Evidence for a Metabolic Limitation of Survival in Hypothermic Hamsters[1] (36658)

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The survival of homeotherms in profound hypothermia has been investigated for many years. There are two main factors which contribute to the ultimate demise of the hypothermic animal: cardiac failure or respiratory failure, both resulting in an anoxic death of the tissues (1). The mechanisms leading to death are highly dependent upon inherent physiological characteristics of the animal itself and the temperature at which it is maintained. Adolph *et al.* (2) have shown that respiration ceases at a higher temperature than the beating of the heart in hypothermic rats, and Fisher *et al.* (3) have shown that a decline in cardiac output is the primary failure in dogs maintained at 23°.

The hypothermic hamster (*Mesocricetus auratus*) is a special case. This animal has the ability to hibernate for periods as long as seven days (4), but will last for approximately 22 hr in helium-cold induced hypothermia at a rectal temperature of 7°. The underlying factor(s) limiting survival in the hypothermic state is the subject of this paper.

Anderson *et al.* (5) have shown that the heart rate and oxygen consumption are stable for several hours in the hypothermic hamster. During this stable period the heart is not subjected to an anoxic stress (6).

Musacchia (7) has shown that cold or heat acclimation prior to the induction of hypothermia affects induction time and survival time in hypothermia. Heat acclimation will shorten induction time and prolong the survival time while cold acclimation has the opposite effect. However, the animals shiver during the induction period thus expending a considerable amount of energy in an attempt to remain homeothermic. If this energy loss has an effect on the survival time in hypothermia it may explain the discrepancy between the heat-acclimated and cold-acclimated hamsters as well as the hypothermic and hibernating hamsters. Thus, an attempt was made in this study to investigate the effect of shortened induction time on the energy stores and the survival of the hamster in hypothermia.

The results of this study may also have an application in the interpretation of experiments on nonhibernators using the Giaja technique (8) or other similar stressful methods of induction (2, 9).

Methods. Hamsters (*Mesocricetus auratus*) of both sexes were inducted into profound hypothermia, rectal temperature (T_{re}) of 7°, using the method of Fischer and Musacchia (10). Briefly, the animals are placed in a 20% O_2 and 80% He atmosphere at 0° until they reach a T_{re} between 10 and 7°. They are then transferred to air (T_a) at 7° until used.

The first series of experiments involved a group of eleven hamsters which were clipped of their fur under light sodium pentobarbital anesthesia (65 mg/kg ip) 16 hr before induction into hypothermia. The control group of 11 hamsters were also lightly anesthetized at the same time. After induction, all of these animals were kept at T_{re} 7° until death, which was determined by monitoring the ecg (lead II) and visual observations.

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TABLE I. Induction Time and Survival Time in Hypothermia, $T_{re} = 7°$, of Control and Fur-Clipped Hamsters. Values are Means ± SE.

	Induction time (hr)	Survival time (hr)
Control ($n = 11$)	7.8 ± 0.7	22.0 ± 2.9
Fur-clipped ($n = 11$)	3.8 ± 0.3 $p < 0.001$	38.7 ± 4.8 $p < 0.01$

A second experiment involved two groups of hamsters; animals in both groups were inducted into hypothermia as described above. Each group contained subgroups of fur clipped and control hamsters. Blood was taken from one group of hamsters immediately following induction. Hamsters in the other group were allowed to remain at T_{re} 7° until the onset of gasping before the blood samples were drawn. Gasping indicates the failure of spontaneous respiration and precedes death by approximately two hr (5). The blood samples were centrifuged and the plasma analyzed for glucose colorimetrically by means of a glucose oxidation reaction using the Glucostat preparations (Worthington Biochemical Corporation, Freehold, NJ.)

A third experiment followed the same procedure as the second except that instead of taking blood samples, a section of the liver was removed and immediately frozen with dry ice. The liver was then homogenized in 10% trichloroacetic acid, filtered, and an aliquot of the filtrate was analyzed for glycogen according to the method of Carroll, Longley, and Roe (11).

Results. The reduction of insulation by clipping the fur from the hamsters resulted in an induction time less than half of that of the control hamsters (Table I). Survival under hypothermic conditions was significantly prolonged when induction time was shortened.

Table II contains the results of the glucose and glycogen analyses. The fur-clipped hamsters had a significantly higher plasma glucose immediately following induction into hypothermia, but at the onset of gasping the plasma glucose levels in animals in both groups were the same.

Similar results were found with the liver glycogen study. The control hamsters depleted more of their liver glycogen during the induction period than the fur-clipped hamsters. At the onset of gasping, animals in both groups were depleted of liver glycogen, but the fur-clipped hamsters were significantly lower than the controls. The gasping control animals had higher liver glycogen levels than control animals immediately following induction.

Discussion. The prolongation of survival in hypothermia by shortening the induction time as demonstrated in these experiments is a new concept in the study of hypothermia. Popovic (12) varied the induction time of rats using several techniques but found no difference in survival times. Instead the survival of the rats was related to the temperature at which they were maintained in hypothermia, the lower the temperature the shorter the survival time. This suggests that there may be a significantly different mechanism leading to failure in the hamster as compared to the rat or else Popovic's different techniques (12) did not alter the stress of induction to a significant degree.

The analyses of glucose and glycogen indicate that the survival of the hamster may be related to the depletion of energy stores dur-

TABLE II. Plasma Glucose and Liver Glycogen of Control and Fur-Clipped Hamsters. Hypothermia, $T_{re} = 7°$. Values are Means ± SE.

	Immediately following induction	Onset of gasping
Plasma glucose (mg %)		
Control	31.3 ± 6.76 $n = 15$	5.48 ± 1.73 $n = 8$
Fur-clipped	73.5 ± 14.7[a] $n = 12$	4.65 ± 2.32 $n = 6$
Liver glycogen (mg/g wet weight)		
Control	.422 ± .11 $n = 9$	.869 ± .14 $n = 8$
Fur-clipped	10.6 ± 2.17[a] $n = 10$	.196 ± .003[a] $n = 5$

[a] Significantly different from controls ($\alpha = .05$).

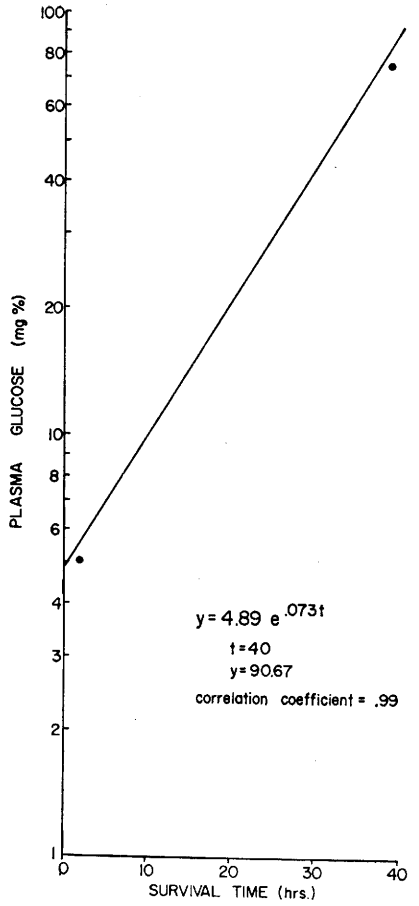

FIG. 1. Survival in hypothermia, T_{re} 7°, as a function of plasma glucose concentration. Average values (●) are used to determine the best fit line.

ing the induction period. The longer the period of stress during induction the greater will be the depletion of carbohydrate stores with a resultant decrease in survival in hypothermia.

The relation between plasma glucose and survival can be easily seen by combining the data from the survival experiment and the glucose experiments in the graph shown in Fig. 1. In this figure the natural log of the average plasma glucose concentration for each group is plotted as a function of the survival time of the corresponding group in the survival experiments. The glucose levels at gasping for control and fur-clipped animals were combined to give one point. If the average values are used to determine the best fit line the result is $y = 4.87$ exponent $.073t$, with a correlation coefficient of .99. The average values must be used since the survival times of the individual animals used for glucose determinations were not measured. Thus, an exponential depletion of plasma glucose allows the prediction of survival time in hypothermia simply by measuring the plasma glucose anytime after induction.

The exponential depletion of plasma glucose, seen in this study, may not obtain in situations in which initial values are high. For example, in the hibernating ground squirrel there is a linear decay in plasma glucose from 150 mg% at the onset of a hibernating period to near 50 mg% at the end of the period (13). It seems reasonable to suggest that at low body temperatures and high plasma glucose levels the metabolism of glucose will depend on enzyme activity. In contrast, at low plasma levels such as we found in the hypothermic hamster, glucose availability might be the rate-limiting factor and result in a first order reaction.

Not only do the fur-clipped hamsters have a higher liver glycogen content immediately following induction than does the control group, but for unknown reasons they also seem to be able to deplete their liver glycogen stores to a lower level before failing. The liver glycogen in the control group tends to be higher at the onset of gasping than immediately following induction. In comparison to the normal liver glycogen levels in these animals this increase in liver glycogen during a hypothermic exposure is very slight. This suggests that the animal's capacity for gluconeogenesis may be limited.

It is seen from the data reported here that the failing organ is extremely dependent upon plasma glucose levels for viability. The brain, including the respiratory centers, is almost completely dependent upon glucose for its source of energy (14). It is also known that when glycolysis and oxygen consumption are blocked, the respiratory center fails before the heart stops beating in neonatal rats (15) Artificial respiration will also prolong survival in hypothermic rats (16).

It appears that the hypothermic hamster maintains a steady state until the depletion

of the carbohydrate stores. The heart rate and oxygen consumption are stable for several hours (5). The homeostatis maintenance of the heart in hypothermia has been well established. Anderson *et al.* (6) have shown that the heart of the hamster is not subjected to anoxic stress during prolonged hypothermia. The metabolism and membrane potentials are maintained in the hearts of hypothermic ground squirrels (17). It is also known that the heart is not dependent upon glucose for its energy supply, *viz.*, the hypothermic rabbit heart will utilize pyruvate in preference to glucose in the presence of endogenous fatty acids (18).

Although the level of oxygen consumption that is maintained during hypothermia is about half of that of a hibernating hamster (5), this does not mean that the tissues are anoxic. Popovic (19), using the Giaja hypercapnic-hypoxia technique, reported that the hypothermic (10°) winter ground squirrel has an oxygen consumption many times higher than the hypothermic summer animal, without any difference in the survival time. Also Fisher *et al.* (3) have shown that in hypothermic dogs (23°) a stable oxygen consumption can be maintained in spite of a declining cardiac output by increasing the A–V oxygen difference. This demonstrates that the tissues could extract more oxygen from the blood if it were necessary to do so.

After the relatively stable period during which the liver glycogen and plasma glucose are depleted, the hamster begins a period of abnormal respiration typified by periods of gasping and apnea and finally respiratory arrest (5). Therefore, these data together with previous reports from this laboratory are consistent with the hypothesis that the primary cause of death in the hypothermic hamster is failure of respiration due to a depletion of energy supplies to the respiratory center.

It is of interest to note that this decline in plasma glucose levels may not be an abnormality of hypothermic hamsters as compared to hibernating ones. Galster and Morrison (13) reported that the plasma glucose levels of hibernating arctic ground squirrels also decline and are restored to higher levels during the arousal periods. They suggest that the decline of carbohydrates in plasma, liver, and muscle may actually initiate the arousal process.

The hypothermic hamster is different only by a matter of degree since it is partially depleted of its carbohydrates at the onset of hypothermia and also because it is unable to initiate the arousal process. If the hamsters are removed from the cold room when the plasma glucose is depleted (onset of gasping) they will rewarm at room temperature and survive with no notable ill effects.

It is probable that the hypothermic hamster would survive for the same length of time that it could hibernate if a means of reducing the body temperature in an unstressful way were possible.

Summary. Two groups of hamsters (*Mesocricetus auratus*) were inducted into hypothermia by the helium-cold method and survival time was determined. The induction time of one group of hamsters was reduced by clipping the fur prior to the induction procedure. Additional groups of fur-clipped and nonclipped control hamsters were used for determinations of plasma glucose and liver glycogen immediately following induction and at the onset of gasping (approximately 2 hr before death). The fur-clipped hamsters had a shorter induction time, a longer survival time, and higher plasma glucose and liver glycogen immediately following induction. At the onset of gasping both groups had similar, extremely low plasma glucose levels and the fur-clipped animals had lower liver glycogen values. Survival time in hypothermia correlates with the plasma glucose levels which decay exponentially during hypothermia. These findings are consistent with the hypothesis that the primary cause of death in the hypothermic hamster is failure of respiration due to the depletion of carbohydrate energy supplies and may explain why survival time in hypothermia is shorter than the normal hibernation time of the hamster.

1. Blair, E., Fed. Proc. **28**, 1456 (1969).

2. Adolph, E. F., Nabershnig, A., and Orchard, D. P., J. Appl. Physiol. **16**, 819 (1961).

3. Fisher, B., Russ, C., and Fedor, E. J., Amer. J. Physiol. **188**, 473 (1957).

4. Lyman, C. P., J. Exp. Zool. **109**, 55 (1948).

5. Anderson, G. L., Volkert, W. A., and Musacchia, X. J., Amer. J. Physiol. **221**, 1774 (1971).

6. Anderson, G. L., Prewitt, R., Jr., and Musacchia, X. J., Amer. J. Physiol. **221**, 1760 (1971).

7. Musacchia, X. J., Amer. J. Physiol. **222**, 495 (1972).

8. Giaja, J., C. R. Acad. Sci. **210**, 80 (1940).

9. Adolph, E. F., and Nail, R. L., J. Appl. Physiol. **15**, 911 (1960).

10. Fischer, B. A., and Musacchia, X. J., Amer. J. Physiol. **215**, 1130 (1968).

11. Carroll, N. V., Longley, R. W., and Roe, J. H., J. Biol. Chem. **220**, 583 (1956).

12. Popovic, V., Amer. J. Physiol. **199**, 463 (1960).

13. Galster, W. A., and Morrison, P., Amer. J. Physiol. **218**, 1228 (1970).

14. Sokoloff, L., "Handbook of Physiology, Neurophysiology," Vol. 3, Sect. 1, Chapt. 77, p. 1843. American Physiological Society, Washington, D.C. (1961).

15. Samson, F. E., Jr., and Dahl, N., Amer. J. Physiol. **188**, 277 (1957).

16. Adolph, E. F., and Goldstein, J., J. Appl. Physiol. **14**, 599 (1959).

17. Timny, M. L., and Taylor, S., Amer. J. Physiol. **208**, 1247 (1965).

18. Pelliveau, R. E., and Covino, B. G., Arch. Int. Pharmacodyn. Ther. **180**, 331 (1969).

19. Popovic, V., Ann. N.Y. Acad. Sci. **80**, 320 (1959)

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