Protein metabolism in hepatic tissue of hibernating and arousing ground squirrels

Whitten, Bertwell K., and George J. Klain. Protein metabolism in hepatic tissue of hibernating and arousing ground squirrels. Am. J. Physiol. 214(6): 1360-1362. 1968.—Protein metabolism in hepatic tissue of hibernating, arousing, and normothermic ground squirrels, Citellus tridecemlineatus, was assessed by three methods: 1) measurement of alanine-U-14C utilization by liver slices; 2) incorporation of methionine (methyl-14C) into protein by microsomal preparations from liver; 3) measurement of arginase activity in liver homogenates. Measurements were made at 6 and 37 °C. In comparison to hepatic tissue of normothermic animals: 1) protein synthesis from methionine and lipogenesis from alanine were markedly lowered in hepatic tissue of hibernating and arousing animals when measured at 37 °C, whereas no change was observed at 6 °C; 2) incorporation of alanine into hepatic glycogen was increased in hibernating animals at 6 and 37 °C. In comparison to hepatic tissue of hibernating animals: 1) oxidation of alanine to CO2 was increased in hepatic tissue of arousing and normothermic animals at 6 and 37 °C; 2) arginase activity was increased in hepatic tissue of arousing animals at 6 and 37 °C and in normothermic animals at 37 °C. These data suggested an increased protein catabolism during arousal.

Methods

Ground squirrels (Citellus tridecemlineatus) were kept at 26 ± 1 °C in individual wire cages and fed a diet of Purina rat chow, carrots, and water ad lib. In November, hibernation was induced by putting animals in a dark room at 6 ± 1 °C with water available ad lib. Three experimental groups were studied: 1) Six hibernating squirrels (115 ± 12 g) which had been in hibernation for 60-70 days were removed from the cold room in January and sacrificed immediately. 2) Six hibernating squirrels (110 ± 20 g) which had been in hibernation for 60-70 days were removed from the cold room in January and allowed to arouse for 2 hr at 26 ± 1 °C before sacrifice with water but no food available. 3) Six normothermic ground squirrels (170 ± 16 g) were removed from the cold room in April after natural hibernation and maintained for at least 60 days at 26 ± 1 °C with food and water available ad lib. until sacrifice in June. Animals were weighed just prior to sacrifice.

Received for publication 13 November 1967.

Several investigators have reported seasonal changes in nitrogenous metabolites and free amino acids in tissues from hibernating, arousing, and normothermic mammals (2, 4, 9, 10, 12, 16, 21). Decreased DNA synthesis in lymphoid tissue of hibernating hamsters (14), increased blood creatine levels associated with periodic arousals in hedgehogs (11), and changes in serum proteins in hamsters (20) were indicative of seasonal alterations in protein metabolism.

During arousal, the respiratory exchange ratio increased from 0.7 to near 1.0 (15). This could indicate a shift from lipid catabolism to carbohydrate catabolism and reflect an increased utilization of protein amino acids as substrates for gluconeogenesis. To support this hypothesis, Burlington and Klain (1) recently demonstrated an increased capacity for gluconeogenesis from amino acids in hibernating and arousing ground squirrels.

At present, protein metabolism in hibernators is incompletely understood. Therefore, in order to assess changes in protein metabolism during hibernation and arousal, protein synthesis, amino acid utilization, and arginase activity were measured in hepatic tissue of hibernating, arousing, and normothermic ground squirrels.
In order to assess the effect of temperature on protein metabolism, all hepatic tissue preparations were incubated at 6 and 37 C. The 6 C temperature approximated body temperature during hibernation and the 37 C temperature approximated body temperature at the time tissues were taken for measurement in arousing and normothermic animals. Approximate temperature characteristics were calculated for arginase activity and protein synthesis by the Arrhenius' equation (13) from data obtained at 6 and 37 C. Analyses of variance were carried out on all data.

RESULTS

Methionine incorporation into protein (Table 1) was three times that found in hibernating and arousing animals when measured at 37 C while no difference was found at 6 C. Arginase activity was elevated in arousing animals at 6 and 37 C when compared to hibernating and normothermic animals and was higher in normothermic animals when compared to hibernating animals at 37 C (Table 1).

Oxidation of alanine to CO₂ at 6 and 37 C was lowest in hibernating animals, higher in arousing animals and highest in normothermic animals (Table 2). When compared to normothermic animals, alanine incorporation into hepatic glycogen (Table 2) was higher in hibernating animals at 6 and 37 C. In contrast, alanine incorporation into lipid (Table 2) was higher in normothermic animals compared to hibernating and arousing animals at 37 C. No difference was observed at 6 C.

The approximate temperature characteristic calculated for methionine incorporation into liver protein was higher in normothermic animals when compared to similar values calculated for hibernating and arousing animals. Approximate temperature characteristics calculated for arginase activity in liver were the same for hibernating, arousing, and normothermic animals (Table 3).

DISCUSSION

Decreased protein synthesis in hibernation and arousing and ground squirrels, a higher arginase activity in liver homogenates of hibernating, arousing, and normothermic ground squirrels, Citellus tridecemlineatus, at 6 and 37 C.

<table>
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<tr>
<th>Table 2. Alanine utilization in vitro by liver tissue from hibernating, arousing, and normothermic ground squirrels, Citellus tridecemlineatus, at 6 and 37 C</th>
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<tr>
<td>CO₂ (micromoles urea formed/g wet tissue/hr)</td>
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<td>Glycogen (micromoles urea formed/g wet tissue/hr)</td>
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<td>Total lipid (micromoles urea formed/g wet tissue/hr)</td>
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Values represent means ± SE of micromoles alanine incorporated/g of wet tissue per 2-hr incubation. N = 5 animals.

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<th>Table 3. Approximate temperature characteristics (μ values) in calories for two rate processes in hepatic tissue calculated from data in Table 1</th>
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<tr>
<td>Protein synthesis from methionine (methyl-¹⁴C)</td>
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<td>Arginase activity</td>
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Calculated for arginase activity in liver were the same for hibernating, arousing, and normothermic animals (Table 3).

Values represent means ± se of micromoles alanine incorporated/g of wet tissue per 2-hr incubation. N = 5 animals.

* Significant at P ≤ 0.05 for arousing and normothermic compared to hibernating.
† Significant at P ≤ 0.05 for normothermic compared to arousing.
be due to a lower liver phosphorylase activity in hibernation (7) which would decrease the capacity for glycogenolysis while not affecting glycogenesis.

A marked increase was observed in the approximate temperature characteristic calculated for methionine incorporation into protein by microsomal preparations from normothermic hepatic tissue when compared to temperature characteristics for hibernating and arousing hepatic tissue (Table 3). This suggested that protein synthesis was qualitatively (i.e., shift to alternate pathway; shift to another rate-limiting reaction; shift in functional difference in entropies of the same enzyme) and quantitatively (i.e., a change in enzyme concentration) altered. In contrast, no difference was found in the approximate temperature characteristics calculated for arginase activity in liver homogenates from hibernating, arousing, and normothermic animals (Table 3). This suggested that protein catabolism was only quantitatively altered.

The effect of temperature on metabolic reactions must be interpreted with caution. South and Heffay (19) found no change in apparent activation energies for O2 consumption by heart mitochondria from hibernating and control hamsters. This would indicate a qualitative change in oxidative processes. In contrast, Chaffee et al. (3) found a temperature-sensitive succinic oxidase system at 7 C in liver mitochondria which would indicate a qualitative shift in this oxidative system. Further research concerning temperature effects on specific processes in protein anabolism and catabolism should contribute to a better understanding of protein metabolism in hibernators.

REFERENCES