Intracardiac Flow Patterns in Early Embryonic Life

A Reexamination

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SUMMARY. Microangiography, using methylene blue injected at eight vitelline vein sites, was performed on 156 developing chick embryos at Hamburger-Hamilton stages 14-22. Two stream patterns were observed. Type A coursed sequentially through the dorsal portion of the sinus venosus, the cranial segments of the primitive atrium and atrioventricular canal, the ventral parts of the primitive ventricle and conus cordis, and, finally, the left branchial arches. Type B coursed through the ventral portion of the sinus venosus, the caudal segments of the primitive atrium and atrioventricular canal, the dorsal parts of the primitive ventricle and conus cordis, and, finally, the right branchial arches. Both streams flowed in parallel fashion in the conus cordis. At Hamburger-Hamilton stages 17-18, the dye stream from the right lateral vitelline vein was chiefly type A, whereas that from the left lateral vitelline vein was type B. At Hamburger-Hamilton stages 19-22, those patterns reversed, i.e., the right lateral vitelline vein stream ran as type B, whereas the left lateral vitelline vein stream assumed type A pattern. The cranial-caudal relationship of the two streams at the primitive atrium and atrioventricular canal is not consistent with the hypothesis that these streams separately expand the future right atrium and left atrium. Their parallel direction at the conus cordis does not support the theory that spiral septation is initiated by two spiral streams. The longitudinal separation of the two streams at and beyond the branchial arches also argues against aortico-pulmonary septation as a consequence of flow streaming. Our observations do not support the traditional flow-molding theory. (Circ Res 53: 363-371, 1983)

It is a traditional view that spiral streams of blood mold the primary heart tube during its development, and play a major role in the formation of the ventricular, bulbar, and aortico-pulmonary septa (Spitzer, 1923; Bremer, 1932; Goerttler, 1955; Barthel, 1960; De Vries et al., 1962; Jaffee, 1962, 1963, 1965, 1966, 1967). Experiments have been carried out to test this hypothesis. Some have induced cardiac malformations in chick embryos after surgical alteration of their blood flow patterns (Stephan, 1952; Rychter, 1962; Gessner, 1966; Clark, 1969; Harh et al., 1973; Clark and Rosenquist, 1978; Sweeney, 1981). Despite evidence in support of the concept, the role of forces generated by the flow of blood through the developing heart, still remains unclear (Manasek and Monroe, 1972; Clark, in Press).

Although considered to be the principal basis of the hypothesis, the normal flow pattern within the embryonic chick heart has not been sufficiently investigated. Two spiral streams have been described, based on observations of the moving blood cells within the primitive heart (Bremer, 1932; Jaffee, 1962, 1963, 1965, 1966, 1967). This method of evaluation, however, is inadequate for tracing the entire course of the streams in the heart tube, and is subject to error. The use of dye indicators injected into segments of the cardiovascular system, is more accurate for this purpose (Rychter and Leméz, 1961).

In this report, we present our microangiographic studies which delineated the intracardiac flow patterns at the early stages of cardiac development in chick embryos. From our data, we question the propriety of the traditional flow-molding theory for cardiac development.

Methods

Fertile White Leghorn eggs were incubated at 38.5°C to 39.5°C at constant humidity, using a standard incubator (Leahy Manufacturing Company). The eggs were positioned blunt end up and were not rotated so that the embryos would be situated beneath the air sac at the blunt end, and thus be easily accessible for our microangiographic studies. The experiments were done after 50 hours to 4 days of incubation at the following Hamburger-Hamilton (H-H) stages of development (Hamburger and Hamilton, 1951): stage 14, stage 15, stage 16, stage 17, stage 18, stage 19, stage 20, stage 21, and stage 22. These developmental stages were chosen because they represent that period of cardiac development which extends from pre-septation to beginning septal formation. The embryos were exposed by creating a window in the shell at the blunt end, and thus be easily accessible for our microangiographic studies. The experiments were done after 50 hours to 4 days of incubation at the following Hamburger-Hamilton (H-H) stages of development (Hamburger and Hamilton, 1951): stage 14, stage 15, stage 16, stage 17, stage 18, stage 19, stage 20, stage 21, and stage 22. These developmental stages were chosen because they represent that period of cardiac development which extends from pre-septation to beginning septal formation. The embryos were exposed by creating a window in the shell at the blunt end, removing the shell membrane, and tearing the chonon and amnion over the embryo. By this exposure, the chick embryo normally lies with its right side up on the yolk, i.e., in a right lateral position.

With a vertical pipette puller, micropipettes were prepared from glass tubes of 1.0 mm o.d. The tip of the micropipettes varied from 1 to 6 μm o.d., depending upon the size of the embryo studied. Each glass pipette was

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filled with a 0.4% methylene blue solution (methylene blue powder, 4 g; 95% ethanol alcohol, 240 ml; 10% potassium hydroxide, 1.0 ml; and distilled water to make a total of 1000 ml), using a vacuum technique. The filled pipette was inserted into a pipette holder into which a polyvinyl tubing connected to a tuberculin syringe (also filled with the dye solution), had been attached. The holder then was attached to a micromanipulator.

Microangiographic studies were performed in the right lateral projection on 134 embryos at the following stages: H-H stage 14 (15 embryos), stages 15-16 (20 embryos), stages 17-18 (49 embryos), and stages 19-22 (50 embryos). Under microscopic guidance and by means of the micromanipulator, the glass pipette was inserted at intervals into all available vitelline veins, as shown in Figure 1. The injection sites were: the three tributaries of the left lateral vitelline veins, the three tributaries of the right lateral vitelline veins, the right and left anterior vitelline veins, and the left posterior vitelline vein. The course of the dye-containing blood stream was checked at the following sites: at the common omphalomesenteric trunk, sinus venosus, right side of primitive atrium, atrioventricular canal, primitive ventricle, conus cordis, truncus arteriosus, right branchial arches, and right and left vitelline arteries.

In another group of 22 embryos, all at H-H stages 17-18, the studies were performed in the left lateral position. After exposure through the same shell window, the embryo was gently turned left side up with forceps, after removing the chorion and amnion. The dye solution then was injected into the same venous sites, and the flow pattern at the left side of the primitive atrium and atrioventricular canal observed.

Approximately 0.003–0.03 μl of the methylene blue solution was injected once at each site, the injection time lasting for 2–5 seconds each time. The total volume injected into each embryo was not determined. However, this was estimated roughly to be less than 0.02 μl in the young embryos (H-H stages 14–16), or not more than 0.2 μl in the older ones (H-H stages 17–22). The dye-containing stream was observed under a microscope and photographed with a 35-mm camera (Olympus, model PM-10-M). Each study took about 10 minutes to complete. During this time, the temperature in the albumen fell gradually from an initial temperature of about 35°C to about 30°C at the end of the study. Initial heart rates varied according to the H-H stage (range: 100–140 beats/min); these also fell during the course of the experiment. In each embryo, if the study could not be completed before onset of severe bradycardia, e.g., to less than half of initial heart rate, accompanied by onset of truncal regurgitation as identified by diastolic backward movements of blood cells and dye particles into the ventricle, the study was discontinued and the results discarded. These circulatory changes are pre-terminal events. Embryos which bled from an injection site before completing the experiments were also excluded from the study.

Results

Blood flow within the primary heart tube was laminar in all of the embryos studied. The orientation of the dye stream within the heart, i.e., the flow pattern, varied with the site of injection. Two general patterns were observed. Type A stream, shown schematically by the dotted line in Figure 2, ran along the dorsal wall of the common omphalomesenteric trunk and sinus venosus, and proceeded to the cranial portion of the future right and left atra (Fig. 3A). This continued through the cranial part of the atrioventricular canal, into the primitive ventricle, where it coursed selectively along its ventral portion, and then proceeded upward along the ventral wall of the conus cordis. It then rotated clockwise about 90° toward the aortic arches, reaching mainly the left branchial arches (Fig. 4A). Type B stream, shown as a solid line in Figure 2, ran along the ventral wall of the omphalomesenteric trunk and sinus venosus, and proceeded to the caudal portion of the future right and left atra (Fig. 3B). This continued into the caudal part of the atrioventricular canal, coursed along the dorsal portion of the primitive ventricle, and then turned upward to run along the dorsal wall of the conus cordis. Finally, it twisted clockwise
about 90°, reaching mostly the right branchial arches (Fig. 4B). Both streams in the right lateral view ran in almost parallel fashion along the conus cordis, and, in the earlier stages, as in H-H stages 14-16, even in the region of the truncus.

Table 1 is a summary of the blood stream patterns observed in the left lateral projection which exposes the left side of the primitive atrium and atrioventricular canal. Type A stream coursed preferentially along the cranial wall of the primitive atrium and atrioventricular canal (Fig. 5A), whereas type B stream preferentially coursed along the caudal walls of the primitive atrium and atrioventricular canal (Fig. 5B). The course of the streams in the conus region was not seen in the left lateral view since the conus lies hidden or beneath the primitive ventricle and left side of the primitive atrium in this projection (Fig. 2).

Table 2 is a summary of the stream patterns observed in the right lateral projection, following dye injection at varying sites and at different stages of development. Injection into the right anterior vitelline vein was done only at H-H stage 14 since this vessel was no longer accessible at the later stages. By the same token, dye injection was not done in the left lateral and right lateral vitelline veins at H-H stages 14-16 since these veins were still poorly developed at this time. An interesting observation was the changing intra-cardiac flow patterns of streams originating from specific segments of the vitelline venous system as cardiac development progressed. Thus, at H-H stage 14, the dye stream from the left anterior vitelline vein assumed type A pattern in 12 of 15 embryos studied, whereas that from the right anterior vitelline veins had type B pattern in 12 of the 15 embryos. At H-H stages 15-16, the dye stream from the left anterior vitelline vein now assumed a type B pattern in 18 of 20 embryos studied. In contrast, the blood stream from the left posterior vitelline vein had a type A pattern in 18 of 20 embryos. At H-H stages 17-18, the dye streams arising from the three tributaries of the right lateral vitelline vein showed type A pattern, as did the stream from the left posterior vitelline vein. By contrast, the dye streams arising from the tributaries of the left lateral vitelline vein and from...
the left anterior vitelline vein had predominant type B pattern. At H-H stages 19–22, the flow pattern from the right lateral and left lateral vitelline veins reversed, i.e., the dye stream from the right lateral vitelline vein was now chiefly type B (Fig. 6A), whereas that from the left lateral vitelline vein was now mainly type A (Fig. 6B). The streams from the left anterior and posterior vitelline veins remained unchanged, i.e., chiefly type B in the former and predominantly type A in the latter. Since both the

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**FIGURE 3.** Microangiograms in the right lateral view from an embryo at H-H stage 19. Part A: dye stream from left lateral vitelline vein (LLVV) passes along dorsal wall of common omphalomesenteric trunk (COT) and sinus venosus (SV), and then along cranial wall of the right side of primitive atrium (RA). Part B: dye stream from right lateral vitelline vein (RLVV) runs along ventral wall of common omphalomesenteric trunk and sinus venosus, and then along caudal wall of the right side of primitive atrium. Ao—aorta.

**FIGURE 4.** Microangiograms in the right lateral view from an embryo at H-H stage 17. Part A: dye stream from right lateral vitelline vein (RLVV) occupies ventral site of conus cordis (C) and then rotates clockwise toward the branchial arches. Part B: dye stream from left anterior vitelline vein (LAVV) passes along dorsal wall of conus cordis and then rotates clockwise toward the branchial arches. The two streams are almost parallel in the conus cordis. Note greater dye density in the right branchial arches (RBA) after the LAVV injection (type B stream) than after the RLVV injection (type A stream), due to preferential flow of the latter to left branchial arches (see also Fig. 5).
left anterior vitelline vein and left posterior vitelline vein continued into the same left omphalomesenteric vein (see Fig. 2), persistence of their differing flow streams indicated absent or poor mixing within the omphalomesenteric vein.

The flow patterns on the branchial arches also were studied. As viewed in the right lateral view, type B stream continued into the right arches, whereas type A stream did not, suggesting that the latter stream preferentially proceeded into the left arches. This was confirmed by the studies at H-H stages 17–18 in the left lateral projection, which showed continuation of type A stream into the left branchial arches (Fig. 5). Finally, selective streaming into the left and right vitelline arteries was observed at H-H stages 14–17, based on the relative densities of the dye in these vessels in the right lateral projection. Type A stream continued preferentially into the left vitelline artery (Fig. 7A). In contrast, type B stream flowed mainly into the right vitelline artery (Fig. 7B).

**Discussion**

According to the flow-molding theory, there are two spiral streams in the primary heart tube which mold the primitive cardiac chambers and play a major role in the septation process (Spitzer, 1923; Bremer, 1932; Goerttler, 1955; Barthel, 1960; De Vries et al., 1962; Jaffee, 1962, 1963, 1965, 1966, 1967). One stream is the so-called right heart stream which flows selectively through the future right atrium, the right part of the atrioventricular canal, right part of the primitive ventricle, and then spirally through the ventral and left portion of the conotruncus to reach the more caudal branchial arches. The other stream is the so-called left heart stream which courses through the future left atrium, the left part of the atrioventricular canal, left portion of the primitive ventricle, and then spirally through the dorsal and right part of the conotruncus to reach the more cranial branchial arches eventually (Fig. 8). The hypothesis assumes that the right heart

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**FIGURE 5. Microangiograms in the left lateral view at H-H stage 17 showing the courses of the two blood streams in left side of primitive atrium and atrioventricular canal.** Part A: type A stream from right lateral vitelline vein (RLVV) occupies cranial part of primitive atrium (LA) and atrioventricular canal (AVC). Part B: type B stream from left anterior vitelline vein (LAVV) flows along caudal walls of primitive atrium and atrioventricular canal. Note greater dye density of the left branchial arches (LBA) after the RLVV injection than after the LAVV injection. PV—primitive ventricle, Ao—aorta.
TABLE 2
Dye Stream Patterns in Right Lateral View after Injection at Different Sites and at Different Stages of Development*

<table>
<thead>
<tr>
<th>H-H stage and no. studied</th>
<th>Stream type</th>
<th>Anterior vitelline vein</th>
<th>Left lateral vitelline vein</th>
<th>Right lateral vitelline vein</th>
<th>Left posterior vitelline vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-H stage 14 (15 embryos)</td>
<td>Type A</td>
<td>Left (g) 12</td>
<td>Right (g') 3</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>H-H stages 15-16 (20 embryos)</td>
<td>Type A</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-H stages 17-18 (49 embryos)</td>
<td>Type A</td>
<td>7</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>H-H stages 19-22 (50 embryos)</td>
<td>Type B</td>
<td>2</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

* Based on 134 embryos. See also Figure 1 for sites a-h.

stream expands the right atrium, while the left heart stream enlarges the left atrium. The endocardial cushion develops between the two streams, resulting in division of the atrioventricular canal into separate right and left atrioventricular orifices. Further, the hypothesis assumes that the spiral streams in the conotruncus promotes spiral septation of the outflow tract and truncus.

Our study confirmed the presence of two blood streams in primitive chick hearts. However, the course of these streams at H-H stage 14 through stage 22 differed greatly from that of the traditional right heart and left heart streams. Type A stream courses along the dorsal wall of the common omphalomesenteric trunk and sinus venosus, continues into the cranial portion of the primitive atrium (future right atrium and left atrium) and atrioventricular canal, then along the ventral wall of the primitive ventricle and then turns cranially to run also along the ventral wall of the conus cordis. From the latter, most of the blood continues into the left branchial arches. Type B stream courses along the ventral wall of the common omphalomesenteric trunk and sinus venosus, continues into the caudal portion of the primitive atrium and atrioventricular canal, then along the dorsal wall of the primitive ventricle, and then turns cranially also along the dorsal wall of the conus to continue preferentially into the right branchial arches (Fig. 2). The type A stream in the left branchial arches continues, for the most part, into the left vitelline artery. On the other hand, the type B stream in the right branchial arches proceeds mostly to the right vitelline artery. Thus, there is evidence for longitudinal separation of both streams, even in the aorta, at these early stages of development (Fig. 7).

Our studies were carried out differently from those of previous investigators, and this may account for the dissimilar observations. Bremer and Jaffee based their conclusions from visual analysis of the forward movements of the blood cells (Bremer, 1932; Jaffee, 1962, 1963, 1965, 1966, 1967). In our experience, it is extremely difficult—in fact, virtually impossible—to delineate the entire course of the two streams by this method. Other microangiographic techniques have been reported. Rychter and Lemez (1961) injected Geigy Blue solution into the right ventricle of 4- to 6-day-old chick embryos and demonstrated preferential flow into the bilateral sixth branchial arches. Nilsen (1981) utilized fluorescein-labeled dextran injected into vitelline veins to visualize the microvasculature of explanted 43- to 76-hour chick embryos. However, the course of the venous streams within the developing heart was not studied.

Methylene blue dye is ideal for outlining blood flow patterns, due to the clear contrast between the dye-containing and non-dye-containing blood columns. Since blood flow is basically laminar, at least during the early stages of cardiac development, the dye particles tend to remain confined within one stream.

All of our studies were performed with the embryo in the shell and, therefore, in a more physiological state than if it were delivered outside the shell. Despite this, a fall in albumen temperature was observed during the course of the experiments, accompanied by gradual slowing of the heart rate. These changes did not alter the position of the intracardiac streams. Dye injection was deliberately slow, lasting for about 2–5 seconds per delivery of a miniscule volume of dye solution, to avoid dis-
FIGURE 6. Microangiograms in the right lateral projection at H-H stage 21. Part A: dye stream from right lateral vitelline vein (RLVV) courses dorsal portion of conus cordis (C), in contrast to that observed in Figure 4A. Part B: dye stream from left lateral vitelline vein (LLVV) flows at the ventral portion of conus cordis. Note better visualization of right branchial arches (RBA) after RLVV injection than after LLVV injection. COT—common omphalomesenteric trunk, RA—right side of primitive atrium, Ao—aorta.

FIGURE 7. Microangiograms at H-H stages 15–16 demonstrating selective arterial filling. Part A: dye stream originating from left posterior vitelline vein (LPVV) occupies central part of conus cordis, and flows mainly into the left vitelline artery (LVA). Part B: dye stream coming from left anterior vitelline vein (LAVV) occupies dorsal part of conus cordis, and appears chiefly in the right vitelline artery (RVA). (See text.)

FIGURE 8. Schematic drawing of the "traditional" spiral streams in the primary heart tube, viewed in the frontal projection. Dotted line is left heart stream, and solid line is right heart stream. For legend, refer to Figure 2.
torting the naturally occurring blood streams. For the same reason, only peripheral injection sites away from the omphalomesenteric veins were used.

Although some molding of the primitive heart by the blood streams probably occurs, based on fluid dynamic principles, many aspects of the traditional flow-molding concept may be questioned in light of our findings. For example, there is no strong basis for the notion that one of the streams selectively expands the future right atrium while the other enlarges the future left atrium on account of the crano-caudal relations of both streams in the primitive atrium. There is no evidence that the cranial part of the primitive atrium ultimately becomes one atrium, and the caudal part, the other atrium. Likewise, since the blood streams at the conotruncus do not follow a spiral course, at least in early embryonic life, the likelihood that these streams initiate conotruncal septation is not great. The unusual streaming pattern in the branchial arches and aorta provides an additional argument against the hypothesis. If the direction of the conotruncal septa was to follow the course of the type A and type B streams along these vessels, septation would be in such a manner that each ventricle could end up with a set of systemic arteries for half of the body. Since our studies were conducted at the early stages of cardiac development, extending from pre-septation to beginning septation, we propose that the streaming of flow during early embryonic life is not primarily responsible for initiating cardiac septation. Our data are more consistent with the idea that the initial septation process is intrinsic or programmed. It is likely that, as the septation process progresses, the streaming pattern may secondarily change in response to the pathway alteration. It may at that point help the process of septation that has already started. Thus, our observations do not necessarily contradict those of Rychter and Lemesz made on comparatively older embryos. Our hypothesis must be corroborated by subsequent circulatory studies at later stages of cardiac development.

The dramatic reversal of the venous return pattern from the right lateral and left lateral vitelline veins, occurring sometime between stages 17–18 and stages 18–22, deserves comment. This phenomenon has not been previously described. As a matter of fact, Bremer (1932) and Goerttler (1955) both presumed that venous return from the right omphalomesenteric vein was always into the right heart stream, whereas that from the left omphalomesenteric vein was always into the left heart stream, throughout embryonic life. The pattern reversal observed by us may be related to the dynamic changes in the vitelline venous system during early embryonic life. At H–H stage 14, most blood reaches the heart through two anterior vitelline veins. At H–H stages 15–16, the left posterior vitelline vein grows larger, while the right anterior vitelline vein disappears. The left anterior vitelline vein gets smaller, and the right and left lateral vitelline system begins to develop at H–H stage 17. Both lateral veins eventually become the main route of venous return during H–H stages 18 to 22. It is conceivable that the reduced venous return from the left anterior vitelline vein, occurring by H–H stage 17 or later, may cause angular shift of the venous streams within the omphalomesenteric veins (see Fig. 2). This could partly account for their reversal from type A to type B streams, or vice versa. Whatever the mechanism, these temporal changes in flow patterns must be taken into consideration in experimental studies of the embryonic circulation.

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