# THE DEVELOPMENT OF THE VASCULAR SYSTEM IN FIVE TO TWENTY-ONE SOMITE DOG EMBRYOS

by

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## INTRODUCTION AND LITERATURE REVIEW

While the dog has been employed extensively as a laboratory animal in various fields of scientific endeavour, the use of this animal in embryology has been neglected. As a consequence, the literature on the circulatory system of the dog was represented only by an unpublished thesis by Duffey (3) on cardiogenesis and the first heart movements. Most literature dealing with the development of the circulatory system in mammals consisted of reports concerning the human by Davis (2), Ingalls (4), Padget (5, 6, 7), and Patten (8). There was one important work on the cat by Watson (11), and a report of lesser application on the ferret by Wang (10).

The proliferation of vasofactive cells from embryonic mesoderm has been reported (11) as the prologue to the development of endothelial-lined vessels including the endothelial lining of the heart. Watson (11) described the presence of vasofactive cells underlying the embryonic mesoderm in the cat. She did not present proof of origin of the cells but indicated the probability that they were derived from the mesoderm under which they were found. Duffey (3) noted that angioblast cells in the dog proliferated from the splanchnic mesoderm, and became arranged into sheets of two or three cells, many of which were connected to the adjoining mesoderm by cytoplasmic processes. Vasofactive cells in the cat were found grouped in four main lines (11); the lateral lines developing into the endocardial heart tubes and the medial lines developing into the dorsal aortae.

Watson (11) proposed that some cells of the aortae originated from the lateral mesoderm and migrated into the aortae lines. The masses of vasofactive cells secondarily acquired lumina (3, 11), forming true endothelial-lined vessels.

Following the formation of amnio-cardiac vesicles by splitting of the lateral mesoderm into somatic and splanchnic layers, the splanchnic mesoderm became thickened to form epimyocardial plates which evaginated dorsally as epimyocardial mantles (3). The endocardial tubes developed ventral to the epimyocardial mantles (3, 11). Davis (2) outlined a similar process in the formation of heart primordia in the human. However, he claimed that the right and left sides of the endocardium were never completely separated although the heart was fundamentally bilateral in origin.

The epimyocardial mantles rotated medially as the splanchnopleuric fold closed posteriorly resulting in fusion of the mantles followed by fusion of the endocardial tubes thus completing the formation of a single median heart (2, 3, 8). In a three-somite human embryo described by Ingalls (4), the heart primordia were stated to be united in a plexus producing a single median mass. Later convolutions of the median heart were described by Davis (2), Duffey (3), and Patten (8).

The dorsal aorta was described by Watson (11) as forming directly from an accumulation of vasofactive cells in the aortic lines. The dorsal aortae were noted as small vesicles within the dense mesenchyme in the early human embryo described by Ingalls(4).

In the human, as described by Patten (8), the dorsal aortae were formed by posterior prolongation of the first aortic arches, added to by knots and cords of cells which later became hollowed out, accumulated along the course of the developing dorsal aortae, forming endothelial lined extensions of the aortae.

The development of the cranial arteries in the human was followed after establishment to adult configuration by Padget (5). In another work on the human, Padget (6) reviewed the literature on the nomenclature and serial numbering of the intersegmental arteries, and discussed their role in the formation of the vertebral artery.

The origin and growth of veins was discussed by Sabin (9) in the chick and by Watson (11) in the cat. Both reported vein origin from vasofactive cells. According to Sabin, veins grew by a process of sprouting. Watson (11) described the formation of veins from lines of grouped vasofactive cells which gradually acquired lumina. She followed the formation of anterior cardinal and umbilical veins to the 15-somite stage. The most recent detailed work on the cranial venous system in the human was done by Padget (7). In this account, he outlined the changes of the cranial veins after their establishment to adult configuration.

The only indication in the literature of the presence of connections between the dorsal aortae and the posterior cardinal veins was in a figure (321-B) used by Arey (1) to illustrate another point.

In an attempt to correlate and clarify the previous efforts at describing the development of a circulatory system in mammals, a detailed study of circulation in dog embryos was undertaken.

## MATERIALS AND METHODS

The 16 embryos used in this study were obtained from dogs of various breeds maintained for that purpose[1] and were aged according to methods developed in that study. Some of the embryos used had previously been taken from the uterine swellings and sectioned for other studies. Additional critical stages were removed from fixed uteri, stained with aceto-carmine, photographed, drawn by projection or camera lucida as whole embryos and subsequently sectioned (Table 1).

The embryos were routinely sectioned at ten microns, stained with a modified Harris' hematoxylin and counter-stained with an Orange-G acid fuchsin combination.

Scaled graphic reconstructions were made from some of the transversely sectioned embryos. Micro-projection outline drawings were made of the longitudinally sectioned embryos and reconstructions of critical regions were made with modeling clay.

The incomplete somite immediately posterior to the tenth cranial nerve was counted as the first somite on each side, and the last somite was considered to be the one anterior to the last intersegmental groove.

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[1]Kansas State Agriculture Experiment Station Project 321; Dog Embryology, under the direction of Dr. H. T. Gier.

Table 1. Embryos studied

| Collection Number | Somite Number | Age (days) | Type of preparation | Length (mm) |
|---|---|---|---|---|
| 260 Lb | 5+ | 17- | Transverse Section | 4.2 |
| 260 Rb | 7 | 17- | Longitudinal Section | 4.5 |
| 45 L-6 | 8 | 17- | Transverse Section | 4.6 |
| 115 La | 10 | 17 | Transverse Section | - |
| 120 Lc | 11 | 17 | Transverse Section | - |
| 120 L | 12 | 17 | Longitudinal Section | 5.3 |
| 120 Rd | 12 | 17+ | Transverse Section | - |
| 115 Rb | 12+ | 17+ | Transverse Section | 5.2 |
| 113 Lb | 15 | 18- | Longitudinal Section | 5.6 |
| 113 La | 17 | 18- | Longitudinal Section | 5.8 |
| 77 L-1 | 17 | 18- | Transverse Section | 5.7 |
| 81 L | 17 | 18 | Transverse Section | - |
| 81 L-2 | 18 | 18 | Longitudinal Section | 6.1 |
| 81 L-1 | 18+ | 18 | Longitudinal Section | 6.1 |
| 121 L | 20 | 18+ | Transverse Section | - |
| 116 Rb | 21 | 19- | Longitudinal Section | 7.2 |

## OBSERVATIONS

### Five-Somite Stage

In the five-somite embryo only primordia of embryonic vessels were found. The lateral mesoderm had split to form the coelom. In the region lateral to the head mesoderm, the coelom (pericardial cavity) extended laterally only to the lateral limiting sulcus and in this region the splanchnic mesoderm was thicker than the somatic mesoderm. Throughout the length of this primitive pericardial cavity to the level of the first somite, the splanchnic mesoderm bulged into the coelom forming an epimyocardial mantle (Plate I, Fig.1).[1]

Ventral to the epimyocardial mantle, the endocardial cell mass was represented by loose strings of vasofactive cells[2] which were generally pressed against the endoderm (Plate I, Fig.1).

Lateral to the somites, a variable string of vasofactive cells was found between the mesial edge of the splanchnic mesoderm and the endoderm. Some sections through the region did not show typical vasofactive cells in this position, but it is possible that interconnecting cytoplasmic processes were present but inevident.

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[1] All Plates in Appendix

[2] The vasofactive cell was first recognizable from surrounding mesodermal cells by its spherical nucleus and the irregularity of the cell membrane. As the cell differentiated the nucleus condensed and elongated while the cytoplasmic extensions became connected with strands from neighboring cells. The long axis of the cell was oriented in line with the path of the future blood vessel. The oval nucleus became reduced to about one-half the original size resulting in some condensation and orientation of the chromatin threads against the nuclear membrane. Such a condensed nucleus appears more darkly stained.

At the level of the first somite, near the posterior extremity of the epimyocardial plate, a scattered row of vasofactive cells extended perpendicular to the endocardial cell mass, mesial to the future position of the dorsal aorta and laterally into the future extraembryonic splanchnopleure.

Posterior to the first somite, the embryonic coelom was continuous laterally with the extraembryonic coelom. Vitelline vessels, with open lumina, were present in the extraembryonic splanchnopleure lateral to the second and third somites. The scattered vasofactive cells ventral to the lateral edge of the embryonic splanchnic mesoderm were continuous into the extraembryonic splanchnopleure where the vitelline vessels were formed. There were discontinuous lumina in the clumps of vasofactive cells in the splanchnopleure ventral to the future body fold lateral to the third intersomitic groove and the fourth somite. These appeared to be vitelline vessels that had extended mesially into the edge of the embryonic area.

From the level of the anterior end of the epimyocardial plate to the fifth somite, there were scattered vasofactive cells along the future line of the dorsal aortae (Plate I, Figs. 1, 2). Most of these cells were between the head or somitic mesoderm and the endoderm, while others seemed to be actually a part of that mesoderm. Isolated vasofactive cells were found against the endoderm between the endocardial mass and the aortae anlagen. Occasional vasofactive cells were identified in the head region off the dorso-mesial edge of the head mesoderm.

Seven-Somite Stage

The epimyocardial plates had expanded into U-shaped mantles, divided by a transverse constriction into an anterior bulbus anlaga and a posterior ventricle anlaga. The endocardial masses had vesiculated forming a tube under each epimyocardial mantle. At the closed ends of the endocardial tubes were grouped vasofactive cells of the endocardial mass. The posterior ends of the endocardial cell masses were connected to the vitelline vessels by continuous strings of vasofactive cells.

Dorsal aortae were present from the posterior edge of the prosencephalon to the level of the potential ninth somite. They consisted of double layers of endothelial cells, the nuclei of one layer alternating with the nuclei of the other layer (Plate I, Fig. 4). The main masses of the dorsal aortae were ventral to the somites, extending laterally to the margins of the somites. There were continuous lumina within the aortae from the level of the future mesencephalon to the middle of the fifth somite. Discontinuous lumina were present posteriorly to the sixth intersomitic groove. The dorsal aortae had no lumina ventral to the mesencephalon. There were lumina between the endothelial layers of the anterior lips of the dorsal aortae, continuous latero-posteriorly about 75 microns into the incomplete first aortic arches which diminished to strings of vasofactive cells connecting to the endocardial mass.

Eight-Somite Stage

This embryo had open endocardial tubes which connected with the vitelline vessels posterior to the end of the epimyocardial plates and anteriorly with the first aortic arches (Plate III, Fig.1). The open side of the epimyocardial mantle had rotated mesially as the splanchnopleuric fold converged, and the anterior ends of the heart primordia were pulled mesially. The endocardial tubes thus approximated each other immediately anterior to the epimyocardial plates with only a few non-endothelial cells separating the two tubes. These extensions of the endocardial tubes were in position to form the future aortic sinus.

Anterior to the point of near junction, the paired endothelial tubes separated antero-laterally and curved dorsally around the anterior end of the foregut on either side of Sessel's pocket as the first aortic arches. The aortic arches were continued posteriorly, dorsal to the foregut, as the paired dorsal aortae (Plate III, Fig.1).

From the anterior curvature of the first aortic arches, the future internal carotid arteries were formed anteriorly a short distance to a position posterior to the optic vesicles. The anterior tips of these arteries were connected through paired capillaries to two endothelial-lined vessels -- the anterior cardinal veins -- located adjacent to the lateral walls of the prosencephalon (Plate III, Fig.1).

The dorsal aortae were flattened or oval tubes between head mesoderm and endoderm, or posteriorly between somites and the endoderm. The vessels had well defined lumina through most of their extent but the lumina were discontinuous posterior to the eighth intersomitic groove. Between the interrrupted segments of lumina in the posterior portion of each dorsal aorta at the level of the anterior end of the segmental plate were from one to four vasofactive cells. These clumps of cells were continuous with other cells which connected laterally into the extraembryonic splanchnopleure with vitelline vessels. At various places along the line of the dorsal aorta in this area, sizable vesicles were present. The dorsal aortae were directly connected laterally to the vitelline vessels through vitelline arteries at several points in the region of somites five, six and seven.

At various places (eight or nine) in the main portion of the dorsal aortae, cytoplasmic processes connecting the dorsal and ventral walls divided the cavity (Plate III, Fig. 1).

In the first intersomitic grooves, intersegmental arteries extended dorsally from the aortae where they ended between the somite and the lateral wall of the neural tube as spindle-shaped lumina approximately 30 microns long. Short intersegmental arteries in the second intersomitic grooves did not reach the position attained by the first arteries.

Ventral to the seventh cranial nerve, the aortae were connected broadly to the anterior cardinal veins. At the level of the middle of the auditory placode on one side, a vessel branched laterally from

the dorsal aorta and coursed meso-dorsally, lateral to the anterior cardinal veins ending near the dorsal surface of the neural tube.

The anterior cardinal veins were continuous from the mesencephalon to the level of the ganglion of the ninth nerve lying close to the lateral surface of the brain (Plate III, Fig.1) and coursing ventro-mesial to the auditory placode. Posterior to the ninth nerve, discontinuous strings of vasofactive cells having an occasional lumen were present on either side of the neural tube as far posteriorly as the fourth intersomitic groove.

The umbilical veins were represented by connected lines of vasofactive cells with lumina in some places. The anterior ends of these lines were just posterior to the lateral edge of the first aortic arches between the somatic mesoderm and the ectoderm dorsal to the bulbus anlagen. At the level of the anterior edge of somite one, the cells were formed into a flattened tube with a lumen. From that point posterior to intersegmental groove three, smaller discontinuous lumina were present. The caudad extent of the vasofactive string in the future umbilical vein position was lateral to somite five. Posterior from somite five to the anterior part of the segmental plate, there was a general scattering of vasofactive cells from the mesial edge of the somatopleure to the lateral limiting sulcus.

The vitelline plexus had connected to the posterior end of the endocardial tubes through two main, and three or four minor vessels posterior to the limits of the epimyocardial mantles. The connecting vessels extended from the embryonic splanchnopleure into the extra-embryonic splanchnopleure where they joined with the anastomosing vitelline vessels.

## Ten-and Eleven-Somite Stage

The ten-and eleven-somite embryos were closely similar. In the ten-somite embryo the anterior end (bulbus portion) of the epimyocardial mantles were fused and the endocardial tubes of the bulbus had joined to make a single lumen. The heart primordia of the 11-somite embryo had fused more extensively, forming definite rounded bulbi and ventricles with a single endocardial tube through these structures.

In both stages, the first aortic arches were present in the same general relationship as described in the eight-somite embryo. The second aortic arches branched laterally from the dorsal aortae at the level of the auditory placode, curved ventro-mesially around the edge of the pharynx anterior to the second pharyngeal pouches and in the 11-somite embryo had continuous lumina into the aortic sinus ventral to the foregut. In the ten-somite embryo, the lumina of the second aortic arches ended just dorsal to the lateral edge of the foregut and were connected to the endocardial tubes by double layered strings of vasofactive cells.

The third aortic arches branched from the dorsal aortae at the level of the potential tenth nerve in both embryos. In the younger stage, the lumina extended only to the lateral margin of the pharynx while in the older stage the lumina extended ventrally around the margin of the pharynx. In both stages the lumina were connected ventrally to the aortic sinus by strings of vasofactive cells. No fourth aortic arch rudiment was found in the 10-somite embryo. In the 11-somite embryo, however, the fourth aortic arches were

represented by pouches from the dorsal aortae laterally to the margin of the pharynx at the level of the first intersomitic groove.

In the 11-somite embryo, internal carotid arteries were short vessels anteriorly from the curvature of the first aortic arches to the base of the prosencephalon ventral to the optic vesicles. Short ventral branches -- the ophthalmic arteries -- extended a short distance under the optic vesicles. Another set of arteries -- the middle cerebral arteries -- branched from the internal carotid arteries dorsally, posterior to the optic vesicles and connected through small vessels to the tip of the anterior cardinal veins. The ten-somite embryo had no opthalmic arteries but the middle cerebral arteries contacted the anterior cardinal veins.

The size and contour of the dorsal aortae were the same as in the previous stage but no cytoplasmic processes were observed dividing the lumina of the arteries. They had lumina to the middle of the segmental plate, and were connected by vitelline arteries to the extraembryonic vessels posterior to somite six. Posterior to somite ten, the aortae were more widely separated so that in the segmental plate area they were ventro-lateral to the plate.

The anterior cardinal veins connected anteriorly with the middle cerebral arteries and had continuous lumina posteriorly to the level of the ninth cranial nerve. They were essentially straight along the lateral wall of the neural tube, passing ventro-mesial to the auditory placode. Strings of vasofactive cells were present in the position of the anterior cardinal veins from the ninth nerves to the first somites.

The lumina of the umbilical veins were continuous from the level of the posterior edge of the auditory placode to the second intersomitic groove. In the middle portion of the vein, the lumina were about the same size as the dorsal aortae. The posterior end of each endocardial tube was connected with several small vitelline vessels lateral to the first three somites.

Twelve-Somite Stage

Vitelline vessels extended into the embryonic splanchnopleure lateral to the second somites, connecting with the posterior ends of the sinu-atrial anlagen. The sinu-atrial anlagen of the heart primordia at this stage had not moved from the lateral orientation as the splanchnopleuric fold had not closed to this posterior extent.

The first aortic arches extended from each side of the aortic sinus antero-laterally a short distance and curved dorsally around the first pharngeal pouches. At the dorsal level of the foregut, these arches were continuous posteriorly with the dorsal aortae.

Ventral to the middle of the auditory placodes, the dorsal aortae were connected to the lateral walls of the aortic sinus by the second aortic arches which curved around the sides of the pharynx anterior to the second pharyngeal pouches.

The third aortic arch anlagen extended as lateral branches from the dorsal aortae curving ventrally around the edge of the pharynx and continuing from there as strings of vasofactive cells mesially toward but not to the posterior margin of the aortic sinus. The fourth aortic arches were approximately in the same stage of development as the third arches were in the 11-somite embryo.

The short internal carotid arteries branched from the anterior extremity into three branches. The ventral branches were the ophthalmic arteries which extended around the ventral wall of the optic vesicles to the point where the vesicles were against the head ectoderm. The dorsal branches (middle cerebral arteries) extended midway around the posterior margin of the optic vesicles and terminated in connections with the anterior cardinal veins. The posterior branches (posterior communicating arteries) were continuous along the lateral walls of the prosencephalon to the mesencephalon and were connected to the anterior cardinal veins by small vessels.

The dorsal aortae had open lumina posteriorly to the potential fourteenth intersomitic groove and consisted of two layers of vasofactive cells from that point to approximately the potential fifteenth intersomitic groove. Isolated single or small groups of vasofactive cells were present in the dorsal aortae line to the end of the neural plate. The dorsal aortae were essentially parallel.

A vertebral artery was observed of either side between the somites and the neural tube with isolated lumina back to the eighth intersomitic groove. The lumina were continuous from about the middle of somite three to the point where the arteries connected with the anterior cardinal veins in front of the first somites. The lumen of the vertebral artery in one embryo was continuous posteriorly to the sixth somite.

Intersegmental arteries branched from the dorso-mesial wall of the dorsal aortae and connected with the vertebral arteries through the first and second intersomitic grooves. At the third intersomitic

grooves, intersegmental arteries branched from the aortae to the vertebral artery line where vesicles were formed within the groups of vasofactive cells. Short intersegmental arteries were present in the fourth intersomitic grooves but caudad, there were only dorsal evaginations from the aortae decreasing in length to the eighth intersomitic groove beyond which no trace of intersegmental arteries could be recognized. In addition to the arteries connecting the vertebral and dorsal aortae, there were vessels from the dorso-lateral wall of the aortae to the posterior cardinal veins through intersomitic grooves two and three.

At least six vitelline arteries connected the dorsal aortae to the vitelline vessels at irregular intervals posterior to somite nine. Caudad to where the dorsal aortae were lumenated the double layered vasofactive cell masses were continuous with the vitelline vessels. Scattered vasofactive cells were present in the embryonic splanchnopleure lateral to the region where isolated cells were seen in the dorsal aorta lines.

The anterior cardinal veins began in the head mesoderm from continuations of the middle cerebral arteries at the anterior end of the mesencephalon connecting with small branches of the posterior communicating arteries. The paths of the veins were essentially straight along the walls of the neural tube, deviating slightly ventral to the fifth and seventh nerves and ventro-mesial to the auditory placodes and ninth nerves. At two places posterior to the ninth nerve, large interconnecting vessels were present between each anterior cardinal vein and the corresponding dorsal aorta. The

anterior cardinal veins were directly continuous with the vertebral arteries between the first somites and the neural tube, but there were lateral connections around the first somites to the posterior cardinal vein which interconnected again with the vertebral arteries through the first intersomitic grooves. The anterior cardinal vein on the right side was constricted in the region between somites one and two and no lumen could be seen for two sections. The anterior cardinal veins were directly continuous posteriorly with the posterior cardinal veins with no point that could be considered to divide the two veins.

Both posterior cardinal veins were open vessels posteriorly to the sixth somite beyond which there were lines of vasofactive cells to the tenth somite with disconnected lumina as far back as the seventh somite on the left and the tenth somite on the right. Connections were present between the posterior cardinal veins and the dorsal aortae in a few places, specifically in one embryo at the fifth and sixth intersomitic grooves on the left and at the third intersomitic groove on the right side.

The umbilical veins were of considerable extent in this stage. The lumina were continuous from somites two to intersomitic grooves five but were discontinuous posteriorly to the eighth somites, being connected by strings of vasofactive cells. On the left side, the lumen was again continuous from somites nine to twelve, continuing caudad as a string of vasofactive cells embracing a few small lumina to the middle of the segmental plate region. The lumen of the right umbilical vein lateral to somite eight was large, decreasing in diameter posteriorly to its termination lateral to the middle of the

segmental plate. At the level of the eighth somite, the right umbilical vein connected ventrally with a vitelline vessel and again at the level of the tenth somite, another umbilical-vitelline connection was imminent. Both umbilical veins were connected by veins through the lateral body wall to the corresponding posterior cardinal veins: two on the left, lateral to somites three and four (Plate II, Fig.1); and three on the right, off somites three, four and eight.

Anterior to the second somites, both umbilical veins curved laterally across the dorsal wall of the sinus venosus where they connected to vitelline vessels (two on the right, and three on the left side) lateral to the anterior ends of the first somites. At the point where the umbilical-vitelline vein crossed the sinus anlagen there was definite erosion on the right side, with only a single cell layer separating the two cavities; while on the left side an opening had been effected allowing blood flow from umbilical into the anlaga (Plate II, Fig.1). The connections between umbilical and posterior cardinal veins described above now permitted blood to move from the cardinal veins to the umbilical veins so an embryonic circulation had become established.

### Fifteen-Somite Stage

The anlagen of the sinus venosus were still separate, connecting laterally with seven or eight vitelline veins lateral to somites two through six. Atrium, ventricle, and bulbus were distinct vesicles, with the ventricle much larger than the atrium and displaced to the right thus forming the sigmoid loop so prominent in later

stages. The aortic sinus formed a central cavity within the floor of the pharynx at the end of the bulbus. The first aortic arches appeared as anterior bifurcations of the aortic sinus, and the second aortic arches appeared as posterior bifurcations.

The first and second aortic arches were well defined and in the same relationship with the pharyngeal pouches and auditory placodes (Plate III, Fig.2) as was described in the previous stage. The second arch was a little larger than before and the connection to the aortic sinus was slightly more anterior than that to the dorsal aortae. The third aortic arches also were about the same as in the previous stage with the lumina continuous around the side of the pharynx and the double-layered strings of vasofactive cells extending to the aortic sinus. The third arches were on the level slightly craniad to the first somite. The fourth aortic arches appeared as in the previous stage with lumina only as small lateral pockets on the aortae.

The internal carotid arteries were turned slightly ventrad due to the angle of the cephalic flexure. Each ophthalmic artery curved laterally from its base and terminated anterior to the wall of the optic vesicles. The middle cerebral arteries were much the same as described for the foregoing stage each connecting by a slender vessel to an anterior cardinal vein. The posterior communicating arteries coursed posteriorly from their bases under the mesencephalon and connected broadly with the anterior cardinal veins at the isthmus (Plate III, Fig.2).

The paired dorsal aortae were a little larger in circumference anteriorly than they were in the 12-somite stage, decreasing in size posteriorly with the lumina obliterated about one-fourth the way back

under the segmental plate. A short string of vasofactive cells continued in the aortic line slightly beyond the middle of the segmental plate. The distance between the aortae had been noticeably reduced at the level of the auditory placodes.

Intersegmental arteries branched from the dorsal wall of the aortae in each intersomitic groove anterior to the fourteenth somite, connecting dorso-mesially with the vertebral artery from the first to the tenth intersomitic grooves (Plate III, Fig.2). Caudad to the eighth intersomitic groove these arteries were progressively shorter. The twelfth intersegmental arteries extended to the level of the vertebral arteries but no sinuses were observed at that point. The thirteenth and fourteenth intersegmental arteries were only dorsal evaginations from the dorsal aortae. The continuous lumina of the vertebral arteries extended slightly posterior to their connection with the ninth intersegmental arteries, followed by isolated sinuses at the dorsal tips of the tenth and eleventh intersegmental arteries (Plate III, Fig. 2).

Vitelline arteries connected to the lateral surface of the dorsal aortae at irregular intervals from the ninth somite to the end of the formed aortae beyond which strings of vasofactive cells connected or almost connected the vitelline plexus with the vasofactive cells in the aortic line.

The anterior cardinal veins were crowded ventro-mesially from their straight course by the increased size of the fifth and seventh cranial nerve ganglia and the auditory placode. Short vessels on each side ventral to the tenth cranial nerve connected the anterior cardinal veins to the dorsal aortae (Plate III, Fig. 2). The

anterior cardinal veins connected with the vertebral arteries anterior to the first somite beyond which the cardinal veins coursed ventro-laterally and continued caudad lateral to the somites into the posterior cardinal veins. Posterior to the middle of somite eight the posterior cardinal veins consisted of strings of vasofactive cells continuous to the fourteenth intersomitic grooves, with 13 small regularly spaced lumina between the eighth and fourteenth somites.

The umbilical veins were connected to the dorsal side of the sinus venosus mesial to the connection of the sinus with the vitelline veins (Plate III, Fig.2). The umbilical veins had continuous lumina to the tenth somites and were represented posteriorly by occasional lumenated clumps of vasofactive cells to the end of the somites; and as isolated vasofactive cells to a point midway between the last intersomitic groove and the tip of the tail.

Seven lumenated vessels connected the umbilical veins to the cardinal veins between the margin of the anterior intestinal portal and the seventh somite. Farther caudad there were strands of vasofactive cells between the umbilical and cardinal veins.

Seventeen-Somite Stage

The unfused portions of the sinus anlagen were connected laterally with several vitelline veins, and met mesially in a single sinus venosus. The connections with the cardinal veins extended antero-dorsally as lateral wing-like projections from the central cavity of the sinus venosus. The sigmoid curvature of the

heart tube was more pronounced than in the 15-somite stage, with the atrium protruding slightly to the left, and the ventricle to the right.

The first and second aortic arches were increased in size in proportion to the rest of the embryo (Plate II, Fig.2). The third aortic arches were still small but the lumina were continuous into the aortic sinus. The fourth aortic arches extended laterally from the dorsal aortae, ventral to the anterior end of the first somite, and curved ventrally around the pharynx. The lumina of the fourth aortic arches ended under the ventral edge of the foregut and the arches were continuous to the posterior edge of the aortic sinus as double-layered strings of vasofactive cells.

The internal carotid arteries extended ventro-laterally from the curvature of the first aortic arches. The middle cerebral arteries extended around the posterior walls of the optic vesicles and ended as small vessels connecting to the anterior cardinal veins.

The posterior communicating arteries branched from the middle cerebral arteries posterior to the optic vesicles, followed the curvature of the prosencephalon to the base of the mesencephalon and were connected by small anterior, dorsal, and posterior vessels with the anterior cardinal veins.

The dorsal aortae in the head region were flattened dorso-ventrally, appearing oval or pear-shaped in cross section with the wider part of the lumina laterally. The sides of the tubes were inclined dorsally bringing the horizontal axis diagonal to the horizontal axis of the embryo (Plate II, Fig.2). The mesial edges were ventral to the neural tube, and as verified by later observations,

the dorsal aortae were moving closer to the medial line. Through the region from the anterior intestinal portal to the tenth somite, the aortae were less flattened and more nearly parallel to the horizontal axis of the embryo. Each aorta terminated in a point of vasofactive cells at the level of the posterior intestinal portal.

Intersegmental arteries branched dorsally from the aortae and connected with the vertebral arteries from somites one to 15 with dorsal evaginations at intersomitic grooves 15 and 16 not yet connected. The vertebral arteries made broad connections with the anterior cardinal veins anterior to the first pair of somites (Plate II, Fig. 2), and extended posteriorly to the seventeenth somites. The lumina were continuous to the eleventh somites posterior to which they were constricted and in most places only strings of vasofactive cells were present. Small vessels connected the vertebral arteries to the posterior cardinal veins at each intersomitic groove anterior to the tenth somite (Plate II, Fig.3). The four anterior-most of these vessels were joined with the middle portion of the intersegmental arteries making a Y-shaped arrangement of vessels with the aortae, vertebral arteries and the posterior cardinal veins.

Vitelline arteries connected from the vitelline plexus to the lateral surface of each dorsal aortae in 12 or 13 places between somites nine and the tail bud. One pair of vitelline arteries extended from the dorsal aortae above the posterior intestinal portal, ventro-laterally through the splanchnopleuric fold, then posteriorly along the ventral surface of the allantois, anastomosing medially, forming a lumen under the expanding allantois. This pair of vessels

was interpreted to be allantoic arteries.

The anterior cardinal veins from their beginning as small vessels dorsal to the telencephalon enlarged posteriorly with dorsal expansions behind the fifth cranial nerves extending over the postero-dorsal sides of the ganglia. Enlargement of the auditory placodes had resulted in constriction of the veins forming small lumina dorsal and larger ones ventral. Posteriorly the lumina rejoined, were crowded mesially by the ninth and tenth nerves, became irregular in shape then curved ventro-laterally from the junction of the vertebral arteries anterior to somite one. From a point lateral to the second somite, each anterior cardinal vein curved ventrally connecting to the posterior cardinal vein then continuing as the common cardinal veins to the sinus venosus. The common cardinal veins connected at an angle ventrally with the antero-lateral portions of the sinus venosus, lateral to intersomitic groove two.

The posterior cardinal veins were connected by two or three small vessels directly to the dorsal wall of the sinus venosus, posterior to which they were gradually reduced in diameter with lumina open to the fourteenth somite. (Plate II, Fig.2) Caudad to that point the veins were double strings of vasofactive cells interspersed occasionally with small lumina back to somite 16.

The umbilical veins (Plate II, Fig. 3) were open from their connection to the postero-lateral wall of the sinus venosus at somite four posteriorly to the twelfth somite, caudad to which they were only strings of vasofactive cells with occasional lumina which terminated in the tail bud. The umbilical and posterior cardinal veins

were interconnected by 17 short veins through the lateral body wall from the fourth to the thirteenth somites, with two more connections forming lateral to somite 14.

Eighteen-Somite Stage

Vitelline vessels had coalesced within the lateral body fold as vitelline veins lateral to the seventh and eighth somites. These veins connected anteriorly with the posterior ends of the sinus venosus anlagen which coursed mesially along the splanchnopleuric fold to the fused portion of sinus venosus. The dorso-lateral portions of the sinus venosus extended anteriorly as wing-like extensions to the common cardinal connections. The dorsal mesocardium had broken over the atrium permitting further increase in the curvature of the heart.

The aortic arches were essentially in the same condition as found in the previous stage. The third aortic arches were smaller than the second but the lumina clearly connected the dorsal aorta with the aortic sinus. The fourth aortic arch lumina did not extend to the lateral margin of the pharynx and the strings of vasofactive cells extended ventrally around the pharynx connecting with the posterior edge of the aortic sinus. The internal carotid arteries maintained the same basic relationship as described above. The ophthalmic arteries had curved further around the anterior wall of the optic vesicles and connected minutely with the tip of the anterior cardinal vein. The middle cerebral arteries were comparable to the foregoing stage and as before connected by small vessels with the anterior cardinal vein dorsal to the posterior

margin of the optic vesicles (Plate II, Fig.4).

The lumina of the dorsal aortae in cross section appeared diagonal to the horizontal axis of the embryo from the aortic arches to the fourteenth somite caudad to which they were relatively parallel to the horizontal axis. The mesial walls of the dorsal aortae were in contact with each other from the tenth somite to about the sixteenth somite, but there was no fusion.

Intersegmental arteries branched dorsally from the dorsal aortae at each intersomitic groove anterior to the eighteenth. The vertebral arteries had continuous lumina from the point of junction with the anterior cardinal vein to about somite 14. Multiple vitelline arteries connected the dorsal aortae with the lateral extraembryonic vitelline vessels from somite ten caudad.

Each allantoic artery was continuous with a rather large vitelline artery at the point where the allantoic artery turned posteriorly into the allantoic mesoderm.

The anterior end of the anterior cardinal veins were curved around the dorsal wall of the optic vesicles and terminated anterior to the vesicles lateral to the tips of the ophthalmic arteries. Behind the ninth nerve, each anterior cardinal vein was subdivided into a plexus with about six passages which again condensed to two and then one vessel a short distance caudad. The veins had lost their simple outline and had formed enlarged irregular sinuses with short pseudopod like branches. Such sinuses were present behind the optic vesicles, lateral to the isthmus, and between the tenth nerve and the first somite.

The lumina of the posterior cardinal veins were continuous to the fourteenth somite. The cardinal veins were connected with the sinus venosus in the same manner as in the previous stage. Umbilical veins had continuous lumina to the sixteenth somite.

Twenty-and Twenty-one-Somite Stage

The extraembryonic vessel plexus fed mesially into the well defined vitelline veins from somites eight to five. The posterior ends of the veins were the same size as the feeder vessels, increasing anteriorly to the size of the posterior ends of the sinus venosus anlagen (Plate IV).

The first and second aortic arches remained prominent in this stage. The third aortic arch had not increased in size from the foregoing stage and the fourth arch was not as distinct as previously described.

The ophthalmic arteries and middle cerebral arteries were developed as in the previous stage. The posterior communicating arteries extended along the lateral base of the prosencephalon from their connection with the middle cerebral arteries (Plate V, Fig.1) to the front edges of mesencephalon and then followed along the base of the mesencephalon to the isthmus.

The dorsal aortae were more rounded in the head region (anterior to somite three) than in previous stages but retained the oval shape posterior to somite three. The mesial walls of the dorsal aortae were in contact and fused with each other from the twelfth to the ninteenth somite. At places the septum formed by the fusion of the

aortal endothelium was broken, specifically in three places in the vicinity of somites 14, 15, and 16.

Intersegmental arteries connected the dorsal aortae to the vertebral arteries through each intersomitic space to the seventeenth somite. There were short dorsal evaginations from the aortae at the seventeenth, eighteenth, and nineteenth intersomitic spaces, but these did not reach the level of the vertebral arteries.

The vertebral arteries had continuous lumina from their continuations with the anterior cardinal veins, anterior to somites one, to the seventh somites (Plate IV). The size of the lumina was smaller than in the 17- or 18-somite stages and the posterior portions were smaller than the anterior ends. Posterior to the sixth intersomitic grooves, the lumina were obliterated between the somitic sclerotomes and the neural tube, remaining open only between somites where connections with the intersegmental arteries were retained (Plate IV). These conditions resulted in the appearance of vesicles at the dorsal terminations of the intersegmental arteries similar to those described at the posterior end of the developing vertebral arteries in the 15-somite stage.

Thirteen or 14 vitelline arteries connected each dorsal aorta with the extraembryonic vitelline vessel plexus posterior to somite nine. The arteries were spaced at intervals of from 30 to 150 microns.

The allantoic arteries were connected with the dorsal aortae as described before. They coursed postero-ventrally from the connections with the aortae into the mesoderm around the allantois, and were fused ventrally (Plate V, Fig.4).

The tips of the anterior cardinal veins had extended around the optic vesicles to a point anterior to the vesicles. Each anterior cardinal vein had extended and branched irregularly, in the region between the optic vesicles and the mesencephalon, forming an anterior venous plexus. Some of the branches of this plexus extended almost to the dorsal median line of the brain where smaller sinuses were forming. Another smaller plexus was formed posteriorly surrounding the fifth nerve. The portions of the anterior cardinal veins between the otocyst and the first somite were enlarged, with projecting branches extending dorsally and ventrally. The anterior cardinal veins curved ventrally under the level of the first somites (Plate IV) into the common cardinal veins which connected with the lateral antero-dorsal wall of the sinus venosus (Plate V, Figs.2,3).

The posterior cardinal veins were smaller than the anterior cardinal veins, opened anteriorly into the common cardinal, and were further connected to the sinus venosus by two small vessels. (Plate V, Figs. 2, 3). The lumina of the posterior cardinal veins were continuous to the fourteenth somite, then discontinuous to the sixteenth intersomitic groove. A vasofactive string continued to the anterior part of the segmental plate region. There were 15 intersegmental veins connecting intersegmental arteries (at the vertebral artery level) with the posterior cardinal veins.

The umbilical veins were continuous from the sinus venousus into the tail region where they were continuous with the allantoic veins which coursed through allantoic mesoderm posterior to the tail, then curved dorsally and anteriorly in the mesoderm extending over the tail (Plate V, Fig.5).

There were 26 vessels horizontally connecting the posterior cardinal veins with the umbilical veins (Plate IV); the first just posterior to the connection of the umbilical veins to the sinus venosus, and the last, lateral to somite 16. There were two incomplete mesial branches from the umbilical vein toward the posterior cardinal veins between the levels of somites 13, and 15 on one side of the younger embryo.

## INTERPRETATIONS AND DISCUSSION

### Vasculogenesis

Although heart development has received special attention in the human by Patten (8) and the origin of the heart in the dog was the subject of a dissertation by Duffey (3), origin of blood vessels has been studied in detail only by Watson (11) whose work seems to have been overlooked or ignored.

Differentiation of blood islands and establishing of the network of vitelline blood vessels has yet to be studied because the vitelline plexus was already present in the youngest embryo available for this study.

Blood vessels were represented in the five-somite embryo only by strands or clumps of vasofactive cells similar to the condition described by Watson (11) in the four-somite cat embryo. Such vasofactive cells occurred principally in two lines on each side between endoderm and mesoderm (Plate I, Fig.1). One line (the endocardial mass) was located under the epimyocardial plate and extended posterior

to the end of the plate a short distance. The second line (anlaga of the dorsal aorta) was not as definite as the first, and in the region anterior to the somites was located lateral to the neural plate continuing posteriorly under the lateral edge of the somites and the mesial margin of the lateral mesoderm. Scattered vasofactive cells occurred between the two lines in the region of the fourth somite, and rows of these cells extended postero-laterally from the endocardial mass to the mesial ends of the vitelline plexus. Scattered vasofactive cells were present off the dorso-mesial margin of the head mesoderm in the position of the future anterior cardinal vein.

The anterior end of each endocardial mass was continued antero-medially by scattered vasofactive cells in the vicinity of the future first aortic arch, joining mesially with the aortic line. The endocardial masses were split by the seven-somite stage forming endocardial tubes nearly the full length of the epimyocardial plates, connected by lines of vasofactive cells posteriorly to the vitelline veins and anteriorly to the first aortic arches which had cavities throughout most of the curves. The dorsal aortae, likewise, had formed cavities as far posteriorly as the seventh somite. By the eight somite stage, the cavity was continuous on each side from the vitelline vein through the endocardial tube, first aortic arch, and dorsal aorta to the anterior end of the segmental plate.

Watson's (11) description of the migration of vasofactive cells from their original scattered positions into lines oriented along future vessel location sites could not be verified nor refuted by the material at hand. The sequence of events in formation of blood

vessels in the dog embryos from five to eight somites closely followed those described by Watson (11) for cat embryos of comparable stages. The sequence appears to be: (1) vasofactive cells increase mitotically; (2) the cells flatten, and shift position such that a double layer is formed in a continuous string (Plate I, Fig. 3); (3) the two layers separate, forming a slit-like cavity (Plate I, Fig.4) which gradually fills with fluid and becomes tubular; (4) separately forming vesicles join by extension of the cavities within the string of vasofactive cells.

As few as two cells at one level in a string could form a vesicle, although in most cases the cross section of a newly formed vessel showed three to six cells in its endothelium. No evidence was found to support Sabin's (9) description of the cavity forming within the cytoplasm of a single cell.

Extension of blood vessels in the early embryos as well as later apparently occurred by differentiation or migration of vasofactive cells beyond the tip of the existing vessel, then separation of the cells in such a way that these cells become directly the endothelium of the extended vessel. Patten (8) described the formation of the endocardium from irregular clusters and cords of mesenchymal cells lying between the splanchnic mesoderm and the endoderm by a similar process as described herein. His (loc.cit.) explanation of dorsal aortae formation varied considerably from Watson's (11) description and the process as seen in the dog. He stated that the dorsal aortae initially formed by cephalic prolongations of the endocardial tubes with extension resulting from hollowing out of cords or knots

of cells of mesodermal origin aggregated along the course of the developing aortae (8).

## Cardiogenesis

Development of the heart in the dog was well covered by Duffey (3) and the present work supports his descriptions. The epimyocardial plates were first visible at the five-somite stage with endocardial masses differentiating by that stage (Plate I, Fig.1). Endocardial tubes opened by seven somites and mesial shifting of the heart anlagen followed concresence of the splanchnopleuric fold, resulting in approximation of the endocardial tubes by eight somites (Plate III, Fig.1). The epimyocardial plates rotated ventrally as they converged mesially bringing the plates together ventrally and dorsally in the region of the bulbus at the ten-somite stage. The endocardial tubes as well as the epimyocardial plates fuse progressively posteriorly such that the ventricle and bulbus were completely fused by 12 somites. The atrial anlagen had fused by 15 somites and the anlagen of the sinus venosus had almost completely fused by 21 somites. The dorsal mesocardium over the bulbo-ventricular junction broke at the 14-somite stage and disintegrated progressively anteriorly and posteriorly. By the 16-somite stage a dorsal mesocardium existed only on the anterior tip of the bulbus and the posterior quarter of the atrium. Loss of the dorsal mesocardium permitted the sigmoid flexure of the heart; the ventricle swung to the right the atrium to the left. By the 20-somite stage, the atrium essentially occupied the left half of the pericardial cavity and the ventricle the right half.

The formation of the sinus venosus and lateral mesocardium are of particular interest. The anlagen of the sinus venosus were the postero-lateral ends of the endocardial tubes (3), overlayed dorsally by extensions of the epimyocardial plate (splanchnic mesoderm) which were separated from the somatic mesoderm by a well defined coelom. The anterior ends of the sinus anlagen converged by the 17-somite stage, but already circulation had become established, the sinus tubes were expanded with blood, and the head fold had progressed posteriorly. These factors resulted in pressure contact and subsequent fusion of the edge of the lateral body wall with the splanchnic mesoderm of the sinus anlaga, thus forming the lateral mesocardium. As the sinus anlaga converged mesially, this lateral connection between the wall of the sinus and the body wall was maintained, and the common cardinal veins developed through it.

## The Origin and Development of Arteries

Aortic Arches. All aortic arches developed from strings of vasofactive cells. The outline of the first aortic arches was distinguishable in the five-somite embryo as discontinuous rows of cells extending mesially to the anlagen of the dorsal aortae under the future mesencephalon and laterally to the anterior end of the endocardial mass. As there was no foregut or splanchnopleuric fold at this stage the endoderm in this region was stretched flat under the embryonic rudiment, so the anlagen of the first aortic arches were vertically flat arcs. Distinct lumina had developed in the mesial anterior curvature of the arches by the seven-somite stage

and became continuous with the cavities of endocardial tubes by the eight-somite stage (Plate III, Fig.1). As the splanchnopleuric fold formed, the endocardial tubes and lateral ends of the first aortic arches were drawn ventrally and mesially, resulting in fusion of the anterior tips of the endocardial tubes in the region of the aortic sinus followed by progressive fusion posteriorly. Thus, mechanically, due to the time of formation and mechanics of folding, the first aortic arches after the 12-somite stage, coursed antero-laterally from the aortic sinus, then curved dorsally and posteriorly around the front of the gut.

The second aortic arches were defined in the ten-somite embryo by lumina extending laterally from the dorsal aortae around the edges of the pharynx, continuous ventro-mesially to the aortic sinus as strings of vasofactive cells. Earlier anlaga of these arches were not recognized. By the 11-somite stage the lumina were continuous from the dorsal aortae into the aortic sinus.

The third aortic arch formed similarly during the time of development of somites ten to 17. The fourth aortic arches were represented by vasofactive cells both dorsally and ventrally in the 12-somite embryo, with lumina from the dorsal aorta to the lateral margin of the gut by 15-somites, but the dorsal and ventral lumina had not yet joined in the 21-somite embryo.

No detailed description of the formation of the aortic arches for any mammal was found in the literature. Watson (11) described the lines of vasofactive cells from which the first aortic arches developed, but the general concept has been "from the aortic sac

"the several aortic arches radiate and curve upward around the pharynx to reach the dorsal aortae"(1).

Cranial Arteries. The first observation of the cranial arteries was made in the eight-somite stage where short internal carotid arteries branched anteriorly from the curvature of the first aortic arches (Plate III, Fig.1). Their first appearance was similar to that described for the nine somite cat by Watson (11).

In the 11-somite stage the internal carotids branched anteriorly ventral to the optic vesicles as the ophthalmic arteries (5,8) and dorsally as the middle cerebral arteries (8) which coursed along the posterior wall of the optic vesicles. An additional pair of branches, the posterior communicating arteries (5,8), from the internal carotid arteries appeared in the 12-somite stage. All three sets of arteries at this stage branched directly from the tip of the internal carotid arteries. The subsequent increase in the angle of the cephalic flexure resulted in mechanical orientation of the internal carotid arteries to an antero-ventral course. Along with this change, the bases of the posterior communicating arteries shifted from the tip of the internal carotid arteries proper to the middle cerebral arteries posterior to the optic vesicles (Plate II, Fig.4).

The ophthalmic arteries gradually increased in length until their distal tips were halted by contact of the head ectoderm with the base of the optic vesicles. With later development these arteries extended around the optic vesicles and in the last stage, ended beyond the anterior wall of the vesicles anastomosing with the tips of the

anterior cardinal veins (Plate IV).

The middle cerebral arteries extended to the dorsal level of the optic vesicles where they connected with the anterior cardinal veins (Plate III, Fig.2). The posterior communicating arteries coursed posteriorly, close to the lateral wall of the prosencephalon to the mesencephalon where they connected through small vessels with the anterior cardinal veins. In the last stages, the posterior communicating arteries continued under the mesencephalon to the isthmus. At this point they turned laterally a short distance and connected through anterior, dorsal, and posterior vessels to the anterior cardinal veins. These connections must be comparable to those reported by Watson (11) by which the "veno capitis medialis" (anterior cardinal vein) communicated with the dorsal side of the apex of the first aortic arch.

The Dorsal Aorta. The anlagen of the dorsal aortae were described above as consisting of irregular lines of vasofactive cells between endoderm and mesoderm, lateral to the midline of the five-somite embryo (Plate I, Figs. 1, 2). These lines of cells were continuous on each side from the level of the prosencephalon to beyond the last somite in the seven-somite embryo, with lumina formed under the rhombencephalon posteriorly to the sixth intersomitic grooves. The lumina were continuous in the eight-somite embryo to the potential ninth somites.

Formation of a blood vessel from vasofactive cells was most clearly seen in the formation of the dorsal aortae. In the caudal portion of the aortae the vasofactive cells were oriented into two

distinct layers and interconnected by long cytoplasmic processes. The nuclei of one layer of cells were alternated with the nuclei of the other layer so that the layers were tightly enmeshed with each other in a gear-tooth-like relationship. Anterior to the enmeshed portion of the aortae, the layers were split apart forming a flattened tube (Plate I, Figs. 3, 4).

The dorsal aortae continued to elongate posteriorly by connection with newly formed cavities within the vasofactive cell layers until they reached nearly to the tail bud of the 18-somite embryo. The cavities of the aortae were continuous in the 20-somite embryo to a point slightly posterior to the posterior intestinal portal, with vasofactive cell lines visible for 40 to 50 microns farther.

The cavity of each dorsal aorta was formed as a transverse slit opening to become eliptical, oval or round in cross section as pressures around the vessel dictate. In general, the vessel was found to be flattened dorso-ventrally (Plate II, Figs. 2, 3).

The paired dorsal aortae were closely parallel from the time of their formation until the 15-somite stage at which time there occurred a convergence of aortae toward the midline in the region of the anterior somites. Convergence continued until the 20-somite embryo showed the aortae in contact and fused in the region of somites 12 to 19 diverging both anteriorly and posteriorly to their original relationships.

Branching of the aortae occurred in the same manner as did elongation of the original artery; strings of vasofactive cells formed in the position of the future artery, then the strings split forming

tubes. Opening of the cavities of new vessels regularly progressed from the aortae outward along the line of cells, but separate vesicles were not infrequently seen within the line of vasofactive cells.

Intersegmental Arteries. Formation of intersegmental arteries began in intersomitic groove one (eight-somite embryo) and progressively more arteries were formed posteriorly. Caudad to the last complete intersegmental arteries were arteries in decreasing stages of development (Plate III, Fig.2). In any particular stage of development, the most posterior indication of a forming intersegmental artery was a slight evagination of the dorsal wall of the dorsal aorta. The ratio of complete intersegmental arteries to the number of somites was low in the first stage in which they were found, but the ratio increased slowly with age until in the last three stages the arteries lagged behind somite formation by only two or three.

Vertebral Arteries. The vertebral arteries differentiate from the original line of vasofactive cells lateral to the neural tube. Lumina seem to be formed subsequent to the connection of the intersegmental arteries with the potential vertebral arterial strings (Plate III, Fig.2 ). The first vertebral arteries appeared as spindle shaped vesicles about 30 microns long at the dorsal termination of the first pair of intersegmental arteries. From the initial formation at the point of connection with the intersegmental arteries, lumina extended both anteriorly and posteriorly so that when a new connection between an intersegmental artery and the string of

vertebral cells was made, the lumina from the preceding connection had extended posterior almost to the new point of contact. The posterior extent of continuous lumina of the vertebral arteries lagged about three or six somites anterior to the formation of the last complete intersegmental arteries. The caudad extent of the strings of potential vertebral artery cells was generally slightly posterior to the last intersegmental arteries.

Vitelline Arteries. Vitelline arteries were first found in the eight-somite embryo although vitelline vessels were present in the extraembryonic splanchnopleure of the five-somite stage with lines of vasofactive cells extending from the plexus mesially almost to the aortic line lateral to the second and third somites. In the eight-somite embryo, there were three or four vitelline arteries on each side lateral to somites five, six and seven. In the 11-somite embryo there were five arteries lateral to somite six to the anterior end of the segmental plate. In the 15-somite and later stages, there were nine to 13 vitelline arteries, all posterior to somite eight. In all cases from the eight- to the 17-somite stages, lines of vasofactive cells from the vitelline plexus made contact with the aortic line posterior to formed vitelline arteries. Obviously, the vitelline arteries were ingrowths from the vitelline plexus to the dorsal aortae forming progressively posteriorly as the embryo differentiated, and becoming reduced from anterior to posterior as circulation became more effectively established (Plates III, IV).

The Allantoic Artery. The allantoic arteries distinctly arose as modifications of vitelline arteries that entered the embryo from the yolk-sac posterior to the tail. Such vitelline vessels were present in the future allantoic mesoderm posterior to the tail bud of the 15-somite embryo; they became involved with the allantoic evagination (17-somite embryo), and were thus extended around the expanding allantois (18-and 20-somite embryos). These vessels had joined the tips of the dorsal aortae by the 17-somite stage, maintaining connections with both the allantois and the yolk-sac throughout the remaining stages covered in this study (Plate V, Fig.4).

## The Origin and Development of Veins

The Anterior Cardinal Veins. Anterior cardinal veins were first observed in the head region of the eight-somite stage in the position occupied by lines of vasofactive cells in the five-somite embryo. Their anterior ends were connected through small vessels (future middle cerebral arteries), to the internal carotid arteries and posteriorly they terminated at the ninth cranial nerves (Plate III, Fig.1). Caudad to this point there were strings of vasofactive cells embracing occasional lumina. The strings of cells extended to the fifth somites, the portions posterior to the first somites representing the future vertebral arteries.

The course of the anterior cardinal veins at their first appearance was straight, within the mesoderm lateral to the neural tube, passing ventro-mesially to the auditory placode. With advances in development, the lumina of the anterior cardinal veins extended both anteriorly and posteriorly. In the last stages studied, the anterior

cardinal veins curved around the optic vesicles and their tips ended anterior to the optic vesicles lateral to the terminal ends of the ophthalmic arteries with which they anastomosed. The veins became enlarged and irregular in outline in advanced stages as associated structures (auditory placode and cranial nerves) enlarged and forced slight deviations from a straight path. In the last developmental stages, anterior, middle, and posterior plexuses formed behind the optic vesicles, lateral to the isthmus, and between the otocyst and somite one respectively (7, 8) (Plate IV). The anterior cardinal veins remained continuous, through the stages studied, with the vertebral arteries and the posterior cardinal veins.

Posterior Cardinal Veins. The posterior cardinal veins developed on each side from a line of vasofactive cells lateral to the somites and dorsal to the nephrotomes. Lumenation of these veins was first seen in the 12-somite embryo with continuous lumina to the sixth or seventh somites and discontinuous vesicles as far posteriorly as the ninth intersomitic groove. The lumina were generally better defined in subsequent stages but in the posterior portions where strings of vasofactive cells were developing into endothelial layers, discontinuous lumina were commonly present except at the caudal extremity (Plate III, Fig.2). The posterior extent of the forming posterior cardinal veins lagged behind somite development but in the last stages the strings of vasofactive cells were seen behind the somites lateral to the segmental plate.

The posterior cardinal veins were connected anteriorly with the anterior cardinal veins medially to the vertebral arteries and laterally to the umbilical veins, producing a considerable plexus of blood

vessels throughout the somite region (Plate III, Fig. 2).

Umbilical Veins. The first appearance of the umbilical veins occurred in the eight-somite stage where they had discontinuous lumina from the level of the anterior end of somite one to the level of intersomitic groove three. Anteriorly and posteriorly strings of vasofactive cells were present in the future umbilical line within the somatic mesoderm mesial to the lateral limiting sulci. The lumina became confluent, larger in diameter, and more extensive posteriorly in later stages. In the 12-somite embryo, the umbilical vein had made connection on one side with the dorsal wall of the sinus venosus anlaga at its postero-lateral limit (Plate II, Fig.1). Connections were made on both sides by the 15-somite stage and were maintained throughout the later stages studied. The posterior extensions of the umbilical veins exceeded that of the vertebral arteries or posterior cardinal veins. In the 12-somite stage, they had proceeded posteriorly one third the length of the segmental plate but the outlines of the lumina were irregular with constrictions in some places and only connecting strings of vasofactive cells between discontinuous lumina in other places. By the last stage studied, the lumina were uniformly large posteriorly to the sixteenth intersomitic groove with constricted but continuous lumina caudad. Lateral to the tail bud within the body stalk, the umbilical veins were continuous with the allantoic veins which curved dorsally into the body stalk mesoderm (Plate V, Fig.5).

Common Cardinal Veins. The common cardinal veins seemed to originate by approximation of the sinus venosus anlagen and the primitive cardinal veins. A functional connection was established in a 12-somite embryo by a rather odd arrangement of veins. An anterior branch of vitelline vein was connected dorsal to the anlaga of the sinus venosus with the anterior end of the umbilical vein. On the right side, an opening between this vitelline-umbilical vein and the sinus anlaga was imminent; the opening was functional on the left side. This connection from umbilical vein to sinus, with the connections between cardinal vein and umbilical vein permitted blood passage from cardinal veins to the sinus venosus anlaga. The 15-somite embryo had no vitelline vein connections with the anterior end of the umbilical veins, but the umbilical veins were connected to the sinus anlagen quite similarly to the connection in the 12-somite embryo.

In the 17-somite embryo the umbilical vein opened into the posterior surface of the sinus anlaga as it did in the 15-somite embryo. A new connection had developed between the sinus and the curve of the cardinal veins lateral to somite two, plus two smaller openings posteriorly between the dorsal wall of the sinus and the cardinal veins. By the 20- and 21-somite stage, the opening between cardinal vein and sinus venosus had been enlarged apparently by further disintegration of the wall separating them (Plate V, Figs.2,3). Further closure of the anterior intestinal portal and concrescence of sinus anlaga had pressed the sinus venosus posteriorly resulting in the part of the sinus that was connected with the cardinal vein

being stretched into wing-like extensions. These developments indicate that the common cardinal vein is formed by contact, fusion, disappearance of the dividing wall, and subsequent stretching of the resultant structure.

No description of the formation of the common cardinal veins in any mammal was found in the literature but Patten (8) and Padget (7) described the common cardinal veins in the human as single vessels connecting at right angles between the cardinal veins and the sinus venosus.

## Interconnecting Vessels

There was no mention in the literature of interconnections of veins to veins, or veins to arteries in mammal embryos, comparable in age and size to the embryos used in this study, except the one description of internal carotid artery to anterior cardinal vein (11). Watson's (11) description of carotid-cardinal connections was expanded in this study to include multiple connections larger than capillary size from all three branches of the internal carotid arteries with the anterior cardinal vein (Plate IV). There were several temporary vessels present in the eight-, 12- and 15-somite stages. The dorsal aortae were connected broadly with the anterior cardinal veins ventral to the seventh cranial nerves in the eight-somite stage and ventral to the ninth cranial nerves in the 12-and 15-somite stages (Plate III, Fig.2). A vessel branched laterally from the aortae and coursed dorso-mesially, lateral to the anterior cardinal veins, ending near the dorsal surface of the neural tube on one side of the eight-somite embryo.

The anterior cardinal veins were found to be directly continuous with the vertebral artery at the level of the first somite, apparently as the result of formation of a vessel lateral to the neural tube from a continuous line of vasofactive cells (Plate II, Fig. 2).

The vertebral arteries served as the terminus for intersegmental arteries, thus forming a secondary longitudinal artery from the eight-somite stage to later than the 21-somite stage.

The vertebral arteries were secondarily connected by intersegmental veins to the posterior cardinal veins with the first connection being formed through the first intersomitic groove in embryos of 12 somites, and progressively posteriorly with seven interconnections in the 15-somite embryo and 15 in the 21-somite embryo (Plate II, Fig.3; Plate III, Fig.2; Plate IV). At first the intersegmental veins connected into the lateral surface of the vertebral arteries. Later, however, they tended to shift to make connection with the intersegmental arteries immediately ventral to the vertebral artery. As the vertebral artery regresses, the intersegmental artery-vein maintains connections between dorsal aorta and posterior cardinal vein.

The umbilical vein and posterior cardinal vein on each side were interconnected by irregularly spaced vessels through the lateral body wall. The first such connections appeared in the 12-somite embryo with two connections lateral to somites three and four. More connections developed posteriorly as the cardinal and umbilical veins progressed, with 15 in the 17-somite embryo and 26 in the 21-somite embryo (Plate II, Fig.1; Plate III, Fig.2; Plate IV).

Vitelline veins sometimes bipassed connection with the ends of the sinus venosus anlagen and joined the anterior end of the umbilical veins as seen in the 12-somite embryo. Secondary connections between the umbilical veins and vitelline vessels were found in the 12-somite embryo; on the right side, an umbilical-vitelline connection was patent lateral to somite eight and in another spot lateral to somite 10, somatic and splanchnic mesoderm were fused and an opening obviously forming between the adjoining umbilical and vitelline veins.

## SUMMARY

A review of the literature revealed inadequate descriptions of the origin or arrangement of blood vessels in mammals.

In a study of vasculogenesis, 16 serially sectioned dog embryos, 17 to 19 days gestation (five- to 21-somites), were examined and reconstructed. All embryonic blood vessels originated from vasofactive cells which accumulated along the future vessel paths. The cells became arranged in double, enmeshed layers which progressively split forming a lumen.

Paired endocardial tubes formed ventral to the epimyocardial plates from the endocardial mass of the five-somite embryo, to vesicles in the seven-somite embryo, to tubes continuous with vitelline veins and first aortic arches in the eight-somite embryos. Fusion of endocardial tubes began in the region of the aortic sinus in the ten-somite embryo and progressed posteriorly, with the sinus venosus not completely fused by 21 somites.

The first aortic arches formed from pre-existing vasofactive cells, completely lumenated by the eight-somite stage. The second aortic arches were outlined in the ten-somite embryo, complete in the 11-somite embryo. The third aortic arches were determined by lines of vasofactive cells in the ten-somite embryo, with the lumina continuous in the 17-somite embryo. The fourth aortic arch was observed in the 12-somite embryo but was not yet continuous in the 21-somite embryo.

Internal carotid arteries in the eight-somite embryo were connected to the anterior cardinal veins through the future middle cerebral arteries. Ophthalmic branches appeared in the 11-somite stage and connected to the anterior cardinals by 20 somites. Posterior communicating branches arose at the 12-somite stage and connected to the anterior cardinals by the 15-somite stage.

The dorsal aortae showed lumina in the seven-somite embryo developed from vasofactive strands present in the five-somite stage. The lumina elongated progressively, reaching the tail region by the 17-somite stage.

Intersegmental arteries developed progressively after the first one showed in the eight-somite embryo until 16 were present in the 21-somite embryo.

Vertebral arteries formed and extended by expansion of vesicles on the intersegmental arteries. They connected broadly to the anterior cardinal veins, and extended to somite 14 in the 18-somite embryo but had become obliterated posterior to the sixth somite in the 20- and 21-somite embryos.

Vitelline arteries connected to the dorsal aorta lateral to somites five, six and seven in the eight-somite stage. New arteries formed posteriorly and the old degenerated anterior to the ninth somite.

Allantoic arteries developed as modifications of the posterior-most vitelline arteries, and maintain multiple connections with the vitelline plexus through the 21-somite stage.

Anterior cardinal veins were lumenated before eight somites, connected to the vertebral arteries and the posterior cardinal veins before 12 somites, and became subdivided into plexuses between the 17- and 21-somite stages.

Posterior cardinal veins were first present in the 12-somite embryo and extended beyond the formed somites in the 21-somite embryo. These veins were connected mesially to the vertebral arteries by intersegmental veins and laterally by irregularly spaced vessels to the umbilical veins.

Umbilical veins consisted of a few isolated vesicles in the eight-somite embryo, and extended posteriorly progressively, connecting to the allantoic veins in the 20-somite embryo. Connection with the sinus venosus anlaga was effected in the 12-somite embryo.

Common cardinal veins were formed by approximation of the curve of the cardinals, lateral to somite two, with the dorsal wall of the sinus venosus in the 17-somite embryo.

Several unusual, probably temporary connections were found; anterior cardinal veins connected to dorsal aortae; vitelline veins to the anterior end of umbilical veins; vitelline vein to umbilical

vein lateral to somite eight; and direct continuity of vertebral arteries and anterior cardinal veins.

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The author wishes to express sincere gratitude to Dr. H. T. Gier for the understanding, guidance and helpful criticism given while this study was conducted and during the preparation of the thesis; also for permission to use his excellent collection of dog embryos as material for this study.

Acknowledgment is made to the Kansas State College Department of Zoology for the various facilities and equipment provided for the accomplishment of the study.

# APPENDIX

## ABBREVIATIONS USED IN THE PLATES

| | |
|---|---|
| All A | Allantoic artery |
| All V | Allantoic vein |
| AA | Aortic arch |
| AA An | Aortic arch anlaga |
| ACV | Anterior cardinal vein |
| ACV-DA | Anterior cardinal vein-dorsal aorta connection |
| ACV- Ve A | Anterior cardinal vein-vertebral artery junction |
| AS | Aortic sinus |
| Bu | Bulbus |
| Bu An | Bulbus anlaga |
| CCV | Common cardinal vein |
| DA | Dorsal aorta |
| DAVC | Vasofactive cells of the dorsal aorta |
| En M | Endocardial mass |
| En T | Endocardial tube |
| Ep M | Epimyocardial mantle |
| ICA | Internal carotid artery |
| ISA | Intersegmental artery |
| ISV | Intersegmental vein |
| MCA | Middle cerebral artery |
| OA | Ophthalmic artery |
| PCA | Posterior communicating artery |
| PCV | Posterior cardinal vein |
| PCV-UV | Posterior cardinal vein-umbilical vein connection |
| PCVVC | Posterior cardinal vein vasofactive cells |
| SP | Segmental plate |
| S-A An | Sinu-atrial anlaga |
| SV | Sinus venosus |
| SV An | Sinus venosus anlaga |
| Som | Somite |
| UV | Umbilical vein |
| UV-SV | Umbilical vein-sinus venosus connection |
| UVVC | Umbilical vein vasofactive cells |
| Ven | Ventricle |
| Ven An | Ventricle anlaga |
| Ve A | Vertebral artery |
| VA | Vitelline artery |
| VP | Vitelline plexus |
| VV | Vitelline vein |

EXPLANATION OF PLATE I

Fig. 1. Transverse section of five-somite dog embryo 260 Lb near the posterior end of the epimyocardial mantle showing vasofactive cells in the dorsal aorta line, and in the endocardial mass.

Fig. 2. Transverse section of dog embryo 260 Lb showing vasofactive cells in the dorsal aorta line ventro-lateral to somite three.

Fig. 3. Longitudinal section of seven-somite embryo 260 Rb showing enmeshed layers of vasofactive cells at the posterior end of the dorsal aorta, ventral to the anterior end of the segmental plate.

Fig. 4. Lumen in the dorsal aorta ventral to somite four in a longitudinal section of embryo 260 Rb. The nuclei of the dorsal endothelium alternate with the nuclei of the ventral endothelium.

PLATE I

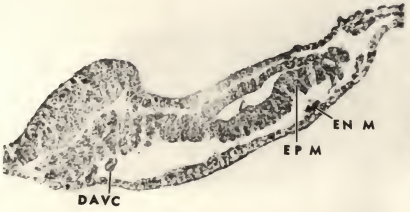



Fig. 1

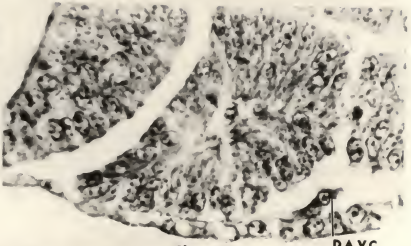



Fig. 2

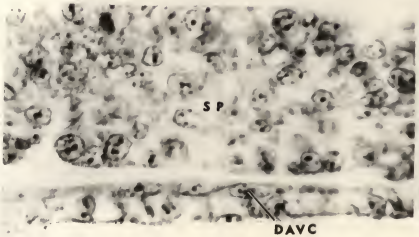



Fig. 3

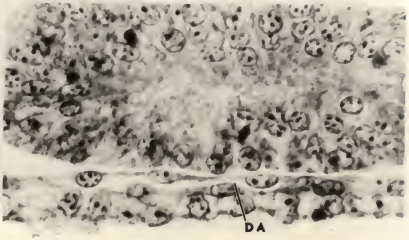



Fig. 4

EXPLANATION OF PLATE II

Fig. 1. Clay reconstruction of the left side of 12-somite embryo 115 Rb illustrating the connections of the umbilical vein to vitelline vessels, the sinus venosus anlaga, and the cardinal vein.

Fig. 2. Transverse section through the anterior cardinal vein-vertebral artery junction anterior to somite one in 17-somite embryo 77 L-1.

Fig. 3. Section through intersomitic groove five of embryo 77 L-1 showing continuity of the dorsal aorta, intersegmental artery, vertebral artery, intersegmental vein, and posterior cardinal vein; the umbilical vein and the posterior end of the vitelline vein are also visible in the section.

Fig. 4 Longitudinal section through the first aortic arch, internal carotic artery, and the bases of the cranial arteries of 18-somite embryo 81 L-2.

PLATE II

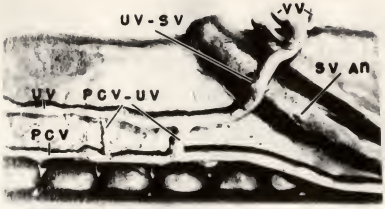



Fig. 1

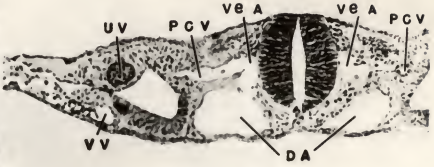



Fig. 3

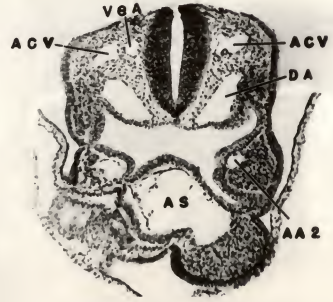



Fig. 2

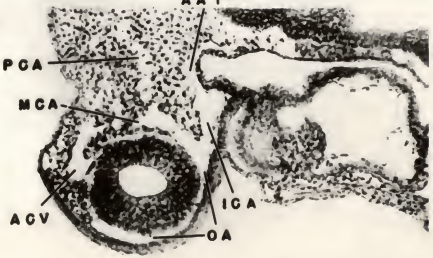



Fig. 4

EXPLANATION OF PLATE III

Fig. 1. Drawing of the vascular system of an eight-somite dog embryo from dorsal view. The outline was made by projection of embryo 45 L-5 and the details filled in from embryo 45 L-6. The original drawings were made at 100X, reduced one half in reproduction.

Fig. 2. Lateral view drawing of the vascular system of an 15-somite dog embryo. The outline made by projection of embryo 120 Rb and the details filled in from saggital sections of 113 Lb. Magnification as in Fig.1.

PLATE III

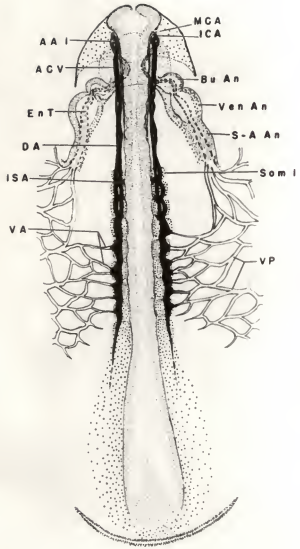



Fig. 1

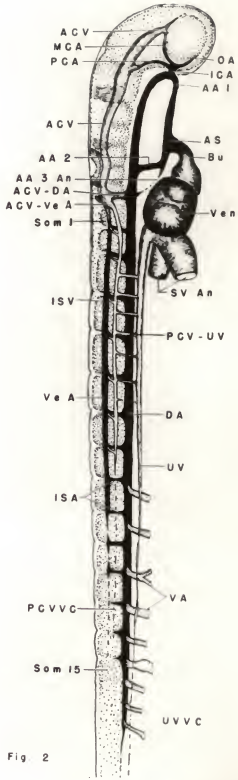
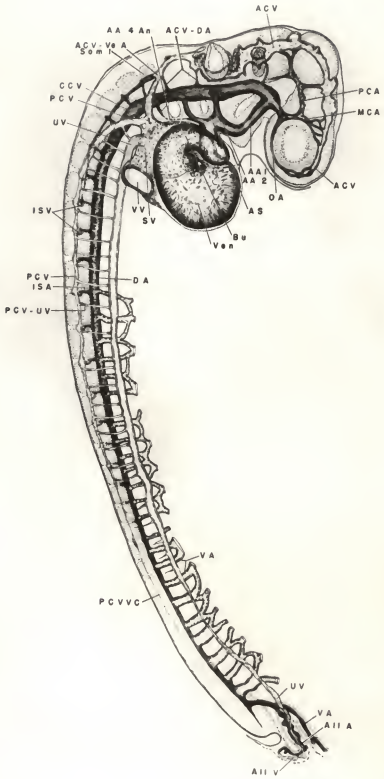



Fig. 2

EXPLANATION OF PLATE IV

Lateral view drawing of a 21-somite embryo. The out-line drawing was made by projection of median saggital section of embryo 116 Rb and the details filled in from the same set of sections and transverse sections of embryo 121 L. The original drawing was made at 100X and reduced to two-fifths in reproduction.

PLATE IV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EXPLANATION OF PLATE V

Fig. 1. Longitudinal section of 21-somite embryo 116 Rb showing the anterior end of the dorsal aorta, the internal carotid artery, the bases of the cranial arteries, and a portion of the anterior cardinal vein.

Fig. 2. Longitudinal section through the heart region of embryo 116 Rb showing the connections of the common cardinal vein, posterior cardinal vein, umbilical vein, and vitelline vein with the sinus venosus.

Fig. 3. Same area as Fig. 2, two sections mesially.

Fig. 4. Longitudinal section through the posterior end of embryo 116 Rb showing the relationship of the allantoic artery with the dorsal aorta and a vitelline artery.

Fig. 5. Longitudinal section through the posterior end of embryo 116 Rb, lateral to the section in Fig. 4, showing the relationship between the umbilical vein and the allantoic vein.

# PLATE V

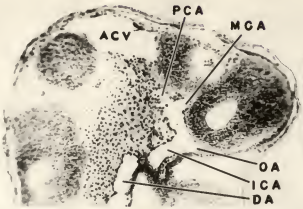


Fig. 1

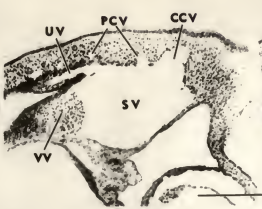


Fig. 2

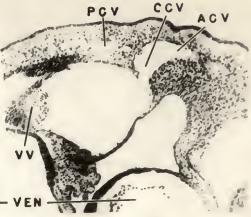


Fig. 3

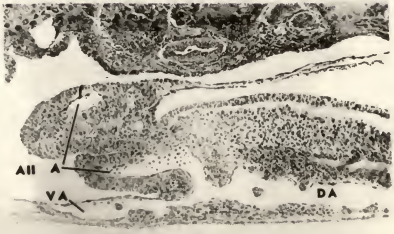


Fig. 4

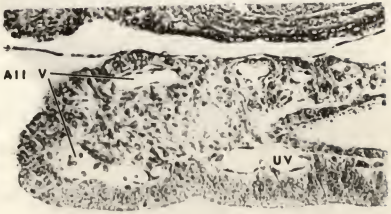


Fig. 5

# THE DEVELOPMENT OF THE VASCULAR SYSTEM IN FIVE TO TWENTY-ONE SOMITE DOG EMBRYOS

by

ELDEN WILLIAM MARTIN

B. S., Kansas State College of Agriculture and Applied Science, 1954

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1958

A review of the literature was made and the literature was found to be grossly inadequate to explain the origin of blood vessels.

In a study of vasculogenesis, 16 serially sectioned dog embryos from five to 21 somites were studied and critical areas reconstructed. Most of these embryos had been studied and drawn as whole mounts before sectioning.

The embryonic vascular system originated from vasofactive cells which accumulated along future vessel paths. The cells became attached to each other by cytoplasmic processes, while arranging into double tightly enmeshed layers which progressively split forming the lumen of the blood vessels.

Paired endocardial tubes formed from loose lines of vasofactive cells ventral to the epimyocardial plates in the five-somite stage by splitting of the masses anteriorly and posteriorly from the center of the primordia. The tubes were formed and continuous anteriorly with the first aortic arches and posteriorly with the vitelline plexus by the eight-somite stage. Fusion of the paired heart primordia and differentiation of the single embryonic heart closely adhered to the pattern set by Duffey (1953) with the exception that the aortic sinus was the first structure formed by fusion of the endocardial tubes. This fusion occurred between the eight-and-ten somite stages in advance of epimyocardial fusion. The sinus venosus was almost completely fused by the 20- and 21-somite stage. Loss of the dorsal mesocardium over the rapidly elongating heart resulted in sharper curvature and displacement in the 17- to 21-somite embryos.

The first aortic arches formed by the seven-somite stage for arcs of scattered vasofactive cells present from the anterior ends of the aortic line to the anterior end of the endocardial mass in the five-somite stage and had continuous lumina from the dorsal aortae to the endocardial tubes in the eight-somite stage. The second aortic arches were forming in the ten-somite embryo and were complete in the 11-somite embryo. The third aortic arches, first present in the ten-somite embryo, were complete by the 17-somite stage. The fourth aortic arches had started to form by the 12-somite stage but were not yet complete in the 20- and 21-somite stage.

Short internal carotid arteries were present and connected with the anterior cardinal veins in the eight-somite stage. Ophthalmic arteries branched ventrally from the internal carotid arteries in the 11-somite stage and their tips connected to the anterior cardinal veins anterior to the optic vesicles in the 20- and 21-somite stage. The connections of the internal carotid arteries with the anterior cardinal veins in the eight-somite stage became directly the middle cerebral arteries. Posterior communicating arteries coursed posteriorly from the internal carotid arteries in the 12-somite stage and communicated around the mesencephalon with the anterior cardinal veins; in the 15-somite stage, their baseshad shifted dorsally to the middle cerebral arteries.

The dorsal aortae showed lumina in the seven-somite stage, developed from the vasofactive strands present in the five-somite stage. The aortae reached the tail bud by the 17-somite stage and

began to fuse in the region of somites 12 to 19 in the 20- and 21-somite embryos.

The progressive formation of intersegmental arteries could be seen first in the eight-somite embryo from posterior to anterior: (1) as slight dorsal evaginations from the dorsal aortae, (2) as evaginations intermediate in length, and (3) as vessels connecting dorsally into the forming vertebral arteries. There were 16 complete arteries in the 20- and 21-somite stage.

Vertebral arteries formed from rows of vasofactive cells which vesiculated upon connection by intersegmental arteries. The vertebral arteries were continuous anterior to the first somites with the anterior cardinal veins. The vertebral arteries extended posteriorly to somites 14 in the 18-somite stage and the lumen were obliterated between the sclerotomes and the neural tube posterior to the sixth intersomitic grooves in the 20- and 21-somite stages.

Vitelline vessels extended mesially, connecting into the posterior portions of the dorsal aortae in the eight-somite stage through rows of vasofactive cells already present in the five-somite stage. Multiple arteries were present posterior to somites nine in the 15-to 21-somite stages.

The allantoic artery arose as a modification of the vitelline arteries posterior to the tail by the 17-somite stage.

Anterior cardinal veins were formed by the eight-somite stage from vasofactive cells present along their future path in the five-somite stage. The veins were continuous anteriorly with the cerebral arteries from the eight-somite stage and posteriorly with the posterior cardinal veins from the 12-somite stage.

The posterior cardinal veins formed from anterior to posterior lateral to the somites from rows of vasofactive cells which were continuous posteriorly to the level of the segmental plate in the 20- and 21-somite stage.

Common cardinal veins formed by approximation of lateral portions of the sinus venosus and the primitive cardinal veins lateral to the second somites, followed by erosion of their adjacent walls.

The umbilical veins originated from rows of vasofactive cells in the somatopleure mesial to the lateral limiting sulci and lumina had developed by the eight somite stage. First connection with the sinus venosus anlaga was made in the 12-somite stage. The posterior ends were discontinuous to the 20- and 21-somite stage where they were continuous with the allantoic veins.

This work represents the most exhaustive effort to date on the analysis of vasculogenesis. Watson's (1924) work on the cat was basically verified, and Duffey's (1953) work on cardiogenesis in the dog was incorporated with the current observations. This report should serve as a basis for future studies in vasculogenesis of other mammals.