THE EFFECTS OF HYPERTONIC MEDIA ON VARIOUS SERUM AND URINE CONSTITUENTS OF THE HELLBENDER, *CRYPTOBRANCHUS ALLEGANIENSIS*

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by

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THE EFFECTS OF HYPERTONIC MEDIA ON VARIOUS SERUM AND URINE CONSTITUENTS OF THE HELLBENDER, CRYPTOBRANCHUS ALLEGANIENSIS

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Introduction

Osmoregulation is essential to aquatic vertebrates. All vertebrates have body surfaces that are exposed to the environment and are permeable to water. In aquatic vertebrates these surfaces can be gills, skin, buccal membranes, gut and cloaca. For animals in fresh water the main problem is overhydration of the body. For animals in brackish water the problem is reversed, the body tends to become dehydrated. Some animals such as sea turtles and marine birds avoid dessication by drinking sea water and excreting the excess salt through special organs. Others, such as the elasmobranch fishes, increase their body osmolality above the osmolality of sea water with the readily available small molecules of urea and trimethylamine oxide (Prosser *et al.*, 1950).

Deyrup (1964) has noted that more information is needed about water balance and osmoregulation in amphibians. One urodele that is common in the Ozarks and is easy to work with is the hellbender, *Cryptobranchus alleganiensis*. Exposure of the hellbender to hypertonic media, and then measuring selected serum constituents should yield valuable information about comparative osmoregulation.

Review of the Literature

The function of the kidney of fresh water amphibians is to remove excess water from the animal's body (Noble, 1954; Deyrup, 1964). Water normally enters the skin by osmosis. When the animals are exposed to an osmotically dehydrating environment they must either increase their body osmolytes or loose body water to the outside (Beadle, 1957).

In this review blood constituents will be organized together without regard to animal species. Gordon (1962) studied a euryhaline toad, <u>Bufo viridis</u>, from Southeast Asia which is able to survive salinities of 500-600 milliosmols by increasing its body osmolytes, mainly Na^+ and Cl^-. The sodium and chloride accounted for 84% of the increase in plasma osmotic pressure. The plasma Na^+ for <u>B. viridis</u> in fresh water was 129 meq/l. In 40% sea water the plasma sodium was 199 meq/l. In 50% sea water the plasma sodium was 249 meq/l, the plasma chloride was 219 meq/l, and the plasma osmolality was 549 milliosmols. Gordon, Schmidt-Nielsen and Kelly (1961) found a similar change in another euryhaline anuran, <u>Rana cancrivora</u>. They found that the plasma Na^+ in this animal was 125 meq/l in fresh water, and 191 meq/l for animals in 50% sea water.

It has been shown that sodium and chloride penetrate the skin of amphibians less quickly than water does (Deyrup, 1964). This occurs as long as the medium concentration is above a low minimal level which is about 10 meq/l for salt depleted Rana esculenta individuals (Krogh, 1937b). Even so, sodium ions pass inward at rates of 20-100 times the rate of movement in the outward direction in isolated frog skin. In these circumstances there is a constant accumulation of sodium ions, and accompanying anions, mainly chloride, from the ambient medium (Krogh, 1937a).

The main nitrogenous waste products of amphibians are ammonia and urea. The aquatic larvae of amphibians excrete ammonia. At metamorphosis most species become ureotelic (Deyrup, 1964). This partially enables the animals to adapt to a terrestrial life (Noble, 1954). Since ammonia is highly toxic a ready supply of water must be available to remove it. Because the water supply must be unrestricted the ratio of ammonia excreted to urea excreted in the adult correlates nicely with the animal's habitat. Cragg et al. (1961) has shown that the more aquatic the animals are, the larger the percentage of waste nitrogen excreted as ammonia (Table 1).

There is also a relationship between the degree of cornification of the skin and the route of ammonia excretion. In Necturus the skin plays a major

Table 1. Correlation of habitat to mode of nitrogen excretion*

SPECIES	HABITAT	% N EXCRETED AS AMMONIA
Salamandra salamandra	Terrestrial	4.7
Triturus cristatus	Terrestrial	4.0
Ambystoma mexicanum	Aquatic	61.9
Xenopus laevis	Aquatic	62.2
Xenopus tropicus	Aquatic	61.7
Hymenochirus spp.	Aquatic	78.0
Pipa pipa	Aquatic	92.5
Rana esculenta	Semiaquatic	9.4
Rana temporaria	Terrestrial	8.2
Hyla aborea	Aboreal	4.6
Bufo bufo	Dry terrestrial	4.8
Bufo calamita	Dry terrestrial	5.7
Crocodylus niloticus	Aquatic	85.5
Caiman crocodilus	Aquatic	92.2

*from: Cragg *et al.* (1961)

role in ammonia excretion. The blood ammonia in Necturus was only 0.7 mg/100 ml, the urinary ammonia was 10.1 mg/100ml (Fanelli and Goldstein, 1964). But only 10% of the total ammonia was excreted by the kidney. The mudpuppy is neotenic and the skin has not metamorphosed. The absense of a cornified epithelial layer of skin allows an easier exit for ammonia than the cornified skin of most amphibians (Fanelli and Goldstein, 1964). On the other hand, Balinsky and Baldwin (1961) found that the aquatic Xenopus laevis excreted 86% of the total ammonia through the kidney. The blood ammonia was 0.05 mg/100ml, and the urine ammonia was 6.1 mg/100ml.

In the euryhaline frog Rana cancrivora the blood urea nitrogen in fresh water was 23.2 mg/100ml. But in 50% sea water the BUN was 162 mg/100ml (Gordon *et al.*, 1961). Urea makes up 5-10% of the plasma osmotic pressure in Bufo viridis (Gordon, 1962). In Xenopus the blood urea nitrogen was 0.17 mg/100ml and the urine urea nitrogen was 19.5 mg/100ml (Balinsky and Baldwin, 1961).

Forster (1954) found that in the Louisiana bullfrog the urine urea concentration equals the plasma urea concentration over a wide range of plasma urea concentrations. In Rana temporaria and Bufo bufo the injection of NaCl solutions into the dorsal lymph sacs caused an antidiuretic response. Injections of urea however, caused diuresis (Eliassen and Jørgensen, 1951).

In toads there is also some indication that the clearance of urea is greater than the glomerular filtration rate (Carlisky, Botbol and Lew, 1967). This may be due to active secretion of urea by the renal tubules. Vogel *et al.*, (1967) felt that this secretion of urea might be coupled with the reabsorbsion of sodium.

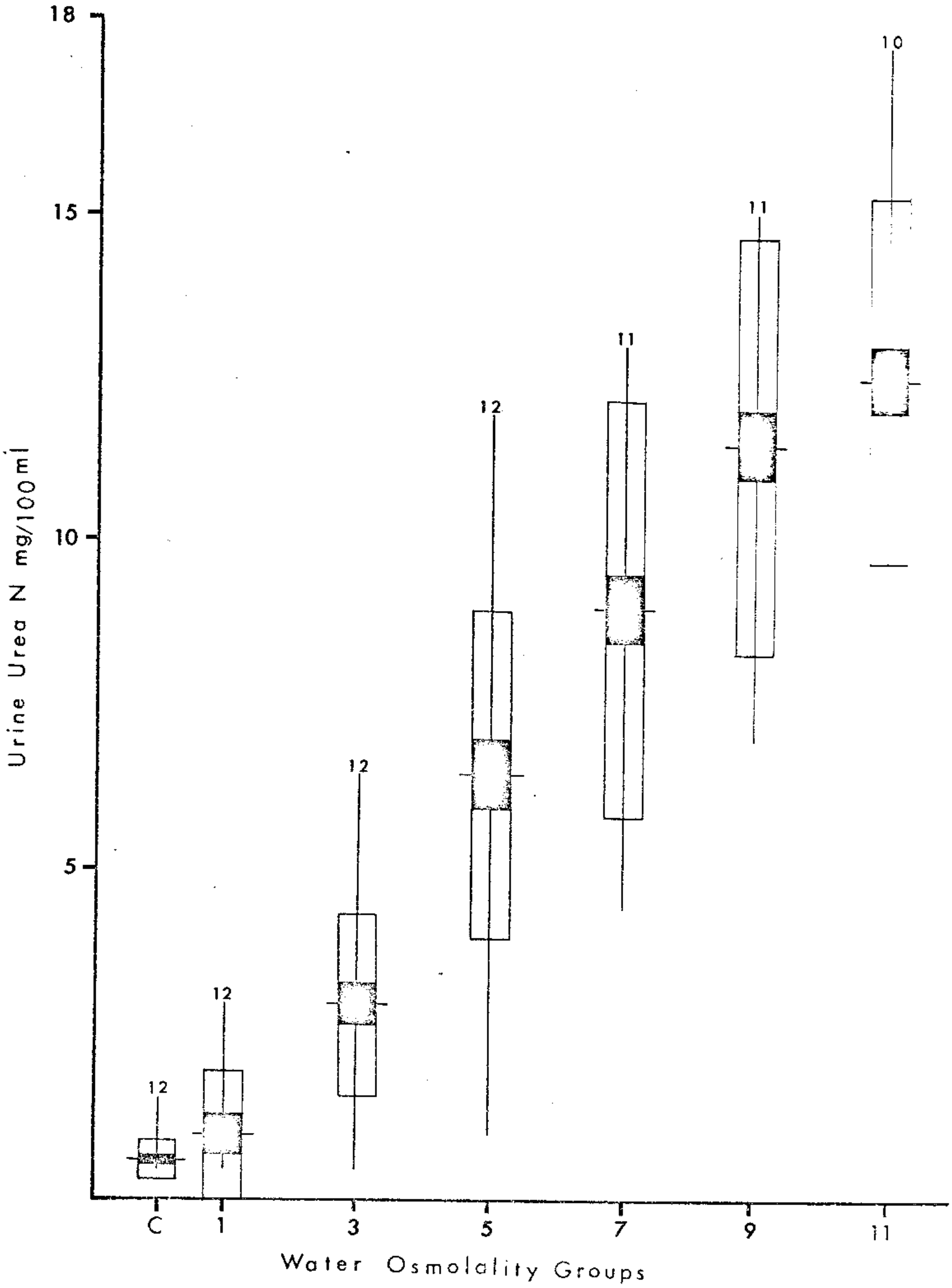
In aquatic amphibians the plasma must be hypertonic to the external environment or dehydration will occur. For animals exposed to hypertonic media the maintenance of the plasma osmotic pressure becomes especially important. The serum osmolality of *B. viridis* in fresh water is 290 milliosmols. In 50% sea water the serum osmolality increases to 560 milliosmols. The urine osmolality also increases, it approaches, but never exceeds, the serum osmolality (Gordon, 1962). *Rana esculenta* individuals exposed to 0.8% NaCl (139 milliosmols) had a serum osmolality of 268 milliosmols. The serum osmolality for this animal in fresh water is 210 milliosmols. The urine remained hypotonic to the serum. The urine osmolality in fresh water was 25 milliosmols, in 0.8% salt water it was 168 milliosmols (Mayer, 1969).

Materials and Methods

Specimens of the hellbender, Cryptobranchus alleganiensis, were collected from the Niangua river below Bennett Springs, Missouri. They were collected during the summer, fall and winter of 1970, and the spring of 1971. They were caught by turning large flat rocks in 2-4 feet of water. As the rocks were turned the animals were carried into a dip net by the current or gently scooped into the net by hand. If they were not unduly frightened they were easily caught.

The animals were transported to the laboratory in moist cloth bags. They were placed in styrofoam coolers or propylene mouse cages containing 3-5 liters of tap water. The animals were kept at room temperature (18-25 C) throughout the experiment. The water was changed once a day, or more if necessary, during a 7-10 day acclimitization period. It was usually necessary because the hellbenders would regurgitate crayfish and small fish during the first few days of captivity. After this acclimitization period a control serum sample was drawn and the animals were placed in the experimental solutions.

The experimental solutions were either NaCl or sucrose. They were mixed in 5.5 gallon (U. S.) (about



18
15
10
5
Urine Urea N mg/100 ml
12
12
12
12
11
11
10
C
1
3
5
7
9
11
Water Osmolality Groups

24 liters) propylene jugs. The osmolality of each 24 liter lot of water was checked with a freezing point depression osmometer (Advanced). The osmolality of the experimental solutions was increased every four days as shown in Table 2. At the end of each four day period a serum sample was drawn. One group of animals was placed directly into 250 milliosmol salt water. Blood samples were drawn at 0.5; 1.5; 4; 8; 19; 30; 48; 70 and 96 hours.

The animals were offered earthworms on the first day in an experimental solution, but not on the other three days.

The Hellbenders were anesthetized in 0.3% Tricaine (Sigma). The osmolality of the anesthetic solution was adjusted to the osmolality of the experimental solutions with NaCl or sucrose.

Two ml of whole blood were drawn by aortic puncture. It was allowed to clot and the serum was removed. Urine samples were obtained by bladder puncture with a 21 gauge needle. If the tests were not performed within two hours the serum was frozen at -10 C.

Blood urea nitrogen, blood ammonia nitrogen, urine urea nitrogen and urine ammonia nitrogen were measured colorimetrically using the urease method of Searcy et al. (1961).

Table 2. A schedule of exposure of Hellbenders to experimental solutions of increasing osmolality

<u>NaCl solutions</u>						
Day	1-4	5-8	9-12	12-16	17-20	
Solution osmolality milliosmols	66-80	127-136	169-182	210-213	249-259	
<u>Sucrose solutions</u>						
Day	1-4	5-8	9-12	12-16	17-20	21-24
Solution osmolality milliosmols	30-32	89-91	151-152	189-202	233-240	266-273

Osmolality of tap water: 9 milliosmols

Fig. 3. Comparison of urine urea nitrogen and blood urea nitrogen. All animals in sucrose.

Sodium and potassium of both the serum and urine were measured simultaneously on a flame photometer (IL). Serum and urine osmolality were measured either in 2.0 or 0.2 ml aliquots with an Advanced osmometer. Serum protein was determined with a protein refractometer if the quantity of serum available was small, or with biuret reagent (bovine albumin standard) if there was sufficient serum.

Serum proteins were applied in 3 microliter aliquots to polycellulose acetate strips. Electrophoresis was performed for one hour at 400 volts. Beckman HR buffer, (Tris-Barbital-Sodium Barbital, Gelman Instrument Co.), pH 8.6, ionic strength 0.75 was used. The strips were stained in ponceau G, cleared in methanol-acetic acid, and scanned in a Gelman densitometer. Percentage area under the curve for albumin and globulins was measured with a planimeter.

Six animals were sacrificed immediately after collection. They were exsanguinated. A two ml serum sample was used to determine calcium; inorganic phosphorus; glucose; BUN; uric acid; cholesterol; total protein; albumin; total bilirubin; alkaline phosphatase; lactic dehydrogenase; and serum glutamic oxalacetic transaminase on an SMA 12/60 autoanalyzer.

Results

The data presented in this section are the combined data from both the NaCl and sucrose solutions. Using Duncan's new multiple range test (Duncan, 1955) no significant difference was noted between the salt and sucrose water baths.

The mean blood urea nitrogen concentration for hellbenders in tap water (9 milliosmols) is 1.4 mg/100ml. This value increases to a mean of 33.6 mg/100ml for animals in 266-273 milliosmols water (Fig. 1). The urine urea is normally low. For animals in tap water it is 0.6 mg/100ml. As the osmolality of the water increases the urine urea also increases to a mean of 12.7 mg/100ml, (266-273 milliosmols water) (Fig. 2). There is a direct relationship between the urine urea nitrogen and the blood urea nitrogen (Fig. 3).

Ammonia is normally found in the blood only in trace amounts. However, the urine ammonia nitrogen is high, 17 mg/100ml for animals in tap water. As the water osmolality increases to 266-273 milliosmols the urine ammonia decreases to 3 mg/100ml (Fig. 4). There is an inverse relationship between the urine ammonia N excreted and the blood urea nitrogen (Fig. 5). The

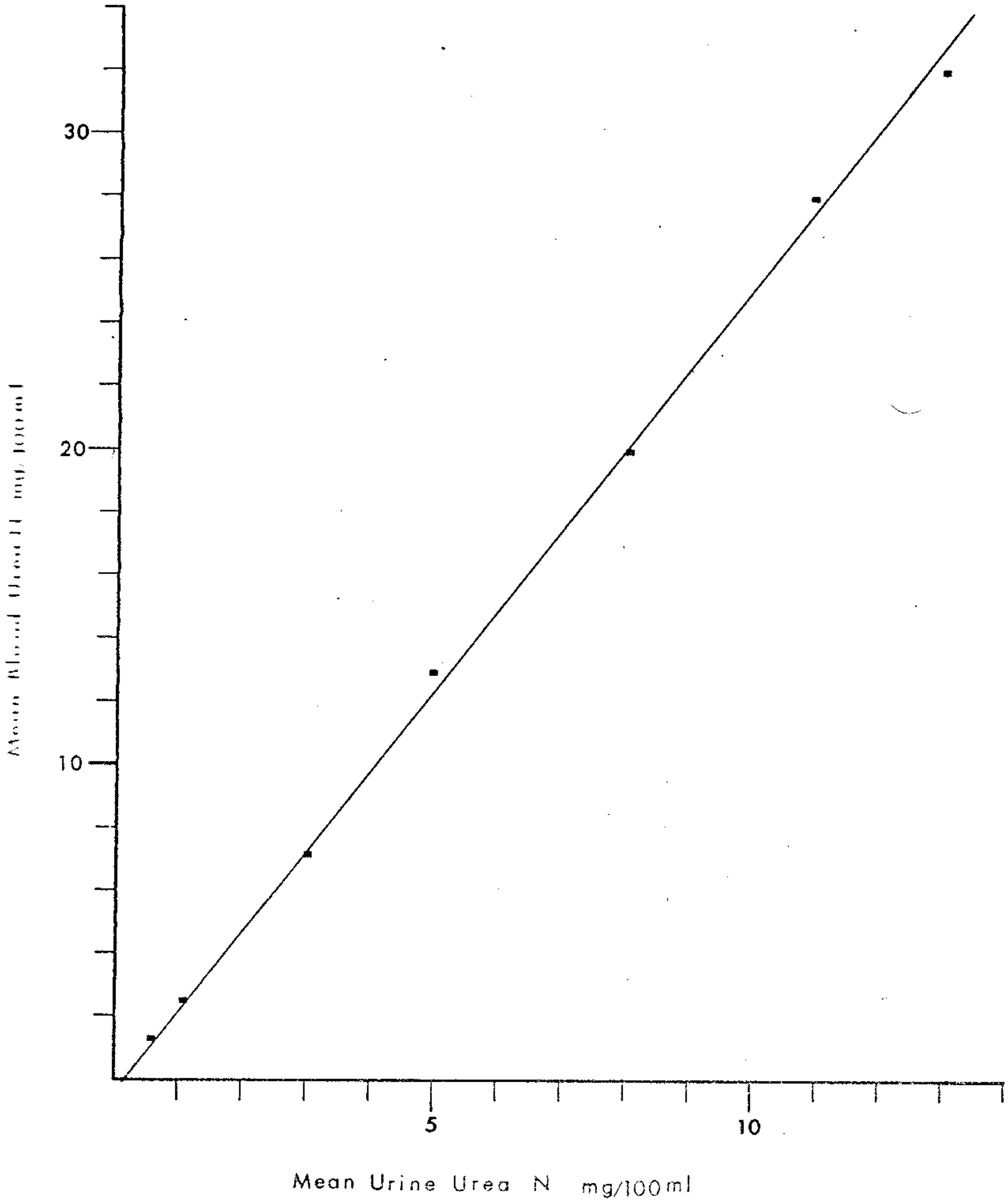

30
20
10
5
10
Mean Urine Urea N mg/100ml

Fig. 1. Effect of increasing water osmolality on blood urea nitrogen. Key to water osmolality in milliosmols. C=9; 1=30-32; 2=66-80; 3=89-91; 4=127-136; 5=151-152; 6=169-182; 7=189-202; 8=210-213; 9=233-240; 10=249-259; 11=266-273. N stands for a group in NaCl, S stands for a group in sucrose. Vertical line indicates range, horizontal line indicates mean, open box is the standard deviation, closed box is the standard error. Numbers indicate the sample size.

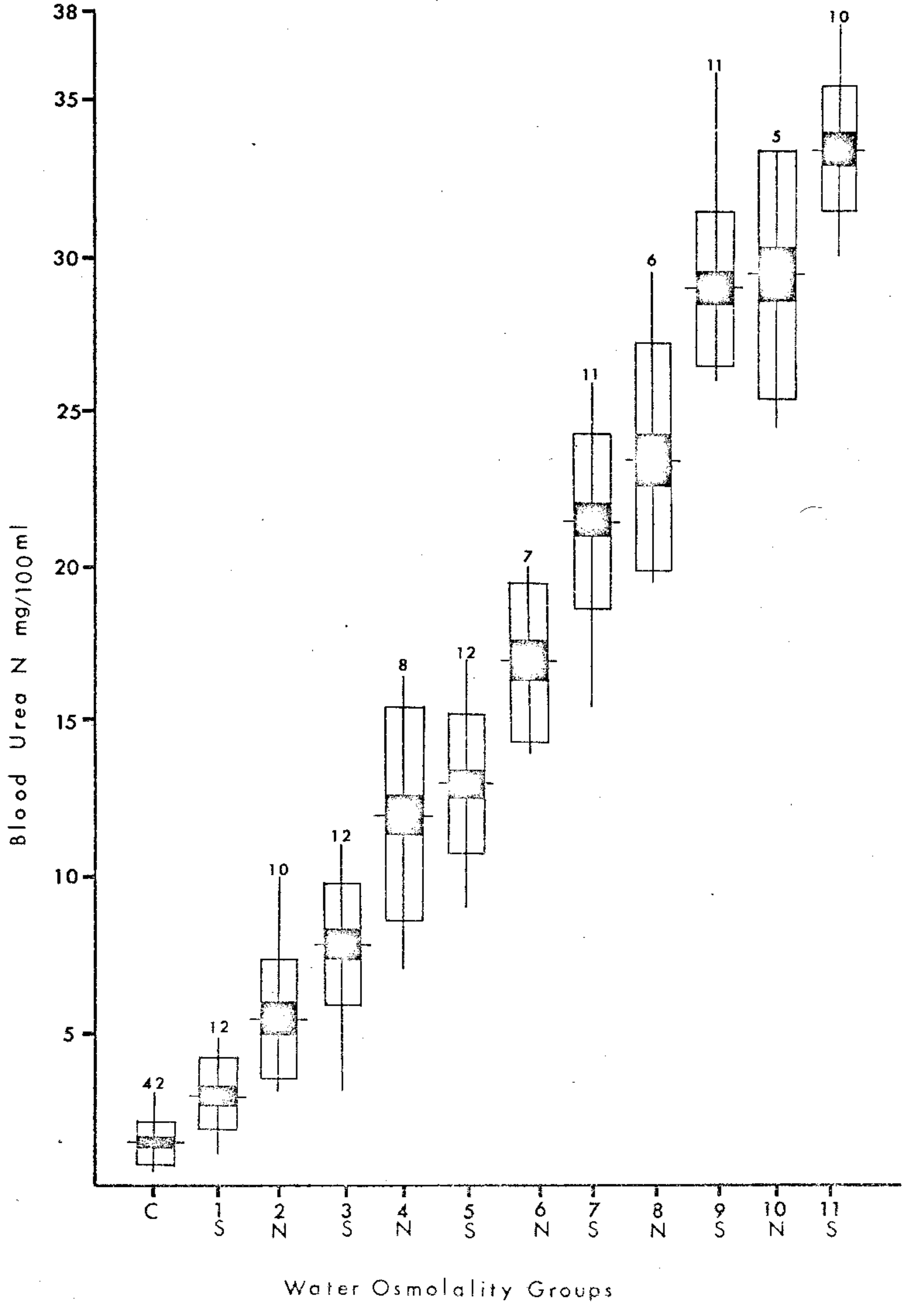
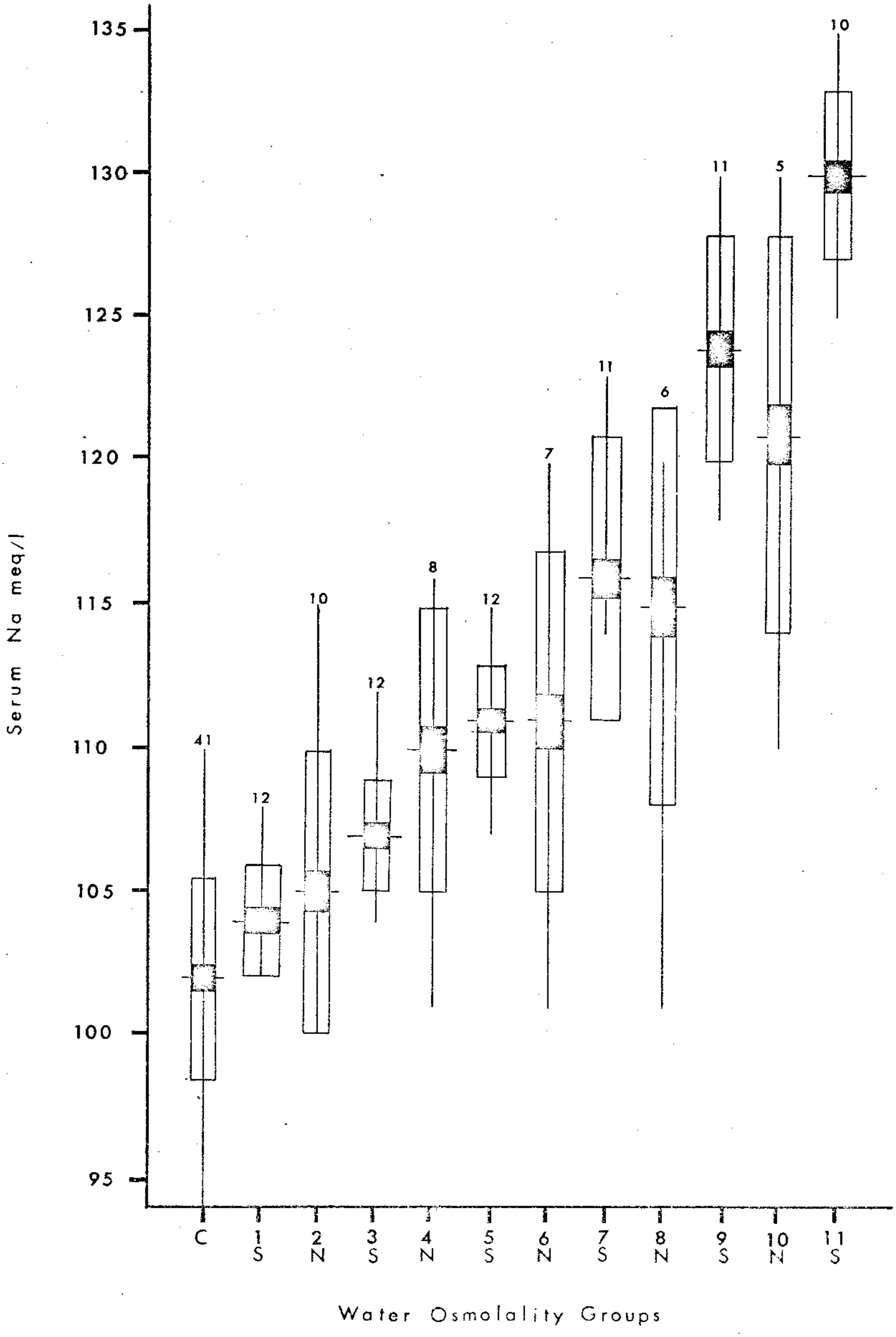

Blood Urea N mg/100 ml
38
35
30
25
20
15
10
5
42
12
10
12
8
12
7
11
6
11
5
10
C
1 S
2 N
3 S
4 N
5 S
6 N
7 S
8 N
9 S
10 N
11 S
Water Osmolality Groups

Fig. 4. Effect of increasing water osmolality on urine ammonia nitrogen. Key to water osmolality in milliosmols. C=9; 1=30-32; 3=89-91; 5=151-152; 7=189-202; 9=233-240; 11=266-273. All animals in sucrose. Vertical line indicates range, horizontal line indicates mean, open box is the standard deviation, closed box is the standard error. Numbers indicate the sample size.

Fig. 2. Effect of increasing water osmolality on urine urea nitrogen. Key to water osmolality in milliosmols. C=9; 1=30-32; 3=89-91; 5=151-152; 7=189-202; 9=233-240; 11=266-273. All animals in sucrose. Vertical line indicates range, horizontal line indicates mean, closed box is the standard deviation, open box is the standard error. Numbers indicate the sample size.



Serum Na meq/l
135
130
125
120
115
110
105
100
95
41
12
10
12
8
12
7
11
6
11
5
10
C
1 S
2 N
3 S
4 N
5 S
6 N
7 S
8 N
9 S
10 N
11 S
Water Osmolality Groups

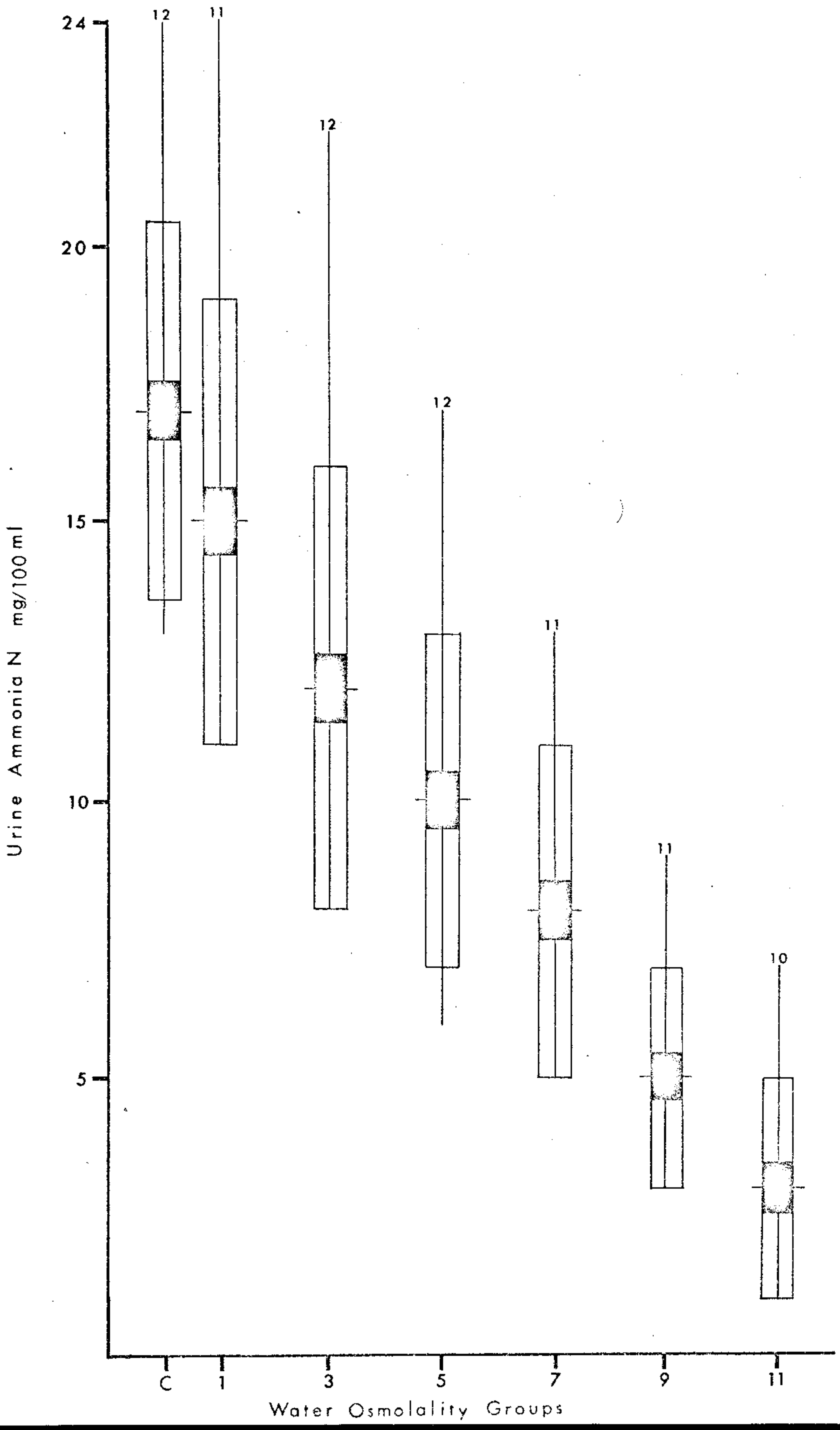
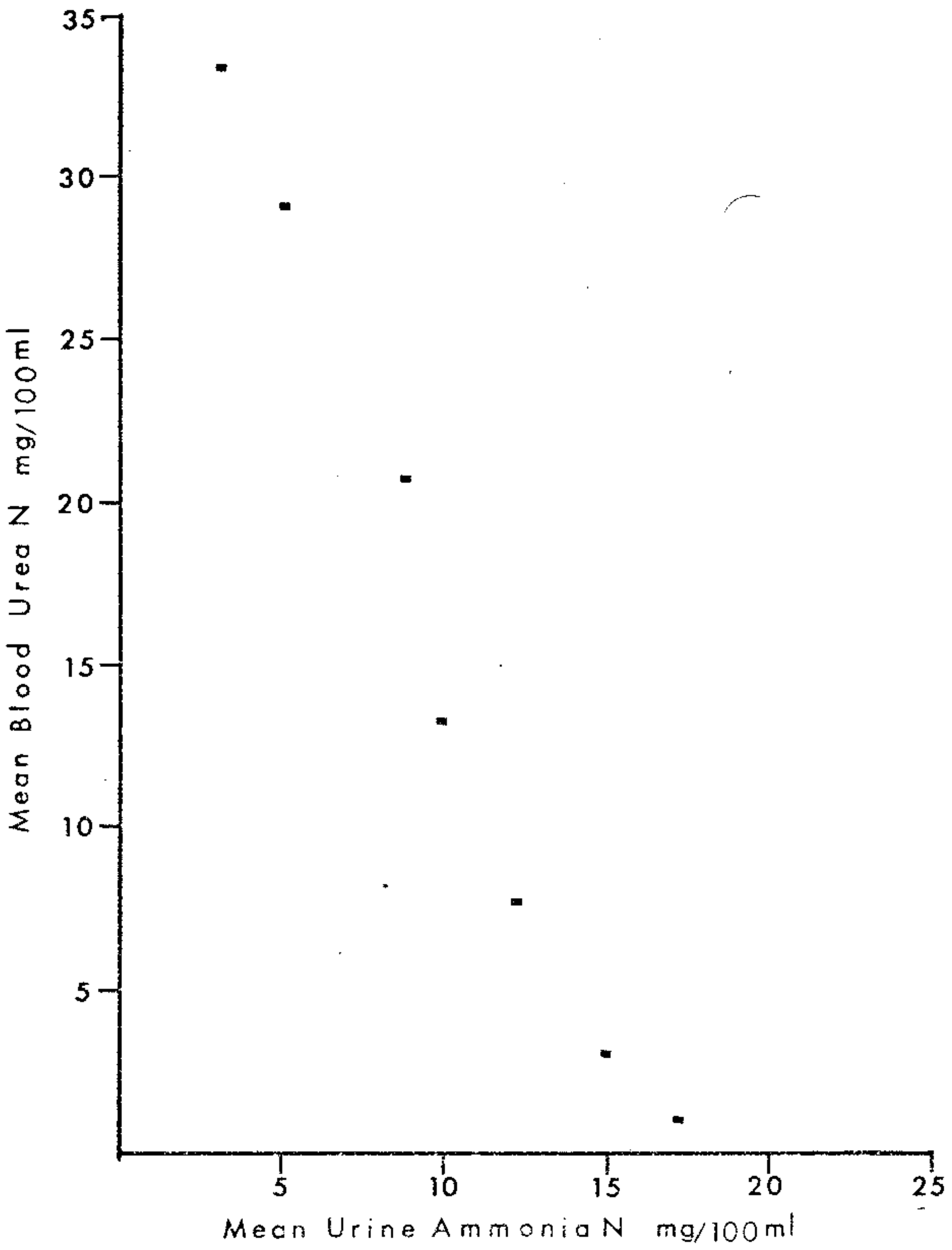

Urine Ammonia N mg/100 ml
Water Osmolality Groups
24
20
15
10
5
C
1
3
5
7
9
11
12
11
12
12
11
11
10

Fig. 5. Comparison of urine ammonia nitrogen and blood urea nitrogen. All animals in sucrose.

Fig. 7. Comparison of urine sodium and serum sodium. All animals in sucrose.


35
30
25
20
15
10
5
Mean Blood Urea N mg/100ml
5
10
15
20
25
Mean Urine Ammonia N mg/100ml

hellbender is ammoniotelic at low water osmolalities and ureotelic at high water osmolalities (Table 3).

The serum sodium is 102 meq/l for animals in tap water. This value increases to a mean of 130 meq/l for animals in 266-273 milliosmols water (Fig. 6). The urine sodium concentration is inversely proportional to the serum sodium concentration (Fig. 7). The urine sodium concentration decreases as the water osmolality increases (Fig. 8).

The serum osmolality reflects the increases in serum osmolytes such as sodium and urea. Animals in tap water had a mean serum osmolality of 200 milliosmols. Cryptobranchus in 266-273 milliosmol water had a mean serum osmolality of 239 milliosmols (Fig. 9). The hellbenders are hypertonic with respect to the external environment up to about 230 milliosmols. After that they are isotonic, or nearly so, with the external environment.

Of the 35 milliosmol increase in serum osmolality, sodium accounts for 25 milliosmols, and urea accounts for 6 milliosmols. The serum potassium shows no significant change over the range of experimental solutions used.

The serum proteins showed no significant change in the albumin to globulin ratio over the range of osmolalities used in this problem. The A/G ratio was

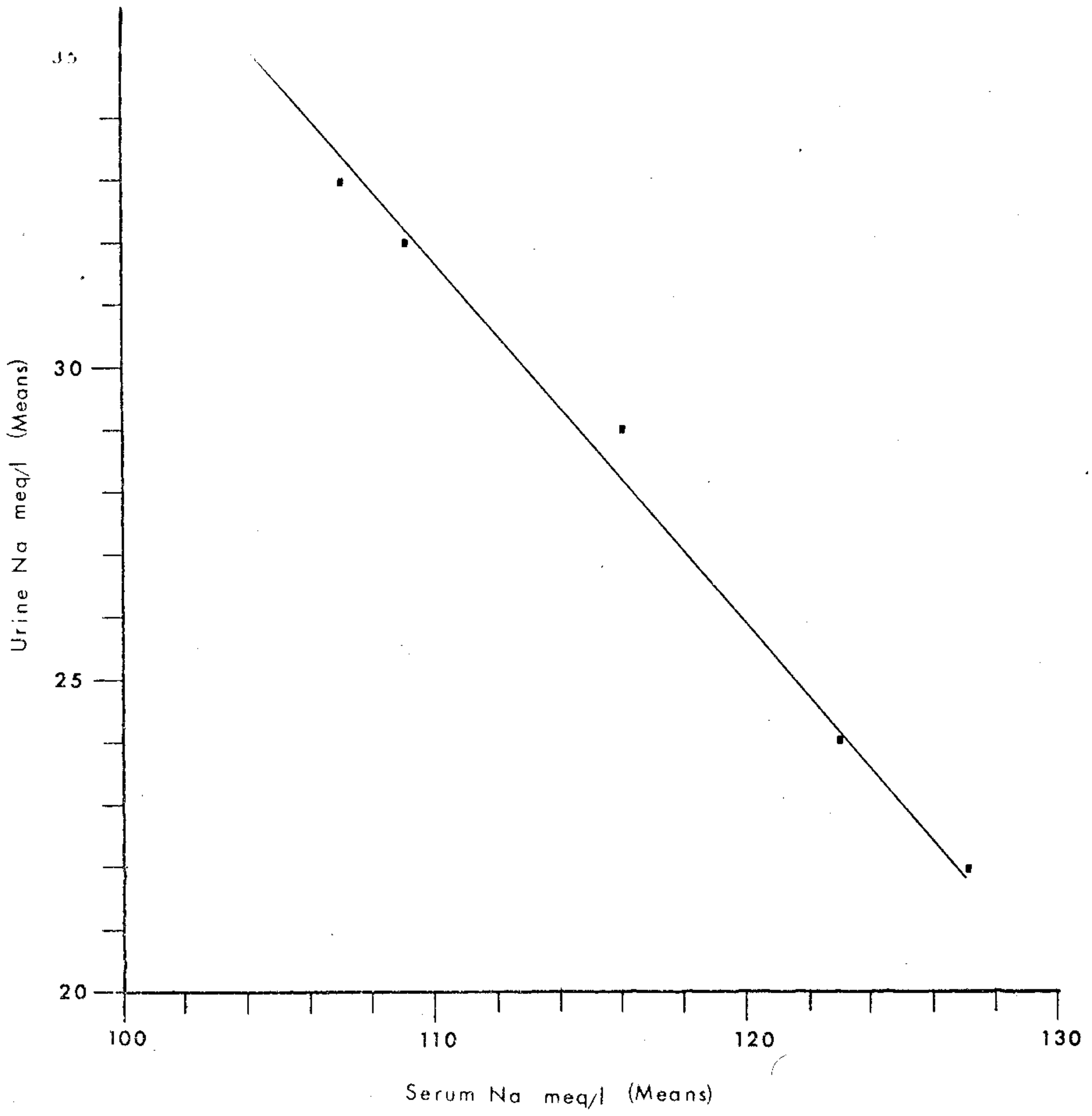

Urine Na meq/l (Means)
30
25
20
100
110
120
130
Serum Na meq/l (Means)

Table 3. Percentage of total nitrogen excreted as ammonia.

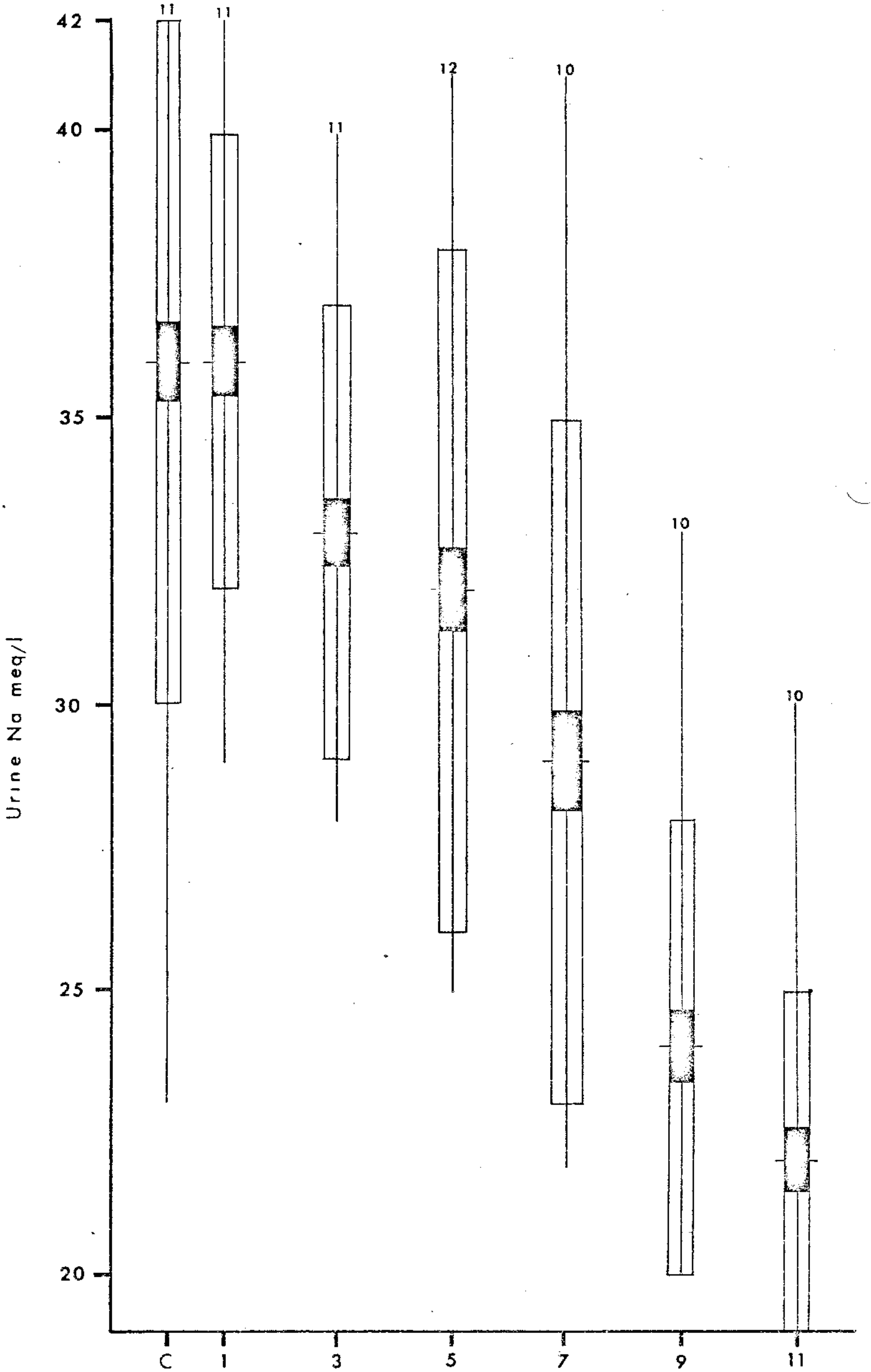
Water Osmolality*	Mean ammonia nitrogen**	Mean urea nitrogen**	Percentage of total nitrogen excreted as ammonia
9	17	0.6	94
30-32	15	1.0	94
89-91	10	6.0	62
189-202	8	9.0	47
233-240	5	11.0	31
266-273	3	13.0	19

*in milliosmols

** in mg/100ml

Fig. 6. Effect of increasing water osmolality on serum sodium. Key to water osmolality in milliosmols. C=9; 1=30-32; 2=66-80; 3=89-91; 4=127-136; 5=151-152; 6=169-182; 7=189-202; 8=210-213; 9=233-240; 10=249-259; 11=266-273. N stands for a group in NaCl, S stands for a group in sucrose. Vertical line indicates range, horizontal line indicates mean, open box is the standard deviation, closed box is the standard error. Numbers indicate the sample size.

Fig. 8. Effect of increasing water osmolality on urine sodium. Key to water osmolality in milliosmols. C=9; 1=30-32; 3=89-91; 5=151-152; 7=189-202; 9=233-240; 11=266-273. All animals in sucrose. Vertical line indicates range, horizontal line indicates mean, closed box is the standard error, open box is the standard deviation. Numbers indicate the sample size.

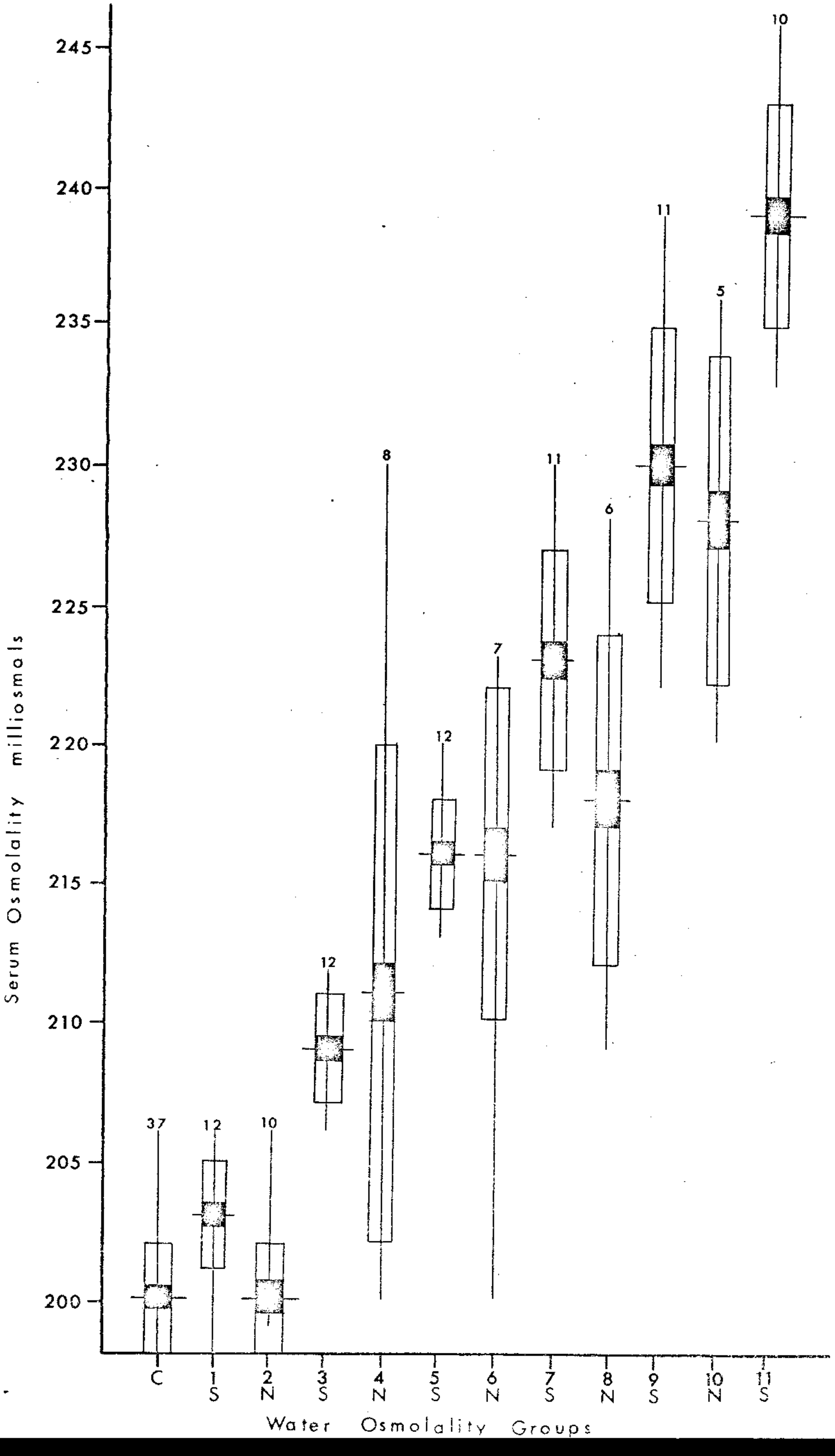

11
11
12
10
11
10
10
42
40
35
30
25
20
Urine Na meq/l
C
1
3
5
7
9
11
Water Osmolality Groups

Discussion

The hellbender, *Cryptobranchus alleganiensis* can regulate its blood osmolality up to about 230 milliosmols. It then becomes an osmoconformer up to about 250 milliosmols. This is approximately 25% sea water. Part of this increase comes from an increase in the blood concentration of the small organic molecule of urea. However, the increase in urea accounts for only 6 milliosmols of the 38 milliosmol total increase in serum osmolality. The value of 33 mg/100ml, although it is much higher than the value for animals in fresh water, may not be a true physiological uremia. Mammals, for instance, can tolerate 150-200 mg/100ml blood urea nitrogen without permanent damage (Prosser *et al.*, 1950). The euryhaline frog *Rana cancrivora* has urea values of 162 mg/100ml in 50% sea water (Gordon *et al.*, 1961). Even though they are not treated in this thesis, questions about even the low value of 33mg/100ml affecting the oxygen binding capacity of hemoglobin and denaturing some enzymes must be asked. If the hellbender was affected in this way it was not apparent.

The excretion of urea in *Cryptobranchus* appears to follow the pattern of the Louisiana bullfrog studied by Forster (1964). There is one significant difference.

Fig. 9. Effect of increasing water osmolality on serum osmolality. Key to water osmolality in milliosmols. C=9; 1=30-32; 2=66-80; 3=89-91; 4=127-136; 5=151-152; 6=169-182; 7=189-202; 8=210-213; 9=233-240; 10=249-259; 11=266-273. N stands for a group in NaCl, S stands for a group in sucrose. Vertical line indicates the range, horizontal line indicates the mean, open box is the standard error, closed box is the standard deviation. Numbers indicate the sample size.


Serum Osmolality milliosmals
245
240
235
230
225
220
215
210
205
200
37
12
10
12
8
12
7
11
6
11
5
10
C
1 S
2 N
3 S
4 N
5 S
6 N
7 S
8 N
9 S
10 N
11 S
Water Osmolality Groups

In the frog the plasma urea concentration must be greater than 15 mg/100ml for the urine urea concentration to equal the plasma urea concentration. In the hellbender the excretion of urea increases as the BUN increases, no matter what the blood concentration is. This indicates that there is no active or facilitated transport into or out of the renal tubules.

Ammonia is the predominate nitrogenous excretory product of the hellbender in fresh water. Since this animal is totally aquatic this is to be expected. Ammonia is formed in the kidney of Necturus (Fanelli and Goldstein, 1964) and is activly secreted into the acid urine of the tubules. It has been sugested by Cragg et al. (1961) that this could aid in the retention of univalent ions by simple exchange. This possibility seems remote in Cryptobranchus under these experimental conditions. As the amount of ammonia excreted decreases the amount of sodium excreted also decreases.

The hellbender is ammoniotelic in fresh water. But as the osmolality of the water increases the animal becomes ureotelic. This switch in the mode of nitrogen excretion resembles the switch that occurs in the estivating South African lungfish, Protopterus (Prosser et al., 1950). The mechanism for this switch is not known. It definitly has selective advantage in situations where the availability of water is restricted. The fact that this

0.39. The serum proteins did show the effects of repeated sampling. The total protein decreased from a mean of 4.0 gm/100ml at the time the first sample was drawn to a mean of 2.8 gm/100ml by the time the seventh sample was drawn 24 days later. This represents a decrease in the total serum osmolality, but the exact amount of decrease is not known.

A summary of the data from the animals placed into 250 milliosmols NaCl is given in Table 4. All the changes in serum constituents take place in 96 hours. The blood urea nitrogen increases after 1.5 hours. The urine ammonia nitrogen shows a decrease after 8 hours. The urine urea nitrogen increases after 19 hours. And the serum sodium increases after 8 hours.

The results of the analysis of the six serum samples by the SMA 12/60 autoanalyzer are seen in Table 5. Most of the values for the hellbender are lower than the accepted values for humans except the enzymes and the inorganic phosphorus. The serum calcium falls within the accepted mammalian value.

Table 4. Mean serum and urine values for animals placed directly into 250 milliosmol water.* N equals 5.

Time in hours	Blood urea nitrogen mg/100ml	Urine urea nitrogen mg/100ml	Serum sodium meq/l	Urine ammonia nitrogen mg/100ml	Total Protein
0.0	1.5 (0.7)	1.1 (0.9)	100 (1)	17 (2)	4.1 (.2)
0.5	1.6 (0.5)	1.1 (0.6)	101 (1)	18 (3)	4.0 (.4)
1.5	2.7 (1.3)	1.1 (0.9)	101 (1)	17 (2)	3.8 (.4)
4.0	2.4 (1.3)	1.4 (0.3)	102 (1)	16 (2)	3.5 (.4)
8.0	3.6 (0.5)	1.6 (0.6)	104 (1)	15 (2)	3.5 (.3)
19.0	6.5 (0.6)	4.3 (1.0)	106 (2)	14 (2)	3.2 (.3)
30.0	9.3 (1.0)	6.9 (1.0)	107 (2)	11 (2)	3.1 (.3)
48.0	11.5 (2.0)	8.0 (1.4)	109 (2)	8 (1)	3.0 (.1)
70.0	17.9 (1.7)	10.6 (0.9)	112 (2)	7 (2)	2.9 (.2)
96.0	23.3 (1.7)	14.1 (2.0)	115 (2)	6 (2)	2.8 (.2)

*Standard deviation of the mean given in parentheses.

switch can occur rapidly is shown by the data from the animals placed directly into 250 milliosmol water. The changes all took place within 96 hours.

The serum sodium represents 80% of the total increase in serum osmolality in the hellbender. The sodium comes in part from the medium, for those animals kept in sodium chloride, and by reabsorbsion from the kidney or bladder for those animals kept in sucrose. Feeding the hellbenders also provided a source of electrolytes and nitrogenous compounds. The animals might have imbibed some salt water, but they were never observed doing so. They probably observed some sodium through the skin although the exact role of the skin is outside the scope of this thesis. The hormones involved in the absorbsion or reabsorbsion of sodium through the skin, kidney or bladder have been studied by several investigators. The "amphibian water balance principle" from the neurohypophysis (Jørgensen, 1950), and the adrenal cortical steroids (Dow and Zuckerman, 1938) are implicated in salt and water balance. Jørgensen, Levi and Ussing (1946) found that neurohypophyseal extracts injected into axolotls increased sodium uptake through the skin. Heller (1941) first studied the water balance hormone from the neurohypophysis of amphibians. He said that this hormone is not the same as the mammalian antidiuretic

Table 5. Mean values of various serum constituents as measured by the SMA 12/60 autoanalyzer* N equals 6.

Serum constituent	Mean value
Calcium	9.4 (1.4) meq/l
Inorganic Phosphorus	5.3 (0.8) mg/100ml P
Glucose	56 (25) mg/100ml
Blood Urea Nitrogen	1.4 (0.7) mg/100ml N
Uric Acid	0.4 (0.1) mg/100ml
Cholesterol	86 (49) mg/100ml
Total protein	3.0 (0.6) g/100ml
Albumin	0.4 (0.1) g/100ml
Alkaline Phosphatase	224 (70) microunits/ml
Lactic Dehydrogenase	265 (219) microunits/ml
Serum Glutamic Oxaloacetic Transaminase	145 (34) microunits/ml
Total Bilirubin	0.1 (0) mg/100ml

*Standard deviation of the mean given in parentheses.

hormone.

The role of the adrenal steroids in electrolyte conservation is far from clear. Crabbe (1964) found that injections of 20 micrograms of aldosterone into Rana esculenta and Rana ridibunda, and 50 micrograms to Bufo marinus caused increased active transport of sodium through the isolated skin and bladder. Dow and Zuckerman (1938) reported a decrease in body weight after administration of deoxycorticosterone acetate (DCA) to the axolotl. This increase was due to the loss of body water. However, Hseih (1950) found an increase in frog weight after DCA injections. In Rana pipiens no effect was observed on water balance after injection of DCA, deoxycorticosterone glucoside, cortisone or ACTH (Sawyer, Travis and Levinsky, 1950).

Dehydration does not appear to play a major role in the response of Cryptobranchus to hypertonic media. If simple dehydration were occuring all serum constituents would increase in about the same ratios. This is not the case. The serum sodium shows a percentage increase of 27%. The blood urea nitrogen increases 25 times and the serum potassium shows no change. Likewise with the urine, if it were merely becoming more concentrated, it would be unlikely that one constituent would increase while two others decreased.

The serum proteins show the effects of repeated sampling with the same animal. The blood proteins are not replaced quickly. Since blood proteins exert an osmotic pressure continued blood collecting will lower serum osmolality. Cryptobranchus shows the typical larval protein pattern of a low albumin to globulin ratio. An increase in albumin or globulins would increase the serum osmolality, but this did not happen.

The results of the analysis by the SMA 12/60 autoanalyzer are interesting. All the values except the enzymes, calcium, and inorganic phosphorus were below the normal human values. The intestinal mucosa contains acid phosphatase. It has been shown that in humans intestinal parasites can cause an increase in acid phosphatase (Cohn and Kaplan, 1966). There were many intestinal nematodes in the Hellbenders from the Niangua river. This might account for the increase in acid phosphatase. The increases in the other enzymes must go unexplained for the present.

Summary

The hellbender, *Cryptobranchus alleganiensis*, is able to regulate its body osmolality up to about 230 milliosmols. It then becomes an osmoconformer up to about 260 milliosmols. There are increases in several body osmolytes.

The blood urea nitrogen increases from a mean of 1.4 mg/100ml in tap water to a mean of 33 mg/100ml for animals in 266-273 milliosmol water.

The serum sodium increases from a mean of 102 meq/l for animals in tap water to 130 meq/l for animals in 266-273 milliosmol water. The urine sodium concentration decreases indicating some method of sodium retention in the hellbender. There is no significant change in the serum or urine potassium concentrations.

The percentage of total nitrogen excreted as ammonia decreases as the water osmolality increases. This indicates a change from ammoniotelism to ureotelism.

The serum osmolality increases from a mean of 200 milliosmols for animals in tap water to a mean of 239 milliosmols for animals in 266-273 milliosmol water.

The serum proteins are larval. There is no change in the A/G ratio throughout the experiment. The

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total protein declines throughout the experiment.

A survey of 12 serum constituents was made by an autoanalyzer. Most values for the hellbender were less than the normal values for humans. The enzymes LDH, SGOT and alkaline phosphatase were elevated.

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Appendix A

Animals placed in
sodium chloride
solutions

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Animal Number	Water Osmolality[a]	BUN[b]	Blood Ammonia[b]	K^{+c}	Na^{+c}	Serum osmolality[a]	Total Protein[d]
C101	9	2.5	0	4.8	94		
C101	9	1.5	0				
C102	9	2.0	0	3.8	102		
C104	9	0.5	0	3.7	105		
C105	9	0.5	0	3.4	104		
C106	9	1.5	0	3.3	104	206	
C107	9	1.5	0	2.8	101	200	
C108	9	1.0	0	3.0	101	204	
C109	9	1.0	0	3.3	103	208	
C110	9	0.5	0	2.6	105	205	
C111	9	1.5	0	3.6	106	209	
C112	9	2.5	0	4.5	98	198	
C113	9	1.0	0	3.4	110	200	
C115	9	2.0	0	2.7	106	208	
C116	9	2.0	0	2.0	102	200	
C117	9	1.0	0	2.6	98	194	
C118	9	1.5	0	2.0	104	201	
C119	9	2.0	0	2.0	104	206	
C120	9	2.0	0	2.1	103	207	
C122	9	2.5	0	2.4	100	199	3.8
	66	5.5	0	2.6	108	204	3.5
	136	13.5	0	3.1	112	217	3.4
	175	14.0	0	2.5	111	220	3.5
C123	9	0.5	0	3.0	97	195	4.2
	66	4.0	0	2.9	103	200	3.7
	136	16.5	0	3.3	112	230	3.9

[a] milliosmols

[b] mg/100ml

[c] meq/l

[d] g/100ml

Animal Number	Water Osmolality[a]	BUN[b]	Blood Ammonia[b]	K^{+}[c]	Na^{+}[c]	Serum osmolality[a]	Total Protein[d]
C124	9	1.5	0	2.5	102	190	3.6
	66	7.0	0	2.5	110	198	3.4
	136	16.5	0	2.8	114	209	3.4
	175	20.0	0	2.9	118	215	3.2
	213	29.5	0	2.9	120	220	3.0
	257	33.0	0	3.1	120	231	2.5
C125	9	0.5	0	3.6	104	204	3.8
C126	9	1.5	0	2.5	98	198	4.0
	73	3.0	0	2.9	100	198	3.5
C127	9	2.0	0	4.1	109	206	4.5
	80	6.0	0	3.1	112	206	4.2
	129	9.0	0	3.3	116	212	4.0
	182	17.0	0	2.5	120	219	4.0
	213	19.5	0	2.9	120	216	3.3
	253	27.0	0	2.5	125	223	3.8
C132	9	0.5	0	3.3	97	180	4.0
	80	4.0	0	3.6	101	195	3.7
	129	11.0	0	2.9	101	200	3.5
	182	18.5	0	3.6	110	207	3.5
	213	22.0	0	2.5	120	209	3.1
	253	29.0	0	4.6	130	220	3.3
C134	9	2.0	0	4.1	109	206	4.5
	75	4.5	0	2.1	100	203	3.5
	127	7.0	0	2.3	104	208	3.5
	170	14.5	0	2.8	108	211	3.0
	210	21.0	0	2.5	101	215	
	249	24.5	0	3.1	110	230	3.8

[a] milliosmols
[b] mg/100ml
[c] meq/l
[d] g/100ml

Animal Number	Water[a] Osmolality	BUN[b]	Blood Ammonia[b]	K^{+c}	Na^{+c}	Serum osmolality[a]	Total Protein[d]
C137	9	2.0	0	3.3	108	180	4.0
	75	4.0	0	2.0	115	200	3.4
C138	9	0.5	0	3.0	98	198	3.5
	75	6.0	0	3.0	101	198	3.2
	127	9.5	0	2.5	110	203	3.2
	170	15.0	0	2.8	109	214	3.0
	210	23.0	0	2.0	115	218	2.9
C141	9	3.0	0	3.8	106	193	4.3
	73	10.0	0	4.1	104	195	4.0
	132	13.5	0	2.5	111	206	3.5
	169	20.0	0	3.1	101	223	3.6
	210	25.5	0	3.0	112	228	3.5
	259	33.5	0	5.2	119	236	3.0

a milliosmols
b mg/100ml
c meq/l
d g/100ml

Appendix B

Animals placed in

Sucrose solutions

Animal Number	Water[a] Osmolality	BUN[b]	Blood[b] Ammonia	K^{+}[c] Serum	Na^{+}[c] Serum	Serum[a] Osmolality	Urine urea[b] nitrogen	urine ammonia[b] nitrogen
C142	9	1.0	0	2.1	101	201	1.5	15
	30	1.5	0	2.1	103	200	1.5	12
	90	8.5	0	2.4	104	206	3.0	10
	152	14.0	0	2.0	110	213	5.0	8
	189	26.0	0	2.6	115	221	7.0	7
	233	30.5	0	2.9	118	222	12.0	4
	266	35.5	0	2.3	126	235	13.5	3
C143	9	1.0	0	2.3	102	200	0.5	19
	30	2.0	0	2.3	102	204	0.5	18
	90	7.0	0	2.0	106	210	2.5	15
	152	14.5	0	2.1	110	215	4.0	13
	189	20.0	0	2.5	117	219	5.5	12
	233	27.0	0	2.5	122	223	9.0	5
	266	33.0	0	2.0	131	233	11.0	1
C144	9	0.5	0	3.0	100	200	0.5	17
	30	1.5	0	3.1	105	198	0.5	14
	90	8.0	0	3.0	106	209	2.0	11
	152	16.5	0	2.9	107	217	4.5	10
	189	22.0	0	2.9	114	217	8.5	8
	233	28.0	0	3.0	121	227	8.0	4
	266	34.5	0	3.3	129	237	9.0	4

a milliosmols

b mg/100ml

c meq/l

Animal Number	Water[a] Osmolality	BUN[b]	Blood[b] Ammonia	K^{+}[c] Serum	Na^{+}[c] Serum	Serum[a] Osmolality	Urine[b] Urea N	Urine[b] Ammonia N
C164	9	1.0	0	2.1	102	200	0.5	15
	32	4.5	0	2.3	102	202	0.5	12
	89	7.5	0	2.4	106	210	2.0	10
	152	13.0	0	2.3	110	215	2.5	6
	197	20.0	0	2.4	116	223	4.5	5
	238	28.0	0	2.2	124	231	7.0	3
	268	33.0	0	2.2	129	237	10.5	2
C165	9	1.0	0	2.2	100	204	0.5	18
	32	3.5	0	2.6	104	202	0.5	
	89	9.0	0	2.4	109	207	4.0	12
	152	17.0	0	2.4	112	220	8.0	9
C166	9	1.0	0	2.4	102	199	0.5	16
	32	2.0	0	2.3	106	200	0.5	15
	89	6.0	0	2.4	108	208	3.0	12
	152	10.0	0	1.3	109	217	6.0	10
	197	21.0	0	2.3	120	223	12.0	9
	238	36.0	0	2.3	130	239	14.5	6
C167	9	1.0	0	6.0	98	202	0.5	13
	32	1.5	0	2.1	105	206	0.5	11
	89	7.5	0	2.1	107	210	3.5	8
	152	13.0	0	2.0	110	214	7.0	6
	197	20.0	0	2.0	120	219	9.0	5
	238	28.0	0	2.3	124	228	13.5	3
	268	32.0	0	2.1	128	237	14.0	3

a milliosmols

b mg/100ml

c meq/l

Animal Number	Water[a] Osmolality	Na^{+}[b] Urine	K^{+}[b] Urine	Total Protein[c]
C142	9	33	1.0	4.3
	30	36	0.3	4.0
	90	30	0.7	3.8
	152	41	0.5	3.5
	189	26	0.6	3.4
	233	23	0.7	3.0
	266	21	0.5	2.8
C143	9	41	0.4	4.1
	30	40	0.5	3.9
	90	35	0.6	3.8
	152	38	0.5	3.5
	189	50	0.5	3.2
	233	33	0.5	3.0
	266	30	0.7	3.1
C144	9	39	0.5	4.2
	30	34	0.3	4.0
	90	33	0.4	3.7
	152	30	0.4	3.5
	189	25	0.5	3.4
	233	24	0.4	3.0
	266	21	0.5	3.0

[a] milliosmols

[b] meq/l

[c] g/100ml

Animal Number	Water[a] Osmolality	BUN[b]	Blood[b] Ammonia	K^{+c} Serum	Na^{+c} Serum	Serum[a] Osmolality	Urine[b] Urea N	Urine[b] Ammonia N
C145	9	1.0	0	2.7	104	204	1.0	26
	30	4.0	0	2.7	108	206	3.0	25
	90	11.0	0	2.7	112	212	6.5	22
	152	13.0	0	2.7	115	218	12.0	17
	189	24.5	0	2.6	123	224	13.0	13
	233	28.0	0	2.4	129	229	15.0	9
	266	37.5	0	2.6	135	240	12.0	7
C146	9	1.0	0	2.4	99	199	0.5	15
	30	3.5	0	2.5	103	203	3.0	13
	90	9.0	0	2.3	106	207	3.0	9
	152	14.0	0	2.1	112	214	5.5	9
	189	22.0	0	3.0	114	220	6.5	7
	233	29.0	0	6.0	119	230	10.0	5
	266	30.0	0	3.1	125	241	12.5	4
C163	9	1.5	0	2.4	101	201	0.5	17
	32	1.5	0	2.3	104	204	0.5	15
	89	3.0	0	2.4	106	208	0.5	12
	152	9.0	0	2.4	109	214	1.0	9
	197	15.5	0	2.3	117	228	6.5	7
	238	26.0	0	2.6	121	233	7.5	6
	268	33.0	0	2.4	133	241	10.0	3

[a] milliosmols

[b] mg/100ml

[c] meq/l

Animal Number	Water[a] Osmolality	Na^{+b} Urine	K^{+b} Urine	Total Protein[c]
C164	9	39		4.0
	32	35		3.8
	89	33		3.6
	152	28		3.3
	197	26		3.1
	238	23		3.0
	268	22		2.7
C165	9	42		3.8
	32	40		3.6
	89	37		3.5
	152	30		3.0
C166	9	37		3.8
	32	34		3.5
	89	31		3.3
	152	28		3.1
	197	24		3.0
	238	25		2.8
C167	9	40		3.9
	32	43		3.7
	89	40		3.3
	152	41		3.0
	197	31		3.8
	238	24		2.6
	268	23		2.4

[a] milliosmols

[b] meq/l

[c] g/100ml

Animal Number	Water[a] Osmolality	Na^{+}[b] Urine	K^{+}[b] Urine	Total Protein[c]
C145	9	37	0.6	4.0
	30	42	0.7	3.8
	90	40	0.5	3.6
	152	38	0.7	3.4
	189	35	0.5	3.0
	233	28	0.5	3.1
	266	25	0.6	2.8
C146	9	23	0.5	4.3
	30	35	0.5	4.1
	90	30	0.5	4.0
	152	25	0.5	3.5
	189	28	0.5	3.3
	233	22	0.5	3.0
	266	20	0.5	3.0
C163	9	37		4.1
	32	35		4.0
	89	33		3.5
	152	29		3.3
	197	26		3.1
	238	24		3.0
	268	23		2.8

[a] milliosmols

[b] meq/l

[c] g/100ml

Animal Number	Water[a] Osmolality	BUN[b]	Blood[b] Ammonia	K^{+}[c] Serum	Na^{+}[c] Serum	Serum[a] Osmolality	Urine[b] Urea N	Urine[b] Ammonia N
C168	9	1.5	0	2.3	101	200	0.5	14
	30	3.5	0	2.4	102	201	1.5	13
	91	7.0	0	4.0	109	207	3.0	11
	151	14.0	0	3.3	112	219	7.0	8
	202	23.0	0	3.1	122	230	12.0	7
	240	29.0	0	3.0	126	237	15.0	5
	273	34.0	0	3.0	131	246	17.5	4
C169	9	1.0	0	3.2	101	198	0.5	19
	30	1.0	0	2.3	104	204	0.5	16
	91	8.5	0	2.4	110	208	2.5	13
	151	12.0	0	2.2	113	216	6.0	11
	202	21.5	0	2.3	121	227	10.0	8
	240	28.5	0	2.3	129	236	14.0	5
	273	33.0	0	2.2	134	244	16.5	2

[a] milliosmols

[b] mg/100ml

[c] meq/l

Animal Number	Water[a] Osmolality	Urine[b] Na^+	K^{+b} Urine	Total Protein[c]
C168	9	35		4.2
	30	30		4.0
	91	29		4.0
	151	26		3.8
	202	22		3.6
	240	21		3.3
	273	20		3.1
C169	9	28		3.1
	30	29		3.6
	91	28		3.3
	151	27		3.0
	202	23		2.9
	240	20		2.8
	273	19		2.5

[a] milliosmols

[b] meq/l

[c] g/100ml

Appendix C

Animals Placed Directly into 250 milliosmol NaCl Solution

Animal Number	Time (hours)	BUN[a]	Blood[a] Ammonia	K^{+b} Serum	Na^{+b} Serum	Total[c] Protein	Urine[a] Urea N	Urine[a] Ammonia N
C148	0.0	2.0	0	2.5	100	4.1	2.5	19
	0.5	2.0	0	3.0	100	3.8	2.0	21
	1.5	2.5	0	3.0	100	3.6	2.5	19
	4.0	2.5	0	3.4	103	3.5	1.5	14
	8.0	4.0	0	2.0	102	3.5	2.5	13
	19.0	7.0	0	5.5	104	3.3	4.5	14
	30.0	8.0	0	2.0	104	3.2	6.0	11
	48.0	8.5	0	2.3	106	3.1	6.0	9
	70.0	16.0	0	2.4	109	3.1	11.5	8
	96.0	21.0	0	2.5	112	3.0	14.0	8
C149	0.0	1.5	0	2.8	99	3.8	0.5	16
	0.5	1.5	0	2.8	99	3.6	1.0	16
	1.5	1.5	0	2.8	101	3.3	0.5	16
	4.0	2.0	0	3.0	103	3.2	1.5	17
	8.0	3.5	0	3.1	103	3.0	1.5	16
	19.0	6.0	0	2.5	105	3.0	3.5	12
	30.0	9.5	0	2.8	106	2.8	6.0	9
	48.0	12.0	0	2.9	109	2.9	8.5	8
	70.0	17.0	0	3.0	113	2.7	9.5	8
	96.0	24.0	0	2.8	115	2.6	12.5	6
C150	0.5	2.0	0	2.5	101	4.4	1.0	15
	1.5	2.0	0	2.5	101	4.2	1.0	15
	4.0	4.0	0	3.4	101	4.1	1.5	15
	8.0	4.0	0	2.5	104	3.6	1.0	15
	19.0	6.0	0	2.5	107	3.5	3.5	15
	30.0	10.5	0	3.0	109	3.5	8.0	10
	48.0	13.0	0	7.6	111	3.0	9.0	7
	70.0	19.0	0	3.0	113	3.1	10.5	6
	96.0	23.0	0	2.7	117	2.8	13.0	5

[a] mg/100ml   [b] meq/l   [c] g/100ml

Animal Number	Time (hours)	BUN[a]	Blood[a] Ammonia	K^{+b} Serum	Na^{+b} Serum	Total[c] Protein	Urine[a] Urea N	Urine[a] Ammonia N
C151	0.5	0.5	0	2.5	100	4.1	0.5	19
	1.5	1.0	0	2.2	102	4.0	0.5	18
	4.0	1.0	0	2.4	101	3.3	1.0	18
	8.0	3.0	0	3.1	105	3.7	1.5	17
	19.0	7.0	0	3.3	107	3.0	5.5	16
	30.0	9.0	0	2.5	109	3.0	7.5	12
	48.0	12.5	0	2.5	110	2.8	8.5	7
	70.0	19.5	0	2.5	112	2.7	11.0	5
	96.0	19.5	0	2.5	112	2.6	17.0	4

a mg/100ml

b meq/l

c g/100ml

Appendix D

Values Obtained from

Electrophoretograms

Animal Number	Serum Calcium[a]	Inorganic Phosphorus[b]	Glucose[b]	BUN[b]	Uric Acid[b]	Cholesterol[b]	Total Protein[c]
C112	8.5	4.5	25	2.5	0.3	40	1.9
C116	10.8	5.4	55	1.0	0.7	70	2.8
C118	9.8	4.5	30	2.0	0.4	63	3.3
C119	8.0	5.9	85	1.0	0.4	75	3.4
C120	11.3	6.5	60	1.0	0.4	97	3.0
C121	8.1	4.9	80	1.0	0.3	180	3.5

[a] meq/1

[b] mg/100ml

[c] g/100ml

Animal Number	Water[a] Osmolality	Percentage Albumen	Percentage Globulin	Albumen to Globulin ratio
C115	9	40	60	.67
C116	9	38	62	.61
C118	9	43	58	.74
C122	9	30	70	.43
	66	24	76	.32
	175	25	75	.33
C123	9	30	70	.43
C124	9	26	74	.35
C125	9	28	72	.39
C127	9	24	76	.32
	129	20	80	.25
	213	38	62	.61
C128	9	25	75	.67
C129	9	29	71	.41
C132	9	28	72	.39
	80	23	77	.30
	127	19	81	.23
	256	30	70	.43
C142	9	14	86	.16
	90	25	75	.33
C143	9	24	76	.32
	30	31	69	.45
	90	40	60	.67
	253	17	83	.20
C163	9	21	79	.27
	89	25	75	.33
	238	29	71	.41
	268	20	80	.25
C164	9	22	78	.28
	89	34	66	.52
	238	24	76	.32
	268	17	83	.20

[a] milliosmols

Percentage values obtained from electrophoretograms

Appendix E

Values Obtained from

SMA 12/60 Autoanalyzer

Animal Number	Albumin[c]	Alkaline Phosphatase[d]	LDH[e]	SGOT[f]	Total Bilirubin[b]
C112	0.2	115	700+	120	0.1
C116	0.5	210	150	175	0.1
C118	0.4	215	165	104	0.1
C119	0.4	210	118	120	0.1
C120	0.4	318	188	181	0.1
C121	0.5	283	270	172	0.1

[b] mg/100ml

[c] g/100ml

[d] microunits/ml

[e] Lactic Dehydrogenase, microunits/ml

[f] Serum Glutamic Oxaloacetic Transaminase, microunits/ml