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ORIGINAL ARTICLE

Impact of Heat Stress on Electrocardiographic Changes in New Zealand White Rabbits

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A study was conducted on twelve clinically normal and healthy New Zealand White rabbits of both sexes, weighing between 2-3 kg, and aged between 1-3 years to observe the normal electrocardiogram and its changes during heat stress. The normal electrocardiogram and changes during heat stress were evaluated for the three bipolar standard limb leads (I, II and III) and three unipolar augmented limb leads (aVR, aVL and aVF). ECG recordings were made in sternal recumbency using a multi channel electrocardiograph. The normal heart rate with a mean of 204±7 beats/min was recorded. The mean amplitude observed was 0.05±0.002 mV for P wave; 0.19±0.008 mV for QRS; 0.14±0.007 mV for T wave. The mean duration observed was 0.03±0.002 sec for P wave; 0.06±0.002 sec for PR interval; 0.05±0.003 sec for QRS complex; 0.13±0.004 sec for QT interval; 0.07±0.002 sec for T wave. During heat stress tachycardia was observed with progressive rise in temperature along with ventricular fibrillation, ventricular extra-systole and atrial fibrillation. At 43°C, ventricular fibrillation was observed in Lead II, III, aVL and aVF. At 45°C, ventricular extra-systole was recorded in Lead III, aVL and aVF. At 45°C (30 mins more exposure), ventricular extra-systole in Lead I and ventricular fibrillation in Lead III was observed. At 47°C, ventricular fibrillation was seen in Lead II, III, aVR, aVL and aVF. At 47°C (30 mins more exposure), atrial fibrillation in all the leads were observed The mean cardiac axis recorded was 90°±0.065 without significant alterations throughout the study.

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Heat stress occurs whenever an animal has excess body heat that it cannot lose. The endocrine system plays an integral part in the animal's response to stress (Ayyat *et al*., 2004). Heat and cold stresses have direct impact on many of the fundamental cardiac control mechanisms of stroke volume, such as preload, afterload, diastolic function or compliance and systolic function or inotropy (Wilson and Crandall, 2011). Tachyarrhythmias and hypotension frequently occur during heat stress (Dematte *et al*., 1998) Hypotension or circulatory shock may result from translocation of blood from the vital organs, including the heart and brain, to the skin in an attempt to dissipate excessive heat (Howorth, 1995). All components of the electrocardiogram (ECG) can be affected, including rhythm disturbances, conduction defects, prolongation of the QT interval and ST segment changes. Rhythm disturbances include sinus tachycardia, atrial fibrillation and supraventricular tachycardia. Conduction defects include right bundle branch block and intraventricular conduction defects. Prolongation of the QT interval may be related to hypocalcaemia, hypokalaemia or hypomagnesaemia. The ST segment changes suggest occurrence of myocardial ischaemia in the territory of a particular coronary artery in 21% of patients who suffered from heat stroke.

MATERIALS AND METHODS

Experimental animals

Twelve clinically normal and healthy New Zealand White rabbits of both sexes, weighing between 2-3 kg and aged between 1-3 years were reared in the Division of Veterinary Physiology, at Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agriculture and Technology, R.S. Pura, Jammu were used for the study. Prior to the study, approval was taken from Institutional Animal Ethics Committee (IAEC), F.V.Sc and A.H, R.S.Pura, Jammu.The animals were individually housed in meshed iron cages. All the animals were fed with fresh green fodder, vegetables (spinach, carrot, cabbage) and pelleted diets in the morning and evening, while water was provided *ad libtum*. All rabbits were subjected to a thorough clinical examination before each study that included examination of conjunctiva, oral mucosa, skin, body temperature, auscultation, general condition and behaviour.

ECG machine

The ECG recordings were made in sternal recumbency as per the method described by Tilley (1979) using a multi channel electrocardiograph (NASAN, NE-3I, India). The machine used for the experiment was a digital, portable rechargeable machine.

Electrocardiographic gel

Cardiac gel (CARDIJELLY, BPL) was used to record the ECG tracings. The gel was applied over the surface of the limbs onto which the electrodes were attached to increase the conduction between the two surfaces and to reduce the resistance produced by the hairs on the body surface.

ECG paper

The thermal ECG paper was inscribed with horizontal and vertical lines spread 1 mm apart, which represent duration and amplitude respectively. The paper speed was adjusted to 50 mm/sec because the heart rate of the rabbit is very rapid *i.e* 205-235 beats/minute. As the tracings were very small in amplitude, the machine was so calibrated that 1 mV gives a deflection of 20 mm.

Position and Restraint of Animal

During electrocardiographic recording, positioning of the animal was standardized and consistent to avoid QRS axis changes. Here, electrocardiographic changes were made in sternal recumbency in all experimental animals as per the method described by Tilley (1979) using a multi channel electrocardiograph. The animal was kept as still as possible on an electrically non-conductive rubber sheet with manual restraint. Hand gloves were used during restraining the animals.

Procedure for recording the normal electrocardiogram

The procedure previously described by Ahmed *et al.* (2008) was followed for recording the electrocardiogram. After restraining the animal in proper position it was allowed to stand for 10-15 minutes so that initial evidence of fear and excitement had subsided (in very excitable subjects, fright could be overcome by putting a light towel over the rabbit's head). Three bipolar standard limb leads (I, II, and III) and three unipolar augmented limb leads (aVR, aVL and aVF) were used to record the electrocardiogram. Site for attachment of electrodes were trimmed with scissors and cardiac gel was applied to increase conductivity. Electrodes were attached with small crocodile clips with flattened teeth and were attached directly to the animal's skin proximal to the olecrenon on the caudal aspect of the appropriate forelimb, and over the patellar ligament on the cranial aspect of the appropriate hind limb. Positioning was consistent to avoid QRS axis changes. The animals were neither sedated nor forcibly restrained, and recordings were traced when they were relaxed and quiet. The ECG machine was calibrated to give 20mm deflection per mv of input and recordings were traced with a paper speed of 50mm/second.

The machine was charged thoroughly each day before recording. The standard nomenclature was used to designate different ECG waves.

Procedure for recording the electrocardiogram under stress condition

After taking the ECG recordings in normal conditions, the animals were subjected to Heat stress. To compare the electrocardiographic changes stress condition, a normal electrocardiogram was recorded beforehand to ensure that the animal was healthy. Thus for each experiment, the normal baseline value was slightly different depending on the other prevailing set of conditions of the day. For example, the 'normal' ECG values before the heat stress study were slightly though not significantly different from the day on which the cold stress experiment was conducted, and the corresponding 'normal values' were used to compare the ECG changed under the induced stressful event.

All the experiments were held on separate days with enough time interval for the animals to recuperate from the previous stressful event.

Heat stress

The ECG recordings in heat stress were taken at a constant temperature of 40°C maintained in a closed chamber and was gradually increased up to 47°C by placing room heaters at different locations in the chamber. The average holding time at each temperature was 60 minutes with recordings taken after every 30 mins (Table 1).

RESULTS

Normal electrocardiographic studies

The normal electrocardiographic parameters in the rabbit were documented (Fig. 1) according to the described methodology in the three bipolar standard and three unipolar augmented limb leads. The heart rate, wave amplitude, their duration, and cardiac electrical axis were recorded.

Heart rate: Heart rate was calculated in standard bipolar leads *viz*., Lead I, II and III by taking an average R-R interval. The values obtained in each lead were averaged and calculated to give heart rate per minute. The heart rate was observed between 167-250 beats/min with a mean of 204±7 beats/min. In all animals a normal cardiac rhythm was recorded.

Amplitude: Average amplitudes (mV) of various ECG waves (in different leads *viz*., I, II, III, aVR, aVL and aVF) are presented in Table 2.

'P' wave: The amplitude of 'P' wave varied from 0.02 mV to 0.10 mV with a mean of 0.05±0.002 mV in all the animals. Slight variations in the amplitude values were recorded between animals. P waves were sometimes not clearly visible or discerned. Interpretatively, minor differences in amplitudes (<0.1 mV) were not considered clinically relevant. No alterations such as atrial or ventricular extra systoles were observed. The highest amplitude for P wave was recorded in Lead II and lowest in Lead aVF.

'QRS' complex: The amplitude of 'QRS' wave varied from 0.10 mV to 0.30 mV with a mean of 0.19±0.008 mV in all the animals. The highest amplitude for QRS wave was recorded in Lead II and lowest in Lead aVF. Notching or slurring of the QRS complex was only occasionally noticed.

'T' waves: The amplitude of T wave varied from 0.05 to 0.25 mV with a mean of 0.14±0.007 mV in all the animals. T waves were sometimes not clearly visible or discerned. The highest amplitude for T wave was recorded in Lead III.

Duration: The mean duration of P wave, P-R interval, QRS, QT interval and T wave in Lead I, II, III, aVR, aVL and aVF are presented in Table 3.

'P' wave: The duration of P wave in rabbits varied from 0.04 sec to 0.06 sec with a mean of 0.03±0.002 sec. Durations varied slightly among rabbits, but differences less than 0.02 sec was not considered clinically relevant.

P-R segment: The duration of P-R segment in rabbits varied from 0.04 to 0.08 sec with a mean of 0.06±0.002 sec in all animals.

QRS duration: QRS complex duration in rabbits varied from 0.04 to 0.12 sec with a mean of 0.05±0.003 sec in all animals.

Q-T interval: The duration of Q-T interval in rabbits varied from 0.08 to 0.16 sec with a mean of 0.13±0.004 sec in all animals.

T wave duration: T wave duration in rabbits varied from 0.04 to 0.12 sec with a mean of 0.07±0.002 sec in all animals.

Mean electrical axis: The mean electrical axis recorded in rabbits was calculated -93° to + 96° with a mean of 90°±0.065.

Electrographic changes during Heat stress

Heart rate: There was a positive correlation of the rectal temperature with the ambient temperature. The heart rate was recorded at various varying ambient temperatures and was found to be elevated inducing a tachycardia in the animals (Table 4).

Amplitude: The amplitude of different ECG waves were recorded in all the rabbits under induced stress conditions. The results of the observations are presented in Table 5.

P wave: No significant change in the P wave amplitude was observed in Lead I.

QRS complex: There was a significant decrease (P<0.01) in the amplitude of QRS complex in Lead I.

T wave: No significant change in 'T' wave amplitude was observed.

Duration: The duration of different ECG waves in all the rabbits were recorded under induced heat stress conditions and the results are presented in Table 6.

P wave: No significant change was observed in 'P' wave duration.

PR interval: There was slight increase in P-R interval from base electrocardiographic tracings, but not statistically significant.

QRS complex: No significant change was observed in QRS complex.

QT interval: Decrease in QT interval was observed in heat stress but not statistically significant.

T interval: No changes were observed in heat stress in T wave interval during heat stress.

Cardiac axis: The cardiac axis in heat stress could not be calculated because of the presence of fibrillation in all other leads except Lead I.

Other notable changes in the ECG tracings during heat stress were ventricular fibrillation, ventricular extra-systoles and atrial fibrillation (Fig. 2). At 43°C, ventricular fibrillation was observed in Lead II, III, aVL and aVF. At 45°C, ventricular extra-systole was recorded in Lead III, aVL and aVF. At 45°C (30 mins more exposure), ventricular extra-systole in Lead I and ventricular fibrillation in Lead III was observed. At 47°C, ventricular fibrillation was seen in Lead II, III, aVR, aVL and aVF. At 47°C (30 mins more exposure), atrial fibrillation in all the leads were observed.

Figure 1 The normal electrocardiographic tracings of the rabbit for six leads

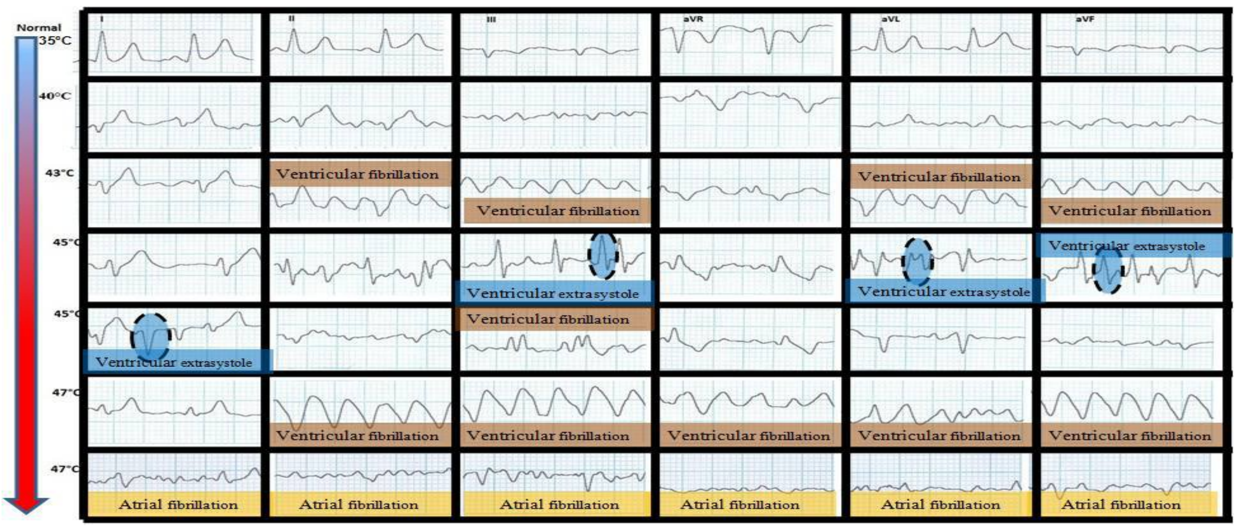

Figure 2. Progressive ECG changes with rising ambient temperature under induced heat stress in rabbits. Note development of ventricular fibrillation, ventricular extra-systoles and atrial fibrillation.

Table 1: Average holding and recording intervals at different ambient temperatures during induced heat stress.

S.No.	Ambient temp. (°C)	Average holding time	Recording intervals
1	40.0	60 min	After 30 and 60 min exposure
2	43.0	60 min	After 30 and 60 min exposure
3	45.0	60 min	After 30 and 60 min exposure
4	47.0	60 min	After 30 and 60 min exposure

Table 2: Amplitude (mv) of various electrocardiographic waves in rabbits under normal conditions (Mean±SE).

Leads	P	QRS	T
I	0.056±0.005	0.202±0.021	0.178±0.015
II	0.059±0.005	0.214±0.016	0.149±0.013
III	0.040±0.006	0.129±0.029	0.053±0.003
aVR	0.056±0.004	0.205±0.011	0.165±0.011
aVL	0.051±0.003	0.183±0.016	0.148±0.011
aVF	0.047±0.009	0.125±0.017	0.091±0.013
Overall mean	0.054±0.002	0.190±0.008	0.149±0.007

Table 3: Duration (sec) of different electrocardiographic waves in rabbits under normal conditions (Mean±SE).

Leads	P	P-R segment	QRS	QT	T
I	0.033±0.003	0.064±0.004	0.060±0.006	0.153±0.004	0.075±0.004
II	0.033±0.003	0.060±0.004	0.052±0.006	0.130±0.007	0.071±0.004
III	0.035±0.010	0.075±0.010	0.035±0.007	0.090±0.017	0.050±0.013
aVR	0.036±0.003	0.059±0.003	0.053±0.005	0.138±0.008	0.071±0.006
aVL	0.031±0.006	0.057±0.008	0.051±0.005	0.134±0.010	0.071±0.005
aVF	0.072±0.008	0.072±0.008	0.048±0.005	0.107±0.018	0.065±0.005
Overall mean	0.034±0.002	0.063±0.002	0.053±0.003	0.136±0.004	0.071±0.002

Table 4: Heart rate in induced heat stress in rabbits.

S. No.	Ambient temperature (°C)	Rectal temperature (°F)	Heart rate (bpm)
1	40.0 (30 and 60 min exposure)	100.0	200
2	43.0 (30 and 60 min exposure)	103.0	230
3	45.0 (30 mins exposure)	103.4	230
4	45.0 (60 mins exposure)	103.8	250
5	47.0 (30 mins exposure)	104.0	272
6	47.0 (60 mins exposure)	104.2	280

Table 5: Amplitude (mv) of the various electrocardiographic waves in rabbits under heat stress (Mean±SE).

Leads	P(B)	P(HS)	QRS(B)	QRS(HS)	T(B)	T(HS)
I	0.050±0.000	0.058±0.004	0.250±0.000	**0.094±0.002****	0.181±0.016	0.150±0.000
II	0.047±0.002	F	0.160±0.023	F	0.149±0.015	F
III	0.020±0.000	F	0.075±0.025	F	0.070±0.000	F
aVR	0.070±0.000	F	0.159±0.026	F	0.163±0.025	F
aVL	0.050±0.000	F	0.123±0.016	F	0.155±0.017	F
aVF	0.038±0.005	F	0.109±0.012	F	0.050±0.000	F

*P<0.05; **P<0.01; F-Fibrillation; B-Base; HS- Heat stress

Table 6: Duration of different electrocardiographic waves in rabbits under induced heat stress (Mean±SE)

Leads	P (B)	P (HS)	PR (B)	PR (HS)	QRS (B)	QRS (HS)	QT (B)	QT (HS)	T (B)	T (HS)
I	0.038 ± 0.004	0.036 ± 0.002	0.057 ± 0.004	0.060 ± 0.000	0.062 ± 0.012	0.056 ± 0.002	0.175 ± 0.008	0.152 ± 0.004	0.080 ± 0.007	0.088 ± 0.004
II	0.041 ± 0.001	F	0.061 ± 0.002	F	0.040 ± 0.003	F	0.173 ± 0.011	F	0.074 ± 0.002	F
III	0.030 ± 0.000	F	0.040 ± 0.000	F	0.040 ± 0.000	F	0.100 ± 0.000	F	0.060 ± 0.000	F
aVR	0.043 ± 0.007	F	0.073 ± 0.008	F	0.043 ± 0.002	F	0.153 ± 0.008	F	0.086 ± 0.008	F
aVL	0.033 ± 0.004	F	0.060 ± 0.003	F	0.040 ± 0.002	F	0.145 ± 0.003	F	0.075 ± 0.006	F
aVF	0.036 ± 0.007	F	0.056 ± 0.007	F	0.043 ± 0.005	F	0.180 ± 0.000	F	0.080 ± 0.011	F

*P<0.05; **P<0.01; F-Fibrillation; B-Base; HS- Heat stress

DISCUSSION

The electrocardiogram was studied only in the sternal recumbency even though recording in other positions (supine and normal) have been reported (Saitanov, 1960) with insignificant differences in ECG values. Similarly, Szabuniewicz *et al.* (1971) reported no changes in cardiac axis when shifted to either of the lateral sides or in the supine position.

Heart rate

The heart rate in rabbits have been variously recorded between 158-250 with a mean of 216±29 beats/min (Ahmed *et al.*, 2008), 198-330 beats/min with lower values expected in acclimatized rabbits (Reusch and Boswood, 2003). Our observations of 167-250 beats/min with a mean of 204±7 beats /min were fairly similar to reported values. Variations in the heart rate may be expected with differences in age and size of animals.

Amplitude

'P' wave: The observed P wave amplitudes were within the range 0.04 to 0.12 mV described for Lead II by Reusch and Boswood (2003) and for Lead I and aVF by Szabuniewicz *et al.* (1971). The P wave amplitude was calculated within a range of 0.04-0.12 mV by Lord *et al.* (2010). However mean values of both bipolar and unipolar leads were 0.06±0.003 mV as reported by Ahmed *et al.* (2008) which is higher than observed values in the present study. In certain cases the P wave could not be discerned on the electrocardiograph. This could be due to lack of coordinated atrial activity so that P waves are not being formed, or that P waves are present, but are not obvious. Lower amplitudes of electrocardiographic waves suggest a high degree of synchronization in depolarization of individual myocardial fibres. Notched and double P waves using high fidelity ECG in rabbits have been observed by Nelson and Waggoner (1964).

'QRS' complex: Lower QRS complex values with a mean of 0.11±0.003 mV have been recorded by Ahmed *et al.* (2008). Meral *et al.* (1998) described higher values for both bipolar and unipolar leads ranging from 0.40±0.63 mV with a mean of 0.50mV, but the range value of 0.03 to 0.39 mV described by Reusch and Boswood (2003) and also by Lord *et al.* (2010) were more in conformity to the observed values. Although the results have been found to correspond to earlier reports, there exists some variability in waveform characteristics. Szabuniewicz *et al.* (1971) while discussing the spontaneous variability particularly of the QRS complexes attribute to changes in vagal tone, intrinsic electrochemical changes in heart and probably looseness of the rabbit's skin that shifts the electrodes during recording.

'T' wave: Lower mean value of 0.07±0.005 mV was described by Ahmed *et al.* (2008) then the observed values in the present study. The T wave amplitudes were within the range 0.05 to 0.17 mV as described for Lead II by Reusch and Boswood (2003) and also by Lord *et al.* (2010) which by and large corresponds with our findings. Similar values of 0.07 mV for Lead I and 0.15 mV for Lead aVF by Szabuniewicz *et al.* (1971) have also been described. The findings of Meral *et al.* (1998) ranged from 0.19 to 0.28 mV with a relatively higher mean value of 0.25 mV than our observation.

Duration

'P' wave: Duration of 'P' wave was slightly lower than previously described mean duration of 0.02±0.001 sec by Ahmed *et al.* (2008), reporting a range of 0.01 to 0.02 sec with a mean of 0.02 sec. Other reports were fairly consistent as those of Lord *et al.* (2010) which are within the range of 0.01 to 0.05 sec, and Szabuniewicz *et al.* (1971) reporting a range of 0.02 to 0.04 sec, much in agreement to present values.

P-R segment: In the literature a similar range of values for the P-R interval has been described by a number of workers, *viz.* mean 0.05±0.002 sec (Ahmed *et al.*, 2008), range of 0.04 to 0.08 sec (Lord *et al.*, 2010). Levine (1942) was of the opinion that the P-R interval never exceeds 0.10 sec, much akin to our findings and those of earlier reports.

QRS duration: Range of 'QRS' duration similar to present studies have been reported by Lord *et al.* (2010) and Ahmed *et al.* (2008).

Q-T interval: Slightly lower (mean 0.11±0.003 sec) Q-T interval as compared to that observed in present studies was reported by Ahmed *et al.* (2008). Present observations coincide with the findings of Reusch and Boswood (2003) and Lord *et al.* (2010) which are within the range of 0.08 to 0.16 sec. Present observations also coincide with the findings of Szabuniewicz *et al.* (1971) which are within the range of 0.10 to 0.15 sec.

T wave duration: The T wave duration mean 0.03±0.003 sec reported by Ahmed *et al.* (2008) was slightly lower than the observations reported in the present study, but similar findings of Meral *et al.* (1998) reporting a range value of 0.05 to 0.12 sec were also observed.

Mean electrical axis: Mean cardiac electrical axis recorded in rabbits indicated that the ventricular axis is from right to left and from front to back in rabbits. Szabuniewicz *et al.* (1971) with orthogonal leads in rabbits described the spatial orientation of ventricular activation to be sinistrad or dextrad, ventrad and caudad. Other workers have variously recorded the axis between -30° to 70° (Ahmed *et al.,* 2008), -20° to +130° (Meral *et al.*, 1998), 45°±20 (Cinar *et al.*, 1999) and -43°±80° (Reusch and Boswood, 2003).

After taking the ECG recordings in normal state, the animals were induced to stress conditions.

It is evident from the present findings that hyperthermia is associated with tachycardia. Hyperthermia produces a hypermetabolic state with increased catecholamine stimulation, tachycardia and possibly an increased risk of ventricular fibrillation and ventricular tachycardia (John and Fleisher, 2006). Epinephrine however, causes vasodilation and vasoconstriction in different regions of the vascular bed and increase in heart rate with little change in mean blood pressure.

No significant change in P and T wave amplitudes were observed. A significant decrease in the amplitude of QRS complex was seen. Duration of P wave, QRS complex and T wave showed no significant changes in heat stress. A slight increase in P-R interval was observed which was not significant. Similarly, Akhtar *et al.* (1993) who also observed increased P-R interval in human heat stroke patients. A slight decrease in the QT interval although not significant was also observed from the basal value. Hyperthermia shortens the QT interval as was observed by Van der Linde *et al.* (2008) in beagle dogs when artificially warmed. This can be explained by the tachycardia which shortens the cardiac cycle associated with hyperthermia, thus decreasing the QT interval. Contrarily, prolonged QT interval was observed by Akhtar *et al.* (1993) in heat stroke human patients.

With the progressive increase in ambient and rectal temperature, ventricular fibrillation, ventricular extrasystole and atrial fibrillation occurred.Ventricular fibrillation has been described as a chaotic asynchronous fractioned activity of the heart. During hyperthermia, there is progressive fall in arterial blood pressure and dilation of the ventricles resulting in an un-coordinated contraction of the cardiac muscle of the ventricles and rapid quivering movement of the myocardium rather than proper contraction of the heart. Ventricular fibrillation was explained by MacWilliam (1887), whose definition still holds today, and is interesting in the fact that his studies and description predate the use of electrocardiography. He described that the ventricular muscle is thrown into a state of irregular arrhythmic contraction, while there is a great fall in the arterial blood pressure, the ventricles become dilated fails to pump blood into the arteries as the rapid quivering movement of their walls is insufficient to expel their contents; the muscular action partakes of the nature of a rapid inco-ordinate twitching of the muscular tissue. The cardiac pump is thrown out of gear, and the last of its vital energy is dissipated in the violent and the prolonged turmoil of fruitless activity in the ventricular walls.

Ventricular extrasystole occurs when heart beat is initiated by the heart ventricles rather than the SA node; the otherwise normal heart beat initiator. In a normal heart beat the ventricles contract after the atria helped to fill them by contracting. In this way the ventricles can pump maximum amount of blood both to the lungs and to the rest of the body. During hyperthermia, it results in a low blood pressure and ventricular dilation, the ventricles contract first, resulting in an inefficient circulation and followed by another systole generated by the SA node after emptying the atria.

In atrial fibrillation, electrical impulses generated in SA node, are overwhelmed by disorganized electrical impulses that originate in the atria and pulmonary veins leading to condition of irregular impulses that generate atrial fibrillation. Vulpian (1874) coined the term *mouvement fibrillaire*, seemingly to describe both atrial and ventricular fibrillation.

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