CARDIOGENESIS IN THE BOVINE TO 35 SOMITES

by

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TABLE OF CONTENTS

INTRODUCTION

Mammalian cardiogenesis is a vast field which so far has not been thoroughly explored. There are few species in which the complete development of the heart has been studied and many in which partial formation has been observed.

The formation of the heart to the functional stage in the dog (14 somites) was studied by Bonnet (1901) and Duffey (1953). Duffey's thesis was used exclusively for comparison in this paper.

The rat has been studied in some detail, from presomite to birth and after, by Burlingame and Long (1939) and early stages by Ravn (1895) and Goss (1942, 1952).

The tubular phase of cardiogenesis in the rabbit of 10-13 somites was described by Girgis (1930, 1933). Dwinnel (1939) worked with early somite stages. Development of the aortic arches was described by Bremer (1912). Hensen (1875), Born (1888, 1889), Strahl and Carius (1889) and Rouviere (1904) have described early cardiac formation.

There are three theories as to the origin of the vasofactive cells (Field, 1946) or angioblasts (Yoshinaga, 1916). One group (Martin, 1902; Parker, 1915; and Wang, 1917) believes they are of endodermal origin and are from the splanchnic endoderm ventral to the epimyocardial plate. Others (Rabl, 1889; Mollier, 1906; and Shulte, 1914) consider that they originate in the splanchnic mesoderm of the cardiac plate and still others (His, 1880; and Bremer, 1912) suggest that the endothelial cells migrate from the yolk sac to take up their function in the vascular scheme.

Yoshinaga (1916) reviewed the evidence, first proposed by Hensen in 1875 that there were two lateral heart anlagen which fuse to form a median secondary heart tube. He quoted Minot's 1900 statement as confirmation of his

interpretation of the process:

> "In mammals by the bending down of the layers and the expansion of the coelom the vorderdarm is shut off and the lateral heart anlagen are brought together in the median line below the vorderdarm and there they fuse into a single thick tubular wall around the double endothelial heart; it is not long, however, before the endothelial tubes fuse into one...."

In cardiogenesis there are several stages of formation. The primordial stage, double tubular stage, single tubular stage, loop stage, fusion of heart chambers and septal formation. Relatively early in embryonic life the first three and part of the fourth stages are completed.

During the primordial stages the epimyocardial plates were formed and endothelial elements seen. Later single primordial tubular elements fuse medially (epimyocardium and endocardium) and then loop formation or falling back of the heart instigated (Yoshinaga, 1916; and Duffey, 1953).

In canine cardiogenesis the heart anlagen were first seen at three or four somites (Duffey, 1953). The medial shifting of the lateral heart tubes began at the six-seven somite stage. At nine-ten somites the epimyocardial mantles fused on the ventral side of the heart and on the dorsal side the beginning of fusion was observed to be at eleven somites. This preceded fusion of the endocardial tubes. Duffey (1953) placed the dog next to the cat in Goss' (1935) series of arrangement. This was based on the relative length of time in which the individuality of the single primordia was still evident and is as follows: rat, sheep, guinea pig, marsupials, ferret, man, rabbit and cat.

The ventral mesocardium was seldom found in the dog but the dorsal mesocardium occurred in the region of the bulbus and anterior ventricle in the 11 somite embryo due to the fusion of the two retrocardiac plates (Duffey, 1953).

At 11 somites the epimyocardium was fused in the region of bulbus and anterior ventricle. This was the consistent site of first fusion in all mammals studied. The epimyocardial plates were in contact but were not fused in the posterior half of the ventricle. The 13 somite embryo had a markedly progressed cardiac loop and the characteristic "S" shape was found at 14 somites (Duffey, 1953). All indications of circulation were found.

In the guinea pig (Yoshinaga, 1916) the endocardial tubes began to fuse and chambering constrictions occurred by the eight somite stage. Chamber constrictions occur in the guinea pig, according to Yoshinaga (1916) after fusion of the myocardial tubes, as opposed to the prefusion constrictions that occur in the canids (Duffey, 1953), the cat (Schulte, 1916), and rabbit (Girgis, 1933). At nine-ten somites the typical bulging to the right was seen as was the dorsal mesocardium (mid one-third of the ventricle to the atrium). According to Wang (in the ferret), the "loop" form and the subdivisions occurred prior to fusion of the endothelial tubes.

In the marsupials, fusion of the endothelial tubes first occur in the 15-16 somite embryos (Parker, 1915). This was observed to begin at the 12 somite stage in the cat, was nearly complete at 14 somites and complete after loop formation was final at 21 somites (Schulte, 1916).

The epimyocardium was found fused at 12 somites in the feline. Loop formation was initiated at 14 somites and nearly complete at 16 somites. The cat embryo of 14 somites was readily comparable to a human of approximately eight or nine somites (Schulte, 1916). Watson (1924) stated that she found the endocardium continuous with the vitelline veins for a short distance in the eight somite cat embryo. She also noted in the seven somite embryo that the myocardium was separate from endothelium by a space bridged by strands of protoplasm which foreshadowed the thick muscular walls of the ventricle. Of

the ten somite stage, she reported formation of the first aortic arch. Further descriptions agree with those of Schulte with regard to endothelium and epimyocardium.

In the rat development was quite rapid. At three somites the heart was a "broad cresent shaped U" (Goss, 1952). The endocardium, epimyocardium and pericardial cavity were quite evident with anterior and posterior limits of the pericardial cavity being greater than those of the other elements. At five somites the atrial anlagen were lateral but not quite in contact. Heart development of the rat corresponded closely to that of the guinea pig in a three somite embryo, although the eight somite stage of the guinea pig was equivalent to that of a five somite rat. Heart development in the ferret was similar to that of the rat and guinea pig. The rat showed constrictions in the heart at five somites (Goss, 1952).

The Burlingame and Long (1938) description of the rat was more complete in that it covered the entire cardiac development. They observed the presence of angioblasts at two somites and the beginning of circulation at eight somites. The rabbit on the other hand presented quite a different situation in that the cardiac anlagen retain functional independence relatively far into their period of development (Girgis, 1933; 1939). The heart tubes were constricted in the ten somite embryo. The endocardial tubes were separate within the epimyocardial plates which were in contact cranially but not fused (Murray, 1919; Girgis, 1933). The posterior two-thirds of the heart anlagen were separated by the "middle cardiac plate." In the 11 somite rabbit, there were myocardial bulges, the left tube craniolaterally and the right tube ventrocaudally. The epimyocardium was separated only by a groove and the endocardial tubes were in contact in the ventricular area.

According to Girgis (1933), rabbit embryos of 12 somites had a heart

double the size of that in the 11 somite stage. The endothelial tubes were in contact the entire length. At 13 somites, the loop was initiated in its own peculiar way and the endocardial tubes began to fuse. The rabbit seemed to form the left chambers of the heart from the left primordium and the right chambers from the right heart tube. The bulbous and ventricle formed from both tubes, atria from the right and partially the left and the sinus venosus from the caudal portion of the right tube and the expanded cranial end of the right vitelline vein. In general these descriptions agreed well with those of Murray (1919).

Watson (1924) worked with the feline and with marsupials of several species. She stated that in the marsupials she found no primary union of the endocardial tubes such as Wang (1917) found in the ferret. Watson stated that she found vasofactive cells in contact with endoderm but did not concern herself with their origin.

Field's (1946) description of the sheep was very vague. A vascular anlage was found at four somites (15 days). There were "fibers in the space between future splanchnic cells and endodermal cells" (Are these vasofactive cells or precursors?). The six somite stage showed cardiac connection in the region of the foregut. During the eight somite stage the endocardial tubes were well formed, the ventral mesocardium was present and the groove between the ventricle and atrial portions of the tube formed.

There is controversy and terminology confusion in the human studies. One of the first descriptions by Dandy (1910) of a seven somite embryo was sketchy and was criticized by later workers. Davis (1927) described three early stages: plexiform, paired tubular, unpaired tubular. In no stages were the endocardial tubes described as being separate. In the one somite stage, he described the endocardial tube as being bilateral angioblastic areas united by slender bands

of mesenchyme. The two and four somite embryos showed strong "interventricular stands" and "slender interbulbular communications." He described a dorsal mesocardium and constrictions during the paired tubular phase.

In man (Davis, 1927) and in the cat (Schulte, 1916), the bulbular part of the left endocardial tube atrophies and disappears. This was not observed in the rabbit (Girgis, 1933) and mention of this condition was not found for any other mammal studied.

There have been many other aspects of cardiology studied by old and recent workers alike. For example Bennett (1936) did descriptive work on pig embryology. He did most of his work on the sinusoids of the muscular wall of the heart but also observed that the early growth of the pig heart was much like that of the rabbit. Therefore, the pig would be placed at the cat end of Goss' (1935) arrangement according to the relative length of independence of endocardial tubes.

According to Grimes (1959) the bovine endocardial tubes fuse at the six somite stage. Her ten somite embryo of 22 days showed fusion of epimyocardium in the atrial area and a well formed endocardial tube. There was a dorsal extension of the bulbus where it entered the ventral aortic roots. Grimes' 12 somite embryo showed a dorsal mesocardium and at 13 somites a distinct "S" shape. In the 20 somite embryo the heart curvature approached maximum and the horns of the sinus venosus were embedded in the septum transversum and opened dorsally into the atria.

By using the cited material, especially that of Martin and Duffey as a basis for general formation and orientation, a more thorough study of bovine cardiogenesis was undertaken.

METHODS AND MATERIALS

The embryos used in this study were previously prepared for other studies,

or prepared especially for this study when stages were missing. They constitute a continuous series from presomite at 20 days to 35 somites at 25 days. They were obtained from cattle maintained by the Dairy Department at Kansas State University and slaughtered at specific days gestation to obtain embryos and other tissues indicated in the overall study of reproductive processes.

The intact uteri were sketched, condition of the ovaries noted and measurement of organs taken. An incision was made in the uterus and the embryo was retrieved via flotation. In this technique, the uterus was placed in a tub of water and the tiny embryos were detected by the filmy white extra-embryonic membranes (serosa and yolk sac) as they floated free. The embryos were fixed in Bouin's fluid, dehydrated in alcohol and stained with aceto-carmine in 70 per cent alcohol. From 95 per cent isopropyl alcohol, they were cleared in oil of wintergreen and photographed intact.

Several Kodachrome transparencies and black and white pictures were made of each embryo. The embryos were then embedded in paraffin, sectioned at 10 microns and stained with hematoxin-acid fuchsin orange-G. Most of the embryos were sectioned transversely although a representative few were sectioned longitudinally. A few embryos were mounted in toto for detailed overall study.

Age gestation was determined by counting the start of day 0 as the morning following insemination. The number of somites, counted on the cleared whole mount, and rechecked on sections correlated closely with the days of gestation. Some of the embryos taken from cows on which there was no available data were aged by somite counts according to work done by Gier (1960).

Somite counts were made in order to place the embryos in a chart in order of age and to better understand the timing of events studied. When sections were destroyed thus causing difficulty in determination of somite numbers, several previously determined land marks were used to make counts. For example,

Embryo number	Days gestation	Somite number	Crown* rump	Contour*	Type section
335	20	0	2.0 mm	2.0 mm	Cross
17	20	0	2.2 mm	2.2 mm	Cross
361	21	6+	5.5 mm	6.1 mm	Cross
304	21	9	5.4 mm	6.4 mm	Cross
743	21	13	5.4 mm	6.7 mm	W. M.
313	21	19	5.0 mm	7.0 mm	Cross
146	22	22	5.9 mm	8.0 mm	Frontal
X	22	24	6.2 mm	9.5 mm	W. M.
314	23	25	6.5 mm	10.3 mm	Long
Y	23	28	6.1 mm	12.2 mm	W. M.
419	23	28	6.0 mm	12.4 mm	Cross
385-1	24	29	6.1 mm	13.5 mm	Cross
232	24	30	7.0 mm	15.0 mm	Obique
254	24	30	7.0 mm	11.1 mm	Cross
206	24	30	6.0 mm	9.0 mm	Long
415	25	33	7.0 mm	18.0 mm	Cross
234	25	33	7.0 mm	16.0 mm	Cross
420	24	34	6.0 mm	16.4 mm	Long
143	25	34	6.0 mm	16.3 mm	Cross
12	25	35	6.1 mm	16.5 mm	Long

* These measurements were taken in formalin or 80% alcohol, and adjusted to normal if not measured when taken.

W. M. whole mount.

the first somite is one and one-half somite widths posterior to the otocyst, and at 24 days gestation the 30th intersomitic groove is at the posterior border of the pelvic limb bud, the 28th intersomitic groove at the anterior end of the bud.

The embryos were studied from the Kodachrome transparencies, whole mounts and sections. Drawings were made and photomicrographs were taken as necessary to provide adequate interpretation. Comparisons were made directly with canine embryos used by Duffey (1953). Terminology adopted was primarily that accepted by Patton (1946, 1951).

OBSERVATIONS

The presomite 20 day embryo was basically a flat disk with surrounding membranes. The foregut was 35 microns in length. One of the embryos of this group had a blind endodermal tube open on both ends into the dorsal midgut. It had a columnar epithelium and extended for 90 microns, 70 microns of which it was closed. The neural plate was open the entire embryonic length.

The lateral mesoderm was split in a few sections indicating the beginning of an embryonic coelom. A distinct row of cells constituted the area in which the cardiac plate was destined to develop 110 to 170 microns posterior to the opening of the foregut. There were well formed vitelline vessels in the yolk sac with some blood cells in the lumena.

The six somite, 21 day embryo was a slightly arched plate covered by amnion, connected on its concave face to the yolk stalk. The neural plate was thickened but was not clearly defined from the epidermal ectoderm. The neural groove was deep in the region posterior to the cardiac area but in no place was there a tubular form. The notochord in the area of the first somite was as large in cross section as the thickness of the neural plate. The

endodermal fold was a wide arc under the region of the first pharyngeal pouch. The foregut was 130 microns long and 180 microns wide at the anterior tip and 350 microns wide just anterior to the MAIP. There were distinct hypopharyngeal and hyperpharyngeal grooves.

The cardiac anlagen consisted of epimyocardial plates (Plate 1, 6, and Fig. 1), 270 microns long, laterally on either side of the open midgut. Each plate consisted of an arc of cells four or five cells thick and 15 to 20 cells broad with the open face of the arc ventral. The endocardial plate was a band of deeply staining cells, one or two cells thick and four or five cells wide, lying within the concavity ventral to each epimyocardial plate for most of its length (Fig. 1). Anteriorly the cardiac plates faced ventro-medially and lay closer together. They dwindled into a pyramid of cells near the AIP and ended 20 microns anterior to the edge of the MAIP. There were cells ventral and anterior to the foregut that were continuous with the endocardial cells and were interpreted to be the vasofactive cells of the first aortic arch.

The yolk sac contained numerous endothelial lined blood vessels in its wall, some of which extended as lines of vasofactive cells toward the embryonic disk, but none of these lines was traceable to the endocardial plate. The embryonic coelom had formed anterior to the MAIP by splitting of the mesodermal mass, and extended posteriorly to the region of the first somite. Further posteriorly there was not always a distinct coelomic lumen, although there was a definite cytoplasmic break where the coelom would form posteriorly beyond formed somites. In the region of the second somite the extra embryonic coelom was narrowly continuous with the embryonic coelom.

The nine somite, 21 day stage was represented by one embryo which was disoriented in processing. Measurements were taken before fixation. The anterior portion formed a U and the tail was bent upon itself. Basically it

EXPLANATION OF PLATE I

Figures on this plate represent the bovine heart form and each stage represented is indicated by the somite number accompanying each drawing.

Stage 6. A representative drawing of the six somite embryo's paired cardiac anlagen showing epimyocardial plates and endocardial tubes.

Stage 9. A nine somite embryo showing the first ventricular fusion. The series runs from posterior to anterior.

Stage 19. The characteristic shape of the heart as seen in the 19 somite embryo, showing the sinusoids of the liver.

Stage 22. The heart form of the 22 somite embryo showing the connection of the left umbilical and vitelline veins and the right and left ducts of Cuvier.

Stage 25. The heart as seen in the 25 somite embryo.

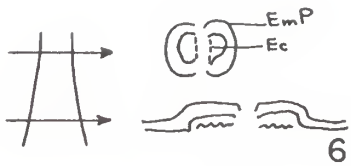
Stage 30. The heart of the 30 somite bovine with internal ventricular structures shown.

Stage 35. The heart of the 35 somite bovine showing internal and external form.

ABBREVIATIONS USED IN THIS PAPER

A-atrium, atria; AA-aortic arch; Au-auricle; AV-Atrio-ventricular notch, foramen, valves; AS-aortic sinus; CC-common cardinal vein; DA-dorsal aorta; DC-duct of Cuvier; Ec-endocardium; EcC-endocardial cushion; EcT-endocardial tube; Em-epimyocardium; EmP-epimyocardial plate; FG-foregut; IaS-interatrial septum; IvS-interventricular septum; L-left; NP-neural plate; MAIP-margin of anterior intestinal portal; MG-midgut; PC-pericardial cavity; R-right; S-sinusoids; SA-sinus anlagen; SV-sinus venosus; SaV-sino-atrial valve; T-truncus ateriosus; TC-trabeculae cordeae; UV-umbilical veins; V-ventricle; VV-vitelline vein(s).

PLATE I



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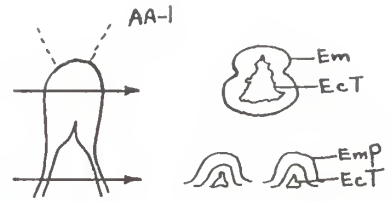

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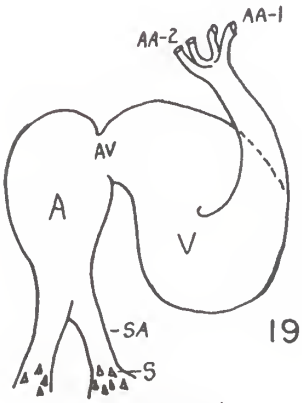

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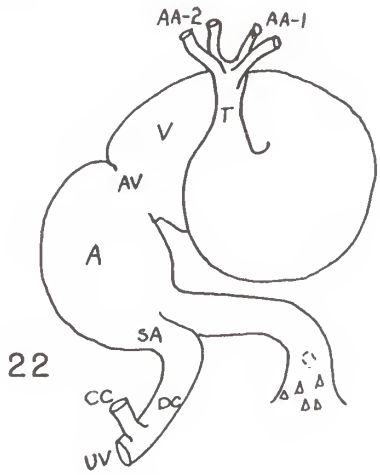

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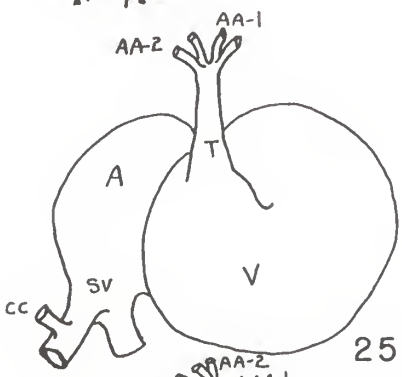

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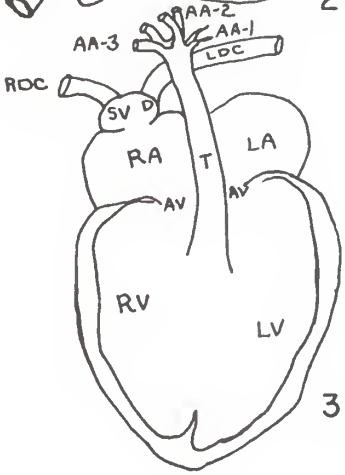

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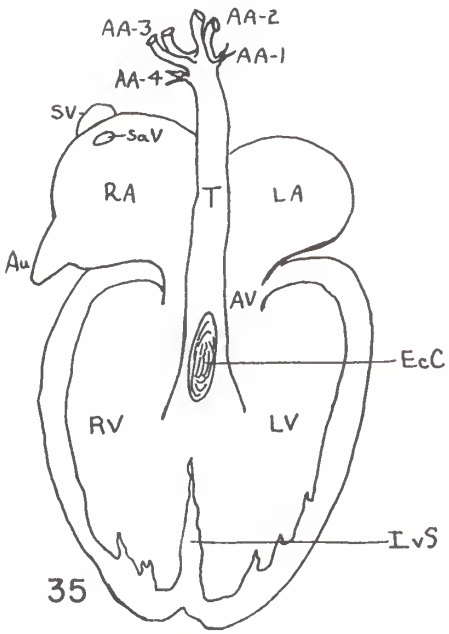

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EXPLANATION OF PLATE II

Fig. 1. Section through the cardiac region of a six somite embryo showing the epimyocardial plate and endocardial cells.

Fig. 2. A series of sections posteriorly to anteriorly, showing the fusion of the epimyocardial plate and the endocardial tubes.

Fig. 3. Section through the atrioventricular foramen region of a 19 somite embryo. The aortic sinus and ventral roots of aortic arch two is also shown.

Fig. 4. A frontal section through the atrial region of a 22 somite embryo showing the connection of the umbilical vein to the left duct of Cuvier and sinus anlagen. The atrioventricular foramen and the dorsal most portion of the ventricle.

Fig. 5. A longitudinal section through a 25 somite embryo showing liver sinusoids, sinus venosus, atrium, ventricle, truncus, aortic sinus, and the second aortic arch.

PLATE II

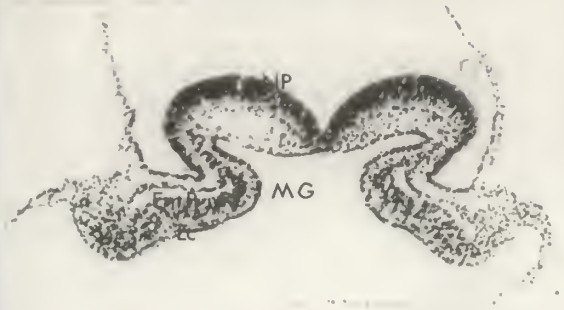
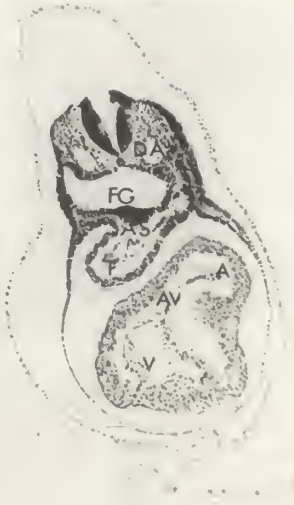


fig 1

fig 2



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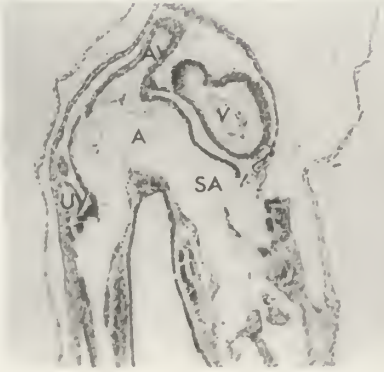

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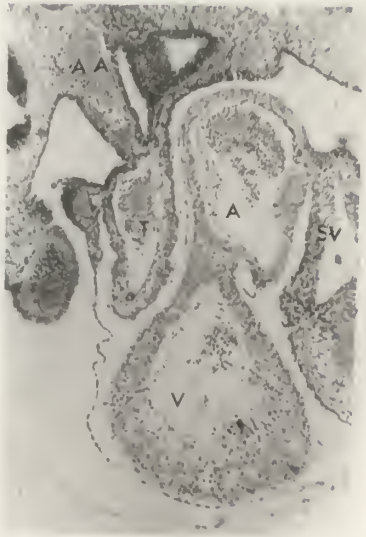


fig 5

EXPLANATION OF PLATE III

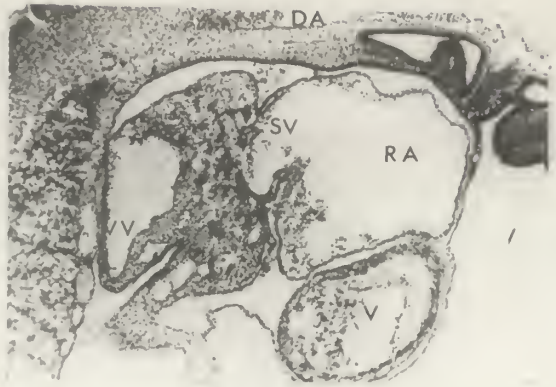
Fig. 6. A cross section through the right and left ducts of cuvier and the sinusoids entering them. The truncus is in the interatrial groove and the left atrium is sectioned through the area of the atrio-ventricular foramen.

Fig. 7. A 30 somite embryo showing a longitudinal section through the liver complex, sinus, atrium, ventricle and dorsal aorta.

Fig. 8. A section through the interatrial septum in the dorsal atrial chamber. The truncus is located in the interatrial groove. This is a 30 somite embryo.

Fig. 9. A 35 somite embryo showing trabecular development, atrioventricular valvular development and the aortic sinus to the far right.

PLATE III



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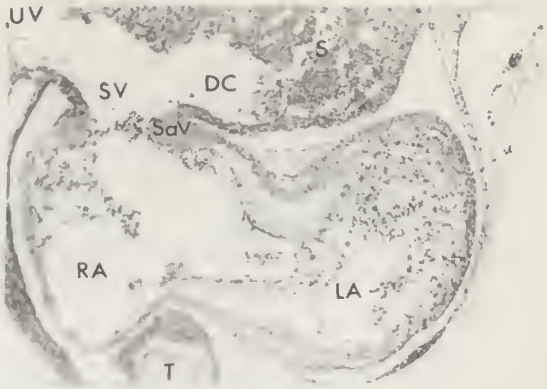

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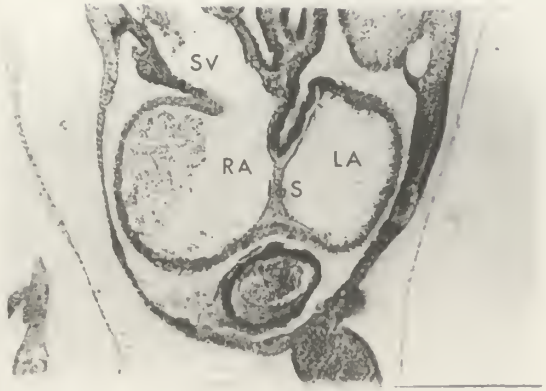

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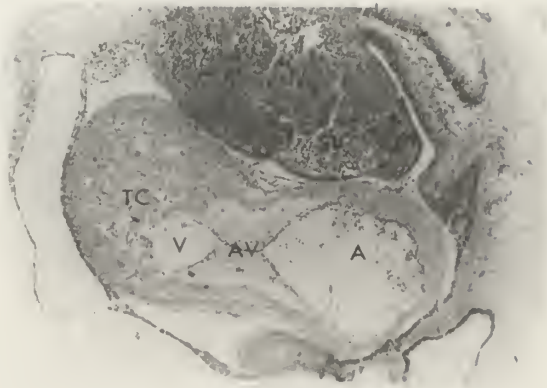

fig 9

had a slightly sharper arch due to beginning closure of the neural tube and formation of the anterior neuromeres. The neural plate ectoderm was differentiated from the epidermal ectoderm by distinct neural folds. The neural groove was open posteriorly and was much deeper than that of the six somite embryo. The notochord was prominent the entire length of the embryo. The foregut was 0.4 mm in length, 290 microns in width and 160 microns in depth just anterior to the MAIP, and 80 microns deep and wide at the tip.

The fused heart tube extended within the pericardial cavity for 80 microns with no connections to the ventral or dorsal cavity walls. There was a groove along the lateral walls of the heart tube beginning 40 microns back of its anterior tip and continuing 70 microns posteriorly (Plate 1, 9). Continuous with it was a "crinkle" in the floor of the foregut. The fused heart tube extended anteriorly as far as the tip of the foregut. The paired cardiac anlagen extended posteriorly beyond the MAIP for 180 microns, of which 100 microns were open endocardial tubes. The posterior portion of each epimyocardial plate was four or five cells thick and 20 to 25 cells wide. It was a broad inverted U. Further anteriorly the epimyocardial plate took on a C-shape with the opening ventral. It was five or six cells thick and approximately 25 cells wide. As the cardiac anlagen moved toward the midline, the face of the arc twisted medially to join the face of the arc on the other side (Fig. 2). The ventral and lateral endoderm fused and were broken down within 20 microns. In the next section anteriorly the dorsal epimyocardium fused and the endocardial tubes were in contact. The medial walls of the endocardial tubes were broken down in the next section (Fig. 2). There was a ventral mesocardium present for 70 microns anterior to the endodermal fold.

There were vasofactive cells present from the region of the truncus along the site of the aortic arch formation. The paired dorsal aortae were formed

from the region of the midbrain posteriorly beyond the somites but there was no sign of blood cells. The small umbilical veins were present in the lateral body wall and the vitelline vessels were a well formed plexus in the yolk sac, but were not yet continuous with the endocardial tubes except as lines of vasofactive cells that overlapped the posterior end of the endocardial cells. The vitelline arteries were represented as a series of lines of vasofactive cells through the splanchnic mesoderm from the open vitelline vessels of the yolk sac to the dorsal aortae.

The 13 somite, 21 day embryo had a slight cephalic flexure. The allantois was eight or nine mm in length. The neural tube was characterized by a swollen forebrain, a large anterior neuropore, and distinct neural folds anterior to the 10th somite. The MAIP was just anterior to the first somite.

The ventricle was fused and bulged ventral and to the right beyond the embryo proper. The atrium was completely fused and with a slight bulge to the left. The truncus was a distinct vesicle with a demarcation between it and the ventricle. The atrio-ventricular and sinu-atrial constrictions were formed after fusion of the cardiac anlagen in the respective areas. The sinus anlagen were not fused but lay in the lateral splachnic mesoderm.

The 19 somite, 21 day embryo had a sharp cephalic flexure and slight cervical and sacral flexures. It was 3.25 mm in greatest length, the neural tube was open its entire length and there was no visible neuromeric constriction in the sectioned specimen. The foregut was 0.5 mm long, and 240 microns wide. The anterior tip of the gut was directly dorsal to the anterior tip of the heart.

The ventricle filled the anterior half of the pericardial cavity. The fused atria entered the ventricle slightly to the left of the medial line and 250 microns posterior to the anterior ventricular tip. The atrium occupied

the left half of the pericardial cavity and extended anterio-posteriorly 240 microns. The sinus anlagen were separate and extended 100 microns posteriorly from the atrium. The ventricle extended posteriorly on the right to a point ventral to the sinuatrial junction. The truncus arteriosus was about 60 microns long extending dorso-laterally from the ventricle then dorso-anteriorly to the aortic sinus.

There were well formed first aortic arches and the second aortic arches were open at their dorsal and ventral extremes but there was no lumen medially (Fig. 3). The dorsal mesocardium was present in the atrial region, ventral to the posterior end of the auditory placode. The pericardial cavity extended to within 10 microns of the anterior tip of the embryo and occupied the widest portion of the embryo for most of its length. The vitelline veins formed a plexus in the yolk sac wall, through the liver to the sinus anlagen. The dorsal aortae were moderate in size, open from the first aortic arch to the vitelline arteries and contained a few blood cells. Cardinal veins were absent.

A 22 somite, 22 day embryo had a nearly closed neural tube with a well defined neural fold and a cephalic flexure bent the tip of the head down sharply. The anterior tip of the neural plate terminated a short distance anterior and dorsal to the atrio-ventricular constriction. The pharynx was 190 microns deep, with two pairs of pharyngeal pouches.

The heart expanded and twisted into a loose spiral (Plate 1, 22); somewhat comparable to that of a 45 hour chick. The ventricle was large and located anterior, right lateral and ventral with the atrium posterior, left lateral and dorsal. The curvature of the heart was such that the ventricular-truncus connection was posterio-medial and the truncus ventral, right lateral to the atrium. The paired sinus anlagen entered the atrium dorsally and posteriorly (Fig. 4). There was a definite atrioventricular constriction in both layers of

the heart tube. The atrial epimyocardium was thinner than that of the ventricle and the endocardium was more closely adhered to the atrial wall. There were minute cytoplasmic strands through the cardiac jelly from the endocardium to the epimyocardium of the ventricle.

The umbilical veins were lateral and ventral to the dorsal aortae, posterior to the fourth somites. The cardinal veins entered the umbilical veins dorso-laterally to the umbilical junction with the sinus anlagen (Fig. 4). The vitelline veins were a plexus from the blood filled vessels of the yolk sac to their connections with the sinus anlagen. The first and second aortic arches were present, functional, and approximately equivalent in size.

The 24-25 somite, early 23 day embryos were concave ventrally due to the closure and flexure of the neural plate and had wide yolk sac connection. There were three pairs of pharyngeal pouches and the liver anlage was established. The MAIP was ventral to the posterior end of the myelencephalon immediately anterior to the first somite. The neural tube had a wide open anterior neuropore, and the otocyst and optic vesicles were formed. There was a large mesonephros which extended anteriorly to the sixth intersomitic groove. The bulk of the nephric material lay in the sixth to 14th somite region with the remainder, a narrow band, tapering to the area of the last somite. The foregut was 160 microns wide and 0.5 mm in length.

The heart had taken on a twisted shape of a tight loop with the ventricle toward the right and the atrium to the left and more dorsal (Plate 1, 25). The pericardial cavity extended 1.3 mm from side to side and was filled by a ventro-cranially located ventricle. The heart had a flat, one cell layered, close lying membrane enclosing its boundaries. There was a medial atrio-ventricular foramen and the cardiac tube showed a definite constriction in the epimyocardium and endocardium of this area (Fig. 5). Also medially but slightly to the left,

the truncus opened from the ventricle on the cranio-ventral surface. The truncus opened medially and dorsally into the aortic sinus (Fig. 5). The second aortic arch was large (Fig. 5), the first small and the third had not developed yet but was represented by a line of vasofactive cells throughout its future course.

The right portion of the atrium had a valve opening from the sinosus venosus. The sinus venosus in turn was entered on either side by the "duct of Cuvier" formed by the vitelline-umbilical liver complex (Fig. 5) and the cardinal veins. The wall of the yolk sac possessed many blood filled vessels which anastomosed gradually toward the embryo proper. Large umbilical veins were present along the lateral edge of the embryo, in close proximity to the endoderm posteriorly, passing into the somatic mesoderm posterior to the coelomic formation. The vitelline network and umbilical vein gradually anastomosed toward the heart and the common cardinal vein joined them lateral to the sinus venosus. The cardinal veins were small and uneven. The umbilical arteries branched from the large aortae near the posterior extreme of the embryo. There were segmental arteries supplying the mesonephros.

The 28-30 somite, early 24 day stage was characterized by an inverted J-shape with a broad cervical flexure, a near 90 degree cephalic flexure, and beginning sacral flexure. The tip of the forebrain fit into the atrio-ventricular notch. The neural tube was 90 microns thick, well developed, and closed the entire length of the embryo. The rhombencephalon was neuromeric and ganglia of the seventh, eighth, ninth, and tenth cranial nerves were established.

The anterior end of the mesonephros was directly ventral to the seventh intersomite groove (Fig. 7) with a little string of pronephros extending anteriorly. The largest portion of nephric material was in the eight to 16 somite region with the thickest portion ventral to the 13th somite. The

mesonephros gradually tapered to the 20th intersomite groove and continued posteriorly only as a darkened ridge. The foregut was about 1.1 mm long, 70 to 110 microns wide, and there were four pharyngeal pouches. The yolk sac had a narrow opening into the midgut posterior to the foregut. There was a small notochord from the forebrain region posteriorly to the limit of the somites.

The heart tube curled into a tight 360 degree coil (Plate 1, 30). The large ventricle was a complete 180 degree arc with the atrio-ventricular notch immediately against the tip of the forebrain. The ventricle was ventral and the atrium was larger and dorsal. The sinus anlagen were fused into a common chamber posterio-dorsal to the atrium at 28 somites.

The sinus venosus was shifted to the right of the midline, and was separated from the atrium by thin flap-like sinu-atrial valves. The right atrium received the sinus venosus immediately ventral to the anterior limb bud. The thin atrial wall was five cells thick and had distinct endocardial and epimyocardial layers. A dorsal mesocardium remained along the posterio-dorsal wall of the atrium and posterio-medial between the sinus anlagen. The 29 somite ventricular wall consisted of a firm outer layer five or six cells thick and a layer of cardiac jelly containing few cells lined with a single cell layer of endocardium. The interatrial septum was forming along the anterio-dorsal midline of the atrium and extended approximately one-fourth the way across the lumen (Fig 8).

The 30 somite ventricular walls were two to three times as thick as the atrial walls. The wall consisted of a five to seven cell layer of epimyocardium; a heavy layer of cardiac jelly which was becoming progressively more cellular, reduced to pockets; an expanded myocardium; and a distinct lining of endocardium (Fig. 7). There was a definite inter-ventricular septum at the posterior tip of the ventricle, possibly developing more by bilateral expansion than by

ingrowth of a true septum. The "endocardial cushion" was barely recognizable as a slight expansion of the dorsal wall of the ventricle at the atrio-ventricular junction (Fig. 6). The atrio-ventricular foramen was half the diameter of the ventricular lumen, bordered by a rim of cardiac jelly at least twice as thick as that in the ventricle proper, thus forming the anlage of the atrio-ventricular valves. The ventricle had extensive trabecular development in which myocardial cells had displaced endocardial jelly in ridges that extended longitudinally within the ventricular wall.

The truncus had a firm muscular wall three or four cells thick and a cardiac jelly layer essentially the same thickness with a firm internal endocardium (Fig. 6, 8). The transition from ventricle to tuncus was gradual with no indication of a definite demarcation or valves. The truncus proper was approximately 150 microns long, from ventricle to aortic sinus, and was pressed for most of its length into the interatrial notch (Fig. 8), thus aiding in separation of the two atria.

The radix aortae were separate and equal anterior to the tip of the mesonephros (Fig. 7). The first aortic arch had undergone constriction with a remnant dorsally and the small mandibular artery ventrally. The paired second and third aortic arches were completely functional and carried most of the blood. There were vasofactive cells where the fourth arch would be formed. There were small segmental arteries the major branches of which supplied the mesonephros. Subcardinal veins were established (Fig. 7), but anastomoses between them were not found.

The umbilical veins were large and had a broad junction posterior to the liver. There was a medial branch into the liver which broke into sinusoids. A small portion of each umbilical vein continued anteriorly lateral to the liver into its respective duct of Cuvier. The single vitelline (hepatic portal)

vein was situated in the left lateral wall of the gut and entered the dorsal liver mass (Fig. 7) where it anastomosed with the liver sinusoids from the umbilical veins. The hepatic segment of the posterior vena cava was identified in the right dorsal liver mass and anastomosed through liver sinusoids with the umbilical and vitelline veins, but contact with the subcardinal vein had not been made. The liver sinusoids emptied by way of many small veins into the ducts of Cuvier (Fig. 6) or directly into the sinus venosus. The ducts of Cuvier were formed by junction of anterior and posterior cardinal veins and umbilical veins within the lateral body wall. The two ducts of Cuvier joined the sinus venosus anteriorly, slightly to the right of the midline, thus making the right duct of Cuvier much shorter than that on the left (Fig. 6).

There was a thin, one cell layer membrane covering the heart (Fig. 7, 8). The ventral half of the pericardial cavity walls was formed by a layer of amnion (Fig. 6, 7, 8) continuous between head fold and lateral body folds. The pericardial cavity was closed posteriorly by the connection between amnion and yolk stalk, so was continuous with the general coelom only dorsally through the pleural cavity and pleuro-peritoneal groove.

The 33-35 somite, 25 day embryos were basically C-shaped with increased cervical flexure and progressing sacral flexure. The allantois was 35 to 50 mm long and with the enlarging allantois the umbilical veins increased and the yolk sac was degenerate except for the bulbular portion near the embryo.

The pharynx had four pharyngeal pouches. The foregut was approximately 1.6 mm in length. There were hypopharyngeal and hyperpharyngeal grooves and a small fourth pharyngeal pouch. The ventral edge of the laryngotracheal groove protruded into the dorsal interatrial groove. There was an internal pleural cavity on the right, but no sign of lung buds. The gall bladder and the dorsal pancreas were established.

The heart was approximately the same size as the head of the embryo and was nestled closely between the tip of the head and the pharyngeal-body region. The heart appeared externally to be three chambered, closely comparable to the heart of a frog, with two atria, separated ventrally by the truncus, and a triangular ventricle that was beginning to show longitudinal separation at the tip.

The sinus venosus was considerably smaller (Fig. 8) than had been observed in previous stages and there were prominent sinu-atrial valves. The sinus venosus entered the dorsal extremity of the right wall to the right of the midline.

The atrial walls differed little from those of the 30 somite embryos. There was some expansion of the atria due to increased blood. The primary interatrial septum was present along the midline of the anterio-dorsal wall of the atrium and extended one-third to one-half the distance across the lumena, tapering off toward the atrio-ventricular junction. The septum extended into the atrial cavity directly ventrally from the dorsal mesocardium, effecting partial separation of the cavity into right and left sides. Auricles were observed on both atria.

The ventricular wall was eight to 25 cells thick, with only a few pockets of cardiac jelly left (Fig. 9). Trabeculae were forming by further expansion of the ridges of myocardial cells that were noted in the 33 somite stage and evagination of endocardium into the spaces between. Most of the bands were longitudinal within the ventricle, attached at both ends, and irregularly separated from the lateral wall by continued undercutting of the endocardium. A few moderator bands were present in the tip of the ventricle formed by the same process described for the trabeculae. The inter-ventricular septum was well formed in the tip of the ventricle, extending anteriorly along both the

ventral and dorsal walls. Both ventricles were expanding posteriorly beyond the inter-ventricular septum, providing an explanation for the double tip appearance of the heart in the whole mount (Plate 1, 35).

The atrio-ventricular foramen was a narrow opening between the atrium and the ventricle surrounded by thickened cellular cardiac jelly constituting the beginning of the atrio-ventricular valves (Fig. 8). The endocardial cushion was nothing more than a slight thickening of the cardiac jelly in the mid-dorsal wall of the ventricle and the atrio-ventricular junction. There was some infiltration of myocardial cells into the "endocardial cushion" area as well as into the cardiac jelly swelling around the atrio-ventricular foramen. Division of the atrio-ventricular foramen was underway in the 35 somite embryo by the interatrial septum and endocardial cushion, but had not progressed far enough to initiate division of the truncus. There was a thin membrane around the heart one or two cells in thickness that probably represent the beginning of the serous visceral pericardium (Fig. 9).

The truncus was 0.5 mm long, and circular in cross section. An expansion of the distal end of the ventricle initiated the ventricular-truncus demarcation. No divisions of the truncus was found in any of the embryos of the 33-35 somite stage. It was pressed firmly into the mid-ventral interatrial groove by pressure of the retaining layer of amnion which formed the ventral wall of the pericardial cavity.

The entire vascular system was expanded tremendously by blood cells. The large umbilical veins received segmental veins from the lateral body wall. There was a broad connection between the umbilical veins within the peritoneal cavity posterior to the liver. A large medial umbilical branch entered the hepatic substance where it broke into sinusoids, anastomosed with the vitelline vein and received the hepatic segment of the vena cava. The original umbilical

veins lateral to the liver joined the common cardinal veins lateral to the anterior margin of the liver, thus forming the ducts of Cuvier, which in turn received many branches of the hepatic veins. The right duct of Cuvier entered the sinus venosus at its anterior extremity. The left duct of Cuvier passed three-fourths the way across the anterior margin of the liver and entered the medial margin of the sinus venosus slightly posterior to the entrance of the right duct. The hepatic segment of the post cava was forming by coalescing of hepatic sinusoids, and fusion with the wall of the subcardinal vein had occurred, even though the blood channels were not yet continuous.

The aortic sinus was short and broad (Fig. 9). The second and third aortic arches were approximately equal and the fourth aortic arch was opening both dorsally and ventrally, but was represented in the midregion only as a cord of vasofactive cells. A tiny vitelline artery branched from the dorsal aortae to the yolk sac. Segmental arteries including the mesonephric branches were considerably enlarged over those observed in the previous stages. Umbilical arteries arose as two or three branches from each side of the paired dorsal aortae, then joined into one allantoic artery on either side of the allantoic stalk. One pulmonary vein had begun formation in the anterio-lateral wall of the left atrium of the 35 somite embryo.

DISCUSSION

The cardiac anlagen began as a distinct row of cells in the cardiac area of the presomite, 20 day embryo. During the formation of the first six somites, the cardiac development progressed to a double arc three or four cells thick and 15 to 20 cells wide on either side of the open midgut. The concave side of the arc faced ventrally with a band of deeply staining endocardial cells between it and the endoderm (Fig. 1). In the nine somite embryo the open arc

of each cardiac plate faced the other medially (Fig. 2), near the embryonic midline. The endocardial cells formed an open tube within each U-shaped cardiac plate (Plate 1, 9, and Fig. 9). Anteriorly, the two cardiac plates fused medially and the enclosed endocardial tubes fused into a single median tube (Fig. 2). Duffey (1953) reported an S-shape of the canine heart prior to fusion of the anlagen. Fusion progressed from anterior to posterior with epimyocardial fusion preceding endocardial fusion. Truncus, ventricle and atrium were completely fused and apparently functional in the 13 somite embryo. Duffey (1953) described first fusion of the heart in the dog embryo as occurring at nine somites and the heart becoming functional by the 11 somite stage, agreeing closely with the observations in the bovine. Although no constriction of the paired anlagen were seen as described by Duffey (1953), all the heart constrictions were evident by the 13 somite stage. As tubular fusion progressed, torsion occurred and by the 19th somite stage the fused tube had assumed the shape of a broad S lying on its side (Plate 1, 19). The atrium expanded progressively anteriorly, to the left and dorso-laterally due to the differential growth rates of the heart and the surrounding tissues, causing a tighter twist in the cardiac contour. By the 25 somite stage the atrium had pressed tightly against the elongated truncus and the atrio-ventricular foramen opened almost directly ventrally, the atrio-ventricular constriction had deepened, and the sinus was shifted dorso-lateral (Plate 1, 25). Maximum curvature of the heart was found in the 28 somite embryo, in contrast to Grimes' report (1959) that such occurred in the 20 somite bovine. The sinus venosus began to regress in size by the 30 somite stage and to orient itself in a dorso-lateral position over the right atrium. By the 30 somite stage the atrium was entirely dorso-anterior to the ventricle and anterio-ventral to the sinus venosus. The interventricular septum formed a visible longitudinal demarcation. The atrium

was partially divided into right and left sides by pressure from the truncus that was pressed into the ventral midline of the atrium (Plate 1, 30; Fig. 6, 8).

The human and dog have cardiac development similar to that found in the bovine. The difference lies in the initiation of certain phases with time. In the human, somite development is slower. By 25 days there are approximately 25 somites (Streeter, 1951) whereas in the cow there are 35 somites by the 25th day. The human cardiac anlagen begin fusion at seven somites (Arey, 1965), and the dog and cow at nine or ten somites (Duffey, 1953). The 14 somite dog embryo heart showed a development much like that of the 19 somite cow, that being the characteristic S-shape (Duffey, 1953). The 21 somite human heart as described by Streeter (1951), was much like that of the 30 somite calf. Therefore even though somite development is slower in the human, heart development is only two days slower than that of the bovine.

The veins have begun to form by the time the ninth somite formed, although no indication of veins were found in the six somite stage. The vitelline veins were a plexus in the yolk sac and the umbilical veins are small veins in the lateral body wall. The sinus anlagen were beginning to fuse by the 19 somite stage. The vitelline veins had formed a hepatic plexus and entered the sinus anlagen from the capillary plexus. The anterior and posterior cardinal veins were not yet present. The posterior cardinal veins appeared by the 22 somite stage, united with the umbilical veins, lateral to the sinus venosus, and received the sinusoids of the umbilical-vitelline capillary plexus of the liver. The sinus venosus progressively fused and shifted well to the right by the time the 25th somite was complete. The sinus was entered on either side by the duct of Cuvier and by the umbilical-vitelline-hepatic complex (Fig. 5). The umbilical joined posterior to the liver thus forming the post-hepatic umbilical sinus that persists throughout fetal life.

The vitelline veins anastomosed as they passed from the yolk sac into the embryo and gradually fused into a single vein that by the 25 somite stage was situated in the left lateral gut wall posterior to the liver. The anterior cardinal veins were small and patchy in the 25 somite embryo and progressed to a small intact vein at approximately 28 somites. The posterior cardinal veins developed slightly faster than the anterior cardinal veins and became continuous channels by the 25 somite stage. Anterior and posterior cardinal veins joined ventro-lateral to the fourth or fifth somite. The umbilical vein joined the posterio-lateral area of the common cardinal vein, thus forming the duct of Cuvier as the major feeder of the sinus venosus. The umbilical veins gave off capillaries into the hepatic tissue throughout their passage posterior and lateral to the liver. The anterior portions of the umbilical veins, the ducts of Cuvier and the sinus venosus received the hepatic capillaries from the liver. This condition was maintained through the 35 somite stage. Due to the position of the sinus venosus near the right body wall, the right duct of Cuvier was much shorter than the left. The left duct of Cuvier received most of the hepatic vessels (Fig. 7). At the 33-35 somite stage the veins of the body were larger due to increased blood flow. The sinus venosus had regressed in size by this time (Fig. 9). The venous system of the human embryos of these stages showed a high comparability to the bovine vascular development. The exception was the right vitelline vein, found to be present in the human embryos (Streeter, 1951). Duffey (1953) and Martin (1958) described two distinct vitelline veins in the dog embryos of 13 somites. There was no mention of a hepatic plexus, possibly because of the age of the embryos observed. The primitive blood cells first appeared as circulating throughout the tubular system at about 22 somites or a little earlier. The number increased gradually at first and then at a faster rate, to the tremendous

amount found in the 35 somite embryo. The early blood cells were large, spherical, mononucleate, granular cells.

The atrial region of the heart was formed by the fusion of the cardiac tubes during the time of development of somites nine to 13. The epimyocardium fused first and the endocardium fused a short time later. The 13 somite embryo showed nearly complete atrial fusion. The endocardium was always tightly adhered to the internal portion of the epimyocardium. The atrial wall was reduced in thickness between the 19 and 22 somite stage (Fig. 4 and 5), and further reduced by 25 somites due to the increased passage of blood through the tubes.

The atrium was beginning to chamber by 28 somites and constriction of the atrial walls had occurred by the 30 somite stage (Fig. 8). The interatrial septum of the human was observed at 6 mm (Arey, 1965). The sinu-atrial valves were well formed by 30 somites (Fig. 7), as flaplike extensions of the atrial wall. The atria continued to develop structural characteristics of the adult heart and by the 33-35 somite stage the right atrium was larger than the left, the atria were three-fourths divided by the interatrial septum and the walls were a thin two or three cell layer.

The endocardial tubes of the ventricle fused shortly after epimyocardial fusion at nine somites and was characteristically approximately one-fourth the luminal distance from the epimyocardial walls at 19 somites (Fig. 3). There was cardiac jelly between the two layers from the time of fusion. The ventricular wall was five to seven cell layers thick. The epimyocardium had expanded and the endocardium was in the same relative position as that of the nine somite embryo. There were cytoplasmic strands between endocardium and epimyocardium. By 25 somites the cardiac jelly was greatly reduced and the endocardium loosely adhered to the myocardial wall (Fig. 5). Cords of

myocardial tissue, the trabeculae, were forming by endocardial evaginations, thus surrounding and beginning to individualize the trabeculae carneae. There were also endothelial lined pockets protruding into the epimyocardium proper. The cardiac jelly continued to be reduced and the trabeculae were well developed by the time the 29th somite had developed. The walls of the ventricle were 12 to 21 cells thick and the endocardium closely covered the trabecular projections and lined the formed pockets. Trabecular development in the human began by 17 somites, 28 days (Arey, 1965).

The 35 somite ventricle wall was up to 25 cells thick. There was an indication that moderator bands had begun to develop; there was also definite atrio-ventricular valvular development (Fig. 9). The endocardial cushion was nothing more than a slight thickening of cardiac jelly from the middorsal wall of the ventricle. The endocardial cushion was better developed in the 6 mm human (Arey, 1965) than in the bovine of comparable heart development.

By 30 somites the interventricular septum was forming and the left ventricular wall was thicker than that of the right ventricle. The interventricular septum was observed in the 5 mm stage (approximately 17 somites) human (Arey, 1965). The 35 somite ventricle had a longer septum and was about three-fourths divided. No direct comparison of either canid or human was possible.

The truncus appeared early in cardiac development (9+ somites), and by 19 somites it was three or four cells thick with an internal layer of cardiac jelly of about the same thickness (Fig. 5). The truncus was about 60 microns in length at this stage. The first and second aortic arches branched from the truncus via the aortic sinus, and emptied into the dorsal aortae. Prior to this stage there were vasofactive cells in the regions where the aortic arches would develop. The 22 somite embryo had a truncus which was lengthened by the

shifting of the ventricle more ventro-laterally. There was not a significant increase in truncus diameter. The first and second aortic arches were functional and of moderate size. The truncus of the 25 somite embryo was situated ventral to the atrio-ventricular notch due to the curvature which the heart had at this time (Fig. 5; Plate 1, 25). The first aortic arch was smaller and there were vasofactive cells where the third aortic arch would develop. Therefore at this stage the second aortic arch carried the major blood flow. The third aortic arch developed by the time the 28th somite had developed. The first aortic arch had regressed to form the mandibular artery. The truncus was nearly 140 microns in length by this stage. It was a compact structure three or four cells thick. The cells were larger and tended to be columnar. The musculature was firm and circular. By the 33 somite stage the truncus was 0.5 mm in length, thicker by two cell layers, and expanding into the interatrial notch (Fig. 8). The truncus continued to increase in length and expand into the ventral interatrial groove caused by shoving of the ventricle ventrally by the relatively stationary atria and the continual expansive growth of the entire cardiac structure. The fourth aortic arch had begun to form in the 35 somite embryo. There were dorsal and ventral roots connected by a string of vasofactive cells. The entire vascular system of the cow and dog was initiated from vasofactive cells such as those first found in the yolk sac as observed by Martin (1958).

CONCLUSION

The bovine heart was formed from two bilateral primordia which fused into a double concentric tube. By tortion and differential growth rates of the heart and the surrounding tissues, the heart tube was twisted into a 360 degree loop from which the adult structure develops. The epimyocardium fuses prior to the endocardial fusion, beginning before the nine somite stage. The heart

of the 19 somite embryo was an S on its side, becoming a tight 360 degree loop in the 25 somite embryo and the 30 to 35 somite embryos showed a heart shaped not unlike that of the adult.

The ventricular and atrial walls began to develop their adult characteristics early and therefore afforded contraction and the needed circulation at the early stage.

The blood cells of a primitive type appeared in the 13 somite stage and were present in great numbers in the 24 somite individual.

The sino-atrial valves formed as thin flaps on the dorso-lateral wall of the atrium at about 29 somites. The primitive atrio-ventricular valves were observed at 33 somites as a cardiac jelly expansion in the atrio-ventricular foramen. The endocardial cushion of the cow is a small layer of cardiac jelly formed on the dorso-medial wall of the atrio-ventricular foramen and appeared to be of relative insignificance in the early division of the bovine heart, unlike the endocardial cushion of other mammals.

The cardiac jelly of the ventricle was gradually infiltrated by myocardial cells, and the trabeculae carneae were formed by concentration of these cells in longitudinal bands situated on the ventricular wall. There were moderator bands observed in the 35 somite embryo formed by undercutting of the myocardium by the endocardium.

There was a thin, one cell layered membrane covering the heart which was first seen in the 19 somite stage and observed through the 35 somite stage where it was two or three cells thick. This is interpreted to be the visceral pericardium of the heart which later is tightly adhered to it.

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