

THE ROLE OF ACETYLCHOLINE IN CARDIO-RESPIRATORY ADAPTATIONS DURING HYPOTHERMIA AND REWARMING*

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INTRODUCTION

During Second World War, hypothermia was known as one of the most important complication of survival at sea. Shipwreck survivors when rescued after prolonged immersion, frequently died because of their lowered body temperature. Although this problem is still far from being resolved yet in recent years hypothermia has acquired great importance in clinical medicine, especially in surgery. Bigelow *et al* (2) pointed out that heart could be clamped off without complications for $1\frac{1}{2}$ minutes at normal body temperature. However, at a body temperature of 28°C , the heart and brain could withstand a stoppage in circulation for 15 minutes. Thus lower the body temperature, longer the period for which the circulation might be arrested. Clamping off the heart allowed intra-cardiac surgery under direct vision; previously such an operation was impossible. Soon it was realised that hypothermia at very low temperatures was incompatible with the life of the animal. In the past few years, evidence has collected which suggest that acetylcholine given in adequate doses may bring about better adaptation of cardiovascular and respiratory systems to hypothermia (1, 7). In the present experimental study, the problem has been tackled by using different rates of acetylcholine intravenous (i.v.) drip during hypothermia and subsequently rewarming.

MATERIALS AND METHODS

A total number of thirty unselected, generally healthy mongrel dogs of both sexes were used as experimental animals. They were anaesthetized with intravenous sodium pentobarbital (30 mg/kg body weight) and tied supine to the animal table. Trachea was exposed by a mid-line incision over the neck between cricoid cartilage and supra-sternal notch. One end of a "y" shaped glass cannula was inserted into the trachea and tied firmly. Rubber non-return valves for inspiration and expiration were fixed in the remaining two outlets of the "y" shaped cannula. The expired air was collected into Benedict's spirometer and accurate measurements of respiratory minute volume, rate and depth of respiration were recorded. Mean arterial blood pressure was recorded by introducing Francois-Frank arterial cannula in the carotid artery which was connected to a mercury manometer. Serial recordings were done by Kymograph. At intervals, drum was run at fast speed to record pulse pressure. Heart rate was recorded by electrocardiograph in Lead II and the body temperature with a mercury thermometer inserted 5-10 cm deep into the rectum.

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Hypothermia was produced by cooling extra-corporeal circulation shunts with the use of ice as described by Delorme (4). A cooling coil of polythene was shunted between femoral artery and vein. The circulation of blood through this coil was maintained by the pumping action of heart. In order to accelerate cooling this procedure was done bilaterally. Clotting inside the coil was prevented by i.v. injection of heparin 150 international units (i.u.) per kg. body weight as an initial dose and 50 i.u. per Kg body weight repeated after every 3 hours.

A summary of the experimental groups is given in Table I. Each dog was cooled to 25°C rectal temperature.

TABLE I

Experimental groups based on rate of acetylcholine intravenous drip used

Group	Number of Dogs used	Weight kg. (mean)	Length cm. (mean)	Surface area M ² (mean)	Acetylcholine i.v. drip * μ g/kg body weight/minute
I	10	12.5	86	0.63	13—15
II	10	13.2	88	0.65	1.3—1.5
III	10	12.0	82	0.58	0.13—0.15

* μ g=Microgram (10^{-3} mg)

Rewarming was achieved by placing the coils in warm water at about 38°C. The procedure was repeated in the same dog with varying dilutions of acetylcholine. Thus each dog formed its own control. As the experiment lasted for about 8-10 hours, the fluid requirements were calculated on the basis of body surface area and given via external jugular vein by continuous slow injector. The fluid used was sterile isotonic saline.

RESULTS

During hypothermia, there was a linear decrease in heart rate, mean arterial blood pressure, respiratory minute volume and rate of respiration. At 25°C, the heart rate decreased to about 30% of the normal value (see Table II). The electrocardiogram showed extension of the P-Q interval, a slight increase in the R-wave and prolongation of the ventricular complex as a whole. Thus transmission of stimulation to the ventricles took almost twice as long as normal (the P-Q interval was increased from 0.05 to 0.1 Sec or more), the ventricular complex lengthened from 0.1 to 0.5 Sec or longer, showing the extremely prolonged ventricular systole (see Fig 1). At a body temperature of approximately 32°C, the T-wave levelled off and subsequently became negative. Mean arterial blood pressure at 25°C was reduced to about 50% of the normal value (see Table II) and showed wide pulse fluctuations amounting to 28 mmHg (see Fig. 2). There was a progressive reduction in the respiratory minute volume and rate of respiration as the hypothermia proceeded (see Fig. 3). The depth of respiration was also

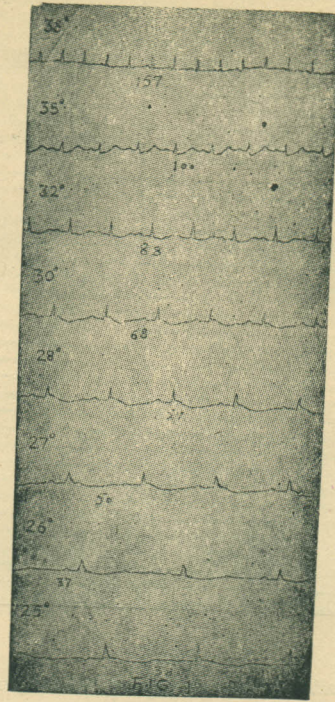


Fig. 1

Electrocardiogram (Lead II) showing the effect of hypothermia on heart rate and ECG pattern. The numerals on the left show the rectal temperature in degrees centigrade. Heart rate per minute is also recorded below each ECG tracing. Note the decrease in heart rate along with prolongation of P-Q interval and ventricular complex as the body temperature fell. Also note inversion of T-wave at very low temperatures.

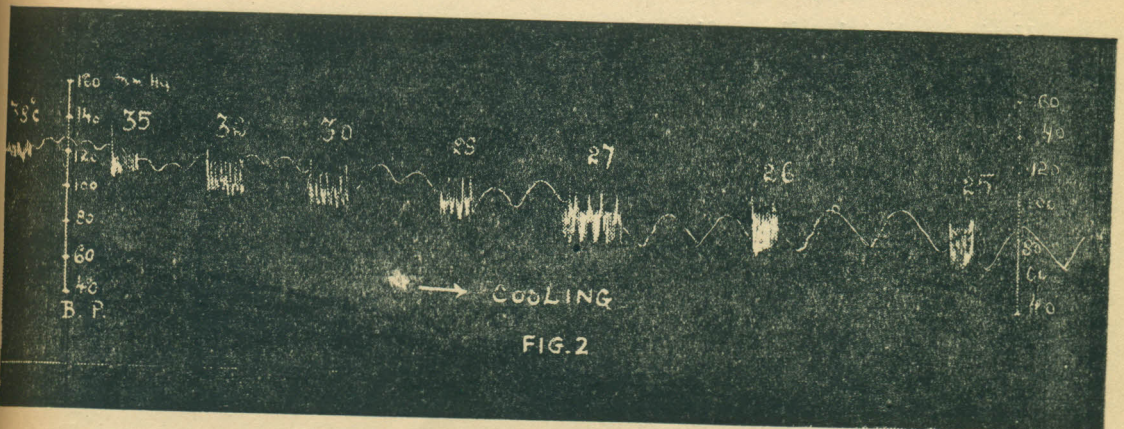


Fig. 2

Kymogram showing the mean arterial blood pressure in mmHg. during cooling. At intervals drum was run at fast speed to record pulse pressure. Note the marked increase in pulse pressure as the temperature fell from 38 through 25°C.

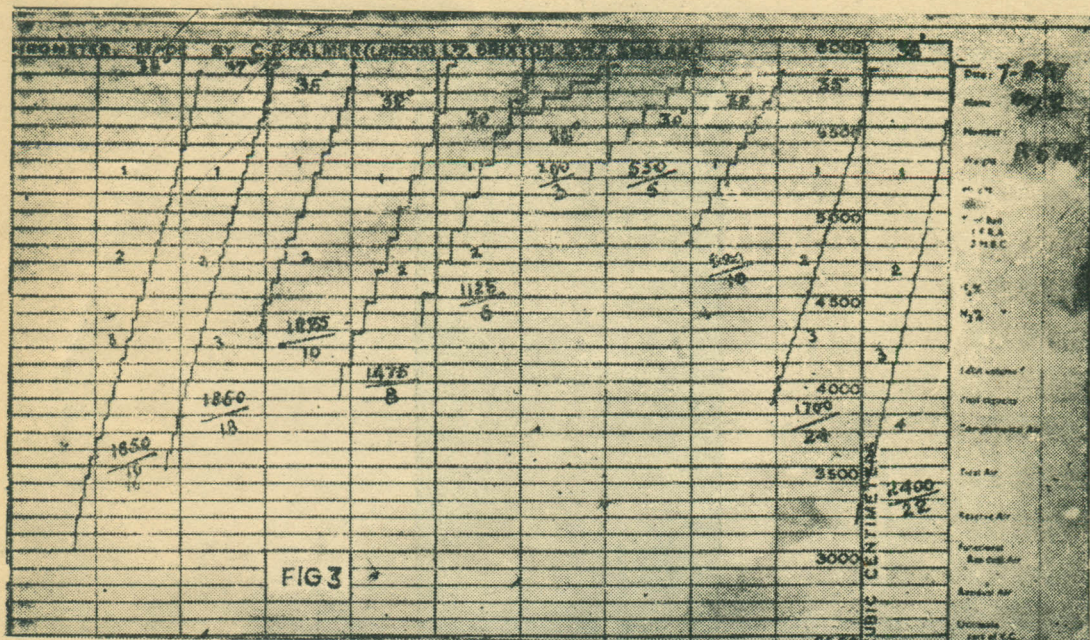


Fig. 3

Kymographic recording of spirometer cooling and rewarming. Respiratory minute volume in ml and rate of respiration per minute is noted for each spirogram. Note the marked reduction in respiratory minute volume and rate of respiration as the body temperature fell. During rewarming, these changes are reversed.

reduced slightly. At 25°C, the respiratory minute volume was 14%, rate of respiration 18% and depth of respiration 83% of the normal value (see Table II).

Having studied the effects of hypothermia alone, the results of the two sets of experiments in the same dog were compared i.e. without and with acetylcholine drip. In group I (Table I) acetylcholine i.v. drip was given at the rate of 13 to 15 $\mu\text{g}/\text{kg}$ body weight per minute. There was marked depression of cardio-vascular system as indicated by extreme slowing of heart rate and fall in B.P. All the ten dogs died of asystole between 31°C to 30°C rectal temperature. In group II (Table I) the rate of acetylcholine i.v. drip used varied from 1.3 to 1.5 $\mu\text{g}/\text{kg}$ body weight per minute. All the dogs survived double cooling and rewarming. Figure 4 (a) & (b) shows the record of cardio-vascular and respiratory responses in a typical experiment. The results of these experiments were compared and Table II shows the mean values, T1 (without acetylcholine) and T2 (with acetylcholine), along with standard error and their statistical significance. It will be seen that during cooling the only significant difference between T1 and T2 values was in the depth of respiration which increased with acetylcholine drip upto 28°C rectal temperature but at lower temperatures no significant difference was noted. During rewarming T2 values for heart rate and mean arterial B.P. were of higher order than T1. The results were of significance statistically. Respiratory minute volume, rate of respiration and pulse pressure did not show any significant difference between T1 and T2 values. In group III

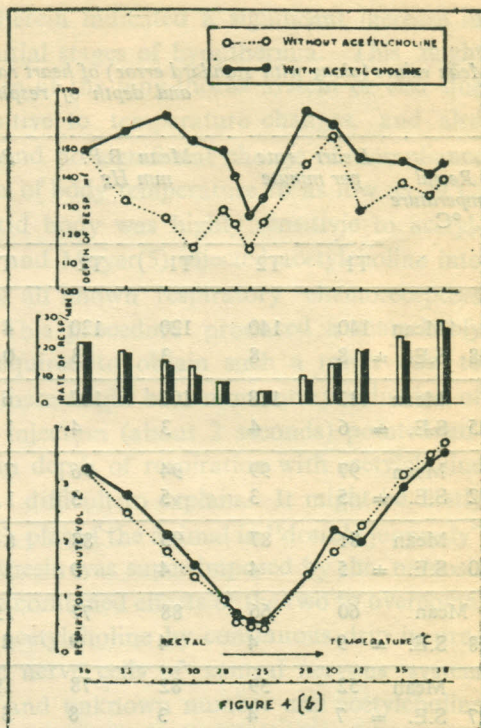
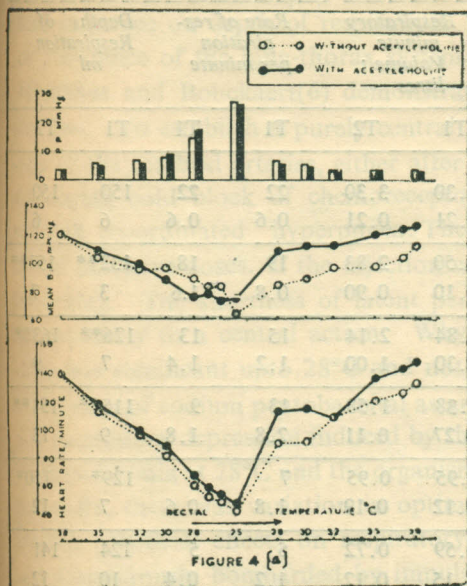


Fig. 4

Experimental record of cardio-vascular and respiratory responses during cooling at 25°C rectal temperature and rewarming (open circles). The same dog was again cooled to 25°C with acetylcholine i.v. drip and subsequently rewarmed (close circles)

- (a) Record of heart rate per minute mean arterial blood pressure (B.P.) and pulse pressure (P.P.) in mmHg.
- (b) Respiratory minute volume in litres, rate of respiration per minute and depth of respiration in ml.

(Table I), the rate of acetylcholine i.v. drip varied from 0.13 to 0.15 $\mu\text{g}/\text{kg}$ body weight per minute. There was no significant difference between T1 and T2 values during cooling and rewarming.

DISCUSSION

The general effect of hypothermia on all body tissues is to reduce progressively all cellular metabolic processes. This was reflected by development of progressive sinus bradycardia which appeared to be the consequence of lowered temperature on the pace maker rather than to extrinsic factors (8). Arterial blood pressure fell due to corresponding decrease in cardiac output but systolic fluctuations in arterial pressure (pulse pressure) registered about 7-fold increase at 25°C. The explanation for the latter could be a considerable reduction in vascular tone in the internal organs combined with reduction in heart rate. All these changes were reversible during the rewarming phase. Moreover, during both cooling and rewarming a close correlation between the respiratory rate and body temperature was found. In almost every case, the lowest respiratory rate occurred at the lowest body temperature.

TABLE II

Mean values (along with standard error) of heart rate, blood pressure, pulse pressure, respiratory minute volume, rate and depth of respiration during cooling and rewarming

Rectal temperature °C	Heart rate per minute		Mean B.P. mm Hg		Pulse Pressure mm Hg		Respiratory minute Volume : litres		Rate of respiration per minute		Depth of Respiration ml	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
38	Mean 140 S.E. \pm 8	140 8	120 3	120 3	4 0.2	4 0.2	3.30 0.21	3.30 0.21	22 0.6	22 0.6	150 6	150 6
35	Mean 113 S.E. \pm 6	118 4	103 3	107 4	6 1.4	5 1.9	2.50 0.10	2.83 0.90	19 0.8	18 1.6	132** 3	157** 7
32	Mean 97 S.E. \pm 5	99 3	94 5	96 3	7 1.7	6 2.2	1.84 0.30	2.14 1.00	15 1.2	13 1.4	126** 7	163** 9
30	Mean 81 S.E. \pm 5	87 4	95 4	88 9	8 1.1	9 1.7	1.38 0.27	1.58 0.11	13 2.8	9 1.8	115** 9	154** 13
28	Mean 60 S.E. \pm 9	66 4	88 4	77 9	15 2.6	18 2.2	0.95 0.12	0.95 0.19	7 1.8	7 0.6	129* 7	150* 12
27	Mean 52 S.E. \pm 7	59 4	82 3	78 8	19 3.9	20 4.3	0.59 0.15	0.72 0.12	5 1.2	5 0.4	124 10	141 12
26	Mean 49 S.E. \pm 7	52 4	82 1	73 9	23 4.2	23 5.0	0.51 0.14	0.62 0.12	5 1.0	5 0.4	115 13	127 6
25	Mean 41 S.E. \pm 1	46 3	61 7	74 4	28 5.3	27 6.6	0.46 0.15	0.56 0.14	4 1.0	4 0.8	124 3	133 6
28	Mean 90 S.E. \pm 9	113 7	85* 7	109* 10	7 1.1	6 2.4	1.38 0.26	1.38 0.29	10 1.4	8 0.2	145 17	165 16
30	Mean 90 S.E. \pm 8	113 8	88 5	112 11	6 0.5	5 1.9	1.78 0.29	2.21 0.36	14 3.2	14 3.4	156 19	160 6
32	Mean 108 S.E. \pm 4	108 13	96* 6	113* 5	4 0.2	4 0.8	2.24 0.33	1.98 0.41	19 2.8	19 2.6	129 7	148 4
35	Mean 120 S.E. \pm 5	134 3	97** 7	121** 5	4 0.2	4 0.4	3.20 0.49	3.00 0.41	24 2.6	22 2.3	139 6	147 5
37	Mean 126* S.E. \pm 6	143* 2	104* 7	125* 5	4 1.1	4 0.7	3.56 0.27	3.46 0.71	28 3.0	26 4.2	135 7	142 5
38	Mean 133* S.E. \pm 6	148* 3	113 7	126 5	4 0.9	3 0.7	3.86 0.68	3.63 0.92	30 3.2	27 4.2	141 8	147 6

T1=Values without acetylcholine.

T2=Values with acetylcholine. S.E.=Standard error.

* = Significant at 5% level.

** = Significant at 1% level (Highly significant).

The evidence from the experiments reported herein indicated a significant increase in depth of respiration with acetylcholine drip during initial stages of hypothermia. This might be possibly due to change of compliance or resistance of the lung-thorax system or else due to depression of Pneumotaxic centre which was sensitive to temperature changes, and also had influence on depth of respiration. Sechzer(9) found no significant change of compliance or resistance of the lung-thorax system with reduction of body temperature to as low as 29°C. Heymans and Bouckaert(6) demonstrated that carotid body was highly sensitive to acetylcholine. To establish a purely central action, Gesell and Moyer(5) injected acetylcholine into one of the cerebral arteries, either after denervation of all known respiratory chemoreceptors or during cold block of chemoreceptors afferents. This procedure produced a remarkably smooth co-ordinated hyperpnoea. The precaution required to obtain such a result was to avoid excessive doses. If the injection was too rapid, there might be a momentary reduction of breathing. The shortness of latent period following injection (about 2 seconds) pointed unquestionably to a central action. Why the increase in depth of respiration with acetylcholine drip was significant upto 28°C and not thereafter is difficult to explain. It might be firstly due to use of sodium pentobarbital as anaesthetic which placed the animal in "double jeopardy" i.e. respiratory depression induced by the initial anaesthesia was super-imposed by the narcosis of hypothermia at 28°C and the organism had therefore combined effects of the two to overcome. Secondly, there was variation in optimal amount of acetylcholine by continuous drip in producing beneficial effects on respiratory centre. The nerve cells of central nervous system are continuously bombarded by impulses of varying and unknown number and acetylcholine is capable of producing diametrically opposite effects (stimulation or paralysis depending upon its concentration) and the inhibition of one reflex may be the expression of another reflex entirely overlooked.

Anand *et al.* (1) demonstrated that an average fall in body temperature by 14.5°C caused significant reduction in acetylcholine content of heart, hypothalamus and frontal lobe of brain. This might be due to lowered synthesis of acetylcholine. Burn and Milton(3) found that at lowered temperature there was marked reduction of choline acetylase activity, as a result of which insufficient acetylcholine was formed. In addition to its function as a cholinergic transmitter, acetylcholine also seemed to protect tissues against anoxaemia. This was done in collaboration with glutathione by facilitating glycolysis during anaerobic metabolism. During hypothermia the threat of anoxaemia was met by resorting to anaerobic glycolysis. Since during hypothermia there was almost *pari passu* diminution of acetylcholine in frontal lobe, hypothalamus and heart, acetylcholine in adequate doses might prevent failure of vital centres in the nervous system and help anaerobic glycolysis.

During the cooled state, it seemed probable that blood was shunted into reservoirs and the reduced cardiac output was adequate. Upon rewarming, the cardiac output and blood flow did not return to normal immediately but a state of circulatory shock developed with continued low output. The mechanism which involved reduction in effective circulating blood volume, might be a failure of homeostatic mechanism to release blood from reservoirs into the effective circulation and an over-expansion of capillary-bed with a peripheral trapping of

blood. The nature of this post-hypothermia circulatory failure is unclear at present. The problem is of great importance however for the proper understanding and management of general hypothermia. In these experiments it was noted that during rewarming, significant increase in heart rate and mean arterial B.P. with acetylcholine drip was found as compared with control values. During recovery phase when body temperature began to rise and the metabolism in the tissues increased, acetylcholine might prevent the development of anoxaemia by facilitating glycolysis anaerobically.

SUMMARY

1. Hypothermia was produced in dogs by cooling extra-corporeal circulation shunts with the use of ice. Each animal was cooled to 25°C rectal temperature and subsequently rewarmed. The procedure was repeated in the same animal with varying dilutions of acetylcholine given intravenously by continuous slow injector.
2. With acetylcholine i.v. drip in adequate doses (1.3 to 1.5 $\mu\text{g}/\text{kg}$ body weight/minute) there was a significant increase in the depth of respiration (upto 28°C rectal temperature). During rewarming heart rate and mean arterial B. P. readings were higher with acetylcholine drip than the control ones without acetylcholine. The results were statistically significant.
3. Higher doses (13 to 15 $\mu\text{g}/\text{kg}$ body weight/minute) of acetylcholine i.v. drip caused marked depression of cardio-vascular system and the dogs died of asystole during induction of light hypothermia (31°-30°C).
4. Lower doses (0.13 to 0.15 $\mu\text{g}/\text{kg}$ body weight/minute) of acetylcholine i.v. drip showed no beneficial effects.
5. The probable action of acetylcholine during hypothermia and rewarming has been discussed.

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