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DEEP HYPOTHERMIA IN THE RAT: STUDIES ON SURVIVAL AND TOLERANCE TO CIRCULATORY ARREST

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Received for Publication Feb. 5, 1963

Surface cooling to deep hypothermia, levels regularly reached by hibernators, has many intriguing clinical and laboratory applications[10]. GIAJA[7,8,5] ANDJUS, and colleagues[6,9,1,21,16,17]. LEWIS and ccolleagues[2,3] have achieved this state in rats ; while Niazi, LEWIS and SMITH[4,23] have succeeded in inducing this degree of cold and reversing the process in monkey and in man. These studies, repeating the techniques of GIAJA and ANDJUS, were designed to determine the following :

(1). The tolerance of rats to deep hypothermia

(2). The tolerance of rats to periods of circulatory standstill during deep hypothermia.

(3). The best technique for achieving reversible deep hypothermia in order to extend the usefulness of this technique for study of toxic agents.

METHODS

The rats were healthy adults of both sexes belonging to the standard Duke laboratory strain, weighing between 150gm. and 200gm.

PROCEDURE FOR COOLING :

The rats were placed in a 2 liter jar with air tight screw lid. Immediately the animal was placed in a large ice-box of -2° to 0°C The rats were observed at intervals ; and, on first studies, they were kept in this environment for 120min. In the latter studies the time in the jar was reduced to 90 minutes.

Access to the jar was possible by means of two rubber stoppers, which permitted in certain experiments sampling of the air for determination of O_2 and CO_2 percentage. This was measured by the SCHOLANDER method[18]. In other experiments pressure determinations were made by means of a mercury mannometer. In some studies a port allowed the attachment of a rubber balloon to equalize the pressure in the jar during the cooling phase.

Second Stage Cooling : After the animal was removed from the jar, a thermometer was inserted into the rectum, and the needle EKG leads were attached to the extremities and standard lead II recorded. In first studies the animal was completely immersed in

This investigation was supported in part by U. S. P. H. S. Grant # H-1782 ; Air Force Contract # AF 41(1657)-312.

cracked ice and observed until cardiac arrest occurred. In the second half of the study the animal was ventilated with room air until the heart stopped. A cup-like mask was fashioned from large tygon tubing and attached to an ordinary blood pressure bulb for insufflating the gas.

After the heart stopped, as shown on the EKG, the animal was maintained in an ice bath for the pre-determined period of complete circulatory arrest. In all experiments there was a short period of arrest. This period was determined by the interval between heart beats, as shown on the EKG.

REVIVAL AND REWARMING

The rat was removed from the ice and placed on a board with head elevated. A rectangle of thick card board was placed over the animals with a 3 cm. hole in it fashioned in such a way that an infra-red lamp would warm the anterior part of the thorax early. By the technique already described, the animal was given continuous artificial respiration until spontaneous breathing occurred. During this time the EKG was continuously monitored ; and as soon as a regular rhythm was established the entire animal was warmed by exposure to the heat lamp. The animals were then observed for evidence of central nervous system damage. A rat who survived for 7 days or longer was considered a long term survivor.

Temperature gradients during cooling and rewarming were determined in 3 experiments with thermistors being inserted in pleural cavity, rectum, braiin and spinal canal.

RESULTS

The cooling and warming pattern followed that previously described by GIAJA, ANDJUS and SMITH[9,8,1]. During the first hour the rat became increasingly lethargic. At the end of the 90 minute period the animal was almost completely anaesthetized. The rectal temperature on removal from jar averaged 20°C. The respiration and heart beat was at this point very slow (Figure 1). The electrocardiogram showed the typical effects of cold with A-V dissociation[26,24] a slow ventricular beat, and long repolarization time. With continued temperature fall the heart went into standstill, usually at 10°C or lower. Spontaneous respiration stopped at 16-18°C temperature. With this technique, ventricular fibrillation was uncommon. In one series, 84 rats were cooled without a single instance of fibrillation. In another series of 64 rats put into standstill for less than 20 minutes, there were no instances of ventricular fibrillation on rewarming. As will be pointed out later, there were more examples of this arrhythmia in animals kept in longer periods of standstill, accounting for ll of 52 deaths when period of standstill was more than 25 minutes.

There were ll animals of 84 that died after the first 24 hours. In the 8 where examination was carried out after death, 3 showed extensive peritoneal fat necrosis thought to be due to pancreatitis. Two died from bleeding in gastrointestinal tract. Only one succumbed from the obvious effects of severe brain damage.

Pressure changes in the jar are recorded in Figure 2. The two factors of low percent of oxygen and decrease in pressure due to lowering of the temperature tends to re-

duce the partial pressure of oxygen during the last 30 minutes of confinement to the jar.

Survival rates are shown in Table 1. These were all rats who were in the first stages of cooling for only 1 1/2 hours. There was a marked difference in the results when the 1st. stage was over 90 minutes. Table 2 compares this 120 min. with the 90 min. group.

The analysis of the percent oxygen and carbon dioxide was very similar to that reported by ANDIUS[16]. Figure 3 represents the observations on this change. The maximum fall in oxygen saturation seemed to occur at 60 minutes. It is obvious that with impaired

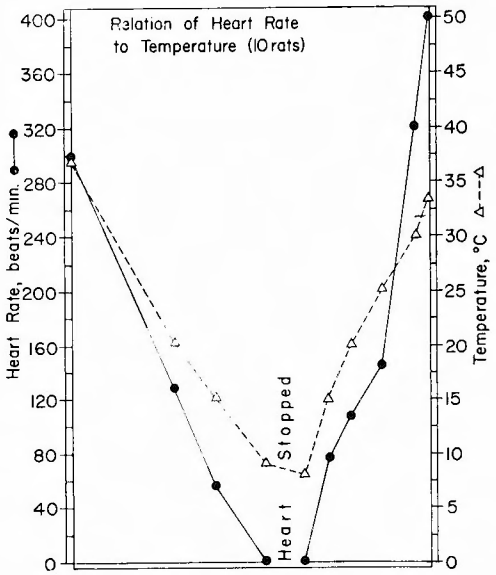

Figure 1. This graph shows relationship of pulse rate and temperature during induction and reversion of deep hypothermia in the rat.

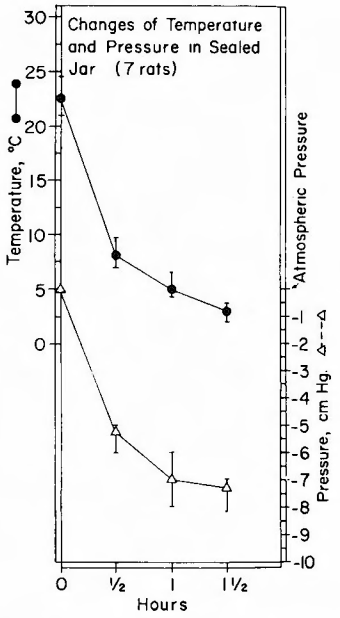

Figure 2. This graph relates temperature to pressure of the jar containing the rat during induction of deep hypothermia.

Table 1 Circulatory Arrest During Profound Hypothermia

No. Rats	Arrest Time	Dead 24 Hrs.	Percent	Dead 1 to 7 Days	Percent	Lived	Percent
30	11 to 20 min.	0	0	5	16	25	83
18	25 to 40 min.	3	16	3	16	12	67
24	50 to 70 min.	3	12	2	8	19	80
10	70 to 90 min.	5	50	1	10	4	40

Table 2

Comparison of 2 Hours with 1 1/2 Hours First Stage Cooling Arrest Time 11 to 38 Min.

No. Rats	1st Stage Time	Dead 24 Hrs.	Percent	Dead 1 to 7 Days	Percent	Lived	Percent
40	90 min.	3	8	8	20	29	72
40	120 min.	14	35	5	13	21	52

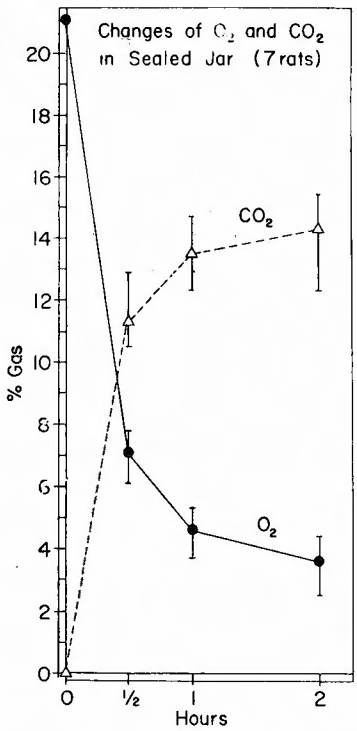

Figure 3. This graph shows the alteration in percent concentration of the CO_2 and the O_2 of the ambient gas during induction of deep hypothermia in rats contained in an air-tight jar.

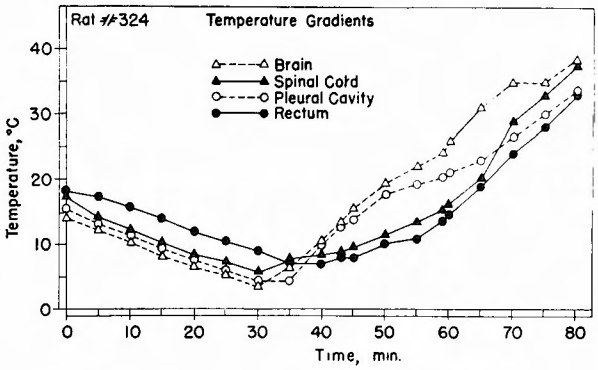

Figure 4. This is representative experiment showing temperature gradients in various body areas of the rat during cooling and warming.

respiration, slow heart rate that this may well have contributed to the poorer survival of the 120 minute group. These various factors all serve to increase the degree of hypoxia.

In ANDJUS' early study he noted that animals carried through the first stage of cooling in a jar not air tight did better than ones in an airtight jar. In our experiments the jar pressure was measured during this stage in 7 experiments. There was a drop (Figure 2) of 70 to 90 mm. of mercury during the temperature drop to 5°C or so. In a series of 20 rats we could not change the survival rate favorably by loosening of the jar lid. In 4 rats in the group without tight lids the O_2 percent was about the same as that in sealed jar.

The CO_2 percent change increased with this reaching maximum concentration at about one hour. The concentration is almost to the anaesthesia level and explains in part the rat's lethargy in the closed jar at the end of 1st. stage.

Table 1 summarizes our findings following circulatory arrest for varying periods of time. This indicates that in the range of one hour or more of circulatory standstill the incidence of non-revival and early death increases.

Figure 4 is one representative experiment of 4 carried out to check the temperature levels in various areas of the body. Fairly uniform cooling occurred. On warming the anterior structures, as represented by the pleura and brain warmed more rapidly than did the posterior aspect of the body.

In the survivors in early studies an occasional rat was noted to have hind quarter weakness for 1 to 5 days after recovery. This usually cleared completely. This same

Table 3 Incidence of Extremity Palsy in Rats

No. Rats	Duration of Arrest	No. with Palsy	Percent
31	11～20 min.	4	13
15	35～50 min.	3	20
13	51～85 min.	7	54

phenomenon had been noted in dogs subjected to deep hypothermia[11,22,27,13] The results of this study are shown on Table 3. In another study where the animals paws and fore leg were left out of the ice and only 30 minutes of arrest permitted, there were no animals with this defect. The long periods of circulatory arrest served to aggravate this as shown by the very high palsy rate present in the long arrested group. Though not shown here, the pattern of the change varied. It was more frequently present in both lower, but was seen in upper or in one lower extremity on occasions.

DISCUSSION

This study was done to establish whether or not rats could be regularly carried to deep hypothermia and then rewarmed without an escessively high mortality rate. ANDJUS and colleagues in series of experiments demonstrated that this was possible ; but when their experiments were duplicated, it was found that a consistent survival rate of above 60% could not be obtained. Such a low rate made it very difficult to devise studies on the protective effect of deep hypothermia to hypoxia or toxic drugs. It soon became evident that hypoxia imposed by a long 1st stage cooling was one important factor that was controllable. When this period was shortened, improvement became evident. An additional period of unnecessary hypoxia occurred at the second second stage of cooling when respiratory movement ceased before cardiac activity. The institution of artificial respiration during this period further reduced the exposure to hypoxia. With these two additional precautions the survival rate was increased to 80 to 90% for challenge with deep hypothermia carried to 10°C or lower.

The mechanisms involved that permits reversible deep hypothermia are not known. GIAJA designed the technique to mimic natural hibernation as seen in other species. By exposing the animals to decreasing supplies of oxygen while in cold surrounding serves to reduce the production of heat by the animal ; therefore permitting it to approach ambient temperature. The increasing levels of carbon dioxide act as a depressant and even an anesthetic, which in turn reduces muscle activity and its accompanying heat production. The effect of carbon dioxide on the heart may possibly play a role in the prevention of ventricular fibrillation as suggested by Lewis[29].

The absence of ventricular fibrillation in these animals is noteworthy, for in surface cooling of man and dogs to deep hypothermia this arrhythmia is the principal cause of inability to get survivors on rewarming. The mechanisms involved in the genesis of the fibrillation are not clearly defined. However, these experiments suggest that if peripheral oxygen need and coronary artery blood supply are kept in reasonable balance with the level of cardiac activity, ventricular fibrillation is not likely to occur.

The cooling and warming patterns are represented in Figure 4 by the pleura and

brain anteriorly and rectum and spinal cord posteriorly. By this technique uniform cooling occurs, but just as in hibernation the anterior areas warm first[1]. This, in effect, permits early warming of the heart which after all is the structure that has to furnish the starting force to warm the remainder of the body.

The clinical usefulness of deep hypothermia is dependent upon the tolerance it provides to long periods of complete or partial oxygen deprivation. In core-induced hypothermia, it is now established that 60 minutes is the upper limits of tolerance to complete circulatory standstill at temperatures in range 10°C or lower [14,26,19,15]. These experiments tend to confirm this tolerance time in the rat. Some degree of metabolism occurs, and without oxygen irreversible cellular damage develops if standstill is too prolonged. The effects of hypoxia are shown by failure to achieve good post hypothermic heart action, occasional overt evidence of central nervous system dissolution, and a high incidence of failure to thrive in first few post-exposure days.

In experiments on dogs subjected to core-induced deep hypothermia, a definite incidence of extremity paralysis was noted in the survivors. This same phenomenon was noted in rats though it occurred in both upper and lower extremities[25,3]. The highest incidence was noted in two situations. The first occurred in those animals completely covered in ice. This incidence could be reduced to zero in some experiments when the animal's extremities were not in contact with the ice bath. The second situation that predisposed to this abnormality were unduly prolonged periods of circulatory standstill. In the latter case the animals were covered with ice except for the distal extremities. This then would indicate that the depth of cold plus time of ischemia working together produced this lesion.

CONCLUSION

Rats can be cooled to deep hypothermia and revived in a high enough percentage to permit quantitative estimates of other experimental situations.

Hypoxia during cooling and warming is apparently important in the improvement of mortality figures.

Circulatory arrest of 60 minutes or less is well tolerated during deep hypothermia.

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和 文 抄 録

白鼠に於ける超低体温：循環停止に対する生存率と耐容力

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体重150～200gm.のDUKE系白鼠を用いてGiajuならびにAnjusのClosed vessel techniqueにより超低体温を来さしめ，i) 白鼠の超低体温に対する耐容力，ii) 超低体温下循環停止時間の限界，更に iii) 本法が有害作用物に対する超低体温の防禦作用の研究に利用出来るか否かを知る目的で本研究を行い，次の結果を得た．

1) 白鼠は超低体温に耐えて高率に生存し得るので，他の実験の定量的評価に利用出来る．

2) 冷却ならびに加温中のHypoxiaは死亡率を左右する重要な要因である．

3) 白鼠は60分又はそれ以下の超低体温下循環停止によく耐えて生存し得る．