Effect of Temperature on the Heart Rate, Electrocardiogram and Certain Myocardial Oxidations of the Rat

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Deep hypothermia in the rat was studied with respect to the sequential changes in body temperature, heart rate, and electrocardiogram. In vitro assays of myocardial metabolic activity were made at temperatures ranging from 5 to 35 C. These assays strongly suggested that the in vivo cardiac dysfunctions observed at low temperatures were attributable to shifts in temperature kinetics of enzyme systems at 20 to 21 C.

In a recent investigation of the in vitro metabolism of ventricular tissue from hypothermic rats, no irreversibly damaging effects were observed. Instead, lowering the body temperature to 15 C led to an increased endogenous metabolism when subsequently measured at 38 C. It was concluded that the only metabolic effect of hypothermia on the rat heart was a possible mild hypoxia and an increased permeability to substrates. It was conceived that the hypoxia led to an accumulation of endogenous substrate and subsequently to an increased endogenous respiratory rate.

During a review of the literature for the study just mentioned, 2 observations were made. The first was that little use has been made of the laboratory rat for deep hypothermic heart studies, and the second was that little effort has been made to study the metabolism of the mammalian heart tissue while it is under the influence of hypothermia.

As a result of these observations the experiments to be described here were undertaken with 2 purposes in mind. The first was to outline generally the response of the rat to standardized hypothermia. In this regard, heart rates, cooling times, and electrocardiographic measurements were made. The second purpose of these experiments was to assay the response of certain aerobic-enzyme systems to various temperatures and to determine whether there were any relationship between in vitro metabolic activity and in vivo myocardial response to hypothermia.

METHODS

Male rats of the Sprague-Dawley strain weighing between 300 and 375 grams were used in all experiments. They were maintained on a diet of "Friskies" dog food and water, fed ad libitum, for at least a month prior to experimentation.

In experiments where hypothermia of the intact animals was studied the rats were first anesthetized by intraperitoneal injection of sodium thiopental (40 to 60 mg/Kg. body weight). Following administration of the anesthesia, the rats were tied in a prone position with legs extended to a small board placed at an angle of about 30 degrees from the horizontal. This board was then placed in an ice and water bath of 1 to 3 C. The level of immersion was adjusted to extend from just under the forelegs and lower chest to over the lumbar region of the back. Temperature was recorded by a "thermistor" rectal probe carefully inserted to a depth approximating that of the caudal parts of the liver. Care also was taken to place this probe as near as possible to the core of the animal, as preliminary experiments utilizing intraventricular thermocouples had shown that this position gave a close approximation of heart temperatures in deep hypothermia.

Electrocardiograms were recorded on a Sanborn Visocardiette, using the number I lead position and a tape speed of 25 mm/sec. The leads were adapted to take no. 20 syringe needles. These were inserted just under the skin on either side of the chest. In addition, when a continuous record of heart rate throughout the whole hypothermic episode was desired, electrocardiograms were made on a Sanborn model no. 150 recorder set at tape speeds ranging from 10 mm/sec. to 1 mm/sec.
In experiments where ventricular metabolic rates were determined, normothermic animals were first killed by a blow on the head and the heart quickly excised and chilled in chopped ice. The atria and A-V septums were trimmed away from the ventricles and the excess blood was pressed out. Following this, the ventricles were weighed on a microtorsion balance and placed in a Potter-Elvehjem type of homogenizer. An ice-cold, 10 per cent homogenate was prepared in a medium consisting of 0.25 M sucrose, 0.0001 M ethylene diamine tetra-acetic acid (pH 7.4) and 0.01 M Tris (hydroxymethyl) amino methane buffer (pH 7.4). After filtering through 4 layers of cheese cloth to remove any large tissue fragments the homogenates were diluted to 3 1/3 per cent with cold homogenizing medium. One-milliliter aliquots were taken from the final dilution and placed in the reaction vessels for the metabolic rate measurements.

The incubation medium for measuring metabolic rates had the following constituents: 0.002 M adenine-triphosphate (pH 7.4), 0.006 M MgCl₂, 1.5 × 10⁻⁵ M cytochrome c, 5 × 10⁻⁴ M diphosphopyridine nucleotide, 0.01 M K-phosphate buffer and 0.01 M succinate, or a mixture of 0.01 M pyruvate and 0.01 M malate. Substrates, where used, were in the form of the potassium salts. The final volume of the medium and homogenate in each reaction vessel was adjusted to 3.0 ml. with 0.25 M sucrose. Isotonic conditions were thus approximated.

Following a five-minute thermaequilibration period, incubations were conducted for 30 min., according to standard manometric procedures. The gas phase in each reaction flask was air. Carbon dioxide was absorbed by 0.2 ml. of 5N NaOH placed in the center well along with a folded piece of filter paper. In a series of separate experiments, oxygen consumption was measured at temperatures of 5, 10, 15, 20, 25, 30 and 35 C. The arithmetical average of 3 animals was used to establish each point.

Metabolic rate was calculated over five-minute intervals during the course of incubation and is expressed as μl oxygen consumed per mg. of tissue. In addition, the respiratory rates over 30 min. of incubation were used for Arrhenius-van't Hoff plots and the calculation of temperature-velocity constants.

These constants were calculated according to the following formula:

\[ \ln \left( \frac{k_1}{k_2} \right) = \frac{\mu}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right) \]

where \( \mu \) represents the temperature-velocity constant, \( k_1 \) and \( k_2 \) the reaction rates at absolute temperatures \( T_1 \) and \( T_2 \) respectively, and \( R \) the gas constant in calories.

RESULTS

Hypothermia in the Intact Rat

The first series of experiments was designed to characterize some of the effects of hypothermia in the rat. Of particular interest were the body cooling rates under a standardized immersion, the changes in heart rate during the course of hypothermia and some of the electrocardiographic anomalies exhibited by the animals.

Figure 1 gives a summary of the data on average body-cooling rates gained from 9 animals under the conditions described (see ‘‘Methods’’). In this figure, curve A shows the average time to cool from a body temperature of 35 C. (cooling occurred a few minutes after immersion in the 1 to 3 C. bath) to various body temperatures as low as to 15 C. Standard deviations of the mean are shown at 5 degree intervals during the course of cooling. Curve B in figure 1 indicates the differential cooling rate of these rats in min./degree fall in body temperature from 35 to 15. Here it can be observed that very early in the cooling process, i.e., while the body temperature is between 35 and 34, there is a relatively slow rate of cooling. This is followed by a sharp increase as the body temperature reaches about 30 C. Thereafter the differential cooling rate becomes progressively slower as body temperatures approach 15 C. Curves
TEMPERATURE AND MYOCARDIAL OXIDATIONS

35 30 25 20

BOOY TEMPERATURE

Fig. 2. Changes in heart rate of lightly anesthetized rats, A, and deeply anesthetized rats, B, during hypothermia.

$\text{A and B taken together, therefore, characterize the nature of the cooling process in the adult Sprague-Dawley rat.}$

The second characteristic studied in the hypothermia rat was heart rate. The results of this study are presented in figures 2 and 3. In figure 2, curve A gives the changes in heart rate in the lightly anesthetized, violently shivering animal. These data were taken from 3 individuals with the range of the 3 values at various body temperatures indicated by vertical lines. Curve B gives the average heart rates of 8 deeply anesthetized animals during the course of hypothermia. Here the vertical lines represent the standard deviations of the means. The obviously more scattered data in the latter curve are felt to be a reflection of the absolute depth of anesthesia in these animals. In agreement with this was the observation of occasional mild shivering in some of the animals used in obtained data for curve B.

In both curves of figure 2, it is important to note the sharp decrease in heart rate at body temperature of 20 to 21 C. The implications of this change will be noted later in relation to the alterations in myocardial metabolic rates at low temperatures.

Following the break at 20 to 21 C, the heart rate, aside from being much lower, became quite erratic, showing considerable variability from animal to animal. Since there were unpredictable degrees of A-V blockade and arrhythmia, rates beyond this point were calculated from only the ventricular portions of the electrocardiograms. Some impression of the variability in the ventricular rate from the breaking point onward can be gained from figure 3. Here, the rates of 2 typical animals are plotted at 0.2 C. intervals from body temperatures of 22 down to 13. These plots, although giving a fairly accurate indication of the break in the rate curve, still do not give a completely true picture of the abnormalities at lower temperatures. This is because the plotting interval of 0.2 degree used here is too coarse to reveal the finer details of the rate fluctuations. In addition, at times it is virtually impossible to tell from an electrocardiographic tracing whether all of the fluctuations actually represent contractions.

Some indications of the difficulty encountered in making rate measurements at lower temperatures can be seen in figure 4, showing electrocardiographic changes typical of those found in the rat. They were recorded at the various body temperatures indicated. Besides the variability of the ventricular rate at temperatures below 20 to 21 C., shown by these tracings, other abnormalities include: shivering patterns at temperatures down to 21, variable degrees of A-V blockage, abnormal and quite often bizarre ventricular complexes, and occasional ventricular tachycardia. Ventricular fibrillation was not observed.
Myocardial Metabolism

The second series of experiments in the present study was concerned with the in vitro activity of certain myocardial enzyme systems at different temperatures. The results of the first of these experiments is shown in figure 5, where succinate oxidation was measured at 5 degree intervals between 5 and 35 C. In the left-hand portion of this figure the series of curves shows the oxygen consumption of the tissue at the various incubation temperatures. As would be expected, the rate of succinate oxidation increases with temperature. In the right-hand portion of the figure an Arrhenius-van't Hoff plot of the data has been made. That is, the logarithm of the rate of oxidation has been plotted against the reciprocal of the absolute temperature. In viewing this plot it should be remembered that temperature decreases from left to right, each point being 5 degrees from its neighbor.

In these data, a sharp break in the rate of oxidation occurs at a temperature of 20 to 21 C. Above this, the temperature-velocity constant indicated by A has a value of 8,490 calories whereas below this point, indicated by B, the constant has more than doubled to a value of 19,960 calories. In other words, what is sometimes called the "activation energy" of the system required for succinate oxidation shows a sharp increase at temperatures lower than 20 to 21 C.

Another, and perhaps more familiar, way of viewing these data is from the standpoint of the Q10 values. When these are calculated, portion A of the Arrhenius plot has a value of 1.59 and portion B has a value of 3.33. Thus the change in rate of succinate oxidation per 10 degree change in temperature at least twice as great below the 20 to 21 transition point as above it.

Similar experiments utilizing other sub-
strates also revealed sharp breaks in the temperature-velocity constant curves in the neighborhood of 20°C. However, in these cases the Arrhenius-van't Hoff plots were curvilinear below the critical point, with a trend toward gradually increasing temperature-velocity constants as the incubation temperature became lower. This will be observed in figure 6, where the rate of endogenous respiration is depicted. For convenience, since, the curve below the break does not yield a single value, the lower portion was divided into 2 sections: one, from 20 to 10 is signified by the letter B; the other, from 10 to 5 by the letter C. From this, temperature-velocity constants were calculated to be 13,520 calories for A, 27,580 calories for B, and 34,920 calories for C. Expressed as Q10, A would be 2.08; B, 5.24; and C, 9.17.

Figure 7 shows data gained from another of these experiments. Here the respiratory rate is seen measured as it might occur under conditions of carbohydrate metabolism, that is, in the presence of pyruvate, an end product of glycolysis, and malate, a component of the tricarboxylic acid cycle. Under normal conditions pyruvate is converted to acetyl coenzyme A and this in turn is condensed with oxalacetate to form citrate. In the present experiment, malate was chosen in preference to oxalacetate because of the chemical instability of the latter. In living systems the more stable malate is rapidly converted to oxalacetate thus assuring an adequate substrate level for the condensation reaction with acetyl coenzyme A.

In the Arrhenius-van't Hoff plot of the right of figure 7 it can be seen that the temperature response of the tissue in the presence of these 2 substrates resembles that observed previously, when only endogenous respiration was measured. In fact, the temperature-velocity constants were quite similar: 10,170 calories for A; 26,300 for B; and 36,570 for C. This corresponds to Q10 values of 1.74, 5.02 and 10.02 respectively. And again, there is a marked shift in the temperature-velocity constant at the breaking point near 20°C.

**DISCUSSION**

The experiments reported here have shown that the response of the rat to hypothermia is quite similar in many respects to that observed in larger mammals, and in many respects similar to the responses in rats previously observed by Crismon. Accordingly, the fall in body temperature during a standardized immersion procedure, although somewhat more accelerated than that observed in animals such as the dog, follows the same general pattern; i.e., a relatively slow initial decline which appears to correspond with the "summit metabolism" discussed by Kayser. This is immediately followed by a more rapid fall until body temperature reaches 30 to 31°C, whereupon the rate of decline gradually decelerates as hypothermia progresses. A second similarity between the larger mammals...
and the hypothermic rat is the behavior of the heart rate. The rate, although not following exactly the van't Hoff-Arrhenius rule as reported by Kayser and Meda, nevertheless was shown to exhibit a sharp break in the neighborhood of 20 to 21 C. This appears to be in approximate agreement, from the standpoint of temperatures, with the onset of severe bradycardia and fibrillation observed in the dog, but a little lower than reported for the guinea pig.

Finally, the electrocardiogram of the rat in deep hypothermia exhibits many characteristics similar to those of other animals. Among these are partial and complete A-V blocks, arrhythmias and bizarre patterns. The only real difference is the absence of ventricular fibrillation in the rat. This difference appears to be secondary to the basic biochemical changes within the tissue that lead to the abrupt fall in rate at around 20 to 21 C. The abnormalities in cardiac function after this point, whether they be arrhythmia, A-V blocks or fibrillation, probably can all be traced ultimately to the same initial source as that leading to the rate discontinuity.

Most, if not all, of the cardiac functions adversely affected by deep hypothermia are dependent upon endergonic chemical activity. That is, they require an input of energy. It seems only reasonable, therefore, that one of the first places to look for the cause of cardiac dysfunction at low temperature would be at the source of this energy, viz., at the exergonic reactions within the tissue where energy is released. In this regard, the reactions of the tricarboxylic acid cycle are of primary importance since they are the major sites of metabolic energy release.

The metabolic studies reported here are the result of this reasoning, and, although somewhat general in scope, they appear to have given a key to the cause of the functional abnormalities observed at low temperature. This key is the similarity in temperature level for the onset of in vivo cardiac malfunction and the in vitro break in myocardial oxidative metabolism when the latter is plotted according to the Arrhenius-van't Hoff formula.

In view of the simultaneous breaks at about 20 to 21 C. in the Arrhenius van't Hoff plot of several different tricarboxylic acid cycle oxidations, it is legitimate to ask what such breaks mean from a physiological standpoint. It is obvious from the data given here that the decline in oxidative rate with a given decrease in temperature is at least twice as great below 20 to 21 C. as above it. Therefore it can be concluded that there is a much sharper reduction per the degree the temperature is lowered, in the amount of energy made available for physiological processes of the tissue at temperatures below this point than above it.

When viewed from the standpoint of the temperature-velocity constant, the doubling of the value below the breaking point, is somewhat difficult to interpret. If the value were representative of a simple chemical reaction it would correspond to the activation energy of the reaction. In such cases, a doubling of the constant would indicate a doubling of the kinetic energy of the reacting molecules before the reaction could proceed. More specifically, in the case of a simple, enzyme-catalyzed reaction, the value is approximately equal to the change in heat content, when the activated complex is formed from reactant molecules.

Biological oxidations such as those reported here are not as simple as this, however, since a whole chain of enzyme-catalyzed reactions is usually involved. In these multienzyme
systems the $\mu$ value may represent the process as a whole. Accordingly, it could result from dominance shared by a number of reactions, each with different temperature characteristics, as suggested by Burton.\textsuperscript{10} On the other hand, one step within the system may be rate-limiting and the constant could reflect the kinetics of this reaction alone.

Carrying this latter reasoning a step further, Crozier\textsuperscript{11} has postulated that the slowest reaction in a sequential series of reactions is the pacemaker or master reaction and hence determines the $\mu$ value for the over-all rate. A shift in the $\mu$ value is then interpreted as indicating that a different step in the sequence of reactions has become the pacemaker. It is noteworthy, with respect to shifts observed in the present studies, that Hadidian and Hoagland,\textsuperscript{12} working with beef heart succinoxidase preparations found a $\mu$ value of 11,200 calories for the succinic dehydrogenase portion of the oxidation and a value of 16,000 calories for the cytochrome-cytochrome oxidase portion. In addition, they were able to shift the $\mu$ value of the system as a whole from 11,200 calories to 16,000 calories through the addition of cyanide to inhibit the oxidase portion. A similar shift, although obviously not caused by cyanide, could be responsible for the change in $\mu$ values of myocardial metabolism.

However, in the present studies it must be remembered that a number of enzymes are active in each of the experiments and the question of which of these, if any, are the pace setters at the different levels of hypothermia will have to await additional studies. The probability of one master reaction establishing the rate above, and another the rate below, the critical temperature of 20 to 21 C. could be considered a debatable point on the basis of the magnitude of shifts in $\mu$ value observed here.\textsuperscript{10}

Finally, it is possible that the same enzyme is controlling the rate of the oxidations at all temperature levels. If this were true, the change in $\mu$ value could be a reflection of some physical or chemical alteration of the protein configuration of this particular enzyme, as has been suggested by Belehradek,\textsuperscript{13} and Kavanau,\textsuperscript{14} or by a reversible heat inactivation above the critical temperature, as suggested by Morales.\textsuperscript{15}

**Summary**

With rats as experimental animals, studies were conducted to establish the nature of the in vivo response of the heart to hypothermia and the in vitro metabolic response of certain enzyme systems to various temperatures. It was found that in vivo responses as assayed by changes in body temperature, heart rate, and electrocardiographic measurements was quite similar to those of larger animals. The only exception insofar as the heart was concerned was the very low incidence of ventricular fibrillation. In the in vitro metabolic studies it was observed in Arrhenius-van't Hoff plots that ventricular hypothermia was associated with a higher temperature-velocity constant below a temperature of 20 to 21 C. than above it. This was characteristic of tricarboxylic-acid cycle substrate oxidations as well as of endogenous respiration. The relationship of cardiac function to myocardial metabolism in hypothermia and the significance of shifts in the temperature-velocity constant are discussed.

**Summary in Interlingua**

Esseva effectuate, in rattos, studios experimental pro stabilire le natura del responsa cardiace in vivo a hypothermia e del responsa metabolic in vitro de certe systemas de enzymas a varie temperaturas non-natural. Esseva notate que le responsas cardiace in vivo, manifestate in alteraciones del temperatura corporee, del frequentia del corde, e de mesurationes electrocardiographic, es satis simile in rattos e in plus grande animales. Le sol exception esseva le bassissime incidentia de fibrillation ventricular in le rattos. In le studios metabolic in vitro, il esseva notate in diagrammas de Arrhenius-van't Hoff que hypothermia esseva associate con un plus alte constante de temperatura-velocitate a temperaturas infra 20 a 21 C que a temperaturas plus alte. Isto esseva characteristic de oxydationes a substrato de cyclo de acido tricarboxylic e etiam de respirasion endogene. Es discutite le re-
lation inter le function cardiac e le metabolism myocardial in hypothermia e le signification de variationes del constante de temperature-velocitate.

REFERENCES
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