HYPOTHERMIA IN SUCKLING RATS: ELECTROCARDIOGRAM OXYGEN UPTAKE AND INTRA-ABDOMINAL TEMPERATURE MEASUREMENTS

A THESIS

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P.M.Z.

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INTRODUCTION

Homeothermy is the ability of higher mammals to regulate central body temperature and provide a stable thermal environment for the functioning of a complex biological system. According to Kleiber (1961) the maintenance of a constant body temperature has advantages for those animals whose survival depends to a considerable extent on the function of a central nervous system, especially a cerebrum, as the rate of nervous processes depends on the temperature.

Various workers have shown that the main adaptation of laboratory animals to cold is an increased ability to produce heat (Hart, 1958).

Most of the detailed studies on thermoregulation have been carried out on adult animals and only a few studies have been made on young mammals. It seems to be generally recognized, but not well-documented, that young mammals are not fully able to maintain a constant body temperature (Cannon, 1929). It would be useful to have more information than is now available concerning when homeothermic responses develop in young mammals. Equally useful would be data on changes in cold tolerance as a function of age. If such information were available it would permit a greater understanding of the ontogeny of the thermoregulatory pattern of mammals in general and the ecological importance of thermoregulation in the success or failure of the particular species studied.

In one of the few available publications on thermoregulation in young mammals, Brody (1943) attempted to define when "homeothermy" developed in suckling rats. For the purpose of the study Brody

defined homeothermy in terms of the difference between body temperature and environmental temperature after a 15-minute exposure to an environment of 15°C. According to Kleiber (1961), this was an unfortunate definition, since if one exposed large and small spheres of any material to a low environmental temperature, one would find that the smaller spheres cool off more rapidly, thus by Brody's definition a larger inanimate sphere would have greater "homeothermy" than a smaller one. Carrying this argument one step further, Pearse and Hall (1928) noted that a dead turtle cools more slowly than a live turtle exposed to the same cold environment. This phenomenon was explained in terms of a faster heat dissipation resulting from the blood circulation of the living turtle. Again, according to Brody's definition, the dead turtle would have greater homeothermy than the living one.

On the basis of Brody's work and Kleiber's criticism and after personal communication with Dr. Dwight L. Spencer (Department of Biology, K. S. T. C.) this more detailed study of responses to cold by young animals was undertaken. The report presents information on responses of white suckling rats (Rattus norvegicus) to cold exposure. O_2-uptake, electrocardiogram and internal temperature were recorded during the cold exposure. This report traces the development of thermoregulatory responses and cold tolerance in the albino rat from the age of 6-7 days to the adult stage (60-70 days).

METHODS AND MATERIALS

Animals

Forty five animals were used in this investigation, ten animals for each of the following age groups, except the last one in which only five animals were studied:

Group 1. 6-7 days

Group 2. 10-11 days

Group 3. 14-15 days

Group 4. 20-21 days

Group 5. 60-70 days

No animal was subjected to experimental conditions more than once in this investigation. Before the animals were used for experimental purposes they were kept in individual cages at 20-25°C. They were exposed to an approximate 12-hour dark-12-hour light cycle and were given water and Purina Lab chow daily. Preparations for the experiments were made in a laboratory with the same temperature and lighting conditions as the animal room.

Instrumentation and Equipment

The experimental set-up (Fig. 1) was assembled from equipment available in the physiology laboratory and from such other items as could be borrowed, improvised and/or purchased locally at little cost.

Cold Environment. The cold environment was made from a regular household refrigerator. On the door a 22 cm by 22 cm window was made to facilitate visual observation of the equipment and the animal's activity during cold exposure within the controlled microenvironment.

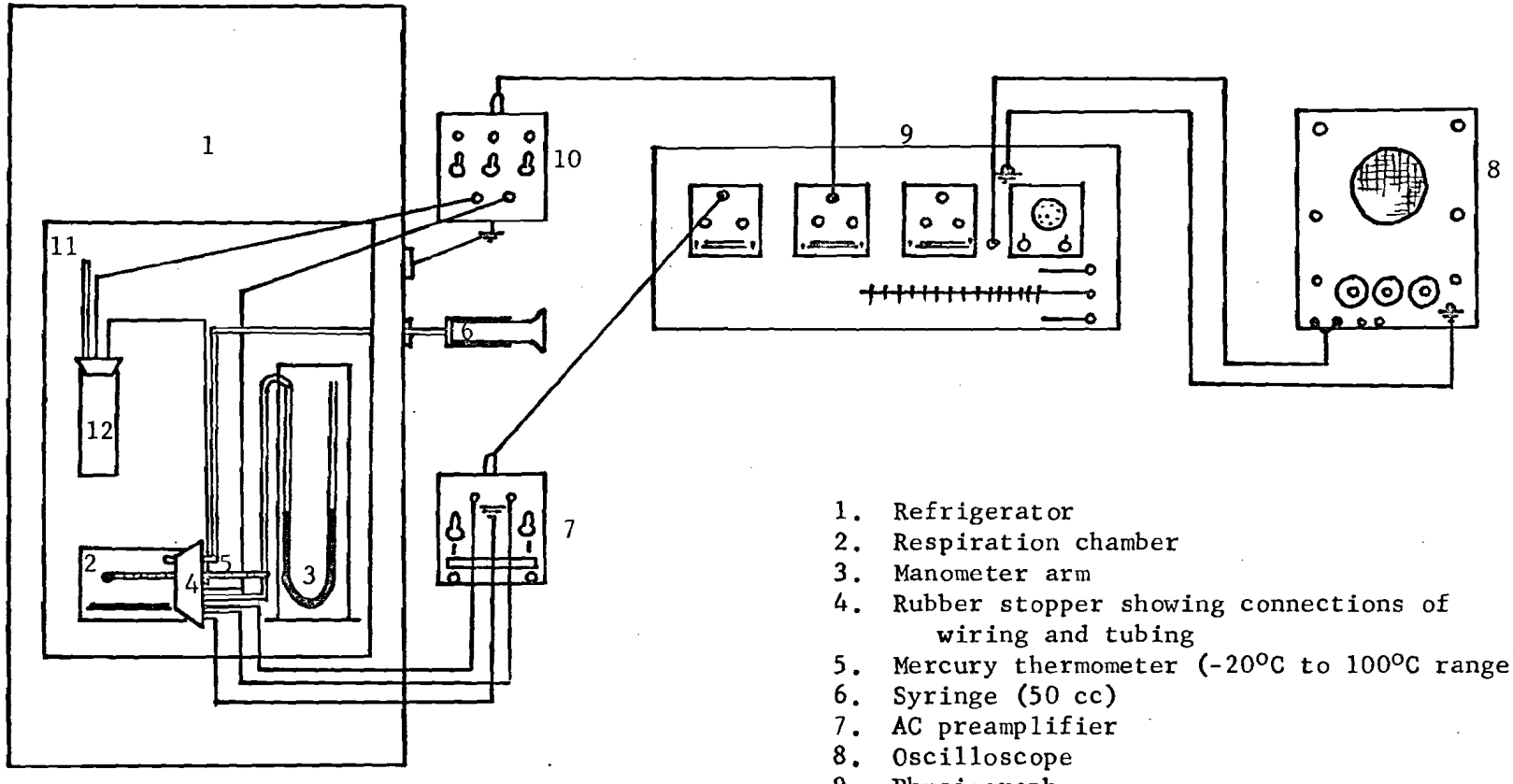

Figure 1. Experimental set-up

1. Refrigerator
2. Respiration chamber
3. Manometer arm
4. Rubber stopper showing connections of wiring and tubing
5. Mercury thermometer (-20°C to 100°C range)
6. Syringe (50 cc)
7. AC preamplifier
8. Oscilloscope
9. Physiograph
10. DC preamplifier
11. 22 X 22 cm window
12. Thermos jug with ice-water for second thermocouple

On one side of the refrigerator an opening was made for the O_2 supply tubing.

During the experiment the animal was fastened in a rat holder and placed in the respiration chamber (800 ml peanut butter jar). The lid of the respiration chamber consisted of a size 15 rubber stopper, with sealed openings for manometer tubing, mercury thermometer, and electrocardiogram and thermocouple wires.

Body Temperature Measurements. Intra-abdominal body temperature was measured by means of the 30 s.w.g. iron-constantan thermocouple. While the experimental animal was under a general ether induced anesthesia, the thermocouple was threaded through the abdomen with a needle and the junction placed between the abdominal muscles and the abdominal organs. In some but not all experiments the position of the thermocouple was checked at post mortem examination.

The thermocouple wires were connected to a DC preamplifier (E & M Instrument Co. MK III). An interconnecting transducer cable was used to couple the output from the preamplifier to one channel of a physiograph recorder (E & M Instrument Co., Model PMP-4A). This temperature signal was then connected through a monitor output to a DC oscilloscope (DuMont Model 401B). The system was calibrated to known temperatures before each series of experiments and the calibrations spot-checked regularly to avoid error due to drift. Temperature readings were taken at 15 minute intervals and were accurate to $\pm$ 0.1°C.

Ambient Temperature Measurements. The ambient temperature (T_a) (respiration chamber temperature) was measured by means of a mercury thermometer (-20° to 100°C range). The thermometer was inserted

through the rubber stopper which served as the lid of the respiration chamber. Readings were taken at 15 minute intervals with an estimated accuracy of $\pm$ 0.1°C.

Oxygen Consumption Measurements. For these measurements a closed-circuit method was used. The system was sealed from the atmosphere except for one manometer arm. As the animal used oxygen in the presence of a CO_2 absorbent, the pressure inside the system fell below the atmospheric pressure. Oxygen then was injected into the chamber, using a 50 ml syringe, until the manometer again provided a reading equal to the atmospheric pressure. The volume of oxygen required to level the manometer arms was read from the syringe. The oxygen volume changes could be read to an accuracy of $\pm$ 0.5 ml. Readings were taken at 15-minute intervals while the animal was under cold exposure. The volume measurements were later corrected to standard temperature.

Electrocardiogram Measurements. While the animal was under anesthesia two chest electrodes were placed subcutaneously, one above the clavicle and one between the second and third rib on the cardiac side. The electrodes were made from number 3 insect pins bent into the shape of a safety pin. The ground electrode consisted of an alligator clamp, modified to reduce tension, fastened to the tail of the subject. The electrodes were connected to a high-gain AC preamplifier (E & M Instrument Co.) which was connected to a channel of the Physiograph. Electrocardiogram recordings were taken every 15 minutes and were accurate to $\pm$ 1.0 beat per minute.

Treatment of Experimental Animals

Each animal studied was removed from the litter and placed under general anesthesia. The thermocouple and electrocardiogram electrodes were quickly implanted and the animal fastened on a rat holder. The subject was allowed to recover from anesthesia, recovery being tested by pinching the tail until reflexes were elicited. The CO_2-absorbent (Collins Inc.) was placed in a container within the respiration chamber. The electrode leads from the rat were connected with extra care to their appropriate lead-in wires on the stopper to secure accurate results. These preparative procedures, all of which were carried out at room temperature, usually required 15 to 20 minutes. The animal was then transferred into the respiration chamber. The chamber was sealed and the refrigerator door closed. The animals were cooled until stabilized conditions were established i.e., the electrocardiogram, O_2-uptake and internal temperature records showed no further drop. The cold exposure time varied among the age groups due to differences in their cooling rates and in the temperature they could tolerate.

RESULTS

During the cold exposure the animals fought their restraints for the first 30-45 minutes and then became less active.

The first readings of cardiac activity, internal temperature and ambient temperature were made as soon as the animal was placed in the respiration chamber (zero time for the experiment) and then recorded at 15 minute intervals. The first reading of oxygen uptake was taken at the end of the first 15-minute interval and repeated at 15-minute intervals thereafter.

Body Temperature

The pattern of decrease in abdominal temperature (T_{ab}) during cold exposure for each age group is shown in Figure 2. Intra-abdominal temperature declined most rapidly in the youngest animals and least rapidly in the oldest animals tested. Comparison of the patterns of T_{ab} drop seen in Figure 2 shows groups 1, 2, and 3 to be similar and to differ from groups 4 and 5 whose patterns are similar. There seems to be a definite difference between the abilities of 14 day old (group 3) and 21 day old (group 4) animals to at least partially regulate internal temperature. The difference between groups 3 and 4 was tested statistically. T-test analysis of the data revealed that the pattern of T_{ab} drop between group 4 (young adults) and group 3 was significantly different ($P<0.05$). The t-test was not applied to the difference between groups 4 and 5 since they differed in sample size.

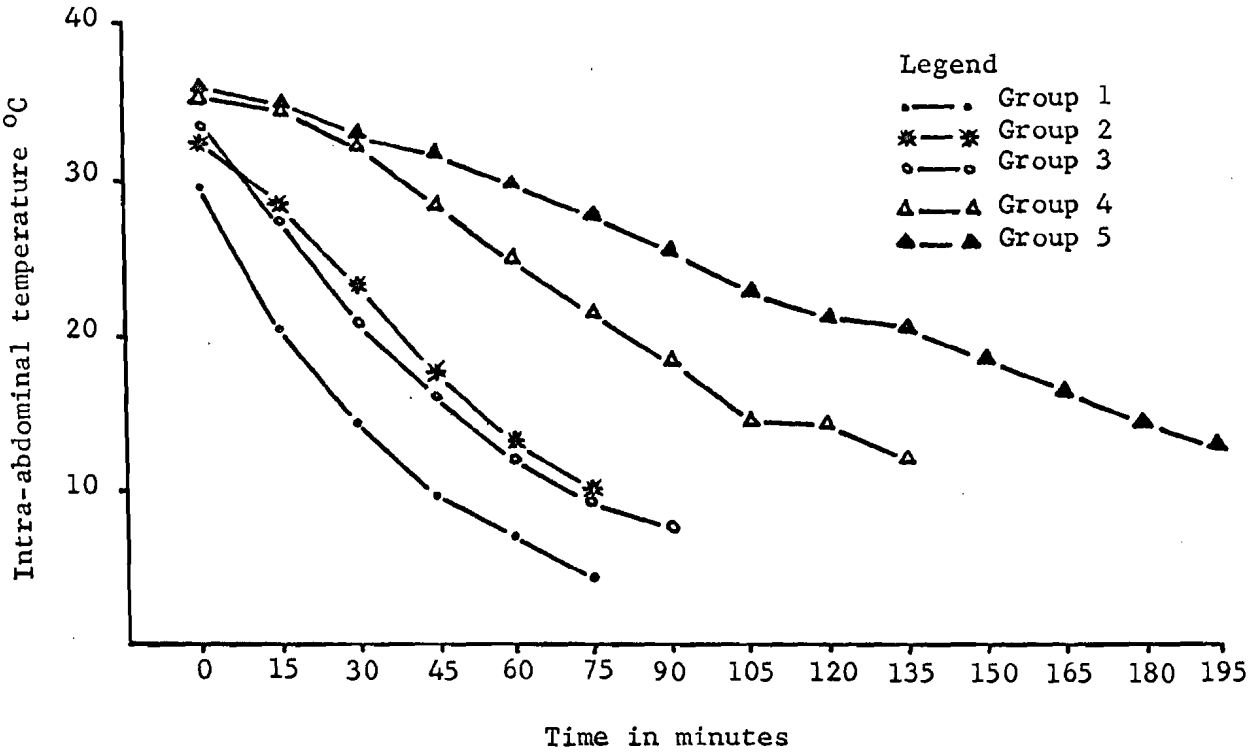

Figure 2. Intra-abdominal temperature (T_{ab}) as a function of cold exposure time for laboratory rats of 5 different age groups. Each point is the mean T_{ab} for 10 experimental runs except group 5 in which only 5 runs were made.

It is important to note in Figure 2 that readings taken at zero time show that the youngest animals had the lowest T_{ab}, the oldest animals had the highest T_{ab}, and the T_{ab} of intermediate groups fell between these two extremes. The trend of these zero time readings is similar to that of Brody's (1943) results which were measured at the end of a 15-minute exposure to 15°C. Since the present results were obtained under generally similar hypothermic conditions (exposure to laboratory temperature for about 20 minutes), the similarity in results is not surprising.

Metabolic Rate

Figure 3 shows data for O_2-uptake for the five experimental groups during cold exposure. Comparing the O_2-uptake of each age group during the first 15-minute exposure (when animals were still in a normal temperature range), it can be seen that they followed the generally expected relationship: the older the animal the greater was their O_2-uptake. Comparing the O_2-uptake curves for the five age groups it can be seen that the rate of O_2-uptake generally decreased with time, presumably as hypothermia caused a decrease in metabolism. However, in the older groups the rate of O_2-uptake declined more slowly, as would be expected from their somewhat better ability to maintain body temperature (Fig. 2). The 30 minute reading for the adult group is higher than the 15 minute reading suggesting a compensatory increase in metabolic rate, *i. e.*, an attempt to balance the heat loss by increased heat production. Further, the 120-minute readings for groups 4 and 5 suggest a possible second zone of thermoregulation.

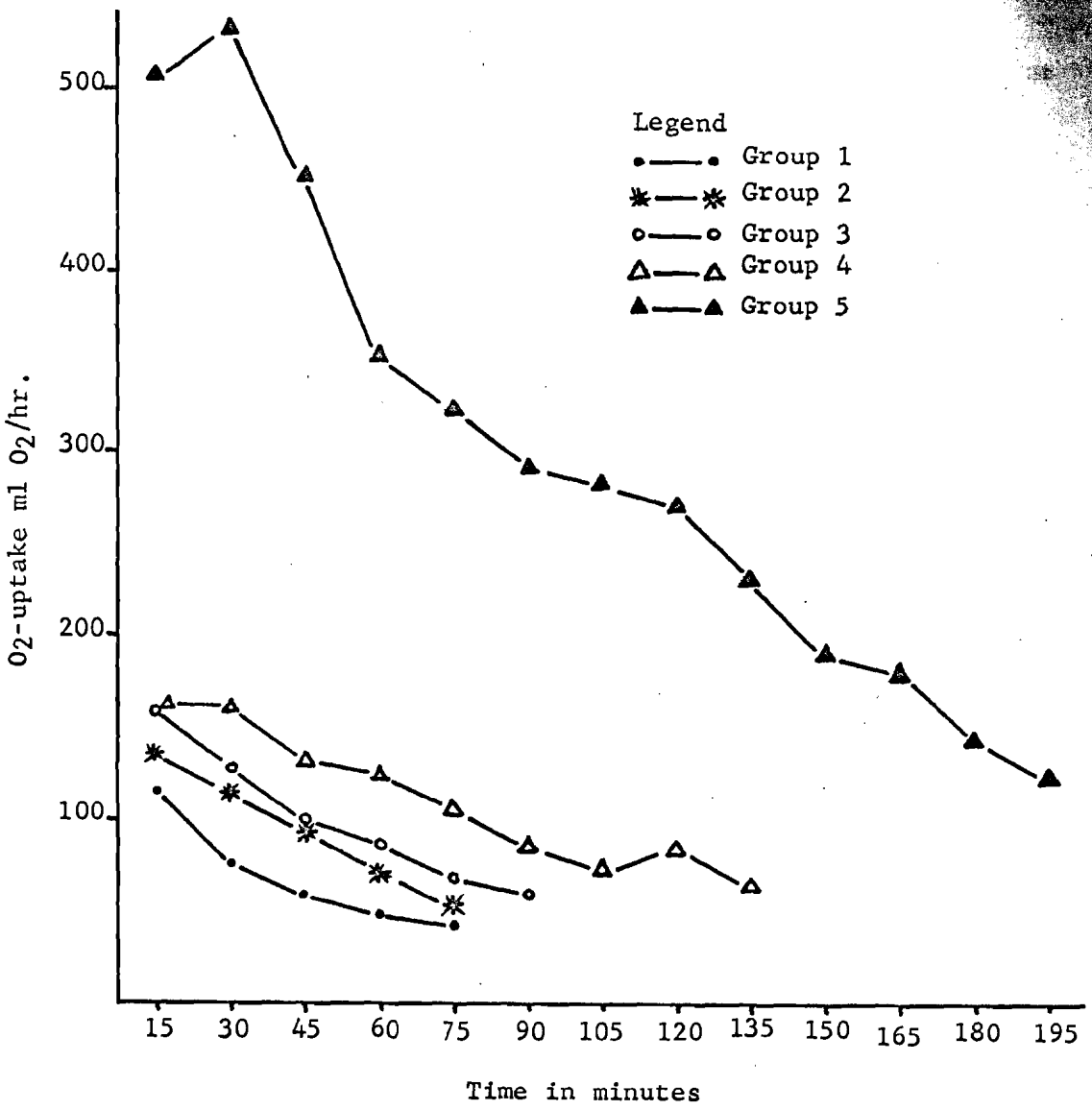

Figure 3. Oxygen consumption in ml/hr as a function of cold exposure time for five groups of laboratory rats.

Figure 4 shows the same O_2-uptake data as presented in Figure 3 but calculated on a per gram body weight basis. As was expected from earlier experiments conducted at room temperature (Kleiber et al., 1956b), the initial weight-specific metabolic rate is higher in young animals and becomes less with increasing age. In all five age groups the metabolic rate decreased during the cold exposure. It can also be seen from these data that the youngest animals showed the most rapid decline in metabolic rate and the oldest animals showed the slowest decline in metabolic rate. This difference seems to indicate, especially during the first 30 minutes of cold exposure, evidence of establishment of thermoregulatory mechanisms in the two older groups. T-test analysis of the data presented in Figure 3 showed that age group 4 was significantly different from groups 1, 2, and 3 ($P<0.05$).

Heart Rate

Heart rate respsonses (Fig. 5) showed a pattern generally similar to oxygen consumption, that is, in all age groups heart rate declined with increasing duration of cold exposure but declined more slowly in older animals. Heart rates obtained at zero time probably represent thermoneutral values for the older animals (age groups 4 and 5), but not for the younger animals. As was explained before, the animals were exposed to room temperature (20-25°C) prior to experimental observation. Since the room temperature was approximately 10°C lower than litter temperature, it probably induced mild hypothermia in the youngest animals.

Another trend that follows the age sequence is the minimum heart rate reached at the end of each experiment. The experiments

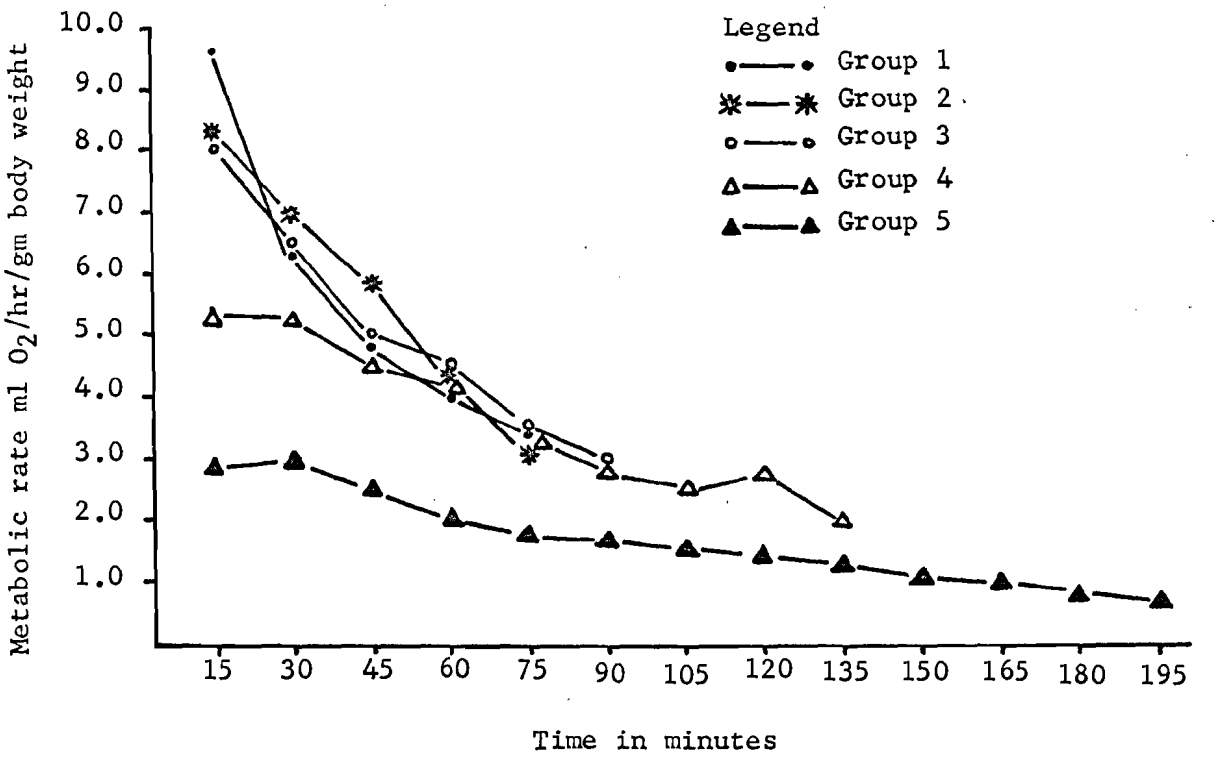

Figure 4. Weight specific metabolic rate (ml O_2/hr/gm body weight) as a function of duration of cold exposure. Data from laboratory rats of five different age groups.

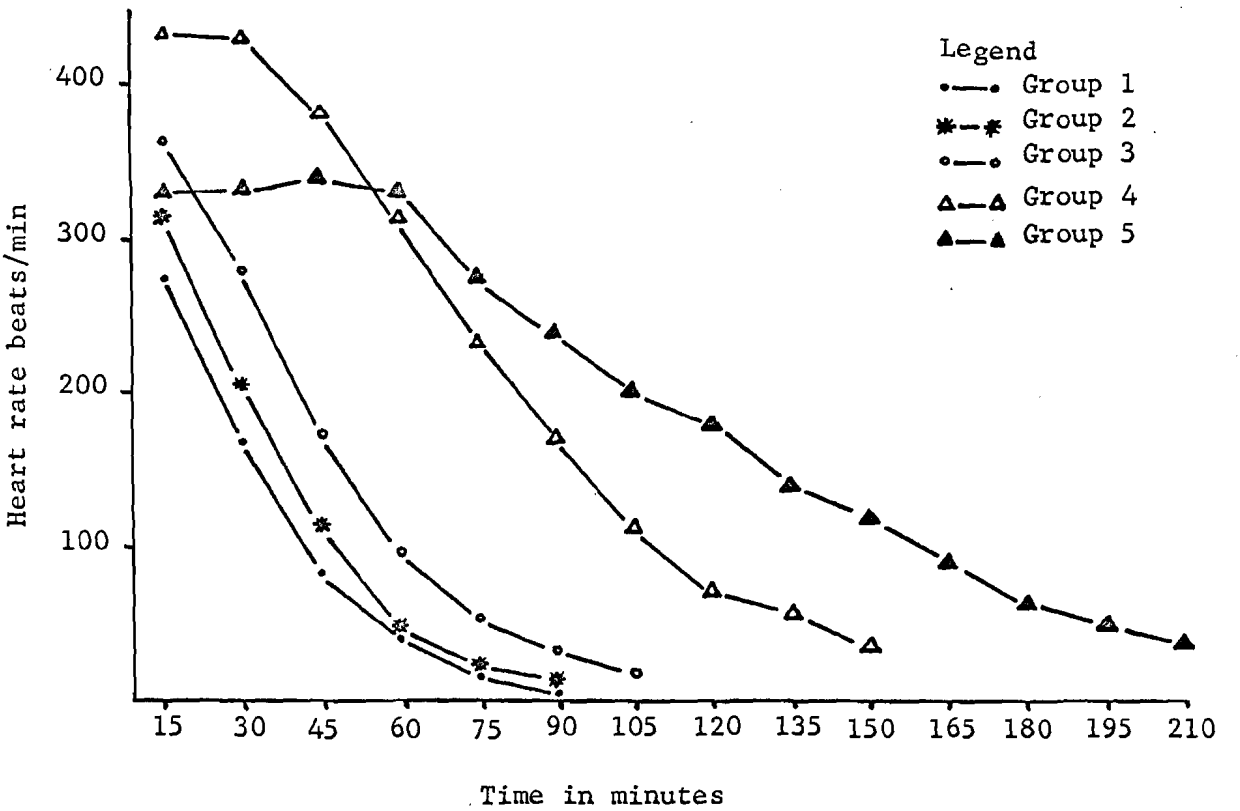

Figure 5. Heart beat rate as a function of cold exposure time for five groups of laboratory rats.

were discontinued when the cardiac potentials fell to such a low amplitude as to make recording difficult. As can be seen in Figure 5 the youngest animals attained the lowest heart rate and the minimum recordable heart rate increased with increasing age. Another difference between the animals of different age groups is that none of the animals in group 5 survived hypothermia but all the younger animals (except one from group 4) survived and recovered from the experimental exposure. The result is more impressive when it is recalled that the younger animals were subjected to deeper hypothermia (see Fig. 2).

The ECG records showed that in the later stages of cold exposure the hearts showed arrhythmias, including possible fibrillations (Fig. 6 & Table 1). Evidences of arrhythmia and fibrillation were noted in all five age groups but were most obvious in the young adults (age group 4) and the adults (age group 5). Keatinge (1969) states that a fall in body-temperature increases the refractory period of cardiac muscle and slows conduction, the two major causes of fibrillations.

Burn (1960) stated that energy is required to maintain the duration of the action potential and thus the long refractory period of cardiac muscle. Therefore, when energy is not available due to lack of oxygen or glucose or to the presence of metabolic inhibitors or because of hypothermic conditions, the action potential is shortened and fibrillation is facilitated. Also the incidence of fibrillation in hypothermia is significantly influenced by the anesthetic agent employed. Covino et al. (1954) investigated the effects of various anesthetics on dogs and concluded that, although cardiac arrhythmias

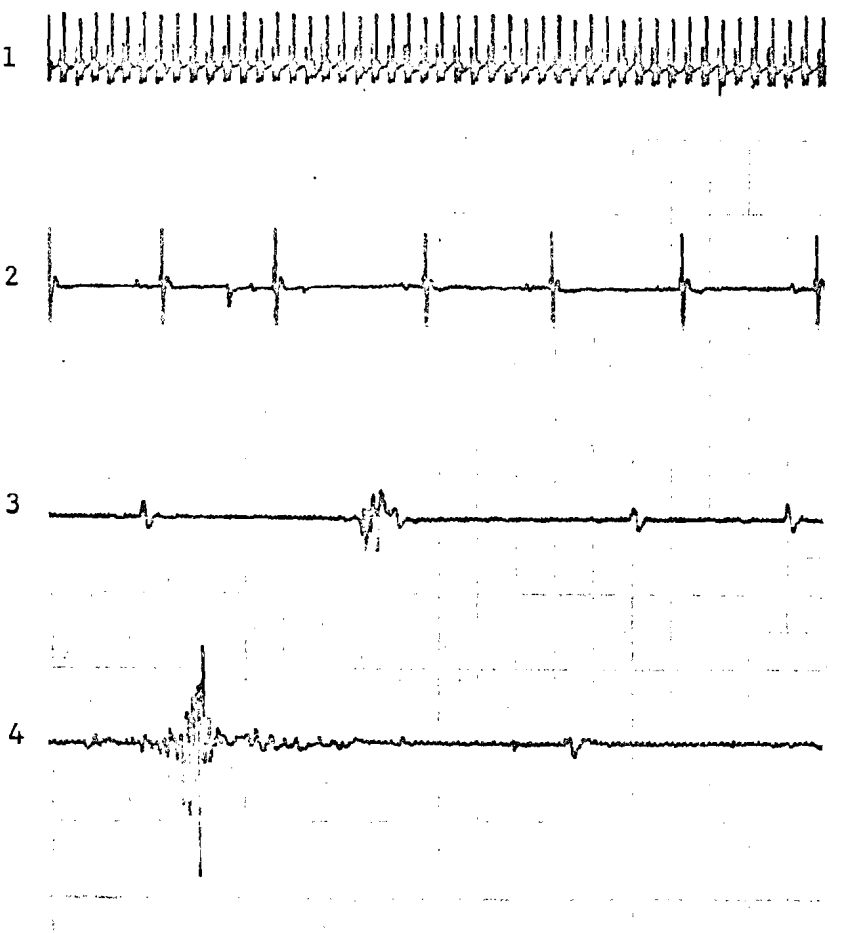

Figure 6. Representative examples from a continuous ECG recording. Data collected from an adult rat during cold exposure.

Record 1. Normal ECG
Record 2. Arrhythmia, T_{ab} = 17.7°C T_a = 7.4°C
Record 3. Arrhythmia and possible fibrillation T_{ab} = 14.8°C T_a = 6.1°C
Record 4. Severefibrillation and arrhythmia T_{ab} = 13.8°C T_a = 5.1°C

Table 1. Conditions under which arrhythmia or fibrillation first occurred in each of 5 age groups. Each number represents a mean value of 10 observations except in age group 5 in which only 5 observations were made.

Age Group (days)	Conditions at time of arrhythmia or fibrillation	
	Body temp. ^{o}C	Heart rate (beats/min)
1. (6-7)	9.3	34
2. (10-11)	15.7	49
3. (14-15)	15.0	86
4. (20-21)	16.0	86
5. (60-70)	19.0	96

and ventricular fibrillation occur with all anesthetics tested, they occur less frequently under thiopental or ether than under pentobarbital. In this study, ether was used as anesthetic and extreme hypothermic conditions were induced. According to Covino et al. (1954) and Burn (1960) both factors facilitate arrhythmia and fibrillations.

Intra-abdominal Temperature as a Function of Ambient Temperature

Refrigerator temperature was set to 0°C before each experiment. During preparation for cold exposure the experimental animal was placed in the respiration chamber at room temperature and the refrigerator opened in order to insert the respiration chamber. Thus the starting refrigerator temperature was above 0°C and the initial ambient (respirometer) temperature was still higher and varied somewhat among the five experimental age groups. Also the ambient temperature dropped more slowly in the experiments with the adult animals; the adults seemed to produce more heat as compared to the youngest ones, and to partially control the temperature of their microenvironment. To remove the variability in rates of cooling of the respiratory chamber, intra-abdominal temperature was plotted as a function of ambient temperature (Fig. 7). The curves, with time now eliminated as a variable, of intra-abdominal temperature as a function of the ambient temperature show some differences between the different age groups. None of the age groups was able to maintain constant body temperature in the face of the imposed cold environment. In order to clarify this information in Figure 7 the earliest three points recorded for each age group were taken under consideration because the more effective thermoregulatory effort would have taken place

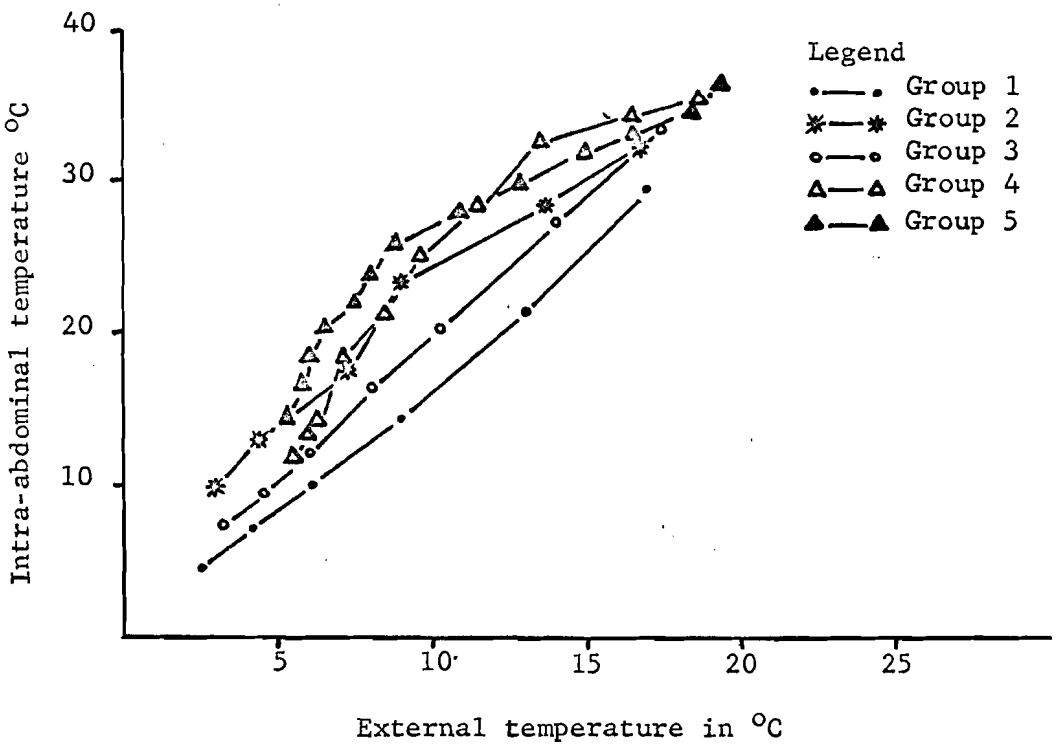

Figure 7. Intra-abdominal temperature as a function of ambient temperature during cold exposure. Intra-abdominal temperatures were obtained from thermocouples in 45 laboratory rats of five different age groups.

within the first 30 to 45 minutes of the cold exposure before thermoregulatory responses were completely overwhelmed. This information was plotted and shown with correlation coefficient lines (Fig. 8). The correlation coefficient lines show in general that the younger the animals the steeper the drop of those lines. Age group 4 appears to have the best level of thermoregulation as its correlation coefficient line drops in a less steep manner than any of the other four age groups.

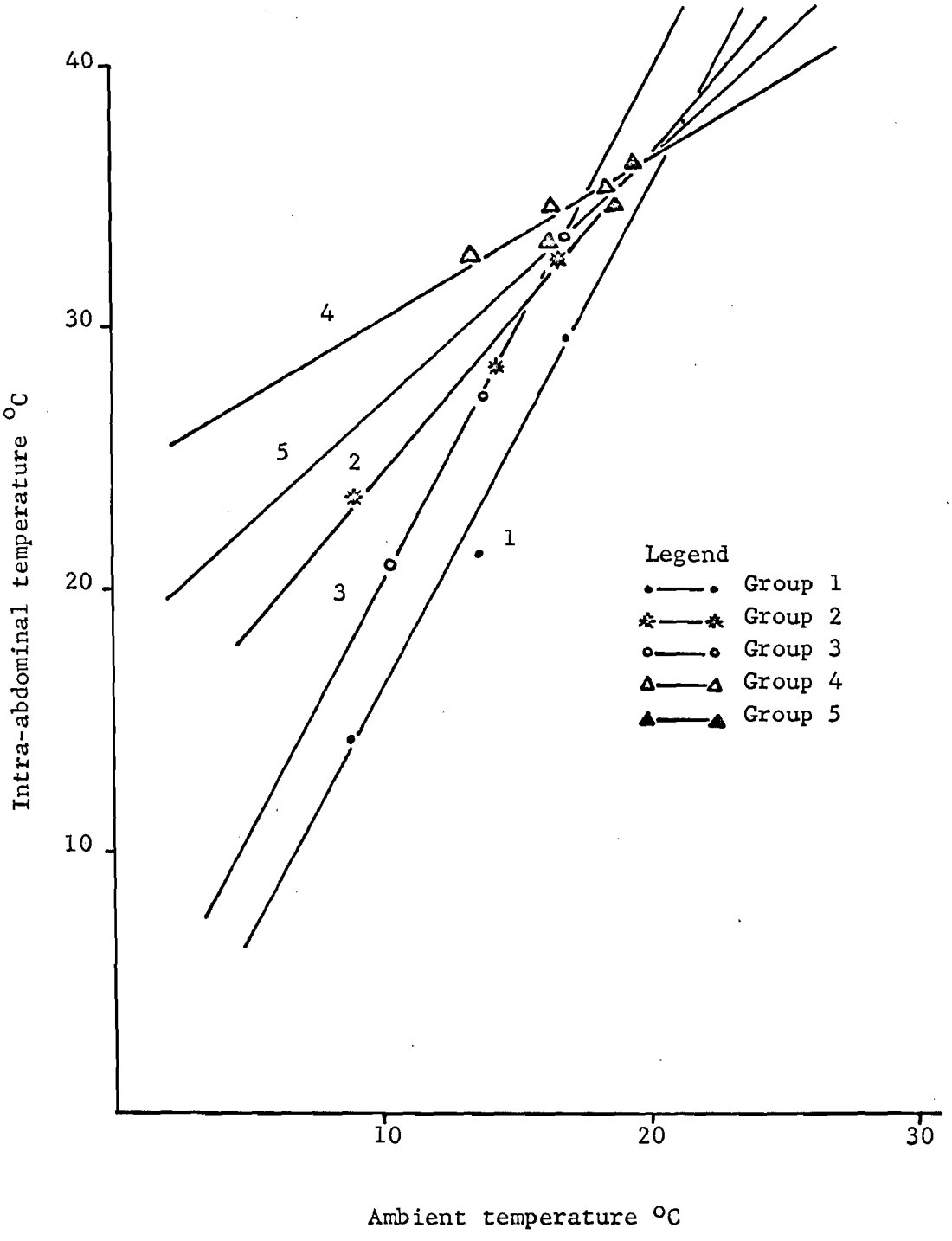

Figure 8. Intra-abdominal temperature (earlier three readings made in each experiment) as a function of ambient temperature. Lines indicate calculated correlation coefficients for each of the five age groups of laboratory rats.

DISCUSSION

Difficulties of Working with Suckling Rats

It was exceedingly difficult to record the data in this study due to the small size and rather delicate condition of the experimental animals. Benedict et al. (1929) stated that the body temperature of small suckling mammals is obtained only with difficulty. The author of this report strongly suspects that difficulty of recording is the main reason that studies on young animals so infrequently appear in the literature. Problems experienced during this study include:

I Due to the size, age range and delicate conditions of the experimental animals an ordinary rectal temperature record could not be obtained since the rectum is not well developed. Both in the present study and in Brody's (1943) study, intra-abdominal temperature was measured by accurately-implanted thermocouples. In other studies thermocouples were chronically implanted in the abdominal cavity or in the dorsal neck muscle between the scapulae (Lawrence et al., 1969).

II Suckling rats are heterothermic (Cannon, 1929). Thus when the young animals were removed from the litter they were exposed to hypothermic conditions. This variable was minimized in the present experiments by allocating the same amount of preparation time for each animal. In future experiments it would probably be better to carry out the pre-recording preparations in a thermostatically controlled environment which would hold the animals at litter temperature until zero time.

III The usage of anesthetic was a critical decision since no data were available on long term effects of ether in suckling rats. Although ether might increase hypothermia and arrythmias, it was felt the animals would be subjected to undue shock if no anesthetic was used.

IV The measurements made for suckling rats, especially the metabolic measurements, are critical because of the small size of the animals. The mean body weight for age group 1 (6-7 days old) was 12.4 grams, so measurements from these animals have to be taken with extra care and constant alertness.

Evidence for Thermoregulation

This study was designed (1) to extend Brody's (1943) experiments in which he drew the conclusion that suckling rats become homeothermic at about the age of 22 days, (2) to meet Kleiber's (1961) objections that by making body temperature measurements only at one exposure time (15 min.), Brody ignored the purely physical effects of body size on cooling rate, and (3) to obtain additional physiological details of responses to cold stress. Three major factors of the response to cold were considered:

I Body temperature was recorded in order to provide a clear picture of how body temperature varies during cold exposure among different age groups. The present data show that in older animals the rate of body temperature decreases more slowly during cold exposure than in young animals and that there seems to be an abrupt improvement in the response between age group 3 (14-15 days old) and age group 4 (20-21 days old). The data are consistent with Brody's (1943) conclusion that

thermoregulatory responses become established around the end of the suckling period (22 days) if account is taken of the difference in the intensity of cold stress in the two experiments.

II Metabolic rate was considered so that one could investigate the overall cost of thermoregulation in animals of different ages during cold exposure. It was expected that the animals at some age would thermoregulate by increasing their metabolic rates but that at younger ages would not thermoregulate and no such increase in metabolic rate would be observed. In these experiments, however, the cold exposure was so intense that none of the animals were able to thermoregulate, but it was shown that the oldest animals (20-21 days old and 60-70 day-old) increased their metabolism during the first half hour of cold exposure. Thus these experiments demonstrated the existence of thermoregulatory mechanisms, but the mechanisms were quickly overwhelmed by the cold.

III Electrocardiograms were recorded in order to follow the cardiac activity and to give information about the overall circulatory activity during cold exposure. The heart rate for all animals in the five age groups of this study showed close parallels to the O_2-uptake. As the metabolic rate, and thus heat production, was elevated in the case of the homeothermic age groups 4 and 5, the heart rate was also elevated, presumably to meet tissue needs for increased oxygen and circulating nutrients.

The results presented in this report on the effects of cold exposure on various ages of white rats show a progressive pattern

of thermoregulation as it occurred during the maturation of the suckling rats. Under the experimental conditions the first evidence of the ability to partially counteract a severe low temperature stress was found in age group 4 (20-21 days old). The three younger groups showed no such level of reactivity. Instead a more uniform decrease was observed with all parameters under study. Also the three younger groups showed statistically significant differences from group 4 in all levels of data collected ($P<0.05$). Furthermore, age group 4 showed some stability in O_2-uptake, heart rate and internal temperature records, in response to the first half hour of cold exposure. In general the response patterns of age group 4 were similar to the data recorded from the adults which are recognized thermoregulators.

Tolerance of Low Temperatures

Since young mammals are not considered to possess well developed homeothermic mechanisms (Cannon, 1929), it was expected that their body temperature would fall more rapidly than in the older animals under the extremely cold environmental conditions of these experiments. Tolerance of low body temperature is a separate question and field observations (personal communication, D. L. Spencer) suggest that the younger animals, although poorer thermoregulators, are more tolerant of low body temperatures. In adults it is known that death from hypothermia occurs at a body temperature not low enough to injure the tissues as such. An adult rat dies when its rectal temperature drops to about 12°C (Crisman et al., 1947). No previous study which reports the lower lethal limit of body temperature in suckling rats has been found by this author. During this study the adults

died at a mean intra-abdominal temperature of 13.3°C while all the younger animals, except one in age group 4, survived and recovered from lower body temperatures (Fig. 2). According to Kleiber (1961), the mechanism of cold death is probably circulatory failure, with resulting irreversible hypoxic damage to the central nervous system; Fuhrman et al. (1944b) have shown that in the hypothermic cat the oxidation rate of epinephrine is lower than it is at normal body temperature. Because of the slower rate of destruction, doses of epinephrine which produce very little effect at normal body temperatures may produce toxic reactions, such as heart block, in the cooled animal.

In discussing the causes of cold death, Samson (1965) pointed out the critical need of the mammalian brain for energy. The need is critical not in the sense of amount of the energy required but because the smallest interruption or even reduction of the rate of energy supply to the brain is almost immediately followed by functional failure.

In addition to cardiac arrhythmia and fibrillation, cold causes metabolic inhibition and, according to Ruhe and Horn (1955), depression of the respiratory centers of the brain. All these causes would contribute to hypoxia and therefore to decreased ATP production. As energy flow is reduced failures would be evident in the most sensitive organ, the brain.

In this study it was found that young suckling rats did not thermoregulate but tolerated the extreme experimental hypothermic conditions that they were exposed to and managed to survive and recover fully. In contrast to this, one young adult animal (age

group 4) and all adult animals (age group 5) failed to survive. According to Samson (1965) young adult and adult rats are more sensitive to the reduction of energy flow to the brain than are suckling rats. Samson's explanation for his difference is based on the following three points: (1) a continuing adequate supply of ATP is necessary for the survival of brain cells, (2) ATP production is suppressed by cold, and (3) the young animals have a better reserve capacity for ATP production. Samson et al. (1960) found that the ratio of mitochondria to rate of energy flow to the brain is higher in the newborn. This indicates that the mitochondria appear sooner in development than the enzymes which will utilize the energy. One particular ATP utilizing enzyme system, Na^{+}-K^{+} stimulated Mg-ATPase, which is probably involved in the critical process of ion transport, is in very low concentration in the one-day-old rat. The system grows primarily in the 10-day to 21-day period (Samson et al., 1964).

The tolerance of low temperatures by suckling rats during hypothermic conditions is a remarkable adaptation which enables these heterothermic organisms to survive until they can develop homeothermic responses. From the ecological point of view it is significant that the attainment of such mechanisms exist at an age range in which the animals can survive without relying on the mother as a source of warmth. In the case of a particular species where maternal behavior is not well developed this could be the factor determining success or failure of that particular species.

SUMMARY

The ability to thermoregulate under extreme hypothermic conditions was studied among various ages of suckling and adult rats, Rattus norvegicus.

For this study three factors were taken under consideration as a function of ambient temperature.

1. Body temperature was recorded in order to provide a clear picture of how body temperature varies during cold exposure among different age groups.
2. Heart rate was recorded in order to follow the cardiac activity and to give suggestions about the overall circulatory activity during cold exposure.
3. Metabolic rate was recorded in order to allow investigation of the overall cost of thermoregulation in animals of different ages during cold exposure.

The results showed a progressive pattern of thermoregulation during the maturation of the suckling rats. Under these experimental conditions, the first evidence of ability to partially counteract a severe low temperature stress was found in age group 4 (20 to 21 days old). All three younger age groups showed no such ability when they were exposed to the same experimental conditions. Instead a steady decrease was observed in all variables under study. Also all the three younger groups showed statistically significant differences from age group 4 in all the variables under study.

It was noticed from the ECG record that in the later stages of cold exposure the hearts showed arrhythmias and evidence of possible fibrillations. These cardiac abnormalities were possibly due to the extreme hypothermic conditions which were imposed in this study but they may have been enhanced by the anesthetic (ether) used during preparations.

It was found in this study that although the young suckling rats did not thermoregulate as well as older rats, they tolerated extreme hypothermic conditions better than did older rats. The present data are consistent with other studies which show that the survival of rats in hypoxia induced by hypothermia decreases as the animals mature and is closely related to the decrease in cerebral ATP concentration.

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