"Water and electrolyte balance in the agamid lizard, *Amphibolurus maculosus* (Mitchell), and the structure and function of the nasal salt gland of the sleepy lizard, *Trachydosaurus rugosus* (Gray)."

by

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma at any University, and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

C O N T E N T S

SUMMARY

The present study consisted of two parts, a study of the mechanisms by which the small dragon lizard Amphibolurus maculosus maintains its water and electrolyte balance, and a study of the structure and function of the nasal salt gland of the skink Trachydosaurus rugosus.

a). A. maculosus inhabits some of the dry salt lakes in the north of South Australia. It is active during summer in temperatures above 40 C, and has a very short water supply the scarcity of which is heightened by the high salt content of the ants on which it feeds. During summer A. maculosus stores excess electrolytes from its diet in its body fluids. The electrolytes are excreted later with water that is obtained from rain.

Water is lost from lizards by two main routes, (i) by evaporation and (ii) through the excretion of wastes. Therefore I studied these two avenues of water loss.

The rate of evaporative water loss (E.W.L.) per gram of body weight for A. maculosus is very low (0.18 mg of H_2O/g per hr.) being the lowest recorded for a lizard of this size. This rate of E.W.L. is about half the rate of E.W.L. of another dragon of similar size Amphibolurus pictus. This lizard inhabits the sand dunes that surround some of the salt lakes.

The reduced rate of E.W.L. of A. maculosus could not

be explained by a lower metabolic rate in A. maculosus compared to A. pictus because both lizards had similar rates of oxygen consumption. Nor could it be explained by A. maculosus having a higher concentration of haemoglobin in its blood than A. pictus and thus being able to absorb more oxygen per unit volume of air inspired since both lizards had similar haemoglobin concentrations. The low rate of E.W.L. of A. maculosus must be due to other respiratory adaptations and also probably to a less permeable skin.

Both A. maculosus and A. pictus can tolerate high temperatures and both possess a weak panting response. However, whereas A. pictus becomes excited at temperatures above 40 C and begins panting at 40.5 C, A. maculosus passively tolerates high temperatures and does not begin panting till about 43.5 C.

In an effort to determine what mechanisms A. maculosus might possess for minimising its excretory water loss, lizards were injected with a hypertonic NaCl solution and blood and voided urine was collected. Similar to what was found for lizards in the field, a significant amount of the injected salt was stored in the body fluids. However, the urine that was voided by the salt-injected lizards was hyperosmotic to the blood; this is the first reptile known to be able to produce a hyperosmotic urine. To determine where the urine was concentrated, urine was collected before

and after it had entered the cloaca. This showed that the urine was concentrated after it left the ureters.

A micro- and ultrastructural study of the cloaca and rectum of A. maculosus and A. pictus showed that for the most part the mucosal epithelium of both organs had a similar structure both within and between species. However, parts of the anterior third or so of the mucosal epithelium of the rectum of A. maculosus were quite different from the rest of the rectum and the cloaca, and closely resembled the structure of the rectal pads and papillae of some insects. It is suggested that A. maculosus can produce a hyperosmotic urine in the anterior part of its rectum.

A study was also made of the water and salt content of the urinary and faecal pellets of A. maculosus. This showed that A. maculosus could produce fairly dry pellets (water composed 71% of the wet weight of the faecal pellet and 34% of the urinary pellet). It was also shown that A. maculosus could excrete significant amounts of Na^+ and K^+ in the urinary pellet, equivalent to excreting these electrolytes in a liquid urine at a concentration of about 2000 mEq/l.

b). T. rugosus were found in the field with a white encrustation around the nares. Subsequent analysis revealed that it was composed of Na^+, K^+ and Cl^- with little if any, $HCO3^-$.

Morphological studies revealed that T. rugosus possessed a nasal salt gland similar in structure to those of terrestrial iguanids.

KCl and NaCl - loading induced the salt gland to secrete and the dried secretion was collected and analysed. NaCl - loading increased the relative Na^+ content of the secretion in all cases, but KCl increased the relative K^+ content in some and decreased it in others. In this respect the salt gland of T. rugosus is similar to those of the terrestrial iguanids, i.e. they are adapted to eliminating that ion which is in excess in the diet.

KCl and NaCl - loading slightly increased the Na^+ and K^+ concentration of the plasma respectively. However, 2 days after the last salt-load there was no significant difference in the ion concentration between the control, NaCl and KCl - loaded animals. Since the salt gland of T. rugosus can secrete salt at a significant rate (about the same rate as the terrestrial iguanids) it is suggested that the gland was the major excretory system controlling the electrolyte concentration of the plasma.

Since there was no significant increase in the electrolyte concentration of the plasma in field animals during summer, I suggest that the salt gland enables T. rugosus at Goolwa to maintain their salt balance. However, the summer during which the samples were taken in the field

was fairly mild. In drier years or drier areas the salt gland may not be able to maintain a constant concentration of electrolytes in the plasma.

It is suggested that the salt gland of terrestrial lizards augments the renal-cloacal system in eliminating ions that are in excess in the diet.

ACKNOWLEDGEMENTS

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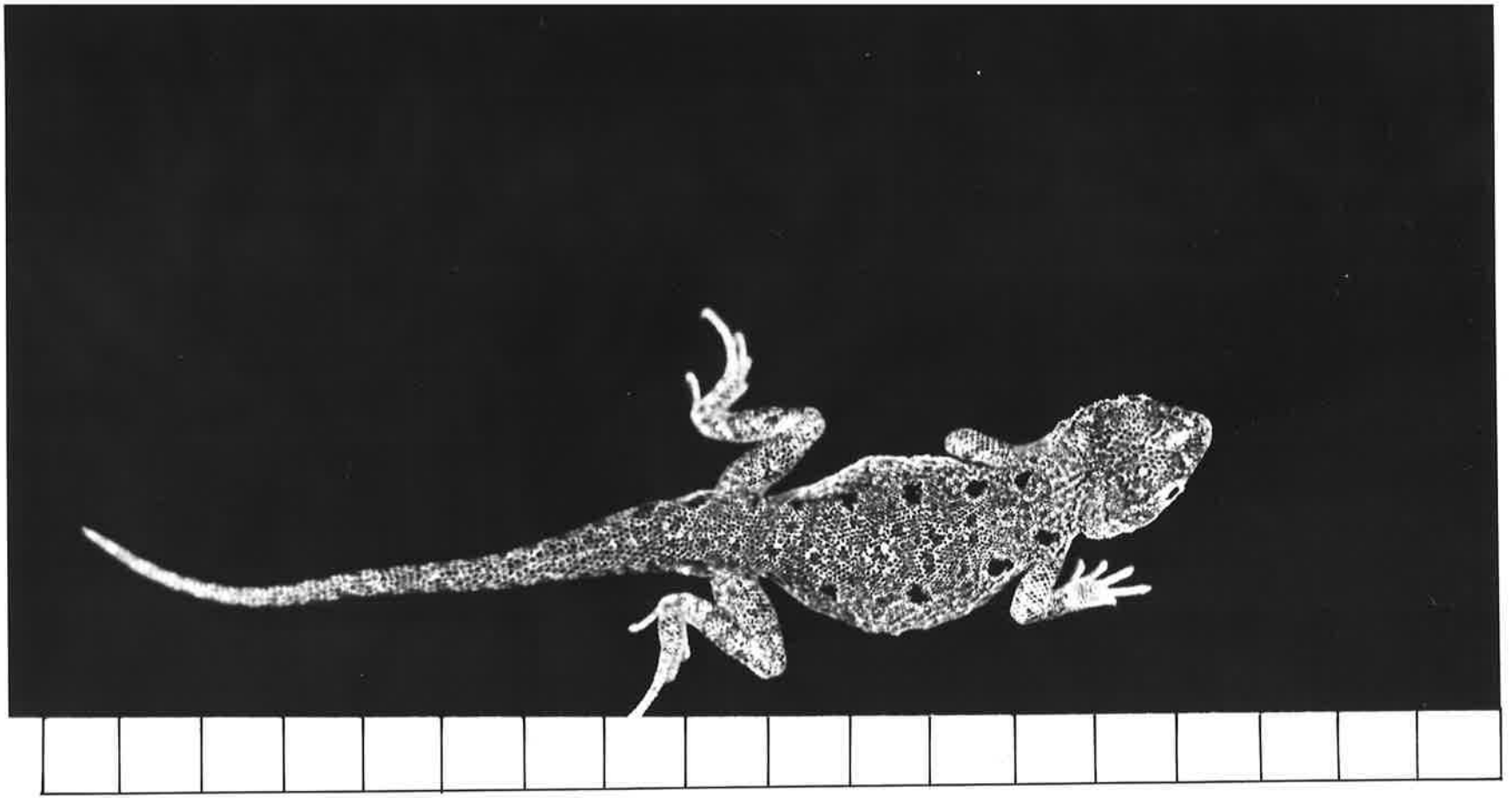
Dr. B. Green provided valuable help and suggestions throughout the project and I wish to thank him and all those who accompanied me on my field trips to Lake Eyre.

I am grateful to the families at Muloorina Station for allowing me to work on their property and for their hospitality. I also wish to thank Mr. M. Hammond and the Weapons research establishment at Salisbury for allowing me to use their vapour pressure osmometer on numerous occasions.

I have been greatly helped in the production of this thesis by Miss C. Mitchell and Mrs. R. Bradbury.

GENERAL INTRODUCTION

Fig. 1. Amphibolurus maculosus (adult male)


cm

GENERAL INTRODUCTION

The present study can be divided into two sections. Firstly a study of the mechanisms that the agamid lizard Amphibolurus maculosus (Mitchell) possesses for conserving water, and secondly a study of the structure and function of the nasal salt gland of the sleepy lizard Trachydosaurus rugosus (Gray).

A. maculosus is a small lizard, about 10 g, that inhabits some of the dry salt lakes in the north of South Australia (Fig. 1). I was introduced to this animal by the late F.J. Mitchel of the South Australian Museum who had been studying the behaviour of this lizard for several years. He found that it spends its whol life either on or under the dry surface of the lake. It feeds mainly on ants (Melophorus spp.) which also inhabit the lake, although it is an opportunistic feeder and will take other insects when they are available. During the summer, when shade temperature regularly exceed 40 C, these animals can be seen defending their breeding territories or foraging up to half a mile out on the lake.

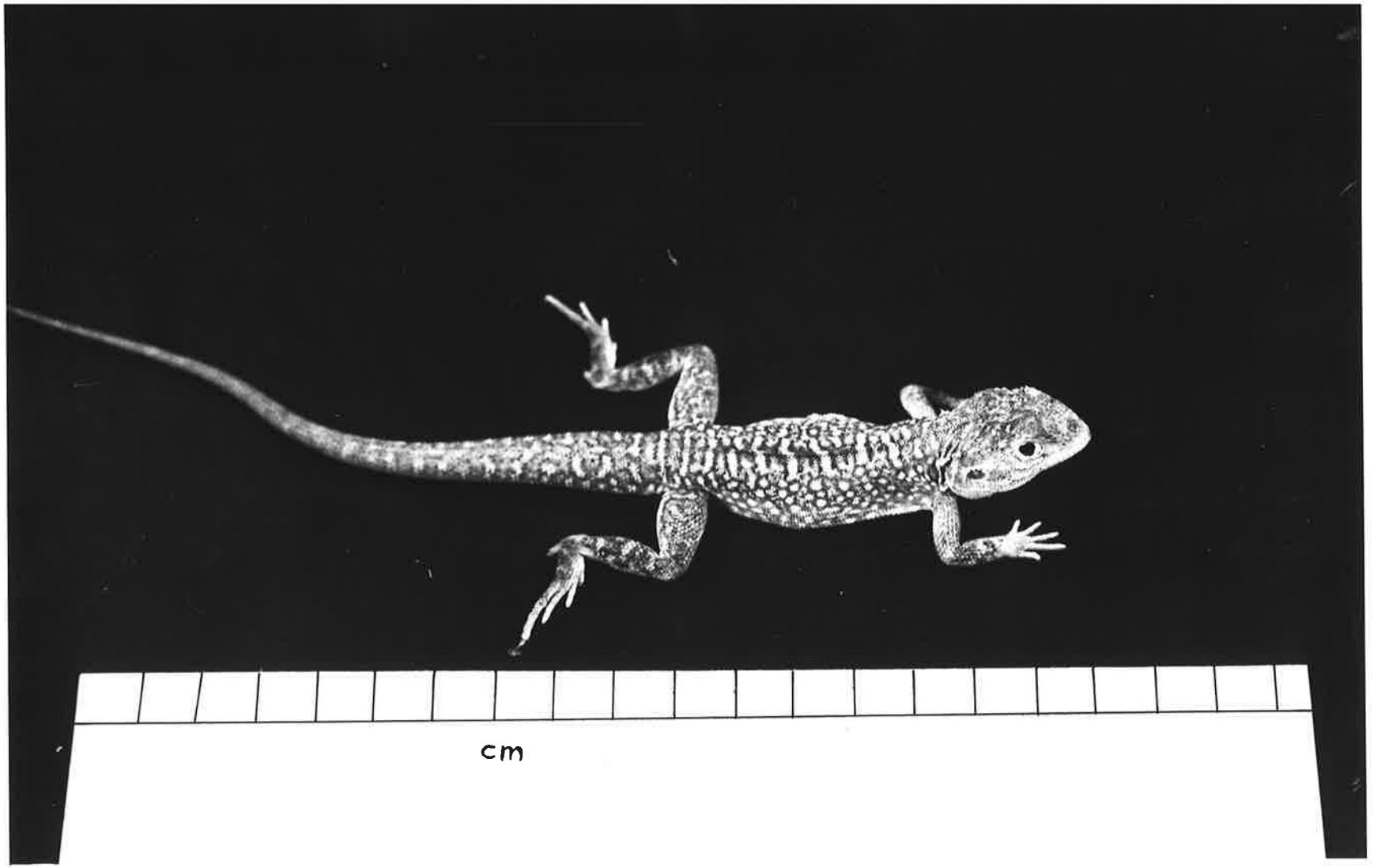
At night, and when temperatures are too high, A. maculosus burrows beneath the surface crust near the shore of the lake and lies quiescent in the moist soil just above the salt water table and thereby greatly reduces its evaporative water loss.

The rainfall in the area where the lizards are found is erratic and less than 12 cm per year, most of which falls during winter when the lizards are inactive. Therefore, it is clear that A. maculosus is exposed to high temperatures and has a very restricted water supply. Thus, I wanted to determine what mechanisms A. maculosus might possess for conserving water, and to compare them with those found for other desert reptiles by other workers.

Initially, I tried to show that A. maculosus was indeed short of water over summer. As an index of water stress I chose to measure the electrolyte concentration of the blood because Bradshaw (1965, 1970) showed that some agamids allow the electrolyte concentration of the plasma to rise when water is scarce. Thus I monitored the electrolyte concentration of the plasma of A. maculosus in the field throughout summer. I also determined the water and electrolyte content of the lizard's food.

Water is lost by two main avenues, (i) through evaporation from the skin and respiratory tract and (ii) through excreting wastes. Thus, next I studied these two avenues of water loss.

Fig. 2. Amphibolurus pictus (adult male)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(a) Evaporative water loss:

Because of the difficulty of comparing the results of this study with published work on other species (see section 2 for a discussion), I compared the rate of evaporative water loss of A. maculosus with that of another agamid of similar size Amphibolurus pictus (Fig. 2). The latter lizard inhabits the sand dunes that surround Lake Eyre, and it has a body temperature when active similar to A. maculosus. It turned out that A. maculosus had a lower rate of evaporative water loss than A. pictus; therefore I measured the oxygen consumption of both lizards to determine whether A. maculosus might have a lower metabolic rate than A. pictus and hence lose less water through respiration. In addition, I measured the haemoglobin concentration of the blood of these two lizards and of another agamid Amphibolurus inermis to determine whether A. maculosus has a higher haemoglobin concentration which might enable it to extract more oxygen from each unit volume of air inspired than the other lizards and so lose less water through respiration.

(b) Excretory water loss

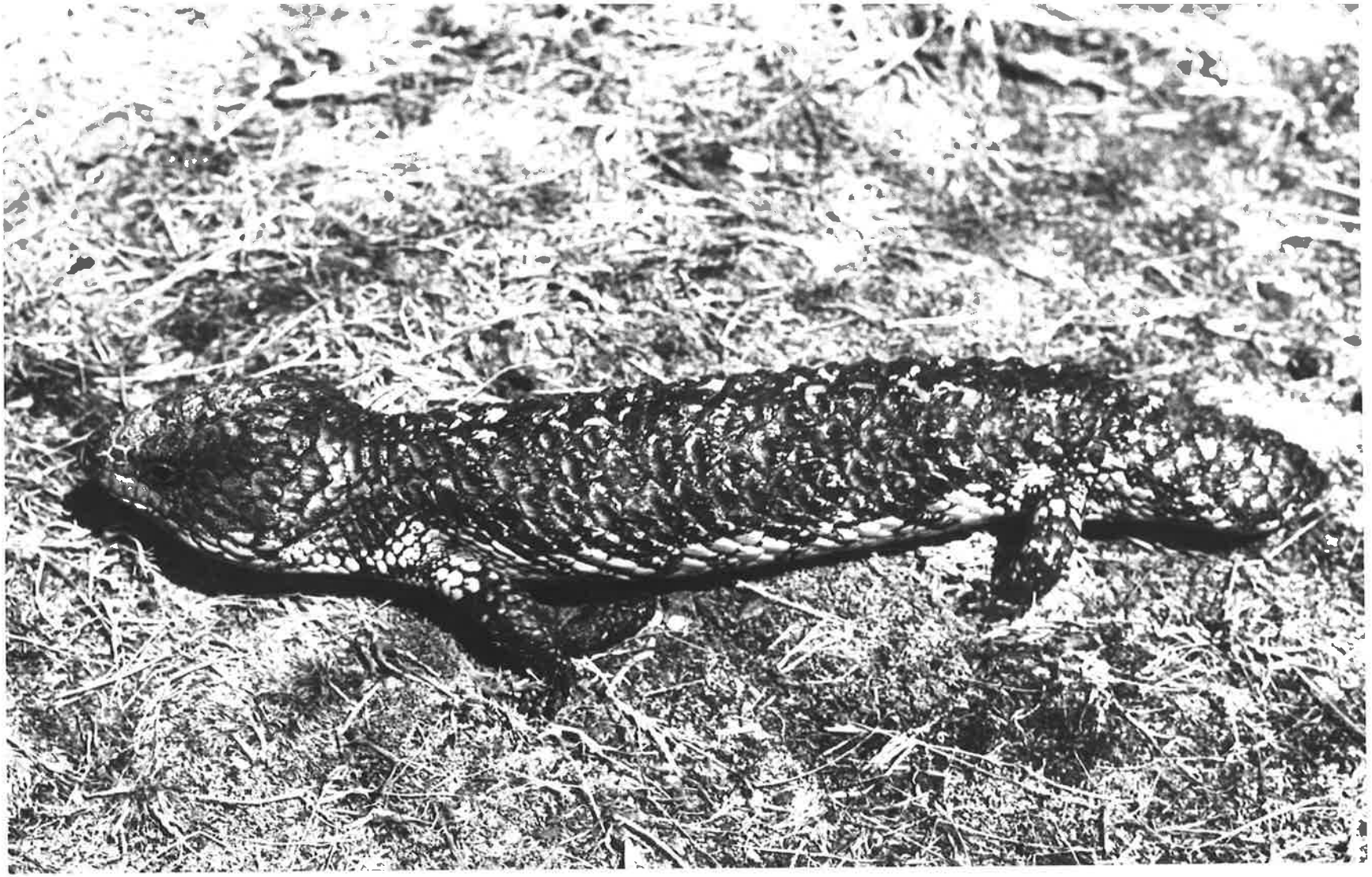
One of the most interesting physiological problems to be uncovered by Mitchell's work was how A. maculosus could cope with all the sodium chloride that it must ingest, since the ants on which it feeds could be expected to have a high salt content (Bradshaw and Shoemaker, 1967). This problem is

heightened by the scarcity of water. Therefore, I injected animals with solutions of sodium chloride in an effort to determine what mechanisms they might employ to deal with excess salt. This showed that A. maculosus could produce a urine that was hyperosmotic to the blood, the first reptile known to be able to do so. Urine could be concentrated in the kidney or in the cloaca or both, hence I attempted to determine how the urine could be concentrated by, (i) collecting urine before and after it had entered the cloaca, and (ii) studying the ultrastructure of the cloaca and kidney.

The second part of the present study was concerned with the structure and function of the salt gland of the sleepy lizard Trachydosaurus rugosus.

Salt glands are organs found in some reptiles and birds (Schmidt-Nielsen et al., 1958; Norris and Dawson, 1964; Templeton, 1964, 1966, 1967; Dunson, 1969; Minnich, 1970b). They can produce a concentrated solution of electrolytes and thus allow the animals that possess them to excrete some excess electrolytes with little loss of water. Until recently salt glands had been found in only one family of lizards, the Iguanids, however work which was conveyed to me subsequent to the commencemen of the present study shows that salt glands occur amongst members o other families as well; notably in a skink Eumeces skiltonianus (Minnich, pers. comm.).

Fig. 3. Trachydosaurus rugosus (Adult)



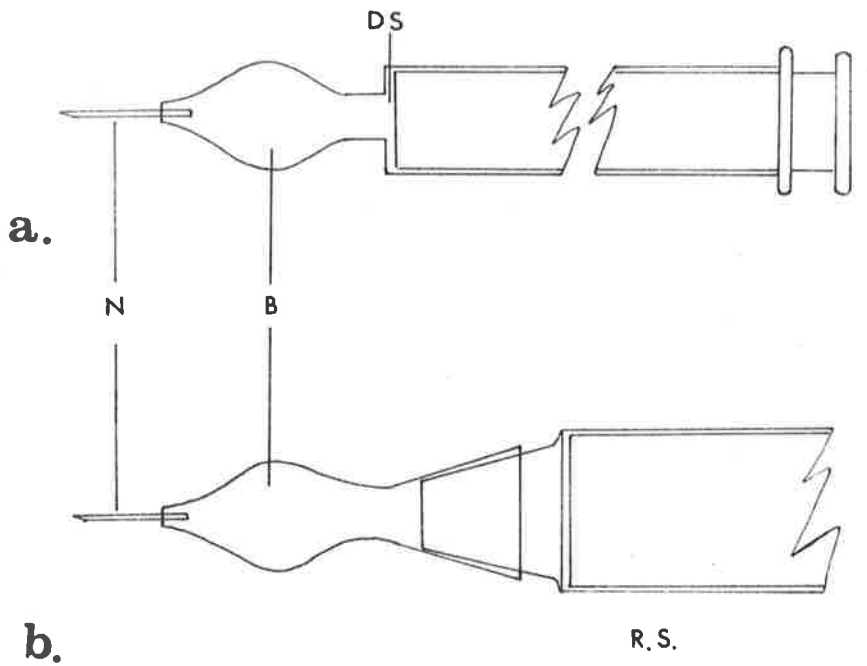
T. rugosus is a skink of about 400 g weight (Fig. 3). It is widely distributed through the southern half of Australia in temperate and desert regions. While collecting these lizards I noticed a white encrustation around their nares, a typical occurrence with animals that possess a salt gland. Although other workers had studied the water and electrolyte physiology of this lizard, they did not report finding a salt gland (Bentley, 1959; Shoemaker et al., 1966). Therefore, I attempted to determine whether T. rugosus possessed a salt gland, and after showing that it had, I studied its structure to determine whether it was similar to that reported for other animals.

The salt glands of most lizards respond to salt loads by increasing the rate of secretion of electrolytes, and also by varying the composition of the secretion depending on whether sodium or potassium loads are given (Templeton, 1966). Therefore, in a further effort to determine whether the gland of T. rugosus is typical of other salt glands, I injected animals with sodium and potassium chloride and collected the secretion and measured its composition.

SECTION 1

Field work on *A. maculosus* at Lake Eyre.

Fig. 4. Diagrams of the modified syringes that were used to take blood samples by heart puncture from small lizards. (B) is a glass bulb of about 0.1 ml that was either fused onto the end of a 1.0 ml glass tuberculin syringe (Fig. 4a) or, as shown in Fig. 4b, adapted to fit onto the end of a disposable syringe (R.S.). A 27 gauge needle (N) was mounted into the end of the bulb. (DS) represents the dead space between the plunger and the barrel of the syringe.


DS
a.
N
B
b.
R.S.

INTRODUCTION

Because A. maculosus lives in a hot place where water is very scarce and feeds on ants which are caught as they forage over salt-encrusted soil, I sought, in the first instance, some measurement that would indicate whether the lizards were obtaining sufficient water during summer for their physiological needs.

Amphibolurus ornatus is a dragon lizard which also lives in a hot dry place, and feeds on ants with a high salt content (Bradshaw, 1965). Bradshaw showed that during summer there was a marked increase in the electrolyte concentration of the plasma of this lizard. This was because the animals stored excess electrolytes from their diet in their body fluids during the hot summer.

I therefore took regular samples of plasma from a population of animals at Lake Eyre in northern South Australia and measured their osmotic, sodium and potassium concentrations. In addition, I estimated the salt and water content of the ants on which A. maculosus feeds.

MATERIALS AND METHODS

The field population consisted of the animals on or adjacent to Campbell's Causeway at Lake Eyre. Blood samples were taken in the field by heart puncture using one of the two modified syringes shown in Fig. 4. Fig. 4a shows how a glass bulb of

about 0.1 ml was fused onto the end of a 1.0 ml tuberculin syringe, while Fig. 4b shows how the 0.1 ml bulb was adapted to fit onto the end of a 1.0 ml disposable syringe. A 27 gauge needle was mounted into the end of the bulb. With these syringes it was possible to determine almost immediately when the needle was in the heart by the appearance of blood at the distal end of the needle, while the bulb prevented the loss of blood in the dead space between the plunger and barrel of the syringe. Before bleeding, a small drop of lithium heparin (50 i.u. units/ml) was placed in the bulb, and then approximately 40 µl of blood was withdrawn and placed in a segment of 'Dural' vinyl tubing. Both ends were sealed in a flame and the blood centrifuged in a hand centrifuge. The plasma was separated from the cells by cutting the tubing at the junction between the cells and plasma, then the tubing was sealed and placed in the refrigerator under mineral oil until it was measured for osmotic and electrolyte concentration.

To test the effect of storage, solutions of known concentrations were sealed and stored under conditions similar to the plasma. These were measured with the plasma.

Ants were collected from nests adjacent to Campbell's Causeway. A nest was excavated with a spade and all the ants that could be found were collected with an aspirator. The

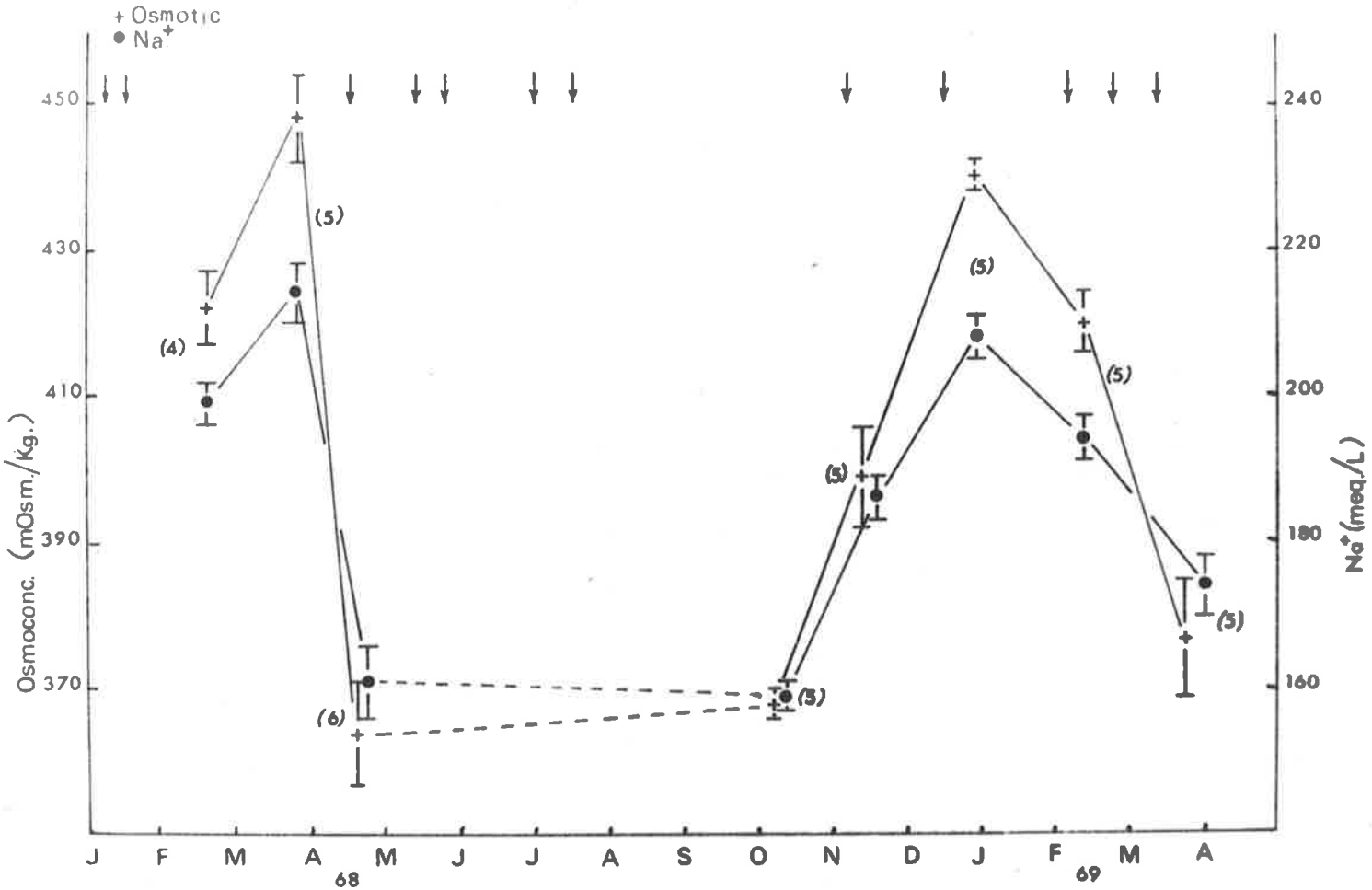
ants were anaesthetized with chloroform and weighed to the nearest 0.2 mg on a Sauter torsion balance (max. capacity 100 mg). They were then dried at 100 C for a week and the water content estimated as the difference between the two weights.

The electrolyte concentration of the body fluids of the ants was measured on only one trip, December 1970, due to technical difficulties. Ants were collected with an aspirator just before I left for the laboratory in Adelaide. They were held at 0 C for the journey. At the laboratory the unwashed ants were crushed and the fluid drawn off and centrifuged in vinyl tubing. The supernatant was sealed in the tubing and stored with the plasma samples until analyzed. The ants were not washed because I did not want to lose any of the salt that they may have picked up from the surface of the lake since this salt would be ingested by the lizards and therefore should be considered in any estimate of the electrolyte concentration of the body fluid.

Plasma samples and ant fluid were appropriately diluted with double distilled water and the sodium and potassium concentration estimated with an Eel Flame Photometer (Evans Electroselenium Ltd., Essex, England.)

Osmotic concentration of the plasma was estimated with a Mecrolab Vapor Pressure, Osmometer Model 302.

Fig. 5. Variation in the sodium and osmotic concentration of plasma samples that were taken from A. maculosus at Lake Eyre. Arrows represent times when substantial rain was likely to have fallen.


+ Osmotic
● Na+
Osmoconc. (mOsm./Kg.)
Na+ (meq./L)
450
430
410
390
370
240
220
200
180
160
(4)
(5)
(6)
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(5)
J F M A M J J A S O N D J F M A
68
69

RESULTS

There was no change in the concentration of the fluid samples that were stored with the plasma, Table 1.

Fig. 5 shows the variation in the osmotic and sodium concentration of the plasma samples taken from A. maculosus at Lake Eyre while the variation in potassium concentration is presented in Fig. 6. The osmotic and sodium concentration increased over summer but fell sharply in April 1968 and January - March of 1969. The potassium concentration showed a similar variation (Fig. 6).

Table 2 shows the rainfall statistics for 1968/69 at Muloorina, a station 30 miles from the study area. It is clear that the sharp falls in the osmotic and electrolyte concentration of the plasma correspond to periods of rainfall (arrows on Figs. 5 and 6). However, care must be taken in relating rainfall statistics at Muloorina to those at the causeway because rainfall is often very patchy and hence gaugings at Muloorina probably do not truly reflect those at the causeway. Nevertheless, the rapid drops in Figs. 5 and 6 seem to be associated with times when substantial rain was likely to have fallen.

Fig. 6. Variation in the potassium concentration of plasma samples that were taken from A. maculosus at Lake Eyre. Arrows represent times when substantial rain was likely to have fallen.

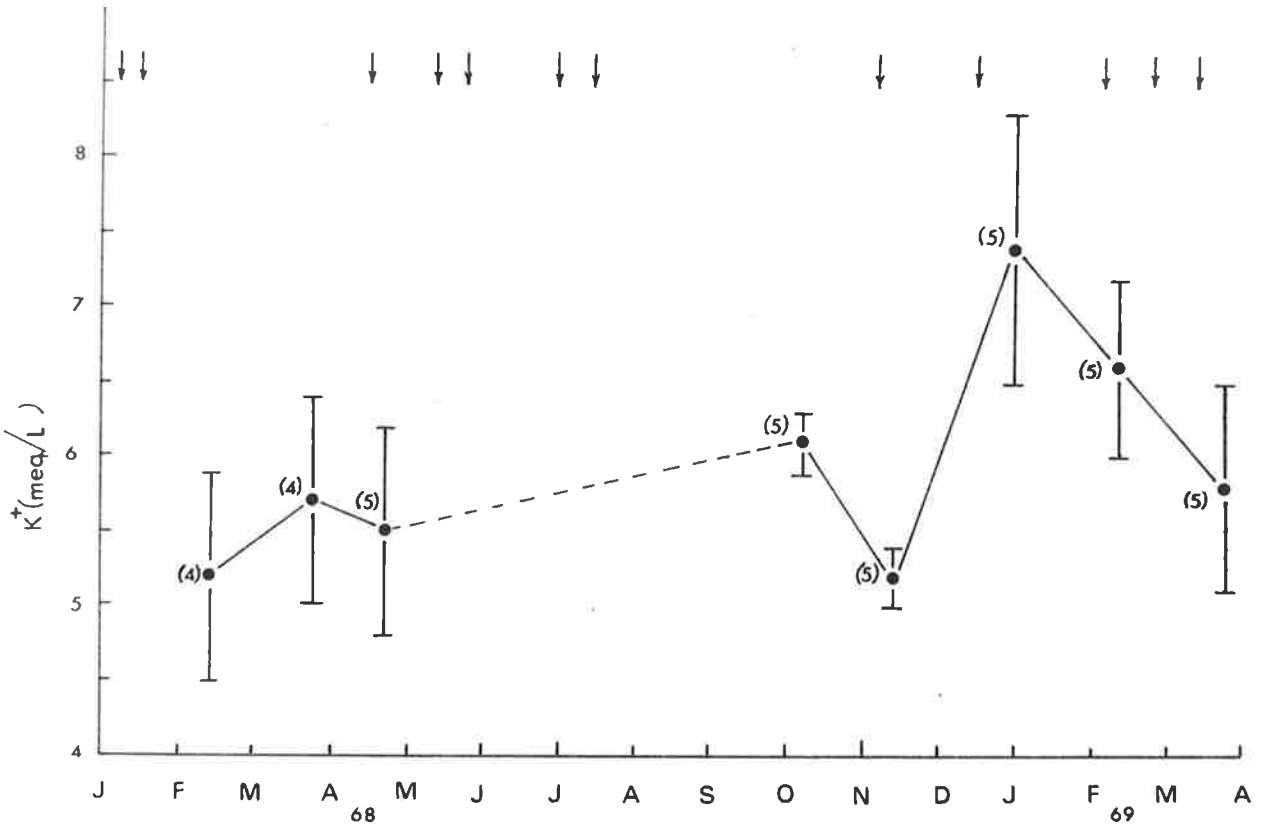

K+(meq/L)
8
7
6
5
4
(4)
(4)
(5)
(5)
(5)
(5)
(5)
(5)
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
68
69

Table 1

The effect on osmotic concentration of storing fluid in vinyl tubing

Tube No	1	2	3	4
Initial osmotic concentration (m Osmols/l)	198	240	300	500
Osmotic concentration after 17 days	198	240	296	498

Table 2

Rainfall at Muloorina Station (mm).

Month							
1968							
	Jan	Feb	Mch	Apr	May	June	July
	3rd - 7.6		2nd - 0.3	18th - 8.9	8th - 1.5	15th - 1.5	1st - 17.5
	4th - 8.4				9th - 2.5	22nd - 0.8	2nd - 23.9
	17th - 6.4				13th - 10.9	26th - 0.3	3rd - 0.3
	19th - 2.0				22nd - 9.9	28th - 1.5	16th - 0.5
	21st - 0.5						17th - 4.8
Total rain (mm)	24.9	Nil	0.3	8.9	24.8	4.1	47.0

Table 2 cont.

	Month							
	1968					1969		
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
	7th - 2.0		6th - 2.0	6th - 12.4	16th - 3.0		4th - 6.6	7th - 2.5
	12th - 0.5		7th - 1.8	7th - 0.5			23rd - 9.7	8th - 3.8
Total	2.5	Nil	3.8	12.9	3.0	Nil	16.3	6.3

Table 3

The water and electrolyte content of the body water of ants from Lake Eyre (*Melophorus* spp.).

Date of Sample	Water content %	Electrolyte Content	
		Na^+ mEq/l	K^+ mEq/l
Oct 1968	72	-	-
Nov 1968	73	-	-
Dec 1970	72	275	44

The variation in the electrolyte concentration of the plasma of A. maculosus at Lake Eyre is similar to what Bradshaw (1965) found for A. ornatus. Similarly, both the ants on which A. maculosus feeds and those on which A. ornatus feeds are high in sodium (Table 3). Therefore, A. maculosus is taking in sodium chloride with its food in quantities which would require more water to excrete via the kidney and cloaca as a solution isosmotic with blood than is contained in the lizard's diet.

DISCUSSION

Although no estimates were made of the variation in the distribution of body fluids in A. maculosus, the fact that both A. ornatus and A. maculosus have a high sodium intake and show similar variations in the electrolyte concentration of the plasma indicates that when water is scarce A. maculosus probably employs a similar mechanism to that proposed by Bradshaw (1965) for A. ornatus.

He suggests that as temperatures rise during summer and evaporative water loss increases A. ornatus can no longer obtain sufficient free water from its food to excrete excess electrolytes. It therefore can take one of two alternatives. Either it can store the electrolytes in its body fluids until sufficient water becomes available to excrete them, or excrete them at the expense of body water. This is assuming that A. ornatus can not produce a

hyperosmotic urine or excrete the electrolytes as a concentrated fluid via an extra renal route such as a nasal salt gland (Templeton 1964).

A. maculosus does not possess a functional salt gland, but, as will be shown later, it can produce a hyperosmotic urine. However, as evaporative water loss increases, it appears to store excess electrolytes, like A. ornatus, instead of excreting them immediately. The electrolytes are excreted later with water obtained from rain.

I was surprised to find that A. maculosus could utilize water from rain (Fig. 5). The soil in the burrow zone contains at least 13% salt (Mitchell pers. comm.), while the surface of the lake is almost pure sodium chloride. Therefore, any rain that falls would soon become brine. However, A. maculosus may be able to use the pools of water before they become salty or else catch the rain as it falls. The lizards will drink water in the laboratory if it is splashed onto their heads. Also, some lizards take up an unusual stance in the rain. They arch their bodies and raise themselves onto the tips of their digits and hold this position while moving the gular region as if drinking.

Bentley and Blumer (1962) showed that the skin of the agamid Moloch horridus acts as a blotting paper so that it can soak up water from pools and channel it to the mouth. The gular movements that they observed when animals were drinking were similar to those that occur in A. maculosus. Also Krakauer et al. (1968) showed that some snakes can take up water from moist sand via the mouth and not through the skin as had been suggested previously. Krakauer et al suggest that the skin of these snakes acts in a manner similar to that of Moloch horridus. A. maculosus probably takes up water in the same way. Thus, they probably raise themselves off the substratum when drinking so that they do not absorb brine from the surface of the lake.

If A. maculosus obtains water from rain in this way it is not surprising that it must eliminate stored electrolytes as a fairly concentrated urine, because it could not obtain as much water by this method as A. ornatus could by drinking from rock pools.

In conclusion, these results show that A. maculosus is short of water over summer. Therefore, the next aspect of the study consisted of examining the two major avenues of water loss, evaporative and urinary water loss.

SECTION 2

EVAPORATIVE WATER LOSS

INTRODUCTION

A good correlation has been demonstrated between the rate of evaporative water loss per gram of body weight* and the aridity of the environment for a number of species of lizards (Dawson et al., 1966; Claussen, 1967; Sexton and Heatwole, 1968; Krakauer et al., 1968; Green, 1969; Bradshaw, 1970). Since A. maculosus is active in very hot and dry conditions, I wanted to determine whether it showed a similar adaptation to aridity.

Evaporative water loss (E.W.L.) varies with metabolic rate (Dawson et al., 1966; Claussen, 1967; Roberts, 1968a; Gans et al., 1968; and Minnich, 1970a). Therefore, a standard of metabolism must be chosen before any comparisons can be made between animals. In most of the earlier work on rates of E.W.L., standard metabolism was taken as that which occurred in a resting animal, but Roberts (1968 showed that this valuc varied with the conditions under which it was measured and was often not repeatable. She suggested that minimum metabolism was a more constant standard. For the diurnal lizard Uta stansburiana steinegeri, this is the level of metabolism that occurs at night in inactive lizards that have been starved and acclimated to the experimental conditions for some time. Using this standard, Roberts (1968b) obtained a rate of E.W.L. for U. stansburiana that was about one half that which Claussen (1967) obtained for a sub-species of this lizard.

*Wherever I use the terms rate of E.W.L. or rate of oxygen consumption, it is implied that I mean rate per gram of body weight.

I have chosen Robert's standard of minimum metabolism for all my measurements of E.W.L. and oxygen consumption, but since only a few workers have used the same standard (Chew and Dammann, 1961; Krakauer et al., 1968; Roberts, 1968a, 1968b; and Minnich, 1970a), it is difficult to compare the rates that I obtained for A. maculosus with those reported for other lizards.

Rates of E.W.L. and oxygen consumption also vary with body temperature (Dawson et al., 1966; Roberts, 1968a; Minnich, 1970a; Crawford and Kampe, 1971). Although the body temperature of lizards varies considerably over 24 hours, it is usually fairly constant while they are active (Licht et al., 1966; Tucker, 1966; De Witt, 1967; Bradshaw and Main, 1968; Pough, 1969a; Heatwole, 1970). In some lizards, because their body temperature when active is fairly constant, physiological processes have adapted to be most efficient at that temperature (Licht, 1964a, b; Dawson, 1967; Licht et al., 1969; Pough, 1969a). Therefore, I measured the rate of E.W.L. of A. maculosus at 37 C which is in the range of the active temperature of this lizard, 37 - 39C. However, since other work on reptiles has been done at a variety of temperatures, often unrelated to the activity temperature of the animal, this further adds to the difficulty of comparing my results with those obtained for other reptiles by other authors.

Consequently, I compared the rate of E.W.L. of A. maculosus with that of another agamid of about the same weight, Amphibolurus pictus. The latter lizard inhabits the sand dunes that surround Lake Eyre and has a body temperature when active of about 37 C (Mitchell, pers. comm.). Shortage of water is probably less of a problem to A. pictus than A. maculosus because A. pictus does not have to forage as far for food and shade is more plentiful in the dunes. Besides A. pictus would probably not have such a high salt intake in its diet.

Two methods are commonly used to measure evaporative water loss. One is the free flow system where dry air is passed over the animal and then passed through drying tubes. Thus the water lost from the animal equals the increase in weight of the tubes. The other is by direct weighing where weight lost by the animal is assumed to be due to evaporative water loss.

I chose direct weighing because this method allows an accurate control of both temperature and humidity. Defaecation is no problem since it is accompanied by a large weight loss and, therefore, these runs can be omitted. Besides, the free flow method has a number of disadvantages. These are discussed fully by Lasiewski et al. (1966).

Fig. 7. A diagram of the apparatus that was used to measure the respiratory quotient of lizards.

A = animal

AT = air tight seal

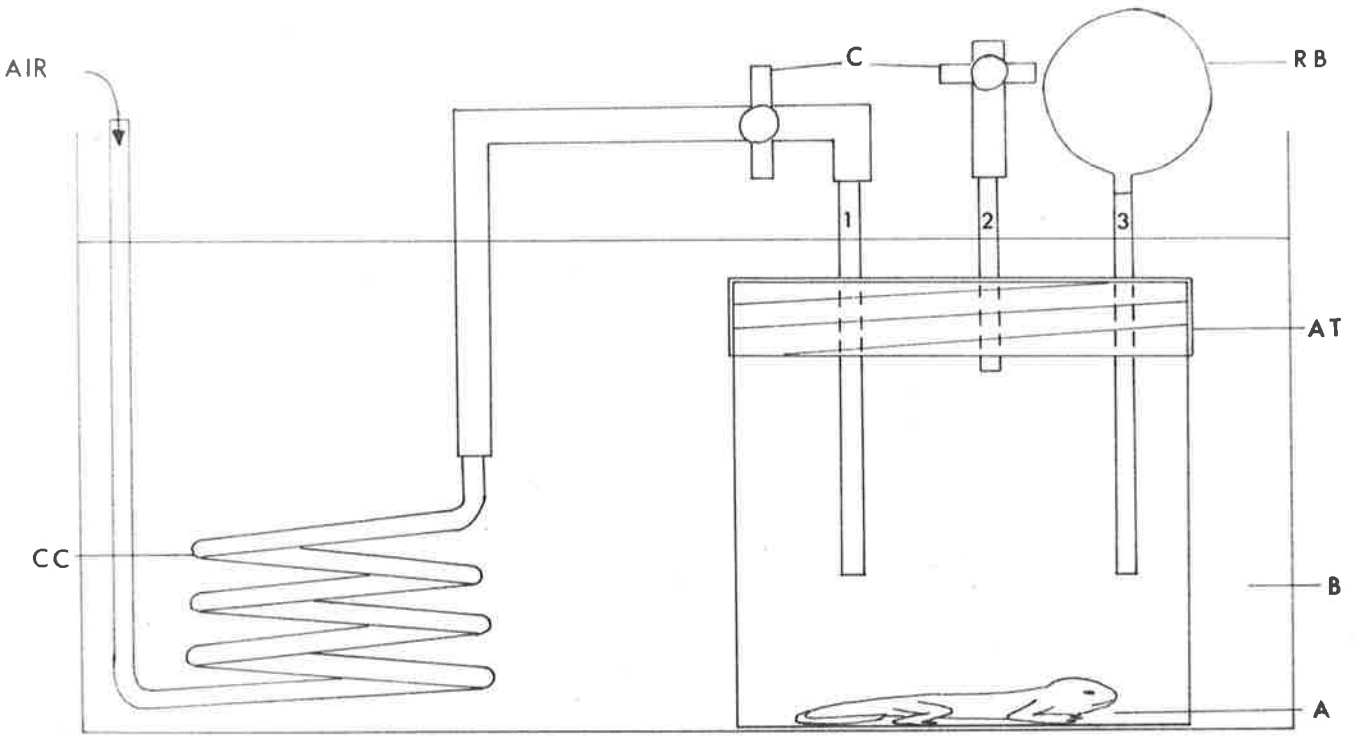
B = water bath

C = screw clamps

CC = copper coil

RB = rubber bulb

1, 2 and 3 = inlet tubes.


AIR
C
RB
1
2
3
AT
CC
B
A

The direct weighing method assumes that the respiratory quotient (R.Q.) is approximately 0.7, that is, that the weight of carbon dioxide expired is equal to the weight of oxygen consumed. I verified that this was true for both A. maculosus and A. pictus. I also determined when minimal rates of water loss occurred and presumably therefore, minimum metabolism. These conditions were used for all subsequent measurements of E.W.L.

MATERIALS AND METHODS

Both A. pictus and A. maculosus were collected from Lake Eyre in South Australia. Each species was kept in a separate pen in the open and they were fed Tenebrio larvae and moths. Heat was supplied with an infra-red lamp for twelve hours a day.

(a) Respiratory Quotient

An animal was acclimated to 37 C for seven days without food or water. It was then placed in the apparatus shown in Fig. 7. The temperature of the water bath (B) was 37 $\pm$ 1 C. The lizard was left for several hours to settle down before the jar was flushed through tube (1) with air that had been heated in the copper coil (CC). A sample of air was taken through tube (2) and then both clamps (C) were sealed. About

two hours later the air in the jar was mixed with the rubber bulb (R.B.) attached to tube (3) after which another sample of air was taken through tube (2).

The samples were measured for carbon dioxide and oxygen concentration with a Lloyd gas analyser, model GC-400 Gallenkamp.

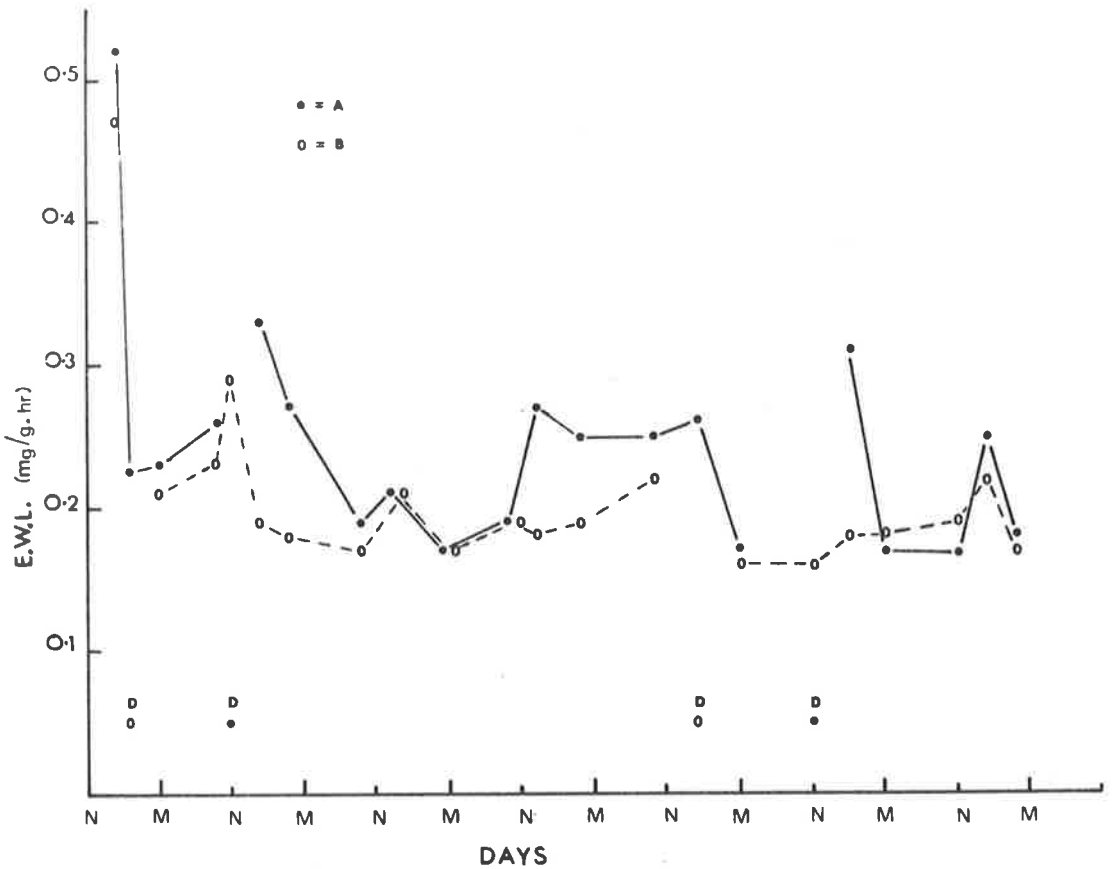
(b) Minimal rates of E.W.L.

Four A. maculosus were placed in a constant temperature cabinet at 37 ± 0.1 C. Relative humidity was maintained below 10% with a tray of silica gel. Light was supplied for twelve hours a day and the animals were not given food or water.

Weight loss was recorded every four hours by removing the lizards from the cabinet and weighing them on a Mettler H5 balance to the nearest 0.1 mg. A. maculosus were hardly affected by handling, especially at night when they would lie quiescent with their eyes closed throughout the weighing procedure. However, A. pictus became very agitated when it was handled. Therefore, to overcome this, a modified weighing procedure was used for later measurements of E.W.L.

The four animals were kept under these conditions for seven days then two lizards were removed. The remaining animals were kept in the cabinet and weighed every day until death to

Fig. 8. A graph of the variation in the rate of E.W.L. for two A. maculosus (A and B) with time. N = noon, M = midnight. D represents times when the lizards defaecated.


E.W.L. (mg/g. hr)
0·5
0·4
0·3
0·2
0·1
● = A
o = B
D
N
M
DAYS

determine the resistance of A. maculosus to desiccation. The weight loss up to death was expressed as a percentage of the initial body weight.

RESULTS

The respiratory quotients for A. maculosus and A. pictus were 0.74 ± 0.01 and 0.70 ± 0.01 respectively. Therefore direct weighing can be used as an accurate measure of E.W.L.

Fig. 8 is a graph of the variation in the rate of E.W.L. with time for two of the A. maculosus. The lizards showed very little activity throughout the period of the experiment and therefore would have at all stages corresponded to the original standard of a 'resting animal' that was used by earlier workers on lizards. Minimal rates of loss occurred at various times, although they tended to occur more frequently at night after the animals had been subjected to the conditions for two or three days. Thus, A. maculosus adapted to the experimental conditions very quickly. In contrast, A. pictus became very excited in strange surroundings and, therefore, is probably more similar to the lizard Uta stansburiana. Roberts (1968b) found that minimal rates of loss usually occurred at night in this latter animal after it had been acclimated to the experimental conditions for about a week. Consequently, both A. maculosus and A. pictus were acclimated to 37 C for six days without food or water before rates of E.W.L. were measured.

The survival time of the two A. maculosus at 37 C and their degree of desiccation at death are presented in Table 4 along with results for other animals. It is clear that A. maculosus can survive hot, dry conditions longer than most of the other lizards that have been studied with the possible exception of the agamid Diporophora bilineata. Bradshaw and Main (1968) found that this lizard could survive for more than six hours at 46 C and for approximately half an hour at 49 C, one half a degree below the temperature at which A. maculosus loses muscle co-ordination, its critical thermal maximum (Mitchell, pers. comm.).

It is difficult to compare the degree of desiccation that A. maculosus can tolerate with values for other lizards because measurements were made at different temperatures and humidities. However, A. maculosus does not seem able to tolerate the degree of desiccation that some lizards can (Table 4), although, admittedly, my results are based only on two animals.

Table 4

The resistence to desiccation and the % weight loss at death for several lizards at various temperatures.

Species	Temperature (C)	Relative humidity (%)	Survival time (days)	% weight loss at death	Reference
Amphibolurus maculosus	37	< 10	(a) 50	38	Present study
	37	< 10	(b) 49	33	
Amphibolurus inermis	37.5	< 10	> 20	?	Warburg (1965b)
Anolis limifrons	21	55	2	27	Sexton and
Anolis auratus	21	55	4	20	Heatwole (1968)
Anolis carolinensis	?	?	?	29 - 46	
Phrynosoma cornatus	?	?	?	19 - 34	Hall (1922)
Sceloporus spinosus	?	?	?	30 - 48	
Mabuya quinquetaeniatus	30	< 10	~ 20	38	Cloudsley-Thompson
Tarentola annularis	34	< 10	47	42*	(1965)

*Not dead - lost a further 10% of its body weight at 26 C.

Fig. 9. A diagram of the apparatus that was used to measure the rates of E.W.L. of lizards.

B = Mettler balance

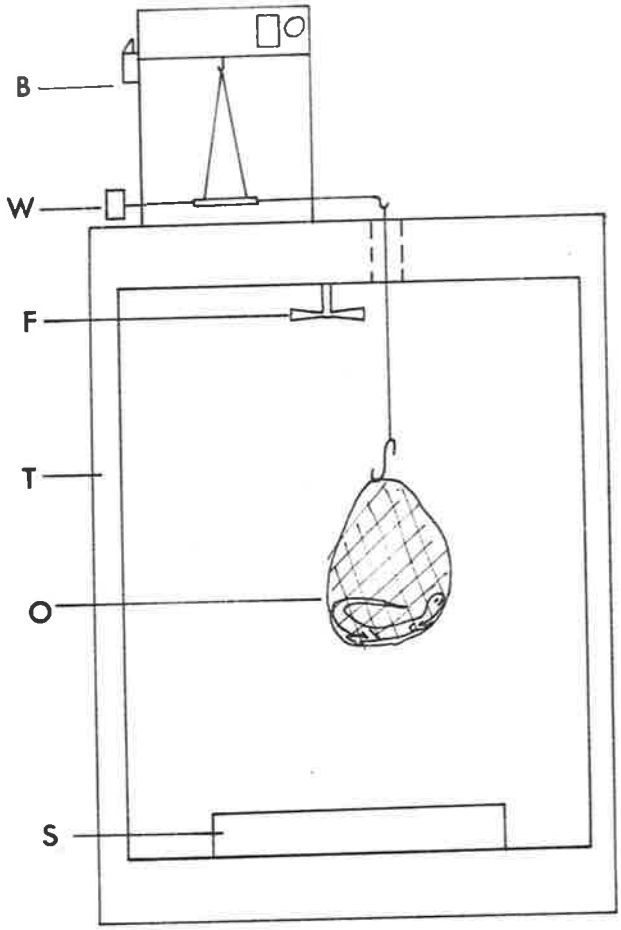
F = fan

O = open mesh bag

S = tray of silica gel

T = temperature cabinet

W = counter balance


B
W
F
T
O
S

Rate of E.W.L. of *A. maculosus* and *A. pictus*

METHODS

The average weight of *A. maculosus* and *A. pictus* was 10.7 ± 0.6 g and 8.5 ± 0.8 g respectively.

Since moulting is associated with increased rates of E.W.L. (Gans *et al.*, 1968; Minnich, 1970a), only non-moulting lizards were used.

A diagram of the apparatus that was used to measure E.W.L. is shown in Fig. 9. An animal was starved for six days and then placed in the open mesh bag (O) inside the constant temperature cabinet (T) which was kept at 37 ± 0.1 C. The bag was attached to a counterbalanced rod (W) which rested on the pan of a Mettler balance (B). A tray of silica gel (S) held the relative humidity at less than 10%. Air was circulated with a fan (F).

Rates of E.W.L. were calculated for each two hour period between 1600 and 2400 over two to three days. The three lowest rates of loss were averaged and expressed as mg of water lost per gram of body weight per hour. Since the rate of E.W.L. varies with body weight (Gans *et al.*, 1968; Green, 1969; and Minnich, 1970a), the weight of the lizard that corresponded to the time at which the rate of water loss was measured was used as the body weight.

Initially, I taped the cloacas of some animals, because Maderson (1965) suggested that this may be an important site of E.W.L. However, there was no significant difference in the rate of E.W.L. between taped and untaped animals, which is similar to what was found by Claussen (1967) and Green (1969). Water may be lost by evaporation when animals defaecate, but since I neglected runs where animals defaecated, the cloacas of the lizards were not taped in subsequent runs.

RESULTS AND DISCUSSION

The rates of E.W.L. for A. maculosus and A. pictus at 37 C are presented in Table 5, along with those obtained for other lizards. Comparisons show that both A. maculosus and A. pictus have very low rates of E.W.L., especially A. maculosus which has by far the lowest rate of any lizard of its size, being about one half the rate obtained for A. pictus ($t_{12} = 15.5$, $P < 0.001$). The advantage for water conservation of such a low rate of E.W.L. is obvious.

However, the difficulty of comparing the present work with that for other lizards is shown again by the fact that I obtained a rate of E.W.L. for A. pictus of about one fifth of that obtained by Warburg (1965b). Although he noted that activity affected the rate of E.W.L., he did not eliminate its effects. Thus, the higher rate that he obtained can probably be accounted for by a higher metabolic rate for his animals.

Table 5

The rates of evaporative water loss for several species of lizards.

Species	Weight (g)	Habitat	Temp (C)	Relative humidity (%)	Rate of E.W.L. $mg.g^{-1}.hr^{-1}$	Reference
Amphibolurus maculosus	11	A	37.0	< 10%	0.18 ± 0.01 (10)*	Present study
Amphibolurus pictus	9	A	37.0	< 10%	0.36 ± 0.01 (4)*	
Amphibolurus inermis	Not given (~ 25)	A	37.5	< 10%	0.85	Warburg (1965b)
Amphibolurus pictus	Not given (~ 10)	A	37.5	< 10%	1.60	
Amphibolurus ornatus	23	T - SA	35.0	< 10%	2.79	Bradshaw (1970)
Tympanocryptis lineata tetraporophora	10	A	37.5	< 10%	0.32	Warburg (1966)
Amphibolurus barbatus	240	T - A	37.5	< 10%	0.59	Warburg (1965a)
Varanus gouldii	1000	T - A	37.0	< 10%	0.38	Green (1969)

Table 5 cont.

Species	Weight (g)	Habitat	Temp (C)	Relative humidity (%)	Rate of E.W.L. $mg.g^{-1}.hr^{-1}$	Reference
Crotaphytus collaris	30	SA	37.0	10%	0.64	Dawson and Templeton (1963)
Trachydosaurus rugosus	320	T - SA	37.5	10%	1.16	Warburg (1965a)
Dipsosaurus dorsalis	50	A	39.0	50%	0.38	Minnich (1970a)

*Sample size in parentheses. These are minimum rates of E.W.L. expressed as means ± S.E.

A = Arid, S.A. = Semi arid, T = Temperate.

Fig. 10. A diagram of the apparatus that was used to partition total E.W.L. into respiratory and cutaneous fractions.

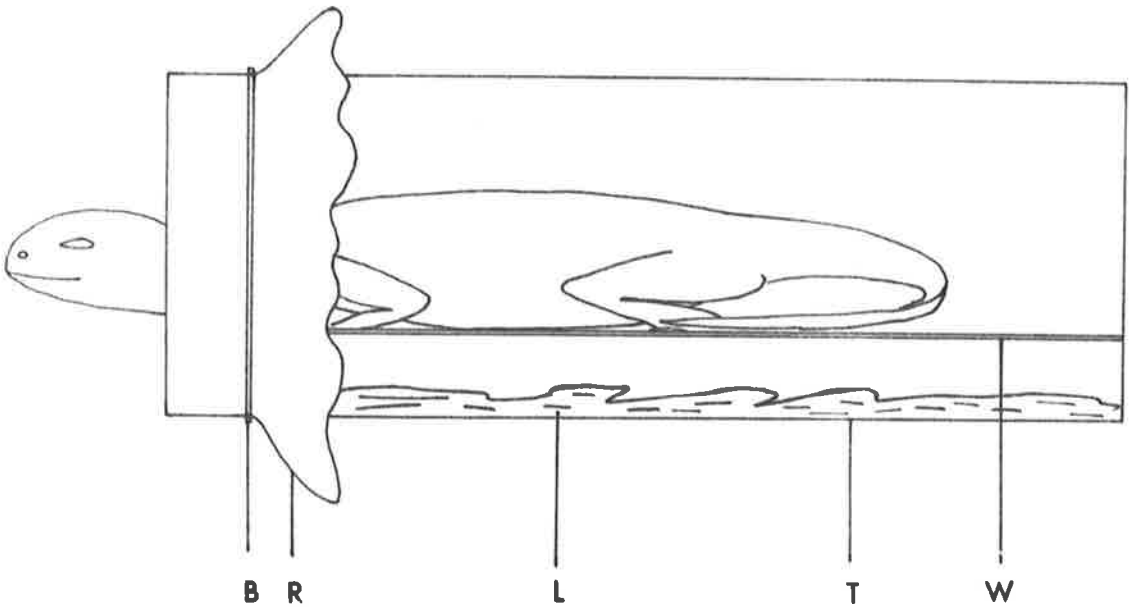
B = rubber band

L = layer of magnesium perchlorate

R = collar made of dental rubber

T = glass tube

W = wire grid


B
R
L
T
W

E.W.L. can be divided into a number of fractions; respiratory, cutaneous and water lost from the eyes. The last was shown by Green (1969) to be a significant site of water loss from the goanna Varanus gouldii. However, it would be difficult to measure this for a 10 g lizard, consequently, it was neglected in this study.

I next wanted to partition total E.W.L. into respiratory and cutaneous water loss to determine what fraction each of these routes represent of the total E.W.L. Prange and Schmidt-Nielsen (1969) showed that the lower rate of cutaneous water loss of desert dwelling snakes accounted for the observed difference in the rate of E.W.L. between them and water snakes. However, Chew and Dammann (1961), Dawson et al. (1966), Claussen (1967), and Bradshaw (1970) showed that both avenues of E.W.L. were reduced in animals that were adapted to more arid environments.

A diagram of the apparatus that was used to partition E.W.L. is shown in Fig. 10. Lizards were acclimated to 37 C for six days before a rubber collar (R) was cemented in place around their necks. The body of the lizard was then placed in a glass tube (T) on a wire grid (W) over a layer of magnesium perchlorate (M). The edges of the collar were sealed to the glass with a rubber band (B) and the whole apparatus was placed in a constant temperature cabinet at 37 C and less than 10% relative humidity.

The weight lost by the animal plus tube was equal to respiratory water loss, while cutaneous water loss was given by the increase in weight of the magnesium perchlorate.

However, using this system the rate of total E.W.L. was approximately double that which I obtained for unrestrained animals no matter how long the animals were left to equilibrate. Thus, the increased rate of E.W.L. was probably due to the greater activity of the restrained lizards. They never completely settled down and would open their eyes at the slightest touch unlike the unrestrained animals. Similarly, both Dawson et al. (1966) and Claussen (1967) observed that restraining animals increased their activity and hence their rate of E.W.L. In contrast, Bradshaw (1970) found no difference in the rate of E.W.L. between restrained and unrestrained animals. This is possibly because the animals were stressed in both situations since he does not state whether he attempted to determine when minimal rates of E.W.L. occurred.

The effect of restraining could be reduced by anaesthetising the lizards, but anaesthetics upset metabolism (Geddes, 1967), and would also therefore, affect water loss. Thus, my attempt to partition minimal E.W.L. was a failure and I cannot suggest a suitable alternative by which this could be done.

An interesting point that was revealed by this attempt to partition E.W.L. was that activity greatly increased cutaneous water loss. This contrasts with the theory proposed by a number of workers that the increased rate of water loss that is observed with activity is due to a higher respiratory water loss (Dawson et al., 1966; Gans et al., 1968; and Green, 1969). This is shown by the fact that the rate of cutaneous water loss I obtained by partitioning water loss was greater than the total rate of E.W.L. obtained for unrestrained animals. Similarly Claussen (1967) found that the rate of cutaneous water loss varied up to 300% at a given temperature within each of the two species of lizards that he studied. Reptiles have no sweat glands, so cutaneous evaporation is a function of vapour pressure deficit across the skin and is limited by permeability (Crawford and Kampe, 1971). It has been suggested that there is a thin layer of relatively moist air next to the skin of resting animals (Gans et al., 1968; Crawford and Kampe, 1971). This layer may be reduced or lost during activity and hence increase the vapour pressure deficit across the skin. Alternatively, during activity there may be physiological changes that decrease the permeability of the skin. However, these suggestions are purely speculative and further work would be needed to substantiate them.

Although I could not test it directly, it is likely that both cutaneous and respiratory water loss are lower in A. maculosus than in A. pictus, and I have assumed that this is true for followin sections of the study.

Maderson (Mitchell pers. comm.) could not find anything unusual about the structure of the dermal layers of A. maculosus. Similarly, Sokolov (1966) failed to find any obvious morphological adaptations for decreasing water loss in the skin of desert animals. Thus, the reduced cutaneous water loss of A. maculosus may be due to structural changes that are sub-dermal or to peculiarities of the biochemical system of the animal. However, I did not pursue these aspects.

A reduced respiratory water loss could be caused by either a lower metabolic rate and hence a lower oxygen requirement, or by a more efficient respiratory system whereby more oxygen could be consumed per unit of air inspired. To determine whether A. maculosus might employ either or both of these mechanisms, I compared the rate of oxygen consumption and the haemoglobin concentration of the blood of A. maculosus and A. pictus.

SECTION 3

OXYGEN CONSUMPTION

INTRODUCTION

Bradshaw (1970) showed that the agamid Amphibolurus ornatus had a metabolic rate about double that of the more arid adapted Amphibolurus inermis. He suggested that this could account for the low respiratory water loss of A. inermis compared to A. ornatus. Also Moberly (1963) showed that during hibernation the desert iguanid Dipsosaurus dorsalis had a reduced metabolic rate which he suggested may have been due to increased anaerobic respiration, or to a general shift to a type of metabolism that required less oxygen.

However, other attempts have failed to show any significant difference in the rate of oxygen consumption between lizards that have different rates of E.W.L. (Dawson and Bartholomew, 1956; Dawson et al., 1966; Claussen, 1967; Sexton and Heatwole, 1968).

Therefore, there seems to be some confusion as to whether the low rate of E.W.L. of some lizards can be due in part to a reduced rate of oxygen consumption. Hence, I wanted to determine whether there was any difference in the oxygen consumption of A. maculosus and A. pictus.

Since metabolic rate is affected by activity (section 2), the same conditions that produced minimal rates of E.W.L. were used to measure oxygen consumption.

Fig. 11. A diagram of the apparatus that was used to measure the rate of oxygen consumption.

F = Pyrex flask

G = Plastic grid

l = Inlet tube

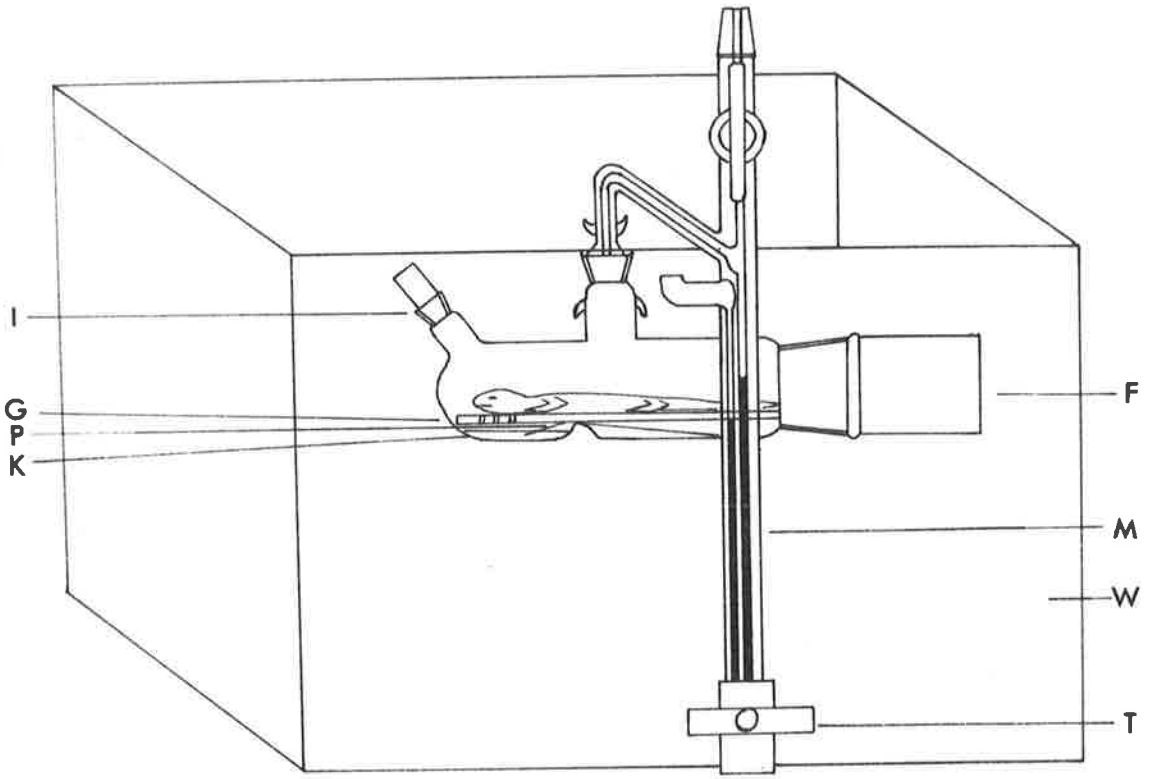
K = 10% KOH

M = Manometer

P = Filter paper

T = Thumb screw

W = Water bath


I
G
P
K
F
M
W
T

METHODS

Both *A. maculosus* (6.9 ± 1.1 g) and *A. pictus* (8.1 ± 0.5 g) were starved at 37 C for six days before their oxygen consumption was measured. The apparatus that was used to measure oxygen consumption (Fig. 11) was similar to that which was used by Alexander and Whitford (1968). A pyrex flask (F) was adapted to fit onto a manometer (M). The lizard rested on a perforated plastic grid (G) with its head above a reservoir of 10% potassium hydroxide which was used to absorb carbon dioxide. The surface area of the potassium hydroxide was increased by a strip of filter paper (P). An animal was placed in the flask at 1200 hours and lowered into the water bath (W) which was kept at 37 ± 0.5 C. The inlet (I) was left open and the whole apparatus was covered with a dark cloth. At 2000 hours 0.5 ml of 10% potassium hydroxide was introduced through the inlet (I) and the inlet was stoppered. After allowing twenty minutes for the carbon dioxide to be absorbed, the level of the manometry fluid was set with the thumb screw (T) and the run was started. All lights were turned off throughout the measuring period to keep the flask as dark as possible. Each run lasted about thirty minutes and three runs were made for each lizard. A thermo-barometer was run during each measuring period.

The volume of the animal was determined by submerging it in a measuring cylinder.

The amount of oxygen consumed per gram of body weight per hour was determined according to the equation

$$X = Y \left(\frac{V_g \frac{273}{T} + V_f \alpha}{Po} \right)$$ (Umbreit et al. (1959)).

X = Volume of oxygen consumed in μl.

Y = Difference in pressure of the two arms (mm)

Vg = Total volume of the system - the volume of the KOH

Vf = Volume of the KOH

T = Temperature in degrees absolute

α = Solubity of oxygen in 10% KOH at 37 C

Po = Density of the manometry fluid.

RESULTS AND DISCUSSION

The rates of oxygen consumption of A. pictus and A. maculosus are presented in Table 6 and they are compared with the results for other lizards in Table 7. There was no significant difference in the rate of oxygen consumption between A. maculosus and A. pictus ($p > 0.1$, Wilcoxon two sample test), although A. pictus tended to have a higher rate. However, I believe that most A. pictus were slightly stressed by the conditions of the experiment and therefore were not at their minimum metabolism, since some had rates of consumption as low as A. maculosus while others had rates up to twice as

Table 6

The minimum rate of oxygen consumption for A. maculosus and A. pictus at 37 C. Rates are expressed as $cc.g^{-1}.hr^{-1}$.

A. maculosus		A. pictus	
Animal	Rate	Animal	Rate
1	0.13	1	0.16
2	0.14	2	0.18
3	0.15	3	0.20
4	0.15	4	0.34
5	0.18	5	0.38
6	0.21		

Table 7

The rate of oxygen consumption per gram of body weight for a number of lizards.

Species	Weight (g)	Habitat	Temp (C)	Rate O_2-consumption $cc.g^{-1}.hr^{-1}$	Reference
Amphibolurus maculosus	7	A	37	0.16	Present study
Amphibolurus pictus	8	A	37	0.25	
Amphibolurus barbatus	370	T - SA	37	0.16	Bartholomew and Tucker (1963)
Varanus spp.	700	T - SA	37	0.21	Bartholomew and Tucker (1964)
Dipsosaurus dorsalis (non hibernating)	51	A	37	0.14*	Moberly (1963)
Dipsosaurus dorsalis (hibernating)	84	A	37	0.10*	
Uta stansburiana	3	SA	35	0.26	Roberts (1968a)
Sauromalus obesus	140	?	37	0.25	Crawford and Kampe (1971)

Corrected from S.T.P. to 37 C.

A = Arid, S.A. = Semi-arid, T = Temperate.

great (Table 6). In contrast, A. maculosus readily adapted to the conditions, as shown by the smaller variation in the rate of oxygen consumption (Table 6). Further evidence that A. pictus was more stressed is given by the fact that it invariably became agitated when it was removed from the flask at the end of the experiment, whereas A. maculosus was rarely aroused.

Allowing for differences in technique and that the rate of oxygen consumption per gram of body weight varies with body weight (Dawson and Bartholomew, 1956; Bartholomew and Tucker, 1964; Roberts, 1968a), there is little difference between lizards in their rate of oxygen consumption (Table 7), with the exception of the hibernating D. dorsalis. This is not surprising since animals that have similar body weights and active body temperatures and which are metabolising similar substrates would at rest require about the same amount of oxygen, unless of course they have an unusual form of metabolism that requires less oxygen.

Thus, it seems clear that no substantial proportion of the difference in the rate of E.W.L. between A. maculosus and A. pictus can be accounted for by a reduced rate of oxygen consumption in the former. Results similar to this using other pairs of spp with different rates of E.W.L. were obtained by Dawson et al., (1966); Claussen (1967); and Sexton and Heatwole (1968).

Table 8 shows the amount of water lost per ml of oxygen consumed for a number of lizards. The value for A. pictus is probably low since the technique that was used to measure oxygen consumption was more stressful than that used to measure E.W.L. Nevertheless, comparisons show that the amount of water lost per ml of oxygen consumed by A. maculosus is less than for any other lizard that has been studied. Even though the value for A. maculosus in Table 8 represents total E.W.L., it is lower than the amount of pulmonary water lost per ml of oxygen consumed for many lizards (Green, 1969), and thus, further illustrates the low rate of water loss of A. maculosus.

The low rate of respiratory water loss of A. maculosus is probably due to a respiratory adaptation whereby less ventilation of the respiratory tract is needed to effect the consumption of a unit volume of oxygen as was postulated by Dawson et al. (1966) for similar findings for three other Australian lizard species. Thus, in the next section I examined whether the proposed increased respiratory efficiency of A. maculosus could be due to a high haemoglobin concentration of the blood.

Table 8

The amount of E.W.L. per ml of oxygen consumed for a number of lizards.

Species	Temp (C)	Weight (g)	mg H_2O lost/ ml O_2 consumed	Reference
Amphibolurus maculosus	37	7	1.1	Present study
Amphibolurus pictus	37	8	1.4	
Dipsosaurus dorsalis	39	50	2.4*	From: Moberly (1963) and Minnich (1970a)
Crotaphytus collaris	36	30	2.2	Dawson and Templeton (1963)
Sauromalus obesus	35	140	1.8	Crawford and Kampe (1971)
Sauromalus obesus	40	140	2.4	

*Calculated from Moberly's (1963) O_2-consumption data and Minnich's (1970a) rate of E.W.L.

SECTION 4

HAEMOGLOBIN CONCENTRATION OF THE BLOOD

INTRODUCTION

Dawson and Poulson (1962) found a large difference between species of reptiles in the oxygen capacity of the blood, but they could find no clear correlation between oxygen capacity and either the altitude or aridity of the habitat. They showed, however, that the oxygen capacity of the blood varied directly with haemoglobin concentration. In contrast, other workers have found a good correlation between the haemoglobin concentration of the blood and the availability of oxygen in the habitat (Hall, 1937; Burke, 1953; Hadley and Burns, 1968; Klicka and Mahmoud, 1971). Thus, the haemoglobin concentration of the blood can vary considerably between species, but the limited studies that have been done do not show that desert reptiles can increase their respiratory efficiency by having a high haemoglobin concentration in the blood. Nevertheless, I compared the haemoglobin concentration of the blood of A. maculosus with that of two other desert dwelling agamids, A. pictus and Amphibolurus inermis.

MATERIALS AND METHODS

A. maculosus and *A. pictus* were collected from the southern end of Lake Eyre in South Australia, while *A. inermis* was collected from Alice Springs in the Northern Territory. Each species was housed in a separate outdoor pen and was fed *Tenebrio* larvae. Heat was supplied for twelve hours a day. All animals were fully hydrated and in good condition before they were used.

I estimated the haemoglobin concentration of the blood by the cyanomethaemoglobin technique (O'Brien *et al.*, 1968). In this method the blood cells are lysed and the haemoglobin that is released is converted to cyanomethaemoglobin the concentration of which can be determined by colorimetry.

Lizards were heated to 37 C, weighed, and killed with an overdose of sodium pentabarbitone (Nembutal). The heart was exposed, the pericardium removed, and about 0.1 ml of blood was removed by heart puncture using a modified syringe (Fig. 4) which contained a small drop of lithium heparin. The haematocrit value of the blood was determined, then 20 µl of blood was placed in 5.0 ml of lysing solution (o.1% w/v sodium bicarbonate, 0.02% potassium ferricyanide, 0.005% potassium cyanide). Three replicates were prepared for each lizard.

After 15 minutes the cyanomethaemoglobin solution was centrifuged and the supernatant was read at 540 nm with an Eel Spectra colorimeter. A standard curve was prepared using standard cyanomethaemoglobin solution, 59.3 mg/100 ml (Acuglobulin, Ortho Pharmaceutical Corporation).

RESULTS AND DISCUSSION

Even though I took blood directly from the heart so that it could not be contaminated by either peritoneal or pericardial fluid, haematocrits (packed cell volume) varied both intra- and inter-specifically from 20 to 30% of the total blood volume (Table 9). Manwell (pers. comm.) found a similar variation in the snake Thamnophis sirtalis and T. elegans as did Green (pers. comm.) in the goanna Varanus gouldii. In contrast, Bradshaw (1965) found very little variation in the haematocrit of A. ornatus, even for animals that had lost 50% of their body weight through dehydration. Part of the variation that I obtained may have been due to age differences since Burke (1953) showed that young animals had low haematocrits. In the present study however, although smaller lizards tended to have lower haematocrits, age differences could not be the major cause of the variation (Table 9). Thus the observed variation in haematocrit of the blood of A. maculosus may occur naturally

Table 9

Variation of haematocrit (packed cell volume) with body weight for three agamid lizards.

Amphibolurus inermis		*Amphibolurus pictus*		*Amphibolurus maculosus*	
Weight (g)	Haematocrit (%)	Weight	Haematocrit	Weight	Haematocrit
6	22	4	18	4	25
6	27	5	27	5	28
9	18	8	32	5	29
10	31	9	22	6	23
17	35				

or it may be an artifact possibly associated with keeping the lizards in the laboratory. With regard to this latter hypothesis, Pough (1969a) found that some blood components changed considerably when lizards were kept in the laboratory.

For the purposes of comparison I divided the haemoglobin concentration by the haematocrit since Burke (1953) showed that haemoglobin concentration varied directly with haematocrit. The results are presented in Table 10. There was no significant difference between the three species in the haemoglobin concentration of the blood (Table 11). Therefore, part of the difference in the rate of E.W.L. between A. maculosus and A. pictus can not be accounted for by a high haemoglobin concentration in the blood of the former.

There are a number of other mechanisms by which desert lizards could reduce their respiratory water loss, although they have not been discussed by other workers, indeed very few hypotheses have been suggested to explain the relatively well accepted fact that desert reptiles have a lower rate of respiratory water loss than more temperate forms. Therefore, I shall discuss a few mechanisms that desert reptiles might employ.

TABLE 10

Ratio of the haemoglobin concentration (mg/100 ml) over the haematocrit (%) for three species of agamid.

Species	Sample size	Mean value	Standard error
A. maculosus	4	1.12	0.043
A. pictus	4	1.07	0.016
A. inermis	5	1.17	0.032

TABLE 11

Analysis of Variance: haemoglobin concentration of the blood of three agamids.

Source	d.f.	S.S.	M.S.	V.R.	p.
Among	2	0.02	0.01	3.00	> 0.5
Within	10	0.04	0.0033		
Total	12	0.06			

It is known that the blood of some reptiles contains significant amounts of methaemoglobin, an oxidised form of haemoglobin that does not react with oxygen (Pough, 1968a; Dessauer, 1970; Manwell and Baker, 1970). Pough showed that the concentration of methaemoglobin varied from 14 to 33% of the total haemoglobin in four species of lizards, but that it was lowest in the most thermophilic species, Dipsosaurus dorsalis. If this compound forms a significant part of the haemoglobin of most reptiles, it may be that A. maculosus has a much lower concentration than A. pictus. However, this is pure speculation at the moment.

The Australian earthworm Spencierella spp. is much larger than the European worm Lumbricus spp. and it inhabits soils that become relatively dry over summer (Blight pers. comm.). Blight showed that these worms were much more resistant to desiccation than Lumbricus spp. and suggested that the large size may be an adaptation to aridity, i.e. that Spencierella spp. can reduce its water loss by reducing its surface to volume ratio. However, reducing the surface area of the body also reduces the area available for respiratory exchange. Blight subsequently showed however, that the haemoglobin of Spencierella spp. had an extremely high affinity for oxygen and that the dissociation curve was very steep over its unloading range. Presumably this would counterbalance the effect of having a reduced respiratory surface.

A similar adaptation may account for the low rate of respiratory water loss of desert lizards. Lizards could reduce their respiratory water loss if the inspired air was not completely saturated in the lung. This could be achieved if the alveolar wall were much thicker or less permeable to water. However, the haemoglobin would have to have a much higher affinity for oxygen to overcome the increased resistence to the diffusion of this gas into the blood. Unfortunately I have been unable to test this hypothesis due to a lack of time and animals although, a cursory look at the lungs of A. pictus and A. maculosus showed that there was not much difference between these two lizards in the thickness of the alveolar wall.

Recent work has shown that some animals can reduce their respiratory water loss by a counter current exchange mechanism whereby expired air is cooled in the nasal passages before it is exhaled (Jackson and Schmidt-Nielsen, 1964; Schmidt-Nielsen et al., 1969; Murrish and Schmidt-Nielsen, 1970). Murrish and Schmidt-Nielsen also suggested that the depression in the nasal passage of Dipsosaurus dorsalis might accumulate fluid from the nasal salt gland which could be used to partially humidify the inspired air. A. maculosus does not possess a nasal salt gland, but it may be able to recover water from expired air. However, I do not have any data which could either support or refute this hypothesis.

Another mechanism that lizards might employ to reduce their respiratory water loss would be to reduce the proportion of the respiratory tract that is not involved in gas exchange, e.g. the trachea.

However, most of the hypotheses that I have discussed above are purely speculative and would require a great deal of work to determine whether they are employed by A. maculosus or other desert lizards.

SECTION 5

RESPONSE TO HIGH TEMPERATURES

INTRODUCTION

Lizards are poikilothermic vertebrates that possess considerable capacity for regulating their body temperature when they are active. They achieve this through an interrelation of discrete behaviour patterns and physiological changes (Bradshaw, 1965; Licht et al., 1966; Bradshaw and Main, 1968; Moberly, 1968; Heatwole, 1970). When the environmental heat load becomes too great and they are unable to maintain tolerable body temperatures during normal activity, most lizards retreat to their burrows or seek shade. However, this is not always possible and sometimes during territorial defense or the evasion of predators, lizards may have to endure large heat loads for extended periods (De Witt, 1967; Heatwole, 1970). Under these conditions lizards can either tolerate high body temperatures, or dissipate some of the excess heat by evaporative cooling.

The two main forms of evaporative cooling that are used by animals are sweating and panting, but since lizards do not possess sweat glands they must use the latter. Although there have been only a few reports of lizards panting in the field (De Witt, 1967; Heatwole, 1970), many lizards possess the physiological capacity to pant (Templeton, 1960, 1970; Dawson and Templeton, 1963; Green, 1969; Richards, 1970; Crawford and Kampe, 1971).

Since A. maculosus forages well out on to the lake where there is little shade, it is probably subjected to high heat loads more often than most lizards. Therefore, I wanted to determine how A. maculosus responds to high temperatures. Again I compared the reaction of A. maculosus with that of A. pictus.

METHODS

All animals were in good condition and well hydrated. They were acclimated to 37 C for one week before they were tested. Several hours before a run was started a thermistor bead (Mullard, type VA 3000, P3K3, 3300 Ω at 25 C) was pushed deep into the rectum and the leads were taped to the base of the animal's tail. The change in resistence of the bead was measured with a Wheatstone Bridge circuit. The air temperature next to the lizard was measured with a Wesco (U.S.A.) fast reading thermometer which was arranged so that the temperature could be read without opening the door of the cabinet. Relative humidity was kept below 10% with a tray of silica gel.

The temperature of the cabinet was raised in steps of 1C, commencing at 37 C. Although the temperature could be raised at about 1 C per six minutes, I waited until the body temperature of the lizard had stabilised before the temperature was raised again. I thus hoped to determine whether the lizards could maintain their body temperature below ambient. The posture

and behaviour of the animal was noted throughout the run, and the number of breaths per minute during periods of normal breathing (eupnoea) was measured at each temperature. I measured breathing rate in this way because lizards breathe irregularly, there being long periods of apnoea interspersed with periods of eupnoea (Schmidt-Nielsen et al., 1966; Pough, 1969b).

RESULTS AND DISCUSSION

Breathing rate changed very little for either lizard over the temperature range 37 to 41 C varying from 24 to 38 breaths per minute for A. maculosus and 32 to 40 breaths per minute for A. pictus. A similar constancy of breathing rate has been obtained for other lizards (Dawson and Templeton, 1963; 1966; Hudson and Bertram, 1966; Pough, 1969b; Heatwole, 1970). However, metabolic rate and hence oxygen consumption increases with increasing body temperature. Thus the supply of oxygen must have been increased by other factors such as increased stroke volume of the heart, greater depth of breathing or an increased arterio-venous difference in the partial pressure of oxygen at the tissues. All those factors have been found to increase the supply of oxygen to the tissues at higher temperatures in a number of lizards; increased breathing rate being only relatively minor in importance (Dawson and Templeton, 1963; Tucker, 1966;

Crawford and Kampe, 1971). Although the breathing rate during eupnoea did not increase much with temperature, the periods of apnoea became shorter till at 45 C A. maculosus was breathing steadily at 60 breaths per minute.

Below 39 C the body temperature of A. maculosus rapidly reached ambient with almost no time lag, but above 40 C body temperature lagged up to four to eight minutes behind ambient. The body temperature at which the change occurred is close to the preferred body temperature of this lizard, 39 C (Mitchell pers. comm.). The lag is probably due to vasoconstriction of the peripheral blood vessels, since it is known that lizards have good control over their peripheral circulation (Bartholomew and Tucker, 1963, 1964; Baker and White, 1970; White, 1970).

A. maculosus exhibited three stages of respiratory cooling which were similar to those that Templeton (1970) observed for the iguanid Dipsosaurus dorsalis. At about 43.5 C (43.3 to 44 C) the mouth was held slightly open and breathing was deep at about 60 breaths per minute, mainly occurring after bouts of activity. At higher temperatures the gape increased while the breathing rate remained about the same, but periods of apnoea became less until about 46 C, where animals were breathing continuously at 65 breaths per minute with their mouths held wide open. Thus, undoubtedly A. maculosus possesses a mechanism for increased

respiratory cooling but it does not show the well developed panting response that is seen in some other lizards (Green, 1969; Crawford and Kampe, 1971).

There is considerable variation between species of lizards in the effectiveness of thermal panting. Some can maintain their body temperature below ambient by panting (Warburg, 1965a, b; De Witt, 1967; Green, 1969), while others can not (Templeton, 1960, 1970; Crawford and Kampe, 1971). The latter workers suggested that panting serves to eliminate most or all of the metabolic heat that is associated with the increased metabolic rates at high temperatures. However, although De Witt (1967) and Templeton (1960, 1970) studied the same lizard, Dipsosaurus dorsalis, De Witt showed that it could lower its body temperature by panting while Templeton obtained contradictory results. Either there is tremendous variation between populations of D. dorsalis, or, as is more likely, the difference is due to variations in experimental procedure. Thus, there is some doubt about the effectiveness of thermal panting in lizards.

The relative ineffectiveness of the respiratory cooling of A. maculosus is shown by the fact that body temperature always reached ambient temperature and at temperatures above 44 C was up to 1 C above ambient.

The body temperature of A. pictus responded to increasing ambient temperature in a manner similar to A. maculosus except that events occurred over a narrower and lower range of temperatures. Body temperature rapidly reached ambient below 39 C but above this temperature lagged several minutes behind. A 'panting' response similar to that of A. maculosus began at about 40.5 C, usually after bouts of activity, and was almost continuous at 42 C. Breathing rate when panting was about 70 breaths per minute.

The bodies of both lizards were dark in colour below 39 - 40 C, but blanched above this temperature range. It is generally considered that blanching is a thermoregulatory device since lighter colours reflect a greater amount of radiant energy, at least in the visible spectrum (Hutchinson and Larimer, 1960; Norris and Lowe, 1964; Norris, 1967; Heatwole, 1970). However, Bradshaw and Main (1968) proposed the interesting hypothesis that blanching may be associated with a decreased flow of melanophore stimulating hormone to the periphery of the body due to vasoconstriction so that blanching is a consequence of vasoconstriction and not an adaptation to reduce heat loss. Thus, it is obvious that more study is required to understand the role, if any, of blanching in thermoregulation.

The main difference between the response of A. maculosus and A. pictus to high temperatures was in their behaviour. Below 40 C A. pictus lay quietly with its eyes closed, on the shelf of the temperature cabinet. Above 40 C it became quite active and at 42 C was almost frantic in its attempts to escape. Heatwole (1970) observed a similar response in Amphibolurus inermis to temperatures above 42 to 44 C, the end result being the death of the lizard when it reached its upper lethal temperature. In contrast, A. maculosus remained quiet with its body flat on the shelf and its eyes closed until about 43.5 C. At this temperature some lizards became moderately active and showed respiratory cooling. From 44 to 46 C most lizards became more active but never frantic like A. pictus.

The difference in the response of these two lizards to high temperatures is probably related to differences in their habitats. Whereas A. pictus is never far from shade or its burrow, A. maculosus forages well out onto the lake where shade is very scarce. Thus, by remaining calm and tolerating high body temperatures A. maculosus can keep its water loss to a minimum while seeking shade. Bradshaw and Main (1968) reported that two other lizards that are exposed to high temperatures for extended periods, the agamid Diporophora bilineata and the gecko Diplodactylus michaelsoni, also passively tolerate high body temperatures.

SECTION 6

RESPONSE OF THE KIDNEY AND CLOACA TO HYPEROSMOTIC SALT INJECTIONS

INTRODUCTION

Reptiles are able to reduce the amount of water lost through excretion by voiding nitrogenous wastes as urates and uric acid which are relatively insoluble. Nevertheless, reptiles must also eliminate their excess electrolytes. This is achieved by the combined action of the kidney and cloaca although some reptiles also possess an extrarenal organ, a nasal salt gland, which can secrete excess electrolytes as a concentrated fluid (Schmidt-Nielsen and Fange, 1958; Templeton, 1964, 1966, 1967). However, no reptile that has been studied can produce a hyperosmotic urine (Smith, 1953). Therefore, unless they have an alternative way of excreting excess electrolytes, large amounts of water would be required to void the ions through the kidney-cloaca system.

Since the habitat of A. maculosus is hot and dry and its diet has a high sodium concentration, I wanted to determine whether this lizard showed any significant adaptations for conserving water when given a salt-load. I assumed that A. maculosus could do one of two things when given a large salt-load without access to food or water. It could excrete the salt as a concentrated solution through a salt gland, or store the salt in its body fluids and excrete it later when sufficient water became available (Bradshaw and Shoemaker, 1967).

Therefore, as a preliminary experiment A. maculosus were injected with a sodium chloride solution to determine which of the above alternatives they might employ.

Experiments were carried out at 37 C for reasons that were given in section 2. This makes it difficult to compare this study with those done with other reptiles because most of the earlier work was carried out at a variety of temperatures often very different from the eccritic temperature of the animal.

MATERIALS AND METHODS

Animals were collected from the southern end of Lake Eyre in South Australia. Five lizards were salt-loaded and four were controls. The animals were placed in separate containers over mineral oil. A wire mesh grid separated the urine from the faeces. Throughout the experiment the lizards were kept in a constant temperature cabinet at 37 $\pm$ 0.1 C with a 12/12 photoperiod. They were not given food or water.

The lizards to be salt-loaded were given intraperitoneal injections of 0.8 ml of 1.0N NaCl/100 g of body weight per day for six days using an Alga micrometer syringe. The salt was injected in small daily doses instead of one large load because Bradshaw and Shoemaker (1967) had shown that large amounts of saline injected intraperitoneally caused disruption of the fluid compartments of the body.

The control lizards were bled by heart puncture at the beginning of the experiment and the salt-loaded lizards were bled on day six. The blood was centrifuged and the plasma drawn off. All urine that was voided during the experiment was collected and this plus the plasma samples were frozen and stored in vinyl tubing under mineral oil until they were measured for osmotic and electrolyte concentration.

To test the effect that evaporation might have on the voided urine, three samples of a 300 mOs/l solution of sodium chloride were dropped into a separate container of mineral oil. These samples were removed after four days, stored in vinyl tubing, and later their osmotic concentration was measured.

The volume of the urine and plasma samples was often too small to measure both electrolyte and osmotic concentrations. Since the measurement of the latter only required a small volume, I measured the osmotic concentration of all the samples and although the potassium and sodium concentrations were measured for some samples they were too few to be meaningful and consequently are not presented.

Osmotic concentration was measured with a Mecrolab vapor pressure osmometer, model 302.

RESULTS

No encrustation of salt was found around the nares or the eyes of any of the salt-injected animals; therefore, A. maculosus does not appear to possess a functional salt gland. Bradshaw and Shoemaker (1967) looked for a salt gland in the agamid lizard, Amphibolurus ornatus, but they also failed to find one. However, Minnich (pers. comm.) found a salt gland in the agamid Uromastix.

The osmotic concentration of the plasma and urine samples is presented in Table 12. The reduced size of the samples was due to several animals dying during the experiment.

The osmotic concentration of the plasma from all the salt-injected lizards was higher than any control. The difference was non-significant when the two groups were compared with a Wilcoxon two sample test ($p > 0.1$) because the sample was small and the variation between salt-loaded lizards was large. Nevertheless, the difference was in the direction that would be expected from the fact that in the field during summer the osmotic and electrolyte concentration of the plasma increases. The observed difference was also consistent with the hypothesis of Bradshaw and Shoemaker (1967) in which they suggest that when water is scarce,

TABLE 12

Osmotic concentration (m Os/l) of individual plasma samples and mean ± S.E. of urine samples, from the preliminary experiment.

Blood Plasma		Voided Urine
Control	Salt-loaded	
355	393	
358	396	
358	461	532 ± 38 (6)
359		

Number of urine samples given in parenthesis.

A. ornatus stores excess electrolytes from the diet in its body fluids. So it seems likely that an experiment with a larger sample size might demonstrate that salt-loading will cause an increase in the osmotic concentration of the plasma.

However, the most interesting result from this experiment was that several samples of voided urine were more concentrated than the plasma (Table 12). The high concentration of the urine samples was not due to evaporation, because none of the test samples of fluid was concentrated by the conditions of the experiment. This result was most unexpected because it has long been held that reptiles are unable to produce a hyperosmotic urine (Smith, 1953).

In reptiles, urine is produced in the kidney and then passed down the ureters into the cloaca or bladder where it may be modified before it is excreted (Bentley and Schmidt-Nielsen, 1966; Junqueira et al., 1966; Roberts and Schmidt-Nielsen, 1966; Schmidt-Nielsen and Skadhauge, 1967; Braysher and Green, 1970). Therefore, urine could be concentrated either in the kidney or the cloaca. The kidney seemed more likely because part of its role in mammals, and to a lesser extent in birds, is to concentrate the urine so that wastes can be excreted with little loss of water; but I made a

histological and experimental study of the cloaca and kidney of A. maculosus is an attempt to determine where and how this lizard concentrates its urine.

EXPERIMENTAL APPROACH

This consisted of salt-loading lizards as before and collecting ureteral, cloacal and voided urine separately so that the electrolyte and osmotic concentration of each fraction could be measured. In these experiments I fed animals after they had been salt-loaded because at this stage I still believed that in the field A. maculosus could obtain water only from its food. Therefore, I wanted to test this by feeding the salt-loaded animals to determine whether they would then excrete the stored electrolytes.

Since I was collecting ureteral urine, I took the opportunity to determine the effect of salt-loading and dehydration on the rate of flow of ureteral urine. Other work on reptiles had shown that salt-loading can cause a large fall in the flow of urine and that dehydration can stop urine production (Dantzler and Schmidt-Nielsen, 1966; Roberts and Schmidt-Nielsen, 1966; Bradshaw and Shoemaker, 1967).

Because I was not trying to study the renal function of this lizard in detail, I did not try to determine glomerular filtration rate (G.F.R.) or whether electrolytes and water were being secreted or reabsorbed by the kidney tubules.

METHODS

Twelve animals that had been collected from Lake Eyre were divided into two groups, seven to be salt-loaded and five to act as controls. They were placed in individual containers in a constant temperature cabinet under conditions similar to those used for the preliminary experiment.

The lizards to be salt-loaded were given intraperitoneal injections of 1.0 ml of 1.0N NaCl/100 g of body weight per day for four days.

The control animals were bled at the beginning of the experiment while the salt-loaded lizards were bled on day four. Each lizard in the latter group was then fed at least three Tenebrio larvae. As soon as a lizard had urinated the fluid was collected and the animal was bled again. Also, any urine that was voided during the experiment was collected from beneath the oil as soon as it was noticed which was never longer than eight hours after it had been voided.

Ureteral urine was collected using the technique of Bradshaw (1965). Clear vinyl tubing (Dural Plastics, England) was marked at 5 µl intervals. The cloaca was drained, cleaned with saline and then dried with tissue paper. The calibrated piece of tubing was then inserted into the cloaca so that its proximal opening lay just posterior to the urinary papillae. The distance that the tubing had to be inserted was judged by eye although most catheters had to be adjusted slightly before they ran freely.

The tubing was then taped into position and the lizards were taped to an inclined board in a constant temperature cabinet at 37 ± 0.1 C. Since the urine flowed directly from the ureters into the catheter, it was probably not modified by the cloaca (Bradshaw, 1965; Roberts and Schmidt-Nielsen, 1966).

The lizards were starved for several days before the experiment which prevented the catheters becoming clogged with uric acid.

The rate of urine flow was measured for each 5 µl or sometimes 10 µl of urine produced. Urine flow rates were measured before the animals were bled, that being at the beginning of the experiment for the control lizards and on

day four for the salt-loaded animals. Another group of five lizards was dehydrated to about 90% of their initial body weight before rates of urine flow were measured.

Cloacal urine was collected from all animals immediately after urine flow had been measured. The cloaca was cleaned, drained and dried; then the animal was left for twelve hours before a sample of fluid was taken from the cloaca.

All plasma and urine samples were frozen and stored in vinyl tubing under mineral oil until they were measured for osmotic concentration and, when there was sufficient fluid, sodium and potassium concentration.

RESULTS

The osmotic and sodium concentrations of the plasma were significantly higher in the salt-loaded lizards than they were in the controls (t_{10} [Osm] = 6.76, $p < 0.01$; t_{10} [Na^+] = 5.08, $p < 0.01$) (Table 13). There was no significant difference in the potassium concentrations ($t_{10} = 1.34$, $0.2 > p > 0.1$). However, plasma samples were taken from the control lizards at the beginning of the experiment, whereas the salt-loaded animals were subjected to four days at 37 C without food or water before they were bled. Nevertheless, any increase in the concentration of the plasma due to dehydration would have

TABLE 13

The effect of salt-loading on the osmotic, sodium and potassium concentration of the plasma of A. maculosus.

	Control day 0	Salt-injected	
		Day 4	After feeding
Osmotic concentration mOs/l	379 ± 8 (5)	452 ± 7 (7)	463 ± 6 (7)
Sodium concentration mEq/l	179 ± 5 (5)	211 ± 4 (7)	201 ± 6 (7)
Potassium concentration mEq/l	7.7 ± 0.7 (5)	6.8 ± 0.2 (7)	7.1 ± 0.8 (6)

Result given as mean ± S.E. Size of samples in parentheses.

been small because the average rate of evaporative water loss of A. maculosus at 37 C is about 0.3 mg/g per hour (about double the minimum rate, see section 2). Therefore, the concentration of the plasma would not have increased more than 8 to 10 mEq/l due to dehydration over the four days and this is without considering the excretion of some electrolytes as a concentrated urine and as urate salts.

I did not use the control group to determine the effects of dehydration after they had been bled because the relatively large volume of blood removed through bleeding would have negated their value as controls. Also, I did not have sufficient lizards for a second control group.

Thus, salt-loading caused a large increase in the osmotic and sodium concentrations of the plasma of A. maculosus, as in the preliminary experiment and similar to what Bradshaw and Shoemaker (1967) found whenthey salt-loaded A. ornatus. Therefore, A. maculosus must have stored at least some of the injected sodium chloride in its body fluids.

Feeding did not cause a significant drop in the osmotic or the electrolyte concentration of the plasma (Table 13) (t_{12} [Osm] = 1.17, $0.3 > p > 0.2$; t_{12} [Na^+] = 1.45, $0.2 > p > 0.1$; t_{11} [K^+] = 0.39, $p > 0.6$). Hence A. maculosus did not obtain sufficient water from its food to eliminate any substantial part of excess electrolytes, at least not within two to three days after feeding.

Table 14 shows the variation in the concentration and composition of the various urine fractions. Voided urine was clearly hyperosmotic to the plasma, up to one and a half times the osmotic concentration of the plasma, whereas ureteral urine was always hyposmotic. Therefore, the cloaca and not the kidney would seem to be the site for the concentration of the urine. Additional evidence for this is found in the data on cloacal urine (Table 14). The concentration of this urine was intermediate between the ureteral and voided urine and this, plus the large variation in the concentration of the cloacal samples, indicates that ureteral urine was being concentrated in the cloaca.

The salt-injected lizards voided urine at various times during the experiment (Table 14), but the concentration of the voided urine did not change appreciably with salt-loading. Thus, the concentration of the urine presented in Table 14 may represent the maximum concentrating ability of the cloaca of A. maculosus. Most mammals and birds however, produce their most concentrated urine when they are dehydrated, but dehydrated A. maculosus produced little or no urine. This is consistent with later data on urine flow which shows that dehydration greatly reduces or almost stops the flow of ureteral urine in A. maculosus. Any that does enter the cloaca is probably quickly reabsorbed.

TABLE 14

The effect of salt-loading on the osmotic, sodium and potassium concentration of ureteral, cloacal and voided urine from A. maculosus.

	Control	Salt-loaded						
	Ureteral	Ureteral	Cloacal before bleeding	Cloacal after bleeding	Voided before bleeding		Voided after bleeding	
					Day 2	Day 4	Day 5	Day 7
Osmotic concentration mOs/l	206 ± 18 (4)	355 ± 21 (4)	489 ± 42 (4)	494 ± 45 (4)	686 ± 25 (5)	671 ± 23 (4)	660 ± 34 (4)	666 ± 11 (5)
Sodium concentration mEq/l	102 ± 11 (5)	160 ± 8 (7)	202 ± 10 (4)	212 ± 9 (4)	282 ± 11 (5)	260 ± 10 (4)	275 ± 23 (4)	244 ± 18 (5)
Potassium concentration mEq/l	10.8 ± 3.1 (5)	7.8 ± 0.5 (6)	44 ± 17 (3)	28 ± 11 (4)	50 ± 5 (5)	63 ± 7 (4)	42 ± 6 (4)	56 ± 11 (5)

Results given as means ± S.E. Size of samples in parentheses.

There was no significant difference in the rate of flow of ureteral urine between the control and salt-loaded lizards ($t_9 = 1.31$, $0.3 > p > 0.2$), although the rate of flow tended to be higher in the salt-injected animals (Table 15).

In contrast, dehydration greatly reduced the rate of flow. The rate of flow was too low to be measured in most of the dehydrated lizards, but measurements were made for two lizards that were less severely dehydrated; they had lost 6.5% of their initial body weight compared to 9 to 13% in the other three animals. The rates are presented in Table 15.

Salt-loading caused an increase in the osmotic and sodium concentration of the ureteral urine while the potassium concentration decreased. This is shown by the urine to plasma ratios (U/P) for these parameters (Table 16). However, since G.F.R. was not measured I can not conclude whether this was due to glomerular or tubular effects.

DISCUSSION

The urine flow rate and the U/P osmotic ratio for a number of reptiles are compared in Table 17. This data is for ureteral urine only and not voided or cloacal urine. All other reptiles except the skink Trachydosaurus rugosus and the water snake Natrix cyclopion have much lower rates of urine flow than

TABLE 15

The effect of salt-loading and dehydration on the rate of flow of ureteral urine from A. maculosus (mean ± S.E.).

	Control	Salt-loaded	Dehydrated
Rate of flow ml/Kg.hr	7.9 ± 1.0 (5)	9.6 ± 0.8 (6)	1.1 ± 0.1 (2)

Size of samples given in parentheses.

TABLE 16

The effect of salt-loading on the urine/plasma ratios for the osmotic, sodium and potassium concentrations of the ureteral urine of A. maculosus.

	Control	Salt-loaded
Osmotic U/P	0.54	0.74
Sodium U/P	0.57	0.76
Potassium U/P	1.40	1.10

TABLE 17

Urine flow rate and the U/P osmotic ratio of ureteral urine for a number of reptiles.

Animal	Temperature C	Experimental Condition	Flow Rate $ml.Kg^{-1}.hr^{-1}$	U/P Osmotic Ratio	Reference
Water snake	Room Temp	Water-loaded	9.0*	-	LeBrie and Elizondo, 1969
Natrix cyclopion		NaCl-loaded	12.8	-	
Freshwater turtle	20 - 22	Normal	1.3	0.6	Dantzler and Schmidt-Nielsen, 1966
Pseudemys scripta		NaCl-loaded	0.4	0.8	
		Dehydration	0.0		
Desert tortoise	20 - 22	Normal	2.0	0.4	
Gopherus agassizii		NaCl-loaded	0.9	0.6	
		Dehydration			
Desert snake		Normal	1.4	0.7β	Komadina and Solomon, 1970
Pituophis melanoleucus	Not stated	NaCl-loaded	3.6	0.7β	
Semi-Arid Skink	37	KCL-loaded	10.0	-	Shoemaker et al., 1966
Trachydosaurus rugosus					

Table 17 cont.

Animal	Temperature C	Experimental Condition	Flow rate $ml.Kg^{-1}.hr^{-1}$	U/P Osmotic Ratio	Reference
Semi-Arid Goanna	30	Normal	3.1	0.5	Green, 1969
Varanus gouldii		NaCl loaded	1.7	0.7	
Tropical gecko		Normal	2.6	0.6	
Hemidactylus spp.		NaCl-loaded	2.4	0.8	
		Dehydrated	1.3	0.7	
Arid Iguanid	24 - 29	Normal	1.9	1.0	Roberts and
Tropidurus spp.		NaCl-loaded	1.2	1.0	Schmidt-Nielsen,
		Dehydrated	0.5	1.0	1966
Arid Iguanid		Normal	2.0	0.9	
Phrynosoma cornatum		NaCl-loaded	0.6	1.0	
		Dehydrated	0.8	1.0	
Arid Agamid	37	Normal	7.9	0.5	Present study
		NaCl-loaded	9.6	0.8	
Amphibolurus maculosus		Dehydrated	1.1	-	

*Rates of flow are double those given in the reference for a single ureter.
βCalculated from data published in the reference.

A. maculosus. This may be due in part to the lower temperatures that were used in other work, but it is doubtful whether temperature could completely account for this difference.

It has been shown that catheterizing the bladders of trout (Hunn and Willford, 1970) and the ureters of pigeons (McNabb and Poulson, 1970) causes a diuresis which lasts up to twelve hours in the trout and from 30 to 70 minutes in the pigeon. However, although the rates of flow of ureteral urine that I obtained for A. maculosus may be elevated due to catheterizing the ureters, it does not explain why the rates of flow were much higher than those that were obtained for other reptiles, because the effect of catheterization was not allowed for in these animals either. Besides it was mentioned earlier (section 2) that A. maculosus remains reasonably quiet during handling so catheterization is hardly likely to have stressed A. maculosus more than the other species mentioned in Table 17.

Thus, A. maculosus and T. rugosus have rates of flow of ureteral urine that are more similar to the chicken (Dantzler, 1966; Skadhauge and Schmidt-Nielsen, 1967) than to most reptiles, although I have no idea why this would be so.

Salt-loading tended to increase the rate of flow of the ureteral urine in A. maculosus, but the increase was not

significant. Most other reptiles respond to salt-loads by decreasing urine flow, although the desertinhabiting snake, Pituophis melanoleucus, and the water snake, Natrix cyclopion, show a significant increase in the flow of urine in response to salt-loading (Table 17). However, A. maculosus is similar to most other reptiles that excrete nitrogenous wastes as uric acid (uricotelic) in that although salt-loading may slightly increase or significantly decrease urine flow, it does not stop or virtually stop urine flow as it does in reptiles and amphibians that excrete nitrogenous wastes as urea (ureotelic) (Schmidt-Nielsen and Skadhauge, 1967; Dantzler, 1970).

In contrast, T. rugosus (Bentley, 1959) and A. ornatus (Bradshaw and Shoemaker, 1967) are uricotelic lizards that are adapted to semi-deserts in which salt-loading causes urine flow to stop. However, these workers, and many others who have studied renal function in reptiles, collected voided and not ureteral urine. Braysher and Green (1970) have shown that the cloaca of lizards is very important in modifying the composition and volume of ureteral urine. Therefore, it is possible that salt-loading did not stop urine flow in T. rugosus and A. ornatus, but that most of the water and electrolytes were reabsorbed in the cloaca or bladder.

Whereas moderate dehydration, 5 to 10% of the initial body weight, decreased urine flow by up to 50% in most uricotelic reptiles, it reduced the rate of flow in A. maculosus much more (Table 17). Thus, in this respect A. maculosus is more similar to the predominantly ureotelic reptiles (Dantzler and Schmidt-Nielsen, 1966; Dantzler, 1970) than to uricotelic animals.

However, it is probable that A. maculosus is never dehydrated in the field. Bradshaw (1970) could divide his population of the lizard Amphibolurus ornatus into two distinct types, fast growers and slow growers. The slow growing individuals did not become dehydrated and did not lose weight over summer. They fed normally and stored excess electrolytes from their diet in their body fluids until they could be eliminated later when water became available. In contrast, the fast growers were not well adapted to hot, dry summers. They lost weight rapidly, mainly as water, and as dehydration increased they ceased to eat; if unfavourable conditions continued too long they died. Bradshaw found no evidence for fast or slow growers among other species of agamids that he studied.

Although I did not have a marked population of A. maculosus that I could weigh regularly, none of the animals that I collected

appeared to be dehydrated. Also these lizards feed throughout summer. Hence, A. maculosus would correspond more closely to Bradshaw's more arid-adapted slow growers than to fastgrowers, and therefore, would become salt-loaded but probably not dehydrated. Consequently, the flow of ureteral urine would not stop.

Dantzler and Schmidt-Nielsen (1966) suggest that to maintain a relatively constant G.F.R. when salt-loaded or dehydrated would be of adaptive advantage to uricotelic reptiles because it would allow the excretion of nitrogenous wastes to continue. In uricotelic reptiles nitrogenous wastes are secreted by the kidney tubules (Marshall, 1932; Dantzler, 1967) probably as soluble urate salts (Dantzler and Schmidt-Nielsen, 1966; Dantzler, 1967, 1968; Minnich, 1970b). The urates are precipitated in the cloaca by the removal of water. Green (1969) suggests that acidification of the urine may also help to precipitate the urates since the solubility of urates decreases with decreasing pH (Minnich, 1970b).

Ramsay (1955) showed that uric acid is excreted in a similar manner in the stick insect Dixippus morosus. That is, potassium urate is secreted in a soluble form in the malpighian tubules and is precipitated in the rectum by removal of water and acidification.

Hence, A. maculosus could continue to excrete nitrogenous wastes over summer since the flow of ureteral urine probably would not stop.

The ureteral urine of A. maculosus was always hyposmotic to the plasma (U/P osmotic ratio < 1), even when lizards were injected with salt, which is similar to what has been found for the desert tortoise (Gopherus agassizii), the semi-desert goanna (Varanus gouldii), the tropical gecko (Hemidactylus spp.) and the desert snake (Pituophis melanoleucus) (Table 17). In contrast, two lizards that inhabit arid areas, the horned toad (Phrynosoma cornutum) and the Galapagos lizard (Tropidurus spp.), always produce an isosmotic ureteral urine (U/P osmotic ratio = 1). Thus, there does not appear to be any strict correlation between the osmotic concentration of the ureteral urine and the aridity of the animal's habitat.

It is commonly accepted that the ability to produce a dilute urine is associated with the need to eliminate large amounts of water (Deyrup, 1964). I can conceive that the other reptiles that produce a hyposmotic urine may have to face this problem, but I would have thought that A. maculosus would never have the problem of excess water. It is more likely that the production of a hyposmotic urine inthis lizard is associated with another function. Certainly A. maculosus is

in no danger of losing excess amounts of water by producing a dilute ureteral urine, because the cloaca can concentrate the urine before it is voided; indeed the cloaca of most reptiles is capable of greatly modifying the composition of the ureteral urine (Bentley and Schmidt-Nielsen, 1966; Junqueira et al., 1966; Roberts and Schmidt-Nielsen, 1966; Schmidt-Nielsen and Skadhauge, 1967; Braysher and Green, 1970; Dantzler, 1970).

Unlike any other reptile that has been studied, A. maculosus can void a urine that is hyperosmotic to the plasma, the urine being concentrated in the cloaca and not the kidney as it is in mammals and birds.

The excretory system of A. maculosus, and for that matter most reptiles, functions in a manner similar to that of insects. The malpighian tubules of insects produce a primary urine probably by a combined secretion - filtration mechanism as compared to reptiles where blood is filtered through the glomerulus (Berridge and Oschmann, 1969; Oschman and Wall, 1969). The fluid, which is usually isomotic to the haemolymph, passes into the hindgut and thence into the rectum where it is modified depending on the needs of the animal (Stobbart and Shaw, 1964). However, insects, unlike A. maculosus, can concentrate their urine to many times the concentration of

the haemolymph. Later, in the section on morphology, I will discuss the similarity of the meachanisms which A. maculosus and insects probably employ to concentrate the urine.

A point that I would like to discuss here is the effect on lizards of intraperitoneal injections of concentrated sodium chloride solutions. The agamid lizard, Amphibolurus ornatus, has a diet that is high in sodium and during summer it stores excess electrolytes in its body fluids, mainly in the extracellular fluid (E.C.F.), causing an expansion of this fluid compartment at the expense of the intracellular fluid (I.C.F.). Bradshaw and Shoemaker (1967) tried to mimic this in the laboratory by progressively salt-loading animals. They injected small doses of sodium chloride intraperitoneally, 0.6 ml of 1.0N NaCl/100 g of body weight per day for six days, because large doses disrupted the distribution of body fluids. The concentration of sodium in the muscles of these salt-loaded animals increased from 28 mEq/l, the value for fully hydrated lizards, to 106 mEq/l, while the sodium concentration of the plasma increased from 150 to 208 mEq/l. In contrast, the concentration of sodium in the muscle of lizards taken from thefield in summer varied from 53 to 73 mEq/l, while their plasma sodium concentration was approximately 250 mEq/l, about 100 mEq/l above the concentration for fully hydrated animals.

Since E.C.F. accounts for only about 10% of the fluid in the muscle of lizards (Chan et al., 1970), and since the concentration of the E.C.F. was about 40 mEq/l higher in the animals taken from the field in summer than in the salt-loaded animals in the laboratory, there must have been considerable penetration of the cells by sodium in the salt-injected lizards.

Therefore, injecting sodium chloride solution intraperitoneally does not mimic the fluid and electrolyte distribution that occurs in salt-loaded animals in the field in that it causes abnormal increases in the concentration of sodium in the cells. Most other workers have used even larger doses than what I and Bradshaw and Shoemaker (1967) used.

I do not know what effect an abnormal increase in the sodium concentration of the cells would have on the animals, but at least some attempt should be made to test it. This could be done by comparing certain parameters such as G.F.R., water and salt excretion, etc. between animals that had been salt-loaded by intraperitoneal injections with others which had been salt-loaded through their diet.

After I had completed the preceding study into some of the mechanisms that A. maculosus might employ to conserve water when given a salt-load, Minnich (1970b; 1968) showed that some

uricotelic lizards could eliminate electrolytes as insoluble potassium and sodium urates. He found, for instance, that the desert iguanid, Dipsosaurus dorsalis, could excrete the equivalent of a 5,000 mEq/l solution of potassium ions as potassium urate. The water conserving advantages of this are obvious, therefore, I wanted to determine whether A. maculosus could excrete ions in a similar manner. The experiments that were done on this aspect of excretion are discussed in a separate section.

SECTION 7

The structure of the cloaca and rectum of *A. maculosus*

INTRODUCTION

It was shown in the preceding section that A. maculosus can produce a urine that is hyperosmotic to the plasma. Collection of urine before and after it had entered the cloaca indicated that the urine became more concentrated after it left the ureters. Further evidence that the kidney of A. maculosus cannot produce a hyperosmotic urine was obtained by examining the structure of the kidney. I cut serial sections of some kidneys of A. maculosus and A. pictus, and I macerated others and looked at the individual nephrons. Both methods showed that the kidney of A. maculosus was similar to that of A. pictus and of other reptiles (Huber, 1917; Davis and Schmidt-Nielsen, 1967). I could find no loop of Henle or any other structure in the kidney of A. maculosus which might enable this lizard to produce a hyperosmotic urine. Therefore, I concentrated my efforts on a study of the cloaca of A. maculosus.

It is now generally considered that the absorption of fluid occurs either by primary active transport of a solute followed by passive solute-linked water-transport, or by passive flow by physical means such as the flow of water due to hydrostatic pressure or along an osmotic gradient (Phillips, 1970; Murrish and Schmidt-Nielsen, 1970b; Wall et al., 1970). Active transport of water as such is not considered plausible for vertebrate epithelia (Ochman and Wall, 1969) although it may occur in some insects (Beament, 1964).

With the advent of the electron microscope many workers noticed the similarity in the ultrastructure of various absorptive epithelia and this promoted attempts to correlate structure with function. In this regard Curran, 1960, 1965; Diamond, 1964; Diamond and Bossert,1967, proposed theoretical models to explain solute-linked water transport. They suggested that solute was actively secreted into the intercellular spaces between epithelial cells and that water followed passively. Recent work on various absorptive epithelia has provided experimental evidence for their hypotheses, e.g. toad bladder (DiBona et al., 1969; Ferguson and Heap, 1970), gall bladder (Kaye et al., 1966; Tormey and Diamond, 1967), kidney tubule (Davis and Schmidt-Nielsen, 1967; Grantham et al., 1969), and insect rectum (Berridge and Oschman, 1969; Oschman and Wall, 1969; Sauer et al., 1970; Wall and Oschman, 1970; Wall et al., 1970). However, of these various epithelia only that of the rectum of insects can produce a hyperosmotic fluid from hypo-osmotic or isosmotic solutions in the lumen (Irvine and Phillips, 1971). Indeed insects can produce very concentrated excreta in their rectum (Ramsay, 1955; Wigglesworth, 1965). Therefore, I wanted to determine whether A. maculosus may concentrate its urine in a manner similar to that of insects.

There is good evidence that the reabsorption of fluid from the cloaca of reptiles is solute-linked (Bentley and Schmidt-Nielsen, 1966; Junqueira et al., 1966; Schmidt-Nielsen and Skadhauge, 1967; Braysher and Green, 1970), while the structure

of the cloaca of reptiles closely resembles that of other absorptive epithelia, at least at the level of the light microscope (Seshadri, 1956, 1957; Fox and Dessauer, 1962; Junqueira et al., 1966; Green, 1969).

The cloaca of reptiles generally consists of three chambers in series. The most posterior chamber is the proctodaeum, which is connected to the second, the urodaeum, while the most anterior chamber is the coprodaeum. Urine from the kidneys enters the cloaca in the urodaeum and moves forward into the coprodaeum where urates and uric acid are precipitated and fluid is withdrawn (Seshadri, 1956, 1957; Roberts and Schmidt-Nielsen, 1966; Green, 1969). However, preliminary observations indicated that the barrier between the coprodaeum and the rectum in A. maculosus and A. pictus was very small. Thus, initially I wanted to determine whether urine moved forward into the rectum of these lizards. I then studied the gross, microstructure and ultrastructure of the cloaca and rectum of A. maculosus and A. pictus and I compared them with those reported for other absorptive epithelia.

This study showed that in A. maculosus and A. pictus, urine could move up into the rectum at least as far as the division between the rectum and the caecum. Micro- and ultrastructural studies showed that the cloaca and the rectum of both lizards was very similar in structure except for the anterior part of the rectum of A. maculosus. Parts of the mucosal epithelium of the anterior third or so of the rectum of A. maculosus have a

structure which is similar to that which occurs in the rectal papillae and pads of some insects. It is suggested that A. maculosus can produce a hyperosmotic urine in the anterior part of its rectum. The anterior part of the rectum of A. pictus, unlike that of A. maculosus, appears to have a structure that is similar to the rest of the rectum and the coprodaeum.

METHODS

a) Movement of urine

Two A. maculosus and two A. pictus from Lake Eyre were starved for a week to eliminate faecal material from the lower intestine then a few drops of India ink were introduced through a piece of plastic tubing into the urodaeum of each lizard. Fifteen minutes later they were killed with an overdose of Nembutal and dissected to determine the position of the India ink.

b) Structure of the cloaca and rectum

A. maculosus and A. pictus were killed with an overdose of Nembutal, then the body cavity was opened and the cloaca and rectum removed and placed in isotonic saline (160 m Eq/l NaCl, 5 m Eq/l KCl).

(i) Light microscope

The cloaca and rectum was fixed for 24 hours in buffered neutral formalin (10%) containing 8% glucose. The tissue was dehydrated in alcohol and embedded in paraffin wax.

Serial sections 6 to 8 μ thick were cut and mounted on glass slides. The sections were stained with alcian blue, Weigert-Lillie alum haemotoxylin and eosin according to the procedure in Preece (1959) and mounted in Canada Balsam.

(ii) Electron microscope

Small pieces of cloaca and rectum were fixed for two hours in 4% gluteraldehyde in Millonig's Buffer (pH 7.4). Glucose was added to make an 8% solution to prevent shrinkage of the tissue. Pieces of tissue were then rinsed in buffer and post-fixed for one hour in 2% osmium tetroxide in Millonig's Buffer. They were then washed in buffer and taken through a graded series of acetone before being embedded in Araldite.

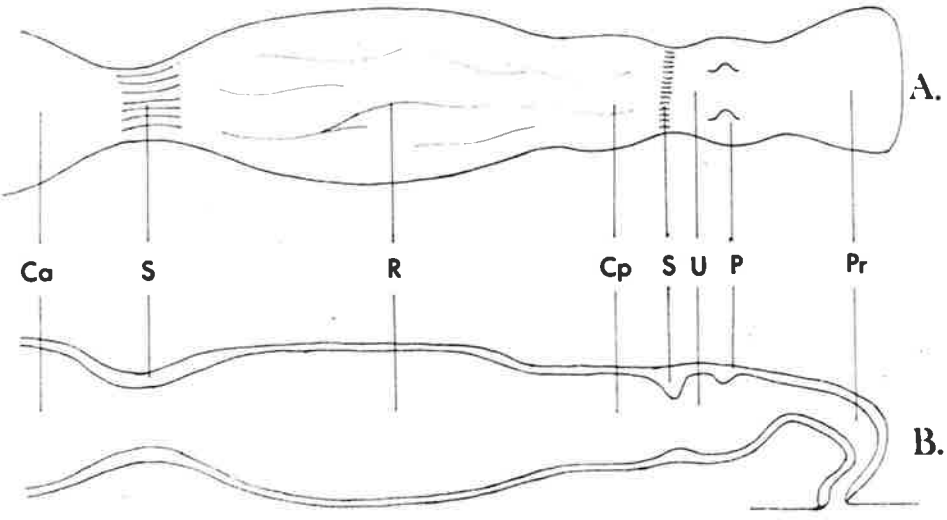
Sections with a gold to pale gold interference pattern were cut with a Porter-Blum ultramicrotome and mounted on 400 mesh unsupported copper grids or 200 mesh carbon-collodion supported grids. They were stained with lead citrate and observed with a Siemen's Elmiskop I.

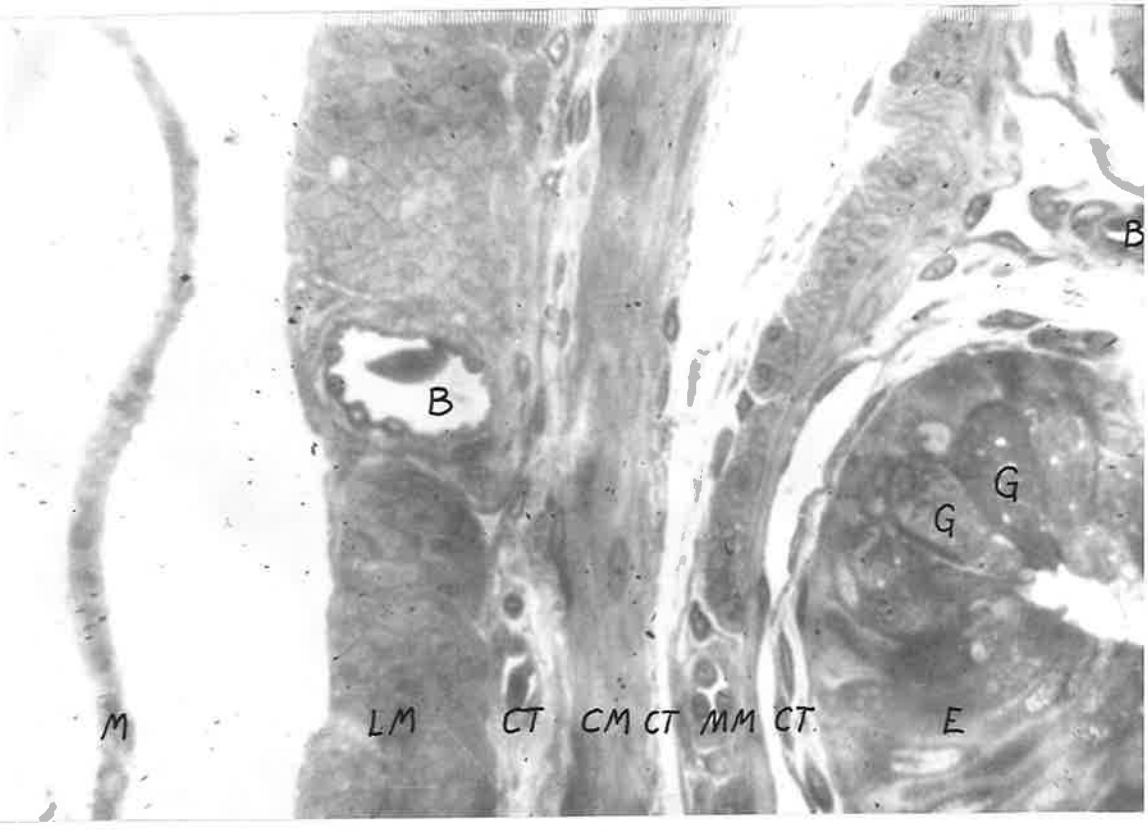
RESULTS AND DISCUSSION

The India ink that was introduced into the urodaeum of A. maculosus and A. pictus moved forward into the rectum. In addition I found deposits of uric acid and urates in the rectum of lizards that had been starved for two weeks. Thus these results provide strong evidence that urine moves forward

Fig. 12. A diagramatic representation of the cloaca and rectum of A. maculosus and A. pictus. 'A' is a ventral view of the structures which have been split along the mid-ventral line. 'B' ia a longitudinal section of the cloaca and rectum. Pr = proctodaeum, P = urinary papilla, S = sphincter, U = urodaeum, Cp = coprodaeum, R = rectum, Ca = caecum.

Fig. 13. T.S. of the wall of the coprodaeum of A. maculosus. The structure of the rectum and the coprodaeum of A. pictus is very similar. E = mucosal epithelium containing goblet cells (G). CT = connective tissue, B = blood vessels, MM = muscularis mucosae, CM = circular muscle, LM = longitudinal muscle. The mesothelium (M) has fallen away from the longitudinal muscle in this section. Tissue is embedded in araldite and stained with toludine blue.


A.
Ca
S
R
Cp
S
U
P
Pr
B.
Ventral


B
B
G
G
M
LM
CT
CM
CT
MM
CT
E
0·1mm

into the rectum of these lizards. However, unlike what has been found in chickens (Skadhauge, 1967; Ohmart et al., 1970), the urinary wastes of A. maculosus and A. pictus are not mixed with the faeces. The urinary pellet is always separate from the faecal pellet and is usually voided attached to the posterior end of the faecal pellet.

I propose that in A. maculosus and A. pictus urine from the ureters moves forward into the coprodaeum and rectum where uric acid and urates are precipitated and fluid is reabsorbed. Then faeces from the caecum move into the rectum to be dried out and in the process they push the urinary pellet into the coprodaeum.

Gross morphology

The rectum and cloaca of A. maculosus and A. pictus are very similar (Fig. 12). The rectum is the largest chamber and lies anterior to the cloaca. It is separated from the caecum by a sphincter and from the anterior chamber of the cloaca, the coprodaeum, by a small transverse fold in some lizards or, as is more usual, by a slight decrease in the diameter of the lumen of the rectum as it merges into the coprodaeum. The urodaeum is smaller and posterior to the coprodaeum and is separated from it by a strong sphincter. The most posterior chamber is the proctodaeum which is connected to the exterior by the overlapping lips of the cloaca and from the urodaeum by a slight increase in the diameter of the lumen. Urine enters the cloaca in the urodaeum by a pair of

dorsally located papillae and moves forward into the coprodaeum and rectum. The mucosal surface of the rectum and coprodaeum appears relatively flat to the naked eye with no large villi or transverse folds, unlike what Seshadri (1959) and Green (1969) found in varanids. The cloaca and rectum of A. maculosus and A. pictus are similar to what Seshadri found for the gecko Hemidactylus flaviviridis (Seshadri, 1956) and the agamid Uromastix hardwickii (Seshadri, 1957). In varanids (Seshadri, 1959; Green, 1969) and in the lizards that Roberts and Schmidt-Nielsen (1966) studied, a sphincter muscle separates the coprodaeum from the rectum.

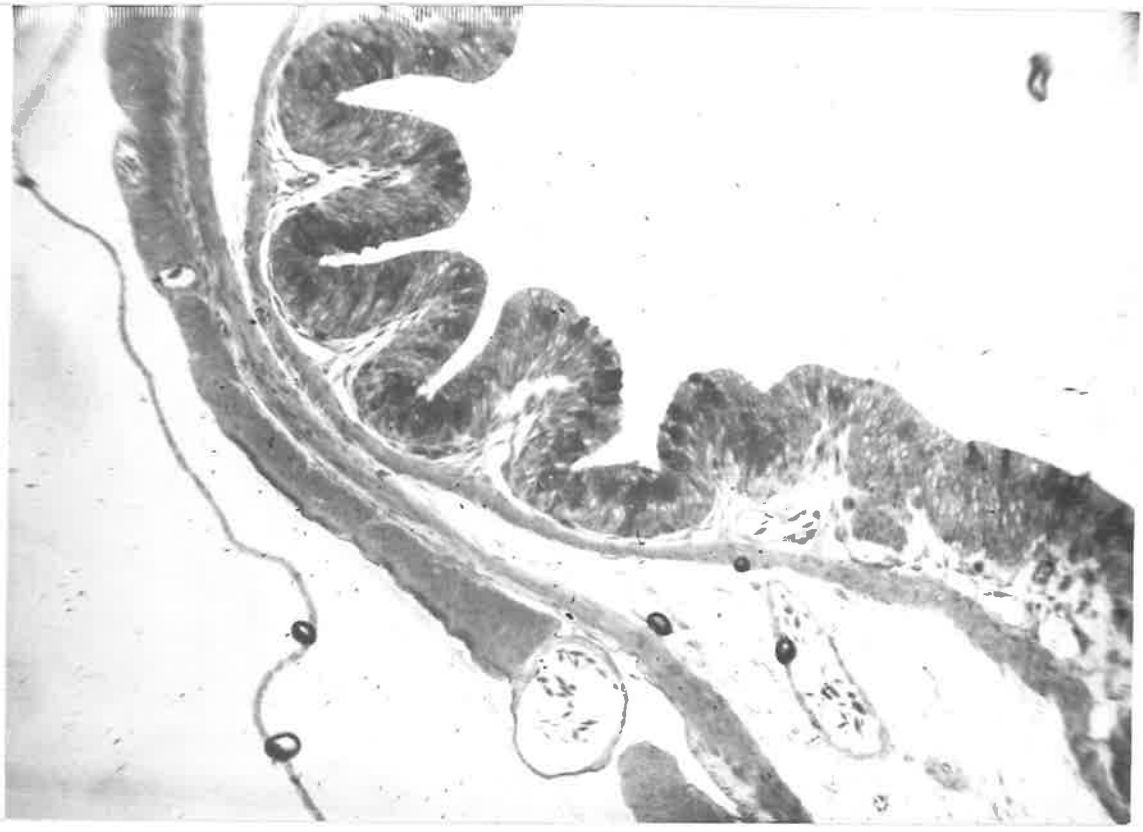
Light microscopy

I shall confine myself to a discussion of the microscopic and ultra-microscopic structure of the rectum and coprodaeum of A. maculosus and A. pictus since these are the structures that appear to be involved in the reabsorption and concentration of the urine before it is excreted.

The microstructure of the coprodaeum and rectum of A. maculosus for the most part is indistinguishable from that of A. pictus, and the following description therefore applies to both species. The coprodaeum and rectum are thin-walled vascular structures that consist of many layers (Fig. 13). The innermost layer is the mucosal epithelium below which is a thin layer of connective tissue, the lamina propria, containing many blood vessels. Beneath this is the muscularis mucosae which has a thin inner band of circular

Fig. 14. Villi of the rectum and coprodaeum. Note the darkly staining mucous or goblet cells in the mucosal epithelium. Araldite - toludine blue.

Fig. 15. Electron micrograph of the mucosal epithelium of the coprodaeum of A. maculosus. Again the ultrastructure of the rectum and coprodaeum of A. pictus is very similar. G = goblet cell, GC = granular cell, N = nuclei, M = mitochondria, MG = mucous granules, V = microvilli. Approx. X2,500.



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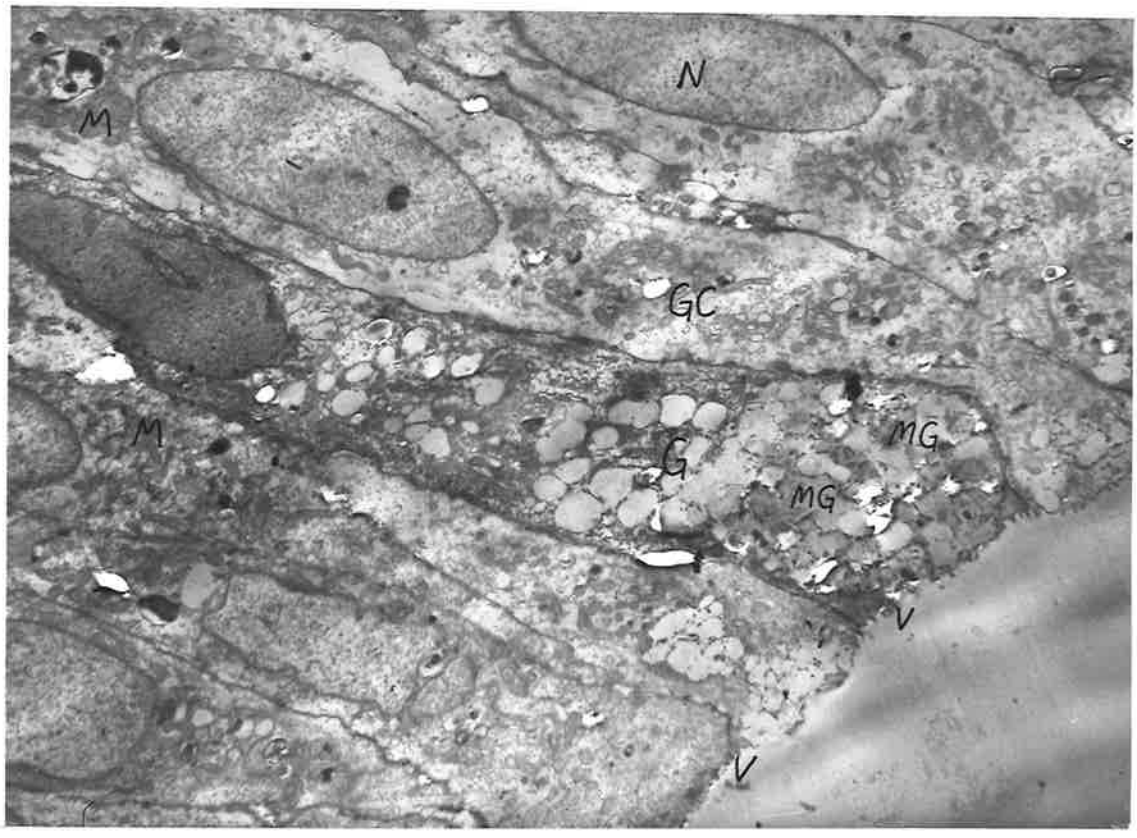


muscle and an outer band of longitudinal muscle. It is bound below by another thicker layer of connective tissue which carries large blood vessels. Beneath this layer is a band of circular muscle, another thin layer of connective tissue and a band of longitudinal muscle fibres. The serosal surface is covered by a thin mesothelium.

The mucosal epithelium and the lamina propria are thrown into numerous small villi (Fig. 14) which presumably increases the surface area available for reabsorption. The epithelium consists of a single layer of large columnar cells which are interspersed with numerous goblet cells (Figs. 13 & 14). The goblet cells contain acid mucopolysaccharide since they stain readily with alcian blue. The nuclei of the columnar cells are centrally located.

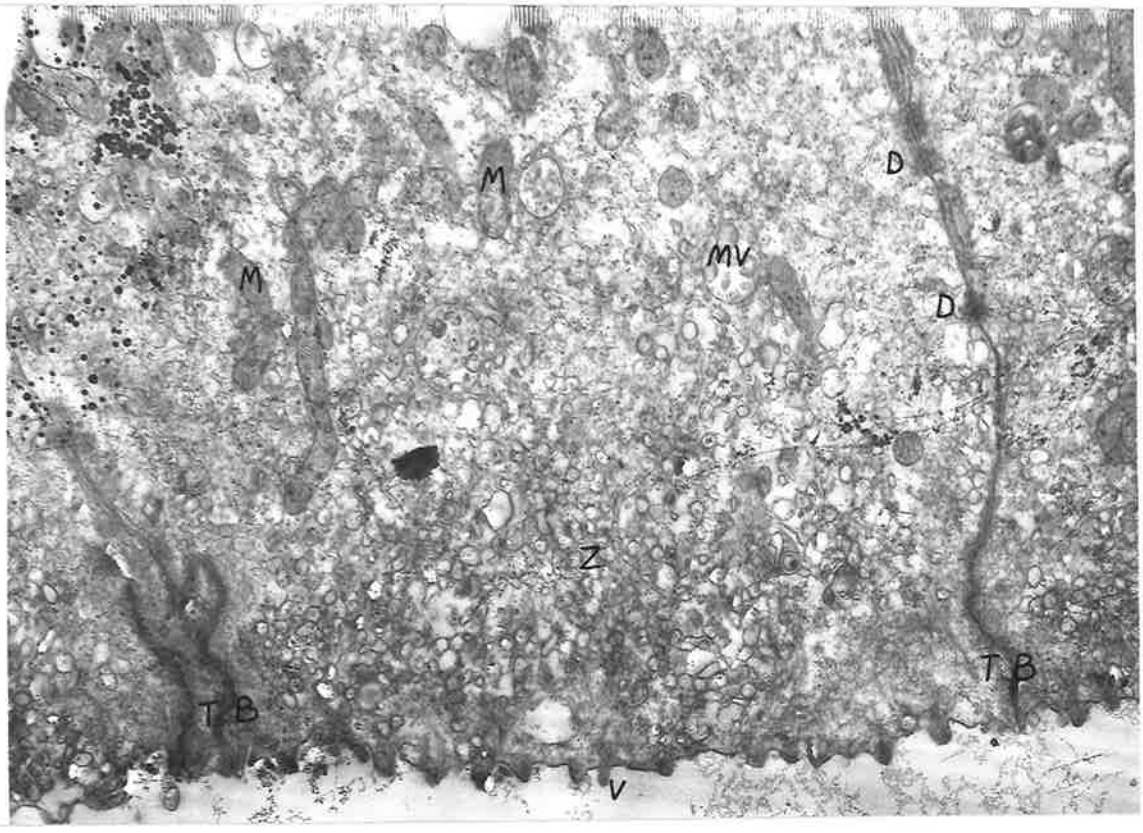
The microstructure of the coprodaeum and rectum of the gecko H. flaviviridis (Seshadri, 1956) and the agamid U. hardwickii (Seshadri, 1957) is similar to that of A. maculosus and A. pictus. Goblet cells have been reported in the cloacas of the lizards mentioned above as well as in the goanna Varanus gouldii (Green, 1969) and some snakes (Junqueira et al., 1966).

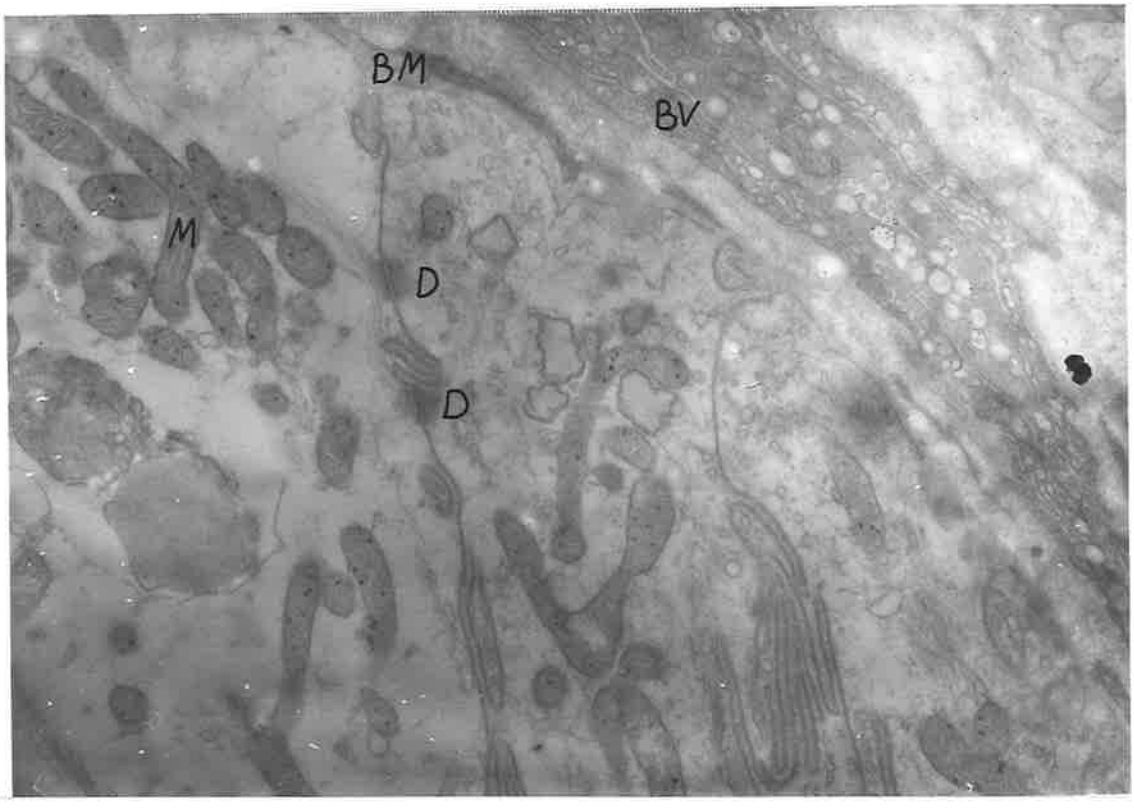
Ultrastructure

The ultrastructure of the walls of the coprodaeum and the posterior half of the rectum of A. maculosus and A. pictus are also very similar. At least two cell types can be distinguished, goblet cells and granular cells (Fig. 15).

Fig. 16. The apical surface of the mucosal epithelium of the coprodaeum showing the terminal bar (TB) and individual desmosomes (D). M = mitochondria, V = microvilli, Z = vesicles, MV = multi-vesicle bodies. Approx. X12,000.

Fig. 17. The basal region of the mucosal epithelium of the coprodaeum of A. maculosus. There is no terminal bar closing the intercellular space as at the apical surface although the lateral cell membranes appear to be 'spot welded' by individual desmosomes (D). Mitochondria (M), basement membrane (BM) and a blood capillary (BV) can also be seen. Approx. X18,000.


M
M
D
MV
D
Z
TB
TB
V


BM
BV
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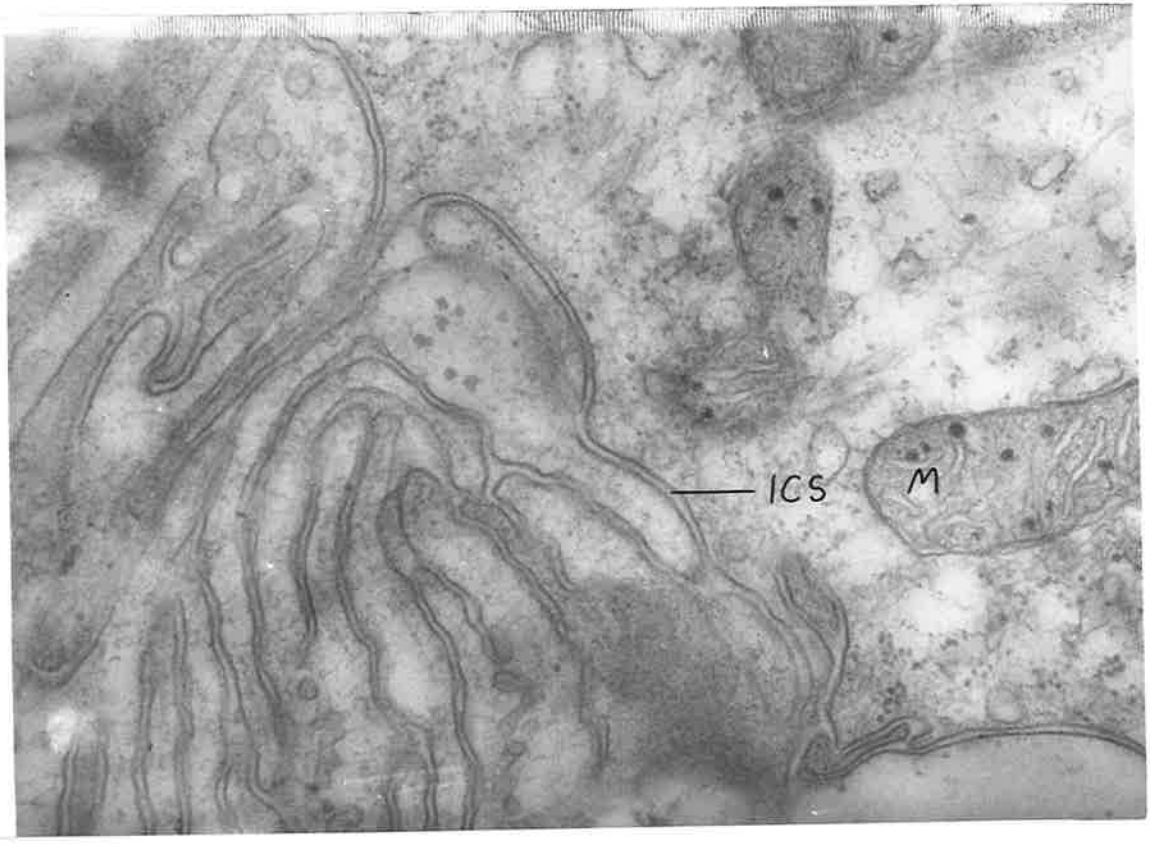
D

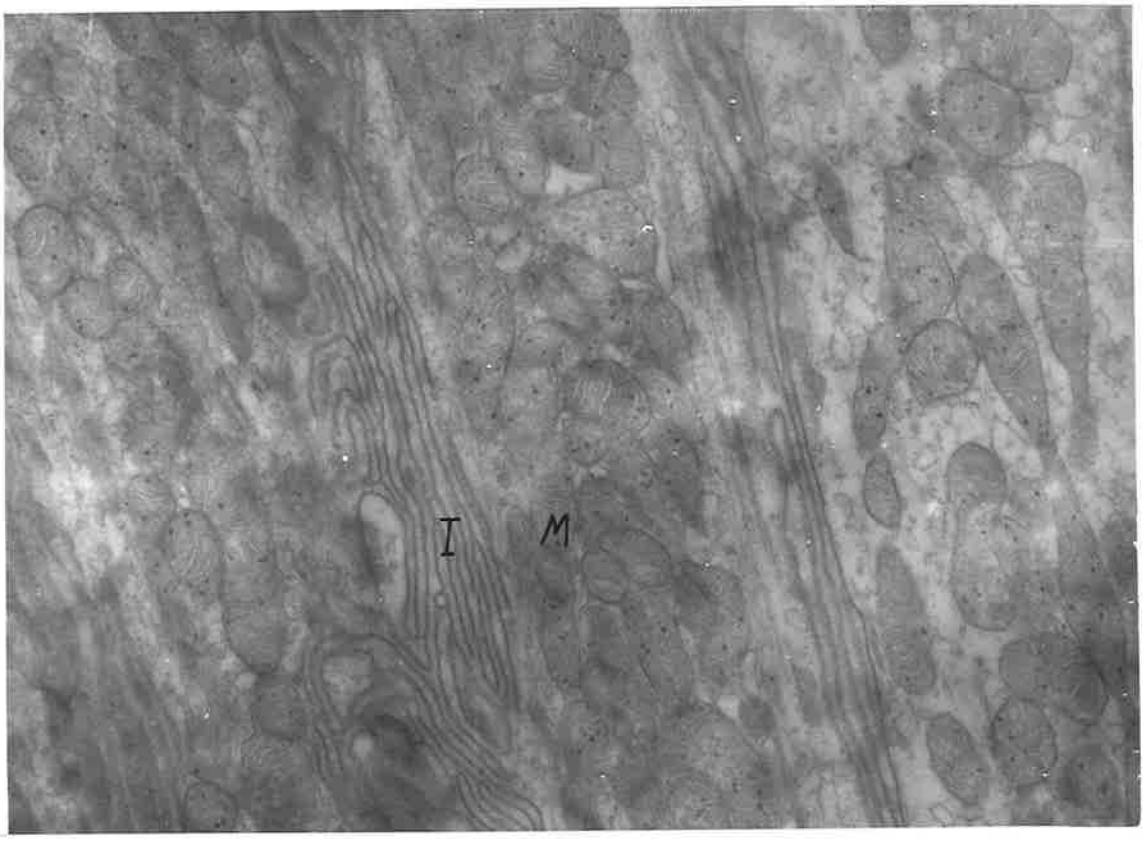
These cells have also been found in the urinary bladder of the toad, together with a third type of cell, the mitochondria rich cell (Peachy and Rasmussen, 1961; DiBona et al., 1969; Ferguson and Heap, 1970). Mitochondria rich cells also occur in the coprodaeum and rectum of A. maculosus and A. pictus but like Ferguson and Heap (1970) I believe that they are probably developing goblet cells.

The granular cells of A. maculosus and A. pictus have an ultrastructure organization that is similar to that of granular cells in the toad bladder and the gall bladders of the rabbit (Kaye et al., 1966; Tormey and Diamond, 1967), mouse (Yamada, 1955; Hayward, 1962) and the dog (Johnson et al., 1962). Near the apical surface the lateral cell membranes are bound together by a terminal bar and several individual desmosomes (Fig. 16). For most of the rest of their length, right to the base, the lateral membranes fall apart except where the cells appear to be 'spot welded' together near the base by a few desmosomes (Fig. 17). Thus an epithelial intercellular space is formed which is closed toward the luminal surface but open at the base (Fig. 18). There are short microvilli on the mucosal surface but they are not as long or as numerous as those of the toad bladder and the gall bladders of other animals. The granular cells have a thin basement membrane while the basal surface is flat and has no extensive basal infoldings. The nuclei of the cells are centrally located while

Fig. 18. Basal region of the epithelial cells of the coprodaeum showing that the epithelial intercellular spaces (ICS) are open to the base. The basement membrane is not discernable in this micrograph. M = mitochondria. Approx. X56,000.

Fig. 19. The prominent cells of the mucosal epithelium of the anterior third or so of the rectum of A. maculosus. Micrograph is taken about halfway down the epithelium. Note the extensive interdigitation of the lateral cell membranes (I) and the large numbers of mitochondria (M). Approx. X18,000.


ICS
M


I
M

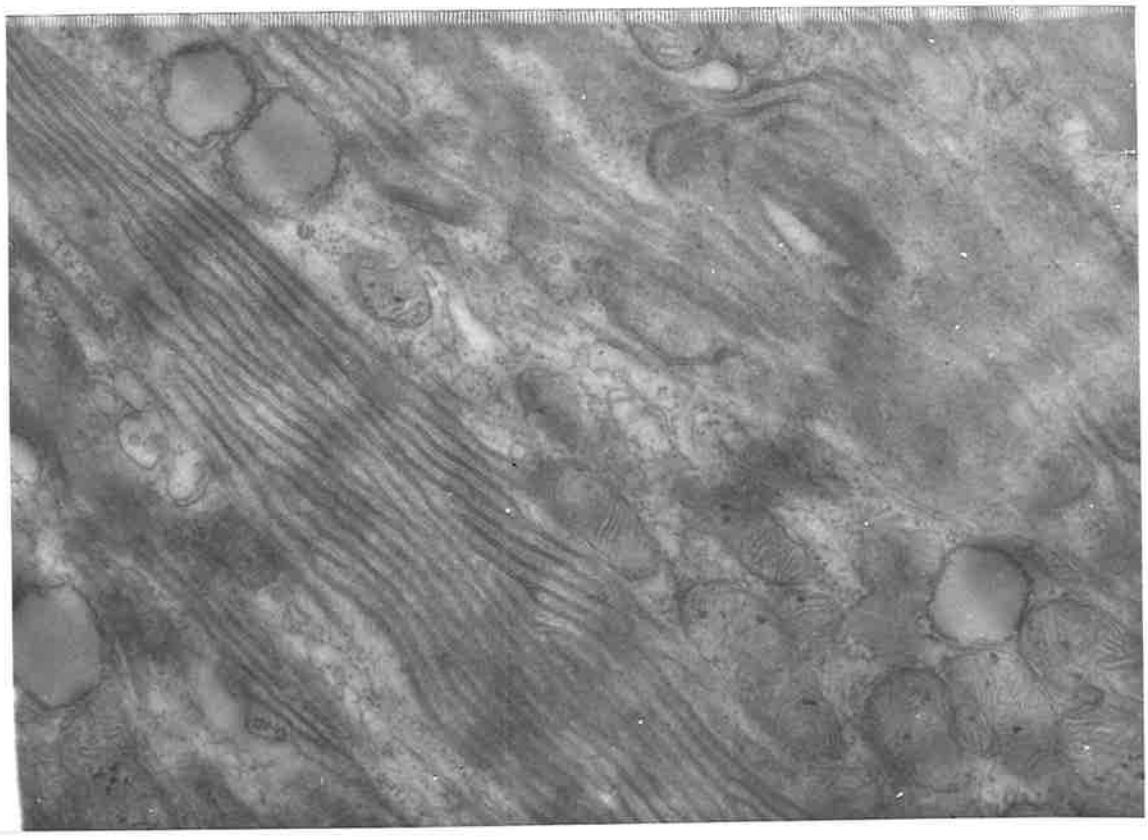
mitochondria are found throughout the cytoplasm except near the apical surface although they tend to be more numerous around and beneath the nucleus. Many small vesicles as well as some multivesicular bodies occur near the apical surface of some cells (Fig. 16).

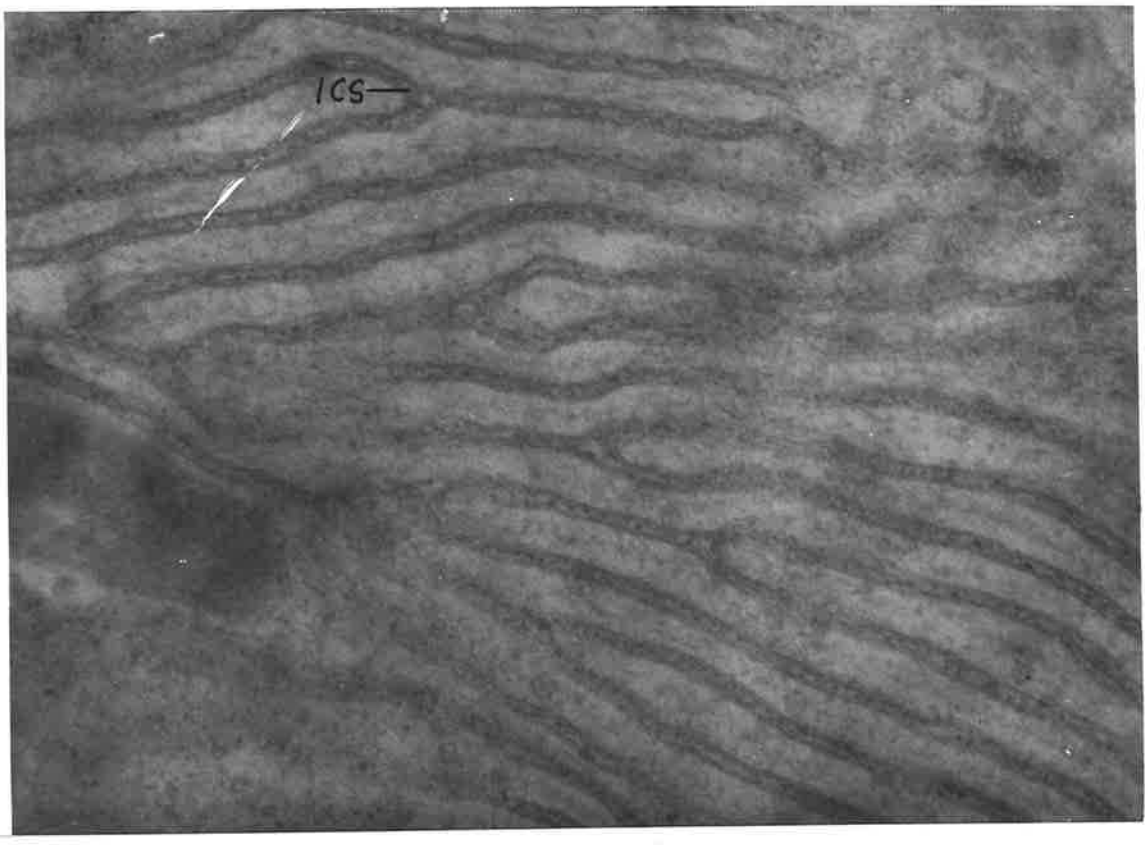
Neither the structure nor the organization of the cells of the mucosal epithelium of the posterior rectum and coprodaeum of A. maculosus is very different from that of other absorptive epithelia. Thus I would suspect that these sections of the rectum and cloaca of A. maculosus could not produce a hyperosmotic fluid in the lumen because other similar absorptive epithelia cannot.

However, although parts of the mucosal epithelium in the anterior third or so of the rectum of A. maculosus are similar to the rest of the rectum and the cloaca, there are large blocks of the epithelium which are quite different. These unusualsegments of the epithelium in the anterior rectum are composed of two types of cells, goblet cells and another more numerous type of cell which is probably a modified form of the granular cell that is found lower down in the rectum. The lateral cell membranes of the epithelial cells in these unusual segments of the anterior rectum interdigitate to an extraordinary degree and the cells are literally packed with mitochondria from the base to just beneath the apical surface (Figs. 19,20,21). Apart from the above differences the components of the cells appear to be

Fig. 20. A further view of the interdigitation of the lateral cell membrane in the anterior rectum of A. maculosus. Approx. X30,000.

Fig. 21. High power view of the interlocking lateral cell membranes or stacks. The dark deposits in the intercellular spaces (ICS) are due to sodium pyro-antimonate. I had hoped to obtain some indication of the concentration of Na in the ICS with this stain (Komnick, 1962) but it was not very successful. Approx. X110,000.




ICS

similar to those which occur in the cells lower down in the rectum. (The lateral cell membranes of the epithelial cells in the anterior part of the rectum of A. pictus do not appear to interdigitate to the degree that they do in A. maculosus nor are the mitochondria as dense. The mucosal epithelium of the anterior part of the rectum of A. pictus appears to be similar to that of the rest of the rectum and the coprodaeum).

The two unusual properties of the epithelial cells of the anterior rectum of A. maculosus - extraordinary interdigitation or stacking of the lateral cell membranes and very abundant mitochondria - are also characteristics of the epithelial cells of the rectal pads and papillae of certain insect recta (Gupta and Berridge, 1966; Berridge and Gupta, 1967; Oschman and Wall, 1969). These two properties are considered to be basic to the mechanism by which it is proposed that insects can concentrate their excreta to many times the concentration of the haemolymph.

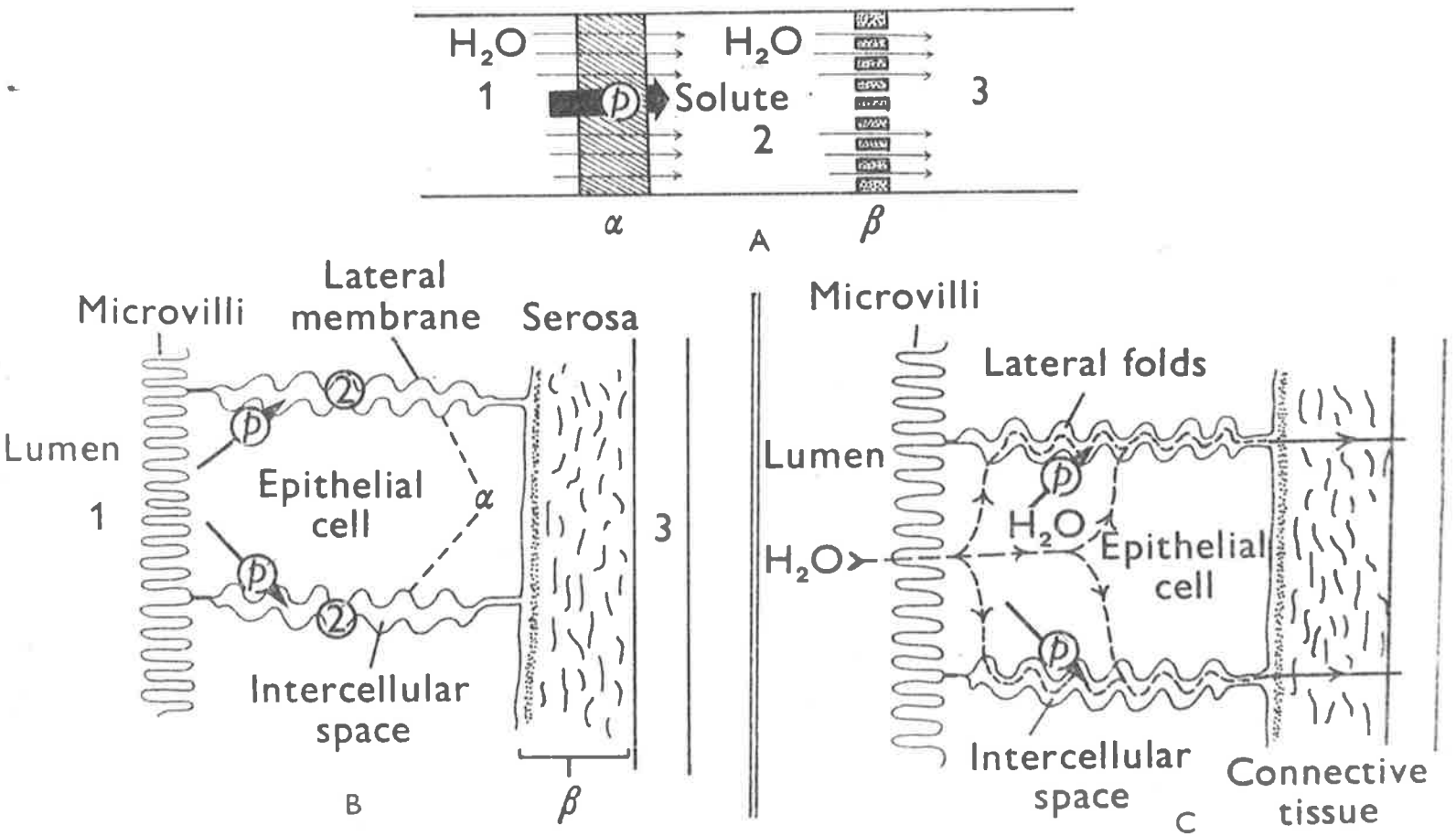
The models that have been proposed to explain how insects concentrate their excreta are based upon Curran's (1960, 1965) and Diamond and Bossert's (1967) models for the transport of fluid across various absorptive epithelia.

a) Curran proposed a double-membrane model (Fig. 22a). The first membrane 'α', has a restricted permeability to solutes but is more permeable to water, while the second membrane, 'β', is non-selective and freely permeable to both.

Fig. 22. (A). A diagramatic representation of Curran's (1960, 1965) model for fluid transport. Compartment '1' is the lumen of the organ, '2' intercellular spaces and '3' lymphatic and blood vessels. If solute is pumped 'p' across the selective membrane 'α' into compartment '2', the solute will exert a greater effective osmotic pressure across the selective membrane 'α' than across the leaky membrane 'β'. Water will thus move into '2', build up the hydrostatic pressure in this compartment and be forced out across the leaky membrane 'β'.

(B). The components of Curran's model can be equated with structures in absorptive epithelia. 1 = lumen, 2 = intercellular space, 3 = lymphatic and blood vessels. Pumps 'p' for the active transport of solute are presumed to be localised on the lateral plasma membranes of the cells (membrane 'α') while the connective tissue etc, on the serosal aspect constitute barrier 'β'.

(C). A diagram representing Diamond and Bossert's (1967) model for fluid transport. As in Fig. 22b, solute is believed to be actively pumped into the intercellular spaces thus making them hypertonic. Water moves from the lumen into the spaces in isotonic proportions. (From Berridge and Gupta, 1967).


H_2O
1
Solute
2
H_2O
3
α
β
A
Microvilli
Lateral membrane
Serosa
Lumen
1
Epithelial cell
α
3
Intercellular space
β
B
Microvilli
Lateral folds
Lumen
H_2O
H_2O
Epithelial cell
Intercellular space
Connective tissue
C

If solute is actively transported across the first membrane into a confined space between the two membranes (Fig. 22a), then the solute will exert a greater effective osmotic pressure across the fine-pored membrane ' α' than across the leaky membrane ' β'. Water thus crosses the first membrane by osmosis, builds up a hydrostatic pressure in the confined space and is forced out of the coarse membrane under this pressure head.

The components of Curran's model can be equated with the various components of absorptive epithelia (Fig. 22b). Compartment '1' = lumen of the organ, '2' the lateral intercellular spaces and '3' the lymphatic and blood vessels. The selective membrane ' α' can be represented by the lateral cell membrane while the non-selective leaky membrane ' β' may be formed by any combination of diffusion barriers at the serosal aspect.

b) Diamond and Bossert suggested a standing gradient model to explain fluid transport. The important characteristics of their model are that there is a long, narrow, fluid-filled space bounded by a cell membrane, and generally open at the end facing the solution towards which fluid is being transported but closed at the end facing the solution from which the transported material originates. Like Curran's model, this model can also be equated with various components of absorptive epithelia (Fig. 22c). If solute is actively transported into the intercellular space (I.C.S.) making its

contents hyperosmotic to the lumen of the organ, water will move into the I.C.S. due to the osmotic gradient. Solute will move toward the bathing solution at the open end of I.C.S. as a result of diffusion down the concentration gradient and of being swept along in the stream of fluid moving down the I.C.S.

There is good evidence that the I.C.S. of various absorptive epithelia are involved in fluid transport (Kaye et al., 1966; Davis and Schmidt-Nielsen, 1967; Tormey and Diamond, 1967; Berridge and Oschman, 1969; DiBona et al., 1969; Grantham et al., 1969; Oschman and Wall, 1969; Sauer et al., 1970; Wall and Oschman, 1970). However neither of these models explains how a hyperosmotic solution can be formed in the lumen of the organ, for they require that the absorbate must be hyperosmotic to the fluid in the lumen, or at best, isosmotic. Thus the fluid in the lumen must become more dilute or remain isosmotic to the bathing solution.

It is generally accepted that sodium or potassium or both is the solute which is actively transported into the I.C.S. to set up an osmotic gradient. However Phillips (1964) showed that the desert locust Schistocerca gregaria could concentrate a sucrose solution in the rectal lumen without any net uptake of ions from the lumen. This observation plus the inability of Curran's or Diamond and Bossert's models to explain how a hyperosmotic solution can

be formed in the lumen led to the hypothesis of ion-recycling within the fluid transporting epithelium (Oschman and Wall, 1969; Phillips, 1970; Wall and Oschman, 1970). In this hypothesis it is proposed that ions are actively reabsorbed from the transported fluid either from the basal region of the I.C.S. or from the haemolymph. The ions can then be actively secreted into the apical half of the I.C.S. A considerable amount of evidence has been obtained with various insect recta to support the ion-recycling hypothesis (Phillips, 1970; Sauer et al., 1970; Wall and Oschman, 1970; Wall et al., 1970; Irvine and Phillips, 1971).

Since the mucosal epithelium of the anterior rectum of A. maculosus has a structure similar to that of rectal pads and papillae of various insect recta, I suggest that a mechanism similar to that operating in these insects is responsible for concentrating the urine of A. maculosus. I propose that solute, probably sodium, is actively pumped into the I.C.S. setting up an osmotic gradient and drawing water from the rectal lumen into the I.C.S. The water then either moves down the I.C.S. toward the blood and lymph vessels due to the diffusion gradient (Diamond and Bossert's model) or due to the increased hydrostatic pressure in the I.C.S. causing the fluid to move through the more leaky barrier, the lamina propria, rather than the lateral cell membrane (Curran's model). Ions can be actively reabsorbed from the extracellular fluid across the base of the epithelial cells

or across the basal region of the lateral cell membrane. I cannot say whether Curran's or Diamond and Bossert's model is more applicable to A. maculosus as indeed it is difficult to determine which of these models is more applicable to the transport mechanisms in various insects.

SECTION 8

Excretion of cations as insoluble urates

INTRODUCTION

Dunson (1969) and Minnich (1969, 1970b) showed that some uricotelic reptiles could eliminate significant quantities of sodium and potassium as insoluble urate salts. The desert iguanid Dipsosaurus dorsalis can form urate pellets with five times the quantity of potassium per unit of water than what the lizard could expect to produce through its nasal salt gland (Minnich, 1970b). Thus the excretion of cations as insoluble salts is an excellent method for eliminating excess sodium and potassium with little loss of water. Therefore, I wanted to determine whether A. maculosus could also excrete electrolytes as urate salts. In addition, I measured the water content of faecal and urinary pellets to determine whether A. maculosus possessed any adaptations for reducing the loss of water by this route.

MATERIALS AND METHODS

Lizards were collected from Lake Eyre and were kept in an outside terrarium for up to six months before they were used. Heating lamps were turned on for twelve hours per day and the lizards were fed Tenebrio larvae and occasionally moths.

a) Water content of the faecal and urinary pellets

Each lizard was placed in a container in a constant temperature cabinet (37 $\pm$ 0.1C) for one week during which it

was given neither food nor water. The animal rested on a wire mesh grid which allowed the pellets to pass through into a bath of mineral oil. Any excreta was removed, cleaned of oil, and dried at 100C for two days. Water content was recorded as a percentage of the wet weight of the excreta.

b) Electrolyte content of the urinary pellets

Lizards were placed in container similar to those that were used to measure the water content of the excreta. The animals were injected with 1.0 ml of 1N NaCl/100 g of body weight per day for three days, then they were left over the oil for a further two days. Urinary pellets were collected, the oil removed and the water content determined by drying it at 100C for two days. The sodium and potassium content of the pellet was determined by the method of Minnich (1970b). The dried pellet was placed in a 10 ml volumetric flask, ground up, then 9 ml of double distilled water was added. Minnich found that after soaking for a week the pH of the solution increased from 5 to 7 which suggested that the cations were originally bound to urates and that there was a cation-hydrogen ion exchange. Since the solubility of uric acid is less than that of urate salts, sufficient acetic acid to lower the pH to 5 was added to foster the cation-hydrogen ion exchange. A week later the volume was made up to 10 ml with double distilled water. Samples of this fluid were appropriately diluted with double distilled water and the sodium and potassium

concentration was measured with an Eel flame photometer.

Table 18

The water content of faecal and urinary pellets of A. maculosus. Per cent measured as per cent of the wet weight (Mean ± S.E.). Sample size in parentheses.

Faecal pellets	Urinary pellets
71 ± 2% (11)	34 ± 2% (9)

RESULTS

The water content of the faecal and urinary pellets is presented in Table 18. The values for the water content of the urinary pellets from the salt-loaded animals were pooled with those obtained for the uninjected animals because I obtained only three urinary pellets from the uninjected lizards.

Table 19 shows that A. maculosus can produce urinary pellets with a high sodium and potassium content. If these cations had been excreted in the fluid contained in the pellet, their concentration would have been about 2000 to 3000 mEq/l. However, all pellets contained more potassium than sodium although the lizards had been injected with sodium chloride. It is known that the salt glands of some lizards are adapted to excrete that ion which is in excess in the diet so that the initial response to the injection of one cation (e.g. sodium) is to increase the secretion of the ion that is common in the diet (e.g. potassium) (Templeton,

Table 19

The water content of urinary and faecal pellets for a number of animals. Values are expressed as a percentage of the wet weight of the excreta.

Animal	Faecal water content %		Urinary pellet water content %	Reference
	Hydrated animals	Dehydrated animals		
Camel *Camelus dromedarius*	52	43.5	-	Schmidt-Nielsen *et al.* (1956)
Kangaroo rat *Dipodomys merriami*		45	-	Schmidt-Nielsen and Schmidt-Nielsen (1951)
Indian python *Python molurus*	State of hydration not mentioned 67		27	Benedict (1932)
Gecko *Hemidactylus flaviviridis*	-		10	Seshadri (1956)
Desert iguanid *Dipsosaurus dorsalis*	59		38 45*	Minnich (1970b) *Murrish and Schmidt-Nielsen (1970b)
Goanna *Varanus gouldii*	77		48	Green (1969)
Amphibolurus maculosus	71		34	Present study

1964, 1967). However, from Table 20 there seems to be no tendency for the concentration of either sodium or potassium in the pellet to change with time. A larger sample however, may reveal a consistent trend since only four lizards were used in the experiment and some dropped only one pellet during the five days.

It was noted in section 6 that intraperitoneal injections of sodium chloride (1 ml of 1N NaCl per 100 g of body weight per day) caused excessive penetration of sodium into cells. In an effort to determine whether this penetration of sodium may have caused the apparently anomalous low content of sodium in the pellet relative to potassium, I repeated the experiment using a much lower dose of sodium chloride. Also, in order to acclimate them to a high sodium diet, the lizards were given orally a slightly hypertonic solution of sodium chloride for two weeks before they were used. Since I had very few animals at this stage with no prospects of obtaining any more, I used the same lizards that I had used in the previous experiment. However, they were left in the terrarium for one month to recuperate before they were used again.

The lizards were given a 6% glucose solution containing 200 mEq/l sodium chloride every two days for two weeks. A syringe was used to drop the solution onto the edge of the mouth of the lizard from where it was lapped up. The animals were also fed Tenebrio larvae. After two weeks the lizards

Table 20

Electrolyte content of the urinary pellets of *A. maculosus* that were injected with 1N sodium chloride.

Animal No	Day pellet excreted	Dried weight of pellet (mg)	Weight K^+ mEq x 10^{-3}	$[K^+]$ mEq/l	Weight Na^+ mEq x 10^{-3}	$[Na^+]$ mEq/l	Total [cation] mEq/l
2	2	9.0	10	2700	2	540	3240
3	2	19.8	3	290	1	100	390
4	2	16.3	10	1370	2	270	1640
1	3	31.0	27	1690	7	440	2130
2	3	13.0	6	1200	2	400	1600
1	5	32.3	22	1330	18	1080	2410
2	5	9.7	9	2200	1.6	390	2590
4	5	11.8	24	3000	4	510	3510

Table 21

Electrolyte content of the urinary pellets of A. maculosus that were injected with 0.5N sodium chloride.

Animal No.	Day pellet excreted	Dried weight of pellet (mg)	Weight of K^+ (mEq) x 10^{-3}	$[K^+]$ mEq/l	Weight of Na^+ (mEq) x 10^{-3}	$[Na^+]$ mEq/l	Total [cation] mEq/l
2	1	17.8	10	1110	7	780	1890
4	1	81.3	31	740	27	640	1380
1	9	37.7	12	630	8	420	1050
3	9	39.3	19	950	14	70	1020
4	9	66.3	37	1090	22	650	1740
1	10	17.7	5	560	8	890	1450

were placed in individual containers over oil as in the previous experiment then 0.2 ml of 0.5 N NaCl per 100 g of body weight was injected intraperitoneally each day for two days. The animals were then left in the temperature cabinet over oil for a further eight days without food or water. Urinary pellets were collected, cleared of oil, and treated as in the previous experiment. I did not estimate the water content of these pellets but assumed that they were 34%, the mean water content that I had obtained for urinary pellets (Table 18). The results are presented in Table 21. Again all the pellets except one contained more potassium than sodium, and, as in the previous experiment, neither the concentration nor the proportion of the cations in the pellet showed any clear trend with time. However, the concentration of the cations tended to be lower than in the first experiment.

DISCUSSION

The water content of the faecal pellets of *A. maculosus* is relatively high in comparison to other desert animals (Table 19). Minnich (1970b) found that the desert iguanid *Dipsosaurus dorsalis* could produce faeces with a water content as low as 59%. However, *D. dorsalis* is mainly herbivorous and digests only 30 to 50% of its food whereas *A. maculosus* is insectivorous and probably digests up to 90% of its food (Minnich, 1970b). Therefore, *A. maculosus* would produce less faeces than *D. dorsalis* and thus would probably lose

less water via this route. Nevertheless, faecal water loss probably would be a major source of water loss in A. maculosus. However, it was shown in the previous section that the structure of the anterior part of the absorptive epithelia of the rectum of A. maculosus is very similar to that found in the rectum of several insects. Since it is believed that this structure plays an intergral part in the mechanism by which insects dry out their faeces, I would have expected that A. maculosus could also produce very dry faecal pellets in its rectum. Maybe the values that are presented in Table 18 do not represent the full capacity of A. maculosus to dry out its faecal pellets.

The water content of the urinary pellets of A. maculosus is about the same as that which Minnich (1970b) obtained for D. dorsalis (Table 19). However, Seshadri (1956) found that the Indian house gecko Hemidactylus flaviviridis can produce urinary pellets containing an extremely low (10%) water content. Thus although A. maculosus does not appear to be able to produce pellets as dry as H. flaviviridis, it can produce urinary pellets with a low water content and this, coupled with the excretion of nitrogenous wastes as insoluble urates and uric acid, enables A. maculosus to eliminate waste nitrogen with little loss of water.

Murrish and Schmidt-Nielsen (1970b) suggested that colloidal osmotic pressure was the main force involved in removing water from urinary pellets in the cloaca of reptiles

and they subsequently showed that the colloid osmotic pressure of the blood was sufficient to dry the urinary pellets of D. dorsalis to 45% water content, which was the value that they obtained for the water content of the urinary pellets of this lizard. (This is a higher value for the water content of the urinary pellets of D. dorsalis than what Minnich (1970b) found. I have no explanation for this discrepancy). However, the water content of the urinary pellets that Minnich (1970b) and I obtained is much lower than can be explained by the colloid osmotic pressure of the blood alone. Some other force, probably involving the active transport of solute, must be acting as well. Structural evidence for such a transport system in the rectum of A. maculosus was presented in section 7.

It was shown that A. maculosus can excrete sodium and potassium as insoluble salts in the urinary pellet. The quantity of cation per unit of water in the pellets was several times that which A. maculosus can produce as a hyperosmotic urine, but well below the concentration that D. dorsalis can produce in its pellets (Minnich, 1970b). However, A. maculosus is insectivorous and Minnich (1969) showed that herbivorous lizards (e.g. D. dorsalis) have a much higher cation content in their pellets than insectivorous or carnivorous reptiles. He also showed that starving lowered the cationic content of the pellets.

Thus, the cation concentrations that I obtained for the pellets of A. maculosus may be lower than what fed animals in the field are capable of producing.

It was also shown that all the urinary pellets except one contained more potassium than sodium even though the lizards had been injected with sodium chloride. The ants on which A. maculosus feeds in the field are high in sodium (Na^+ = 275 mEq/l; K^+ = 44 mEq/l); however, the diet on which A. maculosus was fed in the laboratory was high in potassium and low in sodium. (Fluid from crushed Tenebrio larvae contained Na^+ = 20 mEq/l; K^+ = 75 - 80 mEq/l). Thus, it is possible that the excretory system of A. maculosus had become acclimated in the laboratory to eliminate potassium. It is known that the salt gland of some iguanids often responds to sodium loads by increasing the potassium content of the nasal secretion (Templeton, 1964). Templeton (1967) later concluded that the salt glands of terrestrial iguanids were acclimated to eliminate the excess potassium which results from their herbivorous diet. The salt gland of the sleepy lizard Trachydosaurus rugosus also appears to be acclimated to excrete that cation which is in excess in the diet, in this case sodium (Section 9). Other work has also shown that some mammals can become acclimated to high potassium intakes (Thatcher and Radike, 1947; Berliner et al., 1950). The process of acclimation seems to involve the whole excretory system, both extra renal and renal

components (Alexander and Levinsky, 1968; Wright et al., 1971). Thus the A. maculosus in the laboratory had probably become acclimated to the high potassium diet so that they produced urinary pellets with a high potassium content even though the lizards had been injected with sodium. Although I tried to acclimate the animals to a high sodium diet, I may not have left them on it for long enough, or the amount of sodium may not have been sufficient for the lizards to become completely acclimated. It would be interesting to determine the cationic composition of urinary pellets from A. maculosus in the field.

If this process of acclimation to that cation which is high in the diet occurs in both the salt gland and in the excretion of cations as urate salts, then it is probable that the whole excretory system of reptiles becomes acclimated in a manner similar to that which was found for potassium acclimation in rats (Alexander and Levinsky, 1968; Wright et al., 1971). Therefore, the excretion of sodium and potassium by the kidney and cloaca probably depends to large extents on the electrolyte content of the diet for the previous four or five weeks. Thus, great care must be taken in relating the observed rates of secretion of sodium and potassium in laboratory experiments to that which could be expected for animals in the field unless both laboratory and field animals have the same diet.

Nevertheless, the results show that A. maculosus can excrete significant quantities of cations with little loss of water as insoluble salts in the urinary pellet. However, A. maculosus apparently cannot excrete all the excess electrolytes from its diet by this method since the cation concentration of the plasma increased rapidly during summer (Section 1). The amount that can be excreted as insoluble salts probably depends upon the amount of nitrogenous waste that has to be excreted. Thus, significant quantities of salt from the diet must be stored in the body fluids until it can be excreted later, probably as a hyperosmotic urine, with water obtained from rain (Section 1).

SECTION 9

THE STRUCTURE AND FUNCTION OF THE NASAL SALT GLAND FROM THE SLEEPY LIZARD TRACHYDOSAURUS RUGOSUS (GRAY)

INTRODUCTION

Many reptiles and birds possess a salt gland that is capable of secreting concentrated salt solutions (Schmidt-Nielsen and Fange, 1958; Templeton, 1964; Dunson, 1969). However, until recently, functional salt glands had been reported in only one family of lizards, the Iguanids, although Minnich (pers. comm.) suggests that they occur amongst members of other families, notably in a skink Eumeces skiltonianus. Green (1969) has also reported that the carnivorous sand goanna Varanus gouldii possesses a functional salt gland. Nevertheless, earlier workers failed to find such a gland in the Western Australian sleepy lizard Trachydosaurus rugosus, even though they injected animals with large sodium and potassium loads (Bentley, 1959; Shoemaker et al., 1966).

There is some doubt about the role of the salt gland in terrestrial lizards (Schmidt-Nielsen et al., 1963; Norris and Dawson, 1964; Templeton, 1966; Sokol, 1967), yet there has been only one attempt to study the function of the gland in the field (Minnich, 1970b).

During the present study a white encrustation was noted around the nares of T. rugosus that had been injected with hypertonic sodium chloride. Dried secretion was also found around the nares of lizards in the field. Subsequent analysis

revealed that it was composed of sodium, potassium and chloride ions. A similar encrustation is commonly found around the nares of lizards that possess a salt gland (Templeton, 1964); therefore, attempts were made to locate and study the morphology of the gland in T. rugosus.

Templeton (1964) found that the nasal salt gland of terrestrial iguanids responded to potassium chloride and sodium chloride injections by increasing the relative potassium content of the nasal secretion. He concluded later (Templeton, 1967) that the salt glands of the terrestrial iguanids are adapted to eliminate the excess potassium which results from their herbivorous diet. Since T. rugosus also feeds mainly on plants, I tested the effect of these salts on the composition of the nasal secretion.

It is known that two lizards which do not possess a salt gland, Amphibolurus ornatus (Bradshaw and Shoemaker, 1967) and A. maculosus (Section 1, present study), show large increases in the electrolyte concentration of the plasma over summer, while two lizards which possess salt glands do not, Dipsosaurus dorsalis (Minnich, 1970b) and Varanus gouldii (Green, 1969). Therefore, I measured the concentration of sodium and potassium in plasma taken from T. rugosus in the field during summer to determine whether the sleepy lizard conformed to this pattern. From this and other information obtained from salt loading experiments, I hoped to suggest a role for the salt gland of T. rugosus.

MATERIALS AND METHODS

Animals were collected from coastal dunes at Goolwa, 50 miles south of Adelaide, South Australia. They were kept in an open pen and were fed lettuce and occasionally snails and mince.

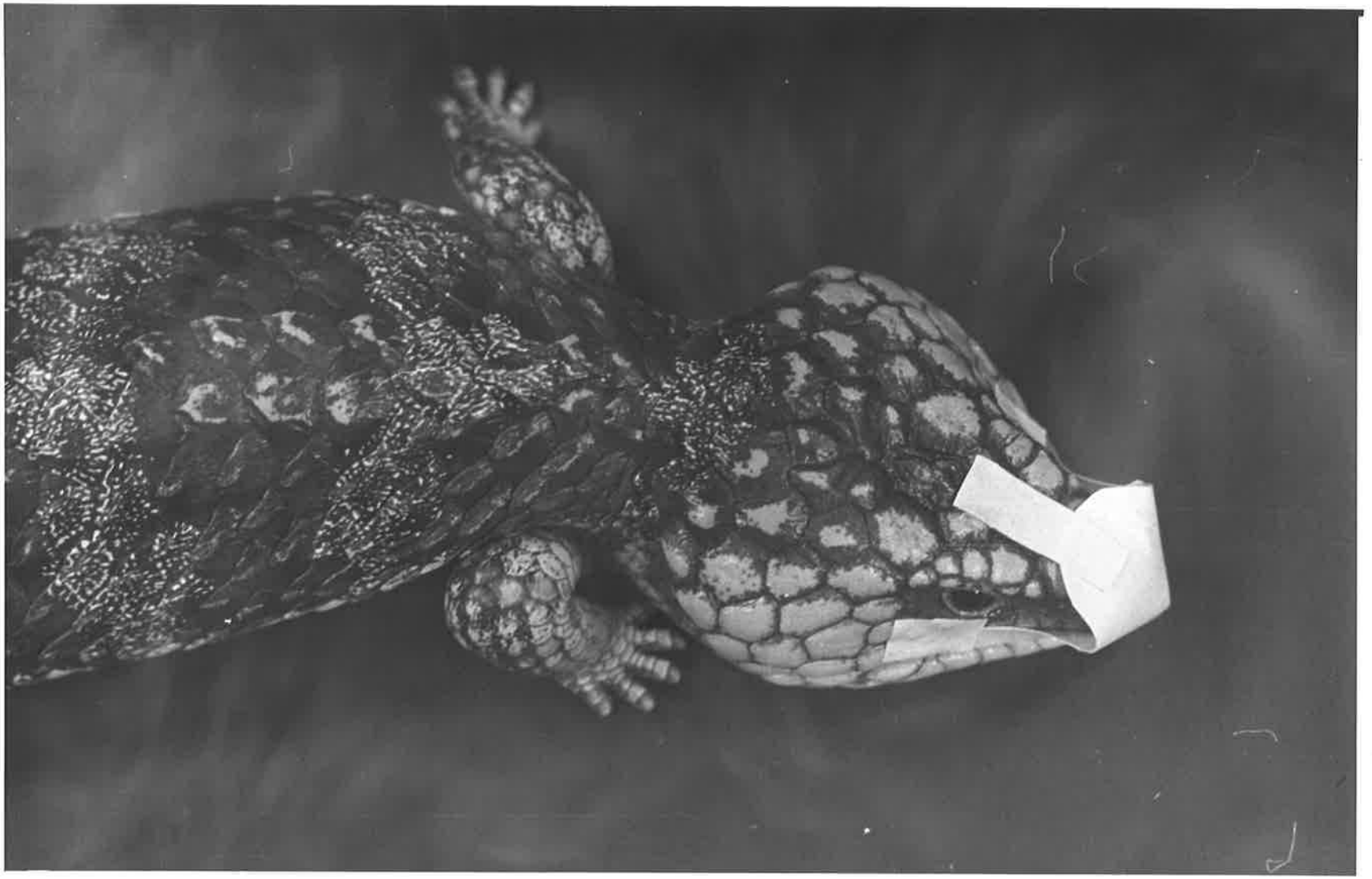
(a) Morphology of the gland

The heads of many sleepies were dissected and a large gland was found in each nasal passage. This gland was removed, fixed in buffered neutral formalin, dehydrated, and embedded in paraffin. Serial sections were cut and stained by one of three staining methods, namely: (i) haemotoxylin - eosin:- to determine whether the cells of the salt gland showed a marked eosinophilia of the cytoplasm, (ii) alcian blue - metinal yellow:- to test for the presence of mucopolysaccharide, and (iii) anilin fuchsin - methyl green:- to determine whether the cells contained abundant mitochondria. These three properties are common to most salt glands (Scothorne, 1959; Ellis et al., 1963; Ellis and Abel, 1964; Philpott and Templeton, 1964).

(b) Salt loading

The average weight of the lizards that were used was 345 $\pm$ 7g. They were divided into three groups, one of 16 to be injected with potassium chloride, one of 16 to be injected with sodium chloride, and a third group of 7 to act as controls.

Fig. 23. A photograph of an adult T. rugosus showing how the mask was fitted to collect the secretions of the salt gland.



The lizards to be salt loaded were given an intraperitoneal injection of 0.1 ml of 1N sodium chloride or potassium chloride per 100 g body weight per day for six days. This was about the same dose that Templeton (1966) used for terrestrial iguanids, except that he injected large loads every few days, whereas the animals in the present experiment were given small daily loads. The latter technique was thought to be more natural; besides, Bradshaw and Shoemaker (1967) showed that large salt loads upset the distribution of body fluids.

Animals were kept two to a pen and heat and light was supplied for twelve hours a day. This allowed the lizards to regulate their body temperature during the day while at night the temperature did not fall below 23C. Neither food nor water was supplied during the experiment.

Blood samples were taken by heart puncture using heparinized syringes. The blood was centrifuged at 3,000 rev./min and the plasma drawn off and frozen until it was analyzed. The control lizards were bled on the first day of salt injections (day 0), then all lizards were bled on day 3 and day 7. Thus the last blood samples were taken two days after the last salt load.

Attempts to cannulate the gland were unsuccessful; therefore, masks made from masking tape were used to collect the dried secretion (Fig.23). The masks were changed each day and the

dried secretion was scraped off and placed in vials. The lizard's head was washed with distilled water before a new mask was fitted. Many animals lost their masks during the experiment, therefore the nasal secretion was analyzed only for those six sodium chloride loaded and nine potassium chloride loaded lizards where samples were collected throughout the experiment. Nasal secretion was dried at 100C for 24 hours, weighed to 0.1 mg and dissolved in 2 ml of double distilled water.

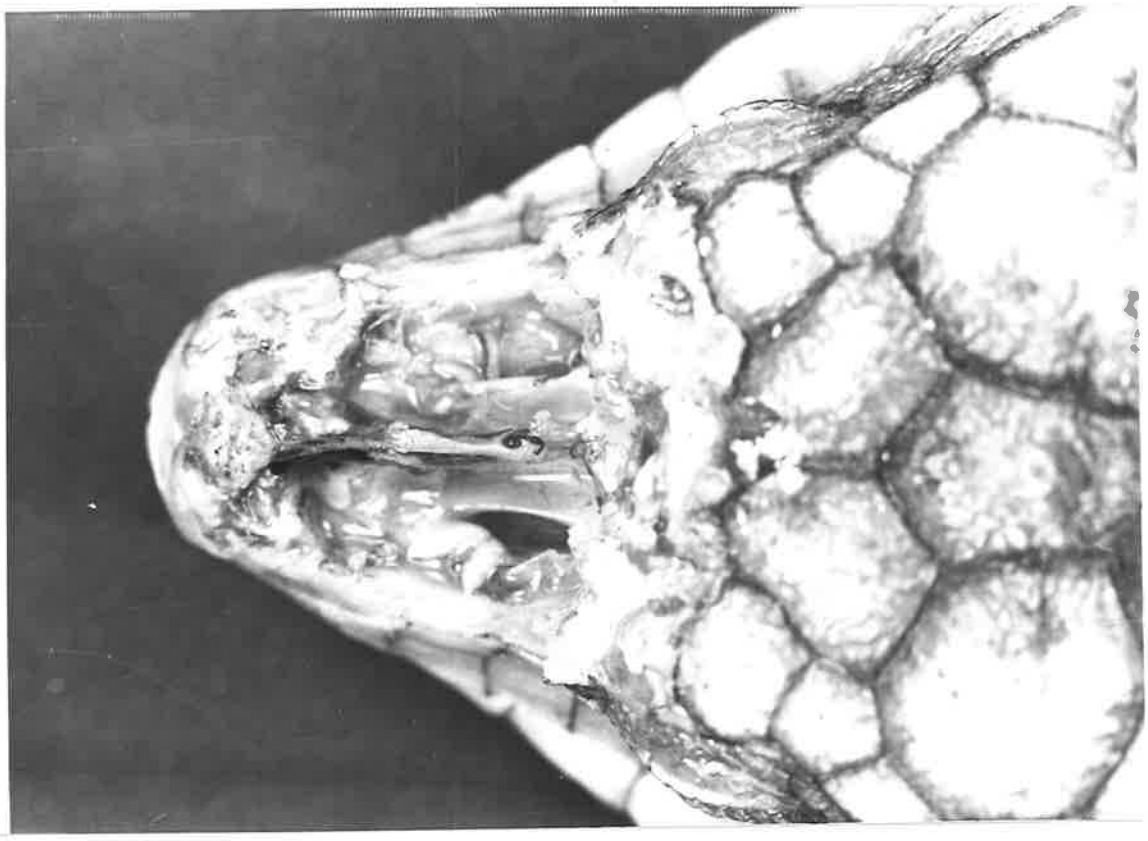
The sodium and potassium concentration of the dissolved secretion and the plasma was measured as well as the chloride concentration of some nasal samples. All sodium and potassium measurements were made with an Eel flame photometer while chloride concentration was measured with an Eel chloride meter.

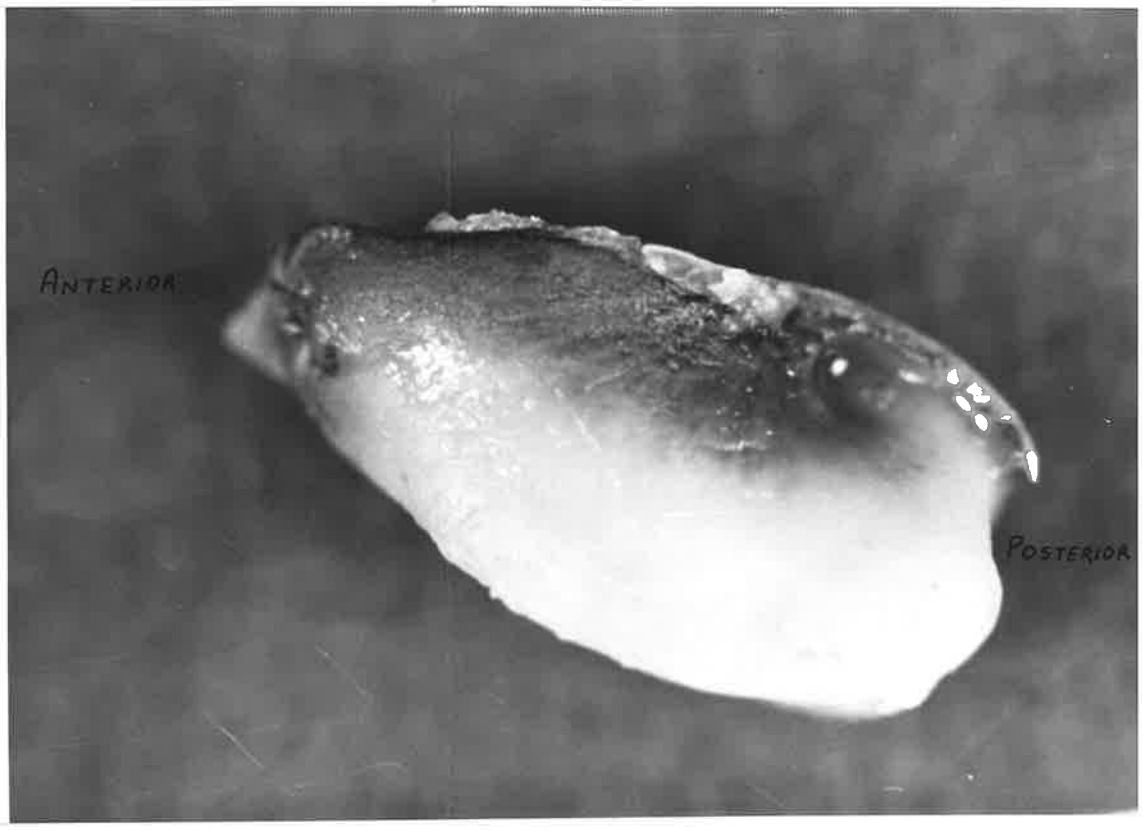
(c) Field work

Blood samples were taken from lizards at Goolwa throughout summer, along with samples of the plant Sonchus megalocarpus on which T. rugosus commonly feeds. Plant samples were crushed and the fluid and the plasma samples were measured for sodium and potassium concentration.

Fig. 24. A dorsal view of the head of a T. rugosus dissected to show where the salt glands are situated. The glands have been removed in this specimen.

Fig. 25. The salt gland from the right hand nasal passage of a T. rugosus. The outer lateral surface is facing away in this photograph.





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RESULTS

(a) Morphology

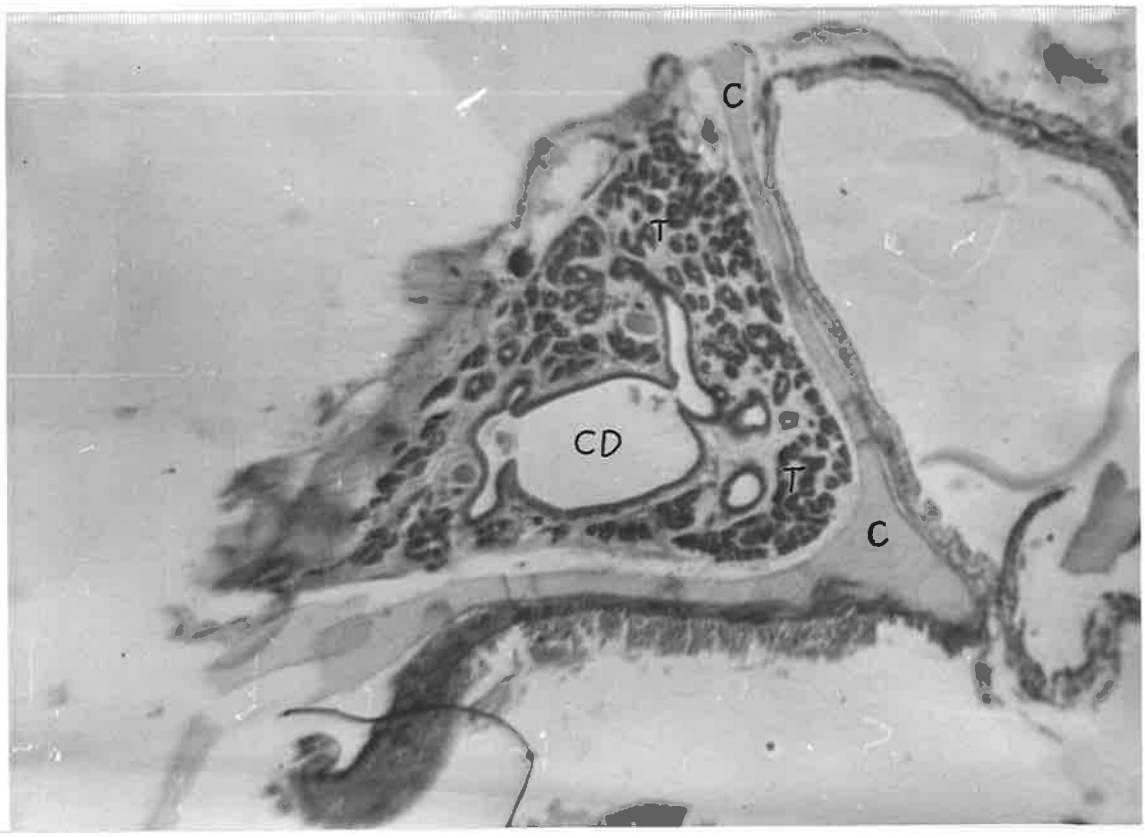
The salt gland of T. rugosus is very similar in structure to those found in other reptiles and in birds, but most closely resembles those of the terrestrial iguanids (Norris and Dawson, 1964; Philpott and Templeton, 1964; Crowe et al., 1970). As in the iguanids the glands are paired, one gland lying dorsally in each nasal passage (Fig.24). Each gland is invested with cartilage which holds the gland in the nasal passage (Figs.25,26)

Like the iguanids, each gland closely resembles a single lobe of an avian salt gland in gross organization (Crowe et al., 1970). There is a large central canal into which flow radial channels which anastomose with secretory tubules that run parallel to the central duct. The tubules are held in a stroma of connective tissue which becomes sparser toward the periphery of the gland. Each tubule is surrounded by a number of blood vessels (Fig.27).

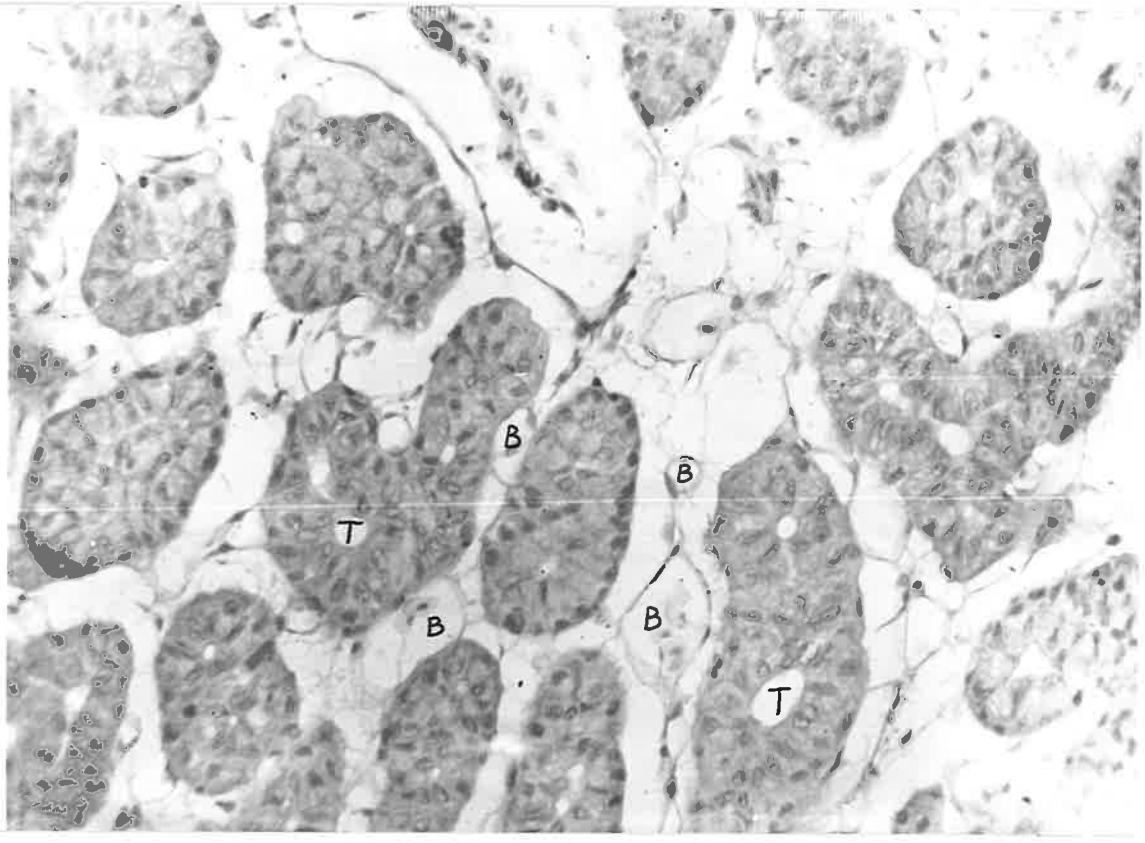
Tall columnar cells form the main and radial ducts, while the secretory tubules are more cuboidal (Fig.28). Anilin-fuchsin staining shows that the secretory cells are rich in mitochondria (Fig.29). The glands also show a marked eosinophilia of the cytoplasm. Alcian blue-metinal yellow staining reveals considerable mucopolysaccharides contained in goblet cells

Fig. 26. T.S. of a salt gland from T. rugosus showing the central duct (CD), secretory tubules (T) and the cartilage which encases the gland (C). Haemotoxylin and eosin.

Fig. 27. T.S. of a salt gland from T. rugosus showing the secretory tubules (T) and the blood vessels (B). Haemotoxylin and eosin.



1·0 mm



0·1 mm

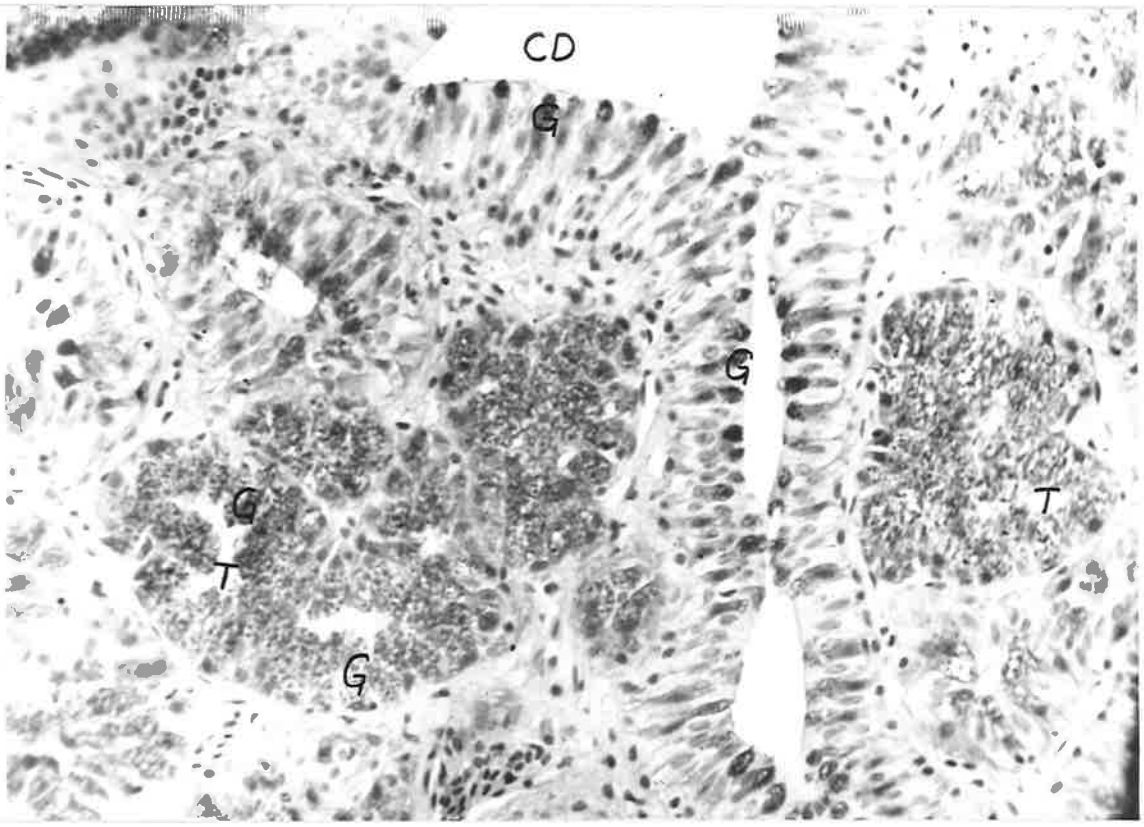
(Fig.28). Mucopolysaccharides have been noted in a number of other salt glands; the lizards Lacerta viridis (Gerzeli and De Piceis Polver, 1970) and Dipsosaurus dorsalis (Philpott and Templeton, 1964), sea snakes (Taub and Dunson, 1967), marine turtles (Ellis and Abel, 1964) and to a lesser extent in the saline loaded duck (Scothorne, 1959; Ellis et al., 1963; Benson and Phillips, 1964). Preliminary observations on the salt gland of the goanna Varanus gouldii shows that it also contains abundant mucopolysaccharides.

(b) Salt loading

The concentration of sodium and potassium in the plasma samples that were taken during the experiment are presented in Table 22 The cation concentration of the three groups were compared by analysis of variance for day 3 and day 7. On day 3, P = .001 for sodium and for potassium P < .001. Subsequent paired "t" tests showed that all but the potassium concentration of the control and sodium chloride loaded lizards ($0.4 > P > 0.3$) were significantly different. However, by day 7, two days after the last injection of salt, the cation concentration of the three groups were just significantly different with respect to sodium but not with respect to potassium (Na^+, $P \triangleq 0.05$; K^+, $0.2 > P > 0.$

Fig. 28. T.S. of a salt gland from T. rugosus showing the central duct (CD) which is composed of columnar cells, and secretory tubules (T) which are formed by cells that are more cuboidal. Large numbers of goblet cells (G) containing mucopolysaccharide are also present. Alcian blue - metinal yellow.

Fig. 29. T.S. of a salt gland from T. rugosus showing secretory tubules (T) which are composed of cells the cytoplasm of which stain deeply with anilin - fuchsin revealing the presence of numerous mitochondria.



0·1 mm

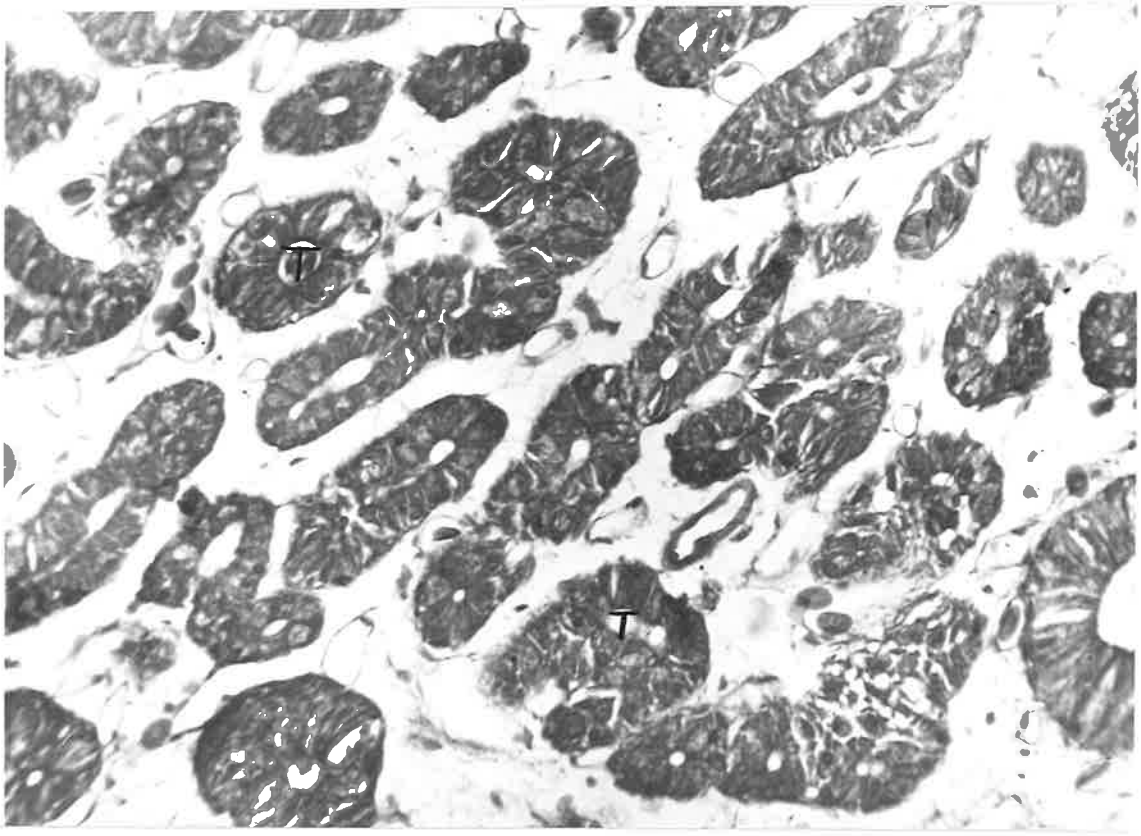
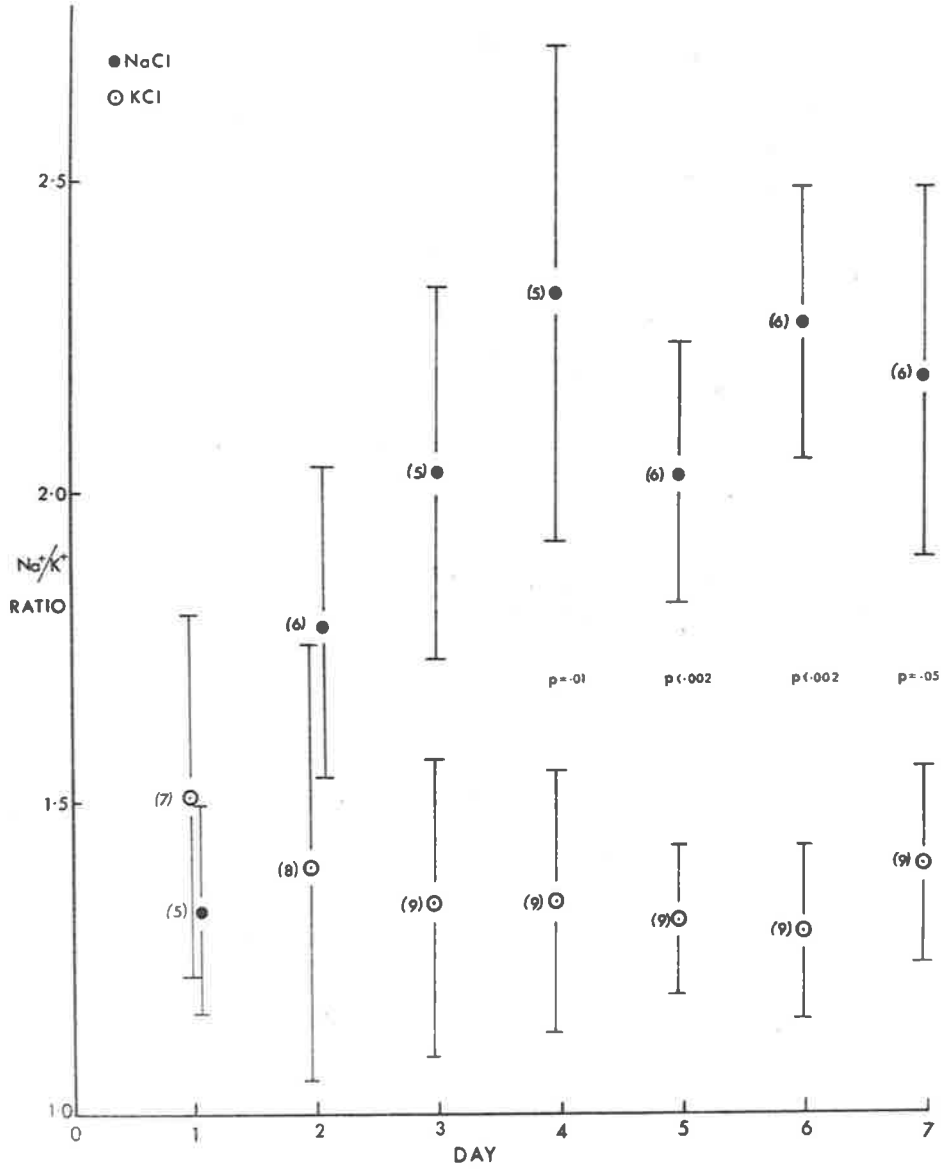


Table 22

Sodium and potassium concentration of plasma samples taken during the experiment. Values given as means ± S.E. Sample size given in parentheses. Units are mEq/l.

Group (Day)		Day of experiment 0	5	7
Control	K^+	3.3 ± 0.1 (7)	3.6 ± 0.2 (7)	3.7 ± 0.3 (7)
	Na^+	160 ± 2.0	154 ± 2	159 ± 2
KCl loaded	K^+		4.6 ± 0.1 (16)	4.1 ± 0.2 (16)
	Na^+		160 ± 1	161 ± 2
NaCl loaded	K^+		3.4 ± 0.1 (15)	3.7 ± 0.1 (16)
	Na^+		166 ± 2.0	167 ± 2 (14)

Fig. 30. Changes in the composition of the secretion with salt-loading. Values given as means $\pm$ S.E. 'p' values given for those groups that are significantly different. Sample sizes are in parentheses.


● NaCl
⊙ KCl
2·5
2·0
1·5
1·0
Na⁺/K⁺
RATIO
0
1
2
3
4
5
6
7
DAY
(7)
(5)
(6)
(8)
(5)
(9)
(5)
(9)
(6)
(6)
(9)
(6)
(9)
p = ·01
p < ·002
p < ·002
p = ·05

Sodium, potassium and chloride accounted for 90 to 95% of the weight of the dried secretion (Table 23). The other 5% or so probably consisted of dirt and pieces of scale. No effervescence occurred when acid was added to the secretion indicating that there was little, if any, bicarbonate. This is in contrast to the secretion of the terrestrial iguanids where the bicarbonate content is relatively high (Norris and Dawson, 1964).

The change in composition of the secretion with saline loading is presented in Fig. 30. The relative potassium content, as shown by the Na^+/K^+ ratio, tended to increase in those lizards that were injected with potassium chloride, while similarly the relative sodium content increased in the animals that were injected with sodium chloride. However, the composition of the two groups was not significantly different until day 4 when they were compared by the Wilcoxon two-sample test ($P = 0.01$).

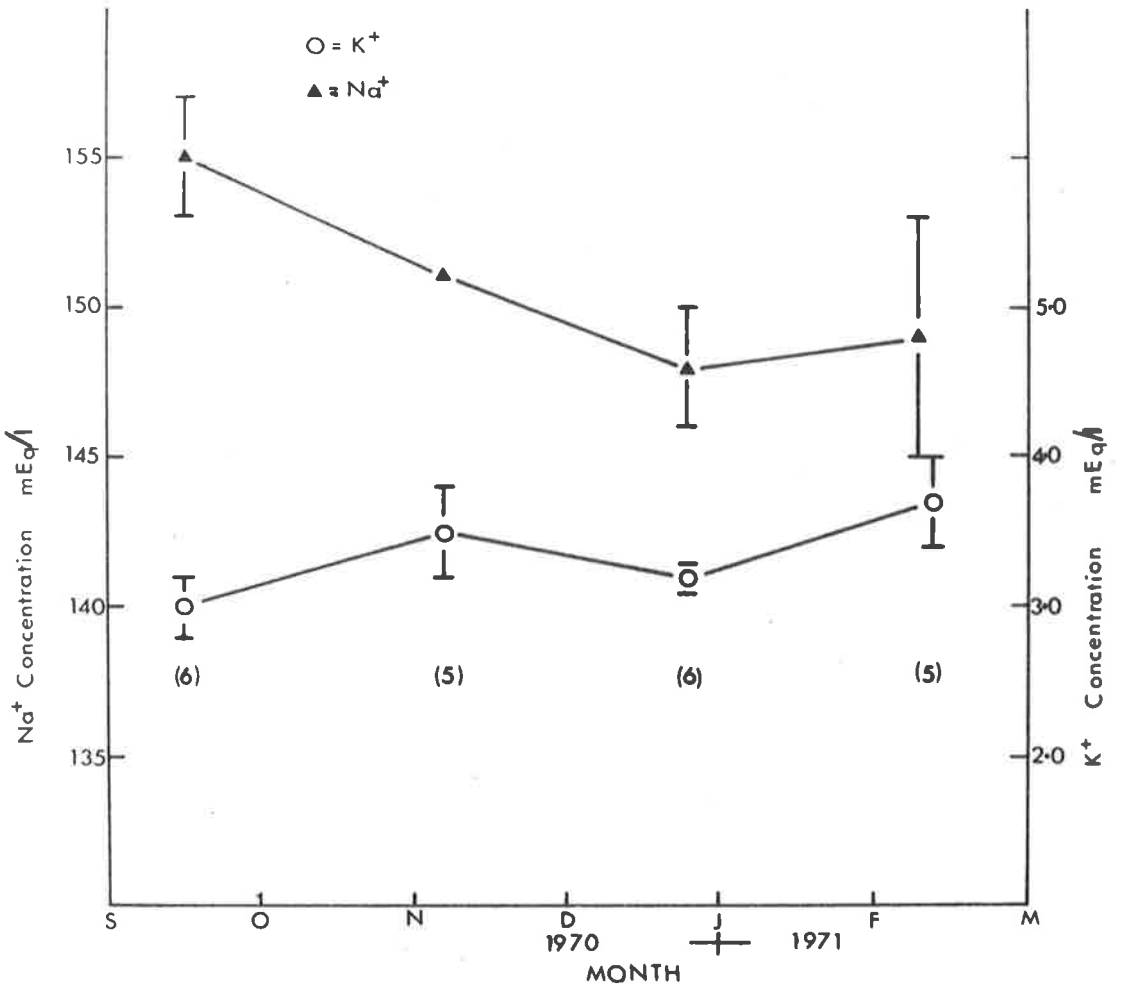
There was considerable variation between the lizards in their reactions to injections of sodium and potassium chloride. This tends to be masked by the way the results are presented in Fig. 30. For example, in one lizard that was injected with potassium chloride the Na^+/K^+ ratio decreased from 2.8 to 1.1 while in another it increased from 0.15 to 0.95. In all cases sodium chloride injections increased the Na^+/K^+ ratio although individuals differed in the amount of the increase.

Table 23

Analysis of dried secretion from T. rugosus.

Salt injected	Weight of secretion. mg	Weight of Na^+ mEq	Weight of K^+ mEq	Weight of Cl^- mEq	Ion weight as % of total
NaCl	1.60	0.015	0.010	0.023	98
NaCl	12.70	0.163	0.048	0.178	94
NaCl	2.60	0.026	0.014	0.037	94
KCl	8.90	0.005	0.103	0.116	93
KCl	2.80	0.001	0.034	0.038	96
KCl	0.75	0.003	0.071	0.009	88

Fig. 31. A graph of the variation in the concentration of Na^+ and K^+ in samples of plasma taken from T. rugosus in the field during summer. Sample sizes are in parentheses.


O = K+
▲ = Na+
155
150
145
140
135
5·0
4·0
3·0
2·0
Na+ Concentration mEq/l
K+ Concentration mEq/l
(6)
(5)
(6)
(5)
S
O
N
D
J
F
M
1970
1971
MONTH

It was difficult to calculate rates of secretion for T. rugosus because the glands were not cannulated. The masks caught an unknown fraction of the total secretion, and therefore it was not possible to calculate directly how much of the inject salt was secreted through the nasal gland. However, in two case most of the secretion was collected from an animal for two hours Both lizards had been injected with sodium chloride. Table 24 shows that the rate of secretion of cations from the two sodium chloride loaded T. rugosus was intermediate between the rates that were observed by Templeton (1964) for the two iguanids Ctenosaura pectinata and Sauromalus obesus over a one hour and ten hour period. Thus, assuming that all the salt that was secreted over the two hour period was collected, the salt gland of T. rugosus can secrete salt at a rate similar to that of the terrestrial iguanids.

There was little variation during summer in the concentratic of sodium and potassium in plasma samples taken from animals in the field Fig. 31. Thus, there is some evidence that T. rugosus at Goolwa can maintain their electrolyte balance over summer. This is in contrast to the high elevation that Bentley (1959) found over summer for T. rugosus in Western Australia.

Table 24

Rate of Na^+ and K^+ secretion from the salt glands of various lizards. Rates are expressed as $\mu Eq\ X^+ \cdot Kg^{-1} \cdot hr^{-1}$.

	Ion	Ctenosaura*	Sauromalus*	T. rugosus$^\beta$ rate for 2 hr. period	
				Lizard a	Lizard b
rate for 1 hr period	K^+	93.9	311	K^+ 20	16.5
	Na^+	9.6	32.5	Na^+ 30	28
rate for 10 hr period	K^+	19.3	27.4		
	Na^+	0.6	2.8		

$^\beta$present study

*Templeton, 1964.

DISCUSSION

These results were unexpected in view of the work of Bentley (1959) and Shoemaker et al., (1966).

Bentley studied animals at 25C, whereas Staaland (1967) has shown that cold decreases and even stops secretion from the salt gland of saline loaded birds. However, it is difficult to equate the effect of temperature on the salt gland of a homiotherm with that of a heliotherm such as T. rugosus. Nevertheless, it was noted in the present study that animals had to be heated to about 30C before the gland would secrete. Thus low temperature may explain Bentley's results, but Shoemaker et al., (1966) salt loaded sleepies at high temperatures and still found no evidence for a salt gland.

Cooch (1964), while looking for functional salt glands in mallards and pintails in North America, found that some glands worked well while others produced little or no secretion. He subsequently showed that birds from salty areas had well developed glands while the gland atrophied in those reared on a low salt diet. Salt loading caused some regeneration in the gland. Ellis et al. (1963) and Schmidt-Nielsen and Kim (1964) suggest that the development of the salt glands of ducks is not complete unless the birds are presented with an osmotic load. It may be that the animals used by Bentley and Shoemaker et al.

had not been previously subjected to relatively high salt intakes so that the glands were not sufficiently developed to respond to a salt load. The animals that were used in the present study, however, have a high salt intake because the area from where they were collected is subjected to an almost continual salt spray. (Fluid from crushed S. megalocarpus, a common food of sleepies at Goolwa, contained sodium, 160 mEq/l; potassium, 65 mEq/l). Thus if the hypothesis of Ellis et al. is correct, the salt glands of these lizards would be maximally developed and ready to respond to a salt load. However, Bentley found that the electrolyte concentration of the plasma of T. rugosus in Western Australia increased steeply over summer, which shows that these lizards were probably subjected to an osmotic stress. Besides, three lizards that were sent to me by C. Taylor from the vicinity of Perth in Western Australia all possessed well developed and functional salt glands. Therefore, I can not explain why Bentley did not find a nasal salt gland in T.rugosus.

Sodium chloride loading increased the Na^+/K^+ ratio of the secretion in all lizards, whereas potassium loading decreased the ratio in some and increased it in others. The reverse was noted in most of the terrestrial iguanids (Schmidt-Nielsen et al., 1963; Templeton, 1964, 1966). However, after acclimation to a high sodium diet, the iguanid Ctenosaura pectinata responded to a sodium load by increasing the Na^+/K^+ ratio of the secretion

(Templeton, 1967). Thus the salt glands of terrestrial lizards, including T. rugosus, are probably similar to that of C. pectinat in that they are labile so that their response to sodium and potassium chloride depends upon whether their diet is high in potassium or sodium. That the salt gland of T. rugosus is acclimated to eliminating sodium in the field is shown by the high sodium content of nasal secretion collected from lizards in the field ($Na^+/K^+ = 3.3$).

Table22 showed that potassium and sodium chloride loading increased the potassium and sodium concentration of the plasma respectively. However, the increase was slight and two days after the last injection there was no significant difference between the groups. Therefore, T. rugosus was almost able to maintain a constant electrolyte concentration in the plasma at the rate of salt loading that was used. Templeton (1964) observe higher elevations in the potassium and sodium concentrations of the plasma in terrestrial iguanids that had been injected with these salts, but this may have been caused by the larger and less frequent salt loads that they were given.

The salt gland of T. rugosus must have been the prime regulator of the cation concentration of the plasma because although urine was not collected, faecal and urine remains were found in only four of the twenty pens. Therefore, most

animals did not urinate during the experiment. If the rate of secretion noted for two lizards can be taken as a guide, then the lizards could secrete at least 70% of the daily salt load through the gland.

There was little change over summer in the sodium and potassium concentration of the plasma of T. rugosus at Goolwa (Fig. 31). Similar results were obtained for two other lizards which also possess salt glands, namely Dipsosaurus dorsalis (Minnich, 1970b) and Varanus gouldii (Green, 1969). In contrast, at least some lizards which do not possess a salt gland show a marked increase in the electrolyte concentration of the plasma (Bradshaw and Shoemaker, 1967; Section 1, present study). When water is scarce, they store excess electrolytes in their body fluids until water becomes available to excrete them. Therefore, a functional salt gland may enable lizards to maintain sodium and potassium balance when water is scarce. However, the summer over which I measured the electrolyte concentration of the plasma of T. rugosus was fairly mild, besides sleepies inhabit regions that are much more arid than Goolwa. Thus, in hot, dry years, or in more arid regions, the salt gland of sleepies may not be sufficient for lizards to maintain electrolyte balance. In this regard, Bentley (1959) observed large increases over summer in the electrolyte concentration of the plasma of the population of T. rugosus that he worked with.

Most reptiles and birds excrete nitrogenous wastes as insoluble urates and uric acid. Waste nitrogen is secreted in the kidney as soluble urates and is precipitated in the cloaca by the reabsorption of water (Dantzler and Schmidt-Nielsen, 1966; Dantzler, 1967, 1968; Minnich, 1970b). Schmidt-Nielsen, et al. (1963) suggest that if, as appears to be likely, the reabsorption of fluid from the cloaca of reptiles and birds is achieved through the active transport of sodium and potassium from the urinary fluid to the blood with water following passivel then an extrarenal mechanism would be necessary to excrete the reabsorbed sodium and potassium. They suggest that the possessic of an extrarenal organ such as a salt gland is probably a pre-requisite for reptiles and birds in arid environments to be able to take full advantage of the water conserving advantages of excreting nitrogenous wastes as insoluble uric acid. However, Minnich (1969, 1970b) showed that some reptiles could excrete significant quantities of electrolytes as insoluble urates; thus cloacal reabsorption of salt does not mean that all salt is reabsorbed. (Preliminary studies have shown that T. rugosus can also excrete electrolytes as insoluble salts).

Some lizards can store excess electrolytes in their body fluids (Bradshaw and Shoemaker, 1967; Bradshaw, 1970; section 1, present study). Thus they can reabsorb salts from the cloaca and precipitate uric acid and still take full advantage of the

water conserving advantage of excreting nitrogenous wastes as insoluble urates and uric acid, even though they do not possess an extrarenal organ for excreting electrolytes. The stored electrolytes can be excreted later when water becomes available.

The probable function of the salt glands of terrestrial lizards is to augment the renal - cloacal system in the excretion of excess electrolytes. This hypothesis is supported by the fact that the gland can acclimate to eliminate that ion which is in excess in the diet.

BIBLIOGRAPHY

Alexander, C.E. and Whitford, W.G. (1968)
"Energy requirements of Uta stansburiana." Copeia (1968): 678 - 683.

Alexander, E.A. and Levinsky, N.G. (1968)
"An extrarenal mechanism of potassium adaptation". J. Clin. Invest. 47: 740 - 748.

Baker, L.A. and White, F.N. (1970)
"Redistribution of cardiac output in response to heating in Iguana iguana". Comp. Biochem. Physiol. 35: 253 - 262.

Bartholomew, G.A. and Tucker, V.A. (1963)
"Control of changes in body temperature, metabolism and circulation by the agamid lizard, Amphibolurus barbatus". Physiol. Zool. 36: 199 - 218.

Bartholomew, G.A. and Tucker, V.A. (1964)
"Size, body temperature, thermal conductance, oxygen consumption and heart rate in Australian varanid lizards". Physiol. Zool. 37: 341 - 354.

Beament, J.W. (1964)
"The active transport and passive movement of water in insects." Advan. Insect Physiol. 2: 67 - 129.

Benedict, F.G. (1932)
"The physiology of large reptiles with special reference to the heat production of snakes, tortoises, lizards and alligators." Carnegie Institution of Washington, Washington. 539 p.

Benson, G.K. and Phillips, J.G. (1964)
"Observations on the histological structure of the supraorbital (nasal) glands from saline-fed and freshwater-fed domestic ducks (Anas platyrhynchus)." J. Anat. 98: 571 - 578.

Bentley, P.J. (1959)
"Studies on the water and electrolyte metabolism of the lizard Trachysaurus rugosus (Gray)." J. Physiol. 145: 37 - 47.

Bentley, P.J. and Blumer, W.F.C. (1962)
"Uptake of water by the lizard, Moloch horridus." Nature 194: 699 - 700.

Bentley, P.J. and Schmidt-Nielsen, K. (1966)
"Permeability to water and sodium of the crocodilian, Caiman sclerops." J. Cell. Comp. Physiol. 66: 303 - 309.

Berliner, R.W., Kennedy, T.J. and Hilton, J.G. (1950)
"Renal mechanisms for excretion of potassium." Am. J. Physiol. 162: 348 - 367.

Berridge, M.J. and Gupta, B.L. (1967)
"Fine - structural changes in relation to ion and water transport in the rectal papillae of the blow-fly, Calliphora." J. Cell. Sci. 2: 89 - 112.

Berridge, M.J. and Oschman, J.L. (1969)
"A structural basis for fluid secretion by malpighian tubules." Tissue Cell 1: 247 - 272.

Bradshaw, S.D. (1965)
"The comparative ecology of lizards of the genus Amphibolurus." Ph.D. Thesis, University of Western Australia.

Bradshaw, S.D. (1970)
"Seasonal changes in the water and electrolyte metabolism of Amphibolurus lizards in the field." Comp. Biochem. Physiol. 36: 689 - 718.

Bradshaw, S.D. and Shoemaker, V.H. (1967)
"Aspects of water and electrolyte changes in a field population of Amphibolurus lizards. Comp. Biochem. Physiol. 20: 855 - 865.

Bradshaw, S.D. and Main, A.R. (1968)
"Behavioural attitudes and regulation of temperature in Amphibolurus lizards." J. Zool. (London) 154: 193 - 221.

Braysher, M.L. and Green, B.F. (1970)
"Absorption of water and electrolytes from the cloaca of an Australian lizard, Varanus gouldii (Gray)." Comp. Biochem. Physiol. 35: 607 - 614.

Burke, J.O. (1953)
"Oxygen capacity in mammals." Physiol. Zool. 26: 259 - 266.

Chan, D.K., Callard, I.P. and Chester Jones, I. (1970)
"Observations on the water and electrolyte composition of the iguanid lizard Dipsosaurus dorsalis (Baird and Girard), with special reference to the control by the pituitary gland and the adrenal cortex." Gen. Comp. Endocrinol. 15: 374 - 387.

Chew, R.M. and Dammann, A.E. (1961)
"Evaporative water loss of small vertebrates, as measured with an infrared analyzer." Science 133: 384 - 385.

Claussen, D.L. (1967)
"Studies of water loss in two species of lizards." Comp. Biochem. Physiol. 20: 115 - 130.

Cloudsley - Thompson, J.L. (1965)
"Rhythmic activity, temperature - tolerance, water - relations and mechanism of heat death in a tropical skink and gecko." J. Zool. (London) 146: 55 - 69.

Cooch, F.G. (1964)
"A preliminary study of the survival value of a functional salt gland in prairie Anatidae." Auk 81: 380 - 393.

Crawford, E.C. Jr. and Kampe, G. (1971)
"Physiological responses of the lizard Sauromalus obesus to changes in ambient temperature." Am. J. Physiol. 220: 1256 - 1260.

Crowe, J.H., Nagy, K.A. and Francis, C. (1970)
"Structure of lizard salt glands." Am. Zoologist 10: 556 - 557.

Curran, P.F. (1960)
Na, Cl, and water transport by rat ileum in vitro." J. Gen. Physiol. 43: 1137 - 1148.

Curran, P.F. (1965)
"Ion transport in intestine and its coupling to other transport processes." Federation Proc. 24: 993 - 999.

Dantzler, W.H. (1966)
"Renal response of chickens to infusion of hypertonic sodium chloride solution." Am. J. Physiol. 210: 640 - 646.

Dantzler, W.H. (1967)
"Stop-flow study of renal function in concious water snakes (Natrix sipedon)." Comp. Biochem. Physiol. 22: 131 - 140.

Dantzler, W.H. (1968)
"Effect of metabolic alkalosis and acidosis on tubular urate secretion in water snakes." Am. J. Physiol. 215: 747 - 751.

Dantzler, W.H. (1970)
"Kidney function in desert vertebrates, p. 157 - 190." In Benson, G.K. and Phillips, J.G. (ed.), Memoirs of the society for endocrinology No. 18, Cambridge Univ. Press, Cambridge.

Dantzler, W.H. and Schmidt-Nielsen, B. (1966)
"Excretion in fresh-water turtle (Pseudemys scripta) and desert tortoise (Gopherus agassizii)." Am. J. Physiol. 210: 198 - 210.

Davis, L.E. and Schmidt-Nielsen, B. (1967)
"Ultrastructure of the crocodile kidney (Crocodylus acutus) with special reference to electrolyte and fluid transport." J. Morph. 121: 255 - 276.

Dawson, W.R. (1967)
Cited by Pough, F.H. (1969b).

Dawson, W.R. and Bartholomew, G.A. (1956)
"Relation of oxygen consumption to body weight, temperature, and temperature acclimation in lizards Uta stansburiana and Sceloporus occidentalis." Physiol. Zool. 29: 40 - 51.

Dawson, W.R. and Poulson, T.L. (1962)
"Oxygen capacity of lizard bloods." Am. Midl. Nat. 68: 154 - 164.

Dawson, W.R. and Templeton, J.R. (1963)
"Physiological responses to temperature in the lizard Crotaphytus collaris." Physiol. Zool. 36: 219 - 236.

Dawson, W.R. and Templeton, J.R. (1966)
"Physiological responses to temperature in the alligator lizard, Gerrhonotus multicarinatus." Ecology 47: 759 - 765.

Dawson, W.R., Shoemaker, V.H. and Licht, P. (1966)
"Evaporative water losses of some small Australian lizards." Ecology 47: 589 - 594.

Dessauer, H.C. (1970)
"Blood chemistry of reptiles: Physiological and evolutionary aspects, p. 1 - 72." In Gans, C. (ed.) Biology of the reptilia Volume 3, Morphology C. Academic Press, London.

De Witt, C.B. (1967)
"Precision of thermoregulation and its relation to environmental factors in the desert iguana, Dipsosaurus dorsalis." Physiol. Zool. 40: 49 - 66.

Deyrup, I.J. (1964)
"Water balance and kidney, p. 251 - 328." In Moore, J.A. (ed.) Physiology of the amphibia. Academic Press, New York.

Diamond, J.M. (1964)
"The mechanism of isotonic water transport." J. Gen. Physiol. 48: 15 - 42.

Diamond, J.M. and Bossert, W.H. (1967)
"Standing gradient osmotic flow: A mechanism for coupling of water and solute transport in epithelia." J. Gen. Physiol. 50: 2061 - 2083.

DiBona, D.R., Civan, M.M. and Leaf, A. (1969)
"The cellular specificity of the effect of vasopressin on toad urinary bladder." J. Membrane Biol. 1: 79 - 91.

Dunson, W.A. (1969)
"Electrolyte excretion by the salt gland of the Galapagos marine iguana." Am. J. Physiol. 216: 995 - 1002.

Ellis, R.A., Goertemiller, C.C. Jr., DeLellis, R.A. and Kablotsky, Y.H. (1963)
"The effect of salt water regimen on the development of the salt glands of domestic ducklings." Devel. Biol. 8: 286 - 308.

Ellis, R.A. and Abel, J.H. Jr. (1964)
"Intercellular channels in the salt secreting glands of marine turtles." Science 144: 1340 - 1342.

Ferguson, D.R. and Heap, P.F. (1970)
"The morphology of the toad urinary bladder: A stereoscopic and transmission electron microscopical study." Z. Zellforsch. 109: 297 - 305.

Fox, W. and Dessauer, H.C. (1962)
"The single right oviduct and other urogenital structures of female Typhlops and Lepto typhlops." Copeia (1962): 590 - 597.

Gans, C., Krakauer, T. and Paganelli, C.V. (1968)
"Water loss in snakes: Interspecific and intraspecific variability." Comp. Biochem. Physiol. 27: 747 - 761.

Geddes, I.C. (1967)
"Recent studies in metabolic aspects of anaesthesia, p. 1 - 41." In Evans, F.T. and Gray, T.C. (ed.). Modern trends in anaesthesia, Volume 3. Butterworths, London.

Gerzelli, G. and De Piceis Polver, P. (1970)
"The lateral nasal gland of Lacerta viridis under different experimental conditions." Monitore Zool. Ital. (N.S.) 4: 191 - 200.

Grantham, J.J., Ganote, C.E., Burg, M.B. and Orloff, J. (1969)
"Paths of transtubular water flow in isolated renal collecting tubules." J. Cell. Biol. 41: 562 - 576.

Green, B.F. (1969)
"Water and electrolyte balance in the sand goanna Varanus gouldii (Gray)." Ph. D. Thesis, University of Adelaide.

Gupta, B.L. and Berridge, M.J. (1966)
"Fine structural organization of the rectum in the blowfly, Calliphora erythrocephala (Meig) with special reference to connective tissue, tracheae and neurosecretory innervation of the rectal papillae." J. Morph. 120: 23 - 82.

Hadley, N.F. and Burns, T.A. (1968)
"Intraspecific comparison of the blood properties of the side-blotched lizard, Uta stansburiana." Copeia (1968); 737 - 740.

Hall, F.G. (1922)
Cited by Sexton, O.J. and Heatwole, H. (1968).

Hall, F.G. (1937)
"Adaptations of mammals to high altitudes." J. Mammal. 18: 468 - 472.

Hayward, A.F. (1962)
"Aspects of the fine structure of the gall bladder epithelium of the mouse." J. Anat. 96: 227 - 236.

Heatwole, H. (1970)
"Thermal ecology of the desert dragon Amphibolurus inermis." Ecol. Monogr. 40: 425 - 457.

Huber, G.C. (1917)
"On the morphology of the renal tubules of vertebrates." Anat. Rec. 13: 305 - 339.

Hudson, J.W. and Bertram, F.W. (1966)
"Physiological responses to temperature in the ground skink, Lygosoma laterale." Physiol. Zool. 39: 21 - 29.

Hunn, J.B. and Willford, W.A. (1970)
"The effect of anesthetization and urinary bladder catheterization on renal function of rainbow trout." Comp. Biochem. Physiol. 33: 805 - 812.

Hutchinson, V.H. and Larimer, J.L. (1960)
"Reflectivity of the integuments of some lizards from different habitats." Ecology 41: 199 - 209.

Irvine, H.B. and Phillips, J.E. (1971)
"Effects of respiratory inhibitors and ouabain on water transport by isolated locust rectum." J. Insect. Physiol. 17: 381 - 393.

Jackson, D.C. and Schmidt-Nielsen, K. (1964)
"Countercurrent heat exchange in the respiratory passages." Proc. Nat. Acad. Sci. 51: 1192 - 1197.

Johnson, F.R., McMinn, R.M. and Birchenough, R.F. (1962)
"The ultrastructure of the gall-bladder epithelium of the dog." J. Anat. 96: 477 - 487.

Junqueira, L.U.C., Malnic, G. and Monge, C. (1966)
"Reabsorptive function of the ophidian cloaca and large intestine." Physiol. Zool. 39: 151 - 159.

Kaye, G.I., Wheeler, H.O., Whitlock, R.T. and Lane, N. (1966)
"Fluid transport in the rabbit gall bladder: A combined physiological and electron microscopic study." J. Cell. Biol. 30: 237 - 268.

Klicka, J. and Mahmoud, I.Y. (1971)
"A comparative study of respiratory pigment in six species of turtles." Comp. Biochem. Physiol. 38A: 53 -58.

Komadina, S. and Solomon, S. (1970)
"Comparison of renal function of bull and water snake (Pituophis melanoleucus and Natrix sipedon)." Comp. Biochem. Physiol. 32: 333 - 343.

Komnick, H. (1962)
"Elektronenmikroskopische lokalisation von Na^+ und Cl^- in zellen und geweben." Protoplasma 55: 414 - 418.

Krakauer, T., Gans, C. and Paganelli, C.V. (1968)
"Ecological correlation of water loss in burrowing reptiles." Nature 218: 659 - 660.

Lasiewski, R.C., Acosta, A.L. and Bernstein, M.H. (1966)
"Evaporative water loss in birds - I. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability." Comp. Biochem. Physiol. 19: 445 - 457.

LeBrie, S.J. and Elizondo, R.S. (1969)
"Saline loading and aldosterone in water snakes Natrix cyclopion." Am. J. Physiol. 217: 426 - 430.

Licht, P. (1964a)
"The temperature dependence of myosin, myosinadenosine triphosphatase, adenosine triphosphatase and alkaline phosphatase in lizards." Comp. Biochem. Physiol. 12: 331 - 340.

Licht, P. (1964b)
"A comparative study of the thermal dependence of contractility in saurian skeletal muscle." Comp. Biochem. Physiol. 13: 27 - 34.

Licht, P., Dawson, W.R., Shoemaker, V.H. and Main, A.R. (1966)
"Observations on the thermal relations of Western Australian lizards." Copeia (1966): 97 - 110.

Licht, P., Dawson, W.R. and Shoemaker, V.H. (1969)
"Thermal adjustments in cardiac and skeletal muscles of lizards." Z. Vergl. Physiol. Physiologie 65: 1 - 14.

Maderson, P.F. (1965)
Cited by Claussen, D.L. (1967).

Manwell, C. and Baker, C.A. (1970)
"Molecular biology and the origin of species: Heterosis, protein polymorphism and animal breeding." Sidgwick and Jackson, London. 394 p.

Marshall, E.K. Jr. (1932)
"Kidney secretion in reptiles." Proc. Soc. Exp. Biol. Med. 29: 971 - 973.

McNabb, F.M.A. and Poulson, T.L. (1970)
"Uric acid excretion in pigeons, Columba livia." Comp. Biochem. Physiol. 33: 933 - 939.

Minnich, J.E. (1969)
"Excretion of urate salts by reptiles." Am. Zoologist 9: 1094.

Minnich, J.E. (1970a)
"Evaporative water loss from the desert iguana, Dipsosaurus dorsalis." Copeia (1970): 575 - 578.

Minnich, J.E. (1970b)
"Water and electrolyte balance of the desert iguana, Dipsosaurus dorsalis, in its natural habitat." Comp. Biochem. Physiol. 35: 921 - 933.

Moberly, W.R. (1963)
"Hibernation in the desert iguana, Dipsosaurus dorsalis." Physiol. Zool. 36: 152 - 160.

Moberly, W.R. (1968)
"The metabolic responses of the common iguana, Iguana iguana, to activity under restraint." Comp. Biochem. Physiol. 27: 1 - 20.

Murrish, D.E. and Schmidt-Nielsen, K. (1970a)
"Exhaled air temperature and water conservation in lizards." Resp. Physiol. 10: 151 - 158.

Murrish, D.E. and Schmidt-Nielsen, K. (1970b)
"Water transport in the cloaca of lizards: Active or passive?" Science 170: 324 - 326.

Norris, K.S. (1967)
Cited by Heatwole, H. (1970).

Norris, K.S. and Dawson, W.R. (1964)
"Observations on the water economy and electrolyte excretion of chuckwallas (Lacertilia, Sauromalus)." Copeia (1968) 638 - 646.

Norris, K.S. and Lowe, C.H. (1964)
"An analysis of background color-matching in amphibians and reptiles." Ecology 45: 565 - 580.

O'Brien, D., Ibbott, F.A. and Rodgerson, D.O. (1968)
"Laboratory manual of pediatric micro-biochemical techniques, 4th edition." Harper and Row, New York 367 p.

Ohmart, R.D., McFarland, L.Z. and Morgan, J.P. (1970)
"Urographic evidence that urine enters the rectum and ceca of the roadrunner (Geococcyx californianus) Aves. Comp. Biochem. Physiol. 35: 487 - 489.

Oschman, J.L. and Wall, B.J. (1969)
"The structure of the rectal pads of Periplaneta americana L. with regard to fluid transport." J. Morph. 127: 475 - 510.

Peachey, L.D. and Rasmussen, H. (1961)
"Structure of the toad's urinary bladder as related to its physiology." J. Biophys. Biochem. Cytol. 10: 529 - 553.

Phillips, J.E. (1964)
"Rectal absorption in the desert locust, Schistocerca gregaria Forskal. I. Water." J. Exp. Biol. 41: 15 - 38.

Phillips, J.E. (1970)
"Apparent transport of water by insect excretory systems." Am. Zoologist 10: 413 - 436.

Philpott, C.W. and Templeton, J.R. (1964)
"A comparative study of the histology and fine structure of the nasal salt secreting gland of the lizard, Dipsosaurus." Anat. Rec. 148: 394 - 395.

Pough, F.H. (1969a)
"Environmental adaptations of the blood of lizards." Comp. Biochem. Physiol. 31: 885 - 901.

Pough, F.H. (1969b)
"Physiological aspects of the burrowing sand lizards (Uma, Iguanidae) and other lizards." Comp. Biochem. Physiol. 31: 869 - 884.

Prange, H.D. and Schmidt-Nielsen, K. (1969)
"Evaporative water loss in snakes." Comp. Biochem. Physiol. 28: 973 - 975.

Preece, A. (1959)
"A manual for histologic techniques." Churchill, London. 219 p.

Ramsay, J.A. (1955)
"The excretory system of the stick insect, Dixippus morosus (Orthoptera, Phasmidae)." J. Exp. Biol. 32: 183 - 200.

Richards, S.A. (1970)
"The biology and comparative physiology of thermal panting." Biol. Rev. 45: 223 - 264.

Roberts, L.A. (1968a)
"Oxygen consumption in the lizard Uta stansburiana." Ecology 49: 809 - 819.

Roberts, L.A. (1968b)
"Water loss in the desert lizard, Uta stansburiana." Comp. Biochem. Physiol. 27: 583 - 589.

Roberts, J.S. and Schmidt-Nielsen, B. (1966)
"Renal ultrastructure and excretion of salt and water by three terrestrial lizards." Am. J. Physiol. 211: 476 - 486.

Sauer, J.R., Levy, J.J., Smith, D.W. and Mills, R.R. (1970)
"Effect of rectal lumen concentration on the reabsorption of ions and water by the American cockroach." Comp. Biochem. Physiol. 32: 601 - 614.

Schmidt-Nielsen, K., Borut, A., Lee, P. and Crawford, E. Jr. (1963)
"Nasal salt excretion and the possible function of the cloaca in water conservation." Science 142: 1300 - 1301.

Schmidt-Nielsen, K., Crawford, E.C. and Bentley, P.J. (1966)
"Discontinuous respiration in the lizard Sauromalus obesus." Federation Proc. 25: 506.

Schmidt-Nielsen, K. and Fange, R. (1958)
"Salt glands in marine reptiles." Nature 182: 783 - 785.

Schmidt-Nielsen, K., Hainsworth, F.R. and Murrish, D.E. (1969)
"Cooling of expired air in birds by counter - current exchange." Federation Proc. 28: 459.

Schmidt-Nielsen, K., Jorgensen, C.B. and Osaki, H. (1958)
"Extrarenal salt excretion in birds." Am. J. Physiol. 193: 101 - 107.

Schmidt-Nielsen, K. and Kim, Y.T. (1964)
"The effect of salt intake on the size and function of the salt glands of ducks." Auk 81: 160 - 172.

Schmidt-Nielsen, B. and Schmidt-Nielsen, K. (1951)
"A complete account of the water metabolism in kangaroo rats and an experimental verification." J. Cell. Comp. Physiol. 38: 165 - 181.

Schmidt-Nielsen, B., Schmidt-Nielsen, K., Houpt, T.R. and Jarnum, S.A. (1956)
"Water balance of the camel." Am. J. Physiol. 185: 185 - 194.

Schmidt-Nielsen, B. and Skadhauge, E. (1967)
"Function of the excretory system of the crocodile (Crocodylus acutus)." Am. J. Physiol. 212: 973 - 980.

Scothorne, R.J. (1959)
"The nasal glands of birds: A histological and histochemical study of the inactive gland in the domestic duck." J. Anat. 93: 246 - 256.

Seshadri, C. (1956)
"Urinary excretion in the Indian house lizard, Hemidactylus flaviviridis (Ruppel)." J. Zool. Soc. Ind. 8: 63 - 78.

Seshadri, C. (1957)
"Water conservation in Uromastix hardwickii (Gray), with a note on the presence of mullerian ducts in the male." J. Zool. Soc. Ind. 9: 103 - 113.

Seshadri, C. (1959)
"Functional morphology of the cloaca of Varanus monitor (Linnaeus) in relation to water economy." Proc. Natn Inst. Sci. India 25(B): 101 - 106.

Sexton, O.J. and Heatwole, H. (1968)
"An experimental investigation of habitat selection and water loss in some anoline lizards." Ecology 49: 762 - 767.

Shoemaker, V.H., Licht, P. and Dawson, W.R. (1966)
"Effects of temperature on kidney function in the lizard Tiliqua rugosa." Physiol. Zool. 39: 244 - 252.

Skadhauge, E. (1967)
"In vivo perfusion studies of the cloacal water and electrolyte resorption in the fowl (Gallus domesticus)." Comp. Biochem. Physiol. 23: 483 - 501.

Skadhauge, E. and Schmidt-Nielsen, B. (1967)
"Renal function in domestic fowl." Am. J. Physiol. 212: 793 - 798.

Smith, H.W. (1953)
"From fish to philosopher." Little and Brown, Boston.

Sokol, O.M. (1967)
"Herbivory in lizards." Evolution 21: 192 - 194.

Sokolov, W. (1966)
"Water content in the tissue of desert animals." Nature 211: 545.

Staaland,H. (1967)
"Temperature sensitivity of the avian salt gland." Comp. Biochem. Physiol. 23: 991 - 993.

Stobbart, R.H. and Shaw, J. (1964)
"Salt and water balance: Excretion, p. 190 - 258." In Rockstein, M. (ed.). The physiology of insecta, 3. Academic Press, New York. 692 p.

Taub, A.M. and Dunson, W.A. (1967)
"The salt gland in a sea snake (Laticauda)." Nature 215: 995 - 996.

Templeton, J.R. (1960)
"Respiration and water loss at higher temperatures in the desert iguana, Dipsosaurus dorsalis." Physiol. Zool. 33: 136 - 145.

Templeton, J.R. (1964)
"Nasal salt excretion in terrestrial lizards." Comp. Biochem. Physiol. 11: 223 - 239.

Templeton, J.R. (1966)
"Responses of the lizard nasal salt gland to chronic hypersalemia." Comp. Biochem. Physiol. 18: 563 - 572.

Templeton, J.R. (1967)
"Nasal salt gland excretion and adjustment to sodium loading in the lizard, Ctenosaura pectinata." Copeia (1967); 136 - 140.

Templeton, J.R. (1970)
"Panting and its control in the desert iguana, Dipsosaurus dorsalis." Am. Zoologist 10: 521.

Thatcher, J.S. and Radike, A.W. (1947)
"Tolerance to potassium intoxication in the albino rat." Am. J. Physiol. 151: 138 - 146.

Tormey, J. McD. and Diamond, J.M. (1967)
"The ultrastructural route of fluid transport in rabbit gall bladder." J. Gen. Physiol. 50: 2031 - 2060.

Tucker, V.A. (1966)
"Oxygen transport by the circulatory system of the green iguana (Iguana iguana) at different body temperatures." J. Exp. Biol. 44: 77 - 92.

Wall, B.J. and Oschman, J.L. (1970)
"Water and solute uptake by rectal pads of Periplaneta americana." Am. J. Physiol. 218: 1208 - 1215.

Wall, B.J., Oschman, J.L. and Schmidt-Nielsen, B. (1970)
"Fluid transport: Concentration of the inter-cellular compartment." Science 167: 1497 - 1498.

Warburg, M.R. (1965a)
"The influence of ambient temperature and humidity on the body temperature and water loss from two Australian lizards, Tiliqua rugosa (Gray) (Scincidae) and Amphibolurus barbatus Cuvier (Agamidae)." Aust. J. Zool. 13: 331 - 350.

Warburg, M.R. (1965b)
"Studies on the environmental physiology of some Australian lizards from arid and semi-arid habitats." Aust. J. Zool. 13: 563 - 575.

Warburg, M.R. (1966)
"On the water economy of several Australian geckos, agamids, and skinks." Copeia (1966): 230 - 235.

White, F.N. (1970)
"Central vascular shunts and their control in reptiles." Federation Proc. 29: 1149 - 1153.

Wigglesworth, V.B. (1965)
"The principles of insect physiology. 6th edition." Methuen and Co., London. 741 p.

Wright, F.S., Strieder, N., Fowler, N.B. and Giebisch, G. (1971)
"Potassium secretion by distal tubule after potassium adaptation." Am. J. Physiol. 221: 437 - 448.

Umbreit, W.W., Burris, R.H. and Stauffer, J.F. (1959)
"Manometric Techniques." Burgess Publishing Co., Minneapolis. 338 p.

Yamada, E. (1955)
"The fine structure of the gall bladder epithelium of the mouse." J. Biophysic. Biochem. Cytol. 1: 445 - 458.

APPENDIX

The following section is a copy of a paper that has been published: M. BRAYSHER and B. GREEN (1970) 'Absorption of water and electrolytes from the cloaca of an Australian lizard, Varanus gouldii (Gray)'. Comp. Biochem. Physiol., 35 : 607 - 614.

This work was done concurrently with the earlier sections and in association with Dr. B. Green.

Abstract - 1. An isotonic solution was introduced *in vivo* into the isolated cloaca of *V. gouldii*. Changes in the volume and composition of the fluid were measured.

2. Fluid was absorbed from the cloaca of *V. gouldii* at a rate of 8.4 ± 0.6 ml/kg per hr at 30°C.

3. AVT (arginine vasotocin) increased the rate of absorption by increasing the permeability to water and the rate of sodium transport.

4. Secretion of potassium into the cloaca was found in some of the control animals.

5. The resorption of fluid from the urine is essential to derive the full benefits of water conservation that are associated with uricotelism.

INTRODUCTION

The cloaca is important in the conservation of water in most terrestrial reptiles. Nitrogen is usually excreted as uric acid; a hard pellet is formed by the withdrawal of fluid by the cloaca.

The resorptive function of the cloaca has been shown in crocodilians (Bentley and Schmidt-Nielsen, 1966; Schmidt-Nielsen and Skadhauge, 1967), snakes (Junqueira *et al.*, 1966) and terrestrial

lizards (Roberts and Schmidt-Nielsen, 1966), but there has been no attempt to measure the rate of resorption fluid. This has been studied only in chickens (Hart and Essex, 1942; Skadhauge, 1967, 1968; Nechay and Lutherer, 1968).

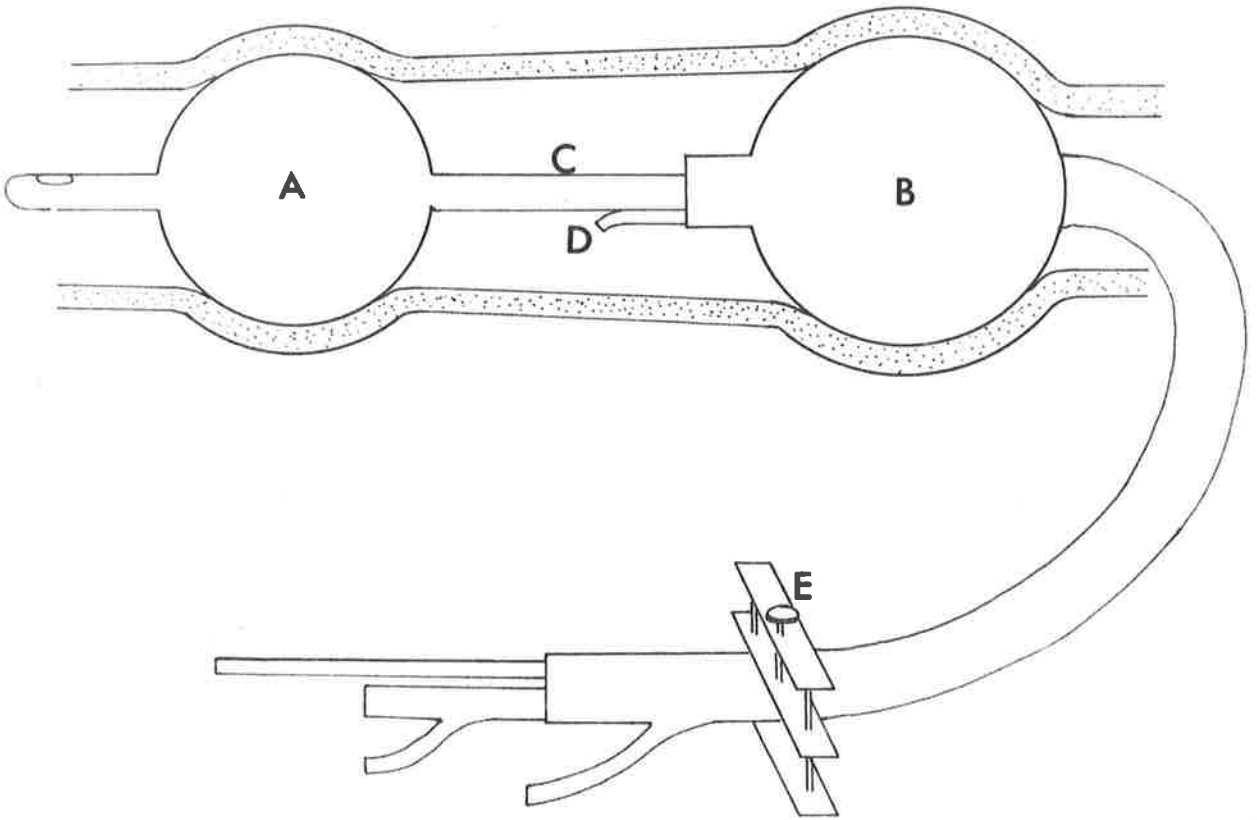
Amphibians and some terrestrial reptiles have a urinary bladder, which like the cloaca is important for the resorption of fluid and electrolytes. Antidiuretic hormones influence the rate of movement of water and sodium across the amphibian bladder (Ewer, 1952; Sawyer and Schisgall, 1956; Sawyer, 1960), but it is not known if the cloaca of reptiles shows a similar response. Consequently, an experiment was designed to measure the rate of resorption of fluid from the cloaca, and the effect of arginine vasotocin (AVT) on the transport of water and electrolytes in the cloaca.

MATERIALS AND METHODS

The goannas used in this study were collected from Kangaroo Island in South Australia and kept in a large open terrarium. Water was always available and they were fed mice. The average weight of the lizards used in the experiments was 1206 g (range: 909 - 1445 g).

The cloaca of the goanna is similar to that of the gecko Hemidactylus (Seshadri, 1956), consisting of three chambers in

Fig. 1. The apparatus for isolating the coprodaeum. This consisted of two Foley catheters (F) positioned in series, the smaller (15 cm^3) anterior catheter (A) passing through the drain canal of the larger (30 cm^3) posterior catheter (B). A sampling tube (D) of Portex polythene tubing (size P.P. 120) opened into the space (C) between two bulbs. This tube passed along the drain canal of the larger catheter and emerged at the far end of the apparatus. A clamp (E) prevented the leakage of saline.


A
C
B
D
E

series, the anterior coprodaeum, central urodaeum and posterior proctodaeum. The ureters drain into the urodaeum and the urine then passes forward into the coprodaeum, where most of the fluid is withdrawn.

Animals were anaesthetized the night before the experiment with an intraperitoneal injection of nembutal (20 mg/kg). The following morning the cloaca and rectum were flushed clean with water before the apparatus shown in Fig. 1 was inserted, deflated, into the cloaca. The catheter bulbs were then inflated with water such that the coprodaeum was isolated from the rectum and urodaeum. An animal was dissected with the apparatus in place to verify the position of the isolated region.

After the apparatus had been inserted, the animals were placed in a constant-temperature cabinet ($35 \pm 0.1^{\circ}C$) until they had recovered from the anaesthetic. They were then transferred to another cabinet ($30 \pm 0.1^{\circ}C$) for the experiment. Here the animals were suspended from racks by rubber straps tied around the thorax and the base of the tail and a hood was placed over each animal to pacify it. All animals had been conscious for at least 2 hr before the experiments were started.

There were six control and seven experimental animals. The experimental lizards were given a cardiac injection of AVT

(100 ng/kg body weight of a solution containing 1 µg/ml), 15 min before the start of the experiment. Then, in both groups, 20 ml of isotonic saline (Na = 164 m-equiv/l., K = 5.4 m-equv/l.) were introduced through the sampling tube into the space between the bulbs. Albumin (0.50 or 0.25%) was used as a volume marker, because preliminary experiments showed that inulin was an unreliable marker.

Samples of about 0.3 ml of the fluid were collected immediately after it was introduced and then at known intervals. They were taken with a 5-ml syringe inserted into the sampling tube. The syringe plunger was moved back and forth until the solution in the cloaca was thoroughly mixed before each sample was taken.

In the control group, samples were taken at 1 hour and then every other hour. Preliminary observations indicated that fluid was absorbed much faster after injection of AVT, and therefore samples from the experimental group were taken every 30 min for the first 2 hr and hourly thereafter.

A blood sample was taken by cardiac puncture at the beginning and end of each experiment. Samples of plasma and cloacal fluid were stored in plastic tubes in a refrigerator until analysed. Sodium and potassium concentrations were measured with an EEL

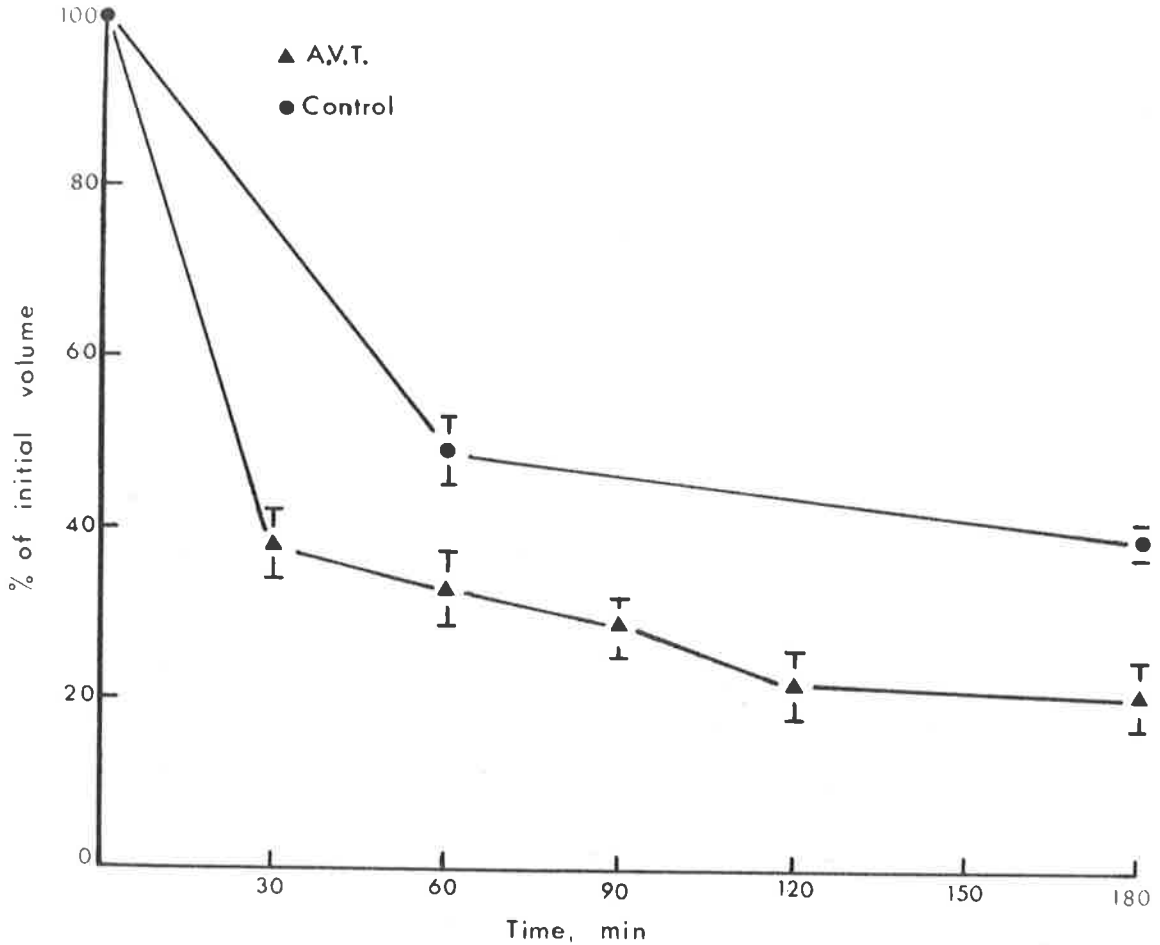
flame photometer. The concentration of albumin in the cloacal fluid was measured with a Unican SP 500 spectrophotometer. For this, 10- or 20-µl aliquots of cloacal fluid were diluted to 1 or 2 ml and read directly at 280 nm in silica cells.

RESULTS

The mean concentrations of sodium and potassium in the plasma were 165 ± 4 m-equiv/l. and 4.4 ± 0.3 m-equiv/l. respectively, and therefore the introduced fluid was almost isotonic to the plasma. There were no significant changes in the electrolyte levels of the blood during the course of the experiments.

The changes in fluid volume for both the control and experimental animals are shown in Fig. 2. Initially, fluid was absorbed rapidly in both groups, but later the rate decreased. The decrease was probably due to a reduction in the surface area of the cloaca to which fluid was exposed. The mucosal surface of the cloaca is increased by folds and villi, and contraction of the cloaca as fluid is resorbed would cause the sides of adjacent villi to come into contact, thus decreasing the absorptive surface area. In normal circumstances, resorbed fluid is continually replaced by urine from the kidneys, and this, together with continual deposition of uric acid in the coprodaeum, would keep the cloaca distended. Consequently, the rates of absorption over the initial periods were assumed

Fig. 2. Changes in the volume of fluid in the cloaca of control and AVT-injected animals: Mean values are given ± S.E.

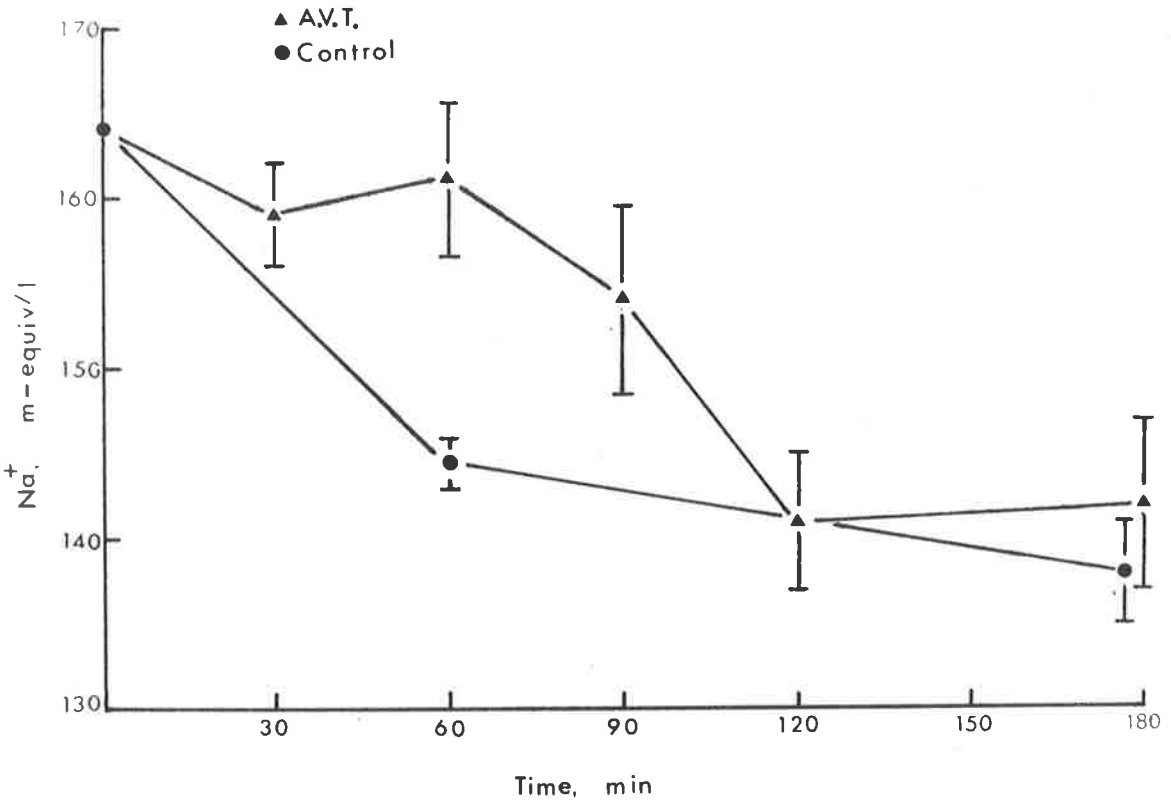

▲ A.V.T.
● Control
100
80
60
40
20
0
% of initial volume
30
60
90
120
150
180
Time, min

more closely to represent normal rates.

No technique for measuring the absorptive surface could be found which would take into account the distensibility of the cloaca and the presence of folds and villi. Consequently, rates of absorption were expressed in absolute terms. Fluid was absorbed at a rate of 20.6 $\pm$ 1.4 ml/kg per hr during the first half-hour in the animals injected with AVT, which is much faster than the rate of 8.4 $\pm$ 0.6 ml/kg per hr during the first hour in the controls. This difference may be partly because samples were not taken as frequently in the control group, so that the control rate may be higher. However, this is probably not a serious error as a check with an extra control animal showed that the rate of absorption was the same in the first and second half-hours. Besides, the rates of absorption in the two groups are still significantly different ($p < 0.02$) if they are compared over the first hour.

Figure 3 shows that after an hour the concentration of sodium in the fluid had fallen significantly from the initial concentration in the control group ($P < 0.01$), but not in the experimental group. Two hours later the concentrations for both experimental and control animals were about the same. Nevertheless, by also taking volume changes into account, it can be calculated that the mean rate of sodium transport in animals

Fig. 3. Changes in the concentration of sodium in the cloacal fluid of control and AVT-injected animals. Mean values are given ± S.E.


▲ A.V.T.
● Control
170
160
150
140
130
Na^+, m-equiv/l
30
60
90
120
150
180
Time, min

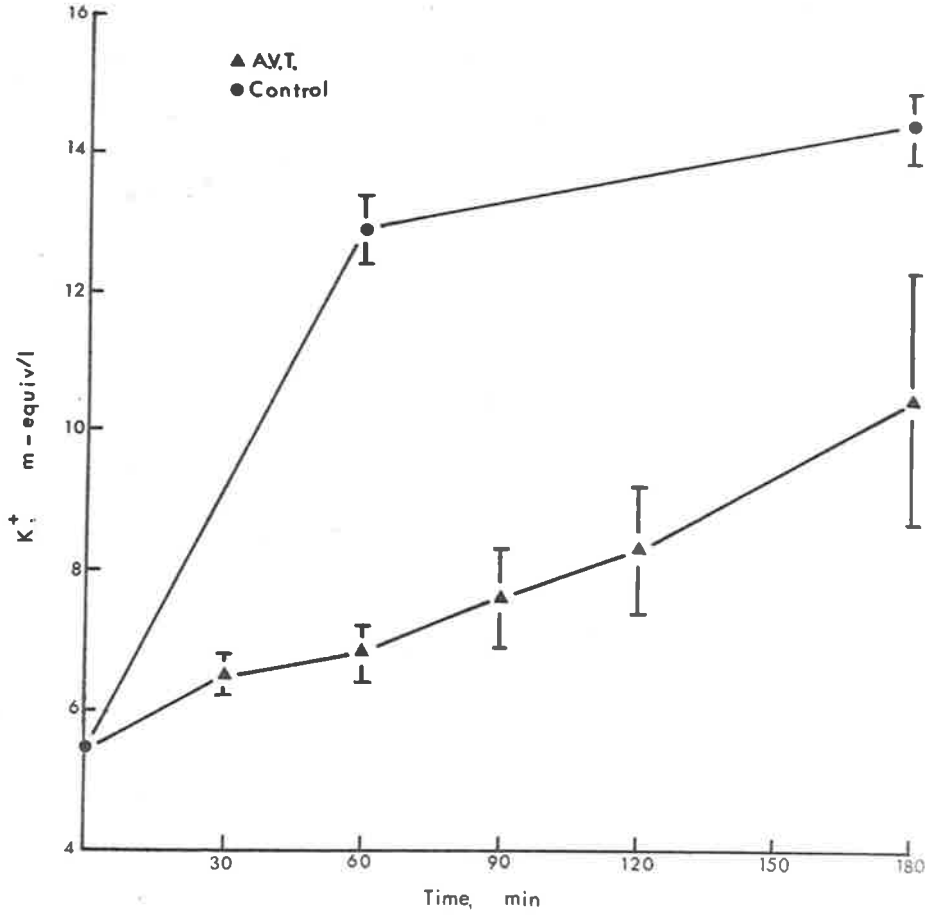
treated with AVT was 4.26 ± 0.25 m-equiv/hr for the first half-hour, more than double the rate of 1.88 ± 0.12 m-equiv/hr for the control group in the first hour.

Figure 4 shows that the potassium concentration of the introduced fluid increased much more rapidly in the controls than it did in the animals treated with AVT. The increase in the control animals was due almost entirely to absorption of fluid, although some animals showed a slight net secretion of this ion. However, potassium was absorbed during the first hour in all the animals injected with AVT, the fastest rate being 131 ± 8 μ equiv. K^+/hr during the first half-hour.

DISCUSSION

These experiments show clearly the resorptive activity of the cloaca and the promotion of this by AVT. The rates of absorption are probably low because some albumin would have been removed by the pinocytotic activity of the epithelial cells (Woodin, 1963). Also, an isotonic solution was used in the experiments, whereas ureteral urine in *V. gouldii* is always hypotonic (Green, 1969). Thus, resorption of water probably would be higher in normal circumstances due to the osmotic gradient between the fluid in the cloaca and the extra-cellular fluid.

Fig. 4. Changes in the concentration of potassium in the cloacal fluid of control and AVT-injected animals. Mean values are given ± S.E.


▲ A.V.T.
● Control
16
14
12
10
8
6
4
K^+, m-equiv/l
30
60
90
120
150
180
Time, min

In Chelonia, fluid is withdrawn from the urine mainly in the bladder (Rogers, 1966; Dantzler and Schmidt-Nielsen, 1966). Dantzler and Schmidt-Nielsen working with the desert tortoise Gopherus found that in $3\frac{1}{2}$ hr approximately 64 ml of fluid was transported out of a bladder containing a hypotonic solution. The animals used in this study weighed between 1.0 and 3.5 kg, which means that the rate of absorption of fluid was approximately 9 ml/kg per hr, which is similar to the rate of absorption in V. gouldii.

Cloacal function has been studied also in chickens. Hart and Essex (1942) found that fowls with exteriorized ureters lost weight dramatically due to the loss of ureteral urine, and that animals with a non-absorptive artificial anus died unless given supplementary salt (1 per cent) in the diet. The volume of urine collected from the exteriorized ureters was between 50 and 188 cm^3/day for birds with a mean weight of 2.3 kg. Presumably most of this fluid would have been resorbed in the cloaca in normal animals; thus, in these birds the rate of resorption from the cloaca was approximately 1 - 4 ml/kg per hr.

Nechay and Lutherer (1968) also worked with chickens. They cannulated one ureter and collected samples of ureteral and cloacal urine from the same animal. They found that urine flowed from the cloaca at a rate 19 per cent lower than from the cannulate

ureter. Taking into account their suggestion that the difference was due in part to a greater rate of urine flow in the cannulated ureter, it can be calculated that cloacal fluid was resorbed at a mean rate of about 6 ml/kg per hr. This is despite the fact that urine was only in contact with the cloaca for between 2 and 10 min. This rate is about the same as that of V. gouldii.

Skadhauge (1967) also studied resorption from the cloaca in chickens. He estimated that water was resorbed at about 1 ml/kg per hr, a much lower rate than that indicated by the results of Nechay et al. However, he worked with anaesthetized animals, and we have observed in our work that fluid is resorbed at much lower rates in anaesthetized goannas.

Skadhauge found that AVT had little effect on the rate of resorption, whereas it greatly enhanced the rate of resorption in V. gouldii. However, it is known that high levels of anti-diuretic hormone have a pronounced vasoconstrictor effect (Walker, 1967). It is possible that the exceptionally high doses used by Skadhauge, about 10^3 times the dose that we used, greatly reduced the blood-flow to the cloaca and hence decreased the rate of resorption of fluid.

On another occasion, Skadhauge (1968) measured cloacal resorption in conscious animals by comparing the composition of

ureteral and cloacal urine. In this study the animals were either water-loaded, salt-loaded or dehydrated. Under these conditions the kidney probably plays a more important role than the cloaca in regulating the final composition of the urine. Even so, in dehydrated animals 0.5 ml/kg per hr of water was resorbed against an osmotic concentration gradient. It is probable that the rate of resorption would be faster in animals that are not producing a concentrated urine or excreting a water load. It has been shown that sodium is actively transported out of the bladder or cloaca of a number of reptiles (Klahr and Bricker, 1964; Bentley and Schmidt-Nielsen, 1966: Junqueira et al., 1966; Schmidt-Nielsen and Skadhauge, 1967). In terrestrial reptiles resorption of water is associated with, and largely dependent on, sodium transport.

AVT greatly enhanced the rate of fluid absorption from the cloaca of V. gouldii by increasing both the rate of sodium absorption and probably the permeability of the cloaca to water. An increase in the permeability of the cloaca to water was indicated by the sodium concentration in the fluid of experimental animals after 1 hr being the same as the initial concentration, whereas in the control animals the sodium concentration was reduced. Thus, in the goannas injected with AVT the withdrawal of water did not lag behind the absorption of solute. This effect lasted for about 90 min after which the

sodium concentration in AVT-injected animals fell to the control level. Antidiuretic hormones are known to have a similar effect on the permeability to water and the transport of sodium in amphibian urinary bladders (Ewer, 1952; Sawyer and Schisgall, 1956; Leaf et al., 1958; Sawyer 1960). In contrast, Bentley (1962) working in vitro found that AVT did not increase the permeability to water or the rate of sodium transport in urinary bladders of the tortoise, Testudo graeca; however, there were increases in response to aldosterone. Therefore, it would be interesting to know how the cloaca and urinary bladder in other reptiles respond to these hormones.

Perhaps the most important physiological mechanism for water conservation in terrestrial reptiles is the excretion of nitrogen as insoluble urates and uric acid. This process involves the tubular secretion of soluble urates by the kidneys and the precipitation of insoluble urates and uric acid in the cloaca or bladder (Dantzler and Schmidt-Nielsen, 1966; Dantzler, 1967, 1968). This is probably achieved by withdrawal of fluid and also by acid conditions in the cloaca. The pH of the fluid in the cloaca of the goanna ranged from 5.2 to 5.8 (pK uric acid = 5.6).

It is surprising that so little attention has been paid to the cloaca in view of its importance in the water conservation of uricotelic reptiles and birds. The resorptive function of the cloaca has been frequently overlooked particularly in renal studies where urine has been collected from the cloaca. Clearly, care must be taken to obtain ureteral urine in renal studies on uricotelic animals.

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REFERENCES

BENTLEY, P.J. (1962) Studies on the permeability of the large intestine and urinary bladder of the tortoise (Testudo graeca) with special reference to the effects of neurohypophysical and adrenocortical hormones. Gen. & compar. Endocr. 2, 323 - 328.

BENTLEY, P.J. and SCHMIDT-NIELSEN, K. (1966) Permeability to water and sodium of the crocodilian, Caiman sclerops. J. cell. comp. Physiol. 66, 303 - 310.

DANTZLER, W.H. (1967) Stop-flow study of renal function in conscious water snakes (Natrix sipedon). Comp. Biochem. Physiol. 22, 131 - 140.

DANTZLER, W.H. (1968) Effect of metabolic alkalosis and acidosis on tubular urate secretion in water snakes. Am. J. Physiol. 215, 747 - 751.

DANTZLER, W.H. and SCHMIDT-NIELSEN, B. (1966) Excretion in fresh water turtle (Pseudemys scripta) and desert tortoise (Gopherus agassizii). Am. J. Physiol. 210, 198 - 210.

EWER, R.F. (1952) The effects of posterior pituitary extracts on water balance in Bufo carens and Xenopus laevis, together with some general considerations of anuran water economy. J. exp. Biol. 29, 429 - 439.

GREEN, B. (1969) Water and electrolyte balance in the sand goanna Varanus gouldii (Gray). Ph.D. Thesis, The University of Adelaide.

HART, W.M. and ESSEX, E.H. (1942) Water metabolism of the chicken (Gallus domesticus) with special reference to the role of the cloaca. Am. J. Physiol. 136, 657 - 668.

JUNQUEIRA, L.C.U., MALNIC, G. and MONGE, C. (1966) Reabsorptive function of the ophidian cloaca and large intestine. Physiol. Zool. 39, 151 - 159.

KLAHR, S. and BRICKER, N.S. (1964) Na transport by isolated turtle bladder during anaerobiosis and exposure to KCN. Am. J. Physiol. 206, 1333 - 1339.

LEAF, A., ANDERSON, J. and PAGE, L.B. (1958) Active sodium transport by the isolated toad bladder. J. gen. Physiol. 41, 657 - 668.

NECHAY, B.R. and LUTHERER, B.D.C. (1968) Handling of urine by cloaca and ureter in chickens. Comp. Biochem. Physiol. 26, 1099 - 1105.

ROBERTS, J.S. and SCHMIDT-NIELSEN, B. (1966) Renal ultrastructure and excretion of salt and water by three terrestrial lizards. Am. J. Physiol. 211, 476 - 486.

ROGERS, L.J. (1966) The nitrogen excretion of Chelodina longicollis under conditions of hydration and dehydration. Comp. Biochem. Physiol. 18, 249 - 260.

SAWYER, W.H. (1960) Increased water permeability of the bull-frog (Rana catesbiana) bladder in vitro in response to synthetic oxytocin and arginine vasotocin and to neuro-hypophyseal extracts from non-mammalian vertebrates. Endocrinology 66, 112 - 120.

SAWYER, W.H. and SHISGALL, R.M. (1956) Increased permeability of the frog bladder to water in response to dehydration and neurohypophyseal extracts. Am. J. Physiol. 187, 312 - 314.

SCHMIDT-NIELSEN, B. and SKADHAUGE, E. (1967) Function of the excretory system of the crocodile (Crocodylus acutus). Am. J. Physiol. 212, 973 - 980.

SESHADRI, C. (1956) Urinary excretion in the Indian house lizard Hemidactylus flaviviridis (Ruppel). J. zool. Soc. Ind. 8, 63 - 78.

SKADHAUGE, E. (1967) In vivo perfusion studies of the cloacal water and electrolyte resorption in the fowl (Gallus domesticus). Comp. Biochem. Physiol. 23, 483 - 501.

SKADHAUGE, E. (1968) The cloacal storage of urine in the rooster. Comp. Biochem. Physiol. 24, 7 - 18.

WALKER, J.M. (1967) Vasopressin. In Hormones in Blood (Edited by GRAY, C.H. and BACHARACH, A.L.), p. 167. Academic Press, London and New York.

WOODIN, A.M. (1963) The passage of proteins and particles across the surface of cells. In The Structure and Function of the Membranes and Surfaces of Cells (Edited by BELL, D.J. and GRANT, J.K.), pp. 126 - 141. Cambridge University Press, London.

Mitchell, F. J. (1973). Studies on the ecology of the Agamid Lizard Amphibolurus-maculosus (Mitchell). *Transactions of the Royal Society of South Australia, 97*, 47-76.