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UREA EXCRETION IN THE HIBERNATING
COLUMBIAN GROUND SQUIRREL,
CITELLUS COLUMBIANUS

By

John Charles Passmore

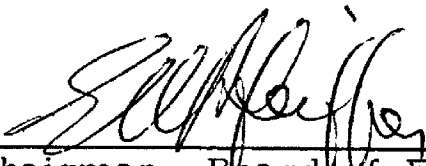
B.A. University of Montana, 1964

Presented in partial fulfillment of the requirements for the degree of
Master of Arts

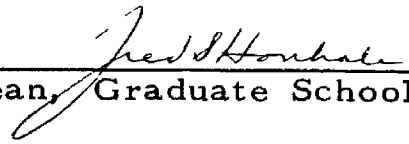
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INTRODUCTION

Shaw (1925c) summarizing the food habits and life history of C. columbianus asserted that the Columbian ground squirrel eats succulent forms of vegetation, such as bluegrass (Poa) and young wheat (Triticum). In late summer or fall these forms of plant life have all become dried. Because in certain localities the Columbian ground squirrel drinks no water they probably cannot maintain water balance on these dried plants, and must therefore begin estivating in the end of summer. They may also remain in dormancy during the winter in those regions where a heavy blanket of snow is found. Therefore, these squirrels have seasons of dormancy which may be 220 days long for females and 204 days long for males (Shaw 1925b).

All ground squirrels of the genus Citellus avoid the stressful external conditions of water and food shortage and low temperatures by hibernating, but in doing so may become faced with stressful internal conditions. For example, how do they handle the nitrogenous metabolic wastes which presumably accumulate in their blood during hibernation? Kayser (1964) found that hamsters which aroused from winter hibernation excreted nearly twice the

urea per day (.35 g per day) than did winter hamsters continually awake (.18 g per day), indicating that urea is stored in the body and subsequently excreted upon arousal. Kristofferson (1963) reported significantly higher urea concentrations in the blood, muscle, and intestinal tissues of hibernating hedgehogs (Erinaceus europaeus) than in that of awakened hedgehogs ($P < .02$). Hong (1957) found that bladder urine collected by catheters from hibernating C. tridecemlineatus was drastically reduced and Pengelly and Fisher (1961) determined by sacrificing squirrels after known intervals of hibernation, that no urine entered the bladder of C. lateralis during hibernation. They found that the bladder urine volume was the same in animals which had hibernated 9 or 10 days, as in those that only hibernated two days.

Recent techniques have elucidated further evidence of kidney non-function during hibernation. Zimny and Rigamer (1966) with the aid of electron microscopy, found structural modifications in the kidney glomeruli of hibernating C. tridecemlineatus which would impede normal filtration. During hibernation the endothelial pores decrease in size and number, the basement membrane of the glomerulus thickens and becomes irregular in contour, and the podocyte foot processes (portions of the glomerular membrane) swell. According to Popovic (1964) cardiac output was reduced.

from 69 ± 5.5 ml per minute in the euthermic C. tridecemlineatus to 1.04 ± 0.1 ml per minute in hibernating squirrels. Bullard and Funkhouser (1962) estimating regional blood flow by Rubidium⁸⁶ distribution found the blood in 24 hours deposited six-fold more Rb⁸⁶ in the heart tissue of hibernating C. tridecemlineatus than in the tissues of the kidney, yet the aroused animal deposited 1.5 times more Rb in the kidney than in the heart. By monitoring renal blood flow, Hong (1957) also showed that the kidney imposes increased resistance to blood flow during hibernation. He felt that constriction of the afferent arterioles might be responsible. Decreased cardiac output and disproportionately reduced renal blood flow plus a heart rate as slow as 1/50 of normal, and a systolic blood pressure of 10-40 mm. Hg. (Lyman, 1959) lead to the conclusion that the kidney does not form an appreciable amount of urine during hibernation.

Upon arousal systolic blood pressure can reach 180 mm. Hg. before the temperature of the heart reaches 37 C° in C. tridecemlineatus (Lyman, 1959). Because the heart is being driven at its maximal rate during arousal by the sympathetico-adrenal system (Chatfield and Lyman, 1950) the animal could soon regain the blood pressure and flow necessary for glomerular filtration during the arousal period.

During hibernation the oxygen consumption of C. tridecemlineatus is reduced from a normal of 271 ml per hr to 4.6 ml per hr (Popovic, 1964; Lyman and Chatfield, 1955). However, it is also known that C. tridecemlineatus (Hoffman, 1964) and C. lateralis (Twente and Twente, 1964; Pengelly and Fisher, 1961) hibernate for longer cycles at lower temperatures and shorter cycles at higher temperatures. This would indicate that perhaps the formation of some glandular or metabolic end product might trigger arousal. Hoffman (1964) suggested that the formation of this product may be temperature-dependent and the product may in some way sensitize nervous receptors.

A lack of kidney function during hibernation would probably mean the unavoidable retention of metabolic waste products. Carpenter (1938) determined that the percent concentration of all nitrogenous excretory products in the urine were proportionally the same in both hibernating and non-hibernating woodchucks (Marmota monax), even though the total quantity was different. Because high blood urea concentration is toxic (Pengelly and Fisher, 1961), urea buildup in the body during hibernation could possibly cause periodic arousals for its removal in the urine. Fisher (1964) determined that blood urea concentrations apparently have an effect on arousal and reentry into hibernation of C. lateralis. He found

that those squirrels with a blood nonprotein nitrogen (NPN) concentration of approximately 152 mg % were all awake. For 34 determinations on blood taken from hibernating animals the average NPN was 89 mg %. In 9 of these instances the animals did not arouse when the blood sample was taken. The average NPN for these 9 samples was 81 mg %.

With such strong evidence against appreciable urine production during hibernation, it seems reasonable to ask if there is urea buildup in the blood of the hibernating ground squirrel and at what rate does this increase in urea concentration take place? The present study is concerned with these questions.

MATERIALS AND METHODS

Citellus columbianus were collected in Pattee Canyon, Missoula County, Montana. In order to determine urea excretion in hibernating squirrels sixteen animals were obtained in the month of June and maintained in 10" x 20" x 11" high screened metal cages until they began, at room temperature, to hibernate in the fall. Four additional squirrels were collected in March to determine the blood urea concentration and the composition of the urine excreted by non-hibernating, active ground squirrels. All of these animals appeared to remain vigorous and healthy on a diet of alfalfa pellets and water ad libitum.

In the hibernation experiments each animal was placed in a specially constructed cage in a refrigerator (Fig. 1). These cages, made of hardware cloth with 0.5" squares and a 2" x 2" wood frame, had internal dimensions of 13" x 10" x 5" high. One half of the cage bottom was covered with hardware cloth mimicking that part of the natural hibernating den which is outside the nest and includes the drain (Shaw, 1925a). The other half of the cage floor was left uncovered and the top 1.5" of an 8" x 6.5" bucket was fitted into this rectangular area (Fig. 1).

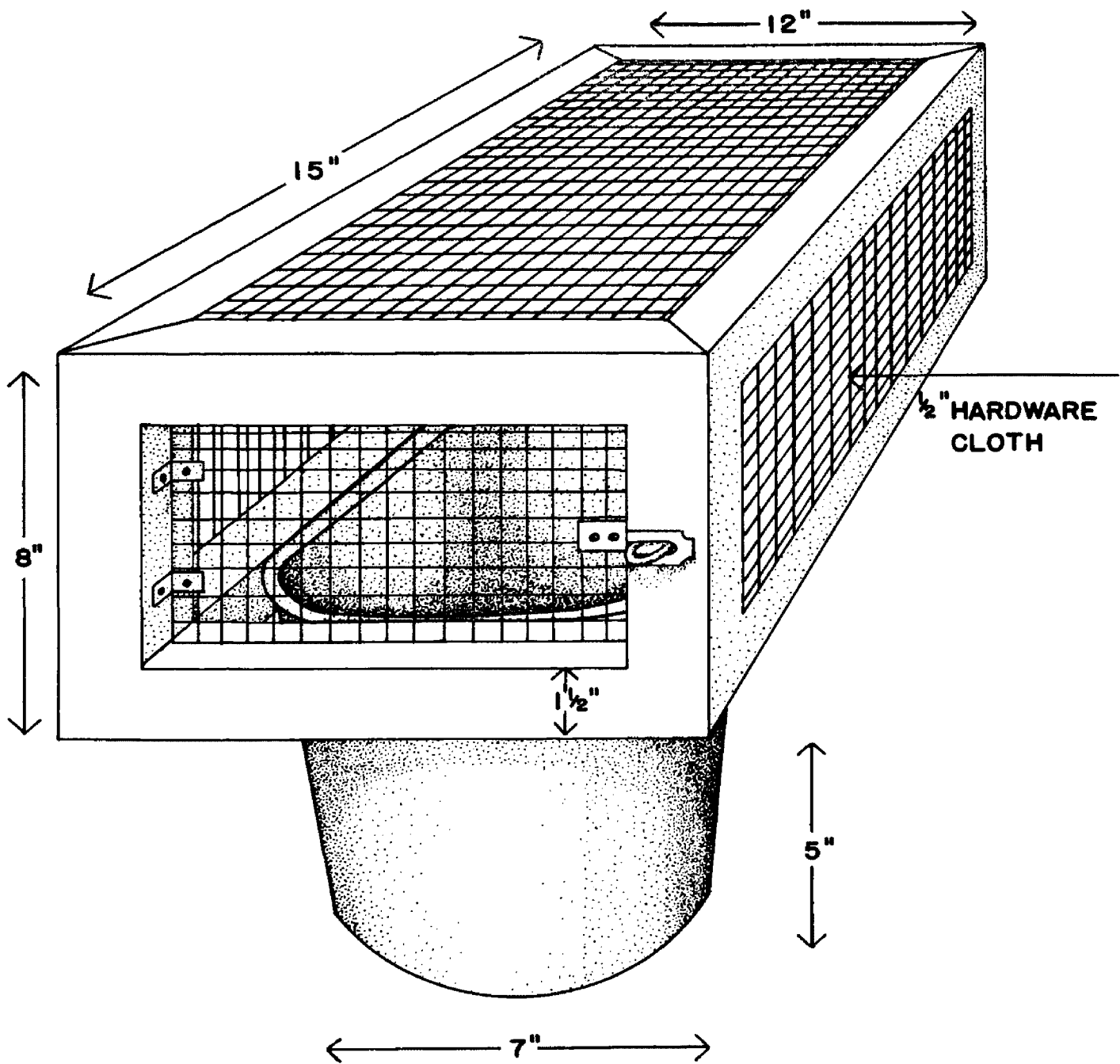


Figure 1. Sketch of refrigerator cage which held C. columbianus.

Pieces of burlap were placed in the bucket for nest material. The refrigerator temperature was maintained at 5-6 C°. At this temperature and with no food or water available, the squirrels curled up in the burlap shreds of the nest bucket and began hibernating within 2 or 3 days. After the squirrel began hibernating an enamel pan¹ containing 0.5" of mineral oil to prevent evaporation of the urine, was placed under the hardware cloth portion of the cage to catch the urine excreted by the ground squirrel during arousal. Wood shavings were then placed on the squirrels back and displacement of these shavings determined the cessation of the hibernating cycle, as previously described by Pengelly and Fisher (1961). Small pieces of red litmus paper were placed in the nest bucket under the nest material to determine if urination occurred in the nest. Thus, if any squirrel urinated in the nest the litmus would change color. In most cases, the squirrels came out of their nest boxes and urinated over the enamel pans. In those few cases when a squirrel urinated in the nest box the trial for that animal was discarded. The pans were observed twice daily and when urine was present it was aspirated from the mineral oil into a syringe. At this time a blood sample was taken by cardiac puncture with a #20 needle. The ground squirrel was allowed to reenter hibernation and then was sacrificed, thus ending the second cycle. At

this time a second sample of blood and any detectable bladder urine were taken. It was assumed that the squirrel would hibernate, if undisturbed, approximately as long as it had in the first cycle. The length of time that the squirrel was allowed to hibernate in the second cycle before it was sacrificed was arbitrarily determined by subtracting one or more days from the length of the first cycle.

Each of the four active non-hibernating ground squirrels was placed in a cage, with no food or water, over an enamel pan containing 0.5" of mineral oil. Blood was taken by heart puncture before the squirrel was put in the cage and all urine was collected from the pan at 9 hours and at 26 hours. A second blood sample was taken at 18-26 hours. One trial was made a week after capture, and a second two months after capture.

Serum urea nitrogen (S. U. N.) concentrations were determined by a Beckman Ultramicro adaption of the method of Fawcett and Scott (1960). Urine urea nitrogen (U. U. N.) concentrations were determined in the same way and no distinction was made between ammonia nitrogen and urea nitrogen in the urine. All urine concentrations were calculated in the following manner:

$$\text{Urea nitrogen mg \%} \times \frac{60}{28} = \text{Urea mg \%}$$

Table 1. Temporal sequence of experimental procedure.

HIBERNATING SQUIRRELS	
TIME	
0 Days	Ground squirrel placed in the hibernating cage in refrigerator.
1-3 Days	When animal was observed hibernating shavings were placed on its back and pan with mineral oil was put under cage.
Day of first arousal	Animal first observed arousing or aroused from first hibernation cycle,
8-10 Hours after arousal	All urine collected from pan under cage and blood sample taken.
1-3 Days after arousal	Animal reentered hibernation in refrigerator cage. Shavings placed on its back.
After hibernation period of 1-3 days less than first	Animal sacrificed, blood sample taken and all urine aspirated from bladder.

NON-HIBERNATING SQUIRRELS	
0 Hours	Blood sample taken and squirrel placed in cage over pan with mineral oil.
9 Hours	All urine collected from pan under cage.
18-26 Hours	Blood sample taken and all urine collected from pan.

$$\text{Urea mg \%} \div 60 = \text{Urea in mMols \%}$$

Osmolalities of all serum and urine samples were determined with a Fiske osmometer.

For statistical analysis of the data a "t" test was used. However, if the two sets were composed of samples taken at an interval from the same group of animals the means were compared with the paired "t" test.

As the length of time in hibernation before sacrifice varied for most squirrels, the urea concentration of the sacrifice serum was extrapolated to a value which was assumed to be that of a squirrel after 8 days of hibernation. This extrapolation was based on the amount of change for the number of days the ground squirrel hibernated as follows:

$$\frac{[\text{Serum Urea (sacrifice)}] - [\text{Serum Urea (arousal)}]}{\text{Days (in second cycle)}} \times 8$$

$$= \Delta \text{ Serum Urea for 8 days}$$

To determine each squirrel's total urea content at the time of reentrance into the second hibernation it was assumed that the sacrifice bladder urea was excreted after the arousal heart puncture but

before reentrance into hibernation. The total amount of urea in each animal's body at the time of heart puncture was calculated and the sacrifice bladder urea was subtracted from this value to give the approximate total urea content of each animal as it entered hibernation. In calculating the total urea content of each squirrel at the time of heart puncture and at the time of sacrifice, it was assumed that, since urea diffuses readily through all body tissues, the urea content of the body fluids was in equilibrium with the urea content of the serum. Kristofferson (1963) found it in the ratio of: 102 mg % in serum to 104 mg per 100 gm muscle to 81 mg per 100 gm intestine, in the hibernating hedgehog (Erinaceus europaeus). Sixty-six percent of body weight was used in calculating the total body fluids (Chesley and Weill, 1956).

The increase in amount of urea per day was calculated and extrapolated for eight days according to the following equations:

$$\text{Estimated Body Urea (entrance) (mMols)} = [\text{Body Fluid (mls.)} \times \text{Serum Urea (arousal) (mMols/ml)}] - [\text{Bladder urea (sacrifice)}]$$

Bladder Urea (sacrifice) = [Bladder Urine Volume (mls)] x
[Urine Urea (sacrifice) (mMols/ml.)]

Body Urea (sacrifice) (mMols) = [Serum Urea (sacrifice)
(mMols/ml.)] x [Body Fluid (mls)]

Accumulated Body Urea = [Body Urea (sacrifice)] - [Body
Urea (arousal)]

[Accumulated Body Urea \div Days in cycle 2] x [8] = Estima-
ted body urea accumulation (mMols) for a cycle of 8 days

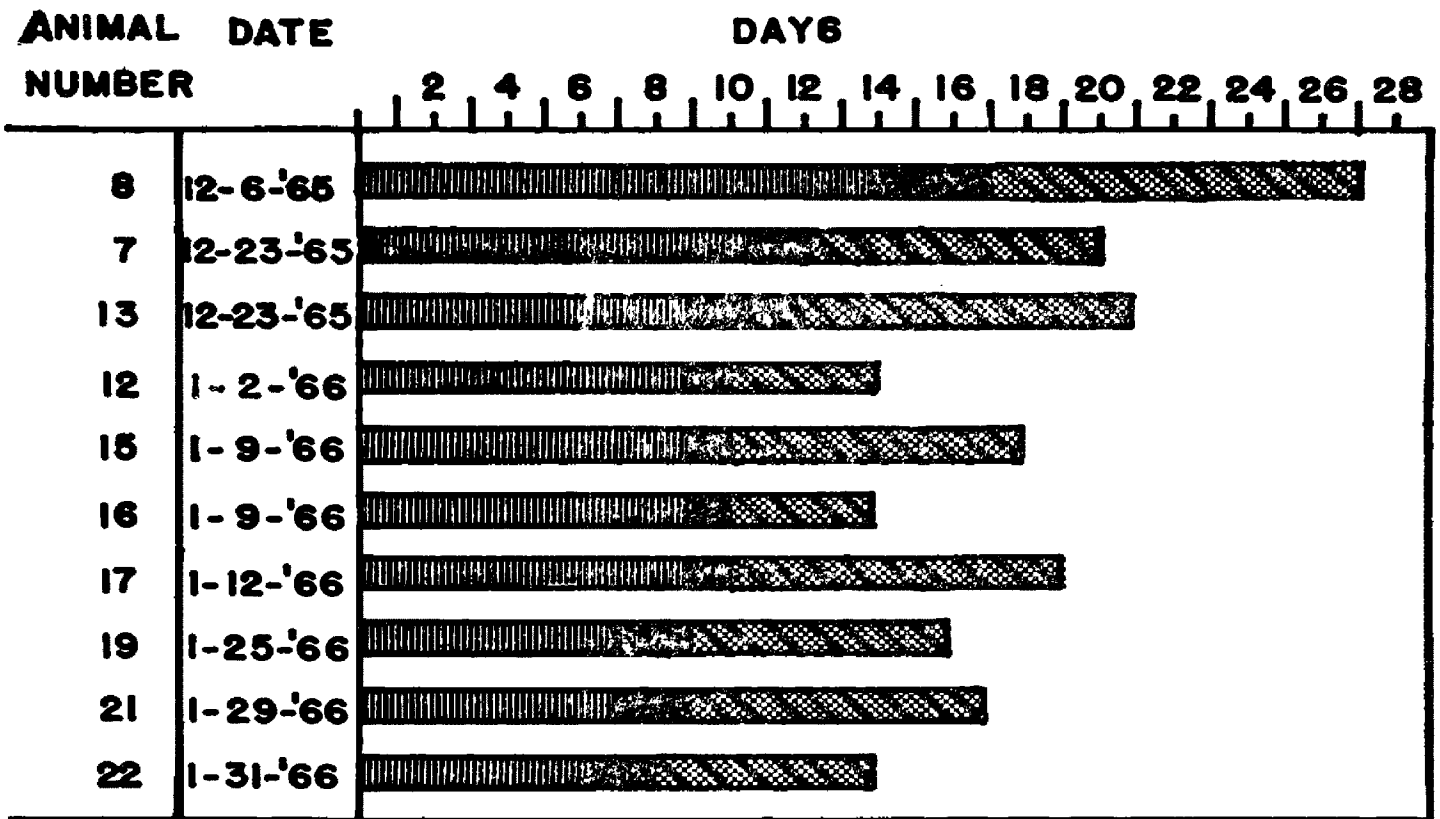
RESULTS

The length of the hibernation period for each animal in the first cycle progressively decreased from December 6 until January 31 (Fig. 2). The longest cycle recorded was for animal #8, which hibernated 14 days beginning December 6, and the shortest cycle was for animal #22, which hibernated 7 days beginning January 31. No animals could be induced to hibernate in February. After spontaneous arousal from the first cycle, each animal remained awake 1-3 days before reentrance into hibernation. The length of the first hibernation and the duration of arousal following hibernation show no correlation (Fig. 2).

The squirrels averaged 345 g at the time of the first arousal (range 275-495 g), whereas sacrificed squirrels weighed 331 g (range 260-482 g) (Table 2). Weight loss ranged from 0.4 % to 6 % of the body weight (mean 3.8 %) between the first arousal and the time of sacrifice.

Serum Osmolality and Urea Concentrations:

The mean osmolality of the sera at the time of arousal was not significantly lower than the mean osmolality of the sera of



Date--The first day on which animal was observed hibernating.
 Days--The number of days from the date of initiation.
 Open bars indicate hibernation cycle #1, at the end of which all squirrels aroused spontaneously.
 Stippled bars indicate days of arousal between hibernation cycles.
 Cross hatched bars indicate hibernation cycle #2, at the end of which all squirrels were sacrificed.

Figure 2. Date and length (days) of hibernation cycle #1, period of arousal and hibernation cycle #2 for each squirrel.

the animals sacrificed during the second hibernation (Table 2).

The mean serum urea concentration of animals at the first arousal was appreciably lower than the mean serum urea concentration of all animals when they were sacrificed. When the mean serum urea of the sacrifice serum was extrapolated to an assumed value for 8 days of hibernation, 7 of the 10 animals had a significantly higher serum urea after hibernation than at the previous arousal ($P < .001$) (Table 2 and Fig. 3). Also, the calculated mean body urea concentration at entrance into hibernation was significantly lower than the mean body urea concentration at the time of sacrifice ($P < .05$) (Table 3).

The mean serum osmolality of the ground squirrels trapped in spring soon after emergence from their hibernating dens did not change appreciably when the animals were dehydrated for 26 hours (Table 4). However, the mean serum urea dropped significantly from 10 mMols per liter to 5.7 mMols per liter ($P < .025$).

When those animals trapped in the spring were maintained in the laboratory for two months and then dehydrated for eighteen hours the mean serum osmolality again did not change appreciably; but the mean serum urea concentration again dropped significantly ($P < .05$) (Table 5).

Table 2. Osmolality and urea concentrations of serum samples from winter Citellus columbianus.

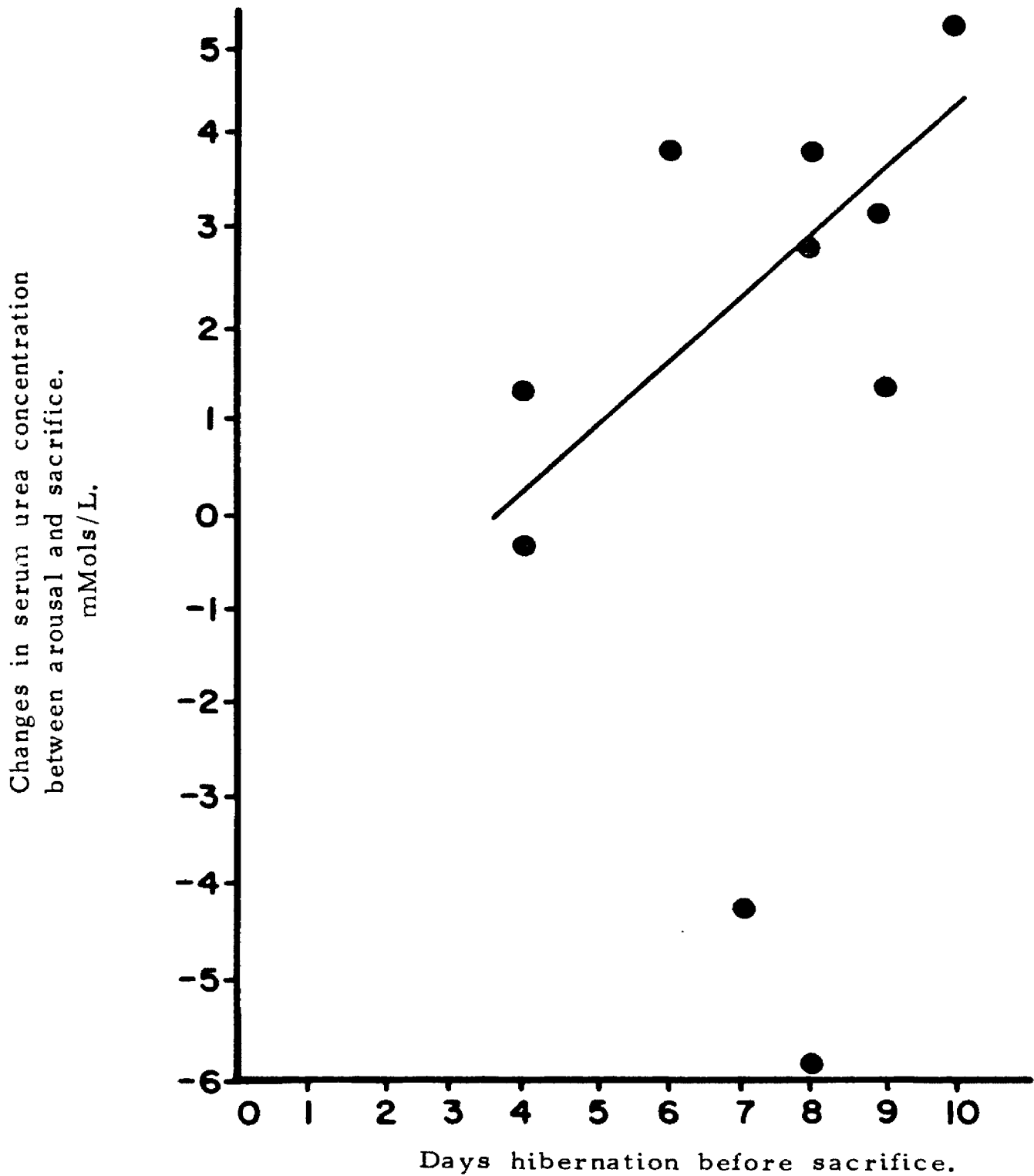
Animal	Wt. grams		Serum Urea mMols./L.		Osmolality mOsm		Days Second Hibernation	Serum Urea ² extrapolated to 8 days mMols/L.
	1	2	1	2	1	2		
7	414	404	7.7	10.3	320	318	8	10.3
8	325	320	6.7	11.8	*	313	10	10.8
12	343	330	5.7	5.3	*	303	4	4.9
13	319	310	5.3	6.7	312	316	9	6.4
15	318	301	12.6	16.4	307	320	8	16.4
16	277	274	6.7	8.0	*	309	4	9.5
17	495	482	7.0	10.0	335	310	9	9.8
19	280	260	16.8	12.6	313	312	7	11.8
21	369	346	18.2	12.3	344	312	8	12.3
22	305	287	7.7	11.5	306	307	6	12.3
Mean			9.2	10.5	320	312		
± S. E.			±.01	±.01	±5.5	±1.6		

1 - at arousal

2. - at sacrifice

* - Indicates sample volume too small for analysis

Figure 3. Changes in serum urea concentration during hibernation.



The line, calculated by the method of least squares can be represented by the equation: $y = .69x - 244$. Calculations exclude squirrels number 19 and 21, lower right, and has a Pearson correlation coefficient of 0.63.

Table 3. Change in total body urea, during Cycle No. 2

Animal	A Body Urea mMols.	B Body Urea mMols.	B-A as % A	△ Body Urea per day in mMols.
7	2.1	2.8	+ 33	.09
8	1.3	2.5	+100	.13
12	1.0	1.2	+ 14	.04
13	.9	1.4	+ 50	.05
15	2.5	3.3	+ 31	.09
16	1.0	1.5	+ 50	.12
17	2.3	3.2	+ 42	.10
19	1.4	2.2	+ 59	.11
21	4.1	2.8	- 34	-.18
22	.8	2.2	+174	.24
Mean				.09
±				±.03

A - reentrance

B - sacrifice

Table 4. Osmolalities and urea concentrations of urine and serum of terminally aroused C. columbianus dehydrated 2 weeks after emergence from their hibernation dens.

Animal	Wt. loss A-B as % of A	<u>Serum A</u>		<u>Serum B</u>		<u>Urine A</u>		<u>Urine B</u>	
		Urea mMols. /L.	Osm. in mOsm.	Urea mMols. /L.	Osm. in mOsm.	Urea mMols. /L.	Osm. in mOsm.	Urea mMols. /L.	Osm. in mOsm.
26	17	13	341	7.5	344	600	1240	408	760
27	14	8.9	293	4.8	310	924	2105	888	1740
28	17	8.9	340	4.8	300	888	1635	556	1050
29	18	9	312	5.3	315	814	1575	666	820
Mean	17	10	322	5.7	315	806	1639	630	1030
± S. E.		±1	±12	±.6	±10	±62	±178	±51	±225

In table 4:

Serum A was collected at the start of dehydration.
Urine A was collected after 9 hours of dehydration.

Serum B and Urine B were collected at the end of dehydration.
* Sample was not of sufficient volume to measure.

Table 5. Osmolalities and urea concentrations of urine and serum of terminally aroused *C. columbianus* dehydrated after being maintained in the laboratory for 2 months.

Animal	<u>Serum A</u>			<u>Serum B</u>		<u>Urine A</u>		<u>Urine B</u>	
	Wt. Loss A-B as % of A	Urea mMols. /L.	Osm. in mOsm	Urea mMols. /L.	Osm. in mOsm	Urea mMols. /L.	Osm. in mOsm	Urea mMols. /L.	Osm. in mOsm
30	9	11.5	345	8	381	*	*	756	2185
31	13	10.4	322	8	337	350	529	*	*
32	8	9.5	330	7.4	325	828	1835	604	1420
33	9	8.9	325	7	305	828	1900	920	1405
Mean	10	9.7	331	7.5	337	668	1421	760	1687

In table 5: Legend as in Table 4.

Table 6. Osmolality and urea concentrations of urine samples from winter Citellus columbianus.

Animal	Urine Urea ₁ mMols. /L.	Urine Urea ₂ mMols. /L.	Osm. ₁ mOsm	Osm. ₂ mOsm	Urine ₁ Volume cc.	Urine ₂ Volume cc.	Urea per day Cycle 1 mMols.	Urea per day Cycle 2 mMols.	No. days Cycle 1	No. days Cycle 2
7	666	74	*	*	.4	.1	.03	.001	11	8
8	360	124	750	260	3.4	1.4	.12	.01	14	10
12	444	666	715	963	6.4	.4	.71	.03	9	4
13	334	598	675	869	2.9	.3	.11	.02	9	9
15	1466	500	1950	1060	2.6	.2	.49	.01	9	8
16	650	784	962	1150	1.3	.3	.21	.03	9	4
17	486	***	743	***	3.2	***	.17	.0	9	9
19	384	1100	689	1430	2.8	1.6	.15	.25	7	7
21	1134	254	1680	643	4.3	.6	.6	.02	7	8
22	1000	1000	1600	1275	3.2	.7	.53	.10	7	6
Mean	692	628	1057	956	3.2	.6	.31	.05		
± S. E.	±122	±120	±155	±133	±.5	±.2				

1 - At arousal
2 - At sacrifice

* - Sample not large enough to analyze on Fiske osmometer.
*** - No bladder urine detectable.

Urine Osmolality and Urea Concentrations:

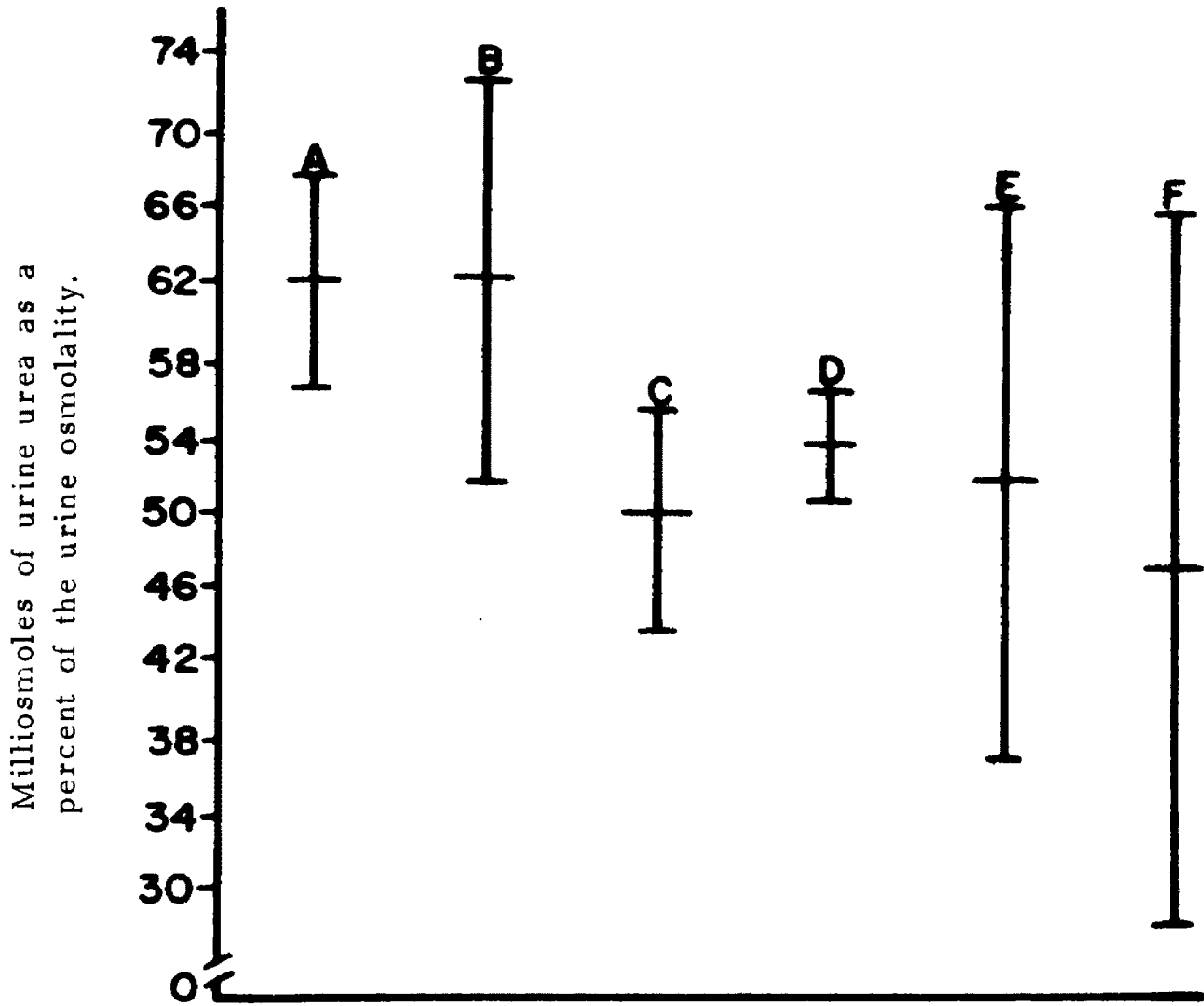
The mean osmotic concentration of the urine collected from the bladders of the sacrificed animals did not differ appreciably from the mean osmotic concentration of the urine normally excreted by the squirrels upon arousal. However, both concentrations varied considerably as shown by the large standard errors (Table 6).

There was no correlation between bladder urine volume or its total urea content and the length of the second cycle. Interestingly, squirrel No. 17 had no detectable bladder urine after a nine-day hibernation cycle (Table 6).

The amount of urea excreted in the urine upon arousal was much higher than the total amount of urea found in the bladder at sacrifice when the amounts were divided by the number of days in hibernation ($P < .01$) (Table 6). The mean amount of urea excreted at arousal was six-fold higher than the mean amount of urea in the sacrifice bladder urine (Table 6).

The urine excreted in the first 9 hours by spring trapped animals initially dehydrated had a significantly higher osmotic concentration (1639 mOsm) ($P < .05$) than that of the winter ground squirrels after the first hibernation (1057 mOsm) or at the time of sacrifice (956 mOsm) (Tables 4 and 6). However, the percentage of this osmotic concentration that was composed of

Figure 4. Urine urea osmolar concentration as a percent of the total osmolar concentration of urine from winter and spring Citellus columbianus.



The horizontal marks at the center of the vertical lines are the means and the vertical lines extend two standard errors each way.

- A - Calculated from urine excreted by aroused winter animals.
- B - Calculated from bladder urine of winter animals sacrificed in hibernation.
- C - Calculated from the urine of spring trapped animals 2 weeks after emergence from hibernating dens, dehydrated for 9 hours.
- D - Same as C except dehydrated for 26 hours.
- E - Calculated from the urine of spring trapped animals dehydrated 9 hours, 2 1/2 months after emergence from hibernating dens.
- F - Same as E except dehydrated 18 hours.

urea was slightly lower (51%) ($P < .025$) than that of the urine excreted by the winter animals at arousal from the first cycle (62%) (Fig. 4). The urine osmolality of those animals dehydrated for 9 and 26 hours in early spring (1639, 1030 mOsm) fell in the same range as the osmolality of both urine samples collected from the winter animals (1057, 956 mOsm) (Tables 4 and 6). The percentage of the osmotic concentration contributed by urea was still significantly lower than that of the urine excreted by the winter animals at arousal ($P < .05$) and the mean was appreciably lower than that of the sacrifice bladder urine (Fig. 4).

When the spring trapped animals, captive for two months, were dehydrated for 18 hours, the osmotic concentration of the urine samples taken at 9 and 18 hours (1421, 1687 mOsm) was in the same range as that of the urine samples taken when they were dehydrated two months earlier (1030 mOsm) (Tables 4 and 5). The mean urea percentage of this total osmotic concentration was also appreciably lower than that of the urine samples taken from winter squirrels (Fig. 4). However, the urea values of these samples varied so greatly that the difference is not significant.

DISCUSSION

The significant buildup of serum urea concentration calculated for 7 of the 10 hibernating squirrels suggests that the kidney is not eliminating nitrogenous wastes while the animal is torpid (Table 2) (Fig. 3). Three of the ten squirrels had a lower serum urea at the time of sacrifice than at arousal. However, they had extremely high serum urea values during the previous arousal which suggests that the blood sample taken at that time was obtained before the animal could clear its blood of urea. Squirrel No. 21 in which body urea decreased had a very high amount of urea in its body at the time of heart puncture (Table 3), but little urea in its bladder at sacrifice (Table 6). This indicates a premature heart puncture and suggests that additional excretion of urine occurred after the heart puncture and before reentrance into hibernation. This urine sample, not caught in the pan, may have been voided in the nest. All squirrels except No. 21 had a significantly higher calculated body urea value at sacrifice than during the previous arousal, ($P < .05$) (Table 3), thereby indicating that body urea rises uniformly during hibernation.

However, a rise in body urea did not occur in spring trapped squirrels which were dehydrated for about as long as the arousal period of winter squirrels. Serum urea also dropped in both active non-hibernating squirrels deprived of food and water and in squirrels aroused between bouts of hibernation. If the kidney were functioning throughout the hibernation cycle, serum urea at sacrifice would presumably be considerably lower instead of higher than the serum urea taken from the aroused squirrel. The results of the two periods of dehydration of the spring trapped squirrel show that the serum urea concentrations always drop significantly.

The present study supports the findings of Pengelly and Fisher (1961) and Hong (1957) that no urine is formed during hibernation. The amount of bladder urine and the length of the hibernation cycle show no correlation. If squirrels constantly deposited urine in the bladder throughout the hibernation cycle, the bladders of animals sacrificed at nine days would supposedly contain more urine than those animals sacrificed at four days. One squirrel actually had no urine in the bladder after nine days of hibernation. The concentration of urea in the bladder urine also bore no correlation with the length of the hibernation cycle (Table 6).

The amount of urea excreted upon arousal from hibernation by the winter squirrels should presumably include that urea stored in the body throughout the hibernation cycle, any urea formed while the animal was arousing, and any urea excreted and stored in the bladder after the animal urinated at arousal and before it reentered deep hibernation. The urine present in the bladder contains the urea deposited after urination at arousal and while reentering hibernation. The difference between the sum of these two values and the amount of arousal urine urea gives that urea presumably formed during arousal and before urination. For example, Squirrel No. 15 excreted 3.8 mMols of urea at arousal (Table 6). At sacrifice the bladder urine contained 0.1 mMols of urea (Table 6), and the change in body urea was 0.8 mMols (Table 3). Therefore, 2.9 mMols of urea were formed during the arousal process which apparently makes up a larger portion of excreted urea in the urine than the other two combined. This indicates that the squirrel is either metabolizing a much larger amount of protein during arousal than during the rest of the cycle, is storing the nitrogenous wastes as a urea precursor during the cycle and then converting to urea at arousal, or is washing out urea accumulated in such tissues as the renal medulla.

Most mammals faced with dehydration conditions similar to those which the summer ground squirrels underwent will reabsorb urea in the kidney to help control water loss (Lever, 1965). This will cause the excreted urine to be highly concentrated, as it was in the summer ground squirrels (Tables 4 and 5). However, the urine of the winter squirrels at arousal contained a significantly higher percent of its solutes as urea ($P < .05$) (Fig. 3) than did the urine of the summer ground squirrels which provides additional evidence that the winter ground squirrel is removing urea from its body tissues during arousal that it accumulated during the previous hibernation cycle.

Hong (1957) indicated that a very small amount of urine was formed while the animal was hibernating. The evidence presented in this study indicates that very little or no urine was formed during hibernation. It seems probable that urine formed during entrance into hibernation was obtained by Hong from catheters in the hibernating animal's bladder.

SUMMARY

Ten hibernation experiments and eight summer dehydration experiments were performed on Citellus columbianus. The hypothesis that the ground squirrel kidney is nonfunctional during hibernation is supported by the following data obtained in these experiments:

1. The mean serum urea concentration of seven of the ten winter hibernation experimental animals at the time they were sacrificed in hibernation was appreciably higher than the mean serum urea concentration of these same animals during their previous arousal.
2. Most squirrels had higher serum urea concentrations when sacrificed during hibernation than when aroused.
3. The animals dehydrated in the summer had a significant drop in serum urea after a day of dehydration.
4. Neither the volume of bladder urine nor its total urea content differed in those squirrels sacrificed after four days of hibernation from those squirrels sacrificed after nine days of hibernation.

5. The percentage of the urine osmolality contributed by urea was higher in the urine of hibernating animals than in that of non-hibernating animals.

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