and perhaps other fatty acids also promise to become powerful agents to prevent the loss of crops by drought, cold, and frost.

PIETER J. C. KUIPER
Department of Soils and Climatology, Connecticut Agriculture Experiment Station, New Haven

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Potassium and Sodium Content of Tissues of Hamsters and Ground Squirrels during Hibernation

Abstract. *Samples of kidney cortex, diaphragm, and atrial and ventricular muscle were taken from hamsters and ground squirrels to determine whether the low body temperature of hibernation caused loss of potassium and gain of sodium. Gradients between tissues and plasma are maintained or even increased during hibernation.*

In most mammalian tissues inhibition of metabolism by low temperature causes a decrease in ionic gradients across cell membranes. The loss of potassium and gain of sodium caused by cold in such tissues as mammalian erythrocytes and parenchymatous tissues (*1*) constitutes a failure of volume regulation and results in swelling (*1, 2*). In excitable tissues such as cardiac muscle the depression of ionic pumping by cold also leads to a lowering of the membrane potential and failure of conduction (*3*). In tissues of mammalian hibernators, however, excitability (*4*) and regulation of water content (*5*) persist at low temperature. Since maintenance of ionic gradients is essential for both of these cellular activities, it is surprising that little attention has been paid to the ionic content of tissues during hibernation. Eliassen and Leivestad have reported (*6*) that in muscles of two species of hibernators, hedgehog (*Erinaceus europaeus*) and bat (*Myotis daubentonii*), potassium concentration in tissue water increased somewhat during the winter months, while sodium concentration remained constant during the year but was greater than in nonhibernating species. They did not specify whether all "winter" bats and hedgehogs were actually hibernating when the tissues were collected. The results described in this report show that the total sodium and potassium content of tissues of hamsters (*Mesocricetus auratus*) and ground squirrels (*Citellus tridecemlineatus*) either remains unchanged during hibernation or changes in a way that would not be consistent with diminished cation transport.

Awake animals were kept in an animal room maintained at about 22°C, whereas hibernating hamsters and ground squirrels were housed in a cold room at 5°C. These animals were placed in the cold on 11 November. The ground squirrels began hibernating on 14 November and were killed between 11 and 24 February. The hamsters, as is typical of the species, delayed hibernation for 4 to 6 weeks (until 6 to 23 December), and 3 to 5 weeks of regular hibernation ensued before these animals were killed (between 5 and 22 January). Records were kept of the length of hibernating periods and frequency of periodic arousal. Of the six hamsters used, three were killed on the 3rd consecutive day of a hibernating period, one on the 2nd day and two on the 1st day. Of four ground squirrels, one was sacrificed on the 6th day of a period, one on the 5th day, and two on the 2nd or 3rd day. Cheek-pouch temperatures were measured prior to killing and were in all cases below 7°C.

After the animal was killed by a blow on the head, the kidneys, diaphragms, atria, and ventricles were removed through a midline incision. The atria and small sections (about 150 mg) of diaphragm and ventricle were rinsed briefly in an ice-cold Krebs bicarbonate-buffered medium to wash out the blood. These tissues were then blotted, placed in a tared tissue beaker, and dried overnight at 105°C for determination of dry weight. The capsule was removed from the kidney, and slices of the cortex were made with a dry razor blade. The outermost slice was rejected because in guinea pigs this has a higher potassium and lower sodium concentration than the rest of the tissue (*7*). The next slice was placed directly into a tared beaker to be desiccated.

Sodium and potassium values of dilutions of nitric acid digest of dried tissue were made by internal standard flame photometry on a Baird flame photometer.

The results of these measurements (Table 1) clearly show that in no tissue was there a significant decrease in potassium concentration or an increase in sodium concentration. Hence the low body temperature of hibernation does not cause the reduction in ionic gradients observed in most tissues of nonhibernating mammals at comparable temperatures.

In kidney cortex of both species, in hamster ventricles, and in ground

Table 1. Cation concentration of tissues of hamsters and ground squirrels. The value given (microequivalents per gram dry weight) in each case is the mean and standard error. Figures in parentheses indicate the number of cases in each column.

Tissue	Concentration			
	Potassium		Sodium	
	Hibernating	Awake	Hibernating	Awake
		Hamster		
	(6)	(8–10)	(6)	(8–10)
Kidney cortex	443 ± 16*	339 ± 6*	239 ± 18	242 ± 17
Diaphragm	332 ± 9	350 ± 6	218 ± 22	177 ± 17
Atria	367 ± 4	377 ± 10	288 ± 18*	393 ± 20*
Ventricles	383 ± 13*	336 ± 3*	144 ±8*	184 ± 5*
		Ground squirrel		
	(3–4)	(6)	(3–4)	(6)
Kidney cortex	469 ± 1*	331 ± 11*	208 ± 32	249 ± 16
Diaphragm	331 ± 12	335 ± 11	185 ± 12	163 ± 12
Atria	314 ± 7*	223 ± 15*	329 ± 40	271 ± 14
Ventricles	350 ± 11	344 ± 11	157 ± 9*	185 ± 6*

* Denotes pairs of values between which the differences between columns are statistically significant ($p < 0.01$).

squirrel atria the potassium concentration actually increased during hibernation. In only the hamster ventricle was this increase accompanied by a reciprocal decrease in sodium concentration. In hamster atria and ground squirrel ventricles, however, sodium content was lower in samples from hibernating individuals while the potassium content was unchanged.

The significance of the changes in sodium and potassium is unclear and may be different for each tissue. The changes do not reflect changes in plasma concentration, for neither plasma sodium (157 m*M* in hamsters and 149 m*M* in ground squirrels) nor plasma potassium (4.1 m*M* in hamsters and 3.9 m*M* in ground squirrels) was measurably altered during hibernation. Change in total tissue content of a cation could reflect either a change in its concentration in the tissue water or an increase or decrease in size of the tissue compartment containing that species of cation. For example, hydration (grams of water/gram dry wt) of hamster atria and ground squirrel ventricles decreases by 10 to 12 percent during hibernation (*5*). If about half of this loss of water represented a decrease in extracellular space, it would be sufficient to account for the observed decrease in tissue sodium. No attempt was made, however, to estimate extracellular space in kidney or the muscular tissues, and the contribution of extracellular sodium to the tissue sodium concentration cannot be stated. Consequently, the tissue potassium concentration is a more useful indication of changes in ionic steady state. (For example, with a plasma potassium concentration of about 4 m*M* and an extracellular space in a tissue of 25 percent the contribution of extracellular potassium to a tissue concentration of 300 microequivalents per gram dry weight would only be 1 percent.)

The possibility that the enormous increase in potassium content of ground squirrel kidney cortex is due to extracellular changes (for example, a great increase in hematocrit) seems to have been ruled out by subsequent experiments in which, as a routine procedure, the slices of kidney cortex were soaked for several minutes at room temperature in a phosphate-buffered Krebs medium containing calcium. In general, this treatment not only washes blood from the tissue but also brings about a decrease in concentration of tissue potassium (*7*, *8*). The slices of kidney cortex from the hibernating ground squirrel, when treated in this way, retained potassium at a concentration about 60 microequivalents per gram dry weight higher than that of slices from nonhibernating ground squirrels. It seems probable, therefore, that the high potassium content of kidney cortex during hibernation reflects a change either in the intracellular concentration of that ion or in the intracellular space.

The results thus demonstrate again the dramatic cold resistance and, therefore, uniqueness of tissues and mammalian hibernators.

J. S. WILLIS

Department of Physiology and *Biophysics, University of Illinois, Urbana*

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Nematode-Trapping Fungi: Evaluation of Axenic Healthy and Galled Roots as Trap Inducers

Abstract. *Larvae of* Meloidogyne hapla *Chit. induced abundant trap formation in the predaceous fungus* Arthrobotrys conoides *Drechs. Under similar aseptic conditions, neither healthy roots of* Lycopersicon esculentum *Mill, nor roots parasitized by the root-knot nematode induced traps.*

Past investigations of nematode-trapping fungi have supplied information about the types of traps, the wide variety of animal tissue extracts that induce traps, the noteworthy absence of such induction by extracts of healthy plant parts, the chemical nature of the inducer (nemin), and the use of the fungi as biological controls of nematode-incited diseases (*1*). Bird (*2*) added greatly to the knowledge of the physiological changes in the host plant tissues brought about by the root-knot nematode, *Meloidogyne javanica* Treub., during pathogenesis. Using sterile conditions to study the formation of giant cells in diseased roots, he concluded that the initiation and growth of the galls depend upon the continuous activity of the endoparasite. Nothing was known, however, about the interaction between such a profoundly altered root and the nematode-trapping fungi. Indeed, it was not known whether intact healthy roots could induce trap formation in those fungi dependent on nemin-like compounds.

The purpose of this investigation was to bring together under aseptic conditions *Lycopersicon esculentum* Mill., *Meloidogyne hapla* Chit., and the nematode-trapping fungus *Arthrobotrys conoides* Drechs. in such a way that the influence of the nematode-parasitized roots on the formation of traps by the fungus could be studied in the absence of free larvae. To do this, a succession of treatments and culture media was required. The surfaces of tomato seeds were sterilized with $Ca(OCl)_2$. Nematode egg masses were collected from tomato plants and

Table 1. Trap formation by *Arthrobotrys conoides* Drechs. when combined in aseptic culture with (i) free larvae of *Meloidogyne hapla* Chit., (ii) healthy *Lycopersicon esculentum* Mill., and (iii) galled *L. esculentum* containing active endoparasitic larvae. Figures represent the average number of traps per 60-mm plate.

Trial 1		Trial 2	
7 days	9 days	7 days	9 days
Lycopersicon, galled*			
2	5	2	9
Lycopersicon, healthy†			
2	5	1	7
Meloidogyne larvae†			
800	850	850	900

* Averages of 41 to 57 cultures. † Averages of 5 cultures.