NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.
BODY TEMPERATURE AND CARBOHYDRATE VALUES IN NORMAL AND ENDOTOXIN POISONED MICE EXPOSED TO LOWERED ENVIRONMENTAL TEMPERATURES

TECHNICAL DOCUMENTARY REPORT AAL-TDR-62-4

August 1962

ARCTIC AEROMEDICAL LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
FORT WAINWRIGHT, ALASKA

Project 8241-32

(Prepared under Contract AF 41(657)-340 by John E. DeTurck and L. Joe Berry
Department of Biology, Bryn Mawr College
Bryn Mawr, Pennsylvania)
NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related government procurement operation, the government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the government may have formulated, furnished, or in any supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

US Government agencies and other qualified ASTIA users may obtain copies of this report from the Armed Services Technical Information Agency, Documents Service Center, Arlington 12, Virginia.

This report has been released to the Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C., for sale to the general public.
A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD_{50} dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C.
| Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. | Body temperature | 1. |
| Unclassified Report | Bacterial toxins | 3. |
| A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD₅₀ dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C, but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C. | Mice | 4. |
| | Exposure | 5. |
| | Starvation | 6. |
| 1. Project 8241-32 | | |
| II. Contract AF41(657)-340 | | |
| III. Bryn Mawr College, | | |
| Bryn Mawr, Penna. | | |
| IV. DeTurck, J. E. and L. Joe Berry | | |
| V. Available from OTS | | |
| VI. In ASTIA collection | | |

| Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. | Body temperature | 1. |
| Unclassified Report | Bacterial toxins | 3. |
| A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD₅₀ dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C, but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C. | Mice | 4. |
| | Exposure | 5. |
| | Starvation | 6. |
| 1. Project 8241-32 | | |
| II. Contract AF41(657)-340 | | |
| III. Bryn Mawr College, | | |
| Bryn Mawr, Penna. | | |
| IV. DeTurck, J. E. and L. Joe Berry | | |
| V. Available from OTS | | |
| VI. In ASTIA collection | | |
ABSTRACT

A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C, and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and, except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD50 dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C, but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle, and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C.

PUBLICATION REVIEW

HORACE F. DRURY
Director of Research
BODY TEMPERATURE AND CARBOHYDRATE VALUES IN NORMAL AND ENDOTOXIN POISONED MICE EXPOSED TO LOWERED ENVIRONMENTAL TEMPERATURES

SECTION 1. HISTORICAL REVIEW

Small homeotherms, such as the rat and the mouse, when exposed to reduced temperatures typically exhibit elevated metabolic rates (see 1 - 10 of Historical Bibliography) which provide the heat required to maintain body temperature within a normothermic range (11 - 14). In these animals the skin (15) and hair (15 - 17) are important in heat conservation, while an adequate food supply (5, 19 - 23) is necessary to support the increased metabolic rate. Shivering resulting from initial cold exposure is recognized primarily as a thermogenic mechanism (10, 24 - 28), yet Cottle and Carlson and other investigators (25, 27, 29, 30) found a nonshivering heat-producing process in rats subjected for prolonged periods to low environmental temperatures. The essential role of the endocrines in raising the metabolic rates of cold-stressed animals is evidenced by increased thyroid (8, 27, 31, 35, 36) and adrenal activity under the directing influence of the anterior lobe of the pituitary gland (36 - 40). An important food reserve which can be mobilized by glycogenolysis during periods of stress such as fasting (41 - 43) and cold exposure (44 - 46) is body glycogen, stored mainly in liver and muscle tissue (28, 41, 42).

Various authors have reported that bacterial endotoxins at various concentrations produce hypothermia in mice (47 - 53). Other effects of endotoxin poisoning in these animals include depletion of liver and muscle glycogen (50 - 54), inhibition of gastric mobility (54), and diarrhea (52 - 55). There is, moreover, an initial hyperglycemia (50 - 55) that is followed by progressively severe hypoglycemia. Berry et al (50) have shown that endotoxins reduce liver, muscle and total body carbohydrate in mice, and perhaps inhibit both glycogenesis and glyconeogenesis. These authors conclude that the aforementioned effects are probably the result of an increased demand by the body for stored carbohydrate reserves without their subsequent replacement.

SECTION 2. INTRODUCTION

Experimentally, endotoxin administration concomitant with exposure to lowered environmental temperatures should doubly stress an animal. Such
treatment might be expected to produce significant reduction in the carbohydrate reserves of the body, even after a limited period of treatment. Evidence that this is partially correct is presented in this paper. In addition, the body temperature pattern of mice exposed to 15°C and 5°C over various periods of time with and without injections of endotoxin has been combined with measurements of carbohydrate reserves under comparable conditions.

SECTION 3. SUMMARY

Mice kept at 15°C and 5°C, if properly housed and fed, can survive at least one week. Body surface temperatures were lower in mice subjected to 5°C than in animals maintained at 15°C or 25°C. Rectal temperatures after 24 hours exposure to 15°C did not vary in either fed or fasted mice more than 2.4°C from animals kept at 25°C. At 5°C rectal temperatures over a 24-hour period did not differ at any time more than three degrees from mice housed at 25°C. After one week exposure to either 15°C or 5°C, rectal temperatures of the cold-treated animals returned to the values of the room temperature controls.

S. typhimurium endotoxin in LD50 doses reduced the rectal temperature of mice kept at 15°C by 3.3 degrees three hours after administration. After an additional two hours the temperature was depressed only one degree below control values. Rectal temperatures of animals maintained at 5°C and given endotoxin were depressed one degree lower than the values of control animals after three hours and five hours exposure.

In comparing fasted and fed animals held at 15°C for three hours, the former group showed no significant glycogen loss in muscle and liver and in total body carbohydrate, whereas two hours later significant decreases were found in muscle and total carbohydrate, but not in liver. At 5°C significant losses in muscle, liver, and total body carbohydrate occurred after three hours in fasted animals, but not in those given food. After five hours exposure only muscle glycogen was significantly depleted when compared to that in fed animals.

Endotoxin poisoned mice exposed to reduced temperatures had significantly lowered liver glycogen after three hours and five hours exposure to 15°C. A significant reduction in the liver glycogen of the animals subjected to 5°C was realized after five hours exposure but not after three hours exposure. Muscle glycogen was not significantly reduced after three hours exposure to either 15°C or 5°C, but was significantly lowered in endotoxin treated mice after three hours at either 15°C and 5°C, and also after five hours at these temperatures.
SECTION 4. MATERIALS AND METHODS

Animals. Female albino mice, CF-1 strain (Carworth Farms), averaging 19 to 23 grams were housed singly in compartmentalized plastic cages, raised from the bench surface to allow excreta and excess food to fall from the wire mesh bottom to the paper below. No bedding was used. Fed animals were given Dietrich and Gambrill's pathogen-free mouse food ad libitum. Tap water was available at all times to both fasted and fed animals.

Two walk-in refrigerated rooms, one at 15° ± 2°C and the other at 5° ± 2°C, were used in these studies. An air-conditioned room kept at 25° ± 2°C was used as the control environment.

Rectal and skin temperatures of mice. Body temperatures were obtained by inserting a rectal probe into a mouse and noting the temperature registered on a calibrated Telethermometer after 30 seconds (Yellow Springs Instrument Co., Yellow Springs, Ohio). Ventral surface temperatures of the sternal region and dorsal surface temperatures at the base of the tail were recorded after 60 seconds, using a flat disc-shaped skin probe. Temperature readings were taken at approximately the same time period with each group of 8 to 12 animals studied.

Carbohydrate determination. The animals were sacrificed by cervical dislocation. Liver and muscle (abdominal body wall) glycogen were determined by the method of Kemp and Kits van Heijningen (1954). Total body carbohydrate was measured after the skin, feet, tail and gastrointestinal tract were removed and the carcass blended for two minutes in 5% trichloroacetic acid in a Waring blender following the technique of Berry et al (1959). Analysis of the glycogen-containing extract followed the procedure of Mendel, Kemp and Myers (1954).

Both of the above techniques rely on the pink color of 5-hydroxymethyl furfural which forms when glucose is treated with hot sulfuric acid. Ten micrograms of glucose equivalents can be reliably measured with this procedure. The Coleman spectrophotometer (Model 14) was used to read the color.

Endotoxin. An endotoxin preparation of heat-killed Salmonella typhimurium, strain SR-11, was obtained by sedimenting in the cold a 17- to 18-hour culture grown in brain-heart infusion broth (Difco). The suspension was washed with isotonic saline and a dilution count made. After pasteurization at 60°C for 30 minutes, the material was tested for contamination and, if sterile, dispensed into test tubes. LD50 determinations of this preparation
were carried out at each temperature and calculated by the method of Reed and Muench (1938). Endotoxin was injected intraperitoneally and observations were terminated after 48 hours.

SECTION 5. RESULTS

Survival Time of Mice at 15°C and 5°C

Mice exposed to each of these temperatures survived at least one week or more, if properly fed and watered. Occasional deaths occurred, however, at 5°C after three days. Frostbite injury was sometimes evident in limb and tail extremities. On the other hand, fasted animals at 5°C showed 100% mortality within 24 hours. At 15°C fasted mice survived 48 hours but began to die at about 62 hours. Only a few were alive after 72 hours.

Rectal and Skin Temperatures of Mice Exposed to 15°C

Rectal temperatures in fasted animals at 15°C, as seen in Figure 1, fell slightly after four hours and continued to decline about two degrees by the end of 24 hours. At this time the temperature was 34.4° ± 1.9°C. Ventral and dorsal skin temperatures showed similar patterns (Figures 2 and 3). In each there was an initial drop of approximately three degrees and then fluctuations around that temperature for about 16 hours. Beyond that time the temperature remained fairly constant up to and including the 24-hour reading. The final ventral skin temperature was 29.0° ± 0.6°C and the dorsal skin temperature was slightly lower, 28.2° ± 0.7°C, each about four degrees lower than that found at room temperature.

Temperatures of fasted mice maintained at 15°C for 48 hours varied from less than 20°C to 35°C for rectal measurements and from less than 20°C up to 28°C for ventral and dorsal skin temperatures. Approximately 50 percent of any group of animals appeared moribund at this time.

The rectal temperature of fed animals at 15°C was never more than about 1.5°C below normal. This can be seen in the data of Figure 4. The maximum drop occurred after only four hours of exposure, and for the remainder of the week the temperature was never more than 1.2°C below that of mice kept at 25°C. At the end of one week (168 hours) the temperature was practically the same as that of control animals.

The ventral skin temperature of fed animals at 15°C was depressed on the average 2.3°C below that of control animals within a two-hour period of exposure. A similar decline was also seen in the dorsal skin temperature.
**RECTAL TEMPERATURES OF FASTED MICE EXPOSED TO 15°C**

**FEMALES 19-23GM CF-1 STRAIN**

* Each point represents the average temperature of a minimum of 8 animals.

**VENTRAL SURFACE TEMPERATURES OF FASTED MICE EXPOSED TO 15°C**

**FEMALES 19-23 GM CF-1 STRAIN**

* Each point represents the average temperature of a minimum of 8 animals.

**FIGURE 1**

**FIGURE 2:**
DORSAL SURFACE TEMPERATURES* OF FASTED MICE EXPOSED TO 15°C
FEMALE 19-23 GMS CF-1 STRAIN

Each point represents the average temperature of a minimum of 8 animals.
Standard deviation

FIGURE 3.

RECTAL TEMPERATURE* OF FED MICE EXPOSED TO 15°C
FEMALES 19-23 GMS CF-1 STRAIN

Each point represents the average temperature of a minimum of 8 animals.
Standard deviation

FIGURE 4.
VENTRAL SURFACE TEMPERATURES OF FED MICE EXPOSED TO 15°C
FEMALES 19-23 GMS CF-1 STRAIN

Each point represents the average of a minimum of 8 animals.

FIGURE 5.

DORSAL SURFACE TEMPERATURES OF FED MICE EXPOSED TO 15°C
FEMALES 19-23 GMS CF-1 STRAIN

Each point represents the average temperatures of a minimum of 8 animals.

FIGURE 6.
These data are given, respectively, in Figures 5 and 6. Beyond this time both temperatures showed a variable pattern up to 24 hours. In each case the greatest deviation from the values obtained at room temperature occurred after 16 hours exposure. A difference of $4.6^\circ C$ was noted in the ventral surface temperature compared to a difference of $3.9^\circ C$ in the dorsal surface temperature. High values were recorded after 24 hours but they declined again after 48 hours and rose slightly at 72 hours. After one week at $15^\circ C$ neither the ventral ($31.0^\circ C \pm 1.1^\circ C$) nor the dorsal ($31.1^\circ C \pm 1.1^\circ C$) surface temperatures had returned to comparable values of the room temperature control mice, in contrast to the findings with rectal temperatures.

Rectal and Surface Temperatures of Mice Exposed to $5^\circ C$

Temperature studies of fasted animals at $5^\circ C$ were omitted since the total number of deaths approached 100 per cent within 24 hours. However, as will be noted later, temperatures of fasted animals after three hours and five hours of exposure to $5^\circ C$ were recorded for comparison with values obtained for endotoxin poisoned mice. Rectal temperatures of fed animals at $5^\circ C$ decreased by approximately three degrees from the temperature of mice kept at room temperature after a two-hour exposure. This can be seen in Figure 7, which also shows a gradually rising temperature 10 hours after cold exposure; it is lowered again at 16 hours and remains more or less constant (between $34^\circ C$ and $35^\circ C$) up to 96 hours in the $5^\circ C$ room. By the end of a week, on the other hand, the temperature of the mice returned approximately to normal levels.

Figures 8 and 9 illustrate the variable patterns obtained for the ventral and dorsal skin temperatures of mice subjected to $5^\circ C$. In both cases there was a considerable temperature drop after two hours exposure to the cold -- from $33.2^\circ C \pm 1.5^\circ C$ to $26.7^\circ C \pm 0.8^\circ C$ (a difference of $6.5^\circ C$) in ventral temperatures, and from $32.0^\circ C \pm 0.7^\circ C$ to $26.1^\circ C \pm 1.1^\circ C$ (a difference of $5.9^\circ C$) in dorsal temperatures. Both sets of measurements showed variations beyond this initial period of adjustment, but after the first 24 hours the fluctuations were within a range of one degree. At no time was the skin temperature more than eight degrees below the initial control value.

**Determination of the LD$_{50}$ Dose of Heat-Killed S. Typhimurium in Mice Placed at the Three Environmental Temperatures**

Mice were injected intraperitoneally with heat-killed _S. typhimurium_ and placed immediately in individual compartments of cages. The cages were then transferred to rooms held at $5^\circ C$, $15^\circ C$ and $25^\circ C$. From the mortality data LD$_{50}$ doses were calculated, with the results shown in Table I.
TABLE I.

LD50 doses of *S. typhimurium* endotoxin injected intraperitoneally into female mice at various temperatures during a 48-hour exposure period.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Dose (viable cells)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>2 x 10⁹</td>
<td>*</td>
</tr>
<tr>
<td>15°C</td>
<td>5 x 10⁸</td>
<td>*</td>
</tr>
<tr>
<td>5°C</td>
<td>8 x 10⁶</td>
<td>*</td>
</tr>
</tbody>
</table>

*Data courtesy of D. S. Smythe and A. A. Jewett.*

At 5°C only 1/250 as many cells are required as at 25°C, and about 1/60 as many as at 15°C. The difference between 15°C and 25°C is only one-fourth. Not shown by the numbers themselves, however, is the shorter time of survival typically seen in most of the mice that died with an LD50 dose at 5°C. The first mortalities occurred at seven to eight hours post-injection and most were completed before 24 hours. This is less apparent at 15°C where most deaths, in agreement with 25°C results, were found between 18 and 24 hours.

Rectal Temperatures of Endotoxin Poisoned Mice at 15°C and 5°C

Figure 10 compares graphically the rectal temperatures of fed and fasted mice exposed to 15°C and 5°C for three hours and five hours with animals also injected with endotoxin. The temperatures shown for fed animals at 15°C and 5°C and for fasted animals at 15°C have been taken directly from Figures 1, 4 and 7. Temperatures for fasted animals exposed to 5°C and for the endotoxin poisoned animals were determined. It is apparent that endotoxin poisoned mice held at 15°C for three hours showed a greater temperature depression compared with control animals than for any other time or environmental condition. After five hours exposure to 15°C the temperatures in the control animals and in the endotoxin group were less than 1°C apart.
RECTAL TEMPERATURE OF FED MICE EXPOSED TO 5°C
FEMALE 19-23 GMS CF-1 STRAIN

*Each point represents the average temperature of a minimum of 8 animals.

Standard deviation

FIGURE 7.

VENTRAL SURFACE TEMPERATURE OF FED MICE EXPOSED TO 5°C
FEMALES 19-23 GMS CF-1 STRAIN

*Each point represents the average of a minimum of 8 animals.

Standard deviation

FIGURE 8.
DORSAL SURFACE TEMPERATURE OF FED MICE EXPOSED TO 5°C FEMALES 19-23 GMS CF-I STRAIN

Each point represents the average temperatures of a minimum of 8 animals.

Standard deviation

FIGURE 9.

RECTAL TEMPERATURE OF FED, FASTED, AND ENDOTOXIN-POISONED MICE EXPOSED TO LOWERED TEMPERATURES

FIGURE 10.

Each point represents the average temperature of a minimum of 8 animals.
At $5^\circ$ C the temperature for all groups after three hours exposure was uniformly depressed. Two hours later each group had a higher temperature, with endotoxin producing no obvious effect.

**Liver and Muscle Glycogen and Total Body Carbohydrate of Mice Exposed to Lowered Temperatures**

Normal fed mice kept at room temperature were used for assay of liver, abdominal wall muscle and total body for carbohydrate content. The results are presented in Table II. The average carcass weight for the animals employed after removal of skin, feet, tail and digestive tract ranged from 11 to 13 grams. In general, mice weighing 20 grams have approximately one gram of liver, 10 grams of muscle and two milliliters of blood. From the data listed it can be calculated on the basis of these weights that liver contains about 57 milligrams of glycogen (5.69 per cent of 1000 mg) and total body muscle has approximately 30 milligrams. This gives a combined total of about 87 milligrams of carbohydrate, excluding that contained in blood and other tissues. Thus the ratio 87 mgs/11 gms or 7.9 mg per gm of carcass compares favorably with the 817 mg/g found by direct analyses of total body carbohydrate (Table II).

Also shown in Table II are the carbohydrate values for liver, muscle and total carbohydrate in fed mice exposed to $15^\circ$ C for three hours. All are slightly higher than those found for mice at $25^\circ$ C but the differences are not statistically significant. Animals fasted for three hours at $15^\circ$ C, on the other hand, had significantly lower carbohydrate reserves than the fed animals. This is apparent also for total body carbohydrate.

The carbohydrate data obtained from fed animals exposed to $15^\circ$ C for five hours are given in the last section of Table II. Liver glycogen was lower than that found for the fed group exposed for three hours, while muscle glycogen was not altered. Total body carbohydrate, somewhat diminished, was not significantly different from that in control animals or those tested after three hours exposure to $15^\circ$ C. The last column of Table II shows that the glycogen content in muscle, liver, and total body after five hours exposure to $15^\circ$ C without food is reduced in each case. All values are significantly lower than those found in the fed animals exposed to $15^\circ$ C for five hours.

Since Berry et al (1959) demonstrated that endotoxin poisoned mice do not generally eat, carbohydrate determinations were performed on fasted animals only. The results are presented in Table III. An LD$_{50}$ dose of endotoxin ($5 \times 10^4$ heat-killed *S. typhimurium*) after three hours did not result in any significant reduction in glycogen content of liver, muscle or total body compared to that of fasted animals exposed to $15^\circ$ C. After five hours at $15^\circ$ C,
TABLE II

Carbohydrate data for CF-1 female mice exposed to 15°C for three and five hours

<table>
<thead>
<tr>
<th></th>
<th>Constant Exposure To 25°C ± 2°C</th>
<th>Three Hours Exposure To 15°C ± 2°C</th>
<th>Five Hours Exposure To 15°C ± 2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fed</td>
<td>Fed</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td>5.69 ± 0.9 (7)</td>
<td>6.84 ± 1.0 (8) &gt; 5%</td>
<td>4.57 ± 1.1 (8)</td>
</tr>
<tr>
<td>(per cent)</td>
<td></td>
<td></td>
<td>3.77 ± 0.9 (7)</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td>0.30 ± 0.05 (7)</td>
<td>0.35 ± 0.1 (8) &gt; 5%</td>
<td>0.35 ± 0.1 (8) &lt; 1%</td>
</tr>
<tr>
<td>(per cent)</td>
<td></td>
<td></td>
<td>0.23 ± 0.05 (7)</td>
</tr>
<tr>
<td>Total body carbohydrate</td>
<td>8.70 ± 1.1 (7)</td>
<td>8.87 ± 1.2 (8) &gt; 5%</td>
<td>7.81 ± 1.8 (8) &lt; 1%</td>
</tr>
<tr>
<td>(mg/g carcass)</td>
<td></td>
<td></td>
<td>4.91 ± 0.7 (7)</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard deviation. The numbers in parentheses are the numbers of separate determinations made for each value. P indicates the degree of significance obtained by comparing the fed and fasted values according to the rank order test.
TABLE III

Carbohydrate determinations of endotoxin poisoned mice exposed to 15°C

<table>
<thead>
<tr>
<th></th>
<th>Three Hours Exposure to 15°C</th>
<th>Five Hours Exposure to 15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted</td>
<td>P</td>
</tr>
<tr>
<td>Liver glycogen (per cent)</td>
<td>4.83 ± 2.2</td>
<td>&gt;5%</td>
</tr>
<tr>
<td>Muscle glycogen (per cent)</td>
<td>0.25 ± 0.09</td>
<td>&gt;5%</td>
</tr>
<tr>
<td>Total body carbohydrate (mg/g carcass)</td>
<td>7.75 ± 3.3</td>
<td>&gt;5%</td>
</tr>
</tbody>
</table>

Values represent the mean ± the standard deviation. Numbers in parentheses are the number of separate determinations made for each value. P indicates the level of significance attained by the rank order test.
however, liver glycogen, muscle glycogen and total carbohydrate were significantly lower than those found in the nonpoisoned animals fasted and exposed to 15° C for the same period of time.

Fed mice after three hours exposure to 5° C, as indicated in Table IV, were able to maintain their liver and muscle glycogen within the range of fed animals quartered at room temperatures and with no significant differences existing between the carbohydrate values of either group. Under fasting conditions, however, significantly lower glycogen values were found in liver, muscle and total body following exposure to 5° C for three hours (Table IV). As might be anticipated, moreover, all values were lower than those found under similar conditions in mice exposed to 15° C for three hours (see Table II).

After five hours exposure to 5° C, mice had muscle glycogen levels in both fed and fasted animals that did not differ significantly from each other or from the values found in mice exposed to the same temperature for three hours. Liver glycogen in the fed animals was greater than that in fasted animals after five hours exposure to 5° C, and each was lower than that found after three hours. Similarly, total body carbohydrate after five hours exposure to 5° C was lower in fed and fasted mice than was previously found in either fed or fasted animals exposed to 5° C for only three hours.

Carbohydrate levels in mice injected with an LD50 dose of endotoxin (8 X 10^6 heat-killed S. typhimurium) immediately before placing the animals at 5° C were determined after three hours and five hours exposure and are shown by the data of Table V. Liver and muscle glycogen and total body carbohydrate after three hours exposure to 5° C are statistically the same in fasted mice and endotoxin poisoned mice. Similarly, no significant differences were noted in the glycogen values obtained with endotoxin treated animals exposed to 15° C for three hours or to 5° C for three hours (Tables III and V). However, five hours after endotoxin and exposure to 5° C, both liver glycogen and total body carbohydrate were significantly lower than in mice only fasted. By contrast, muscle glycogen was not statistically altered under the same conditions. Finally, the carbohydrate levels obtained in endotoxin treated mice exposed for five hours to either 5° C or 15° C (Table III) were not significantly different.
## TABLE IV

Carbohydrate data for CF-1 female mice exposed to 5°C for three and five hours

<table>
<thead>
<tr>
<th></th>
<th>Constant Exposure to 25°C</th>
<th>Three Hours Exposure to 5°C</th>
<th>Five Hours Exposure to 5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fed</td>
<td>P</td>
</tr>
</tbody>
</table>
| Liver glycogen (per cent) | 5.69 ± 0.9  
(7) | 5.83 ± 1.0  
(8) | < 0.1% | 4.07 ± 1.2  
(8) | 4.36 ± 0.9  
(7) | < 1% | 3.63 ± 0.8  
(7) |
| Muscle glycogen (per cent) | 0.30 ± 0.05  
(7) | 0.26 ± 0.1  
(8) | < 0.1% | 0.18 ± 0.5  
(8) | 0.23 ± 0.05  
(7) | > 5% | 0.19 ± 0.08  
(7) |
| Total body carbohydrate (mg/g carcass) | 8.70 ± 1.1  
(7) | 7.77 ± 1.0  
(8) | < 0.1% | 5.00 ± 1.3  
(8) | 6.46 ± 2.1  
(7) | < 5% | 4.80 ± 0.8  
(7) |

Each value is the mean ± the standard deviation. The numbers in parentheses are the number of separate determinations made for each value. P indicates the degree of significance by rank order test.
TABLE V

Carbohydrate determinations of endotoxin-poisoned mice exposed to 50°C

<table>
<thead>
<tr>
<th></th>
<th>Three Hours Exposure to 50°C</th>
<th>Fasted + 8 X 10^6 heat-killed SR-11 cells</th>
<th>Five Hours Exposure to 50°C</th>
<th>Fasted + 8 X 10^6 heat-killed SR-11 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted</td>
<td>P</td>
<td>Fasted</td>
<td>P</td>
</tr>
<tr>
<td>Liver glycogen (per cent)</td>
<td>4.07 ± 1.2 (8)</td>
<td>&gt;5%</td>
<td>3.93 ± 1.8 (8)</td>
<td>3.63 ± 0.8 (7)</td>
</tr>
<tr>
<td>Muscle glycogen (per cent)</td>
<td>0.18 ± 0.5 (8)</td>
<td>&gt;5%</td>
<td>0.22 ± 0.07 (8)</td>
<td>0.19 ± 0.08 (7)</td>
</tr>
<tr>
<td>Total body carbohydrate (mg/g carcass)</td>
<td>5.00 ± 1.3 (8)</td>
<td>&gt;5%</td>
<td>4.80 ± 2.0 (8)</td>
<td>480 ± 0.8 (7)</td>
</tr>
</tbody>
</table>

Values represent the mean ± the standard deviation. Numbers in parentheses are the number of separate determinations made for each value. P indicates the level of significance attained by rank order test.
Fed mice survived temperatures of $15^\circ C$ and $5^\circ C$ for at least one week but the lower temperature required the greater adjustment, as indicated by the temperature graphs. In order for animals to survive this long they must be kept free from excessive moisture and excreta. Neglect results in a striking rise in mortality.

The average body temperature of $36.2^\circ C$ reported in these studies compares favorably with values listed by other authors. Benedict and Lee (1936) found a rectal temperature in mice varying between $36^\circ C$ and $39^\circ C$ depending on the external temperature, while Kennaway and Pembrey (1912) obtained values ranging from $36.1^\circ C$ to $38.6^\circ C$, with an average rectal temperature of $37.4^\circ C$. Sumner (1913) noted a variability in rectal temperature related to the degree of excitement of the animal and the length of insertion of the probe into the rectum. He tabulated the average rectal temperature as being $36.7^\circ C$. Sealander (1953), studying the white footed mouse, lists body temperature, measured intraesophageally, as $38.1^\circ C$ for males and $37.6^\circ C$ for females. He also observed that body temperatures of mice given adequate food and water were not affected by reduced temperature. Morrison and Ryser (1959) noted a variability in response to lowered temperatures in the white footed mouse, listing the normal temperature of these animals as being $37.4^\circ C$ at $22^\circ C$ to $29^\circ C$. Nielsen (1939) found a variability in the response of mice to lower temperatures and believed that between $17^\circ C$ and $40^\circ C$ they were capable of keeping body temperature constant within the limits of normal variability.

The LD$_{50}$ dose of endotoxin employed in these studies depressed the rectal temperature of mice exposed for three hours to $15^\circ C$, but after five hours the temperature was nearer normal. Endotoxin poisoned mice at $5^\circ C$ show at both three hours and five hours a rectal temperature approximately one degree below that of control values. Berry et al (1959) used an LD$_{50}$ dose of endotoxin (0.75mg) to produce the hypothermia noted in their studies at room temperature. Halberg (1961), injecting 1 mg of Brucella melitensis endotoxin in female mice, found a $5^\circ C$ rectal temperature depression when the animals were subjected to $8^\circ C$. Since in both instances the dose of endotoxin was higher than that used in this study, the difference in results may thereby be explained.

Endotoxin treatment along with subjection to lowered temperatures was shown in this study to lower significantly liver glycogen after five hours exposure to $15^\circ C$ but not to $5^\circ C$. Muscle glycogen and total body carbohydrate were lowered after five hours exposure to both $15^\circ C$ and $5^\circ C$, but not after three hours. Stressing the animals with endotoxin and cold (especially at
the $5^\circ$ C temperature) did not, therefore, result in an extensive glycogen loss as might have been anticipated. Since Berry et al (1959) noted a protective role of cortisone in experimental endotoxin poisoning, it might be postulated that a temperature of $5^\circ$ C and to a lesser extent $15^\circ$ C, is initiating increased secretion of glycocorticoids which in turn promote an early glycogenesis, perhaps counteracting the glycogenolytic effect of the injected endotoxin. Smythe and Jewett (1961) injected a group of fasted mice with an $LD_{70}$ dose of heat-killed $S$. typhimurium cells and exposed half of the group to $25^\circ$ C and the other half to $5^\circ$ C. They then observed a greater depletion of liver glycogen in the animals exposed to the higher temperature. This tends to support the above suggestions. However, values from greater numbers of mice injected with larger doses of endotoxin and studied at higher and lower temperatures is needed before any valid conclusions can be made.

Finally, no direct correlation was found between lowered body temperatures and carbohydrate depletion in endotoxin poisoning at the time intervals studied. Even though glycogen and total body carbohydrate are depleted in endotoxin poisoning, the body temperature did not greatly fall and is presumably maintained, at least in part, by these carbohydrate reserves. Again, the use of larger doses of endotoxin and the extension of the time intervals studied might show more correlation between hypothermia and carbohydrate depletion in endotoxin and cold stressed animals.
REFERENCES


HISTORICAL BIBLIOGRAPHY


14. Fregly, M. J. Cross-acclimatization between cold and altitude in rats.

15. Barnett, S. A. The skin and hair of mice living at a low environmental

16. Sumner, F. B. Some effects of external conditions upon the white mouse

17. Sundstroem, E. S. Effects of a tropical climate on growth and pigmentation
   of hair and dependence of these integumental functions on the

18. Pearson, O. P. The oxygen consumption and bioenergetics of harvest

   to environmental temperature and heat and cold stress. Biol.

   I. Technics, activity control, and the influence of fasting. II.
   Influence of environmental temperature, age and sex. Comparison

21. Weiss, B. Effects of brief exposure to cold on performance and food

22. Prychodko, W. Effect of aggregation of laboratory mice (Mus Musculus)


   thermogenesis in the cold-acclimated rat. Arctic Aeromedical
   Laboratory, Technical Note AAL-TN 60-6.


Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash.
Rpt. AAL-TR-62-4. BODY TEMPERATURE AND CARBOHYDRATE VALUES IN NORMAL AND ENDO-
TOXIN POISONED MICE EXPOSED TO LOWERED ENVIRONMENTAL TEMPERATURES. August 1962.
25 p. incl. tables. 12 refs. illus and biblio.
Unclassified Report

A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD50 dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C, but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C.

Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash.
Rpt. AAL-TR-62-4. BODY TEMPERATURE AND CARBOHYDRATE VALUES IN NORMAL AND ENDO-
TOXIN POISONED MICE EXPOSED TO LOWERED ENVIRONMENTAL TEMPERATURES. August 1962.
25 p. incl. tables. 12 refs. illus and biblio.
Unclassified Report

A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD50 dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C, but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C.
A comparison is made between the rectal and the body surface temperatures of fed mice housed in individual compartments without bedding while exposed continuously to environmental temperatures of 5°C, 15°C and 25°C. Surface temperatures of the mice are related to the ambient temperature at which they are held. Rectal temperatures are known to undergo cyclic variations and except for the first 24 hours at 5°C, are within normal limits throughout a week of exposure. Fasted animals at 5°C cannot maintain a core temperature beyond about 6 to 12 hours and all die within 24 hours. Injection of an LD50 dose of endotoxin fails to depress liver and muscle glycogen and total body carbohydrate after three hours at 15°C, but after an exposure of five hours liver glycogen alone remained unchanged. At 5°C, carbohydrate reserves were depleted in liver, muscle and total body after three hours in fasted mice but not in fed mice. After five hours, muscle glycogen alone was lowered. Endotoxin poisoned mice lost carbohydrates after three hours and five hours at both 5°C and 15°C.
UNCLASSIFIED

UNCLASSIFIED

UNCLASSIFIED