"WATER AND ELECTROLYTE BALANCE IN THE SAND GOANNA *Varanus gouldii* (Gray)"

by

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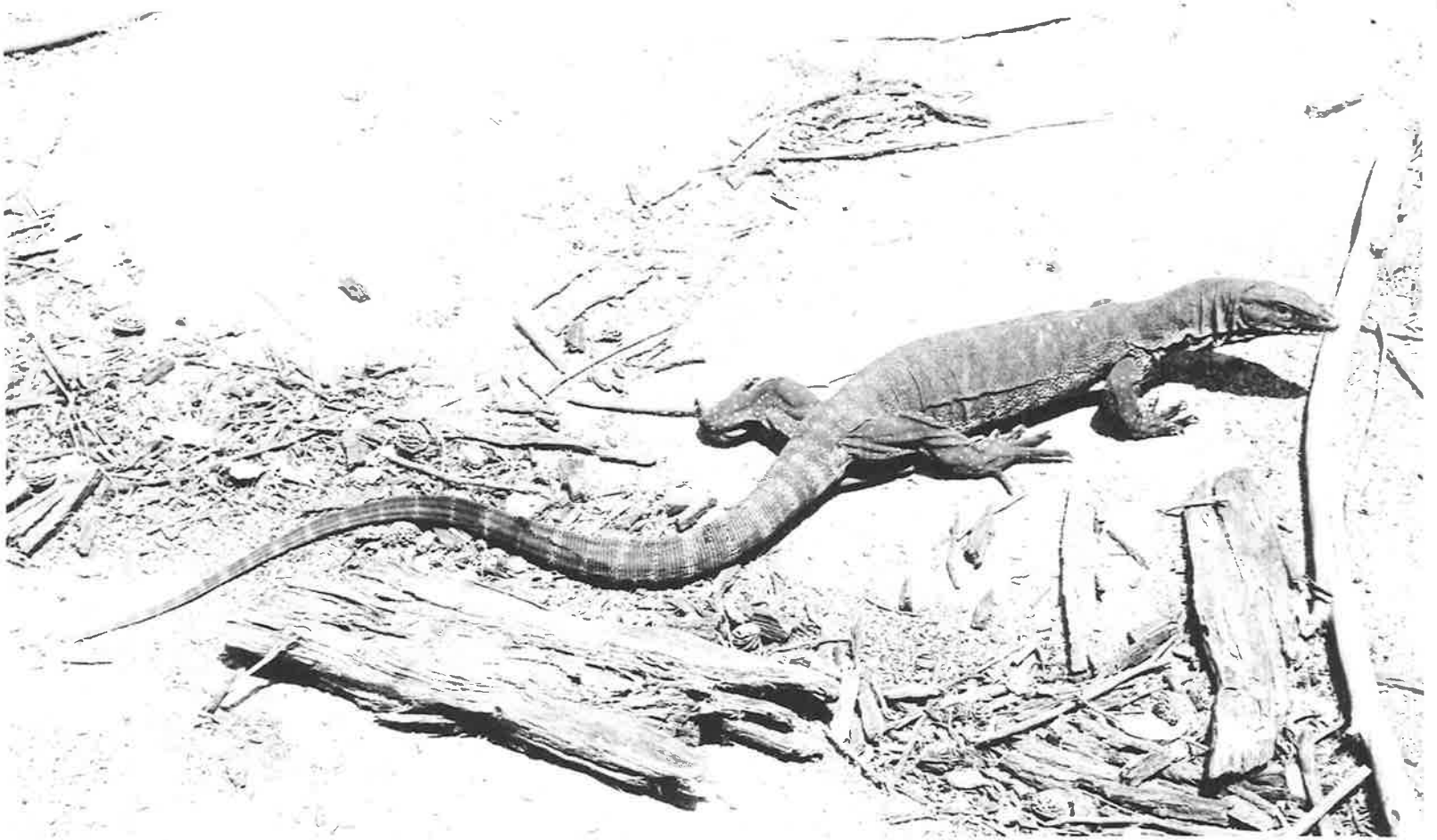

D E C L A R A T I O N

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

(1) A study was made of the physiological and behavioural mechanisms concerned with water and electrolyte balance in the lizard Varanus gouldii.

(2) Evaporative water losses were partitioned in the laboratory and the effects of temperature and size were observed. Approximately 70% of the pulmo-cutaneous evaporation is from the skin in inactive animals, over a wide range of temperatures. There is a significant amount of evaporation from the eyes, but little from the cloaca. Above 40°C panting commences and there is a five-fold increase in the rate of evaporation. Panting allows the dissipation of metabolic heat and to some extent depresses the body temperature below the ambient. Under basal conditions small animals lose relatively more water by evaporation than large animals of the same species. However, this relationship is not necessarily observed in comparing species where the adults are of a different size.

(3) Renal function in V. gouldii is typical of most reptiles in that the glomerular filtration rate (G.F.R.) is low compared with birds and mammals. The G.F.R. is increased by water loading and decreased by arginine vasotocin (AVT). The proportion of the filtered sodium that is resorbed remains constant over a wide range of G.F.Rs, which indicates that G.F.R. is determined by the number of functioning glomeruli. The renal tubules control the osmotic concentration of the urine to a greater extent than in many other reptiles. Over 80% of the filtered sodium is resorbed, but potassium is frequently secreted by the tubules.

(4) The liquid urine is modified in the cloaca by the precipitation of insoluble urates and uric acid. Most of the fluid is withdrawn from the urine, leaving a hard pellet which is then eliminated. Fluid is also resorbed from the faeces. The withdrawal of fluid takes place by the active transport of sodium from mucosa to serosa, and the osmotic diffusion of water. AVT increases the rate of resorption.

(5) Electrolytes are excreted from a nasal gland in V. gouldii. The secreted fluid emerges from the external nares and rapidly forms an encrustation on the snout. The ratio of sodium to potassium in the secretions of animals injected with a solution of sodium chloride ranged from 0.14 to 4.09.

(6) Comparative field studies were made on populations at a temperate and a semi-arid location. The microclimate of burrows was similar at both sites, despite differences in the climate. The diurnal behaviour was quite different in the two populations in Summer, those in the semi-arid region being abroad for much shorter periods. The concentration of electrolytes in the plasma remained low in both areas during Summer which suggests that the nasal glands were excreting electrolytes. Rates of water loss were measured in the field by using tritium, and a seasonal cycle in water loss was found. Animals in the semi-arid region were able to significantly reduce water losses in the Summer by remaining in the burrows for several days at a time. The low rates of water loss in free-living V. gouldii illustrate the efficiency of the mechanisms restricting water loss.

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GENERAL INTRODUCTION

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Reptiles are very conspicuous in hot deserts because they are mainly diurnal, whereas many other animals that live in deserts are nocturnal. Most desert reptiles are therefore exposed for at least part of the day to extremely hot and dry conditions, and this has stimulated research into the mechanisms that enable them to overcome the problems associated with living in arid environments.

Several comparative studies have been made in the laboratory on water loss in reptiles from areas of different aridity. However, most of these investigations have been confined to a particular aspect of water loss, either evaporation or excretion, and have generally been conducted on different species. These studies provide some insight into the physiological mechanisms involved in the regulation of water loss in desert reptiles, but provide only a composite description of overall water balance.

Electrolyte balance in terrestrial reptiles is interesting in that nasal salt glands are functional in some but do not appear to be so in others. When the glands are functional, their capacity to control electrolyte balance is still far from clear. Hypernatremia has been observed in some reptiles (Bentley 1959, Bradshaw 1965), but it is still not known how widespread this occurrence is in reptiles. Therefore, further study of electrolyte balance in terrestrial reptiles is warranted.

No integrated study on the water and electrolyte balance of a

single species of reptile has so far been accomplished, although the study of Amphibolurus spp. by Bradshaw (1965) closely approaches this. In the present study an attempt was made to conduct such an investigation into water and electrolyte balance in a terrestrial lizard, incorporating both laboratory and field studies.

The sand goanna, Varanus gouldii (Gray), is a large monitor lizard that may reach 120 cm. in length and 2 Kg. in weight. The species is characterised by a dark band that extends from the posterior margin of each eye along the sides of the head. Each band is bounded above and below by a light coloured streak. V. gouldii is the most common varanid species in Australia. It is a carnivorous lizard that feeds on a variety of arthropods and small vertebrates, and also carrion.

Mertens (1958) has described three subspecies which tend to occupy habitats of different aridity:

(1) V.g. flavirufus; from the arid zones of Central Australia. (animals used in this study were obtained at Yuendumu, N.T.)

(2) V.g. gouldii; from more coastal regions of the continent, in particular the semi-arid mallee areas. (experimental animals were obtained at Renmark, S.A.)

(3) V.g. rosenbergi; a melanistic form from the extreme S.W. area of W. Australia, and from Kangaroo

Island in S. Australia. This subspecies also occurs on the mainland in the S.E. of S. Australia, and may be generally regarded as inhabiting mesic environments. (animals were obtained from Kangaroo Island.)

The experimental animals used in this study were obtained from the localities shown in Fig. 1.

The wide diversity of habitats which this species occupies was one of the major factors that influenced its selection for study. Thus physiological, behavioural and environmental comparisons could be made to determine whether adaptations differ in animals from different habitats. The large size of the animal was also of considerable value, not only in some of the physiological techniques that were used, but also in the capacity for the animal to carry radio-transmitters for field studies.

In the laboratory, an attempt was made to partition water losses, and this led to the recognition of two major components; evaporative and non-evaporative losses. Evaporative water loss was analysed further according to the different sites from which evaporation occurs, and some of the major influences on evaporation were studied. Evaporative studies are described in Section 1. Non-evaporative water losses are associated with urinary excretion and defaecation, and so investigations were made into renal and

FIGURE 1

Location of areas from which animals were collected


Darwin
N.T.
Yuendumu
Alice Springs
S.A.
Renmark
Adelaide
Flinders Chase

cloacal function in Sections 2 and 3 respectively. Electrolyte excretion by the nasal salt glands was studied in Section 4 to a limited extent, but in sufficient detail to enable useful conclusions to be reached on its probable role in electrolyte balance.

In the field study, described in Section 5, a mesic and a semi-arid area were compared in a number of ways. The macro- and microclimate of each area was studied to determine any differences that might exist between the two habitats. Also, blood samples were taken at regular intervals in both field areas to see if there was much variation in the concentrations of sodium and potassium that might indicate electrolyte imbalance, especially during summer. The most important aspect of field work was the estimation of total water loss in animals under natural conditions. This was done on a seasonal and a locational basis, using tritium turnover techniques. Marked differences in the behaviour of animals at the two localities were observed, and were therefore included in the study.

Section 1

EVAPORATIVE WATER LOSS

INTRODUCTION

Total evaporative water loss has been measured in a number of desert lizards; *Dipsosaurus dorsalis* (Templeton 1960); *Uma notata*, *Phrynosoma solare* and *Dipsosaurus dorsalis* (Chew and Dammann 1961); *Crotaphytus collaris* (Dawson and Templeton 1963); *Tiliqua rugosa*, *Amphibolurus* spp. and *Varanus gouldii* (Warburg 1965a and 1965b); *Amphibolurus* spp. (Bradshaw 1965); *Amphibolurus ornatus*, *Gehyra variegata* (Dawson, Shoemaker and Licht 1966); *Uta stansburiana* (Claussen 1967 and Roberts 1968). Many of these workers have shown that lizards that normally live in arid and semi-arid habitats lose less water by evaporation than lizards from more moist environments when exposed to the same experimental conditions of temperature and humidity.

Measurements of total evaporative water loss have often been assumed to represent pulmonary water loss, as the reptilian skin was thought to be impermeable to water (Andrewartha and Birch 1954, Chew 1961). This view prevailed until recently, despite indirect evidence that suggested evaporation from the general body surface. For example, Benedict (1932) found that at 38°C skin and cloacal temperatures of snakes were as much as 2° or 3° below ambient. Benedict explained this as being due to vaporisation of water from the skin and lungs, but concluded that it was technically too difficult to measure the exact amount of water evaporated from the skin.

Several workers have recently measured evaporation from the skin of a number of different reptiles (Chew and Dammann, 1961; Tercafs, 1963; Bradshaw, 1965; Bentley and Schmidt-Nielsen, 1966a; Claussen, 1967; Dawson et al. 1966, Prange and Schmidt-Nielsen 1969). It was found that the amount of water evaporated from the skin was small in absolute terms if compared, for example, with the amount of water evaporated from the skin of Amphibia, but cutaneous evaporation was still found to represent a major portion of the total evaporative water loss.

Two major sites of evaporative water loss have thus been established; the skin, and the respiratory tract. The effects of temperature on evaporation from these regions was therefore studied in V. gouldii. Two other possible sources of evaporation are the cloaca and the eyes. Little or no information is available regarding water loss from these regions, and so experiments were done to determine the magnitude of evaporation from these areas in V. gouldii.

Previous workers have usually restricted the weight of experimental animals to a narrow range, however, in this study lizards of a wide range of sizes were used to investigate the effects of body weight on evaporative water loss.

Wherever possible, comparisons between V. gouldii and other lizards have been made to determine how well these animals are physiologically adapted to an arid environment.

MATERIALS AND METHODS

Animals were kept in three large terraria each measuring 6' x 18', and fed on mice and raw meat; water was available at all times. Animals were starved for at least two days before the experiments to ensure that they were in a post-absorptive condition, and only animals that were not moulting were used.

Preliminary observations indicated a diurnal rhythm in the activity of animals in the experimental chambers, and rates of water loss became stable only from late afternoon on to early morning. In this period water was evaporated at minimal rates. Therefore in all experiments the animal was placed in the chamber around midday, and left to settle down and equilibrate to the experimental temperature. Readings were taken from 7.30 p.m. onwards, by which time the animal was placid or lightly sleeping.

Technique for Large Animals

Pulmo-cutaneous Water Loss

In this series of experiments the animals were contained in different types of chamber.

In experiments 1 and 3 the chamber consisted of a 40 cm. long, 18 cm. diameter tin can which was sealed air-tight with a metal lid and rubber gasket. For smaller animals a similar chamber 25 cm. long and 10 cm. diameter was used. A raised floor of metal mesh was present in each chamber. The experimental chamber was placed in a constant temperature cabinet which maintained the temperature

to within 0.1°C.

In experiment 2 the experimental chamber (Fig. 2) consisted of an 80 cm. length of 15 cm. diameter P.V.C. tubing, which was permanently sealed at one end. The opposite end was open to allow an animal to be placed inside and the chamber was then sealed off by means of a rubber bung. The chamber had a raised floor of metal mesh and a perspex roof allowed observation of the animal. The chamber was immersed in a water bath thermostatically controlled to within 0.1°C.

An open air flow system was used for all large animals. Compressed air was led through drying columns containing anhydrous $CaCl_2$ and silica gel, and finally through a pair of 'U'-tubes containing magnesium perchlorate. By occasionally checking the weight changes of these 'U'-tubes, spent desiccant in the drying columns could be detected and subsequently replaced. From these tubes, dry air passed through a coil of metal tubing immersed in a water bath, where it was warmed to the temperature of the experiment before being led into the experimental chamber.

In all chambers dry air entered at one end and left at the opposite end. The air emerging from the experimental chamber was led to a second pair of weighed drying tubes containing magnesium perchlorate. At the end of each experimental period the tubes were reweighed to the nearest mg. on a Mettler H.16 balance. The increase in the weight of the tubes was due to evaporation from the

FIGURE 2

Apparatus for measuring evaporative water losses in large animals

A = Drying columns

B = Tubes of $MgClO_4$

C = Thermostatically controlled water bath

D = Metal tubing

E = Experimental Chamber (P.V.C.)

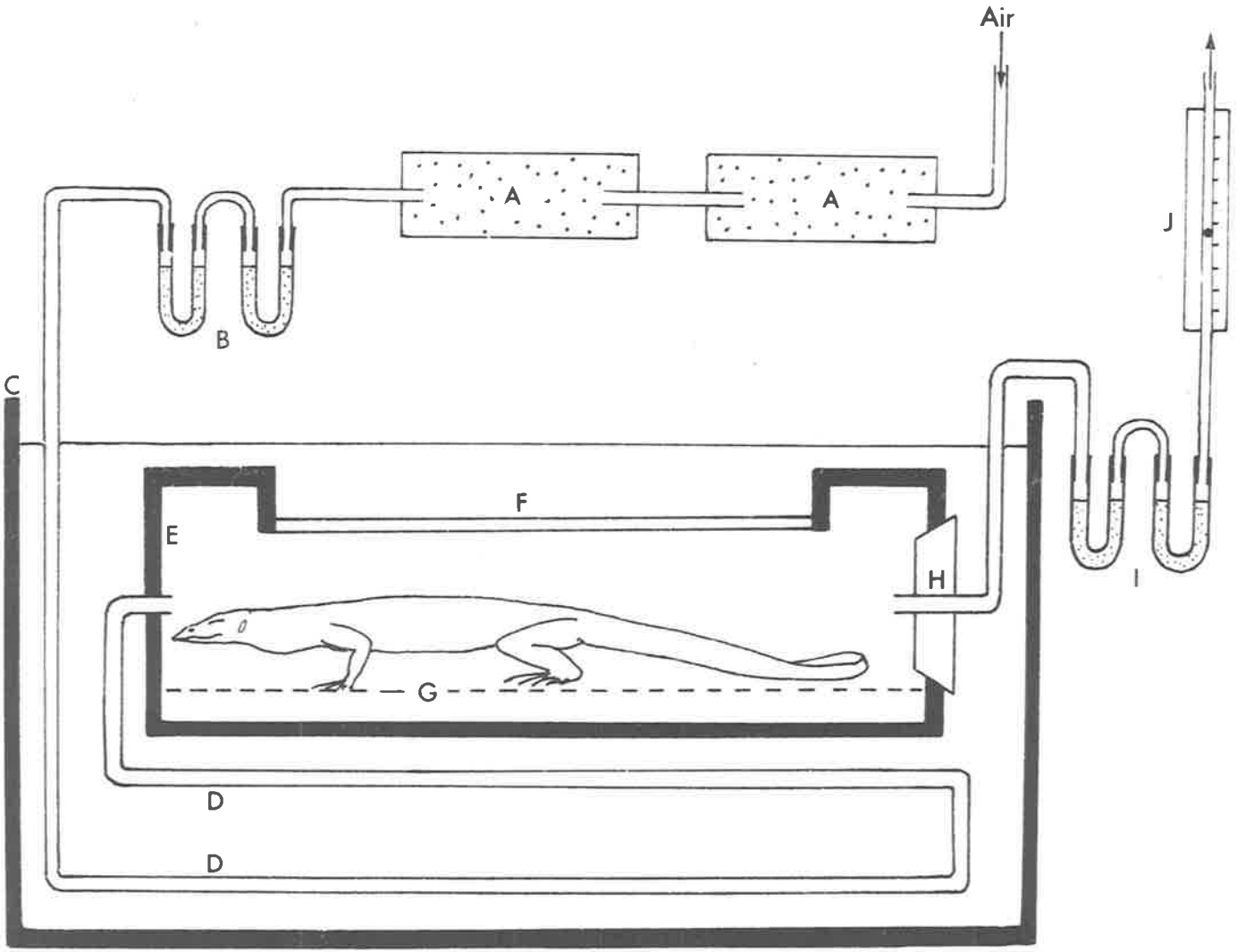
F = Viewing port

G = Metal mesh floor

H = Rubber bung

I = Weighed tubes of $MgClO_4$

J = Flowmeter


Air
A
A
J
B
C
F
E
H
I
G
D
D

animal. The average rate of water loss was calculated from four experimental periods and this mean value was used in all subsequent calculations. Experimental periods were usually 15 minutes long, but were extended to 30 minutes for smaller animals, and reduced to 6 minutes when animals panted.

Cutaneous Water Loss

Cutaneous water loss was measured in the metal chamber described for Experiment 3. The head and neck region were separate from the rest of the body by a rubber diaphragm that was secured at the base of the neck with rubber contact cement. The body was placed inside the chamber which was then sealed with a lid. The head of the animal passed through a central hole in the lid, and the rubber diaphragm also acted as a sealing gasket. With large animals the head was clamped between two plates to prevent tearing of the diaphragm or breaking of the cement seal, but this was unnecessary with smaller animals. The apparatus is shown in Fig. 3a.

Technique for Small Animals

Cutaneous and pulmonary water losses were measured simultaneously in animals weighing less than 50 g. Here, the body of the animal was placed in a glass tube, 3 cm. diameter and 15 cm. long, which was sealed at one end. Magnesium perchlorate desiccant was present beneath a wire mesh floor. The rubber diaphragm about the neck was secured over the open end of the tube by means of a strong elastic band. This whole unit which is shown in Fig. 3b, was then placed

FIGURE 3a

Apparatus for measuring cutaneous evaporation in large animals

A = Clamp for head

B = Rubber diaphragm

C = Metal lid with central hole

D = Experimental chamber (metal)

E = Metal mesh floor

FIGURE 3b

Apparatus for measuring evaporative water losses in small animals

A = Rubber bands

B = Rubber diaphragm

C = $MgClO_4$

D = Experimental chamber (glass)

E = Metal mesh floor

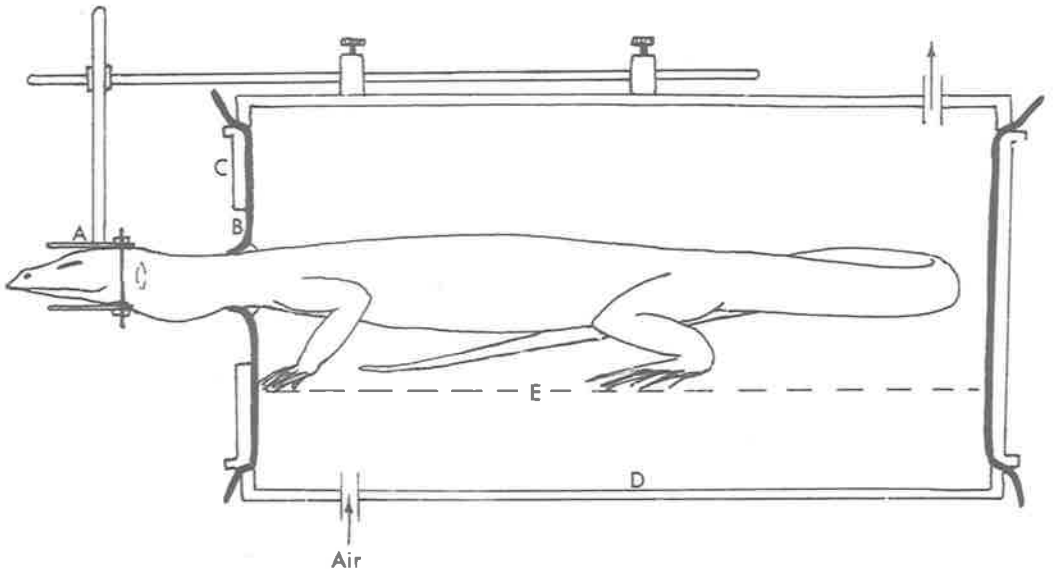


Fig 3a

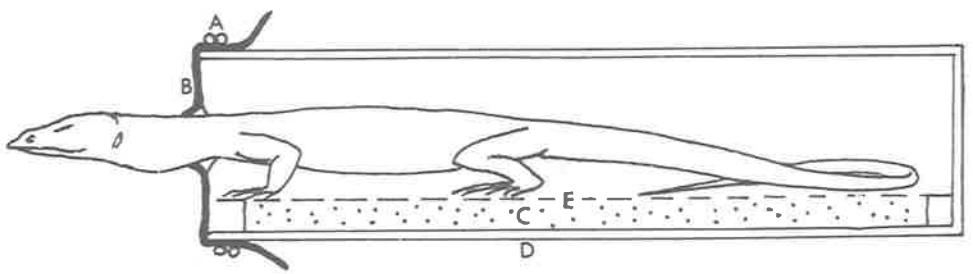


Fig 3b

in a larger chamber containing $CaCl_2$ desiccant, and this chamber was in turn housed in a constant temperature cabinet. Weight changes were measured for the complete unit (as shown in Fig. 3b), the animal plus diaphragm, and the glass tube, and these represented pulmonary, pulmo-cutaneous and cutaneous evaporative losses respectively. Experimental periods were usually of 6 or 12 hours.

In all evaporative water loss experiments body temperature was measured with a rapid response cloacal thermometer immediately after the animal was removed from the chamber. Body temperature could not be measured continuously as the animals remained agitated with a thermister permanently placed in the cloaca. Also, a film of cloacal fluid frequently escaped along the thermister leads despite the use of adhesive masking tape to seal off the cloaca. This would have lead to grossly inaccurate measurements of evaporative water loss.

If an animal defaecated during an experiment the data was rejected.

Surface Area of the Skin

The surface area of the skin was measured by using a silicone-rubber compound, 'Silastomer'. Each animal was anaesthetised by intra-peritoneal injection of Nembutal (20 mg/Kg), and suspended from a clamp by the tail tip. A thin coating of glycerine was applied to the skin with a brush, and excess was removed by blotting with tissue paper. A 20:1 mixture by weight of Silastomer K9161

and catalyst N9162 was then applied to the skin in a thin even coat. Within an hour the compound had set and could be peeled off after making a few cuts with scissors. The various sections of rubber film thus obtained were laid out flat on paper, darts being cut where necessary to achieve a flat surface.

An outline was then drawn around each section of rubber film, and the area enclosed by each outline was measured with a Coradi Planimeter. Surface areas of the head/neck region and body were obtained by summing the component areas. A check on the technique was made against isolated skins and also geometric shapes, and these tests demonstrated that the silicone-rubber was completely elastic.

CLOACAL EVAPORATION

Experiment 1

To Determine the Magnitude of Evaporation from the Cloacal Region

This experiment was conducted at 30°C on four specimens of V. g. rosenbergi. The chamber used for measuring cutaneous water loss in large animals was employed, thus reducing the variability in evaporative water loss associated with respiration. Evaporative water loss from the body posterior to the base of the neck was measured in each animal, the cloaca being covered with adhesive tape. After four experimental readings, the tape was removed and the rate of evaporation measured for a further four periods. The data obtained in this experiment are presented in Table 1. No statistical analysis of the data was attempted as it was quite clear that a negligible amount of water was lost by evaporation from the cloaca.

TABLE 1

Rate of Evaporation with the Cloaca Masked and Unmasked

Animal	Masked (mg/hr)	Unmasked (mg/hr)	Difference (mg/hr)
1	101	101	0
2	119	120	+1
3	173	172	-1
4	170	175	+5

Claussen (1967) found that 47.2% of the total evaporative losses in Anolis carolinensis were non-respiratory if the cloaca was covered,

but this value increased slightly to 48.2% if the cloaca was uncovered. The latter value included losses due to defaecation, urination and cloacal evaporation, but no attempt was made to separate quantitatively these three possible components. The results obtained for V. gouldii would suggest that it is quite likely that urination, defaecation and increased activity during this elimination, could account for the small discrepancy found by Claussen between taped and untaped cloacae. Thus, the suggestion by Maderson (1965) that evaporation of water from the cloacal walls is important has not been confirmed for inactive V. gouldii. When agitated, V. gouldii may be frequently observed with parts of the cloaca evaginated, and in males the hemipenes become erect. On these occasions there will obviously be evaporation of water from the exposed moist mucosal surfaces of these structures, as there would be during faecal and urinary elimination. However these occasions would be quite infrequent and may be considered as unimportant in the water balance of the animal. In all subsequent evaporative experiments no attempt was made to cover the cloaca, and the small error in measurements due to evaporation from this region was disregarded.

PULMO-CUTANEOUS EVAPORATION

Experiment 2

The Effect of Temperature on Total and Pulmo-cutaneous Evaporative Water Loss

Pulmo-cutaneous evaporative water loss was measured in six specimens of *V. g. rosenbergi* with a mean weight of 956 g. (range: 813 - 1060 g). Measurements were made at ambient temperatures of 26°, 30°, 34°, 38°, 40° and 42.5°C. Dry air was passed through the experimental chamber at a rate of 700 cc/min. between 26° and 34°C, but the airflow rate was increased to 1200 cc/min. at 38°C and above to maintain a low humidity in the experimental chamber, despite increased evaporation at these higher temperatures.

The animals could be observed at all times by means of the viewing port in the chamber. Between 26° and 38°C all animals were inactive with the eyes closed, so the data obtained in this part of the temperature range represent basal levels of pulmo-cutaneous evaporation. Measurements at 40° and 42.5°C were made only when animals were still, although the periods of inactivity were of fairly short duration. At both of these higher temperatures the eyes were open almost continuously in every animal and these values therefore represent total evaporative water loss.

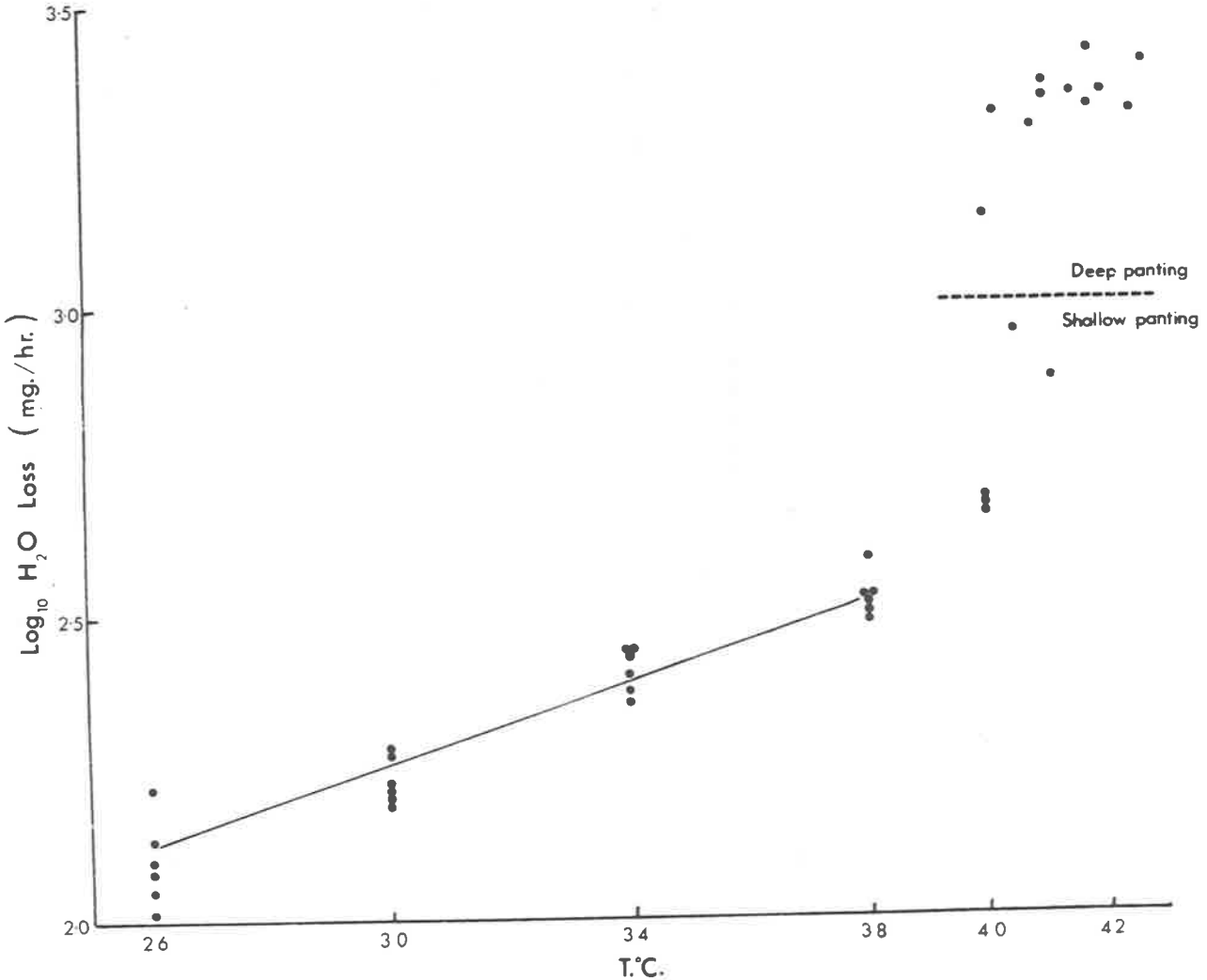
The results of this experiment are displayed in Figure 4, where it is immediately obvious that panting at 40° and 42.5°C resulted in a dramatic increase in the rate of evaporation. It is probably better therefore to consider the results obtained between 26° and 38°C (i.e. prior to panting), separately from those obtained

FIGURE 4

Effect of temperature on pulmo-cutaneous and total evaporation

$$\log_{10} \text{pulmo-cutaneous } H_2O \text{ loss (mg/hr)} = 1.1684 + 0.0358T^{\circ}C$$

$$(S.E.b = \pm 0.0022)$$


3·5
3·0
2·5
2·0
Log10 H2O Loss (mg./hr.)
Deep panting
Shallow panting
26
30
34
38
40
42
T.°C.

during panting. When this is done a regression line may be drawn to fit the data between 26° and 38°C. The slope of this line is described by the equation:

$$\log_{10} \text{Pulmo-cutaneous } H_2O \text{ loss (mg/hr)} = 1.1684 + 0.0358\ T^{\circ}C$$
$$(\text{S.E.b} = \pm 0.0022)$$

which is equivalent to a Q_{10} of 2.35. The regression coefficient was found to be significantly different from zero ($p < 0.001$).

Thus, increase in ambient and body temperatures caused an increase in the amount of water evaporated by the animal. This is due to increased respiratory activity, and also due to the increase in saturation deficit in the chamber associated with the rise in ambient temperature. The effect of temperature on water loss through the skin will be described later.

Panting

When panting V. gouldii holds the mouth open and rapidly flutters the pharyngeal floor by means of the hyoid cartilages and associated musculature. Two panting intensities were observed in V. gouldii.

shallow panting, where the mouth was held only slightly open, with a panting rate between 80 and 88 buccal movements per minute;

deep panting, where the mouth was held wide open with a panting rate in excess of 126 movements per minute.

Transition from shallow to deep panting was quite rapid, and no information could be collected during this transition period.

At 40°C two animals showed occasional shallow panting (panting rates of 81 and 83 per minute), three exhibited continuous deep panting (panting rates of 126, 132 and 152 per minute), and one animal did not pant at all. At 42.5°C, however all animals showed continuous deep panting (panting rates of 137, 159, 173, 184 and 217 per minute) except for one animal which showed continuous shallow panting at the rate of 88 per minute. This latter animal was that which did not pant at 40°C. It thus appears that panting in V. gouldii starts at approximately 40°C, and increases in intensity with increased body temperature. To help clarify the effect of panting on evaporation rate an extra four recordings of evaporation rate, panting rate and body temperature were made, and included in the data displayed in Figure 4. Of these, one animal showed continuous shallow panting at 80 per minute, and the other three continuous deep panting at 132, 148 and 148 per minute.

The evaporation rate increased to such an extent with panting, that the calculated relative humidity in the chamber, assuming complete mixing of the air, increased from below 20% to 50%. Even so, if the regression line for the data between 26° and 38°C is extended to cover the 40° to 42.5°C temperature range, it can be seen that deep panting caused the evaporation rate to rise by at least 4 to 6 times the level expected if no panting was taking place. The effect of shallow panting on evaporation is obscured to some extent since only two animals exhibited continuous shallow panting, but these two

animals showed that shallow panting increased the rate of evaporation to approximately double the non-panting rate.

A close correlation between panting and evaporation rate is therefore apparent. It will be shown later that water is evaporated from the eyes, and therefore a small part of the elevation in water loss at 40° and 52.5°C was due to the eyes being open (< 5% of the total).

The results for V. gouldii agree quite well with those described for Crotaphytus collaris by Dawson and Templeton (1963). These workers found a Q_{10} of 1.8 for total evaporation between 32° and 40°C, and also reported that between 40° and 44°C there was a substantial increase in evaporation, the Q_{10} here being about 10. It is interesting to note that while panting accounted for the major part of this increase in Crotaphytus, the increase commenced about 2°C below the body temperature at which panting occurs in this species. Dawson and Templeton offered no explanation for this, but an attempt will be made later to account for this observation.

Warburg (1965a and b) found for a number of Australian lizards that evaporation increased with temperature. His results indicated that between 37.5° and 40°C there was a substantial increase in evaporation under dry conditions, but he provided little information about the species that panted at temperatures above 40°C. Warburg's results are difficult to interpret as several animals apparently lost more evaporative water at high humidity (95 - 100% RH) than at low

humidity (0 - 5% RH). No explanation was given for this.

The lizard Dipsosaurus dorsalis differs from V. gouldii and C. collaris in that Templeton (1960) found no abrupt increase in evaporation with the onset of panting at 44°C.

Panting is generally regarded as a thermoregulatory mechanism, and this has been demonstrated experimentally by Templeton (1960) in Dipsosaurus dorsalis. Templeton found that the body temperatures of panting animals at 46°C in dry air were within 0.4°C of the ambient temperature, whereas at the same temperature in humid air body temperatures were as high as 1.0°C above ambient. Templeton concluded that panting did not serve to lower the body temperature below that of the environment, but served only to dissipate metabolic heat. Dawson and Templeton (1963) however have shown that panting in Crotaphytus collaris allows dissipation of 1.34 times the calories resulting from metabolism at 44°C.

The cloacal temperatures recorded from V. gouldii at 40° and 42.5°C are shown in Table 2. At 40°C all animals had cloacal temperatures equal to, or slightly above, the ambient, but at 42.5°C five animals had cloacal temperatures equal to, or below, the ambient. These results indicate that it is possible for V. gouldii to not only dissipate metabolic heat by panting, but also to lower the body temperature below ambient temperature.

While panting may be a useful short term method of thermoregulation at high temperatures, it is probably rarely used under

natural conditions. This is because there are usually sufficient areas of shade and also burrows available to enable the animal to avoid and also dissipate heat. Indeed, behavioural thermoregulation is so efficient that it is probably unusual for V. gouldii to ever reach body temperatures at which panting commences. Certainly panting was never observed in the field even on the hottest days. There can be no doubt, however, as to the value of this thermo-regulatory mechanism. It would allow an animal exposed to extremely hot conditions an extended period in which to find a less exacting micro-climate.

TABLE 2

Cloacal and Ambient Temperatures

Ambient Temp.	40.0°C	42.5°C
	40.2	41.8
	40.1	41.1
	40.8	42.5
Cloacal Temp.	40.3	41.4
	40.0	41.8
	40.0	42.7

The Effects of Body Weight on Evaporative Water Loss

It has been suggested by Schmidt-Nielsen (1964) that body size influences evaporative water loss in reptiles in two ways. One way is due to the difference in the metabolic rate of small and large animals, which has been clearly demonstrated for reptiles by Dawson and Bartholomew (1965) and more specifically for varanid lizards by Bartholomew and Tucker (1964).

The other effect of size is due to the well established fact that small animals have a greater surface area to volume ratio than larger animals. It therefore follows that while small animals may show a cutaneous evaporation rate the same as that of adults, they lose a relatively greater amount of water from the skin. However, the magnitude of these effects on the evaporative water loss of reptiles is not known apart from the work of Gans et al. (1968).

An extensive weight range is found in V. gouldii, hatchlings weighing approximately 15 g. while large adults may reach 2 Kg. This species is therefore eminently suitable for investigations into the intraspecific effects of size on evaporative water loss. An even wider weight range exists between the different species of Varanus found in Australia, where there are several species of pygmy monitor that weigh less than 50 g. as adults, as well as V. giganteus which may exceed 10 Kg. in weight. Consequently the interspecific effect of size on evaporative water loss in adults may also be investigated using members of the genus Varanus.

There is a substantial amount of data that indicates the presence of physiological adaptations to reduce evaporative water loss in desert reptiles. This can be seen by comparing genera from xeric and mesic habitats (Dawson et al. 1966; Bentley and Schmidt-Nielsen 1966a; Claussen 1967; Gans et al. 1968; Krakauer 1968) or comparing species within a genus (Warburg 1965b; Bradshaw 1965; Sexton and Heatwole 1968), but there is no information of this type at the subspecific level.

It was decided therefore to conduct an experiment to determine if any differences exist in the rates of pulmo-cutaneous water loss in V. gouldii from mesic, semi-arid and arid habitats, and at the same time test the hypothesis proposed by Schmidt-Nielsen.

Experiment 3

The effect of body weight on the pulmo-cutaneous water loss of three subspecies of V. gouldii

This experiment was conducted at 30°C, in the apparatus previously described. Pulmo-cutaneous evaporation was measured from animals of different weight in each of the three subspecies. Air-flow rates of 250 cc/min. and 750 cc/min. were used, depending on the size of the chamber. These air-flow rates provided a calculated rate of air movement through a chamber of approximately 3 cm/min., based on the cross-sectional area of the chamber. A regression line was calculated for each subspecies and these take the form:

V. g. rosenbergi

$$\log_{10} H_2O \text{ loss (mg/hr)} = 0.0264 + 0.7724 \log_{10} \text{wt. (g)}$$
$$(S.E.b = \pm 0.0679)$$

V. g. gouldii

$$\log_{10} H_2O \text{ loss (mg/hr)} = -0.0465 + 0.8193 \log_{10} \text{wt. (g)}$$
$$(S.E.b = \pm 0.0606)$$

V. g. flavirufus

$$\log_{10} H_2O \text{ loss (mg/hr)} = 0.1374 + 0.7074 \log_{10} \text{wt. (g)}$$
$$(S.E.b = \pm 0.0790)$$

Each of these regression lines was significantly different from zero ($p < 0.001$ in every case). The three regressions were then compared by means of an Analysis of Covariance which is set out in Table 3. The variance ratio had a value which was not significant ($p > 0.2$) indicating that the three samples came from a homogeneous population.

Thus no difference could be shown between the subspecies in the effect of body weight on pulmo-cutaneous evaporative water loss, and also no difference was apparent between subspecies regarding the water loss of animals of similar weight at 30°C. The regression for the pooled data takes the form:

$$\log_{10} H_2O \text{ loss (mg/hr)} = -0.0013 + 0.7797 \log_{10} \text{wt. (g)}$$
$$(S.E.b = \pm 0.0281)$$

and is shown in Fig. 5.

FIGURE 5

The effect of body weight on pulmo-cutaneous evaporation in three subspecies of V. gouldii

$$\log_{10} H_2O \text{ loss (mg/hr)} = -0.0013 + 0.7797 \log_{10} \text{wt. (g)}$$

$$(\text{S.E.b} = \pm 0.0281)$$

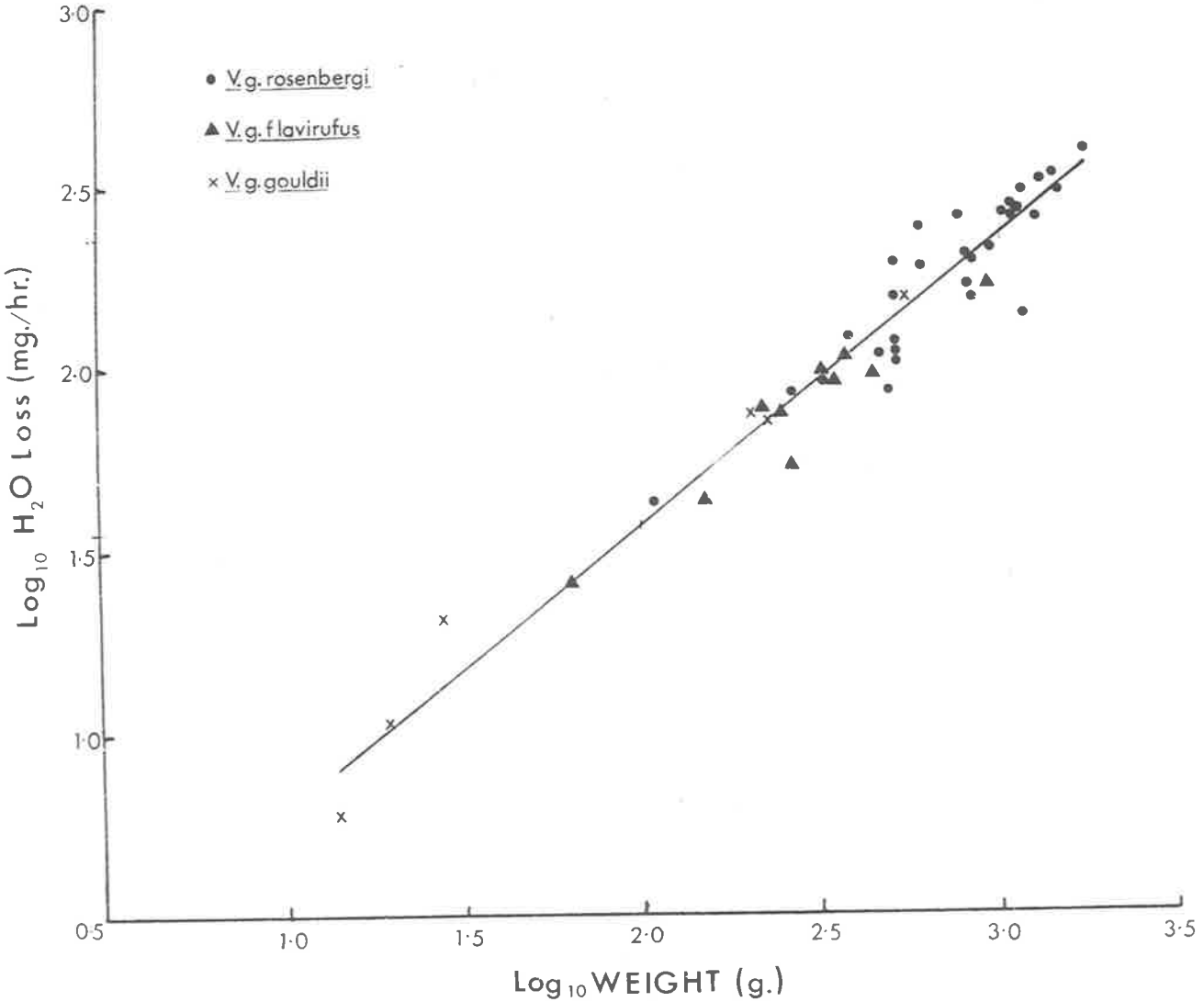

3·0
2·5
2·0
1·5
1·0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
• V. g. rosenbergi
▲ V. g. flavirufus
× V. g. gouldii
Log10 H2O Loss (mg./hr.)
Log10 WEIGHT (g.)

TABLE 3

Analysis of Covariance: Effect of Weight on Pulmo-cutaneous Water Loss in Three Subspecies of V. gouldii

Source	SS.	d.f.	Variance	V.R.	p
Between Groups	0.028884	4	0.007221	0.87	> 0.2
Within Groups	0.333084	40	0.008327		
Total	0.361968	44			

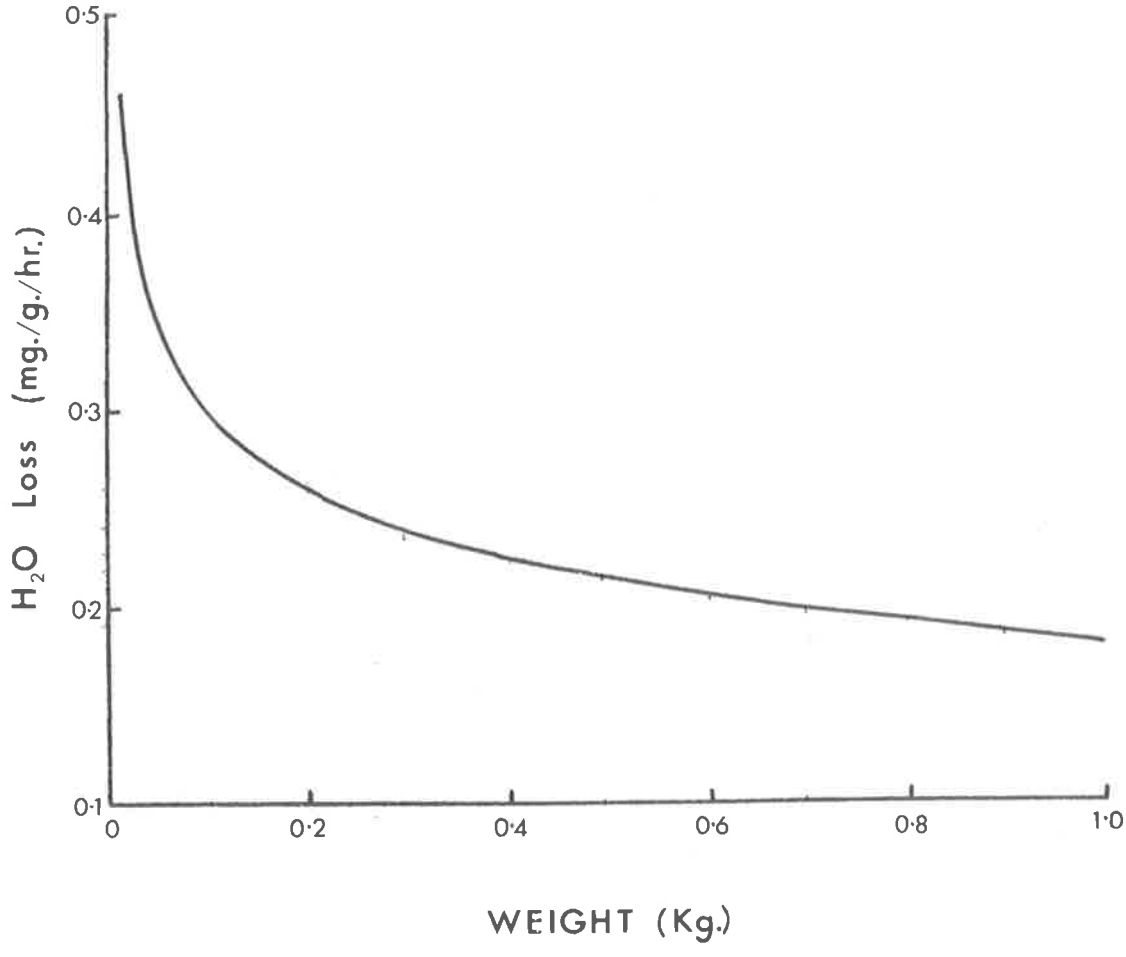
The effect of size on pulmo-cutaneous evaporation is seen more clearly if the loss is expressed in mg.H_2O/g. Body wt/hr., and plotted against body weight, as has been done for the pooled regression in Figure 6. A juvenile of 15 g. loses evaporative water at nearly three times the rate of a 1000 g. adult, thus the predictions of Schmidt-Nielsen regarding the effects of size on water loss are confirmed for V. gouldii.

Warburg (1965a) attempted to demonstrate the inverse relationship of water loss and body weight in Tiliqua rugosa. However, he provided no regression analysis for his data, and the variability in his data suggests that the line drawn in Figure 13 of his paper is not legitimate and also unlikely to be significantly different from a line of zero slope.

The final conclusion that can be drawn from Experiment 3 is that, since no differences exist in the rates of evaporative water loss in

FIGURE 6

Effect of body weight on pulmo-cutaneous evaporation (mg/g/hr) in V. gouldii


0·5
0·4
0·3
0·2
0·1
H2O Loss (mg./g./hr.)
0
0·2
0·4
0·6
0·8
1·0
WEIGHT (Kg.)

animals from mesic, semi-arid and arid environments, it is quite possible that behavioural differences are more important between sub-species in the regulation of evaporative water loss. It will be shown later that this is the case.

Warburg (1965a) measured evaporative water loss in a group of V. gouldii with a mean weight of 80 g. The mean rate of evaporation at 30°C in a dry environment was equal to 0.43 mg/g/hr, a higher rate than that found in this study for animals of similar weight (0.32 mg/g/hr). The discrepancy is probably due to the different techniques that were used in the two studies.

Experiment 4

The effect of body weight on evaporative water loss in Varanus acanthurus brachyurus

This experiment was done to see if the body weight/water loss relationship found in V. gouldii applied to the genus Varanus as a whole, or was characteristic for the species only.

The experiment was conducted at 30°C with nine specimens of this pygmy species that ranged from 2.7 g. - 62 g. in weight. Pulmonary and cutaneous water losses were measured simultaneously by means of the small animal technique in four animals and pulmo-cutaneous loss in another four. Evaporative losses were measured by the large animal technique in the biggest animal.

The results for pulmo-cutaneous evaporative water loss were subjected to regression analysis, the regression equation taking

the form:

$$\log_{10} H_2O \text{ loss (mg/hr)} = 0.4546 + 0.4164 \log_{10} \text{wt. (g)}$$
$$(\text{S.E.b} = \pm 0.0709)$$

The regression coefficient was significantly different from zero ($p < 0.001$). When the water loss was expressed in mg/g/hr and plotted against body weight it was seen that the smallest animal lost water at a rate approximately 5 times that of the largest specimen (Fig. 7).

The regressions of water loss on body weight for V. gouldii and V. a. brachyurus were compared using a 'Students'-t test that is set out in Table 4. There was a significant difference between the slopes of the two regressions shown in Fig. 8 ($p < 0.001$).

TABLE 4

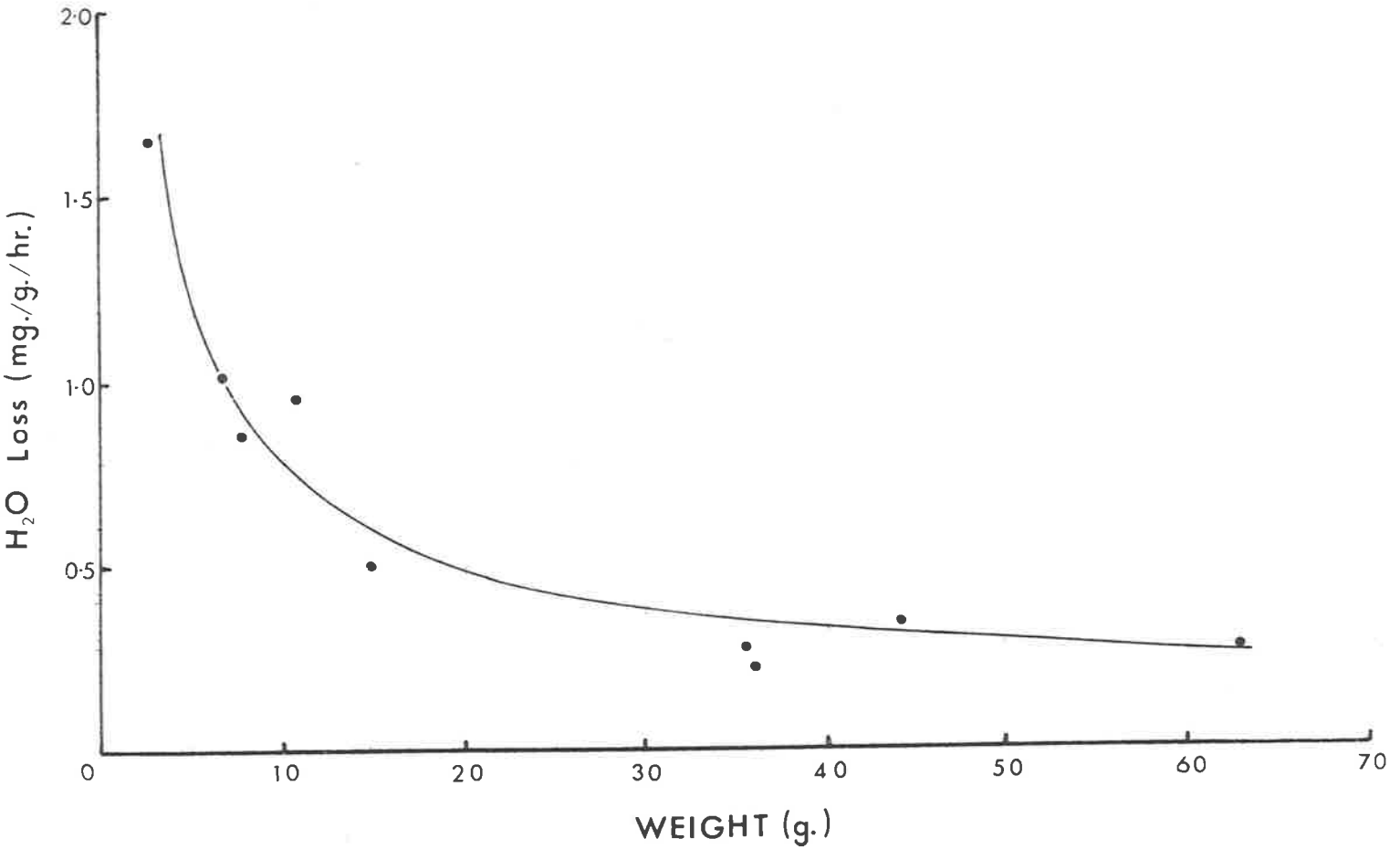
Comparison of body weight/water loss regressions in two species of Varanus

Species	n	b	Var.	V.R.	t_{53}	p
V. gouldii	46	0.779728	0.007753	1.07	4.66	< 0.001
V. a. brachyurus	9	0.416391	0.007215			

Reference to Fig. 8 shows that increased body weight does not affect the absolute rate of evaporative water loss in V. a. brachyurus as much as it does in V. gouldii. Also, where the weight range for each species overlaps (i.e. 20 - 60 g.) the two lines cross, small

FIGURE 7

Effect of body weight on pulmo-cutaneous evaporation in V. acanthurus brachyurus


2·0
1·5
1·0
0·5
0
10
20
30
40
50
60
70
H2O Loss (mg./g./hr.)
WEIGHT (g.)

specimens of V. a. brachyurus losing more water than V. gouldii of the same weight, whereas large V. a brachyurus lose less water than V. gouldii of the same weight. If only adults are compared the relative rate of evaporation is approximately 0.20 mg/g/hr in both species.

These experiments show that within a species the rate of water loss, expressed as mg/g/hr, is higher in small animals, but this is not necessarily true when adults of different sized species are compared. These findings agree with those obtained by Krakauer et al. (1968) for several snakes and amphisbaenians. These workers compared water loss in several species that differed in size, and found that the marked differences in water loss were not dependent on size or classification, but were correlated with the aridity of the natural environment. However, Gans et al. (1968) found an intraspecific effect of weight on evaporative water loss in the snake Elaphe climacophora.

It is therefore clear that physiological adaptations to the environment are responsible for so much variation in the rate of evaporative water loss in reptiles, that intraspecific comparisons of rates of water loss will not necessarily show a relationship to body weight. However, the intraspecific effect of weight has been clearly established for E. climacophora, V. gouldii and V. a. brachyurus.

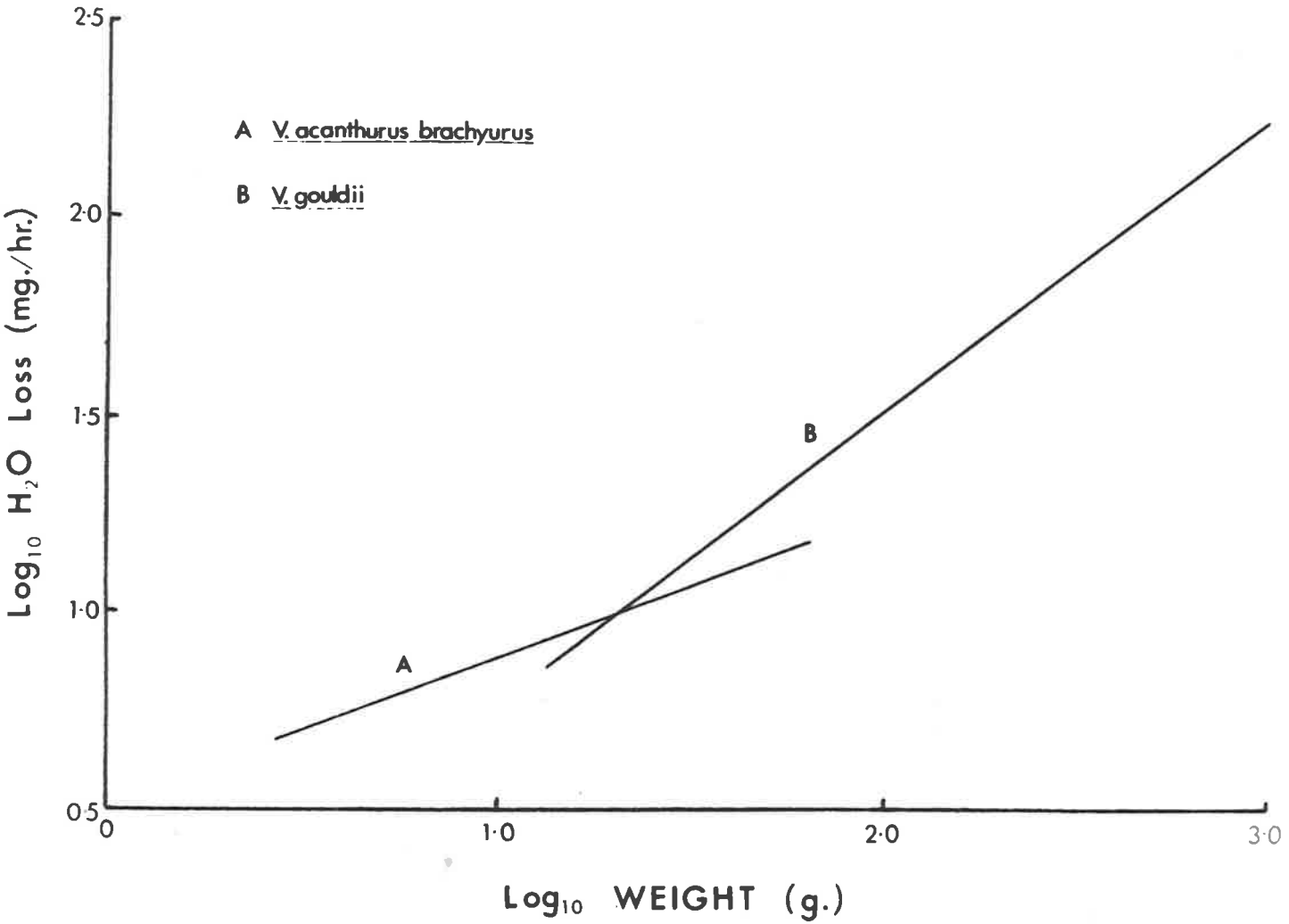
FIGURE 8

Comparison of regression lines for

V. gouldii and V. acanthurus brachyurus

(A) $\log_{10}$ H_2O loss (mg/hr) = 0.4546 + 0.4164 $\log_{10}$ wt. (g)

(B) $\log_{10}$ H_2O loss (mg/hr) = -0.0013 + 0.7797 $\log_{10}$ wt. (g)


A V. acanthurus brachyurus
B V. gouldii
Log10 H2O Loss (mg./hr.)
2·5
2·0
1·5
1·0
0 5
0
1·0
2·0
3·0
Log10 WEIGHT (g.)
A
B

CUTANEOUS EVAPORATION

A necessary prerequisite of any measurement of cutaneous water loss is to know the surface area from which water has evaporated, as well as the total surface area.

The Surface Area to Body Weight Relationship

Total surface area and body weight were measured in 30 animals by means of the silicone rubber technique. The only part of the integument not measured was that of the digits, as it was difficult to remove the rubber from these portions and measure them accurately.

The data were subjected to regression analysis and the following relationship was found.

$$\log_{10} \text{total surface area } (cm^2) = 1.0696 + 0.6673 \log_{10} \text{wt. } (g)$$
$$(S.E.b = \pm 0.0150)$$

The regression, which is shown in Fig. 9 clearly indicates that small animals have a relatively larger surface area than bigger animals.

A general surface area formula for lizards has been suggested by Benedict (1932) and this takes the form:

$$S\ (cm^2) = 10 \times W\ (g)^{0.67}$$

Expressed in this way, $S = 12 \times W^{0.67}$ for V. gouldii. Claussen (1967) measured surface area in two species of lizards and obtained the following relationships:

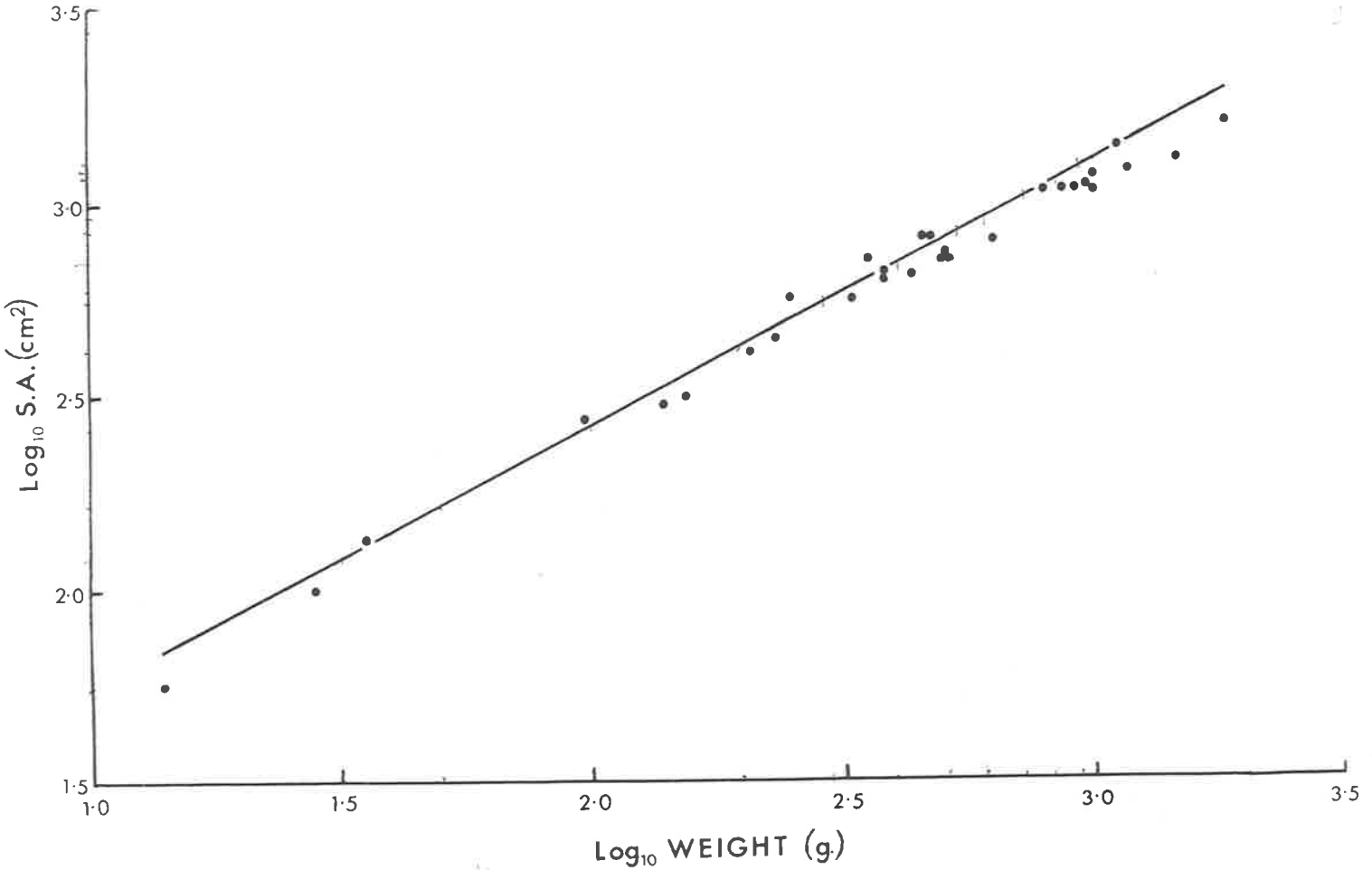
Anolis carolinensis	$S = 11 \times W^{0.83}$
Uta stansburiana	$S = 16 \times W^{0.47}$

FIGURE 9

The relationship of surface area to body weight

$$\log_{10} \text{surface area } (cm^2) = 1.0696 + 0.6673 \log_{10} \text{wt. } (g)$$

$$(\text{S.E.b} = \pm 0.0150)$$


3·5
3·0
2·5
2·0
1·5
Log10 S.A. (cm2)
1·0
1·5
2·0
2·5
3·0
3·5
Log10 WEIGHT (g.)

It can be seen therefore, that there is a great deal of variation in the surface area/body weight relationships of lizards.

Experiment 5

The effect of temperature on cutaneous evaporation

Specimens of V. g. rosenbergi were used in this experiment, which was conducted at 30°, 35°, 38° and 40°C. A stream of dry air flowing at a rate of 500 cc/min. passed through the chamber, shown in Fig. 3a, and weighed drying tubes were connected downstream to the chamber for periods of 15 minutes. The surface area of skin covering the part of the body within the chamber was measured with silicone-rubber.

The results are described by the following regression equation:

$$\text{Cutaneous } H_2O \text{ loss } (mg/cm^2/hr) = -0.3645 + 0.0159T^{\circ}C$$

$$(S.E.b = \pm 0.0022)$$

The regression is significantly different from zero ($p < 0.001$), and is shown in Fig. 10.

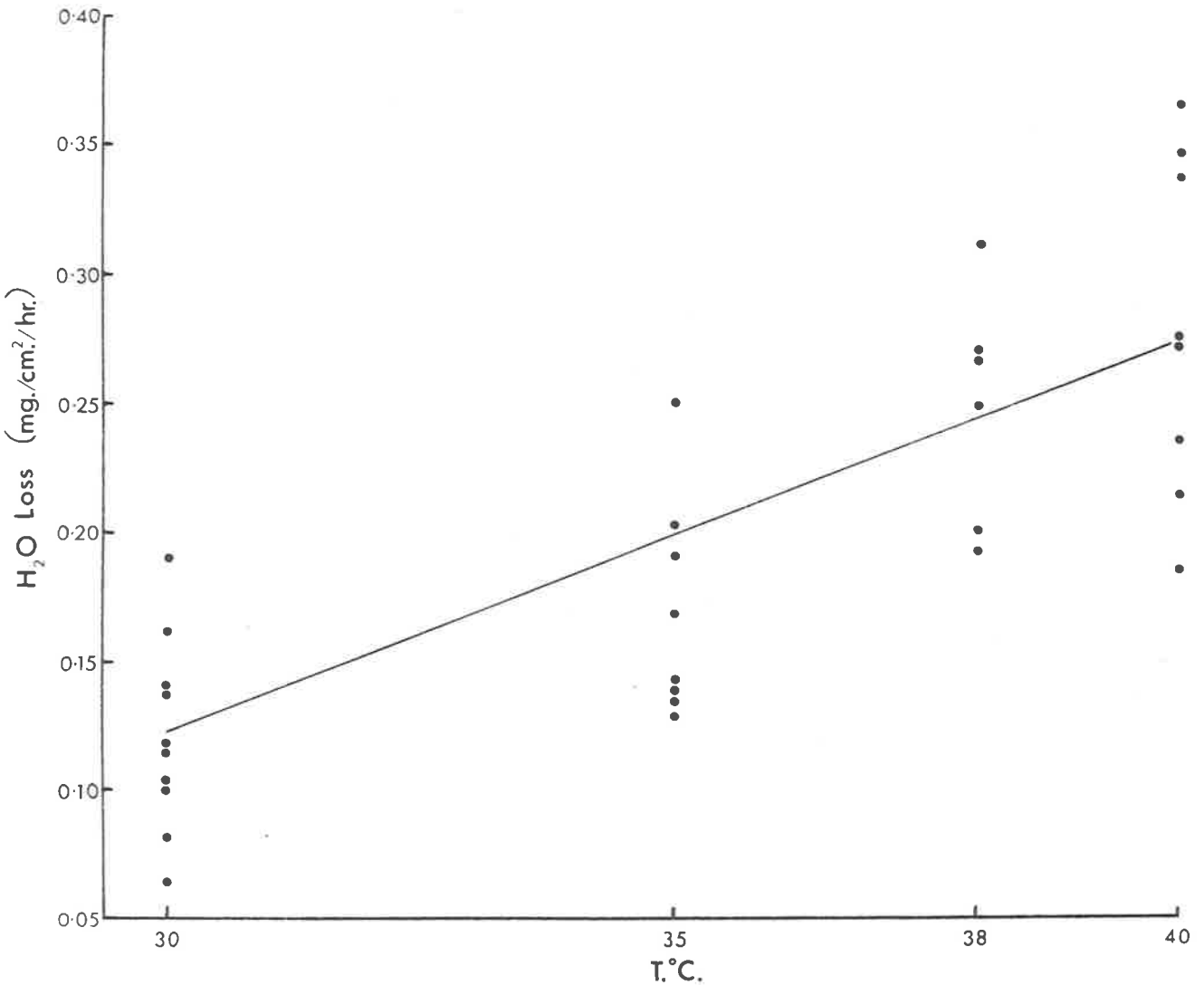
The Q_{10} value for cutaneous evaporation between 30° and 40°C is 2.41 for V. gouldii, which is similar to the high Q_{10} values obtained by other workers. Bentley and Schmidt-Nielsen (1966a) found a Q_{10} of 2.8 for cutaneous evaporation in the lizard Sauromalus obesus between 23° and 40°C, and Gans et al. (1968) found a similarly high Q_{10} of 2.2 for cutaneous water loss in the snake Lampropeltis doliata between 27° and 34°C. However, Dawson et al. (1966) found that cutaneous water loss in A. ornatus was unaffected by temperature

FIGURE 10

Effect of temperature on cutaneous evaporation

$$H_2O \text{ loss } (mg/cm^2/hr) = -0.3645 + 0.0159\ T^{\circ}C$$

$$(S.E.b = \pm 0.0022)$$


0·40
0·35
0·30
0·25
0·20
0·15
0·10
0·05
H2O Loss (mg./cm.2/hr.)
30
35
38
40
T.°C.

between 20° and 30°C, whereas in S. labillardieri the Q_{10} value was 1.8. It is possible that higher Q_{10} values could have been observed if measurements had been made at the higher temperatures used in this and other studies.

The rates of cutaneous water loss obtained in this experiment may be used to estimate the percentage contribution that evaporation from the skin makes to pulmo-cutaneous evaporative water loss. By using the mean weight of animals used in Experiment 2, i.e. 956 g. together with the total surface area formula described on page 27 cutaneous water loss (mg/hr) may be calculated for animals of this weight at different temperatures. It is then found that cutaneous evaporation accounts for 73% and 81% of the pulmo-cutaneous evaporative loss at 30° and 38°C respectively. The slight increase in the cutaneous portion at higher temperatures is more apparent than real when the variability of the data is taken into account, and so it can be concluded that between 30° and 38°C cutaneous evaporation contributes approximately 75% of pulmo-cutaneous evaporation. However, Bentley and Schmidt-Nielsen (1966a) and Dawson et al. (1966) found that as temperature increased the cutaneous contribution to total evaporation decreased in the reptiles that they studied.

Experiment 6

Cutaneous evaporation in three subspecies of V. gouldii

This experiment was conducted at 30°C using the technique described in Experiment 5, but the small animal technique was used

for three animals. The data for cutaneous evaporation in V. g. rosenbergi at 30°C were taken from Experiment 5.

The results that are set out in Table 5 were subjected to an analysis of variance to determine if the data were homogeneous. The results of the analysis appear in Table 6 where the variance ratio is seen to be non-significant ($p > 0.2$). The experiment therefore failed to demonstrate a difference between the mean values of cutaneous water loss for each subspecies.

Consequently the data for all subspecies were pooled to derive a mean value of 0.11 ± 0.01 mg. $H_2O/cm^2/hr$ for cutaneous water loss at 30°C in V. gouldii.

TABLE 5

Cutaneous evaporation ($mg/cm^2/hr$) in three subspecies of V. gouldii at 30°C

V. g. rosenbergi	V. g. gouldii	V. g. flavirufus
0.139	0.062	0.124
0.104	0.065	0.118
0.162	0.096	0.081
0.116	0.125	0.055
0.065	0.162	
0.100	0.169	
0.191	0.072	
0.082		
0.138		
0.118		
$\bar{x}$ = 0.1215	0.1073	0.0945

TABLE 6

Analysis of Variance - Cutaneous evaporation at 30°C in the subspecies of V. gouldii

Source	S.S.	d.f.	Variance	V.R.	p
Between	0.002280	2	0.001140	0.73	$p > 0.2$
Within	0.028105	18	0.001561		
Total	0.030385	20			

This experiment was done at the same time as Experiment 3 in V. g. gouldii and V. g. flavirufus, before the results of that experiment were known. Consequently the results of the present experiment are redundant to a certain extent, as it would be unlikely for the rates of pulmo-cutaneous evaporation to be similar in the three subspecies, and yet the rates of cutaneous evaporation to be different. However, these results do complement those of Experiment 3 quite well.

Experiment 7

Cutaneous evaporation at 30°C in V. a. brachyurus and V. gilleni

This experiment was done to determine the variability in skin permeability between different varanid species.

Data was obtained from five V. a. brachyurus in Experiment 4 and from four specimens of V. gilleni. The data for cutaneous water loss are present in Table 7.

TABLE 7

Cutaneous evaporation ($mg/cm^2/hr$) in two species of Varanus

V. a. brachyrus	V. gilleni
0.137	0.061
0.118	0.058
0.127	0.070
0.135	0.031
0.074	
$\bar{x}$ = 0.1182	0.0550

The data for cutaneous evaporation in V. gouldii, V. a. brachyurus and V. gilleni were arranged in order of magnitude with respect to their mean values, and t-tests were carried out as shown in Table 8. It was not possible to show a significant difference between V. a. brachyurus and V. gouldii ($p > 0.8$), but a significant difference was found between V. gouldii and V. gilleni ($p < 0.01$). V. gilleni therefore has a much less permeable skin than the other two varanids, losing water through the skin at half the rate of the others. This difference in skin permeability is found even though all three species examined are found in the arid zones of Central Australia.

TABLE 8

Cutaneous evaporation in three species of Varanus

Species	n	$\bar{x}$	Var.	V.R.	d.f.	t	p
V.a. brachyurus	5	0.1182	0.000667	2.170	23	0.357	0.8
V. gouldii	20	0.1116	0.001447	5.131	22	2.816	0.01
V. gilleni	4	0.0550	0.000282				

Data on cutaneous water loss in a number of reptiles has been accumulated in Table 9. Comparisons are difficult due to the different techniques and temperatures employed by the various authors. All authors worked with intact skin except Tercafs who worked with isolated skin. Only Tercafs (1963) and Claussen (1967) measured the surface area of the skin under test, while Bentley and Schmidt-Nielsen (1966a), and Schmidt-Nielsen and Bentley (1966) estimated surface area by means of Benedict's formula;

$$S = 10 \times W^{0.67}$$

Bradshaw (1965) and Dawson *et al.* (1966) made no attempt to measure the surface area of the skin, and so, in order to allow comparison of their data with those of other authors, Benedict's surface area formula was used to enable expression of cutaneous water loss in terms of $mg.H_2O/cm^2/hr$. Claussen has expressed his data in terms of surface area derived from direct measurement as well as from

Benedict's formula. The data for V. gouldii and other varanids have been treated in a similar fashion to facilitate comparison with the other data.

Examination of Table 9 reveals that cutaneous water loss in V. gouldii at 30°C is of a similarly low order to that of other reptiles from arid zones e.g. Uta stansburiana, Amphibolurus ornatus, V. a. brachyurus, Sauromalus obesus and Gopherus agassizii. The mean value for cutaneous water loss in V. gilleni appears to be the lowest so far recorded for a reptile. The value obtained for isolated skin of Natrix maura by Tercafs (1963) is also very low, but not described in sufficient detail to allow useful comparisons.

It is not clear what is responsible for the decreased permeability of the skin of desert lizards. An investigation into skin structure and permeability would be most useful and perhaps allow recognition of that part of the skin that acts as a barrier to water loss. Maderson (1964) has suggested that the main site of cutaneous water loss is in the hinge area between the scales where the β-keratin layer is thinnest, but experimental data is necessary to substantiate this suggestion.

The high Q_{10} values that have been described for cutaneous evaporation in several reptiles, suggests that at higher temperatures physical and/or physiological changes may occur in the skin, either by way of increased peripheral perfusion as proposed by Gans et al. (1968), or possibly by change in the structures conferring

impermeability on the skin. It is also possible that the previously mentioned abrupt increase in water loss that takes place before panting in Crotaphytus collaris, is due to a sudden increase in skin permeability.

These suggestions are purely speculative, and further investigation is desirable to help clarify the effects of temperature on cutaneous structure and water loss.

TABLE 9

Cutaneous evaporation in Reptiles

Animal	Gutaneous Loss ($mg/cm^2/hr.$)		T°C	Author
Lacerta	(0.50)*			Tercafs (1963)
Uromastix	(0.70)			
Natrix	(0.0)			
Amphibolurus ornatus		0.41	35°	Bradshaw (1965)
A. caudicinctus		0.24	35°	
A. inermis		0.13	35°	
Iguana iguana		0.20	23°	Bentley and Schmidt-Nielsen (1966)
Sauromalus obesus		0.05	23°	
S. obesus		0.14	40°	
Terrapene carolina		0.22	23°	Schmidt-Nielsen and Bentley (1966)
Gopherus agassizii		0.06	23°	
G. agassizii		0.17	35°	
Sphenomorphus labillardieri		0.25	30°	Dawson et al. (1966)
Gehydra variegata		0.21	30°	
Amphibolurus ornatus		0.10	30°	
Anolis carolinensis	(0.12)*	0.19	30°	Claussen (1967)
Uta stansburiana	(0.08)	0.10	30°	
Natrix taxispilota	(0.70)*		25°	Prange and Schmidt-Nielsen (1969)
Pituophis catenifer	(0.15)		25°	
Varanus gouldii	(0.11)*	0.13	30°	Present study
" "	(0.16)	0.19	35°	
" "	(0.29)	0.34	40°	
Varanus a. brachyurus	(0.12)	0.14	30°	
Varanus gilleni	(0.06)	0.07	30°	

*Figures in brackets represent rates of cutaneous water loss calculated by using a true surface area measurement. All other values have been derived by using Benedict's surface area formula: $S = 10W^{0.67}$.

PULMONARY EVAPORATION

Rates of pulmonary evaporation are easily derived from estimates of pulmo-cutaneous and cutaneous water loss by subtraction. Pulmonary water loss represents approximately 25% of the pulmo-cutaneous evaporation between 30° and 38°C. As it has already been demonstrated that rates of cutaneous water loss are quite low in V. gouldii, this small pulmonary fraction suggests that rates of pulmonary evaporation are also low. This is best seen by expressing pulmonary evaporation as the amount of water lost for each ml. of oxygen taken up during respiration.

No attempt was made to measure oxygen consumption in this study, however, Bartholomew and Tucker (1964) have published data of this type for some Australian varanids, including V. gouldii. Using their results for three specimens of V. gouldii it is possible to estimate the pulmonary evaporation rate in terms of oxygen consumption at 30°C. The estimates of pulmonary water loss were obtained by using the equation from Experiment 3 relating pulmo-cutaneous evaporation to body weight, the surface area formula for V. gouldii, and the mean rate of cutaneous evaporation as in Experiment 6. The relevant estimates are shown in Table 10.

TABLE 10

Pulmonary evaporation in V. gouldii
(mg. H_2O/ml. O_2)

Wt. (g)	Oxygen consumption (ml. O_2/g/hr)	Pulmonary evaporation		
		(mg/hr)	(mg/g/hr)	(mg/ml. O_2)
89	0.074	6.9	0.078	1.1
127	0.119	10.3	0.081	0.7
714	0.092	62.3	0.087	0.9
				$\bar{x}$ = 0.9

The rates of pulmonary evaporation of some other reptiles are shown in Table 11. It can be seen that the estimate for V. gouldii is probably the same as that for Sauromalus obesus at the same temperature, but is lower than the other reptiles shown. A low rate of pulmonary water loss is probably a physiological adaptation for water conservation, as several authors have observed that reptiles from drier habitats exhibit reduced pulmonary evaporation when compared with those from non-arid environments. (Bentley and Schmidt-Nielsen 1966a), Dawson et al. 1966, Claussen 1967). It has been suggested by Dawson et al. (1966) that there might be differences between species in respiratory efficiency that could account for the differences in pulmonary evaporation.

TABLE 11

Pulmonary evaporation in Reptiles

Species	Temp. °C	mg. H_2O/ml. O_2	Authors
Iguana iguana	23	0.9	Bentley and Schmidt-Nielsen (1966 a)
Sauromalus obesus	23	0.5	
S. obesus	40	1.4	
Sphenomorphus labillardieri	20	21.1	*Dawson et al. (1966)
Amphibolurus ornatus	20	4.3	
Anolis carolinensis	30	5.3	Claussen (1967)
Uta stansburiana	30	2.7	
Natrix taxispilota	25	1.6	Prange and Schmidt-Nielsen (1969)
Pituophis catenifer affinis	25	1.3	
Varanus gouldii	30	0.9	Present study

*Own calculation from data presented in the reference.

Although the estimated rate of pulmonary evaporation in V. gouldii is higher than in many mammals (Schmidt-Nielsen and Schmidt-Nielsen 1950a)it is low compared with other reptiles and is therefore important in water conservation in V. gouldii.

EVAPORATION FROM THE EYES

The eyes of most terrestrial vertebrates are kept moist by lachrymal secretions, and the eye surface is continually swept by movement of the lids or nictitating membrane. The eyes would therefore appear to be an important site of evaporation, and so an attempt was made to determine the magnitude of water loss from this region.

Experiment 8

Evaporation from the surface of the eyes (Indirect method)

This experiment was conducted at 30°C with seven animals in the chamber shown in Fig. 2. An airflow rate of 700 cc/min. was maintained throughout the experiment. Each animal was placed in the chamber around midday and by the late afternoon it was usually drowsing. The animal was then aroused by tapping on the side of the chamber. This caused the animal to open both eyes and yet remain inactive, and it was found that shining a lamp into the chamber through the window helped maintain the eyes open. During the experimental periods it was occasionally necessary to tap the chamber to cause the eyes to reopen. Total evaporative water loss was measured for four experimental periods, after which the lamp was removed and the animal was allowed to close its eyes.

Within half an hour after taking readings with the eyes open, the animal was invariably found to have its eyes closed, and a further four measurements of total evaporative water loss were then made.

Subtraction of the mean value for evaporative water loss with eyes closed from the mean value with eyes opened, was taken as a measure of water evaporated from the eyes. The surface area of the exposed part of the eye was calculated by the technique described on page 45.

The results of this experiment are presented in Table 12, where it can be seen that there is a great deal of variation in the amount of water evaporated per unit area of eye surface. This is possibly due to respiratory variation that could have occurred between measurements with the eyes open and closed, but could also be due to differences in the time that individuals spent with their eyes closed during the initial eye-open periods. Some animals kept their eyes open at all times, whereas others had to be roused quite frequently.

TABLE 12

Evaporation from the Eyes (Indirect method)

Exposed Eye S.A. (mm^2)*	mgH_2O/hr	$mg/mm^2/hr$
18.9	41	1.09
23.6	90	1.91
27.5	78	1.42
42.4	101	1.19
37.7	71	0.94
18.9	51	1.35
27.5	54	0.98
		$\bar{x} = 1.27 \pm 0.13$

(*Measurement represents single eye)

Consequently an alternative experiment was performed to measure evaporation from the eyes directly at 30°C.

Experiment 9

Evaporation from the Surface of the Eyes (Direct method)

Direct measurements of evaporation from the eyes were obtained by anaesthetising animals and affixing a plastic cap over each eye with rubber cement. The caps measured 16 mm. in diameter and the bottom was drilled out of each one. Each eye was then prised open and held in this way by means of a small copper clip, the tips of which were covered with a layer of dried rubber cement to prevent damage to the eye. The area of eyeball exposed in this way was diamond-shaped due to tension in the eyelids, and this facilitated surface area measurement of the exposed region. The nictitating membrane was quite active immediately after inserting the metal clips between the eyelids, but after thirty minutes or so, activity of the membrane had returned to normal.

Plastic tubes, which contained magnesium perchlorate as dessicant, were weighed and then connected to the caps affixed to the eyes. After one hour the tubes were removed and re-weighed to the nearest 0.5 mg., and the weight difference was taken as the amount of water evaporated from the eyes. The eyes remained moist throughout the experiment, as the nictitating membrane was able to lubricate them. The experimental apparatus is shown in Fig. 11. The surface area of the eye exposed to the dry atmosphere of the attached tubes, was

FIGURE 11

Diagram of apparatus for measuring evaporation from the eyes directly

A = Contact cement

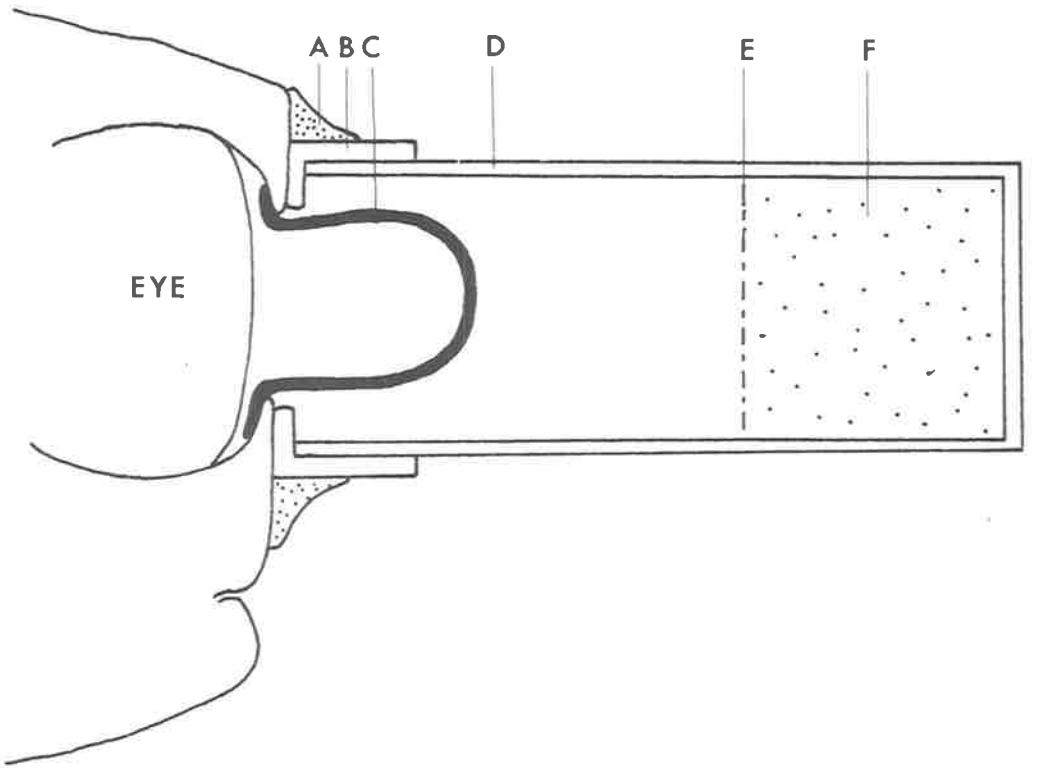
B = Plastic cap

C = Metal clip

D = Plastic tube

E = Plastic gauze

F = $MgClO_4$


A B C
D
E
F
EYE

obtained by measuring the dimensions of the forced eye lids (to the nearest millimeter) with the metal clips in place.

The results of this experiment are presented in Table 13. The mean rate of evaporation from the eyes was 0.67 ± 0.02 mg/mm^2/hr at 30°C.

TABLE 13

Evaporation from the Eyes (Direct method)

S.A. tested (mm^2)	Water loss (mg/hr)	mg. H_2O/mm^2/hr
20	13.5	0.67
20	13.5	0.67
16	10.0	0.63
16	11.0	0.69
16	10.5	0.66
20	12.0	0.59
16	10.0	0.63
16	13.0	0.81
		$\bar{x} = 0.67 \pm 0.02$

The mean values for rate of water loss obtained by the two methods differ quite markedly. The higher values obtained by the indirect method may be due to two factors:

(1) that evaporation was measured in a moving air stream in the first experiment, and consequently a greater evaporative rate than that in still air might be expected.

(2) that in allowing the animals to drowse during the final

periods of the first experiment, it is possible that a depression in respiratory rate, and therefore respiratory water loss, occurred. This would result in the values for optical evaporation obtained by subtraction being too large.

It is more reasonable therefore to accept the values obtained by the direct method as being more reliable than those of the indirect method. These values represent a substantial amount of evaporative water loss and it is surprising that this site of water loss has previously received so little attention. No mention of the eyes as a source of water loss is to be found in the literature, except for a passing reference by Sexton and Heatwole (1968). These workers mentioned that specimens of Anolis limifrons and A. auratus kept their eyes closed after they had become dehydrated "thereby decreasing water loss from these vital organs".

It seems reasonable to consider the exposed surfaces of the eyes as free water surfaces, and as such, for the rate of evaporation to be dependent upon temperature, saturation deficit of the atmosphere and convection rate of the air. The water evaporated from the eyes is obligatory as good visual acuity requires a good interphase between the air and the eye. Reduction of water loss from the eyes could be achieved by means of spectacles as found in Ophidia, Gekkonidae, and some Scincidae, but it is not known if these structures evolved as a mechanism for restricting water loss. The fact that the majority of vertebrates inhabiting arid zones do not possess structures

covering the eyes to restrict water loss, would indicate that visual acuity may be greatly reduced when these structures are present.

The Relationship of Eye Size to Body Weight

It is a general rule that the head and eyes of vertebrates do not increase in size to the same extent as the rest of the body during growth. Consequently eye measurements were made on 40 animals ranging in weight from 13.5 g. to 1632 g., and an attempt was made to calculate the approximate surface area of the exposed surface of one eye in each animal. The distance between upper and lower lids, and between anterior and posterior eye margins was measured with calipers to the nearest millimeter, accuracy greater than this being impossible due to the sensitive nature of the eye.

In calculating the exposed area of the eye, two approximate assumptions were made:

(1) That the exposed surface of the eye was flat,

(2) That the exposed surface of the eye had an elliptical shape the major diameter of which was the distance between the anterior and posterior margins of the eye.

The calculated surface areas were plotted against body weight as in Fig. 12, and the regression

$$\text{Exposed surface area of eye } (mm^2) = 11.0716 + 0.0217 \text{ wt. } (g)$$
$$(S.E.b = \pm 0.0017)$$

was calculated for the data. It is clear that small animals have

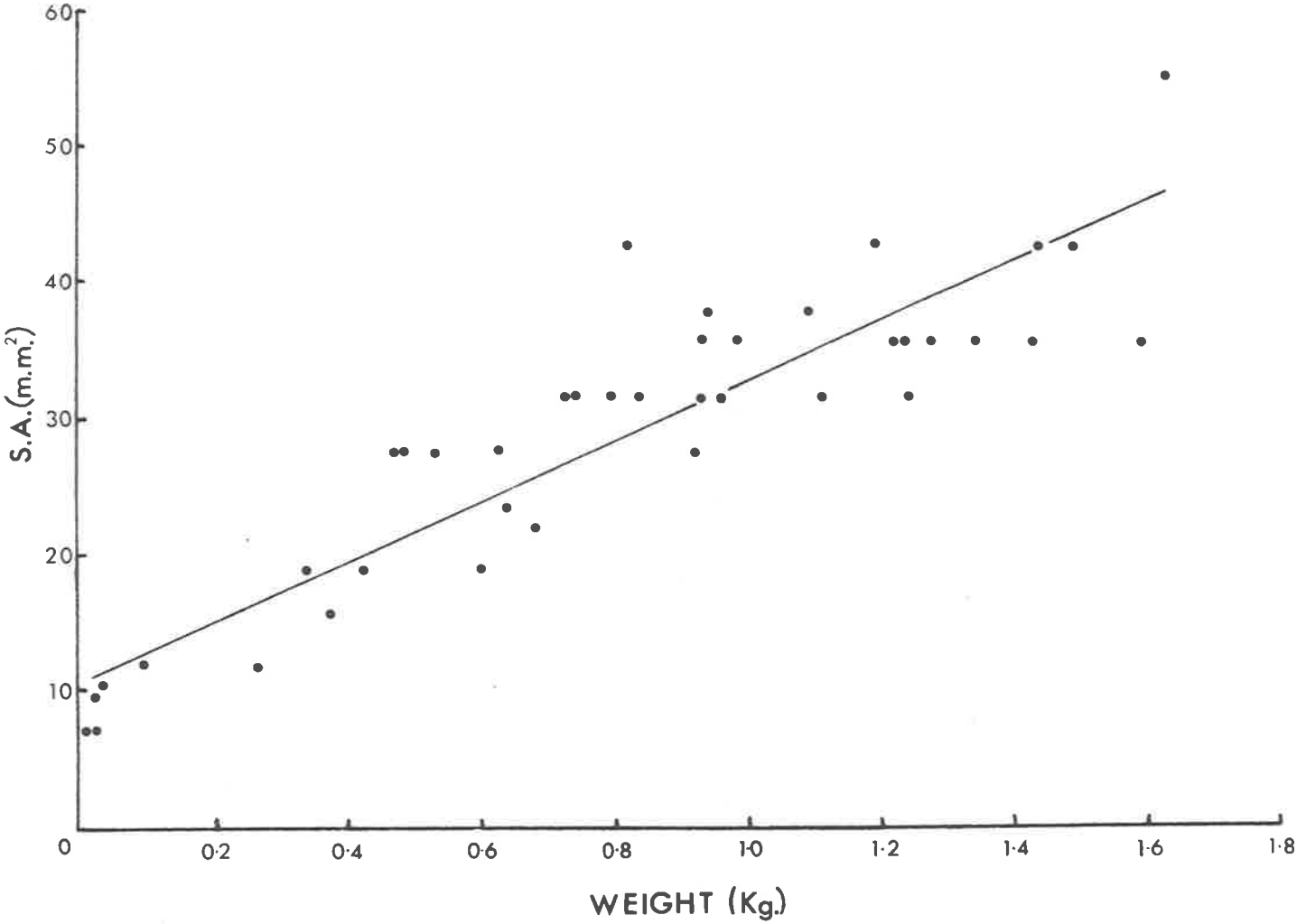
FIGURE 12

Relationship of exposed surface area

of the eye to body weight

Surface area (mm^2) = 11.0716 + 0.0217 wt. (g)

(S.E.b = $\pm$ 0.0017)


60
50
40
30
20
10
0
S.A.(m.m.²)
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1·8
WEIGHT (Kg.)

relatively larger eyes, and so the eyes may be expected to contribute to the total water loss of the animal to a different degree in small and large animals.

An approximate estimate may be made of the contribution made by optical evaporation to the total evaporation of a resting animal at 30°C with open eyes. This can be done by using the eye size/body weight formula and the mean rate of optical evaporation at 30°C (0.67 mg/mm^2/hr). If this calculation is coupled with an estimate of pulmo-cutaneous evaporation at 30°C, obtained from the formula in Experiment 3, the information in Table 14 can be derived.

TABLE 14

Partitioning of Evaporative Water Loss in Juvenile and Adult

Wt. (g)	Pulmo-cutaneous Evap. (mg/hr)	Surface area of eyes (mm^2)	Optical Evap. (mg/hr)	Optical Evap. %	Total Evap. (mg/g/hr)
15	8.24	22.8	15.21	64.9	1.563
1000	217.60	65.6	43.75	16.7	0.261

Under these conditions it is seen that the eyes are a major site of evaporation in a juvenile animal, but this is not so for an adult. When the estimate for optical evaporation is added to that for pulmo-cutaneous evaporation, and expressed as mg/g/hr, the juvenile loses

water at 6 times the adult rate. This compares with the 3 : 1 (juvenile:adult) ratio found when pulmo-cutaneous evaporation only is considered, and emphasizes the importance of eyes in water loss, particularly in juveniles.

SUMMARY

(1) The various sites from which water is evaporated have been shown, as well as the magnitude of water loss from these areas. The cloaca is not an important site of evaporation, but the respiratory tract, skin and eyes are all major regions of water loss.

(2) As temperature rises, evaporation steadily increases (Q_{10} = 2.4). Between 40° and 42°C panting commences and causes a four to six-fold increase in evaporation rate. This high rate of evaporation enables the animal to dissipate metabolic heat and also reduce the body temperature slightly below ambient.

(3) No significant differences were found between the subspecies of V. gouldii in the rates of pulmo-cutaneous and cutaneous evaporation, although they inhabit areas of different aridity.

(4) The rates of cutaneous evaporation in the varanid species studied are low and similar to the rates of other desert reptiles. There are indications that the skin becomes more permeable at higher temperatures.

(5) There are indications that the rate of pulmonary evaporation in V. gouldii is low compared with many other terrestrial reptiles.

(6) The overall effect of size is that small animals lose relatively more water by evaporation than large animals of the same species, but interspecific comparisons do not necessarily show this effect.

(7) Small and large animals differ in partitioning of total evaporative water loss. This is due to relative differences in metabolic rate, cutaneous surface area and surface area of the eye.

Section 2

RENAL FUNCTION

INTRODUCTION

Excretion of nitrogen is accompanied by the loss of water, the amount of which depends on the toxicity and solubility of the excreted compound. It has been known for some time that terrestrial reptiles excrete most of their nitrogen as uric acid, which is a highly insoluble compound. Consequently, little water is lost in excretion. Between glomerular filtration and the elimination of uric acid pellets, however, the urine undergoes many changes, both in the kidneys and in the cloaca.

Investigations were made into renal function under extreme physiological conditions to determine the degree of control over water and electrolyte excretion shown by the kidneys. In particular a study of the renal responses to water loading in V. gouldii was considered important, because observations made by Khalil and Abdel-Messeih (1959) on water-loaded Varanus monitor are anomalous.

All of the reptiles studied so far are unable to produce hyperosmotic urine, due to the absence of a countercurrent multiplier system in reptilian kidneys. Reptiles in general also exhibit limited tubular control over the concentration of the urine. However, renal studies have been made on only a few reptiles, and more research in this field was thought desirable.

It has been demonstrated by Shoemaker et al. (1966, 1967) that temperature is an important factor in the renal physiology of reptiles. These workers pointed out that renal studies should be conducted at

temperatures close to the thermal preferenda of the animals, since at low and high temperatures renal function is irregular. In this study all experiments were carried out at 30°C, which is sufficiently close to the thermal preferendum of 37°C described for V. gouldii by Licht et al. (1966). Working at this temperature also facilitated comparisons with other work that has been done on the renal physiology of reptiles.

MATERIALS AND METHODS

Animals from Kangaroo Island were used in all renal experiments. The animals were kept in a large pen which had heating lamps at one end to provide a temperature gradient. The lamps also provided light and were operated by a time switch to give a 12 hour light and dark cycle. Animals were fed on mice, but starved for at least two days before experiments. In the case of "normal" animals water was available continuously.

At about 10 p.m. on the day before an experiment, animals were anaesthetised with an intraperitoneal injection of Nembutal (20 mg/Kg). The animals were left overnight in a room at about 20°C, and they remained unconscious until after ureteral cannulae were inserted at about 9 a.m. on the day of the experiment. After cannulation the animals were placed in a room at 30°C where they usually regained consciousness within an hour.

Urine was collected by means of ureteral cannulae to prevent changes in volume and constitution of the urine that would occur if the urine entered the cloaca. Each cannula consisted of Portex polythene tubing (Size 52A) to which a skirt of "Araldite" epoxy-resin was attached, as shown in Fig. 13. The tapered part of the skirt facilitated insertion into the ureteral opening, while the sharply stepped rear of the skirt prevented the animal from pushing the cannula out. The ureteral papillae were exposed by opening the lips of the cloaca with retractors. The papillae were easily located in

FIGURE 13

Location of cannula used for collecting urine

C = Coprodaeum

U = Urodaeum

P = Proctodaeum

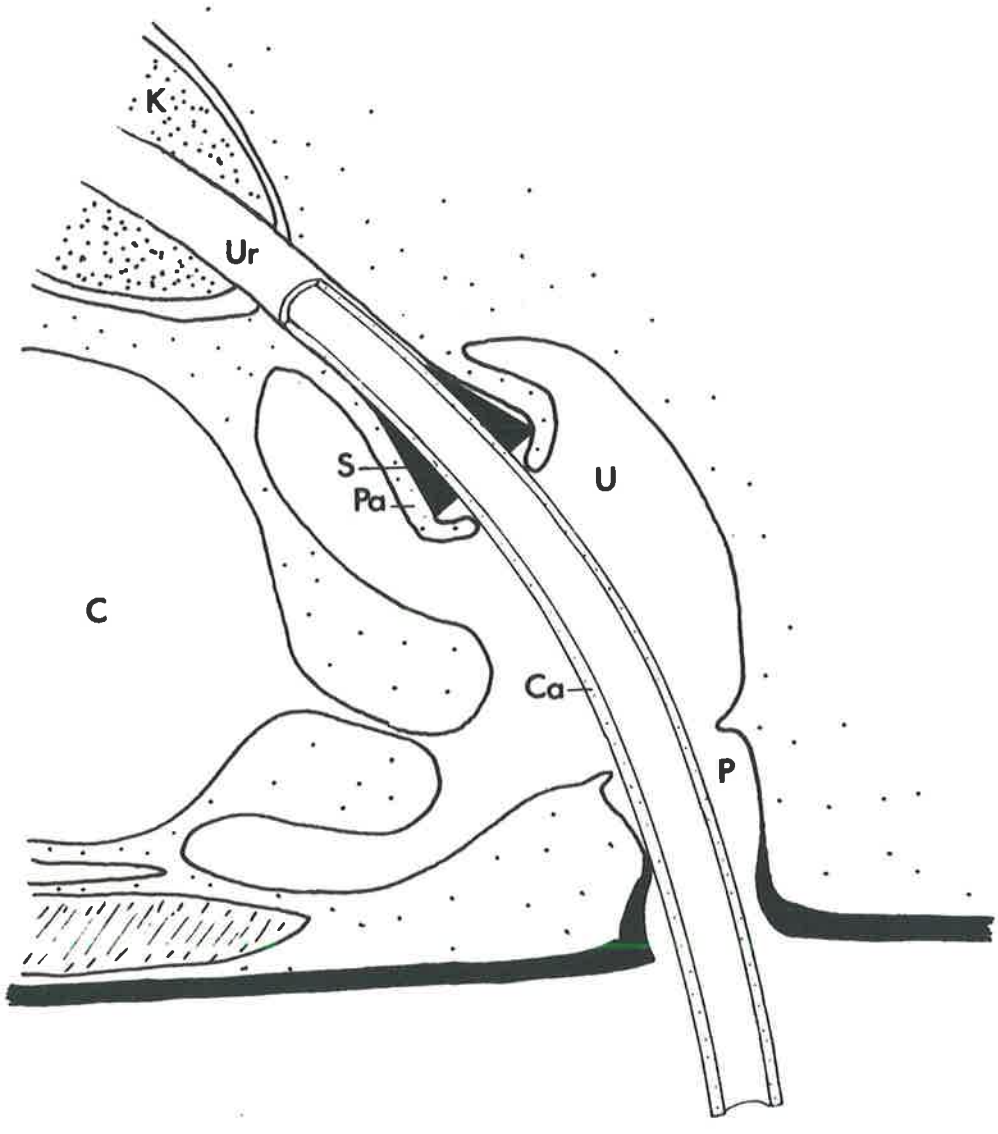
K = Kidney

Ur = Ureter

Pa = Ureteral papilla

Ca = Cannula

S = Skirt of epoxy-resin


K
Ur
S
Pa
U
C
Ca
P

the urodaeum by observing the flow of urine from the ureteral openings. Both ureters were cannulated, and then the retractors were removed. The cannulae were then trimmed back with scissors so that they extended for about $\frac{1}{2}$" outside the cloaca.

The animals were suspended from a raised horizontal bar by straps that were tied around the thorax and the base of the tail. This was the only way in which animals could be restrained. Animals had been conscious for at least three hours before the experiment was started. Urine was collected in graduated centrifuge tubes or, at lower rates of flow, in the barrels of graduated 1 ml. syringes that were plugged at the base. The rate of urine flow was measured in five consecutive periods for each animal, the periods usually being 30 minutes long. The five urine samples obtained from each animal were stored in plastic tubes in a refrigerator until analysed.

When urine was flowing at a low rate, the cannulae occasionally became partially obstructed with uric acid. When this happened the cannulae were cleared by inserting small gauge polythene tubing into them, but clearing was only done at the end of a collecting period.

Glomerular filtration rate (G.F.R.) was assessed by measuring inulin clearance as described by Smith (1956). A single intraperitoneal injection of 10% inulin (5 ml/Kg) provided a pool which maintained a fairly constant level of inulin in the duration of the experiment. All animals were injected with inulin immediately after they were anaesthetised. The use of a single sub-cutaneous injection of inulin

has been shown by Ramsay and Coxon (1967) to be a more reliable technique than the constant infusion of inulin, as the latter technique causes a diuresis. An intraperitoneal injection acts in the same way as a sub-cutaneous injection.

Blood samples were taken by cardiac puncture half an hour before the first collecting period, and immediately after the last. To avoid stressing the animals no blood samples were taken while urine was being collected. Samples of plasma were stored in plastic tubes and kept frozen until analysed.

The concentration of inulin in the plasma and urine was measured by the micro-technique described by O'Brien and Ibbott (1962). The samples were treated with diphenylamine reagent and then read with a Beckman Spinco 151 Spectrocolorimeter. The blood samples included blanks of plasma that were obtained before the injection of inulin.

The osmotic concentration of the plasma and urine samples were measured with a Fiske osmometer (Model H) using 0.2 ml. samples. When there was insufficient urine for this, a Mecrolab vapor pressure osmometer was used.

Sodium and potassium were measured with an EEL flame photometer, and all dilutions were made with double-distilled water.

The concentration of inulin in the plasma at the midpoint of each collection period was derived by interpolation between the initial and final concentrations. The midpoint values for osmotic, sodium and potassium concentrations of the plasma were derived by the same method.

Formulae

The following formulae were used to calculate the data;

$$\text{G.F.R. (ml/Kg/hr)} = \frac{U}{P} \text{ inulin ratio x urine flow rate (ml/Kg/hr)}$$

$$\text{\% filtrate resorbed} = \frac{\text{G.F.R. - urine flow rate}}{\text{G.F.R.}} \times 100$$

$$\text{\% of filtered solute resorbed} = \frac{(\text{Plasma}_{osm} \times \text{G.F.R}) - (\text{Urine}_{osm} \times \text{urine flow rate})}{(\text{Plasma}_{osm} \times \text{G.F.R.})} \times 100$$

$$\text{\% of filtered Na (or K) resorbed} = \frac{(k.\text{Plasma}_{Na} \times \text{G.F.R}) - (\text{Urine}_{Na} \times \text{Urine vol.})}{(k\ \text{Plasma}_{Na} \times \text{G.F.R.})} \times 100$$

where k = Gibbs-Donnan factor. For Na and K the k value is 0.94

The expressions concerned with tubular resorption are only accurate in the absence of net secretion by the tubules. As some secretion does occur, particularly of urates, the estimates of resorption by the tubules may be too low. This is more likely to be true for estimates of the percentage of filtered solutes that are resorbed.

Experiment 10

Renal Function in Normal Animals

Renal function was studied in five normal animals. Normal animals are defined as animals which had free access to food and water. The urine flowed at quite an even rate in these animals, and contained little or no precipitate of uric acid. The osmotic, sodium and

potassium concentrations in the urine and plasma are shown in Table 15. The calculations were based on mean urine and plasma values for each animal. The urine of normal animals was hyposmotic ($U/P_{osm} = 0.45$) and hypotonic with respect to the plasma. The concentration of potassium in the urine was higher than in the plasma in some cases, but lower in others.

TABLE 15

Analysis of the Urine and Plasma of Normal Animals

	Osmotic conc. (mOsm/Kg)	Na (meq/L)	K (meq/L)
Urine (5)	146 ± 24	40 ± 13	4.7 ± 1.1
Plasma (5)	328 ± 5	151 ± 4	3.7 ± 0.3

Values are given ± S.E. The number in parenthesis is the number of animals.

After the concentration of inulin had been determined for the plasma and urine samples, the components of renal function presented in Table 16 were calculated for V. gouldii, and included with data from other reptiles to facilitate comparisons.

Glomerular Function

The mean glomerular filtration rate in normal V. gouldii was 6.79 ± 1.33 ml/Kg/hr. This is approximately the same as the G.F.R. in other terrestrial reptiles as can be seen in Table 16. Strict comparisons between the species is not possible however due to differences in the experimental temperatures.

TABLE 16

Renal Function in Reptiles

T°C	Species	Urine Vol. (ml/Kg/hr)	U/P Osm	G.F.R. (ml/Kg/hr)	% Filtrate resorbed	% Solute resorbed	% Na^+ resorbed	Author
23° - 29°	Natrix sp.	3.49		10.83		89.6		1
25° - 29°	Natrix sipedon	3.49	0.21*	19.38	53.15*		89.96	2
20° - 22°	Pseudemys scripta	1.32	0.62	4.73	72.1*	82.7*	93.4	3
	Gopherus agassizii	2.00	0.36	4.74	57.8*	84.8*	98.0*	
24° - 29°	Hemidactylus sp.	2.55	0.64	10.4	74.2	83.8	84.3*	4
	Phrynosoma cornutum	1.97	0.93	3.54	42.3	47.0	45.1*	
	Tropidurus sp.	1.86	0.96	3.62	47.1	50.0	46.8*	
27° - 29°	Crocodylus acutus	1.23*	0.80	9.6	87.2*	89.7*		5
30°	Varanus gouldii	3.11	0.45	6.79	53.0	77.5	87.7	6

*Calculated from data published in the references.

1. Lebrie and Sutherland (1962)
2. Dantzler (1967a)
3. Dantzler and Schmidt-Nielsen (1966)
4. Roberts and Schmidt-Nielsen (1966)
5. Schmidt-Nielsen and Skadhauge (1967)
6. Present study

Tubular Function

Normal V. gouldii resorbed 53.0% of the glomerular filtrate during the passage of urine along the kidney tubules. The urine was much more dilute than the plasma (U/P_{osm} = 0.45), which indicates that some solute was transported out of the tubules without the accompaniment of water. Roberts and Schmidt-Nielsen (1966) studied the lizards Phrynosoma cornutum and Tropidurus sp., and found the urine of normal animals was almost isosmotic, but hyposmotic urine is produced by several reptiles under normal conditions, as can be seen in Table 16.

More than 87% of the filtered sodium was resorbed in V. gouldii which is a similar value to that found in the water snake Natrix sipedon (Dantzler 1967a), and the gecko Hemidactylus sp. (Roberts and Schmidt-Nielsen 1966). There appears to be a correlation between tubular resorption of sodium and dilution of the urine. When high levels of sodium are resorbed as in the tortoise Gopherus agassizii, the urine is quite dilute (U/P_{osm} = 0.36), but with lower levels of sodium resorption e.g. Tropidurus, the urine is almost isosmotic to the plasma (U/P_{osm} = 0.96). The elevated values for sodium resorption are therefore associated with reduced tubular permeability to water.

The ability of reptiles to produce a hyposmotic urine has been shown by Roberts and Schmidt-Nielsen (1966) to be associated with the structure of the distal tubular cells. In Hemidactylus, cytoplasmic lamellae and elongate mitochondria are present in the contraluminal wall of the tubule cells, and this arrangement is believed to be

associated with sodium transport. It is also considered that the luminal wall of these cells exhibits low permeability to water when dilute urine is produced.

The results obtained for potassium were quite variable, values for resorption of potassium ranging from 93.6% of the filtered load to zero resorption, and in six collection periods tubular secretion of potassium was evident.

After examining glomerular and tubular function in normal animals an experiment was done to study the effects of water and salt loads on renal function.

Experiment 11

The Effect of Water and Salt-loads on Renal Function

In this experiment five animals were used in each treatment, and five urine samples were collected from each animal. The technique for inulin injection and cannulation of ureters was identical to that described for normal animals.

Animals were loaded with salt by an intraperitoneal injection of 5M NaCl (2 ml/Kg/day) on each of three days. Despite this extreme treatment the animals were able to tolerate the load. Animals were left unstressed for one day before the start of the experiment. Over the whole period of loading the animals were kept in individual cages and provided with heating lamps.

After receiving the second or third injection a white encrustation of salt appeared around the external nares of most animals. The

nasal secretion of salt will be considered in Section 4.

Animals were water-loaded by the intraperitoneal injection of distilled water (100 ml/Kg). An initial injection of 40 ml/Kg was given on the evening before the experiment. A second injection of 30 ml/Kg was given at 9 a.m. the following day, and a final injection of 30 ml/Kg was given two hours later. The collection of urine was started about three hours after the final injection of water. An additional injection of inulin was given to water-loaded animals after the second water load (2 ml/Kg of 10% inulin solution).

The osmotic, sodium and potassium concentrations in the urine and plasma of both experimental groups are shown in Table 17.

TABLE 17

Analysis of the Urine and Plasma of Water and Salt-loaded Animals

		Osmotic Conc. (mOsm/Kg)	Na (meq./L)	K (meq./L)
Salt-loaded	Urine (5)	299 ± 16	80 ± 20	16.4 ± 3.4
	Plasma (5)	421 ± 10	202 ± 4	5.0 ± 0.5
Water-loaded	Urine (5)	114 ± 20		4.7 ± 1.1
	Plasma (5)	287 ± 7		3.8 ± 0.5

All values are given ± S.E. The figure in parenthesis is the number of animals.

TABLE 18

Components of Renal Function in V. gouldii

	Urine volume (ml/Kg/hr)	U/P inulin	G.F.R. (ml/Kg/hr)	U/P Osm	% Filtrate resorbed	% Solute resorbed	% Na^+ resorbed
Normal	3.11 ± 0.89	2.09 ± 0.32	6.79 ± 1.33	0.45 ± 0.04	53.0 ± 9.3	77.5 ± 1.6	87.7 ± 3.8
NaCl loaded	1.71 ± 0.38	5.91 ± 0.92	8.37 ± 0.82	0.71 ± 0.02	79.0 ± 4.3	85.1 ± 1.8	89.6 ± 4.8
H_2O loaded	7.72 ± 1.38	1.75 ± 0.16	12.82 ± 1.70	0.39 ± 0.03	38.3 ± 3.8	74.5 ± 3.0	83.0 ± 3.3

All values are given ± S.E. All values are based on mean values of 5 animals.

There were no differences between the controls and water-loaded animals in the osmotic concentration, or in the concentrations of sodium and potassium in the urine. However, in the salt-loaded group the urine was more concentrated than the controls in all respects.

The urine/plasma inulin ratios of the two experimental groups are presented in Table 18, along with the same information for the controls. Also shown are various components of renal function that have been calculated for each group.

Most of the items in each treatment were compared by analysis of variance and t-tests. Analysis of variance was carried out only when variances were not significantly different.

Glomerular Function

The G.F.R. in water-loaded animals was approximately double that of the controls ($p < 0.02$), but there was no significant difference between the salt-loaded and control groups ($p > 0.4$). The relevant statistical tests are presented in Tables 19 and 20.

TABLE 19

Analysis of Variance: G.F.R. (ml/Kg/hr)

Source	d.f.	SS.	Var.	V.R.	p.
Treatments	2	97.7663	48.88315	5.494	< 0.05
Error	12	106.7764	8.89803		
Total	14	204.5427			

The S.E. of the difference between any two means (n = 5) = 1.77961.

TABLE 20

Comparison of G.F.Rs.

Treatment	n	$\bar{x}$	Var.	V.R	t_8	p
Normal	5	6.79	8.8600	1.629	3.196	< 0.02
Water-loaded	5	12.82	14.4373			
Normal	5	6.79	8.8600	2.608	0.838	> 0.4
Salt-loaded	5	8.37	3.3968			

Tubular Function

The rates of urine flow are compared in Tables 21 and 22. As the variances were significantly different the data was transformed to logarithms$_{10}$ before analysis. There was a copious flow of urine in water-loaded animals, at approximately double the rate of the controls ($p < 0.02$). However, there was no significant difference in the rate of flow in normal and salt-loaded animals ($p > 0.1$).

TABLE 21

Analysis of Variance: Rate of Urine Flow ($\log_{10}$ mg/Kg/hr)

Source	d.f.	SS.	Var.	V.R.	p
Treatments	2	1.139450	0.569725	12.589	< 0.005
Error	12	0.543050	0.045254		
Total	14	1.682500			

The S.E. of the difference between any two means (n = 5) = 0.009051.

TABLE 22

Comparison of urine flow rates

Treatment	n	$\bar{x}$	Var. ($\log_{10}$)	V.R.	t_8	p
Normal	5	3.11	0.063717	2.063	3.190	< 0.02
Water-loaded	5	7.72	0.030891			
Normal	5	3.11	0.063717	1.548	1.759	> 0.1
Salt-loaded	5	1.71	0.041154			

The comparisons for the percent of filtrate resorbed are shown in Tables 23 and 24. There was no significant difference between the controls and water-loaded animals ($p > 0.1$) but a significantly greater percentage of the filtrate was resorbed in salt-loaded animals ($p < 0.02$).

TABLE 23

Analysis of Variance: % of Filtrate Resorbed

Source	d.f.	SS.	Var.	V.R.	p
Treatments	2	8255.14	4127.57	20.721	< 0.001
Error	12	2390.34	199.20		
Total	14	10645.48			

The S.E. of the difference between any two means (n = 5) = 39.839.

TABLE 24

Comparison of % Filtrate Resorption

Treatment	n	$\bar{x}$	Var.	V.R.	t_8	p
Normal	5	53.0	431.2275	6.130	1.647	> 0.1
Water-loaded	5	38.3	70.3525			
Normal	5	53.0	431.2275	4.492	2.913	< 0.02
Salt-loaded	5	79.0	96.0050			

The urine/plasma osmotic concentration ratios in the water-loaded group were not significantly different from those in the control group. ($p > 0.5$), but those of the salt-treated group were ($p < 0.05$). The tests are set out in Tables 25 and 26.

TABLE 25

Analysis of Variance: U/P osmotic concentration ratios

Source	d.f.	SS.	Var.	V.R.	p
Treatments	2	0.284490	0.142245	6.913	< 0.05
Error	12	0.246896	0.020575		
Total	14	0.531386			

The S.E. of the difference between any two means (n = 5) = 0.004115

TABLE 26

Comparison of U/P osmotic concentration ratios

Treatment	n	$\bar{x}$	Var.	V.R.	t_8	p
Normal	5	0.453	0.036272	1.849	0.650	> 0.5
Water-loaded	5	0.394	0.019621			
Normal	5	0.453	0.036272	6.22	2.844	< 0.05
Salt-loaded	5	0.711	0.005831			

The percentage of filtered sodium that was resorbed was not found to vary significantly between the treatments ($p > 0.2$), approximately 85% of the filtered sodium being resorbed in all cases. The test is shown in Table 27.

TABLE 27

Analysis of Variance: % of filtered sodium resorbed

Source	d.f.	SS.	Var.	V.R.	p
Treatments	2	114.50	57.250	0.700	> 0.2
Error	12	980.42	81.702		
Total	14	1094.92			

The increased resorption of filtrate in the salt-loaded animals

appears to be inconsistent with the observations that G.F.R. and rate of urine flow were not significantly different from the controls. However, the G.F.R. tended to be higher in salt-loaded animals while the rate of urine flow tended to be lower than the controls. This indicates that there was in fact increased tubular resorption in the salt treated group. The increase in U/P osm suggests that resorption of extra water accounted for most, if not all, of the increased resorption of filtrate. Certainly sodium was not resorbed at a greater relative rate than in the controls. Although there was an increase in resorption of water, the tubules were not completely permeable to water as the urine remained hyposmotic.

Thus the renal response to salt-loading in V. gouldii is confined to the tubules, which become more permeable to water. A similar response occurs in Crocodylus acutus when subjected to hyperosmotic salt loads (Schmidt Nielsen and Skadhauge 1967). Hemidactylus sp. also exhibits no change in G.F.R. when salt-loaded, but this animal also shows no change in tubular permeability (Roberts and Schmidt-Nielsen 1966). In other reptiles, however, the response to salt loading consists of a decrease in G.F.R. as well as an increase in tubular resorption e.g. Tropidurus sp. and P. cornutum (Roberts and Schmidt-Nielsen 1966), P. scripta and G. agassizii (Dantzler and Schmidt-Nielsen 1966).

Little difference could be detected between the three groups regarding tubular resorption of potassium as the data were so variable.

Values for potassium resorption ranged from over 80% of the filtered load through to tubular secretion in all three groups. Tubular secretion of potassium has also been shown in the sleepy lizard Tiliqua rugosa by Shoemaker et al. (1966), and mammals (Berliner et al. 1950).

The diuresis induced by water is produced solely by an increase in G.F.R. in V. gouldii. The absence of a tubular response is indicated by U/P osm. ratios and filtrate resorption values in water-loaded animals that are similar to those in control animals. This diuretic response is similar to that of other reptiles treated in the same way, for example Natrix sp. (Lebrie and Sutherland 1962), T. rugosa (Shoemaker et al. 1966), P. scripta and G. agassizii (Dantzler and Schmidt-Nielsen 1966). However, there is a tubular response to water-loading in Hemidactylus sp. (Roberts and Schmidt-Nielsen 1966) where there is a decrease in the amount of filtrate that is resorbed. A similar reduction is indicated in C. acutus by the lower U/P osm. ratios in water-loaded animals (Schmidt-Nielsen and Skadhauge 1967).

It has therefore been demonstrated that V. gouldii responds to water-loading with a marked diuresis. However, Khalil and Abdel-Messeih (1959) reported that Varanus griseus could be loaded with an amount of water corresponding to 15% of the body weight "without inducing urinary output". These workers did not define what was meant by urinary output, but they mentioned that urine was collected for two

weeks before the water-loading experiments. It is unlikely that ureteral urine was collected over this long period of time, and so it can be inferred that these authors meant urine elimination by "urine output". If this is the case, then the diuresis shown by V. gouldii is compatible with the observations of Khalil and Abdel-Messeih. Three water-loaded V. gouldii were each placed in a cage over a tray of oil, but no urine was eliminated by the animals over a period of several days. This shows that although there is a diuresis when these animals are water-loaded, the urine is later resorbed in the cloaca. In this way the animal is able to store extra water in the tissues, as suggested by Khalil and Abdel-Messeih.

Some misunderstanding has arisen in the interpretation of the work of Khalil and Abdel-Messeih. Shoemaker et al. (1966) assumed that "urine output" meant urine flow rate, as they cite V. griseus as an example of a reptile that does not exhibit the diuresis shown by other reptiles when water-loaded. This citation seems unfounded in the light of present observations.

Even when water loaded, 38% of the filtrate is resorbed by the tubules. This value probably represents obligatory resorption of water in the proximal tubular region, and compares with a value of 40% found in T. rugosa (Shoemaker et al. 1966) and 60% in Natrix sp. (Lebrie and Sutherland 1962), under similar conditions of hydration.

Over the whole range of G.F.Rs observed in the treatments, the same relative amount of sodium was resorbed. This indicates that variation in G.F.R. is achieved by changes in the number of functioning

glomeruli, as suggested by Lebrie and Sutherland (1962) and later corroborated by Dantzler and Schmidt-Nielsen (1966) and Dantzler (1967a). In T. rugosa, however, it has been found that the percentage of sodium resorbed varies inversely with G.F.R. (Shoemaker et al. 1966).

Antidiuresis

Arginine vasotocin (AVT) has been found in neurohypophyseal extracts of the turtle Chelonia mydas (Sawyer et al. 1959) and the snake Crotalus atrox (Munsick 1966) and is generally regarded as being the antidiuretic hormone of reptiles and most other non-mammalian vertebrates. In order to study antidiuresis, an experiment was set up to determine the effects of A.V.T. on glomerular and tubular activity.

Experiment 12

The Effects of Antidiuretic Hormone on Renal Function

The water-loaded animals used in Experiment 11 were also used in this experiment. After the final blood samples had been taken in the previous experiment, the animals were left for half an hour to settle down. Each animal was then injected in the heart with 0.05 ml/Kg body weight of a solution containing 1.0 ug. AVT/ml.

The response to the AVT injection was quite dramatic; urine flow ceased within a minute in two animals and within four minutes in another. In two animals the urine flowed for slightly longer and here it is possible that the injection was not made completely into the heart.

However, all animals were anuric within 15 minutes of injection, and remained so for up to 45 minutes before urine began to flow again. Here, the urine was thick in consistency, and milky in appearance due to the presence of precipitated uric acid and urates.

The results of the urine analyses are set out in Table 28. The changes in renal function induced by the hormone were so marked that little statistical treatment of the data was required.

TABLE 28

The Effects of AVT on Renal Function

Treatment		Urine vol (ml/Kg/hr)	U/P Inulin	G.F.R. (ml/Kg/hr)	U/P osm.	% filtrate resorbed	% Na resorbed
-AVT	$\bar{x}$*	7.72	1.75	12.82	0.39	38.3	83.0
	S.E.	0.60	0.16	1.70	0.06	3.8	3.3
+AVT	$\bar{x}$	0.86	2.83	2.54	0.55	51.5	86.1
	S.E.	0.20	0.56	0.80	0.09	8.3	2.2

*All means are based on the results of 5 animals.

The major effect of AVT in V. gouldii appears to be at the glomerular level. At first the hormone caused glomerular activity to cease, but when filtration started again the G.F.R. was much lower than the pre-injection rate in every animal. The tubular effects of the hormone, were not as pronounced, but there was an increase in the permeability

to water. This was indicated by increases in the percentage of filtrate resorbed and the osmotic concentration of the urine after AVT was injected. The percentage of filtered sodium resorbed was unchanged by the injection, however there was a significant increase in the number of collecting periods in which secretion of potassium occurred ($p < 0.05$). The data for potassium secretion are presented in Table 29.

TABLE 29

Number of Urine Samples Showing Net K Secretion

Treatments	Net K secretion		Totals
	(+)	(-)	
-AVT	6	19	25
+AVT	14	11	25
Totals	20	30	50

$\chi^2_1 = 5.333$ $p < 0.05$

The results of this experiment agree in part with those obtained by Dantzler (1967a) for the water snake N. sipedon, where it was found that AVT reduced the G.F.R. and increased tubular resorption. The tubular effects persisted long after G.F.R. had returned to normal, but the present experiment was not continued for long enough to see if a similar response prevailed in V. gouldii.

Dantzler found that sodium resorption was increased when AVT was injected, but this effect was not seen in V. gouldii. In addition, Dantzler showed that AVT increased the percentage of potassium resorbed in N. sipedon whereas in V. gouldii it has been shown that AVT promoted secretion of potassium by the tubules.

Injection of vasopressin caused a decrease in G.F.R. in Alligator mississippiensis (Burgess et al. 1933, Sawyer and Sawyer 1952) and Natrix sp. (Lebrie and Sutherland 1962), but there was no effect on the tubules. However, vasopressin is not the naturally occurring antidiuretic hormone in reptiles, therefore care must be taken in interpreting these results.

The glomerular and tubular effects of antidiuretic hormone have been studied in other reptiles by using dehydrated animals. Some reptiles, when dehydrated exhibit reduced G.F.R. and increased resorption by the tubules e.g. T. rugosa (Shoemaker et al. 1966), P. scripta (Dantzler and Schmidt-Nielsen 1966), whereas others show a marked reduction in G.F.R. but little tubular response e.g. Hemidactylus sp. and Tropidurus sp. (Roberts and Schmidt-Nielsen 1966). Some reptiles show only modest tubular and glomerular responses to dehydration e.g. C. acutus (Schmidt-Nielsen and Skadhauge 1967) and P. cornutum (Roberts and Schmidt-Nielsen 1966). It is clear therefore that reptiles are quite variable in their renal response to dehydration or administered antidiuretic hormones.

It appears that V. gouldii is capable of controlling the osmotic

and ionic composition of the urine to a greater extent than many other reptiles that have been studied. However, the urine emerging from the ureters bears no resemblance to the urinary waste that is finally eleiminated, which indicates that the cloaca is a resorptive structure capable of modifying the urine to a marked extent.

Nitrogen Excretion

No attempt was made in this study to investigate nitrogenous excretion by the kidney. The main excreting compound in lizards and snakes is uric acid but small amounts of other nitrogenous compounds such as allantoin and creatine may also be excreted (Khalil 1948a and b, 1951; Seshadri 1956, 1959).

Marshall (1932) has shown that only 6% of the uric acid is filtered by the glomeruli in Iguana iguana, tubular secretion accounting for the rest. This finding has been substantiated in other reptiles by Dantzler and Schmidt-Nielsen (1966) and Dantzler (1968). The secreted urate is probably in the form of soluble sodium and potassium salts, as Dantzler (1967b) has shown by stop-flow technique that concentration peaks for sodium, potassium and urate coincide in N. sipedon.

Ureteral urine in normal and water-loaded animals was invariably a clear fluid containing little, if any, precipitate of uric acid or urates. The mechanism of precipitation will be discussed in Section 3.

SUMMARY

(1) Glomerular filtration rate in V. gouldii is similar to that of other reptiles, but is low compared with birds and mammals.

(2) G.F.R. increases in water-loaded animals, but decreases when AVT is injected. Salt-loading has no significant effect on G.F.R.

(3) A constant percentage of the filtered sodium is resorbed in all treatments, indicating that G.F.R. is determined by the number of functioning glomeruli.

(4) The rate of urine flow varied between the treatments, being lowest in salt-loaded and AVT-treated animals, and highest in water-loaded animals.

(5) The renal tubules of V. gouldii control the osmotic concentration in the urine to a greater extent than those in some other reptiles, U/P osmotic concentration ratios ranging from 0.18 to 0.91.

(6) The resorption of 38% of the filtrate in water-loaded animals probably represents obligatory resorption of water in the proximal region of the tubules. Facultative resorption of water probably occurs in the distal tubules and is under the control of AVT.

(7) Over 80% of the filtered sodium is resorbed by the tubules, but potassium is frequently secreted, probably by the distal tubular segments as in Mammals.

Section 3

STRUCTURE AND FUNCTION OF THE CLOACA AND RECTUM

(The experiments on resorption of fluid were conducted in association with Mr. M. Braysher, Dept. of Zoology, Adelaide University)

INTRODUCTION

In terrestrial vertebrates the urine that emerges from the ureters is not immediately expelled, but is carried into a storage organ. The storage organ may be a urinary bladder or a cloaca, and in some animals both structures are present. These organs may also serve the ancillary function of regulating salt and water loss in non-mammalian tetrapods. The renal tubules of most amphibia and reptiles exhibit limited control over the composition of urine, but the cloaca and bladder have been shown in many cases to modify the composition of ureteral urine to a marked extent.

In amphibia, dehydration results in increased resorption of fluid from the bladder (Steen 1929, Ewer 1952, Sawyer and Shisgall 1956, Sawyer 1960) and it has been shown that part of the fluid movement is associated with active transport of sodium out of the bladder (Leaf et al. 1958). Chelonians also have urinary bladders which are able to resorb fluid (Bentley 1962, Rogers 1966, Dantzler and Schmidt-Nielsen 1966). Dantzler and Schmidt-Nielsen found movement of sodium into the bladder of Pseudemys scripta, while Klahr and Bricker (1964) have shown that sodium is transported out of the bladder in the same species. The reason for this disagreement in the direction of sodium transport in P. scripta is not known. Sodium is transported out of the bladder of the tortoise Testudo graeca (Bentley 1962).

The resorptive function of the cloaca has been studied in the crocodilians Caiman sclerops (Bentley and Schmidt-Nielsen 1966) and

Crocodylus acutus (Schmidt-Nielsen and Skadhauge 1967). In both of these aquatic reptiles the urine is stored in the cloaca for several hours before it is voided. During this period sodium is resorbed as well as some water, and therefore the cloaca assists in preventing salt depletion, although some electrolytes are still lost in the liquid urine that is finally voided.

Sodium and water are resorbed in the cloaca of chickens (Hart and Essex 1942 and Skadhauge 1967, 1968), and similar activity is shown by the cloaca of a snake Xenodon sp. Junqeira et al. (1966).

It is generally recognised that the cloaca is a resorptive structure in terrestrial lizards as uric acid is normally eliminated as hard pellets. However, no attempt has been made to measure the rate at which fluid is withdrawn by the cloaca.

Fluid is also resorbed from the faeces in the rectum, and so a study was therefore made of the structure of the cloaca and rectum in V. gouldii, and also of the physiological processes involved in the resorption of fluid. It will be shown that the rectum and cloaca are important in conserving water in desert reptiles.

MATERIALS AND METHODS

The animals used in this study on cloacal structure and function were from Kangaroo Island. Histological material was fixed in 10% formalin, dehydrated and embedded in paraffin wax. Secretions were stained with alcian blue, haematoxylin and metanil yellow (Humason 1967), and mounted in Depex after dehydration and clearing.

The mean weight of animals used in experiments to measure the absorption of cloacal fluid was 1206 g. (range: 909 - 1445 g). The animals were anaesthetised with an intraperitoneal injection of Nembutal (20 mg/Kg), and the cloaca and rectum were then cleared of all faecal and urinary material by flushing with water. Retractors were used to keep the cloacal lips open while this was in progress. After the cloaca and rectum had been cleared, the apparatus shown in Fig. 14 was inserted into the cloaca and the catheter bulbs were then inflated with water. The apparatus, which was designed by M. Braysher, consisted of two Foley catheters arranged in series, the smaller (15 cc.) anterior catheter passing through the drainage canal of the larger (30 cc.) posterior catheter. There was a space of 3.5 cm. between the two bulbs into which a sampling tube of polythene (Portex, size PP.120) opened. This tube also passed along the drainage canal of the large catheter to the far end of the apparatus.

The insertion and inflation of the apparatus isolated part of the coprodaeum from the rectum and the urodaeum. The location of the isolated region was verified by dissecting an animal with the apparatus in place.

FIGURE 14

Apparatus for isolating part of the coprodaeum

A = Anterior catheter bulb

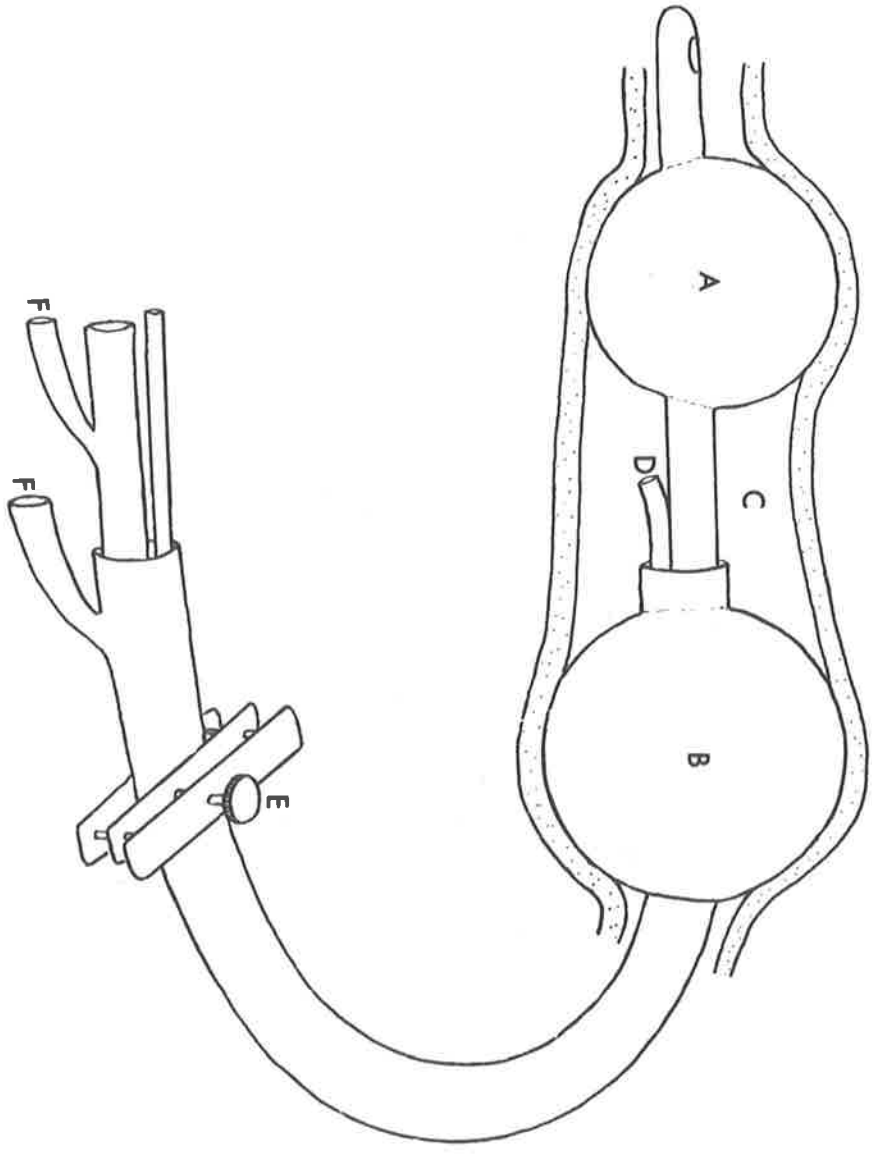
B = Posterior catheter bulb

C = Isolated part of coprodaeum

D = Sampling tube

E = Screw clamp

F = Valves for inflating catheter bulbs


A
B
C
D
E
F
F

After the apparatus had been set up, the animals were placed in a constant temperature cabinet ($35^{\circ} \pm 0.1^{\circ}C$) to speed up recovery from the anaesthetic. The animals recovered after an hour or so and they were then transferred to another temperature cabinet ($30^{\circ} \pm 0.1^{\circ}C$). Here the animals were suspended from racks by rubber straps tied around the thorax and the base of the tail. A hood was placed over each animal to pacify it. All animals had been conscious for at least two hours before experiments were started.

At the start of each experiment, 20 ml. of isotonic saline was injected through the sampling tube into the space between the catheter bulbs.

The saline contained bovine serum albumin (0.50 or 0.25%) which was used to estimate changes in the volume of fluid. After saline had been introduced a clamp was closed to prevent the fluid from escaping along the drainage canal of the larger catheter. A piece of metal tubing was placed inside the sampling tube to prevent the clamp from occluding it.

Samples of fluid were taken at regular intervals with a 5 ml. syringe to which was attached a blunt 17 gauge needle. The needle was inserted into the sampling tube and the syringe plunger moved to and fro, to thoroughly mix the fluid in the cloaca. When this had been done a sample of approximately 0.3 ml. was removed. The end of the sampling tube was sealed by a spring clip. A blood sample was taken by cardiac puncture at the beginning and end of each experiment,

and the 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, and the concentration of albumin in the cloacal fluid was measured with a Unicam SP.500 Spectrophotometer. For albumin measurements, 10 or 20 µl of cloacal fluid were diluted to 1 or 2 ml., and read directly at 280 mµ in silica cells. All dilutions of sodium, potassium and albumin were made with double distilled water.

CLOACAL AND RECTAL STRUCTURE

The cloaca in reptiles usually consists of three chambers positioned in series; the anterior coprodaeum, central urodaeum, and posterior proctodaeum. The chambers are separated by sphincters, and there is also a strong sphincter between the rectum and the coprodaeum.

The cloaca of *V. gouldii* is different in gross morphology to that in *V. monitor* (Seshadri 1959). The arrangement of the chambers is identical in male and female *V. gouldii*, whereas the urodaeum is dorsal to the coprodaeum in female *V. monitor*. The cloaca of *V. gouldii* is similar to that of *Hemidactylus flaviviridis* (Seshadri 1956).

The coprodaeum is the largest of the chambers, and is a highly

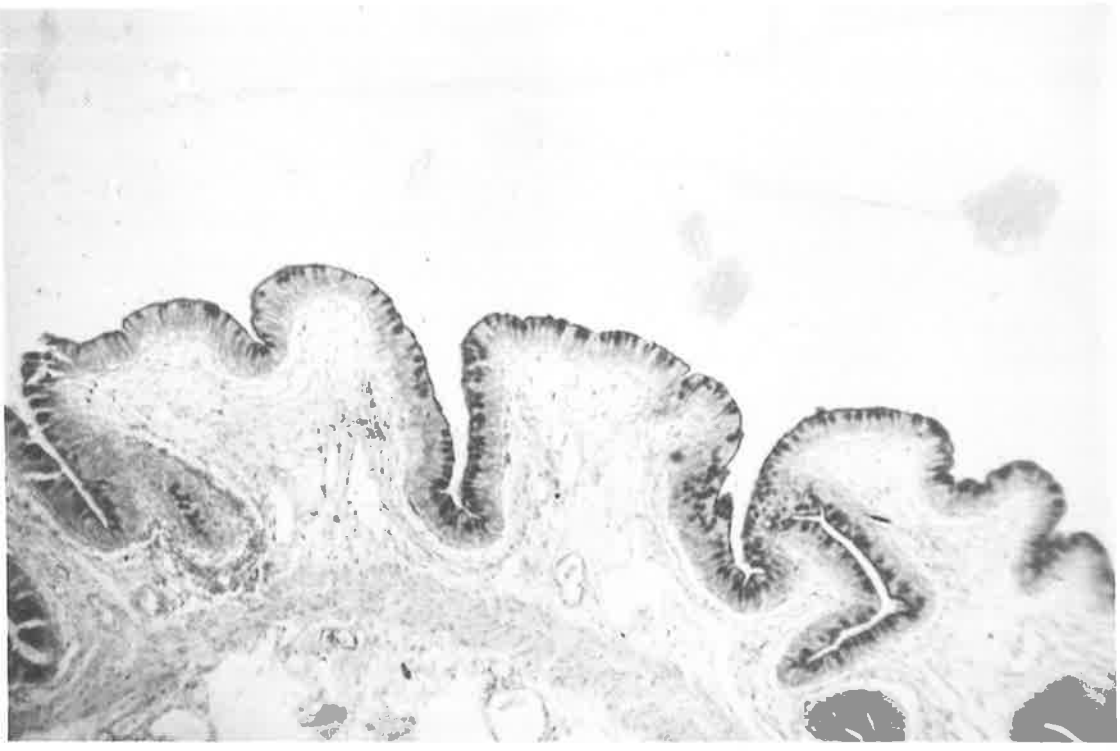
vascular, thin-walled structure. The mucosal surface is extended into folds which may be quite large. The urodaeum is smaller than the coprodaeum and the walls are not as folded. The ureters open into the urodaeum through a pair of urinary papillae positioned in the dorso-lateral walls, and urine passes forward from the urodaeum into the coprodaeum. The proctodaeum is the smallest chamber, and is located just inside the lips of the cloaca.

Microscopic examination reveals that the walls of the rectum and coprodaeum bear villi that are similar to those of the small intestine. Infoldings of the mucosa also occur in the rectum and coprodaeum and these are similar to the crypts of Lieberkühn of the intestine. Villi and crypts are also present in the large folds of the coprodaeal walls.

Typical villi are shown in Fig. 15. The mucosa consists of columnar epithelial cells, interspersed with numerous goblet cells. The columnar cells have a brush border of microvilli. The contents of the goblet cells stained green after treatment with alcian blue and metanil yellow, indicating the presence of acid polysaccharides. Similar goblet cells have been reported in the cloaca of a snake, Xenodon sp., (Junqueira et al. 1966). Beneath the mucosa is a region consisting mainly of connective tissue, blood vessels and muscle fibres. A muscle coat is present beneath this, with circular muscle lying towards the mucosa and longitudinal muscle towards the serosa. Where folds are present the muscle layers extend into them.

FIGURE 15

Villi of the coprodaeum and rectum



It can be seen therefore, that the structure of the rectum and coprodaeum is well suited to the resorptive function of these structures. The surface area over which resorption takes place is greatly increased by the presence of folds, villi and microvilli, and the rich blood supply to these regions ensures rapid transport of fluid away from the resorptive areas.

CLOACAL AND RECTAL FUNCTION

Experiment 13

Absorption of Fluid by the Cloaca

This experiment was conducted at 30°C with six animals. Samples of the introduced cloacal fluid were collected immediately after the introduction of fluid, and one hour later. Subsequent samples were taken every two hours. Changes in the volume of cloacal fluid were calculated from the formula:

$$V_2 = \frac{\text{Albumin conc. at } T_1}{\text{Albumin conc. at } T_2} \times V_1$$

where V_1 = fluid volume at T_1

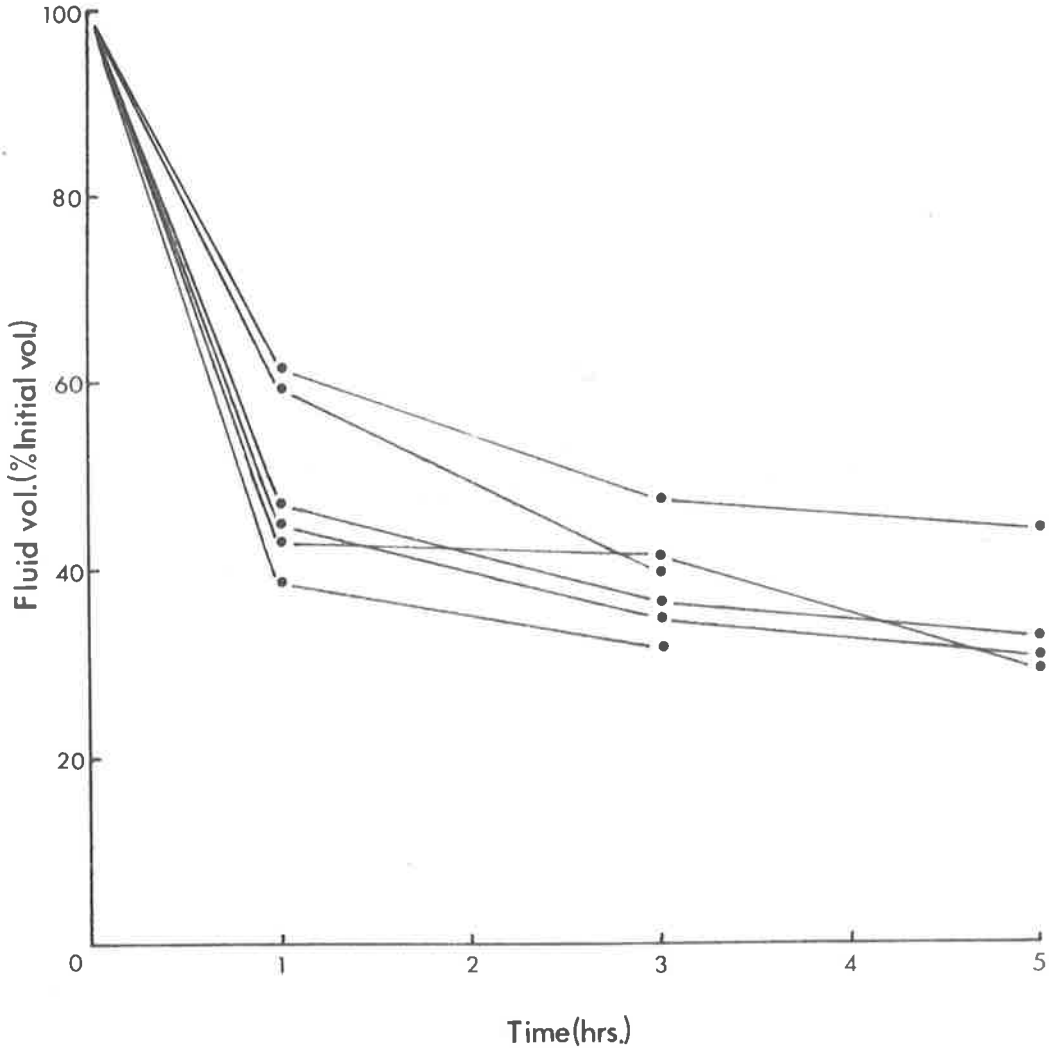
and V_2 = fluid volume at T_2

The changes in fluid volume that occurred during the experiment are shown in Fig. 16. Absorption of fluid was rapid at first, but after an hour or so the rate decreased. This decrease was probably due to a reduction in the internal surface area of the cloaca. When distended, the complete mucosal surface of the villi and folds would be exposed, but contraction of the cloaca as fluid is withdrawn would

FIGURE 16

Changes in the volume of fluid

in the cloaca of control animals


100
80
60
40
20
0
1
2
3
4
5
Fluid vol.(%Initial vol.)
Time(hrs.)

cause the sides of adjacent villi to become adpressed, thus decreasing the surface available for absorption. Fluid that is resorbed by the cloaca is normally replaced by urine from the kidneys, and this, together with continual deposition of uric acid would tend to keep the cloaca distended. Consequently, the changes in volume in the first hour were assumed to more closely represent normal rates of resorption than subsequent changes.

The surface area over which absorption occurred was not measured, as no technique could be found that would take account of cloacal distensibility and the presence of septa and villi. Consequently absorption was expressed in absolute terms, the mean rate in the first hour being 8.42 ± 0.63 ml/Kg/hr.

The initial concentration of sodium in the cloacal fluid was 164 meq./L, but after an hour the concentration had decreased to 145 ± 1 meq./L and after three hours to 138 ± 3 meq./L, as shown in Fig. 18. When volume changes are taken into account, sodium was resorbed at a mean rate of 1.88 ± 0.12 meq./hr. during the first hour.

The initial concentration of potassium in the cloacal fluid was 5.4 meq./L, but after one hour the concentration had risen to 12.9 ± 0.5 meq./L, and after three hours to 14.4 ± 0.5 meq./L, as can be seen in Fig. 19. These increases may be explained by change in fluid volume for the most part, but reference to Table 30 shows that in some cases potassium may have been secreted. This is seen more clearly in the samples taken one hour after commencement. After

three hours every animal but one showed that net resorption of potassium was in progress. The reason for this change from secretion to resorption is not clear. Secretion of potassium by the cloaca has also been demonstrated in chickens by Skadhauge (1967).

TABLE 30

The Amount of Potassium in the Cloacal Fluid

Time (hrs)	K (µeq)					
0	108	108	108	108	108	108
1	93	133	161	105	112	161
3	131	116	144	79	83	118

This experiment clearly demonstrates the resorptive function of the cloaca in V. gouldii. The withdrawal of fluid by the cloaca was quite rapid considering that the water movement was solute linked. Faster rates of resorption might be expected under normal conditions as ureteral urine is always hyposmotic. This would presumably result in rapid diffusion of water from the cloaca in response to an osmotic gradient. Even so, the rates of absorption measured in this experiment are sufficient to accommodate the rates of urine flow that were measured in Section 2, and thereby ensure the formation of urinary pellets containing only small amounts of water.

There is not much data available on *in vivo* fluid resorption from cloacae or urinary bladders. Only a few comparisons can therefore be made between *V. gouldii* and other animals.

Dantzler and Schmidt-Nielsen (1966) found that approximately 64 ml. of water diffused from the bladder of a tortoise, *Gopherus*, in 3 ½hours. The weight of this animal was not given, but was somewhere between 1.0 and 3.5 Kg. Thus the rate of resorption was between 5 and 18 ml/Kg/hr, which is of the same order as the rate measured in *V. gouldii*. However, it should be pointed out that a very dilute fluid was used in *Gopherus* whereas isotonic solutions were used in *V. gouldii*.

Nechay and Lutherer (1968) investigated the function of the cloaca in *Gallus domesticus* and concluded that the cloaca appeared to have no active role in salt and water transport. They cannulated one ureter and then collected ureteral and cloacal samples from the same animal. Despite their conclusions, their results show that urine flowed from the cloaca at a rate 19% lower than from the cannulated ureter, and the osmotic concentration in the cloacal urine was 18% higher than in ureteral urine. Since the G.F.R.'s on both sides were equal it can be assumed that the cloaca was resorbing fluid. In fact the difference in flow rate of ureteral and cloacal fluid was 0.25 ml/min., or 15 ml/hr., which represents a resorption rate between 4 and 8 ml/Kg/hr, again a similar rate to that in *V. gouldii*. However, strict comparisons between *V. gouldii* and *G. domesticus* are not possible as the birds were slightly water-loaded, and were also at a higher temperature.

Skadhauge (1967) studied cloacal absorption in anaesthetised chickens by *in vivo* perfusion, and estimated that about 1 ml/Kg/hr of fluid was absorbed, but this low rate could be due to the anaesthetic. On another occasion Skadhauge (1968) measured resorption from the cloaca in unanaesthetised animals by comparing ureteral and cloacal urine. In this study the animals were either water-loaded, salt-loaded or dehydrated, and under these conditions the kidney probably plays a more important role in regulating the final concentration of the urine. Even so, 0.5 ml/Kg/hr of water was resorbed against an osmotic concentration gradient in dehydrated animals. It could be that resorption by the cloaca is more evident in animals that are not producing a concentrated urine or excreting a water load.

The work concerned with cloacal function in birds is confusing, as some workers have found no resorptive activity by the cloaca (Hester *et al.* 1940; Dixon, 1958). However it is surprising that more attention has not been paid to the fact that many birds are able to eliminate a semi-solid mass of urine that is totally different from ureteral urine in appearance and volume. There can be little doubt that the cloaca in many birds is capable of modifying the urine to a great extent.

As the cloaca in *V. gouldii* is concerned with conservation of water, an experiment was designed to determine the effects of arginine vasotocin on the resorption of fluid by the cloaca.

Experiment 14

The Effect of Antidiuretic Hormone on the Absorption of Fluid by the Cloaca

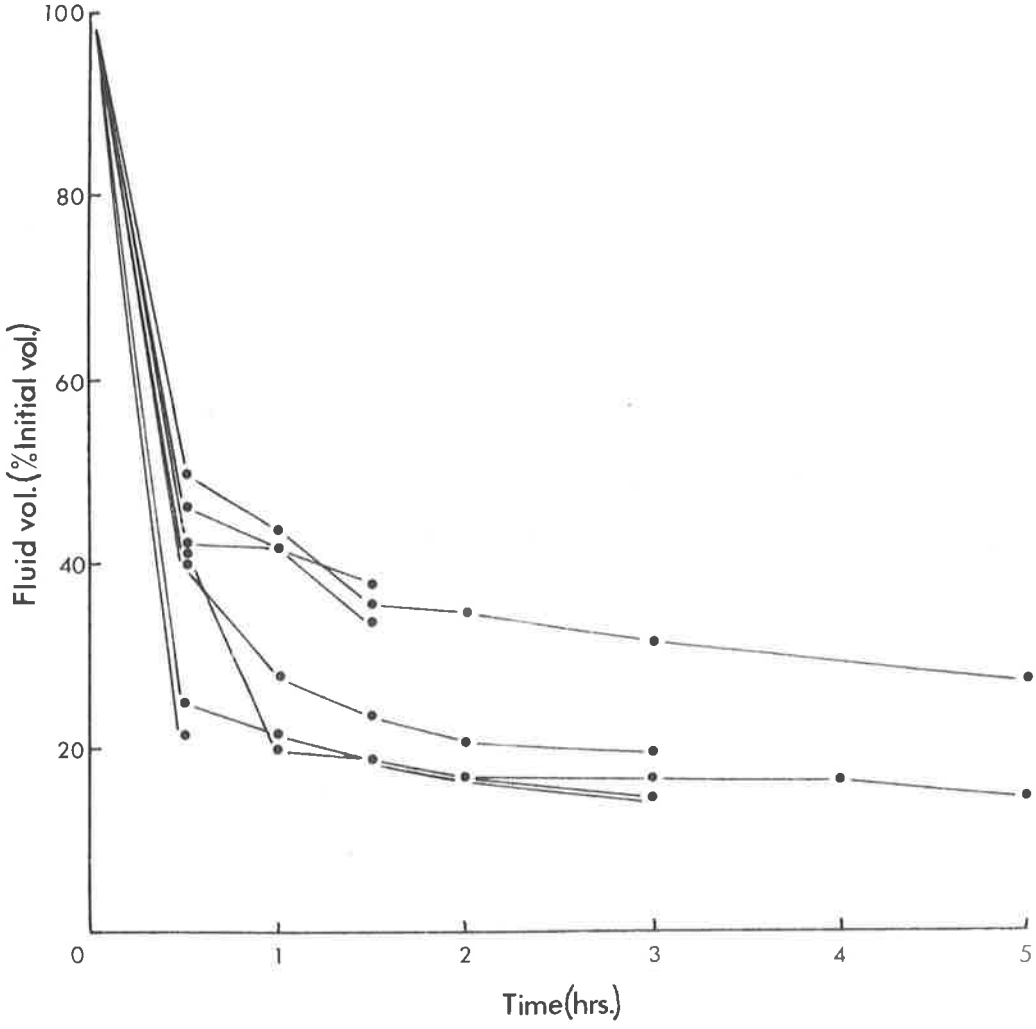
Seven animals were prepared and set up the same way as in the previous experiment. The results for the animals in Experiment 13 were used as control values for this experiment. Before the introduction of saline into the cloaca, each animal was given an injection of AVT into the heart, (0.1 ml/Kg. body weight of a solution containing 1 µg/ml). Fifteen minutes were allowed for circulation of the hormone and then saline was introduced into the cloaca.

Preliminary observations indicated that the rate of absorption in AVT injected animals was much faster than in control animals, therefore samples of cloacal fluid were taken every half-hour for two hours, and then every hour. In some cases only two or three samples could be obtained as little fluid remained in the cloaca after this time.

The changes in fluid volume in the hormone treated animals are shown in Fig. 17. Over half of the initial volume was absorbed within the first half-hour, after which the rate of absorption decreased rapidly. This decrease was similar to that seen in control animals after half of the fluid had been absorbed. Fluid was absorbed at a rate of 20.57 ± 1.34 ml/Kg/hr during the first half-hour which is a much greater rate than in the controls. This is partly because samples were not taken as frequently in the

FIGURE 17

Changes in the volume of fluid in the cloaca of AVT-injected animals


100
80
60
40
20
0
1
2
3
4
5
Fluid vol.(%Initial vol.)
Time(hrs.)

control group, so that the control rate may actually be a little higher. However this is not thought to be 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-hour.

Even so, there is still a significant difference between the two groups in the amount of fluid absorbed within the first hour ($p < 0.02$) as shown in Table 31.

TABLE 31

Volume of Fluid Absorbed After 1 Hour

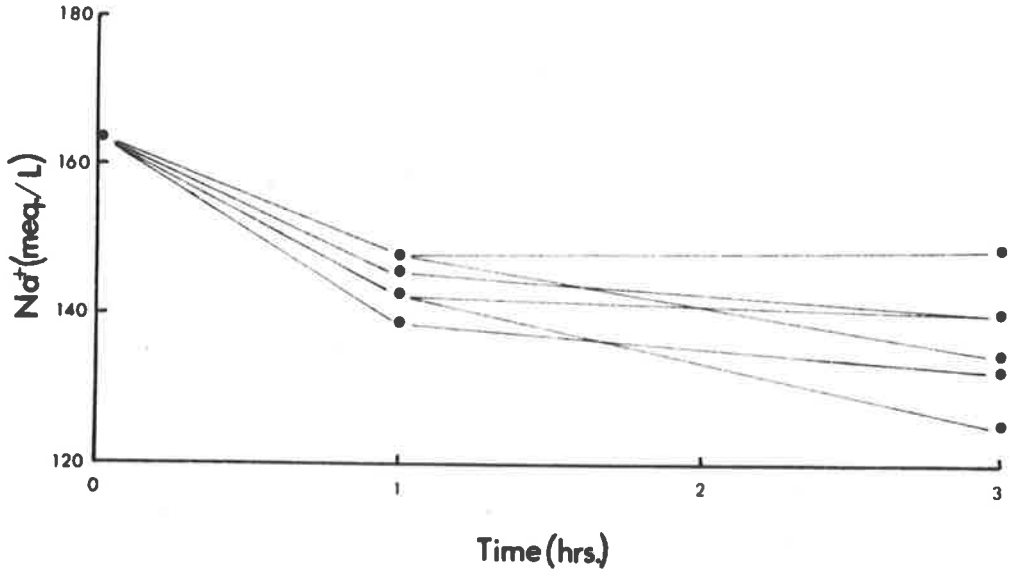
Treatment	n	$\bar{x}$	Var.	V.R.	t_{10}	p
Control	6	10.13	3.506	1.273	3.167	<0.02
AVT	6	13.37	2.754			

Changes in the concentration of sodium in the cloacal fluid of AVT injected animals are shown in Fig. 18. After one hour the mean concentration was 161 $\pm$ 5 meq./L which is not significantly different from the initial concentration. However, after three hours the mean concentration of sodium had fallen to 142 $\pm$ 5 meq./L. The concentrations of sodium in the cloacal fluid of both groups of animals were compared with t-tests that are set out in Tables 32 and 33. In fact a modified t-test (d-test) was used in comparing the samples taken at one hour, as the variance ratio was significant.

FIGURE 18

Concentration of sodium in the cloacal fluid of control animals

Concentration of sodium in the cloacal fluid of AVT-injected animals


180
160
140
120
Na+(meq./L)
0
1
2
3
Time (hrs.)

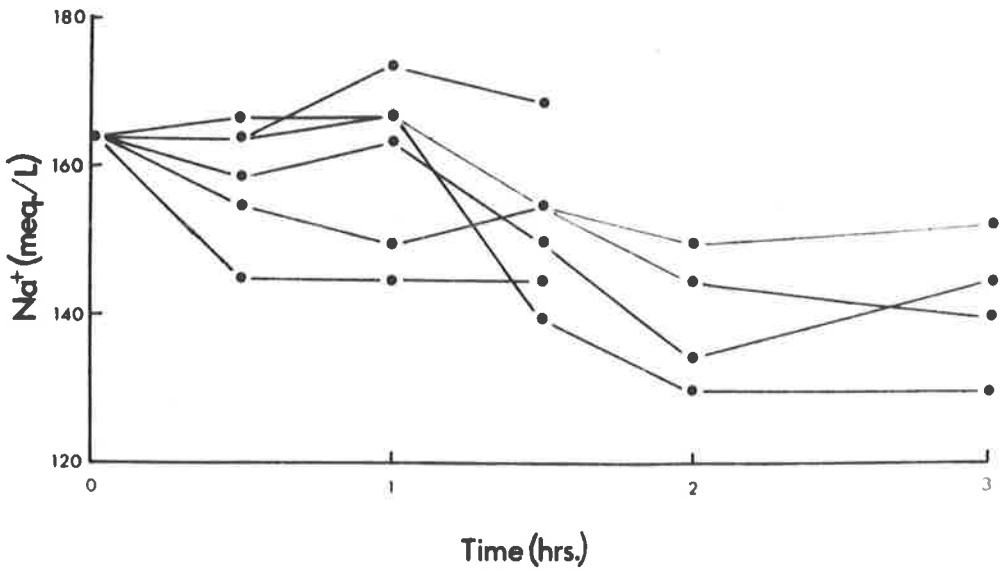

180
160
140
120
Na+ (meq./L)
0
1
2
3
Time (hrs.)

TABLE 32

Concentration of Na at 1 Hour

Treatment	n	$\bar{x}$	V.R.	t_9*	p
Control	6	144.5	10.279	3.487	< 0.01
AVT injected	6	161.2			

* f = 9.35 (u = 0.63142)

TABLE 33

Concentration of Na at 3 Hours

Treatment	n	$\bar{x}$	V.R.	t_8	p
Control	6	137.7	1.939	0.872	> 0.4
AVT injected	4	142.0			

A significant difference was found between the treatments at one hour ($p < 0.01$), but the difference was not significant at three hours ($p > 0.4$). Therefore, the injection of AVT not only increased the rate of sodium transport but also increased the permeability of the cloaca to water. This is indicated by the sodium concentration at one hour being the same as in the initial sample in AVT injected animals, whereas in the controls, the sodium concentration had

decreased. After an hour or so the effect of the AVT disappeared, as the sodium concentration decreased to the same level as the controls.

Sodium was transported at a rate of 4.26 $\pm$ 0.25 meq/hr., in the first half-hour which is more than double the rate found in control animals.

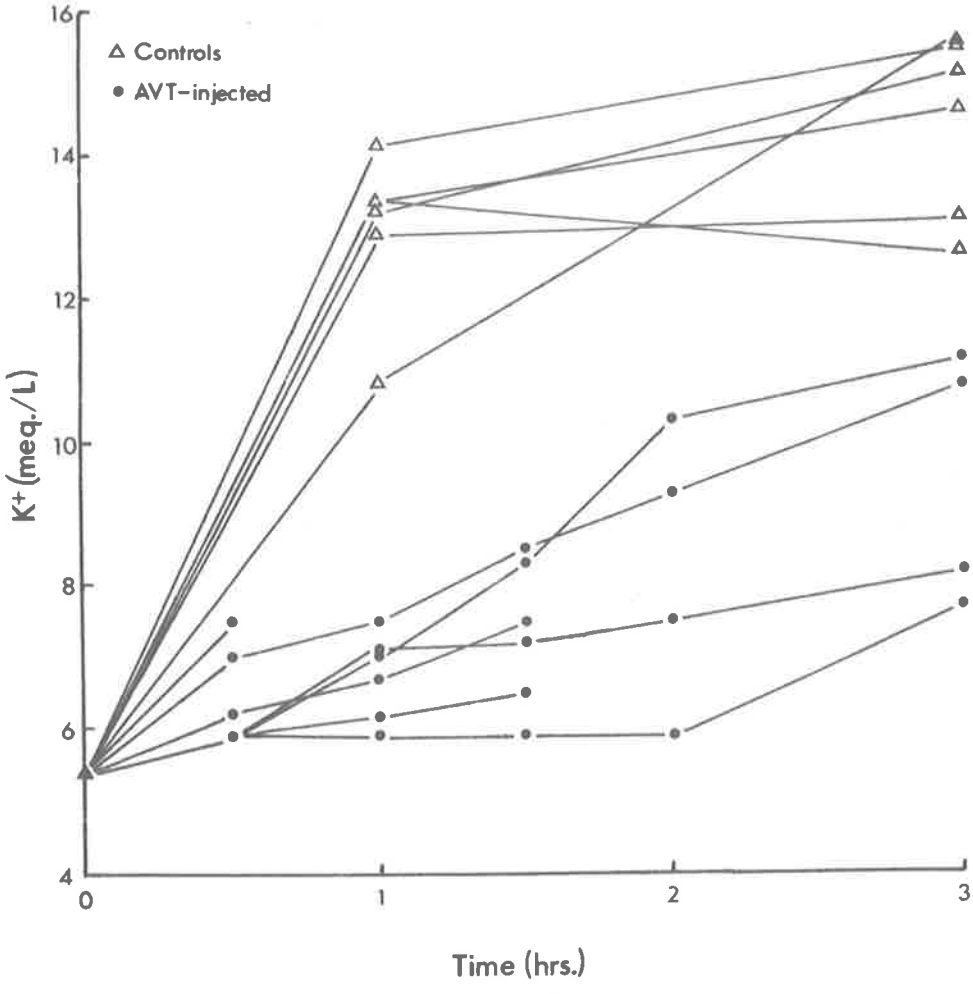
The concentration of potassium in the cloacal fluid of the AVT injected animals increased only gradually as shown in Fig. 19. Statistical comparisons between the two treatments were not undertaken, as the concentrations of potassium were higher in the control group in every case. The slow increase in the potassium concentration in the AVT treated animals was due to absorption of potassium in the first hour or so of the experiment. Most activity took place in the first half-hour when the mean rate of transport was 131 $\pm$ 8 ueq/hr.

This experiment, therefore shows that AVT enhances sodium transport across the cloacal wall. The effect of the hormone on water permeability was not studied in detail, but there were indications that the cloaca becomes more permeable to water in the presence of AVT.

In contrast, Bentley (1962) found that AVT did not increase the rate of sodium transport or the permeability to water in isolated urinary bladders of the tortoise Testudo graeca, however there were increases in response to aldosterone. It would be interesting to see how the cloaca and urinary bladder in other reptiles respond to both AVT and aldosterone.

FIGURE 19

Concentration of potassium in the cloacal fluid of control and AVT-injected animals


△ Controls
• AVT-injected
16
14
12
10
8
6
4
K+ (meq./L)
0
1
2
3
Time (hrs.)

It is surprising that so little attention has been paid to the cloaca in view of its importance in water conservation. The resorptive function of the cloaca has often been overlooked or ignored, particularly in renal studies where urine has been collected from the cloaca. Dawson et al. (1966) estimated urinary water loss in three Australian lizards by measuring the weight of urine accumulated in the cloaca over a known period of time. Consequently, these estimates are probably higher than rates of urinary water loss, and lower than rates of urine flow because of resorption of fluid by the cloaca. Shoemaker et al. (1967) estimated rates of urine production in Phyllurus milii and Amphibolurus barbatus in a similar way.

Bentley (1959) and Bradshaw (1965) have both suggested that reptiles in arid regions become almost anuric during summer on the basis of the difficulty experienced in collecting urine from the cloaca or bladder at this time. It is probable that fluid is rapidly resorbed from the urine in summer, and therefore the absence of urine does not necessarily indicate cessation of renal function.

Another field of research where cloacal function has been disregarded is that of extra-renal excretion of electrolytes. Dunson and Taub (1967) used indwelling cloacal catheters in their experiments on Laticauda to determine the excretion of electrolytes from the cloaca. In this situation there was no opportunity for the cloaca to resorb electrolytes from the urine, and therefore the values for extra-cloacal excretion given by Dunson and Taub may be too high.

Clearly then, care must be taken in the interpretation of results that are obtained without due regard to the effect of the cloaca on the composition and volume of urine.

Precipitation of Uric Acid

Dantzler and Schmidt-Nielsen (1966) found that ureteral urine in Pseudemys scripta and Gopherus agassizii was devoid of uric acid precipitate, but the presence of uric acid was indicated by a positive murexide reaction. They concluded that the uric acid was probably in the form of soluble urates, as the pK value for uric acid is 5.6 whereas the pH of the urine was about 6.7. The ureteral urine of V. gouldii is also clear in normal and water-loaded animals, the pH ranging from 6.0 - 7.1. In salt-loaded animals, however, the pH was between 5.8 and 6.4 and here some precipitated uric acid was present.

The pH of urine in different regions of the cloaca was measured in five V. gouldii. Each animal was injected with a fatal dose of Nembutal and dissected while the heart was still beating. The different regions of the cloaca were then isolated by tying ligatures, and when this had been done the cloacal wall in each region was slit open and small strips of pH indicator paper (B.D.H. narrow range) were pushed into the lumen of the cloaca. The results are presented in Table 34.

TABLE 34

pH of Rectal and Cloacal Contents

Rectum	Ant. coprodaeum	Post. coprodaeum	Urodaeum
	5.8	5.8	
7.0	5.2	5.8	
5.5	5.8	6.1	6.4
4.5	5.5	6.1	6.7
6.7	5.8	5.8	6.4

It is therefore clear that urine is acidified as it passes into the coprodaeum. The reduced pH of the coprodaeum is probably due to secretion of acid by the numerous goblet cells of the mucosal epithelium. Precipitation of uric acid is promoted by acidic conditions and resorption of fluid in the cloaca. A similar situation has been described in the insect Dixippus morosus by Ramsay (1955). Where soluble potassium urate is secreted into the lumen of the Malpighian tubules, and on reaching the rectum, uric acid is precipitated by resorption of water and acidification of the urine.

Therefore nitrogen excretion in terrestrial reptiles consists of the tubular secretion of soluble urates which are converted into insoluble urates and uric acid in the cloaca before elimination. In this way the kidney tubules are not occluded by uric acid crystals, and yet the full benefits of water conservation associated with uricotelism are retained. Clearly, therefore, the resorptive function of the cloaca is of prime importance in the excretion of

uric acid.

Experiment 15

Water Content of Faecal and Urinary Pellets

Urinary and faecal pellets are only expelled after most of the fluid has been resorbed, but some water still remains in the pellets. The amount of water lost with the pellets was measured in the following way.

Pellets were collected between 9.00 and 10.00 a.m. each day from open terraria. Collection was confined to cool days and in this way pellets were obtained in a fairly fresh condition without excessive evaporation having taken place. Pellets voided after this time were not collected, and were discarded at the end of each day.

The faecal and urinary pellets were quite distinct and easily separated. Each pellet was immediately weighed on a Mettler H16 balance to the nearest 10 mg. in a glass petri-dish, and then placed in a drying oven at 103°C for 24 hours.

The petri-dishes and their contents were reweighed, and the difference in weight represented the water content of the pellets. Sand particles from the terraria that adhered to the pellets were removed after reweighing the dried pellets. These said particles were then weighed with the petri-dish to determine the dry-weight of each pellet.

The values obtained are presented in Table 35.

TABLE 35

Water Content of Urinary and Faecal Pellets

Faeces			Urine		
Dry Wt. (g)	Water (g)	Water (% Fresh Wt.)	Dry Wt. (g)	Water (g)	Water (% Fresh Wt.)
3.06	10.66	77.7	1.82	1.17	39.2
1.59	4.60	74.3	1.90	1.36	41.6
2.03	6.24	75.5	1.83	1.34	42.3
1.85	5.54	75.0	0.59	0.64	52.0
1.08	3.16	74.5	1.60	1.30	44.8
0.31	1.81	85.4	0.68	0.97	58.8
2.11	5.99	74.0	1.43	1.76	55.2
		$\bar{x}$ = 76.6 ± 1.5%			$\bar{x}$ = 47.7 ± 2.9%

The water content of the urinary part of the pellets is quite high compared, for example, with the gecko Hemidactylus flaviviridis, where only 10% of the fresh weight is due to water (Seshadri 1956). However the V. gouldii used in these observations had access to food and water at all times, and presumbably were not dehydrated. It is possible that dehydrated animals remove more water from the pellets.

Even so the amount of water lost in the excretion of uric acid is very small. This can be best shown by comparing the amounts of water required to excrete a given weight of nitrogen in the form of either urea or uric acid, the two major excretory products of terrestrial vertebrates.

It has been shown that the urinary pellets of V. gouldii contain water to the extent of 47% of the fresh weight. Approximately 90% of the dry weight of urinary pellets is due to uric acid in Varanus monitor and 5% is due to creatine and creatinine (Seshadri 1959). The composition of urinary excreta is probably similar in V. gouldii. However, to simplify some approximate calculations it will be assumed that the dry weight of urinary pellets represents uric acid only.

It can then be calculated that each gram of uric acid that is eliminated is accompanied by the loss of 0.9 ml. of water. As each gram of uric acid contains 0.33 g. of nitrogen, it follows that each gram of nitrogen excreted is associated with 2.7 ml. of water. It was pointed out earlier that it is quite likely that when dehydrated even more water is withdrawn from the pellets before expulsion. If this is the case this estimate of urinary water loss is probably maximal. The urinary pellets of Hemidactylus contain water equivalent to only 10% of the fresh weight (Seshadri 1956), and here only 0.3 ml. of water is lost with each gram of nitrogen.

Each gram of urea contains 0.47 g. of nitrogen, and if this compound was excreted by a reptile in isosmotic form over 100 ml. of water would accompany each gram of nitrogen excreted. Clearly then, urea can only be efficiently excreted by terrestrial vertebrates if a concentrating kidney is present.

Although mammals are able to excrete urea in high concentrations, the nitrogen in urea still requires more water for excretion than the nitrogen in uric acid. The highest concentration of urea in the urine

recorded so far is that of the rodent Notomys alexis, where concentrations as high as 5429 mM urea/L are found (Macmillan and Lee 1969). Thus each gram of nitrogen eliminated by Notomys involves the loss of only 7 ml. of water or so, but this is still more than double the amount lost by V. gouldii for each gram of nitrogen, and 20 times that lost by Hemidactylus.

The water content of faecal pellets is high compared with the faeces of a snake Constrictor constrictor (Benedict 1932) and mammals such as the camel Camelus dromedarius (Schmidt-Nielsen et al. 1956) and the kangaroo rat Dipodomys merriami (Schmidt-Nielsen and Schmidt-Nielsen 1951). Again it is likely that the water content of faeces could be reduced in dehydrated animals, as Benedict's data shows that the water content of faeces in C. constrictor ranged from about 77% down to 47%. Therefore, although the rectum resorbs fluid from the faeces, it does not appear to be specially modified in any way as an adaptation to arid environments.

SUMMARY

(1) The cloaca and rectum are structurally adapted, by the presence of folds, villi and microvilli, to provide a large surface area for the resorption of fluid.

(2) Fluid is withdrawn from the cloaca at a rate that can accommodate the renal production of urine.

(3) Fluid moves out of the cloaca by the transport of sodium from mucosa to serosa, and the osmotic diffusion of water. This is probably true for rectal resorption also.

(4) The antidiuretic hormone AVT increases the rate of sodium transport and therefore enhances the withdrawal of fluid. The permeability of the cloaca to water is also increased by the hormone.

(5) Potassium is normally secreted to a limited extent, but AVT leads to resorption of this ion immediately after administration.

(6) The acidity of the cloaca and the resorption of fluid promote precipitation of uric acid from the urine. The deposits of uric acid are stored in the cloaca until a hard pellet is formed.

(7) A considerable amount of water is conserved by excreting pellets of uric acid.

Section 4

EXCRETION OF ELECTROLYTES

BY THE NASAL GLANDS

INTRODUCTION

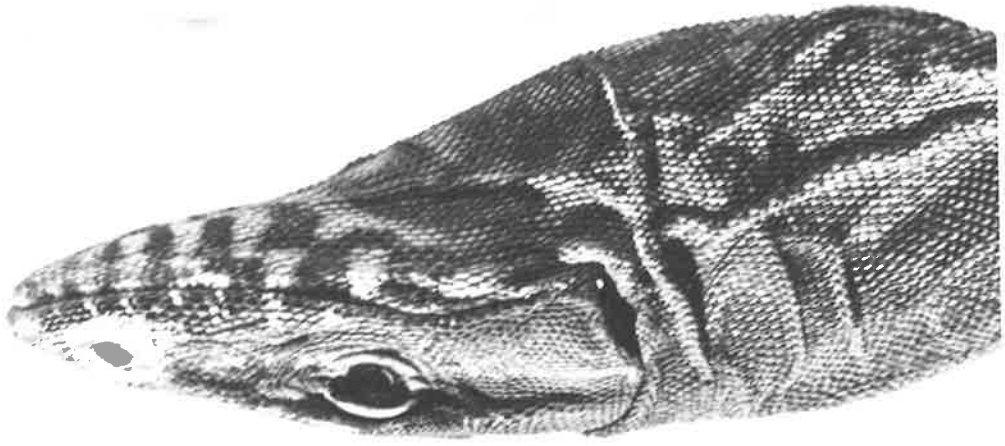
The extra-renal excretion of electrolytes via salt glands has been reported for many marine birds and reptiles. Functional nasal salt glands have also been found in several terrestrial reptiles; Iguana iguana, Dipsosaurus dorsalis and Uromastyx aegyptus (Schmidt-Nielsen et al. 1963), Ctenosaura pectinata and Sauromalus obesus (Templeton 1964, 1967), Sauromalus spp. (Norris and Dawson 1964), Dipsosaurus dorsalis and Sceloporus cyanogenys (Templeton 1966) and Conolophus suboristatus (Dunson 1969).

In the course of salt-loading V. gouldii in one of the renal experiments, a discharge of fluid was noted around the external nares of several animals. The fluid rapidly changed into a white encrustation, as is shown in Fig. 20. These encrustations were collected and analysed for electrolytes.

The nasal salt gland in V. gouldii consists of two lobes that are separated from each other by a thin septum in the midline of the nasal chamber. The gland lies immediately anterior to the eyes, and each lobe drains into the nasal chamber through a large duct. No attempt was made to study the finer structure of the gland in V. gouldii.

FIGURE 20

Encrustation of salt around the external nares



MATERIALS AND METHODS

Seven animals were each given an intraperitoneal injection of a 5M NaCl solution (2 ml/Kg.) on each of four days. The animals were housed in cages and provided with heating lamps. The lamps were found to be necessary for promoting secretion from the glands. In most animals the nasal gland started to secrete after the second injection, but others did not do so until after the fourth.

Attempts were made to collect the fluid directly from the nares so that the concentration of electrolytes in the secretion could be determined. However, the volume of fluid was never sufficient to allow this, and so the secretion was allowed to evaporate to dryness, and the resulting deposits around the external nares were scraped off with a scalpel. The scrapings were collected on a clean piece of stiff paper, and transferred into small glass vials.

The vials were placed in an oven at 103°C for twenty-four hours, to completely dry the samples. When this had been done the samples were allowed to cool before they were weighed on a Mettler H16 balance to the nearest 0.1 mg.

Each sample was then dissolved in 1 ml. of double distilled water, and 10 µl of this solution was diluted in another 1 ml. of double distilled water. The concentrations of sodium and potassium were then measured with an EEL flame photometer. The chloride concentration was determined with an EEL chloride meter using 200 µl portions of the initial solutions.

Experiment 16

An Analysis of Secretion from the Nasal Gland

A total of eleven samples of nasal secretion were obtained from the seven animals, and the results of the analyses are shown in Table 36. It can be seen that chloride is probably the only anion in the secretion. The discrepancy between the total weight of ions measured and the original weight of the secretion might indicate the presence of small amounts of other electrolytes, but it is more likely due to particles of skin that were scraped off with the secretion and were therefore included in the initial weight.

The sodium/potassium ratios for the nasal secretions are shown in Table 36. The amount of sodium in the samples exceeded potassium in all but two cases, one where the amounts were equal and the other where potassium predominated. In many samples the ratio was close to unity despite the fact that the animals were loaded with sodium. It is therefore possible that in other circumstances potassium is the major cation in the nasal secretion of V. gouldii.

TABLE 36

Analysis of Nasal Secretions

Animal	Wt. of sample (mg)	Na (mg)	K (mg)	Cl (mg)	Total wt. of electrolytes (mg)	Na/K
1	3.0	0.67	0.42	1.56	2.65	1.60
	3.0	0.62	0.50	1.70	2.82	1.24
2	3.7	0.82	0.82	1.95	3.59	1.00
	1.5	0.39	0.26	0.85	1.50	1.50
3	3.5	0.93	0.34	2.02	3.29	2.74
	3.5	1.08	0.46	2.09	3.63	2.35
4	3.0	0.90	0.22	1.60	2.72	4.09
	3.7	0.95	0.46	1.99	3.40	2.07
5	2.1	0.10	0.70	0.64	1.44	0.14
6	5.2	1.18	0.88	2.88	4.94	1.34
7	7.4	1.54	1.24	3.98	6.76	1.24

It was not possible to measure the concentration of electrolytes in the secreted fluid immediately after secretion, or for that matter the fluid emerging from the external nares. However, in view of the rapid deposition of enonisation on the outer surface of the snout, it can be inferred that the nasal secretion is highly concentrated. Where direct measurements have been made on nasal secretion by

cannulation, as in Caretta caretta (Schmidt-Nielsen and Fange 1958) and C. pectinata and S. obesus (Templeton 1964, 1967), the secretion has been shown to be much more concentrated than the plasma.

In most terrestrial reptiles that have been studied so far potassium is the major cation that is secreted from the salt glands under normal conditions. However, the land iguana Conolophus mainly secretes sodium under natural conditions (Dunson 1969).

Even when loaded with sodium chloride solutions, some terrestrial reptiles still produce a secretion in which potassium is the predominant cation e.g. Sauromalus spp. (Norris and Dawson 1964), S. cyanogenys (Templeton 1966) and C. pectinata and S. obesus (Templeton 1967), but others secrete mainly sodium under those conditions e.g. I. iguana (Schmidt-Nielsen et al. 1963), D. dorsalis (Templeton 1966), although the Na/K ratios were close to unity. It has been shown that V. gouldii also secretes mainly sodium when loaded with NaCl solution.

It seems therefore that terrestrial reptiles vary considerably in their ability to regulate the composition of the nasal secretion. Some animals are able to secrete the cation that predominates in the load, whereas others continue to secrete potassium even when loaded with sodium.

The predominance of potassium in the nasal secretions of many terrestrial reptiles is believed to be due to the high concentrations of potassium in the plant food of the animals studied (Norris and Dawson 1964, Schmidt-Nielsen et al. 1963). However, V. gouldii is

carnivorous and therefore might not be expected to encounter potassium loads as readily as a herbivorous reptile would. A nasal gland that secreted mainly sodium or potassium, depending on the relative concentrations of these cations in the plasma, would be of greater advantage in this situation. The results of this experiment indicate that a variable nasal gland such as this is present in V. gouldii. Further experiments on the salt gland of V. gouldii would be most interesting, particularly the response to KCl loading.

The amount of sodium excreted in the few days over which samples were collected, was only a small fraction of that which had been injected. However, it must be remembered that the animals were loaded with a large amount of salt over a very short period of time, and this in no way represents the normal situation under which electrolyte loading occurs. Under natural conditions the salt gland probably functions in a quite discrete fashion.

Section 5

FIELD STUDIES

INTRODUCTION

Studies of V. gouldii in the laboratory allowed useful comparisons to be made with other species. However, it was necessary to study the animal in the wild in order to obtain a more relevant description of water and electrolyte balance in the animal. The populations in two widely different areas were compared in this study.

The amount of water that an animal loses in the field is dependent upon the severity of the environment, the length of time that the animal is exposed to these conditions, and the activity of the animal. Therefore, it is important to understand the nature of the climate and micro-climate of the situations where the animal is found, as well as the behaviour of the animal. These facets of the biology of V. gouldii were consequently studied.

In addition, an attempt was made to determine the water requirements of the animal in the field. It was anticipated that the ability to maintain electrolyte balance would be revealed by comparing the concentrations of electrolytes in the plasma throughout the year and at different localities.

By making regular field trips sufficient comparative data was obtained to indicate the relative importance of the various mechanisms controlling water and electrolyte balance.

FIELD STUDY AREAS

Field studies were conducted at two localities in South Australia; at Flinders Chase and at Renmark. Flinders Chase is a flora and fauna reserve occupying approximately 220 square miles of the extreme western end of Kangaroo Island. The study area was centered around the Rocky River Homestead where a permanent research station was established. Animals were mainly collected on a system of firebreaks that surround the homestead and extend along both sides of a road leading to the West. The firebreaks around the homestead were between 50 and 60 yards wide and extended for the same distance along the sides of the road. The area of firebreak that was regularly searched measured approximately 50 acres, but an undetermined area of scrub adjacent to the firebreak was also examined. Searching was also carried out in about 110 acres of scrub and cleared paddock that were enclosed by the homestead firebreak.

Field work at Renmark was conducted on a part of Calperum sheep station about 7 miles WNW of Renmark. There was no permanent research station at this site. The study area measured approximately 4 square miles and was composed of parallel sand hills between which were hard sandy flats. Searching was confined mainly to the sand hills, as this was where most animal activity was seen.

The vegetation of both localities has been described by Wood (1937). The vegetation at Flinders Chase is mainly dry sclerophyll

forest, the dominant species in the association being Eucalyptus diversifolia. At Renmark the vegetation is sparse and of the mallee scrub type with little ground vegetation. The dominant species in this association are E. oleosa and E. dumosa.

The aspects shown in Figs. 21 and 22 are typical of the field areas.

CLIMATE

The climate of Kangaroo Island has been described by Gentilli (1948) as warm and subhumid with temperate dry summers. The mean annual rainfall at Flinders Chase, based on recordings over 24 years is 28.34". Monthly rainfall data for the years in which field work was conducted are presented in Table 37. It can be seen that the winter of 1967 was quite dry while that of 1968 was much wetter. The summer of 1967-68 was much drier than that of 1966-67.

Renmark is in an area described as semi-arid by Gentilli (1948) and Meigs (1954). The summers are hot with little precipitation and the mean annual rainfall, based on records for 75 years is 10.33. Rainfall data for Renmark is also included in Table 37, where it can be seen that 1967 was a year of very low rainfall.

FIGURE 21

General aspect of field area at Flinders Chase

FIGURE 22

General aspect of field area at Renmark

TABLE 37

Rainfall at the Two Study Areas

Renmark

Year	J	F	M	A	M	J	J	A	S	O	N	D	Total
1966	26	127	10	21	50	36	84	51	40	134	40	268	8.87"
1967	46	209	11	0	35	29	56	88	20	8	0	12	5.14"
1968	93	20	44	102	102	146	108	159	27	69	43	155	10.68"

Mean Annual Rainfall (75 years) = 10.33"

Flinders Chase

Year	J	F	M	A	M	J	J	A	S	O	N	D	Total
1966	25	33	179	128	372	426	766	443	512	195	88	645	38.12"
1967	46	379	90	99	145	84	570	358	264	78	44	64	22.21"
1968	63	89	376	601	608	351	486	735	283	227	153	93	40.65"

Mean Annual Rainfall (24 years) = 28.34"

(Data from Commonwealth Bureau of Meteorology)

Information on the mean monthly maximum temperature was not available for either of the field areas and so data for Kingscote and Berri are presented in Fig. 23, although Kingscote is approximately 60 miles East of Flinders Chase, and Berri about 13 miles West of Renmark, the climate does not differ much from that at the field localities. The data for both sites show that the winter of 1967 was slightly warmer than that of the following year, and also that the summer of 1967-68 was hotter than the preceeding summer.

The rainfall and temperature data presented here show quite clearly how the two study areas differ in climate. This, together with the obvious difference between the seasons in the successive years in which this study was made, provided an excellent opportunity to investigate the effects of climate on the behaviour and physiology of V. gouldii under natural conditions.

FIELD TECHNIQUES

Field trips at Flinders Chase were generally one week long, and were conducted at intervals of between four and six weeks throughout the year. At Renmark field work could only be done during Spring and Summer, and trips were generally longer than at Flinders Chase.

Capture Techniques

Four methods were used to capture animals in the field:

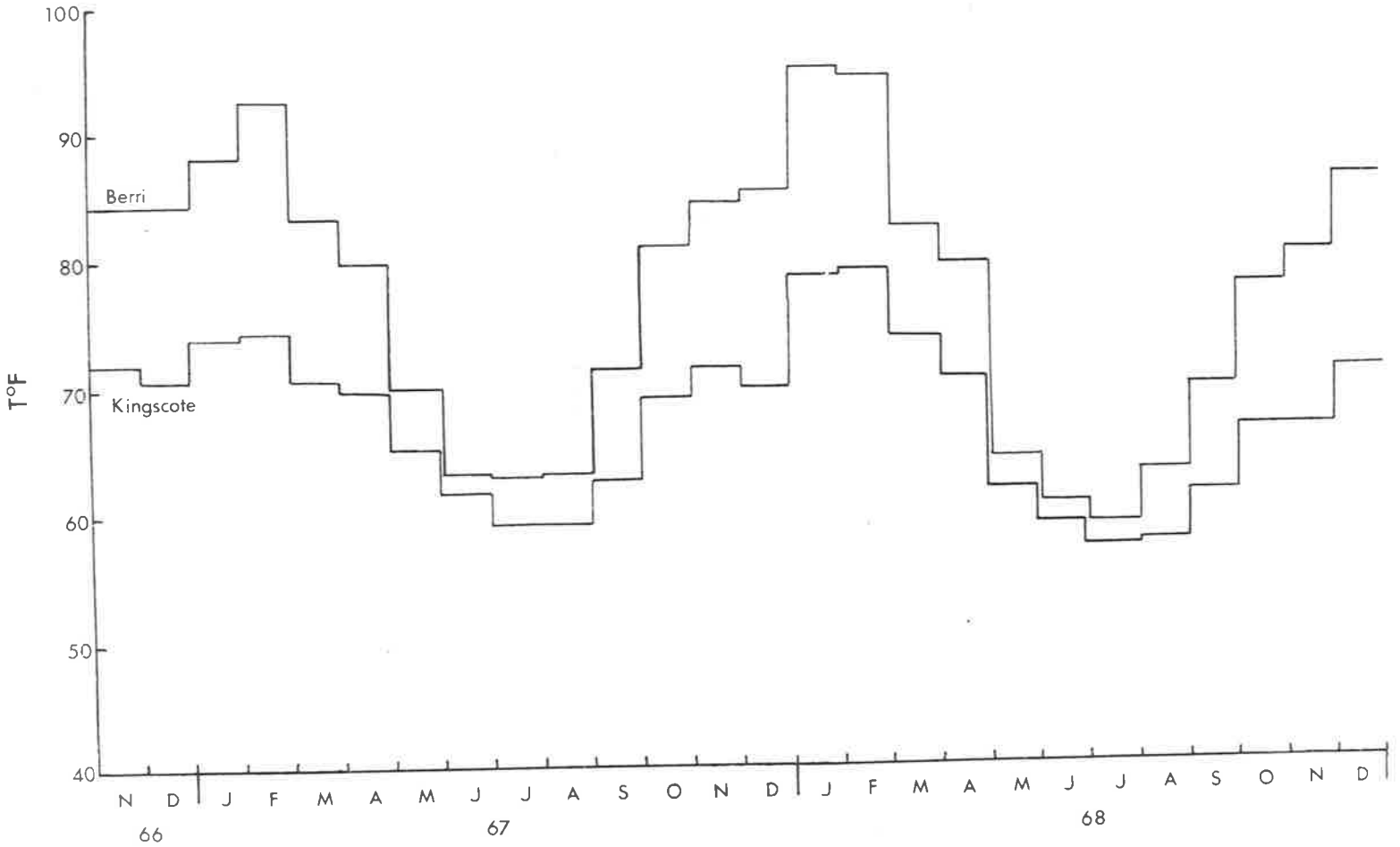
(1) Bait-trapping
(2) Burrow-trapping
(3) Digging out burrows
(4) Noosing

FIGURE 23

Mean monthly maximum temperatures at localities

near to the two study areas

(Commonwealth Bureau of Meteorology)


100
90
80
70
60
50
40
T°F
Berri
Kingscote
N D J F M A M J J A S O N D J F M A M J J A S O N D
66
67
68

There was a tendency for different techniques to predominate in each field area mainly due to the differences in behaviour of animals at the two sites.

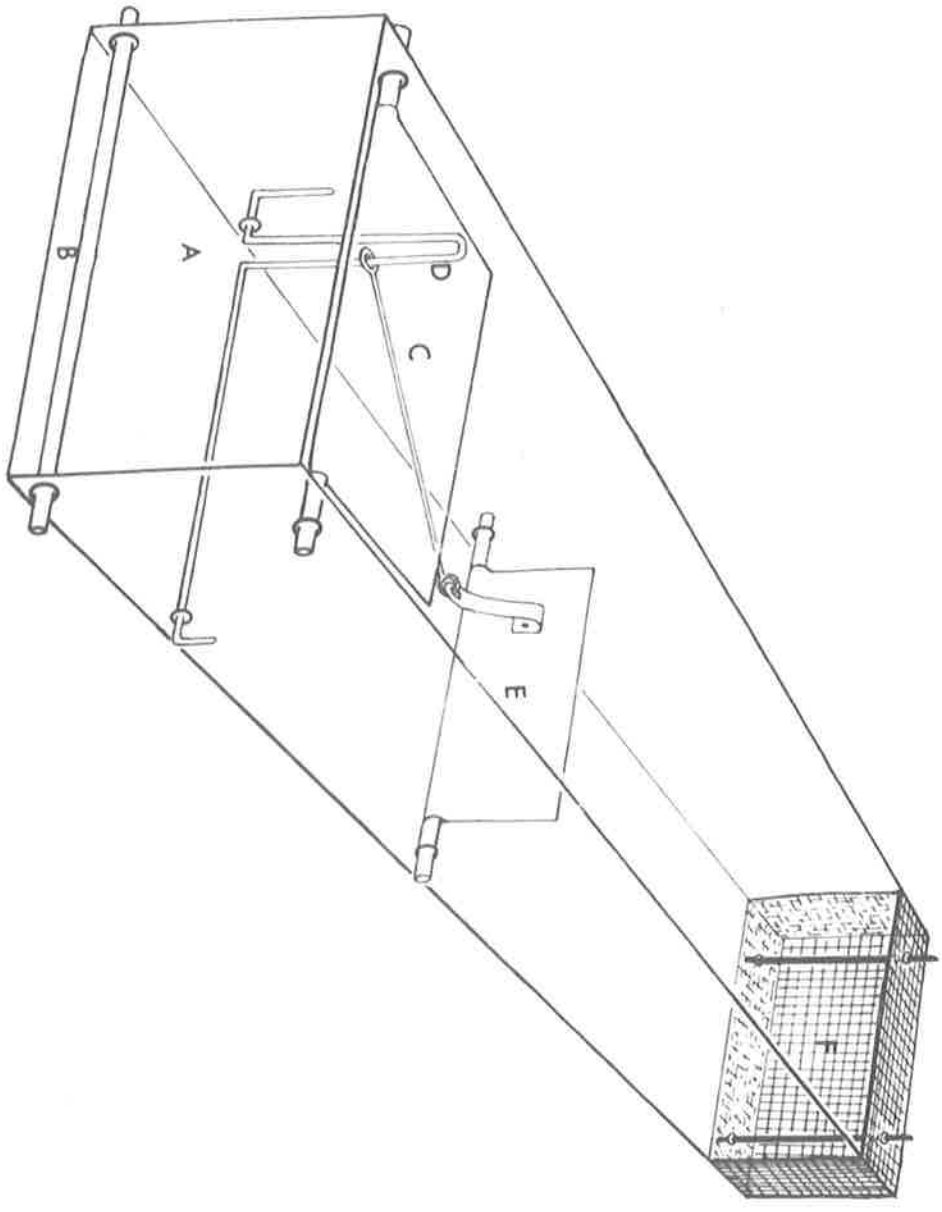
The traps used for bait and burrow-trapping were identical. They consisted of 3" x 4" rectangular metal piping, cut into 3' lengths. One end was sealed over with fine gauge wire mesh, held in place with two metal rods. At the other end a metal door, hinged at the roof, was held open by means of a vertical wire pillar at one side. The pillar was connected by a length of wire to a metal trip-lever that was positioned halfway along the floor of the trap. A diagram of the trap is shown in Fig. 24. An animal moving into the trap depresses the trip-lever, and therefore pulls the pillar inwards allowing the door to drop shut.

When used for bait-trapping the trap was held open with the mouth upward and a piece of bait was dropped deep inside. The trap was then laid on the ground with the pillar supporting the door open. The bait was made by cutting up fish into small pieces, and then allowing the fish to putrefy. This usually took only a day in the hot conditions at Renmark.

For burrow-trapping no bait was used. Here the trap was placed over the entrance of a burrow, care being taken to make sure the door was held open. It was usually necessary to prop the rear end of the trap up with a rock or stump because of the ascending angle of the burrow. Any spaces around the edge of the trap at the burrow mouth were filled with small stones and earth. Animals would crawl straight

FIGURE 24

Diagram of a trap


A
B
C
D
E
F

into the traps as they emerged in the morning.

Sometimes it was not possible to use traps over burrows, or it was unnecessary, and on these occasions animals were captured by digging them out of their burrows. Extra care was required in digging at Renmark because here the animals tended to dig an accessory escape tunnel.

The other major method of capturing animals was by using a 9' pole with a noose of fine nylon rope. On cool days this was a fairly efficient technique, although approaching an animal still required extreme caution. However, this technique was not very successful on hot days as the animals were extremely wary and would run away on approach to within 20' or so.

At Flinders Chase the main methods of capture were burrow-trapping and digging, as here the animals dig their own burrows at all times and the burrows are fairly conspicuous. At Renmark however bait-trapping was used as it was quite difficult to locate active burrows and deep rabbit warrens were commonly used for refuge.

Marking Techniques

Animals were individually identified by removing claws in various combinations with scissors. In most cases only one claw was clipped on any limb, and wherever possible claws on the hind-limbs were clipped in preference to those on the fore-limbs. This was done to prevent too much interference with fossorial behaviour, in which the fore-limbs are mainly used.

Some animals were equipped with radio-transmitters, which were

modified versions of telemetry devices described by Tester et al. (1964). An NPN transistor was substituted in the circuit, and the loop antenna was made into a more convenient size by forming a double loop. The transmitters operated on a frequency of 27.05 MHz, and could be individually identified by the pulse rate of the signal. The components of the transmitters were covered with wax for water-proofing, and then covered with a layer of Araldite epoxy resin to give extra protection. Each transmitter was powered by two Mallory RM1H mercury cells connected in series to provide 2.8 V. The batteries gave a minimum life of five weeks to the transmitters and a range of 100 - 150 yards.

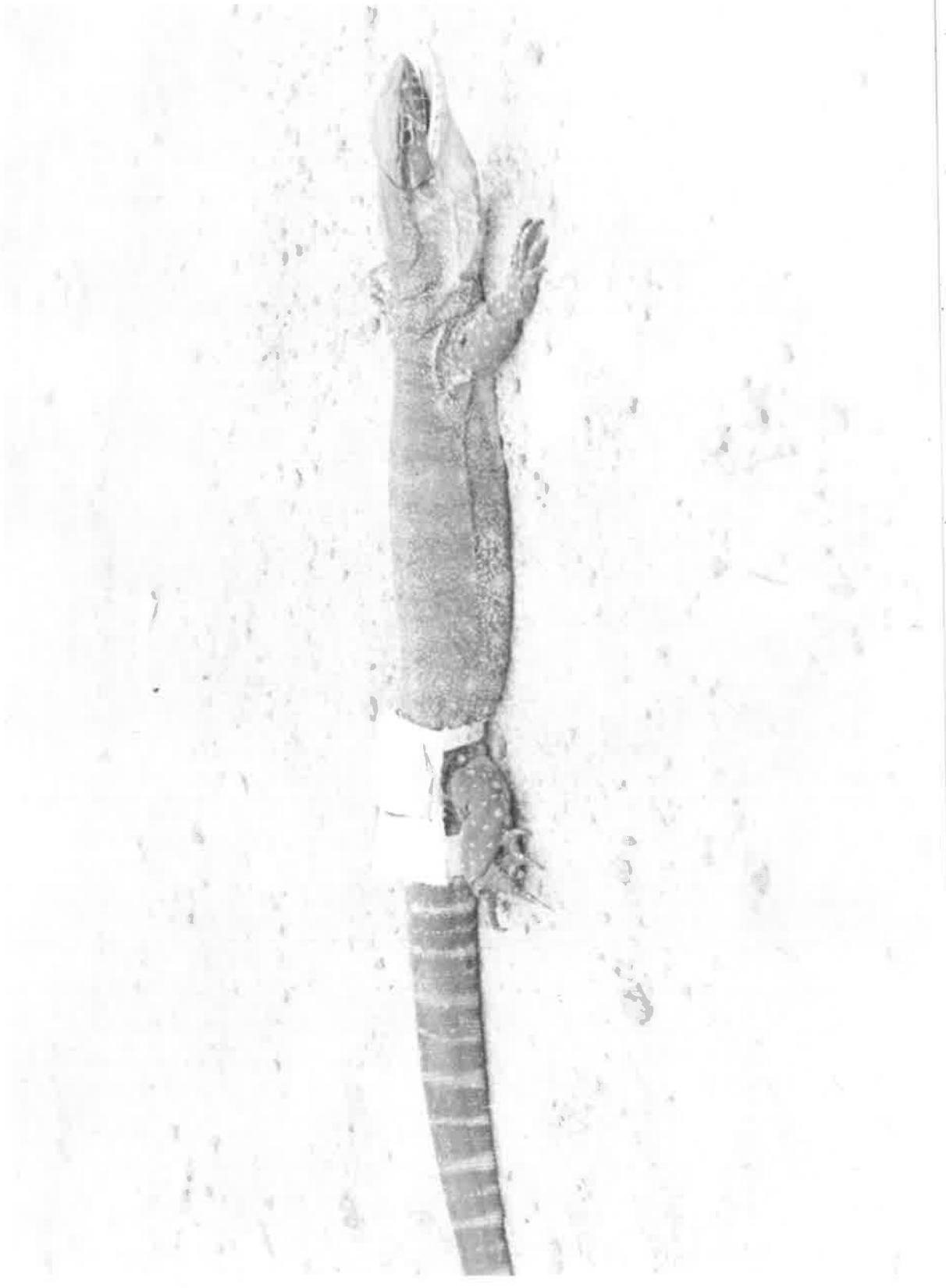
Each transmitter was stitched into a denim harness that had two straps of elastic; the anterior strap of $\frac{1}{4}$" elastic passed around the body of the animal just in front of the hind-limbs, and the posterior strap of 1" elastic passed around the tail immediately behind the cloacal opening. The all-up weight of the transmitter, batteries and harness was about 46 g.

The straps were fastened by hooks and eyes, and the complete transmitter was stuck into place above the pelvic girdle with contact cement. The bond between the skin and the transmitter was very strong and lasted for several months, but transmitters were readily discarded along with the old skin during moulting. The complete transmitter is shown in situ in Fig. 25.

Portable two-way radio receivers were used for locating the transmitters. A system of parallel traverses was followed, each

FIGURE 25

Transmitter in situ



traverse about 200 yards from the next. Animals were usually located within a few minutes of first hearing a signal.

The transmitters did not restrict locomotion in any way, and the animals appeared to carry the extra weight without effort. The only obvious restriction imposed by transmitters was the difficulty encountered by the animals in entering burrows, however, within a few days the animals were found to accommodate the increased height when constructing new burrows. In cases where rabbit warrens were being used this restriction was not imposed.

BURROWS.

Burrows are used by V. gouldii for nocturnal refuge, evasion of predators and thermal refuge. The burrow entrance is usually quite conspicuous with a mound of excavated earth lying immediately outside the entrance. The burrow mouth has a low profile and is semi-elliptical in shape.

The burrow runs in and down at a shallow angle, and usually curves slightly to one side. At the far end of the burrow there is a small chamber where the animal curls up on retiring into the burrow. The chamber also allows the animal to turn around so that it can leave the burrow head-first.

BURROW DIMENSIONS

The length and depth of burrows at Renmark and Flinders Chase were measured to see if there were any differences in the burrows at

these two areas. Burrow length was measured from the mouth to the back wall of the chamber, and depth was measured from the soil surface to the roof of the chamber. All measurements were made to the nearest cm. The data are presented in Table 38, where it can be seen that there is an obvious difference in the depth of burrows at the two areas. No significant difference was found in the length of burrows at the two localities ($p > 0.05$).

TABLE 38

Dimensions of Burrows

Depth (cm)		Length (cm)	
Renmark	Flinders Chase	Renmark	Flinders Chase
19	10	152	78
21	10	122	100
25	6	76	80
27	10	64	75
46	12	102	120
27	10	147	83
30	13	76	90
	10		70
	15		122
	10		107
	10		76
$\bar{x} = 27.68$	10.55	105.57	91.00
S.E. $\pm$ 3.34	$\pm$ 0.68	$\pm$ 13.48	$\pm$ 5.57

TABLE 39

Length of Burrows

	n	$\bar{x}$	Var.	V.R.	t_{16}	p
Renmark	7	105.57	1271.95	3.724	1.155	> 0.05
Flinders Chase	11	91.00	341.60			

THE CLIMATE IN BURROWS

The temperature and relative humidity in burrows was studied at both field localities to determine the nature of the microclimate. Temperature was measured with G.T.14 thermistors and a portable wheatstone bridge, while relative humidity was measured with electro-humidity sensors (Phys-Chemical Research Corp. type 11) and a wide-range resistance bridge. The sensors consisted of a 1.5" x 1" polystyrene plate, the surface of which was impregnated with lithium chloride. Electrical contact with the sensor surface was made by printed carbon electrodes on each side of the plate. Changes in relative humidity caused adsorption or desorption of water from the LiCl, and resulted in corresponding changes in surface resistance.

The humidity sensors were protected with a perspex sheath which was perforated at many points to allow access for the surrounding atmosphere. To prevent the contamination of the sensor with dirt when inserting it into burrows, the sheath and sensor were placed inside a metal cylinder that was open at both ends. The temperature

and humidity sensors were held together with an elastic band, and pushed into the burrow with a rod. Relative humidity and temperature was measured at a point between 45 and 60 cm. from the burrow entrance, as it was not possible to push the sensors deeper than this because the burrows were usually curved.

The P.C.R.C. sensors were calibrated against saturated solutions of known relative humidity (Winston and Bates 1960). The sensors are affected by temperature and also show hysteresis, but allowing for these factors have an accuracy of $\pm$ 3% R.H.

Relative Humidity in Burrows

The relative humidity in burrows was fairly constant throughout the day at both sites, as can be seen in Fig. 26. Although there was little variability in individual burrows, there was great variability between burrows. The relative humidity of several burrows was measured between 1 p.m. and 3 p.m. during Summer. The mean relative humidity in burrows at Renmark was 80.6 $\pm$ 3.8%, (range = 62% to 93%) and at Flinders Chase was 84.8 $\pm$ 2.3%, (range = 69% to 97%). There was no significant difference in the relative humidity of burrows at the two sites ($p > 0.3$) as can be seen in Table 40. On some occasions it was noticed that there was condensation of water on the metal shields surrounding the P.C.R.C. sensor which indicated a saturated atmosphere in the burrow.

FIGURE 26

Relative humidity of burrows throughout the day in Summer

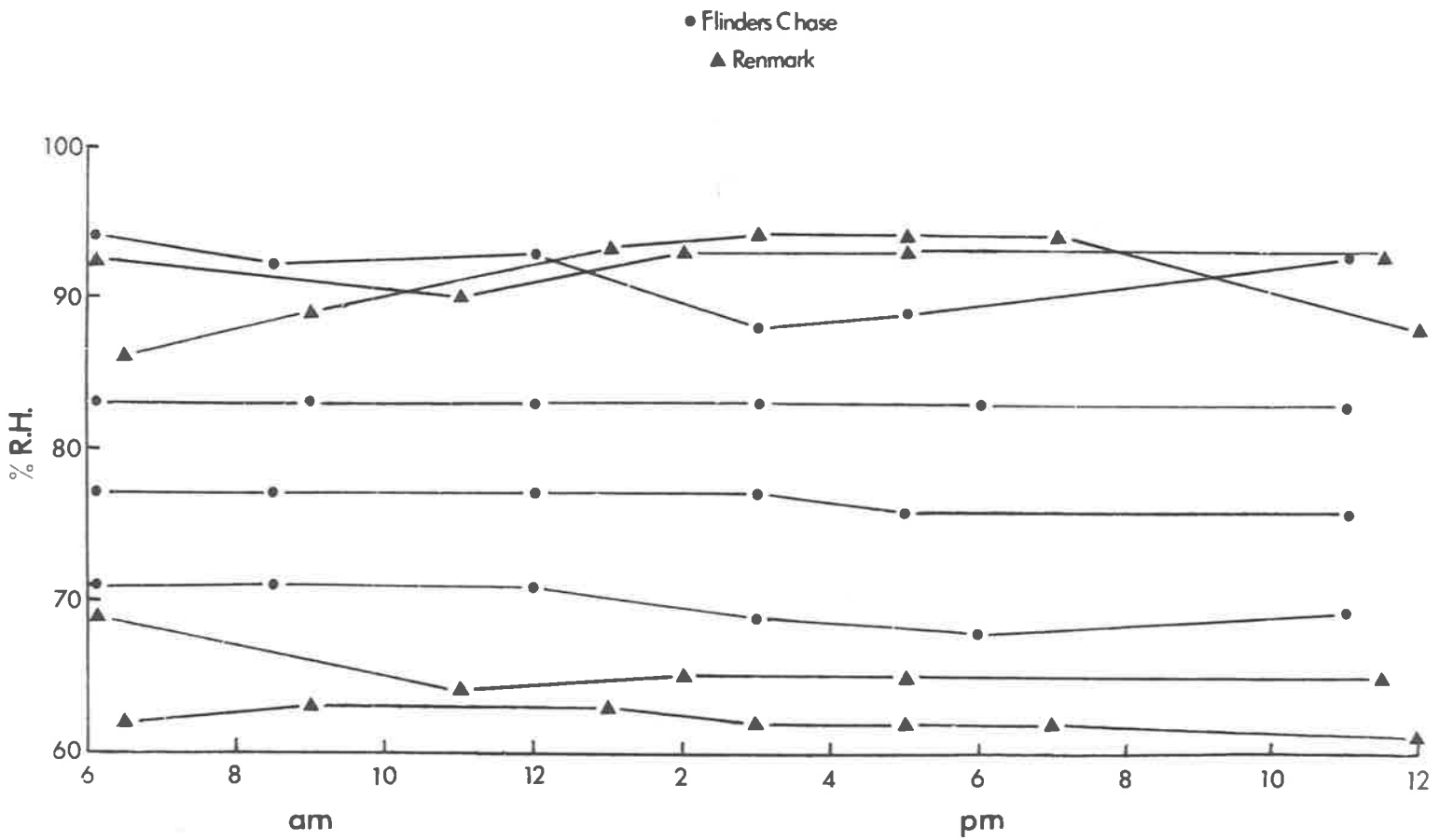

● Flinders Chase
▲ Renmark
100
90
80
70
60
% R.H.
6
8
10
12
2
4
6
8
10
12
am
pm

TABLE 40

Relative Humidity of Burrows

	n	$\bar{x}$	Var.	V.R.	t_{23}	p
Renmark	9	80.6	132.78	1.631	1.012	> 0.3
Flinders Chase	16	84.8	81.40			

Temperature in Burrows

Temperature was recorded in four burrows at each locality, and the data is shown in Figs. 27 and 28. These records were taken in the summer of 1966-67, and include shade temperatures taken 5 cm. above the soil surface immediately outside the burrow.

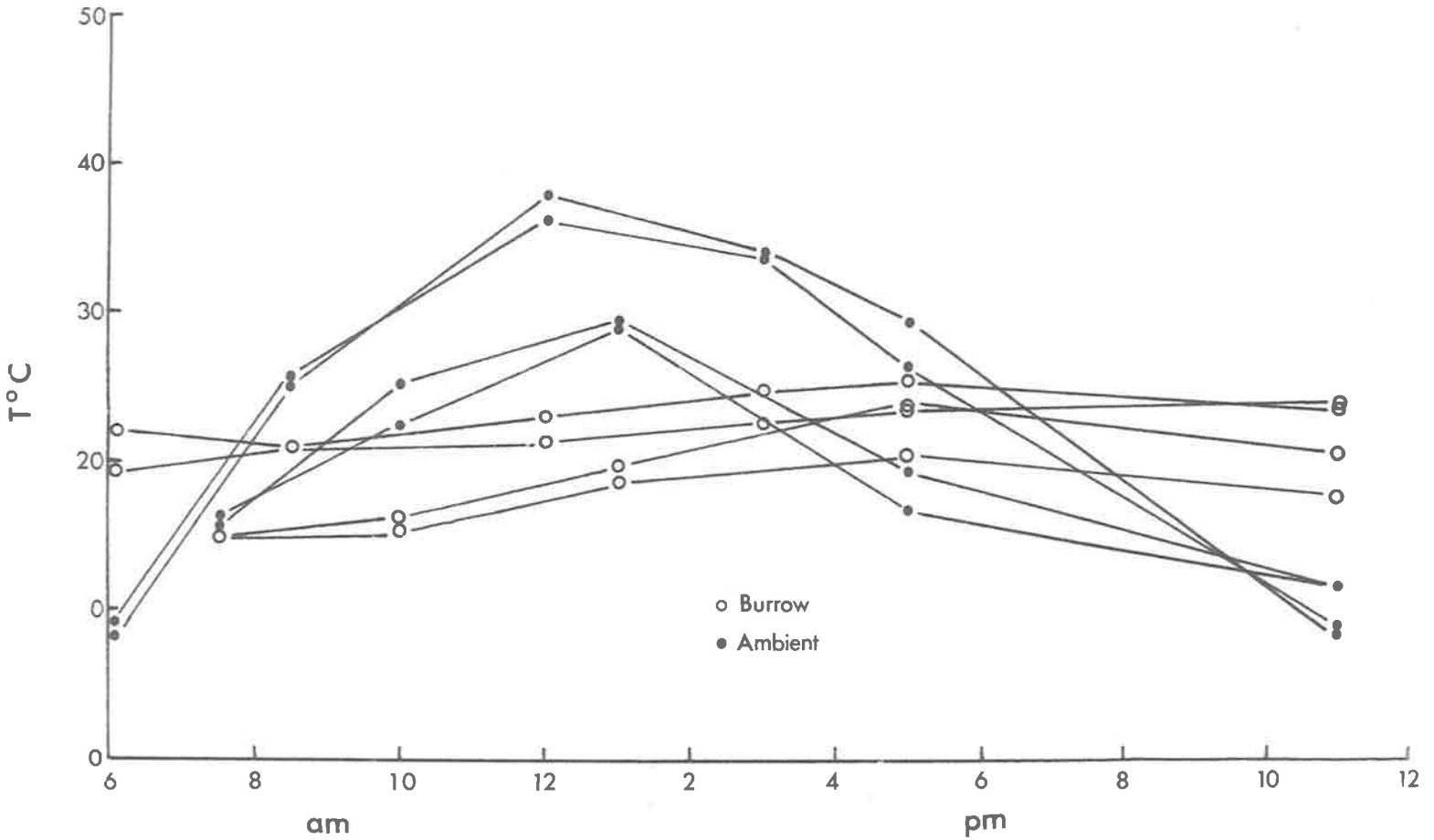
Burrow temperatures were very stable throughout the day, but there were great fluctuations in the temperature of the external environment. Burrow temperatures in summer were as much as 15-25°C cooler than the air outside the burrow during the hottest part of the day at Renmark, while the temperature difference was between 10°C and 15°C at Flinders Chase. During the night and early morning however, the burrows were warmer than outside.

Burrows therefore offer thermal refuge from both high and low external temperatures. A warm burrow would allow an animal to avoid high temperatures during the day and also reduce the time required for an animal to reach its eccritic temperature on emergence in the morning.

FIGURE 27

Burrow and ambient temperatures at

Flinders Chase in Summer


T°C
50
40
30
20
0
0
6
8
10
12
2
4
6
8
10
12
am
pm
o Burrow
• Ambient

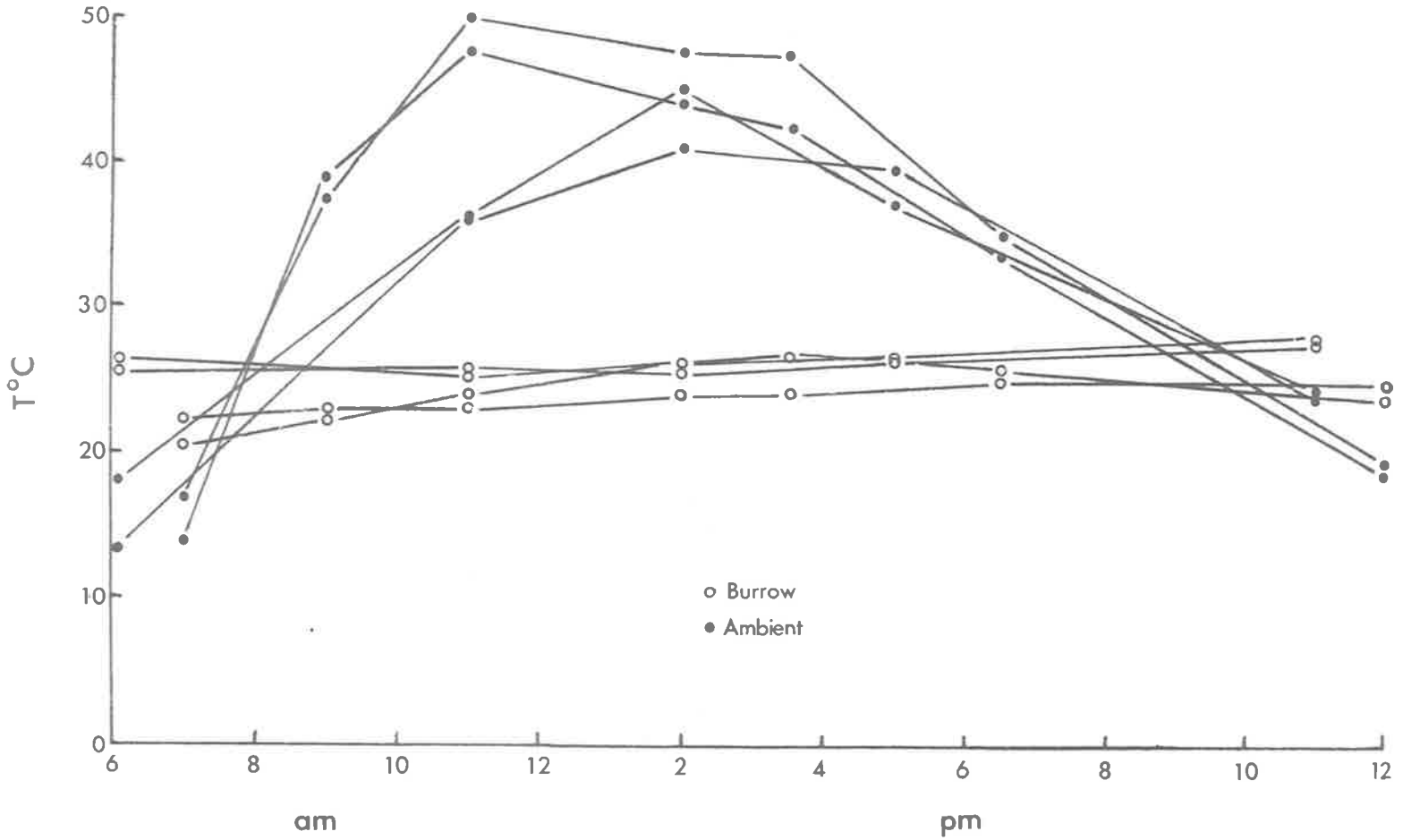
The burrows at Renmark were significantly deeper than those at Flinders Chase, while in an arid region of South Australia burrows were excavated to an even greater depth of 50 cm. (Warburg 1965b). This indicates that in more arid regions it is necessary to dig deeper to acquire the same degree of thermal insulation from the soil.

Temperature recordings from burrows support this suggestion. There is little difference between the burrow temperatures at Renmark and those published by Warburg, although the climate of these two regions is quite different. Warburg did not measure relative humidity in the burrows, but it is possible that they were deep enough to reach a region of the subsoil with a high water content, as Schmidt-Nielsen and Schmidt-Nielsen (1950b) found that in a desert region of Arizona during summer, the air in soil at a depth of 30 cm. was saturated with water vapour. It is not clear if soil temperature, water content of the soil or both of these factors, govern the depth to which burrows are excavated.

The burrows of V. gouldii are very humid compared with those of the snake Aspis sp. (Warburg 1964) where relative humidities between 30 and 40% are found in Summer. Schmidt-Nielsen and Schmidt-Nielsen (1950b) reported a mean relative humidity of 30% for burrows of the rodents Dipodomys spectabilis and D. merriami although readings as high as 91% were obtained. However, the humidity recordings for V. gouldii burrows were made in less arid areas than those for Aspis and Dipodomys.

FIGURE 28

Burrow and ambient temperatures at Renmark in Summer


T°C
50
40
30
20
10
0
6
8
10
12
2
4
6
8
10
12
am
pm
o Burrow
• Ambient

BEHAVIOUR

A number of different techniques were used to study the behaviour of animals. Direct observation of animals provided information on the time of emergence and the times of the day that animals were errant, while obliteration of old tracks outside burrows and observations on the formation of new tracks indicated movement into and out of burrows. Movements to new locations were studied by recapturing marked animals, and radio-tracking.

Diurnal Behaviour

The daily behaviour of animals in summer was quite different at the two field areas. At Flinders Chase animals emerged from their burrows every day at about 8.30 a.m. and spent all day in the open, retiring to their burrows in the late afternoon. Even on the hottest days animals could be observed moving around in the afternoon, although much of the time was spent in the shade. On only two occasions when the temperature was above 100°F were animals found in burrows in the early part of the afternoon, but it is possible that these animals retreated underground on the approach of the observer.

At Renmark, however, animals emerged at about 8 a.m. and retreated underground around midday, a pattern similar to that seen in many desert reptiles. No animals were ever seen or trapped in the afternoon in summer. The retreat pattern is probably a thermoregulatory response to high ambient temperatures that is not usually elicited at Flinders Chase. Some animals emerged for a short while in the late afternoon, but this was not usual.

It was also common for animals to spend the whole day underground, and not emerge at all. This was particularly the case on hot windy days that were also overcast, although no explanation for this behaviour on these days can be given. However, there are obvious benefits to be derived from this type of behaviour, as will be discussed in reference to water turn-over rates measured at Renmark.

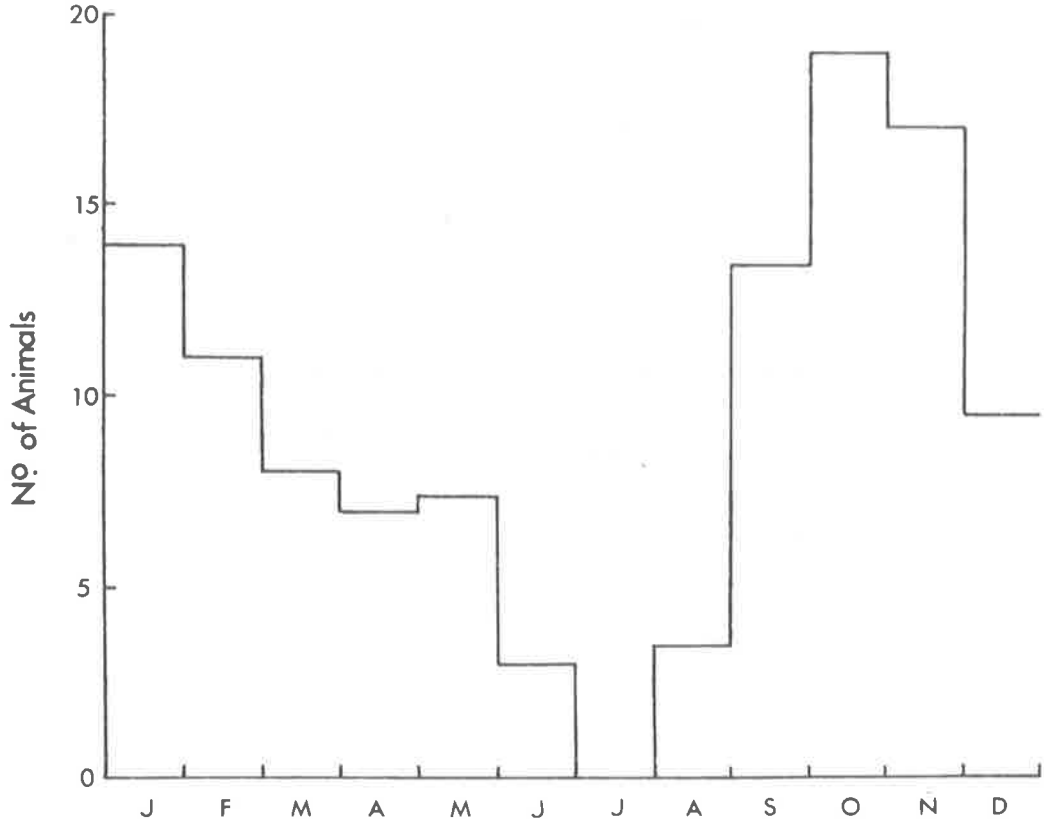
Population Movements

The animals at Flinders Chase represent a fairly static population with respect to animal movements. Approximately forty per cent of all animals marked were recaptured on at least one subsequent occasion, and the distance between the points of capture was usually between 100 and 200 yards. The evidence is that animals do not move great distances at Flinders Chase, however seasonal movements of the population appear to take place. In winter animals are found in the scrub, and then only with great difficulty, but spring is marked by a dramatic increase in the number of animals caught on the firebreaks. This is perhaps best demonstrated by comparing the number of animals captured on field trips throughout the year.

The average number of animals caught at various times of the year, over 2 years, is shown in Fig. 29, where a sharp increase in the number of animals caught is evident in September and October. All field trips were of approximately the same duration, and a uniform searching procedure was followed. Searching was only made more

FIGURE 29

Mean number of animals captured at different times of the year at Flinders Chase


20
15
10
5
0
Nº of Animals
J
F
M
A
M
J
J
A
S
O
N
D

thorough in winter when animals were difficult to find. Only two animals were found on the firebreaks during winter, and both of these occasions were in the mild winter of 1967. All of the other animals found during winter were found in the scrub.

At Renmark however there are strong indications that the population is not stationary, and that animals move much greater distances. The evidence for this is mainly indirect. The failure to recapture any animals in baited traps is a point in favour of this inference, and additional evidence is provided by the inability to relocate radio-equipped animals after intervals of a month or so. It is unlikely that the transmitters had ceased to function as on four occasions when animals had discarded the harness, the transmitters were easily located with the receiver. On each of these occasions the transmitters were found up to half a mile from the point of release. It thus appears that at Renmark V. gouldii may move over quite great distances, in a similar way to V. varius (Stebbins and Barwick 1968).

ELECTROLYTE STUDIES

Introduction

Increased concentrations of sodium in the plasma (hypernatraemia) was first reported for reptiles in the lizard Trachysaurus (Tiliqua) rugosus by Bentley (1959). He recorded sodium concentrations in the plasma of 195 meq/L. during Summer, and also reported that T. rugosa could tolerate concentrations as high as 230 meq/L, induced by injections of NaCl solution, without apparent ill effect. A similar situation has been described in lizards of the genus Amphibolurus by Bradshaw (1965) and Bradshaw and Shoemaker (1967).

Hypernatraemia in these animals results from the inability to excrete a hypertonic urine, consequently the animals are forced to retain electrolytes in the absence of sufficient water to excrete them in isotonic form. This situation prevails in Summer when water losses are probably equal to, or in excess of, water intake. The condition is aggravated in T. rugosus by the high electrolyte levels of the plant diet, and in A. ornatus by the high sodium content of the ants on which this species feeds. Seasonal variation in the plasma electrolyte levels has also been reported in the desert lizard Varanus greseus by Haggag et al. (1965).

Measurements were therefore made of the osmotic, sodium and potassium concentrations in plasma obtained from animals at the two field areas, to see if these concentrations vary with the season, and with locality.

Materials and Methods

Blood samples of approximately 1.5 ml. were taken within 24 hours of capture by cardiac puncture. Disposable 5 ml. syringes with 23 gauge needles were used, and the blood was transferred to 2 ml. plastic tubes and sealed with plastic caps. The hypodermic needles were heparinised and a globule of heparin (approximately 5 µl) was placed into each tube. After shaking, the tubes were placed in a centrifuge and spun at 1,000 r.p.m. for one minute. The samples of plasma were pipetted into fresh 2 ml. plastic tubes, and after sealing, frozen in a refrigerator. During transportation from the field areas to Adelaide, a duration of approximately four hours in both cases, the samples sometimes thawed, but there was no appreciable evaporation during this time. On arrival at Adelaide the samples were once more frozen until analysed.

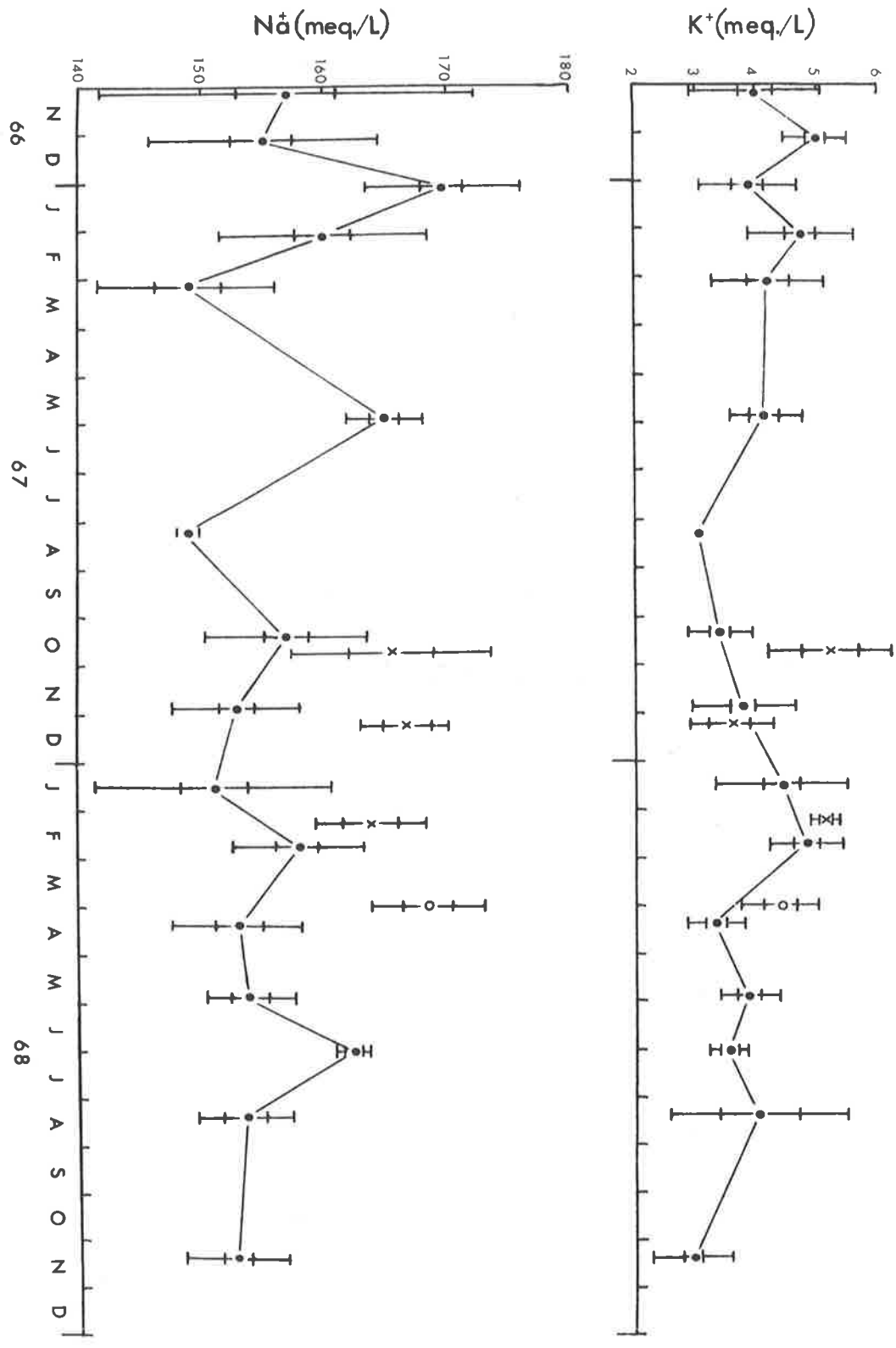
Osmotic concentration was measured in 0.2 ml. samples with a Fiske osmometer (Model H). Sodium and potassium were measured with an EEL flame photometer, the appropriate dilutions being made with double distilled water.

Concentration of Sodium in the Plasma

Over the two years in which blood samples were taken at Flinders Chase there were no great changes in the concentration of sodium in the plasma as can be seen in Fig. 30. The mean concentrations of sodium were maintained between 150 and 160 meq/L. over the whole period except on three occasions; January 1967, June 1967 and July 1968. No explanation can be advanced for the higher concentrations

FIGURE 30

Concentrations of sodium and potassium in the plasma


Na+(meq./L)
K+(meq./L)
140
150
160
170
180
2
3
4
5
6
N D J F M A M J J A S O N D J F M A M J J A S O N D
66
67
68

on these occasions, especially those in midwinter of each year.

However, compared with the seasonal elevations in sodium seen in some other reptiles, these values are quite small. Certainly the plasma values over the hot dry summer of 1967-8 were fairly constant and showed no elevation whatsoever.

This is in contrast to the situation in Varanus gresseus, where the concentration of sodium in the plasma reached 181 meq/L. in summer, and decreased to 131 meq/L. in winter (Haggag et al. 1965). These workers suggested that the low level in winter was due solely to hibernation. However these results could be interpreted as a combination of slight elevation in the concentrations of sodium in summer and a slight depression during the inactive period in winter.

Blood samples were collected from Renmark in the Spring, and early and late Summer of 1967-8. These results are also shown in Fig.30 for comparison. The sodium concentrations in the plasma during Spring and early Summer were significantly higher at Renmark ($p < 0.05$ and $p < 0.001$ respectively), but in late Summer there was no significant difference ($p > 0.05$). These tests are set out in Tables 41, 42 and 43. Although these differences in sodium concentrations are statistically significant, they are nevertheless slight, the differences being of only 10 meq/L. or so.

Blood samples collected at Yuendumu in April 1968 by Baverstock (1969) also had significantly higher sodium concentrations in the plasma than those taken at Flinders Chase at the same time ($p < 0.001$), but again the difference was slight. The environment at Yuendumu is even more

extreme than at Renmark. The t-test for this comparison is set out in Table 44.

TABLE 41

Concentration of Sodium in the Plasma

October 1967

Locality	n	$\bar{x}$	Var.	V.R.	t_{17}	p
Renmark	5	165.6	62.8	1.485	2.420	< 0.05
Flinders Chase	14	156.9	42.3			

TABLE 42

Concentration of Sodium in the Plasma

December 1967

Locality	n	$\bar{x}$	Var.	V.R.	t_{16}	p
Renmark	3	166.7	12.5	2.248	4.269	< 0.001
Flinders Chase	15	152.9	28.1			

TABLE 43

Concentration of Sodium in the Plasma

February 1968

Locality	n	$\bar{x}$	Var.	V.R.	t_{11}	p
Renmark	4	163.8	21.0	1.348	1.948	> 0.05
Flinders Chase	9	157.8	28.3			

TABLE 44

Concentration of Sodium in the Plasma

April 1968

Locality	n	$\bar{x}$	Var.	V.R.	t_{10}	p
Yuendumu	5	168.2	21.7	1.323	5.102	< 0.001
Flinders Chase	7	153.0	28.7			

Concentration of Potassium in the Plasma

No consistent changes in concentration of potassium in the plasma were apparent over the period that samples were taken at Flinders Chase as is shown in Fig. 30, although values may be minimal in the October to November period. Overall, the mean

concentration of potassium was maintained between 3 and 5 meq/L. The concentrations at Renmark were quite similar to those at Flinders Chase in early and late Summer, but were slightly higher in the Spring.

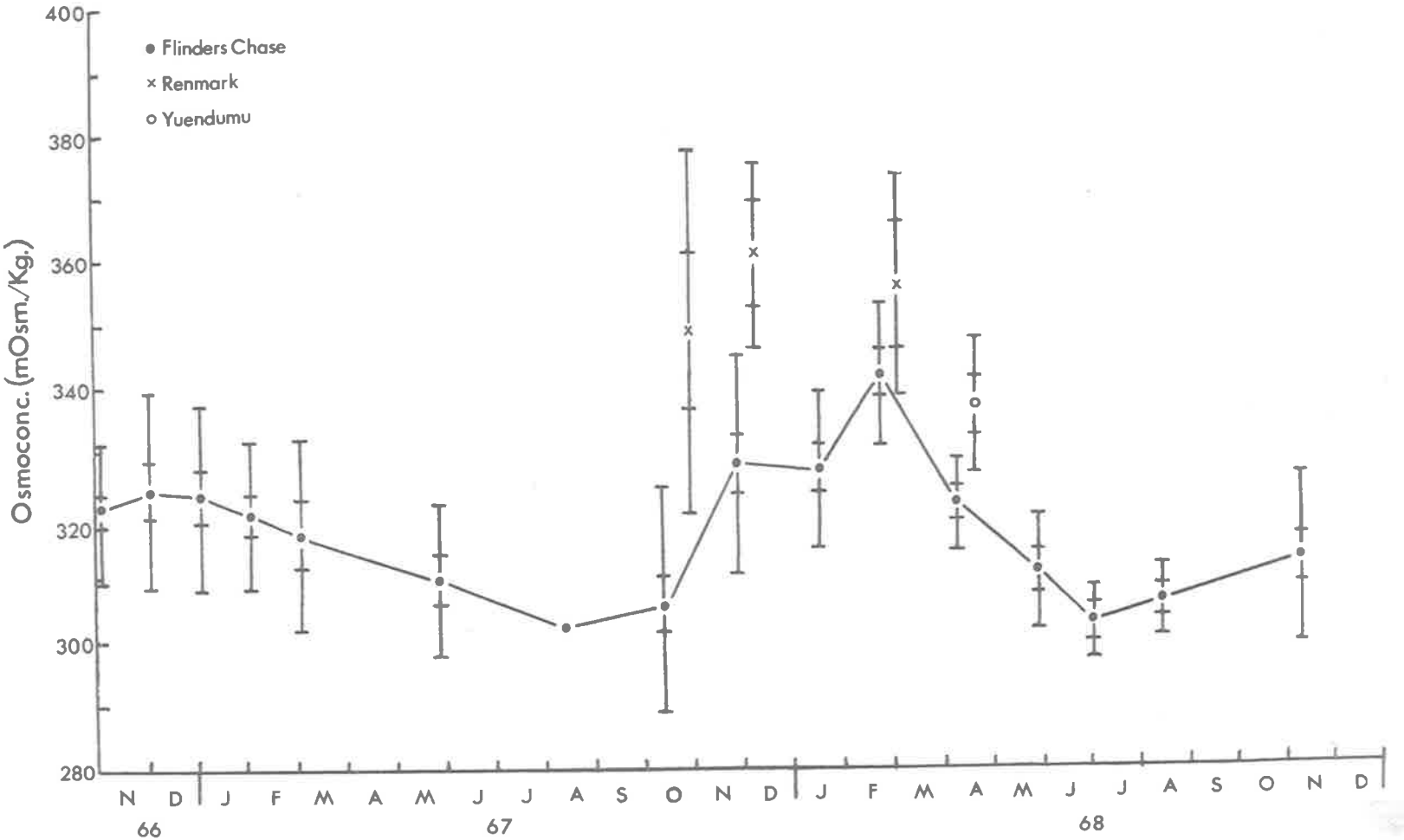
Osmotic Concentration of the Plasma

Seasonal variation in the total osmotic concentration of plasma from Flinders Chase is shown in Fig. 31. The highest osmotic concentrations occurred during the summer period, the lowest during Winter. This variation is possibly due to seasonal variation in plasma glucose concentrations that have been shown to occur in several reptiles (Miller 1961, Coulson and Hemandes 1964, Moore 1967). Again the values for Renmark are higher than those for Flinders Chase.

Thus hypernatraemia does not seem to occur in V. gouldii during Summer, even in quite arid situations where it is doubtful that sufficient water is available for the cloacal elimination of electrolytes. It seems reasonable therefore to interpret these results as showing that the nasal gland does function in the wild to maintain electrolyte levels fairly constant. Encrustation of salt around the nares was never observed in animals captured in either field area, but this is not surprising as the snout is used for digging and therefore any encrustation that developed would abrade off. However, small amounts of encrustation have occasionally been noted in animals kept in captivity, and also in a specimen of V. acanthurus brachyurus.

FIGURE 31

Osmotic concentration of the plasma


400
380
360
340
320
300
280
Osmoconc. (mOsm./Kg.)
• Flinders Chase
× Renmark
o Yuendumu
N D J F M A M J J A S O N D J F M A M J J A S O N D
66
67
68

Bradshaw (1965) found no evidence of extra-renal electrolyte excretion in Amphibolurus ornatus, and Bentley (1959) made no reference to the nasal secretion of salt in Trachysaurus rugosus. Presumably the absence of extra-renal excretion of electrolytes explains the condition of hypernatraemia in these reptiles during Summer.

Obviously the only way of determining the importance of the nasal gland in electrolyte balance under field conditions would be to surgically ablate the gland in a number of animals, and then follow the blood electrolyte levels over a period of time to see whether or not these animals could control the electrolyte concentrations of the body.

WATER PHYSIOLOGY IN THE FIELD

Introduction

Turnover of tritium has been measured in a number of mammals, including *Mus musculus* (Thompson 1952), *Dipodomys deserti* (Richmond *et al.* 1960), Mouse, Rat, *Dipodomys deserti*, Rabbit, Dog, Man and Horse (Richmond, *et al.* 1962), Sheep (Morris *et al.* 1962) and *Odocoileus hemionus* (Knox *et al.* 1969). Tritium turnover has also been studied in the fowl *Gallus gallus* by Chapman and Black (1967), but there is no published information of this kind for reptiles.

The use of tritium turnover as an estimate of water turnover has mainly been confined to the laboratory or to studies on domesticated animals. There are obvious restrictions in using the technique on wild animals, the major one being the need to recapture animals at reasonable time intervals to obtain samples of fluid. In the case of small mammals where the turnover rate is very high, recaptures would be needed within a few days. It is perhaps for this reason that the technique has so far been exploited to a very limited extent in the field.

Materials and Methods

Total water content of animals was estimated by dilution of injected tritium oxide (HTO). Animals were weighed to the nearest g. and then given an intraperitoneal injection of tritiated water in 0.9% saline (1 ml. containing 100 μc activity). Care was taken to remove the syringe slowly from the body cavity to prevent leakage of

water from the needle wound.

After complete distribution and equilibration of the tritiated water, approximately 400 µl of blood was taken from each animal by cardiac puncture. The blood samples were stored in screw-topped glass vials.

The animals were then released at the point of capture, and the recapture of animals at a later date allowed the water turnover rates to be determined. Release and recapture dates were carefully noted. Upon recapture, a second blood sample was taken prior to re-weighing the animal and injecting a further 1 ml. of tritiated water. After equilibration a third blood sample was taken. The second injection enabled estimation of the total body water (T.B.W.) at the time of recapture, and allowed adjustments to be made for any change that occurred in the volume of water in the body between release and recapture.

The blood samples were sublimed to apparent dryness using the technique of Vaughan and Boling (1961). The separated water samples were then thawed and 250 µl of each was added to 5.0 ml of scintillation fluid. The scintillation fluid consisted of 100 g. napthalene, 9.0 g. PPO (2, 5-diphenyloxazole) and 250 mg. POPOP (1, 4-bis-/2-(5-phenyloxazolyl)/ benzene) in 1 litre of dioxane. Counting was done with a Packard Tricarb scintillation spectrometer. Every sample was counted for three periods of 5 minutes, to give an average 5 minute count. Standards of 25 mµc/ml. were also counted in the same way to enable expression of the tritium

concentrations in terms of mμc/ml.

Total body water was calculated from the expression:

$$\frac{\text{HTO conc. injected (m}\mu\text{c/ml)}}{\text{HTO conc. (m}\mu\text{c/ml) after equilibration}} = \text{T.B.W. (ml)}$$

The HTO concentration in the second blood sample was subtracted from that of the third sample before the T.B.W. was calculated at recapture.

Equilibration Time

An experiment was conducted with four animals to determine the time required for complete and equal distribution of tritium throughout the water in the body i.e. the equilibration time. The experiment was done in a field laboratory under the same conditions that field measurements of T.B.W. and water turnover were made. The temperature in the laboratory was usually about 20°C. Blood samples were taken 3, 6, 12 and 24 hours after an injection of HTO (100 μc), and the concentrations of HTO in the samples at these times are shown in Table 45.

TABLE 45

Concentration of HTO (mμc/ml) at Various Times After Injection

Animal	3 hrs.	6 hrs.	12 hrs.	24 hrs.
1	126.3	124.8	114.4	130.0
2	245.5	239.0	245.0	233.0
3	64.7	93.0	90.0	92.8
4	130.0	134.0	134.5	136.0

Equilibration was assumed to have occurred when the concentration of HTO in the plasma was stable and at a level that was equivalent to complete dilution by the water in the body.

The results show that equilibration was reached within 3 hours in three animals (1, 2, and 4), and within six hours in the other animal. Consequently at least six hours were allowed to pass before equilibrated blood samples were taken in all estimations of T.B.W.

Total Body Water

No consistent changes in T.B.W. (ml/100 g.) were apparent in V. gouldii over the two years in which estimations were made. The mean water content of 57 animals from Flinders Chase was found to be 76.7 $\pm$ 0.2 ml/100 g. Similar measurements on 13 animals from Renmark gave a mean value of 77.0 $\pm$ 0.9 ml/100 g., which is obviously no different from that of animals from Flinders Chase.

These T.B.W. values are higher than those reported by Bradshaw (1965) and Thorson (1968) for a number of reptiles. Bradshaw (1965) measured T.B.W. in Amphibolurus ornatus and found a mean value of 73.5 ml/100 g. Thorson found T.B.W. values for Constrictor constrictor imperator, Pituophis catenifer annectans, Iguana iguana and Gopherus polyphemus were similar, and represented about 70% of the body weight.

Both Bradshaw and Thorson estimated T.B.W. by desiccating carcasses at 105°C, a technique that may be regarded as fairly accurate. However, HTO is known to give T.B.W. estimates up to 4% greater than those obtained by desiccation, due to incorporation of

HTO into organic compounds (Pinson and Langham, 1957; Foy and Schneiden, 1960). If this factor is taken into account the true T.B.W. values for V. gouldii are probably of the same order as those reported by Bradshaw (1965) and Thorson (1968).

The T.B.W. values for V. gouldii are between 10% and 15% greater than those found for a number of mammals by the HTO dilution technique (Pace et al. 1947, Thompson 1952, Pinson and Langham 1957, Richmond et al. 1960, 1962).

Water Turnover and Water Loss

Water turnover rates are obtained by measuring a biological half-time ($t_{\frac{1}{2}}$) for the equilibrated HTO. The $t_{\frac{1}{2}}$ is read from a semi-logarithmic plot of HTO concentration against time (days), and is taken as the point where the regression line reaches 50% of the initial concentration.

The mean water turnover rate is then derived from the expression,

$$\frac{0.693}{t_{\frac{1}{2}}\ (\text{days})} = \%\ \text{T.B.W. (ml.)/day}$$

Water turnover has been measured mainly in mammals, where the turnover rates are high, and therefore the experiments generally last only a few days. During this time changes in weight and size of the T.B.W. pool are assumed to be small and are generally disregarded.

The dilution of equilibrated HTO results from the loss of HTO and water to the environment, and the replacement of these losses by unlabelled water from food and metabolism.

In the situations described above the water turnover rate

provides a good approximation to the rate of water loss, and where no change in the absolute size of the water pool (ml.) takes place, the turnover rate is exactly equal to the rate of water loss.

However, an increase in the absolute size of the water pool would cause additional dilution of the HTO and yield a higher turnover rate than the rate of water loss. With a decrease in pool size however, the HTO would not be diluted sufficiently for the turnover rate to approximate to the rate of water loss. Consequently water turnover rates measured under these conditions are of limited value as no information on water loss or water balance is imparted by these measurements.

Where changes occur in the absolute size of the water pool, it is possible to convert water turnover rates to rates of water loss. This is done by changing the HTO measurements from concentrations (mμc/ml.) to absolute values (μc animal). To do this, estimates of T.B.W. (ml.) are required for points at the beginning and end of the experimental period. Absolute HTO values (μc) are then obtained by multiplying the HTO concentration (mμc/ml.) by the pool size (ml.) for both initial and final points.

In this way the actual amount of HTO that leaves the body is measured, rather than the simple dilution of the isotope. Consequently, to take account of changes in pool size, absolute HTO values were plotted to determine the $t_{\frac{1}{2}}$, the latter being read to the nearest half-day.

The mean body weight and mean T.B.W. (ml.) was calculated for

each animal from release and recapture measurements. These mean values were used in subsequent calculations of water loss (ml/day and ml/Kg/day).

Sometimes the estimates of T.B.W. at recapture were too high. This was due to leakage of the injection in some cases but this explanation does not apply to others.

A similar effect has been observed on reinjecting sheep (Howard 1969). It is not clear what is responsible for the error in T.B.W. estimation, but either the pre-injection count is too high, or the post-injection count too low. It is more likely to be the latter, as the rates of water loss in animals where accurate final T.B.W. measurements were made are similar to those where erroneous final T.B.W. values were found. Even so, the cause of this error would be worth investigating. Until an explanation is found for this error this technique remains approximate, with the estimated rates of water loss being minimal.

In all cases where T.B.W. estimates were obviously too high, it was assumed that the T.B.W./body weight ratio for each animal had not changed over the period of release. None of the erroneous estimates of T.B.W. were used in calculating the mean T.B.W. values previously described.

Water loss under natural conditions was measured at Flinders Chase over a two year period, from November 1966 to December 1968, but only during January 1969 at Renmark.

Water Loss at Flinders Chase

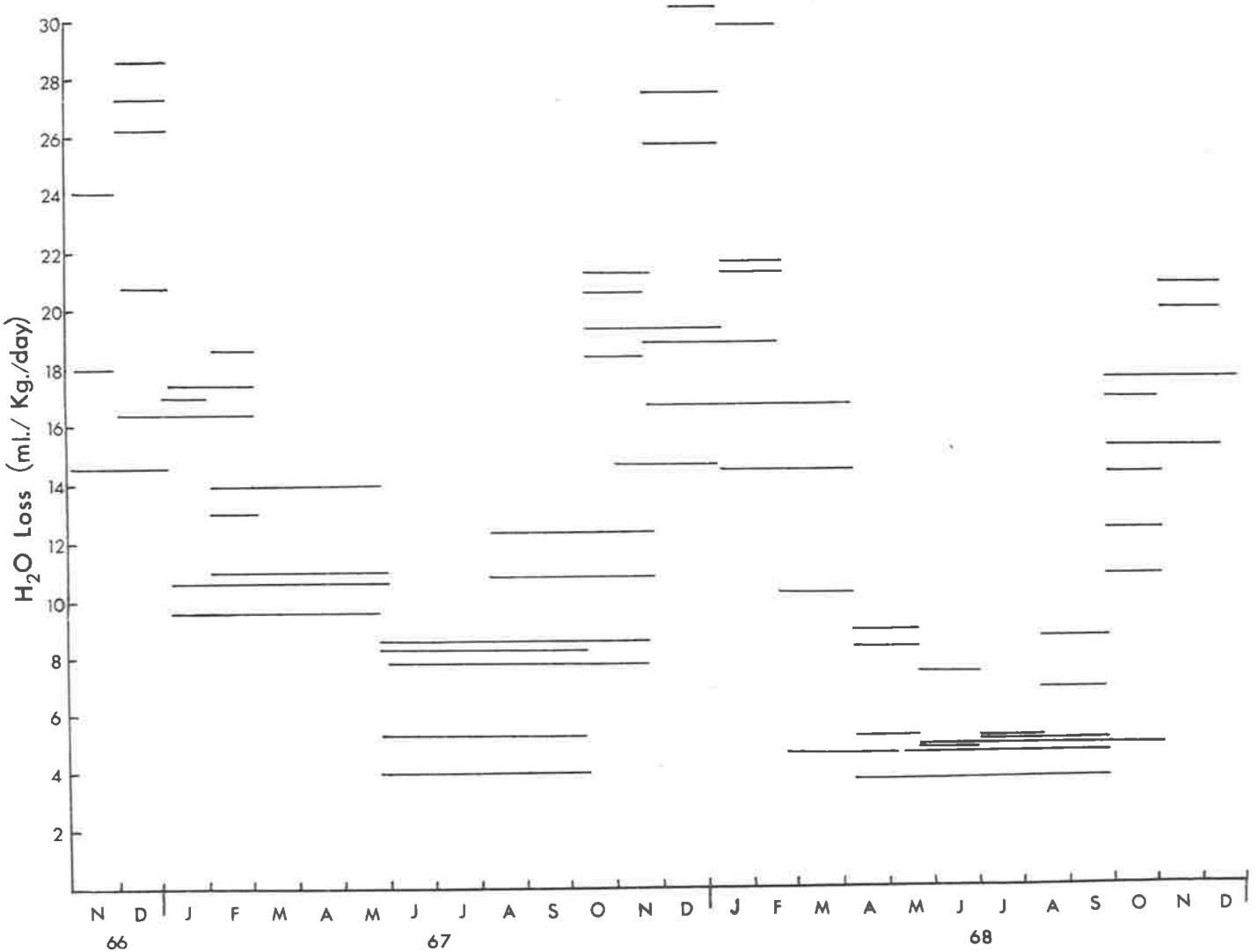
The rates of water loss (ml/Kg/day) measured over two complete years are shown in Fig. 32. A circannian cycle is quite clearly present, high rates of water loss occurring in late Spring and early Summer, and low rates during Winter. These results reflect seasonal differences in animal activity and the climate. The cycle of water loss was quite similar in both years, although the rates in Winter of 1967 seem to be slightly higher than those of 1968. It may be that animals were more active in the Winter of 1967 which was very mild with low rainfall, than in the colder wet Winter of 1968.

The rates of water loss in the Summer of 1967-68 appear to be higher than those measured in the Summer of 1966-67, and this may be attributed to the hotter and drier conditions that prevailed throughout the Summer of 1967-68.

The differences between rates of water loss in the two years are not great, however, and so to facilitate analysis, the data for the two years were pooled. The data were classified into seasons; Summer (1st December - 28th February); Autumn (1st March - 31st May); Winter (1st June - 31st August) and Spring (1st September - 30th November). Some overlap of these dates was allowed, but where the overlap was too great the data were omitted. This classification resulted in the exclusion of 10 items, the remaining 47 items being distributed as follows: Summer (17), Autumn (5), Winter (8) and Spring (17). The mean rate of water loss (ml/Kg/day) in each season was 22.04 ± 1.35 (Summer), 9.84 ± 1.42 (Autumn), 5.45 ± 0.56 (Winter

FIGURE 32

Rates of water loss over two years at Flinders Chase


H_2O Loss (ml./ Kg./day)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
N D J F M A M J J A S O N D J F M A M J J A S O N D
66
67
68

and 15.75 $\pm$ 1.19 (Spring). Because the variances were unequal the data were transferred to logarithms and then subjected to an analysis of variance that is set out in Table 46. This showed that the population was not homogeneous ($p < 0.001$), and so t-tests were conducted to determine any differences in water loss between the seasons. Significant differences were found between Summer and Spring ($p < 0.01$), Spring and Autumn ($p < 0.02$) and Autumn and Winter ($p < 0.02$). It followed therefore that all seasons were significantly different from each other. The t-tests are presented in Table 47.

TABLE 46

Analysis of Variance: Water Loss in Different Seasons

Source	d.f.	SS.	Var.	V.R.	p
Seasons	3	2.169927	0.723309	40.050	< 0.001
Error	43	0.776562	0.018060		
Total	46	2.946489			

TABLE 47

Differences Between Seasons

Season	n	$\bar{x}$	Var.	V.R.	t	p
Summer	17	1.329538	0.012840	1.810	3.374	< 0.01
Spring	17	1.174126	0.023240			
Spring	17	1.174126	0.023240	1.089	2.588	< 0.02
Autumn	5	0.971616	0.025311			
Autumn	5	0.971616	0.025311	1.807	3.257	< 0.01
Winter	8	0.721698	0.014005			

The differences in rates of water loss between the seasons are probably best explained by differences in climate and activity of the animals, the latter of course being dependent to a great extent on the former. However, no reasonable explanation can be provided for the difference between Spring and Autumn. It is possible that micro-climate differences may exist between these two seasons, and therefore affect rates of water loss, but this is not known. It is thought more likely that there are differences in the behaviour of the animals in Spring and Autumn that might account for the difference, certainly it has been shown that the location of the population is different on these occasions. However, until a detailed behavioural study is performed on these animals, no firm explanation can be provided for these results.

The Effect of Size on Water Loss in the Field

In view of the results obtained in the laboratory, the rates of water loss for Spring and Summer were examined to see if there was any correlation between water loss and body weight. The data for both seasons were subjected to regression analysis, the lines of best fit being described by the expressions;

Summer: Water loss (ml/Kg/day) = 25.4737 - 0.0032 Wt. (g)
(S.E.b = $\pm$ 0.0046)

Spring: Water loss (ml/Kg/day) = 6.3707 + 0.0086 Wt. (g)
(S.E.b = $\pm$ 0.0044)

Neither line was significantly different from zero ($p > 0.05$ in both cases), and it was therefore not possible to show an effect of body weight on water loss in the field. The regression lines and data are shown in Fig. 33.

Therefore factors other than body weight are responsible for such variability in water loss that the influence of size on water loss is masked. These undetermined factors must therefore be regarded as major influences on water loss, whereas size is probably not as important a factor as laboratory experiments indicate.

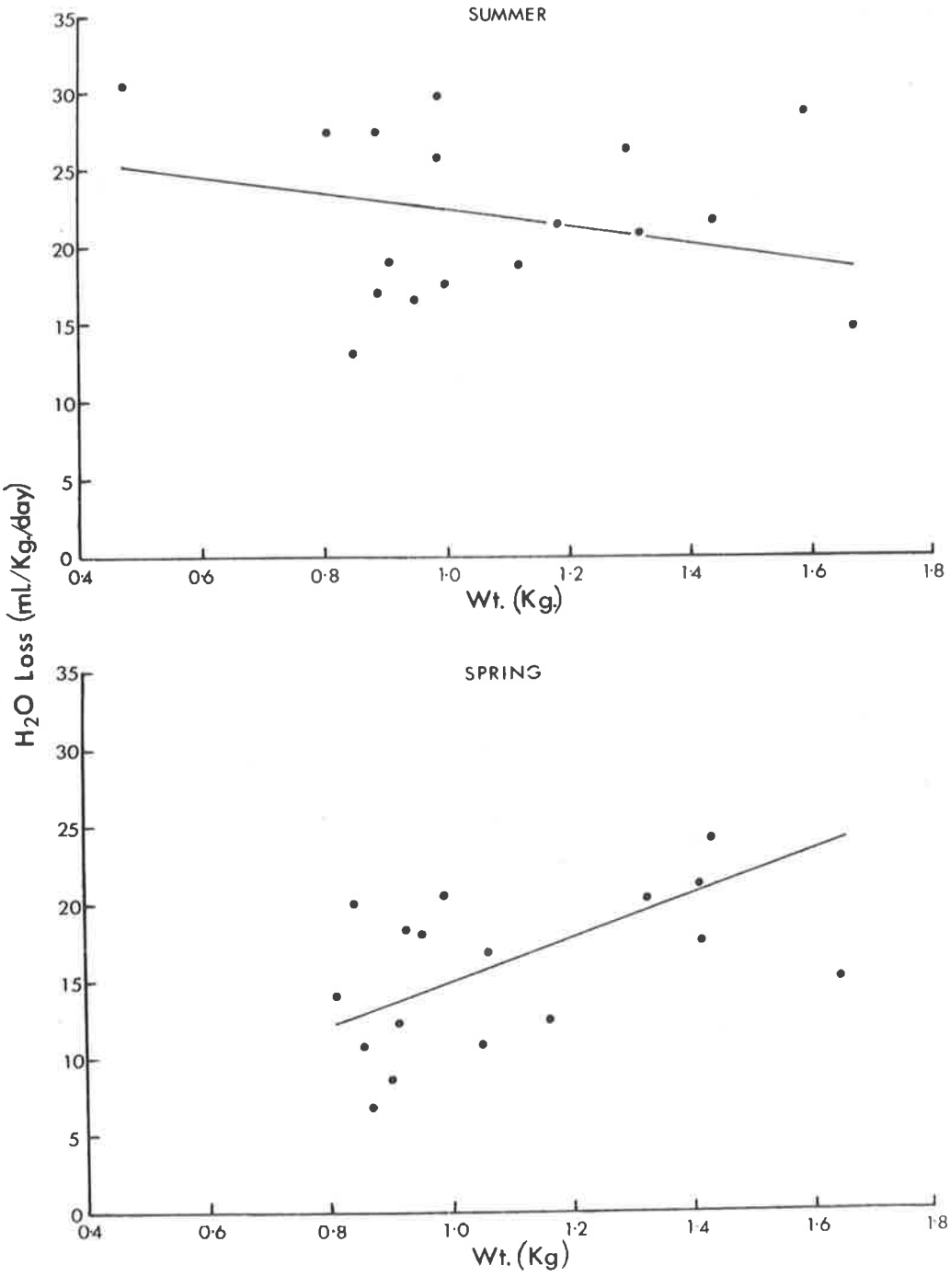
Water Loss at Renmark

Attempts to measure the seasonal variation in water loss at Renmark were unsuccessful, because it was not possible to recapture animals during the summers of 1967 and 1968, despite the use of radio-transmitters. It is probable that the animals at this location were

FIGURE 33

Effect of size on the rate of water loss in Summer

Effect of size on the rate of water loss in Spring


SUMMER
SPRING
H_2O Loss (ml/Kg.day)
Wt. (Kg.)
Wt. (Kg)
0
5
10
15
20
25
30
35
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1·8

moving over much greater distances than those at Flinders Chase, and consequently in the period between field trips moved beyond the area that could be covered with radio-receivers.

Rates of water loss were successfully measured in January 1969 however, by locating the position of seven radio-equipped animals every day over a period of about two weeks. The position of each animal was recorded at mid-day and in the evening, and frequently at other times. At the end of the release period the animals were recaptured by digging them out of burrows and rabbit warrens. Only one animal was not recaptured as it had retreated deep into a warren and could not be reached despite extensive digging.

The rates of water loss at Renmark were highly variable, ranging from 7.90 ml/Kg/day up to 40.33 ml/Kg/day. The activity shown by each of the animals was also variable. An index of activity was obtained by determining the number of days on which each animal emerged from the burrows. Emergence and activity were indicated by movement to different burrows, direct observation of emergence, and reading track signs. The number of active days was expressed as a percentage of the total number of days the animal was free. A significant correlation was established between activity and water loss ($r_4 = 0.87$, $p < 0.05$). The data is presented in Table 48, and also in Fig. 34.

FIGURE 34

Activity of animals and rates of water loss

at Renmark during Summer

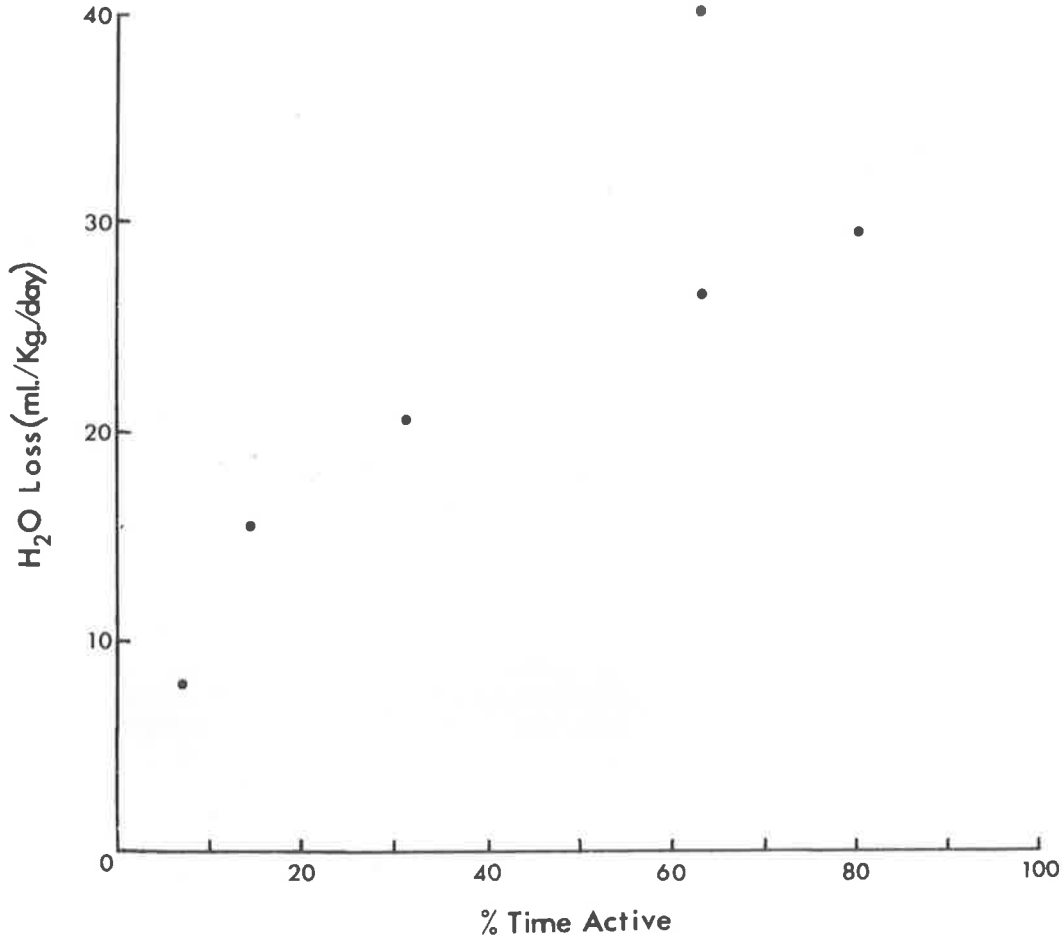

40
30
20
10
0
20
40
60
80
100
H_2O Loss (ml./Kg./day)
% Time Active

TABLE 48

Animal Activity and Water Loss

Active days (% of total days free)	7.1	14.3	31.2	64.3	64.3	80.0
Mean H_2O loss (ml/Kg/day)	7.90	15.48	20.56	26.88	40.33	29.66

Exchange of Water

During respiration there is some exchange of water molecules by diffusion at the respiratory surface. When a saturated atmosphere is breathed there would be water movement from the body to the air, and a corresponding amount would move from the air into the body. Thus, in this situation water is lost, and replaced at the same time. It has already been shown that the burrows are quite moist, and with an animal respiring inside, the air may be close to saturation. Water losses determined with HTO take into account the water lost in pulmonary water exchange, and there is presumably some cutaneous water exchange as well.

The least active animal at Renmark emerged on only one day out of fourteen. If account is taken of the extra water lost on that one day, approximately 30 ml/Kg., the mean rate of water loss in this animal can be regarded as an estimate of pulmonary and cutaneous exchange of water in the burrow, i.e. 6 ml/Kg/day. This estimate will be used later in an attempt to partition water losses under natural conditions.

It is difficult to compare the highly variable estimates of water loss at Renmark with the more stable estimates at Flinders Chase in Summer. However, although the animals at Renmark were abroad for shorter periods, the most active animals still lost water at a greater mean rate than those from the more temperate area. This demonstrates the effect of the more extreme environment at Renmark on water loss. The data also illustrates how effective behavioural control of water loss can be, as the less active animals at Renmark were able to reduce water loss considerably by remaining in the burrows.

It could be argued that these animals were not behaving normally due to the presence of transmitters. However, animals without transmitters showed similar behaviour at Renmark, and animals with transmitters at Flinders Chase emerged every day in the normal way.

The mechanisms controlling the activity of animals at Renmark, and the factors responsible for the variation in activity, are unknown. Further study on the comparative aspects of behaviour in the different subspecies of V. gouldii would be most interesting and might provide explanations for the observations described above.

Information on tritium turnover and water loss in V. gouldii and a number of other animals is presented in Table 49. The rates of water loss in the mammals and fowls were measured under laboratory conditions, and so strict comparisons with V. gouldii

are not possible. Even so, the rates of water loss are much lower in V. gouldii, this clearly shows the efficiency of the mechanisms involved in the conservation of water in V. gouldii.

It is to be expected that other terrestrial reptiles also show low HTO turnover rates, and would therefore be ideal subjects for utilising this technique in the field in view of the long periods that could be allowed between captures.

TABLE 49

Comparative water losses of some Vertebrates

	Animal	Wt (g)	Pool Size (% Wt)	$t_{\frac{1}{2}}$ (days)	H_2O Loss (ml/day)	H_2O Loss (ml/Kg/day)
(1)*	Mouse	21.4	58.5 ± 4.0	1.13 ± 0.14	7.22 ± 1.15	337
	Dipodomys	93	62.2 ± 2.4	11.82 ± 2.96	3.75 ± 0.95	40
	Rat	298	59.6 ± 4.0	3.53 ± 0.40	34.54 ± 4.90	116
	Rabbit	3,159	58.4 ± 5.3	3.87 ± 0.21	338 ± 62	107
	Dog	10,582	66.0 ± 1.4	5.14 ± 0.18	946 ± 124	89
	Man	67,302	55.3 ± 5.3	9.46 ± 0.88	2,747 ± 519	41
	Horse	398,533	65.7 ± 0.7	8.41 ± 0.53	21,722 ± 3,247	55
(2)	Gallus gallus	2,600	64.1 ± 2.9	7.3 ± 0.3		60.0
		1,700	62.0 ± 4.2	3.6 ± 0.3		120.0
(3)	Varanus gouldii	1,004	76.69 ± 0.56	26.0 ± 1.7 (summer)		22.04 ± 1.35
				61.2 ± 12.0 (autumn)		9.84 ± 1.42
				103.9 ± 10.3 (winter)		5.45 ± 0.56
				37.3 ± 3.3 (spring)		15.75 ± 1.19

(1) = Richmond et al. (1962)
(2) = Chapman and Black (1967)
(3) Present study.

* Mean values given ± S.D., all other authors quote ± S.E.

SUMMARY

(1) Although the climate of the two field areas was different and subject to large variations, the micro-climate in burrows at both sites was similar and uniform throughout the day.

(2) The diurnal behaviour of animals was different at the two study areas, those in the semi-arid region emerged for only a few hours each day while those in the mesic environment were abroad all day. This was probably due to differences in the heat stress to which the animals were subjected at the two localities.

(3) The concentrations of electrolytes in the plasma did not increase much at either field area in Summer, which indicates that the nasal salt gland is able to excrete excess electrolytes.

(4) There was a circannian cycle in the rates of water loss in the field. The more active animals at the semi-arid location showed a higher rate of water loss than those at the mesic site.

(5) There was a strong correlation between activity and water loss, and animals in the semi-arid region were able to reduce water loss by remaining in the burrow for long periods.

GENERAL DISCUSSION

GENERAL DISCUSSION

The aim of this discussion is to draw together the information obtained in the laboratory and the field on the various aspects of water and electrolyte balance in Varanus gouldii. The general statements that will be made on the homeostatic mechanisms involved and their relative importance apply to V. gouldii in particular, but probably to many other desert reptiles as well.

Survival in arid environments is largely dependent on the ability to conserve water, and this is achieved by a number of physiological and behavioural adaptations. These adaptations allow the animal to maintain water balance in regions where the only reliable source of water is that in the food.

Evaporative water loss from the skin and respiratory tract is low in V. gouldii in comparison with many other reptiles, presumably as a result of structural adaptations in the skin and increased respiratory efficiency. No significant differences in the evaporative water losses of the three subspecies of V. gouldii, were observed in the laboratory, although they occupy areas that range from temperate to desert. However, it is probable that behavioural differences between the subspecies influence the rate of evaporation in these different areas. During Summer, the animals in the semi-arid field area spend most of the day in burrows, and frequently do not emerge for several days. The value of this evasive mechanism is clearly

illustrated by the low rates of water loss in the less active animals. It is expected that V. gouldii in arid regions behave in a similar way to those in semi-arid areas.

The nature of the microclimate is important in this behaviour pattern. It is likely that an animal curled up inside a burrow would create a pocket of air saturated with water vapour. Once this pocket was established there would be little net water loss from the animal while it remained in the burrow.

Perhaps the most important physiological mechanisms for water conservation in V. gouldii is the excretion of nitrogen as insoluble urates and uric acid. This excretory process involves the secretion of soluble urates by the renal tubules and the precipitation of insoluble urates and uric acid in the cloaca. The precipitation is partially dependent upon the withdrawal of fluid by the renal tubules and the cloaca, and the rate of resorption is rapid enough to ensure that little water remains in the pellets of uric acid that are thus formed. Arginine vasotocin increases the rate of resorption by the renal-cloacal system.

These physiological and behavioural mechanisms for water conservation are very efficient, but the resulting restriction of water loss creates problems for the animal in thermoregulation, electrolyte excretion and general behaviour.

Terrestrial reptiles can maintain a fairly constant body temperature by moving between exposed and shaded areas. This mode of regulation is efficient only while the temperature in the shaded areas is low

enough to allow dissipation of heat from the body, otherwise the animal is unable to prevent the body temperature from rising. Although V. gouldii is able to dissipate heat by panting, when exposed to high temperatures in the laboratory, this is only achieved by losing a great deal of water. There is insufficient water available to employ this means of evaporative cooling except in extreme circumstances, therefore the animal is forced to seek the thermal refuge afforded by a burrow when behavioural thermo-regulation is no longer possible. Consequently the animals in hotter environments are limited to only a few hours each day for activities outside the burrow.

The other major problem imposed by the conservation of water is that of electrolyte excretion. Because electrolytes must be resorbed from the urine in order to extract as much fluid as possible, there is only a limited excretion of electrolytes during elimination of pellets from the cloaca. Therefore, the animal must either retain these resorbed electrolytes and tolerate increased concentrations in the body, or excrete them as a hypertonic fluid from an extra-renal gland. It is now clear that some terrestrial reptiles such as V. gouldii have functional nasal glands to excrete electrolytes, whereas others such as Amphibolurus and Tiliqua apparently do not and consequently store electrolytes during periods of water shortage (Bentley 1959, Bradshaw 1965). Therefore, an extra-renal mechanism for electrolyte excretion is not necessarily a pre-requisite for cloacal resorption, although

this has been suggested by Schmidt-Nielsen *et al.* (1963).

The nasal salt gland of *V. gouldii* probably functions in a discrete fashion under natural conditions, and that it functions at all can only be inferred from the comparatively low sodium and potassium concentrations in the plasma of animals during Summer, when electrolyte imbalance is known to occur in other lizards. It would be interesting to study the concentrations of electrolytes in the plasma of reptiles that readily show nasal secretion of electrolytes in the field. In this way the ability of the nasal gland to control electrolyte balance could be determined more definitely.

The combined action of the kidneys, cloaca and nasal salt gland provides *V. gouldii* with a most impressive excretory system. It is frequently stressed that reptiles cannot produce a hyperosmotic urine and that electrolytes can only be excreted by the kidneys to a limited extent. However, the extra-renal excretion of electrolytes obviates the need of a concentrating kidney, and it has been suggested by Schmidt-Nielsen *et al.* (1963) that an improvement in the concentrating capability of avian and reptilian kidneys would only lead to occlusion of the renal tubules with uric acid.

The excretory system in *V. gouldii* and other reptiles with nasal salt glands should therefore be regarded as an alternative system for water conservation, and electrolyte excretion to that found in mammals, rather than an inferior system. The latter view is based

purely on a basic difference in the function of the kidney in these animals.

The laboratory conditions under which measurements of evaporative water loss and renal and cloacal function were made are totally artificial, and the results are only useful for comparative purposes. No information can be obtained on the actual water losses and water requirements of animals in their natural environment by using these techniques. This is why the use of HTO in the study of wild populations is valuable, even though the actual partitioning of water losses is not revealed by this technique.

However, the discrepancies between rates of water loss measured in the laboratory and in the field are of sufficient magnitude to allow some approximate conclusions to be drawn. The animals at Flinders Chase spend at least 14 hours in burrows each day during Summer, during which time pulmo-cutaneous exchange of water takes place. An estimate of the magnitude of this exchange that was obtained at Renmark probably applies to Flinders Chase also, as the microclimate of the burrows in the two areas was similar. Therefore, about 2.5 ml. H_2O/Kg body weight is exchanged in the burrow with little or no net loss of water.

During the 10 hours or so spent outside the burrow the body temperature is maintained at about 35°C, while the ambient temperature varies. The humidity of the air is low, and so the climatic situation in the field is roughly similar to laboratory conditions at 35°C.

In this 10 hour period therefore, the amount of water lost from the skin and the eyes would be approximately 2.5 and 0.8 ml/Kg body weight, respectively. These values, which are shown in Table 50, were calculated from laboratory data, and are maximal estimates.

The remaining 16.2 ml/Kg of the daily water loss is made up of pulmonary evaporation, urinary excretion and defaecation while the animal is errant. The partitioning of this amount of water is difficult, as the cloacal loss depends on such variable factors as the water content of pellets and the frequency of elimination, and no information of this type could be obtained in the field studies. However, even if half of this amount is from the cloaca, an over-estimation from observations made of animals in terraria, about 8 ml. H_2O/Kg is lost from the respiratory tract in the period when the animal is active.

Allowing that this value for pulmonary evaporation is minimal it still represents 71% of the total evaporative water loss in the field, while cutaneous and optical losses represent no more than 22 and 7% respectively. Thus the respiratory tract is a much more important site of evaporation than laboratory experiments indicate because of the increased ventilation of the lungs that is associated with activity.

TABLE 50

Approximate Partitioning of Water Losses in V. gouldii at Flinders Chase

Location	Source	H_2O Loss (ml/Kg/day)	
Inside Burrow	Water exchange	2.5	
Outside Burrow	Cutaneous evaporation	2.5 = 22%	% of total evaporative water loss
	Optical evaporation	0.8 = 7%	
	Respiratory evaporation	8.1 = 71%	
	Cloacal elimination	8.1	
	Total	22.0	

This study of water balance has been concerned mainly with water losses and factors controlling them, but little has so far been mentioned of the sources from which the animal obtains water.

Surface water is an unreliable source as it is not always available, particularly in the more arid habitats. However, V. gouldii does drink in the laboratory and so it is likely that these animals drink when water is present in the field. Norris and Dawson (1964) have suggested that Sauromalus spp. store extra fluid in abdominal sacs when there is plenty of water available. It is

possible that V. gouldii might store extra water in the tissues under these circumstances as injected water-loads are retained in the body despite increased renal output. However, with water-loads greater than 15% of the body weight excretion of water commences in V. griseus (Khalil and Abdel-Messeih 1959) and the same is probably true for V. gouldii. Presumably the dilute ureteral urine is diverted away from the cloaca in a similar way to the diversion of urine from the bladder in water-loaded Gopherus agassizii (Dantzler and Schmidt-Nielsen 1966). In this way the diuresis would become effective in excreting the water-load.

It is unlikely that V. gouldii would drink sufficient water to voluntarily load the body to the extent produced by injection in the experiments. Therefore, the counteraction of the water diuresis by sustained cloacal resorption may be an artefact caused by excessively large and unphysiological water-loads.

If some water is naturally stored when surface water is available, it would presumably assist the animal in achieving water balance in drier periods. However, in the more arid regions where extreme droughts may last for many years, V. gouldii is still able to survive without employing this mechanism.

The major source of water available to V. gouldii is that incorporated in the food. Since this animal is carnivorous approximately 60 to 70% of the fresh weight of food is in the form of free water. In addition to this, water is also derived from the food

when it is metabolised. If only half of the dry weight of food is digested and resorbed, a conservative estimate, it can be calculated that the amount of metabolic water formed represents between 9 and 19% of the fresh weight of the food, depending on the substrate oxidised. Therefore between 70 and 90% of the fresh weight of food constitutes an accessible source of water for the animal, of which between 12 and 22% is metabolic water.

The animal also receives some water from the atmosphere by exchange. In a saturated atmosphere the water lost and gained by diffusion would be equal, consequently there would be no net loss of water. However, a similar diffusion of water would take place in a drier atmosphere, except that here there would not be an equal flux of water in each direction and a net water loss from the animal would occur.

The animals at both field areas were never dehydrated during Summer as the water content of the body was no different from at other times of the year. Therefore, the animals were able to obtain sufficient water to completely replace water losses.

To conclude, this study shows that the sand goanna, Varanus gouldii is well adapted to living in arid environments. It exhibits several physiological and behavioural adaptations that enable it to achieve water and electrolyte balance when water is scarce.

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