OBSERVATIONS ON ISOLATED HEART AT LOWERED TEMPERATURE

By

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Since the advent of artificial hibernation much work has been done to explore possibilities of using induced hypothermia in surgical procedure. In fact the early work by Bigelow *et al* (1950) gave an impetus to study the possible physiological changes that are produced in many vital organs subjected to cooling.

Various ograns under condition of hypothermia undergo a marked change in their response, both to physiological and pharmacological stimuli although the exact mechanism underlying this alteration yet remains unexplained. As a result of more generalised use of induced hypothermia as an adjuvant in cardiac surgery, it was noted that lowering of body temperature predisposes to cardiac arrhythmias and surgical shock. Jouvenelles *et al* (1954) observed a definite involvement of the heart during cooling, and it was a common experience of the workers in the field that one of the real dangers in hypothermia was the tendency on the part of myocardium to go into ventricular fibrillation.

Swan and his associates (1954) tried to implicate factors like lowering of serum potassium, accumulation of $Co_2$and thus a rise of *Ph*, and haemoconcentration to explain the altered reactions of myocardium at lowered temperatures. However there was no unanimity of opinion on the biochemical changes produced by cooling. Thus Bigelow *et al* (1950) and Elliot and Crimson (1947) actually noted in their experiments a rise of serum potassium. Lewis *et al* (1956) tried to study extensively the biochemical changes in dogs under conditions of hypothermia and the possible role of potassium, glucose, ventillation and haemoconcentration in prevention of cardiac arrhythmias. They noted a marked haemoconcentration and loss of plasma albumin, associated with a regular depressions of plasma potassium levels. Lars Erik

Gelin and Lofstrom (1954) think that one of the important problems in hypothermia is a failure of capillary circulation.

Satoskar *et al* (1956) working on digitalis found that there was an alteration in the reaction of myocardium under hypothermia to the normal dose of digitalis.

Rindani and Merchant (1957) have brought out interesting evidence that cold perfused heart of frogs and dogs did not show the normal phenomena of inhibition to vagal stimulation and to acetyl choline administration.

Since there is an accumulating evidence pointing to the altered behaviour pattern of myocardium at lowered temperatures we decided to investigate the responses of an isolated mammalian heart severed from the other circulatory and nervous influences at lowered temperatures especially with respect to electrolyte imbalances.

METHODS AND MATERIAL

All experiments were performed on rabbits bred in our own Laboratory. A few observations were also made on guinea-pig heart. Rabbits weighing from 1.5 to 2.5 kg. were used. Each Rabbit was killed by a blow on the head, the chest was opened and heart excised. It was dipped in Ringer solution and gently squeezed to remove blood from the ventricles. Aorta was dissected from its attachments to the plumonary artery and was then tied on to a cannula. The heart was perfused with an oxygenated Ringer solution at a constant head of pressure (70 mm. of Hg) and at a temperature of 34-35°C.

A record was obtained of the amplitude of ventricular contraction by fixing a small bent entomological pin in the tip of the ventricles and this was tied over a system of pulleys to a Starling heart lever which recorded the amplitude and rate of contraction of ventricles.

The rate of perfusion was adjusted initially to 80-85 drops per minute, as the heart was found to beat normally at this rate of perfusion. The rate was initially adjusted by a screw clamp placed immediately above the perfusing cannula.

After obtaining a record at normal temperature the perfusing fluid was cooled gradually by putting ice or ice-salt freezing mixture around the coils. The reaction of the heart was noted at various lowered temperatures.

Drugs like ACh., adrenaline and eserine were injected initially and at lowered temperatures to note their effects and alterations in effects, if any.

The volume of fluid injected did not exceed ½-1 cc. and the dose varied from 5 to 10 micrograms. The drugs were injected very slowly so as not to cause any marked alteration in the hydrostatic pressure and temperature.

In most of the experiments when the heart came to a stand still the reaction of the heart to modified perfusing fluids was studied. The fluid was modified by changing the composition of electrolytes, especially that potassium and calcium. The modified fluid was perfused by means of another reservoir at the same head of pressure as the initial and at the same rate of perfusion. The oxygenation of the modified Ringer was done as before. The drugs were also studied for their effect on myocardium perfused with modified Ringers.

In all, three groups of experiments were performed.

The first group, consisting of six experiments, comprised a study of the behaviour of heart at lowered temperatures. The effects of drugs were noted at normal temperature and then again when the heart ceased to beat at lower temperature. In two experiments drugs were injected at intermediate temperatures to note the effects, if any. In all the cases heart was revived by raising the temperature.

In the second group, consisting of four experiments, effect of potassium chloride solution injected or perfused in various concentrations was noted after the stoppage of the heart at low temperature.

Third group of four experiments were performed to study the effect of high or low calcium Ringer. In three, heart was revived at low temperature by perfusing ¼ calcium ringer.

In four experiments of the first group, coronary flow was noted before and after cooling, and was not found to alter significantly and therefore was not noted in subsequent studies.

RESULTS

The heart which beats normally at 36°C, is found to show a progressive diminution in force of contraction as the temperature is lowered below 36°C and also there is a lowering of the rate. At 22°C only ventricular beats are seen and there is a marked brady-cardia. The heart stops in diastole at 20°C and in some cases at 19.5°C. (Figure 1). At this stage 5 and 10 micrograms of adrenaline, acetyl-choline or eserine fail to produce any effect. We have noted a similar behaviour of the isolated heart of a guinea pig.

In a few experiments, we have studied the effects of adrenaline and acetyl-choline at intermediate temperatures. Thus we noted that response

of the heart to adrenaline or ACh did not alter qualitatively at 24, 23 or 22°C, (Figure 2).

Since the heart stopped faithfully between 19.5 and 20°C, we studied the effects of potassium chloride on the mycardium at lowered temperature. Heart which is arrested at 19.5°C fails to respond to an injection of 1 ml. of normal saline but 1 millilitre of potassium chloride (5 milliequivalent of K/litre) produces a powerful contraction with a prolonged diastolic relaxation. (Figure 3). The same effect can be demonstrated repeatedly by administration of half ml. of the same strength of potassium chloride solution;

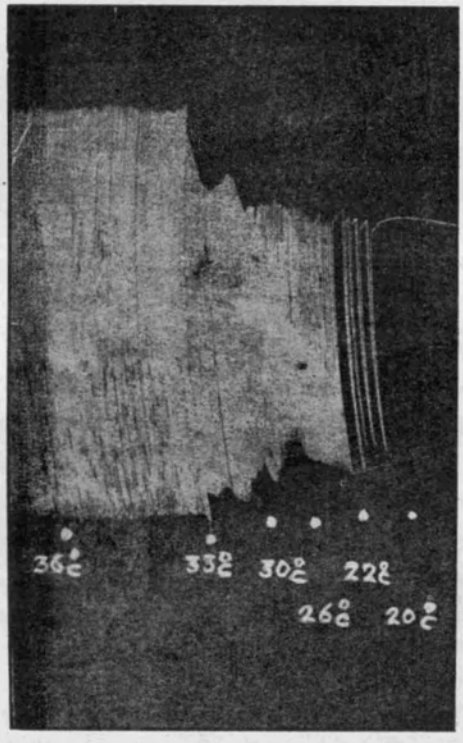

Fig. 1. Progressive diminition in force rate of heart as temperature is lowered. The heart stops in diastole.

5 to 10 mcg. of adrenaline or acetly-choline injected before or with potassium chloride fail to produce the drug effect, although potassium chloride effect is seen (Fig. 4 and 5).

We have noted in most of our experiments that the myocardium which has ceased to function at 20°C is capable of beating regularly and with a sufficient force of contraction when the temperature is brought back to 34°C,

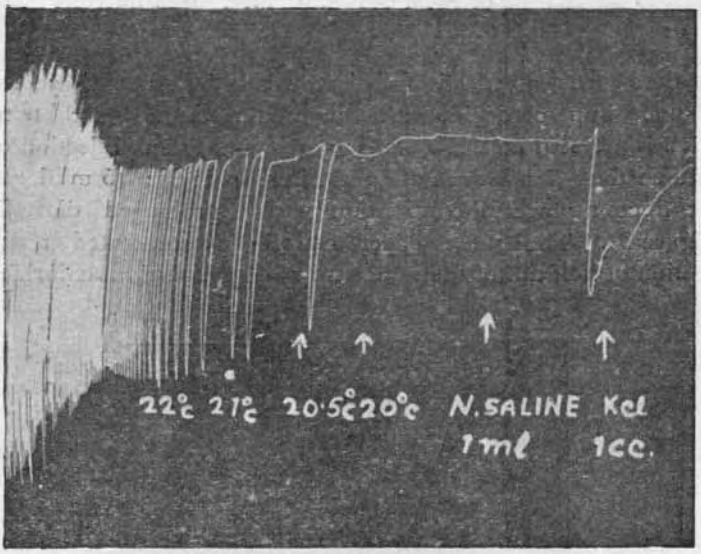

Fig. 2. Response of heart to adrenaline and Ach. at lowered temperature.

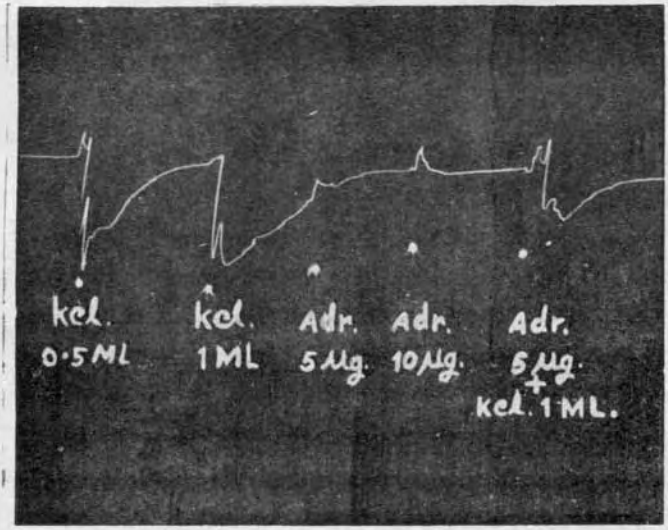

Fig. 3. Effects of Kcl. on myocardium at lowered temperature.

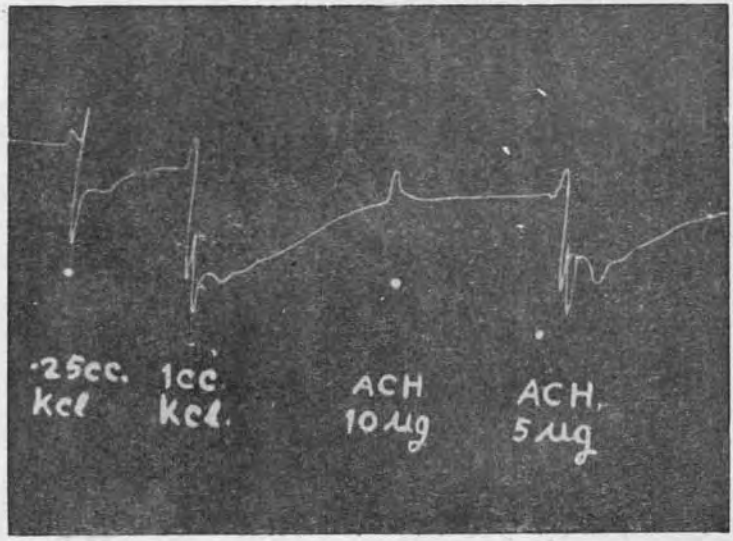

Fig. 4. Effects of Ach. on heart injected before or with Kcl.

proving very vividly that there is only a cessation of the physiological function and there is probably little organic damage done as a result of artificial hypothermia. In all our experiments the period of cessation of cardiac activity has ranged from 10 minutes to ½ hour and inspite of this period of

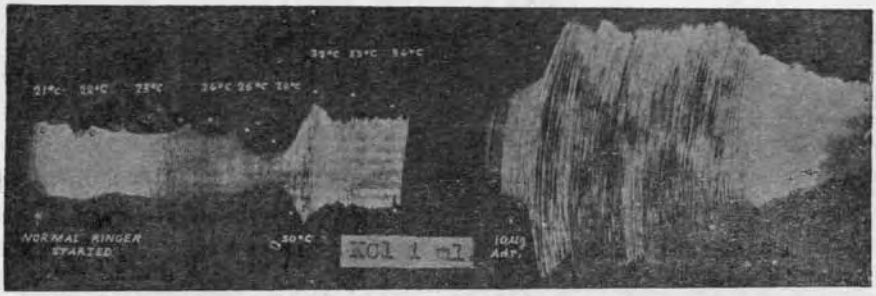

Fig. 5. Effects of adrenaline on myocardium injected before or with Kcl.

inactivity, it has been possible to revive the heart. This is well illustrated in Fig. 6, in which the cooled heart has been revived by raising the temperature back to normal. It can be seen further that 0.5 ml. KCl (5 m. Eq. k/per litre) produces a diastolic arrest of the myocardium at normal temperature, and the heart revives after the drug is washed off. Further, 10 micrograms of. adrenaline produces a very powerful increase in rate and force of contraction

In fact we feel that the myocardium becomes a little more responsive to the effect of adrenaline when previously subjected to the influence of potassium in such a preparation.

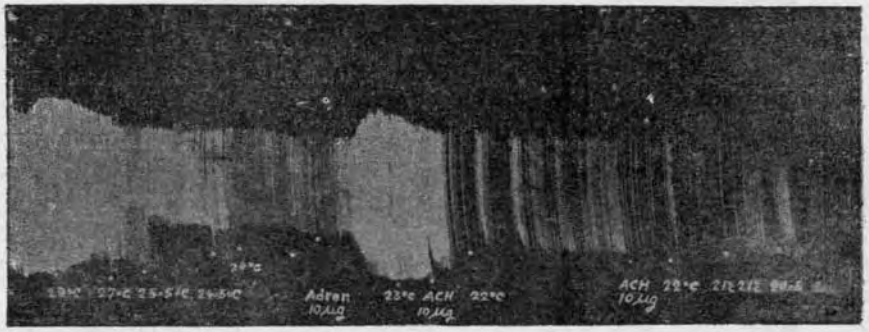

Fig. 6. Cooled heart revived by raising the temperature back to normal.

Since it was found that administration of KCl produces a change in the myocardium at lowered temperature we decided to study the effects of hyper-potassium Ringer. Thus Ringer solution containing potassium varying in strength from 0.5 M. Eq. to 5 M. Eq. per litre was perfused continuously after the heart ceased to contract under hypothermia. In spite of perfusing KCl solution the heart fails to revive at low temperature. It was also noted that 25 micrograms of digoxin, 10 micrograms of adrenaline, 20 micrograms of acetyl-choline and 10 micrograms of eserine all fail to produce any action

Fig. 7. Revival of heart under the influence of 1/4 Calcium Ringer.

inspite of the presence of hyper - potassium. Further such a heart fails to revive with perfusion of Normal Ringer when the temperature is slowly raised to 34°C. This clearly proves that hyper-potassium when perfused continuously at low temperature results in permanent damage to the myocardium.

Since increased potassium was not effective we tried to study the effect of relative changes brought about in potassium concentration when calcium content of Ringer solution was lowered to one fourth of normal. We noted that the heart which ceases at 19.5°C is revived under the influence of 1/4 Calcium Ringer. Initially the beats are irregular but later on Ventricles start beating regulary at 19.5°C, (Fig. 7).

Although the heart continues to beat, it does not respond to adrenaline or ACh in as high a concentration as 10 mcg/ml. It is further observed that the heart which started beating with 1/4 calcium Ringer, stops at 19.5°C, when perfused with normal Ringer. This heart could be revived by raising the temperature. It reacts to acetyl-choline in a normal way and also to adrenaline at higher temperatures. The heart can be made to stop at low temperature, revived with 1/4 calcium and made to stop again when perfused with normal Ringer at 19.5°C.

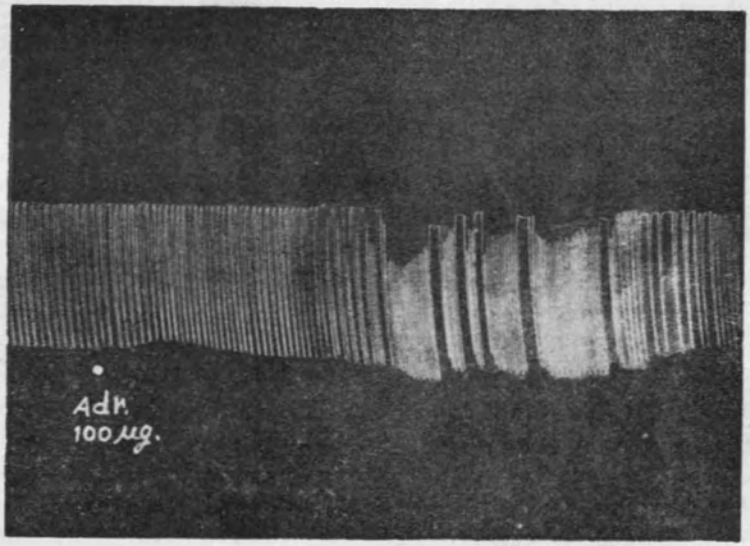

Fig. 8. Response to adrenaline of heart at 19.5°C perfused with 1/4 Calcium Ringer.

This was noted in two experiments. Further we found that the heart which was beating at 19.5°C perfused with 1/4 calcium Ringer, responded to 100 mcg. of adrenaline, by a little acceleration and irregularity of rhythm. (Fig. 8). Thus, under these conditions, the myocardium did respond to higher concentration of adrenaline.

DISCUSSION

We have confirmed that hypothermia produces a change in the reaction of the myocardium to pharmacological stimuli. In this connection it may be pointed out that the mammalian heart continues to respond to acetylcholine even at 22°C. The heart stops everytime at 20 or 19.5°C. Cold produces bradycardia progressively and heart comes to a standstill in diastole. We have not seen ventricular fibrillation in any of our experiments. In fact, even after the heart ceased to beat or at higher temperature, there was an absence of arrhythmia inspite of injection of 10 mg. of adrenaline. This aspect is quite interesting in view of the fact that in an intact animal one of the dangers of hypothermia is the precipitation of ventricular fibrillation.

It is pertinent to note that the heart which was made to stop under hypothermia, revived on raising temperature and continued to beat powerfully. It also responded to pharmacological stimuli. Thus it seems that during the period of observation, which ranged from 10 minutes to a little over half an hour, there were little irreversible changes in the myocardium. We have not done any histopathological studies. But we feel that the changes produced due to cooling, which resulted in the altered behaviour of myocardium, are probably temporary and could be easily reversed by altering the environmental conditions.

The altered behaviour of myocardium at low temperature is thought to be due to changes in electrolyte concentration. We found that injection of potassium chloride, brought about a quick contraction under hypothermia. This effect is opposite of what is seen at a high temperature. This reversal of response may be due to altered polarity or changed permeability of myocardium. Further studies on electrolyte concentrations and E.C.G. are in progress. We have been able to revive the ventricular beats at low temperature by perfusing low calcium Ringer. Whether this effect is due to optimum relative changes in potassium concentration or a change in membrane permeability, is difficult to assess. In fact we found that under these conditions heart responded to larger doses of adrenaline. We are trying to study various factors like electrolyte changes, and gaseous tensions in perfusing fluids, so as to establish optimum conditions required to revive a mammalian heart that stops at low temperature.

SUMMARY

(1) Effect of lowering the temperature on the isolated heart of rabbits and guinea-pigs has been studied. Isolated heart stops beating at 20°C or 19.5°C and can be made to restart by raising the temperature.

(2) Drugs like acetylcholine, adrenaline and eserine continue to act normally at as low temperature as 22°C. They fail to produce any action after the heart stops.

(3) Hyper or Hypo-potassium ringer perfused continuously at low temperature after the arrest of the heart fails to produce the revival of the heart at low temperature or when the temperature is raised.

(4) Continuous perfusion with low calcium ringer revives the heart beats at low temperature and the myocardium responds under this condition to action of adrenaline in a large dose of 100 mcg.

(5) Further work on electrolyte composition, E.C.G. and gascous tensions is in progress with a view to assess the relative importance of these factors in reviving the heart after it stops beating at low temperature.

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