PRACTICALS FOR CARDIOVASCULAR PHYSIOLOGY*

Practical 1:

1.1. The automatism of the heart. Stannius ligatures on the frog heart.

Objectives:

1. Define and discuss the **automaticity** and **rhythmicity** as properties of the cardiac muscle.
2. Describe the frog heart; discuss the **Stannius ligatures** on the frog heart and the hierarchy of automatism centers.

Automaticity is the intrinsic property of the heart muscle to depolarize spontaneously, in the absence of an external stimulus**.** The cycles of depolarization/repolarization are repeated regular, a property known as **rhythmicity.**

In the human heart, the sinoatrial (SA) node has the highest automaticity (72/min.), followed by the other structures of the excito-conductive system: the atrio-ventricular (AV) node (40/min), bundle of His and its branches and the Purkinje system (25-30/min). Normally, the highest rate of SA node paces the excitatory drive in the whole heart, consecutively suppressing the spontaneous depolarization of other cardiac structures.

In this practical, you will observe these properties of the cardiac muscle in a computer simulation.

The frog heart consists in three chambers: two atriums and one ventricle (Fig. 1.a). The amphibian heart receives the blood from the systemic circulation through the cava veins via the *sinus venosus*, a thin walled sac empting into the right atrium and contracting at the same intrinsic rhythm as the heart chambers. Also, part of the blood is received in the left atrium directly from the lungs. The ventricle pumps blood out through a large artery (truncus arteriosus).

Preparation of the experiments on the frog heart:

The spinal cord of an unconscious frog is destroyed by inserting a needle through the foramen magnum, a procedure called *pithing*. The frog is pinned on a dissecting pad and the thorax is opened to allow the observation of the beating heart. After dissecting the pericardium, the heart is maintained moist by adding Ringer solution at room temperature from time to time during the investigation. A 'heart clip' can be attached to the apex of the ventricle, for better manipulating the heart or to directly record the movements of the heart as it pumps, by means of a recording pen in contact with a kymograph. When needed, the vagus nerve is also dissected, as descends close to the carotid artery.

Stannius ligatures are experimentally used to demonstrate the presence of automatism centers and their hierarchy in the frog heart (Fig. 1.b). Hermann F. Stannius was the first physiologist who performed this experiment.

Finding the location from where the heartbeat originates was a challenge for the cardiac physiology late in the nineteenth-century. Known at that time was what William Harvey observed in 1651, that "the pulse has its origin in the blood . . . the cardiac auricle from which the pulsation starts, is excited by the blood" (Fye WB. The origin of the heartbeat: a tale of frogs, jellyfish and turtles. Circulation 1987;76:493–500.). In 1839 Robert Remak discovered the presence of groups of ganglion cells in the sinus venosus of the frog (Gaskell WH. The contraction of cardiac muscle.

In: Schafer EA, editor. Textbook of Physiology. Edinburgh and London: Young J. Pentland, 1900;2:169–227.), that were further supposed to be the one that initiate and perpetuate the heartbeat in response to sympathetic stimulation (Acierno LJ. The History of Cardiology. London: The Parthenon Publishing Group, 1994:248.). In 1852 Hermann F. Stannius tied a ligature around the sinoatrial junction of a frog's heart, causing standstill of the atria and ventricle while the sinus portion still contracted (H. F. Stannius: Two series of physiological experiments; 1. experiments on the frog heart; 2. experiments with prussic acid. Müller's Archiv für Anatomie, Physiologie und wissenschaftliche Medizin, Berlin, 1852: 85-100). Three decades later Walter Gaskell investigated the nature of the heartbeat and the sequence of contraction showing that the impulse travels from the sinus venosus, the dominant generator of the heartbeat with the highest cardiac rhythmicity, to the atrium and then to the ventricle. Gaskell was the one to conclude that the heart muscle itself possessed rhythmicity independent of the ganglia, at different paces (Walter Gaskell and the understanding of atrioventricular conduction and block. Mark E. Silverman, and Charles B. Upshaw, Jr, *J. Am. Coll. Cardiol.* 2002;39;1574-1580).

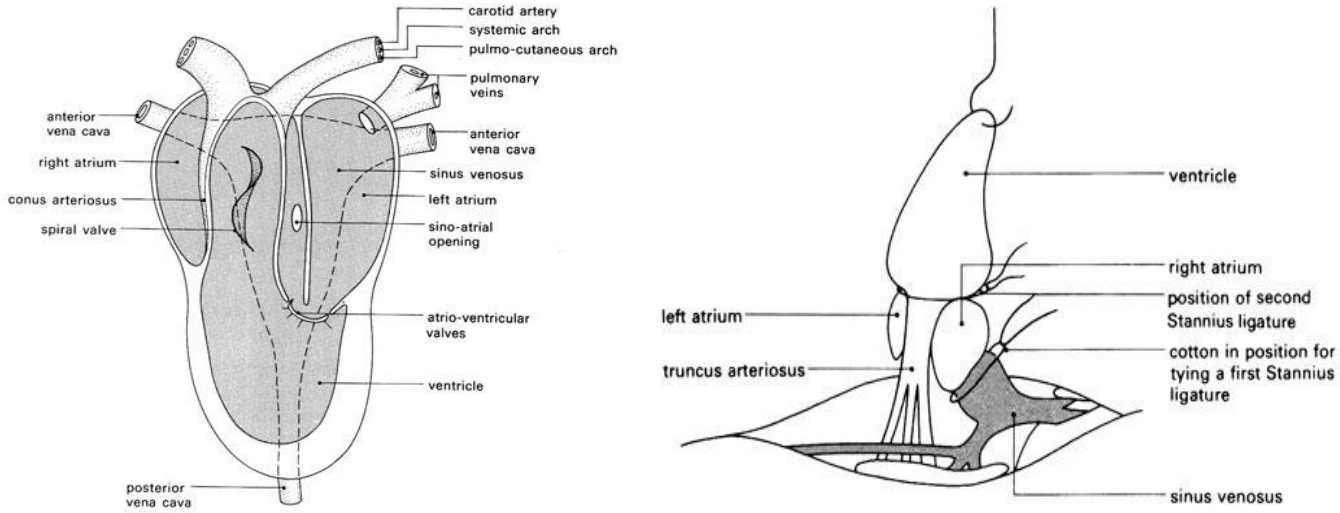

Fig. 1. a) the frog heart (left); b) Stannius ligatures on the frog heart (right).

First Stannius ligature: A piece of cotton thread is tied firmly around the heart at the junction of the sinus venosus and the right atrium, to isolate the sinus from the other chambers of the heart (Fig. 1.a). The ligature physically disrupts the conducting bundles, and both atria and ventricle stop contracting.

Second Stannius ligature: Keeping the first ligature on, a second one is placed and tied around the atrioventricular junction. After a while, the ventricle begins to beat again, but at a slower rate than the sinus venosus (idioventricular rhythm), whilst atriums remain still. If the second ligature is applied immediately below the atrioventricular junction, the ventricle is not contracting.

Taking off the first ligature and keeping the second one will determine the beating of atriums at the same rate as sinus venosus, while the ventricle will continue to contract at its own slower rate.

Conclusions: 1) the pacemaker of the frog heart (Remak ganglion) is located in the sinus venosus; 2) the cardiac impulse is conducted from sinus venosus to atriums and then to ventricle; 3) when atriums are disconnected from the sinus venosus, they are not contracting; 4) the ventricle possesses its own automaticity center located at the lower limit of the atrioventricular junction; 5) atriums exhibit an inhibitory influence on the ventricular center.

1.2. Action potential in the cardiac fibers. The law of periodic inexcitability of the heart.

Objectives:

1. **Excitability** and **resting potential**
2. The **action potential** in the myocardium
3. The **law of periodic inexcitability** of the heart - computer simulation (PhysioEx 8.0)

Excitability and resting potential

Define the resting potential and the mechanisms that maintain it.

The action potential in the myocardium

Define the threshold value and initiation of the action potential.

a. Slow response fibers
b. Fast response fibers

Define the refractory period: the absolute and relative refractory period.

Law of periodic inexcitability of the heart

This law states that while depolarized, the heart doesn't react to any other stimulus, being in the absolute refractory period. There is a short period of time, at the end of the action potential, when the cardiac cell can respond to additional stimuli, generating a premature depolarization and a premature contraction, called extra-systole or premature beat, which is usually followed by a compensatory pause.

In the lab, the cardiac mechanical activity changes in volume and shape of the heart and its response to different electric stimuli can be recorded on the frog heart as a *direct cardiogram*, using the Marey cardiograph (called direct because the cardiograph is connected directly to the heart and named after the French physiologist who created it). Marey cardiograph is composed of two paddles, one fixed and one mobile connected to a pen; you will place the beating heart between these two paddles, in order to observe the changes in volume and shape of the heart, by means of a kymograph that rotates to record the movements of the mobile paddle. Single or multiple electric stimuli of different frequencies can be applied during systole and diastole (during different phases of the action potential) and the recording is then observed. The heart will not respond during the absolute refractory period. Extra-systoles and the compensatory pauses that follow can be observed when the stimulus is applied during the relative refractory period. The experiment can be simulated on a computer using the PhysioEx 8.0 software.