EFFECT OF COLD ON THE ISOLATED RAT HEART

By

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Electrocardiographic changes in the intact and isolated hypothermic heart have been studied in various mammals (Richards *et al*, 1953; Nardone *et al*, 1955, Siem *et al*, 1955; Fleming and Muir, 1957; Covino and Beavers, 1958). As the temperature drops there is a slowing of the heart with prolongation of the PR interval, the QRS complex and alterations in the T wave. Although other organs in the body suffer little damage from hypothermia, the intact heart becomes more irritable and shows the tendency to develop cardiac arrhythmias, particularly ventricular fibrillation in the higher mammals. The increased irritability of cooled canine and human hearts has been explained on the basis of anoxia of the myocardium (Bigelow *et al*, 1950) hypercapnea (Swan *et al*, 1953), overwork caused by overloading from increased venous return (Bigelow *et al*, 1950) and increased susceptibility to the endogenous adrenaline (Cookson *et al*, 1952). However, it is difficult to evaluate the individual contribution of these different factors in the production of cardiac arrhythmias in hypothermic animals. In contrast to canine and human hearts, rodent hearts are far less prone to develop ventricular fibrillation on cooling (Richards *et al*, 1953; Editorial, Brit. Med. J. 1955; Covino and Beavers, 1958). The explanation for the differences in myocardial irritability observed in different species on cooling is far from clear.

The chief electrolyte changes associated with hypothermia are a fall in serum potassium level, a smaller and less consistent rise in serum sodium level (Swan *et al*, 1953; Olsen *et al*, 1955; Mavor *et al*, 1956; Lewis *et al*, 1956; Covino and Beavers, 1958; Munday *et al*, 1958) together with a fall in the blood pH (Swan *et al*, 1953; Munday *et al*, 1958). These changes in the serum electrolyte pattern on cooling are in the same direction in the different species studied. It is known that the kidney does not excrete excess potassium to account for the fall in serum potassium of intact animals during cooling (Swan *et al*, 1953). Munday *et al* have attributed these changes to excess secretion of the adrenal corticoids (Munday *et al*, 1958).

The electrocardiographic changes in the isolated and intact hearts on cooling are similar (Reismann and Van Citters, 1956, Covino and Beavers, 1958). However, little information is available on the alterations in cation exchanges in the cooled isolated heart. This work was therefore undertaken to study the changes in the electrocardiogram of the isolated perfused rat heart, on cooling. Alterations in the coronary outflow per minute and the cationic content of the perfusate, at low temperatures, have also been investigated.

METHODS

Albino rats weighing 120·200 G. were used. The animals were anesthetised with ether, the chest was opened and the heart removed rapidly together with a sufficiently long portion of the aorta. It was washed with the perfusing fluid and the aorta tied to the perfusion cannula. The perfusion apparatus was of the Dale type such as is used for the Langendorff heart preparation (Burn, 1952). The perfusing fluid had the following composition NaCl, 9 gm.; KCl, 0·42 gm.; CaCl2, 0·24 gm.; NaHC03, 0·5 gm.; Dextrose, 1 gm. and distilled water to make 1l. The fluid could be heated or cooled during its passage through a coil from the reservoir to the cannula. The temperature of the perfusing fluid was measured on a thermometer placed inside the cannula just above its tip. The method of recording electrocardiograms was essentially that described by Johnson and Vendsalu (1957). Recordings were made on a direct writing EK II Burdic Electrocardiograph. Lead I recorded the potential difference between the left ventricle and the aorta, Lead II that between the right ventricle and the aorta. Higher potential over a ventricle gave upward deflection. Hearts with initial irregularties were rejected.

The samples of perfusate from the heart during cooling were collected at various temperatures; and the volume of the outflow per minute was noted. Electrocardiographic tracings were taken simultaneously.

The perfusate samples were analysed for their contents of sodium, potassium and calcium by flamephotometry (EEL Flamephotometer). In the analysis of calcium by flamephotometry, higher values are obtained in the presence of sodium due to intensification of calcium spectral bands (Zak *et al.*, 1953). Therefore calcium from the samples was estimated as described by Powell, (1953). The changes in the cationic concentrations of the perfusate are expressed as percentage fall or rise taking the initial values as hundred percent.

RESULTS

Normal electrocardiographic pattern of isolated rat heart.

Cardiac rate:—In intact rats the heart rate varies from 300 to 600 beats per minute. In isolated perfused hearts the rate is much slower. The mean

initial heart rate at 36° to 37°C. was 254 per min. (S. E. ±11.7) in 12 experiments and at 33° to 35°C. it was 219 per min. (S.E. ± 4.7) in 24 experiments. These findings are similar to those reported in rabbits where at 38°C. the intact heart rate was 218 per min. while the isolated heart rate was 150 per min. (Covino & Beavers, 1958). In this study hearts beating at the rate of 180 per min. or less at the initial temperature were rejected.

P Wave and PR Interval :—The P wave appeared as an upward, downward or biphasic deflection (Fig. 1). The mean initial PR interval in 12 experiments at 36° to 37°C. was 0.038 sec. (S. E. ± 0.0017) and in 24 experiments at 33° to 35°C. it was 0.049 sec. (S, E. ± 0.0039).

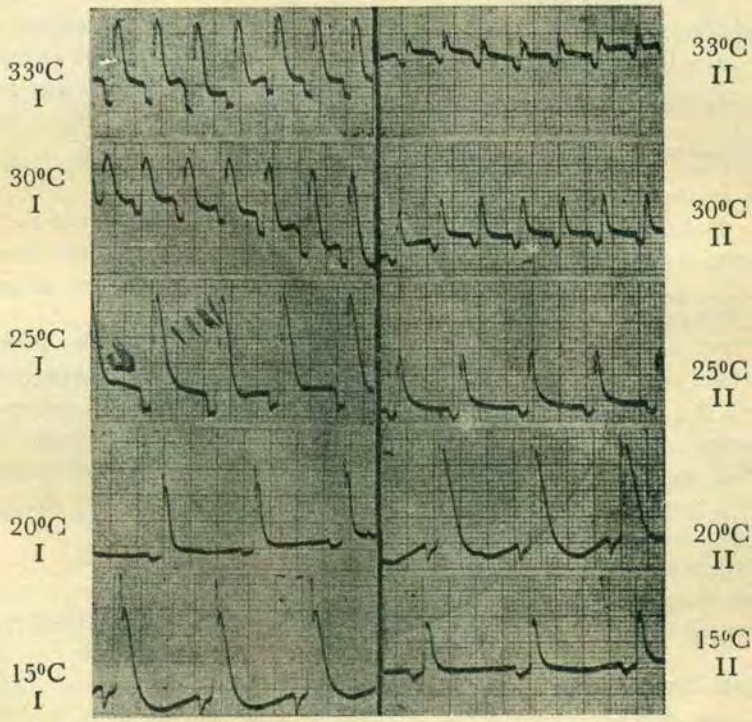

Fig. 1.

QRS Complex and QT Interval :—The begining of the ventricular depolarization was usually marked by a sharp upstroke. The end of depolarization could be made out by return of the stylus to the baseline. The QT interval was measured between these two phenomena. Its mean initial value in 12 experiments at 36° to 37°C. was 0.097 sec. (S. E. ± 0.0069) and in 24 experiments at 35° to 33°C. it was 0.114 sec. (S. E. ± 0.0066). It was not always possible to measure the QRS complex separately for reasons mentioned below.

ST Segment and T Wave:—In small mammals it is difficult to discern a separate T wave in the electrocardiogram due to overlapping of the depolarization and repolarization processes (Hundley *et al*, 1945; Lombard, 1952; Richards *et al*, 1953). It is usually characterized by a small notch following directly the downstroke of the R wave, often before this has reached the base line. In our experiments a distinct "T notch" was often replaced by a gradual return of the stylus to the baseline, in sharp contrast to the rapid deflections of the QRS complex. Hence it was not possible to measure separately changes in the QRS complex, the ST segment and the T wave.

Perfusion of the heart at a constant temperature.

Six hearts were perfused at constant temperature (37° to 32°C) for one hour. The volume of the coronary outflow per minute, the cation content of the perfusate and the electrocardiographic pattern checked at regular intervals showed no significant changes during this period, which was longer than that required for an average experiment.

Electrocardiographic changes during hypothermia.

Table I summarises the changes in the rate, the PR interval and the QT interval on cooling, in 40 experiments. It also shows the percentage changes in the volume of the coronary outflow per minute and its potassium content.

As the temperature was lowered the hearts showed a progressive slowing, along with increase in the PR and QT intervals (Fig. 1). In 13 experiments the PR interval could not be measured at temperatures below 25°C either due to absence of the P wave or the development of atrio-ventricular block. Distinct intraventricular block at various temperatures below 25°C. was observed in 24 experiments. Out of 13 hearts cooled below 17°C., 3 hearts stopped between 14° and 16°C. but could be revived on rewarming.

The T wave tended to separate out on cooling. The ST segment showed no consistant changes. It should be emphasised that ventricular fibrillation was not observed in any of the experiments.

The coronary out flow and cation content of the perfusate on cooling.

The volume of the coronary outflow per minute diminished progressively with the fall in temperature (Table 1).

The perfusate samples collected at different temperatures were analysed for their contents of sodium, potassium and calcium in 10, 17 and 12 experiments respectively.

TABLE I

Changes in the Electrocardiogram, Coronary outflow per minute and Potassium content of the perfusate of the isolated perfused Rat's Heart During Hypothermia

	37°—36°C	35°—33°C	32°—30°C	29°—27°C	27°—24°C	23°—21°C	20°—18°C	17°—15°C	14°—12°C
Rate	254	219	165	137	104	75	52	44	52
± S. E.*	11.7	4.7	17.8	20.3	9.0	9.9	5.0	7.6	—
n **	12	24	10	6	17	10	22	12	2
P R	0.038	0.049	0.062	0.085	0.090	0.110	0.150	0.150	0.170
± S. E.	0.0017	0.00039	0.0054	0.0274	0.0007	0.0118	0.0155	0.0176	—
n	12	24	10	6	16	7	18	7	2
Q T	0.097	0.114	0.155	0.148	0.304	0.657	0.703	0.606	0.580
± S. E.	0.0069	0.0066	0.0195	0.0173	0.0324	0.1345	0.0660	0.0678	—
n	12	24	10	5	17	11	22	10	2
% Fall in Cor. outflow Per min.	0	0	8.5	—	13.5	—	28.7	34.3	—
± S. E.	—	—	4.0	—	2.7	—	4.3	3.6	—
n	14		10	—	14	—	9	12	—
% Fall in 'K' Content of Perfusate	0	0	1.08	1.50	2.31	1.25	3.67	4.42	4.30
± S. E.	—	—	0.41	—	0.65	—	1.03	0.81	—
n	17		9	3	17	2	11	13	2

* S. E. = Standard Error
** n = Number of observations.

TABLE II

Comparision of Electrocardiograms of the Isolated heart during cooling and warming.

	35° — 33°C		32° — 30°C		26° — 24°C		20° — 18°C	
	Cooling	Rewarming	Cooling	Rewarming	Cooling	Rewarming	Cooling	Rewarming
Rate	211	180	148	153	102	124	72	70
± S. E.	7.9	12.9	16.0	20.1	12.2	6.7	7.7	8 3
n	8	8	6	7	8	7	8	6
P R	0.045	0.050	0.066	0.070	0.100	0 080	0.123	0.140
± S. E.	0.0025	0.0100	0.0063	0.0123	0.0118	0 0077	0.0105	0.0142
n	8	8	6	7	8	7	8	5
Q T	0.120	0.130	0.160	0.150	0.260	0.190	0.390	0.280
± S. E.	0.0145	0.0187	0.0179	0.0247	0.0458	0.0142	0.0414	0 0985
n	8	8	6	7	8	7	8	6

The sodium or calcium content of the perfusate showed no significant change on cooling. As contrasted with this the potassium content showed a definite fall which was related to the drop in temperature of the perfusing fluid (Fig. 2). The results were statistically significant (Cor. Coeff. = −0.825: $P < 0.05$)

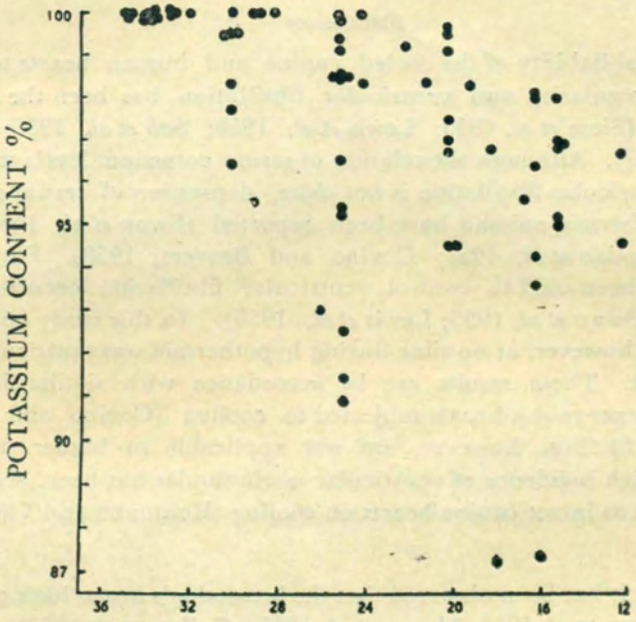

Fig. 2.

Effect of rewarming.

In 8 experiments the perfusion fluid was rewarmed and similar observations were made on the electrocardiogram, the rate of coronary outflow and its cationic contents at various temperatures.

Table 2 shows the progressive return of the electrocardiographic values to normal levels. The various figures on rewarming havs been compared with corresponding ones, from the same hearts, at identical temperatures, during cooling. The two sets of values are not significantly different ($P > 0.1$).

The volume of the coronary outflow per minute and its potassium content also returned to the initial levels.

Perfusion of fluids with Altered Potassium content

In some experiments the cooled hearts were perfused with fluids containing amounts of potassium higher or lower than normal for about 20 mins.

the electrocardiogram being recorded before and after changing the fluid. The high potassium fluid perfused in 6 experiments contained 1¼ to 2 times the normal amount of potassium. The low potassium fluid perfused in 7 experiments contained 0 to ½ times the normal amount. Comparision of the results showed no significant alterations in the electrocardiographic pattern of the hypothermic heart after either of these procedures.

DISCUSSION

The special liability of the cooled canine and human hearts to develop ventricular irregularity and ventricular fibrillation has been the subject of much enquiry (Siem *et al*, 1955; Lewis *et al*, 1956; Sen *et al*, 1956; Fleming and Muir, 1957). Although the relation of serum potassium levels to the production of ventricular fibrillation is not clear, depression of serum potassium levels in hypothermic animals have been reported (Swan *et al*, 1953; Lewis *et al*, 1956; Gollan *et al*, 1957; Covino and Beavers, 1958). Furthermore potassium has been used to control ventricular fibrillation occuring during hypothermia (Swan *et al*, 1953; Lewis *et al*, 1956). In this study on the rat's isolated heart, however, at no time during hypothermia was ventricular fibrillation observed. These results are in accordance with similar findings in isolated and intact rabbit hearts subjected to cooling (Covino and Beavers, 1958). These findings, however, are not applicable to higher mammals. Thus a very high incidence of ventricular arrhythmias has been reported in isolated as well as intact canine hearts on cooling (Reismann and Van Citters, 1956).

Previously it has been observed that the intact dog's heart loses potassium on cooling (Olsen *et al*, 1955: Mavor *et al*, 1956; Gollan *et al*, 1957). But the cooled isolated perfused rat's heart does not lose any potassium (Taylor, 1955). Our studies indicate that the hypothermic isolated rat's heart takes up extra potassium, without any change in the sodium or calcium contents of the perfusate. This change in potassium content was related to the fall in temperature. Furthermore the progressive increase in corrected QT interval—($QTc = \frac{QT}{\sqrt{C}}$, where C represents the cycle length) correlates well with percentage fall in potassium content of perfusate (Fig. 3; Cor. Coeff = 0·619, $P > 0{\cdot}05$). This is in contrast with the loss of intracellular potassium and shortening of the corrected QT interval in hearts treated with cardiac glycosides, (Rayner and Weatherall, 1957; Vick and Kahn, 1957; Wood, 1957). The decreased sensitivity of the heart to toxic doses of digitalis observed previously in cats under hypothermia might have been due to a similar change in potassium content of the heart (Satosker and Trivedi, 1956). This shift in potassium therefore, could explain the absence of ventricular fibrillation in cooled isolated rat hearts. It should be noted that the fall in

serum pH and potassium which have been implicated in the genesis of ventricular fibrillation in hypothermic dogs was also found to occur in hypothermic rabbits which did not develop ventricular fibrillation on cooling (Covino and Beavers, 1958). However, the possibility of a similar shift of potassium occuring in the cooled rabbit heart cannot be ruled out. A species difference may also account for the variation in cardiac response in different animals subjected to cooling.

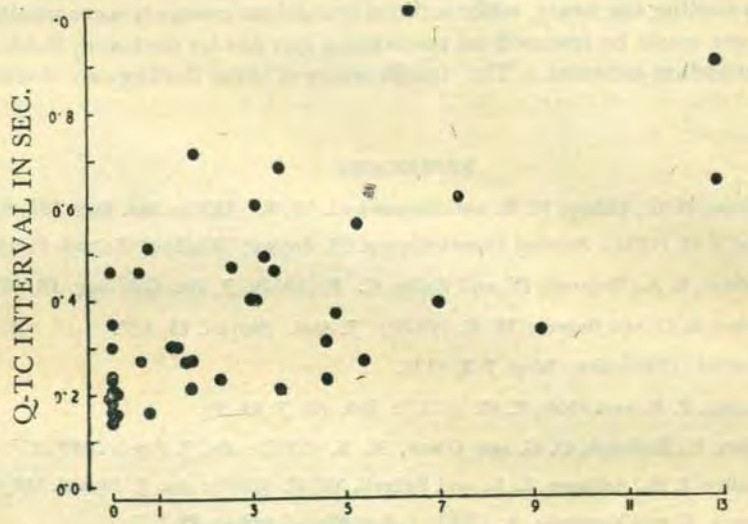

Fig. 3

The results from the experiments in which the cooled hearts were perfused with fluids containing increased or decreased amounts of potassium indicate that the rat's heart is refractory to the effects of these, at low temperatures.

Most of the hearts that were cooled below 25°C. developed some type of conduction defect but no ventricular fibrillation. These changes were reversible on rewarming as seen from the electrocardiograms and restoration of the coronary outflow per minute and its potassium content to initial levels. This agrees with the findings of Covino and Beavers in rabbits (Covino and Beavers, 1958).

These studies on the isolated heart indicate that the changes following hypothermia are not directly dependent on any extrinsic humoral or nervous factors.

SUMMARY

The effect of cold on the perfused isolated rat heart was studied by recording the electrocardiogram, the coronary outflow per minute and the changes in the cation content of the perfusate. The electrocardiogram showed progressive slowing of the heart rate and prolongation of the PR and QT intervals with fall in temperature. Ventricular fibrillation did not occur in any of the hypothermic hearts. The rate of coronary outflow fell progressively with the temperature. The potassium content of the perfusate also diminished on cooling the heart, while sodium or calcium contents were unaltered. The changes could be reversed on rewarming but not by perfusing fluids with altered potassium contents. The implications of these findings are discussed.

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