Electrocardiographic Observations on the Heart of the Guppy (*Lebistes reticulatus*).* (27483)

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In the pursuit of a simple laboratory bioassay procedure for cardiac glycosides, the authors have characterized the electrocardiogram and vectorcardiogram of the common guppy, *Lebistes reticulatus*, and the electrocardiographic changes produced in this animal by digitalis glycosides. Although our bioassay proved unsuitable for clinical use because of its relative insensitivity(1,2), the methods and electrophysiologic observations are considered sufficiently unique to merit reporting and may have application elsewhere.

Investigations have been made on the electrophysiology of hearts of larger fish(3-10). However, we are not aware of any such studies on the hearts of small fish approaching the size of the guppy. Few of the earlier investigators utilized sensitive recording apparatus with high gain, high frequency response, and fast paper speed. No description of the vectorcardiogram of any fish could be found in the literature.

The heart of the guppy is similar to the hearts of the larger teleosts as described by Kisch(8). It has a single ventricle and a single atrium. In the guppies studied the ventricle measured between 1.5 and 2.0 mm in length, varying directly with the size of the animal.

Materials and methods. 33 Female guppies averaging 2.5 cm in length (2.3-4.1) and 0.5 g in weight (0.29-1.70) were selected for the study. The specimens were obtained from local commercial sources and were maintained in apparent good health in a glass 10 gallon aquarium at room temperature (approximately 24.5°C) until the time of the study.

Satisfactory anesthesia was produced by placing the test fish in a small glass dish containing 5.0 ml of 2% urethane (ethyl carbamate) in water. In 15 seconds the fish lost its equilibrium and within 1-2 minutes response to tactile sensation was abolished. The fish was then removed from the urethane solution and secured ventral side superior in a small plastic trough by means of a small metal tail clip (Fig. 1).

The gills of the fish were perfused by hydrostatic pressure with aerated water at room temperature through a #22 needle inserted into the mouth of the animal. A barb on the needle tip served to secure the needle in place as well as to steady the cephalic end of the fish. The water flow was regulated by a stopcock so that the fish continued to respire regularly and easily at a rate which varied from animal to animal, but averaged approximately 300 respirations per minute as observed through a 7× dissecting microscope or recorded by an electrocardiographic lead placed near the gills. Care was taken to avoid overperfusion as evidenced by pallor and edema of the gill membranes, or underperfusion, characterized by 'gasping' motions of the gills or bradycardia. Once the fish was secured, further anesthesia was unnecessary as relatively little muscle movement occurred, even after long periods of observation.

Three German steel insect pins with tip diameters of 50 μ and insulated to within 0.2 mm of their tips served as electrodes. After the superficial scales had been removed with tweezers, the pins were inserted through the precordial skin, by means of Brinkman micromanipulators to a depth of approximately 1.0 mm and held in place by these same instruments. The tips of the electrodes formed a 2 mm equilateral triangle with its base directed cephalad and with the heart in its approximate center. The tail clip and intubation needle were both grounded.

The electrodes were connected to a lead se-

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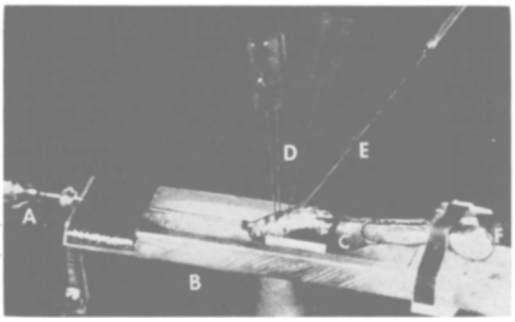


FIG. 1. Photograph of apparatus used to study the electrocardiogram of extra-aquatic guppy. (A) Hub of perfusion needle. (B) Plastic trough. (C) Anesthetized fish in place. (D) Three recording electrocardiograph electrodes attached to micromanipulators above. (E) Glass capillary needle inserted through abdominal wall for administration of test solutions. (F) Metal clip for restraint of fish and grounding. White strip in trough is one cm in length.

lector box coupled to a Grass P5 AC preamplifier. Scalar electrocardiographs were recorded from this amplifier by a Sanborn Visocardiette (Model 51). By connecting the electrodes directly to a Sanborn Twin Beam photographic recorder (Model 62) simultaneous records at 75 mm per second paper speed were recorded. Higher speed (200 mm per second) photographic electrocardiograms and frontal plane vectorcardiograms were obtained by means of a Sanborn Polybeam and Visoscope (Model 550M). The apparatus, exclusive of recorders and amplifiers, was enclosed in a 'Faraday cage.'

Before insertion of the electrodes a small puncture wound was made through the abdominal skin of the fish and the tip of a glass capillary needle was inserted into the resulting orifice. This needle was connected by means of a polyethylene tube to a Micrometric syringeburette capable of delivering accurately a volume of 0.02 ml. Through this system cardiac glycosides or other drugs could be delivered to the peritoneal cavity of the guppy.

Observations were made on the endurance of the guppy in the test apparatus, the control electrocardiogram and vectorcardiogram of the guppy, and the changes induced in these records by various cardiac glycosides.

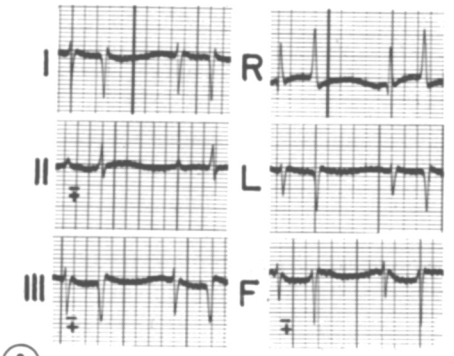
Results. The heart rate averaged 165 beats per minute (S.D. 20) initially and 169 beats per minute (S.D. 11) after 120 minutes in the apparatus. All but 2 of 13 fish tested for endurance in the apparatus survived in good condition for at least 2 hours. One fish survived for 6 hours, when the experiment was terminated. The initial and final heart rates in this fish were both 162 and a maximal rate of 176 occurred in the second hour. There was no relationship found between heart rate and size (weight or length) of individual fish.

Electrocardiogram. Scalar electrocardiograms were obtained on 29 of the 33 guppies. The control electrocardiograms resembled those of larger fish except for a more rapid rate. The initial vectors of the QRS complex were directed predominately rightward in 83% of the fish (24 of 29). Of these 67% tended toward the tail (16 of 24), 21% tended toward the head (5 of 24), and 12% were directed approximately perpendicular to the midline (3 of 24). The terminal forces were directed about equally to the right or left, but tailward in 70% of the fish. Average QRS duration was 0.024 second (0.020 to 0.032) as determined from high speed photographic tracings on 8 fish. By the same method the PR interval was found to average 0.115 second (0.100 to 0.125), (Fig. 2, 3).

The complexes with the largest magnitude were found in lead I since the X forces exceeded the Y forces (AVF) in 85% of the fish. The maximum QRS amplitude ranged from 25 to 830 microvolts and averaged 370 microvolts.

The contour of the P wave varied more than that of the QRS complex and was biphasic with an intrinsic deflection probably due to the proximity of an 'arm' reference electrode to the atrium. For this same reason the P wave varied more markedly than the QRS during vertical movement of the electrodes. Large atrial T waves were occasionally recorded and were identical with those of man. These atrial T waves modified the ventricular ST contours in some instances to such a degree that ST displacement was obscured.

Frontal plane vectorcardiograms were recorded from several fish (Fig. 4). The mean axis of the QRS loop was located in the quadrant between 90 and 180 degrees. Rotation



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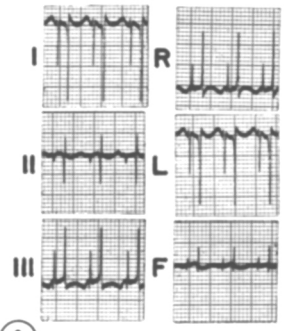

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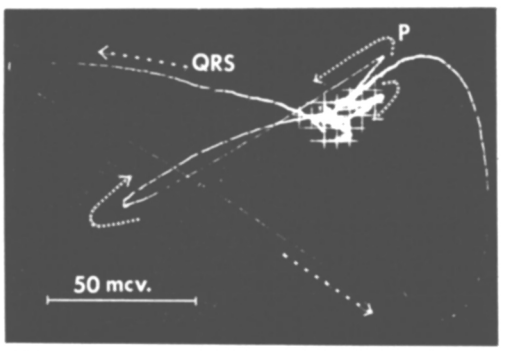

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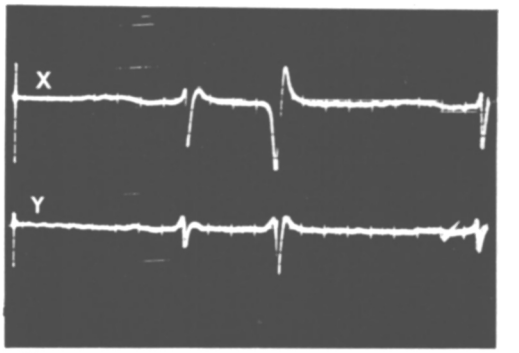

4B

FIG. 2. Electrocardiogram of normal guppy. 75 mm/sec. paper speed. Calibration 1.0 millivolt produced 20 mm deflection.

FIG. 3. Electrocardiogram of normal guppy. 25 mm/sec. paper speed. Calibration 1.0 millivolt produced 20 mm deflection.

FIG. 4. Frontal plane vectorcardiogram of guppy (part A) and X and Y components swept at 100 mm/sec. (part B). The P vector loop shows counterclockwise rotation of initial portion and is directed cephalad and leftward; middle portion is directed caudad and rightward with a clockwise rotation; terminal portion continues in a clockwise rotation cephalad and leftward. QRS loop has a counterclockwise rotation, forming a Qr complex in the X axis scalar lead and an rSr′ complex in the Y axis scalar lead. Amplitude of the T vector loop and T wave is low. T loop is directed leftward.

of the QRS loop in the frontal plane was counterclockwise.

Other observations. Anoxia produced by reducing water flow through the gills caused sinus bradycardia progressing to A-V block, prolongation of QRS duration, standstill, and STT changes similar to those produced by anoxia in larger fish(8) and man.

In 13 experiments on endurance, complex arrhythmias occasionally occurred spontaneously in dying or damaged hearts. These included atrial flutter, A-V dissociation, and premature ventricular beats.

As observed by Kisch(8), pressure of the electrode on the atrium or ventricle produced a monophasic injury potential.

The heart rate of the guppy varied directly with the temperature of the perfusate, a fact consistent with observations on larger fish by von Skramlik(11).

Intra-abdominal injection of 2 μg of digitoxin (29 fish) produced in every case pronounced typical 'digitalis effects' on repolarization (STT), A-V conduction, and on cardiac rhythm.

Significant arrhythmias, second degree A-V block, Wenckebach phenomenon, and/or third degree A-V block were produced in each case within 5 to 23 minutes (average 14.6 minutes). In all instances these effects were preceded by ST displacement and first degree A-V block. The frequency of occurrence of

aberrant intraventricular conduction in the presence of Wenckebach or other A-V nodal conduction defects was striking.

Discussion. The electrocardiogram of large fish has been extensively studied by Kisch(8). He found that voltages from the surface of the fish heart were in the same range as surface voltages of mammalian hearts and the P wave was similar to the mammalian P, having a slow deflection and often being split or bifid. Auricular T waves were observed. Excitation proceeded from the atrium to the ventricle. The P-R interval ranged from 0.2 to 0.6 second in bone fish. The ventricular electrocardiogram consisted of an R, S, and T wave with an occasional Q wave also present. QRS duration was 0.04 to 0.06 second. Rates varied from 20 to 100 beats per minute.

The electrocardiogram of the common guppy which can be easily and reproducibly recorded is similar to that of larger teleost fish, except for a faster rate and shorter intervals. It consists of the same components found in the human electrocardiogram (P, QRS, and T). The rapid heart rate observed in these small fish extends the observations of Kisch(8) and emphasizes the presence of an inverse relationship of heart rate to body weight in the poikilotherms similar to the conditions existing in warm blooded vertebrates. Many of the arrhythmias found to occur in anoxic and dying fish and mammals may occur spontaneously or may be produced in this small animal.

The heart of the guppy responds to the digitalis glycosides in the classical manner. It also shows responses to anoxia, and temperature similar to other fish and mammals.

Summary. 1. A method is described whereby the electrocardiogram of a small fish may be easily and reproducibly recorded after urethane anesthesia. 2. The anatomy of the heart of the common guppy (*Lebistes reticulatus*) was found to be similar to the hearts of larger teleosts. 3. The electrocardiogram and vectorcardiogram of the common guppy were characterized by the use of high gain and high speed recording apparatus. The electrocardiogram was found to consist of the same components present in the electrocardiograms of larger teleosts and mammals (P, QRS, and T). 4. Intraperitoneal injection of digitoxin (2.0 micrograms) produced typical changes in the electrocardiogram consisting of first, second, and third degree A-V block as well as Wenckebach phenomenon and ST displacement within 5 to 23 minutes (average 14.6 minutes).

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