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Aspects of thermogenesis in a seasonal hibernator, *Spermophilus richardsonii*

by

© Bruce Abbotts

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled, "Aspects of thermogenesis in a seasonal hibernator, Spermophilus richardsonii," submitted by Bruce Abbotts in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

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Date April 23, 1979

Abstract

The maximum thermogenic capabilities (HPmax) and the maximum capabilities to produce heat via non-shivering thermogenesis (NSTmax) were estimated at several times throughout the year in a seasonal hibernator, Spermophilus richardsonii. The following eight groups were tested: APRIL-ADULT (Adult animals tested within one week of capture in April.), JUNE-ADULT (Adult animals tested within one week of capture in June.), JUNE-YOUNG (Young of the year animals tested within one week of capture in June.), AUG-YOUNG (Young of the year animals tested within one week of capture in August.), HIB-77 (Ground squirrels in hibernating phase, tested late in the hibernation season, February-March.), HIB-78 (Ground squirrels in hibernating phase, tested early in the hibernation season, October-November.), COLD-ACCL (Animals acclimated to 5°C), WARM-ACCL (Animals acclimated to 20°C). Maximum thermogenesis was estimated by exposing the animal to acute cold in Helium (79%)-Oxygen (21%), and monitoring oxygen consumption ($\dot{V}O_2$). To estimate NSTmax, isoproterenol, a beta-adrenergic agonist, was used to stimulate non-shivering thermogenesis (NST) in anesthetized animals at thermoneutral temperatures (20°C). Dose-response curves were generated for each group of animals to determine the maximum metabolic response which could be elicited by the drug. This value was used as an estimate of NSTmax. The dynamic changes in NSTmax and HPmax could then be examined as the animals progressed through the yearly cycle

of activity and hibernation. Since NST and shivering thermogenesis (ST) are additive, the two parameters measured, HPmax and NSTmax, also gave an indirect estimate of ST.

Although values for HPmax ranged from 101.1 (±6.3) cal/wt$^{0.73}$/hr in the JUNE-YOUNG group to 142.1 (±14.7) cal/wt$^{0.73}$/hr in the JUNE-ADULT group, no clear relationship was observed between HPmax and the time of the year. However, NSTmax was observed to increase during the hibernation season, from values of 40 to 50 cal/wt$^{0.73}$/hr in groups WARM-ACCL, AUG-YOUNG, JUNE-ADULT and APRIL-ADULT, to 66.5 (±2.7) and 79.2 (±6.8) cal/wt$^{0.73}$/hr in groups HIB-78 and HIB-77, respectively. No significant increase in NSTmax was observed when cold-acclimated animals (Group COLD-ACCL) were compared to warm acclimated animals (Group WARM-ACCL). These results suggest that the increase in NST observed in hibernating animals is a response to the increased demands for heat production during periodic arousals from hibernation. Since no increase in HPmax was observed, these results also indicate that the animal's capacities to produce heat via ST are somehow reduced during the hibernation season.

Acknowledgements

I would like to thank Dr. L.C.H. Wang for his time spent toward the careful guidance of this research. In addition, I would like to acknowledge the funds and facilities provided by him. Without either of these this research would not have been completed.

I would also like to thank the members of my supervisory commitee, Dr. W.R. Kaufman and Dr. J.O. Murie, for their input into this research.

Acknowledgement is due to the Department of Zoology, University of Alberta, which provided financial support in the form of a teaching assistantship.

In addition, I would like to thank the many friends, who contributed to this project with their thoughts and friendship. Several people deserve special recognition. Magi Nietfeld often provided much needed moral support and editorial remarks. Euclid played a critical role in obtaining the ground squirrels for this study. Susan Greene and Stilgar also deserve mention for their important role in keeping me in working condition throughout the final stages of preparation.

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I. Introduction

Mammals maintain a constant body temperature (Tb) by precisely balancing heat production (HP) and heat loss (HL) in the face of changing environmental temperatures. Homeothermy must be maintained within narrow limits to allow normal function of many vital processes (Prosser 1973). To achieve this, mammals have evolved a finely-tuned temperature sensing and regulating system (see Hammel 1965; Carlson 1973; Heller et al. 1978 and Satinoff 1978 for reviews).

In response to decreasing ambient temperature (Ta) mammals increase their HP by increasing metabolism. It can be seen from Fig. 1, that at some low temperature (Tc), maximum heat production (HPmax) is reached. For temperatures below Tc, the animal can no longer compensate for decreased Ta with increased HP. Prolonged exposure to temperatures lower than Tc, therefore, results in hypothermia and eventually death.

At any given temperature the total HP consists of obligatory HP (basal metabolism) and regulatory HP (Girardier 1977; see Fig. 1). Regulatory HP is the result of two processes, shivering thermogenesis (ST) and non-shivering thermogenesis (NST). Shivering thermogenesis is the result of small contractions of skeletal muscles and is mediated by somatic motor neurons (Jansky 1977). Non-shivering thermogenesis is mediated by

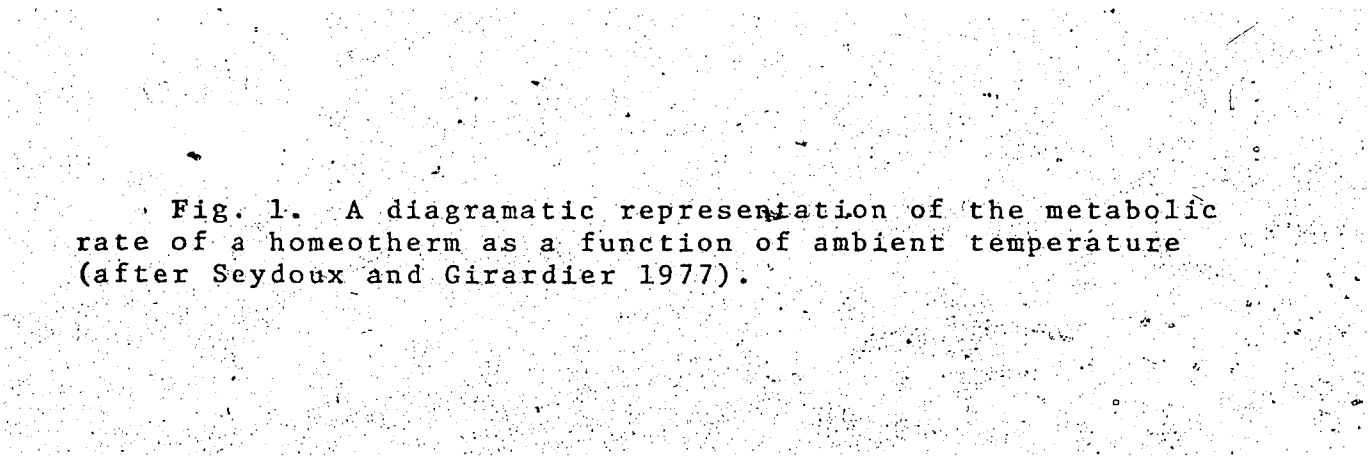
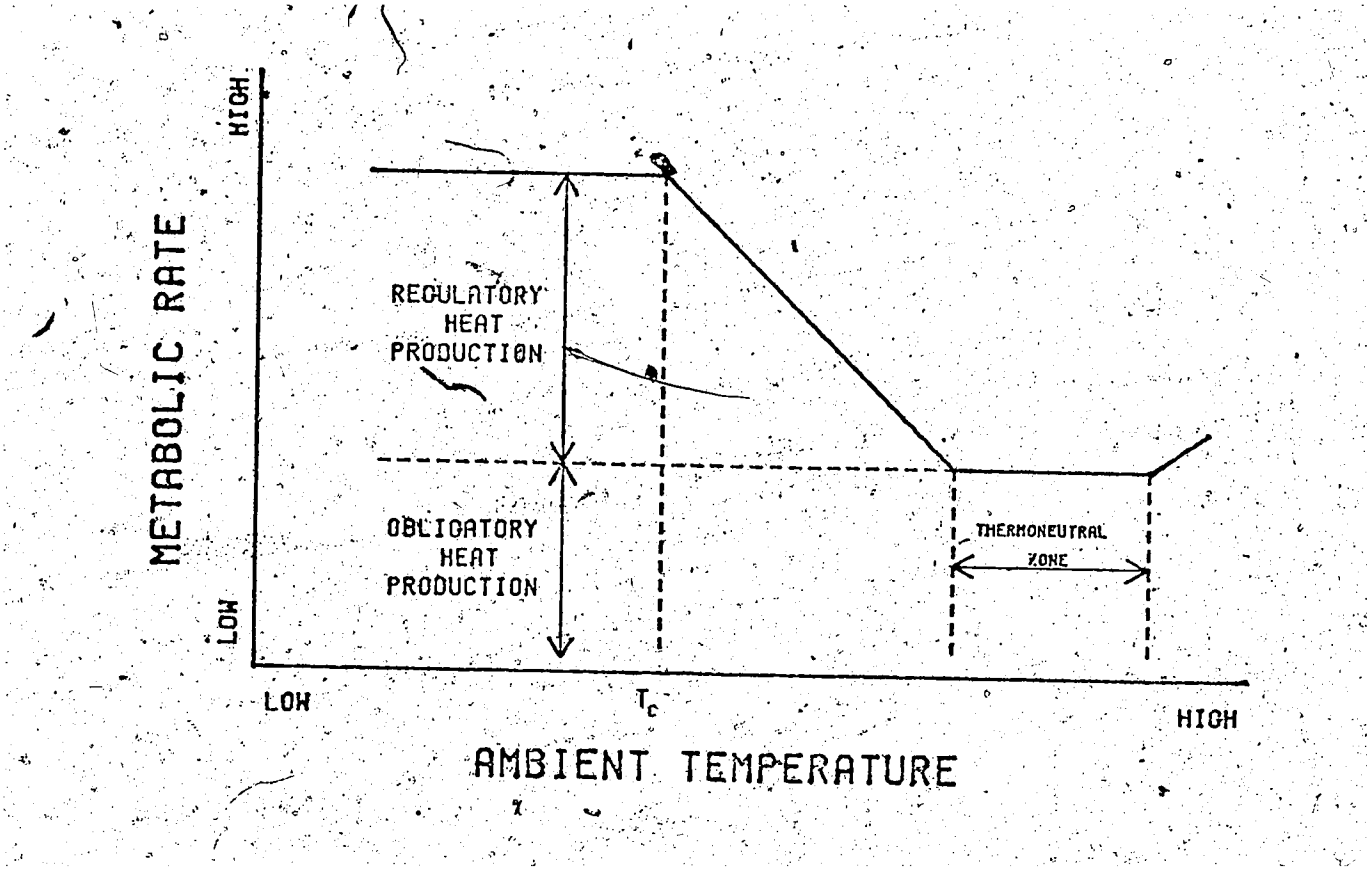


Fig. 1. A diagramatic representation of the metabolic rate of a homeotherm as a function of ambient temperature (after Seydoux and Girardier 1977).


METABOLIC RATE
HIGH
LOW
REGULATORY HEAT PRODUCTION
OBLIGATORY HEAT PRODUCTION
THERMONEUTRAL ZONE
LOW
T_c
HIGH
AMBIENT TEMPERATURE

norepinephrine (NE) released from sympathetic nerve endings, acting primarily on beta-adrenergic receptors (LeBlanc 1976; Horwitz 1978). Although the mechanism of NST is not fully understood, it appears to involve the uncoupling of oxidative phosphorylation and/or increasing ATP utilization of the Na^+-K^+ ATPase pump (see Seydoux and Girardier 1977; Himms-Hagen 1978; Horwitz 1978 for reviews). Brown adipose tissue (BAT) seems to be the primary effector organ of NST, although supportive organs (heart, diaphragm, etc.) and skeletal muscles are also thought to be of importance (Jansky 1977; Foster and Frydman 1978).

In addition to short-term changes in temperature, requiring minute to minute adjustment of HP and HL, animals experience longer-term, seasonal changes in thermal demands. This can be especially important to small homeotherms, such as rodents, which have large surface area to mass ratios and, therefore, high rates of HL per unit weight. Consequently, many rodents make adjustments in behavior (e.g. nesting, huddling, and, seeking less severe microenvironments) and/or physiology (e.g. increasing insulation, and increasing capacities to produce heat) to allow their survival through the colder seasons (see Hart 1971 for review of thermoregulation in rodents). Many species develop a higher degree of NST when cold-acclimated (CA) than when warm-acclimated (WA) (Cottle and Carlson 1956, rat; Cottle 1963, rabbit; Hemingway et al. 1964, cat; Pohl and Hart 1965, 13-lined ground squirrel; Nagaska and

Carlson 1965, dog; Williams 1968, golden hamster; Jansky et al. 1969, mouse; Bartunkova et al. 1971, rat; Portet et al. 1971, rat; Feist and Rosenmann 1976, red-backed vole). Because NST does not interfere with muscular activity, the increased development of NST is viewed as an adaptive response to combat cold, freeing the muscles from the interference of shivering (Cottle & Carlson 1956; Horwitz 1978).

Often winter is a time of low food supply, making it difficult to meet the energy requirements for HP. One strategy to cope with this problem is to store energy in the form of seeds, other non-perishable food items, or fat. Also, energetic demands can be reduced, as in hibernation. In hibernation, Tb is greatly reduced as are bodily functions, thereby decreasing the energy requirements. For reasons which are not fully understood, animals in hibernation periodically arouse (i.e. they rewarm from Tb which is near ambient (which can be as low as 0°C) to normal Tb (37°C). Wang (1979) estimates that the energetic cost of hibernation for the Richardson's ground squirrel averages 12.2% of the normothermic energy requirements and reaches a low of 4% in January. The bulk of the energetic cost during the hibernation season is required for maintainence of normal Tb between bouts of torpor (51.6%). The remaining cost is divided between the stages of entrance into torpor (12.8%), deep torpor (16.6%) and arousal (19.0%) (Wang 1979). Although the largest total cost is due to the

inter-torpor periods, the rate of HP during the arousal process is greater than that required during the inter-torpor periods of normothermy (Wang 1979; personal observation). Clearly, the overall energy savings during hibernation are great, however, the demands made on the mechanisms for HP during the arousal process are high.

Seasonal hibernators (ground squirrels, marmots, woodchucks, etc.) breed in spring and remain active throughout summer, fattening for the coming winter season. In early fall, they enter hibernation for the year, arousing periodically, as previously mentioned. In the golden-mantled ground squirrel, this cycle has been shown to be under endogenous control, i.e., it persists for several years with a periodicity of approximately one year even when the animals are kept in the laboratory without any apparent seasonal cues (Pengelley and Fisher 1963). Annual cycles of body weight parallel the endogenous hibernation cycles with peak weight occurring just prior to the onset of hibernation (Mrosovsky and Fisher 1970; Heller and Poulson 1970; Mrosovsky 1975).

The present study investigates the seasonal changes in HPmax and the maximum capabilities to produce heat by NST (NSTmax) in a seasonal hibernator, the Richardson's ground squirrel, Spermophilus richardsonii. The HPmax and NSTmax were estimated at different times throughout the year, in active, non-hibernating phase ground squirrels and ground squirrels in hibernating phase, to ascertain the specific

adjustments which are made to meet seasonally changing demands for HP. In view of the demand for HP at reduced Tb during rewarming from torpor, the animal may meet this demand by increasing NSTmax, HPmax, or both. Alternatively, the capacities for HP may be maintained at high levels throughout the year without any special adjustments for the hibernation season.

II. Materials and Methods

A. Animals

The Richardson's ground squirrels used in this study were live-trapped approximately 15 km south of Edmonton, Alberta, Canada. Upon capture, the animals were taken to the animal holding facilities at the University of Alberta, where they were housed individually in shoe-box type cages (45 x 25 x 20 cm). Ground squirrels maintained in the laboratory from June 1977 to May 1978 were fed Vitamite cubes (Northwest Feeds, Ltd. Edmonton, Alberta) ad libitum. After May 1978, all animals were fed a mixture of Purina rat chow (Ralston-Purina Co.) and Wayne dog food pellets (Allied Foods Inc.) ad libitum.

The following groups of animals were tested for NSTmax and HPmax to determine changes in thermogenic capabilities:

- APRIL-ADULT. Ground squirrels tested within one week of capture in mid-April, prior to birth of young.
- JUNE-ADULT. Ground squirrels determined to be adult animals on the basis of weight and tested within one week of capture in mid-June.
- JUNE-YOUNG. Ground squirrels determined to be young of the year on the basis of weight and tested within one week of capture in mid-June.
- AUG-YOUNG. Ground squirrels tested within one week of capture in early August. Since adult animals enter

hibernation from late June to early-July (Wehrell 1973; Michener 1974), all animals captured at this time would be young of the year.

- HIB-77. This group of animals was captured in the summer of 1977 and maintained in a walk-in environmental chamber at 5 (±1) °C until the time of testing. At the time of testing (February), these animals had been in hibernation for several months. Animals which are undergoing bouts of torpor are referred to as hibernating phase animals.
- HIB-78. This group of animals was captured in the summer of 1978 and maintained in a walk-in environmental chamber at 5 (±1) °C until the time of testing. At the time of testing (October-November), these animals had gone through at least 3 bouts of torpor.
- COLD-ACCL. This group of ground squirrels consisted of animals which were in the active, or non-hibernating phase (i.e. they had not exhibited torpor for at least 1 month) and were acclimated to 5 (±1) °C for at least 4 weeks prior to testing in April.
- WARM-ACCL. These ground squirrels were acclimated to 20 (±1) °C in a walk-in environmental chamber for at least 4 weeks prior to testing in October-November. None of these animals had shown signs of torpor at this Ta.

B. Testing of Thermogenesis

Estimation of Non-Shivering Thermogenesis

Catecholamine-induced thermogenesis was used to estimate NST. There is considerable evidence which indicates that NST in rodents is mediated primarily via beta-adrenergic receptors (Horwitz 1978). Exogenous beta-adrenergic agonists mimic this action and thus have been used as a method to estimate NST (Portet, et al. 1971; Himms-Hagen 1978; Horwitz 1978). For this study, isoproterenol, a beta-adrenergic agonist (Rotenberg 1977; Horwitz 1978), was used to induce NST. Heat production was measured by monitoring the rate of oxygen consumption ($\dot{V}O_2$). Using an RQ of 0.7, $\dot{V}O_2$ could be converted to its caloric equivalent using a conversion factor of 4.7 cal/ml O_2 (Kleiber 1961). Preliminary data, obtained when CO_2 production ($\dot{V}CO_2$) and $\dot{V}O_2$ were monitored simultaneously, indicated that this was a reasonable value for the RQ.

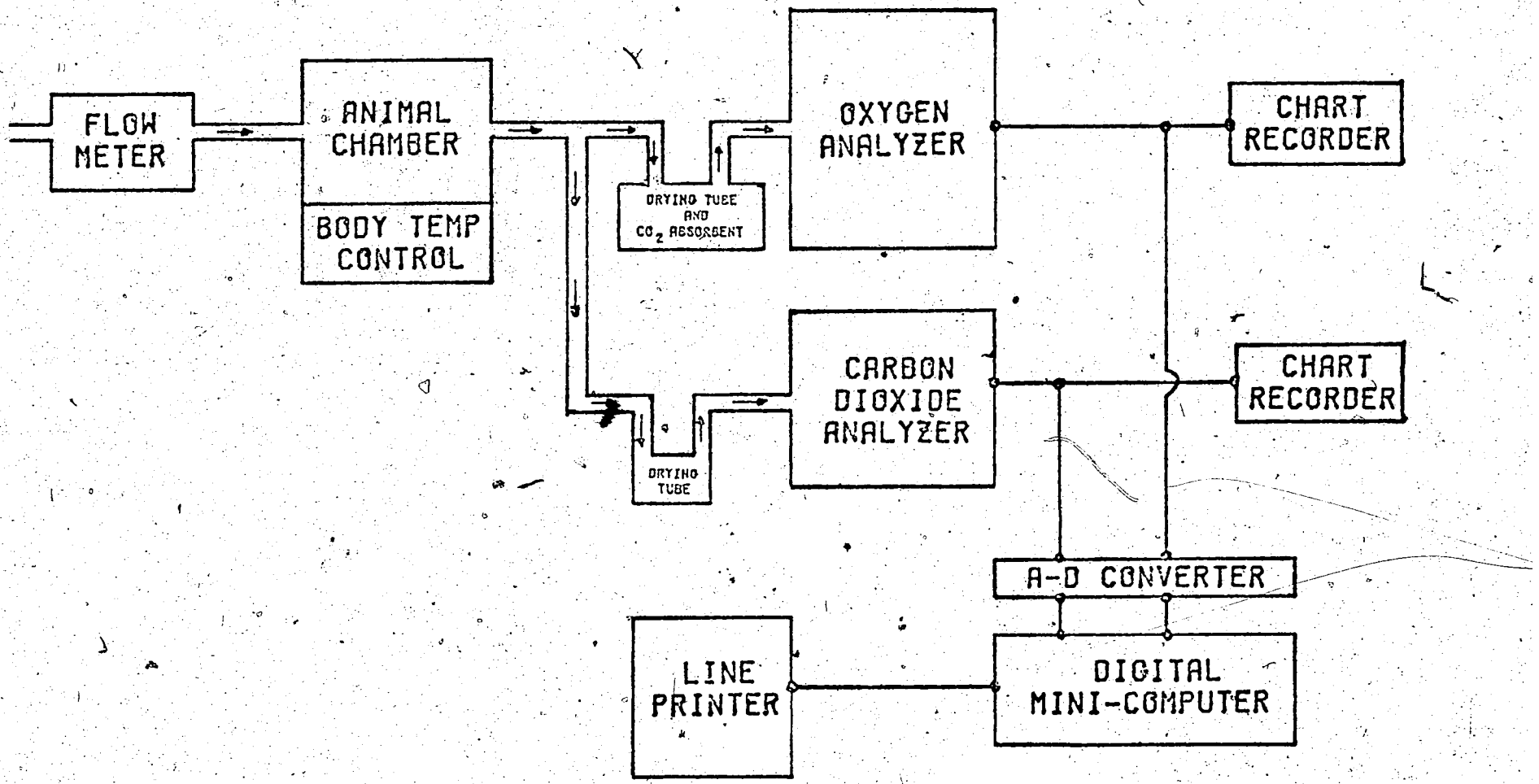
In order to measure catecholamine-induced thermogenesis, the animals were cannulated in the jugular vein with polyethelene tubing (PE10, Clay-Adams Inc.) while under sodium pentobarbital anesthesia (50 mg/kg Nembutal, Abbott Labs Inc.). Once the cannula was secured, animals were placed in a plexiglass metabolism chamber (20 x 15 x 15 cm). The animals rested on a bed of copper tubing through which hot (35°C) or cold (5°C) water flowed

to heat or cool the animal and thus maintain its Tb. Water flow was controlled by a YSI temperature controller (Yellow Springs Inc., Model 63RC) through a 3-way electric solenoid valve (Skinner Electric Valve Division). A rectal thermistor probe (Yellow Springs Inc., No. 402) inserted to a depth of 4 cm and taped to the tail was used to measure Tb and to control switching of the solenoid valve. This system generally controlled Tb within a range of 36-39°C. Data was not used on the occasions when Tb exceeded this range.

The flow of compressed air through the metabolism chamber, depressurized to 5 psi, was regulated at 1000 ml/min (STP) by a Matheson (Model 8240) flow controller (Fig. 2). The exhaust gas from the metabolic chamber was divided into two streams (Fig. 2). One stream was dried with CaSO4 (Drierite) and the CO2 removed (Ascarite) for analysis by a Beckman G-2 paramagnetic oxygen analyzer. The other stream led through a drying tube (Drierite) to a Beckman (Model 864) infra-red carbon-dioxide analyzer. The $\dot{V}O_2$ was calculated automatically by a Texas Instruments (Model 980A) digital minicomputer as described by Wang & Peter (1975). Values for $\dot{V}O_2$ were printed out every 60 sec on a Texas Instruments (Model 733) data terminal. For some initial trials when CO2 was analyzed, $\dot{V}CO_2$ and RQ were calculated and printed along with $\dot{V}O_2$. This allowed the confirmation of RQ values of 0.7 for the conversion of $\dot{V}O_2$ to the calcric equivalent.

Once the animal was situated in the metabolism chamber,

Fig. 2. Schematic representation of the data acquisition system for measuring metabolism.


FLOW METER
ANIMAL CHAMBER
BODY TEMP CONTROL
DRYING TUBE AND CO_2 ABSORBENT
OXYGEN ANALYZER
CHART RECORDER
CARBON DIOXIDE ANALYZER
DRYING TUBE
CHART RECORDER
A-D CONVERTER
LINE PRINTER
DIGITAL MINI-COMPUTER

infusion of the drug-carrying vehicle (1 mg/ml ascorbic acid (Fisher Chemical Co.) in double distilled, deionized water) was begun. All tests were conducted at room temperature (20°C), which is within the thermoneutral zone of this species (Wang, unpublished). Flow rate for the infusion of the vehicle and all doses of isoproterenol was controlled at 1.0 ml/hr by a Sage syringe pump (Model 352). Isoproterenol solutions of the appropriate concentrations were prepared fresh before each trial by dilution of a stock solution of isoproterenol (10 mg/ml vehicle). The stock solution of isoproterenol was prepared from d,l isoproterenol-HCl (Sigma Chemical Co.) prior to each trial. Isoproterenol was infused at doses of 1.0, 2.5, 5, 10 and 20 ng isoproterenol-HCl/wt(in grams)$^{0.74}$/min (equivalent molar doses are 4.0, 10.1, 20.2, 40.2, and 80.7 pM/wt$^{0.74}$/min). All 5 doses of isoproterenol were administered to each ground squirrel if the animal's condition permitted. The order of drug administration was varied. Each dose was infused until a plateau level of $\dot{V}O_2$ was obtained, approximately 20 to 30 min. Infusion was returned to the vehicle and $\dot{V}O_2$ allowed to stabilize several times throughout the experiment to be certain that the basal level had not changed. Each trial lasted 3-1/2 to 4-1/2 hours. Supplementary doses of Nembutal (10 to 20 mg/kg) were administered periodically via the infusion cannula to maintain the level of anesthesia.

Estimation of Maximum Heat Production

To estimate HPmax, animals were placed in a paper-lined metal metabolism chamber (modified from a 1 gal paint can) and exposed to cold temperatures (-15 to -25 (±1) °C) under helium (79%) and oxygen (21%) gas mixture (Helox). Because the conductivity of helium is approximately 6 times greater than that of nitrogen, Helox has been used to increase heat loss for the induction of hypothermia (Mussachia 1972; Mussachia & Jacobs 1973; Wang & Pet[illegible] 1975) and to elicit HPmax (Rosenmann & Morrison 1972; Rosenmann et al. 1975; Wang 1978). These conditions were thought to have elicited maximum thermogenesis because VO2 did not increase any further when the temperature was decreased within the test range (-15 to -25°C) or when HL was increased by wetting the animal with water. Exhaust gases were treated as mentioned previously, and $\dot{V}O_2$ was printed out every two minutes along with Ta. Body temperature was measured by insertion of a YSI thermistor probe 4 cm into the rectum after each trial to ascertain if hypothermia had been induced. The maximum $\dot{V}O_2$ sustained for at least 5 min was used to estimate HPmax.

C. Heat Production during Arousal from Hibernation

The $\dot{V}O_2$ was measured during induced arousal from hibernation in 15 ground squirrels to determine the maximum rate of HP during arousal (HPmax:ar) and the time to HPmax:ar. Values for HPmax:ar were obtained using the peak $\dot{V}O_2$ sustained for at least 5 min during the rewarming process.

Animals were removed from their hibernaculae, a copper-constantan thermocouple inserted 4 cm into the rectum and taped securely to the tail. The animals were then placed in a paper-lined metabolism chamber in a temperature controlled cabinet (±1°C). Temperatures at which arousal was induced are as follows: -1°C (n=1), 1°C (n=7), 3°C (n=2), 4°C (n=3), 5°C (n=1), 10°C (n=1). By arousing animals at different temperatures the effect of Ta on HPmax:ar could be determined. Rectal temperature (Tr) and Ta were printed out automatically every 5 min along with $\dot{V}O_2$.

D. Data Analysis

The basal metabolic rate of animals of different body size does not vary with weight but with some fractional power of the weight (Brody 1945). Brody (1945) terms this the "metabolically effective body size" and suggests that this fractional power of weight is a more appropriate reference than weight alone. Data from many sources compiled by Hart (1971) shows the standard metabolism of a variety of rodents to vary with weight (in grams)$^{0.73}$. Since the weight of Richardson's ground squirrels varies greatly at different times of the year, from 200 to 550 gm (personal observation; Wang, unpublished), metabolic rates for this study are expressed in terms of weight$^{0.73}$ to correct for this variance in body weight.

All data were analyzed using programs in the "Statistical Package for the Social Sciences" (SPSS), which is available to computer users at the University of Alberta. The particular tests used, analysis of variance with Duncan's multiple range test, t-test and linear regression, are mentioned where applicable in the results.

III. Results

A. Estimation of Non-Shivering Thermogenesis

Time Course of Drug Action

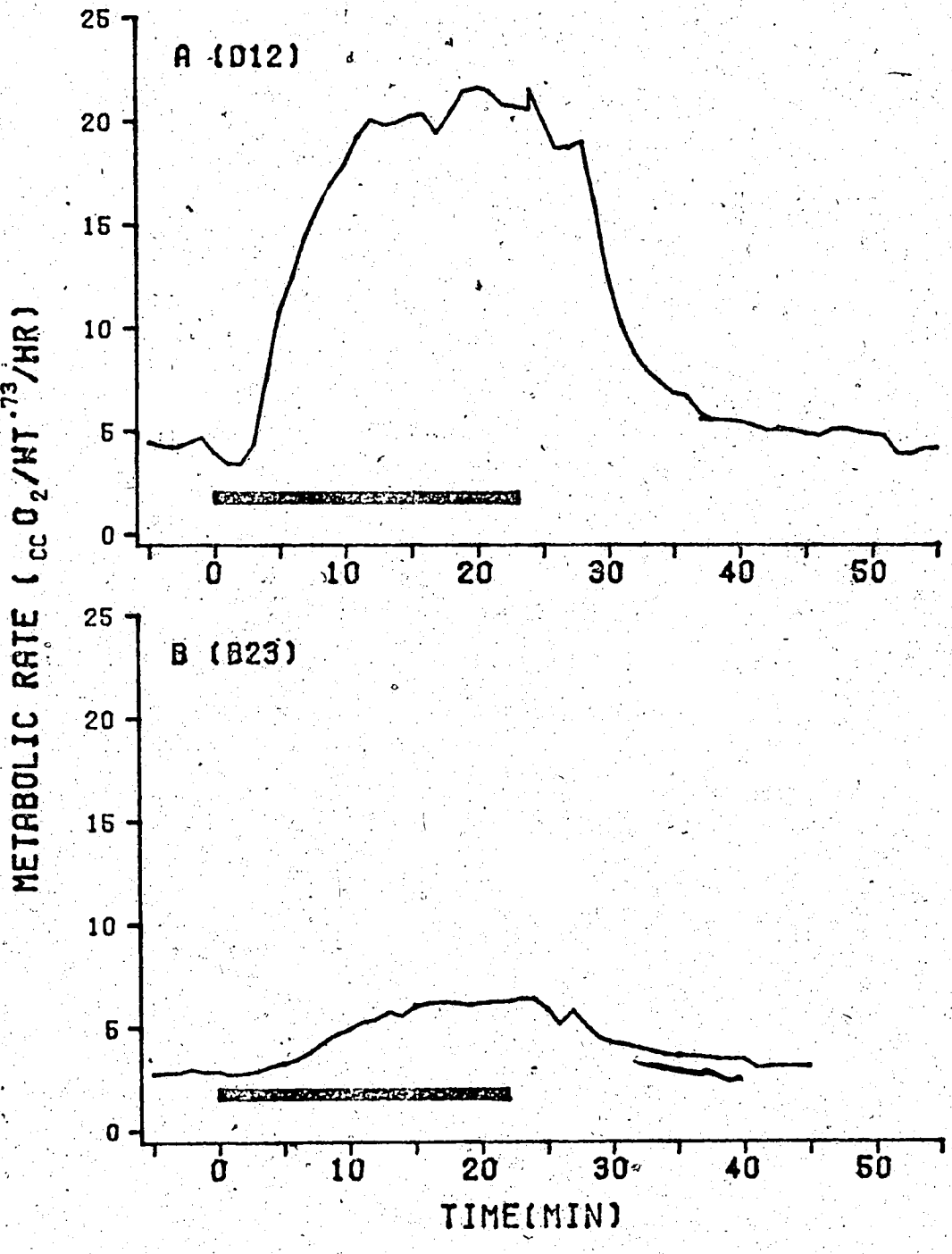
Fig. 3 shows the typical time course of response to isoproterenol. The upper graph (A) indicates the response of a male in hibernating phase to a dose of 10 ng/wt$^{0.74}$/min/min. The lower graph (B) shows the response of a female in hibernating phase to a much lower dose, 1.0 ng/wt$^{0.74}$. Shortly (2 to 5 min) after the onset of isoproterenol infusion, the metabolic rate of the animals began to increase. After 20 to 30 min of infusion the metabolic rate had reached a plateau. It remained at this level until drug administration ceased. When infusion was returned to the vehicle, metabolic rate began to drop within several minutes. When sufficient time was allowed between doses, metabolic rate returned to near baseline levels, as shown in Fig. 3.

Fig. 3. Typical time course of response to isoproterenol.

A). Response of a male (D12, wt=488 g) in hibernating phase to a dose of 10 ng/$wt^{0.74}$/min.

B). Response of a female (B23, wt=292 g) in hibernating phase to a dose of 1.0 ng/$wt^{0.74}$/min.

Black bars indicate duration of drug infusion.


A (D12)
B (B23)
METABOLIC RATE (cc O2/WT.73/HR)
TIME(MIN)
0
5
10
15
20
25
30
40
50

Dose-Response Relationships

All groups exhibited sigmoid dose-response relationships between HP and the dose of isoproterenol administered. Increasing doses elicited greater responses until a maximal response was attained. This is shown for two groups, COLD-ACCL and HIB-77, in Fig. 4. The curves for all groups are roughly parallel, differing only in the maximum response.

The mean metabolic response of each group to all doses of isoproterenol is listed in Table 1. Metabolic response to a dose of 1 ng/$wt^{0.74}$/min was significantly greater than basal levels in groups COLD-ACCL, JUNE-ADULT, AUG-YOUNG and HIB-78 (one-tailed t-test, $p < 0.05$). Administration of all higher doses of isoproterenol elicited responses significantly greater than basal rates in all groups (one-tailed t-test, $p < 0.05$). Within each group the higher doses (5, 10 and 20 ng/$wt^{0.74}$/min) were not significantly different from each other (two tailed t-test, $p < 0.01$), indicating that a maximal response had been attained for doses of 5 ng/$wt^{0.74}$/min and greater.

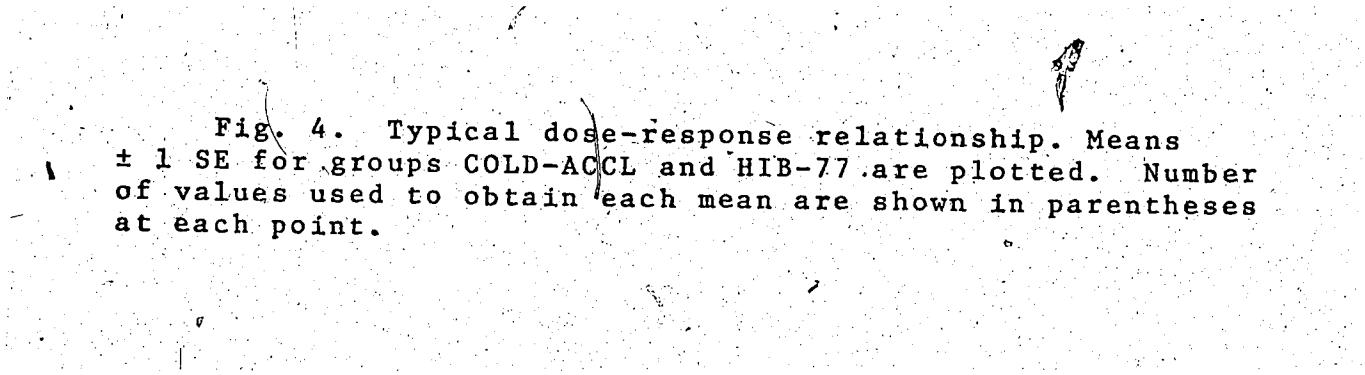


Fig. 4. Typical dose-response relationship. Means ± 1 SE for groups COLD-ACCL and HIB-77 are plotted. Number of values used to obtain each mean are shown in parentheses at each point.

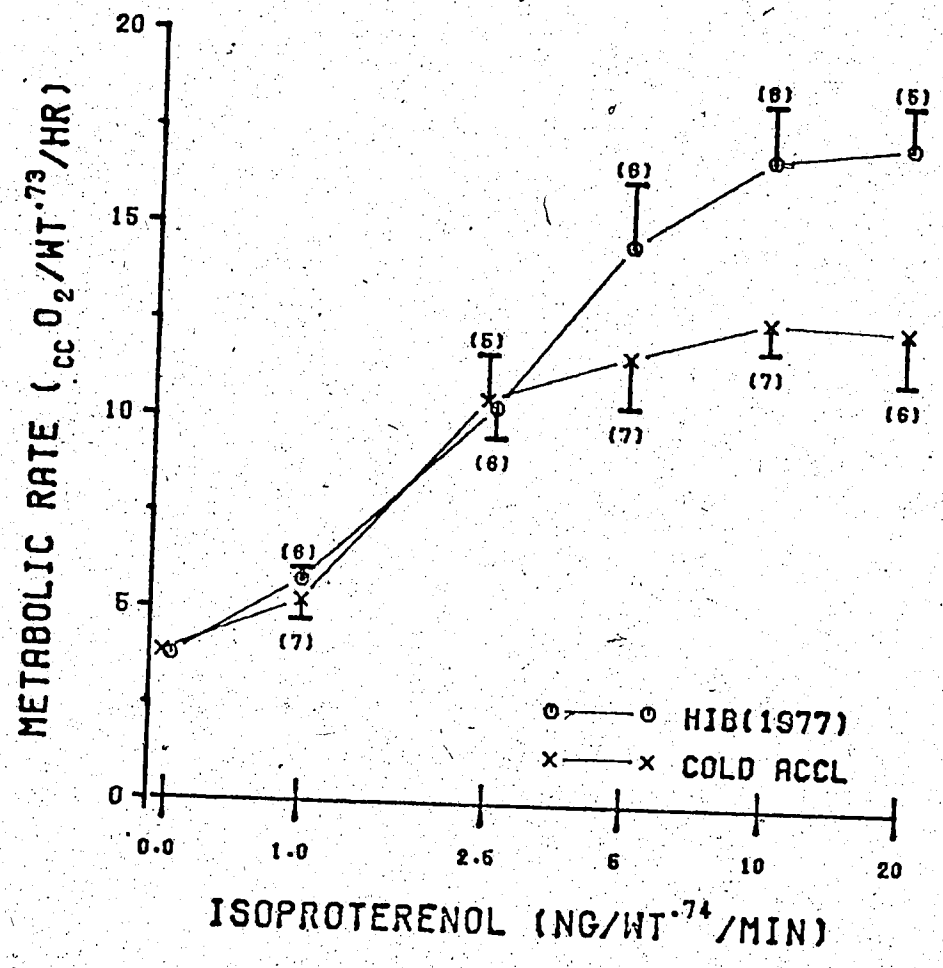

METABOLIC RATE ($_{cc}O_2/WT^{.73}/HR$)
20
15
10
5
0
(6)
(5)
(6)
(5)
(6)
(7)
(7)
(6)
(6)
(7)
(7)
(6)
HIB(1977)
COLD ACCL
0.0
1.0
2.5
5
10
20
ISOPROTERENOL (NG/WT$^{.74}$/MIN)

Table 1. Mean metabolic response of each group to all doses of isoproterenol. Means ± 1 SE are indicated in ml $O_2/wt^{0.73}/hr$. Number of experimental values used to determine each mean is shown in parentheses.

GROUP	BASAL HP	Dose of Isoproterenol (ng/$wt^{0.74}$/min) 1.0	2.5	5.0	10	20
APR-ADULT	3.83±.13 (6)	4.29±.27 (6)	7.41±1.02 (6)	10.64±.80 (6)	10.51±.50 (6)	10.29±.51 (6)
JUNE-ADULT	3.46±.05 (5)	5.32±.79 (5)	7.34±1.10 (5)	9.29±.69 (5)	9.69±.86 (5)	9.42±.73 (5)
JUNE-YOUNG	4.27±.18 (5)	4.47±.23 (5)	6.47±.15 (5)	7.86±.40 (5)	8.11±.31 (5)	8.23±.25 (5)
AUG-YOUNG	3.64±.21 (6)	4.16±.15 (6)	9.13±.72 (5)	10.03±.69 (6)	10.54±.60 (6)	11.14±.76 (6)
HIB-78	3.31±.24 (7)	5.30±.56 (7)	11.37±.65 (7)	14.18±.58 (5)	14.15±.58 (7)	14.42±.32 (5)
HIB-77	3.75±.26 (10)	5.75±.32 (6)	10.31±.78 (6)	14.55±1.68 (6)	16.85±1.46 (8)	17.26±1.09 (5)
COLD-ACCL	3.83±.18 (8)	5.21±.48 (7)	10.50±1.24 (5)	11.65±1.28 (5)	12.62±.74 (7)	12.47±1.34 (6)
WARM-ACCL	3.13±.26 (6)	5.37±1.24 (5)	9.64±1.07 (5)	9.82±.84 (5)	10.27±.82 (6)	10.79±.82 (5)

Maximum Non-Shivering Thermogenesis

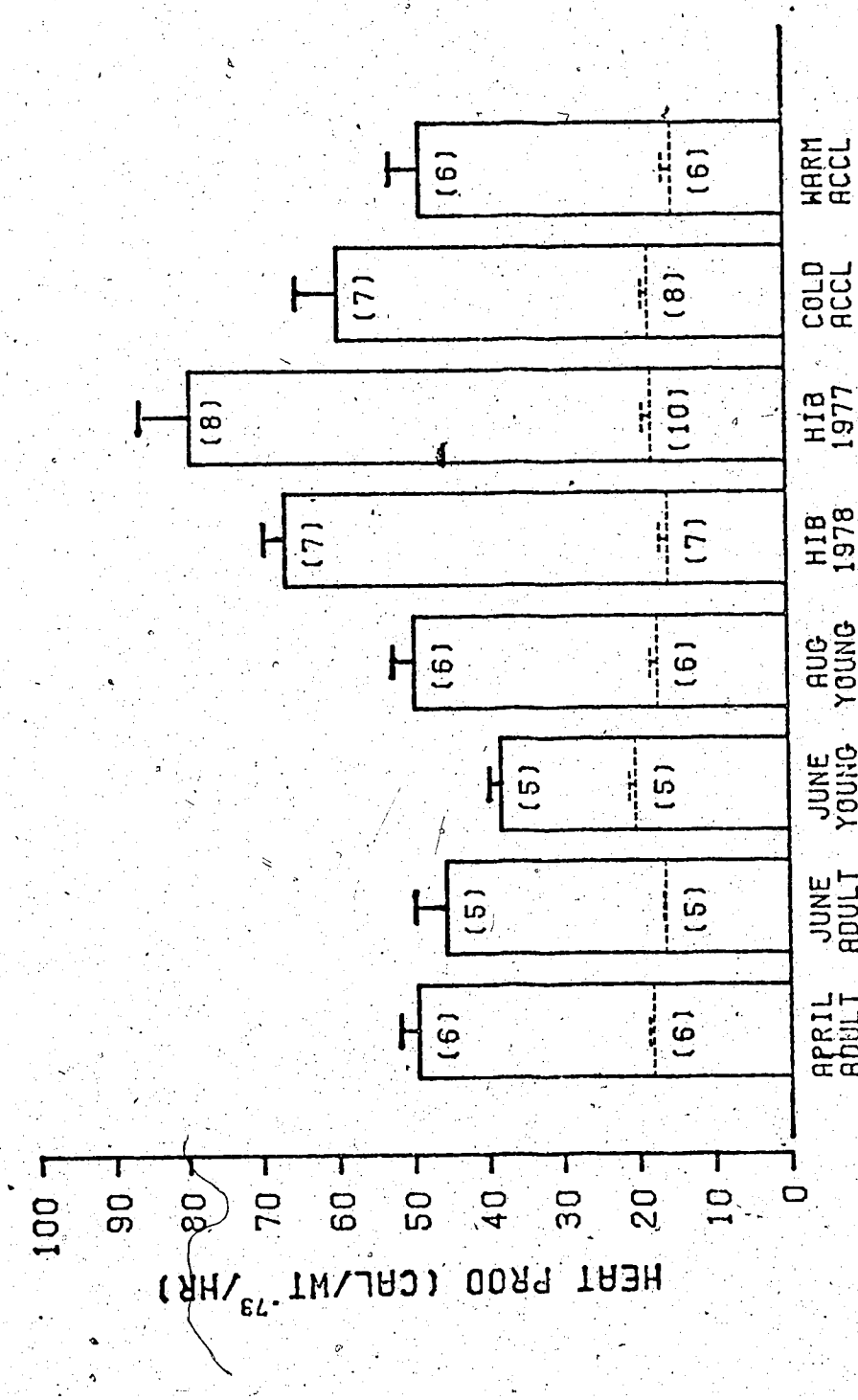
Since doses of 5, 10 and 20 ng/wt$^{0.74}$/min all elicited maximal responses, the response to a dose of 10 ng/wt$^{0.74}$/min was used to estimate NSTmax. Fig. 5 shows mean values for NSTmax for each group. Basal rate of HP was estimated using the stable rate of HP when no drug was infused and is also shown in Fig. 5. Analysis of variance and Duncan's multiple range test ($p < 0.01$) revealed that the animals in group HIB-77 had significantly greater NSTmax than all other groups except HIB-78. HIB-78 showed greater NSTmax than all other groups except COLD-ACCL. COLD-ACCL animals showed higher NSTmax than the animals in group JUNE-YOUNG. NSTmax was not significantly different in all other groups (see legend Fig. 5). The only significant differences in basal HP between groups is between the groups with the lowest (WARM-ACCL) and the highest (JUNE-YOUNG) basal HP (see legend Fig. 5).

Fig. 5. Comparison of mean maximum response to isoproterenol between groups. Histogram plots mean responses to a dose of 10 ng/wt$^{0.74}$/min (+ 1 SE) as an estimate of NSTmax. Dotted lines indicate mean basal HP. Number of values used to obtain each mean is indicated for each mean in parentheses.

Significance with Duncan's multiple range test ($p < 0.01$) is shown below. Groups which are not significantly different are underlined by a common line.

BASAL HP	WARM ACCL	HIB 77	JUNE ADULT	APRIL ADULT	HIB 78	COLD ACCL	AUG YOUNG	JUNE YOUNG

NSTmax	JUNE YOUNG	JUNE ADULT	WARM ACCL	APRIL ADULT	AUG YOUNG	COLD ACCL	HIB 78	HIB 77


HEAT PROD (CAL/WT.73/HR)
100
90
80
70
60
50
40
30
20
10
0
APRIL ADULT
JUNE ADULT
JUNE YOUNG
AUG YOUNG
HIB 1978
HIB 1977
COLD ACCL
WARM ACCL
(6)
(6)
(5)
(5)
(5)
(5)
(6)
(6)
(7)
(7)
(8)
(10)
(7)
(8)
(6)
(6)

B. Estimation of Maximum Heat Production

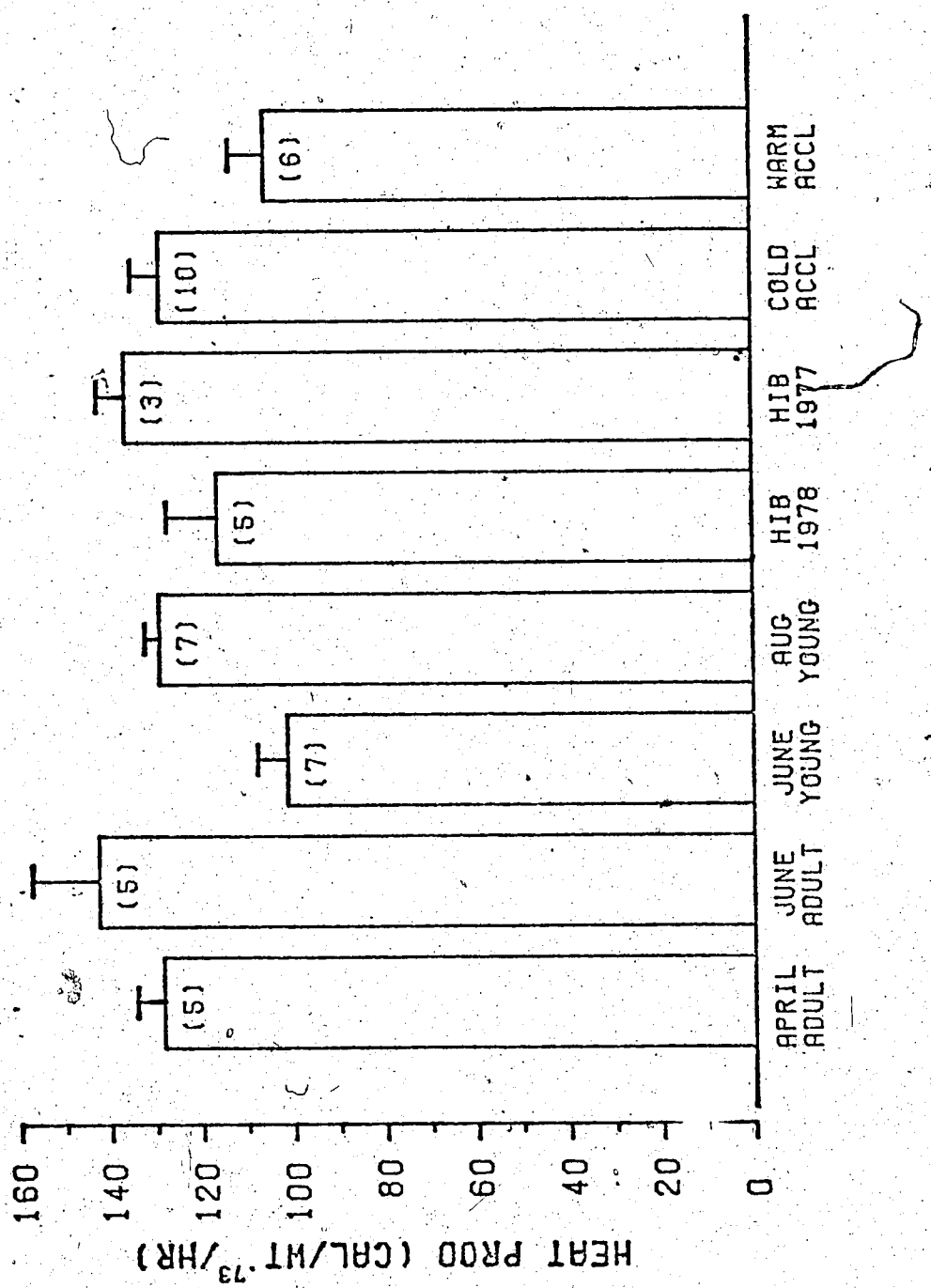
The mean HPmax for each group is shown in Fig. 6. Analysis of variance and Duncan's multiple range test ($p < 0.01$) indicated that the highest group (JUNE-ADULT) exhibited significantly greater HPmax than the lowest group (JUNE-YOUNG), however neither of these groups were significantly different from any of the other groups (see legend Fig. 6).

JUNE-YOUNG animals, in addition to showing the lowest HPmax, became hypothermic very rapidly after the onset of Helox exposure at -10°C. Adult animals in all groups, however could maintain homeothermy for the two hours tested in temperatures down to -25 °C unless the fur was soaked prior to exposure. The Tb of animals which were thoroughly soaked with water prior to exposure to test conditions dropped to between 28 to 34°C in 6 of 11 cases. Whether animals were tested wet or dry had no effect on the observed values of HPmax, suggesting the attainment of HPmax under these experimental conditions.

Fig. 6. Comparison of mean maximum heat production between groups. Histogram plots mean HPmax (+ 1 SE) for each group of animals. Number of values used to obtain each mean is shown in parentheses.

Significance with Duncan's multiple range test ($p < 0.01$) is shown below. Groups which are not significantly different are underlined by a common line.

JUNE YOUNG	WARM ACCL	HIB 78	COLD ACCL	APRIL ADULT	AUG YOUNG	HIB 77	JUNE ADULT


HEAT PROD (CAL/WT.73/HR)
0
20
40
60
80
100
120
140
160
APRIL ADULT
JUNE ADULT
JUNE YOUNG
AUG YOUNG
HIB 1978
HIB 1977
COLD ACCL
WARM ACCL
(5)
(5)
(7)
(7)
(5)
(3)
(10)
(6)

C. Heat Production during Arousal from Hibernation

Maximum rate of HP during arousal from hibernation was taken from $\dot{V}O_2$ records of 15 animals during induced arousal. The mean HPmax:ar was 93.3 (±3.2) cal/wt$^{0.73}$/hr. No significant relationship was observed between HPmax:ar and the temperature at which the animal was aroused ($r=0.124$). Average Tr at which HPmax:ar was observed was 8.2 (±0.7) °C ($n=13$), with a range of 6 to 13°C. Mean time to HPmax:ar was 165 (±9.7) min with a range of 110 to 249 min.

IV. Discussion

A. Non-Shivering Thermogenesis

Increase in NST capacities with cold-acclimation has been well documented in many species (Cottle and Carlson 1956, rat; Cottle 1963, rabbit; Hemingway et al. 1964, cat; Pohl and Hart 1965, 13-lined ground squirrel; Nagaska and Carlson 1965, dog; Williams 1968, golden hamster; Jansky et al. 1969, mouse; Bartunkova et al. 1971, rat; Portet et al. 1971, rat; Feist and Rosenmann 1976, red-backed vole). For example, Bartunkova et al. (1971) report that i.m. or i.v. injection of NE at room temperature more than doubles the HP in CA (5°C) rats while WA (28°C) rats show a much smaller increase (20 to 30%). Similar increases of different magnitudes were observed in all the species cited above, ranging from 2 to 3-fold increases above basal HP in ground squirrels (Pohl and Hart 1965) to a 7-fold increase in red-backed voles (Feist and Rosenmann 1976). In this study, a 3.3 fold increase above basal HP was observed in CA (5°C) animals. Increased NST with cold acclimation is thought to be important because the use of NST for HP allows the animal to produce sufficient heat to maintain Tb without hampering voluntary movements with shivering (Cottle and Carlson 1956). Similarly, investigators have reported increases in NST with seasonal acclimatization from summer to winter (Lynch 1973; Feist and Rosenmann 1976). This response too

can be viewed as an adaptive response of the animal to increased demand for HP.

Petrovic et al. (1972) tested ground squirrels (species unreported) for NST by monitoring VO2 at 30°C before and after i.p. injection of NE. As was observed in this study, they reported increased responsiveness to NE (indicative of increased NST) in animals hibernating at 5 to 10°C in February-March (166% above basal, 2,112 cal/m²/24hr) when compared to WA (25 to 26°C) animals (115% above basal, 1,559 cal/m²/24hr) or CA (5 to 10°C) animals (32% above basal, 1,391 cal/m²/24hr). A much greater basal rate of HP was obtained in the CA group, accounting for the low percent increase which they observed. No such change in basal rate was observed in the COLD-ACCL group in this study. Petrovic et al. (1972) reported no significant differences in total HP elicited by NE between the CA group and the WA group, similar to the results observed in the Richardson's ground squirrel. These results suggest that the seasonal change in NST is primarily an adaptation to aid in the arousal process in ground squirrels, rather than an adaptation to cold, as in the rat. It should also be noted that Petrovic and Markovic-Giaja (1975) observed a greater response to NE in ground squirrels acclimated to 36°C than in rats acclimated to the same temperature. Although non-hibernators, such as rats, develop NST as a response to cold, ground squirrels seem to maintain high levels of NST even in the warm, and are consequently well prepared at all times for the cold.

During the hibernation season, there is a great demand for HP during the periodic arousals. The animal must be able to produce sufficient heat at depressed Tb to raise it from near Ta (which can be near freezing) to normothermy. In addition, the animal must also produce sufficient heat to maintain normal Tb once they have reached it. Non-shivering thermogenesis has been shown to be important as a heat source during arousal, especially in the early stages (Ball 1965; Hayward and Lyman 1967; Hayward 1971; Jansky 1973). Therefore, the increases in NST observed with hibernation can be viewed as an adaptation to meet the increased demand for HP during hibernation.

In addition to increased abilities to produce heat at normal Tb, the ability to produce heat at lowered Tb sets hibernating animals apart from non-hibernators. While ground squirrels can produce sufficient heat to rewarm from lowered Tb at Ta as low as 0°C during arousal (personal observation), rats cannot rewarm from Tb lower than 20°C at Ta of 19°C without the aid of exogenous heat sources (Wang and Peter 1975). Although further studies are needed to compare the effects of induced hypothermia between rats and ground squirrels in hibernating and non-hibernating phases, this increased power of HP at depressed Tb suggests qualitative differences in cellular function between hibernators and non-hibernators. This is also suggested by studies which compare isolated tissues and cells of hibernators and non-hibernators. Tissues from hibernating

species generally can remain viable at lower temperatures than tissues from non-hibernating species (see Willis 1978 for review).

Qualitative differences between the membranes of hibernators and non-hibernators have been suggested as an explanation of the increased ability of hibernators to function at low temperatures. Lyons and Raison (1970) and Raison and Lyons (1971) reported sharp breaks in Arrhenius plots of succinate oxidase activity, a membrane-bound enzyme, in the rat and non-hibernating phase golden-mantled ground squirrels, but not in hibernating phase ground squirrels. Also, Raison et al. (1971) found that these breaks correspond to the temperatures at which the membrane undergoes phase change from fluid to crystalline. These results suggest that membrane function is impaired at lower temperatures in non-hibernating animals due to loss of fluidity of the membrane (Raison and Lyons 1971).
Aloia et al. reports that golden-mantled ground squirrels in hibernating phase have a greater proportion of phospholipids in the membrane than non-hibernating phase animals. Since this would increase the fluidity of the membrane at lower temperatures, they suggest that this increase in phospholipid may allow the normal function of the membrane, and thus normal cellular function, at lower temperatures.

B. Non-Shivering Thermogenesis in Arousal

Although NST has been shown to be important during arousal, expecially during the early stages (Ball 1965; Hayward and Lyman 1967; Hayward 1971; Jansky 1973), the quantitative role of NST in arousal is difficult to determine and has only been indirectly calculated. Ball (1965) calculated the contribution of NST to HP during the first two hours of arousal in the following manner:

1. Using the data of Joel (1965) on in vitro metabolism of BAT, he estimated the maximum HP of BAT to be 0.34 kcal during the first two hours of arousal.
2. Assuming a specific heat of 1 kcal/kg/°C, he estimated that 0.555 kcal nwould be needed to warm the thoracic region (approximately 30% of the body weight) of a 185 gm 13-lined ground squirrel from 5 to 15°C.
3. Using 1) as an estimate of the HP due to NST and 2) as an estimate of the total heat required during the first two hours, he calculates the contribution of NST to be 0.34/0.55 of 61%.

This value of 61% is not accurate for several reasons. HL of the animal was not taken into account. No attempt was made to account for temperature effects on BAT metabolism that would occur as arousal progressed. In addition, the temperature of the thoracic area would probably increase much more than the 10°C change that Ball assumes. For example, in the Richardson's ground squirrel, a similar species, brain temperature, and therefore core body

temperature, increases from 5 to 30°C during the first two hours of arousal. In a similar manner, using cytochrome c data for the same species (Joel 1965), Smith and Horwitz (1969) estimate the contribution of NST to HP to be 46.7% during the first two hours of arousal. Without further verification the application of *in vitro* data to processes occurring in an intact animal, as was done in the above studies, is of questionable accuracy and should be viewed with that in mind.

For the little brown bat, *Myotis lucifugus*, Hayward (1968) calculates the percent of total HP during arousal by dividing the mean maximum NE-induced HP by the mean HPmax:ar. This yields a value of 81% for the contribution of NST to HP during arousal in this species. When shivering was blocked by curare during arousal, the rate of arousal in bats was unaffected, while in dormice and hamsters the rate was decreased 20% and 40%, respectively (Hayward and Lyman 1967). One might expect, therefore, a larger contribution of NST to HP in arousal in bats than in rodent hibernators, such as dormice and hamsters, and perhaps ground squirrels.

The method of Hayward (1968) outlined above may be a reasonable method to estimate the contribution of NST in arousal in bats, assuming that the relative contributions of NST and ST do not change throughout the process of arousal and that the maximum rate of NST during arousal is equal to the maximum rate of NST when the animal is normothermic. These assumptions may indeed be true for bats, as the

contribution of the peripheral tissues in the form of shivering is low (Hayward and Lyman 1967) and the temperature of the interscapular region (and thus the primary effector organ for NST, BAT) is near normothermic temperatures when HPmax:ar is achieved (Hayward 1968). In the Richardson's ground squirrel, however, the brain temperature, and therefore core body temperatures is approximately 30°C when HPmax:ar is attained (Glass 1979). Non-shivering thermogenesis at this time will be reduced due to temperature effects on the metabolism of BAT. Joel (1965) reports the Q10 of BAT in the 13-lined ground squirrel to be 1.4. Applying this to the average NSTmax at normal Tb (37°C) obtained in this study for both hibernating groups (81.1 in HIB-77, 67.8 in HIB-78, average = 74.5 cal/wt$^{0.73}$/hr), a value of 58.7 cal/wt$^{0.73}$/hr can be calculated for the maximum NST at Tb = 30°C. Dividing this value for the maximum NST during arousal (58.7 cal/wt$^{0.73}$/hr) by the mean HPmax:ar (93.3 cal/wt$^{0.73}$/hr), a value of 63% is obtained for the contribution of NST to HP during arousal.

Unlike bats, where the contribution of ST to arousal HP is small, ST does play a significant role in arousal of rodents (Hayward and Lyman 1967), including the Richardson's ground squirrel (Wang, unpublished). Clearly, as the peripheral circulation opens up and peripheral tissue is warmed, the contribution of shivering could change drastically. However, during the first two hours of

arousal, circulation is primarily restricted to the body core, and little increase in temperature is observed in the peripheral tissue. Since HPmax:ar occurs shortly after or concurrent with this warming of the peripheral tissues (mean time to HPmax:ar observed in this study was 165 (±9.7) min), this estimate of 62.9% for the contribution of NST to arousal probably reflects only the contribution of NST arousal at this time. It seems likely that the contribution of NST is somewhat higher during the early stages of arousal prior to the opening of the peripheral circulation. As has been pointed out by Smith and Horwitz (1969) and Hayward (1971), these indirect measures are far from satisfactory. In addition, at least for rodents, the relative contribution of ST and NST may change during the course of arousal and this factor must be considered in any study of the relative contributions of NST and ST to HP during arousal.

C. Analysis of Dose-Response Relationships

The concept of drugs interacting with specific receptors to exert their actions is well established in pharmacology. The first attempts to quantify the relationships between dose and response using this concept is credited to Clark in 1937 (Goldstein et al. 1974). Clark's formulation, however, cannot explain adequately some aspects of dose-response relationships. Presently, the magnitude of response is thought to be a function of 3 properties:

- the affinity of the drug for the receptor
- the efficacy of the drug, a term which describes the effect or biological activity of the drug
- the total number of receptors available for binding (McKay 1966; Goldstein et al. 1974).

It is well known that changes in drug-receptor affinity manifest themselves by shifts of the dose-response curves to the right or left (Goldstein et al. 1974). Results of this study show no such shifts, thereby eliminating changes in affinity as an explanation of the observed changes in the dose-response relationships. Changes in catecholamine breakdown (which in the intact animal would appear to have the same effect as changes in affinity) also cannot explain the results of this study. Therefore, the observed increases in the maximal response to isoproterenol are probably due to either an increase in the efficacy of the drug and/or an increase in the number of receptors.

Clearly, changes in the efficacy of the drug must be due to biochemical changes in the animal as the drug itself would not change. Chaffee et al. (1966) reports an increase in the metabolic activity of BAT with the winter season in the golden-mantled ground squirrel. Also, Joel (1965) reports increases in the cytochrome c content of BAT during the hibernation season in the 13-lined ground squirrel. These observations seem to suggest that some biochemical changes are occurring concurrently with the onset of the hibernation season and that these changes may account for the increased efficacy of the isoproterenol. Changes in the total number of receptors can be accomplished by increasing the number of receptors per cell or simply by increasing the mass of the active tissue, in this case BAT. Increases in the mass of BAT accompanying increases in NST have been reported for several species (Didow and Hayward 1969, meadow vole; Feist and Quay 1969, golden hamster; Lynch 1973, white-footed mouse; Feist and Rosenmann 1976, red-backed vole). Consequently, it appears that changes in NST accompanying hibernation are not a result of increases in drug-receptor affinity, but due to biochemical changes causing an increased efficacy in isoproterenol and/or increases in BAT mass. However, speculations such as these, based solely on indirect evidence and dose-response curves are dangerous, and are not conclusive. Further investigations are necessary to determine the actual changes which are responsible for the observed increase in NST during the

hibernation season.

D. Maximum Thermogenesis

No clear relationship can be seen in this study between the stage in the seasonal cycle or acclimation temperature and HPmax (Fig. 6). This is contrary to what has been reported for other species of small rodents. Rosenmann et al. (1975) report, for the red-backed vole, HPmax in the summer to be 9 times the standard metabolic rate, while in winter the animal is capable of producing heat at 14.5 times its standard metabolic rate. Voles are known to venture above ground and above snow cover even in the coldest months (Rosenmann et al. 1975) and would therefore be exposed to a wider range of temperatures than a hibernating ground squirrel in its burrow. Consequently, the voles may need increased abilities for HP to survive over winter.

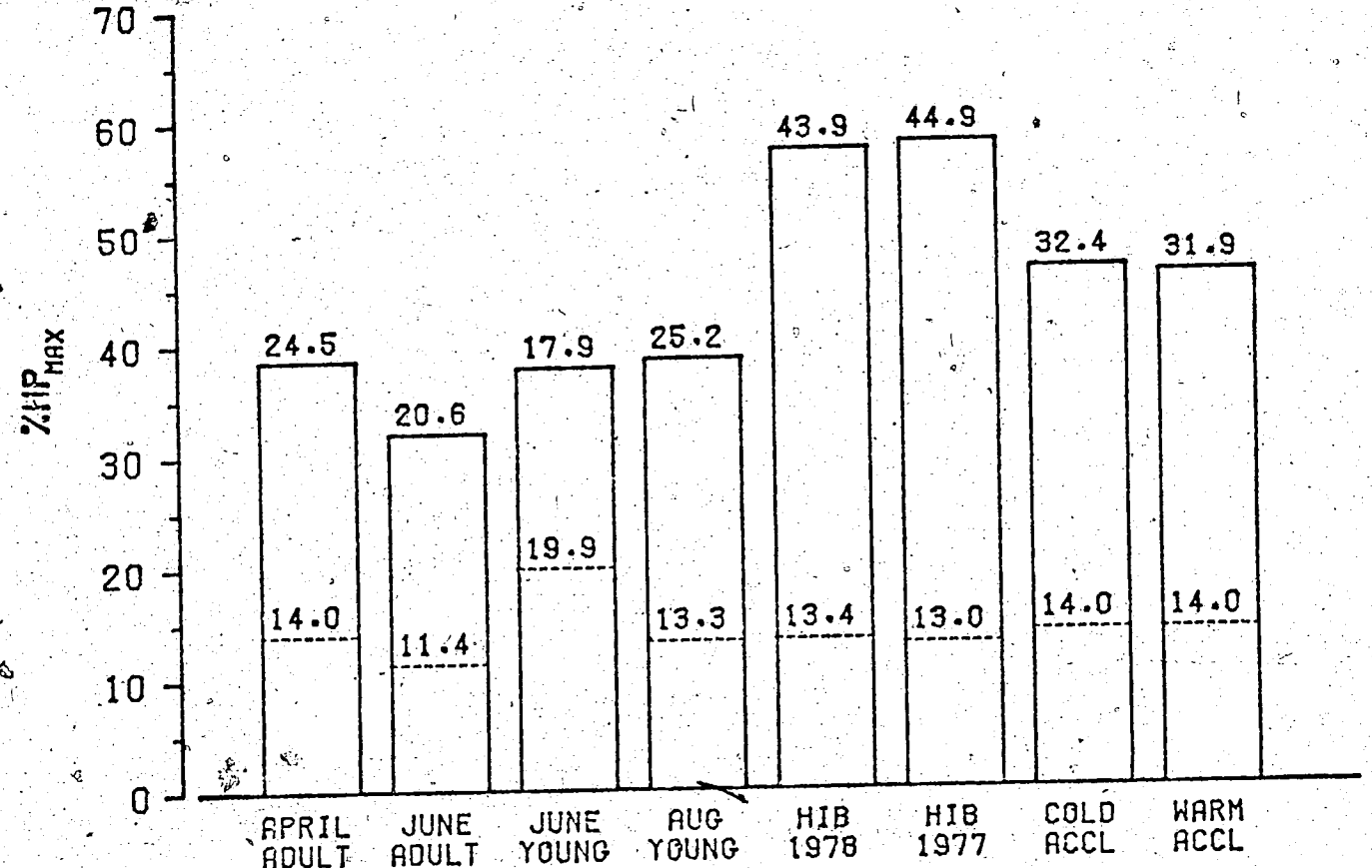
In the 13-lined ground squirrel, Pohl and Hart (1965) report that the HP at -30°C is greater for CA (6°C) than for WA (28°C) animals, and that HPmax is reached under less severe conditions in WA animals. Similar results have been shown by Pohl (1965) for hamsters acclimated to 6 and 28°C. The 13-lined ground squirrel, like the Richardson's ground squirrel, hibernates underground throughout the winter. Their distributions are similar, although the range of the 13-lined ground squirrel extends considerably further south (MacClintock 1970). For this reason, one would expect the two species to be exposed to the same range of temperatures.

Consequently, one might expect a similar repertoire of adaptations in these two closely related species. The disparity between the results of Pohl and Hart (1965) and the results in this study can perhaps be explained by differences in acclimation temperature. The acclimation temperature used in this study for WA animals (20°c) is only slightly above the lower critical temperature of the thermoneutral zone (Wang, unpublished), while WA animals in the study of Pohl and Hart (1965) were acclimated to a considerably higher temperature (28°C). Consequently, WA animals in this study (WARM-ACCL) may not have been sufficiently "driven" to show decreased HPmax, as they might have been if acclimated to higher temperatures such as 28°C.

E. Relationship Between Non-Shivering Thermogenesis and Maximum Heat Production

Since at HPmax, NST would be employed at its maximal levels, the relative contribution of NST to HPmax can be calculated Fig. 7. The relative contribution of NST was greater in the hibernating phase, while that of basal HP remained the same. Since NST and ST are additive (Jansky 1973), the fraction of HPmax not accounted for by either NST or basal HP is due to the process of shivering. Data presented earlier in this study (Fig. 6) indicates that HPmax does not change significantly with the onset of the hibernating phase. For the Richardson's ground squirrel, therefore, not only does the relative contribution of ST

Fig. 7. Relative contribution of basal heat production and non-shivering thermogenesis to maximum heat production. Dotted lines indicate the percent contribution of basal HP to HPmax. Solid lines indicate the percent contribution of NST to HPmax corrected for the contribution of basal HP: ((NSTmax - basal HP) / HPmax). Numbers above lines indicate exact percentages.


%HP MAX
70
60
50
40
30
20
10
0
24.5
20.6
17.9
25.2
43.9
44.9
32.4
31.9
14.0
11.4
19.9
13.3
13.4
13.0
14.0
14.0
APRIL ADULT
JUNE ADULT
JUNE YOUNG
AUG YOUNG
HIB 1978
HIB 1977
COLD ACCL
WARM ACCL

decrease with hibernation but so does its absolute magnitude. This is a surprising result in that, were the capacity of the animal to produce heat via ST to remain the same, we would expect to see an increase in HPmax with increases in NSTmax, since NST and ST are additive (Jansky 1973). This appears not to be the case. This finding indicates that 1) the capacity of the muscle tissue to produce heat via shivering is decreased during the hibernating phase, or 2) the HP due to shivering is somehow limited by factors which set the upper ceiling for HPmax in the cold. The first possibility seems unlikely as shivering depends on muscular movement and therefore the maximum magnitude of ST should depend only on the muscle mass. Galster and Morrison (1975) report in the arctic ground squirrel (Spermophilus undulatus) a decrease of 31 g in body protein over the whole hibernation season, possibly indicating a loss of muscle tissue. However, this decrease should not manifest itself early in the hibernation season and cause a decrease in shivering. It also seems unlikely that a small decrease such as that observed by Galster and Morrison would cause the large decrease in the ability of the muscles to shiver which was observed in this study. The second possibility requires further experimentation before it can be established as the underlying mechanism. However, the results of Wang (19 , suggest that, at least in the rat, the availability of substrate to the cells may limit HPmax.

F. Conclusions

In this study, I observed increases in isoproterenol-stimulated NST with the onset of the hibernating phase in a seasonal hibernator, Spermophilus richardsonii. In view of the role of NST in periodic arousal from hibernation, this increase in NST is interpreted as an adaptation to aid the animals in producing heat at depressed Tb. Analysis of dose-response curves of isoproterenol-stimulated HP suggests that this increase in NST is not due to increased drug-receptor affinity but due possibly to biochemical changes which increase the efficacy of the drug and/or increases in the total number of receptors. Furthermore, the fact that hibernators can produce enough heat at depressed Tb to rewarm to normothermy, while non-hibernating species cannot, suggests that there may be qualitative differences in mechanisms of HP in these two types of animals.

Maximum HP, on the other hand, did not change with the increases in NST observed during hibernation. These results contradict those reported for other species of rodents. This disparity can perhaps be explained by the differences in acclimation temperatures between this study and those which reported changes in HPmax. These results do suggest that HPmax, and therefore ST, are possibly limited by energy or substrate availability rather than the capability of the cellular oxidative mechanisms.

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