consisted of Ringer's solution containing 7% gum arabic and coloured with cyanol blue. Intermittent pressures were exerted manually on the rubber tubing connected to the arterial canulae and at the rate of 140 per minute thus imitating normal pulsations in the cord.

The intermittent pressure waves exerted manually on the rubber tubing while injection is taking place causes the filling and distention of all cotyledons under the fluid surface in which the placenta is immersed. On looking through the basal plate of the placenta Spanner was unable to see any villous movement within the cotyledons. Even by shutting off the fluid outflow through the umbilical vein by forceps, and the raising up of the burette to increase the pressure of the fluid injected, no villous movement could be seen.

If a window was now cut through the basal placental plate, villi would be seen to come out through that window and float in the Ringer's solution in/

in which the placenta was immersed, swimming in the current created by the fluid injected. That is, the villous movement in Ringer is a passive movement and not an active one. The rhythmic intermittent pressure exerted manually on the tube while injection is taking place causes the villi to distend without exhibiting any movement.

The above experiment drove Spanner to the belief that in vivo villi do not exhibit any form of active movement, and therefore in his opinion no villous movement as claimed by Mikulicz, Wagner, and Hofbauer, could play a part in driving the maternal blood through the intervillous space. On the other hand, Spanner, who believes in the existence of muscular sphincters round the lumina of small and medium villous veins, states that this sphincteric mechanism when it contracts causes a holding up of the foetal circulation within the villi thus allowing of better gaseous and nutrient exchange between mother and child. This holding up of the foetal circulation would cause turgescence and distension of the villi and this is not to be mistaken for any active villous movement. Spanner admits that the increase in volume of the villi subsequent to sphincteric action is very hard to notice with the naked eye.

MATERIAL.

The material at my disposal consists of the following:-

(i) Full term placenta injected with celloidin and then macerated to obtain a cast of the foetal villous vessels within the placental cotyledons for the purpose of studying their course and general architecture.

(ii) A uterus removed at caesarean hysterectomy with the placenta in situ and injected with carmine-gelatine through both uterine arteries in an endeavour to follow the course of the afferent blood flow into the placenta, its circulation within the intervillous space, and its efferent flow out of the placental organ.

Such points as the placental septa and marginal sinus were also kept in mind.

(iii) A uterus removed by caesarean hysterectomy in a case of chronic nephritis and injected with carmine-gelatine through the ovarian arteries to compare and contrast results with the previous specimen.

(iv) Full term placenta injected with carmine-gelatine through both umbilical arteries. Sections cut for microscopic study of the course and mode of termination of the villous vessels, also anastomoses, if/

if any; and general arrangement of these villous vessels were looked for.

(v) Full term non-injected placenta, the umbilical cord having been tied soon after delivery so as to keep the foetal blood within the placental organ. Sections were then cut and stained by methods used for the study of brain capillaries in an attempt to approach the villous vessels and their architecture from another angle.

(vi) Placental upper chorial wall removed and fixed in formalin and ordinary paraffin sections cut and stained by Weigert's iron haematoxylin and Van Gieson; to demonstrate the existence, if any, of plain muscle elements in the subchorial connective tissue layer.

TECHNIQUE OF EXPERIMENTS.

(1) CELLOIDIN CORROSION INJECTION METHOD.

This has been done along the same lines as recommended by Mr F.W. Pettigrew in this University.

Celloidin in acetone was used as injection mass made up in solutions of two varying strengths as follows:-

(A)	Acetone	100 c.c.
	Celloidin	4 g.m.
	Camphor	2 g.m.
(B)	Acetone	100 c.c.
	Celloidin	8 g.m.
	Camphor	2 g.m.

The acetone used was chemically pure and the camphor was added to make the celloidin less brittle. Alkanin which is a resin dye soluble in acetone and unaffected by strong hydrochloric acid was used as a colouring agent. The amount of dye added depending on the depth of colour required.

Bacsich, Smout, and others recommend the use of old X-ray films, that have been washed thoroughly to remove emulsion and then dried, in place of pure celloidin for injection purposes; but in our experiments only pure celloidin was used as it proved far superior.

The pressure used for injection comes from a compressed/

compressed air tap, is indicated by a mercury manometer and is regulated by means of a Woulfe bottle carrying a safety screw clamp (Fig 8).

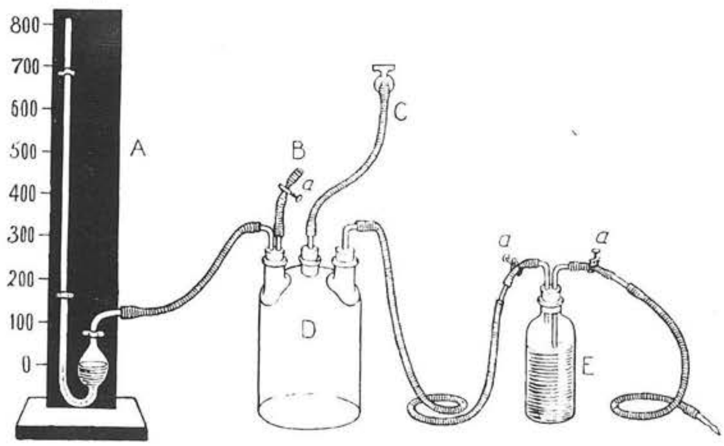

FIG. II.
A. Manometer. B. Safety Valve. C. Source of Pressure. D. Woulfe Bottle. E. Pressure Bottle. a. Screw Clamps.

Fig 8. Illustration representing the apparatus used for the celloidin corrosion injection method.

Soon after the placenta is delivered, it is immersed in a warm citrate bath and the blood is milked out of the umbilical cord vessels.

A 3.8% sodium citrate solution is now run into the placenta through a glass canula inserted into the umbilical vein and at a pressure of 300 m.m. Hg. This serves to wash away the blood from the vessels within the placenta. A big brass hand syringe would serve/

serve the purpose if only gentle pressure is put on its piston.

The placenta is now removed from the warm citrate bath and after washing is put into a big rounded glass dish containing 13% gelatine warmed to body temperature. It is put into the dish with its basal surface downwards so that gelatin when set would seal off any little tears that there may be between the cotyledons and thus prevent leakage when injection under pressure took place. The placental chorionic surface with its emerging umbilical cord is uppermost.

The vessels within the umbilical cord are dissected out. A few c.c. of pure acetone are run into the placenta through the glass canula inserted into the umbilical vein. This is followed by about 150 c.c. of the weak 4% celloidin injection mass because the thin celloidin could penetrate into the fine terminal villous capillaries. Lastly the thick 8% celloidin is allowed to run in so as to fill the big villous trunks and main divisions of the umbilical vessels. A pressure of about 500-600 m.m. Hg. is maintained throughout the injection. We have had a return flow of celloidin via the umbilical arteries on a few occasions.

All vessels in the cord below the level of the canula are now tied to keep the injection mass retained/

retained within the placenta. An hour or two after the injection, the placenta is removed from the gelatin dish and is immersed in a bath of cold running tap water for 12 - 24 hours. The submergence of the injected specimen in cold water serves to solidify the celloidin within the vessels. Thereafter the specimen is placed in pure hydrochloric acid and left for 12 hours during which the acid is changed once. This digests all the soft tissues and thus macerated tissue is now removed from the celloidin cast by washing the specimen under a gentle spray or a fine stream of water till all the debris is cleared thoroughly.

(II) CARMINE GELATINE INJECTION OF UTERUS REMOVED AT CAESAREAN HYSTERECTOMY.

This has been done in the Rotunda Hospital under Dr Falkiner. The placenta was left in situ and the uterine wound was stitched. The uterus after removal was then placed in warm citrate solution. Immediately the process of dissecting out the uterine arteries and introducing a fine metal canula into each artery was undertaken. A 3.8% sodium citrate solution was run in through the canulae at a pressure of 200 - 300 m.m. Hg. so as to wash away all blood from the vessels in the specimen. Should the citrate solution come out through the cut vessels in the incision in the uterine wall then the incision should be carefully and rapidly stitched up in such a way as to occlude these vessels.

The citrate was kept running in through the uterine arteries until the return flow through the ovarian and uterine veins was clear. The citrate solution surrounding the uterus needs to be constantly changed not only to maintain the temperature but to keep it comparatively clear.

The carmine gelatine solution was then run in at a pressure of 300 - 400 m.m.Hg. until it could be seen clearly coming back through the veins. Pressure was cut off while the veins were tied, care being taken/

taken that the carmine mass was not lowered so as to prevent solidification. Pressure was resumed again so as to allow the specimen to be filled with the injection mass. The injection should be carried out with the specimen and the carmine bottle immersed in the warm citrate bath. It is to be noted that no air bubble should be in the circuit while the injection is being carried out.

After the specimen is well injected as indicated by its filling up and by the change in the colour of its peritoneal coat, the injection is stopped, the canulae removed and the arteries tied. Immerse in cold water before fixation to solidify the gelatine, then the specimen is fixed for 10 - 14 days in ordinary methylated spirit or Carnoy's fluid. The latter is preferable and consists of:-

Methylated spirit	60%
Chloroform	30%
Glacial acetic	10%

Preparation of the carmine-gelatine injection mass.

It is to be used for warm injections only.

Prepare the following solutions:

Solution A -	Gelatine	250 grms.
	Distilled water	750 c.c.
	Dissolve on the water bath at 60°C.	
Solution B -	Carmine rubr. opt.	150 grms.
	Distilled water	75 c.c.
	Ammonia	25 c.c.

Add/

Add B to A, and stir thoroughly. Then cautiously add 50 per cent. acetic acid, about 1 c.c. at a time. Stir thoroughly after each addition of the acid. The mass will change from the transparent to the opaque, and the smell of ammonia nearly or completely disappears. Immediately this occurs stop adding the acid, as the neutral point has been reached.

The neutralisation of the mass is rather delicate. If the carmine gelatine is acid, it will form lumps in the vessels; and if alkaline, it will diffuse through the vessel walls.

Finally, filter the hot mass through flannel into a one litre bottle to which 2 grammes of crystallised phenol have been added.

The following illustration represents the apparatus used:

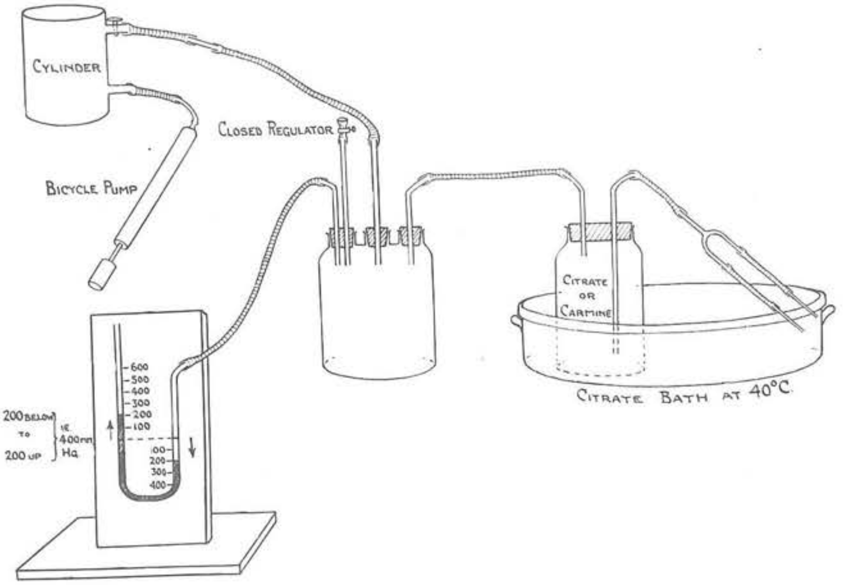

Fig 9. Illustration representing the apparatus used for the carmine-gelatine injection method.

(III) CARMINE - GELATINE INJECTION OF THE PLACENTA.

The technique used here is the same as that used for carmine injections of the uterus; only the placenta has to be carefully chosen as one that exhibits no tears between the various cotyledons or otherwise the carmine gelatin mass injected under 400 m.m. Hg. pressure would leak out through those tears.

The placenta must be fresh and kept in warm citrate as soon as delivered so as to keep its tissues alive. The viability of the blood vessels within the placental organ is of prime importance as otherwise the injection mass would not permeate deep enough into the fine villous capillary network. Preliminary washing of the vessels with 3.8% citrate solution before injection is commenced is essential for removal of all contained blood.

(IV) FUSCHIN AZUR PARAFFIN "F.A.P." METHOD FOR DEMONSTRATING THE VILLOUS VESSELS.

This method is still unpublished but kindly lent to me by Dr Pickworth and permission given for its description in this work. It is devised and used by him for the demonstration of pathological haemorrhagic lesions in the brain; but used here in an endeavour to follow the course of foetal villous vessels within the placenta. It was considered for our purpose because it differentially stains the vessel walls blue while the red blood corpuscles take up a red colour so that the vessels can be easily studied. Ordinary paraffin sections of a placenta previously fixed in formalin are all that is required in this method. This we found to be a great advantage over the benzidine sodium nitro-prusside method, also used in brain capillary studies, which only stains the red blood corpuscles a deep Prussian blue without differentially staining the vessel walls; also the tedious procedure of having to use celloidin sections for the latter method, to hold the villous branches together and in position, was dispensed with. Another point was that in using the benzidine sod. nitro prusside method which only stains blood corpuscles one often lost trace of the course of the foetal red blood corpuscles that were contained within the villous vessels/

vessels amongst the masses of similarly stained maternal blood that lay between the villi.

To overcome this latter difficulty, we tried the soaking of the placenta in 4% acetic acid, for 24 hours prior to fixation in 10% formalin in an attempt to destroy the maternal red blood corpuscles between the villi; in other words, using a similar solution to the one used for a white blood count. This, however, was found to penetrate through the vessel walls and destroy to a large extent the red blood cells contained within them. With the F.A.P. stain, which is a differential method, such a difficulty was overcome.

F.A.P. Method:-

The placenta is fixed in 10% formalin, the umbilical cord having been tied to prevent the foetal blood escaping from the umbilical vessels. This fixation was carried out for 48 hours. Then a single cotyledon was cut out and fixed separately in formalin for two weeks so as to get even fixation. At the end of that period it is dehydrated, cleared, impermeated and embedded in paraffin. Paraffin sections were cut at 20μ thickness and stained as follows:

1. Xylol - alcohol - spirit - water.
2. Phenol fuchsin 5 min.
3. Rinse in water.

4./

4. Decolorise in weak Gram's iodine "diluted $\frac{1}{3}$ strength with warm tap water".
5. Wash in water.
6. Rinse rapidly in 70% alcohol "until R.B.C. are bright red"; or leave in 0.5% hypo till red.
7. Rinse in doubly distilled water.
8. Dilute azur "1 in 5" 2 - 5 min.
9. Wash in water 2 min.
10. Differentiate in methyl spirit.
11. Absolute alcohol; xylol; mount in Canada balsam.

Phenol Fuchsin.

Phenol 1 in 20	....	100 c.c.
Acid fuchsin	....	5 g.m.

Azur "Stock Stain".

Azur 11	....	0.05 g.m.
Methyl alcohol		75.00 c.c.
Glycerine		25.00 c.c.

Dilute 1 part with 4 parts of doubly distilled water for use.

(V) DEMONSTRATION OF PLAIN MUSCLE TISSUE IN THE PLACENTAL CHORIONIC PLATE.

The placental upper chorial wall is removed and fixed in 10% formalin for 24 hours, dehydrated up through ascending grades of alcohol, cleared in benzol, impermeated with and embedded in paraffin.

Sections were then cut at the usual thickness of 5μ and stained by Weigert's iron haematoxylin and Van Gieson's methods.

This stains cell nuclei brown, collagenous connective tissue fibrils red, the muscle and red blood cells yellow.

In our preparations a lot of yellow staining matrix was seen in the chorionic layer as well as in the villous stems. This to our mind did not have any cellular outline or the structural appearance of muscle tissue. The iron haematoxylin and Van Gieson stain which is the differential stain generally used for muscle also stains yellow the red blood cells and epithelial tissue besides the muscle tissue.

Despite Jisuka demonstrating plain muscle element in the subchorial connective tissue; also Spanner demonstrating the same in both the chorial plate and the villous stems reinforced by his observation of contraction waves occurring along the chorial surface of the placenta, nevertheless in our studies no plain muscle tissue could be demonstrated.

DESCRIPTION OF SPECIMENS

AND

OBSERVATIONS.

(1) CORROSION SPECIMENS OF CELLOIDIN INJECTED PLACENTAE.

This method was found to be more serviceable for the purpose of studying the general architecture of the foetal vessels than ordinary dissection under a binocular microscope, because it provides a cast of the villous vessels which could be handled. Also the use of X-Rays as recommended by Fraser was thought to provide no real advantage in a stereoscopic study of the course of these vessels.

In our specimens some were injected through the umbilical vein alone (Fig 10 A & B);while others were injected through both arteries and the vein (Fig.11). They all show that the main arterial branches of the placenta lie in a horizontal plane as was first pointed out by Spanner (1935) and also seen by Bacsich and Smout in their corrosion specimens (1938). Following Schordania's classification, it was noted that in most of our injected placentae the arrangement of the umbilical arteries was of the disperse type (Fig.1) in which the two arteries divide dichotomously; and to which Fraser has applied the term "spokes of a wheel" like arrangement.

As was pointed out by Bacsich and Smout, we found that both umbilical arteries were always of equal calibre in the cord irrespective of the relation of the/

the areas supplied by them.

The corrosion specimens also show that as the umbilical vessels run in a horizontal plane between the amnion and the chorion, they send off their main villous branches at right angles to their course in that plane. The branches, therefore, are perpendicular to both chorionic and basal placental plates.

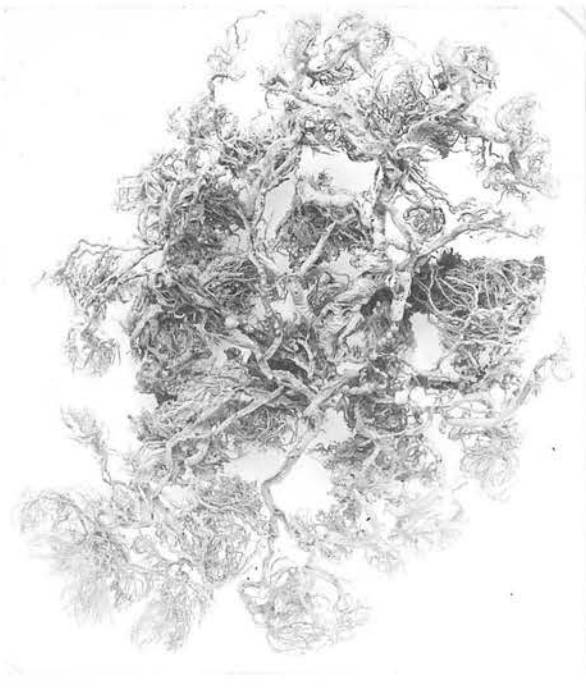

Fig.10 A. Celloidin corrosion specimen of a placenta injected through the umbilical vein alone, "viewed from the chorionic surface".

Fig.10 B. Celloidin corrosion specimen of a placenta injected through the umbilical vein alone. "Viewed from the decidual surface".

Fig.11. Celloidin corrosion specimen of a placenta injected through the umbilical vein as well as through both the umbilical arteries.

We next studied the specimens for the beaded appearance of the villous vessels which was first described by Fraser in 1923 through X-Ray studies of placentae injected through the umbilical vessels with barium in gelatine and thus rendered radio-opaque; but which he interpreted as a criterion of physiologic senescence in the "mature or ripe placenta". Spanner in 1935 demonstrated this beading in his celloidin corrosion preparations and he attributed it to the presence of muscular sphincters along the course of the small and medium-sized veins. In our celloidin specimens this beaded appearance is shown (Fig. 12) but they were not found in such frequency or uniformity along the course of the villous vessels as claimed by Spanner. They were only found occasionally. Later microscopic studies of villous vessels stained by the F.A.P. method showed the existence of sphincters along the course of villous veins. Therefore, these findings led us to attribute the beading to the presence of sphincters as held by Spanner. It will be noted that Bacsich and Smout in 1938 could not demonstrate this beading appearance in their celloidin preparations.

Fig.12. Celloidin corrosion specimen of a placenta showing a beaded appearance in some of the villous vessels.

The manner of division and architecture of the villous tree was next studied. It was always claimed that the villous tree was comparable to a tree with its branches arranged like a conifer - most intricate and most complex. Spanner, however, brought forward the conception that the villous trunk with its branches was like the trunk of an inverted drooping ash or an old-fashioned chandelier. This he claims to have been able to observe both with the naked eye study of celloidin casts of the villous tree; and by examination under a binocular microscope. Although this arrangement sounds suited to the direction of the blood/

blood stream yet in our celloidin casts we could not see that such a state of affairs existed. Both naked eye and microscopic study, as advocated by Spanner, were used for the purpose. In fact in well injected specimens, the celloidin cast of the capillary network of an individual cotyledon resembles "a ball of cotton wool" (Fig. 13). Bacsich and Smout got similar results in the study of their corrosion specimens. Falkiner who is a great believer and supporter of Spanner's conception of the placenta has never been able to demonstrate such a manner of division of the villous tree.

Fig.13. Celloidin cast of a villous tree exhibiting "a ball of cotton wool" appearance.

That the villous vessels are end arteries and do not anastomose with each other is a view held by Spanner supported by Bacsich and Smout from a study of their corrosion preparations. Our specimens show similar results reinforced by microscopic study of our carmine-gelatine injected placentae. Fraser, Hyrtl, Holl and Stieve, however, believe in the existence of a free anastomosis between the villous vessels deep within the substance of the cotyledon.

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(2) UTERUS REMOVED AT CAESAREAN HYSTERECTOMY WITH PLACENTA IN SITU AND INJECTED WITH CARMINE GELATINE THROUGH BOTH UTERINE ARTERIES.

This specimen was obtained and prepared in the Rotunda Hospital under Dr Falkiner's supervision. It is seen in (Fig. 14) and affords a good study of the various placental vascular components and their various connections with the uterus.

It will be noted that the shape of the placenta in this specimen is not the flattened deflated organ one is accustomed to see after delivery but is much more rotund. The maternal and foetal blood is always completely or partially drained from the separated placenta. It is this fact that makes the subchorial space difficult to recognise in the separated placenta.

The utero-placental arteries are seen entering the intervillous space, being irregularly distributed throughout the base of the placenta as maintained by Spanner; and where they enter through the basalis they do so at right angles to that surface.

In (Fig.15) the placental septa will be seen standing out between the red carmine-gelatine mass filling the placental cotyledons. They are seen arising/

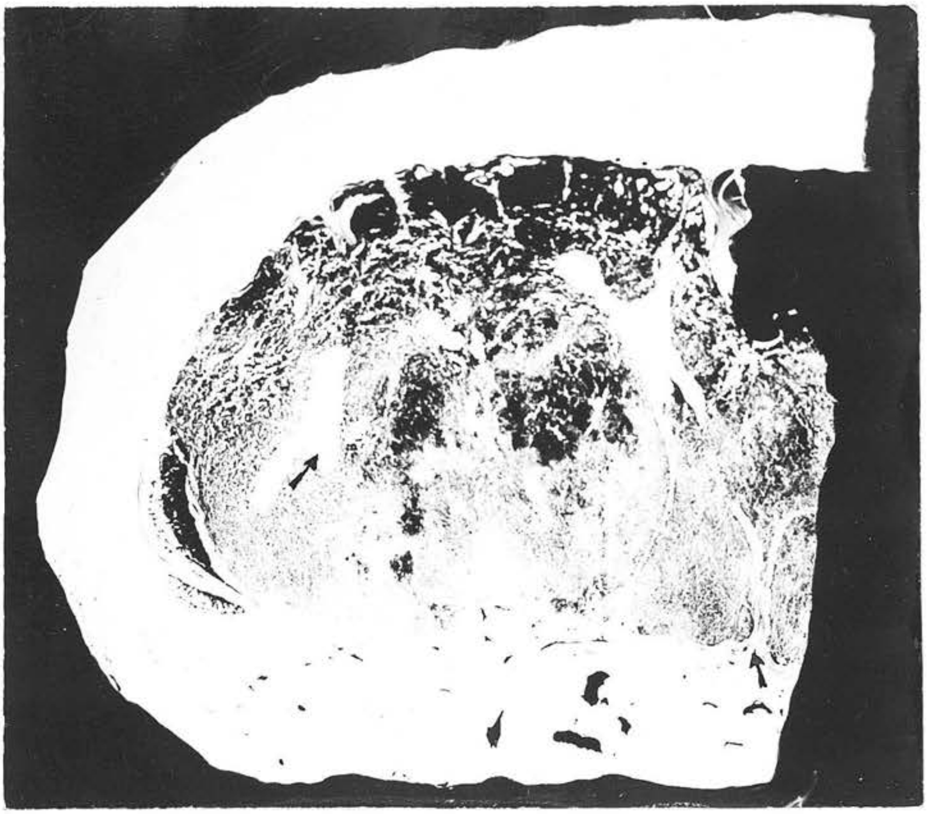

Fig. 14. Microphotograph of a section of full term placenta in situ. The distribution of the carmine-gelatine mass in the I.V.S. is shown. The subchorial blood space and marginal sinus are filled with injection mass. The section also shows the villous trunks (arrow) and the placental septa (arrow).

arising from the basal placental surface running upwards perpendicularly or slightly obliquely towards the chorionic surface but stopping some distance away from that surface. The carmine-gelatine mass is seen in the basal part of the placenta to come up to the various cotyledon boundaries and limited there from further/

further lateral flow by the presence of these septa. Subchorially, however, the septa are absent and the carmine-gelatine mass has accumulated into one big solid mass - in the subchorial blood lake as described by Spanner. In other words, the intermingling of carmine-gelatine from one inter villous space to another in the basal placental portion is being prevented by the presence of the septa; whereas subchorially, above the level of the septal limits, all inter villous spaces open into the one common space where the carmine-gelatine has accumulated into one big mass.

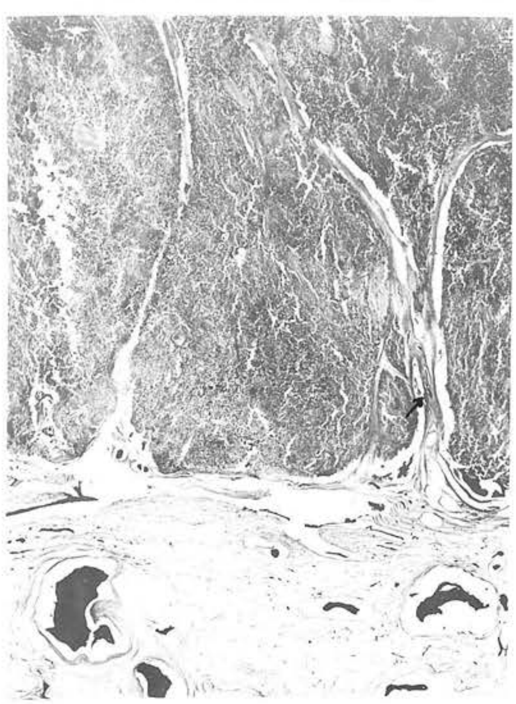

Fig 15. Microphotograph of a placental septum showing a maternal vessel coursing upwards and containing injection mass (arrow).

Therefore, one may conclude from this septal arrangement the following:-

(1) The septa prevent any connection between neighbouring cotyledons in the basal portion of the placenta.

(2) Above the limits of septal level, all inter villous spaces communicate with each other in the subchorial space.

(3) Each cotyledon besides forming an independent unit as regards its foetal vessels, also forms an independent unit in as far as its maternal blood circulation is concerned.

(4) The maternal blood stream in the one cotyledon has no influence on other streams in neighbouring cotyledons as such an influence would be prevented by the presence of placental septa.

The above findings, therefore, fall into agreement with Spanner; and the view held by Stieve that the placental septa reach the chorion exhibiting openings in them to allow of blood flow from one intervillous space to another can be no longer accepted.

The distribution of the villi in this specimen is very striking; namely, they are very dense almost packed together in the basal portion of the placenta as shown by the sparse distribution of the carmine-gelatine mass in that part. Subchorially, however, the/

the villi are very scarce and uninterrupted injection mass is seen filling up that subchorial space except where it is traversed by the main villous trunks.

The marginal sinus is clearly shown at the chorio-decidual angle of the placenta. It is devoid of villi and runs along the margin of the placenta. It will be seen in the specimen to be divided by a partition wall and the lower part of the sinus can be seen communicating with the uterine veins (Fig.16). This relationship of the marginal sinus to the subchorial blood space, the intervillous space and the uterine veins is, therefore, in agreement with Spanner's conception of the maternal circulatory path through the placenta.

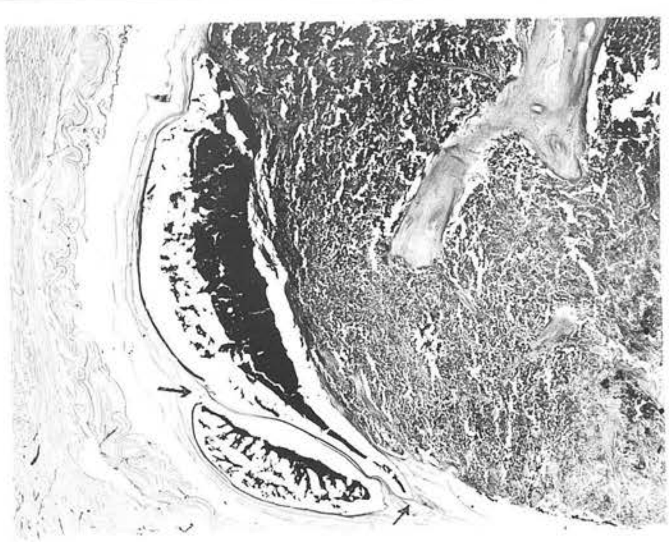

Fig 16. Microphotograph showing the marginal sinus and its relationship. The sinus is divided by a partition wall (arrow) and the lower part of the sinus can be seen communicating with the uterine veins where the injection mass can be traced (arrow).

The point, therefore, to be brought out in this specimen and which is clearly shown, is that the injection mass had run up in the utero-placental arteries into the intervillous space, as far as the subchorial blood space and thence to the circular sinus and back to the uterine veins - a view in which we, therefore, are in full agreement with Spanner.

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(3) UTERUS REMOVED BY CAESAREAN HYSTERECTOMY AND INJECTED WITH CARMINE GELATINE VIA THE OVARIAN ARTERIES.

The canulae were inserted into the ovarian arteries and the injection carried out in a similar manner to the previous specimen after a preliminary washing out with citrate solution. Unfortunately, the injection mass did not reach the intervillous space; however all anatomical points brought out in the previous specimen can be readily recognised by the naked eye in this specimen (Fig 17).

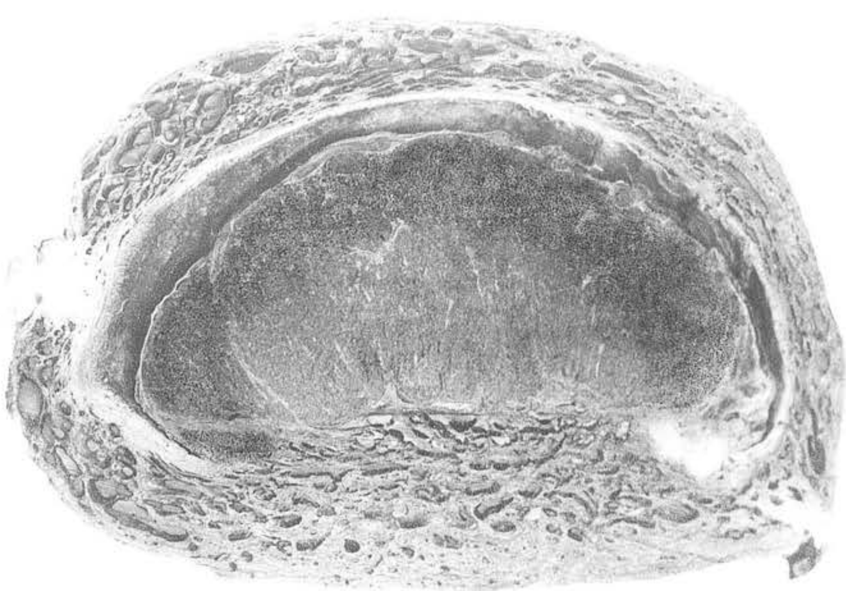

Fig 17. Photograph of a full term uterus with placenta in situ. This specimen was injected through the ovarian arteries, but the injection mass did not fill the I.V.S. The subchorial blood space and the marginal sinus are clearly shown.

The subchorial blood lake and marginal sinus are seen filled with dark brown mass which is the maternal blood altered by fixation. Placental septa can also be seen as fine whitish filaments dividing the basal placental portion into intervillous spaces.

A longitudinal section taken at the utero-placental junction of this specimen (Fig 18) shows certain interesting points. Two intervillous spaces are seen separated by a septum. The septum shows maternal vessels filled with carmine-gelatine. A coiled utero-placental artery, the lumen of which is partially filled with the injection mass, can be seen in the basal part of the placenta and can be traced to one intervillous space. Some amount of the injection mass had made its way between the villi.

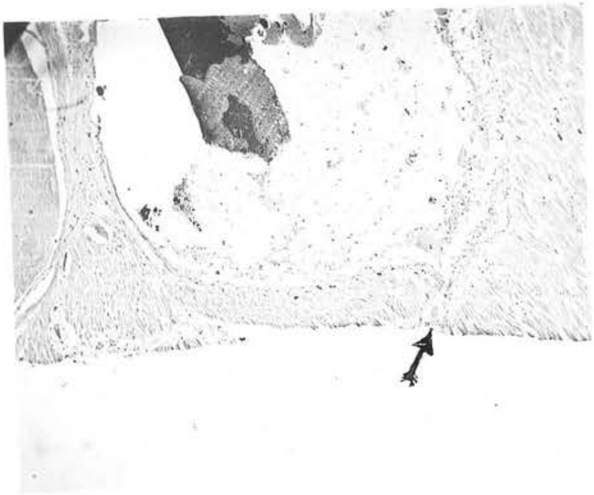

Fig 18. Microphotograph of the basal portion of the placenta from the specimen in Fig 17. A utero-placental artery (arrow) is shown opening into an I.V.S. and partially filled with injection mass.

(4) FULL TERM PLACENTA INJECTED WITH CARMINE GELATINE THROUGH BOTH UMBILICAL ARTERIES.

These specimens were made and sections examined microscopically in an attempt to follow the course of the villous vessels, their architectural pattern, and anastomoses, if any, between the various branches of the villous tree.

It must be said outright that by this method we were unable to demonstrate the pattern and manner of division of the villous vessels that had been likened by Spanner to the shape of an inverted drooping ash or an old-fashioned chandelier. Our own observations showed that the villous tree divided in the most intricate and complex manner like the branching of a tree. It is to be remembered that our celloidin corrosion specimen casts of the villous tree did not conform to Spanner's claim in that respect either.

The question was; could serial sectioning of such carmine-gelatine injected placentae and reconstruction of the villous tree help to throw more light on the subject? It might do so! But we thought it unlikely as our celloidin injected placentae gave such a fine cast of the villous tree, already reconstructed to handle for both naked eye and microscopic examination, and to our mind each villous tree looked like "a ball of cotton wool" that no better purpose might be served/

served by this tedious reconstruction technique!

The question of the villous vessels being end arteries as held by Spanner, Bucsich and Smout, on the one hand; and being freely anastomotic with each other as held by Hyrtl, Holl, and Fraser, on the other hand next came to our notice.

Our specimens (Fig 19 A & B) of the carmine-gelatine injected placentae show that the villous vessels undoubtedly terminate as end arteries. They illustrate that the terminal villous veins loop back on themselves to become arteries with no anastomosis whatsoever between the various branches.

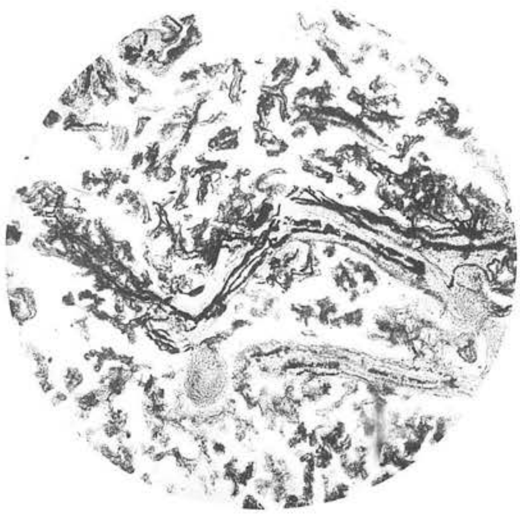

Fig 19 A. Microphotograph showing the mode of termination of the villous vessels in a carmine-gelatine injected placenta through both the umbilical arteries.

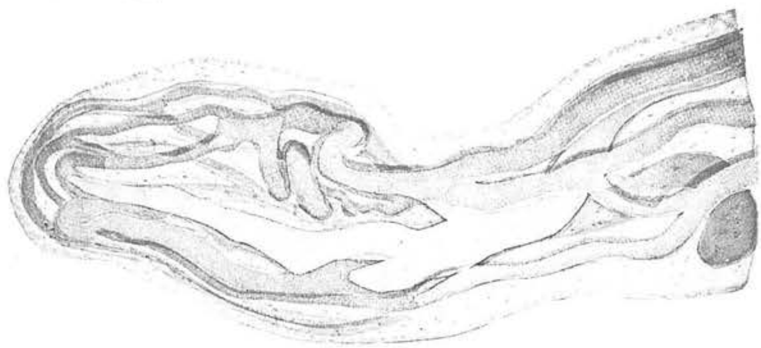

Fig 19 B. Illustration showing the mode of termination of the villous vessels.

(5) FULL TERM PLACENTA WITH ITS CONTAINED FOETAL BLOOD FIXED AND STAINED WITH F.A.P. STAIN.

This method was intended to attack from another angle the question of studying the course and arrangement of the villous vessels.

The results we got with this method were rather unexpected. It did not help to throw any more light on the structural arrangement of the villous tree than the carmine-gelatine injected specimens; but it showed the existence of sphincters in the small villous veins (Fig 20 and Fig 21). These sphincters are seen to take a blue stain like the rest of the general vessel wall, thus contrasting in colour with the contained red blood cells which show red. The sphincters are seen constricting the vein lumen with the red blood cells being in the form of a filament threaded through the sphincteric opening.

The presence of these sphincters thus seen microscopically together with the beaded appearance we got in the celloidin corrosion casts of the placental villous vessels make us attribute the presence of the latter to the existence of these sphincters.

This/

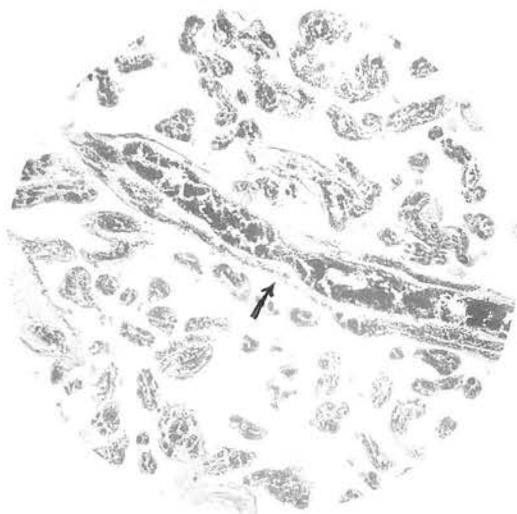

Fig 20. L.P. microphotograph of a small villous vein showing local thickening in its muscular wall to form a sphincter.

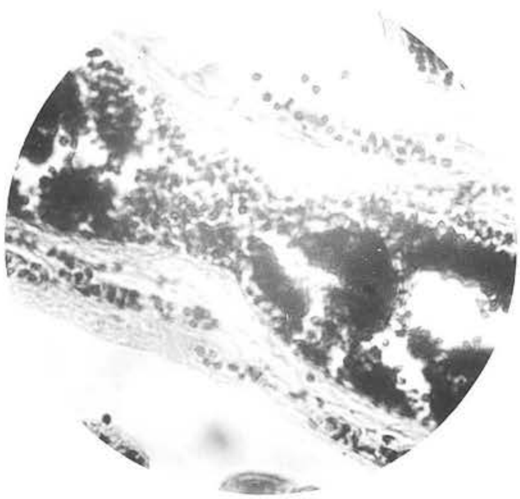

Fig 21. H.P. microphotograph of a sphincter in the muscular wall of a small villous vein.

This is in agreement with Spanner who first demonstrated the existence of sphincters along the course of small and medium villous vessels in placentae/

placentae injected with Indian ink through the foetal vessels. His microscopic studies of these specimens showed constrictions along the course of the black Indian ink mass within the vessel lumen.

CONCLUSION.

The microscopic study of the villous vessels with the fuchsin azur paraffin method together with the examination of celloidin casts of the villous tree in the placenta, confirms Spanner's belief in the existence of sphincters along the course of the small and medium-sized villous veins. They seemingly play a similar role to valves in veins elsewhere in the body, namely in regulating the blood flow along these vessels.

Our various attempts at studying the general architecture of the villous tree did not throw any more light on the old belief held that the villous tree was comparable to a tree with its branches arranged like a conifer. In other words, Spanner's description of the villous trunk as being like the trunk of an inverted drooping ash or like an old fashioned chandelier could not be demonstrated in our work.

Microscopic study of the carmine-gelatine injected placentae shows that the villous vessels are undoubtedly end arteries; the veins looping back on themselves to become arteries with no anastomosis between them deep in the substance of the cotyledon - a fact that probably explains the frequent occurrence of placental infarcts.

Despite Jisuka demonstrating plain muscle element in/

in the subchorial connective tissue; also Spanner demonstrating the same in both the chorial plate and the villous stems reinforced by his observation of contraction waves occurring along the chorial surface of the placenta; nevertheless in our studies no plain muscle tissue could be demonstrated by using the iron haematoxylin and van Gieson stain which is the stain generally used for the purpose.

The distribution of the carmine-gelatine injection mass in the uterus removed at Caesarean hysterectomy with the placenta in situ supports Spanner's contention with regard to the placental circulation.

Firstly, the utero-placental arteries can be demonstrated and their communications with the intervillous space shown. Secondly, in the basal portion of the placenta, the injection material is rather sparsely distributed amongst the numerous villi. Tracing the intervillous space more chorialwards, the injected carmine-gelatine material becomes greater in amount because the villi are less numerous. Whereas immediately underneath the chorionic plate all the intervillous spaces are seen to coalesce opening into the one large subchorial space where a solid mass of injection material is present.
At the outer end of this subchorial mass of the injected carmine material the marginal sinus is clearly shown which itself communicates with the uterine/

uterine veins. Such a distribution of injected material would fit in with Spanner's interpretation.

The placental septa arising more or less perpendicularly from the basalis do not reach the chorial plate of the placenta. They act as limiting boundaries to the intervillous space in their basal portion preventing any lateral flow of blood between the cotyledons. Subchorially, however, where the placental septa stop short, the blood from each cotyledon merges into the common stream in that part of the intervillous space above the level of the septa.

The blood having traversed the intervillous space now forms a large lake or pool immediately subjacent to the chorion which drains out to the periphery, into the marginal sinus and thence to the uterine veins.

Although Spanner concludes that the chief driving force in the maternal circulation through the placenta is the maternal arterial pressure; it is very difficult to assess what effect the intermittent uterine muscular contractions have on the placental circulation. They may act by altering the pressure on the arterial inflow or venous outflow through the placenta. They may also act by varying the pressure on the subchorial blood space. There remains the other suggested factor in the circulation of maternal blood through the placenta, namely the villous pulsations. It is difficult/

difficult to imagine that the villous vessels would differ in their properties in that respect from vessels elsewhere in the body; as nowhere else do the vessels in the body exhibit any form of active movement such as swaying.

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