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Unique Pathologic Findings in the Heart of a Red-Eared Turtle (Trachemys Scripta Elegans)

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SUMMARY

The necropsy of a turtle (Trachemys scripta elegans), which was euthanized after rapid loss of vitality, revealed multiple unique abnormalities in the heart.

We found areas of atrophy in the wall of the ventricle. These may be connected with aneurysmata at the same locations.

A prominent hypertrophic, disc-like cartilago cordis was present. It may have disturbed the flow of blood leading to local atrophies. Gradually, the excessive size may have impeded the flow of blood by compression of large blood vessels at the heart base resulting in ischemia and acute cell death in the myocardium on one side of the ventricle. In one of the larger blood vessels there was an ulcus covered with a thrombus. Thrombi were found on various places and filled the larger part of the ventricular chamber.

It can be assumed that these abnormalities were linked and fatal in the end.

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Introduction

We believe the paucity of reports on cardiac pathology in testudines reflects the unspecific nature of clinical symptoms in cardiovascular disease [1]. Postmortem examinations of over 4000 reptiles revealed pathologic changes in the cardiovascular system in only ± 2% of cases and most of those changes were in the pericardium [2].

The present case involved various parts of the cardiovascular system.

This paper describes rare and unknown pathologies in the heart of a red-eared turtle (Trachemys scripta elegans). Pathologies of the cartilago cordis have not been described in turtles. Hypertrophy of the cartilago cordis is a unique event.

A different pathology in this turtle is acute cell death in a larger part of the ventricle. To the best of our knowledge this is an undescribed event in chelonians.

An ulcus in one of the larger vessels near the heart was covered with a thrombus. This may have initiated the production of thrombi in the ventricle and elsewhere in the heart.

In man, free-floating venous thrombi in the heart are well known as stasis or coagulation thrombi but fixed ventricular thrombi are considered rare [3]. In contrast, only fixed thrombi have been reported in turtles [4].

Case History

Material and Methods

The patient, a female red-eared slider turtle (Trachemys scripta elegans), had been housed in a full glass terrarium at a thermostat-controlled water temperature of ± 26 °C. Water depth was 40 cm. A plateau was available for basking. At daytime focal heat was provided with a 60 W tungsten bulb suspended 40 cm above the plateau. The animal was 12 years old, weighed 276 grams at a carapace length of 117 mm. Feeding was with Mazuri Freshwater Turtle Diet ® which was readily accepted.

Several superficial lesions were present on the skin, the carapace and the plastron. A topical treatment with Terramycin ointment was started.

Fourteen days after the first presentation, the clinical situation suddenly deteriorated dramatically. Within a few hours the animal was lethargic. It was euthanized by administering 4 ml of T61 (Intervet Canada Corp.) intracoelomically.

At postmortem, samples of organs were fixed in 4% neutral-buffered formaldehyde. For histology, tissues were embedded in

paraffin and sectioned at 4 µm. Tissue sections were mounted on glass slides and stained with hematoxylin and eosin, Alcian blue and by the Lawson-Van Gieson method.

Post Mortem

Gross Findings

The specimen was in fair condition, showing well-developed musculature and sparse abdominal fat.

The heart revealed remarkable changes. The epicardium on the left side of the ventricle showed a whitish area but otherwise it appeared to be normal. The surface was smooth and subepicardial blood vessels were present over the white area (Figure 1).

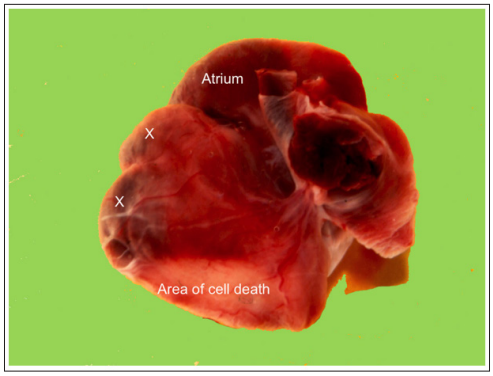

Figure 1: Heart Showing a Whitish Area of Acute Cell Death on the Epicardium, Two of the Aneurysmata (X) and One of the Atria

The heart (16 mm in length) was cut longitudinally, over its maximal width, including the center of the whitish area. It appeared that this area corresponded with a sharply delineated band of homogenously altered heart muscle. It extended from just below the left atrium to the apex of the ventricle with a maximal width of 4 - 5 mm. It comprised both the thin compact outer layer and the spongiform center. The changed area was paper-white, free of blood and the tissue looked necrotic. There were no indications of an inflammatory reaction.

On the right side of what appeared as the normal remains of the ventricular muscle, three sharply delineated, bulging aneurysms measuring 2 - 3 mm in diameter, were present (Figure 2). All three were covered with a normal, smooth epicardium. The cut surfaces of the three aneurysms were identical. They contained w few muscle bundles and dark blood, present as flaccid clots. This contrasted with the normal colored, well-contracted remainder of the adjoining intact spongiform ventricular muscle.

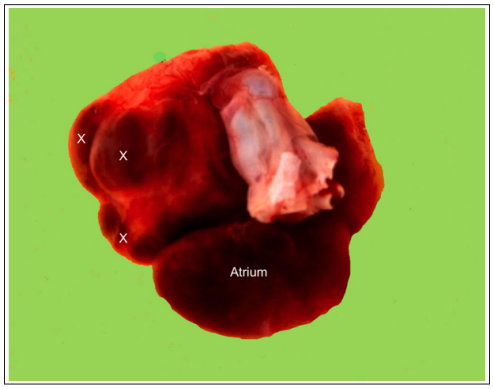

Figure 2: Heart Showing the Three Aneurysmata (X7) and One Atrium

A second cut was made transversally between the atria and the ventricle, over the atrioventricular fibrous plane. Adjacent to the area of white discoloration was a large, irregularly delineated field of glassy, pearly bluish tissue interpreted as cartilage.

Microscopy

Atrophy of the Myocardium

Atrophy was present in three circumscribed areas of the spongiform part of the ventricle. Each atrophic area was surrounded by normal myocardium (Figure 4). The remaining muscle bundles in an atrophic area, were randomly distributed and widely spaced. They were short, slender and, revealed a normal cross striation.

Larger blood-filled spaces between these muscle fibers contained isolated thrombi. Connective tissue was absent. Two of the atrophic areas were partially contiguous. Originating at the epicardium, a wedge-shaped area of the original myocardial tissue had remained between them (Figure 4).

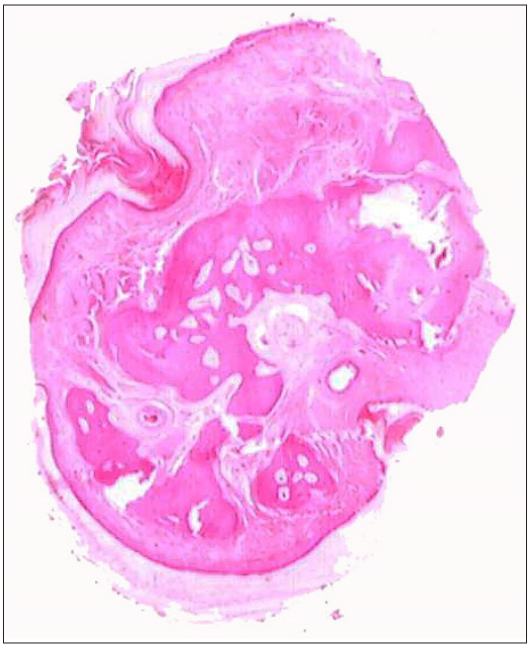

Figure 3: The Proliferated Cartilago Cordis. A Very Irregular Field Of Cartilage in the Trigone Between Atria and Ventricle. Stain HE, Slide Scanned.

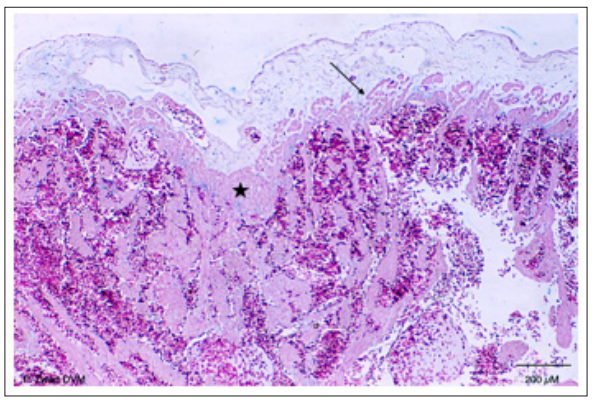

Figure 4: The 2 atrophic areas situated on both sides of the wedge of normal myocardium(*). Over the atrophic areas, the compact layer also shows some atrophy (arrow). HE Obj. 4x.

A remarkable feature was the presence of rather small, but distinct muscle projections, originating from the compact layer, extending in the hollow atrophic areas. These projections were slightly variable in shape and dimension. Some were cone-shaped with either sharp or blunt endings; others were longitudinal with parallel margins. Only a few projections contacted the muscles in the atrophic area.

Ventricular Aneurysms

Three well-delineated epicardial dilatations were present as gently curving domes. The epicardium over the domes appeared normal. These corresponded exactly with the atrophic areas. (Figure 7) As mentioned above there were no connective tissue fibers observed in or around the atrophic areas.

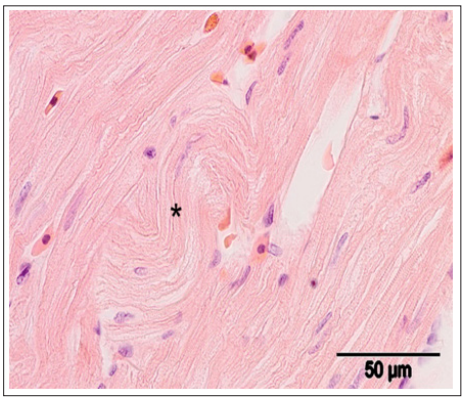

Figure 5: Undulation of myofibrils in the myocardiocytes (*). HE Obj. 25x.

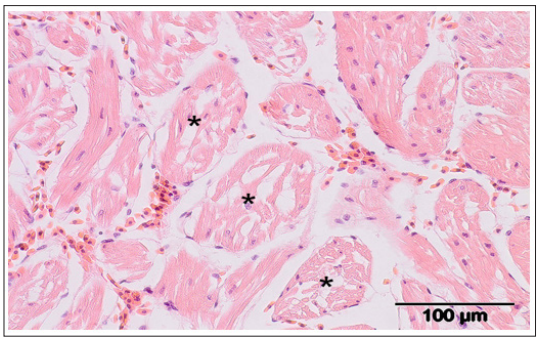

Figure 6: Longitudinal separation of myofibrils in a muscle-fibre (*). HE Obj. 25x.

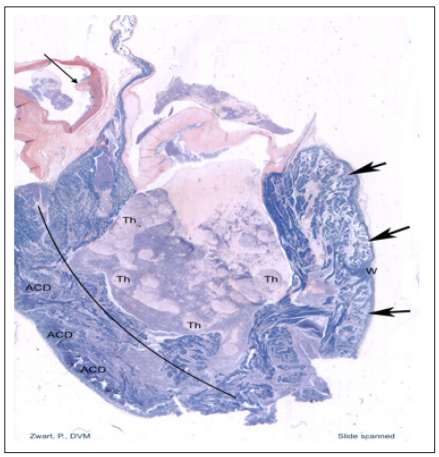

Figure 7: Three bulging ventricular aneurysms (Arrows). Indicated are also the area of acute cell death (ACD) approximately indicated by a line and the parietal thrombus (Slender arrow). PTHA. Slide scanned.

The Cartilago Cordis

In view of the location, the large, irregular field of cartilage in the cut over the atrioventricular plane, was diagnosed as the Cartilago Cordis (CC). This CC was surrounded with a broad zone of connective tissue.

The outer boundary of the process in part was in contact with the epicardium and the adventitia of the larger artery (mentioned above), and for the remainder to the ventricular muscle.

The CC consisted of a large disc-like central area of cartilage. It was surrounded by a corona of irregular protrusions. The central area of cartilage was interspersed with bands of loosely structured connective tissue which were continuous with and identical to the surrounding broad zone of connective tissue. The surrounding connective tissue also contained some isolated, free islets of cartilage (Figure 3). A well-delineated perichondrium was not recognized. There were no indications of neoplasia.

The CC was a typical alcianophilic, hyaline cartilage with a somewhat cloudy inter-territorial matrix. There were no signs of calcification. Chondrocytes were haphazardly distributed. They appeared mainly as individual cells, enclosed in their respective lacuna. Some were seen as a set of two cells separated by a thin septum of matrix.

Though a perichondrium could not be identified, slender chondroprogenitor cells were spread along the perimeter.

At the basis of larger blood vessels and near the heart valves a number of isolated pieces of cartilage were present. These were elongate; in general about 3-4 times longer than wide; bordered by a smooth surface: free of chondroprogenitor cells; lightly vascularized and covered with a thin perichondrium.

Ulcus in a Bloodvessel

In one of the large arterial vessels, close to some valves, a lesion was present. At its basis, the arterial intima was lost. The media was largely lost only a few elastic fibres had remained. In part, these fibres stood up, producing a flat crater. The top was covered with fibrin identical with the formations in the ventricle. Cellular inflammatory exudate failed. This was identified as an ulceration covered with a thrombus (Figure 8).

Acute Cell Death in the Myocardium

The whitish area was present on the left side of the heart. It comprised both compact and spongy heart tissues which appeared to have undergone acute cell death. It had a thin, microscopically recognizable hyperemic border without a cellular demarcation. It contained minimal, if any, blood in the trabeculi. At the side of the epicardium, muscle fibres were separated from each other. The spaces between the muscular trabeculi contained a loose structured network of wrinkled connective tissue fibres. Locally, a delicate network of fibrin fibres and an isolated rounded thrombus were present. In the trabeculi, muscle fibres showed normal cross-striation. Contraction band necrosis, a sign of myocardial infarction, was rarely seen. Karyolysis was irregular and karyomegaly was absent. An undulation of myofibrils in the myocardiocytes was occasionally found (Figure 5). A more pronounced alteration was the longitudinal separation of myofibrils in muscle-fibres. In this area, there was a paucity in endothelial cells covering the muscle bundles (Figure 6). Connective tissue was not recognized.

Most coronary vessels traversing the area of acute cell death were intact, and contained erythrocytes. However, one contained a free-floating blood clot.

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In one of the large arterial vessels, close to some valves, a lesion was observed. At its basis, the arterial intima was lost. From the media, only a few, elastic fibres had remained. In part, these fibres stood up, producing a flat crater. Minimal invasion of inflammatory cells was observed. The top was covered with fibrin which was identical with the formations in the ventricle. Inflammatory exudate failed. This was considered to be an ulceration covered with a thrombus (Figure 8).

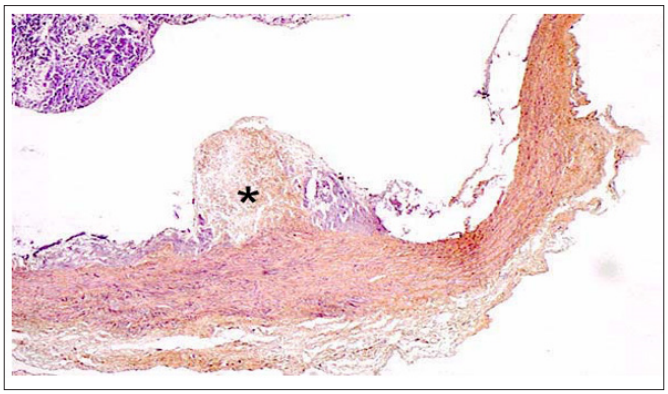

Figure 8: Arterial lesion covered by a thrombus (*). PTHA Obj. 2.5x, Photo M. Tersteeg

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Ventricular Thrombi

Most of the rather wide ventricular chamber was filled with circumscribed rounded or more elongate thrombi. They impinged upon each other, were similar in diameter and composed of well-preserved erythrocytes mixed with a meshwork of fibrin fibres. The number of fibrin fibres was judged to be low. Remnants of thrombocytes or white blood cells were not observed. Identical thrombi were found in the spongy areas of the heart as well as in a coronary vessel.

No thrombi were found in the liver or the lungs.

Other Organs and Tissues

Hepatic parenchyma revealed some pale staining around veins. In the pancreas, a small abscess surrounded by an early, loose structured capsule of connective tissue was present. The carapace and skin showed unspecific ulcerations. The kidneys, spleen, trachea, esophagus, stomach, intestine, ovary and oviduct were histologically normal.

Discussion

Various pathologic changes were localized in the cardiovascular system. They were judged to be unique.

Regional atrophy of the myocardium is a salient finding in this animal. It differs completely from atrophy in man. In man, atrophy affects the ventricle wall as a whole [5]. In man, extended periods of extreme starvation, bed rest, microgravity and cancer are found to be related to cardiac atrophy [6, 7]. In reptiles, especially in snakes, local atrophy may occur at unpredicted places without a sign of reaction or inflammation. A multitude of pathways including disruption of the balance of protein metabolism may be causative [7]. We detected three isolated affected regions. Contiguous areas of atrophy at various locations are known in the heart of reptiles [8]. In the present case, three isolated foci of atrophy are described.

In view of the subtle regulation of intracardiac circulation in reptiles, minor regional disturbance may cause an imbalance in metabolism. In our opinion, the proliferation of the CC may have influenced the stream of blood in the heart, leading to a local disruption of the balanced metabolism.

Ventricular Aneurysm

Ventricular aneurysm is defined as a regional bulging of the myocardium. This generally is the result of local impairment of the ventricular wall [9]. In our case the bulgings correlate exactly to the thinning of the ventricular wall at the sites of atrophy of the spongiosa and the compact ventricular wall.

Myocardial aneurysms in man are known to occur following infarction. Thinning of the ventricular wall combined with scar tissue results in regional weakness of the ventricular wall [10]. The development of this pathology results in man at systolic pressures of ± 140 mm Hg. The arterial blood pressure in normoxic red-eared turtles at 22 °C has been recorded as 17 ± 0,9 mm Hg [11]. In our case, there were no indications of local increase of connective tissue (which eventually could have reinforced the affected area).

As there was no production of connective tissue at these sites, the remnants of the ventricular wall under the atrophic site were fully exposed to the (low) forces of blood pressure. To the best of our knowledge, ventricular aneurysms have not been described in reptiles. We did not find reports where atrophy of myocardium

has been recorded as a cause of ventricular aneurysm. We propose this possibility.

Hypertrophy of the Cartilago Cordis

The hypertrophy of the CC is of particular interest. The Anlage of the CC develops from a small mesenchymal condensation of pleuripotent stem cells. It is located between the atria and the ventricle in the atrioventricular horizontal septum. After hatching, it converts into hyaline cartilage [12]. The CC has been described in 2 chelonian species (Mauremys japonica and M. reevesii) as an isolated, homogenous structure provided with a perichondrium [13-16]. In these species, further pieces of cartilage were located at other sites in the heart [16]. This is in agreement with the observations made in the turtle under study.

Most probably, the proliferation of the CC was three dimensional. It will have resulted in a massive piece of tissue leading to a compression of adjoining blood vessels a reduced supply of blood and some regional degree of ischemia in the heart. This may also have led to the broad left-sided area of necrosis in our turtle. In man, focal ischemia is a known cause of myocardial necrosis [17].

In the turtle, a possible additional aspect is the blood supply of the heart. It differs markedly from the supply in a four-chambered heart of mammals and birds. In general, acute myocardial infarction can occur from increased myocardial oxygen demand and / or reduced supply [18]. India ink perfusion preparations have demonstrated that only about 10% of the ventricular myocardium, namely the compact outer layer and only a few of the thicker muscle bundles in the spongiform area, are perfused by a short coronary artery. The major part of the myocardium (the spongiform part) receives oxygen by diffusion from blood in the ventriculum [19]. A somewhat comparable anatomic situation may be found in the anthropoids: it is in the placenta where maternal blood bathes the villous trees and percolates between them [20, 21]. These villi have no true end-artery. Any reduction in the blood flow can cause placental infarction in man [22].

The microscopic characteristics of the whitish area of the ventricular wall were analogous to acute anemic infarction in man [23]. This was supported by the minimal changes recognized in the myofibrils, the narrow hyperemic zone delineating the area and the absence of a cellular demarcation. The absence of connective tissue supports the acute development of the process.

Anemic infarction in man is ascribed to the blocking of a coronary artery. It is fascinating, though inexplicable: acute cell death in our patient affected both the compact as well as in the spongy area of the heart. Some authors describe an adaptation of the heart muscle of reptiles to moderate to severe hypoxia [24]. However, this physiological adaptation occurs at lower temperatures. Our patient was kept at the preferred optimal temperature of ± 26 °C.

The occurrence of numerous thrombi in the heart of the red-eared turtle is quite unique. A thrombus is a blood coagulum formed in the blood vessel of a living creature [25]. Virchow formulated three factors in thrombus formation intravascular vessel wall damage, stasis of flow, and the presence of a hypercoagulable state [26]. In addition, blood coagulation in vivo is a spatially nonuniform, multistage process.

In the turtle under review, part of these factors can be recognized. We found an ulcus covered with a similar thrombus and extensive damage of endothelium in the necrotic part of the heart. The nonuniform aspect is illustrated by the irregular localization of the thrombi.

In man, (left) ventricular thrombosis is one of the most feared complications in myocardial infarction. In our case, possible causes of the intraventricular thrombi are the underlying multiple cardiac pathologies. Virchow has recognized three factors as prerequisites for the formation of a thrombus. These are: 1) blood stasis, 2) endothelial injury and 3) hypercoagulability [27].

It may be assumed that the acute cell death of a considerable part of the left ventricular wall will have resulted in a disturbed circulation or even blood stasis. In chelonians there is a cardiac shunt enabling recirculation of insufficiently oxygenated blood returning from the lungs again into the pulmonary circulation [28]. We suppose that massive thrombus formation in the ventricle, as seen in this case, will have severely interfered with the normal functioning of the cardiac shunt.

It can readily be accepted that the injured endothelial surface in the spongiform structure of the ventricle is much larger than the surface of the endothelium covering the ventricle wall in mammals and birds. Thus, degeneration of muscle fibres in the spongiform part and the related dysfunction of the endothelial covering will have distinct effect on the elements of the blood. In man it is known that patients with an acute coronary syndrome develop a hypercoagulable state, resulting in thrombus formation [27].

In man, free-floating venous thrombi are well known (stasis or coagulation thrombi) but fixed ventricular thrombi are considered rare [3]. In contrast, only fixed thrombi have been reported in turtles [4]. We observed a parietal coagulum/thrombus in a large arterial vessel close to the valves. The origin of this damage remains unknown, and indications of an infectious cause were not found.

Thrombosis

The endothelial lesion and the accompanying formation of a superposed thrombus, in combination with the proliferation of the cartilago cordis may explain the ischemic infarction of a larger part of the ventricle. The lack of inflammatory reaction underlines this pathogenesis.

Overviewing the various pathologies found, a pathogenesis could be suggested. Most probably the oldest change is the proliferation of the CC with the larger masses of cartilage and connective tissue produced.

The proliferation of the CC, the focal atrophy of the heart muscle and the presence of aneurysmata in itself may have led to a dysfunction, possibly resulting in hypoxia and acute cell death in a larger area of the heart including both the compact and the spongy tissue.

Acute cell death in muscle bundles will also have affected the endocardial covering, leading to the formation of thrombi.

This patient was rich with challenging problems in pathologies and fluid mechanics involving three-dimensional, pulsatile flows [29-32].

Acknowledgements

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Competing interests
The authors declare that they have no competing interests.

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