Cardiol Young 2001; 11: 322–328

ISSN 1047-9511

The Paediatric Cardiology Hall of Fame

Maria Victoria de la Cruz (1916–1999)

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ON THE EVE OF A NEW MILLENNIUM, THE international cardiovascular, scientific community lost one of its foremost embryologists, Maria Victoria de la Cruz. Just two months before her death in 1999,[1] she had presented, at an international colloquium in Rome, her views on the future of cardiac development in the new millenium. Her message that day, as it has been in the more than 90 papers published from 1953 until 1999, was that the development of the heart could not be fully understood only by studying "snapshots of frozen time" collected from serially sectioned embryos fixed after death. Rather, with characteristic passion and singularity of focus, Maria Victoria reaffirmed that cardiac development is a dynamic, progressive, sequential and irreversible process. She emphasized that the future progress would largely rest with those who could integrate the dynamics of living morphogenesis with molecular mechanisms. This was the overarching theme of her academic life, and was the focus of her most recent book, *Living Morphogenesis of the Heart.*[2] During her long and fruitful life, she received many honors and awards, such as the "Premio Nacional de Cardiologia", an honorary doctorate degree from the University of Barcelona. She was designated as a national emeritus scholar from the National System of Research of Mexico. These awards were richly deserved and, together with the respect and admiration in which she was held by her colleagues internationally, fully justify her induction to The Paediatric Cardiology Hall of Fame.

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The scientist

Maria Victoria de la Cruz Toyos (Fig. 1) was born in April 24, 1916 in Sancti Spiritus, Cuba. Her father, a scholar and lawyer, introduced to her a desire for discovery, an appreciation for literature, culture, history and human aspiration, and an unwavering commitment to integrity and truth. On reflecting upon her life, she concluded that each of us, as a human being, is the result of the socioeconomic and

Figure 1.
Photograph of Maria Victoria taken in March 1999 during the celebration to recognize the publication of her new book "Living Morphogenesis of the Heart", held at Children's Hospital, Federico Gomez, Mexico City.

https://doi.org/10.1017/S1047951101000361 Published online by Cambridge University Press

historical environment in which we develop, or, as she eloquently expressed in it her native language, "Pienso que el ser humano es el producto de la interaccion entre el y el contexto socioeconomico e historico en que vive". She attributed to her father, and to her late husband, H. Losado, also a medical doctor, the support and encouragement that created the opportunities for her to develop her career.

She began her career by pursuing a medical degree, which she received from the University of Havana in 1943. Because of a natural curiosity for biology, she became drawn to the basic sciences and, ultimately, to the attention of Demetrio Sodi Pallares, founder of the Mexican School of Electrocardiography. Dr. Pallares urged her to move to the National Institute of Cardiology in Mexico City, the first such center in the world to be devoted entirely to basic and clinical studies of cardiology. There, under the influence of an extraordinary group of exiled Spanish professors, she focused her energies on the new and emerging field of cardiac embryology.

With encouragement from the Institute, and a grant from the Rockefeller Foundation, she went to the United States in 1949 to continue studies in experimental embryology and the anatomy of normal and malformed human hearts. This she achieved at the University of Michigan in Ann Arbor, at Columbia University in New York, and the Carnegie Institute and Johns Hopkins University in Baltimore. She met famed pioneers of cardiac embryology such as C.L. Davis and C.T . Kramer, and was given the opportunity to review all the human embryological material collected by George L. Streeter. At Johns Hopkins, she met Mary Rawles, who introduced her to the study of embryology in living embryos. Through the use of living explants, Dr. Rawles was able to trace the origin of the heart to a pair of heart-forming fields. She taught these techniques to Maria Victoria, as well as the concepts behind the techniques. On many occasions, Maria Victoria expressed how the influence of Dr. Rawles shaped her thinking throughout her scientific career. Dr. Rawles also introduced her to another leading woman scientist of the time, Dr. Helen Taussig, internationally recognized as the pioneer of paediatric cardiology and surgery.[3] Rawles and Taussig impressed upon her the need to understand normal cardiac development, arguing that new information could then be applied to understanding the aetiologies of cardiac malformations, as well as their diagnosis and surgical treatment. When her fellowship ended, she was invited to continue her research in collaboration with Dr. Taussig at Johns Hopkins, but declined in order to honor the agreement she had made to return to Mexico. Maria Victoria eschewed making gender an issue in science, considering it just "a genetic random game", yet it would seem remiss not to note that over a half a century ago, at a time when science and medicine were largely male professions, three extraordinary women of different backgrounds would briefly come together in the same city to initiate studies that would have life long impact upon both the basic and clinical aspects of their field in ways that continue to be recognized today.

Upon her return, in 1951, to the National Institute of Cardiology she began a long collaboration with Jorge Espino-Vela, founder and chief of the service for Paediatric Cardiology. Together, they organized a museum of congenital cardiopathies. This became an integral part of the new laboratory she established in experimental cardiovascular embryology. Her goal was to integrate her new laboratory with developmental cardiovascular pathology and surgery. In this way, she became one of the first to advocate a bench-to-bedside approach for advancing medical research and treatment. Together with Dr. Espino-Vela, she would train a large number of cardiologists, anatomists and embryologists, including several who later became departmental or division heads in both Mexico and the United States of America. Among them were Bernardo Nadal-Ginard, who introduced molecular biology into pediatric cardiology at Boston's Children's Hospital, and Paolo Angelini, who continued to collaborate with Maria Victoria until her death, working on innovative and fundamental studies into the anomalies of coronary arteries at Baylor University and the Texas Heart Institute. In 1976, Maria Victoria left Mexico to work two years in Venezuela. She then spent four years in Spain, where she started the Department of Experimental Embryology at the Ramon y Cajal Hospital in Madrid.

Homesick for family and friends in Mexico, she declined a departmental chair at the University of Madrid, and returned to her adopted homeland in 1983. As noted in a recent tribute to her by Dr. Espino-Vela, "Mexico welcomed her, and gave her full support which she well deserved". When asked why she had adopted Mexican citizenship, she said she did it "because the prehispanic past of Mexico bestows dignity on those born in America". Indeed, a close friend, and former Mexican Ambassador to Cuba, once pointed out to her that church records from the city of Puebla de los Angelos, Mexico, revealed that her great grandfather was born there in the 18th century.

"Home" once again in Mexico, she created yet

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another new department in experimental cardiac embryology, first at National Institute of Pediatrics, and then at the Children's Hospital of Mexico City "Federico Gomez". Calling together her pupils, former students, and colleagues, she began one of the most productive periods of her career. This latter stage continued literally up to the very moment of her final illness. In these final days, she was still actively working on a manuscript, the message of which was directed at the young practicing paediatric cardiologist. This final word of Maria Victoria has been submitted to "Cardiology in the Young", and is in the final stages of preparation for publication.

The science

Maria Victoria is best known for her elegant mapping studies using "in vivo" markers, or other experimental manipulations, to trace the temporal sequence of the origin and fate of the structural precursors for the anatomically mature, four-chambered, heart. These studies, which have changed how we view normal and abnormal cardiac development, can be grouped into three major categories.

The segmental basis of cardiac development

As illustrated in one of her best known drawings (Fig. 2), the straight or linear heart tube is formed by the fusion of the paired heart fields. It is still a

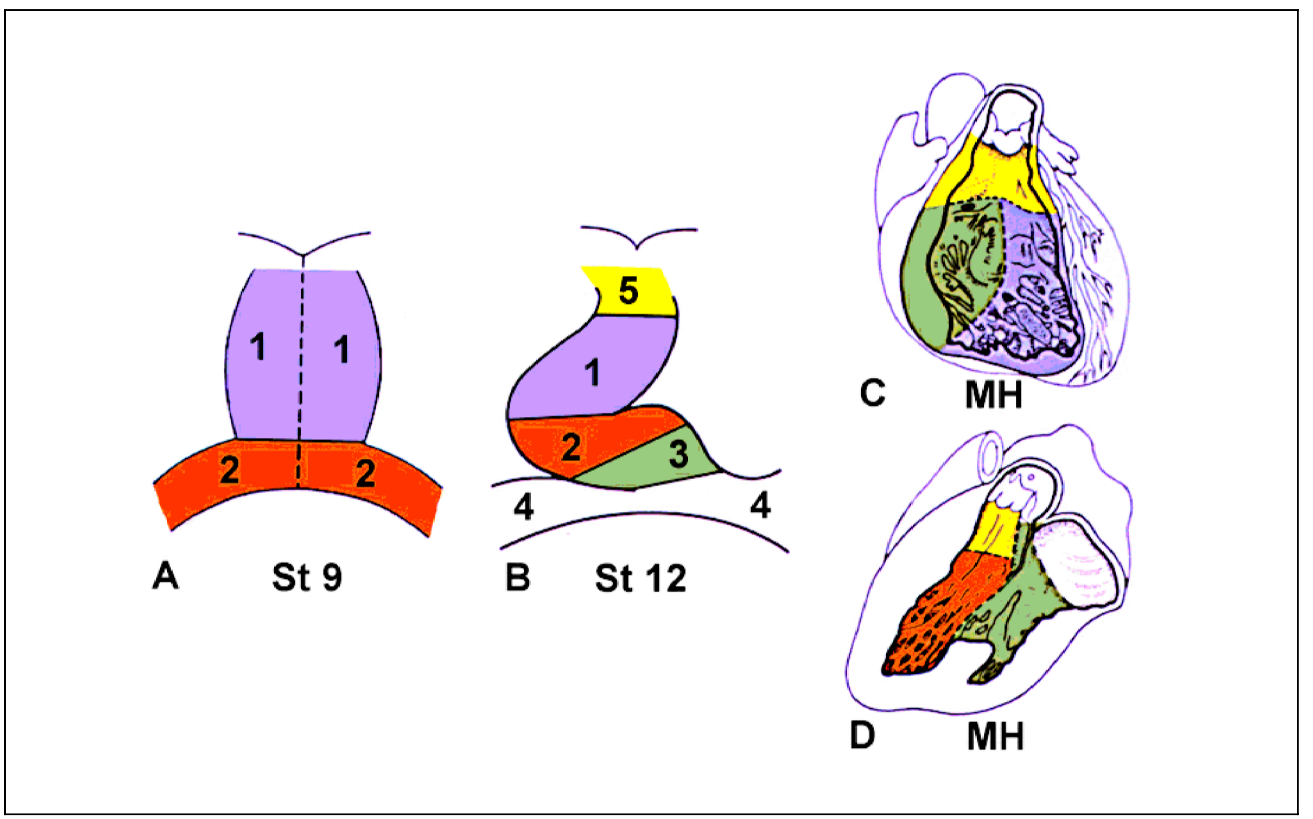

Figure 2.
This well-known illustration on the formation of the embryonic segments of the chick heart summarizes almost 20 years of study using in vivo labelling techniques. In this diagram, Maria Victoria emphasized that segments numbered 1 through 4 were formed "progressively, sequentially, dynamically and irreversibly" by the fused heart-forming fields. Panel A shows that, at stage 9, the straight tube heart is comprised mostly of the first segment (# 1), the future trabeculated region of the right ventricle (D). Note that that the second segment (# 2) still lies within the fused heart fields at this stage. Once fully established by the heart fields, at stage 12), as shown in Panel B, this segment will constitute the future trabeculated region of the left ventricle of the mature heart (MH, in panel C). Looping occurs between stages 9 and 12 after completion of the second segment 2. The third segment (# 3), the atrioventricular canal, is formed between stages 11 and 14. It will form the vestibules to the inlet regions of both ventricles in the mature heart (Panels C,D). At stage 12, the fourth segment, or the future atrium (# 4), remains a part of each heart field. It will be the last segment to be established at the venous pole. The fifth and final segment is established at the arterial pole (Panel B), and will form the outlet for each ventricle (Panels C,D). One of the important contributions of Dr. de la Cruz was the demonstration that the outlet segment had a different origin than the rest of the heart. Molecular studies have confirmed her work, and reveal that this fifth segment is derived from an anterior heart field located at the distal (arterial) end of the heart tube (see ref. 2).

https://doi.org/10.1017/S1047951101000361 Published online by Cambridge University Press

common misperception that the straight-tube heart gives origin to all future chambers of the heart. As was shown by Maria Victoria in 1989,[4] nothing could be further from the truth. Markers placed in the linear heart tube trace only to the trabeculated region of the morphologically right ventricle. The outlet and inlet portions of the right ventricle are not even represented within the heart at the stage when it is a straight tube. Indeed, over time, the paired heart fields progressively add "segments" to the primitive heart. With the addition of a second segment, the heart loops. Thereafter, two more segments are added to the future venous pole. The fate of the four segments is shown in Figure 2, with the initial segments becoming the trabeculated regions of the morphologically right and left ventricles, respectively, the third segment the atrioventricular canal, and the fourth segment the primary atrial component. A fifth, and final, segment is added to the distal, or arterial, end of the primary heart tube and forms the outlet segment of the ventricular component, often termed the "conotruncus", although there is still no consensus on this latter terminology. She was the first to propose that this final segment did not derive from the original heart fields as defined by Rawles, but from an unidentified source of cells located at or near the arterial pole.[5] Using molecular markers, and real time imaging, it has recently been shown that the progenitor of this ventricular outlet component is an anterior heart-forming field located in the posterior wall of the aortic sac and the persisting part of the dorsal mesocardium found in this region.[2] The outcome of years of performing carefully crafted experiments, conducted with the highest rigor and attention to detail, was to show unequivocally that the anatomically mature chambers of the heart develop by the integration of embryonic segments that appear sequentially over time.

Her concept of a segmental basis for cardiac development is currently giving meaning and rationale to molecular observations. Thus, for those who understand that heart is put together piece by piece, it is not surprising that regulatory genes like the *hand* genes, which encode basic helix loop helix proteins which bind DNA, are expressed in the right and left ventricles, respectively. The *heart defect* gene, and the *bone morphogenetic proteins*, in contrast, are expressed in the atrioventricular and outlet segments that contain cushion tissues[2]. A particularly graphic molecular validation of her segmental hypotheses is the "cardiosensor" mouse. Animals of this type are created as insertional mutations, in which specific *cis* elements within the promoter of a gene are used to drive expression. In such mice, specific, patterns of gene expression[6,7] are observed that reveal lineage relationships remarkably consistent with those originally demonstrated by the "in vivo" mapping studies of Maria Victoria. The power of her studies to reveal lineage relationships is further exemplified by her experiments on the origin of the primary muscular ventricular septum. Based on studies in post-mortem embryos, it has traditionally been believed that this septum is formed by the coalescence of myocardial trabeculations. Working with Moreno-Rodriguez and other collaborators,[8] Maria Victoria recently demonstrated in living embryos that a specific cluster of cells derived from precursors located at the original ventral fusion line of the linear heart tube develop into the muscular septum, an observation now supported by the expression pattern of the *tolloid-like* gene.[9] The full significance of her tracing studies, nonetheless, probably awaits future molecular studies. For example, her observation that the right and left sides of the original heart tube become the dorsal and ventral axis following looping and torsion may prove a valuable key to understanding the expression of genes potentially involved in the determination of laterality.

Fate of the atrioventricular cushions

Maria Victoria focused much of her investigative attention on the endocardial cushions that form as mesenchymal expansions, or swellings, into the lumens of the atrioventricular and outlet segments.[10] By means of in vivo labeling,[11] she directly demonstrated that these swellings did differentiate into valvar tissue (Fig. 3), a finding that had once been challenged,[12] but later confirmed by specific molecular approaches.[13] She also demonstrated that the inferior and superior cushions fused to form a septum that divided the atrioventricular canal. At least in the chick, she showed that this septum, particularly that part derived from the inferior cushion, became muscularised. It then served to anchor the primary atrial and ventricular muscular septums, at the same time closing all communications across the partitions developing between the atriums and the ventricles. As shown in Fig. 3, she also demonstrated that the cushions do more that just form valves and septums. She traced the superior cushion into the area of fibrous continuity between the leaflets of the aortic and mitral valves, a structure which serves as the posterior wall of the outlet of the left ventricle,[1,11] whilst contributing also to the valves and inlet of the left ventricle. In turn, she showed that the cushions of the proximal region of

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the outlet segment formed part of the walls of the right ventricular outlet. The latter was particularly difficult to accept, because it is well recognised that the mature walls of the right ventricular outlet are cardiac muscle, yet the cushions are mesenchymal. While she admitted the paradox, she, nevertheless, stood by her data that the proximal cushions of the ventricular outlet formed the primordium of part of the right ventricular outlet. She suggested, therefore, that they became muscularized by some unknown process. That she was right was eventually substantiated by the observation that the myocardium of the parietal ventricular walls actively invades the cushions, replacing the mesenchyme with cardiac muscle, a process subsequently dubbed "myocardialisation" by Frits de Jong and his colleagues from Amsterdam and London.[14,15]

Cardiovascular developmental anatomy and pathology

On many occasions, Maria Victoria expressed the view that she thought the most important contribution of her in vivo labeling experiments performed over three decades was to give assistance to the diagnosis of congenital cardiopathologies, and to their surgical repair.[16] Understanding the destiny of the original segments, their internal components, and the interfaces that separated them within the embryonic heart, provided valuable morphological clues to the aetiology of a

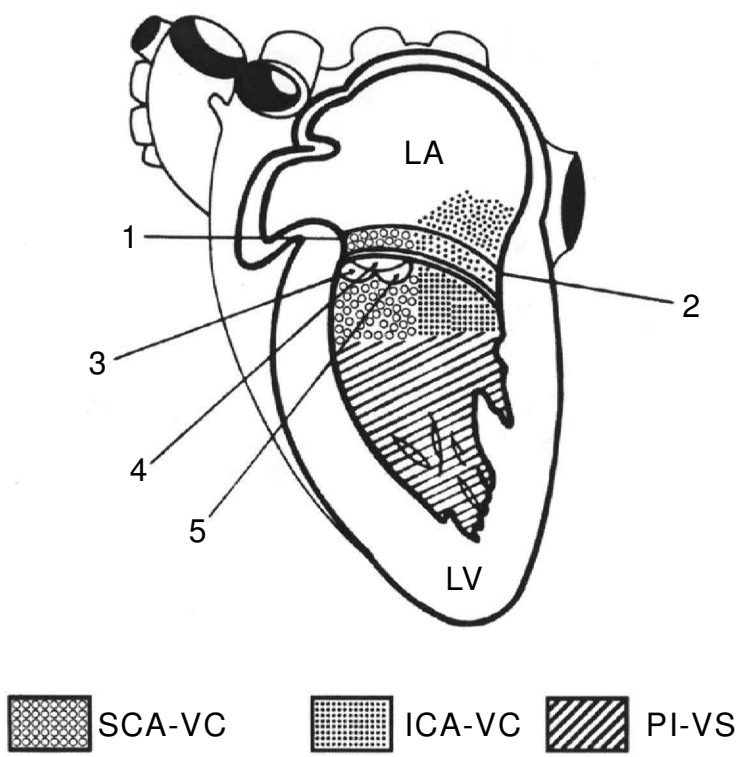

Figure 3.
Diagram summarizing in vivo labelling studies in chick embryos to reveal the fate of the major cushions of the atrioventricular (AV) canal. LV (left ventricle), LA (left atrium). SCA-VC (superior cushion of the atrioventricular canal), ICA-VC (inferior cushion of the atrioventricular canal) and PI-VS (muscular portion of primitive interventricular septum). The superior cushion contributes to the area of mitroaortic continuity, a structure which Maria Victoria considered central to understanding how segments were aligned and integrated to form the mature heart. 1 = that part of the mitroaortic continuity which contributes to the anteroseptal leaflet of the mitral valve. 2= that portion of the anteroseptal leaflet of the mitral valve that inserts onto the muscular part of the septum. 3, 4 and 5 = the outlet of the left ventricle, specifically, the right, left and non-coronary aortic leaflets, respectively. By this diagram, she also intended to illustrate how the superior cushion, through the area of mitroaortic continuity, could serve both as part of the inlet and outlet for the left ventricle. She also wished to demonstrate how both atrioventricular cushions formed the membranous portion of the primitive interventricular septum, albeit that this stucture is not represented in the chick heart. For the outlet of the right ventricle (not shown in the diagram), she conducted many dissections and labelling studies which suggested to 3 origins: the free anterior wall of the right ventricle, the dorsal dextral cushion (the future supraventricular crest) and the sinistral ventral cushion of the proximal region of the outlet segment. Here, she challenged tradition by stating that she could find no evidence that the septal wall of the right ventricle contributed anything to the outlet.

https://doi.org/10.1017/S1047951101000361 Published online by Cambridge University Press

particular defect. For example, knowing that the anatomical ventricles are established by the interaction of three embryonic segments makes it easy to grasp why the mature ventricle can have developmental pathologies related specifically to either its inlet, apical trabecular, or outlet regions. She believed that her findings produced the context for using the concept of sequential segmental analysis[17] to identify a specific anomaly.[16] This approach is widely considered one of the most important tools available to the pediatric cardiologist for identifying complex cardiac dysmorphology. At a major meeting devoted to the atrioventricular junctions, and organized by Maria Victoria in Mexico City in 1992, Robert Anderson, one of the originators of sequential segmental analysis,[17] paid tribute to Maria Victoria as a pioneer whose work helped to formulate and substantiate the segmental method of analysis.[18]

Another principal contribution in this area was her studies on double outlet right ventricle.[19] Interestingly, if a cardiac anomaly happens to occur from a particular gene knockout in the mouse, it is frequently double outlet from the right ventricle.[2] The gene in question is usually expressed normally in the proximal part of the ventricular outlet segment, either by resident cells or cells migrating in from the neural crest. The resulting phenotype from knocking out the particular gene can be presumed to have affected the alignment or integration of the proximal outflow tracts with the primary muscular ventricular septum. Here again, before the availability of molecular tools, the pioneering in vivo studies of Maria Victoria in living embryos pinpointed the candidate structure for formation of double outlet right ventricle. Using dynamic experimental procedures, she showed that an irreversible error in the integration of the proximal outlet into the supporting morphologically right ventricle resulted in double outlet and, moreover, that the proximal part of the outlet segment was the only component in which perturbed development would cause this defect.

Double outlet right ventricle, however, was but one anomaly studied by Maria Victoria. Beginning in 1953, she and her students and colleagues studied the anatomy of most of the congenital heart defects. In all of these studies, she sought, through careful description and experimental genetic and environmental manipulation, to integrate anatomical findings with evolving developmental concepts. As with her work on double outlet, her insights have provided a wealth of information which will continue to serve as a legacy to guide us in our search for genetic and molecular understanding for years to come.

Epilogue

I had the privilege of working with Maria Victoria for almost 10 years. I considered her mentor, and my "mother in science". On the many occasions we would greet each other at airports in Mexico City or Charleston, she would first ask about family and friends but soon, usually after a minute or two, she would then say "now Markwald, about the conus…" And, for days thereafter, we would work long and intensely on a project. But always, with a little prompting, she would take time to share some of the experiences of her century in science, for she was a bridge to the past and an inspiration at the beginning of a new millennium. As stated so eloquently by Paolo Angelini in her obituary,[20] "Like others who knew and admired Dr. de la Cruz, I can testify to her intensely focused personality and disciplined intellect. Throughout her career, she had a great respect for the scientific method and structure and a remarkable ability to formulate comprehensive, unifying hypotheses. She will be remembered not only for these qualities but also for her wit, humor, generosity, diplomatic skill and tenacious faith in humankind."

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