Electrophysiological and anatomical observations on the heart of the African lungfish

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THE AFRICAN LUNGFISH, Protopterus ethiopicus, continues to inhabit the lake regions of central Africa (23). It uses lungs or gills alternatively for breathing, and has accordingly retained transitional features of the heart and circulation which evolution has erased in the higher vertebrates. The heart of the lungfish resembles that of tetrápods in having a discrete sinus venosus, a partially septate atrium and ventricle, and a distinct conus arteriosus (6). Moreover, the bulbus cordis persists in the lungfish as a distinct chamber instead of being incorporated into the right ventricle. Since electrophysiological adaptations must have accompanied the morphologic evolution of the mammalian heart, the lungfish heart provides an unusual opportunity for gaining insight into comparative electrophysiology.

Comparative electrophysiology; atrioventricular conduction; temperature effect; sinus venosus rhythm; ventricular excitation; atrial rhythm; electrical stimulation; sinoatrial conduction; bulbus cordis atriosinal conduction

Previous studies in this laboratory of the heart and circulation of *P. ethiopicus* have focused on hemodynamic and morphologic correlations (9, 16). In the present report, the electrophysiology and histology of the *Protopterus* heart, particularly those aspects dealing with impulse formation and conduction, are considered.

METHODS

Lungfish weighing 3–5 kg were netted in Lake Victoria, Uganda, and flown in special cartons, as air breathers, to Chicago. On arrival in the laboratory, they were returned to freshwater and maintained at ambient temperatures of approximately 25°C. All fish were eating well and appeared to be thriving at the time of our studies. We used three kinds of preparation for the electrophysiological studies: unanesthetized fish with chronically implanted pericardial electrodes, anesthetized fish with exposed hearts, and isolated hearts immersed in Ringer solution.

Unanesthetized lungfish with chronically implanted electrodes. Lungfish that were to be studied in the free-swimming, unanesthetized state were prepared by prior surgical implantation of electrodes. For this purpose, 11 lungfish were anesthetized by immersion in a 1% solution of tricaine methanesulfonate (MS-222) in tap water for 15 min. This procedure provided adequate anesthesia for 2–3 h. As soon as the anesthetic took effect, the lungfish were wrapped in moist towels to prevent desiccation, exposing only enough of the surface to provide access to the heart. Because the thick pericardium acted as an insulator (transpericardial resistance to direct current ranged from 30,000 to 40,000 Ω), cardiac electrical activity could not be recorded from peripheral electrodes. To obtain suitable records, we exposed the pericardium via a midventral incision and attached electrodes to the pericardium facing the pericardial cavity directly. After obtaining satisfactory records, the proximal ends of the leads were channeled subcutaneously and exteriorized on the dorsal surface of the fish. The wound was then closed in layers, and after the lungfish had resumed normal breathing, they were returned to water in their tanks. In two of these fish, polyethylene
catheters were also implanted in a branchial artery for administering atropine (80-200 μg). A thermistor probe was inserted deep in the rectum.

Bipolar records of cardiac electrical activity were taken from the isolated pericardial electrodes after recovery while the lungfish were resting quietly at the bottom of their tanks, as well as when they surfaced for air breathing. In addition, observations were made during stress which was induced either by poking or by placing a plastic cage in the water so that the lungfish could not surface to breathe air.

Anesthetized lungfish with exposed hearts. In 10 anesthetized lungfish, the pericardium was excised via an elliptical ventral flap, and the exposed heart was studied in situ. In these lungfish, the origin and propagation of electrical activity were determined from surface electrograms obtained directly from the heart using unipolar and bipolar platinum electrodes. For unipolar recording, the indifferent electrode was placed in the subcutaneous tissue of the tail. Bipolar tracings were obtained using platinum electrodes (Grass Instrument Co., E2B) that were closely spaced (0.5 mm apart).

Method of recording. Electrodes were connected by grounded shielded wires to ECG amplifiers contained in either a photographic oscilloscopic recording apparatus (Electronics for Medicine; amplifiers EEP-8) or in a direct-writing recording apparatus (Sanborn; amplifiers 350-2700). Frequency limits were set between 0.1 and 500 Hz. The water tank and fish were grounded to the recording instrument. Rectal temperatures were registered using thermistor probes (telethermometer; Yellow Springs Instrument Co.).

Isolated hearts. In two anesthetized lungfish, the hearts were excised and immersed in a bath containing Ringer solution made up as follows (meq/liter): sodium 130; potassium 4; calcium 3; chloride 109; and lactate 28. Compressed air was bubbled slowly through the solution; its pH was 7.1. Recording electrodes were attached to the sinus venosus, atrium, and ventricle. The method of recording was as described in the preceding paragraph.

The sinus venosus, atrium, ventricle, and bulbus cordis were subsequently dissected free and their intrinsic electrical activity was also recorded.

Preparation for detailed histologic studies. After each study, the heart was perfused with a 10% solution of Formalin before immersion in the same solution. After fixation, these hearts were examined grossly and by conventional histologic techniques. In addition, two hearts were sectioned serially, one in the sagittal and the other in the coronal plane; serial sections were alternately stained with hematoxylin and eosin and with Weigert van Gieson stains. All sections were examined using light microscopy.

RESULTS

Electrocardiogram. In two anesthetized lungfish in which the pericardium remained intact, the electrocardiogram was recorded using electrodes attached to the ventral pericardial surface at points corresponding to the four limb leads in tetrapods. As illustrated in Fig. 1, the heart rate was 20 beats/min, the cycle was initiated by depolarization of the sinus venosus (S), followed by atrial (P) and then ventricular (V T) electrical activity. The bulbus cordis did not produce discrete deflections. The electrical activity of the sinus venosus was not always discernible, because at usual heart rates, its activity generally coincided with the repolarization wave of the preceding ventricular beat. Sinoatrial and atrioventricular conduction times were identical and measured 0.4 s, respectively. In one fish, studied in the horizontal position, the mean ventricular depolarization vectors of the sinus venosus, atrium, and ventricle were oriented at 35°, 30°, and −130°, respectively. The mean ventricular repolarization vector was at 50°. The initial forces of ventricular activation pointed caudally and to the left. Similar orientation of the vectors was found in the second fish.

Origin and propagation of electrical activity during sinus venosus rhythm. In six other lungfish, the origin and propagation of electrical impulses were delineated by direct epicardial surface electrograms recorded from the exposed heart in situ. Earliest electrical activity was recorded from the sinus venosus. In five of the six lungfish, this activity originated near the junction of the sinus venosus with the left cardinal vein (Fig. 2, left; Fig. 3, D and E); in the other, depolarization began at the junction with the right cardinal vein. The records in the right panel of Fig. 2 were obtained from electrodes located at the right and left cardinal vein junctions of the isolated sinus venosus from the same fish as

![Fig. 1. Electrocardiogram during sinus venosus rhythm from an anesthetized fish with intact pericardium. Electrodes were attached to ventral pericardial surface at points corresponding to limbs in tetrapods. Tracings show from above leads I, II, III, a VR, a VL, and aVF. S, sinus venosus electrical activity; P, atrial electrical activity; V, ventricular depolarization; T, ventricular repolarization. Vertical bar = 1 mV; horizontal = 1 s. Body temperature = 23°C.](image)
FIG. 2. Left and right panels: electrocardiogram and local electrograms from an anesthetized Protopterus with open pericardium during sinus rhythm. Body temperature = 26°C. Left panel: simultaneous records from a left-to-right bipolar pericardial lead and from unipolar leads on sinus venosus, close to entrance of right (rt) and left (lt) cardinal veins. Right panel: simultaneous records from similarly located electrodes on isolated sinus venosus of same fish immersed in a bath of Ringer solution. Voltage and time calibrations shown at lower right corner. Arrows indicate prepotentials recorded at left sinus venosus-cardinal vein junction. Trace on right bottom was recorded with a reversed polarity and at a higher sensitivity; note slow (negative) repolarization; P, atrial depolarization; QRS, ventricular depolarization; T, ventricular repolarization; S, sinus venosus depolarization.

in the left panel. The contours and sequence of activation are similar to those observed in the intact heart.

The earliest sinus venosus potential was of low amplitude (Fig. 2, arrows). Such potentials have been attributed to impulse generation from the site of a pacemaker (4). The absence of these potentials in the record from the isolated sinus venosus (Fig. 2, right) could be due to a shift of pacemaker site away from the recording electrode. In the intact heart, these potentials are followed by larger polyphasic potentials averaging 0.18 mV in amplitude (Fig. 2). Electrical activity of the sinus venosus was occasionally followed by deflections that occurred within 0.45 s in the pericardial electrogram and in records obtained from electrodes at the sinus venosus posterior vena cava junction (Fig. 3, A–C and Fig. 5, top record). These polyphasic waves were present in the isolated sinus venosus (Fig. 3F) as long as the posterior vena cava and cardinal veins were attached. However, when the veins were separated from the sinus venosus, only a single broad negative repolarization wave was recorded (Fig. 3G). Negative repolarization waves were also recorded from the sinus venosus and atrium in situ (Fig. 3E).

The isolated sinus venosus contracted at slightly higher frequency than that observed in the intact heart (25 beats/min). The isolated atrium contracted at a lower frequency than the sinus (15 beats/min), whereas the isolated ventricle had the lowest frequency of contraction (8 beats/min). The isolated bulbus lacked spontaneous activity.

Heart rates, depolarization times, duration of electrical activity of the different chambers and the conduction intervals between them during sinus rhythm are given in Table 1. Not included in this table are data from lungfish that did not recover well after surgery and anesthesia. These "sick" fish appeared lethargic and had difficulty in maintaining normal position. Heart rates before and after anesthesia were similar, ranging...
LUNGFISH HEART

between 20 and 29 beats/min; they varied with body
temperature. In four unanesthetized lungfish, heart
rates ranged from 12 to 48 beats/min as body tempera-
ture was varied in each fish over a range from 15 to
35°C, respectively \((Q_{10} = 3.77)\). Depolarization time was
shortest in the atrium, slightly longer in the sinus
venosus, and longest in the ventricle. The duration of
electrical activity was similar in the sinus venosus and
atrium but was much longer in the ventricle. Sinoatrial
conduction time (SACT) was shorter than the atrioven-
tricular conduction time (AVCT).

<p>| TABLE 1. Heart rates and activation times during sinus venosus rhythm |
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<table>
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<th>T, °C</th>
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<th>Rate, beats/min</th>
<th>S</th>
<th>S-T</th>
<th>S-A</th>
<th>P</th>
<th>P-T</th>
<th>A-V</th>
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<td>0.6</td>
<td>0.7</td>
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<td>26</td>
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T, body temperature; S-S, sinus venosus cycle; S, sinus venosus
depolarization time; S-T, duration of sinus venosus electrical activity;
S-A, sinoatrial conduction time; P, atrial depolarization time;
P-T, duration of atrial electrical activity; A-V, atrioventricular conduction
time; QRS, ventricular depolarization time; Q-T, duration of ventricular electrical activity. * Isolated sinus venosus.

Electrocardiograms from estivating lungfish were
used for comparison. These records showed sinus venosus
rhythm occasionally punctuated by bouts of atrial rhythms.
They did not differ from records described above and below (atrial rhythms) for nonestivating lungfish.

Atrial rhythms. In one unanesthetized lungfish, spontaneous
prolongation in both the SACT and sinus cycle length (Fig. 4, upper) was followed by an escape atrial rhythm shortly after submersion in a plastic cage (Fig. 4, lower). The first two cardiac cycles in the upper part of Fig. 4 illustrate a regular sinus venosus rhythm of 18.8 beats/min. Atrial activation occurred after a SACT of 0.4 s; the AVCT was 0.9 s. The third sinus cycle increased by 0.15 s and the SACT by 0.10 s, thus increasing the atrial cycle by 0.25 s. A further prolongation in the SACT was followed by the appearance of an irregular atrial escape rhythm at an average rate of 14 beats/min. Atrial activation occurred after a SACT of 0.4 s; the AVCT was 0.9 s. The third sinus cycle increased by 0.15 s and the SACT by 0.10 s, thus increasing the atrial cycle by 0.25 s. A further prolongation in the SACT was followed by the appearance of an irregular atrial escape rhythm at an average rate of 14 beats/min (Fig. 4, lower); the contour of the P wave had changed from a poly- (Fig. 4, upper) to a diphasic deflection (Fig. 4, lower). The AVCT and contour of the ventricular complexes remained the same. During atrial rhythm, the sinus venosus was activated retrogradely by the atrium after an atriosinal conduction time of 2.95 s and the sinus deflections became more pointed (Fig. 4, lower).

FIG. 4. Upper and lower traces: sinus rhythm in an unanesthetized Protopterus. Upper: tracing obtained using a bipolar pericardial lead shortly after submersion in water enclosed in a plastic cage. Vertical arrows that point downward indicate beginning of depolarization of sinus venosus (s) and atrium (p); upright arrows point to beginning of ventricular depolarization (QRS) and repolarization (T). Upper traces (black background) are enlargements to show details of T, S, P deflections (×2.8). Ladder-diagram symbols: S, sinus venosus depolarization; S-A, sinoatrial conduction time; A, atrial depolarization; A-V, atrioventricular conduction time; V, ventricular depolarization. Numbers in seconds. At heavy vertical bar prolongation of sinus cycle length and sinoatrial conduction time occurred. Horizontal line = 1 s. Lower: ectopic atrial rhythm occurring after 20 min of submersion in same fish. A-S, atriosinal conduction time. Note change in shape of P wave.
Figure 5 illustrates an ectopic atrial rhythm in an anesthetized lungfish. The cycles were initiated by atrial depolarization (P) occurring at a rate of 17.5 beats/min. The sinus venosus (S) and the ventricle (QRS) followed the atrium after a delay of 0.32 and 0.63 s, respectively. Also in this lungfish, a bout of paroxysmal atrial tachycardia occurred at a rate of 43 beats/min.

In another anesthetized lungfish (Fig. 6), sinus tachycardia was observed at a rate of 46 beats/min (arrows), completely dissociated from a slower ectopic atrial rhythm. The atria contracted at a rate of 20.5 beats/min and activated the ventricle after a delay of 0.5 s. One premature ventricular complex (PM), similar to the dominant in contour but following a short P-R interval, was followed by a fully compensatory pause. Its origin appears to be in the A-V junctional tissue. An atrioventricular junctional parasystole occurred spontaneously in another anesthetized lungfish during sinus rhythm that followed electrical stimulation of the ventricle.

The heart rates, depolarization times, and duration of electrical activity of the different chambers and the conduction times between them during ectopic atrial rhythm are given in Table 2. The atrial rhythm in an unanesthetized lungfish was markedly irregular; the mean rate (± SE) was 14.5 ± 2.08 beats/min, values that differ significantly (P < 0.01) from those during sinus rhythm (24.3 ± 4.5 beats/min). In the anesthetized lungfish, however, the atrial rhythm was less irregular and at a faster average rate of 23.7 beats/min.

In the unanesthetized lungfish, the atriosinal conduction time during ectopic atrial rhythm was longer than the sinoatrial conduction time during sinus rhythm. However, in the anesthetized lungfish, the two were similar (Tables 1 and 2). The long atriosinal conduction time in the unanesthetized fish is probably due to the high vagal tone that is also responsible for the depression of impulse formation in the sinus venosus. In the anesthetized fish, the escape atrial rhythm is presumed to be by 10.220.33.

**TABLE 2. Heart rates and activation times during ectopic atrial rhythm**

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<tr>
<th>TCO</th>
<th>P-P</th>
<th>Rate</th>
<th>S</th>
<th>ST</th>
<th>A-S</th>
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<td>15±</td>
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<td></td>
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<td>17±</td>
<td>0.3</td>
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<td></td>
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<td>14±</td>
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<td>2.96</td>
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<td>0.5</td>
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Anesthetized with open pericardium

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<td>1.4</td>
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<td>0.37</td>
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P-P, atrial cycle length; A-S, atriosinal conduction time. Other abbreviations as in Table 1. * Average rate. t During paroxysmal atrial tachycardia.
ably due to a nonvagally mediated decrease in sinus venosus frequency possibly induced by the anesthetic. The anesthetic was without effect on the sinoatrial junction. Atrioventricular conduction times did not differ significantly before and after anesthesia during sinus or ectopic atrial rhythm. The duration of the electrical activity of the different chambers was generally shorter at the faster heart rates.

**Arrhythmia induced by stress in nonanesthetized lungfish.** Following mechanical stimulation of the body surface, transient arrhythmias were consistently observed (Fig. 7). These consisted principally of atrial slowing and atrioventricular block resulting in a temporary ventricular asystole and changes in the pattern of atrial and ventricular activation. This response could not be elicited in anesthetized lungfish, and was readily diminished or abolished by atropine. In Fig. 7, sinus venosus activity could not be clearly identified and was probably superimposed on the ventricular repolarization wave. Thus the first two cycles probably illustrated sinus rhythm. Mechanical body surface stimulation produced a change in configuration of the repolarization wave of the third ventricular beat (because of separation of the sinus component) and prolongation of the third and fourth (escape) atrial cycles with abrupt atrioventricular block lasting for 9 s. Following the second nonconducted P wave, the atrial cycles shortened progressively and an accelerating rate of impulse formation in the sinus allowed resumption of its function as the cardiac pacemaker with gradual merging of sinus and T-wave deflections. Atrioventricular conduction times were longer during the first two cycles following the ventricular pause and then gradually returned to the original value of 0.5 s.

The first ventricular complex after the pause lacked a prominent R' in the upper tracing and an S wave in the lower; the contour of the initial part was preserved and the R wave was taller in the upper record and smaller in the lower. These alterations suggest aberrant ventricular conduction which gradually disappeared as the heart rate increased.

**Sequence of epicardial activation of ventricle.** The sequence of electrical activation was mapped on the ventral ventricular surface in three anesthetized lungfish in which the pericardia had been opened. Simultaneous records were obtained from a bipolar pericardial reference lead and from closely spaced bipolar electrodes located at different points on the ventricular surface. Activation times were measured from the beginning of ventricular electrical activity on the reference electrocardiogram to the beginning of the fastest deflection in the local electrograms. Earliest activity was recorded on the right, near the ventricular apex, 114 ms (± 2 SE) after the beginning of the peripheral ventricular electrogram. The apical area followed with an activation time of 134 ms (± 2 SE). Activation then proceeded in a basal direction with the latest activation time of 168 ms (± 3 SE).

**Bulbus cordis.** Discrete electrical activity from the bulbus cordis could not be ascertained on the electrocar-
diograms. However, as illustrated in Fig. 8, electrograms recorded from the exposed proximal and distal bulbus indicate that the proximal muscular part of the bulbus contributes to the terminal portion of the ventricular depolarization complex and propagated electrical activity from the distal, arterial end of the bulbus appeared after a delay of 0.4 s. The activation times of the ventricle, proximal bulbus, and distal bulbus were 0.2, 0.28, and 0.68 s, respectively.

**Ventricular response to electrical stimulation.** Bipolar electrical stimulation, using rectangular pulses of 2 ms duration and of variable intensity, failed to elicit responses from the sinus venosus. Attempts to stimulate the atrium were unsuccessful but these unsuccessful efforts are difficult to interpret because the fragile atrial wall was easily injured by the stimulating electrodes. Although the heart did not respond to stimuli applied across the pericardium, the ventricle did not respond to stimuli applied across the pericardium, the ventricle did respond to direct electrical stimulation as shown in Fig. 9. The upper panel shows a spontaneous rhythm of 17.6 beats/min. The lower two panels illustrate the ventricular response to electrical stimuli. The upper record in each of the lower two panels is a bipolar ventricular electrogram. The lower record is a display of the driving stimulus that is also seen in the upper record. The left-hand tracings of the middle panel show a 3:1 ventricular response with a latency of 0.18 s. The second ineffective stimulus falls well after the inscription of the T wave, indicating that restoration of the excitability has been delayed with respect to ventricular repolarization. When the intensity of the driving stimulus was increased (middle trace), the heart responded to every second stimulus with a shorter latency time of 0.12 s. As stimulation was continued using the same stimulus and frequency, a second-degree exit block of alternate effective stimuli appeared within 1 min. The pattern was that of a Wenckebach periodicity, manifested by progressive prolongation in the stimulus response latencies: the conduction ratios before an expected ventricular response was dropped were 6:5 and 5:4 in the lower panel and 3:2 in the middle panel, right-hand side. During the Wenckebach periodicity, the Q-T interval measured 1.2 s. The latest stimulus (S) in the ventricular cycle that was not followed by a propagated response (end of the ventricular effective refractory period) had a Q-S interval of 1.55 s. Thus recovery of excitability was delayed by 0.35 s after repolarization. The shortest interval obtained between two ventricular responses to electrical stimulation, which is an estimate of the functional refractory period, was 2 s.

**Morphologic findings.** The gross and histologic anatomy of Protopterus has been described in earlier publications from this laboratory (9, 16, 25) and others (6).

![Fig. 9. Effect of bipolar ventricular stimulation in an anesthetized fish with open pericardium. Upper panel shows a spontaneous rhythm in which upper record is a bipolar pericardial and lower is a ventricular electrogram. Numbers are in seconds. In lower half of this figure, upper trace is a ventricular electrogram and lower shows driving stimuli delivered to ventricle (indicated by short vertical lines above records. Numbers below panels indicate ratio of total stimuli to effective ones (vertical arrows point to ventricular responses). W, Wenckebach periodicity. Numbers preceded by W indicate ratio of half of number of stimuli to number of ventricular responses during Wenckebach periodicity. Numbers above panels indicate stimulus-response time (latency) in seconds.](http://ajpheart.physiology.org/)

Present considerations are confined to the relevant histology of the impulse-forming and conducting areas of the heart, i.e., the sinus venosus; the sinoatrial, atrioventricular, and ventriculobulbar junctions; and the atrial and ventricular musculature.

We could find no identifiable nodal structures or discrete conduction bundles in the heart of *Protopterus* despite serial section examination in both the sagittal and coronal planes. Myocardial cells were generally similar in structure regardless of their location in the heart. The cytoplasm of the cells of the sinus venosus was paler staining than that of the cells of the atria, which was, in turn, paler than the cytoplasm of the ventricular and bulbar cells (Fig. 10). The type of pale cells seen in the sinus venosus was also found in the posterior wall of the atrium and atrioventricular canal and at the base of the posterior sinoatrial junction. Thus there was a continuum of pale cells between the sinus venosus and the posterior wall of atrium and the atrioventricular canal (Fig. 11). In addition, the myocardium of the superior (cranial) wall of the sinus venosus was continuous with that of the superior wall of the atrium, which led into the superior wall of the atrioventricular canal to the ventricle. Thus musculature of the supremaledial wall of the atrioventricular canal met the bulbus musculature in the bulbo bulboauricular spur rather than the ventricle (Fig. 11). Pale cells were not seen within the ventricular walls nor at the junction between the ventricle and bulbus. However, at this ventricular bulbar junction, there was an abrupt change in orientation of muscle fibers from the generally longitudinal ventricular pattern to the circular pattern of the bulbus (Fig. 12).

**DISCUSSION**

The primitive heart of *Protopterus* allowed us to examine certain aspects of cardiac electrophysiology in a precursor of the terrestrial vertebrates. Of particular interest to the present study were 1) the sinus venosus, which is a discrete cardiac chamber that initiates the cardiac impulse; 2) the absence of an anatomically distinct conduction system; and 3) the opportunity for combining electrophysiological and histological studies in a lower vertebrate that possesses relatively constant sites of impulse formation and patterns of conduction, despite the absence of the familiar anatomical counterparts of the conduction system seen in the higher vertebrates. It was also possible to examine the electrocardiographic behavior of the intact, unanesthetized fish living under nearly natural conditions in water. By recording simultaneously the peripheral electrocardiogram and local surface electrograms, we were able to analyze the specific contribution of each cardiac chamber to the overall cardiac cycle, including successive sites of impulse formation and changing patterns of conduction between chambers during stress.

Our anatomical studies using serial histologic sectioning in two planes failed to reveal organized conductive tissue in *P. ethiopicus*. Instead, we found pale-staining cells which others have implicated in pacemaker function (26) in other animals. These were found in the sinus venosus which usually functions as the normal pacemaker in the lungfish heart. The atrioventricular ectopic beat (Fig. 6) and the parasystole could have originated in the posterior wall of the atrial canal where similar pale cells were observed. Our morphological findings resemble those described by McWilliams (19) in the eel heart and Gaskell (10) in the tortoise heart. Similar cells with pale cytoplasm were described by Robb at the sinoatrial junction of the eel heart and in the frog sinus venosus (24). Examination of serial sections did not reveal tracts of specialized conduction tissue. We did identify direct muscle continuity on both the inferolateral and the superior heart walls between...
ranged from 19.5 to 28.5 beats/min, rates that are similar to those reported by others for the elasmobranch and reptile (7, 14). We found that the heart rate of unanesthetized lungfish changed with a Q10 of 3.77 for a change in body temperature between 15 and 35°C; this value is similar to the Q10 of 3.24 found by Hutton et al. (12) in unanesthetized turtles for a change between 10 and 25°C. Barcroft and Izquierdo (1) and a Q10 between 1.55 and 2.2 for the change in heart rate of excised frog hearts with change in temperature between 17 and 27°C. Whether the difference between their results and ours is due to differences in species or in technique, or both, remains unanswered.

In the unanesthetized submerged lungfish, with no access to air, the appearance of an atrial rhythm was preceded by a slowing of the sinus rate and prolongation of sinoatrial conduction time. The ensuing atrial rhythm was irregular and slow; however, the A-V conduction time remained the same as during sinus rhythm. In contrast, following body surface stimulation, the transient arrhythmias were characterized not only by sinus slowing but also by A-V block and the appearance of atrial escape beats. Similar responses to stress have been reported in the eel (19) and in the sturgeon, Acipenser sturio (15). These arrhythmias seem to be related to increased vagal tone since they were regularly abolished by atropine. The atrial rhythm in the unanesthetized fish is reminiscent of the sinus bradycardia and arrhythmia with escape of subsidiary pacemakers that occurs in man. It is interesting to speculate that these pacemaker sites, which are poorly differentiated histologically, may be particularly sensitive to alterations of vagal tone, thus making the heart in these animals particularly prone to pacemaker arrest and takeover by lower pacemaker sites. In the anesthetized lungfish, the atrial rhythm was faster and more regular than in the unanesthetized fish and the atrioventricular conduction time was shorter, suggesting that the escape rhythm was not a consequence of heightened vagal tone.

The sinoatrial (SA) conduction time in lungfish with intact pericardium during sinus rhythm was 0.4 s, a value similar to those reported in the frog and eel (7) and in the common king snake (27). The SA conduction time increased markedly at lower temperatures, reaching 3.4 s at 10°C. To our knowledge, the effect of temperature on sinoatrial conduction time in poikilothermic vertebrates has not been previously reported. The A-V conduction time ranged from 0.4 to 0.9 s in different fish. These values are similar to the range previously reported by others for dogfish, turtle, and eel (7), and the prolongation of A-V conduction time at low temperatures that we observed in the lungfish was also similar to findings reported in other cold-blooded vertebrates (5, 8).

Although we only studied the ventral surface of the Protopterus ventricle, activation appeared similar to that described in the toad ventricle (17). Our observed mean ventral plane axis of ventricular depolarization was −130° as determined from the electrocardiogram, and as such was in accord with our finding that earliest ventricular activation was at the right ventral apical
area of the heart and proceeded in a basal direction.

The conduction delay between the ventricle and the muscular bulbus cordis and its proximal arterial part may be attributed to the abrupt change in myocardial fiber direction between the ventricular circular arrangement of the fibers in the former. However, the delay may, in part, be due to a narrow junctional area between the two anatomically different parts of the bulbus, and bulbar cells, similar to observations made in the ventricular canal junction of the frog heart (13).

An interesting anatomical observation in the present study was a potential dual antegrade pathway emanating from the atrium, going to the ventricle via the atrioventricular junction, as well as a direct pathway between the atrium and the bulbus cordis called the bulboauricular spur (Fig. 11), which bypasses the ventricle. These dual pathways provide the anatomical potential for a "fusion type" of ventricular and bulbar activation demonstrated possibly in Fig. 7, although an alternate explanation for the latter observation could be an additional conduction pathway within the atrioventricular junction itself.

The ventricular response to electrical stimulation revealed an interesting phenomenon of 2.1 response, with Wenckebach periodicity in the stimulus-response intervals of the effective beats. Such a Wenckebach period of alternately conducted beats has been described in the human A-V conduction system (3, 11). Our observation directly demonstrates one mechanism that may underlie this conduction disorder. In the presence of a long effective refractory period and a cycle length of stimulation equal to about one-half of the ventricular effective refractory period and a cycle length of stimulation equal to about one-half of the ventricular effective refractory period, every even stimulus (2nd, 4th, 6th, 8th, and 10th) falling during the effective refractory period is blocked. The odd stimulus (3rd), occurring during the relative refractory period, elicits a response with a prolonged stimulus-response time. Thereby, the subsequent odd stimulus (5th) occurs earlier within the relative refractory period and has a more prolonged stimulus-response time. In this manner, Wenckebach periodicity is initiated, which terminates with the first potentially effective odd stimulus (11th) occurring within the effective refractory period of the ventricle. Thus the pauses separating the groups of ventricular responses contain two ineffective stimuli in succession.

To our knowledge, Wenckebach periodicity of alternately effective stimuli applied to the ventricle has not been observed before. Its occurrence raises the question of whether the mechanism responsible for this periodicity, i.e., marked delay in recovery of the ventricular excitability after repolarization, is an inherent feature of myocardial tissue early in phylogenesis. If so, it would operate to safeguard the ventricle from premature sinoatrial excitation and excessively rapid rates, a protection that is afforded to the mammalian ventricle by the equivalent electrophysiologic behavior of the A-V node (21).

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