Certain specific types of cheese contain relatively high concentrations of tyramine (*4*). Depending on the concentration of the tyramine in the cheese and the amount of cheese ingested, it is quite conceivable that a sufficient amount of tyramine could be ingested to precipitate a marked hypertensive response in a patient whose monoamine oxidase is blocked. Indeed, a number of clinical investigators (*5*) have already called attention to the fact that patients being treated with monoamine oxidase inhibitors have experienced hypertensive crises after ingestion of cheese known to contain large amounts of tyramine.

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References and Notes

1. R. M. Cares and C. Buckman, *Diseases Nervous System* **22**, 97 (1961).
2. R. F. Maronde and L. J. Haywood, *Ann. N.Y. Acad. Sci.* **107**, 975 (1963).
3. J. H. Burn and M. J. Rand, *J. Physiol.* **144**, 314 (1958).
4. F. V. Kosikowsky and J. Dahlberg, *J. Dairy Science* **32**, 316 (1949).
5. B. Blackwell, *Lancet* **1963-II**, 414 (1963).
6. The technical assistance of P. J. Fowler and T. Fujita is gratefully acknowledged.
7. After this manuscript was submitted for publication, reports by Blackwell and Marley and by Natoff were published in *Lancet* **1964-I**, 530 (1964). These reports are in agreement with the conclusions drawn here.

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Hypothermia, Asphyxia, and Cardiac Glycogen in Guinea Pigs

Abstract. *Cardiac glycogen was not affected by cooling guinea pigs for short periods. In normothermic animals it was reduced 75 percent or more at the time of death from asphyxia. Quickly cooled animals asphyxiated until the time of death of warm controls showed no significant losses of cardiac glycogen; animals cooled while breathing 10 percent oxygen plus 5 percent carbon dioxide showed slight reductions. Therefore, hypothermia spares cardiac glycogen during asphyxia, but there are factors other than cardiac glycogen which influence survival of asphyxiated animals.*

Hypothermia has been found to be the most effective treatment we have tested in preventing death from asphyxia in experimental animals (*1–4*). For example, puppies at 15°C body temperature live on the average 7½ times as long as normothermic littermates and many recover without assistance from 2½ hours in 95 percent N_2 plus 5 percent CO_2 (10 times the lethal exposure for warm littermates) (*3, 5*). Cooling also has been tried successfully on more than 130 asphyxiated human infants that had previously failed to respond to the usual resuscitative measures (*5, 6*). As indicated by the learning and memory of a conditioned avoidance response in rats, cooling, if adequate, not only saves lives but also protects brain function as well. Some impairment was found in the rats in which cooling was minimal, that is, lowest temperatures were above 32°C. None was found in those whose temperatures fell to 31°C or less (*7*).

Because of the precise relationship found by Stafford and Weatherall (*8*) between cardiac glycogen and the length of survival of rats in nitrogen, these workers suggested that the limiting factor in anoxic survival might be the initial carbohydrate concentration in the heart. Accordingly, it became important to ascertain whether or not the protection conferred by hypothermia against asphyxia was reflected by a similar protection against the depletion of cardiac glycogen caused by the asphyxia.

The experimental material consisted of unanesthetized young adult male guinea pigs (weighing 300 g) which had been fasted for 24 hours, and day-old neonates of the same species. The animals were asphyxiated in a bell jar through which was flowing a stream of 95 percent N_2 plus 5 percent CO_2 at a rate of 10 liters or more per minute. Both the warm and the cooled experimental animals were killed immediately after the time of last gasp of the warm animals. The adult animals were killed by a blow on the head and the neonates by decapitation with a guillotine. At the time they were killed the body temperature of the warm controls was 37°C and that of the cooled animals was between 23°C and 25°C. While one investigator rapidly dissected the gastrocnemii, another excised the liver, the heart, and the diaphragm. The liver and the heart were quick-frozen immediately after removal; the gastrocnemii and diaphragm were frozen simultaneously somewhat later. Rapid freezing was accomplished by compression between two blocks of CO_2 ice (minus 79°C) and the freezing time was recorded as seconds after decapitation. Since guinea pigs cooled under hypoxia-hypercapnia live approximately 50 percent longer than those cooled in air (*5, 9*), in the experiments with adults two additional groups of animals were cooled to 25°C in an atmosphere of 10 percent O_2 plus 5 percent CO_2 (while subjected to hypoxia-hypercapnia). The animals in one group were killed without further treatment in order to test the effects of the method of cooling on glycogen content. Those in the other group were asphyxiated for the same length of time as the normothermic experimental animals.

Litters of three were used for the experiments on newborn animals. One animal in each litter, which served as the control, was killed while normothermic. The second was asphyxiated while normothermic and decapitated immediately after its last gasp. The third was cooled to approximately 25°C and killed after asphyxiation for the same length of time as in the case of the second animal.

Glycogen was determined by the anthrone method of Morris (*10*) as modified by Russell and Bloom (*11*) and by use of Russell's "routine reagent A" (*12*).

The effects of cooling and of cooling combined with hypoxia-hypercapnia on cardiac glycogen of adult animals are summarized in Table 1. The table shows that rapid cooling by immersion in ice water was not associated with loss of glycogen from the heart. However, when the cooling was accompanied by exposure to 10 percent O_2 plus 5 percent CO_2 plus 85 percent N_2 (hypoxia-hypercapnia) there was a statistically significant fall in cardiac glycogen of 30 percent (from 6.3 $\pm$ 0.72 mg/g to 4.4 $\pm$ 0.27 mg/g). The normothermic animals which were asphyxiated until their last gasp (hearts frozen 70 seconds later) lost 75 percent of the glycogen from the ventricles (a reduction from 6.3 mg/g to 1.6 mg/g). The quickly cooled hypothermic animals asphyxiated for the same length of time showed no loss of cardiac glycogen when frozen 74 seconds after stunning. Under the same experimental conditions, the cardiac glycogen of the animals which were cooled with hypoxia and hypercapnia averaged less than that of the group which was cooled while breathing air, but the difference was not statistically significant (5.9 $\pm$ 0.43

mg/g as compared with 6.6 ± 0.22 mg/g).

Our determinations also showed that neonatal guinea pigs have less cardiac glycogen than do adults of the same species. Since they gasp for a longer period than do adults, it was not surprising that at the time of last gasp the glycogen content of the ventricles likewise was lower than in the case of adults (0.7 mg/g as compared with 1.6 mg/g). The percentage loss of glycogen during asphyxiation also was somewhat greater in the newborn than in the adult (82 percent as compared with 75 percent).

The protection against asphyctic loss of cardiac glycogen conferred by hypothermia which has been found in the experiments with adults also was evident in the experiments on the newborn animals. At the time of death of the normothermic animals, their cooled littermates showed no significant reduction (from 3.9 mg/g to 3.6 mg/g, a mean loss of 8 percent).

Hypothermia has been shown to prolong greatly the survival time of newborn animals under asphyxia, to permit spontaneous recoveries without sequelae from exposures which were lethal for littermates, and to permit retention of a previously learned, conditioned avoidance response (*2, 3, 7*). The results reported here demonstrate the benefits of hypothermia in asphyxia with respect to another parameter of measurement. Since there is general agreement that oxygen lack causes marked breakdown of carbohydrate in the heart (*13, 14, 15*), the postponement of this process in cooled animals until after the time of death of normothermic controls is evidence of the efficacy of hypothermia in protecting the heart from anoxemia. Although, as will be seen later, there are reasons for believing that the crucial organ in asphyxia is the brain rather than the heart, it is entirely possible that a relatively small and completely reversible reduction in cardiac output during asphyxia may cause profound and irreversible changes in the central nervous system. Recent studies, which have shown that oxygen content of brain tissue is very low (*16*), support the concept that under ordinary conditions this organ receives barely enough oxygen to maintain normal function.

The data of Stafford and Weatherall (*8*) show remarkable parallels between length of anoxic survival and levels of cardiac glycogen. This has been interpreted as evidence that resistance to anoxia is dependent upon cardiac glycogen (*14*). However, with the facts now at hand, it would be premature to assign a cause-effect relationship between these two phenomena. Although it is highly improbable that the events are unrelated, the possibility that they both have a common cause and are not directly related to each other has not been excluded in the published experimental results. Indeed, the latter possibility may represent the true state of affairs.

Indications that factors other than cardiac glycogen are important in the resistance of animals against asphyxia are found in the data reported here. First, although newborn guinea pigs are approximately twice as resistant to asphyxia as are adults at the same body temperature, the glycogen content of their ventricles is only approximately one half that of adults. Secondly, exposure to 10 percent O_2 plus 5 percent CO_2 during cooling has been found to increase by 50 percent the survival times over controls at the same body temperature (23° to 25°C) which had been cooled while breathing air. The determinations reported here showed that, instead of increasing the glycogen content of the heart by approximately 50 percent, this method of inducing hypothermia resulted in appreciable decreases.

Thirdly, species differences in resistance to anoxia do not correlate well with cardiac glycogen. Thus, although the piglet has three times as much cardiac glycogen as the guinea pig at birth [Fig. 3 of Shelley (*15*)], it has nearly the same tolerance to asphyxia as the guinea pig (*5*). Likewise, the kitten and rabbit, which have less glycogen than the piglet [Fig. 3 in (*15*)] are approximately four times as tolerant of asphyxia as the guinea pig and piglet (*5*).

Table 1. Effects of hypothermia, hypoxia-hypercapnia, and of asphyxia on cardiac glycogen in guinea pigs.

Experimental conditions	Control series (no asphyxia)			Asphyxiated until time of death of normothermic control		
	Time to freeze (sec)*	Cardiac glycogen (mg/g)	Glygogen content of warm control (%)	Time to freeze (sec)*	Cardiac glycogen (mg/g)	Glycogen content of warm control (%)
		Young adult males†				
Normothermic	93	6.3 ± 0.72	100	70	1.6 ± 0.54	25
Hypothermic	84	6.1 ± 0.27	97	74	6.6 ± 0.22	105
Hypothermic (hypox. + hypercap.)	78	4.4 ± 0.27	70	68	5.9 ± 0.43	94
		Neonates‡				
Normothermic	82	3.9 ± 0.54	100	107	0.7 ± 0.18	18
Hypothermic				94	3.6 ± 0.36	92

* Time interval in seconds between stunning or decapitation and freezing of the ventricles between two blocks of dry ice. † Means of five animals in each category. ‡ Means of five litters of three animals each.

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References

1. J. A. Miller, Jr., *Science* **110**, 113 (1949); ——— and F. S. Miller, *Surgery* **36**, 916 (1954); J. A. Miller, Jr., in *Influence of Temperature on Biological Systems*, F. H. Johnson, Ed. (American Physiological Society, Baltimore, 1957), pp. 229–257.
2. J. A. Miller, Jr., in *Neurological and Psychological Deficits of Asphyxia Neonatorum*, W. F. Windle, Ed. (Thomas, Springfield, Ill., 1959), pp. 105–122.
3. J. A. Miller, Jr., *N.Y. State J. Med.* **61**, 2954 (1961).
4. J. A. Miller, Jr., and F. S. Miller, *Am. J. Obstet. Gynecol.* **84**, 44 (1962).
5. J. A. Miller, Jr., F. S. Miller, B. Westin, *Biol. Neonatorum*, in press.
6. B. Westin, R. Nyberg, J. A. Miller, Jr., E. Wedenberg, *Acta Paediat. Suppl.* **139**, 1 (1962).
7. B. Westin, J. A. Miller, Jr., A. Boles, *Acta Paediat.* **52**, 49 (1963).
8. A. Stafford and J. A. C. Weatherall, *J. Physiol.* **153**, 457 (1960).
9. F. S. Miller and J. A. Miller, Jr., *Anat. Rec.* **136**, 244 (1960).
10. D. L. Morris, *Science* **107**, 254 (1948).
11. J. A. Russell and W. Bloom, *Endocrinology* **58**, 83 (1956).
12. J. A. Russell, personal communication.
13. G. Evans, *J. Physiol.* **82**, 468 (1934).
14. J. C. Mott, *Brit. Med. Bull.* **17**, 144 (1961).
15. H. J. Shelley, *ibid.*, p. 137.
16. P. W. Davies and D. W. Bronk, *Federation Proc.* **16**, 689 (195➞ D. B. Cater, S. Garattini, F. Marina, I. A. Silver, *Proc. Roy. Soc. London Ser. B.* **155**, 136 (1961).

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