False Narratives Drive Heart Looping Research in Early Cardiac Morphogenesis

A Critical Analysis of Methodology and Interpretation

Francis J Manasek

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Contact: embryo@cardiac-looping.org

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Abstract

Cardiac looping, the morphogenic event transforming the embryonic tubular heart to a curved C-shape, is essential for normal continued development. A defect in looping may contribute to congenital heart defects. Looping is also an example of morphogenesis that should be amenable to experimental analysis.

The past three decades of research into the mechanism of cardiac looping have been based on a cascade of flawed data that created a false narrative and paradigm. The present analysis of these decades' published studies of heart looping demonstrates that science is not inherently self-correcting. Showing how false narratives influenced subsequent research, this work also documents the ensuing scientific consensus that created a literature of contradictory or incorrect data. It may be impossible to unravel the resulting morass of inaccuracies.

Careful reading of original papers demonstrates that many published studies of heart looping used highly selected data. Most computer models examine only the material properties of the myocardium and largely ignore the other components of the heart tube although it has been established that they are critical to the generation of normal anatomy. Some studies have attributed fabricated physical properties to functionally important components of the tubular heart.

Although fundamental material properties of the embryonic tubular heart wall had been ascertained experimentally much of this data was not incorporated into later computer models and its omission resulted in erroneous results. The cascade of publications based upon questionable data or incorrect analysis of existing data established an alternative narrative.

Several publications that had profound effects on later research are shown to contain misleading data or conclusions inconsistent with the published results. Throughout this period some striking contradictions, or anomalies, in experimental findings were overlooked or misreported and significant earlier findings ignored or discarded to facilitate finite element modeling. The present work also documents a pernicious phenomenon: misuse of papers published earlier, either by overt misstatement of their contents or by selective inclusion in (or exclusion from) bibliographies.

As a historiography, this is not intended to survey the literature nor does it attempt to propose a definitive mechanism for cardiac looping. Rather it explores the nature of evidence, the role of anomalous findings, the assembly of discrete experiments into cohesive models, the introduction of biases, and how the historical record of published papers may be manipulated. Recent developments in the philosophy of development are shown to be relevant to the study of cardiogenesis. These philosophical contributions, particularly in modularity and generative entrenchment, suggest fundamental conceptual errors in many contemporary studies. It is argued that no defined morphological endpoint has been articulated and if we cannot articulate the problem, we cannot answer it.

The conclusion that a false narrative has been imposed upon the field of early embryonic cardiogenesis is a disturbing outcome of this study. The false narrative distorts future research into embryogenesis as well as corrupting studies of the developmental basis of congenital heart disease.

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Hanover, NH
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Introduction

In this study I explore the development of data, its incorporation into mechanistic models and the manner in which both data and models become part of a disciplinary matrix by establishing a consensus of veracity. The time period of this study (roughly seventy years) is short enough to permit conceptual threads to be rigorously followed in detail and provides the opportunity to examine the epistemology and historiography of ideas in experimental developmental biology.

The general theme is the epistemology of the modern investigation of tubular heart looping. It explores the general issue of morphogenic mechanisms, especially that of epithelial deformation by extracellular matrix hydrostatic force. Hydrostatic epithelial deformation is demonstrably part of the repertoire of experimental embryology that has unfortunately become largely ignored. The present work challenges the concept that science is self-correcting (see Sagan 1980; Ioannidis 2012).) and shows, using specific examples, that scientific studies on cardiac development were not self-correcting and how a parallel false narrative was created. I also discuss the nature of experimentation and publication in this area and the ease with which scientific trajectories can be altered.

Using evidence from the developing chick heart, I summarize, sometimes cursorily, some of the experimental and descriptive work that enters into the quest for the morphogenic machine (q.v.) that constructs and bends the tubular heart using substituent modules. The narrative revolves around the specific shape change early in cardiogenesis, called looping, but has much broader implications regarding conceptually new mechanisms of embryonic morphogenesis and ways of framing questions regarding development. While the apparently modular construction of the tubular heart broaches quite an evolutionary range with minor phenotypic variation, the general format is highly conserved providing an example of generative entrenchment (see Wimsatt 2002). Ignoring generative entrenchment has hindered greatly the development of coherent models of looping based upon actual data.

From about the middle of the 20th century to the present time (2024) many mechanisms to explain looping have been proposed. I will primarily trace the history of one mechanism, that of deformation by hydrostatic pressure regulated by anisotropy and contextually refer to several other proposals such as differential cell division (see Sissman 1969; Chacko 1973; Icardo and Ojeda 1984). I shall concentrate on how the hydrostatic pressure concept was developed experimentally and how its trajectory was deflected as it traversed the research environment through time. Another purpose of this essay is to explore the nature of experimentation and publication in this area and the ease with which scientific trajectories can be altered.

I also explore the parallel evolution of some other proposed mechanisms contributing to looping. However, none of these alternative mechanisms have succeeded in presenting a simple unifying mechanism that explains bending and rotation in the presence of known concomitant cytologic and matrix changes. More recently, many assumptions have been tested by computer assisted modeling and, indeed, at least several seemingly plausible albeit remarkably complex models have emerged (see for example Shi et al. 2014b). It is noteworthy that these models consider organ shape change almost exclusively with respect to the myocardium, with little regard for the contributions of other components of the tubular heart.

Much of the search for mechanism was conducted within the context of the long-held dominating paradigm of "differential growth" and intracellularly-mediated changes in cell shape being predominant drivers of looping. This dominance, based upon the historical general acceptance of such mechanisms within the embryology community and their success in explaining

several diverse morphogenic events made them obvious candidates to explain looping. Working within this paradigm to explain the developing heart loop may have hindered the development and acceptance of alternative morphogenic processes.

The bulk of experimental studies on cardiogenesis has been done with chick hearts with the tacit assumption of complete mechanistic homology with mammal (rat and mouse) hearts. There are fundamental morphological differences between early rat and chick heart formation (Kaufman and Navaratnam 1981. See also review by Männer 2024). Hundreds of millions of years ago amniotes diverged into synapsids (clade Synapsida) from which mammals evolved, and sauropsids (clade Sauropsida) from which birds evolved. Their hearts, similar in appearance, are the result of evolutionary convergence.

Data from chick and rat experiments do not fit together neatly and in order to resolve the conflict investigators needed to discard or ignore a crucial mass of experimental chick data. Discarding this data forever altered the research trajectory. I traced the first example of chick data cancellation to a single publication (Taber et al. 1992) that created a hypothetical tubular organ with fictitious biomechanical properties. These hypothetical properties have since been used to model chick heart development despite the fact that they do not represent those of the actual developing organ. The fictitious properties acquired their own reality and are fundamental to the creation of the new false narrative.

Chapter 1

Early Embryonic Cardiac Morphogenesis: A *Very* Brief Survey

Embryos develop from a single cell. As the number of cells increase, they assemble to form discrete shapes: tissues, organs, and systems. Some of the means by which cells assemble into more complex higher-level configurations that in turn morph into even more complex configurations have been elucidated and fall into a few general categories.

First, some definitions

An **epithelium** is a sheet of cells tightly connected by means of specialized junctions. Epithelia have a *basal surface* resting on a substratum of *extracellular matrix*. The other side is the *apical surface* and generally faces a body cavity or the exterior.

Mesenchymal cells, in contrast to epithelial cells, are free to migrate. They are anatomically independent of each other and although they may exhibit leading and trailing edges do not have apical/basal polarity.

The extracellular matrix (ECM) is the material that fills intercellular spaces between mesenchymal cells and underlies epithelia. The extracellular compartment in early embryos is relatively large compared to the cellular component and provides support to the various cellular components. This compartment contains glycosaminoglycans (GAGs, formerly known as mucopolysaccharides) largely hyaluronate (HA or hyaluron or hyaluronic acid), chondroitin sulfate (ChS), and, as the embryo matures, increasing amounts of various collagens as well as a myriad of other macromolecules. The ECM received relatively little attention until the 1970s when analytic techniques other than histochemistry (very unreliable when demonstrating GAGs) evolved and were applied to embryos. Although appearing relatively homogeneous in sections examined with the light microscope, cardiac ECM is filamentous and highly anisotropic, a fact largely ignored in recent work and a subject to which I shall return.

Much recent work with ECM has focused upon its interactions with cells on a signaling level and with specific interaction with the interior of the cell via transmembrane integrin receptors and affecting the cytoskeleton (for brief summary see McCain and Parker 2011) but has largely ignored the physical/mechanical properties described earlier that can profoundly determine shapes.

The basement membrane (BM) is a layer of extracellular material underlying epithelia. Basement membranes are complex, nanoporous structures containing Type IV collagen, laminin, glycoproteins, and other components. The *basal lamina* is a substructure of the basement membrane and is not visible in the light microscope. Basement membranes are highly conserved and have multiple functions.

Morphogenesis, or the shape changes undergone during embryogenesis

As understood here, a mechanism comprises the interactions, dependent upon the chemistry and physics of existing antecedent parts, that produce a change in morphology.

Historically, morphogenesis was a descriptive anatomical subject and many detailed sequences of embryological development have been carefully observed and recorded. Epithelial cell shape changes and mesenchymal migration are histologically observable events and are readily relatable to embryonic form. Many biochemical and cytological events have been discovered and explored as possible contributors to morphogenic phenomena, but until relatively recently these connections were speculative. It is more difficult to connect a specific biochemical event (or the result of gene activation) to a discrete structural event than it is to relate one shape change to another. There is conservation both at the molecular/genetic level and at the anatomic level.

If some of the cells in an epithelial sheet change their shape the entire sheet deforms. This is a known phenomenon involving active contraction of one side, or end (either apical or basal), of columnar or cuboidal cells and is a proven morphogenic mechanism. (For a concise summary of the modeling of this phenomenon see Clausi and Brodland 2001.) Of course, there are fundamental levels of mechanism involved, for example the signal that "tells" cells to change shape and the intracellular machinery such as the cytoskeleton and contractile elements (much of which has been elucidated) that provides the force and the direction for the force to act. Epithelial shape change lends itself very well to quantitative experimental study and seems intuitively obvious.

Most well-described epithelial deformations result from contraction of different parts of constituent cells, essentially setting up a "pulling in" of adjacent regions. This, of necessity, creates areas where some cells are under tension and others under compression. Less obvious (or likely) is the possibility that cells flatten and push aside adjacent cells to deform an epithelium.

Not all cell shape changes result from intrinsic cell activities. Epithelia can also be deformed by extrinsic forces such as those from the extracellular matrix (ECM). When hyaluronate-rich ECM swells, it creates a hydrostatic force that is directed by anisotropy in the containing epithelia. This mechanism largely disappeared from the literature in the 1990s when the dogma of intrinsic cell shape change gained ascendancy, By the early 2000s, deformation as a morphogenic mechanism and ECM as a deforming force had seems to have disappeared from the literature (for an extensive overview see Davies 2013) although mentioned briefly by Taber (2009). In some systems it appears that the ECM may act to stabilize epithelial-originated tissue changes, not initiate them (see Nematbakhsh et al. 2020) but the architecture of those systems is quite different from that of the looping heart. Much of the present essay is related to understanding the role of deformation and anisotropy in cardiac morphogenesis.

Many commonly described morphogenic mechanisms invoke *additive* and *subtractive* activities. Cells proliferate, hypertrophy, and increase mass. If this occurs unequally in different regions, a shape change will occur because mass is added unequally. Mass can also be added by migration and accumulation of mesenchymal cells creating new multicellular mass. The extracellular compartment can also be modified by addition (by secretion) and reorientation of components such as collagen. Subtractive mechanisms, involving such events as cell death (see Pexieder 1975), are known to play a morphogenic role. A well-known example is the involvement of cell death in the formation of the digits.

Growth is a term used rather liberally. Clearly if one part of a structure enlarges faster than another, there will be a proportionate change in shape. In many models, "growth" is synonymous with a dimensional change whether that change is cell-based or tissue based. Is an isovolumic

change in length growth? Should any alteration of shape be considered as growth? Is hydration of the ECM with attendant volume/shape changes considered growth? When hydrated, molecular domains do increase in size (grow) and this can be translated to shape change.

The fuzziness of the term "growth" has been exploited in many studies, with authors sometimes employing different meanings in the same publication. The term is often used to mean cell division, cell hypertrophy, addition of cells, elongation, and sometimes even complex multifactorial morphogenesis depending upon convenience. Adding more cells requires mitosis and if cell division is to change organ shape (instead of isomorphic enlargement) it must occur at different rates in different regions. But getting bigger, or "growth," must involve the addition of mass or volume, be it cellular or extracellular. As used here "growth" is descriptive, simply meaning getting bigger and has no intrinsic mechanistic connotation.

Components of the Tubular Heart

We will examine a seemingly simple yet vexing problem: that of shape change in the early embryonic tubular heart. In all vertebrate embryos, two bilateral sheets of epithelial cells, the precardiac splanchnic mesoderm, migrate toward the midline, join and form a tubular structure known as the *tubular heart*. This structure then bulges and develops a "C" shape that rotates (usually) to the right side of the embryo. This dramatic phenomenon called "looping" has been most studied in the chick where it occurs within the period of a few hours.

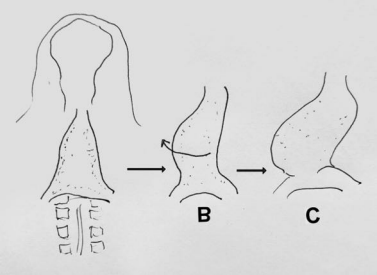


Figure 1. The left drawing shows the pre-loop midline chick heart in relation to the surrounding embryo. The rectangles are somites and the "head" region is at the top. The middle drawing shows the **C-** looped heart. It has bulged and begun to rotate to the right (this is a ventral view with the embryo on its back so the embryo's right is to your left. The diameter of the heart at this stage is approximately 0.1 to 0.2 mm). The rightmost drawing shows a later stage where the heart is beginning to develop the post-loop "S" shape. The developing murine heart does not look like this. The diameter of the chick tubular heart shown here is roughly 0.1-0.2mm. **All illustrations show a ventral view of the embryo. Accordingly, the embryo's right side is toward the viewer's left.**

If we are going to study morphogenesis of the early heart it is necessary first to know the structure's constituents.

The early embryonic chick heart, the major experimental model of cardiogenesis studies, is a layered system that is best described roughly as a tubular structure comprising three major layers: the outer epithelial layer of developing muscle, the myocardium; the inner layer, the endocardium, lining the lumen; and the thick intervening sleeve of extracellular matrix, the cardiac jelly. There is another
layer, the basement membrane (BM), not often discussed nor made a part of FEM models. The BM separates the basal layer of the epithelial myocardium from the cardiac jelly. Both the CJ and BM are secretory products of the myocardium

The myocardium

The embryonic myocardium is an epithelium. It is derived from bilateral inward migration of splanchnic mesoderm through the dorsal mesocardium. At the early tubular heart stage the undifferentiated myocardium does not have a smooth continuous basal surface, rather it is irregular

with large intercellular spaces. It does not have a continuous basal lamina (BL). The BL becomes more extensive as the myocardial basal layer becomes more regular.

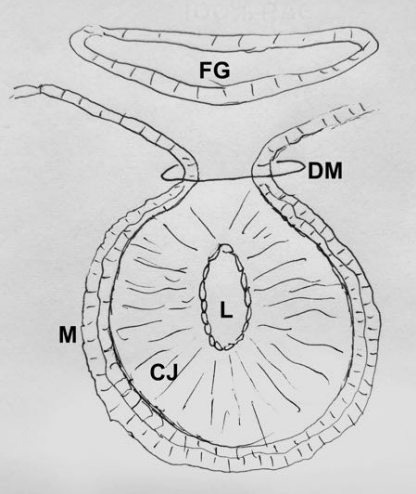


Figure 2. Diagrammatic transverse section midway through the tubular heart. The central space is the lumen lined by simple squamous endothelium. The radial filaments that contribute to anisotropy of the cardiac jelly are shown diagrammatically. Omitted from this diagram is the BM, which is present along the basal surface of the myocardium facing the CJ. FG=foregut, DM=dorsal mesocardium, M=myocardium, CJ=cardiac jelly, L=lumen, lined by endocardial cells.

In the chick, developing muscle of the myocardium secretes the underlying cardiac jelly. Presumably, the murine myocardium does also but this has not been established. The histology of the early chick myocardium strongly suggests, on morphological grounds, that it is a transport epithelium. If form and function are linked and the early myocardium is indeed a transport epithelium, then it should have the ability to regulate hydration of the ECM that it produces. It has been reported that inhibiting the Na^+K^+ ATPase pump with oubain (Linask and Gui 1995) had effects on cardiogenesis but it was not made clear if there were any effects on CJ hydration.

In the chick the myocardium *differentiates,* that is, it acquires the cytological characteristics of heart muscle developing abundant, highly aligned myofibrils after the tubular heart forms and begins to inflate with CJ. The chick heart then begins to beat. In murine development there is a different sequence. Myocardial cells differentiate and beat before the tubular heart is formed and the single heart is the product of fusion of two already functioning primitive "hearts" (for summary see Männer and Yelbuz 2019).

Every developing striated muscle cell becomes anisotropic because of its myofibrils. Cardiac muscle is no exception. In the developing myocardium, muscle cells in different regions have different orientations. *Therefore, the entire developing myocardium has regions of directionally different anisotropy, hence regional forces are directed in different directions.* All the muscle cells of the myocardium are firmly connected by intercalated discs and other cell-cell junctions. In the chick myocardial differentiation is essential for looping to occur. If myofibrils do not form, hearts do not loop but continue to inflate.

It had been shown some time ago that there are essentially no differences in right–left-sided contributions or mitotic rates (see Sissman 1988; Stalsberg 1969a; Soufan et al. 2006) and that hearts loop in the presence of colchicine (Icardo and Ojeda 1984). Nonetheless, a great deal of work has concentrated on employing differential "growth," in quantitative models of heart looping (Ebrahimi et al. 2022a).

In the chick, myocardial cells exhibit different shapes and orientations in different regions of the looping heart (Manasek et al. 1972). At the time it was enticing to try to invoke changing cell shapes as a morphogenic mechanism of heart looping but this has proven unsuccessful. Unfortunately, there are no comparable studies of cell shape changes in murine hearts. Published SEM images (see Layton and Manasek 1980; Kaufman and Navaratnam 1981; Camenisch et al.

2000; Marcela et al. 2012) do not have sufficient resolution to be able to discern regional differences in cell shapes.

Developing chick myocytes also have an anatomically demonstrable extensive, non-myofibrillar cytoskeleton (for early examples see Manasek et al. 1984; Isobe et al. 1984; Linask and VanAuker 2007) and much has been learned about mechanotransduction. (McCain and Parker 2011) as a regulator of cytoskeletal architecture with attempts being made to link this to heart shape. Studies of cytoskeletal responses to the underlying matrix have proven fruitful in elucidating the mechanism of cell surface receptors, but not of looping.

The dorsal mesocardium

The tubular chick heart is more or less midline along the embryo's long axis and connected to the embryo's long axis by a remnant of the splanchnic mesoderm, the *dorsal mesocardium*. The dorsal mesocardium, resulting from the inward movement of the splanchnic mesoderm, initially forms, before it fuses, a "U" channel along the dorsal length of the tubular heart and becomes part of the inner curvature.

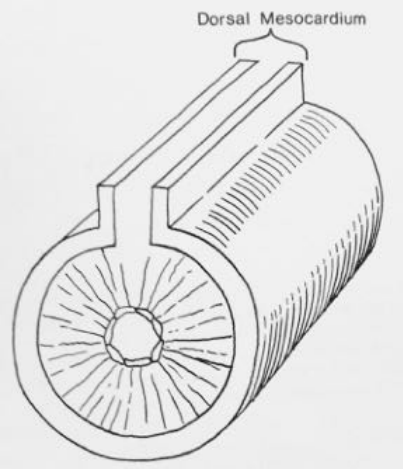


Figure 3. Schematic drawing of a tubular heart showing relative position of the dorsal mesocardium. (From Manasek 1983)
This figure should be compared to Figure 1 in Taber et al. (1995) and to that in Taber & Perucchio (2000).

Such a structure running the length of a tube increases rigidity along the that side of the tube making it stiffer and more resistant to bending. This physical property holds true for all tubes be they structural steel supports or the tubular heart. The concept that the dorsal mesocardium adds rigidity seemed intuitively obvious to us and we demonstrated that longitudinal stiffness was necessary to effect bending and incorporated that physical attribute inro our morphogenic machine (q.v.). It remains essential to any looping morphogenic machine. The DM may also be stiffened by an intensely argyrophilic ECM component **(**discovered by Nakamura and described in Manasek et al. 1986), running longitudinally and comprising part of the complex anisotropic CJ filament system. Of course, insight might be easily obtained by looking at histological sections that show the myocardium of this region to be thicker, hence less flexible than elsewhere.

Our finding that the dorsal mesocardium adds stiffness (Manasek et al. 1984b), later adopted by Taber et al. (1995), measured directly by Zamir et al. (2003), and adopted by others (for a thorough discussion see Shi 2014), became an important element in most subsequent models of looping. Le Garrec et al (2017) explored the role of the dorsal mesocardium to great length and provide an extensive review of the concepts behind dorsal mesocardial influences on looping as

well as models employing that concept. Zamir et al. (2003) suggested that such stiffness "combined with internal driving forces" "could theoretically cause the heart to bend." This is not theoretical. It had been demonstrated experimentally decades earlier (Manasek et al. 1984a). The DM's contribution to bending was also resurrected by Taber and Perucchio (2000) invoking "myocardial activation" as the trigger event but it had already been shown that chick hearts loop in the absence of myocardial contractility (Manasek and Monroe 1972).

The Cardiac Jelly (CJ or Extracellular Matrix)

The developing chick myocardium is a secretory epithelium and elaborates the thick sleeve of CJ interposed between the endothelial lining of the nascent lumen and the myocardium. This sleeve of matrix has biomechanical properties that had been explored decades ago and partially elucidated. Despite all evidence to the contrary, the CJ is considered as isotropic in finite element modeling (FEM).

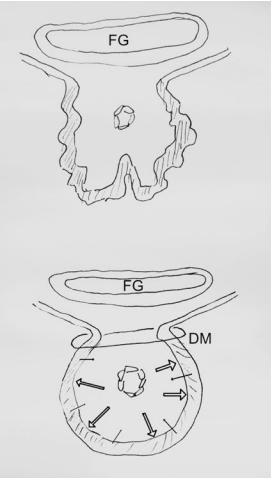


Figure 4. Schematic drawing of transverse sections through an early tubular chick heart (top) and somewhat later when the cardiac jelly has increased in volume by additional synthesis and hydration. The outward pressure of the CJ, indicated by arrows (bottom) deforms the now-anisotropic myocardium.

In experiments where the myocardium is removed, the intact naked CJ retains the original shape it had *in situ* (Nakamura and Manasek 1978), demonstrating that CJ has structural integrity and is not simply a viscous isotropic filler. Such isolated CJ was shown to swell and shrink in response to changes in the ionic environment indicating its regulatory capacity.

Cardiac jelly has its own unique anatomic substructure (Davis 1924; Nakamura and Manasek 1978a,b; Hurle et al. 1980. For review see Männer and Yelbuz 2019). This fibrillar substructure was hinted at by early microscopic observation and it has been long established unequivocally that the matrix filaments are real and not artifacts of preparation (for discussion see Männer and Yelbuz 2019). However, their function in morphogenesis was speculative until enzymatic studies (Nakamura and Manasek 1981) showed them to be essential to controlling heart shape, a finding entirely ignored in subsequent modeling and ignored in all reviews (see for example Little and Rongish 1995) and not considered as a morphogenic regulator by Davies (2013).

We therefore do know a great deal about the chemical and physical nature of CJ and its physical role in forming and maintaining the shape of the early chick heart loop.

We know very little about its role in murine hearts, which have a different developmental sequence. Rat embryos developing without appreciable hyaluronate are abnormal. The cardiovascular system is malformed and in the case of the $Has2^{-/-}$ rat embryos the outflow tract is

occluded. (Camenisch et al. 2000). Much recent work with ECM has focused upon its interactions with cells on a signaling level and with specific interaction with the interior of the cell via transmembrane integrin receptors and affecting the cytoskeleton (for brief summary see McCain and Parker 2011) but has largely ignored physical/mechanical properties described earlier that can profoundly determine shapes.

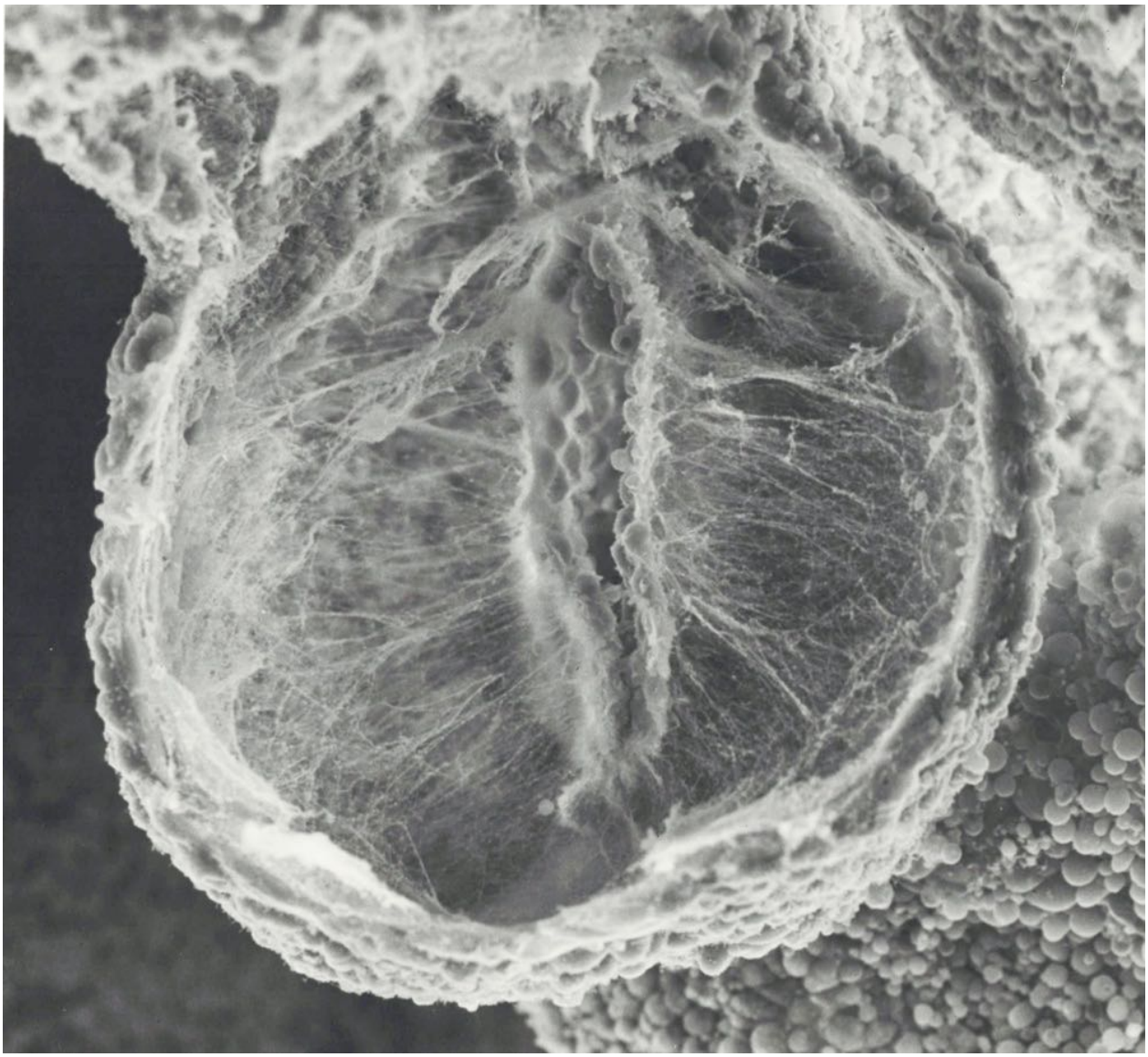


Figure 5 (above). This is a freeze-fracture scanning electron micrograph made ca. 1979 by Dr. Atsuyo Nakamura in my laboratory. It shows a transverse section of the pre-loop embryonic chick heart like that shown in Figure 2. A preparation such as this demonstrates the enormous anisotropic complexity of the very large extracellular matrix, the cardiac jelly. The endocardium, the CJ, and the myocardium comprise an integrated structural system with the components acting in concert during morphogenesis. (Manasek Laboratory Archives)

CJ is responsible for inflating and maintaining turgor in the chick heart tube. This function was discovered decades ago in our laboratory by injecting hyaluronidase (HyAse) directly into the CJ (Nakamura and Manasek 1981) and confirmed by Ramasubramanian et al. (2013) or by adding the enzyme to the culture medium (Linask et al. 2003) and noting collapse of the heart tube. It is a true example of a morphogenic process but has since been largely ignored as a force in deformational morphogenesis. Although Taber alludes to inflation and extracellular matrix swelling (see Taber 2006; 2009) and describes the inflation in textbook examples (Taber 2020 p445) he generally ignores it as a prime morphogenic force and does not include ECM swelling as a major driver of cardiogenesis. The concept of the ECM as a force-generator in cardiac looping remained peculiarly obscured in later studies seemingly to be reintroduced as a new concept (Taber and Perucchio 2000).

Enzymatically collapsed hearts can reinflate rapidly (Nakamura and Manasek 1981) presumably by secreting replacement CJ.

Of particular importance to any valid model is the fact that *CJ is highly anisotropic and enzymatic alteration of the glycosaminoglycans or the non-collagenous proteinaceous filaments profoundly alters heart shape.* It appears obviously necessary to include these physical characteristics in any looping model and to ascertain the mechanical role of the proteins targeted in the enzyme studies. They too, need to be integrated into any morphogenic system.

The endocardium

The lumen of the heart is lined by the simple squamous cells of the endocardium. The CJ filaments also anchor to these cells. Endocardial cells are polarized and have specific alignments relative to the presumptive blood flow (see Icardo et al. 1982). Cardiac jelly also provides a scaffolding for migration of mesenchymal cells in cushion regions (Markwald et al. 1978; Camenisch et al. 2001).

The basement membrane

The cardiac BM is first seen in the pre-loop heart as a discontinuous basal lamina that becomes denser and continuous as the myocardium develops. This component of the CJ, indeed of the whole early heart tube, has been largely ignored except in morphological descriptive studies (see however Kitten et al. 1987) and general increasing knowledge of BM components (see Kefalides and Borel 1987). Little is known of the physical properties of the early cardiac BM but basement membranes in general are anisotropic with some known physical characteristics and play a role in epithelial morphogenesis. As pointed out, such anisotropy may well determine shape change **(**for discussion see Khalilgharibi and Mao 2021) and they perhaps should not be disregarded as anisotropic elements of the cardiac tube. However, collagenase has no effect on the shape of the heart tube (Nakamura and Manasek 1981).

As Figure 5 shows, these components are intimately connected, or linked, and together comprise a four-compartment (myocardium/BM/CJ/endocardium) physical system. *It is this system as a whole that undergoes shape transformation, not just one or another of the substituents. It is not just the myocardium that changes shape, and the entire system is not isotropic.*

Cardiac Looping: The Definition Used in this Essay

The anatomic events that occur between the first two drawings in Figure 1 occur within a time period of a few hours encompassing the period when the chick myocardium differentiates and the extracellular matrix expands rapidly placing the myocardium under tension. If either is interfered

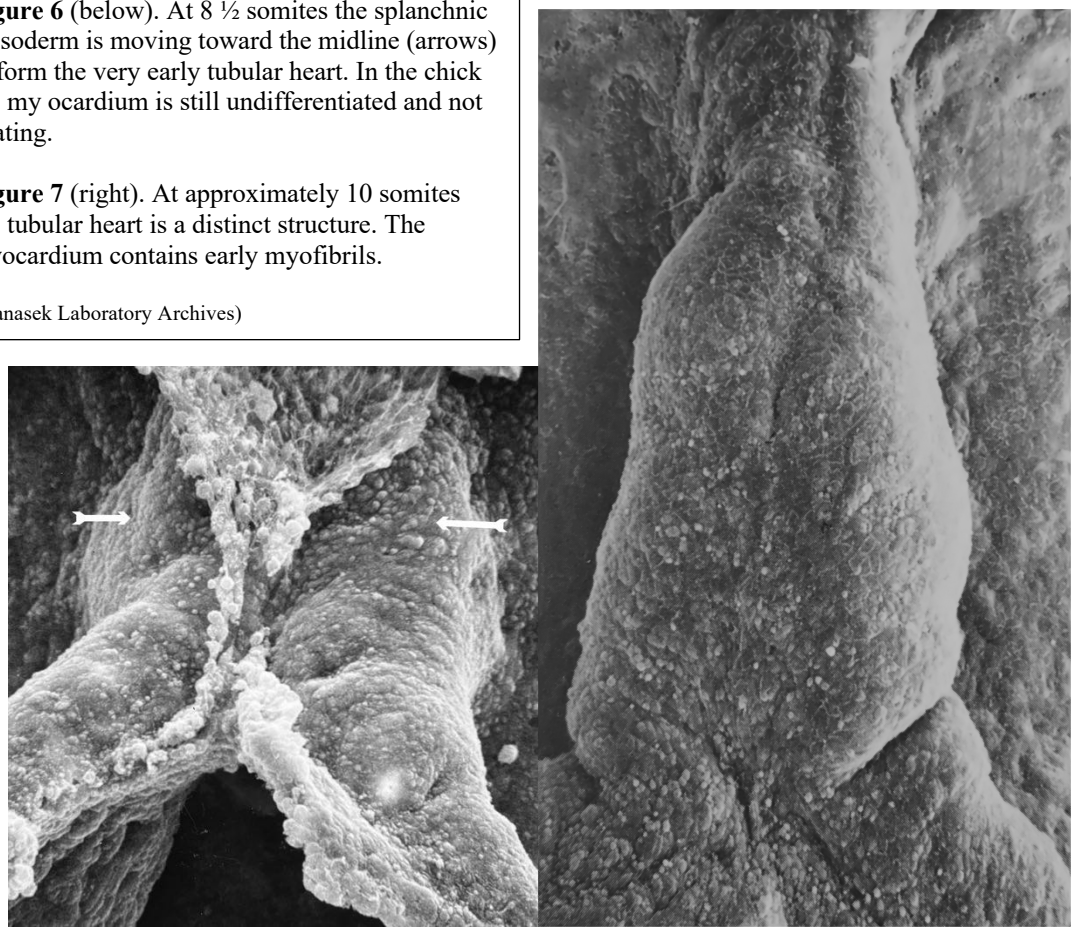
with, the chick heart does not loop. Cytodifferentiation occurs much earlier in murine hearts and internal hydrostatic pressure may not be due entirely to the CJ. All the layer of the tubular heart described in the last section bend together and bending requires certain properties of each layer. Heart shape change is not restricted to the myocardium.

In the carefully restricted sense used here, *"looping" refers only to the very rapid changes occurring to the tubular heart wherein it acquires first a ventral bulge, or aneurysm, forming a distinct "C" shape*. The heart then rotates to one side, causing bilateral asymmetry. The "C" shape occurs both in hearts *in situ* and in isolated cultured hearts. In 1952 Butler, for his master's thesis, (Butler 1952) showed that the heart rudiment loops when grown in culture, free of longitudinal constraints. This observation has been confirmed in numerous publications and demonstrated again by Männing and McLachlan (2000). These *in vitro* studies demonstrated that the ability to form the C shape is intrinsic to the heart. Nonetheless, many aspects of post-looping may be dependent upon the tubular heart being constrained in the pericardial space. A very simple mechanism had been proposed (see Patten 1922): the heart tube *in situ* grows in a confined space, then lengthens and perforce kinks because it becomes too long for its space. In the mouse, the heart tube was shown to elongate faster than the accommodating space (LeGarrec et al 2017). Under these conditions any tubular organ will bend or kink. Indeed, even a defective and deformed heart tube, if left to grow, will bend upon itself and kink *in vivo* (Baldwin and Solursh 1989; Camenisch et al. 2000) because it gets too long for its compartment. Some complexities of the buckling phenomenon were reviewed by Ebrahimi et al. 2022).

Figure 6 (below). At 8 ½ somites the splanchnic mesoderm is moving toward the midline (arrows) to form the very early tubular heart. In the chick the my ocardium is still undifferentiated and not beating.

Figure 7 (right). At approximately 10 somites the tubular heart is a distinct structure. The myocardium contains early myofibrils.

(Manasek Laboratory Archives)



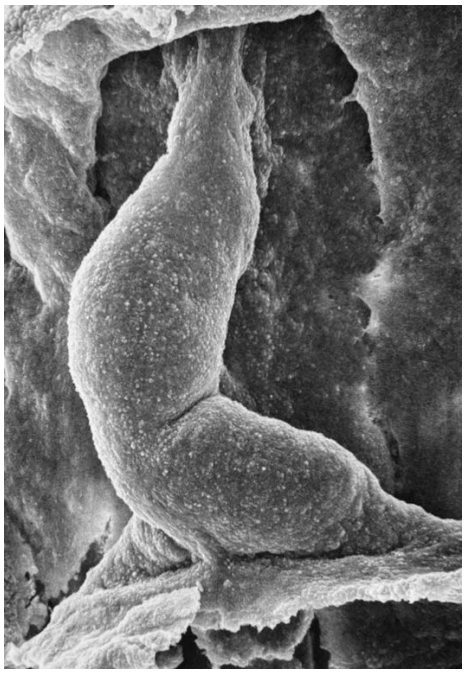


Figure 8 (left). At 11 ½ somites the heart has developed its haracteristic "C" shape loop. Myofibrils are abundant and the heart is beating. The diameter is approximately 0.1mm.

(Manasek Laboratory Archives)

Later convolution of the heart into its "S" form is *not* the subject of this essay and is *not* part of the initial looping process. That transformation requires different mechanisms. For a discussion of this distinction see Ramasubramanian et al. (2013). The review by Männer (2000) is exceptionally thorough and the minute complexities of later convolutions are reviewed by Männer (2004). The transformation to the S may require continued growth of the tube in a restricted space. It is certainly a hierarchical cascade dependent upon successful earlier formation of entrenched modules.

Since the "C" phase of looping occurs in isolated hearts grown in culture, the driving force must be intrinsic to the heart and I shall concentrate on this phenomenon. Many papers conflate C-looping, *sensu strictu*, with S-looping, cushion formation, trabeculation, valvulogenesis, chamber expansion, and many other developmental events and consider them all under the rubric of looping. Such conflation continues to cloud the experimental approach to looping since it inhibits the articulation of discrete mechanistic endpoints making it more difficult to uncover the underlying biological mechanisms.

There is little disagreement concerning the anatomic shape changes comprising looping in the chick and a recent metareview (Ebrahimi et al. 2021) has presented an excellent overview. The very early morphogenic sequence is quite different in avian and murine hearts and I shall indicate wherever necessary to which organism I refer. I also stress (as have others), based upon personal visual examination of developing hearts in well over a thousand chick embryos over several decades, that there is no "typical" loop. Each is slightly different, some fat some skinny, but eventually they (most) revert to a generic norm. Some workers suggest this indicates a redundancy of mechanisms, and others an intrinsic feedback regulation of shape. It also suggests that fussing over extrinsic and intrinsic developmental mechanisms that might cause trivial variations of some idealized loop shape is probably irrelevant. It may very well be that loop shape is evolutionarily robust and mechanisms of normal hierarchal assembly using highly conserved modules overcome perturbations, construct a single basic shape and oft-cited redundancy has nothing to do with it.

There is, to my knowledge, no empirical evidence for any redundant or parallel mechanisms. Such would imply a multiplicity of parallel morphogenic machines, probably an evolutionary absurdity.

Not every asymmetric embryonic heart seen in transverse section is an example of looping. Several published papers show light micrographs of sectioned embryos with the heart tube displaced to one side of the embryonic axis and call this "looping." For example, Linask et al. (2003) incubated chick embryos in HyAse and confirmed our earlier findings that the hyaluronate-deficient hearts collapsed (as did much of their embryos). Their hearts were displaced to one side and were therefore considered by some as having "looped." A collapsed heart squished to one side of a partly-collapsed embryo is not evidence of looping! Notably, Ramasubramanian et al. (2013) also had reservations about whether such hearts had actually looped.

Chapter 2
What Is a "Morphogenic Machine?"

Mechanisms are entities and activities organized such that they are productive of regular things from start or set-up to finish or termination conditions. *Craver and Darden*

Some pre-existing embryonic "stuff" comprises the physical products of gene expression and any subsequent post-translational modification. This "stuff," both cellular and extracellular, is inevitably going to be involved in changing the shape of tissue and organs as the embryo matures and develops. In a developing organism "stuff" is constantly being synthesized, modified, removed and replaced, somehow resulting in anatomic changes.

A morphogenic machine is the biomechanical mechanism that can create new shapes of a developing embryonic structure by reassembling the "stuff" into organized structures, thereby increasing the complexity of the developing organism.

Many of a morphogenic machine's attributes are elucidated in the extensive work by Marcel Weber (2022). The distinction between machine and mechanism has been argued by philosophers (see Craver and Darden 2013) but I shall use the terms rather interchangeably, mostly because the distinctions are minor and I disagree with many of them. The morphogenic machine is a higher-level biological *mechanism* that may comprise a number of lower-level machines or mechanisms. The machine we discuss here is the combined interaction of existing modules to effect looping

Mechanistic causality has a long history in biology (for discussions see Craver and Darden 2013; Glennan and Illari 2018) and has matured into a philosophy that is uniquely capable of influencing concepts of developmental organogenesis. The morphogenic machine is a fundamental part of Developmental Systems Theory (DST) and is evolutionarily conserved to produce modules (see Oyoma et al. 2001; Callebaut and Rasskin-Gutman 2005) that themselves are highly conserved.

Not merely a conceptual construct, the morphogenic machine must exist as a physical assembly of biomaterials known to be present. With metaphorical connection to the pre-silicon "age of the machine" a morphogenic machine employs these materials in a mechanical way to physically influence downstream shape or construct an embryonic structure. At this level a morphogenic machine does not deal with the gene expression that produces the "stuff."

A description of events, no matter how detailed, is not a morphogenic machine—it does not deal with mechanism nor does it yield explanation or prediction. For example, extremely detailed studies of valvulogenesis, (see Markwald et al. 1978) while necessary in order to design a valvulogenic morphogenic machine (we need to know *what* to build), are not mechanisms and are not part of any valvulogenic machine, but rather tell us what that machine must accomplish and in what order.

In building a morphogenic machine each part should have been (or can be) tested experimentally for its influence on the end product. Thus, the end product of the machine is the result of coordinated activities of its known individual working parts. The machine supplies the structure that mediates causal variables to produce a predictable event and should use a minimum number necessary (for discussion of machines in general see Weber 2022).

Abstraction

A morphogenic machine, much like any model is of necessity an abstract. A model need not be considered a solution but rather a way to state a problem and requires abstraction to make the problem tractable. Models neither confirm nor falsify the validity of the input nor the hypothetical mechanism(s) invoked, Abstraction must be done judiciously since a morphogenic machine cannot

simply ignore an integral anatomic part that, if modified or removed, would result in a defective product. The machine needs to include all materially active, relevant parts. For example, the morphogenic machine responsible for creating the C-loop must use both the CJ and the myocardium since both have been shown experimentally to play essential roles. If it incorporates fictitious physical properties ascribed to either, the machine is excessively abstracted and no longer represents the biological system *regardless of the results produced*. These constraints are often mentioned (see Clausi and Broadland 2001; Wimsatt 2007b; Craver and Darden 2013) but are equally often disregarded.

Many heart FEM studies are restricted to the myocardium, myocardial strain and myocardial cell shapes and ignore other major components of the tubular heart. Morphogenesis is a symphony not the result from one player playing a single note and heart shape is not just myocardial shape. Reductionism is an attempt to separate the different components and abstract them to make them amenable to experimentation but there is an inherent conflict between such reductionism and holism in analysing a developing organ. This issue was addressed in part by Ebrahimi et al. (2021)

Sometimes, too, blinders induced by a maldirected information trajectory (or an existing paradigm, e.g. Kuhn's sense of a "disciplinary matrix") will prevent the recognition of very obvious events. For example, the myocardium differentiates and develops myofibrils at the time of looping in the chick. This is the most dramatic cytological event undergone by developing muscle, and appears to be necessary for looping to occur, yet myofibrillar anisotropy has been largely ignored in silicon modeling of chick cardiogenesis.

Chapter 3
Morphogenic Mechanisms at the Beginning of the 21st Century

Following the period of descriptive embryology, the major thrust of the investigation of organogenesis is to uncover the biological mechanisms responsible for changing the shape or building the organ as anatomically described. Biological mechanisms, formal constructs based upon experimental data, have been abstracted and deconstructed in a most thorough manner by Craver and Darden (2013) and their analyses have stiffened the backbone of the present work.

The general mainline thinking among embryologists and the various existing morphogenic paradigms were surveyed comprehensively by Jamie Davies in his *Mechanisms of Morphogenesis* first published in 2005 and revised in 2013 (Davies 2013). This thorough work presented a very clear overview of the main areas of understanding what "mechanisms" meant in the first part of the 21st century. This was a watershed time period in the history of embryology, yet the emphasis was still on cells and intracellular activity as drivers of morphogenesis. Davies does mention the concept of molecular machines being able to construct embryo parts autonomously.

It is noteworthy that Davies' *Mechanisms* included neither "deformation," nor "hydrostatic pressure," as morphogenic mechanisms, and hyaluronate is mentioned only in the context of a substrate for mesenchymal aggregation. Epithelial tension is typically considered as a function only of individual cell cytoskeletons and the influence of adjacent cells, not of external deforming matrix forces. The physical properties of ECM were ignored, even though they had, at the time of publication of both editions, been already well-explored in the 1970s and 1980s in at least one system, the heart. Davies' work was well received and is an influential and important contribution serving as an intellectual baseline for morphogenic knowledge. Unfortunately, being far outside the paradigm of "put-and-take" morphogenesis, hydrostatic force of the ECM did not gain intellectual traction nor even parenthetical inclusion into Davies' otherwise comprehensive survey.

The consequence of omitting a major morphogenic mechanism from this work may have helped obliterate it from future generations of investigators and the entire body of work done on cardiac hydrostatic deformation seems to have been bypassed by the rest of developmental biology. The concept that hydrostatic pressure is deformative really is not difficult to comprehend. Perhaps the difficulty was in accepting the very presence of hydrostatic pressures in developing embryonic systems although it was measured directly in the chick CJ (Manasek et al.1984b). ECM pressure is also known to be present in many mature organisms. When hydrostatic pressure was included in computer modeling (Hosseini et al. 2009) pressures vastly lower than the published measured ones were used. The reason for using lower pressures is unclear since measured pressures had already been published.

Icardo (1996; 1998) published carefully documented summaries of the actual knowledge of mechanisms of early heart development at the end of the 20th century, chronicling an earlier type of analytical approach to a complex problem of biology, an approach that has perhaps been supplanted by powerful analytical techniques often applied without fully considering the basic anatomy of the system. The necessity of correlating experimental data and models has been well noted (Ebrahimi et al. 2021). Icardo had also noted the discrepancy between murine and avian looping but his inclusive attempt to integrate the activities of different components of the heart into a functional system apparently did not find resonance in later FEM analyses.

A seismic shift occurred to the direction of research. The coming of age of molecular genetics (see Brand 2003) initiated a concerted search for molecular and genetic changes during development. When did specific genes turn on and what were their products? A great deal of fundamental information was derived from this effort and our understanding of the cell surface, receptors, and intracellular dynamics exploded. While much of this work discovered and described events in isolation, it was not immediately apparent how it affected understanding of morphogenesis. Reductionism in embryology became almost synonymous with the search for genetic regulation. Still wanting remained the mechanisms that actually connect gene regulation and cytodifferentiation to physical changes in anatomy (see Bartman and Hove 2005). This was also the time when computer assisted analysis of morphogenesis became a reality and FEM was applied to the surface of the heart.

In a very recent foray into the looping problem passing notice was given to "a number of hypotheses" but concentrated on differential growth. How is "differential growth" (again, the oft-stated problem of what the term "growth" means) reconciled with existing experimental data such as elucidated in the coherent narratives by Icardo. Ebrahimi's work (Ebrahimi et al. 2022) is perhaps the pinnacle of shape analysis divorced from the corpus of literature on the whole system. It appears to be the logical progression from earlier FEM modeling to where the model has become the end point and the actual biology of the system irrelevant. However elegant this analysis, the abstractions used in the study are sufficient to divorce the findings from the actual developing organ. It is as though histogenesis and cytodifferentiation no longer exist as part of the problem or solution.

We are now about one-quarter through the 21st century and studies of looping have lost the path to coherence they seemed to once have had. To me, the field has become intellectually chaotic with internal contradictions, and use of incorrect physical properties that inhibit the emergence of a comprehensive theoretical framework. Ebrahimi et al. attempted to produce a coherent narrative of studies of looping (Ebrahimi et al. 2021) and indeed summarized the field quite well but by giving credence to studies that do not hold up under close scrutiny they, and other reviewers, have created a new "ground zero" upon which future investigations will rely. This phenomenon is revisited later.

Chapter 4
Old Paradigms

Some models that look at single parameters, for example "growth," can come close to approaching the actual shape of the real heart but these all have a common failure: they do not include all the physical components that have been empirically shown to influence shape of the developing system. In many models experimentally proven properties are ignored and convenient substitution made (we will see this later when I discuss ascribing isotropy to the myocardium and CJ). Selecting specific input characteristics and omitting others which likely would give a different result violate the criteria Wimsatt and others developed (see Wimsatt 2007) when discussing false models.

Many contemporary studies of cardiac morphogenesis derive from the long-held central core concepts in embryology that actin polymerization or actomyosin-mediated cell shape change and differential growth, in various combinations, provide the basis upon which to build a morphogenic machine. As a consequence of this strongly embedded philosophically embraced *elan locomotif*, many silicon models of cardiac morphogenesis are constructed with a strong fixation on cytoskeletal mediated myocardial cell shape change, differential cell division and the near-vitalistic notion of "growth." If one reads many papers very carefully, "growth" is found to have many meanings, sometimes several in the same paper.

The imperative to use the paradigmatic contractile cytoskeletal model to produce organ shape change had led some investigators to interject this mechanism as another complexity into looping. For example, Taber (see Taber 2020, 433-445) recognizes that the CJ inflates the heart as do Shi et al. (2014a). Kawahira et al. (2020) acknowledge that cardiac jelly exerts pressure but interpret this pressure as effecting myocardial shape change via a cytoskeletal response to stress. They seem not to consider the more direct possibility that the pressure itself is deformative and the cells are deformed directly. By adding the hypothetical intermediary of the cytoskeleton their model conforms to their existing paradigm. Nonetheless, inhibition of non-muscle myosin (presumed to be part of the cytoskeleton) does not prevent looping in the chick heart (Rémond et al. 2006).

If one were to consider only the myocardial epithelium and not the rest of the layers, cytoskeletal-mediated deformations of cuboidal or columnar epithelia might be considered as a driver of cardiac looping Such a mechanism is enticing. Force generators within each cell are reasonably well understood and documented both biochemically and structurally and cytoskeletal elements restricting or directing shape changes were known and visualized in other epithelia (see Pourati et al. 1998). In such cases, modeling invagination, neurulation and other epithelial deformations was straightforward.

The success and simplicity of intracellular morphogenic mechanisms reinforced the idea that cell shape changes modulated by intracellular forces (see McCain and Parker 2011) were the universal, predominant factors in epithelial morphogenesis. Most workers modeled differential growth and myocardial cell shape change without a full recognition of the tubular heart as an integrated, multilayered anatomical system (see Figure 5). Such a single-component morphogenic machine has never "worked" to build a multi-tissue organ. In another system, *Drosophila* (Nematbakhsh et al. 2020), while a complex relationship may exist between intracellular-mediated cell shape changes and the underlying ECM the anatomy is quite different creating a different physical system.

Differentiating chick myocardial cells change their shape as the heart loops. These changes have been observed and measured on actual looping hearts by several investigators (Manasek et al. 1972a, see Kawahira et al. 2020). Several quantitative studies have suggested, however, that cell shape changes by themselves are inadequate as a mechanism of looping (see also Shi et al. 2014; Ebrahimi et al. 2022).

In their modeling of single-mechanism effecting looping, Shi et al. (2014) used myocardial cell aspect ratios that differ dramatically from those actually measured. They concluded that cell shape changes do not account for the bending mechanism. This is an impossible conclusion. If one measures the size, orientation of myocardial cells in pre-loop and post-loop hearts, as we did much earlier (Manasek et al. 1972), they are qualitatively and quantitatively different. The myocardial cells of the looped heart will not fit a pre-loop anatomy. One cannot argue that the cell shapes seen in the looped heart can only partially fit the looped heart. They do so exactly even for each slight anatomic variant. Consider the outlines of the myocardial cells as pieces of a jigsaw puzzle. If disassembled and then reassembled they will form exactly the same topology. They cannot create a greater or lesser curvature; they define exactly the shape they were traced from. Therefore, it is not possible that the measured shapes of all the cells cannot account for the entire shape of heart bend but only for some percentage of it.

Shiraishi et al. (1995), examined phalloidin stained specimens and ascribed polygonal shapes to surface myocardial cells but these shapes do not match those actually seen using SEM. Cell shapes and alignments in pre and post looped hearts have ben quantitatively measured and analysed statistically (Manasek et al. 1972). Cell outlines were observed both by silver impregnation and SEM and the two methods corroborate each other. None of the polygonal cell shapes indicated by Shiraishi and colleagues (1995) correlate with anything seen in the actual heart. Nonetheless, the Shiraishi data has been used in later models.

Chapter 5

The Basis of the Morphogenic Machine Discussed in this Essay

A long series of studies continuing through the 1980s added substantially to a new body of knowledge about the different components of the very early chick heart. These studies elucidated many characteristics of early heart muscle cells and the extracellular matrix they secrete and suggested different contributions to the developing functional anatomy.

I consider the experiments that demonstrated the following points to be foundational in the ontogeny of the concept of hydrostatic deformation as a morphogenic process. These represent successive distillations, or dissections—anatomical, biochemical, and biophysical—of an object (the heart) seen as separate mechanisms that in concert operated as a morphogenic machine.

1. **C**–looping is intrinsic to the heart rudiment and independent of external anatomic constraints. Pre-looped hearts grown in culture completely free of surrounding tissue developed the typical C shape. Pre-looped hearts grown in culture in the presence of hyaluronidase do not develop the C shape.
2. There is no significant difference in mitotic rate between right and left sides of the myocardium. Hearts loop in the presence of colchicine.
3. Digesting the matrix with the enzyme hyaluronidase (HyAse) collapses the chick heart. This demonstrates hydrostatic support and the presence of hyaluronate-based hydrostatic pressure within the tubular heart. It explains why the myocardium is under tension, much like the wall of an inflated balloon. The mouse or rat heart may expand in the absence of cardiac jelly suggesting sufficient pressure is developed by other means.
4. The CJ is not isotropic but has a complex array of filamentous components. Models assuming an isotropic matrix are inherently false because the matrix is *not* isotropic.
5. Disruption of some the proteinaceous, non-collagen, CJ filaments causes the chick heart to lose its shape. This finding demonstrates that heart shape depends also (or primarily) upon demonstrable cardiac jelly filamentous substituents. *This experimental finding has been ignored in all subsequent modeling or experimental work on heart development.*
6. Isolated cardiac jelly, free of the myocardium, retains the shape of the heart from which it was derived. It swells and contracts in response to ionic changes. It is capable of exerting force. The hydrostatic pressure developed by the cardiac jelly *in situ* has been measured quantitatively. These studies proved the presence of internal pressure within the heart tube.
7. Myocardial cytodifferentiation is required if the heart is to loop rather than expand symmetrically. Myofibrillogenesis is observed to occur along expected lines of stress during the looping process.
8. Blocking myofibrillogenesis (for example by cytochalasin B, or actin polymerization by cytochalasin D) prevents looping but not heart growth. The heart continues to enlarge but does not develop a curvature.

The evidence derived from numerous experiments that internal pressure is a mechanistic morphogenic force driving the morphogenic machine was tested by constructing a physical analogue (Manasek et al. 1984a; see Manasek 1981). This is a true morphogenic machine that can create the changing shapes of the looping heart by simulating known normal developmental events. This qualitative analogue model based on the following known parameters mimicked looping:

1. The elastic modulus of the developing myocardium is unequal, a function of cytodifferentiation and consequent anisotropy. The dorsal mesocardium stiffens the dorsum, increasing its elastic modulus and preventing elongation along this axis.
2. The internal pressure causes outward bulging of the less rigid ventral side, creating an aneurysm.
3. Anisotropy within the myocardium, probably resulting from cytodifferentiation and the acquisition of myofibrils, directs this force and results in rotation as well a stabilization of the looped form. Formation of new myofibrils along new stress lines stabilizes the myocardial shape.

4. The amount of deformation is limited by the physical properties of the myocardium, presumably due in part to the orientation of newly formed myofibrils and by the action of cardiac jelly fibrils.

The rubber balloon in our morphogenic machine showed a dramatic rearrangement of the rubber molecules along lines of stress, mimicking rather precisely what is seen with myofibrils in the looping heart. A comparison of Figure 17 (Manasek et al. 1984a) and the polarized light studies in Nakamura et al. 1980) demonstrates very nicely the relationship between myofibrils and stress. This observation has been ignored.

As noted by Taber (2020 p5) simple qualitative geometries may be more important than quantitative precision. Our semiqualitative analogue model was perhaps the first example demonstrating cardiac looping to be the result of *physical deformation* by extracellular matrix (CJ) forces, rather than by differential growth or cell shape changes. *Moreover, this is perhaps the only looping model that incorporates known properties of the cardiac jelly and does not try to model early heart morphogenesis by studying the myocardium alone. It also includes rotational consequences of anisotropy and myocardial cytodifferentiation which later models seem to ignore.*

In this model cell chape change is a consequence of hydrostatic deformation. It demonstrates the likelihood that cell shape changes result from, but do not cause the loop. As such it is a departure from the rigidly held idea that all epithelial tensions are the result of intracellular activity.

By 1981, of all the data pointing to hydrostatic deformation as a morphogenic mechanism had been published. For reasons explored in this monograph the deformation model lay dormant until 2021 when our basic hydrostatic mechanism was independently rediscovered. (Munjal et al. 2021)

Curiously, the mechanism of our looping model was resurrected twenty years after its publication (see Figure 11, Taber and Perucchio 2000). These authors diagrammed our mechanical analogue retaining the helical anisotropic fiber-wound myocardium and a dorsal mesocardium. However, they postulate something they term “muscle activation” as the force generator causing the bend and rotation. It is unclear what this term means. It cannot refer to heartbeat since it is known that looping is independent of contractions. No experimental data was provided and this illustration seems to be simply a redrawing of our chick model and the paper implies novelty. They do reintroduce cardiac jelly swelling as a potential motive force, perhaps not shutting that window entirely (see their figure 14).

Experiments are essential to generate the data needed for formulating morphogenic hypotheses. I will discuss several experiments upon which the hydrostatic deformation model of morphogenesis is based and concentrate on those that may be considered as *crucial* experiments. (See Giere 1996 268-302).

The very concept of a crucial experiment (*experimentum crucis*) is old and often attributed to Francis Bacon (see Schwartz 2017). A crucial experiment is one designed to clarify unambiguously which of two competing alternatives is the more likely one. By using lower-level attributes a crucial experiment creates a condition wherein a hypothesis is supported or disproven using an experimental intervention that clearly targets a causal variable (see Woodward 2003). Such effort may result in the discovery of anomalies. The resolution of anomalies requires diagnosing the nature of the failure and the consequences. Responses to anomalies may involve discarding the entire hypothesis, falsifying the anomaly itself, or modifying the hypothesis to retain valid parts while discarding invalid. A detailed discussion can be found in Craver and Darden (2013 144-160).

Complex hypotheses such as hydrostatically driven looping are hierarchical and contain lower-level modules, or propositional assumptions. If every assumption is valid then no single higher-level experiment can falsify the higher-level construct without changing (discarding or adding)

one or more demonstrable foundational proposition (see Wimsatt 2007). Anomalies will arise as additional data is gathered. A well-known example often used to illustrate this point is the discrepancy between the observed orbit of Uranus and the orbit predicted from Newtonian laws. The failure of Uranus to follow a predicted orbit did not falsify Newton's laws of motion and gravity! Newtonian physics had a long history of demonstrated robustness and the discrepancy was solved not by discarding Newton but by the discovery of Neptune by Adams and Leverrier. This discovery added a hitherto unknown gravitational body to the propositional assumptions, an addition that resolved the anomaly.

Chapter 6
Controlling Deformation: Anisotropy

If the expansion of the looping chick heart is deformation, as experimental evidence suggests, then in order for the chick heart to deform asymmetrically *in vitro* there must be intrinsic anisotropy. We know that the chick heart myocardium is under tension from the pressure of cardiac jelly because when the cardiac jelly is degraded enzymatically the myocardium collapses and from the behavior of cuts made in the myocardium (see Shi et al. 2014). There can be no other plausible explanation for the enzymatic result which has been repeated often and ignored by many. The internal pressure essentially "blows up" or inflates the myocardium. If we just inflate an isotropic balloon (or an isotropic tubular heart), it will expand uniformly. If we inflate a cylindrical isotropic balloon it will preferentially increase in diameter not length because circumferential stress is twice longitudinal stress. We demonstrated that adding longitudinal higher elastic modulus anisotropy (i.e., the dorsal mesocardium) to the balloon will cause it to bend (Manasek et al. 1984) upon inflation. Adding pitched, oriented fibers of higher elastic modulus were shown to effect rotation as well if the ends of the tube are fixed as they are *in situ.*

What is the source of embryonic cardiac anisotropy? The early heart has no fibrillar collagen that could act as a stressed filamentous skeleton; purified collagenase has no effect upon heart shape (Nakamura and Manasek 1981). The myocardium of the tubular heart in the chick, as opposed to the rat, is initially undifferentiated and has no myofibrils at the pre-loop stage although it does have a very complex, extensive cytoskeleton (for examples see Isobe et al. 1984; Manasek et al. 1984b). The architectural complexity of this cytoskeleton has not been investigated further with respect to the whole heart or whether it dictates regional anisotropy. The structural complexity of this cytoskeleton makes integrated orientational analysis difficult.

Striated muscle cells are not isotropic.

Myofibrillogenesis is the most dramatic cellular event visible in the myocardium during looping yet is ignored in computer models that treat the myocardium as isotropic.

Coincident with th formation of the C shape is formed in chick (but not murine) hearts the myocardium differentiates, acquires myofibrils, and changes from an isotropic epithelium to one profoundly anisotropic.

Myofibrils are the most prominent anisotropic elements in cardiac muscle cells and are anatomically organized with respect to the tubular heart. Although early myofibrils can be visualized with the electron microscope, it is not possible to discern their orientation within the heart tube by TEM and I was mistaken in thinking so (Manasek 1968). However, they can be seen easily with polarized light microscopy and their distribution and orientation appreciated (Nakamura et al. 1980; see also Fig. 9). Initially circumferential, the first longitudinal myofibrils appear on the bulging convex region, altering the anisotropy of that area. The concave region develops a concentration of circumferential fibers. Collectively, these fibers must introduce anisotropy to the myocardium within the space of a few hours while the chick heart is looping. All the studies regarding myofibril orientation during looping were done using the chick heart. There are no comparable studies for murine heart development during which looping occurs after myocardial cytodifferentiation.

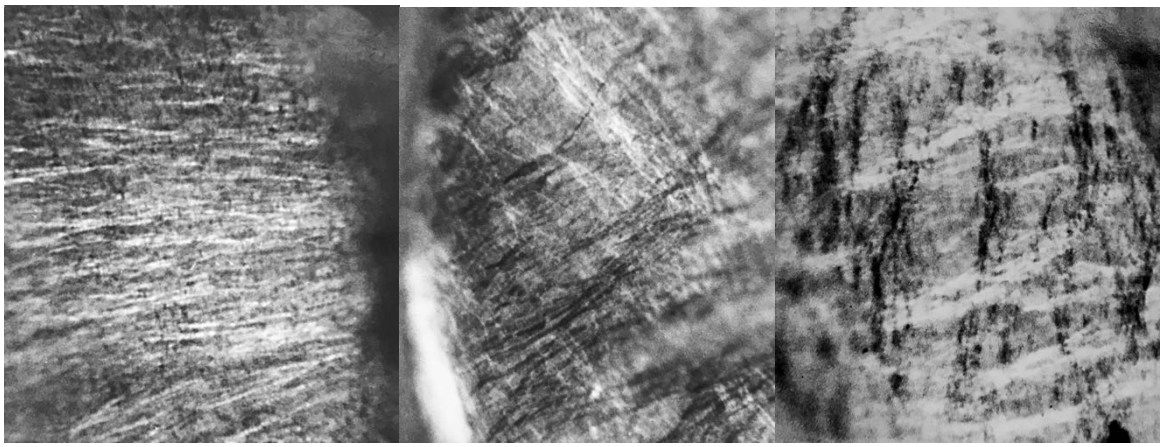


Figure 9. Polarized light microscopy demonstrated developing myofibrils in differentiating myocardium at the time of looping. The lefthand panel shows the predominantly circumferential orientation of the myofibrils at the inner curvature. Virtually no longitudinal myofibrils, which would appear dark, are present. The left-hand side of the tube is seen in the center and there are fibers running in several directions. Looking *en face* at the greater curvature we note a basket-weave of myofibrils. (Manasek Laboratory Archives)

Other elements may also introduce anisotropy. Filaments of f-actin have been implicated (**see** Latacha et al. 2005; Kawahira et al. 2020) and many investigators favor f-actin as a myocardial shape regulator (Itasaki et al. 1991). It is very tempting to implicate f-actin since it fits nicely into the well-accepted, conventional cytoskeleton story of epithelial cell shape change and has been modeled extensively and, indeed, interfering with actin polymerization does interfere with normal development. However, we do need to keep in mind that f-actin is a component of myofibrils and what many investigators see, when looking at f-actin, may really be myofibrils!

Histologically, myofibrils dominate and their orientation determines the polarity, or anisotropy of muscle cells. F-actin filaments may also be mediators via the cytoskeleton, but myofibrils are overwhelmingly the dominant linear elements in the normally developing myocyte and are intimately related to the fibrillar cytoskeleton (Isobe et al. 1984). The complexity of the entire cytoskeleton as demonstrated by *in situ* detergent extraction of the cytosol far exceeds that suggested by Latacha et.al. (2005) and other subsequent work.

Experiments in my laboratory in 1972 showed that cytochalasin B, interrupting myofibril assembly, (Manasek et al. 1972b; Burnside and Manasek 1973) prevents looping (Figures 10, 11), consistent with the later findings of others using cytochalasin B as well as other agents. In these embryos the myocardium never developed myofibrils, the hearts did not beat and did not loop.

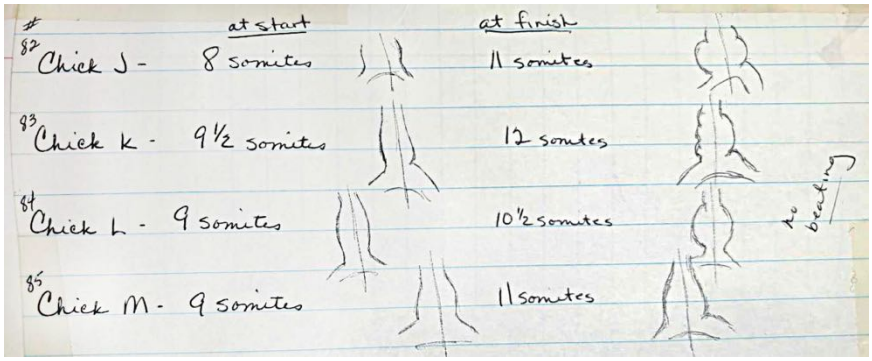


Figure 10 (left). Photograph of an entry in my laboratory notebook of 1972 showing a series of embryos incubated with cytochalasin B and the heart outline at start and end of incubation.

(Manasek Laboratory Archives)

Figure 11 (left).
Polaroid record of a horizontal plastic section through the heart of an embryo that had been cultured with cytochalasin B, showing the continued bilateral symmetry even though he heart had swelled.

(Manasek Laboratory Archives)

Striated muscle cells are not isotropic. This is obvious to anyone who has ever looked at a muscle cell through a microscope or examined their birefringence through a polarizing microscope (or consulted a histology textbook). The histogenesis and ultrastructure of early myocardial cytodifferentiation has been well-described since the1960s. In addition to being intuitively obvious at a qualitative level, changes in physical characteristics of differentiating striated muscle have been measured quantitatively (see Collinsworth 2002). *It is simply not possible to argue that the differentiating myocardial epithelium is isotropic.* Yet, Taber's stunning assertion of tubular myocardial isotropy (Taber et al. 1992) has gained sufficient traction to override the realities of simple histological examination. Moreover, these authors continued to ignore myofibrillogenesis and seem to be concerned only with f-actin. They quite erroneously claim that our studies involve f-actin even though we studied myofibrillogenesis and the orientation of myofibrils in the looping heart and *not* f-actin. There is no ambiguity on this matter in our published work. Others (see Lindsey et al. 2013) have also made similar claims despite the fact our laboratory never investigated actin polymerization.

It is germane to reiterate that the circumferential orientation of the earliest myofibrils is along the greatest lines of stress since circumferential (hoop) stress is always twice that of longitudinal stress. Anisotropy is defined by myofibrillar orientation and varies among different regions of the heart since myofibril alignment is different.

Even though polarization microscopy permits the easy demonstration of myofibrils I think we were the last to look at the tubular heart with a polarizing microscope. The recent work by Kawahira et al. (2020) repeats many of our findings but has reported that f-actin aligns much the same way we reported myofibrils to align as the heart develops. They have also repeated published cytochalasin-B demonstration that treated hearts do not loop by using cytochalasin-D.

Cardiac muscle has intercalated discs

In concert with myofibril formation the myocardium develops intercalated discs—specialized, complex cell-cell junctions into which the myofibrils insert. The possibility that intercalated discs define the deformation limits of the myocardium or even introduce an overall anisotropy to the myocardium has not been investigated nor, apparently, has it been suggested. But it would be predicted that since the myofibrils are aligned within the myocardium there would be a non-random pattern to the distribution of developing intercalated discs. Intercalated disc formation occurs along with myofibrillogenesis at the time of myocyte differentiation. Accordingly, one might expect intercalated discs to be present in the contracting pre-tubular myocardial cells of the murine heart.

Latacha et al. (2005) in a most interesting paper did a very thorough study of C-looping using several different actin polymerization inhibitors. They suggested that actin-mediated cytoskeletal changes mediated myocardial cell shape changes and thus heart shape. Despite the fact that their work involved only the chick C-loop, they invoked the rat study by Baldwin and Solursh (1989) as disproving the role of cardiac jelly's internal pressure in the chick. If one examines the Latacha et al. photographs carefully one notices that the hearts still got bigger in the presence of the actin inhibitors even when they didn't loop (see also Figure 10 above). A reasonable explanation is that when the anisotropy is disrupted, the cardiac jelly simply inflates an isotropic myocardial balloon. Latacha et al. made the salient point that cytoskeletal f-actin polymerization stresses can be of the order of magnitude of a few hundred pascals, enough to deform the myocardium. What they ignore is the measured cardiac jelly pressure (Manasek et al.1984b) and the potential of a much greater Young's modulus (yet unknown) for the developing basement membrane. Basement membranes (not measured in early embryos) have a modulus of elasticity ranging from tens of kilopascals to several hundreds of kilopascals. The reported ranges are enormous (see Chang and Chaudhuri 2019) whereas the Young's modulus of epithelial cells governed by actin filaments ranges from 0.5 to 2.5 kPa.

The study by Abu-Daya (2009) showing that inhibition of myofibril assembly does not prevent looping in *Xenopus tropicalis* with the mutation *muzak* is intriguing and would seemingly contradict the cytochalasin and BrDU studies. I think polarized light microscopy would be able to determine quickly and easily if there is or isn't any higher order to fibrillar components in such hearts. Perhaps this model would be ideal for exploring the role of intercellular junctions in developing myocardial anisotropy. It is quite possible that the distribution of zonulae adherentes creates an effective anisotropy. This model may be well-suited to further explore the relationship between cytodifferentiation, anisotropy and morphogenesis. It may also suggest some fundamental differences in mechanisms among vertebrates.

The cardiac jelly is also anisotropic and highly structured

As already discussed in detail, the cardiac jelly introduces yet another source of anisotropy into the developing tubular heart. This thick sleeve of extracellular matrix is not homogeneous, or isotropic, but contains a complex set of radial filaments of different diameters and composition (see Nakamura et al. 1980). Their principal arrangement is much like that of a bristle test-tube brush and would not inhibit bending, although a dense longitudinal argyrophilic structure in the dorsal mesocardium is thought to stiffen that structure. This exceptionally complex anisotropic structure to cardiac jelly is also overlooked today by most investigators who persist in describing and modeling the CJ as isotropic. Despite having been in the literature for decades *none of the*

anisotropic substructures of ECM have been incorporated into any mathematical or computer model. For example, Shi et al. (2014) specifically state that their model utilizes *both* an isotropic myocardium and cardiac jelly. Bevilacqua et al. (2021) modeled the entire heart tube as a homogeneous, isotropic structure, again ignoring the anatomic evidence that there at least two components, the myocardium and the CJ, that have remarkably different physical characteristics and different, developing anisotropies. Munjal et al., in another system, (Munjal et al. 2021) specify that the ECM in their studies is isotropic but present no supporting data. Ramasubramanian et al. (2008) had also based their work on the assumption that all layers are isotropic. This legacy of inventing properties for the tissue under investigation can be traced back to 1992 when Taber and his colleagues made the decision to ignore all work to the contrary and arbitrarily declare both myocardium and CJ isotropic. The reason for doing this is still mystifying.

The shape of the heart tube also depends upon the integrity of cardiac jelly fibrils

Dissection of the CJ using highly specific enzymes has a profound effect on heart shape. Enzymatic studies (Nakamura and Manasek 1981) indicate conclusively that some of the matrix proteinaceous fibers are required to maintain the shape of the heart. When these fibers are disrupted (leaving the glycosaminoglycans intact) the hearts lose their original outlines. *The intriguing findings that disruption of cardiac jelly fibrils by specific proteolytic enzymes injected into the cardiac jelly disrupt heart shape have not been investigated further nor have they been incorporated into any subsequent silicon models.* Completely ignoring this morphogenic phenomenon has certainly influenced the synthesis of viable explanations of the looping mechanism.

Clearly, under these circumstances, shape changes of myocardial cells or "growth" cannot cause heart shape changes or the hearts would have retained their general shape (or even continued to loop) when the cardiac jelly fibers were enzymatically disrupted. The enzyme studies alone make it difficult to accept any conclusions that active cell shape changes cause heart shape change.

Our group in the 1980s seems to have been the last to investigate systematically the consequences of enzymatically altering cardiac jelly or its substituent fibers on the shape of the looping heart despite having demonstrated dramatic effects. Most later studies involving cardiac jelly involve cushion formation and hemodynamics, not heart shape.

I reiterate that the tubular heart is a multilayer anisotropic organ, not just an isotropic myocardial epithelium undergoing shape changes.

Chapter 7
A Single Experiment and Ensuing Confusion

The basic tenet of the hydrostatic morphogenic machine, that internal pressure inflates the tubular heart and deforms the myocardium, is derived from *in situ* experiments on the chick embryo.

Baldwin and Solursh (Baldwin and Soliursh 1989) incubated whole rat embryos in medium containing HyAse, resulting in a reduction of cardiac jelly in their later developing hearts. Their paper presented photomicrographs of transversely sectioned specimens. Unfortunately, the investigators did not provide any photographs of intact hearts at any stage of their experiment and their conclusion that CJ-deficient rat embryo hearts loop is based upon a single histological section of one embryo. The entire subsequent thirty-five-year impact this paper had on chick looping studies rests upon interpretation of that single histological section from a single rat embryo.

There is no stage at which there is morphological and physiological equivalence between tubular hearts of rodents and chicks (see le Garrec 2017). As noted by Baldwin and Solursh, the tubular heart in their rat specimens had already formed and were contracting at the time incubation began (see also Baldwin et al. 1991). The 9½ day pc embryos were almost a day after early tubular heart inflation has already occurred (see Marcela et al. 2012) and the heart is already beating. It is also well known, and has been mentioned in many papers, that there is significant variability of developmental stage among rodent littermates at early PC times (see Tyser et al. 2020). This necessitates staging *each* embryo. Baldwin and Solursh did not present photographic evidence showing whole control and experimental hearts at any stage. It remains questionable whether or not the Baldwin and Solursh data shows normal looping or, indeed, looping at all. Certainly, C–looping was *not* documented at all although, oddly, many later workers claimed without basis that the paper showed normal C–looping.

How to interpret the Baldwin and Solursh data?

Baldwin and Solursh studied rat hearts in intact embryos. They (and subsequent workers) assumed their rat work applied also to chicks and their work is widely claimed to falsify data acquired from chick embryo studies despite uncertainty regarding what they actually demonstrated.

Available evidence indicates that mammals and birds branched off a common ancestor over 300 million years ago. Mammalian and avian cardiac embryogenesis share many similarities and most investigators of early cardiogenesis using either the chick or rodent as a model organism appear to assume mechanistic identity or homology. But chick hearts are not rat hearts.

Although both birds and mammals have a generally similar four chambered heart there are significant adaptive variations. General phenotypic similarity is the result of convergence and does not imply that the hearts evolved from in identical fashion. If we compare the morphologies of very early tubular heart formation in rats (mammals) and chicks (birds) we find distinct anatomic differences as well as a difference in sequence of developmental events. Much less is known about early rat (Synapsida clade) heart development than about the chick (Reptilia clade) but such differences may reflect the several hundred million years of divergence. These differences indicate the morphogenic machines differ, suggesting conserved differences in modularity. The apparent similarities when examined closely refute homology. Much of the implausibility resident in the literature seems to result from trying to align avian studies with a single murine study.

Alternative explanations to the Baldwin and Solursh conclusions abound and are more consistent with the paucity of their data. It is entirely possible the Baldwin and Solursh hearts did not loop, but kinked. Normally, the rodent tubular heart *in situ* grows faster than the pericardial space and its length soon exceeds physical boundaries (Le Garrec et al. 2017). In such a situation the tube will kink, the same way Bayraktar and Männer's elastic cylinder kinked (Bayraktar and Männer 2014) when they applied force. Such kinking, while causing a convolution, is not looping and has nothing to do with cardiac jelly or internal hydrostatic pressure. The Bayraktar and Männer kink, a modern confirmation of earlier proposals, strongly suggests any cardiac asymmetry in the Baldwin and Solursh study could well be the result of the heart growing in length within a confined space. This latter observation emphasizes the logical error of trying to falsify a holistic hypothesis in such manner.

Inflation and CJ

Baldwin and Solursh interpreted their findings broadly. They dismissed the idea of myocardial deformation by an internal force from CJ and indicated that looping was therefore intrinsic to the myocardium. Such a conclusion is a bit overreaching since they had not investigated internal pressures or myocardial strain. In their single micrograph of a section through a HyAse treated heart the lumen is clearly patent and the heart inflated, seemingly plump and turgid.

The early injection studies by Nakamura and Manasek (1981) indicated that looping does not progress in flaccid hearts. Over twenty years later, it was confirmed by others that chick hearts at the S-loop stage also deflate and interrupt development when injected directly with HyAse (Ramasubramanian et al. 2013). Moreover, stage 8 chick embryos grown in the presence of HyAse do not inflate (Hosseini et al. 2017). Most convincingly, the actual pressure generate by the CJ had been measured (Manasek et al. 1984b) so its presence was not hypothetical.

Shi, et al. (2014a) published data suggesting that isolated chick hearts incubated with HyAse continue morphogenesis to develop a C shape. Careful comparison of their Figure 4, showing an isolated chick heart incubated with HyAse for 24 hours with earlier published data reveals a striking contradiction which the authors did not discuss, attempt to reconcile or even acknowledge by citing the earlier data. It had already been demonstrated (Nakamura and Manasek 1981) that isolated chick hearts in HyAse medium shrink rapidly after only ten minutes' incubation, causing the heart to shrivel compared to controls. Published, clear, properly focused images (Figure 5, Nakamura and Manasek 1981) show approximately a 40% diametric shrinkage after a bit less than two hours' incubation when the cardiac jelly is digested. This raises the likelihood that the Shi hearts, plump after 24 hours' incubation, did *not* have their CJ digested. Although unfortunately out of focus, their 24-hour incubate appears to be as plump as the control suggesting that this specimen was still fully inflated with cardiac jelly. Further, it had been confirmed that hearts *in situ* injected with HyAse collapse, assuming the appearance of a collapsed balloon (Ramusambran 2013). Hearts do not resume development until reinflated (Nakamura and Manasek 1981).

It would seem prudent and worthwhile to revisit the finding of Shi et al. that hearts explanted to medium with HyAse remain plump and unaffected and also to determine if their Figure 4 was not published in error. Errors of attribution are rife in the Shi et al. paper. Again, the assertion that Baldwin and Solursh (1989) used chick hearts (they did not) and the omission of references to contradictory HyAse digestions make it difficult for someone not already familiar with the literature to reconstruct a historical scientific record.

Linask et al. (2003) provide credible microscope evidence of growth of collapsed, flaccid, laterally displaced chick hearts without the thick layer of CJ (see their figure 1). Whether this

represents looping in the strict sense or simply a lateral collapse (the neural tube is collapsed to the right and the entire embryonic architecture is distorted) is difficult to determine from the microscope evidence presented. The myocardium is of equal thickness all around in the single section shown, suggesting that the heart did *not* loop (the myocardium of the ventricular-bulge loop is *always* thinner than that of the inner curvature). Unfortunately, incubating embryos in hyaluronidase medium affects all parts of the embryo and distorts anatomy in general, whereas injections directly into the cardiac jelly are specifically targeted.

We know what looping looks like and it is distinct from kinking or "S" curvature. I am most familiar with scanning EM studies of mouse heart looping (see Layton and Manasek 1980; Icardo 1999; Camenisch et al 2000)) that documented the true period of loop formation in the murine heart. The early published sequences (Layton and Manasek 1980) providing a true, normal baseline for looping were already available and yet Baldwin and Solursh failed to show any comparable images of looping hearts in their own study. Detailed SEM illustrations of mouse heart looping demonstrated a clear difference between early mouse and chick loop morphologies. This was confirmed by the later study of Marcela, et al. (2012) who also provided timeframes for the rat heart and a comparative timetable.

The extensive micrograph data in the paper by Camenish et al. (2000) is perhaps the most well-documented study of CJ-deficient murine hearts, in this case in genetically altered *Has2*$^{-/-}$ mouse embryos, that fail to produce CJ. Unlike other studies, this paper presented clear, light microscope images of critical sections and used SEM to look at the early looping hearts lacking CJ, comparing them to normals. The CJ-deficient hearts beat, looped, but were not normal. Most interesting is that the lumens clearly were patent. The myocardium had not collapsed even though the hearts had no CJ and there was no discernable ECM support.

Contradictions

We are left with a contradiction: chick studies show myocardial collapse, or deflation, when the CJ is demonstrably destroyed and rat studies show obviously "inflated" myocardia in hearts devoid of any CJ. Are they also hydrostatically supported? If so, what is the source of the pressure and if not, why don't they collapse? We must keep in mind that rodent myocardia already have myofibrils and their hearts are already beating at the time of tubulation. Chick myocardia differentiate and beat after the tube is formed and inflated.

There is no ready explanation for these contradictory results (most reports are not sufficiently well illustrated and it is difficult to interpret the detail in many of the published micrographs) but it is very clear that the Camenisch embryo lumens were patent. Is this a murine vs avian difference? One might speculate that since the murine hearts were beating and there was already blood circulation that the hemodynamic pressure-maintained patency.

Formation of the cardiac loop, part of normal heart development in all vertebrates, is a highly conserved event demonstrating both modularity (see Callebaut and Rasskin-Gutmann 2005), generative entrenchment and the consequences of developmental systems theory (DST) (see Oyama et a. 2002). These latter concepts reached philosophical maturity in the late 1900s but all concurrent work on heart looping seems curiously unaware of them and their intellectual impact on the experimental analysis of organogenesis.

At no time in chick and rodent tubular heart ontogeny are the hearts identical either morphologically or functionally (see Männer and Yelbuz 2019). The absence of homologous stages implies that the morphogenic machine that builds chick hearts differs from the mammalian machine. It may use similar highly conserved modules but the hearts are not assembled in the same

sequence even though the overall process of looping is hierarchal, evolutionarily constrained, and results in great stability **(see** Simon, 1962). The hierarchy would need to be elucidated "top down" but the mechanism building each module would likely be similar in all vertebrates although there would be evolutionary pressure at all hierarchical levels. It is not obvious that the assembly of entrenched modules would be identical in tube formation in birds and mammals since tubular heart ontogeny is different.

Nonetheless, the Baldwin and Solursh data, comprising a single light micrograph, does not support the claim that it shows normal looping or has evidence of a myocardial basis for shape change. Craver and Darden (2013 144) have defined an anomaly "…as an empirical finding that appears to conflict a how-possible/how-plausibly schema." The Baldwin and Solursh data is a false anomaly and probably has nothing to do with chick heart looping.

Textual Confusions Distort the Scientific Narrative.

Erroneous citations add to the confusion surrounding looping, inflation, rats and chicks. Linask et al. (2003) falsely claimed that Nakamura and Manasek (1981**)** demonstrated normal looping in *rat* hearts after degradation of CJ and they juxtaposed that claim to the Baldwin and Solursh claims. *This is a complete misrepresentation of our published work*. In the same paper, Linask and co-workers also stated that "as previously reported for the rat embryo" the (hyaluronidase treated) heart collapsed into a flat structure. Although true for chick embryos, *there are no such previous rat studies.* Equally false is the claim by Linask et al. (2002) that Nakamura and Manasek (1978) demonstrated that looping "does not require the presence of cardiac jelly." This work demonstrated precisely the opposite! Shi, et al. (2014a) claim that the Baldwin and Solursh hearts became flaccid as a result of HyAse incubation. They did not. In the same paper, Shi et al., indicate that our physical model supports our hydrostatic looping hypothesis and that Taber and Perucchio's (2000) computer models do the same thing. Taber and Perucchio did no such thing. They presented a new drawing of the helical windings we originally proposed in 1984 (Manasek et al. 1984a) but added nothing new, ignoring our data relating torsion to the helical angle. Their entire analysis depends upon stated assumptions that all elements of the tubular heart are isotropic, has misstated the relationship between myofibrillogenesis and looping and does not indicate an understanding that myofibrils dictate anisotropy.

Many historians would consider that such errors of attribution lend credence to the accumulating evidence that the Baldwin and Solursh paper may not have been read by every subsequent investigator, but rather cited by rote in successive publications. As it was passed along the citation chain it acquired different attributes, much like the childhood game of "telephone."

Unfortunately, the Ramasubramanian et. al. papers also contain sufficient numbers of incorrect statements relating to the Baldwin and Solursh results that once again a historian may a conclude that either they did not read the paper or they misread it profoundly. For example, they state: "experiments of Baldwin and Solursh (1989), who found that hearts whose CJ was ablated by the enzyme hyaluronidase looped normally." and "It is curious that CJ removal has no influence on c-looping (Baldwin and Solursh 1989; Linask et al. 2003)". *Neither Baldwin and Solursh nor Linask showed any data regarding C-looping nor did they discern the normality of the purported loop.* Those are some examples of phantom attributions seemingly in support of their conclusions. Linask et al. showed a transverse section through a HyAse treated embryo (their Figure 1). The heart was completely collapsed as previously shown and the entire embryo was grossly distorted. No "normal" loop was documented by either Baldwin and Solursh or by Linask. These are citation

errors: neither Baldwin and Solursh nor Linask and colleagues classified normal looping based upon a C loop. Neither paper contains any data on the C-loop stage.

Whatever the reasons eliciting them, conceptual errors inserted into the literature affected subsequent investigation. The corrupting influences of the extrapolations from the Baldwin and Solursh rat heart paper are pervasive and too numerous to catalogue in this monograph. In many cases it appears possible that only the Baldwin and Solursh abstract was read or that the references to Baldwin and Solursh were simply transmitted from one paper to another without adequate consideration of the original data.

Consequences of the Baldwin and Solursh paper

The Baldwin and Solursh paper has been repeatedly cited by investigators studying chick hearts as "disproving the hydrostatic model" whereas it has nothing to do with hydrostatic pressure, myocardial intrinsic shape or, quite likely, looping. It may well be unrelated to anything having to do with the development of the chick tubular heart.

While not a crucial experiment in the sense of satisfying the criterion of providing substantive data that decides between two alternatives, the Baldwin and Solursh study became critical to the history of later studies of chick heart development. From a historical standpoint the fact that it is cited widely in most subsequent studies and reviews as negating hydrodynamic effects of cardiac jelly has probably placed it in the category of published works that have had profoundly deleterious, probably irremediable, effects on subsequent scientific endeavors (see however Perrit 2025).

Also disturbing, and what many may consider directly relevant to scientific integrity, is the fact that micrographs from the 1989 (Baldwin and Solusch 1989) paper were repurposed in an entirely separate paper (Baldwin et al. 1993) fouryears later that purportedly used a completely different group of embryos in a completely different study. Figure 3a (control) in their 1993 paper is identical to Figure 2a (also control) in their 1989 paper. Moreover, the similarities between Figure 3b (1993) and Figure 2b (1989) are such that anyone familiar with serial sections would reasonably infer both to be of the same embryo, perhaps only a single section apart. *The same embryo cannot represent controls in two different cohorts!* This hitherto unnoticed reattribution of data used in one study to a control group in a different study is contrary to scientific practice and may well suggest a cavalier attitude toward scientific veracity and perhaps even explain why whole-heart photographs of control and experimental specimens were not presented in either paper. The use of false images is a major breach of scientific integrity (see Perritt 2025).

A survey of the cardiogenesis literature of the period following the Baldwin and Solursh rat paper confirmed that indeed their conclusions regarding cardiac jelly had become accepted as a valid universal falsification of the hydrostatic deformation model. Seemingly no one critically studied the Baldwin and Solursh paper but rather accepted their conclusions without considering whether they were supported by data. As a result, hydrostatic deformation was no longer a central part of the morphogenic process.

The antecedent chick experimental data upon which the hydrostatic model is based were not disproven. They were ignored or flatly contradicted without evidence.

Unraveling the skein of garbled citations and trying to comprehend the relatively few and often contradictory data points is difficult and may never take place. Recent investigations have resulted in a patchwork quilt of observations, ideas and measurements that cannot, as currently presented, be synthesized into a mechanistic whole.

Chapter 8
Criticism of the Hydrostatic Morphogenic Machine

In 1995 Taber and colleagues accurately described (Taber et al. 1995) our hydrostatic deformation model and cited the single anomaly described by Baldwin and Solursh (1989) as falsifying it. They did not attempt to deny the validity of any the published data that provided the experimental foundations of the hydrostatic model but instead selected a single study on rat hearts, based upon a single light microscope section, as refuting a conclusion based upon the antecedent work done on chicks. The Baldwin and Solursh paper (1989) is a dramatically poor choice to use to try to falsify our findings since there is no stage in mouse/rat heart development equivalent to the chick midline tubular heart with its undifferentiated myocardium, no blood flow and no heartbeat. Both rat and mouse myocytes begin contracting before the tubular heart is established (Hirota et al. 1985; Nishii and Shibata 2006). There are no experimental studies in the rat or mouse where morphogenesis has been examined in the absence of heartbeat, blood flow and where hydrostatic pressure had been measured.

It is worth quoting an abbreviated version of the Taber group's 1995 argument since it appears to be the foundation of all subsequent work from this group:

1. The heart loops when removed from the embryo.
2. Arresting myocardial contraction with potassium does not prevent looping.
3. Inhibiting contractile protein synthesis or myofibril assembly prevents loping.
4. Globally disrupting actin filaments prevents looping.
5. Disrupting microtubules with colchicine does not prevent looping.
6. Dissolving cardiac jelly with hyaluronidase does not prevent looping. (this is a highly suspect argument)
7. Growth is relatively uniform over the myocardium.
8. The dorsal mesocardium is located along the inner curvature.
9. The myocardium of the inner curvature thickens and the outer thins.
10. Actin filaments in the looped ventricle are oriented circumferentially.

Any logical attention to the data should have questioned the single aberrant rat finding and not discarded the many propositional assumptions that collectively went into the hydrostatic hypothesis using chick studies (see Craver and Darden 2013 144-160).

Importantly, Taber's group (Taber et al. 1995) specifically state that they ignored the effects of the cardiac jelly. They simply canceled the findings (see above, Number 6) that digesting CJ with HyAse causes hearts to collapse, stop looping and only begin to loop again after recovery. This sequence had been specifically described for injected chick hearts (Nakamura and Manasek 1981) and much the same effect was confirmed later by Ramasubramanian et al. (2013).

The well-known investigator, Jörg Männer, has published several reviews of the cardiogenesis literature that have had a strong influence. In 2000 he published a review (Männer 2000) that concluded:

> Based on 15 years of experience in research on early cardiac morphogenesis, Manasek et al. (1984) presented the most elaborate model of the biomechanics of dextral looping. The model took account of the "ventral" bending and the rightward flapping/torsion of the primitive ventricular region. It was proposed that these two components of the looping process both resulted from regulated responses of the myocardial envelope of the heart tube to the deformative forces originating from the internal pressure of the expanding cardiac jelly. The looping heart tube was compared with a cylindrical balloon that becomes inflated with air. Such balloon models have shown that "inflation" of the myocardial envelope of the straight heart tube with cardiac jelly would result in ventral bending if, due to the presence of the dorsal mesocardium, the elastic modulus of its dorsal wall were higher than that of its ventral wall. Expansion of the heart tube

> would additionally result in a rightward torsion if a handed helical arrangement of myofibrils in the myocardium were to convert the increase in diameter into rotation. *Manasek's model, however, was disproven by experimental embryology. Baldwin and Solursh (1989) showed that the degradation of the cardiac jelly with hyaluronidase did not disturb dextral-looping in cultivated rat embryos.* (Italics mine.)

This is a very accurate and concise summary of our work and how it was synthesized to form a deformation model of organogenesis. But it ignores the basic data that form the propositional assumptions of our hypothesis. Männer only suggested that we "presented" the model yet our hypothesis was not plucked out of thin air but was derived logically from rigorous experimental data. In this review, Männer simply ignored those data, uncritically taking the findings of Baldwin and Solursh (1989) at face value *even though that work reported on a different system*. Männer's dismissal of our model meant that he, too, had to cancel all the basic science that formed the propositional assumptions of the chick morphogenic machine and select an anomaly drawn from a single photomicrograph in a single rat heart study as a crucial experiment that falsified all the other published data. The looping hypothesis so aptly described by Männer is a complex hypothesis and one that cannot be falsified by any single experiment involving a lower-level propositional assumption. Attention is drawn to the compelling arguments of Wimsatt and Schanck (2004) and Craver and Darden (2013).

Unfortunately, the Männer review contributed to the growing consensus of veracity of the Baldwin and Solursh work conferred by numerous citations in subsequent literature. By giving its conclusions such primacy over all earlier experimental results and ignoring the fundamental differences in rat and chick heart development, the Baldwin and Solursh paper exerted undue influence future investigation of heart looping, distorting it for at least a scientific generation.

In 2006 Taber (2006) again reported accurately our morphogenic machine and again cited Baldwin and Solursh as falsifying it. In that paper Taber cited the paper by Linask et al. (2003) as also having falsified our model. Linask had subjected chick embryos to hyaluronidase resulting in a collapse of the heart, actually confirming(!) our findings that it is normally supported by CJ hydrostatic pressure. Upon careful reading the Linask report and studying the published illustrations, it is apparent that the flaccid, collapsed heart, lacking its internal hydrostatic support, was deflected to one side and this was interpreted, perhaps uncritically, as "looping," a reasonable explanation briefly explored later by Ramasubramanian et al. (2013). Although the data shown in the Linask et al. paper actually confirmed our report of a hydrostatically supported myocardium, their study *also had nothing to do with hydrostatic pressure being related to* looping. It is also hard to interpret the histological sections shown in their Figure 1 as demonstrating normal looping. Additional confusion is presented on page 216 where they state that Nakamura and Manasek (1981) showed that HyAse treatment had no effect on rat heart looping. Linask et al. made a completely false and confused statement: our laboratory never studied rat hearts but did show that HyAse digestion interferes with chick heart looping. In yet another misattribution Yu et al. (2006) confused the work done in my laboratory with studies relating actin to looping and incorrectly cites our papers. These confused claims, juxtaposed to yet additional references to Baldwin and Solursh continue to support my historiographic conclusion that many researchers, including reviewers, had not read the papers they cited, or at least not with much understanding. I think such an interpretation is preferable to inferring intent. It would be unfortunate to propose that a scientist would deliberately misstate others' findings.

Despite its logical flaws, the 2006 Taber paper contains perhaps one of the most thoroughly argued presentation of the chick looping problem, drawing upon careful selections of much solid, earlier evidence while ignoring other. But the finality of Taber's conclusion relating a rat heart

paper to chick hearts also contributed to the virtual extinction of the hydrostatic deformation model of looping. Taber notes after correctly and succinctly summarizing our model:

> Unfortunately, this clever idea apparently was put to rest by Baldwin and Solursh (1989) and later by Linask et. al., (2003) who found that digesting the CJ with hyaluronidase does not prevent looping."

Although it does not contribute any new or relevant experimental evidence, Taber's 2006 paper has been subsequently widely cited as support for the claim regarding HyAse digestion.

Since there is no evidence refuting any of the published experimental evidence from which our model is derived one can but conclude the model's validity has not been disproven by Taber's work nor by his assertions. *In point of fact no study of Taber's or anyone else's has ever refuted our experimental discovery that the chick tubular heart is hydrostatically supported or that hydrostatic pressure directly deforms (inflates) the myocardium.*

The 2006 Taber paper sealed the case for ignoring masses of data regarding the properties of the developing myocardium and cardiac jelly and in the process created a fantastical tubular heart. Events of cytodifferentiation, myocardial anisotropy, and the physical properties of cardiac jelly were largely excluded from future analyses and virtually disappeared from the literature. It might appear to any historian familiar with the actual data that Taber's 2006 paper supported an entirely new alternative narrative not requiring established evidence. It introduced (certainly without realizing it) the fallacy that a complex hypothesis based upon a spectrum of propositional assumptions can be analysed independently of its decomposable parts.

To date, our 1984 mechanical analogue remains the only demonstration that the propositional assumptions we selected can act in concert to bulge, bend and twist the heart tube. This is truly a morphogenic machine that generates the cardiac loop and twists it by using known properties of the myocardium, the dorsal mesocardium, cytodifferentiation, anisotropy, and cardiac jelly in modular fashion.

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Chapter 9
The Artificial Heart: How to Create a New Paradigm

Modeling Needs its Own Data

Beginning about 1990 computer assisted modeling permitted quantitative analysis of shape changes. Computer models also continue to explore numerous variations of the "differential growth" and "differential cell shape-change" school of morphogenesis (for example see Ebrahimi et al. 2022). These analyses reexamined earlier unifactorial explanations of looping while canceling others but reach the same inconclusive end.

Whatever the reasons, most computer assisted analyses of looping have used models of the tubular heart with one or more spurious characteristics inconsistent with actual experimental and descriptive anatomy. I traced the use of such models based upon entirely artificial properties to 1992 (Taber et al. 1992), seemingly the first time an invented organ was used in computer-assisted analysis of heart shaping. The authors gave false attributes to the anatomic layers:

> The model is a thick-walled, pseudoelastic cylindrical shell composed of *three isotropic layers:* the endocardium, the cardiac jelly, and the myocardium. (Italics mine.)

By defining, contrary to evidence, the heart as isotropic these authors explicitly exclude from consideration cytodifferentiation and myofibrillogenesis (See Collingsworth et al. 2002) as well as the established physical properties of cardiac jelly. These are so profoundly obvious components that ignoring them is hard to understand. I personally knew the late Ed Clark during the time our own studies were ongoing, had many conversations, attended many international conferences with him and know that Ed understood the cytological changes that are part of cytodifferentiation. That Taber and his group created false physical properties of the heart tube upon which to base their modeling remains baffling. Equally baffling is that Taber continued the obviously erroneous claim of isotropy, a claim repeated so often that it gained credibility in the complete absence of any data supporting it and incontrovertible evidence against it.

The issue of use of false material properties was raised in an exchange of letters (Taber and Perucchio 2001; Tobita and Keller 2001). The discussions were well articulated and very worthwhile reading. I have already noted that, among others, Shi et al. (2014 a,b) and Ramasubramanian et al. (2008) have made incorrect assumptions about isotropy, cellular aspect ratios, even the architecture of the tubular chick heart (Taber et al. 1992).

It is germane to note that all the papers that use the cell outline data from our 1972 work (Manasek et al. 1972) fail to take into account that both scanning EM and silver impregnation provided the outlines of the outer layer only. We still have no information about the shapes of the subjacent myocardial cell layer, their aspect ratios and orientations.

When Observation Conflicts with Theory

I discussed the observation that hyaluronidase-collapsed chick hearts can reinflate and then continue to develop (Nakamura and Manasek 1981). One would predict on that basis that subsequent development would continue normally as CJ was replaced by myocardial elaboration. The continued elaboration of hyaluronate has not been exploited in later modeling, yet it could explain aberrant findings in such studies (Ramasubramanian et al. 2013). These workers also injected HyAse directly into hearts, confirmed their collapse to flaccid state and the subsequent

maldevelopment of the further "S" loop in some injected specimens. This is one of the few papers to express some concern about the influence of the CJ on their modeling, but the concern did not carry over to others' such studies. It is also one of the very few studies where HyAse was injected directly into the CJ thereby avoiding abscopal effects and clearly demonstrating the dependence of turgor on CJ. No such studies have ever been done on rat or mouse embryos. The clarity of the results of injecting directly into the CJ compared to those from whole embryos incubated with enzyme also seems to have been overlooked.

It has been argued by some philosophers of science that investigation requires an existing theoretical basis (for discussion see Hacking 1983). The work of Ramasubramanian et al. (and others) was constructed within an existing theoretical framework. Viewing their work as a historian or philosopher of science, one can interpret it as having been heavily influenced by theoretical constraints, or the impetus of the existing paradigm. Their theoretical construct was firmly rooted in the concept that CJ was unimportant and that the myocardium dictated shape. However, when their own data created an anomaly, or tension, by showing the effects of HyAse injection to be contrary to the dominant theoretical framework, they did not interpret their findings as refuting theory. Instead, they tried to integrate them into the existing paradigm and not violate it. This is akin to what happened to Joseph Priestly who was so unable to break away from the phlogiston theory that he could not recognize the oxygen he discovered as anything other than phlogisticated air.

The paper by Ramasubramanian et. al. (2013) was almost certainly influenced by the social imperatives of science. A scientist must decide which data to accept and which to discard or ignore. The decision is often based upon whether the experimental result is "correct" or not. The "correctness" of data is a complex issue and I refer the reader to chapter 2 of Barnes et al. (1996) for an excellent analysis of this issue. Pragmatically, it is difficult to work outside a consensus-driven narrative (see Pillar 2025).

Remarkably, Ramasubramanian et al. (2013) may be the only ones *not* to have unquestionably accepted the conclusions of the Baldwin and Solursh (1989) paper nor to have ascribed a similar conclusion to the Linask et al. paper (2003). They carefully noted, puzzled, and ruminated over their own observed effects of HyAse injection, the resulting degradation of cardiac jelly, and the flaccid collapse of their injected hearts. Nonetheless, they refrained from drawing the most direct conclusion that CJ is probably the prime inflator. The Baldwin and Solursh paper had already exerted an undue influence on the field and as noted, created an intellectual trajectory that was too entrenched to refute. As an example, in their 2006 paper, Ramasubramanian et al. struggle with the expansion of CJ during looping and agree that it alters myocardial stress, causing a net increase in tensile stress. They suggest, although without evidence, that this stress "enhances the structural integrity of the myocardium." I do not know precisely what this statement means, but if the CJ is increasing stress in the myocardium at the time it undergoes substantial strain, why ignore it as a morphogenic force? They came very close to having sufficient confidence in their observations to carry them through to confirming our fundamental hydrostatic morphogenic machine.

A final excerpt from the Ramasubramanian paper illustrates their lurking concerns:

> Third, enzyme-treated hearts are flaccid and we found it somewhat challenging to determine if they had looped or not. By our criteria, a heart is not considered normal unless the loop had the same morphology as control *at the same time point*. It is possible that Baldwin and Solursh (**1989**) and Linask et al. (**2003**) found similar distortions due to flaccidity, but nonetheless classified looping as normal based purely on whether the heart acquired a c-loop at the end of culturing.

It does seem that Ramasubramanian et al. struggled with the apparent contradictions between their own observations and what they perceived to be consensus in the literature (or, perhaps one can

say the dominant paradigm relevant to their work). It is unfortunate that they did not continue their hyaluronidase injection studies to the point of performing a crucial experiment. They cite Taber (2006) as negating the hydrodynamic model despite the fact that Taber's 2006 paper (or any of his others) had no experimental data on hydrostatic pressure and looping. Taber's authoritative statement had indeed become established fact.

The Voice of Images

When reading published literature, we need to keep in mind the great rhetorical power images have in scientific literature. It is absolutely fair, and necessary, to examine published images as closely as one would look at numbers in a table. One may well also ask why authors choose not to simply photograph, by light or SEM, an embryonic heart claimed to have looped or not looped. Such a direct approach asks a discrete question and provides a definitive answer. A microscope section through a heart can only give rise to a secondary interpretation. I have indicated this flaw in a number of studies where a single photograph of an intact heart would have provided an unambiguous result.

The quality of an image speaks to its veracity. Indistinct or out of focus images are open to misinterpretation and if severely deficient can justifiably be disregarded. I have questioned several such images, especially when they purportedly demonstrate something contrary to what has been reported and substantiated by clear images (data).

Chapter 10
Epistemology: Impetus of Research Trajectories and Ideas

Ignoring published findings to promote one's favorite ideas might well be career-driven but it is poor science (as well as bad behavior). What are the imperatives that drive some ideas from scientific consciousness without having been disproven, and ignored in subsequent work? Is such impetus intrinsic or extrinsic? Scientists are often pretty smart. But we do have egos that sometimes intrude.

What if "disappeared" work reappears in later publications without attribution? Scientific knowledge can but shouldn't be ephemeral, to disappear and reappear as a result of either legitimate scientific effort or as a result of the social nature of every scientist's research.

Conservation of intellectual trajectories

Once an idea gets implanted, has some experimental success and "explains" the problems contemporary embryologists are working on, it begins its trajectory with an almost Aristotelian impetus.

A good example is induction. About a hundred years or so of anatomical experience, along with the entire model of primary and secondary induction gaining dominance, had shaped the thinking of embryologists. When induction became the dominant paradigm there was a long search for inducing tissues and defining the developmental window when induction could take place. Cytodifferentiation, or the change from a generic undifferentiated cytology to one phenotypically specific to a cell with a particular function, was a major problem and "induction" by an inducing tissue seemed to provide an answer. Studies of induction resulted in a phenomenology often difficult to replicate and curiously resistant to reductionist investigation. Nonetheless many papers reported studies, often lacking adequate controls, agreeing with and perpetuating classical induction.

Intellectual trajectories based upon solid experimental evidence developed over a long time persist for a long time. This conservation of ideas has fed the tendency to force the conventionally known and successful morphogenic mechanisms to be applied to the heart, regardless of whether or not they are appropriate. The certitude of the applicability of these mechanisms diminished contradictory information. Findings that were interpreted to support an existing paradigm were often based upon selective data.

In the case of looping, hard data (see Icardo 1996; 1997; 1998) that had led to discrete models was arbitrarily discarded or ignored and research took a direction that supplanted the existing paradigm. Perhaps this is why the hydrostatic deformation model was ignored and countered by an intense effort to develop models that have not contributed to a plausible solution to the problem after almost forty years of effort. By ignoring or discarding years of evidence and attributing false characteristics to the various cardiac compartments many FEM studies have essentially analysed a non-existent biological structure.

Conservation of Scientific Momentum

Marketable results are those that get papers accepted for publication, get grants renewed and that lead to academic promotion and accolades.

If a research program is producing marketable results, it will continue along the same trajectory. Impetus is not dissipated. Marketability can be influenced by how fashionable or "modern" the

work appears and excellent science belonging to a previous paradigm is often eschewed even if it answers important questions. A laboratory riding such a trajectory will be perceived by the scientific community as successful and productive. Such perception will not necessarily depend upon the quality of the science. Scientific quality or relevance and marketability are not linked in any obligatory way. The trajectory horse will be ridden to exhaustion, its path often unaltered by competing data. If, as documented here, one is successfully producing marketable models of any morphogenic event focused on a limited repertoire of cell activities while avoiding other parameters that may influence results, there is little incentive to include such additional complexities. Marketable results are often those supporting current concepts and present few anomalies. Complexities risk diverting the horse and losing the market. This phenomenon has been recently described by Pellin (2025).

An example of the inability to reverse the trajectory of a confusing implanted idea once it has become incorporated into the body scientific is the case of *Ambystema mexicanum* that has a mutation in which the homozygous (c/c) larvae do not develop a beating heart. Experiments of the classical induction sort by Humphrey (1972) suggested it was a failure of cardiac muscle induction and several studies began to appear on the biochemistry of the mutant's myocardium. This mutation interested our laboratory and we obtained fertilized eggs from the colony maintained at Indiana University. We were planning a very basic series of analyses on the c/c hearts. An undergraduate working in my laboratory was set the task of harvesting hearts from the homozygous larvae, identifiable because, in the absence of circulation, they were swollen and edematous compared to the heterozygous larvae. His job was to harvest the hearts and place them into Holtfreter's balanced salt solution. He came running into my office, very excited, exclaiming "They're beating!" Indeed, they were, after just a short while in the balanced salt solution. A quick look with polarized light microscopy showed birefringent myofibrils, clearly demonstrating that these myocardial cells had indeed been induced. The failure to beat *in situ* was most likely due to an ionic imbalance in the pericardial fluid preventing cardiac depolarization and contraction. This finding was independently confirmed (Justus 1978). It was not a failure of cardiac muscle induction as claimed in the literature.

It is highly unlikely that anyone will ever go back and reinvestigate Humphrey's original induction experiments, including making parabiotic embryos. It is more likely that it is convenient and supportive of one's career to ignore contrary findings or any of the earlier literature on the c/c heart and soldier on, riding the existing intellectual trajectory. In all likelihood the narrative as now known and with its own reality will persist as truth unlike the California-as-an-island cartographic story. This is perhaps another example of the hypothetical posed by Feyerabend that investigators will continue to investigate phenomena they know to be false (Machamer 1973) although in this case Feyerabend, in his own selectivity, was mistaken about Galileo.

Of Reviews and Summaries

Reviews are highly focused historical narratives, often written by people with intimate knowledge of a scientific field and with broad, respected, first-hand experience. Although review papers are secondary sources, they should reflect the critical analysis of their content rather than only citing a chronology of conclusions. Reviews, as well as monographs (some also in the realm of historiography) are extremely useful in "getting one up to speed" in an area. By summarizing the work of the past few years, reviews can, by default, serve as a baseline to which further investigation ascribes veracity, and create a presumptive foundational knowledge that is supposed

to define the current status of knowledge (not necessarily research). Such a baseline may be given agency on the basis of the reviewer's authority rather than critical analysis of the works cited. A good review should avoid that trap but few do. The practical considerations of necessary brevity preclude a deep critical analysis of reported results. Nonetheless there should be unbiased critical judgments, providing an adequate window into the earlier literature upon which current research is based and not, in essence, erasing it or perpetuating fallacies.

But sometimes, in addition to acting as a service directing readers to the primary literature, a review becomes conflated with primary literature, becoming a surrogate. Consequently, a review can establish a new (the surrogate) reality by creating, defining, or supporting an orthodoxy and failing to consider logical or philosophical contradictions is methodologies. As a surrogate, a review may then be cited in a new research paper as the source of earlier experimental data without reference to the original papers. The source and the nuances inherent in the original publication have the potential of becoming either lost or clouded especially if the review only refers to a selected portion of the original results. The review can create the appearance of a synthetic cohesion between disparate descriptive experiments, giving the impression of a unified system or collectively accepted paradigm. The reader of a review will generally not go and read critically each cited reference, especially if they all seem functionally correlative. Thus, a review can become a *de facto* primary source obliterating all nuance and caveats originally expressed and interrupting the historic record.

Reviews, too, are disseminated widely, perhaps more so than any of the individual papers they include. Rarely is a review critically neutral. Often it contains overtly or covertly a particular perspective. This is in no way a condemnation of review articles, rather it acknowledges the historiographic nature of the works and as such recognizes the sociological imperatives inherent in any historical interpretation. Nonetheless we must always consider the possibility that the simple omission or misinterpretation of earlier work which thereby helps to establish a false narrative is based upon the review author's biases, misinterpretation, deliberate attempts to manipulate the narrative or simply an inability to comprehend the nuances of the problem. This is, I believe, a problem with the well written review by Ebrahimi et al. (2021). This review, as others, constructs a reality drawn exclusively from the conclusions of earlier investigators and thereby validates the deficiencies leading to those conclusions and erases much salient data. Equal validity is given to all conclusions except those that are ignored or cancelled. As noted in the present work those deficiencies are sufficient to cast doubt on a large number of studies whose results have unfortunately been made part of the scientific narrative by being legitimized in reviews. I am not aware of a single review article that has tackled the issues raised in the present monograph.

As a historical document, reviews are subject to the social and economic pressures that all historians face and with the flaws inherent in all historical accounts (I write this wearing my historian's hat). One may compare reviews to maps that historically are not neutral representations of known data. The viewer of a map, much like the reader of a review article, is presented with a reality resident in the map (or review) itself: the physical map becomes the reality. There is an extensive literature on the geopolitical uses of maps, their propaganda value, and, especially exploiting their ability to create a surrogate reality (Russell 2006). It's probably safe to argue the generality that when a large body of information is assembled into a unifying document, review or map, human biases cannot be avoided. The larger a body of work becomes the more difficult it is to identify and weed out seemingly coherent fallacies.

An extensive review carries with it a danger that original works cited recede behind the edifice of the review and will not be critically read anymore. But these original studies may still get cited

as primary sources, "lifted" directly from the review despite not having been read, successively perpetuating a review's misconceptions.

A review paper can also alter the scientific narrative, and, hence the trajectory of future research, by giving imprimatur to the reviewer's currently accepted construct, citing as fact an earlier result that is questionable, materially false, or misunderstood. As an example, the world of cartography has many instances where a surrogate reality does not interface well with true geographic representation. A well-known example is the depiction of California as an island. The earliest such depiction (de Herrera 1622) found its way into mainstream cartography and was still shown even when competing geography (the peninsula) was known to be correct.

Many literature reviews also become a bit of a "thought stew" (see Lindsey et al. 2014) and comingle different concepts of looping (variously defined or not defined at all), do not distinguish between rat and chick hearts, deal with trabeculation, cushion formation and valvulogenesis, hemodynamics, stroke volume and regurgitation, septation and a myriad of other considerations that are not part of the same looping morphogenic machine, thus diluting whatever clarity is needed for analysis of discrete problems. A review of looping should be just that and not get involved with the rest of cardiogenesis lest it create an intellectual fog and support laxity of thought. We can see just such a fog permeating many research papers wherein authors wander down many diverticulations and "throw the kitchen sink" at their problem, often distorting earlier findings.

References Can Be Selectively Omitted or Corrupted: Ideology, Dogma, and Bibliography

Selective citing essentially alters the intellectual history of the subject and can introduce irremediable biases and errors into the understanding of a phenomenon. It essentially creates untruths in the literature. It is safe to state that absolutely everything humans express contains some bias. What we think is important and unimportant is our bias and our bias may be dictated by some of the drivers I've discussed. What we write is limited by what we know, or choose to know and that determines what we write. We can never be free of bias, but we can try to be honest and confront it.

There is yet another way to deform the literature. It is particularly insidious to insert ghost citations in the reference list. One can include papers in the bibliography yet not incorporate their findings into the body of the paper so they don't form part of the conceptual basis of that paper. Yet, since they do appear in the bibliography, they give any reader (or reviewer!) the idea that they are part of the paper's scientific pedigree. Very few readers will check to see where, or how, a citation has been used in the paper. As a historian of ideas, I have examined citation use in the context of this monograph and am genuinely surprised at the frequency of ghost citations, misattributions, and convenient omissions as well as false claims regarding the actual content of a cited paper. In almost every case, these deviations from reality seem to be in favor of the author's thesis.

Building a heart from existing modules is an exercise in engineering as well as construction. A knowledge of physical properties of components is essential. In his generally comprehensive volume, *Continuum Modeling in Mechanobiology,* Taber (2020) explores many possible examples of mechanobiology. The major utility of *Continuum Modeling* lies in the careful and rigorous analyses of several biomechanical processes, essentially serving a didactic function. Many forces applied to known biological form are mathematically described and, in classical textbook manner, problems and solutions are offered. But its great weakness is that it largely ignores the fact that the biology so analysed is the end result of a hierarchy of evolutionarily determined upstream

selections and an engineering analysis of the end product is often but a descriptive transform and may have limited heuristic value. It is not unexpected that Taber, who did not include cardiac jelly function in his published studies of looping, also deals with matrix in a most cursory manner in this book. *Continuum Modeling* has an excellent introduction to morphogenesis, concentrating on the role of epithelial cell changes with the author seemingly deriving much of his understanding from Davies (2013), who, as noted earlier, seemed completely unaware of the morphogenic role of ECM. Although the role of matrix swelling in gastrulation is mentioned in *Continuum*, matrix swelling was not considered a driver of other morphogenesis. Embedded in this work is the implicit notion that mechanisms of biological shape changes are explained by elucidating the biomechanical properties of substituent cells. We must be aware of biological reality (see Wimsatt 2007). We must also be aware of the false authority of mathematical analysis overshadowing actual experimental findings.

In *Continuum Modeling* Taber cites (page 449) one of our papers on the hydrostatic model (Manasek et al. 1984b) but neglects to include the citation that explicitly demonstrates the analogue model that actually works (Manasek et al. 1984a). It is unlikely that many readers will make the effort to track down this missing paper. Oddly, in the introduction Taber mis-cites a paper giving me co-authors I never had, thereby creating another false reference. It appears that this citation received yet more spurious authors when listed in the bibliography.

Despite its apparent thoroughness, this work on mechanobiology ignores the experimentally derived relationship between hydrostatic pressure and anisotropic elements as a general morphogenic process. As mentioned before, it is apparent that all of Taber's published looping analyses seem to rely entirely on the false premise that all the components are isotropic. In *Continuum* he seems to acknowledge that the myocardium becomes anisotropic as it differentiates (page 136), yet this is never incorporated into his models although striated muscle is among the most anisotropic of all phenotypes.

Ideological approaches to a scientific inquiry are not necessarily unique to studies of cardiogenesis. Once a trajectory has become marketable, it often gets locked into its course until the market changes. Additionally, once a laboratory has established its dogma it may be very difficult for acolytes, trained and immersed in that tradition, to break free. Ultimately sufficient tensions, or discrepancies appear that should cause a paradigm shift in the Kuhnian sense, but that can be delayed by judicious interpretation of the literature.

Old Wine in New Bottles

Every generation or so, historians reexamine and perhaps reinterpret history according to the need of the current culture or the new and sexy emerging philosophical and cultural fashion. This kind of relativistic perspective should not dominate science, yet it does intrude to some extent. Ideas and concepts that were derived from earlier methodologies can be transposed to new technological matrices and given the appearance of newness. The newness may become believable to those unfamiliar with older methodologies and whose intellectual formative years occurred after some major paradigm shift. As a result, if something buried in older literature is revived it can seem new and exciting after undergoing a transform to a new intellectual matrix. Computer modeling, particularly FEM, provided new bottles. As a corollary, perfectly valid and significant scientific concepts not included in FEM may well disappear or simply be presented again with minimal attribution.

If a discovery is too far from the mainstream, or its contemporary scientific milieu, it will often be trivialized or ignored or simply considered an anomaly. Later, when its significance becomes

apparent it becomes subsumed and possibly resurrected as “new” knowledge. This is especially true if much time has elapsed and scientific change is rapid. I use the term “change” instead of “advance” since I do not wish to introduce presentist or anachronic teleology.

Throughout this monograph we have seen examples of earlier ideas and studies reexamined by newer methods (for one example see Zamir et al. 2003) sometimes discarding the provenance of the original. However, as Icardo noted (Icardo 1997) “…translating well-known facts into modern biological language does not improve our knowledge overall.”

Deletions from Scientific Memory

An unfortunate side effect of diminishing the agency of the anisotropy-dependent looping model was the diversion of attention away from the hydrostatic deformations occurring in other developing epithelial systems, thus the hydrostatic deformation model did not become part of the known repertoire of general morphogenic mechanisms. The deletion of the matrix-mediated mechanism from the scientific memory was so complete that in 2021 it was possible to publish papers claiming hydrostatic deformation to be a newly discovered morphogenic phenomenon (Munjal et al. 2021). This work (followed quickly by a review (Chugh et al. 2022)) consolidating both the concept and the purported novelty of Munjal’s work) has reintroduced the concept of hydrostatic deformation as part of the repertoire of morphogenic mechanisms. One might hope that this time the concept gains more traction and is not again subject to selective deletion or reattribution.

Chapter 11
Philosophical Perambulations

Philosophy of science is as useful to scientists as ornithology is to birds. *Attributed to Richard Feynman.*

Feynman's alleged quote in the epigraph is amusing, may have some truth, but is dated. The past thirty or forty years of enquiry into the philosophy of development, especially with the deepening understanding of evolutionary mechanisms, has added a new dimension to the philosophy of science relating to development. This more recent work is directly applicable to studies of heart development. I believe it is absolutely essential if we are to sort through the morass of models, arguments and theories, many of which are contradictory, incompatible with data from actual embryos, or contain distortions of the existing literature. This more recent work in the philosophy of science is far more relevant than many of the earlier philosophical concepts that were predominantly drawn from physics or astronomy. Despite that, much of the interpretation I include in the present work relies heavily on Kuhn's original philosophical contributions that I consider quite valid even though there appeared a cottage industry reinterpreting Kuhn soon after the publication of *Structure*. The morphogenic machine dominates the overall narrative and the mechanistic perspective is its driving force.

In this regard, especially since I deal with models, I urge the reader to study the work of the Chicago philosopher William Wimsatt, whose insightful studies of models should influence everyone working in the field (see Wimsatt 2007; also Craver and Darden 2013; Glennan and Illari 2018).

The very term, *science*, has origins in the general meaning of "knowledge," hence "the science of music" or the "science of perspective." What we today generally regard as science, the study of the natural world, was known earlier as natural philosophy. The modern term "science" replaced "natural philosophy." Early in the 19th century the term "scientist" replaced "natural philosopher."

The philosophy of science of the 1970s and 1980s was neither a driver of our scientific enquiry nor an invisible hand guiding and directing us. Rather it was a distinct and separate field derivative of science and dependent upon it for its very existence as a branch of knowledge. In my personal experience as a working wet-lab scientist in the period between the mid-1960s and into 1990s I never heard a discussion about the philosophy of science as related to the work at hand among my scientific colleagues during working hours. In fact, none of us ever even bothered to try to define "science" and did not fret over its definition since we had no operational need to define it. We worked in microcosms, not macrocosms, and philosophical demarcations had no relevance. Philosophy of science was perhaps most interesting in a retrospective sense than in a prospective one. It provided a critical framework for conceptualizing a historical narrative. Principles articulated by philosophers are of value in deconstructing some published papers and providing an ontology for conceptual ontogeny. But philosophy cannot, by itself, discern ontogenesis: that is the role of the historian, and is what I have tried to do in the present work.

When I began to practice science, the field reflected much of the flavor of Karl Popper, Norwood Russell Hanson, Michael Polanyi, and Paul Feyerabend to mention a few. The times I tried to engage the philosophy of science, thinking it could impact my own work I became discouraged, getting the feeling that philosophy exhibited a certain lack of tacit knowledge regarding the actual practice of science and that the two did not interface well, at least in embryogenesis. I think this changed somewhat when I read Thomas Kuhn (who had a seemingly

good grasp of what constituted the practice of science) or perhaps when I acquired a more formal knowledge of the history of science

The relevance of philosophy to the study of morphogenesis changed later in the twentieth century with the integration of modern evolutionary concepts into the philosophy of development. Developmental Systems Theory (DST), (see Oyama et al. 2001) integrates a vast amount of recent work and is particularly valuable in considering evolutionary perspectives. Wimsatt noted (Wimsatt 2002) that "DST is a broad research perspective rather than a specific model." Accordingly, DST opens a thought gateway into considering more directly the manner in which an embryo can utilize its own evolutionarily determined internal resources to direct future development. It permits formulating concepts that help ask questions (explicandum) most likely to result in explicit answers (explicans) and avoids the chaos of innumerable randomly selected questions tested experimentally.

The overarching paradigm of all biology is evolution. This is true for all levels, from viruses to herds of elephants. However, by and large, evolution does not intrude into the daily activities of the bench scientist and neither experiment nor model generally consider evolutionary forces. This, of course, should have changed.

The concept and mechanisms of modularity (see Callebaut and Rasskin-Gutman 2005) in biological development has become sufficiently refined to be highly relevant to cardiogenesis at the time of looping. Every organ at every stage of development is hierarchal (see Simon 1962). It is assembled from upstream subsystems at many levels, each evolutionarily determined. The assembly of subunits into more elaborate structures follows a stochastic imperative. Successful modules are highly conserved evolutionarily and can be found across a wide taxonomy.

Conservation of essential modules forms the core of generative entrenchment (GE) which has made its way very neatly into modularity and DST (see Oyama 2001; Wimsatt 2002). Any organized biological structure has hierarchical modules that are essential to generate a downstream final structure. Evolution selects organisms and the successful completion of an organism requires innumerable entrenched generative elements. Since alteration of any vital upstream module will have downstream consequences, (generally deleterious) modules must be highly conserved if they are to be successful. Modularity, DST and GE have altered the understanding of enquiry into organogenesis, hopefully diverting it from its focus on ever more detailed reexamination of singular events. These philosophical developments have perhaps elucidated the conceptual errors in the design and interpretation of many investigations over the past thirty years. Works that looked at specific, singular events in an attempt to model looping were doomed because of their failure to incorporate the hierarchal complexity of the morphogenic machine and its substituent mechanisms. Many discrete studies produced factoids that are not part of any morphogenic sequence. Singular events need to coalesce into machinery but many events are corrupted by dominant false narratives and are either irrelevant or deleterious.

In the absence of broad, sometimes highly nuanced knowledge a reader may well accept an author's conclusions without truly understanding the limitations presented by methodology or recognizing that the conclusions drawn by the author(s) are incompatible with the data presented. It is this breadth of knowledge in adjacent or even peripheral fields that can influence the understanding of any work. The ability to see "resemblances between seemingly disparate problems" (Kuhn 1977 p306) is perhaps the most important characteristic of an innovative scientist. Scientists require a strong, almost intuitive understanding of what makes sense (these days it might be called a "spidey sense"). Such intuitive understanding is, in reality, a manifestation of practical knowledge/wisdom; an almost Aristotelian sense of *phronēsis*. There is a subjective

human element to research at the laboratory bench level that partly depends upon the intuition, experience of the investigator and the breadth of their general knowledge.

These attributes were typically acquired by laboratory apprenticeship, not by reading philosophers. The quality and rigor of that apprenticeship (called post-doc) had a lasting effect upon an individual's later work and often continued the intellectual trajectory of the parent laboratory, including, unfortunately, the continuation of false dogma.

Balloons, sticks and rubber bands.

A turning point in my thinking about how the heart tube can change shape came about suddenly in the mid-1970s during a talk given by a colleague at The University of Chicago, Michael LaBarbera, who had been a student of Steve Wainwright at Duke University. Wainwright had the unique advantage of being familiar with evolution, organisms, cells and tissues as well as having hands-on knowledge of the properties of materials and used biomechanical principles to understand their physical interactions (see Wainwright et al. 1976). This approach requires a holistic understanding of the interacting parts that create phenomena.

My colleague, LaBarbera, was discussing the hydrostatically supported tubular organisms and the mechanical restrictions to their possible flexure. I had been aware of hydrostatically supported tubular organisms such as ascarids and hydras, but the demonstration that it was anisotropy of the tubular wall as well as internal pressure that regulated the organism's shape was new to me and afforded immediate insight into possible mechanisms of tubulation and looping in embryonic development. Hydrostatic support and modulation by tensile elements are widespread evolutionary strategies and it should come as no surprise that similar evolutionary strategies are present during embryonic development.

Since ascarids and hydra both are closed-end systems they are isovolumic and their shape changes result from redistribution of fluid contents, not of addition or removal nor of active changes to cell shape. These organisms have muscles in their walls but are basically epithelial-bounded tubes. In addition, they are fiber-wound with fibers of greater elastic modulus than the more readily-deformable epithelia. Such fibers make the wall anisotropic and the angles the fibers make with the long axis determines the possible elongation (see discussion of fiber-wound systems in Wainright et al. 1976; see also Wainright 1988). Koehl et al. (2000) have more recently investigated fiber winding: this is a universal phenomenon and mechanism that dictates deformation. It is a successful evolutionary product that is highly conserved and ubiquitous.

It seemed obvious to me given evolutionary conservation, that similar hydraulic forces must prevail in anatomically similar embryonic tubular structures. The chick heart, with its thick sleeve of extracellular matrix, is a prime example of a hydrostatically supported embryonic structure. Fibers of greater elastic modulus, either in the subjacent matrix or within the myocardium, are permissive in the sense that they allow shape change in specific directions but restrict it in others. These fibers do not *cause* the deformation. From an evolutionary standpoint the biological elements of tension are more efficient, therefore more economical, than those of compression. A system such as this will have been conserved. The concept of helical winding as relevant to looping has been resurrected by Taber and Perruchio (2000) and more recently in an oblique manner by Männer (2013).

Historically, for didactic purposes mechanical concepts were widely demonstrated by relatively simple mechanical models. Until a few decades ago Newtonian physics was demonstrated in classrooms by using relatively simple mechanical devices. The mechanical demonstration model, long retired from most physics courses, enabled students to connect abstract principles with actual,

tangible working machines that were not abstract. Principles of biological machinery can also be visualized directly by mechanical analogues. But perhaps this approach belongs more to the age of machine than to the age of silicon. I suspect it can be easily dismissed by generations of younger workers who often no longer perceive the world through mechanical, tangible imagery, yet it is just such mechanical elements that comprise morphogenic machines (for an interesting insight see Nersessian 2009). Our very simple model of looping (Nakamura et al.1980; Manasek et al.1984a) is just such a didactic instrument integrating experimental and anatomical data to show how actual events effect bending and rotation.

If the hydrostatic mechanism is incorrect then perhaps the evolutionary forces responsible for other tubular biological structures do not apply to embryonic tubes. In such case we would have to postulate the unlikely evolution of entirely new mechanisms that support and change the shape of hydrostatic embryonic tubular structures. This is most improbable. Hydrostatically supported tubes and tube bending in all biological organisms are subject to the same biomechanics simply as a consequence of their shape and material composition. Old mechanical demonstration models show this very neatly.

Despite the remarkable similarity between avian and mammalian hearts, they have evolved independently of each other and are the result of evolutionary convergence over hundreds of millions of years. There are important ontogenic differences between rat and chick tubular heart formation and modularity differs despite similarities between the mature organs. If there are ontogenic differences between two different organisms perhaps we must look for different sequences of action in morphogenic machines, but recognizing that these machines use very similar conserved modules. Experimental findings from rodents and birds should not be forced to corroborate each other and if they differ, they do not necessarily contradict.

Theory, discovery and models.

When Galileo's initial telescopic astronomical observations were made in 1610 Kepler had not yet published his theory on optics and there was no theoretical basis for the telescope nor for the images it produced. It was a true "black box" whose results existed completely independent of theory and paradigm. The struggle to make sense of the images and information produced by this instrument should be kept in mind by all modern scientists. Similarly, Fleming had no theoretical basis directing him to his discovery of penicillin. Early discovery is often independent of theory.

In general, in every field, especially those made "new" by advances in technology, there is a relatively brief period when low-hanging fruit can be harvested and this period is important in providing a new database for later integration and assembly. In effect, the work of such a period begins as tactical, not strategic. In my own scientific lifetime, the development of the transmission electron microscope as a practical laboratory instrument along with vastly improved fixation protocols provided just such a harvest.

Such a non- or pre-paradigmatic theoretical approach often yields information that, although seemingly disconnected, is interesting and provides a chronology of independent descriptive events, morphological as well as biochemical. These can serve, via induction or abduction, to alter the intellectual trajectory and cause a paradigm shift or creation of entirely new paradigms. In such instances a burst of new information hopefully becomes integrated to form the basis of a theoretical model. Complex models may be incapable of having preexisting theory. Very often work on the model progresses, and then must fall back for correction, something intuitive to anyone who has designed a mechanism or even a child who is attempting to build a windmill using Tinkertoy parts..

The inability of theory to account for, or to accommodate unexpected complexity has been discussed by Simon (1996) among others.

And there are instances where the theoretical underpinnings of a new paradigm are simply wrong and lead to irrelevant or dubious work. The general notion that myocardial cell shapes regulate heart morphogenesis is, at best, incomplete. At worst it is completely false. Myocardial cells on the convex surface are markedly flatter and thinner than those on the concave side (Manasek et al. 1972), a change that occurs as the heart loops. The demonstration of CJ hydrostatic pressure makes it most likely that the observed cell shape changes in this 1972 study are the result, not the cause of the loop. It has always bothered me that we had suggested otherwise in the title of that paper! It seemed very logical at the time, since cell shape change was the morphogenic mechanism *du jour* and we thought this to be an unequivocal, quantitative example. Don Fawcett, who kindly read our manuscript, suggested that the shape changes were perhaps secondary to the deformation but the intellectual dogma of that moment dictated that cell shape changes altered tissue morphology. We were also unaware, at that time, of the existence of cardiac jelly's hydrostatic pressure. In retrospect, it seems naïve to have thought that the flattening of a small number of cells could push against their neighbors with sufficient force to bend a much larger viscoelastic tube. Such a mechanism would be incompatible with the observations that the bulge is in tension, as evidenced by the enzyme studies and observing slits (Shi et al. 2014) made in the myocardium. *A flattening cell expanding its surface area at the expense of height and pushing aside neighbors in a boundaried epithelium would not create tension but rather would create compressive forces.*

Our initial rush to ascribe myocardial cell shape change as a probable cause of looping probably is an example of the conservation of an intellectual trajectory to which we fell victim. It might be also an example of dismissing an interpretation that would introduce an anomaly into an existing paradigm, as described by Kuhn (1962). Unfortunately, it also seized the imagination of future investigators who seemed unable to let go of this concept despite its physical implausibility. The persistence of this model has further distanced hydraulic forces from consideration as drivers of deformation and its continued intrusion is an example of the consequences of an absence of tacit knowledge and an intuitive grasp of mechanics.

As a scientist with decades of direct laboratory experience, I know firsthand that much research is also conducted in the absence of any preconceived "ideal" outcome. Much is done in an ad-hoc fashion, perhaps best described as a "What if?" experiment, especially if a laboratory is skilled in a specialized technique that can be easily transferred to many systems. Much of the research in early cardiogenesis has also been done as discrete question-answering activity and many studies, experimental or descriptive, are done independent of theory or even without any apparent locus in an existing paradigm. For example, "What is the origin of the ventricular progenitor cells?" Such works are interesting, but do not readily self-assemble into a greater hypothesis (for a fuller discussion of this problem see Weber 2022). Despite the publication of dozens of sophisticated examples of FEM, recent reexamination of older procedures, minute redescription of morphogenic events, these still cannot be assembled into a coherent, comprehensive morphogenic machine. The tacit understanding of the machine as a modular, decomposable hierarchal system seems to have vanished.

If known, measured parameters are put into a model, and the model then behaves like the structure from which the known parameters were taken it is a (more or less) accurate model. If it does not, then it isn't a model. A poor model is not a model any more than de Vaucansin's *Canard Digérateur* is a model duck with heuristic value. But what are we to make of a model that is known

to be qualitatively and quantitatively unlike the system studied yet may even appear to mimic parts of normal ontogenesis? Such models violate Winsatt's dictum (2007) that

> "A model may give a totally wrong-headed picture of nature. Not only are the interactions wrong, but also a significant number of the entities and/or their properties do not exist."

I think I have shown in my historical analysis that many of the FEM models are based upon physical characteristics that have no counterpart in nature. Many of the material they investigate are not those actually found in the biological system. It is not possible for such studies to have any meaning–any predictive output to the actual organism would be pure happenstance. Such studies are examples of *underdetermination* since the propositional assumptions are known to be incorrect.

Chapter 12
Do We Know When We Get There? How Do We Know?

"Would you tell me, please, which way I ought to go from here?" "That depends a good deal on where you want to get to," said the Cat. "I don't much care where—" said Alice. "Then it doesn't matter which way you go," said the Cat. "—so long as I get SOMEWHERE," Alice added as an explanation. "Oh, you're sure to do that," said the Cat, "if you only walk long enough."

Lewis Carroll

Can we articulate what we are looking for? Are we seeking the equivalent of the *Homeowner's Guide to Building a Garden Shed* or something else? Perhaps a new set of postulates whose conditions must all be satisfied?

We must ask, "How do we recognize the terminus?" Wenning (2009) presented a nice summary of the criteria we can use to decide when a problem in morphogenesis has been solved. We may also ponder the seemingly absurd question, "Do we ever know when a problem is solved?" We can only do this if we know what comprises a solution.

If a problem is discrete enough, the answer is a resounding "Yes." For example, the question, "Does the myocardium secrete hyaluronate?" can have a definitive answer but it is neither a model nor a mechanism. In the case of a complex hierarchical system such as morphogenesis the "yes" must be acknowledged as being an asymptote acceptable to the immediate. We can build a working morphogenic machine and keep refining it, each iteration is a "yes" if not viewed from a presentist perspective. The hierarchal nature of the machine, comprising decomposable modules, each of which has lower-level modules, (for discussion see Simon 1962; Povich and Craver 2017) may mean that we can only achieve mechanistic understanding at the modular level. It is also important to recognize that a solution may not be unique and may also be suboptimal (see Simon 1962). Both the Ptolemaic and Tychonic system were quite adequate models to predict the behavior of the solar system at the contemporary level of resolution and it took more than fiddling with seeming irreleventia to get to the next step.

Of course, it is widely argued that phenomenological knowledge can never be complete (see for example, Pingree 1992). Accretion will become asymptotic, approaching stasis unless there is a major paradigm shift in the Kuhnian sense. And then we start all over. To be somewhat lighthearted about all this, quoting *Beyond the Fringe*, (see Wikepedia 2023) "there's always a little piece in the corner you can't get out."

Knowledge of the morphogenic machine does not imply knowledge of the synthesis of its upstream parts nor the downstream consequences of its construct. We can only know for a very brief period of development, perhaps one module at a time, and must frame our expectations of knowledge rather narrowly and precisely.

The intellectual trajectory of developmental biology changed dramatically and our own working morphogenic machine, built upon an earlier paradigm, would probably not resonate with adherents of the new silicon paradigm. The morphogenic machine might well be discarded and a new machine attempted using bricks of a different shape. But the phenomenon within the actual organism remains unchanged; what changes is our scientific culture and how we perceive, interpret, and translate the organism's shape changes. We always have to use the organism's bricks, not those we fantasize.

Are we asking the right questions? Raised much earlier much earlier (Icardo 1998), this vexing issue can only be decided retrospectively, but I'm convinced that the past few decades have seen

old questions resurrected and re-answered using contemporary methodology with the resulting answers often implied as new data. As such, these last several decades are a disappointment if old information is simply being reconfigured, sliced and diced into new derivatives of already-documented mechanisms or altered willy-nilly to fit invented physical characteristics.

Do we understand what we are looking at? If we have no mechanical understanding and are looking at any mechanical device, say, a steam engine, the actual device will make no sense however carefully we might measure any component or however accurately we measure stress on any component. The role of valves and pistons would not be understood. I believe that the post machine-age culture in which the vast majority of today's active scientists live has made it difficult to visualize intermediate developing stages as integrated mechanical systems. They are not just a collection of discrete postage stamps nor a computer printout of myocardial strain.

Not surprisingly, if we are to ask most investigators how they would know if the morphogenic mechanism of looping had been "discovered" they would probably not be able to answer. We would likely be met with a morass of descriptive detail, most of it highly selected to illustrate some point and, to be sure, omitting some annoying contradictory evidence. A recognition that in evolved structures stability is introduced by decomposable systems would likely be absent.

We have many dozens of papers, reviews, and chapters contaminated with conceptual errors, so much misinterpretation regarding actual data, and so much potentially false data that the collective mass no longer has a direction. New investigation attempting coherence within this textual construct will likely be based upon faulty antecedents and will simply perpetuate the problem and make the web more tangled.

Of course, the ultimate, true morphogenic machine is the embryo itself. Our job of understanding is to strip away all those parts except the ones directly involved in building a particular structure. This may well be impossible without the recognition of the questionable veracity of a large mass of the published data.

I think this is where studies of looping are right now: a morass of ever-increasing data points that surround but cannot deal with the main issues in the absence of theoretical guidance. Too many of the assumptions used are either overtly false or dubious. A more rigorous application of the philosophical imperatives that emerged over the past thirty years is needed.

Summary and Conclusions
What Does This Essay Show about Research?

Study of the morphogenic event of cardiac looping is, and has been, the purview of relatively small numbers of scientists, many with pedigrees from an even smaller number of laboratories. Intellectual consanguinity is high and intellectual trajectories can be readily discerned and followed.

Science is not always self-correcting. The present historical analysis of several decades of research documents concepts and models of cardiogenesis that, once corrupted by erroneous data, do not self-correct. Rather, the false paradigm that was created to accommodate erroneous data continues to be perpetuated by reliance upon works whose foundational data do not support their conclusions.

Such distortions introduced by biopolitical, cultural and sociological factors have, to my knowledge, not been considered in earlier philosophical analyses of scientific activity but the relatively small number of actors has certainly made detection easier. The positivist view, as expressed by Sarton (1927), is that science becomes more accurate as knowledge is accreted and, we may presume, becomes closer to the truth. This assumes the integrity of the accreted knowledge, a conceptual restriction not considered by Sarton and the succeeding philosophers; an assumption proved incorrect by the present monograph.

Careful examination of the bibliographies of many post-1990s studies show that the investigators ignored published research papers presenting evidence contradictory to their study, often omitting them from bibliographies (and discussion). In several instances data in published papers was misrepresented and spurious "results" were added to them, invariably to strengthen an argument. It is a reasonable opinion that these spurious attributions were done in order to lend more credence to the work that (mis)quoted them. Several of the studies analyzed in the present monograph repeated earlier work but used different, often more recent techniques. By omitting or obfuscating reference to the earlier works it was possible to impute originality to such material. To be sure, at some point in their career, every scholar will make an error and mis-cite a reference, omit an important paper from their bibliography, or make an erroneous attribution. But I know of no other area of investigation where these have occurred with the regularity and specificity documented in the present work.

By repeating selective interpretations of earlier published studies a series of influential review articles also served to reinforce the intellectual legitimacy of the new paradigm. The sheer volume and interdependence of publications expressing somewhat idiosyncratic interpretations of existing data makes it virtually impossible for the field to now "self-correct." It is impossible for any individual reviewer of any manuscript to slog through the errors compounded over several generations of publications. The false reality has become the reality but the new reality is such a jumbled stew of complex contradictions, impossibilities and confusions that verifiable coherence can never result.

If we divide the research trajectory of this field into pre and post computer modeling we can discern a specific point in time when a dramatic paradigm shift occurred. In 1992, one of the first computer assisted analyses of looping was proposed by Taber and his colleagues. They specifically defined both the developing myocardium and the cardiac jelly as being isotropic and thereby created a mythical anatomical structure. In order to create this fictitious organ, the authors needed

to disregard the earlier basic studies demonstrating fundamental material properties of both the differentiating myocardium and the cardiac jelly. This set a precedent.

All subsequent quantitative modeling used isotropy as the baseline characteristic of the myocardial/cardiac jelly system and all results depend upon the starting assumption, known to be incorrect, that all components are isotropic. Hydrostatic force as a possible driver of looping was dismissed on the basis of a single microscope section in a single 1989 rat paper, which upon careful reading really has nothing to do with the hydrostatic model of chick heart looping and which contains images that were fraudulently misrepresented in a later publication. This rat paper was often cited with completely spurious findings added. Generative entrenchment would suggest that the hydrostatic tube model is valid across a wide taxonomic spectrum. Unfortunately, in this case, the wide evolutionary distance between the Synapsida and Reptilia clades makes phenotypic homology more speculative especially when early tubular heart ontogeny is so different between chicks and rats.

The net result has been the removal from the working scientific armamentarium, without any evidentiary reasons, a corpus of experimental data thereby altering the direction of research. Canceling material realities obscured the presence of highly conserved, entrenched morphogenic modules effectively altering further understanding of the process of looping. Studies since 1992 have ignored DST, modularity and GE to the disadvantage of mechanistic understanding.

No new coherent morphogenic machine has emerged since 1984.

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Coda

Commenting critically on any scientific paper requires, of the critic, substantial familiarity with the techniques and validity of conclusions made from the published results. It requires challenging the authors' conclusions by examining their published results in detail. In those situations where I have examined the results and commented, I have that credential. When a micrograph is misinterpreted or when an aberrant micrograph is shown or where perhaps an incorrect image was used, I am certain.

I have reread the papers from my laboratory and after half a century I stand behind their results and conclusions. Many of the issues addressed experimentally or analytically in those papers were issues of the day and are issues no longer, having been absorbed into common knowledge. But the major trajectory of the collective output resulted in a series of observations that logically could be assembled into a morphogenic machine cognizant of evolutionary principles.

The hydrostatic morphogenic machine is a construct. The data upon which it is based is not. That data can be replicated by anyone capable. No one else has tried; it has not been falsified and it stands. That data and logic can be used by anyone to assemble our morphogenic machine.

I am in my mid 80s and it would be nice to see an answer—one way or the other.

But forty years of begging the question gives me doubt.

About the Author

I am a long-retired investigator of early cardiac morphogenesis. I began studying very early heart development in the chick embryo in 1966 while a post-doctoral fellow at Harvard Medical School. In 1968 I published the first systematic electron microscope study of the myocardium during the initial formation of the very early heart. I continued my work on early cardiogenesis at Harvard Medical School, Boston Children's Hospital, the University of Chicago and ultimately at Dartmouth Medical School in Hanover, New Hampshire, where I also worked with William Layton and his colony of iv/iv mice.

During my decades with an active laboratory career I was fortunate to have had the advice and assistance of many collaborators and close colleagues, notably Atsuyo Nakamura, José Icardo, Joan Lacktis, Mel Lieberman, Glenn Rosenquist, Bob Waterman, Beth Burnside, L.H.S. (Bob) Van Mierop, Page Anderson, Yukio Satow, Don Fawcett, Bob Kulikowski, Bill Layton, Tomas Pexieder, Grier Monroe, Betty Hay, Nick Sperelakis, Yutaka Shimada, and Richard van Praagh, as well as the many colleagues in adjacent laboratories with whom I shared innumerable cups of coffee while bouncing around ideas. I also treasure and acknowledge those scientists with whom I attended scientific meetings, had dinners and sipped a few too many martinis. I very much miss my close colleagues, most of whom are gone, from those decades past.

In about 1984 I brought my experimental work on morphogenesis to what I considered a satisfactory end: explication of a likely mechanism of early heart looping (referred to here as the *hydrostatic deformation model*). Deformation as a morphogenic mechanism had not been defined earlier. My scientific interests then gradually shifted.

I decided to retire early and I turned to other pursuits including my Vermont apple orchard, stone walls, and working on several projects, most notably scientific historiography. I matriculated in Oxford University and obtained a master's degree in History of Science in 2000, acquiring skills useful to the present work, as well as in others. The older I get the more I realize my debt to the scholars and fellow students at Oxford who shared their extraordinary depth of knowledge and helped me refine my critical abilities.

Having made a conscious effort upon retirement not to become a wistful, sidelined hanger-on, I stopped reading all journals and publications regarding heart development and let my memberships in various professional societies lapse. My reasons for returning to the cardiac arena are made clear in this essay.

Parenthetically however, I remain a Fellow of the Royal Microscopical Society (FRMS) and a Fellow of the Royal Astronomical Society (FRAS).

NOTE I have not intentionally made misattributions, misquoted anyone, nor have I invented false references. I was sorely tempted to include some benign ghost citations to demonstrate important points made in this monograph but seriously entertained reconsiderations.

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