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Experimental Marine Studies of Liquid Respiration Technology for Diving/Surfacing of Biological Objects in Capsule to Depth of up to 1500 Meters

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Experimental Marine Studies of Liquid Respiration Technology for Diving/Surfacing of Biological Objects in Capsule to Depth of up to 1500 Meters

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Abstract. The article considers the study of the liquid respiration technology for diving/surfacing of biological objects in the Black Sea to the depth of 500, 1,000, and 1,500 meters. The authors focus on safe diving/surfacing modes and depths. The authors developed and tried an autonomous capsule attached to a winch with a cable that can be used for the diving and surfacing of the immersed bioobject. The authors provide the key diving/surfacing speeds, as well as maximum diving depths for a dog under spontaneous breathing. The authors present the results of medical research of the bioobject before, during, and after diving involving electrocardiogram, electroencephalogram, as well as ultrasound, x-ray, and instrumental tests.

1. General statement of the topic

Liquid respiration technology is used to prevent barotrauma and decompression disease resulting from fast surfacing during the free-ascent submariner rescue operations from the depth of over 250 meters. The use of liquid respiration technology, as well as partial liquid respiration devices (PLRD) and total liquid respiration devices (TLRD) in medicine, has been researched and described extensively in medical books – [1-15]. Unfortunately, almost all of the suggested approaches are theoretical, and the practical application cases of the liquid respiration technology are almost absent from open publications available today, which can be attributed to the lack of hardware and software support required [9]. There are no guidelines on the use of liquid respiration in the Black Sea taking into account the parameters of its water (salinity, hydrosulfuric impurities, etc.). Therefore, the problem of the establishment of modern technical complexes to facilitate the application of liquid respiration during a rescue mission, especially in the Black Sea, remains relevant and pressing, especially in the context of the order of the President of Russia concerning the creation and introduction of high-technology to facilitate import substitution in industry, medicine, and other economic sectors.

This work reviews the results obtained in experiments with the liquid respiration technology that could be potentially used for the diving/surfacing of biological objects in the Black Sea to/from the depth of 500-1500 meters. The article consists of two major parts and an annex. In the first part, we describe the technical solutions required for the implementation of the technology in question. The second part is dedicated to the result presentation. Since the experimental data are cumbersome, they are given in the annex to this article.



2. Description of the research object

Due to the difficulties of practical implementation of liquid respiration during real dives, as well as the lack of such experience in the Black Sea, we selected small animals for the research: dachshunds aged 6-8 months weighing 5-7 kg. We used perfluorodecalin and carbohal as the respiratory liquid. Test animals were quarantined for 30 days before the experiment and underwent all the key tests, such as blood, urine, ECG, EEG, ultrasound, etc. The animals were monitored for 30 days after the experiment.

The goals of the experiment included the following:

- identifying the maximum safe diving depth for a bioobject with spontaneous breathing immersed in the respiratory liquid in a special capsule;
- monitoring the key vital function parameters of the bioobjects during diving/surfacing (breathing rate, heart rate, body temperature);
- identifying safe diving/surfacing modes.

To conduct field experiments with the survival suit technology demonstrator with the life-support module for the test animal (dog) at the depth of 500-1500 meters, we developed the capsule shown in Figure 1. The capsule consists of body 1, lid 2, upper fairing 3, and lower fairing 4. Lid 2 is attached to body 1 by three locks 5. We manufactured two capsule options: - Option 1 with the camera and light source located at the bottom of the capsule, and Option 2 with the camera and light source located in the lid of the capsule. The capsule body is a welded shell with a header flange at the top and the bottom plate at the bottom. In Option 1 (Figure 2), the capsule bottom features sockets for the camera and the light source.

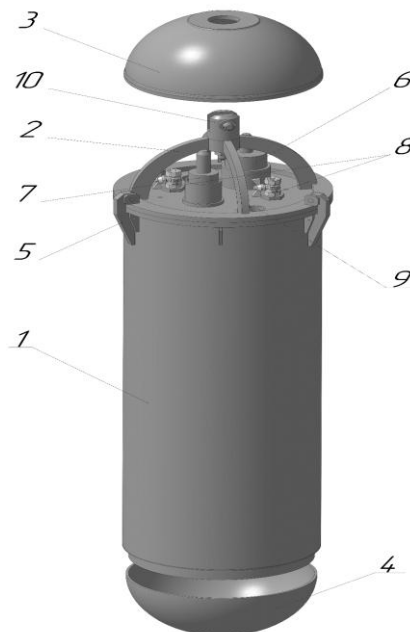


Figure 1. The 3D model of experimental devices.



Figure 2. The head element of experimental devices.

The camera and the light source are sealed in their sockets with standardized rubber rings. The capsule body and lid are connected through flanges with quick-release locks. To prevent accidental unlocking, the locks are equipped with retainers. The flanges are sealed with a special rubber-tube ring.

In Option 2, video camera 6 and light source 7 are installed in the sockets in the lid. The respiratory liquid (RL) is poured via one of the ball valves 8, and the air is ousted via the other. After that, valves 8 are closed. The external pressure is transferred to the respiratory liquid inside the capsule using two pressure compensator valves installed in apertures 9.

The body of the capsule consists of four steel beams 2 connected to the flange on one side and the sleeve on the other. Swivel 10 connecting the capsule with the speed winch cable and preventing the axial rotation of the capsule during diving and surfacing is mounted on the sleeve.

Mobile support was produced to move the rigged capsule on the deck of a vessel. The capsule on the mobile support is shown in Figure 3. The frame of the mobile support is made of a 30×30 mm shaped tube. The base of the support has swivel casters with brakes and a centering ring. To facilitate the loading and unloading of the capsule on/off the support, the top of the capsule is designed so that it could open on hinges and be locked closed using a quick-release splint.



Figure 3. The photo of experimental devices.

The bioobject (dog) should be immobilized for marine field tests. To do this, we designed a cage to be placed inside the capsule. The cage is a cylindrical metal structure made of 6-mm round rods. The interior of the cage was covered in 12×12-mm metal mesh. To facilitate the bracing of the test animal, the cage is made of two semi-cylinders connected by special semi-flush hinges (Figure 4). After the object is braced, the semicylinders are rotated on hinges until they close and are locked in this position. In this position, the cage can be placed in the capsule.



Figure 4. The photo of experimental devices

The field test capsule assembly with fairings and covered in thermoinsulating material (isolon) to maintain the temperature of the respiratory liquid (RL). We used perfluorodecalin and carbohal as the respiratory liquid. Figure 5 shows the capsule without the upper fairing and thermal insulation. You can see RL and air valves, as well as the apertures for pressure compensators in the capsule lid. Figure 6 shows the capsule without the lower fairing and thermal insulation. You can see the autonomous video camera and the light source, as well as the RL drain valve in the capsule bottom. The autonomous operation time for the camera and the light source is at least 1 hour with the video capture dimension of 1920x1080.

The marine tests of the liquid respiration technology using a capsule with an animal (dog) were carried out in the Black Sea at the depth of 500-1500 meters near Sevastopol on the research vessel Professor Vodyanitsky from November 07, 2018, to November 09, 2018.



Figure 5. The photo of experimental devices.



Figure 6. The photo of experimental devices.

During the preparation for marine tests, the vessel was loaded with research equipment, as well as researchers and test animals. After that, it left the port of Sevastopol and sailed to the diving location with a depth of 1800 meters near Sevastopol. The equipment was installed, prepared, and tested, and test animals were examined. During the main testing stage, the capsule was submerged to the depths of 500, 1000, and 1500 meters without the animals using the ocean-grade winch of the research vessel. The submersion rate was 2 m/s/

3. The main body of the article

The first submersion of the capsule with an animal to the depth of 1000 meters is shown in Figure 7. During the test, the autonomous camera and pressure and temperature sensor recorded a video file along with the pressure and temperature readings. The dynamics of temperature and pressure are shown in Figure 8. The submersion zone on the graph is shown by two graphic dependencies of temperature and pressure on the right side of Figure 8. The RL temperature increases up to 32°C. Submersion and surfacing are reflected by the pressure sensor as the peak of linear pressure increases and decreases, while the RL temperature is not decreasing linearly.

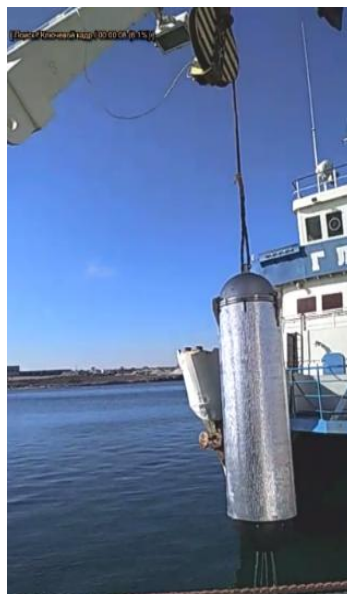


Figure 7. The photo of experimental devices.

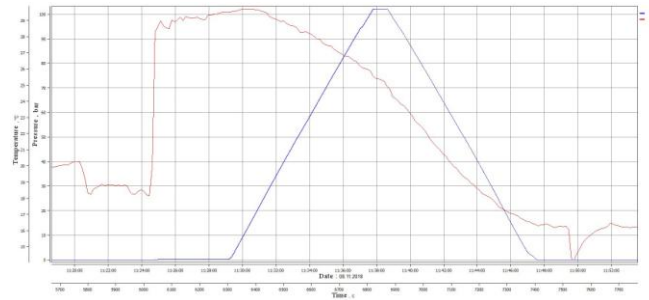


Figure 8. Pressure and temperature graphs for diving to a depth of 1000 m.

The second submersion of the capsule with an animal to the depth of 500 meters with the record of pressure and temperature inside the capsule is shown in Figure 9. The submersion is shown by the RL pressure and temperature graphs in the central part of Figure 9. After the experiment, the test animal survived and was placed in the oxygen tent for further monitoring.

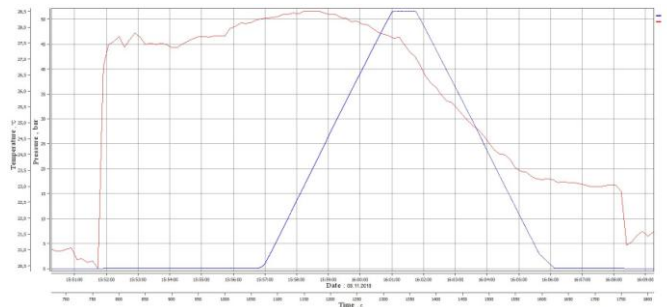


Figure 9. Pressure and temperature graphs for diving to a depth of 500 m.

The third submersion of the capsule with another animal to the depth of 1000 meters with the record of pressure and temperature inside the capsule is shown in Figure 10. After the experiment, the test animal recovered spontaneous breathing. The animal was placed in the oxygen tent for further monitoring.

The fourth submersion of the capsule with an animal reached the depth of 1500 meters. By the end of the experiment, the temperature inside the capsule reduced significantly. The dynamics of temperature and pressure inside the capsule are shown in Figure 11. The test animal died after the experiment.

During the final stage of the experiment, the vessel sailed from the submersion area to the port of Sevastopol, and the research equipment was removed. After that, research equipment, personnel, and test animals were unloaded at the port. The health parameters of the surviving animals were monitored for 30 days, including blood and urine tests, ultrasound, lung x-ray, ECG, etc.

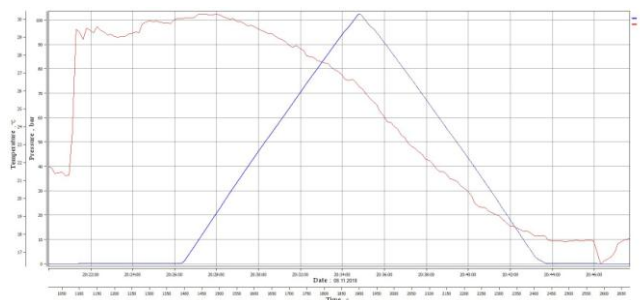


Figure 10. Pressure and temperature graphs for diving to a depth of 1000 m.

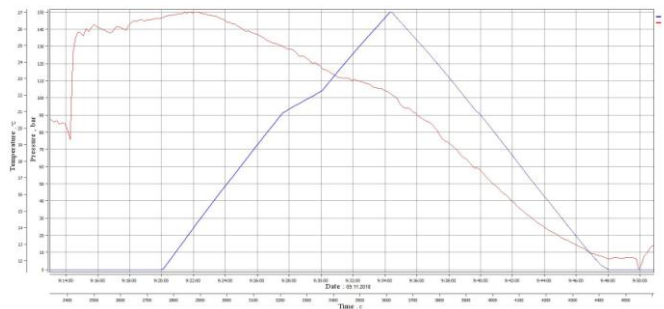


Figure 11. Pressure and temperature graphs for diving to a depth of 1000 m.

The selective results of medical research of the functional status of the cardiorespiratory system of the test animals under LR and high pressure and after that for two out of four surviving animals are shown in Tables 1 and 2 in the annex [10,11].

Before the experiment, test animals had a heart rate of 121.44 ± 3.02 bpm and a rectal temperature of 37.54 ± 0.03 °C. Respiration parameters of the test animals were within the physiological standard, the RR of 47.20 ± 3.32 bpm. Before bioobjects were placed in the capsule, the ECG showed a regular sinus rhythm with moderate tachycardia caused by the excitation of the CNS. All the intervals and complexes were within the normal reference ranges. During the submersion, test animals had a stable suppression of the SA-node with the dominance of the vagal atrial rhythm (floating pacemaker). As the submersion depth increase, the rhythm changed to spastic ventricular. Some animals experienced ventricular fibrillations (with subsequent cardiac arrest). After the test animals were ejected from the capsule, their awareness was suppressed due to drug sedation. The skin covers were pale and cold, visible mucous membranes were pale. The rectal temperature of the test animals was 31.24 ± 0.67 °C. The respiratory rate of the bioobjects after decompression was 28.40 ± 3.61 bpm. After the test animals were ejected from the capsule, their heart rates were different: we registered both normocardia (up to 110–120 bpm) and bradycardia (66–70 bpm).

The results of the gas composition and blood acid-alkaline balance dynamics of the bioobjects show that after the test animals were ejected from the capsule (after diving to 500 meters), the pH level reduced. We established that the unidirectional changes in blood pH and pCO₂ with reduced HCO₃⁻ value can be linked to the development of the metabolic acidosis in the test animals. The registered buffer base deficit reflects a metabolic malfunction. Because of that, there is a deficit of bicarbonate needed to maintain the optimum level of carbon dioxide in the blood. Carbon dioxide accumulated in the blood of the test animals. Thus, the pO₂ figures reflect the development of hypoxia. The lack of oxygen is complemented by the increased production of lactate. Thus, the results of blood gases and metabolites speak for the development of metabolic acidosis in test animals. We established, that the body temperature of the bioobjects decreased significantly during the submersion (e.g. they developed hypothermia) caused by the rapid cooling of the RL. To counter this, we put on more thermal insulation on the capsule, which helped significantly reduce the heat-loss rate of the RL. The studies the correlations between the bioobject parameters (heart rate, body temperature, ECG data) and the ambient parameters (RL temperature, excessive pressure) during the submersion of the animals. We established that during the 500-meter submersions, the heart rate of the test animal does not depend on the reduction of body temperature and reduced to 46 bpm as the diving depth increased. During surfacing, heart rate gradually increased to 66 bpm despite the continued reduction of body temperature. When analyzing the results of the ECG, we noticed that there was no connection between the body temperature and cardiac abnormalities. The latter correlated with the changes in pressure (diving depth). During the submersion, cardiac abnormalities included the development of the sinus bradycardia turning into the spastic sinus rhythm against the ST-segment depression. During the surfacing, the heart rate gradually increased, while the signs of myocardial ischemia persisted. After

the animal was ejected from the capsule, it could recover spontaneous breathing and heart rate, and the electrocardiographical signs of ischemia disappeared, even though the signs of hypothermia persisted.

When the test animals were submerged to the depth of 1000 m, the changes in the heart rate and the ECG abnormalities of the test animals had different character: the heart rate rapidly reduced from 120 to 66 bpm, and bradycardia (16-60 bpm) remained until the end of the submersion with the lowest value of 16 bpm registered at the maximum depth of 1000 meters. During the surfacing, heart rates increased up to 26 bpm and then 39 bpm. The cardiac abnormalities registered by the ECG during the submersion and at the beginning of surfacing included bradycardia with spastic sinus rhythm (wandering pacemaker) turning into bradycardia with spastic supraventricular rhythm and signs of myocardial ischemia. After that, due to the resuscitation activities, heart rhythm and rate recovered to standard levels. During the submersion, the cardiac abnormalities registered by the ECG included bradycardia with spastic sinus rhythm (wandering pacemaker) turning into bradycardia with spastic supraventricular rhythm and signs of myocardial ischemia. At the depths of 800 and 1000 meters, the rhythm couldn't be identified. During the surfacing, single supraventricular complexes were registered. When the animal was back on the deck, we registered ventricular fibrillation. We performed resuscitation procedures (intubation, ALV, defibrillation, adrenaline, and atropine injections) for 20 minutes without result and pronounced the animal dead. We could not register the parameters of the bioobject submerged to the depth of 1500 meters due to the failure of the remote sensor caused by high pressure. When the animal was ejected from the capsule, no cardiac electrical activity was registered. We performed resuscitation procedures (intubation, ALV, defibrillation, adrenaline, and atropine injections) for 20 minutes without result and pronounced the animal dead.

4. Conclusions

The results of the research show that during the submersion, the test animals experienced hypothermia due to the differences between their body temperatures and the ambient temperature. The degree of hypothermia depended on the original temperature of the respiratory liquid, marine ambience, and the duration of the submersion. Even the most favorable abnormalities of body temperatures registered by us can be classified as profound hypothermia. Taking this into account along with the results of the electrocardiographic research, we can say hypothermia during submersions caused significant disruption of hemodynamics caused by the myocardial ischemia that can result in adverse outcomes. The myocardial ischemia is probably caused by hypoxia due to the disruption of external respiration. However, we could not register respiration parameters during the submersion due to the rocking of the capsule during the descent and ascent. The basic results of the medical research for high-pressure submersion/surfacing are confirmed by the experiments conducted under normal pressure [12-15].

The analysis of the bioobject organism state under spontaneous respiration in respiratory liquid conducted before the submersion (quarantine, monitoring, and tests for 30 days), during the submersion/surfacing (500 m), and after the submersion (monitoring and tests for 30 days) shows that the test animals have a high survival rate without any health effects after marine experiments. For submersions/surfacing to the depth of 1000 meters, the survival rate reduced to 50% (one dog survived, and one died), and the bioobject shows some neural activity disorder during the first 10 days. The liquid respiration technology is essentially safe for the immersed bioobjects during submersions to the depth of 500 meters both at laboratories and at sea (100% survival rate, three at lab and one at sea). The depth of 1000 meters is the maximum permissible depth of submersion/surfacing of a bioobject immersed in a respiratory liquid and breathing spontaneously. The survival rate is between 50% and 67% (1 out of 3 lab submersions and 1 out of 2 sea submersions ended in the bioobject death). During the marine tests, the cooling of the respiratory liquid and the bioobjects (resulting in hyperthermia and death) occurs more rapidly due to the insufficient thermal insulation of the capsule and the lower temperatures of the seawater (by 3-5°C) compared to the lab tests, especially at the depths of 1000–1500 meters. Safe descent speed is 3-5 m/s, and safe ascent speed is 2-3 m/s. This research was carried out under the SevSU grand ID 26/06-31, contract No. 62/16 of 01.07.2016.

5. Annex

Table 1. The dynamics of ECG parameters of the test animals at various research stages, ($X \pm m$).

Parameters	Quarantine	Research stages							
		Experiment							
		Fixation	Compression (100 m)	Compression (500 m)	Compression (1500 m)	Decompressio n (300 m)	Decompressio n (150 m)	Decompression (50 m)	Ejection
Heart rate (strokes/m minute)	109.5±5.5	90.5±9.4	119.3±8.2	106.0±26.5	183.0±25.7	97.5±0.84	106.5±17.27	92.5±9.57	134.8±21.1
R - R min (ms)	468.5±3.5	579.36±72.7	327.0±121.2	331.63±145.5	133.25±27.7	389.75±169.82	424.5±183.92	465.8±107.8	278.86±36.3
R-R max (ms)	659.0±99.0	912.3±107.7	613.33±403.07	105.3±192.0	1229.5±139.1	1355.5±70.01	1036.0±153.2	1089.9±154.5	960.14±47.55
R-R average (ms)	549.0±29.0	752.8±67.1	506.3±34.4	649.0±146.0	341.25±59.55	714.5±161.97	617.0±114.15	691.5±83.5	497.14±57.97
P (ms)	52.5±0.5	49.83±10.1	48.25±12.86	48.57±11.91	41.75±12.53	64.0±3.04	62.33±3.62	64.6±4.86	72.40±8.71
P-R (P-Q) (ms)	98.0±3.0	84.0±18.4	86.0±29.3	84.71±20.18	47.5±12.28	73.3±6.29	100.0±6.87	103.8±3.60	113.4±3.66
QRS (ms)	74.5±4.5	57.3±3.76	61.0±8.39	56.38±7.65	41.7±5.21	67.75±9.12	62.5±6.74	69.67±5.15	60.71±5.33
Q-T (ms)	216.5±7.5	236.6±16.7	253.0±30.14	266.14±46.87	176.75±55.75	273.25±49.16	248.0±48.11	253.0±22.4	271.8±20.59
Q-Tc (ms)	290.5±5.5	287.3±14.3	308.0±117.2	292.57±71.01	274.25±71.54	307.0±47.5	292.25±85.32	318.67±59.0	310.0±44.1
QRS axis (°)	33.0±27.0	-90.0±0	-90.0±0	-90.0±0	-90.0±0	-90.0±0	-90.0±0	-90.0±0	-90.0±0
P (mv)	0.2±0.03	0.16±0.06	0.13±0.06	0.09±0.07	-0.01±0.03	0.14±0.05	0.12±0.05	0.10±0.03	0.11±0.03
R (mv)	1.08±0.32	0.79±0.10	0.68±0.13	0.68±0.16	0.41±0.14	0.93±0.24	0.81±0.19	0.68±0.13	0.82±0.14
S (mv)	-0.14±0.03	3.20±0.02	-0.21±0.05	-0.18±0.04	-0.15±0.02	-0.19±0.08	-0.18±0.07	-0.17±0.03	-0.05±0.03
R' (mv)	0.02±0.02	0.01±0.01	0.01±0.01	0.03±0.04	0±0	0.16±0.01	0.11±0.04	0.08±0.01	0.09±0.05
S' (mv)	-0.03±0.03	-0.15±0.01	0±0	-0.01±0.01	0±0	0.12±0.01	0.12±0.01	0±0	-0.03±0.01
STj (mv)	-0.03±0.01	-0.04±0.03	-0.09±0.07	-0.03±0.05	-0.05±0.05	-0.01±0.07	0.03±0.05	0±0.01	0.03±0.07
STj+60 (mv)	-0.01±0.03	0.08±0.01	-0.09±0.14	0.02±0.07	-0.04±0.04	-0.03±0.1	0.04±0.07	0.04±0.22	0.01±0.05
T (mv)	0.14±0.03	0.21±0.07	0.02±0.31	0.14±0.06	-0.06±0.17	0.28±0.15	0.27±0.16	0.15±0.05	0.07±0.09
R (ms)	27.5±0.5	31.71±1.52	34.5±31.9	30.38±1.27	27.0±0.58	33.5±3.84	32.0±9.14	37.5±4.06	36.57±4.52

Table 2. The dynamics of ECG parameters of the test animals at various research stages, ($X \pm m$).

Parameters	Research stages	Experiment				
		Day 3 after the experiment	Day 5 after the experiment	Day 8 after the experiment	Day 14 after the experiment	Day 28 after the experiment
Heart rate (strokes/minute)		138.5±5.61	127±1.34	129.0±0.53	128.6±16.3	150.67±5.2
R - R min (ms)		345.5±7.75	327.50±4.01	347.50±6.68	351.3±15.0	341.0±5.5
R-R max (ms)		728.5±45.70	760.38±21.38	712.5±37.15	723.6±131.9	546.33±86.2
R-R average (ms)		436.0±18.17	390.0±5.35	466.0±2.14	484.0±79.5	398.67±12.9
P (ms)		52.50±5.61	60.0±1.07	50.50±4.54	52.3±1.2	44.33±2.3
P-R (P-Q) (ms)		78.50±0.27	74.0±1.07	77.0±1.07	82.6±0.33	74.67±4.4
QRS (ms)		59.0±0.53	57.0±0.33	60.0±1.07	52.0±2.08	58.67±2.0
Q-T (ms)		189.0±0.53	178.0±1.87	184.0±3.21	211.0±22.3	224.67±48.7
Q-Tc (ms)		271.5±10.42	261.0±4.81	263.5±6.15	278.6±17.9	335.0±52.9
QRS axis (°)		-55.5±57.46	59.50±1.87	52.50±0.27	-90.0±0	73.33±34.4
P (mv)		0.22±0.010.19	0.19±0.01	0.18±0.01	0.21±0.03	0.25±0.02
R (mv)		-0.32±0.17	0.23±0.14	0.45±0.08	0.23±0.10	0.42±0.32
S (mv)		-0.15±0.01	-0.17±0.01	-0.16±0.01	-0.10±0.03	0±0.10
R' (mv)		0±0	0±0	0±0	0±0	0±0
S' (mv)		0±0	0±0	0±0	0±0	0±0
STj (mv)		0.08±0.02	0.47±0.23	0.06±0.02	-0.02±0.01	0.03±0.06
STj+60 (mv)		0.08±0.02	0.45±0.19	0.08±0.01	0.03±0.02	0.09±0.07
T (mv)		0.14±0.01	0.12±0.01	0.13±0.01	0.15±0.05	0.10±0.1
R (ms)		15.00±7.48	14.50±7.22	13.50±6.68	35.0±1.73	36.0±2.89

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