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Antimetabolic Extract from the Brain of the Hibernating Ground Squirrel *Citellus tridecemlineatus*

Abstract. *Extracts of subcortical brains from hibernating ground squirrels, when injected intravenously into rats, caused a mean decrease in oxygen consumption of 35 percent and a decline in body temperature of 5°C. The effects lasted from 75 minutes to 30 hours. Brain extracts of nonhibernating squirrels caused no significant changes in these parameters.*

Lyman and Chatfield (*1*) have presented the evidence that upon entry into hibernation a reduction in metabolic rate precedes any drop in the body temperature. Keller (*2*) showed that a 40 percent reduction in basal metabolism followed ablation of certain areas of the tuberal and anterior hypothalamus in dogs. He stated "the normally elevated basal energy metabolism is a function of an endocrine hypothalamus." Subsequently, Swan and Hall (*3*) presented data that the metabolic rate of estivating lungfish in nature drops independent of a change in body temperature. This has been directly confirmed by Rienhard (*4*). Thus, evidence is accumulating that metabolic rate can be reversibly lowered in mammals and poikilotherms by mechanisms independent of the depression of biochemical reaction rates associated with decreasing temperature.

The existence of a specific endocrine factor in the torpor of hibernation was first suggested by Kroll (*5*) and Dawe and Spurrier (*6*) later showed that injections of a filtrate of blood from hibernating ground squirrels could induce onset of torpor in alert ground squirrels.

The first evidence that metabolic rate might be lowered in a homeotherm (rat) by an agent extractable from the brain of a torpid animal was that published by Swan, Jenkins, and Knox (*7*). When injected intravenously into white rats, extracts of estivating lungfish brains caused a 35 percent decline in CO_2 production followed by a decrease in body temperature of 5°C. The effect lasted only a few hours. They theorized that the extract might contain a specific antimetabolic hormone and suggested the name "antabolone" for it.

In 1972 Swan reported data derived from studies of comparative metabolism, which supported Keller's classic concept of "a *normally elevated* basal metabolism" (*8*). And that portion of the resting metabolic rate of homeotherms, which normally exists primarily to produce heat rather than energy biologically convertible to work, was suggested by Swan to be the expendable or viably depressable fraction of metabolism when accompanied by alterations in thermoregulatory mechanisms (*9*).

We now confirm that metabolic rate may be reversibly decreased in rats without a preceding drop in the level of body temperature and describe experiments in which the response of white rats to intravenous injection of an extract of the brain of hibernating ground squirrels is compared to the response following injection of a similar extract from the nonhibernating ground squirrels (*Citellus tridecemlineatus*). Oxygen consumption ($\dot{V}_{O_2}$), electrocardiograms (EKG), and body temperature (T_B) were monitored.

Ground squirrels were obtained (commercially) in October. To encourage hibernation they were housed in a quiet dark room at 6°C with adequate food and water. Most of the animals were hibernating by late November. During the next 3 months, torpid squirrels (T_B = 6° to 8°C) were killed without arousal by

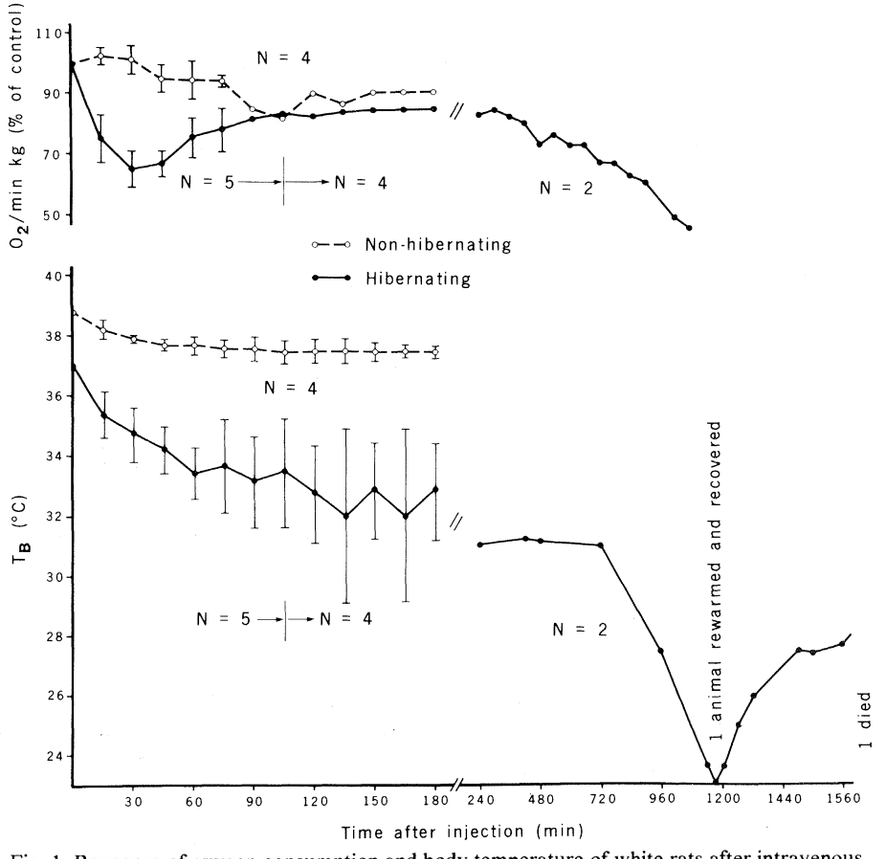

Fig. 1. Response of oxygen consumption and body temperature of white rats after intravenous injection of brain extract (300 mg/kg) from nonhibernating and hibernating ground squirrels. The ambient temperature was 24° ± 2°C.

decapitation, the brain was rapidly dissected, the superficial cortex was excised, and the remainder of the brain was plunged into liquid nitrogen. The entire procedure took approximately 90 seconds. The tissue, consisting of the entire brain (except a thin layer of the cortical hemisphere), especially the hypothalamus and the pituitary gland, was stored for varying periods in liquid nitrogen prior to extraction. Nonhibernating squirrels were killed in May. Extracts were prepared by the method of Guillemin *et al.* (*10*) as modified by Swan, Jenkins, and Knox (*7*). The method is designed to extract and partially purify polypeptides by means of acetone extraction, homogenization, multiple extraction in acetic acid and acetone, and removal of lipids with ether. The resulting dry powder, about 8 to 10 mg per brain, was stored at − 20°C. Immediately prior to use, the extract was reconstituted in 1.75 to 2.5 ml of acetate buffer at a final *p*H of 5.2.

Holtzman white rats (250 to 350 g), with an inlying cephalic vena caval catheter of P-50 tubing and with stainless steel wires implanted in the extremities, were used as subjects for biological assay. From 2 to 4 days elapsed between surgery and testing.

At about 0900 hours on the day of experiment, the rat was placed in a restrainer, a recording (Yellow Springs) thermistor was positioned in the colon, EKG leads were attached to the wire sutures, and the jugular catheter was attached to an extension that led through a sealed aperture in the front door of the metabolic chamber in which the rat was placed.

The temperature in the chamber was regulated between 22° and 26°C by running water through a surrounding jacket. Oxygen consumption was monitored with a Beckman F3 analyzer and strip chart recorder. Body temperature and heart rate were recorded every 15 minutes throughout the experiment. After a 3-hour preinjection period, the test material was injected slowly over a 15-minute period. Observations were routinely continued for the next 3 hours. In two instances, the response was monitored for 26 and 30 hours.

The results of injections of extract from hibernating and nonhibernating squirrel brains at a dose of 300 mg of extract per kilogram of body weight are shown in Fig. 1. Oxygen consumption was calculated as milliliters of O_2 per kilogram per minute at standard temperature and pressure and expressed as a percentage of the preinjection value, a mean of the last three observations before injection.

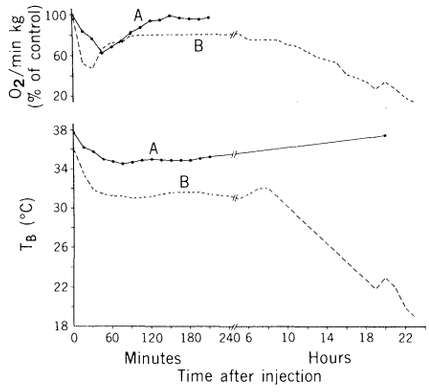

Fig. 2. Oxygen consumption and body temperature of two rats injected with brain extract of hibernating ground squirrels. Differences in the response patterns after 2 hours are shown.

Oxygen consumption after injecting extract of brain from hibernating animals decreased to 65 percent of control values at 30 minutes. After 45 minutes, the effect diminished; and by 90 minutes it had returned to the same level as that of rats injected with brain extract from nonhibernating animals. The observed difference between the two groups was significant up to 75 minutes ($P \leq .05$; Student's *t*-test).

The body temperature response of the two groups was equally distinctive. After injection of brain extract from nonhibernating animals, the rats showed a mean decline of 1.5°C in T_B in 3 hours. In contrast, hibernating extract induced an average decrease in T_B of 5.25°C so that, after 1 hour, the mean temperature was 31.7°C. We cannot account for the fact that the mean body temperature of rats injected in winter with brain extract from hibernating squirrels was lower than that of rats injected in May and June with brain extract from nonhibernating squirrels except for the difference in season. There was no evidence of shivering.

From 60 minutes on, there was increasing variability in the responses (Fig. 2). Rat A was typical of three of the animals in this group; rat B was typical of the other two. These subtleties are lost in the statistical presentation of the data.

All the rats had initial rapid suppression of metabolic rate, and all showed a tendency for both $\dot{V}_{O_2}$ and T_B to return toward control levels in the period between 60 and 120 minutes. It is presumed the depressive effect of the antimetabolic agent wore off at about 1 hour, and that more normal metabolic and thermoregulatory cybernetics returned. We believe rat A was able to generate sufficient heat to return its body temperature to normal. The animal then survived with no apparent ill effects.

However, rat B was apparently unable to initiate sufficient thermogenesis to rewarm its depressed T_B. The animal, with its metabolism thermally suppressed, became progressively hypothermic until body temperature approximated ambient temperature. This rat followed the classical course of irreversible, induced hypothermia. Death occurred at 26 hours. The other rat, experiencing progressive hypothermia similar to rat B, was able to rewarm at 20 hours when T_B was 22°C and eventually recover. Since the two rats with the dramatic responses were injected by extracts of brains obtained late in the hibernating season, we believe that the antimetabolic agent or agents may increase in either quantity or potency with increasing duration of hibernation.

We conclude that hibernation of *Citellus tridecemlineatus* may be associated with the elaboration in subcortical brain tissue of an extractable metabolically depressant agent or agents. The active extract, designated antabolone, can be cryogenically stored. When the agent is injected intravenously into rats, there is a decrease in oxygen consumption followed by a decrease in body temperature lasting from 75 minutes to 30 hours. The data suggest that antabolone directly suppresses both metabolic rate and the subsequent thermoregulatory response.

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