CARDIOGENESIS AND OBSERVATIONS OF THE FIRST
HEART CONTRACTIONS IN CERTAIN CANIDS

by

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INTRODUCTION

Mammalian cardiogenesis occurs by a series of events, each of which are rapidly succeeded by more advanced stages of development. Ultimately, the single, tubular heart, slightly modified by bulges and twistings, and possessing an inherent capacity of contraction comes to be the prime mover of the early embryonic nutrients.

To arrange these events into a general pattern characteristic for all mammals is a herculaen task often complicated by optimistic folly. However, by comparing observations of cardiogenesis in certain *Canids* with other mammals, definite similarities do occur. Irrespective of specific chronological relationships, mammalian embryos in general possess bilateral heart primordia composed of epi-myocardial tissue and endocardial tissue, which by shifting toward the midline of the embryo and there undergoing fusion, come to form the heart. At this point, generalities end and intricacies begin. Gross variations have been reported on the mode of and the factors responsible for the formation of the heart between the orders of *Mammalia* which have been studied. These variations are justifiable if for no other reason than the diversity of the particular orders. Less chance of pronounced variations seems possible between families or species. The dog and the coyote have been found to have identical cardiogenic processes and, likewise, these have been found to differ in no essential respect in the cat.

Reference has been made on numerous occasions to the work of others who have studied mammalian cardiogenesis. Particular attention has been directed to the works of Schulte (1916) and Watson (1924) on cardiogenesis in cat embryos having three somites to those having twenty-one somites. Comparisons were also made with conditions in rat embryos of the pre-somite stage to the eight somite stage as described by Goss (1935, 1938, 1942, 1952), in early rabbit

embryos having two to three somites as described by Dwinnel (1939), in guinea pig embryos from the pre-somite stage to those having nine somites as described by Yoshinaga (1921) and in the human embryo of twenty somites as described by Davis (1927). Occasional reference was made to Bonnet's (1901) description of the dog.

Thus, by comparing the observations of canid cardiogenesis with the above stated works, it was possible to insert the dog into the general pattern of mammalian heart development.

METHODS AND MATERIALS

The canid embryos were obtained from mixed dog stock and wild coyotes.

The dogs were maintained under supervised breeding conditions at the Small Animal Laboratory at Kansas State College. In order that timed stages of embryos might be obtained, ovulation times were determined by examinations of vaginal smears (using the technic of Newberry and Gier, 1952) during the estrus period, and breeding was allowed at the optimum condition.

The coyote embryos were removed from freshly killed bitches obtained during organized coyote hunts common to this region. Before the removal of the embryos, measurements were made of the swellings of the pregnant uteri. This data and the state of development of the embryos were the criteria for placement of the coyote embryos in the series.

The pregnant uteri were removed via abdominal incision, and the swellings measured and immediately placed in warm Ringer-Locke's solution. Dissection of the swellings was begun directly by making a longitudinal incision through the myometrium directly opposite the line of attachment of the broad ligament. The endometria were then peeled out of the myometria. The endometrial portion of each swelling was transferred to fresh Ringer-Locke's solution and opened

by making an incision along a line opposite the line of attachment of the
broad ligament. The endometrium was then spread flat exposing the embryo
intact and anchored by its extra-embryonic membranes. After preliminary obser-
vations, the embryos were fixed in half-strength Bouin's solution and trans-
ferred to 70 percent isopropyl alcohol. The whole mount preparations were
stained either with borax-carmine or hematin. Sectioned embryos were stained
with a progressive stain of 8 percent Harris' hematoxylin. Drawings were made
by micro-projection or camera lucida.

The embryos used in this study make a continuous series from pre-somite
early 16 day to 14 somite, late 16 day, or a span of only 20 hours and 14
somites. These embryos are briefly characterized below.

Dog 16a. This was a pre-somite embryo removed early on the sixteenth day
of pregnancy from a uterine swelling measuring 7mm x 10mm. The embryo
had distinct neural folds, anterior and lateral limiting sulci, and marked
proliferations of the intra-embryonic mesoderm ventral to the lateral limiting
sulci. It was sectioned transversely at 10 microns and stained with Harris'
hematoxylin. A micro-projection drawing was made of a representative section
through the head process (Plate I, Fig. 2).

Dog 16b. This was a two somite embryo removed from the same uterus as
embryo 16a. The embryo had three complete inter-somite grooves, anterior and
lateral limiting sulci, and neural folds extending posteriorly from the tip
of the head process to the second inter-somite grooves. Blood islands were
present in the extra-embryonic membranes. The embryo was stained with hematin
and mounted in toto. A micro-projection drawing was made of the embryo (Plate
I, Fig. 1).

Coyote 761a. This was an embryo having five somites from a uterine swel-
ling measuring 13mm x 16mm. Three primary vesicles were present in the brain

and the neural folds extended posteriorly to the end of the tail process. The
neural folds were in contact in the region of the hind brain. The embryo was
stained with borax-carmine and mounted in toto. A micro-projection drawing
was made of the embryo (Plate I, Fig. 3) and a more detailed drawing was made
of the heart primordia (Plate I, Fig. 6).

Dog 117Lb. This was an embryo having five somites. The embryo was removed
early on the sixteenth day of pregnancy from a uterine swelling measuring 13mm
x 16mm. This embryo, a mirror-image of embryo 761a, was sectioned transversely
at 10 microns and stained with Harris' hematoxylin. Micro-projection drawings
were made of representative transverse sections (Plate I, Figs. 4 and 5).

Coyote 761b. This was a six somite embryo removed from the same uterus
as embryo 761a. The embryo had a shallow fore-gut and neural folds which had
fused in the posterior portion of the midbrain and the anterior portion of the
hind brain. The embryo was sectioned transversely at 10 microns and stained
with Harris' hematoxylin.

Dog 117La. This was a four somite embryo removed from the same uterine
cornu as 117Lb. The embryo had a shallow fore-fut, head fold, tail fold, and
a precocious hind-gut. Two neuromeres were present in the fore brain which had
flexed ventrally through an angle of 90 degrees. The mid-brain and hind brain
were distinct. Numerous blood islands were present in the extra-embryonic
tissues adjacent to the embryo. The embryo was stained with hematin and
mounted in toto. A micro-projection drawing was made of the embryo (Plate II,
Fig. 1).

Dog 45R. This embryo had seven somites and was removed during the middle
of the sixteenth day of pregnancy from a uterine swelling measuring 12mm x 14mm.
The embryo had a deep fore-gut extending posteriorly from the hind region of

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the fore brain to the constriction between the midbrain and the hind brain,
a slight tail fold of the amnion, and a head fold slightly posterior to the
anterior margin of the fore-gut. The fore brain was flexed ventrally. The
embryo was stained in borax-carmine and mounted in toto. A micro-projection
drawing was made of the embryo (Plate II, Fig. 2) and a drawing was made of the
heart primordia (Plate II, Fig. 5). A drawing was also made of the region of
junction of the embryonic endothelium and the extra-embryonic vascular elements
(Plate II, Fig. 4).

Dog 115L. This was an eight somite embryo removed early on the sixteenth
day of pregnancy from a uterine swelling measuring 12mm x 16mm. The embryo
had a fore-gut extending posteriorly from the constriction between the fore
brain and the midbrain to the anterior part of the hind brain, optic vesicles
nearly contacting the head ectoderm, and a shallow hind gut. The head fold
was slightly anterior and ventral to the anterior margin of the fore-gut. The
embryo was stained with hematin and mounted in toto. A micro-projection drawing
was made of the embryo (Plate II, Fig. 3) and a drawing was made of the primordia
(Plate II, Fig. 6).

Dog 120Rd. This was an embryo having eleven somites. It was removed
from a uterine swelling measuring 13mm x 17mm during the middle of the six-
teenth day of pregnancy. The embryo had a wide fore-gut extending posteriorly
from the floor of the diencephalon to the first inter-somite grooves and cephalic
flexure of ninety degrees. Rathke's pouch was in contact with the infundibulum
and the oral plate had formed ventral to the posterior part of the diencephalon
and the anterior part of the mesencephalon. The optic vesicles were in contact
with the head ectoderm; however, there was no thickening of the ectoderm in-
dicative of the lens primordia. The tail fold of the amnion had proceeded
anteriorly as far as the anterior region of the segmental plate, still there

was no indication of a head fold of the amnion. The embryo was sectioned transversely at 12 microns and stained with Harris' hematoxylin. Microprojection drawings were made of representative sections (Plate III, Figs. 3, 4, 5 and 6).

Dog 120Lc. This was an embryo having twelve somites. It was removed from a uterine swelling measuring 13mm x 20mm during the last hours of the sixteenth day. The fore-gut extended posteriorly from the floor of the diencephalon to the posterior edge of the head mesoderm. The five divisions of the brain were well formed and cephalic flexion had directed the fore brain ventro-posteriorly through an arc of approximately 100 degrees. The tail fold of the amnion was directly over somite twelve; however, there was no indication of a head fold of the amnion. The embryo was maintained for several minutes in Ringer-Locke's solution at normal temperature and observations were made of the origin and rate of the myocardial contractions. The embryo was then fixed, sectioned transversely at twelve microns and stained with Harris' hematoxylin.

Dog 118Lb. This embryo had thirteen somites and was removed from a uterine swelling measuring 13mm x 18mm during the latter part of the sixteenth day. The embryo had the same general characteristics as 120Lc. It was sectioned logitudinally at twelve microns and stained with Harris' hematoxylin. A micro-projection drawing was made of a representative section (Plate IV, Fig. 2).

Dog 120Rb. This embryo had thirteen somites. It was removed from a uterine swelling measuring 14mm x 18mm during the last hours of the sixteenth day of pregnancy. The general characteristics were the same as for the previously described embryo except for further development of the tail fold of the amnion and the appearance of a head fold of the amnion. The embryo was

maintained in Ringer-Locke's solution at normal temperature for several minutes and observations were made of the origin and rate of the myocardial contractions. It was then fixed, sectioned transversely and stained with Harris' hematoxylin.

Dog 118La. This was a fourteen somite embryo obtained during the latter part of the sixteenth day from the same uterine cornu as 118Lb. The embryo had a head fold of the amnion enclosing the head posteriorly to the middle of the midbrain. Cranial flexure was present in nearly the same degree as in 120Lc; however, cervical flexure had begun in the hind brain. The margin of the anterior intestinal portal was at the level of the first inter-somitic grooves. The hind gut was only a shallow pocket. The embryo was stained in hematin and mounted _in toto_. A micro-projection drawing was made (Plate IV, Fig. 1).

Dog 120Ra. This was a fourteen somite embryo removed from a uterine swelling measuring 12mm x 19mm during the last hours of the sixteenth day. The general characteristics of the embryo were the same as for the previously described embryo. It was maintained in Ringer-Locke's solution at normal temperature for observations of the nature and rate of the myocardial contrac- tions. It was then fixed, sectioned logitudinally and stained with Harris' hematoxylin.

OBSERVATIONS

The first embryos considered were a pre-somite embryo and a two somite embryo.

In the pre-somite embryo (Plate I, Fig. 2) the intra-embryonic mesoderm had undergone marked proliferation and was five to six cells thick ventral to the lateral limiting sulci. The cells of these thickened areas were more

compact than the mesoderm medial and lateral but no differentiation had oc-
curred indicative of the bilateral cardiac primordia.

The two somite embryo (Plate I, Fig. 1) had primitive blood islands
clustered around the pellucid area surrounding the embryo. Otherwise, it
resembled the pre-somite embryo.

In embryos having five somites (Plate I, Fig. 3), the amnio-cardiac
vesicles had developed as cavities in the lateral mesoderm under the lateral
limiting sulci, extending cranio-medially and uniting in the extra-embryonic
mesoderm immediately anterior to the head fold. The lateral limbs of the amnio-
cardiac vesicles were wide and of considerable depth (Plate I, Figs. 5 and 6)
but in the cranial arc where confluence of the lateral limbs had occurred,
the lumen was restricted to a narrow slit. The splanchnic mesoderm forming the
floor of the amnio-cardiac vesicles had thickened ventral to the lateral limit-
ing sulci and had bulged dorsally into the vesicles forming ridges extending
from the level of the posterior limits of the head mesoderm anteriorly to the
midbrain. These ridges, the epi-myocardial mantles which later give rise to
the epicardium and myocardium of the adult heart, were bordered on both sides
by folds, the epi-myocardial folds. Posteriorly, these folds were well defined,
fading out toward the anterior end of the primordia. With the increased dorsal
curvature of the mantles, the concavity ventral to it increased as a definite
epi-myocardial furrow. The mantles were connected medially to the wall of the
amnio-cardiac vesicle by the retro-cardiac plates and were limited from the
retro-cardiac plates by the medial epi-myocardial folds. The splanchnic
mesoderm forming the floor of the amnio-cardiac vesicles lateral to the mantles
is known as the pre-cardiac plates and are limited from the mantle by the lateral
epi-myocardial folds. The anterior ends of the heart primordia were directed
antero-medially although no fore-gut was present to account for this curvature.

Endocardial primordia were present and extended the full length of the epi-myocardial furrows, except for the most anterior and posterior limits where the endocardial masses fused indistinguishably with the epi-myocardial mantles. Toward the middle of the primordia, the endocardial masses had differentiated by a progressive formation and confluence of vesicles from the middle toward the extremities to form endothelial tubes with discontinuous lumina (Plate I, Fig. 6).

Certain primordial endocardial cells, the angioblasts, had proliferated from the ventral side of the retro-cardiac plate and from the undifferentiated mesoderm medial to the plates. The angioblasts were arranged singly or in sheets of two to three cells, many of which were connected to mesoderm by cytoplasmic processes. The angioblasts were most prevalent ventral to the retro-cardiac plate. Some scattered angioblast sheets were in contact with the endocardium by cytoplasmic processes.

In the embryo which had six somites, the lumen of the cranio-median portion of the amnio-cardiac vesicle was still shallow; the heart primordia lay in the splanchnopleuric folds bordering the anterior intestinal portal; and the openings of the epi-myocardial furrows, having been rotated through approximately thirty degrees, were directed ventro-medially. Anteriorly, the mantles were almost flat with very slight furrows; however, posterior to the anterior intestinal portal, the mantles were curved into the shape of a horse shoe, opening ventrally, by the convergence of the epi-myocardial folds.

The endocardial masses extended beyond the mantles anteriorly and became lost in the splanchnic mesoderm, but posteriorly, the endocardial masses ended within the dorsal wall of the mantles. The middle half of the endocardial elements had differentiated to endocardial tubes.

The heart primordia of a four somite embryo were next considered (Plate II, Fig. 1). Although the embryo was somewhat precocious in certain phases of its development, as compared with the five and six somite embryos previously described, the state of development of its heart primordia fitted satisfactorily into the series of heart stages between the six and seven somite stages. The primordia, directed anterio-medially, lay lateral to the margins of the wide, shallow fore-gut and only the posterior ends of the primordia lay in the splanchnopleuric folds. Expansions of the epi-myocardia were indicative of the future divisions of the paired primordia. The expansions were bounded by constrictions, namely, the atrio-ventricular and the incomplete bulbo-ventricular sulci. The expanded portion of the left primordium was more prominent than that of the right.

The endocardium, completely differentiated, closely followed the contour of the epi-myocardium. Endothelial sprouts, the anlagen of the first aortic arches, projected beyond the anterior margins of the mantles. Posteriorly, the embryonic endocardial elements were extended toward but not fused with the endothelial elements of the extra-embryonic splanchnic mesoderm.

The paired heart primordia of seven somite embryos (Plate II, Figs. 2, 5) were more expanded and were distinctly divided into the bulbi arteriosus, ventricles, and atria. The paired primordia had shifted toward the midline; the bulbi and the ventricles lay directly ventral to the lateral margins of the fore-gut while the atria lay in the splanchnopleuric folds, directed toward the yolk sac. The outer wall of the left ventricle was considerably thicker than that of the right. The mantles had rotated through approximately sixty degrees, progressively less posterior, so that the epi-myocardial furrows were directed ventro-medially, preparatory of later fusion. The anterior ends

of the lateral and medial epi-myocardial folds had progressed and joined
anterior to the bulbar primordia as the anterior epi-myocardial folds. The
cup-like ends of the bulbar primordia were directed slightly posterior as
well as medially. The position of the head fold by direct obstruction of the
forward expansion of the primordia was probably responsible in part for the
posterior curling of the anterior ends of the bulbi.

The endocardial tubes closely followed the contour of the epi-myocardia.
Anteriorly, sprouts of endothelium (bases of the ventral aortae) turned ven-
trally in the bulbi and then projected anteriorly and dorsally beyond the epi-
myocardial folds (Plate II, Fig. 5). Posteriorly, the endocardial tubes had
joined the vascular elements in the yolk sac to such extent that the lumina
of the endocardial tubes were confluent with the lumina of the vessels formed
from blood islands (Plate II, Fig. 4).

The bulbi and ventricles of the heart primordia of embryos having eight
somites lay directly ventral to the wide fore-gut. The openings of the ventric-
ular epi-myocardial folds had formed to the extent that the walls of the bulbi
extended laterally and anteriorly beyond the folds, thus, directing the openings
of the anterior portions of the bulbar epi-myocardial furrows slightly posterior
as well as ventro-medially. The pressure of the head fold on the tissues
supporting the anterior ends of the primordia, together with the forces exerted
by the proliferative activity of the epi-myocardial cells, had caused lateral
sacculations of the bulbi to lap over the anterior ends of the ventricles.
The ventricles had reacted in like manner in relation to the atria protruding
below the atria. The primordia, then, were in the shape of a crude S, reversed,
when viewed from the ventral aspect. In effect, the cardiac loop had been
instigated before the fusion of the primordia (Plate II, Fig. 6).

The endocardial tubes loosely followed the contour of the epi-myocardia. Anteriorly, endothelial sprouts twisted from beneath the bulbar epi-myocardia and followed a course dorsally around the anterior margin of the fore-gut as the first aortic arches. Dorsal to the fore-gut, these sprouts continued into the dorsal aortae; however, no signs of a functional circulation were present.

In eleven somite embryos, the right and left mantles had come together and fused both dorsally and ventrally in the posterior part of the bulbus and the anterior part of the ventricle so that the respective epi-myocardial furrows were confluent. The mantles at the anterior ends of the bulbi had not fused dorsally due to the passage of the ventral aortic roots. In the anterior end of the atrium, the mantles were fused ventrally but not dorsally and from the middle of the atria posteriorly, the mantles were unfused and diverged around the anterior intestinal portal. The fused portion of the atrium was much compressed dorso-ventrally and the epi-myocardial furrows faced each other preparatory to complete fusion (Plate III, Fig. 5).

Fusion of the two retro-cardiac plates to form the dorsal mesocardium (Plate III, Fig. 3) had progressed throughout the posterior part of the bulbus and the anterior half of the ventricle. In the posterior half of the ventricle and the anterior end of the atrium, dorsal fusion of the epi-myocardia and the retro-cardiac plates had not yet occurred (Plate III, Fig. 5). Some difference of size existed in the lateral walls of the atrial epi-myocardia, the left being more extensive and thicker than the right. The median ventral wall of the anterior part of the atrium, the middle cardiac plate, had formed by fusion of the medial parts of the pre-cardiac plates. If there was a median cardiac plate anterior to the atrium, it had lost its identity by disappearance of epi-myocardial folds. Posterior to the anterior intestinal portal, the atrial

primordia and the omphalomesenteric veins lay in the folds of the splanchno-
pleure bordering the portal. The size and thickness of the left atrial pri-
mordium was considerably greater than the right. The rotation of the atrial
primordia was approximately ninety degrees so that the openings of the epi-
myocardial furrows were directly opposite each other preparatory to fusion.

The right and left endocardial tubes were completely fused in the region
of the bulbus and anterior half of the ventricle (Plate III, Fig. 3). Also,
a short fused region was present in the aortic sinus just anterior to the bul-
bus. In the posterior half of the ventricle, the endocardial tubes were in
contact but the lumina were not confluent, thus, a septum composed of the
median walls of the endocardial tubes was present (Plate III, Fig. 4). The
endocardial tubes of the atrium remained separate and diverged posteriorly.
In the anterior region of the atrium, cytoplasmic processes connected the
endocardium to the epi-myocardium. No cardiac jelly was detectable. The
endocardial tubes of the paired atrics were flattened to conform to the dorso-
ventral compression of the epi-myocardium. As the endocardial tubes diverged
posteriorly, a sheet of angioblast cells connected the two tubes (Plate III,
Fig. 5). These angioblasts probably has originated during the four to five
somite stage, and added to the endocardial tubes as well as forming the median
sheet which extended anteriorly to the site of fusion of the atrial primordia
and posteriorly to the middle of the omphalomesenteric veins. Posteriorly,
the angioblast sheets projected medially from the endothelia of the omphalo-
mesenteric veins and extended below the retro-cardiac plate to the inner walls
of the splanchnopleuric folds (Plate III, Fig. 6).

The heart of thirteen somite embryos had undergone marked progress in the
formation of the cardiac loop. The ventricle had been forced ventrally and dex-
trally; the atrium was directed ventrally into the ventricle, and the ventricle

opened dorsally into the bulbus (Plate IV, Fig. 2). The anterior dextral wall
of the ventricle was slightly extended; however, not so sharply as the left
wall. The anterior end of the atrium was projected slightly over the posterior
portion of the ventricle but lateral bulges had not formed in the atrial walls.
The pericardial coelom had enlarged in volume reaching from the fold poster-
iorly behind the atrium and extending laterally confluent with the extra-
embryonic coelom. Cardiac jelly was present in all regions of the heart. The
epi-myocardium was fused dorsally and ventrally throughout the extent of the
ventricle and anterior atrial region. In the anterio-dorsal end of the bulbus,
the epi-myocardial folds remain continuously separate for the departure of the
ventral aortae. The epi-myocardial folds had not fused on the dorsal side
of the median portion of the atrium, although the endocardial tubes had fused.
There were erythrocytes present in the heart, indicative of a functional cir-
culation at this stage.

The embryo having fourteen somites (Plate IV, Fig. 1) had a cardiac loop
which was in the form of an S from the dorsal perspective. The right wall
of the ventricle was conspicuously distended into the right half of the peri-
cardial coelom. The left wall of the ventricle was also expanded but not so
much as was the right. The anterior end of the atrium had bulged dorsally
and anteriorly over the atrio-ventricular sulcus. Approximately two-thirds
of the atrium was fused. The unfused portions were directed sharply around
the margin of the anterior intestinal portal toward the extra-embryonic
splanchnopleure. The first pair of aortic arches were in the prime of func-
tion. Erythrocytes were present in the heart and dorsal aortae indicative of
a complete circulation.

Four embryos were studied in vivo and observations were made of the site and rate of the myocardial contractions. The eleven somite embryo was the first considered. The waves of myocardial contractions originated at the atrio-ventricular sulcus and proceeded anteriorly through the left wall of the ventricle as far as the bulbo-ventricular sulcus. The right wall of the ventricle was not involved in the contraction. The actual contractions consisted of rhythmical beats occasionally giving way to rapid fibrillations. The rate of the rhythmical contractions irrespective of the fibrillations was 98 beats per minute.

A twelve somite embryo, quite similar to the thirteen somite embryo presented in Plate IV, Fig. 2, had a rhythmical beat interrupted on rare occasions by fibrillations. The contractions occurred in the left wall of the ventricle and originated at the atrio-ventricular sulcus. The atrial region had not yet formed and contractions were absent in the laterally directed omphalomesenteric veins. The rate of the contractions was 114 beats per minute.

The thirteen and fourteen somite embryos had myocardial contractions of similar nature to the twelve somite embryo. Erythrocytes were observed in the ventricle of the fourteen somite embryo suggesting a functional circulation between the embryo and the extra-embryonic splanchnopleure.

DISCUSSION

The three most important components of the embryo which have a direct role in the process of cardiogenesis are the amnio-cardiac vesicles which later form the pericardial cavity, the endocardial masses which form the endocardium of the heart, and the epi-myocardial mantles which become the epicardium and myocardium of the heart. Some writers (Goss, 1952; Yoshinaga, 1921) have

stated that these occur embryonically in the order in which they have been
listed. The work which has been done on the cardiogenesis of certain canid
embryos has resulted in findings which in part verified this long standing order
for the Canidae. There is a question as to which is formed first, the amnio-
cardiac vesicles or the epi-myocardial mantles. By the careful study of the
cells of the lateral mesoderm of pre-somite embryos (Plate I, Fig. 2), it was
evident that the proliferations directly ventral to the lateral limiting sulci
were in effect the sites of the future bilateral cardiac primordia. Then by
comparing the two somite embryo with five somite embryos it could be seen that
the amnio-cardiac vesicles arose as a result of a split which had formed in
the proliferated lateral mesoderm. In embryos having five somites, the amnio-
cardiac vesicles were well developed posteriorly; however, anteriorly, opposite
the fore-brain they were slit-like and finally, farther anterior at the level
of the head fold, they did not exist at all. In the region opposite the fore-
brain, the vesicles had formed independent of any marked proliferation of the
mesoderm indicative of mantle formation. Thus, the vesicle formation was
instigated at the site of the mesodermal proliferation in three to four somite
embryos and immediately preceded the formation of the epi-myocardial mantles.

    Likewise, the proliferation of the angioblasts had not occurred prior to
mantle formation. These cells proliferated from the ventral side of the
retro-cardiac plates and the lateral mesoderm medially to the notochord.
After proliferating from the mesoderm, they became arranged in sheets one to
two cells thick which (Plate III, Fig. 5) at the time of the close approxi-
mation of the primordia connected the endocardial tubes and brought about their
fusion by the successive formation of vesicles (angiocysts). Goss (1938)
in describing the rat and Yoshinaga (1921) in describing the guinea pig state
that the angioblasts arose in pre-somite to one somite embryos; however, in
the cat (Schulte, 1916) this occurred later.

In a six somite embryo (761b), the lateral amnio-cardiac vesicles had become confluent immediately anterior to the head fold. Thus, the vesicle was U-shaped, the cranio-median arc corresponding to the curve of the U. The fluent description of Bonnet's (1901) observations of the Pleuro-Pericardialhohle (amnio-cardiac vesicles) in the dog was supported by this study.

Consideration of the relationships existing between the endocardia and the epi-myocardia of the dog has resulted in the placement of the epi-myocardial mantle formation prior to the formation of the endocardial masses. In the six somite embryo (761b), the masses were connected to and later lost among the cells of the mantles, both in the anterior and posterior limits of the mantles. This indicated that in the dog the mantles were present before endocardial tissue had appeared. Yoshinaga (1921) in describing the guinea pig and Goss (1952) in describing the rat, stated that the reverse was true and their statements were well supported with detailed drawings; however, endocardial cells were not observed before mantle formation in the dog.

The differentiation of the endocardial masses occurred by a successive formation and coalescence of vesicles which began in the median part of the primordia of four to five somite embryos and progressed both anteriorly and posteriorly. Anteriorly, differentiation of the masses to endocardial tubes progressed until the endothelial sprouts, the primordia of the ventral aortae formed in embryos having seven somites (Plate II, Fig. 5). Posteriorly, endocardial tubes had formed to the limits of the mantle (Plate II, Fig. 1) and had fused with the vitelline capillaries in embryos having seven somites (Plate II, Fig. 4).

The mantles, as observed in embryos having five to six somites, were relatively parallel to the midline of the embryo and unmarked by expansion;

however, in embryos having seven somites (Plate II, Fig. 5), the primordia had shifted medially and were divisible into bulbar, ventricular, and atrial parts. Several workers have described sub-divided mantles prior to fusion in the cat (Martin, 1902; Schulte, 1916) and the ferret (Wang, 1917). Bonnet (1901) went on to describe a dog embryo in which the bilateral primordia were sub-divided into ventricle, atrium, and sinus venosus; however, it is the opinion of the writer that Bonnet mistook the bulbi for the ventricles, the ventricles for the atria, and so on.

The median shifting of the primordia was quite pronounced in embryos having eight somites. Considerable rotation had also occurred causing the mantles to be tipped on their sides, the epi-myocardial furrows opened toward each other preparatory to later fusions. By comparing embryos having eight somites with those having eleven somites, it was evident that actual fusion occurred in the nine to ten somite stages. The first parts which underwent fusion were the posterior part of the bulbi and the anterior part of the ventricles. The fusion of the original lateral walls of the mantles and the pre-cardiac plates occurred first, forming the ventral wall of the heart; later, the original medial epi-myocardial folds fused forming the dorsal wall of the heart; by thirteen somites (Plate IV, Fig. 2), both the dorsal and ventral walls of the heart were complete posteriorly to the diverged posterior atrial primordia.

Fusion of the endocardial tubes occurred subsequent to the formation of the ventral wall of the bulbus and of the anterior end of the ventricle. The fusion was effected by the angiocysts (vesiculated angioblast tissue), the endocardial tubes becoming confluent by a progressive coalescence of vesicles.

The formation of the dorsal mesocardium by the fusion of the retro-cardiac plates was first apparent in eleven somite embryos and was complete from mid-bulbus

to the fused atrium in embryos having thirteen somites. The ventral meso-
cardium failed to persist except for a short area in the wake of the margin
of the anterior intestinal portal, thus substantiating Robinson's (1902) declara-
tion that the ventral mesocardium is absent in all mammals. Schulte (1916)
in describing the fusion of the mantles in the cat said there were two factors
which influenced the formation of the mesocardia, the width of the fore-gut
and the original position of the bilateral cardiac primordia. The sheet of
splanchnic mesoderm intervening between the mantle and the medial angle of the
amnio-cardiac vesicle is designated the retro-cardiac plate, so, that between
the mantle and the lateral angle, the pre-cardiac plate (Plate I, Figs. 4, 5).
If the retro-cardiac plates are narrow relatively to the fore-gut, the mantles
will be widely separated upon fore-gut closure and the ventral mesocardium
will precede the dorsal in formation. This type occurs in the dog, and cat,
with some peculiarities recorded below. If, however, the retro-cardiac plates
are broad relative to the width of the fore-gut, the formation of the dorsal
mesocardium will be accelerated. The guinea pig is an example of the latter
type. The third type, as occurs in the chick where the retro-cardiac and pre-
cardiac plates are of such proportions that the dorsal and ventral mesocardia
are formed at the same time, is a modification of the two previous types.

Considering the dog in relation to the types mentioned, it is evident that
due to the late formation of the dorsal mesocardium and rather early closure
of the ventral wall of the heart, the dog belongs to the first type, also
characterized by the cat. Schulte failed to completely explain the absence of
the ventral mesocardium and the presence of the middle cardiac plate which
forms the floor of the heart becoming increasingly wider toward the posterior
(Plate III, Fig. 5). If Schulte had simply observed that the greater part of
the pre-cardiac plates go into the formation of the ventral wall of the heart,

no problem would have resulted. With the greater part of the pre-cardiac plate
involved in the heart formation, a relatively small amount, not enough, was
present for the formation of the ventral mesocardium. Thus, with insufficient
tissue available for its formation, the ventral mesocardium was progressively
eliminated as soon as it was formed in the wake of the anterior intestinal
portal.

The dorsal mesocardium persists until fourteen somites when the formation
of the cardiac loop necessitates its obliteration. It breaks first at the
bulbo-ventricular junction, proceeding posteriorly to the atrium in embryos
having fifteen somites.

For the most part, cardiogenesis in the dog corresponds closely with the
conditions prevailing in the cat. Further consideration of canid cardiogenesis
in relation to the general pattern of mammalian heart development as inter-
preted by Goss (1952) resulted in the placement of the dog at the end of a
series beginning with the rat which has unusually large bilateral primordia
fusing early and ending with the cat which has smaller, more independent pri-
mordia which persist for a relatively long interval prior to fusion. The
tentative arrangement of Goss, as amended by the writer, begins with the rat
and continues with the sheep, guinea pig, marsupials, ferret, man, rabbit,
cat and finally the dog which has the most persistent and individualistic
bilateral cardiac primordia.

The earliest myocardial contractions which have been observed in the dog
occurred in eleven somite embryos in which fusion of the epi-myocardia and
endocardia had commenced. Myocardial contractions have been observed much
earlier in the paired primordia of the rabbit (Dwinnel, 1939) and the rat
(Goss, 1938). In both the rabbit and the rat, the epi-myocardia were con-
siderably expanded and according to Dwinnel, this is a criterion for the

determination of the onset of contraction in the paired primordia. It is evident then that contraction of the primordia of the dog occurs in embryos having seven to eight somites.

The myocardial contractions of the eleven and twelve somite embryos were occasionally interrupted by fibrillations. Nordmann and Ruther (1931) explain interruptions of the heart rate as due to nutritional variance. Patten and Kramer (1949) in studying the chick came to the conclusion that unfavorable culturing conditions were the cause of changes of the rate.

So it appears, therefore, the rate of the heart of embryos is extremely variable, the variance attributable to a multiplicity of extrinsic and intrinsic factors. Probably more important than the rate is the site of and the origin of the contractions. In the dog, the contraction originates first in the posterior part of the ventricle--the atrio-ventricular sulcus. The first beats occur only in the left wall of the ventricle.

Further work along this line is contemplated.

SUMMARY

1. Canid cardiogenesis is instigated in early three to four somite embryos.

2. The amnio-cardiac vesicles, the epi-myocardial mantles, and the endocardial masses occur embryonically in the order stated.

3. The median shifting of the bilateral cardiac primordia begins in six to seven somite embryos.

4. The epi-myocardial mantles fuse first on the ventral side of the heart in embryos having nine to ten somites. Fusion on the dorsal side begins directly in embryos having eleven somites.

5. Epi-myocardial fusion precedes fusion of the endocardial tubes.

6. The ventral mesocardium is practically absent in canids.

7. The tentative arrangement of the types of heart formations of mammals, as arranged by Goss, based on the relative persistence and individuality of the primordia has been amended by the dog, adjacent to the cat in the arrangement.

8. The earliest myocardial contractions observed originated at the atrioventricular sulcus and proceeded anteriorly throughout the left wall of the ventricle in embryos having eleven somites.

ACKNOWLEDGMENTS

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APPENDIX

PLATE I

Explanation of Figures

Fig. 1. Drawing of dog embryo 16b. Dorsal view. 17x.

Fig. 2. Drawing of a transverse section of dog embryo 16a. The section corresponds to line N. F. on Fig. 1. 50x.

Fig. 3. Drawing of coyote embryo 761a. Dorsal view. 17x.

Fig. 4. Drawing of a transverse section of dog embryo 1171b. The section corresponds to line A.C.V. of Fig. 3. 50x.

Fig. 5. Drawing of transverse section of dog embryo 171b. The section corresponds to line END'C. of Fig. 3. 50x.

Fig. 6. Detail drawing of the cardiac primordia of coyote embryo 761a. Dorsal view. 50x.

ABBREVIATIONS FOR ALL FIGURES

A.C.V., Amnio-cardiac vesicle
A.I.P., Anterior intestinal portal
A.L.S., Anterior limiting sulcus
ATR., Atrium
B.A., Bulbus arteriosus
BL. I., Blood island
C., Pericardial coelom
C.J., Cardiac jelly
D.AO., Dorsal aorta
DIENC., Diencephalon
END'C., Endocardium
END'C.S., Endocardial septum
F.G., Fore-gut

PLATE I

Fig. 1 — labels: A.L.S., L.L.S., N.F., S.I, N'CH., BL.I.

Fig. 2 — labels: L.L.S., N.G., N.F., H.MES., L.MES.

Fig. 3 — labels: F.B., M.B., A.C.V., END'C., END'C. MASS, EPI-MYOCARD., H.B., BL.I., S.I

Fig. 4 — labels: N.F., EPI-MYOCARD., END'C., END'C. MASS, A.C.V.

Fig. 5 — labels: EPI-MYOCARD., N.G., N.C., A.C.V., END'C., R.P., F.P.

Fig. 6 — labels: DIENC., MESENC., METENC., END'C. MASS, EPI-MYOCARD., END'C., END'C. MASS, MYELENC.
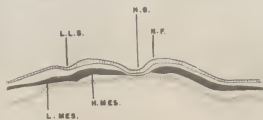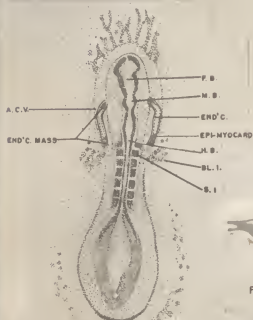
PLATE II

Explanation of Figures

Fig. 1. Drawing of dog embryo 117La. Dorsal view. 17x.

Fig. 2. Drawing of dog embryo 45R. Ventral view. 17x.

Fig. 3. Drawing of dog embryo 115L. Ventral view. 17x.

Fig. 4. Detail drawing of the fusion of the endocardium with the extra-embryonic vascular elements. The drawing corresponds to the area outlined on the drawing of embryo 45R. 225x.

Fig. 5. Detail drawing of the cardiac primordia of dog embryo 45R. 50x.

Fig. 6. Detail drawing of the cardiac primordia of dog embryo 115L. 50x.

ABBREVIATIONS FOR ALL FIGURES (cont.)

F.B., Forebrain
H.B., Hindbrain
H.F., Head fold
H.MES., Head mesoderm
L.L.S., Lateral Limiting Sulcus
L.MES., Lateral mesoderm
M.B., Midbrain
M.C.P., Middle cardiac plate
MESENC., Mesencephalon
METENC., Metencephalon
M.G., Midgut
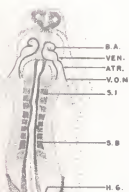M'S'C.D., Dorsal mesocardium
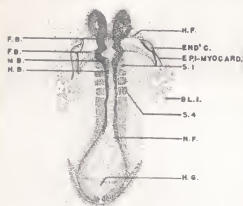
PLATE II

Fig. 1
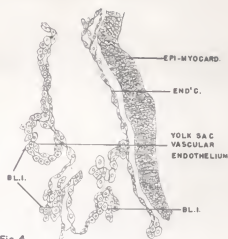
Fig. 2

Fig. 3

Fig. 4
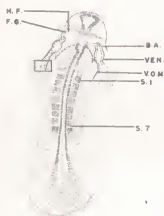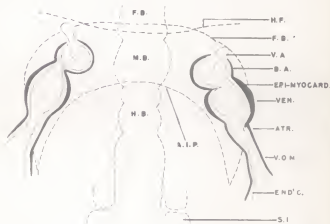
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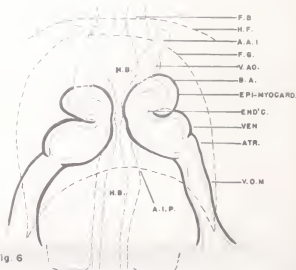
Fig. 6

Explanation of Figures

Fig. 1. Sketch of an eleven somite dog embryo for a guide to the transverse section of dog embryo 120Rd. 17x.

Fig. 2. Sketch of the heart of an 11 somite dog embryo. 120Rd. 50x.

Fig. 3. Drawing of a transverse section of dog embryo 120Ld. The section corresponds to line AA' of the guide. 50x.

Fig. 4. Drawing of a transverse section of dog embryo 120Ld. The section corresponds to line BB' of the guide. 50x.

Fig. 5. Drawing of a transverse section of dog embryo 120Ld. The section corresponds to line CC' of the guide. 50x.

Fig. 6. Drawing of a transverse section of dog embryo 120Ld. The section corresponds to line DD' of the guide. 50x.

ABBREVIATIONS FOR ALL FIGURES (cont.)

M'S'C.V., Ventral mesocardium
MYELENC., Mylencephalon
N.C., Neural crest
N'CH., Notochord
N.F., Neural folds
N.G., Neural groove
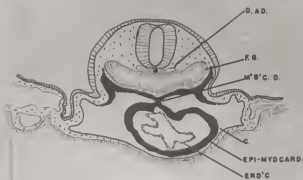PH., Pharynx
P.P., Precardiac plate

PLATE III

D. AO.
P. B.
M'B'C. D.
C.
EPI-MYOCARD.
END'C.

Fig.3

D. AO.
P. B.
M'B'C. D.
C.
EPI-MYOCARD.
END'C.

Fig. 4

D. AO.
P. B.
C.
EPI-MYOCARD.
END'C.
M'B'C. V. M C.P.

Fig. 5
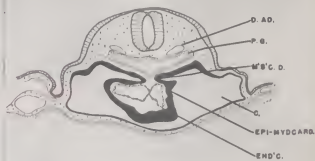
D. AO.
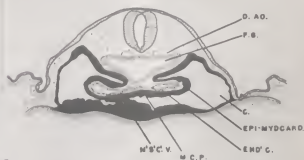M. B.
C.
EPI-MYOCARD.
END'C.

Fig. 6

A
B
C
D
B.1

Fig.1

Fig 2

PLATE IV

Explanation of Figures

Fig. 1. Drawing of dog embryo 118La. Dorsal view. 20x.

Fig. 2. Drawing of a longitudinal section of dog embryo 118Lb. 50x.

ABBREVIATIONS FOR ALL FIGURES (cont.)

R.P., Retrocardiac plate
S.1, S.2, etc., Somite and number
T.F.AM., Tail fold amnion
V.AO., Ventral aorta
VEN., Ventricle
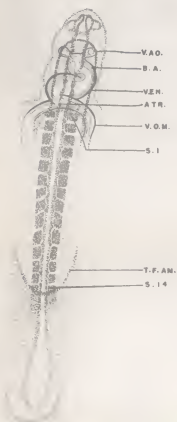V.O.M., Omphalomesenteric vein
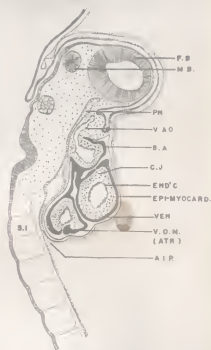
PLATE IV

FIG. I

FIG. 2

CARDIOGENESIS AND OBSERVATIONS OF THE FIRST
HEART CONTRACTIONS IN CERTAIN CANIDS

by

LOWELL MYERS DUFFEY

B. S., Maryville College, Maryville, Tennessee, 1951

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ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

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OF AGRICULTURE AND APPLIED SCIENCE

1953

ABSTRACT

This study of canid cardiogenesis was made on a series of pre-somite, early sixteen day embryos to fourteen somite, late sixteen day embryos, over a span of only twenty hours and fourteen somites. Results were compared with descriptions of cardiogenesis in the cat, guinea pig, ferret, rat, rabbit, and man.

In the dog embryos studied, cardiogenesis began early on the sixteenth day of gestation in three to four somite embryos. The primordia were located in the splanchnic mesoderm ventral to the lateral limiting sulci and consisted of epi-myocardial folds but without differentiated endocardial tubes. In five and six somite embryos, the primordia consisted of epi-myocardia and endocardial tubes which extended from the midbrain to the posterior limits of the head mesoderm. By comparing the five somite embryos with two somite embryos, it was evident that the amnio-cardiac vesicles (coelom primordia), the epi-myocardial mantles (primordial layer later differentiating to epicardium and myocardium in the adult) and the endocardial tubes (primordia of the adult endocardium) arise embryonically in the order named. The median shifting of the primordia was evident in six to seven somite embryos taken from uteri during the middle of the sixteenth day of gestation. The epi-myocardial mantles fused ventrally in embryos having nine to ten somites. Progressive fusion of the primordia was evident--most pronounced in the bulbo-ventricular regions and continuing both anteriorly and posteriorly in older embryos. Fusion was complete in the bulbus, ventrice, and anterior half of the atrium in embryos having fourteen somites removed from uteri during the latter part of the sixteenth day of gestation. It was evident that epi-myocardial fusion preceded the fusion of the bilateral endocardial tubes. The fusion of the epi-myocardia ventrally, associated with the flooring-in

of the fore-gut, resulted in the absence of a ventral mesocardium, an observation which coincided with previous descriptions of mammalian cardiogenesis. It was observed that the cardiac primordia persist in the unfused condition for a much longer time than in the rat, rabbit, sheep, marsupial, or ferret and human embryos. The observations have made possible the inclusion of the dog in the series of mammalian cardiogenesis based on the relative persistence of the bilateral condition of the primordia. The tentative arrangement of the series proposed by Goss now is as follows: rat, sheep, guinea pig, marsupials, ferret, man, rabbit, cat and dog.

The study of canid embryos _in vivo_ has revealed that the early myocardial contractions occur in the left wall of the ventricle proceeding anteriorly from the atric-ventricular sulcus of the single tubular heart. The rate increases from 98 beats per minute in eleven somite embryos to 114 beats per minute in fourteen somite embryos. Further work is contemplated pertinent to the physiology of the embryonic heart contractions.