STUDIES ON THE ELECTRICAL POTENTIALS OF LIVING ORGANISMS: II. EFFECTS OF LOW TEMPERATURES ON NORMAL UNANESTHETIZED MICE*

RALPH G. MEADER AND CLYDE MARSHALL

In a preceding paper (Marshall and Meader[6]) we reported a study of base-line bio-electric potentials in normal unanesthetized mice, preliminary to the investigation of the effects of altered external and internal environmental factors. In the present communication are presented the records of electrical potentials obtained during the depression of body temperature and its return to higher levels, together with observations on some physiological concomitants of the process.

Material and Methods

The methods used in securing and recording the data on potentials were, in general, similar to those described in the previous paper. Leads were taken from the nape of the neck and the dorsum of the sacrum of the mice by means of saline bridges connected with silver-silver chloride electrodes. The magnitudes of the potential differences were continuously and automatically indicated by a Burr, Lane, Nims microvoltmeter operating a photo-electric recording galvanometer. On a second similar recording galvanometer were obtained simultaneous, continuous, graphic readings of the internal temperature of the mouse as detected by a copper-constantin thermocouple. The thermocouple was inserted into the rectum of the mouse for a distance of 2 to 3 centimeters, so that only the insulated portions of the wires were exposed. Environmental temperatures were observed by means of a suspended mercury bulb thermometer with the bulb resting on the cork platform with the mouse.

The environmental temperature was depressed by placing a cooling chamber around the suspended platform on which the mouse

* From the Laboratory of Neuro-Anatomy, Yale University School of Medicine. Aided by grants from the Research Funds of the School of Medicine.

was fastened. The chamber consisted of an uncovered metal box set in another open box. The space between the walls of the two boxes contained dry ice. The environmental temperature was controlled by regulating the depth of immersion of the mouse in the cooling chamber. Temperatures as low as −25° C. could be obtained in a few minutes with the platform bearing the animal inserted into the chamber 3½ inches, even though the upper opening of the chamber was not closed. In practice, it was found desirable to secure an environmental temperature of approximately −10° C., although occasionally readings as low as −23° C. were reached before the condition of the mouse required elevation of the temperature of the surrounding air. The period of exposure, therefore, varied considerably, covering a range of 25 to 65 minutes.

After the insertion of the thermocouple and the establishment of electrical contacts through saline bridges, records of the body temperature and electrical potentials at room temperature (24° to 26° C.) were obtained as a base-line with which to compare the effect of lowered environmental temperature. The mouse was then placed in the cooling chamber until the body temperature reached 15° C., or lower, and the mouse showed marked physiological changes. At that time the cooling chamber was removed and heat from lamps was supplied.

The mice were of the two genetic strains used previously and came from the colony of Dr. L. C. Strong. There were 10 CBA and 10 C_3H mice, all males. At the time of the experiments, the ages of the CBA's ranged from 45 to 75 days while the C_3H's were between 45 and 109 days.

PHYSIOLOGICAL OBSERVATIONS

Base-line Period

The internal temperatures of the mice at the beginning of the base-line period were not uniform. They ranged from 33° to 38° C. During the course of time the temperature varied within narrow limits. At the end of the base-line period the temperature was sometimes found to be lower or higher than at the beginning, but it was more often lower. The greatest range covered was 3.5° C. The change of temperature was not proportional to the time the animal was stretched out on the board. Occasionally the thermal

level dropped during part of the base-line period but returned later to the previous level.

At the beginning of the experiment the animals often struggled considerably, but soon quieted except for occasional intermittent periods of activity. The struggles were massive movements involving the whole body. They were usually spontaneous but could be elicited by almost any type of stimulus. The respiratory rate during this time ranged from 180 to 260 excursions per minute. This rate was obtained by actual counting and timing with a stop-watch; hence, the higher rates should be regarded as only approximate.

Period of Chilling

When the mouse was placed in the cooling chamber, the body temperature began to fall with a latency of no more than a minute or two. The temperature steadily declined, often producing a perfectly straight line on the record. In the course of 25 to 65 minutes the internal temperatures were reduced to levels between 14.5° and 8.5° C. at which time the animals were in a condition such that it seemed inadvisable to expose them longer to cold. In a certain number of animals the temperature continued to drop for a minute or two after removal from the cooling chamber.

The immediate effect of chilling on bodily movement was usually a marked increase of spontaneous struggling, although in a few cases no such accentuation occurred. As the internal temperature became lower, the bodily movements became less frequent, less violent, and they involved a progressively smaller amount of the body. Usually the last movements to disappear were those of the head and tail. No shivering movements were observed. During the early stages of chilling, touching the animal or even the click of a stop-watch might initiate a severe paroxysm of massive struggling. Later, when the spontaneous movements began to subside, the response to environmental stimuli subsided also. Finally, after all spontaneous movements had ceased, touching the vibrissae, face, ears, or eyes produced small head, ear, or lid movements. Eventually even these could not be evoked, the lid movement usually being the last to disappear. The body temperatures at which these events took place were all in the low end of the range. Spontaneous movements were maintained until the temperature fell to a point below 20° C. In a

few cases they were still present below 15° C., but none was noted below 13.5° C. The reflexes mentioned disappeared between 16° C. and 11.5° C. It is difficult to correlate with precision, temperatures and movements, spontaneous or induced, because of the variation from animal to animal and because the same animal occasionally gave a response at a lower temperature than that at which it had previously given none. Glazing of the cornea and watering of the eyes frequently occurred at low temperatures.

The respiratory rate usually increased immediately upon putting the mouse into the cooling chamber, reaching, in some cases, over 300 a minute. It returned to the base-line level after 10 to 20 minutes of chilling, during which time the body temperature was depressed 8° to 18° C. below its base-line value. Respiratory movements continued to decrease steadily in rough proportion to the loss of body temperature. The decrease of respiratory rate was approximately 10 per minute per degree of temperature lost, until the colonic temperatures approached 16° to 12° C. From that point on, the loss in rate increased to about 20 per degree, until respiration appeared about to stop and the mouse was removed from the cooling chamber. Cheyne-Stokes breathing was sometimes noted in the lower temperature ranges.

Warming Period

When the mice were removed from the cooling chamber a few were allowed to remain in the environmental temperature of the room, about 25° C., while the others were supplied with heat from one or two lamps which raised the environmental temperature varying amounts, up to a maximum in one case of 44° C. The immediate effect of removal on the body temperature was variable. In some cases the temperature began to rise promptly but more often it continued to drop for one to two minutes before starting upward. The rate of increase from this point was slow and steady. When no additional heat was supplied, more than two hours were required to reach the normal level. With extra heat the time was materially shortened so that in one case, for example, the body temperature was elevated from 11° to 37° C. within 30 minutes. In no case was the body temperature raised significantly above normal with the environmental temperature used.

The reflexes tested usually returned promptly upon removal from the cooling chamber, and often could be elicited at temperatures lower than those at which they had disappeared during the cooling process. The lid reflex was often the first to reappear. The earliest spontaneous movements were usually those of the lids and vibrissae. They were followed by slight movements of the head from side to side. Stroking of the vibrissae, at first elicited a response confined to the vibrissae themselves but, later, at higher temperatures brought about turning of the head toward the side stimulated. Still later it aroused generalized struggling. The massive bodily activity so characteristic of the early part of the chilling process was slow in developing and was never as great. Even when the body temperature had returned to normal the animals were sluggish in comparison to their previous state. All of the animals recovered with no permanent recognizable disabilities.

The respiratory rate continued to decrease for the first few minutes after the mouse was released from the cold, and then began a rapid rise which paralleled, at a lower level, the rate at comparable temperatures during the cooling process. During the period of observation it never equalled the base-line rate.

MEASUREMENTS OF BIO-ELECTRIC POTENTIALS

Base-line Period

The base-line potentials at the onset of chilling and for the 30 minutes preceding are shown in table I. The averages were calculated by two methods: 1) considering magnitude only, without regard to polarity; 2) taking polarity into account, allowing + and — values to cancel each other. Plus and minus here indicate that the head was positive or negative to the sacrum. The two averages thus determined are measures of different things. The first is essentially a measure of distribution, and shows the average deviation from the neutral point. The second is the true mean and, therefore, gives no indication of distribution.

When these two measures are compared in the two strains some interesting differences appear. The mean and the average deviation of the C_3H strain are relatively constant, while in the CBA group the mean is variable and the average deviation shows a progressive

increase. The probable error of the average deviation for the CBA group is around ± 1.3 mv. and that for the C_3H strain is around ± 0.9 mv.

TABLE I

Minutes before chilling	30	25	20	15	10	5	0
CBA							
No. of cases	8	8	8	8	10	10	10
Av. deviation from 0 in mv.	7.6	7.7	7.3	8.2	8.2	9.2	9.4
Mean potential in mv.	—2.6	—1.8	—0.8	—0.7	—1.8	—2.8	—2.7
C_3H							
No. of cases	9	9	10	10	10	10	10
Av. deviation from 0 in mv.	4.9	4.9	4.5	4.5	4.5	4.5	4.5
Mean potential in mv.	—3.7	—4.0	—3.5	—3.3	—3.2	—3.1	—3.2

Cooling Period

The average potentials during the cooling period are shown in table II. A striking difference in the average deviation of the e.m.f. is again evident in the two strains. The CBA average dropped off markedly during the first 10 minutes of chilling, after which it tended to become more steady. The average for the C_3H's rose slightly during the first 5 minutes and increased very slowly thereafter. The probable errors of the average deviations of the two strains are around ±1 mv. The mean potentials varied in the same manner as the average deviation, but, in view of the base-line variations, it is doubtful if any significance can be attached to them.

TABLE II

Minutes of chilling	0	5	10	15	20	25	30	35
CBA								
No. of cases	10	10	10	10	10	10	6	
Av. deviation from 0 in mv.	9.4	6.3	4.7	4.4	4.2	4.5	3.6	
Mean potential in mv.	—2.7	—0.9	—0.2	—0.5	—0.4	—0.7	—1.2	
C_3H								
No. of cases	10	10	10	10	10	10	9	9
Av. deviation from 0 in mv.	4.5	5.2	5.2	5.4	5.4	5.6	5.8	6.0
Mean potential in mv.	—3.2	—4.2	—4.7	—4.6	—4.6	—4.4	—4.4	—4.3

Warming Period

During the warming period the average potentials were as in table III. The duration of the period is measured from the time of removal from the cooling chamber and since the animals were not kept identical lengths of time in the chamber the 0 time column does not correspond to any one of table II. In the CBA's there was a small increase in the average deviation of the potentials during the first 15 minutes, after which they remained fairly constant. The C_3H's also exhibited a slight rise followed by an equally slight fall. The probable error of the average deviations of the two strains is again around ± 1 mv. The mean potential exhibited the same strain difference in variability as during the base-line period.

TABLE III

Minutes of Warming	0	5	10	15	20	25	30	35	40	45	50	55	60
CBA													
No. of cases ...	10	10	10	10	10	10	10	9	8	6			
Av. deviation in mv. ..	4.2	4.4	4.9	5.2	5.3	5.1	5.2	5.0	5.5	4.9	-		
Mean potential in mv. ..	0	—0.2	—0.8	—1.4	—1.5	—1.3	—1.2	+0.2	+1.1	—0.1			
C_3H													
No. of cases ...	10	10	10	10	10	10	10	10	10	8	8	6	6
Av. deviation in mv. ..	4.9	4.8	5.2	5.4	5.7	5.5	5.3	5.2	5.1	5.2	5.0	4.9	5.0
Mean potential in mv. ..	—3.3	—3.4	—3.3	—4.4	—4.4	—4.4	—4.3	—3.9	—3.5	—3.9	—4.0	—3.4	—3.5

Discussion

In this analysis of the effects of a depressed environmental temperature the observations may be discussed under the following headings: (1) the internal temperature, (2) the bodily activity and reflexes, (3) the respiratory rate, and (4) the bio-electric potentials.

Internal body temperature. The initial temperatures of the mice under base-line conditions were not uniform. They ranged from 33° to 38° C. During the continued observations at room

temperature, lasting 30 minutes or more, the internal temperatures sometimes varied, up to a maximum of 3.5° C. Occasionally this change was an elevation, but more often a depression. Variations of similar magnitude have been noted in pigs, asses, cattle, dogs, and poultry, according to Deighton.[1] There was no apparent correlation of bodily activity and body heat, for the temperature rose, dropped, or remained steady in the absence or presence of massive struggling movements. The disparity of these observations with those of Hamilton,[2, 3] who found an increase in temperature with struggling and excitement, may be due to the possibly greater restriction of movement in our animals.

When the mice were placed in the cooling chamber the temperature began to drop almost immediately, with a latency of no more than a minute or two. The active attempt of mice to maintain the body heat level which Pembrey[7] noted, undoubtedly occurred in our animals, as was indicated by the rise in the respiratory rate. They were unable, however, to overcome, even by struggling, the effects of the severe cold. The loss of heat proceeded with remarkable uniformity, the speed of which appeared to be roughly proportional to the intensity of the environmental cold. There were no plateaus which indicated a sustained body temperature at any time during the chilling period beyond the first minute or two. A similar linear decrease in body heat was observed by Hamilton, Dresbach, and Hamilton[5] in rats. In a few of our cases the temperature record did show sudden drops with prompt returns which were, apparently, associated with relaxation of the anal sphincter and the admission of cold air into the rectum. The prompt return in these cases gave definite indication that the thermocouples were recording actual body temperatures, not environmental air temperatures.

It was found possible to reduce the body temperatures to extremely low levels, in one case reaching 8.5° C., with subsequent recovery of the animals. So far as we know this is the lowest temperature on record to which non-hibernating warm-blooded animals have been reduced and have survived. Every animal in the experiments here reported recovered without artificial aid other than heat from a lamp. Simpson and Herring[10] found temperatures below 16° C. lethal for cats; Hamilton[4] found that rats did not survive below 12° C. and that life was precarious even up to 16° C., artificial respiration often being necessary to assist recovery. It should be noted, however, that these latter experiments are not strictly comparable with ours, for, in them, the animals were exposed over a longer period (3 to 5

hours) to a less intense cold (4° to 9° C.). Hibernating mammals have been brought to much lower temperatures according to Pembrey and White.[8]

An interesting phenomenon was the frequent continuance of the lowering of body temperature for a minute or two after the animal was removed from the cooling chamber. This latency, as well as part of that observed at the onset of chilling, may well be the expression of the thermal inertia of the mass of tissue measured by the colonic thermocouple. The same effect was figured by Hamilton[4] without comment.

Following the latent period, the temperature curve rose steadily until it approached the normal level, when it became progressively less steep. The time required for this process could be shortened by the application of more external heat.

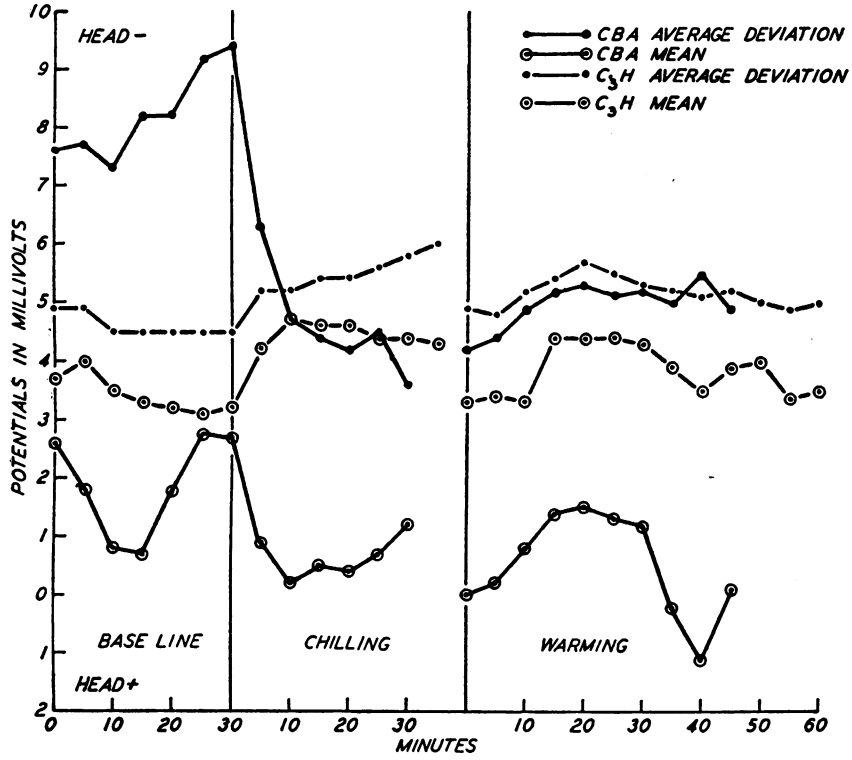
Body movements and reflexes. With the lowering of body temperatures to 15° C. or less, spontaneous body movements were gradually reduced in magnitude and number until they eventually disappeared, the movements of the head and tail being the last to go. The response to the reflexes tested, likewise, altered from a generalized pattern to a more restricted one, so that, for example, touching the cheek at first led to extensive movements involving a large part of the body while later there followed only a slight turning of the head toward the stimulus. Eventually, even this small response disappeared. The same course of events took place with touching other parts of the body, as well as with visual and auditory stimuli. Approximation of the eye-lids on stroking the skin near the eye was usually the last reaction to disappear and was not always lost. The temperatures at which these more discrete responses failed were usually below 15° C. The return of these functions was more or less in the inverse order of their disappearance. These observations are in harmony with those of Simpson and Herring[10] and of Hamilton.[4] Shivering movements were not seen during the chilling period, perhaps because they were masked by the coarser, struggling activity of the animal. They were noted, however, during the warming period after spontaneous movements had appeared.

Respiration rate and metabolism. The initial effect of chilling on respiratory activity was an acceleration from the normal rate of approximately 200 per minute to a rate of 250 or more per minute. This lasted during the first 8° to 18° C. of heat-loss and was followed by a diminution at a fairly steady gradient of about 10 excursions per minute per degree of heat-loss until a temperature of

approximately 12° to 16° C. was reached. The subsequent drop in frequency was much greater, being about 20 excursions per degree of heat-loss. At the thermal level at which the respiratory decline became accelerated, the spontaneous movements ceased and the individual reflexes began to disappear. The decline in the number of respiratory excursions continued for a minute or so after removal of the mouse to a warmer environment and then rose in a manner more or less similar to the temperature curve. The frequency was usually slower during the warming period than during the cooling period at comparable temperatures. These observations on the correlation of body temperature and respiratory rate are in agreement with those of Hamilton, with the exception of the initial increase and the more rapid decline at lower temperatures which he did not report.

It is interesting to speculate whether the rate of respiration and the temperature are indicative of the metabolic level. Within the non-lethal limits of human temperature fluctuations, it may not be safe to assume a positive correlation between body heat and metabolic rate according to Deighton,[1] although it has been emphasized "that in fevers metabolism rises with body temperature in accordance with van't Hoff's law" (pp. 429-30). In rodents, however, where the induced temperature changes may be great there is much to suggest a closer correlation of respiratory rate than of body temperature to metabolic rate. Pembrey and White[8] found that CO_2 production of the dormouse during marked chilling first increases and then decreases. There is, moreover, a continued decline in CO_2 production during the first few minutes of warming, followed by a rise during the subsequent increase in body heat. Unpublished observations of Herrington indicate that when mice are chilled there is an initial acceleration of metabolic activity and cardiac rate, followed by a decline. These phenomena resemble the respiratory curves of our experimental animals and suggest, therefore, that the respiratory rate under certain conditions may be an index of the metabolic rate. The findings of Hamilton[4] and of Hamilton, Dresbach, and Hamilton[5] are somewhat at variance with this, for they reported that the respiratory and heart rates in kittens and rats fall linearly with cold until a level of 65° F. (18.5° C.) is reached, when cardiac arrhythmias appear. Further experiments are needed to determine this point. Regardless of the question just raised, there can be little doubt that at the low temperature reached with resultant loss of spontaneous movement and of reflexes, the metabolic rate was definitely reduced.

Bio-electric potentials. In our previous study of base-lines it was pointed out that the mice should be segregated into genetic strains in order to give valid results in dealing with the potential differences. Our present observations confirm this point. The aver-



Average deviations and means of electrical potentials in millivolts of CBA and C_3H mice. Head− and head+ refer to means only. The curve from chilling to warming is discontinuous, since the period of cold was not equal in all cases. The recovery period was calculated from the beginning of warming in all cases in order to make them comparable.

age deviations and the means of the potentials for the two strains during the base-line, chilling, and warming periods are shown in Fig. 1. During the base-line period the average deviations for the CBA's for the 30 minutes prior to chilling were greater than those of the C_3H's. They also increased with time while those of the

C_3H's did not, as was shown in our previous report. Although this increase is not much more than the probable error, it is consistently in the same direction (i.e., greater magnitude). It should be stated, also, that these figures correspond in some cases to the last 30 minutes only of longer base-lines, the other parts of which show a similar consistent increase in potential with time.

During the cooling period the potential differences of the CBA's rapidly declined for the first 10 minutes to reach a level commensurate with that of the base-line of the C_3H's. Here they remained. This drop in the average deviations of the potential differences is slightly more than four times the probable error and is, therefore, probably a reliable indication of a change associated with the experimental situation. The C_3H's on the other hand, showed a slight and probably not significant increase. During the warming period the potential differences of the two strains were more or less constant and equal in magnitude. The means of the C_3H's varied within narrow limits during all phases of the experiment. Those of the CBA's, on the other hand, ranged widely and with no particular pattern.

It is apparent from these observations that there is no direct effect of cold on the e.m.f. of the mouse as determined by our method, for there is no consistency appearing in both strains. Nevertheless, there is a marked reduction in the average deviation of the bio-electric potentials of the CBA's which is even more striking in contrast with the increasing magnitude of the base-line period and the relatively unchanging level of the warming period. It raises the question whether there is some factor responsible for the greater magnitude and variability of the CBA's which is reduced or obliterated by chilling so that the two strains are left more or less equal.

The possible relation of these bio-electric potentials to metabolism is a question that has frequently been raised. Purdy, Johnson, and Sheard[9] reported a correlation between basal metabolism and skin conductance which could be stated in mathematical terms so that $BM = \frac{\log y - \log 0.005}{-0.0396}$ when y is the electrical potential difference between the area of skin over the articulation of the ulna and radius at the wrist and that at a point 12 cm. more proximal. According to them, the higher the basal metabolism the lower the potential difference and the higher the skin conductance. They found the rate of blood flow to be the medium whereby the metabolic

rate affects the potential. Since the Burr, Lane, Nims microvoltmeter was constructed so as to be independent of skin resistance, our results would hardly be expected to coincide with those of Purdy, Johnson, and Sheard.

Although we have no direct observations on the metabolism of our experimental animals with which to correlate the data obtained on their electrical potential differences, the evidence available offers no support for the thesis that these potentials are directly related to metabolism. If it can be assumed that a mouse has a lowered metabolism when its body temperatures are so low that its respiratory rate is markedly reduced and many of its spontaneous activities and reflexes are eliminated, then there is seen to be no distinct relationship between the metabolic rate and the electrical potential differences of the C_3H mice as recorded by our method. Since there is no evidence that the metabolic rate differs in strains, it should hold for the CBA mice as well. Metabolism, therefore, would appear to be not effective in the production or alteration of bio-electric potentials as determined by the procedures used here.

Summary

The effects of a markedly depressed environmental temperature on 20 young, normal, unanesthetized male mice of two genetic strains are discussed. The experiments reveal the following facts:

1. A normal variability exists in the base-line temperatures and a linear drop appears on exposure to severe cold. The animals are able to survive an internal temperature of 8.5° C.

2. Chilling produces an initial acceleration of bodily activity followed by an obliteration of spontaneous movements and of certain reflexes which return, approximately, in the inverse order of their disappearance.

3. Chilling produces an initial acceleration in the respiratory rate followed by a reduction. The rate diminishes approximately 10 excursions per minute per degree of heat-loss, until an internal temperature of 12° to 16° C. is reached, after which it diminishes approximately 20 excursions per minute per degree.

4. Strain differences in the bio-electric pattern of potentials in mice are confirmed. These differences appear both in the base-line and in the mode of reaction to chilling. The electrical potentials of the C_3H strain remain relatively constant throughout all condi-

tions of the experiment. Those of the CBA strain increase during the base-line period, drop sharply with cold, and remain at this new level during warming.

References

1 Deighton, T.: Physical factors in body temperature maintenance and heat regulation. Physiol. Rev., 1933, *13*, 427-65.

2 Hamilton, J. B.: Visceral temperatures in the intact and unanesthetized animal. II. The uterus of the rat. Endocrinology, 1936, *20*, 788-94.

3 Hamilton, J. B.: Visceral temperatures in the intact and unanesthetized animal. I. A new technique for measurement. J. Lab. Clin. Med., 1937, *22*, 466-76.

4 Hamilton, J. B.: The effect of hypothermic states upon reflex and central nervous system activity. Yale J. Biol. & Med., 1937, *9*, 327-32.

5 Hamilton, J. B., Dresbach, M., and Hamilton, R. S.: Cardiac changes during progressive hypothermia. Am. J. Physiol., 1937, *118*, 71-76.

6 Marshall, C., and Meader, R. G.: Studies on the electrical potentials of living organisms: I. Base-lines and strain differences in mice. Yale J. Biol. & Med., 1937, *10*, 65-78.

7 Pembrey, M. S.: The development of the power of regulating heat production. J. Physiol., 1894, *17*, Proc. Physiol. Soc., 27-28.

8 Pembrey, M. S., and White, W. Hale: Heat regulation in hybernating (sic) animals. J. Physiol., 1895, *28*, Proc. Physiol. Soc., 35-37.

9 Purdy, C., Johnson, A. F., and Sheard, C.: The relationship between electrical differences of potential in the skin and normal basal metabolism. Science, 1931, *73*, 46-49.

10 Simpson, S., and Herring, P. T.: The effect of cold narcosis on reflex action in warm-blooded animals. J. Physiol., 1904-05, *32*, 305-11.