Comparative Studies on the Cardiovascular System in the Wistar Rat (Rattus norvegicus) and Agama Lizard (Agama agama)

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ABSTRACT: This paper is a comparative investigation on the cardiovascular system in the Wistar rat (Rattus norvegicus) and Agama lizard (Agama agama). The structural development of the heart differs from the two chambered heart of the fish, to the three chambered heart of the lizard and four chambered heart of the rat. This difference in structure also affects their mode of blood circulation. The purpose of this study was to determine if the anatomical structure of the heart of mammal (Wistar rat) and reptile (Agama lizard) differs and affects their mode of blood circulation and muscular activities. Five adult Wistar rats and Five Agama lizards were used for this Study. Result showed differences in the anatomical structure of the heart of these vertebrates explains why the lizards interrupt their movement when compared to the rat.

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Two classes of vertebrate were compared on the basis of their circulatory systems: the mammal (Wistar rat) and the reptile (Agama agama). The circulatory system consists of the cardiovascular and the lymphatic systems. The cardiovascular system comprises: the heart which is a muscular pump; a series of efferent vessels called the arteries, through which blood is pumped and have its bore reduced in size from large bore nearer the heart to small bore farther from the heart; a network of tubes – capillaries -which end in tissues bringing oxygen and removing carbon dioxide and other metabolites; afferent vessels carrying deoxygenated blood and other metabolites from the tissues towards the heart (Mescher, 2010).

Structures of the heart vary among different vertebrates in the animal kingdom. In most vertebrates, the heart lies in the anterior part of the body cavity. It is always surrounded by a pericardium (Romer et al., 1986). The heart (like other blood vessels) is mesodermal in origin and develops from splanchnopleuric mesoderm lying immediately cranial to the prechordal plate. This mesoderm constitutes the cardiogenic area. It is closely related to the pericardial plate, which is derived from part of the intra-embryonic coelom (Singh, 2007). Signals from the anterior (cranial) endoderm induce a heart forming region in overlying splanchnic mesoderm by inducing the transcription factor NKX 2.5. The signal requires secretion of BMPs 2 and 4 secreted by the endoderm and lateral plate mesoderm. The activity of WNT proteins (3a and 8) secreted by the neural tube, must be blocked because they normally inhibit heart development (Saddler, 2010).

The wall of the heart chambers consists of three layers or tunics: the internal endocardium, the middle myocardium and the external epicardium. The endocardium consists of a single layer of squamous endothelial cells on a thin basal lamina layer of loose connective tissues. The myocardium is the thickest of the tunics and consists of cardiac muscle cells arranged in layers that surround the heart chambers in a complex spiral. The myocardium is much thicker at the ventricles than in the atria. The heart is covered externally by simple squamous epithelium (mesothelium) supported by a thin layer of basal lamina. These constitute the epicardium and it corresponds to the visceral layer of the pericardium, the serous membrane in which the heart lies (Mescher, 2010). The mammalian four-chambered heart has double circulation with an interior circuit within the thorax. Blood enters the heart, from which it leaves to the lung to be oxygenated. Oxygenated blood is pumped back to the heart before being released into the body and this process is known as double circulation. The four-chambered heart pumps blood...
with much more pressure and vigor than single chambered heart. Though the four-chambered heart has two atria-ventricular pairs, both pairs do not exert the same pressure (Simon et al., 1996). The inter-atrial and inter-ventricular septa are complete in the hearts of mammals providing two separate circulatory systems: pulmonary and systemic circulation (Kimball, 1994). Most reptiles have a three-chambered heart consisting of two atria, one variably partitioned ventricle and two aortas that lead to the systemic circulation. The degree of mixing of deoxygenated and oxygenated blood in the three-chambered heart varies depending on the species and physiological state. Under different conditions, deoxygenated blood can be shunted back to the body or oxygenated blood can be shunted back to the lungs (James, 2002). In reptiles, the inter-ventricular septum of the heart is incomplete and the pulmonary artery is equipped with a sphincter muscle. This allows a second possible route of blood flow — instead of blood flowing through the pulmonary artery to the lungs, the sphincter may be contracted to divert this blood flow through the incomplete inter-ventricular septum into the left ventricle and out through the aorta. This means the blood flows from the capillaries to the heart and back to the capillaries instead of to the lungs. This process is useful to ectodermic (cold blooded) animals, for regulation of their body temperature (Bailey, 1990). Reptiles lack the capacity to run and breathe simultaneously but can run fast for a short time. This is so because they first use up the oxygen stored in their lungs and blood, then switch to anaerobic glycolysis. It has been shown experimentally that the stops made during running are for breathing as lizards cannot breathe as they run (Cowen, 1987). Taking this into view, it was considered necessary to investigate the heart of these vertebrates and explain why the lizard has to interrupt its movement in relation to that of the rat to gain distance.

RESULTS AND DISCUSSION
The study showed differences in structure and histological appearance of the heart of the Wistar rat and Agama lizard. The heart of the rat appeared pinkish, bigger and more conical in shape (figure 1) when compared to that of the lizard which appeared dark red and conical in shape (figure 3). The physical examination of the heart of the Wistar rat revealed that it has a structure typical of mammalian heart.

Five adult Agama lizards (Agama agama) were obtained from the surroundings of the University of Benin 5 days before the sacrifice and were kept in cages in the animal house of the Department of Anatomy to acclimatise. They were fed with leaves, insects and water. The animals were accessed and certified to be in a healthy condition before they were used for the experiment.

METHOD OF SACRIFICE AND TISSUE COLLECTION: The experimental animals were anaesthetised in an air tight plastic bucket containing cotton wool soaked in chloroform. The unconscious animals were dissected and the hearts perfused with formal saline, which were later harvested and placed in specimen bottles containing the formal saline for long term fixation.

Tissue preparation for light microscopy: The heart of each experimental animal was prepared for microscopy: Starting from the 8th day of fixation, the heart tissues were dehydrated in ascending grades of alcohol using ethanol (70%, 90% and two successive changes of absolute alcohol). They were cleared in two changes of xylene followed by infiltration in the oven at 60°C. The tissues were embedded in wax and then blocked out using an embedding mould. Sections of the tissues were obtained using a rotary microtome cutting at 5microns thick and sections were mounted on clean glass slides for staining with haematoxylin and eosin stains. The sections were treated for microscopy.

It has been shown experimentally that reptiles cannot run and breathe at the same time. They can run fast for a short distance and stop. It was also showed that the stops are for breathing (Cowen, 1987). In this research, attempts have been made in view of this discovery to ascertain that the reason for reptiles (e.g. lizard) not being able to run for a long distance without stopping and mammals (e.g. rat) being able to do so is due to the differences in the structures their heart. The lizard has a three chambered heart consisting of two atria and one ventricle, which allows mixing of oxygenated and deoxygenated blood while the rat has a four chambered heart consisting of two atria and two ventricles which help to separate oxygenated blood.

MATERIALS AND METHODS
Materials: This study was carried out with the aid of the following materials: Chloroform, cottonwood, distilled water, air tight bucket, tissue processor, buffered formal saline, hot plate, ethanol, xylene, specimen bottle, measuring glass cylinder, rotary microtome, water bath, oven, disposable gloves, dissecting kit and cardboard, slides and cover slips, binocular microscope, cages and digital camera.

Animals: Five adult male Wistar rats (Rattus norvegicus) were gotten from the animal house of the Department of Anatomy, University of Benin, Benin City, Nigeria. They were fed with water and commercial grower’s mash ad libitum before sacrifice.
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through the pulmonary trunk from deoxygenated blood through the aorta.

Fig 1: Photomicrograph of the longitudinal section of the heart of the rat showing the Lumen (L), Endocardium (E), Myocardium (M), Intercalated discs (I) and Nucleus (N). Stained with H & E (×1000)

Fig 2: Photomicrograph of the smear of the rat’s blood showing the Erythrocyte (E), Leukocyte (L-Lymphocyte) with kidney shaped nucleus and Basophils (B) with bi-lobed nucleus. Stained with H&E (×1000)

Fig 3: Photomicrograph of the longitudinal section of the lizard’s heart showing the Myocardium (M), Intercalated discs (I) and Nucleus (N). Stains with H & E (×1000)

This research supports the work of Foster and Smith, 1997 which stated that the four chambered heart in mammals (e.g. rat) ensures that the tissues of the body are supplied with oxygen saturated blood cells to facilitate sustained muscular activities and that the four chambered heart has a more efficient circulatory systems when compared to that of the reptile (e.g. lizard) which has three chambered heart. The mixing of oxygenated and deoxygenated blood in the three chambered heart does not provide enough saturated oxygen blood to the tissues of the body to facilitate sustained muscular activities. In the course of this research, it was seen that the heart muscle of the rat showed cardiomyocytes that run parallel to each other. Intercalated discs were visible. It showed the presence of spindle shaped and elongated nuclei with dense chromatic material. Cross striation was not visible. The heart muscle of the lizard showed oblong nuclei (figure 3) with less chromatin materials when compared to that of the rat (figure 1). Cross striations were also not visible. Intercalated discs were present. It was demonstrated under the microscope that erythrocytes of mammals lack nuclei while that of the lower species of vertebrates (e.g. fishes, amphibians, reptiles and birds) have a DNA bearing nucleus (Tagliasachi and Carboni, 1997). In the course of this research study, it was seen that the erythrocytes of the rat lacked nuclei (figure 2) while that of the lizard were nucleated (figure 4) which agrees with the report of Tagliasachi and Carboni, 1997.

Conclusion: Reptiles have a smaller body when compared to mammals. It is thus possible that the structure of the heart and blood cells of animals conform to its energy requirement. The heart of the lizard is thus structured enabling mixing of oxygenated and deoxygenated blood resulting in the
presence of nucleated erythrocytes unlike the a-
nucleated erythrocytes of mammals. This buttresses the
advancement of mammals over reptiles in evolutionary trend.

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