

Effect of Hibernation and Cold Torpor on Tissue Catecholamine Content.* (27671)

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Activity of the sympatho-adrenal system in hibernators changes with transition from hibernation to arousal. Much of this evidence has been reviewed by Kayser(1). The storage of epinephrine and nor-epinephrine in the adrenal medulla, in active and hibernating animals, has been investigated(2,3,4).

To our knowledge little attention has been directed toward the changes in catecholamine content of extra-adrenal tissues during hibernation. Studies of variations in total catecholamine content of the heart, liver, and kidneys of the ground squirrel during activity and hibernation, and of the turtle during activity and cold torpor are reported here.

Material and methods. The ground squirrel, *Citellus tridecemlineatus*, and the turtle, *Chrysemys picta*, were used. The control groups of ground squirrels and turtles were active and their body temperatures averaged $37^{\circ} \pm 1^{\circ}\text{C}$ and $23^{\circ} \pm 2^{\circ}\text{C}$ respectively. The experimental group of ground squirrels

were hibernating for 7 to 10 days and body temperatures at time of sacrifice were 6 to 8°C . The turtles were in cold torpor from 12 to 14 days during which time their body temperatures ranged from 4 to 8°C .

Animals were spinalectomized; liver pieces, kidney and heart were excised and immediately placed on dry ice until analyzed. Catecholamines were assayed by the method of Crout *et al.*(5). Oxidations were carried out at pH 4.9 in order to obtain equivalent readings for epinephrine and nor-epinephrine. Tissue nitrogen was determined by the micro-Kjeldahl method with nesslerization.

Results. In the hibernating ground squirrel, the catecholamine content of the liver and kidney showed significant increases, 109% and 66% respectively, over the values found in non-hibernating controls. On the other hand, the catecholamine contents of the hearts in hibernating and non-hibernating squirrels were essentially the same (Table I). The

TABLE I. Relationships between Catecholamine ($\mu\text{g/g}$) and Tissue Nitrogen (mg N/g) in Liver, Kidney and Heart (wet weight) of Active and Hibernating Ground Squirrels and Active and Cold Torpid Turtles.

			Catecholamine, $\mu\text{g/g}$	Nitrogen, mg/g	CA/N, $\mu\text{g/g}$
<i>Citellus tridecemlineatus</i>					
Liver	(9)	A	$.35 \pm .16$	36.0 ± 1.8	$6.5 \pm .98$
	(10)	H	$.73 \pm .11$ ($P < .01$)	30.2 ± 1.5	23.6 ± 3.85 ($P < .01$)
Kidney	(9)	A	$.34 \pm .08$	26.7 ± 1.1	12.7 ± 2.68
	(7)	H	$.57 \pm .06$ ($P < .05$)	24.3 ± 1.3	25.5 ± 2.87 ($P < .01$)
Heart	(10)	A	$.91 \pm .13$	26.8 ± 1.3	32.2 ± 4.38
	(9)	H	$1.05 \pm .13$	26.4 ± 2.7	45.0 ± 6.71
<i>Chrysemys picta</i>					
Liver	(20)	A	$.12 \pm .04$	19.7 ± 4.5	$.47 \pm 1.25$
	(18)	C.T.	$.04 \pm .09$	18.2 ± 1.8	4.20 ± 8.64
Kidney	(19)	A	91.16 ± 11.71	23.2 ± 1.7	$1.73 \pm .98^*$
	(19)	C.T.	62.81 ± 11.13	24.9 ± 2.6	$4.16 \pm 2.36^*$
	(20)	A	$.48 \pm .24$	18.4 ± 1.5	28.9 ± 16.05
	(20)	C.T.	$.34 \pm .20$ ($P < .02$)	16.2 ± 2.5 ($P < .02$)	27.0 ± 19.13

* mg/g N . † A, active; H, hibernating; C.T., cold torpid. () No. of specimens.
P < .05 represents statistically significant difference between means of samples.

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nitrogen values for each organ, in both hibernating and non-hibernating squirrels were comparable, thus suggesting little or no change in water content of the 3 organs. When each analysis of catecholamine content was evaluated per g N, rather than $\mu\text{g/g}$ wet weight, the same points of significant differences emerged. This further substantiated that the catecholamine content of liver doubled during hibernation, that the kidney content was significantly greater in these animals, and that the myocardial levels did not differ between active and hibernating animals.

The catecholamine content of livers from turtles in the torpid state was not different from those of livers from active turtles. In the torpid turtle, kidney catecholamine levels tended to be somewhat lower, but were not statistically different from those in active turtles. Myocardial catecholamine contents expressed as $\mu\text{g/g}$ wet weight were lower in torpid specimens although the difference was not statistically significant (Table I). The myocardial nitrogen values in cold torpid turtles were significantly lower. When analysis of catecholamine content was evaluated per g N, differences in myocardial catecholamine content were not evident. Liver and kidney nitrogen values were comparable in both active and cold torpid turtles (Table I).

Discussion. These data indicate that in the ground squirrel catecholamine accumulated in extra adrenal sites in organs the functional activity of which is depressed during hibernation. The functional significance of the accumulation of catecholamine in tissue is controversial(1). The accumulation in the liver and kidney during hibernation offers an opportunity to study synthesis and degradation under naturally induced conditions of lowered metabolism.

Release of stored catecholamines may facilitate arousal through the calorogenic potential of epinephrine and by the analeptic action of the sympathetic agents on the central nervous system(6). The unaltered levels of myocardial catecholamines appeared to be related to the remaining cardiac activity, during hibernation.

The lack of significant variations in tissue nitrogen during the period of hibernation sug-

gests that little or no change in hydration has taken place in these organs.

Catecholamine contents of tissue from the turtles were noted to vary with the handling of the animal prior to or during spinalectomy. This is seen in the wide variation in values recorded (Table I). Consequently, kidney and liver values were lower in those animals spinalectomized with rapidity.

The catecholamine values for turtle kidney were considerably greater than those for any other organ or tissue studied. The possibility that kidney samples included adrenal tissue was not supported by histological examination. Another consideration is the role of a venous connection between adrenals and kidney. This would enable high concentrations of catecholamine of adrenal origin to be deposited in the kidney without traversing the systemic circulation.

The catecholamine data presented above do not preclude the possibility of changes in ratios of epinephrine to nor-epinephrine in the extra-adrenal sites during hibernation. This may pertain to those organs in which the total catecholamine contents did not differ significantly from those observed in active animals.

Summary. Hibernation in the ground squirrel is accompanied by increases in hepatic and kidney catecholamine content and no change in cardiac content. Cold torpor in the turtle was not accompanied by significant changes in catecholamine content of heart, liver or kidney.

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1. Kayser, C., *The Physiology of Natural Hibernation*, Pergamon Press, New York, 1961, p325.
2. Allara, E., *Boll. Soc. Ital. Biol. Sper.*, 1957, v33, 259.
3. Suomalainen, P., *Biochem. Z.*, 1938, v295, 145.
4. Suomalainen, P., Uuspaa, V. J., *Nature*, 1958, v182, 1500.
5. Crout, J. R., Creveling, C. R., Udenfriend, S., *J. Pharmacol. Exp. Therap.*, 1961, v132, 269.
6. Arbutov, S. Ya, *C. R. Acad. Sci., USSR (N.S.)* 1950, v74, 859.

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