

AN ABSTRACT OF THE THESIS OF

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Title GLYCOGEN UTILIZATION IN NEWBORN RATS SUBJECTED
TO COLD STRESS

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Newborn rats exhibit a torporous response to cold stress at 2°C from which they revive after several hours exposure even though respiratory, cardiovascular, and muscular movements have ceased. At less severe temperatures (20 to 25°C) the rats respond with a non-torporous decrease in metabolic and physiologic activity and a lowering of body temperature to that of the environment. This experiment was an attempt to determine if anaerobic glycolysis contributed to the energy required during the period of anoxic torpor at 2°C, as well as the role of glycogen at 22°C. Control animals were kept at 32°C (nest temperature).

After newborn rats had been exposed to one of the three experimental temperatures (2, 22, or 32°C) for a specified time interval up to ten hours, they were killed by quick-freezing. Samples of liver and muscle tissue were analyzed for glycogen concentration or for lactic acid. Survival time at 2 to 6°C was indicated in a separate

group of animals by signs of life during rewarming.

It was found that glycogen was depleted to a greater extent during cold exposure than at nest temperature. This indicates that the effects of cold inhibited those factors maintaining glycogen more than those factors utilizing it. The degree of lactic acid increase at 2°C indicated anaerobic glycolysis, while more complete oxidation of glycolytic intermediates was suggested at 22°C. Older animals were found to have a significantly higher liver glycogen concentration than younger ones, yet they succumbed to cold more readily than did the younger rats. This indicates that maturation affected resistance to cold more than did glycogen concentration.

GLYCOGEN UTILIZATION IN NEWBORN RATS
SUBJECTED TO COLD STRESS

by

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GLYCOGEN UTILIZATION IN NEWBORN RATS SUBJECTED TO COLD STRESS

INTRODUCTION

Several workers observed that newborn rats showed a "torporous" response to near-freezing temperatures. In this condition, cardiovascular, respiratory, and voluntary muscular movements came to a standstill. Body temperature fell to that of the environment, and metabolic activity nearly ceased. Thus, the animals were in a partially anoxic condition. They were capable of surviving several hours of this stress, after which, when warmed, they regained the ability to make voluntary and involuntary movements, and externally appeared to be normal (12, p. 4-5; 5, p. 356-364; 1, p. 99-100).

Newborn rats exposed to moderate cold stress (20 to 25°C) on the other hand, were not torporous and the activity of the cardiovascular system, and presumably of respiration, was depressed but not completely stopped. The metabolic rate was higher than in those rats at 2°C but lower than normal. Body temperature eventually fell to that of the environment (5, p. 356-360).

Nest temperature for newborn rats has been reported to be about 32°C (17, p. 457). This was between the "neutral environmental temperature zone" of 33 to 38°C (the range of no increased oxygen consumption), and the temperature of maximum oxygen consumption

at about 30°C (18, p. 167). Metabolism, then, seemed to be "normal" at 33 to 38°C environmental temperature. It heightened at about 30°C, and successively decreased as the animals were exposed to lower temperatures.

The primary purpose of the present investigation was to determine if glycogen served as an energy source in the newborn rats during cold-stress conditions. It was expected that anaerobic glycolysis would contribute to the energy requirements during anoxia at 2°C, particularly since iodoacetate and fluoride, which inhibit glycolysis, were found to shorten the survival period of newborn rats in nitrogen (8, p. 389). The role of glycogen at 22°C and at 32°C was also to be studied and survival time at 2 to 6°C was to be observed.

METHODS AND MATERIALS

Newborn rats were removed from the mother and nest, weighed, and subjected to one of the three experimental temperatures for a specific interval of time up to ten hours. One group was exposed to the temperature of 2°C, another group to 22°C, and still another to 32°C. To maintain the temperature, a refrigerator was used at 2°C, the laboratory at 22°C, and an electric bulb at 32°C. The temperature was measured continuously by a thermometer and was held within a fairly narrow range. During the course of the experiment the rats were separated by cardboard partitions into compartments about one inch wide, two inches long, and one inch deep. At the appropriate time, each rat was removed, its sex usually determined, and then killed by quick-freezing in a solution of dry ice and acetone.

A sample of liver weighing between 150 and 300 milligrams was taken from each animal. Likewise, a sample of ventral abdominal and sometimes pectoral musculature usually weighing from 75 to 150 milligrams was obtained. The tissues were carefully weighed and then quickly digested in separate tubes of hot potassium hydroxide. The glycogen was precipitated by alcohol, centrifuged, and dissolved in water. These last three steps were then repeated. The method of Seifter, et al. (15, p. 191-200) was used for glycogen determination in which there was produced, in a boiling water bath, a colorimetric

reaction between the aqueous solution of glycogen and an anthrone-sulfuric acid reagent. The intensity of the color produced by this reaction was determined on a Klett colorimeter. By comparison of this with the color intensity produced by anthrone reagent in a standard glucose solution, the milligrams of glycogen per gram of tissue were calculated. Two portions of the solution derived from the original sample were compared in the majority of cases. A total of 386 rats were used in the glycogen analyses.

Liver and muscle lactic acid analyses were made using other rats. They were exposed to experimental and analytical procedures similar to those upon which glycogen determinations were made. However, following treatment of the tissues with potassium hydroxide, the Barker-Summerson method of lactic acid analysis was used (2, p. 535-554). The color intensity of two portions of the solution derived from each tissue was determined on a Klett colorimeter. Then the milligrams of lactic acid per 100 grams of tissue were calculated by comparison with a standard solution. A total of 24 rats was used.

A series of experiments was performed on survival times of 97 newborn rats subjected to temperatures of 2 to 6°C. After exposure to the cold, the rats were rewarmed at room temperature, and survival was determined by signs of obvious movement and response to pinching. Death was indicated by either a lack of movement or by no response to pinching. A comparison was made between those rats

12 to 24 hours old, and those 24 to 36 hours of age in regard to their ability to survive the cold.

RESULTS

Exposure to 2°C

The initial concentration of liver glycogen was about ten milligrams per gram of tissue (Table 1 and Figure 1). A slight, insignificant rise was observed within one hour of exposure to the cold, followed by a rather large and sudden decline to about five milligrams per gram by the second hour of exposure. The amount of glycogen remained near this level until six hours, with an increase in the final determination at ten hours.

Table 1. Liver Glycogen

Hours Exposure	Temperature: 2°C		Temperature: 22°C		Temperature: 32°C	
	No. of Animals	*Glycogen (mg/gm)	No. of Animals	*Glycogen (mg/gm)	No. of Animals	*Glycogen (mg/gm)
0	36	9.8±0.93	36	9.8±0.93	36	9.8±0.93
.25	10	10.0±1.16	12	11.7±1.30	13	9.5±1.49
.50	9	9.6±1.35	10	4.1±1.04	10	13.0±1.10
1	16	12.6±1.17	11	11.9±0.76	10	15.8±0.98
2	18	5.0±0.58	13	9.9±1.57	11	12.4±0.88
3	16	6.8±0.89	12	6.3±1.18	16	12.5±1.41
4	9	3.8±0.88	21	6.0±1.34	14	16.2±1.91
5	14	3.9±1.08	10	0.9±0.33	19	7.9±1.60
6	15	4.6±0.80	10	2.6±0.60	14	9.0±0.77
10	15	7.4±1.27	10	1.4±0.36	12	7.3±1.36

* Average values with standard error indicated S. E. = $\sqrt{\frac{S^2}{n}}$

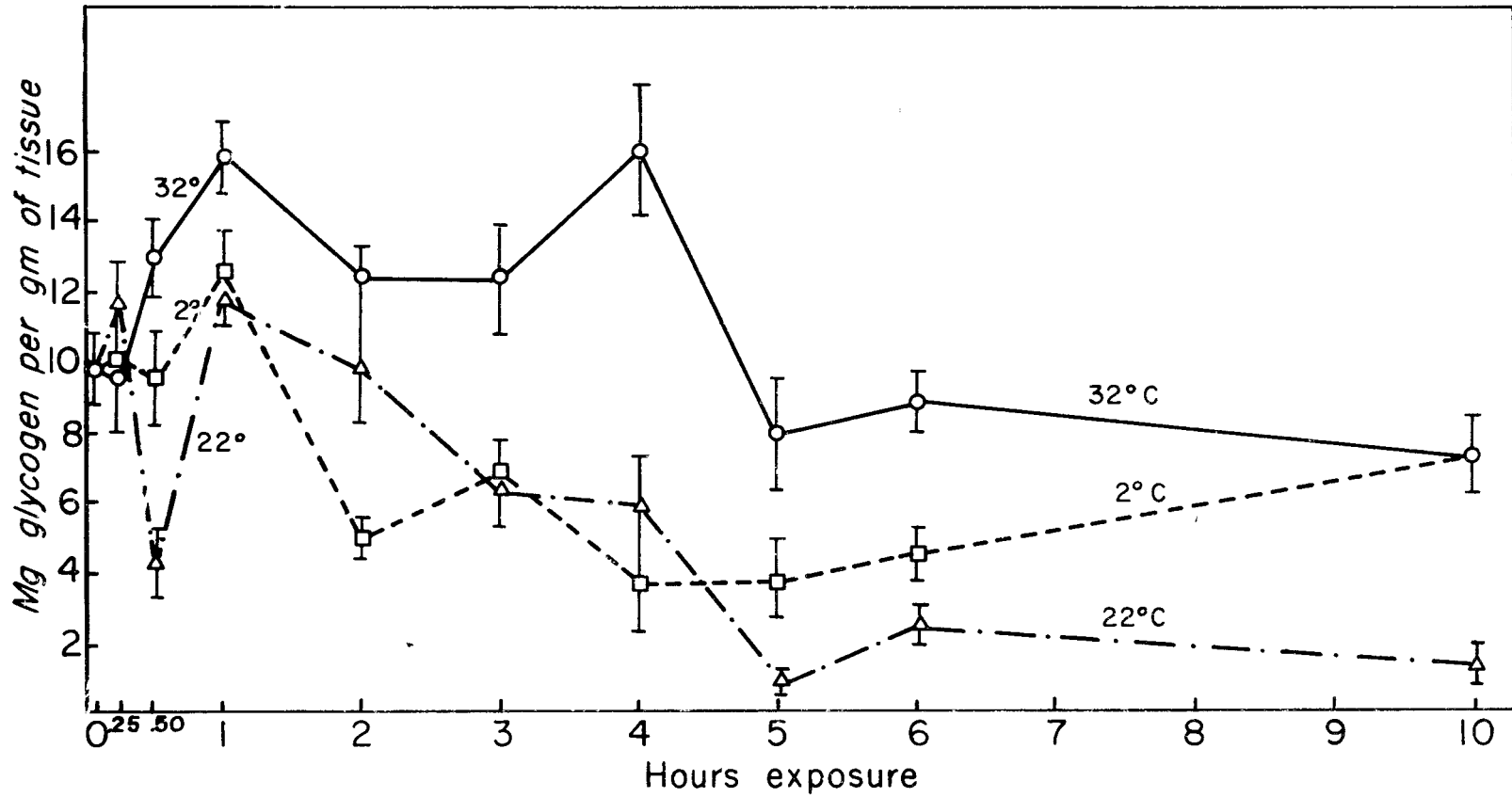


Fig 1 Liver glycogen variation in newborn rats with respect to temperate and time

The muscle glycogen concentration (Table 2 and Figure 2) was about 2.8 milligrams per gram of tissue in the unexposed animal. After an initial decrease and then rise to above normal, the value steadily declined to about one milligram per gram at five hours. It remained near this low level through the tenth hour. Liver and muscle lactic acid showed a very large increase up to six hours, whereafter, there appeared a decline to near-normal values (Table 3).

Table 2. Muscle Glycogen

Hours Exposure	Temperature: 2°C		Temperature: 22°C		Temperature: 32°C	
	No. of Animals	*Glycogen (mg/gm)	No. of Animals	*Glycogen (mg/gm)	No. of Animals	*Glycogen (mg/gm)
0	30	2.8±0.18	30	2.8±0.18	30	2.8±0.18
.25	10	2.1±0.53	12	2.1±0.41	13	3.3±0.19
.50	8	3.6±0.14	8	2.9±0.29	10	3.3±0.21
1	16	3.4±0.15	13	3.6±0.35	13	3.6±0.16
2	18	2.7±0.26	14	3.3±0.29	9	3.9±0.26
3	26	2.1±0.14	14	4.0±0.23	19	3.4±0.18
4	8	1.5±0.25	18	4.2±0.28	11	3.6±0.18
5	14	1.0±0.15	9	2.3±0.37	19	2.8±0.30
6	10	1.3±0.28	11	3.9±0.18	16	3.5±0.13
10	15	0.6±0.19	10	3.3±0.60	12	3.3±0.17

* Average values with standard error indicated

$$S. E. = \sqrt{\frac{S^2}{n}}$$

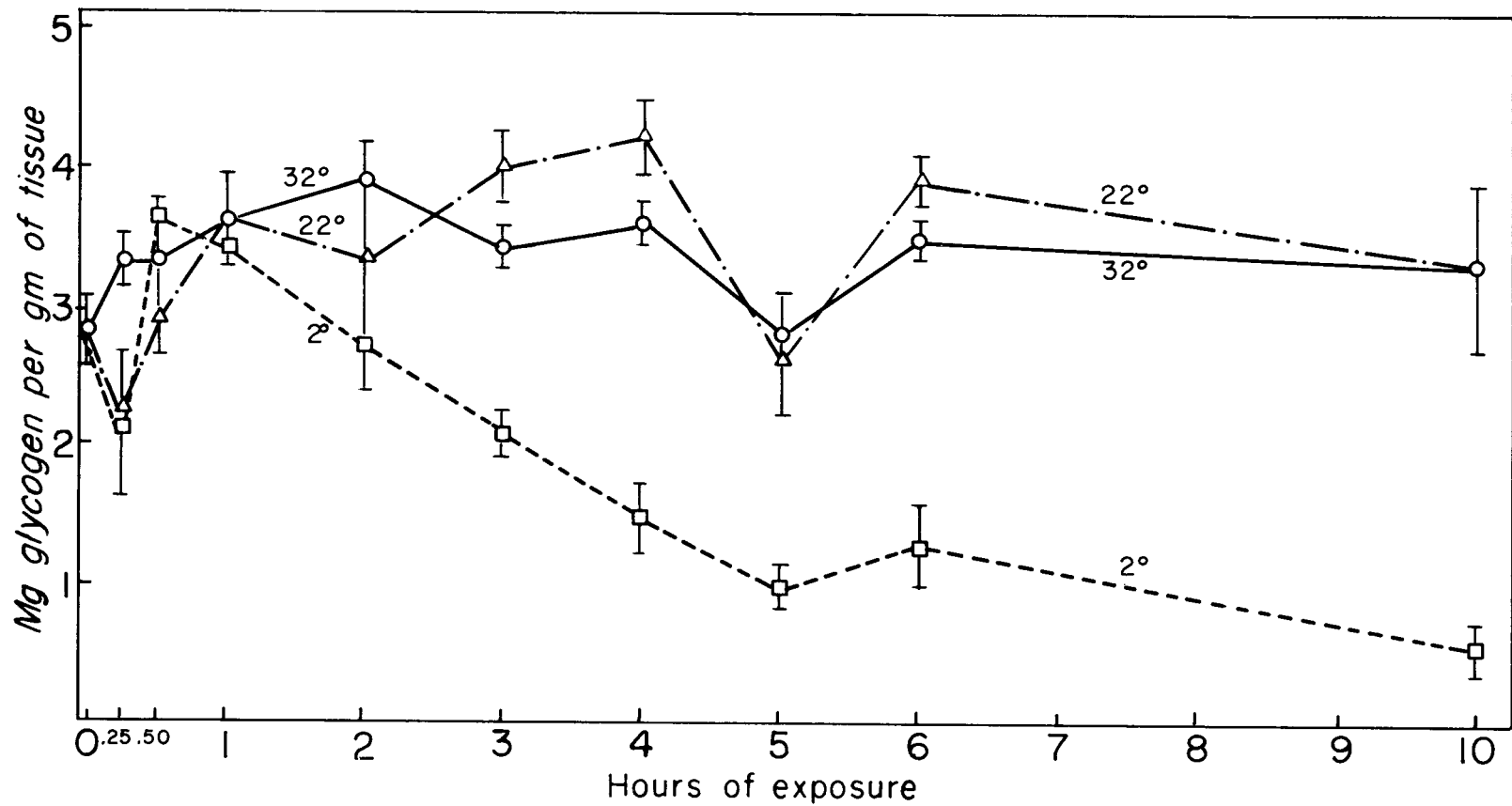


Fig 2 Muscle glycogen variation in newborn rats with respect to temperature and time

Table 3. Lactic Acid: Liver and Muscle

Hours Exposure	Temperature: 2°C		Temperature: 22°C		Temperature: 32°C	
	Liver (mg/100 gm)	Muscle	Liver (mg/100 gm)	Muscle	Liver (mg/100 gm)	Muscle
0	28	40	28	40	28	40
1	43	53	16	39	31	63
4	65	83	24	28	20	31
5	117	112	29	49	55	74
6	281	492	19	70	63	141
7	196	174	51	62	-	-
10	46	33	19	19	27	29

(Note: since there are only one to two animals per point, the standard error was not computed)

Exposure to 22°C

Liver glycogen (Table 1 and Figure 1) dropped temporarily from the initial value of ten milligrams per gram to about four milligrams per gram at 30 minutes of exposure. It returned to a value slightly higher than normal at one hour. Then it steadily declined to about one milligram per gram by five hours, near where it remained through the last determination at ten hours.

The level of the muscle glycogen (Table 2 and Figure 2) displayed an initial and transient drop from 2.8 milligrams per gram to 2.1 milligrams per gram, similar to that seen in those animals at 2°C. After this, it steadily rose to a value higher than normal at four

hours. Thereafter, the content fluctuated somewhat above and below 3.3 milligrams per gram which was the final determination at ten hours. Lactic acid levels in liver and muscle remained relatively low throughout the ten hours of exposure, with some increase at seven hours (Table 3).

Exposure to 32° C

The level of liver glycogen between one and four hours of exposure was higher than that in the unexposed animals. It reached about 16 milligrams per gram during this period, compared with the initial value of about ten milligrams per gram (Table 1 and Figure 1). At five hours the glycogen level suddenly dropped to about eight milligrams per gram, near where it remained through the end of the experiment at ten hours. Muscle glycogen (Table 2 and Figure 2) remained at a fairly constant level slightly above normal throughout the test. Lactic acid in liver and muscle showed some increase at five and six hours of exposure (Table 3).

Survival

The results of the experiment on survival following exposure to temperatures of 2 to 6° C were as follows:

(a) Animals 12 to 24 hours of age showed 100 percent survival throughout eight hours of exposure and only 50 percent survival at

nine hours (Table 4 and Figure 3).

(b) Rats 24 to 36 hours old showed less ability to recover from cold stress than did the younger animals. Survival dropped to 67 percent at five hours, to 20 percent at seven hours, and zero percent at ten hours (Table 4 and Figure 3).

Table 4. Survival Following Exposure to Temperature of 2 to 6°C

Hours Exposure	Age: 12 to 24 hours old			Age: 24 to 36 hours old		
	Number of Animals: Exposed	Survived	Percent Survival	Number of Animals: Exposed	Survived	Percent Survival
1	5	5	100			
2	5	5	100			
3	5	5	100			
4	10	10	100	5	4	80
5	6	6	100	6	4	67
6	6	6	100	6	4	67
7	9	9	100	5	1	20
8	12	12	100	5	1	20
9	4	2	50			
10				8	0	0

Analysis of Data

In order to determine what affect each of the numerous variables in the experiment had upon glycogen content, a stepwise multiple regression analysis was made of the data. This was done by the use of an IBM 709 high speed digital computer, using program BIMDO9.

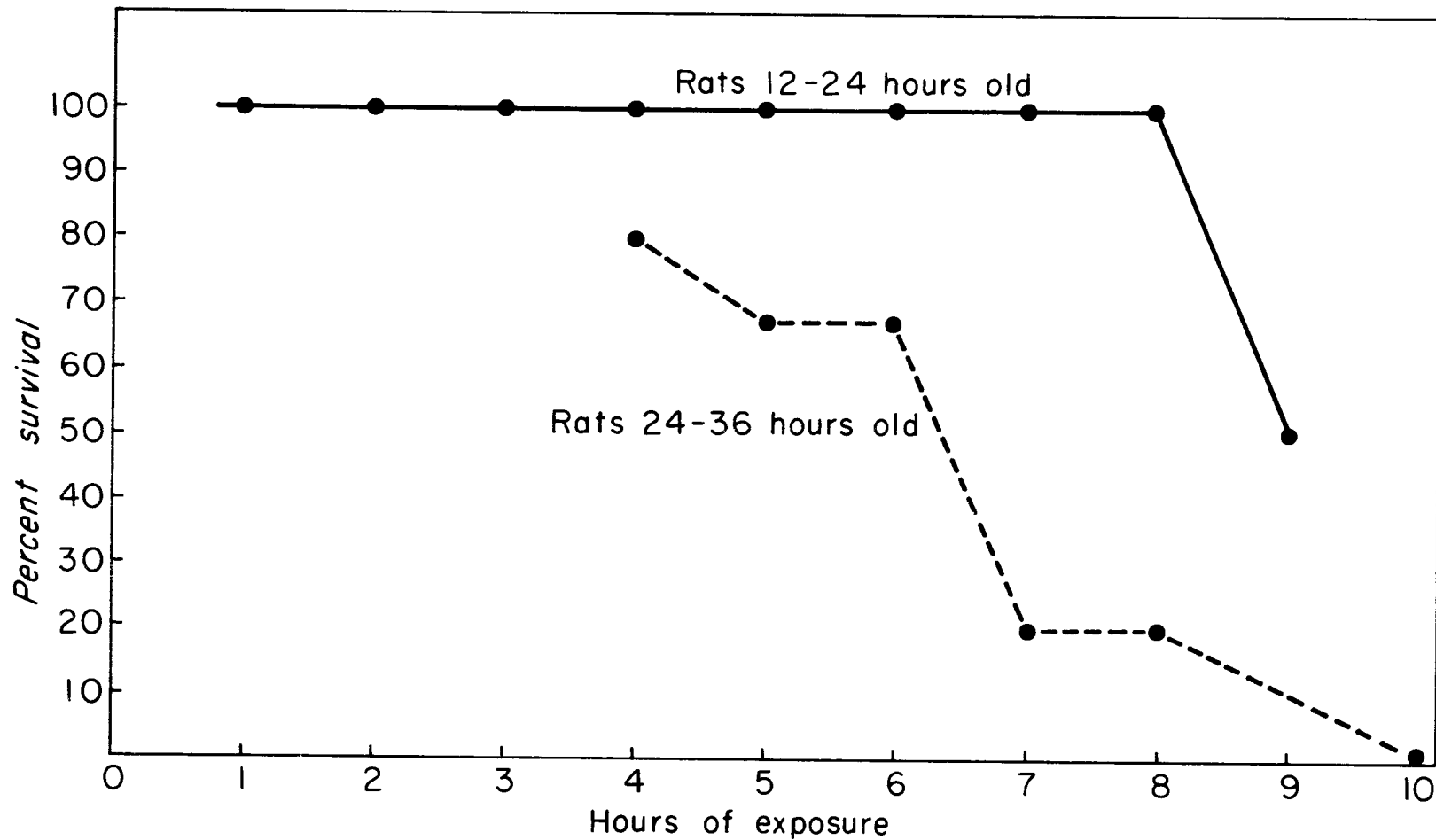


Fig. 3 Survival of newborn rats following exposure to temperature of 2°-6° C

Glycogen concentration was chosen as the dependent variable (Y), and the several independent variables as (X). The square of each X was also used in order to obtain possible correlation, in the event that the relationship between X and Y was nonlinear. The following X variables were used:

- X(1) = age of animal in hours
- X(2) = age of animal, in hours, squared
- X(3) = temperature of exposure in degrees C
- X(4) = temperature of exposure in degrees C, squared
- X(5) = hours of exposure
- X(6) = hours of exposure, squared
- X(7) = body weight of animal in grams
- X(8) = body weight of animal in grams, squared

The affect of sex upon glycogen was also analyzed separately.

It was decided that the X variable which had the highest correlation coefficient with glycogen (Y), would be selected first by the computer and the equation constructed which would define Y. Then other X values, with decreasing correlation to residual Y would be selected in order, and algebraically added to the first, one at a time.

The results of the data analysis were as follows:

Liver Glycogen. Liver glycogen was most significantly affected by age (Figure 4) according to the equation:

$$(1) \text{ Glycogen} = 0.099 (\text{Age}) + 4.58$$

Next, glycogen was affected by the square of the temperature. This factor was added to age to obtain the equation:

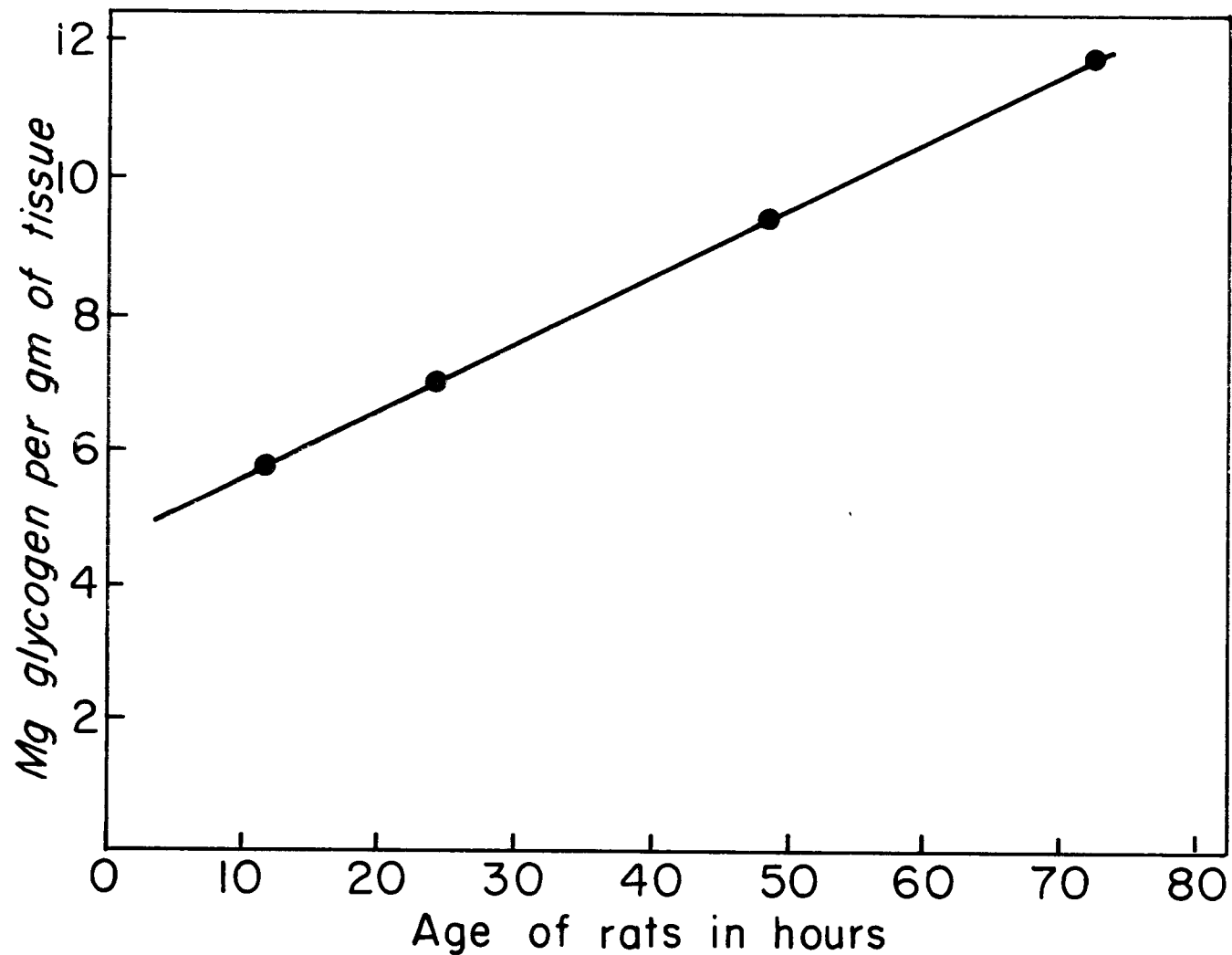


Fig 4 Effect of age upon liver glycogen in newborn rats according to equation: $\text{glycogen} = 0.099(\text{age}) + 4.58$

$$(2) \text{ Glycogen} = 0.11 (\text{Age}) + 0.004 (\text{Temp.}^2) + 1.45.$$

The negative hours of exposure was the third variable to affect glycogen. It was found that glycogen decreased significantly with time.

When this was added to the former factors, the following equation was constructed:

$$(3) \text{ Glycogen} = 0.12 (\text{Age}) + 0.004 (\text{Temp.}^2) - 0.60 (\text{Hrs}) + 3.48.$$

It was found that sex had no significant affect upon glycogen.

The difference between males and females was considered insignificant if the difference was less than three σ , where $\sigma = \sqrt{\frac{\sum x^2}{n - 1}}$.

(See Table 5.)

Table 5. Average Glycogen Concentration According to Sex

Tissue	No. Animals	Sex	Av. Glycogen (mg/gm)	σ Value*
Liver	167	Males	8.66	5.55
Liver	145	Females	8.92	6.25
Liver	72	Undetermined	6.63	5.18
Liver	384	Average: All	8.38	---
Muscle	177	Males	2.85	1.22
Muscle	152	Females	3.02	1.41
Muscle	56	Undetermined	2.73	1.25
Muscle	385	Average: All	2.90	---

$$* \sigma = \sqrt{\frac{\sum x^2}{n-1}}$$

Muscle Glycogen. Muscle glycogen was found to rise significantly and linearly with increasingly higher temperatures. However, the next most significant variable was the square of temperature. These two factors in the same equation produced the curve in Figure 5. The equation constructed was:

$$(4) \text{ Glycogen} = 0.141 (\text{Temp}) - 0.003 (\text{Temp}^2) + 1.77.$$

The square of body weight and the square of hours of exposure also significantly affected glycogen. Sex had no significant affect according to the factor noted above. (See Table 5)

Averages. The average age of the rats was about 40 hours post-parturition at the beginning of exposure. The average body weight was about 6.1 grams, and the average number of hours of exposure was 3.3 hours. The average temperature of exposure was 20.0°C.

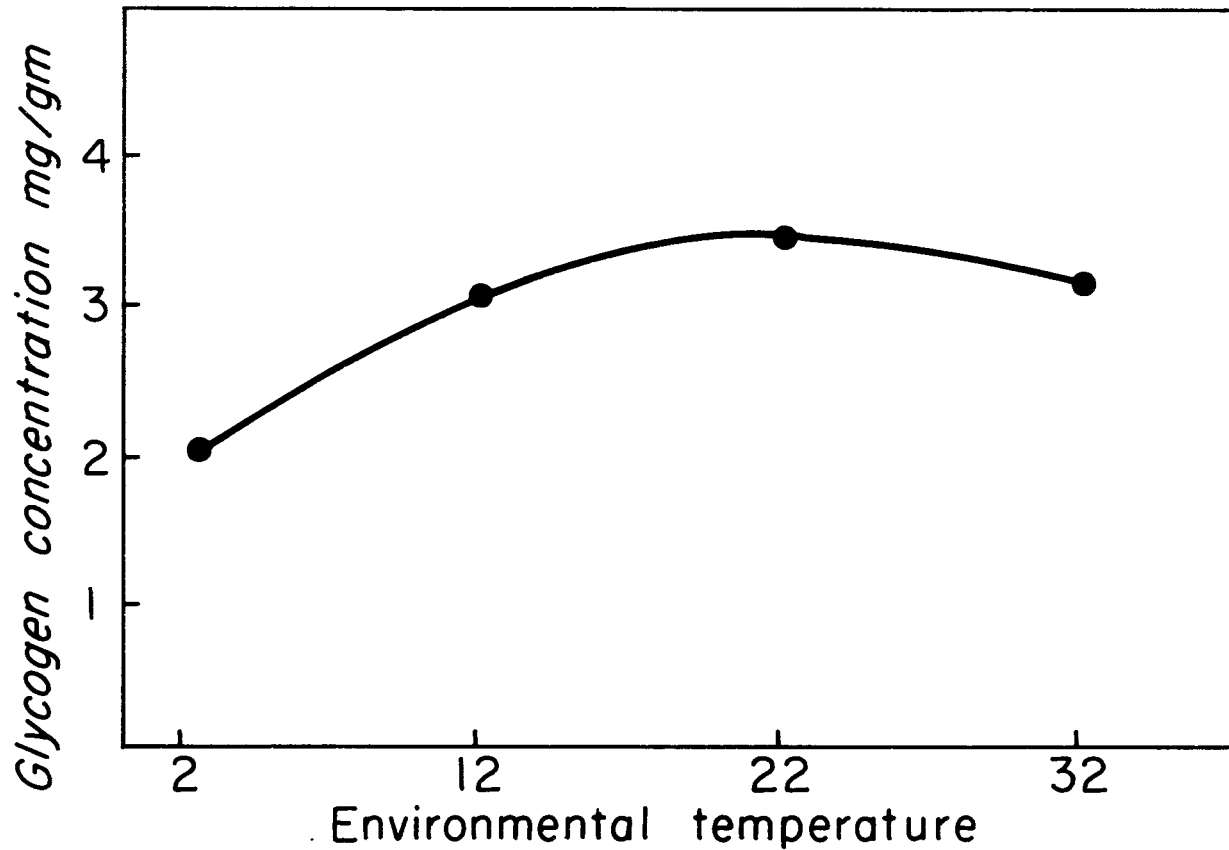


Fig.5 Effect of temperature upon muscle glycogen in newborn rats according to the equation:
$$\text{Glycogen} = 0.141 (\text{temp.}) - 0.003 (\text{temp.}^2) + 1.77$$

DISCUSSION

The most obvious observation from this series of experiments was that liver glycogen was depleted to a greater extent, and more rapidly, when newborn rats were exposed to the cold temperatures of 2°C and 22°C than when they were kept at the nest temperature of 32°C (Table 1 and Figure 1). This indicated that either synthesis of glycogen* and/or absorption of carbohydrate from the digestive system were severely inhibited, or else that utilization of glycogen was unusually high at the low temperatures. The latter could not be true because the metabolic rate was below normal during cold exposure as explained previously (5, p. 358-359). Therefore, it must have been that, first, the inhibition of synthesis and absorption was great enough to account for the failure to maintain glycogen; and second, there was enough demand for energy, even though the metabolism was low, to utilize existing glycogen stores.

It is important to remember that one would not necessarily expect these results. It might be thought that the low metabolic rate would decrease energy requirements to such an extent that glycogen would be maintained for a longer period of time during cold exposure

* There is some evidence that at least part of the mechanism for de novo synthesis of glycogen--glyconeogenesis--is present at birth (9, p. 476-477; 13, p. 986-987).

than at higher temperatures. Because that was not the case, it would seem that torpor, or any decrease in metabolic rate occurring during cold stress, is not a good way to prolong life indefinitely, at least not in newborn rats. Rather, it is a means of extending life somewhat longer than if the metabolic rate had been increased in an attempt to maintain a high body temperature. In that case, the limited glycogen stores of the newborn would have been very quickly exhausted. At any rate, the animals were in a more precarious position during cold exposure than at the temperature of the nest.

The need for a source of energy even during torpor was seen in the anaerobic glycolysis that occurred during exposure to 2°C. This was indicated by the increases in lactic acid (Table 3) and the depletion of liver glycogen (Table 1, Figure 1). Lactic acid per se, however, does not indicate anaerobic glycolysis. Some lactic acid increase was observed even at 32°C (Table 3) when the rats were not anoxic. Also, Domonkos (4, p. 138) reported that certain rabbit muscles produced lactic acid in aerobic conditions.

The magnitude of lactic acid accumulation noted herein at 2°C supports the conclusion that anaerobic glycolysis did occur. Likewise, Heath's finding indicates this (7). He recorded an increase of lactic acid in whole-body determinations of newborn rats at 2°C but no increase at 30°C.

The anaerobic glycolysis occurring in the liver of newborn rats

during torpor is probably the same thing that was reported in adult muscle following death (3, p. 110-113). In both instances, there was an increase in lactic acid and a depletion of glycogen. Following death, there was a temporary production of ATP (adenosine triphosphate). It was probably the production of ATP during torporous anaerobic glycolysis that supplied the energy enabling these newborn animals to survive long periods of anoxia.

Some doubt exists that skeletal muscle of newborn rats can metabolize glycogen due to the lack of phosphorylase (16, p. 403; 17, p. 469). The need for ATP anaerobically suggests that metabolism of muscle glycogen might have occurred possibly via some route not requiring phosphorylase. Otherwise, the muscles would have been limited to non-glycogen ATP production since blood circulation ceased during torpor (5, p. 360). This would make it impossible to transport ATP to the muscles from the liver where glycolysis occurred. Furthermore, the large decrease in muscle glycogen (Table 2, Figure 2) and the large rise in lactic acid (Table 3) would indicate anaerobic glycolysis in the muscle at 2°C. However, the failure of muscle glycogen to fall at 22°C (Table 2, Figure 2) might suggest that it is not normally metabolized.

It is apparent that the rats at 22°C did not rely upon the anaerobic metabolism of glycogen. This was evident by the low lactic acid level (Table 3) and by the overt observation that respiratory :

movements continued during exposure at this temperature. This meant that glycogen underwent more complete oxidation than that taking place anaerobically at 2°C. The result would be a higher production of ATP per mole of glycogen. This would be advantageous since the animals at 22°C had a higher metabolic rate and hence a higher energy requirement than those at 2°C.

The very large transient decrease in liver glycogen at one-half hour exposure to 22°C deserves comment (Table 1 and Figure 1). It may have been the result of a litter with an abnormally low value, since most of the animals were from the same litter in this case. Normally more than one litter was represented at each point. On the other hand, it may have been caused by an initial but brief attempt to maintain body temperature by increased activity, as by shivering. This would have increased the metabolism and placed a demand upon liver glycogen. It is further possible, though not confirmed in newborn rats, that a type of non-shivering thermogenesis occurred through the mediation of nor-adrenalin utilizing non-esterified fatty acids (6, p. 786; 10, p. 9-10; 14, p. 324-325). This would spare glycogen and, thus, would not be reflected in the glycogen decrease noted above.

It was implied previously that newborn rats passively permitted body temperature to vary with the environment. The question might arise as to whether there is any other evidence than that indicated in

the preceding paragraph that the animals were capable of regulating their body temperature. There is some reason to believe that they do make a rather feeble attempt to maintain body temperature during cold exposure. Poczopko (11, p. 180) found that newborn rats, if slowly cooled to 25°C maintained a body temperature 3.5°C above that of the environment for about 40 minutes. Salhanick (12, p. 4) observed increased locomotor activity when newborn rats were first exposed to 3°C. On the other hand, Fairfield (5, p. 358) observed no increased oxygen consumption at 20°C in rats under three days of age when the temperature was lowered abruptly. In the present investigation it was observed that liver glycogen declined to a much lower level at 22°C exposure than at 32°C (Table 1 and Figure 1), even though metabolism was reportedly depressed at 22°C. This might indicate that there was a demand for energy which was needed in an attempt to regulate body temperature. It could, however, mean that the cold inhibited those mechanisms maintaining glycogen.

Rats exposed to 32°C exhibited a reaction opposite to those at 22°C. That is, liver glycogen initially increased instead of decreasing (Table 1, Figure 1). Possibly this was a response to the stress of handling. However, the liver glycogen at 32°C returned to near-normal levels at five hours of exposure. This may have been caused by the depletion of digestible carbohydrate initially present in the digestive tract. If this were so, then the failure of glycogen to fall

after five hours would indicate that other factors maintaining glycogen were functioning, or that utilization suddenly diminished. It is apparent that not all the factors involved in glycogen balance were studied.

It seemed clear from these experiments that the content of liver glycogen was not the only factor involved in resistance to cold stress by newborn rats. All rats at 22°C were alive after ten hours of exposure even though liver glycogen content was extremely low during the last five hours. However, rats at 2°C did not survive ten hours of cold stress although their liver glycogen level seemed to be higher (Table 1 and Figure 1). It was also indicated that younger animals survived cold better at 2°C than did older ones (Table 4 and Figure 3), but that older animals had a higher concentration of liver glycogen (Figure 4)*. Salhanick noted that one day old females survived prolonged cold exposure better than did males of the same age (12, p. 38). However, the present investigation showed only a slightly greater glycogen content in females than in males, which was not significant (Table 5). One might conclude that while glycogen utilization was important as an energy source to newborn rats under varying temperature

* It is important to know if the apparent affect of time and temperature upon glycogen levels was invalidated by the fact that the experimental animals varied somewhat in age. The stepwise multiple regression analysis showed that glycogen concentration was significantly correlated with temperature and time of exposure, as well as with age (Equations 3 and 4, and Figures 4 and 5). This strengthened the conclusions stated herein.

conditions, certain intrinsic factors, such as the maturation process, were even more important in determining the ability to withstand cold exposure.

SUMMARY

Glycogen and lactic acid levels were determined in the liver and muscle of newborn rats exposed to one of the environmental temperatures: 2°C, 22°C, or 32°C. After removal of each animal from exposure, it was killed by quick-freezing in dry ice and acetone. Samples were taken of liver and muscle tissue, some of which were analyzed for glycogen using the method of Seifter, et al. (13, p. 986-989). Lactic acid determinations were made on other samples using the Barker-Summerson method (2, p. 535-554). In another set of animals, survival times were determined following exposure to temperatures of 2 to 6°C. The results of the experiments were as follows:

1) In general, during exposure to cold stress, the metabolic rate and body temperature decreased (as reported in the literature). It was determined in this work that glycogen levels fell (Table 1, Figure 1). This indicated a greater inhibition upon those factors maintaining glycogen than upon those utilizing it.

2) The animals exposed to 2°C showed a decrease in liver glycogen (Table 1, Figure 1) and in muscle glycogen (Table 2, Figure 2) with time of exposure, and also a rise in lactic acid concentration (Table 3). These results indicated that anaerobic glycolysis occurred in these animals, which were in a state of anoxic torpor.

3) At 22°C a decrease in liver glycogen was noted with time

(Table 1, Figure 1), while muscle glycogen (Table 2, Figure 2) and lactic acid (Table 3) remained relatively unchanged. This indicated that marked anaerobic glycolysis did not occur and that the animals oxidized glycogen more completely than did those at 2°C.

4) Liver (Table 1, Figure 1) and muscle glycogen (Table 2, Figure 2) at 32°C showed initial increases above normal with subsequent decreases to about normal level. Lactic acid did not accumulate (Table 3). The animals may have depleted their store of digestible material, while other undetermined factors apparently contributed to glycogen balance.

5) Analysis of the glycogen data showed that the liver glycogen was significantly affected by age (Figure 4), square of temperature, and hours of exposure. Muscle glycogen was significantly determined by temperature (Figure 5), square of hours of exposure, and square of body weight.

6) All rats less than 24 hours old survived eight hours of exposure to 2 to 6°C. Of those animals between 24 and 36 hours of age, only 20 percent survived eight hours of similar exposure (Table 4, Figure 3).

7) Sex had no significant affect upon glycogen levels (Table 5).

8) Maturation, and perhaps other factors, apparently had more affect upon the ability to withstand cold than did glycogen levels.

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