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CUTANEOUS WATER LOSS IN REPTILES

by

Albert Zucker

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1977

ALBERT ZUCKER

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

CUTANEOUS WATER LOSS IN REPTILES

by

Albert Zucker

Advisor: Professor Paul F. A. Maderson

Cutaneous water loss (CWL) in several reptiles was measured with a compartmentalized chamber technique and with in vitro and in vivo capsule techniques. Special attention was paid to the selection of the materials used in the construction of the apparatus, and the measuring procedure itself. Such considerations reduced the measuring errors which characterized previous studies in this area. In squamates, the stage of the epidermis in the shedding cycle was determined by taking skin biopsies at the time of measurement.

The rate of CWL does not significantly change after death, anesthesia, or water immersion during the renewal phase of the shedding cycle; and does not vary greatly among the body sites measured. CWL increases after acute hydration, water immersion during the post-shedding stage of the shedding cycle, and acute cloacal taping: it probably decreases after prolonged dehydration and exposure to a dry environment.

Several morphological observations aided interpretation of the relationship between CWL and the squamate shedding cycle. In several lizards the shedding cycle is not synchronized over the entire epidermis, and in many squamates, especially in snakes, external stimuli may alter the shedding frequency and affect epidermal histology. Generally, CWL begins to increase sometime during the re-

newal phase, peaks at approximately stage 5 of the shedding cycle, remains high during shedding, and decreases thereafter until the perfect resting condition of the shedding cycle is attained.

The squamate integument was analyzed as a complex diffusional barrier with heterogeneous horizontal (hinges and outer scale surfaces) and vertical (principally the beta and alpha layers) components. By a variety of experimental protocols, it was shown that the hinges are not more permeable than the outer scale surfaces, although it may take longer for the rate of water loss from the hinges to equilibrate in the measuring systems used in this study. The results of experiments involving cellophane stripping the epidermis, an analysis of the relationship between the shedding cycle and CWL, and several other indirect protocols show that the alpha layer is probably the primary permeability barrier while the beta layer is primarily a mechanical barrier.

The position is advocated that the measurement of CWL in reptiles as determined in this or any other study, is only meaningful in an analysis of the functional morphology of the integument, and that such studies do not have quantitative ecological applicability.

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Finally, I thank my wife, Liba, who unselfishly volunteered to sacrifice part of her dreams to help me fulfill mine.

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This Thesis is Dedicated to:

Sean Maderson

(1962-1977)

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INTRODUCTION

SECTION I. COMPARATIVE ANATOMY AND PHYSIOLOGY OF THE AMNIOTE INTEGUMENT

A. MAMMALS

The skin or integument of all vertebrates consists of an outer epidermis and an inner dermis separated by a basement membrane. The mammalian dermis can be subdivided into a superficial papillary layer and an underlying deep layer. The mammalian epidermis consists of a population of germinal cells which produces the more superficial keratinizing cells. The keratinizing cells die as they mature and the outermost layer, which consists only of such dead cells, is the stratum corneum. The epidermal cells of mammals only produce the alpha type keratin.

Human skin is relatively impermeable to water, that is, water can pass only slowly through it, 0.1-0.6 $mg/cm^2/hr$ (depending on the region of skin measured and the physical properties of the measuring system), as compared to 13 $mg/cm^2/hr$ for water evaporation from a free water surface at 37°C and 0% relative humidity (Bettley and Grice, 1965; Idson, 1973; Kligman, 1964; Lamke and Wedin, 1971; Scheuplein and Blank, 1971). Cutaneous water loss (CWL) in man is limited by the rate of diffusion through the skin, not by evaporation from the skin's surface (Scheuplein and Blank, 1971). Most authors feel that the stratum corneum is the most impermeable layer of the integument and therefore determines integumentary permeability. Baker and Kligman (1967), Idson (1973), Kligman (1964), and Scheuplein and Blank (1971),

all feel that all vertical components of the stratum corneum are uniformly impermeable, while Bettley (1970), Mali (1956), Malkinson (1964), and Wills (1972) feel that the most proximal part of the stratum corneum, the stratum compactum, is most impermeable.

Water movement thorugh the stratum corneum occurs mainly by intracellular paths. The small cross-sectional area of the intercellular spaces keeps them from being significant paths of water movement. Similarly, sweat glands and hair follicles are also unimportant for the water lost during long term measurements (Scheuplein, 1972; Scheuplein and Blank, 1971). Permeability is related to the activation energy of the molecule (water) and the system through which it diffuses (keratin and its matrix). In hydrated human skin the activation energy is 13-20 Kcal/mole (Idson, 1973; Spruit, 1971).

Hattingh (1973) feels that the stratum corneum is not the only layer that limits water loss. He finds that dried tongue epithelium is as impermeable as keratinized skin. However, he feels that the barrier (whatever it may be) has the ability to rapidly change its structure and permeability in response to environmental stimuli. Spruit and Herweyer (1967) feel that the barrier can be conditioned. Therefore, depending on the previous experience of the skin, the same physical treatment may cause a different quantitative effect on percutaneous water loss. Thiele and van Senden (1966) found a strong correlation between cutaneous circulation and cutaneous water loss. Hattingh (1972c) investigated the effect of some definite cutaneous vascular changes on percutaneous water loss. The relationship between cutaneous circulation and water loss is complex, but he concludes that the blood supply to the skin determines transepidermal water loss.

Thus, diminished cutaneous blood supply (cutaneous vasoconstriction) limits CWL, while increased capillary pressure allows greater CWL. Therefore, Hattingh feels the barrier is not a physical diffusion barrier but rather a physiological barrier. Although the integrity of the barrier is dependent on its lipid composition, no specific lipid has been linked in a causal manner to cutaneous permeability (Sweeney and Downing, 1970).

Temperature, keratin hydration, wind velocity, and ambient humidity all have an effect on the rate of CWL. It is difficult to manipulate one factor without affecting another. Thus, an increase in temperature will decrease the relative humidity, and also have a drying effect on the keratin. Changing wind velocity must also change ambient humidity. Even after a factor has been shown to have a direct effect, it is of interest to know if the effect changes linearly, or otherwise, with a change in the magnitude of the factor. Critical analysis of the factor's real effect is hampered, because some investigators measure equilibrated effects, while others measure short term effects (Goodman and Wolf, 1969; Grice, Salter, Sharratt, and Baker, 1971; Grice, Salter, and Baker, 1972; Hattingh, 1972a; Idson, 1973; Johnson and Shuster, 1969; Kligman, 1964; Lamke and Wedin, 1971; Mali, 1956; Rieger and Deem, 1974; Schmid, 1972; Spruit, 1971). Based on Scheuplein's (1972) analysis: temperature and keratin hydration would have direct effects, wind velocity would only act by affecting ambient humidity, and the ambient humidity would have a small direct effect by changing the concentration gradient, and an indirect effect by altering the keratin's state of hydration.

Although the structure and function of human skin have been stu-

died intensively, there have been few studies on the permeability of the skin of other mammals (Bartek, 1972; Hattingh, 1972b) or the relation between CWL and pelage (Cena and Monteith, 1975; Hattingh, 1973). It is not clear if desert mammals have lower rates of CWL than related mesic forms, nor if CWL decreases during dehydration (Allen and Roddie, 1973; Chew, 1955; Haines and Shield, 1971; Haines, Ciskowski, and Harms, 1973; Haines, Macfarlane, Setchell, and Howard, 1974; Hudson et al., 1972; Hulbert and Dawson, 1974; Hulbert and Rose, 1972; Macmillan and Lee, 1967; Schmidt-Nielsen, 1969; Shkolnik and Borut, 1969; Sokolov, 1962).

B. BIRDS

The body integument of birds is thinner than that of mammals, it lacks glandular specializations, and has feathers (composed of feather keratin) instead of hair (Cane and Spearman, 1972; Maderson, 1972ab; Stettenheim, 1972). As in mammals, the keratinization of the interfollicular epithelium is a continuous process (Parakkal and Alexander, 1972). Generally the rhamphotheca (bill integument) is hard and is composed of feather keratin, however, its leathery areas are composed of alpha keratin (Frasier et al., 1972). The podotheca (foot integument) is scaled, the outer scale surface is composed of feather keratin, while the inner scale surface and hinge are composed of alpha keratin (Baden and Maderson, 1970).

In birds total evaporative water loss (EWL) increases with an increase in temperature or rate of air flow. However, in both cases the increase is mainly due to a decrease in ambient water vapor pressure (Lasiewski et al., 1966ab). Although it was once thought that the ma-

jor source of evaporative water loss in birds is respiratory water loss (Crawford and Schmidt-Nielsen, 1967; Lasiewski et al., 1966ab), it now seems that at least in some birds CWL (through the skin and plumage) is larger than the respiratory component (Bernstein, 1969, 1971; Lasiewski et al., 1971). Lee and Schmidt-Nielsen (1971) showed that zebra finches can drastically reduce CWL during dehydration, and although Bernstein (1971a) contends that CWL decreases with an increase in temperature, Smith and Suthers (1969) and Bouverot et al. (1974) claim the reverse.

C. REPTILES

1. Non-lepidosaurs

Generally the entire integument in the two recent orders of non-lepidosaurian reptiles (the crocodilia and the testudinata) is characterized by scales. Scales always have dermal and epidermal components. Some scales are strong protective units (for example, the leg scales of tortoises, or the back scales of crocodiles), while in other cases (for example, neck and inguinal scales in all turtles and the leg scales of sliders), they are as pliable as non-scaled skin. Scales range in shape from strongly overlapping or imbricate (tortoise and sea turtle scales), to cuboidal (crocodile belly scales), to granular (slider leg scales).

The dynamics of the keratinization process in these two orders has been described as continuous, the cornified layers are thick over the entire integument and shedding is generally restricted to single scale units or less (Alexander, 1970; Matoltsy and Huszar, 1972; Parakkal and Alexander, 1972; Spearman and Riley, 1969).

On the basis of X-ray diffraction, Baden and Maderson (1970) showed that when epidermal alpha keratin synthesizing regions alternate with feather keratin synthesizing regions, the outer scale surface (OSS) produces the feather type, and the inner scale surface (ISS) the alpha type. Baden and Maderson (1970) also showed that this situation characterizes the imbricate podotheca scales of birds (but not those on the plantar aspect of the foot; Sawyer, personal communication). The keratin distribution in the Chelonia is more complex; the relatively hard "tortoise shell" consists of feather keratin. Scales on the rest of the integument of Testudo and Erytmochelys have feather keratin on the OSS and alpha keratin on the ISS. The integument of the extremities and neck of the slider, and the entire integument of the softshells and the leatherback, contain only alpha keratinizing cells. Much of this analysis was independently verified ultrastructurally by Alexander (1970), who also showed that alpha-containing cells do not lose their membranes during keratinization, while the loss of cell membranes occurs frequently during maturation of feather keratin containing cells. In fact, the feather-containing corneous layer over the carapace of hardshelled turtles has no remains of cell membranes.

2. Squamates

The integument of all squamates is scaled. There is more variation in scale form in squamates than in any other group of reptiles. Scales can be highly imbricate (for example, Sceloporus back scales), cuboidal (for example, Tupinambis belly scales), granular (for example, Anolis back scales), spiny (for example, Phrynosoma back scales), annular (for example, amphisbaenids), or any intermedi-

ate condition. Generally, squamates shed their outer epidermal generation (see below) several times a year. In snakes and many lizards this event is synchronized so that the entire outer generation is removed as one piece (Maderson, Mayhew, and Sprague, 1970). Several species of lizard eat the old generation as they remove it (Bustard and Maderson, 1965).

The histological changes during the shedding cycle have been described both at the light and electron microscopic levels (Alexander and Parakkal, 1969; Bryant et al., 1967; Maderson, 1965a, 1966; Maderson, Chiu, and Phillips, 1970b; Maderson, Mayhew, and Sprague, 1970; Maderson et al., 1972; Roth and Jones, 1967, 1970; Spearman and Riley, 1969). The following description of the histological changes occurring on the OSS in the tokay during the sloughing cycle, emphasizes those points most germane to the factors affecting the rate of CWL. The shedding cycle is a continuum, and it is only divided into arbitrary stages to facilitate presentation.

Just after shedding the epidermis is in the post-shedding condition and contains from without inwards, an Oberhautchen, beta, mesos, and alpha layers, several layers of immature cells and a stratum germinativum (figure 1). While at the time of shedding the three outermost layers are fully mature and do not change throughout the remainder of the cycle, the alpha layer is usually thin when shedding occurs. During the next few days the immature cells directly beneath the alpha layer become steadily incorporated into the maturing tissue. Beneath these presumptive alpha cells are less flattened cells which do not completely keratinize until just before the following shed. At about the time that the last presumptive alpha cells are

incorporated into the alpha layer, the germinal population becomes mitotically quiescent. During this perfect resting condition (which is prolonged in infrequent shedders) the epidermis consists of one epidermal generation.

As the renewal phase begins, the epidermis is reactivated and produces cells which will eventually constitute a new inner epidermal generation. The first cells produced, which synthesize feather keratin, form the new Oberhautchen and beta layers (figures 2 and 3). These cells synchronously lose their nuclei and cell membranes a few days before shedding (figure 4). Usually a day before shedding the beta layer shrinks and its staining characteristics change. By this time the beta layer is separated from the germinal layer by many living cell layers. Most of these cells will contain alpha keratin when they cornify (before or after shedding).

Shedding involves the separation of an outer mature generation from a partially mature inner generation.

The shedding cycle described above can be divided into several stages, numbered 0 to 6 (Maderson, Chiu, and Phillips, 1970b). In the present study many of these stages have been further subdivided into early, mid, and late conditions, for finer resolution in association with the presentation of physiological data (see figures 15-29, 42, 43, 45-47, and 49).

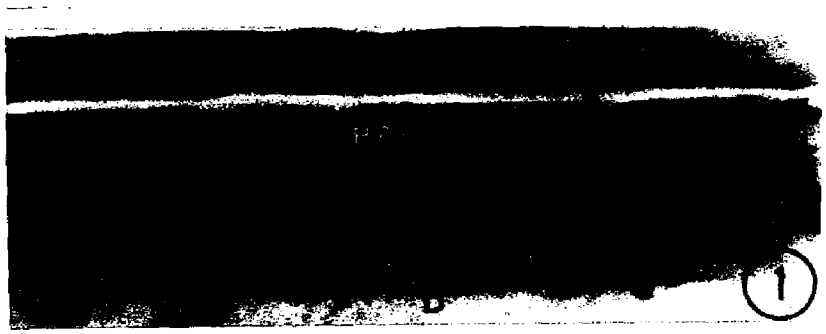
In snakes it has long been known that the texture of the integument, especially over the eye, undergoes characteristic changes during the shedding cycle (Maderson, 1965b). Although the relationship between gross changes in the spectacle and epidermal histology is inexact (Chiu, Maderson, and Zucker, unpublished observations),

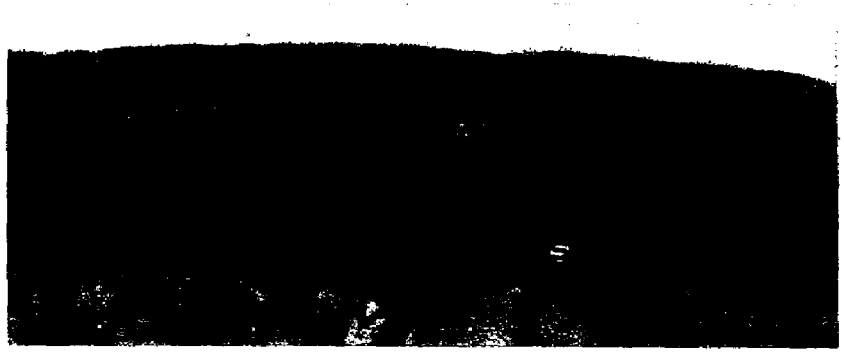
Figure 1. Photomicrograph through the outer scale surface of Gekko gecko skin just after shedding (the post-shed condition). BO - beta layer, AO - alpha layer, PAO - presumptive alpha cells, I - immature cells, SG - stratum germinativum, D - dermis. The Oberhautchen layer on top of the beta layer is not discernible at this resolution. The mesos layer is a very thin layer which is usually obscured by the artifactual split between the alpha and beta layers. The alpha layer is thin but becomes thicker as more presumptive alpha cells mature. (X 400).

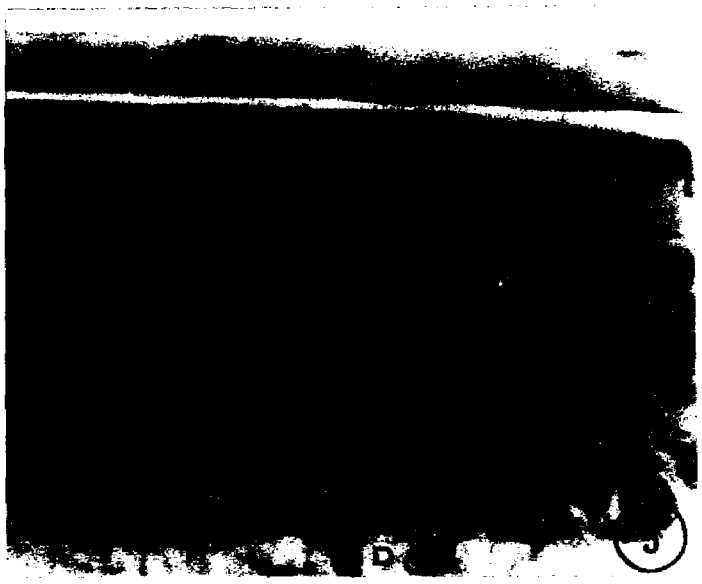
Figure 2. Photomicrograph through the outer scale surface of Gekko gecko skin during early renewal (stage 2). CLO - clear layer. By this stage the alpha layer has attained maximal thickness. For other symbols see figure 1. (X 400).

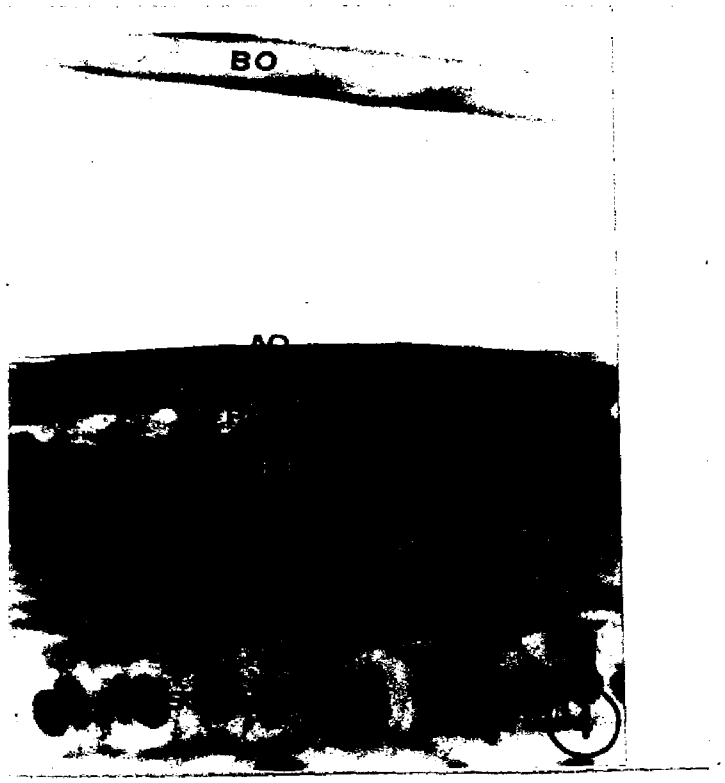
Figure 3. Photomicrograph through the outer scale surface of Gekko gecko skin during mid renewal (mid stage 4). POBI - presumptive inner Oberhautchen, PBI - presumptive inner beta layer. The inner beta layer is in the midst of keratinization. For other symbols see figures 1 and 2. (X 400).

Figure 4. Photomicrograph through the outer scale surface of Gekko gecko two days prior to shedding (stage 5). PMI - presumptive mesos cells, PAI - presumptive alpha cells. S - indicates the plane where the separation of the outer generation from the inner generation occurs during shedding. The beta layer (PBI) is still thick and chromophilic although almost all traces of its nuclei have been lost. For other symbols see figures 1-3.





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such changes do indicate the general epidermal condition. Usually, the spectacle starts to become cloudy during early renewal (stage 2 to early stage 3), becomes opaque at mid renewal (late stage 3 to early stage 4), and becomes clear during late renewal (late stage 4 to stage 5). Similar, but more subtle, changes have been noticed for several lizards (Zucker, unpublished observations).

SECTION II. CWL AND THE REPTILIAN INTEGUMENT

In hyperosmotic or dry environments, the skin is an avenue of water loss, while in hypoosmotic habitats it may be an avenue of water uptake (Bentley, 1971). Since reptiles lack cutaneous glands over most of their body surface (that is, mucous or sweat glands), the terms: cutaneous water loss, transepidermal water loss, percutaneous water loss, cutaneous evaporation, cutaneous perspiration, and insensible cutaneous water loss, are synonymous.

A. METHODS OF ANALYSIS - COMPARTMENT STUDIES

In two fundamentally different approaches to the problem of measuring CWL *in vivo*, the animal is usually maintained in a tightly sealed container. Some workers have sealed the subject's cloaca (Bentley and Schmidt-Nielsen, 1966a; Dmi'el, 1972; Prange and Schmidt-Nielsen, 1969) but Roberts (1968) suggests that if the animal does not urinate or defecate during the experiment, no significant loss occurs through this part of the body.

In two compartment systems, water derived from the head and the body region are measured separately. The two regions of the animal

may be exposed to a current of predried air, or maintained in stagnant air. The drying technique for reptiles usually involves passage of incoming air over Drierite (trade name for calcium sulfate) or calcium chloride. The amount of water derived from the body may be determined gravimetrically following absorption over Drierite or calcium chloride, or the exhaust air's water vapor may be monitored by an infrared analyzer or a sensitive hygrometer. Bettley and Grice (1965) and Neuman et al. (1941) feel that calcium chloride absorption is not adequate for either initial drying or capture of transepidermal water. They feel that more complete drying can be obtained by passing air through aluminum or glass tubing immersed in a dry ice-alcohol solution (this causes water to condense in the tubing, which can be weighed). Crawford and Kampe (1971) used this technique for a lizard. However, drying agents are more convenient and they leave a constant amount of moisture in the effluent air. Since most previous work done with reptiles involved such drying agents, and since the absolute rate of CWL is not important, their use is preferred. Absorbents generally flush, that is, lose absorbed water, and using humidity sensors circumvents this problem. Lasiewski et al. (1966a) and Licht and Bennett (1972) indicate good agreement between measurements made with hygrometers and absorbents. Hattingh (1972c) severely criticizes long term studies using dehydrated skin; however, this is the only way to get repeatable results of water lost through the skin.

If the animal is placed into a single container (one compartment system), it is usually exposed to a current of predried air. In such systems the total weight lost by the animal is due to three

components: metabolic, respiratory, and cutaneous. The first refers to the loss of metabolic carbon brought about by oxygen-carbon dioxide exchange in the lungs. Fasting squamates at rest have respiratory quotients (RQ, volume of carbon dioxide produced divided by the volume of oxygen consumed) of about 0.7 (Benedict, 1932; Claussen, 1967; Roberts, 1968), which means that metabolic weight loss will usually only account for approximately 5% of the total weight loss (Claussen, 1967). Therefore, this avenue of weight loss is often considered negligible (Claussen, 1967; Lasiewski et al., 1966b; Minnich, 1970; Munsey, 1972; Roberts, 1968). The remaining weight loss is called evaporative water loss (EWL). Respiratory water loss refers to the water lost as a result of exhaling air which has become saturated in the lungs and respiratory passages. The magnitude of this component can be estimated by determining pulmonary minute volume, ventilation rate, nasal and ambient temperature and ambient humidity. However, it is usually estimated by measuring oxygen consumption, since in the system outlined, ambient temperature and body temperature are equal, and ambient humidity of the predried air is almost zero (Bentley and Schmidt-Nielsen, 1966a). Dmi'el (1972) and Krakauer (1970) showed that extrapolations based on oxygen consumption may be erroneous. Labial and lingual water loss can make up a large percentage of the total water loss, and Cloudsley-Thompson (1971, p. 70) cites Reichling's finding that 20% of EWL in Lacerta agilis (at 3°C) was from its eyes. However, these routes of water loss (labial, lingual, and ocular) have not been taken into consideration in determining CWL in one compartment systems. Thus, one compartment systems may overestimate CWL, while two compartment systems would overestimate pulmo-

nary water loss. Once metabolic and respiratory water loss are accounted for in one compartment systems, CWL can be derived since it makes up the remainder of the total weight loss. Single compartment systems might seem preferable in that they put less strain on the animal; however, Bradshaw (1970) found similar results for one and two compartment systems.

B. METHODS OF ANALYSIS - OTHER TECHNIQUES

Gans *et al.* (1968) and Krakauer (1970) measured CWL in *L. doliata* and a *Natrix* species, respectively, with a different *in vivo* system. The snakes were injected with tritiated water and then placed in a water-filled tube which reached from the mouth to the cloaca. Water flux was determined by scintillation counting the surrounding water and the plasma. However, in these cases they were probably measuring water exchange with keratin rather than trans-cutaneous water loss.

There have been only a few attempts to measure squamate skin permeability *in vitro* (George, 1947; Pettus, 1958; Tercafs, 1963; Tercafs and Schoffeniels, 1965). In such studies a piece of skin separates two compartments differing in osmolarity or water vapor pressure. Permeability can be measured by a change in fluid volume (osmometer), solute concentration, water vapor pressure, or concentration of tritiated water.

C. THE PROBLEM OF SURFACE AREA

At best, *in vivo* techniques measure total CWL, which should be proportional to surface area (SA). Most investigators rely on a relationship between surface area and body weight (W): for example,

$SA = kW^{2/3}$ or $SA = kW^{0.67}$ or $\log SA = \log k + 0.67 \log W$ (where k is a constant). This formula also represents the surface to volume or weight relationship of many regular geometric objects; for a sphere (the geometric object with the lowest surface to volume ratio) k = 4.83, and for a cube k = 6. For most animals, from unicellular to multicellular homeotherms, k is between 9 and 11.8, or about twice the value of k for a sphere (Hemmingsen, 1960).

Currently, several different equations are used for reptiles. Hemmingsen (1960) says that although k in general is twice that of a sphere, there are body types that are exceptions to the rule. Thus, for serpentine forms (Ascaris spp.; snakes, based on Benedict, 1932) k is three times that of a sphere or $SA = 12.5W^{0.67}$. Benedict (1932) arrived at this formula by assuming the exponent is equal to 0.67, and then found the average k for snakes with a weight range of 3.5-12.2 kg. Hemmingsen (1960) includes a 32 kg snake which Benedict (1932) rejected. Dmi'el (1972) also used the formula $SA = 12.5W^{0.67}$ for her snakes (body weight range 0.14-0.58 kg). Gans et al. (1968) computed the surface area of 21 Elaphe climacophora, ranging in weight from 8-600 gms. Their method for determining surface area was to multiply the snake's circumference anterior to the midbody by its total length; that is, SA = CL (where C is circumference and L is length), which is the formula for determining the surface area of cylinders. Since a snake is not a cylinder, their regression equation, $SA = 25.05W^{0.63}$, has a constant approximately double that used by Benedict (1932) and others. However, Gans et al. (1968) attribute the difference to Benedict's (1932) use of heavy-bodied snakes. Elick and Selander (1972) determined the surface area of small snakes

(average weight for all three species was below 6 gms) by skinning the animals. The regression equations for their three species are: $SA = 12.4W^{0.74}$, $SA = 9.8W^{0.76}$, and $SA = 10.5W^{0.76}$. Benedict (1932) also attempted to determine the surface area of snakes by skinning, but he felt that this results in considerable stretching of the integument in all directions.

The surface area weight relationship used most often for other reptiles is: $SA = 10W^{0.67}$ (Benedict, 1932; Bentley and Schmidt-Nielsen, 1966ab). Tercafs and Schoffeniels (1965) used the equation $SA = 8W^{2/3}$, but their source for this equation was a study on anurans and thus seems inappropriate. Norris (1967) feels that the formula $SA = 10W^{0.67}$ is not adequate for lizards and he also feels that stretching is less of a problem with lizards than with snakes. He determined the surface area for different species of lizards by skinning, but he did not compute a regression equation from his data for lizards as a whole. I computed a least squares regression from his data (his table 4). The resulting equation, $SA = 10.6W^{0.69}$, is not significantly different from the accepted equation. Claussen (1967) measured the surface area of 5 Uta stansburiana and 6 Anolis carolinensis by skinning. He arrived at the equations: $SA = 16W^{0.47}$ and $SA = 11W^{0.83}$ for Uta and Anolis, respectively. He does not say how he arrived at these equations nor if they are significantly different from the accepted equation. Bartlett and Gates (1967) measured the surface area of one 18.4 gm lizard by making a silver cast of its body and measuring some electrical properties of the cast. They determined this animal's surface area to be 75.8 cm^2 or about 10% greater than would be estimated with $SA = 10W^{0.67}$ (but see below).

With turtles the relationship is even more complex. Benedict (1932) admits that he is not sure if the plastron and carapace weight or surface area should be included for physiological reasons. Hurlbert (1966) agrees, but Bentley and Schmidt-Nielsen (1966ab) feel there is little alternative but to include both in determining the surface to weight relationship. Benedict (1932) used 12.6 as the value of k for the alligator. The alligator seems more heavy-bodied than most lizards and therefore should, in comparison, have a smaller constant. In fact, Spotila et al. (1972) assign k a value of 10, however, they incorrectly cite Benedict as using such a value. Bentley and Schmidt-Nielsen (1966b) and Schmidt-Nielsen (1973, p. 91) feel that an accuracy of greater than 10-20% cannot be achieved in measuring surface area.

Both skinning and the silvercast technique only give the exposed surface area. However, up to 50% of the surface area of a scaled vertebrate may lie in the inner scale surface and hinge region, which is not exposed to the surface. Since several authors (Claussen, 1967; Gans et al., 1968) have discussed the possibility that the inner scale surface-hinge region may be especially permeable to water, the surface area of these regions should certainly be considered when presenting data in terms of water loss per unit body area. Bartlett (cited in Norris, 1967) found that as far as heat transfer is concerned, effective surface area was only 89% total surface area (very close to that predicted by $SA = 10W^{0.67}$). Many reptiles have membranous areas (for example, the gular fan of iguanids and the webbed feet of many aquatic forms). Dawson and Hulbert (1970) found in marsupials that such areas may give a misleading total surface area

for expressing physiological parameters. Unlike mammalian skin, or even that of most other scaled vertebrates (for an exception see Fishelson, 1973), the lepidosaurian integument is covered with keratinaceous projections, the Oberhautchen spinules (Maderson, 1965a; Maderson and Licht, 1967; Maderson, Mayhew, and Sprague, 1970; Ruibal, 1968; Stewart and Daniel, 1972, 1973). Should their surface area be included when water loss data are presented? Kleiber (1961, p. 185) asks what should be measured in determining surface area, but reaches no conclusion.

Finally, these regression equations simply describe a relationship between two parameters as long as the relationship does not change (for example, the relationship between surface area and volume in a series of spheres). But if there is a non-linear relationship between two parameters (using our example, as the object becomes larger - it becomes ovoid) a simple regression is inappropriate.

D. RESULTS OF MEASUREMENTS OF REPTILE CWL

1. In Vitro and Associated Techniques

Tercafs and Schoffeniels (1965) and Gans et al. (1968) showed that hydrated squamate skin is more permeable than dehydrated skin. Since the skin of terrestrial squamates is relatively dehydrated (Khalil and Abdel-Messeih, 1954, 1959ab, 1961, 1962), CWL is minimized. Although agamid skin is very impermeable when dehydrated (for example, compared to skinks and geckos; Dawson et al., 1966), it becomes disproportionately permeable when hydrated (Tercafs and Schoffeniels, 1965). In fact, Tercafs (1963) found that in vitro, the skin of Uromastyx at 200 mosmole concentration difference is as

permeable as amphibian skin and responds to ADH as does amphibian skin.

Several studies have tested the bidirectional permeability of the squamate integument, and whether it has the ability to change its permeability in some circumstances. George (1947) and Dunson and Robinson (1976) found that squamate skin is rectified, in that under the same conditions water leaves the integument (exoosmosis), more easily than it enters (endoosmosis). Tercafs and Schoffeniels (1965) also demonstrated that lizard skin is rectified, but in the opposite direction. Krakauer (1970) showed that Natrix skin is slightly less permeable in seawater than in fresh water. Pettus (1958) was unable to find any osmotic movement across the skin in two species of Natrix.

2. Chamber Technique

Chew (1961) and Chew and Damman (1961) measured CWL in various squamates. They found that squamates exhibit lower CWL at any given ambient temperature than mammals. Although Chew (1961) concluded that squamate skin is nearly waterproof, subsequently it has been shown that CWL usually accounts for more than 50% of the total EWL (Bentley and Schmidt-Nielsen, 1966a). Thus, although absolutely small, CWL would seem to be of great relative importance to the water economy of squamates.

Studies on total water loss in reptiles have shown that, for the most part, EWL is inversely related to the aridity of the animal's natural habitat and shows little phylogenetic correlation. Thus, at a given temeprature and humidity, Bentley and Schmidt-Nielsen (1965, 1966a), Bogert and Cowles (1947), Cloudsley-Thompson (1967, 1969), and Diefenbach (1973) found that crocodilians have

very high rates of water loss. Aquatic turtles (especially soft-shells; Bentley and Schmidt-Nielsen, 1966a, 1970; Bogert and Cowles, 1947) and amphisbaenians (Bogert and Cowles, 1947; Krakauer et al., 1968) also have high rates of EWL. Desert tortoises (Bentley and Schmidt-Nielsen, 1966a) have lower rates of EWL than semiaquatic turtles. Mesic squamates have higher rates of EWL than most xeric forms (Bentley and Schmidt-Nielsen, 1966a; Bogert and Cowles, 1947; Claussen, 1967; Cloudsley-Thompson, 1965; Dawson et al., 1966; Dmi'el, 1972; Elick and Selander, 1972; Gans et al., 1968; Krakauer, 1970; Munsey, 1972; Prange and Schmidt-Nielsen, 1969; Roberts, 1968; Sexton and Heatwole, 1968; Warburg, 1966).

There are several conditions in in vivo systems which may modify experimental results: 1) air humidity or, more correctly, water vapor pressure; 2) air flow rate; 3) air temperature; and 4) activity of the subject.

a. The effect of humidity

Schmidt-Nielsen (1969, p. 290) states that "cutaneous evaporation can be expected to increase in relation to the increasing water saturation deficit". Krakauer (personal communication) also believes the drying power of the air to be the important factor. From Fick's Law (see discussion, pp. 197-198), it is obvious that the concentration difference (that is, ambient water vapor pressure minus membrane water vapor pressure) is important. In reptiles, unlike mammals, the temperature of the skin in the measuring system usually closely approximates ambient, so that the saturation deficit is equal to the concentration difference.

Benedict (1932) measured insensible weight loss of sev-

eral boas at various high ventilation rates (2.5-8.0 liters/minute). Air was predried by passage through sulfuric acid. Benedict feels that the higher the ventilation rate the lower the humidity around the animal, because the air current will wash away evaporated water from the animal. He concludes that ambient humidity has no effect on total water loss. However, at high ventilation rates, the air exiting from the drying agent is not as dry as when it goes through it slowly. In addition, the slowest air flow rate he used is an order of magnitude greater than that used currently and his ventilation rates have only marginal physiological relevance. Minnich (1970) found that in lizards total water loss is inversely proportional to ambient humidity, and Warburg (1965, 1966) found that lizards lose water more rapidly in dry air than humid air. Bogert and Cowles (1947) and Diefenbach (1974) found this to be true for crocodilians. Snyder (1971) found that total water loss increased with an increase in temperature. However, when ambient water vapor pressure was held constant, an increase in temperature from 27-36°C had little effect. This indicates that normally it is the increased saturation deficit which accompanies the increased temperature that causes the observed higher rates of water loss. This has been confirmed by Krakauer (1970), but conflicts with Warburg (1966).

b. The effect of convection

Benedict (1932, p. 121) found that the air flow rate did not have any effect on total water loss. However, Benedict (1932, pp. 124-126) also reported that increased ventilation caused increased water loss (from 4 to 5.24 and from 7.3 to 8.43 gm/kg/day, in two snakes). Claussen (1967) found that increased air flow (convection)

did not increase total EWL of Uta but did that of Anolis. However, Claussen could not find any pattern as to which component of total EWL was more dependent on convection rate. Tregar (see discussion in Johnson and Shuster, 1969) has argued that linear velocity may be more important than actual flow. Claussen (1967) considered this possibility, but his data did not allow confirmation of this point. Gans et al. (1968) found no correlation between total EWL and convection rate in a variety of snakes. They suggested that even if CWL is limited by evaporation, a general increase in air flow may not increase air flow where most cutaneous evaporation occurs, presumably the hinge region (but see: Licht and Bennett, 1972; and Bennett and Licht, 1975). Crawford and Kampe (1971, p. 1257) state that increased air flow "reduces the boundary layer surrounding the animal, thus providing greater opportunity for evaporation at higher temperatures". They do not give their air flow rate nor any data relating to convection rates. Cohen (1975) provided evidence that CWL increases with flow rate, but the relationship was not linear. In cases where evaporation is not limiting, it would seem that air flow rate should have little effect other than via changing ambient humidity.

c. The effect of temperature

An increase in temperature will both increase the metabolic rate of an ectotherm, and increase the saturation deficit of the air. Increased metabolism causes greater pulmonary exchange. Thus, increased temperature will greatly increase respiratory water loss, because the animal will be breathing more frequently and the difference between the water vapor pressure of inhalent and exhalent air is increased. Often, at higher temperatures, a very dramatic in-

crease in EWL is found, which represents either salivation or panting (Crawford and Kampe, 1971; Morgareidge and Hammel, 1975). CWL is usually found to increase with increased temperature, but not to the same extent as the respiratory component (Bentley and Schmidt-Nielsen, 1966a; Crawford and Kampe, 1971; Snyder, 1971). However, Warburg (1966) found that increased temperature increased total water loss. He says that temperature may have a greater effect on respiratory water loss, while humidity would have a greater effect on CWL. He offered no support for this statement since he did not compartmentalize water loss. Cohen (1975) found a large increase in EWL between 30 and 40°C, which could be explained on the basis of a change in the air's saturation deficit, but this could not explain why there was only a small increase in EWL between 20 and 30°C. It seems likely that at 40°C he stimulated some panting (Crawford and Kampe, 1971), so that temperature did not have a linear effect. Snyder (1971) feels that the change in saturation deficit is more important than the direct thermal effect. Krakauer (1970) also found no relationship between CWL and temperature if differences in the saturation deficit at the different temperatures are accounted for. Dawson et al. (1966) feel that the increased saturation deficit, brought about by the increased temperature, should only affect CWL, if CWL is limited by evaporation, for example, in a skink (Sphenomorphus labillardieri). However, they feel that a change in saturation deficit should not affect CWL if CWL is limited by diffusion, for example, in a desert gecko (Gehyra variegata) and an agamid (Amphibolorus ornatus). Krakauer (1970) concurs with this viewpoint. However, these conclusions are incorrect, since an increase in temperature will give more water molecules

enough energy to overcome the activation energy for skin diffusion (Idson, 1973; Scheuplein, 1972). Of course, it is possible that the integuments of G. variegata and A. ornatus were so impermeable, and the measuring system used by Dawson et al. (1966) so relatively insensitive, that even an increase in the energy of the system would not lead to detectable changes in CWL. An increase in temperature has been found to increase CWL in Sauromalus obesus (Crawford and Kampe, 1971) and in several snakes (Dmi'el, 1972). Using tritiated water Gans et al. (1968) found that increased temperatures caused greater amounts of labelled water to be lost to the external solution, again indicating that the skin itself is more permeable at higher temperatures. However, Gans et al. (1968) attributed their results to changes in cutaneous circulation.

d. The effect of activity

Activity is an important determinant of total EWL. Benedict (1932) found that snakes lose more insensible weight when they are active. Active animals can lose four times the amount of water lost by resting animals (Chew and Dammann, 1971; Minnich, 1970). This explains the relatively large initial (first two hours) total water loss of animals placed in an in vivo apparatus (Warburg, 1965). Gans et al. (1968) showed that activity greatly increased only the respiratory component of EWL in snakes. Krakauer (1970) feels that since CWL is not related to metabolism, the increased CWL during activity is due to the flushing of pockets of high humidity in the hinge regions. While Dmi'el (1972) found that activity did not affect CWL in some vipers and colubrids, Claussen (1967), working with lizards, concluded the opposite.

e. Literature survey

As will be evident from the foregoing, and from Materials and Methods (pp. 51-59), previous studies of reptilian CWL contain such a variety of debatable theoretical premises and practical shortcomings that detailed comparison with the data presented in this thesis is impossible. In this section, an attempt has been made to normalize the results of a number of previous studies to permit at least empirical comparison, and to indicate the type of methodology which has been employed.

Table 1 presents values for CWL obtained from the literature for a variety of reptiles measured at or near 30°C. The regression used to relate surface area to body weight was $SA = 10W^{0.67}$ for the lizards and the caiman, and $SA = 12.5W^{0.67}$ for the snakes, irrespective of the formulas used by the original investigators.

Some indication of the method of measuring CWL is given in column 5. No attempt was made to differentiate values obtained by the *in vitro* desiccating capsule technique from those using the *in vitro* osmometer technique (both type A procedure). Also, no attempt was made to differentiate the studies grouped in category C on the basis of the accuracy of the technique. However, this type of procedure is indirect, and no matter how much attention the investigator pays to other technical aspects, these values are less reliable than those involving type B proceudres. Blank spaces indicate places where this reviewer was unable to acquire this information for the survey.

3. Organismic Factors Affecting the Rate of CWL

a. Regulation

Table 1. Rates of CWL reported for reptiles.

Species	1 Temp. (°C)	2 Wt. (gm)	3 CWL (mg/cm^2/ hr)	4 CWL (mg/ gm/hr)	5 Method of Study
Sauromalus obesus[4]	23	134	0.05	0.11	C
Sauromalus obesus[7]	30	140	0.13		B
Iguana iguana[4]	23	124	0.20	0.40	C
Amphibolorus ornatus[1]	37			0.5[a]	C
Amphibolorus ornatus[8]	30	2.8[a]		0.71	C
Gehyra variegata[8]	30	2.8[a]		1.52	C
Sphenomorphus labilliardieri[8]	30	2.8[a]		1.79	C
Anolis carolinensis[6]	30		0.19	0.93	B
Anolis carolinensis[15]	20		0.18		C
Uta stansburiana[6]	30		0.10	0.74	B
Lacerta viridis[16]	30		0.54		A
Uromastyx acanthinurus[16,17]	30		0.73		A
Snake[11]	20		0.92		A
Coluber ravergieri[9]	30	136	0.32		B
Spalerosophis cliffordi[9]	30	218	0.27		B
Vipera palaestinae[9]	30	581	0.10		B
Aspis cerastes[9]	30	125	0.06		B
Diadophis punctatus[10]	25	4.89	4.4		C
Carphophis vermis[10]	25	6.08	2.5		C
Virginia valeriae[10]	25	4.51	0.1		C
Crotalus atrox[5]	26-7			0.05	B
Crotalus scutellatus[5]	26-7			0.05	B
Pituophis catenifer[13]	25	570		0.23	C

Table 1. Rates of CWL reported for reptiles.
(continued)

Species	1 Temp. (°C)	2 Wt. (gm)	3 CWL (mg/cm^2/ hr)	4 CWL (mg/ gm/hr)	5 Method of Study
Natrix taxispilota[13]	25	673		1.07	C
Natrix maura[16,17]	b		0		A
Natrix f. compressicauda[12]	32	136.5		1.60	B
Natrix f. pictiventris[12]	32	159.9		2.43	B
Natrix sipedon[2]	20	9.6	0.42	2.45	B
Gopherus agassizi[14]	23	1770	0.063		C
Pseudemys scripta[4]	23		0.51		C
Terrepene carolina[4]	23		0.22		C
Terrepene carolina[15]	20		0.54		C
Caiman sclerops[4]	23	60	1.06		C

A. In vitro

B. Partition

C. Total - respir

a. Approximate value

b. Room temperature

1. Baverstock, 1975

2. Bentley and Licht, 1975

3. Bentley and Schmidt-Nielsen, 1965

4. Bentley and Schmidt-Nielsen, 1966a

5. Chew and Dammann, 1961

6. Claussen, 1967

7. Crawford and Kampe, 1971

Table 1. Rates of CWL reported for reptiles.
(continued)

8. Dawson *et al.*, 1966
9. Dmi'el, 1972
10. Elick and Selander, 1972
11. Hattingh, 1972a
12. Krakauer, 1970
13. Prange and Schmidt-Nielsen, 1969
14. Schmidt-Nielsen and Bentley, 1966
15. Spotila and Berman, 1976
16. Tercafs, 1963
17. Tercafs and Schoffeniels, 1966

Claussen (1967), Gans et al. (1968), Whitford and Livezey (1973), Cohen (1975) and others imply that squamates can control their rate of CWL. For mammals, such a position is presently only advocated by Hattingh (1972ab). In human studies it has been extensively documented that cadaver skin, and vasodilated and vasoconstricted skin, all have the same rate of CWL as normal in vivo skin (Ainsworth, 1960; Baker and Kligman, 1967; Burch and Windsor, 1946; Jelenko, 1967; Mali, 1956; Malkinson, 1964; Onken and Moyer, 1963; Pinson, 1942). Even the lowered rate of CWL during severe dehydration is explained by a decrease in skin temperature (brought about by a decrease in cutaneous blood flow) not a change in the permeability constant of the membrane itself (Yoshimura, 1964).

Pettus (1958), Krakauer (1970), and Dunson and Robinson (1976) found that CWL remains constant after animal death, cell death, or even in excised skin. It can be inferred from the experimental protocols used by Snyder (1971) and Elick and Selander (1972), that their studies showed that CWL remains constant after death. However, a living animal can behaviorally control its rate of CWL, either by selecting a more appropriate microhabitat, or by adopting a different posture (Cohen, 1975; Krakauer, 1970).

b. Skin shedding

Maderson, Chiu, and Phillips (1970a) concluded that there is no proven relationship between shedding and habitat, stress, regeneration, growth, or excretion. They suggest that shedding might be a pleiotropic effect of changes in the hormonal milieu selected for other, unknown reasons. Kropech and Soule (1970) suggest that shedding in aquatic snakes may be an anti-fouling mechanism.

Benedict (1932) found that snakes which were about to shed had very high rates of total water loss. He ascribed this increased water loss to an increase in cutaneous permeability during, or just before, shedding. Bogert and Cowles (1947) found large total evaporative loss in a shedding indigo snake. Claussen (1967) found that the cutaneous component of total water loss increased greatly in a shedding Anolis, and therefore suggested that the permeability of the skin increases at this time. Gans et al. (1968) found that total EWL of a shedding L. doliata was greater than it was between sheds. The next time the animal was about to shed, they removed the presumptive shedding skin and found no subsequent increase in total EWL. They concluded that the increased rate of water loss during shedding was due to increased activity, which causes mainly an increase in respiratory water loss. Minnich (1970) found an increase in total water loss even in sleeping D. dorsalis. Minnich attributed this to an increase in cutaneous permeability. Cohen (1975) found that the rate of CWL increased the day before shedding, was slightly lower during shedding, and decreased to normal levels a day or so after shedding. Dunson and Robinson (1976) found that the skin just after shedding was more impermeable than skin taken at a later, unknown, time during the cycle. Bradshaw et al. (1972, p. 623) reported that "Animals which accidentally came in contact with (paraffin) oil usually died from the effects of acute dehydration unless they were able to shed their skin whereupon the cutaneous water loss returned to normal". They definitely feel that the increased water loss was due to an increase in cutaneous permeability since, "48 hours after (their) first exposure to the oil, handling the lizards

resulted in scale loss, the scales detached themselves readily from the skin with handling and bare patches developed at points of abrasion" (Bradshaw, personal communication).

c. Scales

Rose (1969) separated the epidermal scutes (scales) from the bony elements of the plastron and carapace of Terrapene ornata. He found that animals without epidermal scutes over a large part of their body lost water much faster than control animals. The amount of water lost by the skinned animals was much greater than the water content of the skinned regions, thus indicating that epidermal scutes prevent loss of internal body water. Dunson and Robinson (1976) found that shed skin was as impermeable as whole skin. Licht and Bennett (1972) and Bennett and Licht (1975) reported respectively on mutant gopher and water snakes, which lacked scales over large portions of their bodies. The epidermis of the mutants was irregular, being morphologically similar to the hinge region of normal scales, and the dermal structure was also unusual. The mutant gopher snake's oxygen consumption and EWL were normal, as were EWL and CWL for the mutant water snake, leading the authors to conclude that the absence of normal scales does not affect CWL.

The relationship between scale size and CWL is contested. Claussen (1967) feels that granular scales (Anolis) have higher rates of CWL than overlapping scales (Uta). Soule (1966) and Soule and Kerfoot (1972) feel that since large scales have keels and mucronation, their surface area is increased, and therefore such scales should have high rates of CWL. Munsey (1972) also feels that large scales cause higher rates of CWL because the hinge regions are larg-

bounded real valued functions defined on X .

8.2 For a sequence of uniformly bounded finite measures $u_1,u_2,u_3,\ldots$ defined on X any Banach limit $u_*(\cdot) = B_j(u_n(\cdot))$ will be a finitely additive set function on the σ-field Σ .

8.3 A finitely additive set function $u(\cdot)$ defined on a compact Hausdorff space X is called regular if for each closed set $F \subset X$ and any $\epsilon > 0$ there exists an open set $0 \supset F$ such that $u(0-F) < \epsilon$.

8.4 Proposition 8.4: Let $u_1,u_2,u_3,\ldots$ be a sequence of uniformly bounded finite measures defined on the compact Hausdorff space X then for each Banach limit functional B_j there exists a unique finite regular meas $B_j(\int_X f du_n) = \int_X f du^*$ for all $f \in C_0(X)$

Proof: For each $f \in C_0(X)$ set $\varphi_j(f) = B_j(\int_X f du_n)$. Then φ_j will be

Proof: For each $f \in C_0(X)$ set $\varphi_j(f) = B_j(\int_X f du_n)$. Then φ_j will be

a bounded linear functional defined on $C_0(X)$. Hence by the Riesz representation theorem (see Dunford-Schwartz, p. 265) there exists a unique finite regular measure $u^*(\cdot)$ such that $\varphi_j(f) = \int_X f du^*$ for all $f \in C_0(X)$.

Q.E.D.

8.5 We refer to u^* in Proposition 8.4 as the weak Banach limit (WBL) of the sequence $u_1,u_2,u_3,\ldots$ under the Banach limit functional B_j (denoted by $u^*(\cdot) = WB_j(u_n(\cdot))$).

8.6 Let $P_1,P_2,P_3,\ldots$ be a sequence of probability measures and assume $\lim_{n\to\infty} \int_X f dP_n$ exists for all $f \in C_0(X)$, then the Riesz representation theorem assures that there exists a unique finite regular probability

measure $\bar{P}$ such that

$$\lim_{n\to\infty} \int_X f dP_n = \int_X f d\bar{P} \quad \text{for all} \quad f \in C_0(X) .$$

In such a case we will call $\bar{P}$ the weak limit of the sequence of measures $P_1, P_2, P_3, \ldots$ (denoted by $P_n \Rightarrow \bar{P}$) .

8.7 Proposition 8.7: Given a sequence of probability measures $P_1, P_2, P_3, \ldots$ defined on the compact Hausdorff space X. If

$\limsup_{n\to\infty} P_n(F) \leq P(F)$ for all closed sets F (P some probability measure) then there exists a unique regular probability measure $\bar{P}$ such that

(a) $\lim_{n\to\infty} \int_X f dP_n = \int_X f d\bar{P}$ for all $f \in C_0(X)$

and

(b) $\int_X f dP = \int_X f d\bar{P}$ for all $f \in C_0(X)$.

(Hence $\bar{P}$ will be the weak limit of the sequence $P_1, P_2, P_3, \ldots$).

Proof: (a) implies (b) by the remarks made in 8.6. Part (a) generalizes Theorem 2.1 of Billingsley (Billingsley 1, p. 11) to a compact Hausdorff space. For the sake of completeness we repeat the applicable part of Theorem 2.1.

By a linear transformation we can restrict the proof to only those $f \in C_0(X)$ such that $0 \leq f \leq 1$. Let

$$F_i = \{x \mid \tfrac{i}{k} \leq f(x)\} \qquad i = 0,1,2,3,\ldots,k .$$

Then

$$\sum_{i=1}^{k} \tfrac{i-1}{k} P\left[x \mid \tfrac{i-1}{k} \leq f(x) \leq \tfrac{i}{k}\right] \leq \int_X f dP < \sum_{i=1}^{k} \tfrac{i}{k} P\left[x \mid \tfrac{i-1}{k} \leq f(x) < \tfrac{i}{k}\right] . \quad (8.7\text{-}1)$$

The right hand side of (8.7-4) can be expressed as

$$\sum_{i=1}^{k} \tfrac{i}{k} \left[P(F_{i-1}) - P(F_i)\right] = \tfrac{1}{k} + \tfrac{1}{k} \sum_{i=1}^{k} P(F_i) .$$

A similar transformation of the left side (8.7-1) yields

$$\frac{1}{k}\sum_{i=1}^{k} P(F_i) \le \int_X f dP < \frac{1}{k} + \frac{1}{k}\sum_{i=1}^{k} P(F_i) \ .$$

Since $\limsup_{n\to\infty} P_n(F_i) \le P(F_i)$ we obtain

$$\limsup_{n\to\infty} \int_X f dP_n \le \frac{1}{k} + \frac{1}{k}\sum_{i=1}^{k} \limsup_{n\to\infty} P_n(F_i) \le \frac{1}{k} + \frac{1}{k}\sum_{i=1}^{k} P(F_i)$$

$$\le \frac{1}{k} + \int_X f dP \ .$$

Hence,

$$\limsup_{n\to\infty} \int_X f dP_n \le \int_X f dP \ .$$

Applying the above argument to $-f$ yields

$$\liminf_{n\to\infty} \int_X f dP_n \le \int_X f dP \ .$$

Therefore

$$\lim_{n\to\infty} \int_X f dP_n = \int_X f dP = \int_X f d\bar{P} \ .$$

Q.E.D.

8.8 The following proposition is a consequence of Alexandroff's theorem (see Dunford-Schwartz, p. 316) and is included in order to give a complete presentation.

Proposition 8.8: In a compact Hausdorff space X the sequence of probability measures $P_1, P_2, P_3, \ldots$ converges weakly to some $\bar{P}$ ($P_n \Rightarrow \bar{P}$) if and only if

$$\limsup_{n\to\infty} P_n(F) \le \bar{P}(F) \quad \text{for all closed sets } F.$$

Proof: Assume $P_n \Rightarrow \bar{P}$, hence $\bar{P}$ is regular (see 8.6). Consequently for a given closed set F and any $\epsilon > 0$ we can choose an open set

$0 \supset F$ so that $\bar{P}(0-F) < \epsilon$ (see 8.3). Let $0 \le f \le 1$ be a continuous function so that $f = 1$ on F and $f = 0$ on the complement of $0 \supset F$. Then

$$P_n(F) \le \int_F f dP_n \le \int_X f dP_n \quad \text{and} \quad \int_X f d\bar{P} = \int_X f d\bar{P} \le \bar{P}(0) \le \bar{P}(F) + \epsilon.$$

Hence,

$$\limsup_{n\to\infty} P_n(F) \le \lim_{n\to\infty} \int_X f dP_n = \int f d\bar{P} \le \bar{P}(F) + \epsilon .$$

Since ϵ is arbitrary we have

$$\limsup_{n\to\infty} P_n(F) \le \bar{P}(F) \quad \text{for all closed sets } F.$$

Conversely, assume $\limsup_{n\to\infty} P_n(F) \le \bar{P}(F)$ by proposition 8.7(a) we have that

$$\lim_{n\to\infty} \int_X f dP_n = \int_X f d\bar{P} .$$

If $\bar{P}$ is regular then the weak limit will be $\bar{P}$; if not, then by Proposition 8.7(b) there exists a $\hat{P}(\cdot)$ which is the weak limit and by the arguments given above

$$\limsup_{n\to\infty} P_n(F) \le \hat{P}(F) \quad \text{for all closed sets } F.$$

Hence we originally could have chosen $\hat{P}$ instead of $\bar{P}$ and then our original statement in Proposition 8.8 would remain valid.

Q.E.D.

er. Gans et al. (1968), Krakauer (1970), and Horton (1972) feel that large overlapping scales decrease CWL by covering the hinges or by reducing the number of hinges.

4. Location of the Permeability Barrier

For both mammals and reptiles, there is evidence that the dead corneous material is the permeability barrier. However, the outstanding question is of the relative effectiveness of alpha versus feather keratin containing tissues as permeability barriers in reptiles.

The work of Bennett and Licht (1975), and Licht and Bennett (1972) indicates that the beta layer is of relatively minor importance as far as water impermeability is concerned, since their mutants had thin beta layers, and normal rates of water loss. The only other keratinized epidermal component which could be the permeability barrier is the alpha layer. In non-squamate reptiles when alpha and feather keratins are spatially separated (one occurring in the OSS and the other in the ISS and hinge), feather keratin always occupies the place where greater abrasion occurs (the OSS). Furthermore, although reptiles are exposed to hard surfaces, they rarely have hypertrophied alpha material, as do mammals on their palmar and plantar surfaces. If, in fact, the two types of keratin do have different functions (feather keratin - counteracting abrasion, alpha - controlling permeability), a rationale for their stratification and for shedding in squamates may be found. Thus the outermost beta layer prevents abrasion of the permeability barrier (the alpha layer), and skin shedding is a method of restoring the abrasion retarding material on the outer scale surface. Hinge regions cannot be worn thin

by abrasion, and thus need little feather containing tissue.

Krakauer (1970) cites two reasons for assuming that the beta layer is the permeability barrier. A recently fed Thamnophis sauritis was so engorged that its scales did not overlap; its rate of CWL was five times that of post-absorptive snakes (its oxygen consumption was only twice that of post-absorptive snakes, so that activity could not be the cause of the increased CWL). Also, Krakauer feels that the maximal scale overlap occurs when a snake is straight and least overlap when the animal is sigmoidal (but not coiled) in shape. He found that CWL was greater when the animal was sigmoidal. He feels that this is strong evidence that the hinge (where the beta layer is thin) is more permeable than the OSS.

CWL is higher for softshell turtles than for other freshwater turtles, while the latter have higher rates of CWL than desert tortoises (Bentley and Schmidt-Nielsen, 1966a, 1970). Softshells have only alpha keratin, Pseudemys (a freshwater hardshell) carapace scutes are composed of feather keratin while its limbs have only alpha keratin, while the desert tortoise's (Gopherus) carapace and OSS of its limb scales are composed of feather keratin (Baden and Maderson, 1970). Thus animals which have low rates of CWL have more feather keratin in their integument, suggesting that feather keratin is a less permeable material.

MATERIALS AND METHODS

SECTION I. INTRODUCTION

The following text describes the experimental protocol for most of the experiments. For ease of presentation, exceptions and details for individual experiments are provided when that experiment's results are presented. All statistical tests were performed according to the procedures outlined in Sokal and Rohlf (1973).

SECTION II. GENERAL MATERIALS

Data were obtained on the following animals: tokay geckos (Gekko gecko), leopard geckos (Eublepharis macularius), green iguanas (Iguana iguana), desert iguanas (Dipsosaurus dorsalis), jeweled lacertes (Lacerta lepida), black tegus (Tupinambis nigropunctatus), yellow rat snakes (Elaphe obsoleta quadrivittata), boa constrictors (Constrictor constrictor), American crocodiles (Crocodylus acutus), spectacled caimans (Caiman sclerops), slider terrapins (Pseudemys scripta), a jaboty (Geochelone denticulata), a leopard frog (Rana pipiens), a Norway rat (Rattus norvegicus), and man (Homo sapiens).

Most animals were kept on a 12:12 light:dark photoperiod at 30°C, and the relative humidity was measured daily with a sling psychrometer.

Throughout the course of investigation, most squamates and the jaboty were housed in wire cages. The American crocodiles were kept in a large wooden pen (3 feet by 6 feet), with a small children's

pool (28 inches square) at one end. The caimans were kept in an inclined 20 gallon tank which contained 2 inches of water at its deepest point, with an adjacent dry substrate. The sliders and the leopard frog were kept in large damp bins in a cold room (11°C). The rats were kept in an animal room. All reptiles, except the sliders, had access to food and water. The lizards were squirted with water daily and the snakes were provided with fingerbowls of water, too small for them to enter. The tokays, leopard gecko, and lacertas were fed crickets and mouse pups, and the lacerta's diet was occasionally supplemented with chicken liver and fresh fruit. The iguanas and the jaboty were fed lettuce; the black tegus were fed eggs; and the crocodiles and snakes were given mice, rats, and chicks. Apart from the green iguanas most individuals adapted well to captivity.

Shedding records were kept for all squamates. All lizards which eat their shed, and all squamates which were not housed individually, were marked with a waterproof marker. A shed was indicated by the absence of a mark on these animals, or the presence of a molt in the cage of individually housed squamates. Some rat snakes were thyroidectomized according to the procedures described by Chiu and Lynn (1970ab).

SECTION III. MEASURING TECHNIQUES

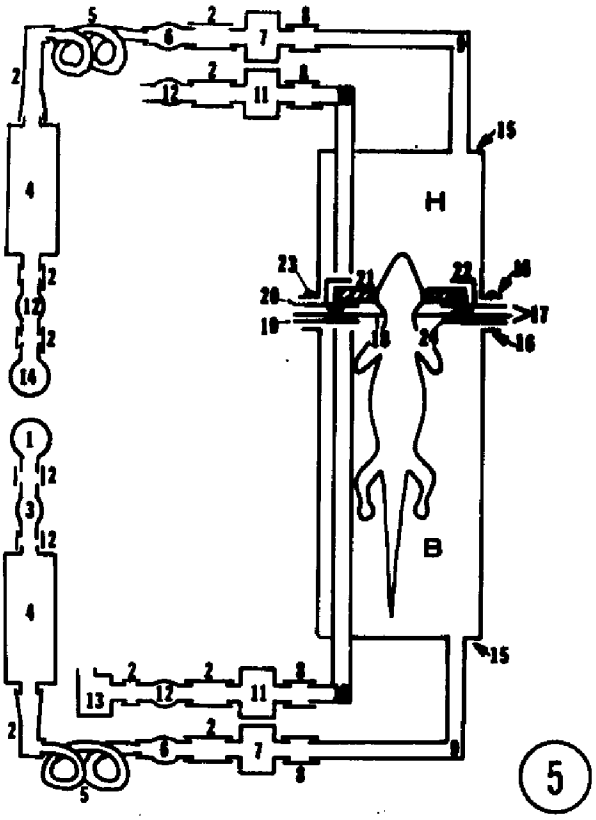
A. COMPARTMENT STUDIES

1. Description

The apparatus is illustrated in figures 5 and 6. Compressed

Figure 5. Schematic diagram of the chamber system which was used to compartmentalize water loss (H - head compartment, B - body compartment, 1 - dry air cylinder, 2 - Nalgon tubing, 3 - pressure regulating flowmeter, 4 - drying column containing 6 mesh Drierite, 5 - coiled copper tubing, 6 - stopcock, 7 - pre-drying tube containing 10-20 mesh Drierite, 8 - 20 mm length of neoprene tubing, 9 - brass inlet tube, 10 - brass exhaust tube, 11 - post-drying tube containing 8 mesh Drierite, 12 - flowmeter, 13 - wet test meter, 14 - school air line, 15 - solid end plate, 16 - doughnut shaped end plate, 17 - collar, 18 - neoprene membrane, 19 - lower brass plate, 20 - upper brass plate, 21 - pillory, 22 - brass clips, 23 - neoprene '0' ring, 24 - neoprene gaskets).

Figure 6. Surface view of collar assembly (Fig. 5, 17-24) in situ (1 - upper plate of collar, 2 - 1/8 inch screws, 3 - 1/4 inch clearance holes, 4 - pillory, 5 - brass clips, 6 - screws, 7 - hole for animal's neck, 8 - neoprene membrane, 9 - edge of hole through brass plate).



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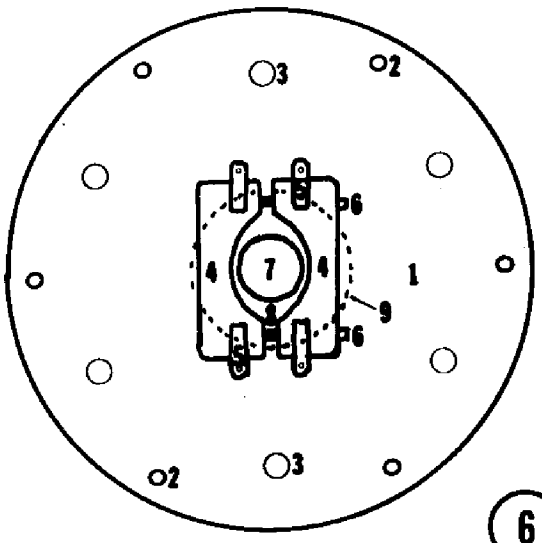

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air from a dry air cylinder (1) was passed through a flowmeter (3) and then dried by passage through a one meter column (25 mm ID) containing Drierite (4). Nalgon tubing (2), 3/8 inch OD, 1/16 inch wall, was used for all connections in the system unless otherwise specified. Flow rate was always regulated upstream to prevent a pressure buildup in the chamber. The air then passed into a 30°C BOD box being first warmed to that temperature by passage through a one meter length of coiled copper tubing (5). It then passed through a ground glass stopcock (6) and a small stoppered glass tube (16 mm OD) containing fine (10-20 mesh) indicating Drierite (7). The ends of the tube were plugged with cotton to prevent dust from escaping. The pre-drying tube was connected to the body compartment inlet via a short length (less than 20 mm) of neoprene tubing (8), 5/16 inch OD, 1/16 inch wall. Air pressure within the chamber was 3-4 mm mercury above ambient. Air passed through the chamber and left via a large cotton-plugged tube filled with 8 mesh indicating Drierite (11), which was attached to the chamber by a short length (less than 20 mm) of neoprene tubing (8). Air then passed out of the BOD box, through a flowmeter (12) and a wet test meter (13), and was finally released into the laboratory. A parallel system passed air through the head compartment, except that a school air line was used as the air source (14) and a wet test meter was not attached.

In the two compartment system a 4 inch (OD) brass cylinder formed the chambers. A solid end plate (15) was soft-soldered to each compartment. The inlet (9) and outlet (10) brass tubes extended through the end plate and were attached to it by soft solder. At the other end of each compartment a doughnut shaped end plate (16) was

attached by soft solder. The latter connection was the only one which required periodic repair. The doughnut end plate attached to the body compartment had six 1/4-20 tapped holes, while the one attached to the head compartment had six 1/4 inch clearance holes. A machined groove in each of the doughnut shaped end plates housed a neoprene '0' ring (23). A collar (17) was constructed of two pieces of brass (19 and 20), each with a central hole. Screws were tapped through the lower piece of brass. A large neoprene membrane (18) was sandwiched between two flat neoprene gaskets (24), this membrane complex was located in the collar. A pillory like structure (21) was made of brass and rested on top of the upper collar. The pillory was prevented from moving by 4 brass spring clips (22) attaching it to the upper surface of the collar (see figure 6).

2. Procedure

Food was withheld from lizards and snakes for at least 3 or 7 days (respectively) prior to compartment measurement. The cloacal contents of the tokays were emptied by applying gentle pressure to the abdomen, but this was not effective for the other squamates used in this study. Depending on their size, animals were weighed on a Mettler P-10 or P-1200 balance to the nearest 1.0 or 0.01 gram, respectively.

The animal's head was passed through the neoprene membrane and stopcock grease was liberally applied as a sealant around the animal's neck. The pillory was tightened around the animal's neck and posterior end of the jaw, and then attached to the collar by spring clips. The pillory prevented the animal from falling into the body compartment. Some animals would gape when the pillory was

tightened. While this increased head water loss, attempts to hold the mouth closed with rubber bands killed many animals. Furthermore, it was impossible to establish whether the mouth was opened during the experimental runs. Therefore, while head water loss was measured, it was not analyzed. Cloacas were taped shut with 1/2 inch Curity adhesive tape (#7650, The Kendall Co., Chicago, Ill.). Taping does not always prevent urination or defecation, but such events can usually be detected by examining the chamber or the tape.

Although Roberts (1968, p. 584) stated that, "Measurements of taped and non-taped animals were similar in the absence of excretory loss", in retrospect some of my data indicate that taping may increase the rate of CWL. CWL was determined on 23 dehydrated tokays, only 4 of which were not taped. CWL of 18 out of the 19 taped animals was higher than that of any non-taped animal, the difference being highly significant (Mann-Whitney U test, $p < 0.001$). The one exception was later measured without tape, and was found to have the same rate of CWL as with tape. Also two tokays were measured while their cloacas were not taped and then again after they were taped. In both cases CWL was higher when the animals were taped (17.35 compared to 20.80 and 10.9 compared to 11.3 mg/0.5 hr). There are several possible reasons why an acute taping could cause CWL to increase. Taping might decrease tail venous return and increase tail blood pressure, which might increase CWL (see Hattingh, 1972c). Taped animals may be more active than non-taped animals, and the tokays might strip off some keratin as they remove the tape with their legs (for other effects of taping see pp. 165-194 and discussion).

The animal was then placed into the body compartment and

the head compartment was bolted on. The chamber was placed in a BOD box and the inlet and outlet lines connected. The seal between the two compartments was then checked by closing the inlet stopcock to one compartment and reading that compartment's exhaust flowmeter. This procedure was repeated for the other compartment. If both flowmeters read zero it was assumed that a good neck seal existed, and the run was continued. The seal was also checked in this manner after each weighing. Usually the exhaust flowmeters were watched for about five minutes to make sure the animal was breathing regularly. Drierite tubes were weighed to the nearest 0.1 or 0.01 mg with a Sartorius 2463 or Mettler H-20 balance, respectively. After the runs a skin biopsy was usually taken from the gular or lower jaw region, and the specimen was prepared for histological examination as described by Maderson, Chiu, and Phillips (1970b).

3. Comments

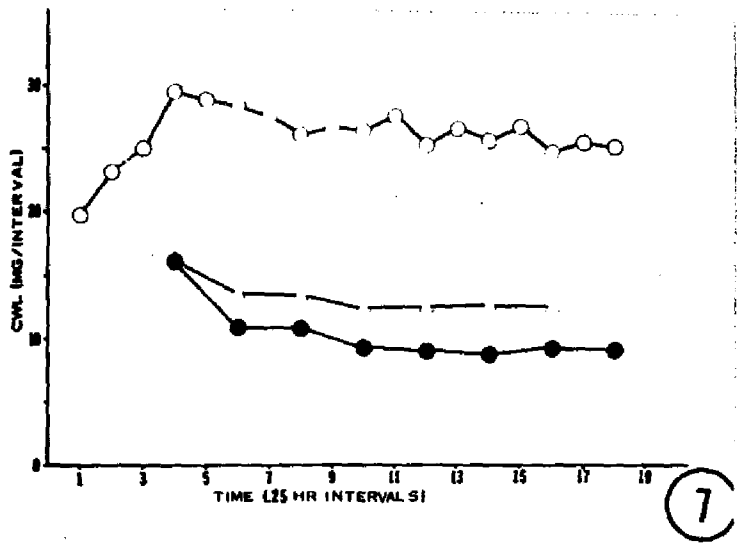
The size and shape of the chamber are important parameters. It is impossible to design a two compartment system similar to the one compartment system advocated by Lasiewski et al. (1966a). Their formula for determining the time needed for a chamber to reach 99% of equilibrium, underestimated the time needed for the system discussed above. For example, according to their formula, at an air flow rate of 200 cc/min, equilibrium should be obtained in 15 minutes, and at 400 cc/min, equilibrium should be obtained in 7.5 minutes. I found that after drying an animal free system for one hour at 200 cc/min, during the next 0.5 hr, 2.65 mgs of water were picked up (n = 3). At 400 cc/min, after 0.5 hr drying the following amounts of water (in mg) were picked up for each of the next three

0.5 hour periods: 1.15 (n = 4), 0.55 (n = 3), and 0.35 (n = 3). This indicates that the air flow in this two compartment system was not laminar (possibly this could have been eliminated by the use of baffles). When animals were placed in the chamber, equilibrium values were achieved and maintained after 1.5 hours pre-drying at 200 cc/min (tokay 1, figure 7) and after 1 hour pre-drying at 400 cc/min (tokays 2 and 3, figure 7). Values fluctuate less at the higher flow rate (figure 7).

The inlet and exhaust tube's ends were positioned as far apart as possible, hopefully minimizing dead air space. At a given air flow and rate of CWL, it takes longer for a large chamber to equilibrate, and the equilibrium humidity will be lower than in a small chamber (Lasiewski et al., 1966a). A narrow chamber would increase the linear velocity of the air at the skin's surface, thus possibly decreasing the skin's boundary layer and therefore, increasing CWL. It might seem preferable to have many chambers each suited to a different body shape and size. However, results obtained from a snake in a long slim chamber would not be directly comparable to those from a heavy-bodied lizard obtained in a short wide chamber. Also, it would not make sense to measure a 50 cm iguana and a 150 cm iguana in different chambers and then compare rates of water loss. The original chamber's size was designed to comfortably hold medium-sized Iguana iguana and still fit into the BOD box. This design was not changed for the other species. Animals were always suspended by the neck for two reasons: first, such an arrangement exposes the greatest surface area of the animal to a free flow of dry air; second, the animal cannot obtain locomotory purchase and pull out of the

Figure 7. CWL as a function of time for three tokays (open circles - tokay 1, measured at 200 cc/min; open triangles - tokay 2, measured at 400 cc/min; shaded circles - tokay 3, measured at 400 cc/min).

Figure 8. Photograph of a large capsule *in situ* on a tokay.



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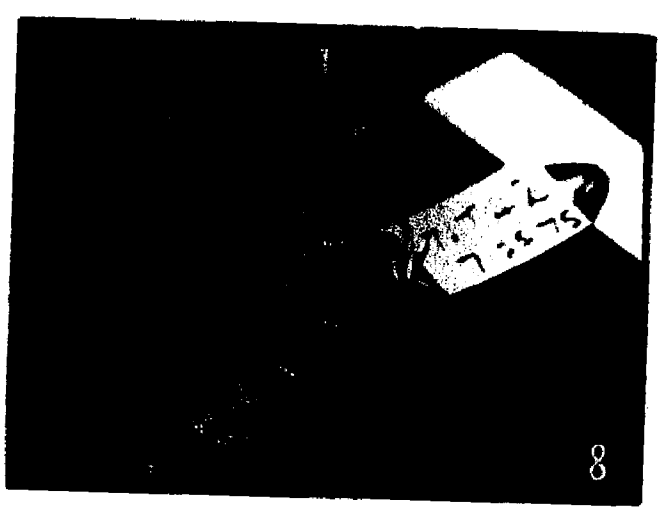

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head seal.

Dry air cylinders were usually used as the body compartment's air source, since such air contains less moisture than Drierite filtered air. The major advantage in using an air cylinder is that relatively fine control of air flow can be achieved. Erratic pressure fluctuations in the school compressed air line affected the flow rate, but this was not critical for head compartment measurements. For most experiments the cylinders were adjusted to give flow rates of approximately 200 cc/min or 400 cc/min. Due to the rigorous method by which the head-body compartment seal was checked (see page 41), very high flow rates could not be employed. Furthermore, had lower flow rates been used, equilibration time for the system would have been too long. Assuming that the animal's rate of CWL remains fairly constant, the flow rate would determine the relative humidity of the chamber. Thus attempts might have been made to control the chamber's humidity for animals with different rates of CWL by varying the flow rate (Bennett and Licht, 1975). However, the rate of CWL must first be approximately known or the humidity sensor must be installed next to the animal and be connected to a flow regulator. Finally, in nature, humidity varies diurnally and seasonally, and different species are exposed to different microhabitat humidities within the same general habitat. Therefore, even if one wanted to regulate humidity, it is not obvious what level should be regulated.

B. CAPSULE STUDIES

1. In Vivo

The rate of CWL should be proportional to the animal's sur-

face area. The relationship between weight and surface area can only be approximated (mainly due to skin stretching) within a species. Species with different body proportions certainly have different weight specific surface areas. Probably the physiological importance of some regions of skin is not directly related to their surface area. Therefore, the only way to directly compare different animals or body sites is by the use of capsules (Hattingh, 1973).

The errors involved in using capsules (mainly due to weighing and handling) are approximately constant regardless of capsule size. Therefore, greater accuracy is achieved by using large capsules, although they are more difficult to seal. Four different capsules were employed with measuring areas (in cm^2) of: small capsule (S) = 1.29, small to medium capsule (#4) = 3.60, mid-sized capsule = 4.52, and large capsule (L) = 8.40. The average rate of water loss in dry run situations was highly variable, the mean for all capsules was: 0.348 ± 0.289 (SD, n = 17) mg/hr.

The distance between the surface of the skin and the end of the inlet tube is not as critical in flow through systems as it is in stagnant air systems (Hattingh, 1973), but it was always approximately 3.0 mm. Very flexible light tubing had to be used to take air towards, and away from the capsule, in order to prevent the capsule from falling to one side due to the weight of the tubing alone, and also to prevent undue pallor as a result of pressure on the animal's skin. The tubing had to be of relatively large diameter to prevent pressure buildups in the capsule (which would cause it to pop). For the same reason a wet test meter was not connected to the exhaust line and the exhaust Drierite tube was kept small. The re-

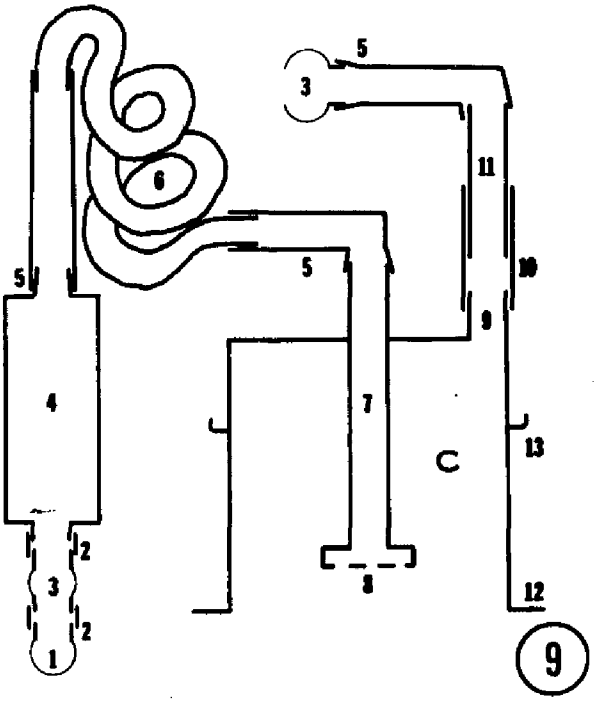
sulting capsule internal pressure was 2.0 mm water above ambient.

The capsule apparatus is illustrated in figures 8 and 9. Air for capsule determinations came from a dry air cylinder (1), and flow was regulated at 25-75 cc/min by the cylinder's two stage regulator. Air passed through a flowmeter (3), and drying column (4), and then into a BOD box set at 30°C. The air then passed through a one meter copper coil (6) and through latex tubing (5, 1/4 inch OD, 1/16 inch wall). The flexible tubing was connected to a glass tubular extension of the capsule (7). The extension was filled with 10-20 mesh indicating Drierite. At the bottom of this tube was an expanded perforated disc (8). After picking up moisture, the air left another glass extension (9), through a Teflon connecting tube (10) and then through a small glass tube (3.0 mm OD) filled with 10-20 mesh indicating Drierite (11). Air then passed through latex tubing, out of the BOD box, and through a flowmeter (3), before being released.

Animals were usually anesthetized and restrained in a supine position to prevent sudden random movements. Attempts were made to measure animals restrained in a prone position, since it was felt that there might be unusual cutaneous blood flow in the supinated animal. However, when the animal was placed in a prone position the capsule had to hang upside down from the animal, instead of resting on the animal. Thus rubber bands were needed to hold the capsule onto the animal's belly. The bands had to be on so tight that invariably pallor developed under the capsule's rim. Attempts were also made to hold the capsule on the prone animal's belly by hand. This did not produce pallor, but many movements, including respira-

Figure 9. Schematic diagram of the capsule system used for the measurement of CWL (C - capsule, 1 - dry air cylinder, 2 - Nalgon tubing, 3 - flowmeter, 4 - drying column containing 6 mesh Drierite, 5 - latex tubing, 6 - coiled copper tubing, 7 - pre-drying glass inlet tube containing 10-20 mesh Drierite, 8 - perforated disc, 9 - glass outlet tube, 10 - Teflon tube, 11 - post-drying tube containing 10-20 mesh Drierite, 12 - flange, 13 - glass retaining hooks).

Figure 10. Schematic diagram of the capsule system used to measure CWL <u>in vitro</u> (B - beaker, C - capsule, S - stir plate, 1 - inlet tube, 2 - perforated disc, 3 - exhaust tube, 4 - flange, 5 - glass retaining hooks, 6 - rubber band, 7 - saline, 8 - magnetic stir bar, 9 - vacuum grease, 10 - filter paper, 11 - skin).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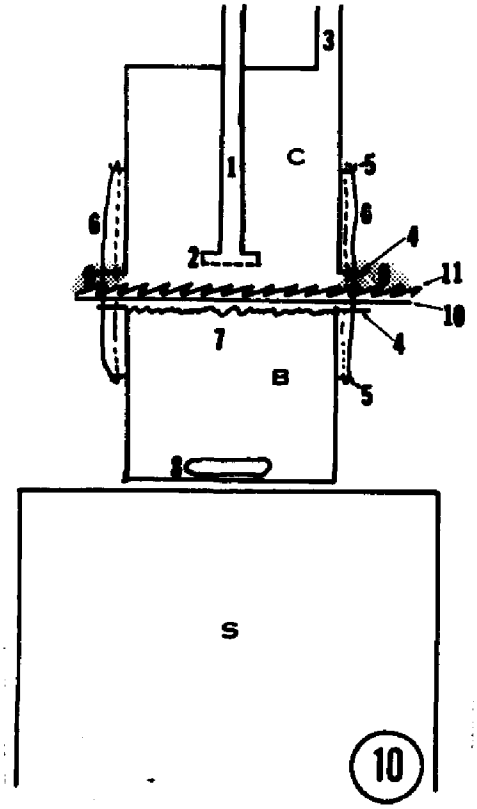
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tion, often caused the seal to break. However, two paired tests were satisfactorily completed; the one on crocodile belly skin showed that CWL was higher in the prone position (8.04 versus 6.00 mg/hr), while measurements on tokay belly skin indicated the reverse (0.78 versus 1.01 mg/hr).

The bottom rim of the capsule had a flange (12) to which high vacuum grease could be evenly applied. The capsule was then placed on the animal (outside the BOD box), and the entire area surrounding the capsule's rim was covered with high vacuum grease. The capsule was then secured in place with a loosely fitting rubber band, and then the animal was placed in the BOD box. The exhaust Drierite tube was weighed on a Mettler H-20 balance to the nearest 0.01 milligram, and the tube was then attached to the exhaust latex tubing and to the capsule. The inlet latex tubing was then attached to the capsule and a stopwatch started. The seal was checked by comparing flowmeter readings upstream and downstream from the capsule. The balance was zeroed and the exhaust Drierite tube weighed after two minutes. This was repeated for the next four 5-minute intervals, except that the inlet latex tube remained connected to the capsule throughout. Generally water loss decreased during the first twelve minutes and stabilized for the last two 5-minute runs. The average of the last two runs was used to present the data, unless otherwise specified. Biopsies were taken from various areas and prepared for histological examination.

2. <u>In Vitro</u>

It was difficult to keep the capsule attached even to anesthetized animals. Capsule determinations on skin, while still at-

tached to a dead animal, caused the skin to dehydrate. Therefore, skin was excised from freshly-killed animals, and measured *in vitro* in an agitating system (which kept the dermis moist).

The *in vitro* capsule system is illustrated in figure 10. Excised skin (11) was supported on filter paper (10) moistened with physiological saline. The capsule (C) was placed on the skin, and then the capsule and filter paper were placed on a beaker (B) of physiological saline which contained a magnetic stir bar (8). The capsule was attached to the beaker by rubber bands which spanned glass flanges on both parts (5). The entire complex was put on a stir plate (S) in a BOD box. The rest of the procedure was exactly the same as in *in vivo* capsule determinations, discussed above. After the runs a large central necropsy was taken and prepared histologically.

SECTION IV. MATERIALS TESTING

A. TUBING

Several studies which measured the permeability of skin indicated that some materials are inappropriate for use in such measuring systems (Baker and Kligman, 1967; Burch and Winsor, 1946; Cohen, 1975).

Originally latex tubing was used throughout, but early results indicated that all organic tubing is considerably permeable to fluids. I was able to obtain the manufacturer's tested permeability values for two types of tubing (table 2). These values did not agree

Table 2. Manufacturer's quoted permeability values of two types of tubing to several fluids.

	H_2O mg/100in^2/mil/day	CO_2 cc/100in^2/mil/day	O_2 cc/100in^2/mil/day
Tygon	194	970	7
Teflon	4000	1670	750

with my observations. Therefore, three procedures were designed to test the relative permeabilities of various types of tubing. Tests were made in parallel on at least two different types of tubing.

Procedure One: Knots were tied in water filled flexible tubing and the rate of water loss determined by direct weighing at approximately daily intervals (tubing tested - Tygon 3/8 inch OD, latex 3/8 inch OD).

Procedure Two: Air was passed through tubing used to connect two Drierite tubes. After the tubing was thoroughly dried, the air flow was shut off and the system allowed to remain overnight. It was then flushed with dry air, and the gravimetric difference in the exhaust Drierite tube determined (tubing tested - 3/8 inch: latex, Nalgon, neoprene, and silicone).

Procedure Three: Tubing was used to connect two Drierite tubes, and air was constantly metered through. The gravimetric change in the exhaust Drierite tube was determined for several intervals (tubing tested - 3/8 inch OD: latex, Nalgon and silicone; and 1/4 inch OD: latex and Teflon).

The results of some of these tests are given in table 3, and may be summarized as follows. Procedure One showed that Tygon tubing lost weight twice as fast as a similar length of latex tubing, this was confirmed by Procedure Three. From Procedure Two, one may infer that silicone tubing is highly permeable to water vapor; and that latex and neoprene tubing were slightly less permeable than Nalgon tubing. But Procedure Three clearly indicated that Nalgon has a lower permeability than latex (see table 3). By Procedure Three it was also determined that silicone tubing was highly permeable to water

Table 3. Post Drierite gain (in mg) from 73 cm lengths of latex and Nalgon tubings.[a]

	Length of consecutive time periods (in minutes).					
	33	30	45	100	60	60
Latex	0.3	0.6	1.0	1.6	0.7	0.4
Nalgon	0.2	0	0.5	0.6	-0.1	0

[a]ambient conditions 25°C, 50% RH, flow: 200 cc/min.

vapor, that thin latex was relatively permeable to water, and that thin Teflon tubing was not. Therefore, in places not requiring flexibility Teflon tubing was used. When flexibility was required either latex or Nalgon tubing was used, depending on specific requirements.

B. MEMBRANES

The permeability of membrane materials (1/64 inch neoprene and latex) was determined by separating two Plexiglas chambers with these materials. One of the chambers had dried air metered through, while humidified air (bubbled through water) was metered through the other chamber. The amount of moisture absorbed by the dry air chamber's exhaust tube approximately equals the amount of water vapor penetrating the membrane. Neoprene and latex have approximately the same permeability, but since neoprene was stronger, and did not distort after application of grease, it was therefore the material of choice.

C. COMPARTMENT

Originally the compartments were constructed of Plexiglas and other acrylics, but they were found to be unsatisfactory. With plastics, all seals were made by melting the opposing surfaces with chloroform or other plastic bonding agents before connecting them. The connection between the doughnut shaped end plates and the cylinders often cracked whilst the two compartments were being bolted together. The perspex chambers were checked for leaks by immersion in water while a high rate of air flow was passed through them. Cracks were difficult to seal permanently and generally the two

parts had to be separated and refinished before reattachment. The brass chamber was also periodically checked for leaks in the same manner.

The manufacturer's stated permeability of Plexiglas to water is: 3.2×10^{-8} gm cm/cm^2/mm Hg/hr at 73°F. A closed cylindrical chamber was constructed of acrylic and two holes were drilled at one end through which glass inlet and exhaust tubes were firmly attached by means of a rubber bung. Drierite tubes were connected to the inlet and exhaust tubes via a very short length of Nalgon tubing, and air was metered through at 200 cc/min. After equilibration the post Drierite tube gained 0.5 mg/hr. This gives a calculated Plexiglas permeability of 2.2×10^{-7} gm cm/cm^2/mm Hg/hr at room temperature (approximately 23°C). At 28°C the rate went up to 4.6×10^{-7} gm cm/cm^2/mm Hg/hr. If the chamber was dried and left overnight it accumulated 0.63 mg/hr. Plexiglas permeability would account for an artifactual rate of CWL of 8 mg/0.5 hr using a standard sized chamber under normal experimental conditions. If the animal's rate of CWL were 20 mg/0.5 hr, almost 30% of the effluent water picked up by the Drierite would have come into the chamber through its walls.

Periodically Plexiglas and brass chambers were tested under dry run conditions; that is, an empty chamber with the normal experimental setup, except that a solid neoprene membrane separated the two compartments. Two experimental procedures were employed:

Procedure One: The chamber was thoroughly dried and left overnight and then flushed the next morning with dry air.

Procedure Two: The rate of water loss was determined by weighing the exhaust Drierite tube at regular intervals.

Since the Plexiglas and brass chambers were the same size and shape, the results are directly compared in table 4. Since brass is much more impermeable than the acrylic, it is the material of choice.

D. ABSORBENT

Indicating Drierite of either 8 mesh or 10-20 mesh was used to capture water vapor. There are several obvious advantages in using Drierite: 1) it is cheap; 2) it is readily available in convenient mesh sizes; 3) it is safe to handle; 4) it is easily regenerated, losing absorbed moisture when it is heated; and 5) the procedure is simple. Large Drierite tubes absorbed 96-99.9% of the total water presented, and at moderate flow rates (200-400 cc/min) a mean of 99% was obtained. If small Drierite tubes are fresh, they absorb an equally high percentage of presented water. Flushing (that is, the loss of absorbed moisture with the continued movement of dry air) seems to occur only when the Drierite absorbed considerable moisture, although it was more common at high flow rates.

E. FLOWMETER

The flowmeters (Vis-Flo "150" series, Kontes, Vineland, N. J.) did not accurately reflect the actual flow of gas as determined by water displacement. An oily film often developed on the flowmeter's stainless steel ball, and caused it to stick. In addition, the metering ball had a tendency to settle even if the flow rate was held constant. Therefore, body compartment air flow was passed through a wet test meter (Precision Scientific Model #63111) before being released into the laboratory. The total flow of air passing through

Table 4. Mean "water loss" for acrylic and brass chambers under dry run conditions.

	Procedure One (total accumulation)	Procedure Two (allowed at least 2 hr pre-dry)
Plexiglas	34.6 mg (n = 3)	5.55 mg/0.5 hr (n = 11)
Brass	2.74 mg (n = 3)	0.15 mg/0.5 hr (n = 15)

the wet test meter was divided by the time interval, and thus the average flow rate was determined. Although the manufacturer's stated range was 56.6-566.0 liters/hr (2-20 cubic feet/hr), in fact the meter was accurate at much lower flow rates as determined by water displacement (see table 5).

SECTION V. REMOVAL OF EPIDERMAL CORNEOUS MATERIALS

A. INTRODUCTION

A number of investigations on human skin have used cellophane tape to remove parts of the epidermis (Eriksson and Lamke, 1971; Matoltsy et al., 1962; Spruit, 1970). This procedure allows determination of which layer(s) constitute the barrier against moisture loss (Scheuplein, 1972).

B. PROCEDURE

Of a number of brands tested, Tuck 1/2 inch wide cellophane tape (Tuck Industries Inc., New Rochelle, N. Y.) was the most effective. The tape was applied sticky side down onto the skin surface, and then pushed into crevices to the greatest degree possible with the fingernail. The tape was removed from posterior to anterior, and better strips were effected by rapid rather than by the slow procedure recommended for human skin (Weigand and Gaylor, 1973). The tape was examined after removal to check for the presence of corneous materials; some pieces of stripped material were examined microscopically according to the technique of Jenkins and Thesise (1969). As will be described below, the cellophane stripping tech-

Table 5. Air flow determined by a wet test meter and by water displacement.

	Flow rate (cc/min)			
Calculated from wet test meter	109	178	207	786
Measured by water displacement	101	180	219	820

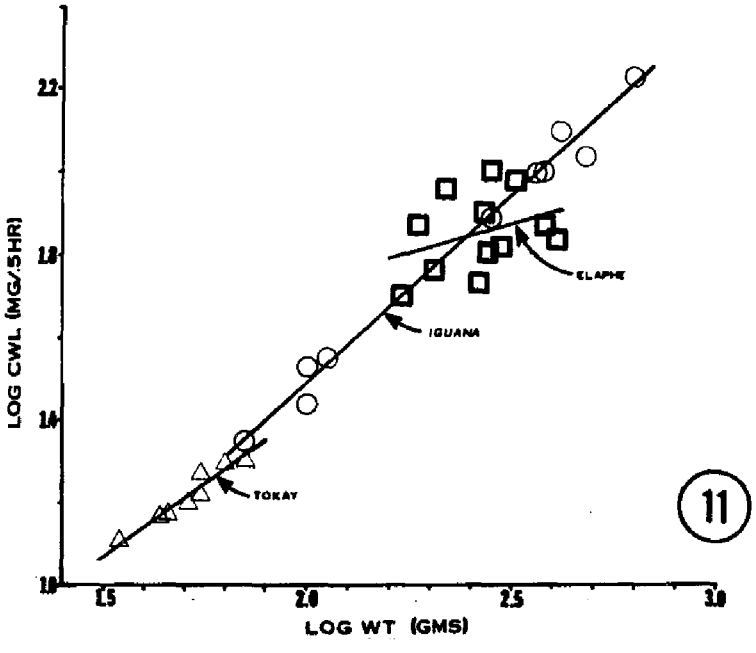
nique is inherently variable when applied to reptile skin, but usually after 40 applications of tape an adequately large number of scales are affected.

RESULTS

SECTION I. THE RELATIONSHIP BETWEEN BODY WEIGHT AND CWL

The relationship between CWL and body weight was determined by calculating a least squares regression for each of eight tokays, each measured once per cycle during the resting phase (3-5 days post-shed), for several cycles (mean: 5.9 cycles). Biopsies taken during the day the animals were measured always revealed a resting condition (perfect or late resting stages). Runs were carried out at a flow rate of 200 cc/min. The animals' cloacas were taped, and they had access to water, but not food, from the time of shedding. The data used were the single half hourly runs, or the mean of the first two half hourly runs following a 1.5 hour pre-drying period. Weight and CWL were log transformed for the analysis. The slope for the regressions ranged from -1.768 to 3.925 (mean: 0.673). None of the regressions accounted for a significant portion of the variance in CWL within each animal. A regression was then calculated for the group as a whole using the log mean CWL and the log mean weight for each animal. The results of this regression are reported in figure 11 and table 6. The exponent of this equation is not significantly different from the exponent used to relate surface area to body weight. Individual measurements were expressed in terms of surface area by using the equation: $SA = 10W^{0.67}$ (where SA is surface area in cm^2, and W is weight in grams). A Model II ANOVA on the transformed data showed that the group variance was not significantly greater than the individual (intercycle) variance ($F_S = 0.837$).

Figure 11. The relationship between log CWL and log body weight for Iguana iguana (open circles), Elaphe obsoleta quadrivittata (open squares), and Gekko gecko (open triangles).


LOG CWL (MG/5HR)
2.2
1.8
1.4
1.0
1.5
2.0
2.5
3.0
LOG WT (GMS)
TOKAY
IGUANA
ELAPHE

11

Table 6. Log CWL/log body weight regression parameters for three squamates.

Species	N	Regression coefficient (b)	95% confidence limits of b	Y intercept (a, in log units)	Significance of regression
Gekko	8	0.707	0.628 - 0.786	0.007	$P < 0.001$
Iguana	10	0.893	0.815 - 0.971	-0.290	$P < 0.001$
Elaphe	12	0.271	-0.269 - 0.811	1.196	$0.2 < P < 0.4$

Table 6 and figure 11 also show a regression for a log mean CWL and log mean weight for 10 iguanas. On the basis of weight, these animals fall into two groups: small (n = 4, 70-113 grams), and mid-sized (n = 6, 282-624 grams). Also reported is a regression of log CWL on log weight for Elaphe, however in this instance each point represents only one measurement. For both Iguana and Elaphe the flow rate was 200 cc/minute, cloacas were taped, and the stage in the epidermal shedding cycle in each animal was determined.

The different values and variability of the regression coefficients for these three squamates will be explained in the discussion (pp. 207-210).

SECTION II. SHORT TERM FACTORS AFFECTING THE RATE OF CWL

A. ANESTHESIA, DEATH, AND KERATIN HYDRATION

Table 7 summarizes several separate experiments in which the effect of a particular variable on an animal's rate of CWL was examined by comparing results obtained before and after the treatment. The significance of these results was tested by a paired t-test. The results show that neither anesthesia nor even recent death significantly affected the rate of CWL. Anesthesia was given as a 0.5% or 2.5% Nembutol solution (25 mg/kg) in saline (0.15 M NaCl). Some animals also received 5 mg/kg of a 0.5% solution of Valium to potentiate the effect of Nembutol. The anesthetics were injected subcutaneously in the gular region. To determine the effect of death on CWL, anesthetized animals were killed either by an overdose of Nembutol (additional 75 mg/kg) and/or by occluding their glottis

Table 7. CWL before and after various treatments for two reptile species.

Species	Treatment	N	Mean CWL before	Mean CWL after	Probability
Gekko[a]	Anesthesia	7	21.4	19.8	$P > 0.05$
Gekko[b]	Recent death	15	1.38	1.59	$P > 0.05$
Gekko[a]	Hydration-rest	8	25.05	38.96	$P < 0.001$
Gekko[a]	Hydration-renewal	6	19.12	22.48	$0.1 < P < 0.2$
Elaphe[a]	Anesthesia	8	104.2	104.4	$P > 0.05$
Elaphe[a]	Recent death	3	142.1	126.9	$P > 0.05$

a. Chamber determinations, mg/0.5 hr

b. Capsule determinations, mg/hr

with a glass plug.

The animals used for the tokay capsule determinations were studied at the end of an unrelated endocrine experiment. These animals were either intact, thyroidectomized, hypophysectomized, or thyroidectomized and hypophysectomized; and acclimated to either 28°C or 32°C. The surgical treatments were not obviously correlated to the absolute difference in CWL nor to the difference in CWL before and after sacrifice.

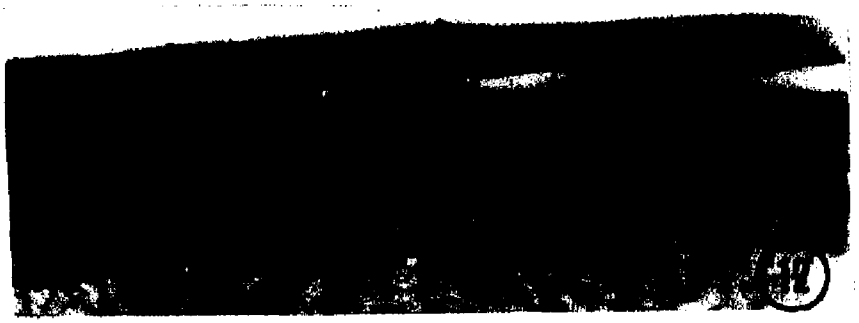
The hydration experiments reported in table 7 involved first measuring normal CWL, then immersing the animal's body in distilled water at 30°C for two hours, blotting the animal dry and then remeasuring CWL. The animals were arbitrarily divided into two groups according to the phase in the shedding cycle: rest (0 to 4 days post-shed) and renewal (14 to 2 days pre-shed, stages 3-5). The range in the change in CWL for animals in the resting stage was 14.2-280% increase, while for the animals in the renewal phase the change ranged from a 6.5% decrease to a 66.8% increase. A one tailed Mann-Whitney U test showed that the animals in the resting phase have a significantly greater ($P \simeq 0.025$) increase for this treatment than animals in the renewal phase. There was no relation between the magnitude of change and the specific stage in the renewal phase nor to body weight.

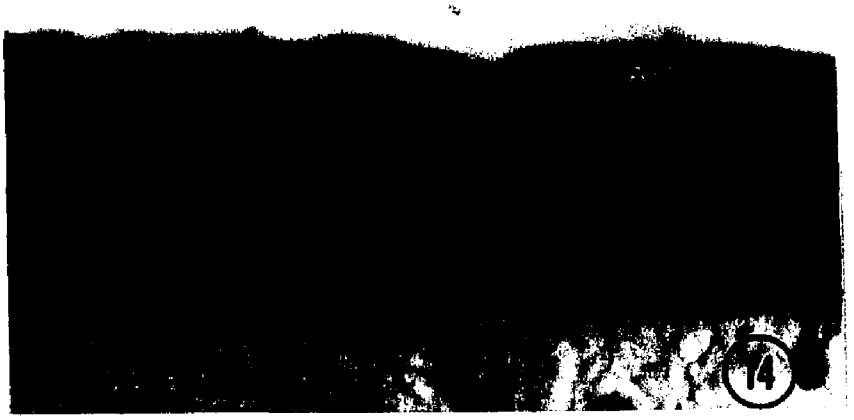
Histological samples were taken of hydrated skin sites in a different group of tokays during many stages of the shedding cycle. Hydration did not have any obvious effect on the histology of the renewal material (figures 12 and 13). However, animals in post-shed or perfect rest conditions had swollen alpha tissue and suprabasal living layers (figure 14). Biopsies taken a day later still showed

Figure 12. Photomicrograph through the outer scale surface of G. gecko skin (stage 2) after a two hour immersion in water. For symbols and control material see figure 2. (X 400).

Figure 13. Photomicrograph through the outer scale surface of G. gecko skin (mid stage 4) after a two hour immersion in water. For symbols and control material see figure 3. (X 500).

Figure 14. Photomicrograph through the outer scale surface of G. gecko skin (post-shed condition) after a two hour immersion in water. The beta layer has artifactually split away from the underlying epidermis and is not represented in this figure. Note the irregular contour and swollen condition of the alpha layer and the presumptive alpha cells. For symbols and control material see figure 1. (X 500).





signs of an unusual condition.

B. AMBIENT HUMIDITY

1. Changing Flow Rate

The relationship between ambient humidity and CWL can be deduced by varying the rate of air flow. This indirectly changes the ambient water vapor pressure. Table 8 shows the rate of CWL for a number of animals measured at an air flow rate of 200 cc/min, and at least one other flow rate. In all cases the neck seal was intact during the measurement. Since doubling the flow rate halves the ambient water vapor pressure in the chamber, this would seem to double the concentration gradient and therefore should double CWL.

Although CWL was often greater at the higher air flow rate, a simple relationship between air flow (and thus indirectly ambient water vapor pressure) and CWL was not found.

2. Changing Measuring Humidity

a. Chamber

An attempt was made to ascertain the relationship between CWL and ambient humidity, by humidifying the incoming air by bubbling it through various saturated salt solutions of known relative humidity (Winston and Bates, 1960; see table 9).

The procedure was to bubble dry air through an agitated three liter flask of a supersaturated salt solution which was thermally equilibrated at 30°C. This air was passed into the chamber for 1.5 hours to allow equilibration. The exhaust Drierite's change in weight for the next half hour was recorded. This change was due to water entering the chamber from the salt solution plus the ani-

Table 8. CWL (mg/0.5 hr) at several different flow rates for two squamate species.

	Approximate flow rate (cc/min)				
Animal #	50	200	400	800	1000
Tokay 1	16.3[a]	21.0			
Tokay 2		25.9	30.5		
Tokay 3		24.9	40.8	34.1	
Tokay 4		19.7	30.5		
Tokay 5		12.6	11.8		12.2
Elaphe 1		65.8			68.9

a. Not fully equilibrated

Table 9. The effect of changes in measuring humidity on chamber determinations of CWL.

Solution	1	2	3	4	5	6	7	8	9	10	11	12
KAC	22	193.3	5.4	0.0056	18.5	177.6	55.6	0.0104	34.3	0.0048	0.0200	0.24
$Ca(NO_3)_2$	47	-	13.1	0.0140[a]	46.7[a]	168.9	103.9	0.0205	67.5	0.0065	0.0099	0.66
$NaNO_2$	63	184.8	19.3	0.0208	68.5	180.0	126.7	0.0235	77.4	0.0027	0.0069	0.39
NaCl	75.5	179.6	20.9	0.0233	76.8	179.2	134.6	0.0250	82.4	0.0017	0.0054	0.32
K_2CrO_4	86.5	181.9	25.7	0.0283	93.2	175.0	151.9	0.0289	95.2	0.0006	0.0015	0.40
Dry Air	0	180.0	0.05	0.0001	0.3	181.5	19.6	0.0036	11.9	0.0035	0.0303	0.12

1: Winston & Bates RH at 30°C. 2: 5 min flow (cc/min). 3: Water gain (mg/5 min). 4: 5 min water vapor content (mg/cc). 5: 5 min calculated RH. 6: Run flow (cc/min). 7: Water gain (mg/0.5 hr). 8: Exit vapor (mg/cc). 9: Exit RH. 10: CWL (mg/cc). 11: ΔC[b]. 12: K[c].

a. Assuming 5 min flow of 185 cc/min

b. Concentration gradient mg/cc air

c. Permeability constant, cm/hr

mal's CWL. After the run, a five minute measure of the water content of the air leaving the salt solution was determined by placing a Drierite tube just before the air entered the chamber. Since the flow rate was measured, the water content of the air and therefore, the relative humidity of the air could be calculated. The water vapor content of the air entering the chamber was subtracted from the water content of the air passing out of the chamber. The difference is the water vapor lost by the animal per cc of air. Since the exit humidity is approximately the same as that surrounding the animal (Lasiewski et al., 1966a), one can determine the concentration gradient, if one assumes that the lowest layers of the barrier are saturated with water vapor. The total CWL would be given by:

$$J_s = K\Delta C$$

where ΔC is the concentration gradient and K is the permeability constant. K can be determined for the animal's skin for each solution. Unfortunately no measurement for the five minute air flow for the calcium nitrate run was made. For all calculations concerning this solution a flow rate of 185 cc/minute was assumed.

The results of this experiment indicate that if these assumptions are valid, the permeability constant differs with each solution or humidity.

b. Capsule

In vitro capsule determinations of CWL were made on post-shed skin at two different measuring humidities. For all runs, the system was pre-dried for 20 to 25 minutes, and the mean of the next four 5-minute runs was used. The flow rate was determined with a wet test meter only once during this series of measurements. The

results are reported in table 10. Note that in this case the permeability constants are approximately the same in the two solutions, and differ substantially from those calculated from the chamber data. This discrepancy, and the relationship between CWL and the measuring humidity, will be explained in the discussion (p. 199).

3. Changing Acclimation Humidity

Animals were placed in plastic boxes on wire mesh supports over Drierite, water, or air. Calcium nitrate was also tried, but all four animals placed in this environment died within a week in spite of the fact that contact with the solution seemed practically impossible. Thus, only one measurement was obtained on an animal acclimated to calcium nitrate, and its rate of CWL was normal. Animals did not have access to food from the time they were placed in the box, which was at least three days prior to the first measurement. Water was originally supplied by a rodent type watering bottle. This was discontinued when it was realized that tokays did not make use of this source of water. Therefore, after a daily set of measurements, their mouths were sprayed with water.

Some indication of the relationship between CWL and ambient humidity can be obtained by examining table 11. CWL was transformed to $mg/cm^2/hr$ by using the regression equation: $SA = 10W^{0.67}$. These data were acquired from two separate experiments. In the first, the animals were isolated randomly during the shedding cycle, but in the second all animals were isolated just after shedding (PS). CWL measurements of the animals of the first series began three to four days after isolation, but in the second series there was an interval of one cycle between the time of isolation and the first measurement.

Table 10. The effect of changes in measuring humidity on capsule determinations of CWL.

Solution	1	2	3	4	5	6	7	8	9	10	11	12
Dry air	0	44	0	0	0	44	3.93	0.0015	4.9	0.0015	0.0289	0.052
$Ca(NO_3)_2$	47	44	49.78	0.0189	62	44	51.39	0.0195	64.1	0.0006	0.0189	0.056

1: Winston & Bates RH at 30°C. 2: Flow[a] (cc/min). 3: Water gain (mg/hr). 4: Vapor content (mg/cc). 5: Calculated RH. 6: Run flow[a] (cc/min). 7: Water gain (mg/hr). 8: Exit vapor (mg/cc). 9: Exit RH. 10: CWL (mg/cc). 11: ΔC[b]. 12: K[c].

a. Estimated from only one determination

b. Concentration gradient mg/cc air

c. Permeability constant, cm/hr

Table 11. CWL ($mg/cm^2/hr$) measured during the resting phase[a] for tokays acclimated to various humidities for several cycles.

Animal #	Acclimated environment	Stage isolated	First cycle	Second cycle	Third cycle
Tokay O	water	e3	0.597	-	-
Tokay S[e]	water	PS	-	0.198	0.127
Tokay T[e]	water	PS	-	0.212[b]	-
Tokay N[e]	water	m4	0.365	0.640	0.886
Tokay U[e]	water	PS	-	0.395[c]	0.134
Tokay W[e]	none	PS	0.257	0.103	-
Tokay Q	Drierite	6	0.367	-	-
Tokay P	Drierite	m4	0.158	-	-
Tokay V[e]	Drierite	PS	-	0.081[d]	-
Tokay X[e]	Drierite	PS	-	0.112	-
Tokay Y[e]	Drierite	PS	-	0.114	0.125
Tokay R	Drierite	5	0.235	0.221[d]	-

a. All measurements during pR unless otherwise specified

b. Measurement immediately after shedding

c. Measurement 2 days after shedding

d. Cloaca not taped during this measurement

e. Second series

Tokays acclimated to a humid environment had higher rates of CWL in the desiccating chamber, than those acclimated to a dry environment. CWL was higher in animals placed in a dry environment late in the cycle, than those isolated early in that cycle or isolated in the previous cycle.

In several instances, CWL for the initial cycle was considerably different than the value for a later cycle. CWL was measured in tokay N and tokay R more than one cycle after they were returned to their regular ventilated cages. In both cases this later rate of CWL was more moderate (tokay N lower, and tokay R higher) at comparable stages, than when the animals were held at constant humidity. The animals which made up the second experimental series were used elsewhere, when they were housed in ventilated cages. All measurements of the animals held over Drierite, and several of the measurements of the animals held over water, were lower than their ventilated values. No histological differences in the skin were found which would account for these differences associated with acclimation. However, grossly it was noted that during the second cycle of tokay N, hyperkeratotic welts developed, which lasted until the experiment was terminated.

C. STATE OF WATER BALANCE AND CWL

1. Non-Paired Comparisons

The relationship between an animal's water balance and its rate of CWL ($mg/cm^2/hr$) was investigated. Several tokays were kept in good water balance by frequently spraying them, while water was withheld from others for three to seven days prior to measuring CWL.

The cloacas of all animals were taped, and animals were measured at an air flow rate of 400 cc/minute. Some animals were anesthetized, but since results from such animals did not apparently differ from the non-anesthetized group, all results were combined. Comparison between animals of different weights was made possible by expressing absolute CWL as a function of surface area using the regression equation: $SA = 10W^{0.67}$. The two groups were not significantly different (t-test, P = 0.3, table 12).

2. Before and After Studies

a. Gekko gecko

Another approach was used to determine the relationship between an animal's state of water balance and CWL. CWL was determined for anesthetized and awake tokays, which did not have access to water for three to seven days prior to being measured. The flow rate was approximately either 200 cc/min or 400 cc/min; the results for the two flow rates were similar and therefore they were combined. The animals were then injected with air, water, or saline; or not injected. The injected volume was either a small percentage (0.1 or 0.5) or a large percentage (5) of the body weight; and given IP, subcutaneously, or via a stomach tube. The tokay was then remeasured later that day and again the next day. Some animals were not measured later that day, and others were not injected, but rather placed overnight in a cage in which water was available. Many of the protocols performed on the dehydrated lizards were repeated on hydrated lizards. The immediate change in CWL (same day) is given in table 13. Table 14 presents the change in the next day's measurement relative to the first day's measurement. For both tables, a value great-

Table 12. CWL ($mg/cm^2/hr$) in hydrated and dehydrated tokays.

Condition	N	CWL	Standard Deviation
Hydrated	7	0.067	0.013
Dehydrated	9	0.060	0.012

Table 13. Effect of hydration state on CWL in tokays expressed as the ratio of CWL after treatment to CWL before treatment.

		Anesthetized		Non-anesthetized	
Solution	Injection size	Dehydrated	Hydrated	Dehydrated	Hydrated
Saline	small	-	-	0.96, n = 1	0.97, n = 2
	large	0.88, n = 3	0.92, n = 2	-	1.12, n = 3
Water	small	-	-	0.93, n = 4	-
	large	0.98, n = 2	0.98, n = 1	1.70, n = 2	0.94, n = 1
Air	large	0.89, n = 2	-	1.03, n = 2	-

Table 14. Effect of hydration state on CWL in tokays expressed as the ratio of CWL a day after treatment to CWL before treatment.

Solution	Injection size	Anesthetized		Non-anesthetized	
		Dehydrated	Hydrated	Dehydrated	Hydrated
Saline	small	-	-	1.33, n = 1	-
	large	1.24, n = 3	0.97, n = 1	1.87, n = 1	1.13, n = 2
Water	small	-	-	1.35, n = 3	-
	large	1.36, n = 2	1.18, n = 1	2.12, n = 4	1.76, n = 1
Air	large	1.25, n = 2	-	1.24, n = 2	-
Nothing	-	1.38, n = 1	-	1.05, n = 3	-

er than 1.0, indicates that the second measurement was greater than the first; a value less than 1.0, indicates that the first measurement was higher than the second; and a value of 1.0, indicates no change.

None of the injections caused an immediate increase in CWL in the anesthetized animals. Small doses of water or air had no immediate effect on awake animals. However, an increase was observed when large doses of saline or water were given to hydrated or dehydrated tokays, respectively.

CWL during the next day was usually considerably higher for the control animals, animals given air, and animals given small doses of saline or water, irrespective of anesthesia. The unanesthetized dehydrated animals which received large doses of saline or water; and the unanesthetized, hydrated animals given water, had a much larger increase than the groups mentioned above. This was not true for the anesthetized animals. The smallest change was found for the hydrated animals given large doses of saline, irrespective of wakefulness.

b. Iguana iguana

A small, dehydrated, unanesthetized green iguana was given an injection of water (6.5% body weight) via a stomach tube. Its rate of CWL increased from 33.7 mg/0.5 hour to 40.3 mg/0.5 hour following the injection. The animal was returned to its cage and provided with water. After two days CWL returned to its original value (33.5 mg/0.5 hour). The animal was again dehydrated and its rate of CWL dropped to 30.5 and 27.6 mg/0.5 hour, after three and six days, respectively.

c. Lacerta lepida

CWL was measured in a few lacertas from which food and water were withheld for several days. The animals were given access to water overnight (invariably they showed an increase in weight), CWL was measured the next day, and occasionally thereafter. There was no evidence that hydration affected the rate of CWL.

SECTION III. THE SHEDDING CYCLE AND CWL

A. THE SHEDDING CYCLE

1. Shedding Frequency

Table 15 is a synopsis of the shedding data for the lepidosaurs used in this study. All the tokays and Elaphe were kept at 30°C. Some of the Lacerta were kept at higher temperatures, but (unlike the tokays) this did not seem to affect their cycle length, and therefore, all Lacerta data were pooled. The black tegus and the green iguanas were kept for some periods in a 30°C controlled temperature environment, while at other times they had access to heat lamps. There were no obvious differences regarding shedding between these two environments, and their data were therefore combined.

The tokay data are divided into six groups, each consisting of animals purchased at about the same time. Tokays had various acclimation histories preceding the time that their sheds were recorded for use in this table. There was an added variance component among tokay groups for shedding cycle length ($P < 0.01$). Using a Model II ANOVA it was shown that the great majority of the variability was within groups (95%), with only a smaller among group component (5%).

Table 15. Shedding frequency for several of the squamates used in this study.

Species	Group	Number of animals	Number of intermolts	Range of mean cycle length (in days)	Mean cycle length (in days)	Individual[a] CV range	Group CV
Gekko	Total	85	510	17.1 - 31.7	22.01	0 - 67.39	26.61
	A	4	31	21.0 - 23.7	22.42	11.35 - 21.30	14.24
	B	13	84	20.6 - 26.3	23.19	8.56 - 22.64	16.51
	C	23	142	17.1 - 25.7	20.57	4.38 - 21.78	15.27
	D	9	63	18.6 - 25.0	21.68	6.13 - 40.53	28.66
	E	22	71	17.3 - 26.0	21.42	0 - 19.25	18.50
	F	14	119	21.3 - 31.7	23.33	11.63 - 67.39	29.38
Tupinambis		5	16	21.5 - 47.3	33.19	b	39.18
Lacerta		45	157	30.7 - 150.0	61.57	9.32 - 84.20	56.08
		48	205[d]	1/174 - 1/43.8[e]	79.81[e]	b	36.64[c]
Iguana		22	18[d]	0/82 - 1/9[e]	98.17[e]	b	b
Constrictor		2	20	44.4 - 57.4	50.25	34.99 - 39.19	40.94
Elaphe		11	20	28.5 - 165	53.40	b	64.54
		35	44[d]	0/142 - 1/8[e]	75.41[e]	b	b

Table 15. Shedding frequency for several of the squamates used in this study.

(continued)

a. Only for individuals with three or more intermolts

b. Not enough data

c. CV of mean molts/time (mean, one molt every 89.7 days)

d. Total number of molts

e. Number of molts during total time in captivity

Records were kept on only two boa constrictors (original weight: 1.43 kg and 1.55 kg), and both seemed to have the same cycle length when not stressed (approximately 60 days). When their rate of CWL was measured (see pp. 136-145, table 34), their cycle length was shorter (CC 1: 24, 43, 16, 38, and 34 days; CC 2: 48, 37, 54, and 32 days). It is possible that the measuring procedure caused a shortening of the cycle, however biopsies revealed that the renewal phase was of normal duration (approximately fourteen days).

Two shipments of black tegus were received: the first consisted of large animals (1.26 to 1.95 kg) and the second of smaller animals (0.44 kg). Animals in both groups had access to water, were fed eggs ad libitum, and could thermoregulate via a heat lamp. On this regime animals in both groups gained weight rapidly. After two months the large animals ranged in weight from 2.07 to 3.07 kg, and after one month the small animals weighed 0.56 kg. The cycle length increased during this acclimation period for each animal (for example; TT 1: 20, 27, 30, and 34 days; TT 2: 32, 33, and 33 days; TT 3: 18 and 25 days; and TT 5: 22 and 28 days). When their rate of CWL was measured, cycle length dramatically elongated (TT 1: 43 and 74 days; TT 2: 43 days; TT 4: more than 73 days; and TT 5: more than 32 days).

The rat snakes, lacertas, and iguanas shed infrequently and unpredictably. The two lizards did not molt in one piece, although usually their back and belly were shed in one or a few large flakes. It was not possible to report a meaningful intermolt length for these animals, since some lacertas and rat snakes did not shed twice. The data are therefore presented as: number of sheds for all ani-

mals of that species divided by the total number of days those animals were in captivity. Thus frequent and infrequent shedders were pooled. For Lacerta it was possible to compute the total number of sheds divided by the total number of days observed for each animal (since all animals shed at least once). These data are presented in the group CV and as a footnote to table 15.

2. Water Content of the Shed

In animals which shed piece-meal, the shed is dry when it comes off (see Lacerta, table 16). However, in animals which shed in one piece or in large flakes, the shed may be moist (table 16). Material was removed from lizards as soon as it was apparent that a shed was imminent, that is when the outer generation no longer tightly adhered to the inner generation. The snakes were allowed to shed by themselves, and the shed material was used immediately (boas) or after a twenty minute delay (Elaphe).

After removal the moist shed was quickly weighed on a Sartorius 2463 balance. It was impossible to get a precise reading for this material since it sublimated water very rapidly. Also, much water was lost from the time the outer generation's integrity was broken until all the material was on the balance pan. The moist shed was allowed to dry over Drierite at room temperature for two weeks before being reweighed. Although not all the shed material was recovered from all the gekkonids, in no case did the amount recovered seem to be less than 75% of the total. Therefore, the values for dry shed weight as a percentage of body weight is an underestimate for tokays and Eublepharis.

Two tokays were held over Drierite, and another over water,

Table 16. Percent water content and percent dry weight of the shed for several squamates.

Species	N	Percent water in shed	SD	Shed dry wt as a percent body wt	SD
Gekko[a]	9	16.88	7.18	0.45	0.15
Gekko[b]	2	12.56	5.18	0.52	0.16
Gekko[c]	1	19.71		0.35	
Eublepharis[a]	1	54.28		0.32	
Lacerta[a]	1	10.26		0.57	
Constrictor[a]	3	46.84	11.33	0.33	0.12
Elaphe[a]	1	51.31		0.95	

a. Ambient humidity uncontrolled

b. Held over Drierite

c. Held over water

for a complete cycle before shedding. Results from these animals show that the percentage water in the shed is related to the animal's habitat humidity.

B. THE SHEDDING CYCLE AND CHAMBER DETERMINATIONS OF CWL

1. Gekko gecko

Several separate experiments were conducted to elucidate the relationship between the shedding cycle and CWL in tokays. These epxeriments are presented in the chronological order in which they were performed, in order to facilitate subsequent discussion.

a. Tokays measured several times per cycle

In this section, the protocol for each of the separate experiments is first presented and later (p. 93) the results are presented.

i. Protocol

α. Animals measured once per week (figures 15-19): Food, but not water, was withheld from these animals from the time they were isolated, three to four days prior to measurement. Air flow was regulated at approximately 200 cc/minute, their cloacas were taped, and the animals were not anesthetized. The chamber was pre-dried for 1.75 hours and the average of the next two 45 minute runs was used in presenting the results. Gular biopsies were taken after each day's measurement.

β. Animals measured up to three times weekly (figures 20-23): Animals which were measured frequently (tokays J, L, and M) were anesthetized with a 0.5% solution of Nembutol, dissolved in 0.15 M NaCl, at a dose of 17.5 mg/kg. It was hoped that anesthe-

sia would prevent struggling and therefore, decrease stress and possible wounding. Chambers were pre-dried for 1.5 hours, and the next half hourly run, or the mean of the next two half hourly runs was used in presenting the results. Biopsies were not taken since it was feared that such stress might affect the rate of CWL. At the time it was thought that CWL only changed during the act of shedding, and not during the renewal phase. Since the time in the shedding cycle at which the measurements were made was known, it was possible to deduce the probable histological condition of the epidermis (table 17). In all other aspects of protocol these animals were treated in the same manner as the first experimental group.

γ. Animals maintained at constant humidity (figures 24-30): For the first series (tokays N, O, P, Q, and R), unanesthetized animals were measured at approximately 200 cc/minute, and for the second series (tokays S, T, U, V, W, X, and Y) anesthetized animals were measured at 400 cc/minute. In both groups biopsies were taken after each measurement, and cloacas were usually taped. All animals were held on wire mesh supports: tokays N, O, S, T, and U over water, the rest over Drierite, except tokay W which stood in an empty cage. Animals were isolated without food, but with water, for the entire duration of the experiment. It was originally felt that if the animals were acclimated to a constant humidity there would be less variation or fluctuation within a cycle than would normally be the case. The second or repeat series was an attempt to determine whether unstressed animals (immobilized and often without tape) would show the same relationship between the shedding cycle and CWL.

Table 17. The relationship between the days pre- and post-shedding and the microscopic stage of the skin for G. gecko.

Histological stage	PS	pR	lR	e3	m3	l3	e4	m4	l4	e5	5	6
Days post-shed	0-2	*	*	*	*	*	*	*	*	*	*	*
Days pre-shed	*	*	*	12-10	9	9	8-7	7-6	5-4	3	2	1-0

*Variable, depends on length of cycle

ii. Analysis

From the results presented in figures 15 to 29, the general relationship between the shedding cycle and CWL can be described as follows. Just after shedding CWL is high, it quickly decreases during the first few days following shedding (post-shed stage) and reaches a low value as the epidermis attains the perfect resting condition, usually four days after shedding. The rate of CWL remains fairly constant through the early renewal phase (stage 2). As the renewal phase proceeds, CWL increases, gradually at first, and then more sharply. The most marked increase generally occurs at early stage 5. The rate of CWL peaks just prior to normal shedding and remains at this high level until just after shedding. There are several instances where an individual pattern differed from this typical pattern, these are enumerated below as Exceptions #1-18, and these are discussed on pages 214-215.

Excep. #1. Fig. 16, tokay B, first cyc., incr. Op-1p;

Excep. #2. Fig. 17, tokay C, second cyc., decr. pR-14;

Excep. #3. Fig. 19, tokay G, second cyc., decr. e3-e5;

Excep. #4. Fig. 20, toaky I, second cyc., decr. pR-2;

Excep. #5. Fig. 21, tokay K, third cyc., incr. 1p-pR;

Excep. #6. Fig. 22, tokay L, second cyc., incr. 1p-pR;

Excep. #7. Fig. 23, tokay M, first cyc., incr. Op-pR;

Excep. #8. Fig. 23, tokay M, first cyc., decr. pR-pR;

Excep. #9. Fig. 23, tokay M, first cyc., decr. 13-e4;

Excep. #10. Fig. 23, tokay M, first cyc., decr. e5-6;

Excep. #11. Fig. 23, tokay M, second cyc., decr. e4-14;

Excep. #12. Fig. 24, tokay N, third cyc., incr. 1p-1R;

Excep. #13. Fig. 24, tokay N, fourth cyc., incr. 0p-1R;

Excep. #14. Fig. 25, tokay O, second cyc., incr. 1p-2;

Excep. #15. Fig. 26, tokay P, second cyc., decr. pR-pR;

Excep. #16. Fig. 26, tokay P, second cyc., decr. 2-13;

Excep. #17. Fig. 26, tokay P, second cyc., decr. m4-14; and

Excep. #18. Fig. 29, tokay W, first cyc., decr. pR-e3.

An attempt was made to support the general pattern by setting up the data so that any apparent trends could be revealed by statistical analysis.

Data from animals measured several times per cycle were divided into four periods: period 1 - 0 to 2 days post-shed; period 2 - pR to 1R; period 3 - stages 2 to 13; period 4 - stages 4 to 6. Several intervals were defined by using these arbitrary periods. If the second measurement in an interval was greater than the first, a plus was assigned; if the first measurement in the interval was larger a minus was assigned (see table 18).

For intervals 3 and 5 the sample sizes were too small to draw any conclusions. For intervals 4, 6, and 7 the trend was clearly for an increase to occur. For intervals 1 and 2 no clear trend was apparent since both increases and decreases were seen. Inspection of the data reveals that the latter situation is related to how soon after shedding the first period's measurement was made. If it was taken soon after shedding the trend was for decreases to occur.

b. Tokays measured once per cycle

i. Eight tokays, measured for several cycles

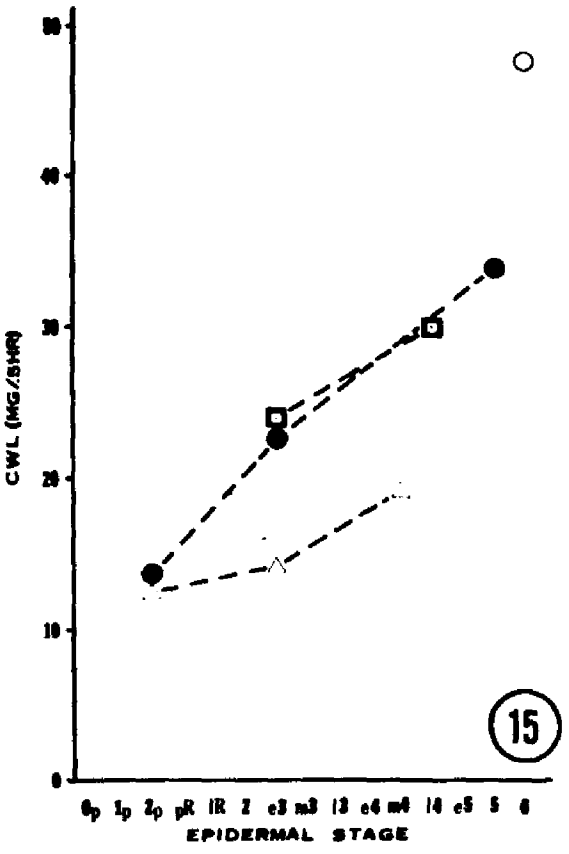
Eight tokays from a single shipment were measured

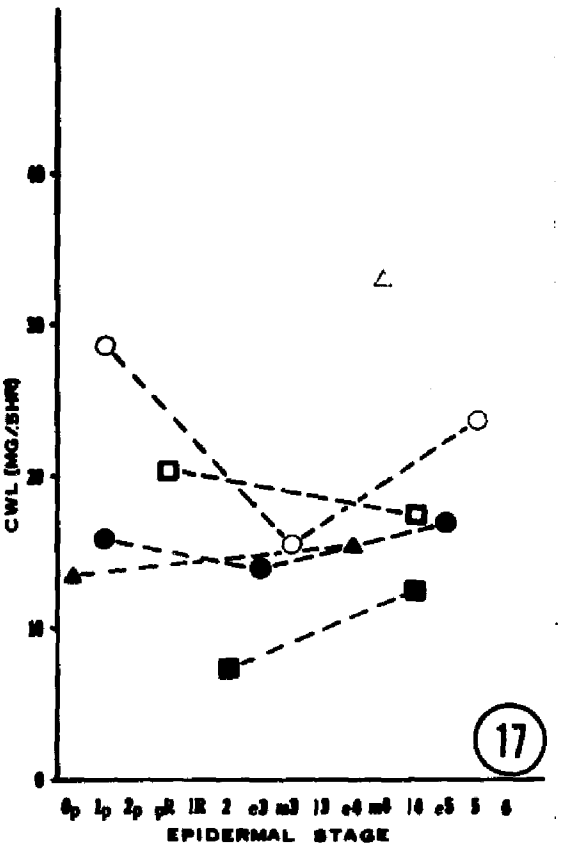
Figure 15. The pattern of CWL during the shedding cycles of Tokay A (open circle - first cycle, open squares - second cycle, open triangles - third cycle, shaded circles - fourth cycle; 0p - just after shedding, 1p - one day after shedding, 2p - two days after shedding, pR - perfect resting condition, lR - late resting stage, 2 - stage 2, e3 - early stage 3, m3 - mid stage 3, l3 - late stage 3, e4 - early stage 4, m4 - mid stage 4, l4 - late stage 4, e5 - early stage 5, 5 - stage 5, 6 - stage 6).

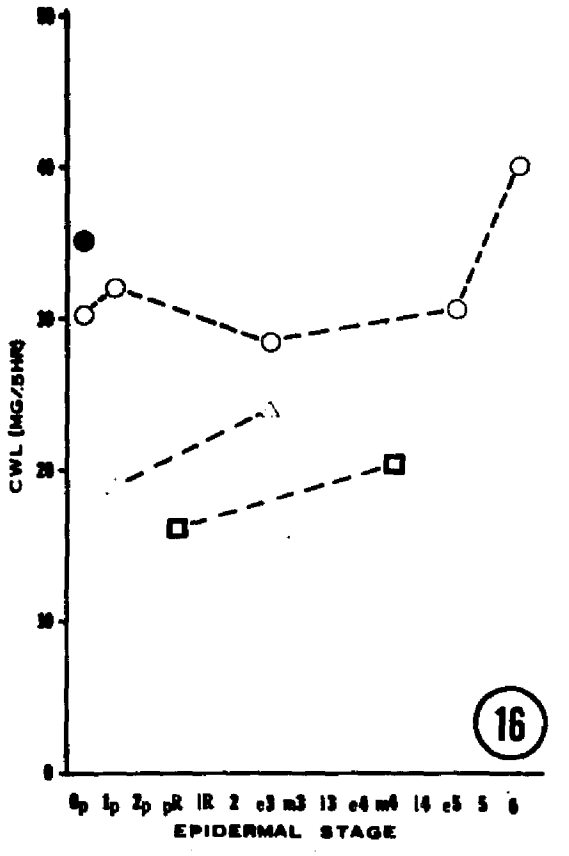
Figure 16. The pattern of CWL during the shedding cycles of Tokay B (open circles - first cycle, open squares - second cycle, open triangles - third cycle, shaded circle - fourth cycle; for other symbols see figure 15).

Figure 17. The pattern of CWL during the shedding cycles of Tokay C (open circles - first cycle, open squares - second cycle) and of Tokay D (open triangle - first cycle, shaded circle - second cycle, shaded squares - third cycle, shaded triangles - fourth cycle; for other symbols see figure 15).

Figure 18. The pattern of CWL during the shedding cycles of Tokay E (open circle - first cycle, open squares - second cycle, open triangles - third cycle) and of Tokay F (shaded circle - first cycle, shaded squares - second cycle; for other symbols see figure 15).


CWL (MG/SHR)
0
10
20
30
40
0p 1p 2p pR IR 2 e3 m3 I3 e4 m4 I4 e5 5 6
EPIDERMAL STAGE
15


CWL (MG/SHR)
0
10
20
30
40
0p 1p 2p pR IR 2 e3 m3 I3 e4 m4 I4 e5 5 6
EPIDERMAL STAGE
17


CWL (MG/SHR)
0
10
20
30
40
0p 1p 2p pR IR 2 e3 m3 I3 e4 m4 I4 e5 5 6
EPIDERMAL STAGE
16

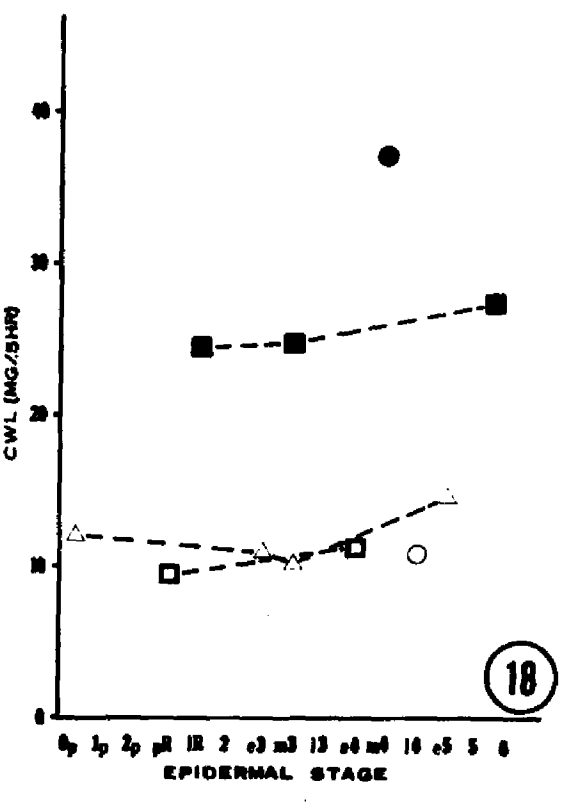
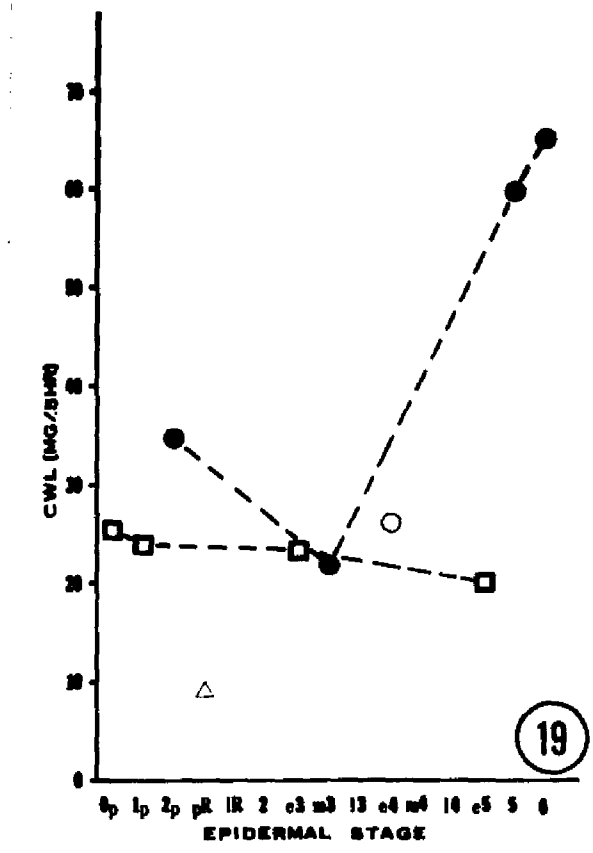

CWL (MG/SHR)
0
10
20
30
40
0p 1p 2p pR IR 2 e3 m3 I3 e4 m4 I4 e5 5 6
EPIDERMAL STAGE
18

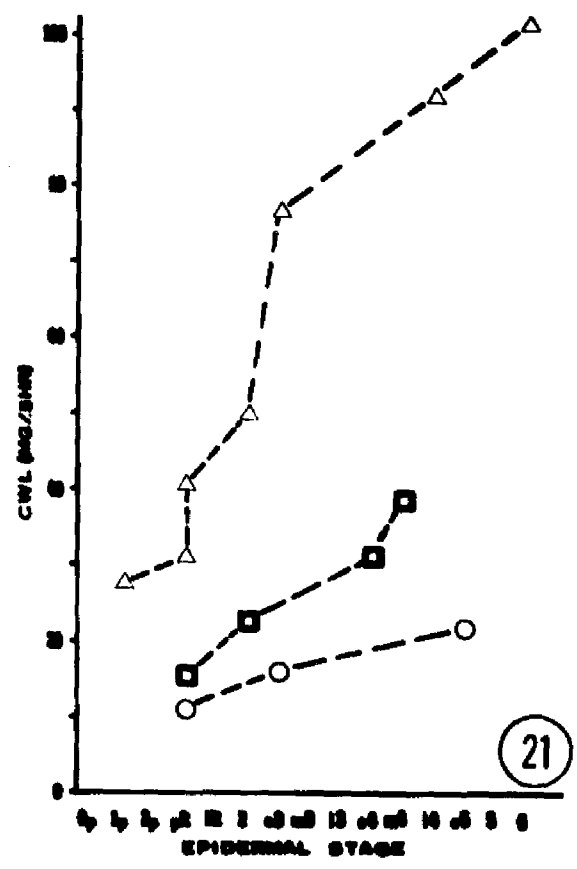
Figure 19. The pattern of CWL during the shedding cycles of Tokay G (open circle - first cycle, open squares - second cycle, open triangle - third cycle) and a shedding cycle of Tokay H (shaded circles; for other symbols see figure 15).

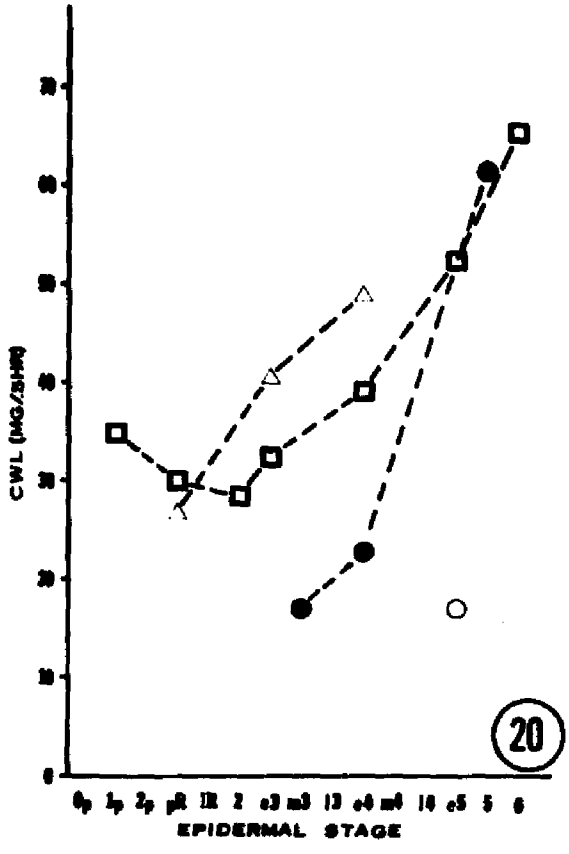
Figure 20. The pattern of CWL during the shedding cycles of Tokay I (open circles - first cycle, open squares - second cycle, open triangles - third cycle) and the shedding cycle of Tokay J (shaded circles; for other symbols see figure 15).

Figure 21. The pattern of CWL during the shedding cycles of Tokay K (open circles - first cycle, open squares - second cycle, open triangles - third cycle; for other symbols see figure 15).

Figure 22. The pattern of CWL during the shedding cycles of Tokay L (open circles - first cycle, open squares - second cycle, open triangles - third cycle; for other symbols see figure 15).


CWL (MG/SHR)
EPIDERMAL STAGE
19


CWL (MG/SHR)
EPIDERMAL STAGE
21


CWL (MG/SHR)
EPIDERMAL STAGE
20

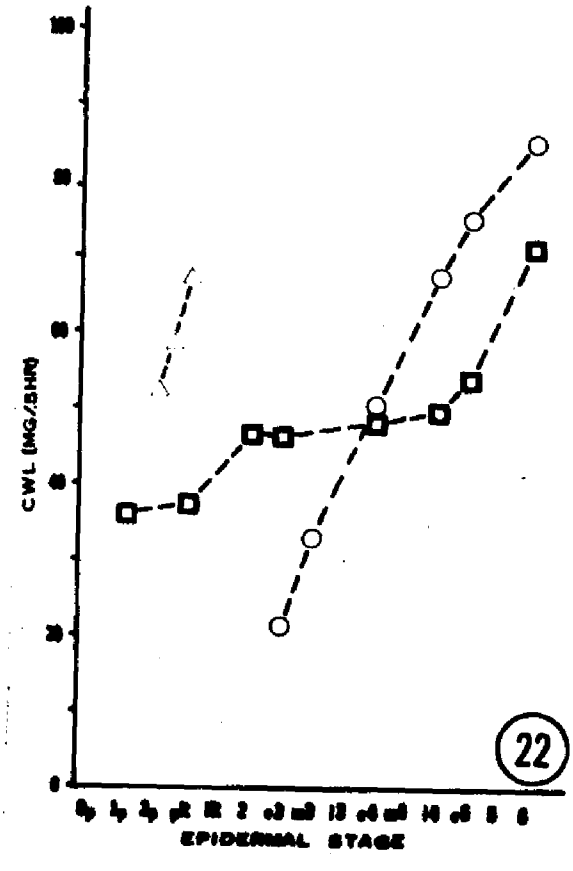
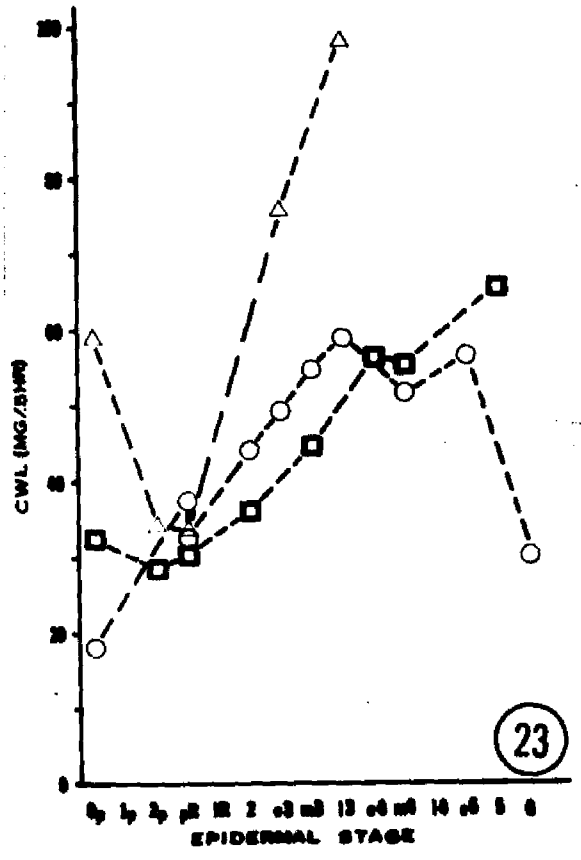

CWL (MG/SHR)
EPIDERMAL STAGE
22

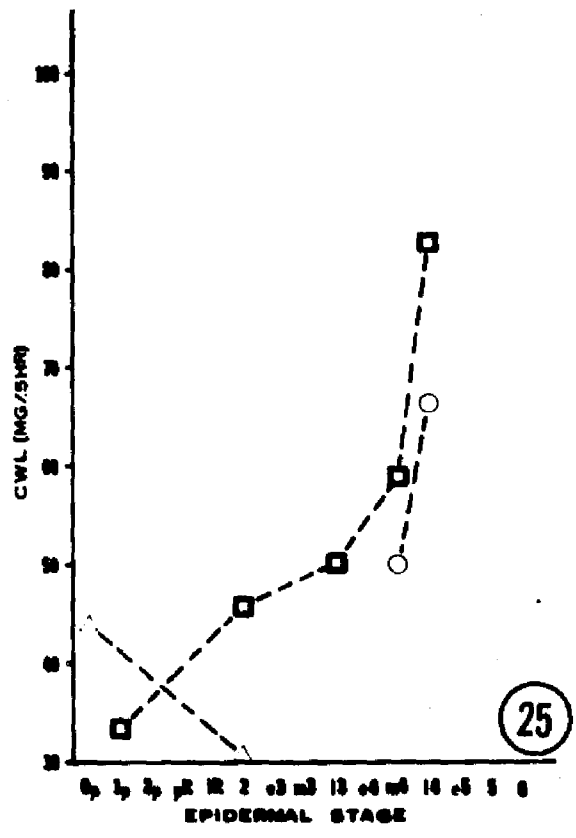
Figure 23. The pattern of CWL during the shedding cycles of Tokay M (open circles -first cycle, open squares - second cycle, open triangles - third cycle; for other symbols see figure 15).

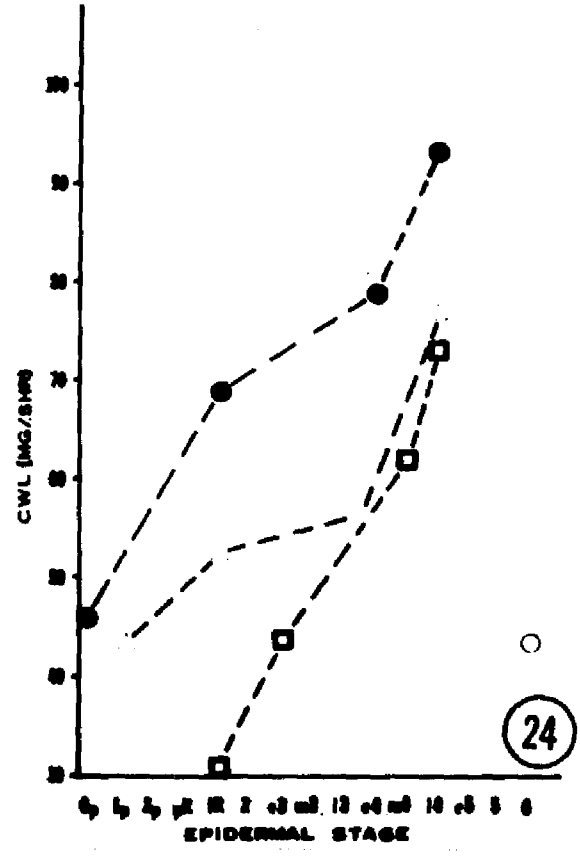
Figure 24. The pattern of CWL during the shedding cycles of Tokay N (open circle - first cycle, open squares - second cycle, open triangles - third cycle, shaded circles - fourth cycle; for other symbols see figure 15).

Figure 25. The pattern of CWL during the shedding cycles of Tokay O (open circles - first cycle, open squares - second cycle, open triangles - third cycle; for other symbols see figure 15).

Figure 26. The pattern of CWL during the shedding cycles of Tokay P (open circle - first cycle, open squares - second cycle) and the shedding cycle of Tokay Q (open triangles; for other symbols see figure 15).


CWL (MG/SHR)
EPIDERMAL STAGE
23


CWL (MG/SHR)
EPIDERMAL STAGE
25


CWL (MG/SHR)
EPIDERMAL STAGE
24

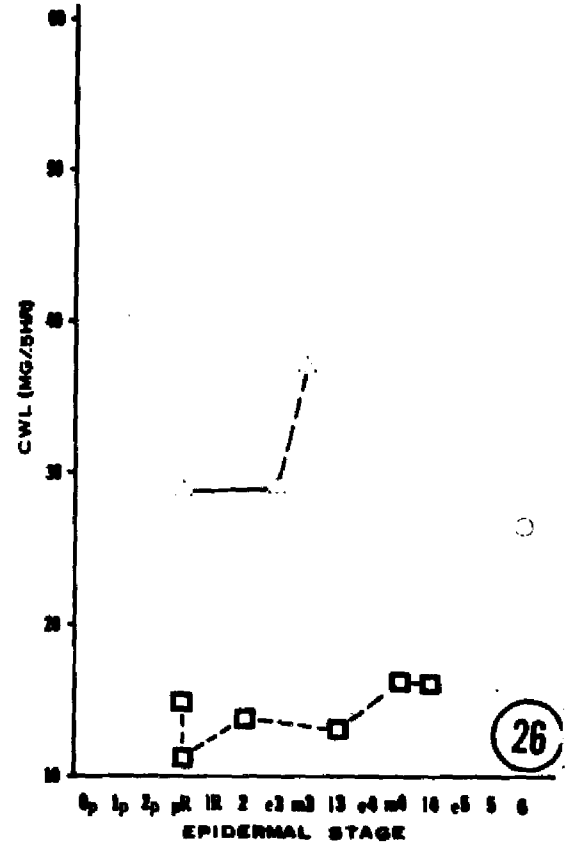
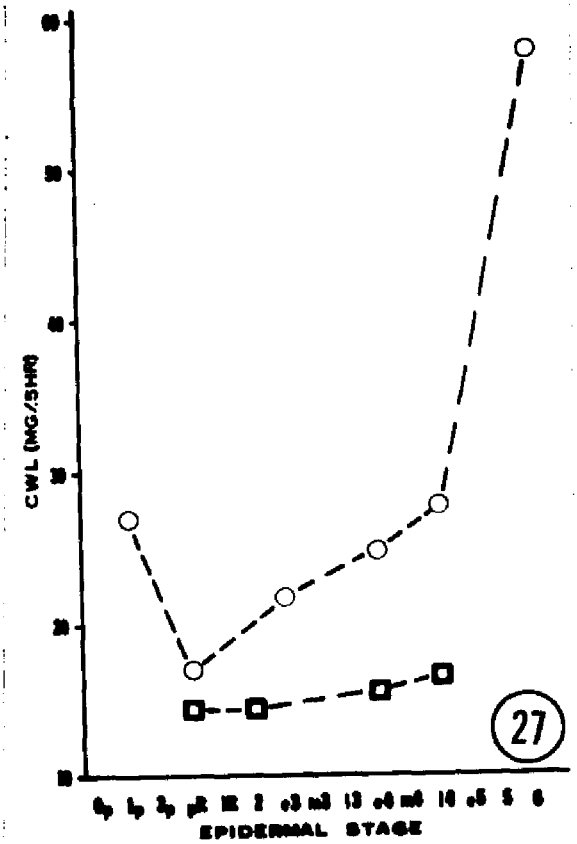
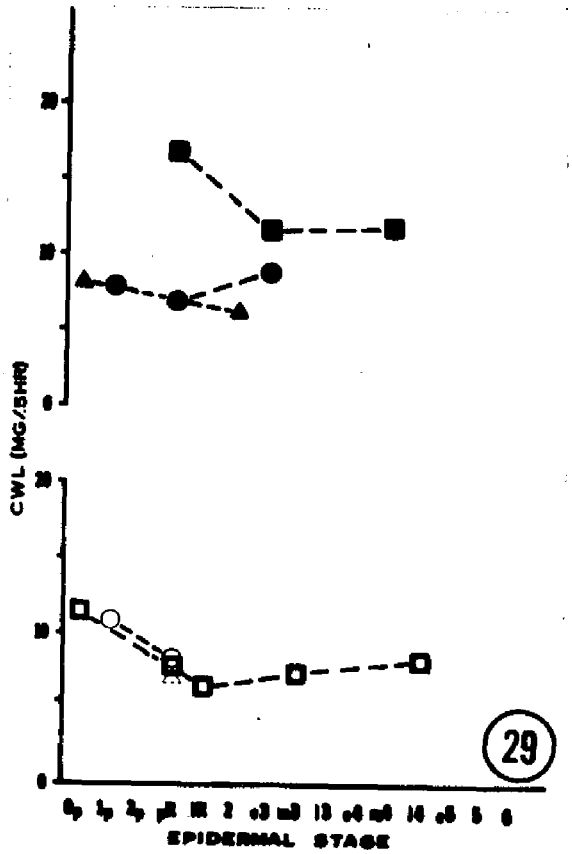

CWL (MG/SHR)
EPIDERMAL STAGE
26

Figure 27. The pattern of CWL during the shedding cycles of Tokay R (open circles - first cycle, open squares - second cycle; for other symbols see figure 15).

Figure 28. The pattern of CWL during the shedding cycles of Tokay S (open circles - first cycle, open square - second cycle), the shedding cycle of Tokay T (open triangle), and the shedding cycle of Tokay U (shaded circles - first cycle, shaded squares - second cycle; for other symbols see figure 15).

Figure 29. The pattern of CWL during the shedding cycle of Tokay X (open circles), the shedding cycles of Tokay Y (open squares - first cycle, open triangle - second cycle), the shedding cycle of Tokay V (shaded circles), and the shedding cycles of Tokay W (shaded squares - first cycle, shaded triangles - second cycle; for other symbols see figure 15).


CWL (MG/SHR)
EPIDERMAL STAGE
27


CWL (MG/SHR)
EPIDERMAL STAGE
29

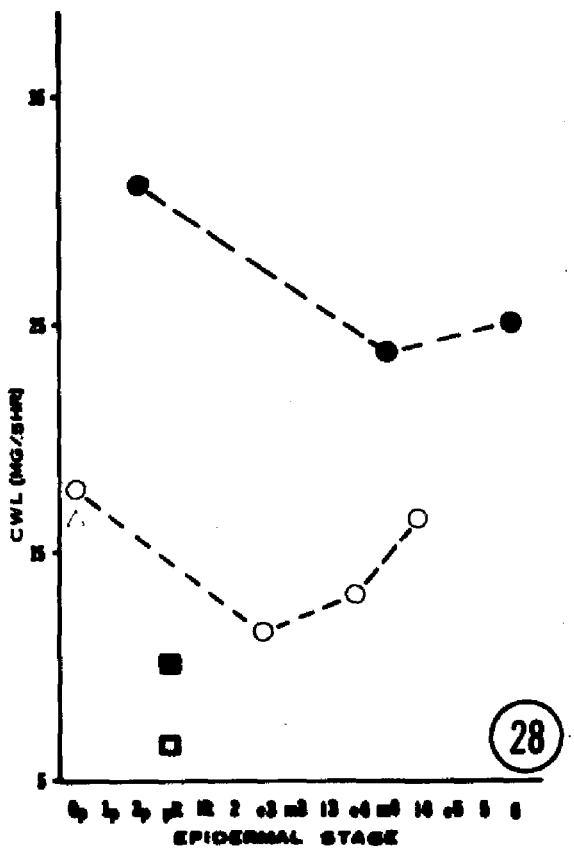

CWL (MG/SHR)
EPIDERMAL STAGE
28

Table 18. Changes between successive measurements during the shedding cycle, for tokays measured several times per cycle.

Interval	Period(s)	+	-	Chi Square
1	1 to 2	7	9	$0.5 < P < 0.9$
2	1 to 3	4	9	$0.1 < P < 0.5$
3	1 to 4	1	1	-
4	2 to 3	16	2	$P < 0.005$
5	2 to 4	3	1	$0.1 < P < 0.5$
6	3 to 4	28	2	$P < 0.005$
7	within 4	10	0	$P < 0.005$

only once per cycle, usually four days post-shed (preliminary results indicated that almost all tokays were either in a perfect resting condition or late resting stage at this time). Interspersed with these measurements were cycles in which runs were made only during the renewal phase. These animals were not anesthetized, but in all other respects had the same experimental protocol as the second group (mentioned above, p. 90). Table 19 presents these data.

From these data it would seem that CWL remains low, or possibly decreases through mid renewal (stage 4), is probably higher at stage 5, is definitely high post-shed, and remains high for several days after shedding.

ii. Many tokays, no specific regime

The relationship between the shedding cycle and CWL was also investigated by pooling all tokay data in which an animal had not been previously stressed or measured during that cycle. CWL was compared by transforming the raw data to CWL in terms of $mg/cm^2/hr$, by use of the regression: $SA = 10W^{0.67}$. The measurements were divided into four arbitrary parts of the cycle: 1 - 0 to 1 day post-shed ($n = 14$, mean = 0.212); 2 - post-shed, 2 days post-shed until the end of the histological post-shedding condition ($n = 21$, mean = 0.162); 3 - pR to 1R ($n = 26$, mean = 0.109); and 4 - stages 3 to 4 ($n = 18$, mean = 0.129). The stages were compared with a Mann-Whitney U test, using a t-test for $n > 20$. The differences between the first and second part, and the second and third part were statistically significant ($0.002 < P < 0.05$ and $0.001 < P < 0.01$, respectively), while the difference between the third and fourth parts of the cycle

Table 19. CWL (mg/0.5 hr) during resting and renewal phases for tokays measured only once per cycle.

	4 d Post							
	N	Mean CWL	SD	LR-2	3	4	5	6	0-PS
Tokay S	4	12.1	1.3	8.0		9.3			25.4
Tokay Z	7	19.9	2.8			21.1	51.0		55.7
									44.7
Tokay V	7	15.8	3.3			16.5			53.1
Tokay AA	4	20.2	1.2		20.0				
Tokay X	5	19.6	4.0	13.2		16.3			
				17.9					
Tokay T	5	13.4	2.9	17.6		12.2	18.5		25.1
Tokay U	5	16.5	2.1	16.4		13.5			37.6
						13.0			17.8
Tokay Y	5	15.6	4.1	10.3		12.0			
						12.5			

were not statistically significant ($0.05 < P < 0.1$). Although the group means confirm the trend mentioned earlier, it is only significant for the decrease following shedding.

c. Intercyclic trend

The shape of a curve representing the rate of change of CWL during a cycle varies between cycles. Usually such a curve is consistently higher, or consistently lower, than another curve (cycle) for that animal. Many animals (excluding those held at constant humidity, which were analyzed separately, p. 78) were measured for most of three cycles, thus it can be determined if there is a general trend of intercyclic water loss changes within individuals. For three cycles there are six permutations as to which cycle will have the highest rate of CWL and which will have the lowest rate. Of these six possibilities one would be interpreted as a general increase, another as a general decrease, and four as no discernible trend. A chi square test was carried out on eight (Tokays A, B, C, G, I, K, L, and M) groups of curves (3 increase, 1 decrease and 4 no trend), and the result indicates that there is no statistically significant trend ($0.1 < P < 0.5$) between cycles.

2. Other Lizards

a. Iguana iguana

Gross observations of shedding in I. iguana revealed that the head and neck shed several days, to a week, before the trunk. The proximal portions of the tail generally started shedding together with the trunk, while the distal portions of the tail might finish shedding up to a week later. Green iguanas were biopsied weekly from their lower jaw or gular region. While wounds in these

areas would not affect the body compartments' water loss, they were not accurate indicators of the developmental stage of the trunk epidermis at the time of measurement. Thus, for green iguanas measured in brass chambers, the renewal phase was assessed as lasting from the time the gular biopsy showed an early renewal phase histology until after the body shed. This definition makes the renewal phase approximately one week longer than it was at any one skin site.

Many measurements of green iguanas were made with acrylic chambers. Although the values of the Plexiglas runs were more variable than brass chamber runs (see the respective standard deviations in table 20), they can be used to supplement the more precise data. Most Plexiglas measurements were two hours in duration, and a day's runs with one animal usually lasted seven hours (one hour pre-drying and three 2 hour runs). Three animals were measured often enough at various times during the renewal phase for their data to be subdivided (see table 20). These data show that CWL increased during the renewal phase of body epidermis and was high during shedding.

b. Tupinambis nigropunctatus

Compartment studies were performed on a single black tegu (2.13 kg). A larger head compartment, and a new pillory were constructed to fit this animal. The animal was isolated just after most of its body had shed, the extremities shed by the time the animal's second set of runs were performed (day three, figure 30), and the tail shed much later. Throughout the experiment the animal was held in a control temperature room (30°C) with an alternating 12 hour light:dark cycle; water was provided, although food was with-

Table 20. CWL and the shedding cycle in Iguana iguana.

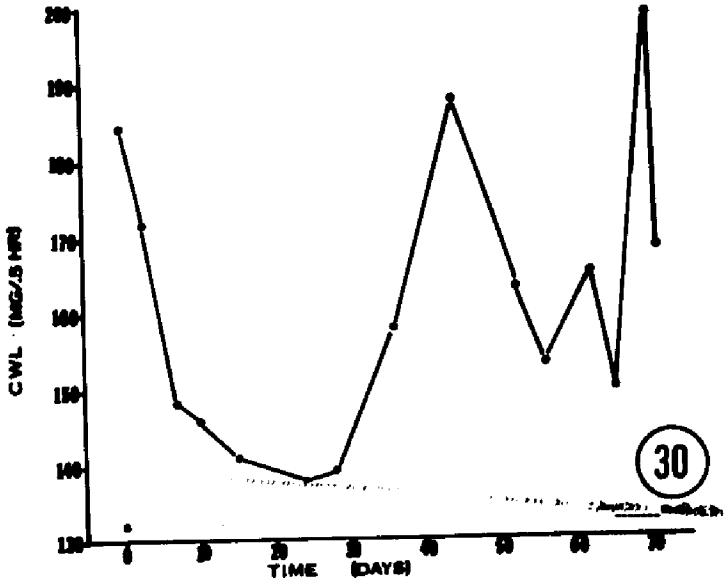
Animal number	Mean CWL during the resting phase	n[c]	SD	Mean CWL during the renewal phase	n[c]	SD	Comments
II3[a]	99.83	5	2.5	100.47	6	10.17	General renewal
II2[b]	307.20	3	14.39	337.23	3	33.30	Late gular, early body renewal
II3[b]	419.59	6	44.14	352.75	2	1.63	Mid gular renewal
				424.15	8	22.86	Mid body renewal
II5[b]	352.34	7	49.71	386.58	4	49.16	Late body renewal - body shedding

a. Brass, mg/0.5 hr

b. Plexiglas, mg/2 hr

c. Number (of days) measured

Figure 30. CWL in Tupinambis nigropunctatus (TT 4) as a function of the number of days after the initial measurement (the dashed line indicates the time the epidermis was in the renewal phase and 'S' indicates the time of shedding). Measurements started when this animal was just finished shedding, and continued until the animal died just prior to the next shed, for further details see text.


CWL (MG/L HR)
TIME (DAYS)
30

held. The animal was anesthetized with Nembutol (8.3 mg/kg) combined with Valium (2.5 mg/kg) one hour prior to starting the runs, and its cloaca was not taped. It proved difficult to work with this animal, since its weight would often pull its head down through the collar and the animal's slightest movements invariably broke the head-body seal. If the pillory was made too tight (thus forcibly supporting the body) the animal would stop breathing (whenever this happened, the engorged tongue was outstretched and it seemed to physically interfere with respiration). Several times the run was aborted and the animal was artificially revived. This animal often urinated whilst in the chamber, and unlike tokays, this could not be prevented by emptying the cloaca with gentle pressure before the run. The chamber was pre-dried for one half hour and usually the next three half hourly runs were recorded. The mean of the last two runs, or of the second run (if the last was aborted), was used for all comparisons.

CWL was high after shedding and initially decreased during the resting phase (figure 30). During the following prolonged resting phase, CWL gradually increased. Although no measurements are indicated on the graph for days 40 and 48, measurements were in fact taken, however only the first measurement following the predrying period was not aborted. In both cases, this value was almost exactly the same as the first measurement on day 36. Thus it would seem reasonable to believe that the next two measurements would have been similar to those reported for day 36. The peak on day 44 is related to the removal of old shed from the tail just prior to the runs. This peak probably corresponds to the dehydration of some

keratin in addition to transepidermal water loss. This also explains the peak in the first measurement on day 19 (the second and third measurements that day were aborted and therefore, there is no corresponding point on the graph), since some of the old shed was removed just prior to the runs. A sharp dip in CWL was recorded on the day the neck biopsy showed a stage 2 condition (day 65). No technical explanation can be offered for this sudden drop. A sharp increase in CWL was found when the neck epidermis was in a stage 5 condition. This increase was actually too steep: the head slipped down from the head compartment (although the seal was still perfect), so that some water loss which would have been normally associated with head compartment figures was recorded as body water loss. The last point on this graph represents a post mortum measurement. The neck necropsy revealed a post-shed condition. The first measurement after death was as high as the preceding day's first measurement, but the last two (which were averaged) were considerably lower. This is probably due to cutaneous dehydration following death. However, CWL at this stage was higher than it was in the normal resting condition, despite the fact that the animal was dead.

c. Lacerta lepida and Dipsosaurus dorsalis

Some lacertas were measured weekly (LL 9 and LL 48), while other lacertas and the desert iguanas were measured biweekly. Animals were housed in a control temperature room (30°C), with an alternating 12 hour (light:dark) photoperiod. Cloacas were not taped (except where indicated) and all animals were anesthetized with Nembutol (17.5 mg/kg) combined with Valium (5 mg/kg). Chambers were pre-dried for one half hour and the next three half hourly runs

were recorded.

Figures 31-34 show the relationship between CWL and three sequential half hourly runs for Lacerta and Dipsosaurus. In almost all cases the first measurement was higher than the second and the second higher than the third. This impression was statistically confirmed by performing a Wilcoxon's signed rank test for paired observations (for: runs 1 versus 2, and runs 2 versus 3), for all Lacerta and Dipsosaurus (Lacerta 1 X 2, $P < 0.005$; 2 X 3, $P < 0.005$; Dipsosaurus 1 X 2, $P < 0.005$; 2 X 3, $P < 0.005$). The differences between the runs are too large to be ascribed to residual moisture in the chamber (see p. 56). This situation was rarely found in tokays except when the animals had relatively low rates of CWL. Due to this relation between CWL and time in the chamber, only the third run was used for all comparisons.

The stage in the animal's shedding cycle could not be predicted from its shedding record. Shedding in Lacerta was approximately as asynchronous as it was in I. iguana. Therefore, the stage of the body epidermis at the time of measurement was prorated from the corresponding gular biopsy (table 21).

The epidermis of both Dipsosaurus remained in the perfect resting condition throughout the duration of their experiment.

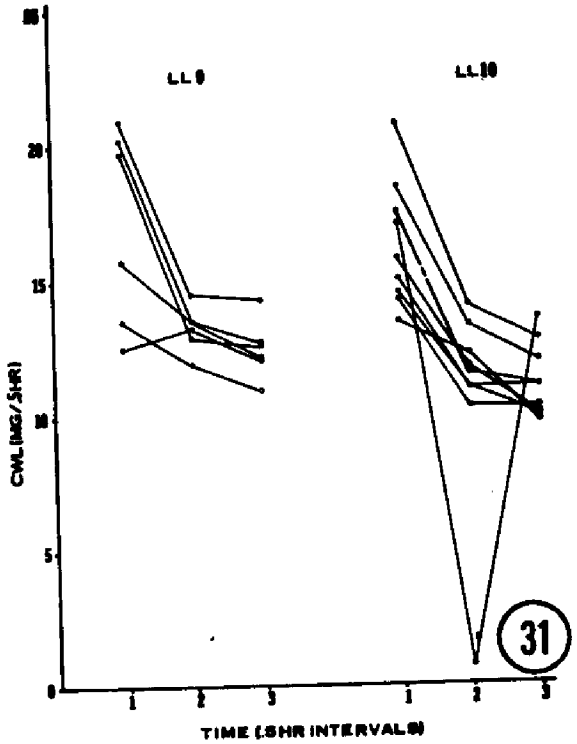
Food, but not water, was withheld from five Lacerta (LL 9, LL 10, LL 11, LL 15, and LL 48) throughout the time they were measured. One Lacerta (LL 10) did not enter a renewal phase. Its second measurement was made just after a claw broke off and a high rate of CWL was recorded at this time. The last day this animal was measured, the second run had a very low value and the third run had

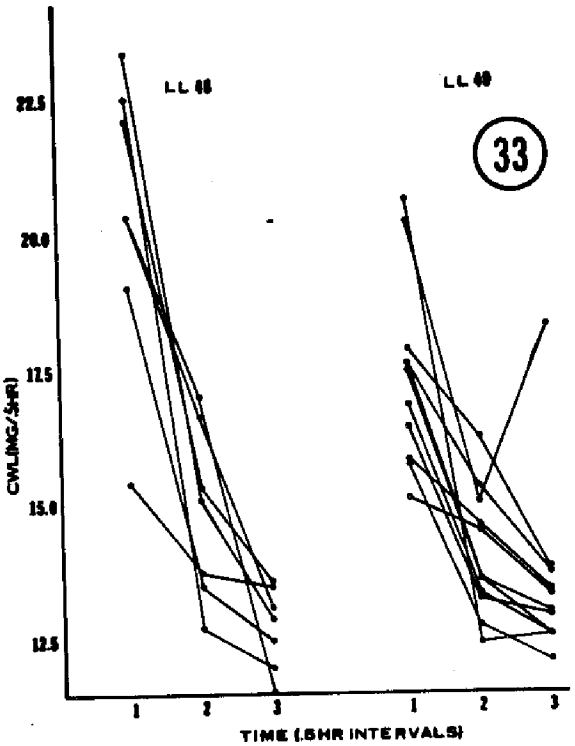
Figure 31. Consecutive half hourly measurements of CWL on several different days for two Lacerta lepida.

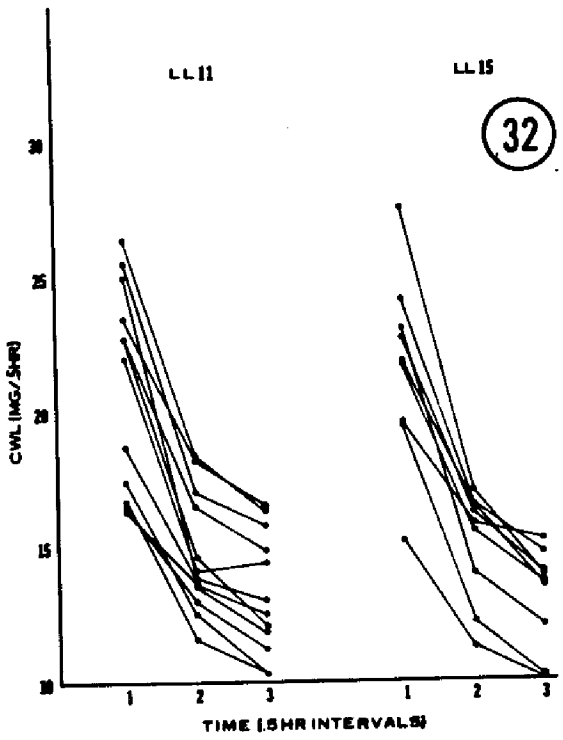
Figure 32. Consecutive half hourly measurements of CWL on several different days for two Lacerta lepida.

Figure 33. Consecutive half hourly measurements of CWL on several days for two Lacerta lepida.

Figure 34. Consecutive half hourly measurements of CWL on several different days for two Dipsosaurus dorsalis.


LL 9
LL 10
CWL (MG/5HR)
TIME (5HR INTERVALS)
31


LL 48
LL 49
CWL (MG/5HR)
TIME (5HR INTERVALS)
33


LL 11
LL 15
CWL (MG/5HR)
TIME (5HR INTERVALS)
32

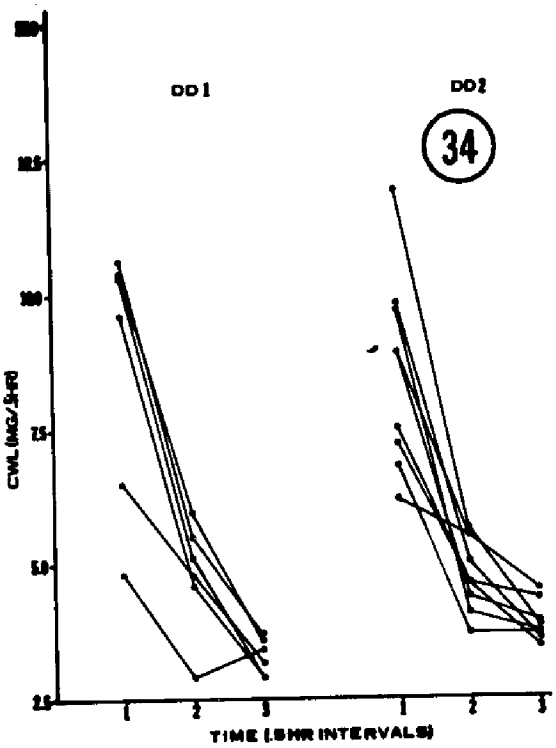

DD 1
DD 2
CWL (MG/5HR)
TIME (5HR INTERVALS)
34

Table 21. Histological stage of the skin taken the same day from various body sites, for jewelled lacertas and a black tegu.

Species	Neck	Pectoral belly	Mid back	Pelvic belly	Mid dorsal tail
Lacerta 1	PS	pR	pR	pR	5
Lacerta 2	pR	2-3	15	5	PS-pR
Tupinambis	PS[a]	2	3		pR

a. Outer generation still attached

a very high value. This latter situation indicates a balance misfunction during the second measurement. If the runs for the second day and the last day are rejected this animal's coefficient of variation (CV) reduces to 7.55% (table 22). Another Lacerta (LL 15) also did not enter a renewal phase during the measuring period.

One lacerta's (LL 9) rate of CWL during the resting phase was low and constant (mean, 11.98 mg/0.5 hour; CV = 5.72%), and was higher when the neck was in a stage 2 (12.7 mg/0.5 hour) and when the neck was shedding (14.3 mg/0.5 hour). These neck stages probably correspond to belly epidermal stages, pR and m4, respectively. Another Lacerta (LL 11) had high rates of CWL during various stages of the renewal phase (14.9, 16.3, 15.8, and 14.4 mg/0.5 hour). The neck biopsy corresponding to the last measurement showed a keratinizing inner alpha layer. The old generation was retained on several regions for several weeks after the epidermis showed a perfect resting condition. During this period the rate of CWL was lower (range, 10.3 to 13.0 mg/0.5 hour, n = 7). However, another Lacerta (LL 48) had approximately the same rate of CWL during the resting and renewal phases, as at the time of shedding.

The dietary and fluid intake of three other lacertas was manipulated to gain some insight into the relation of such factors to CWL (these results were reported on p. 84). One of these animals (LL 49) entered a renewal phase. Its rate of CWL during the renewal phase while it was hydrated was: 14.2 mg/0.5 hour. This Lacerta was deprived of water for several weeks. In the interim it entered a renewal phase and subsequently shed. Its rate of CWL during these periods was lower than those measurements taken during the

Table 22. The mean rate of CWL (mg/0.5 hr) and its coefficient of variation for all measurements taken for eight jewelled lacertas and two desert iguanas.

Animal #	N	Mean CWL	CV	Mean wt (gms)
LL 9	6	12.48	8.62	82.8
LL 10	9	11.10	12.09	76.8
LL 11	11	12.96	16.29	100.3
LL 15	9	12.99	14.31	96.3
LL 48	7	12.73	6.10	109.0
LL 49	11	13.47	12.26	69.4
DD 1	6	3.28	10.45	42.5
DD 2	10	3.92	9.75	43.5

preceding resting phase.

Although the hydration state of the animal normally has no effect on CWL in Lacerta (p. 84), it is possible that dehydration can diminish the increase in CWL which usually accompanies the renewal phase in other lacertilian species. Because the epidermal condition is not synchronized throughout the body, it is impossible to determine the exact relationship between CWL and the shedding cycle.

3. Snakes

a. Elaphe obsoleta quadrivittata

i. CWL during the resting phase

Many of the Elaphe which were measured weekly to thrice weekly did not enter the renewal phase even after long periods. With these animals it was possible to consider temporal changes in CWL while the subject's epidermis was in the resting condition.

Each graph in figures 35 and 36 has two curves. One relates absolute CWL (mg/0.5 hour) to elapsed days since the initial measurement. The other curve relates percentage changes in CWL between successive measurements to elapsed days since the initial measurement, and thus allows visualization of the magnitude of the fluctuations between measurements with time.

CWL increased in Elaphe #5 (figure 36) at a rate of 0.76 mg/day or 2.37 mg/measurement, throughout the entire experimental period. An odd measurement occurred on day 29; the individual half hourly runs for that day were: 124.4, 102.5, 91.6, and 83.3 mg/0.5 hour. For the sake of consistency, the average of the second and third measurements was used, although it is obvious that the rate of CWL was constantly decreasing throughout the measuring peri-

Figure 35. Absolute CWL (open circles) and percent daily changes in CWL (open squares) as a function of time since the initial measurement, for four Elaphe obsoleta quadrivittata (the dashed line indicates the time the epidermis was in the renewal phase, the dotted line indicates the time that the epidermis showed the "odd" resting condition, the solid line indicates the time in which both an "odd" resting condition and a "trauma" reaction were found, 'T' indicates the time of thyroidectomy, 'S' indicates the time of shedding).

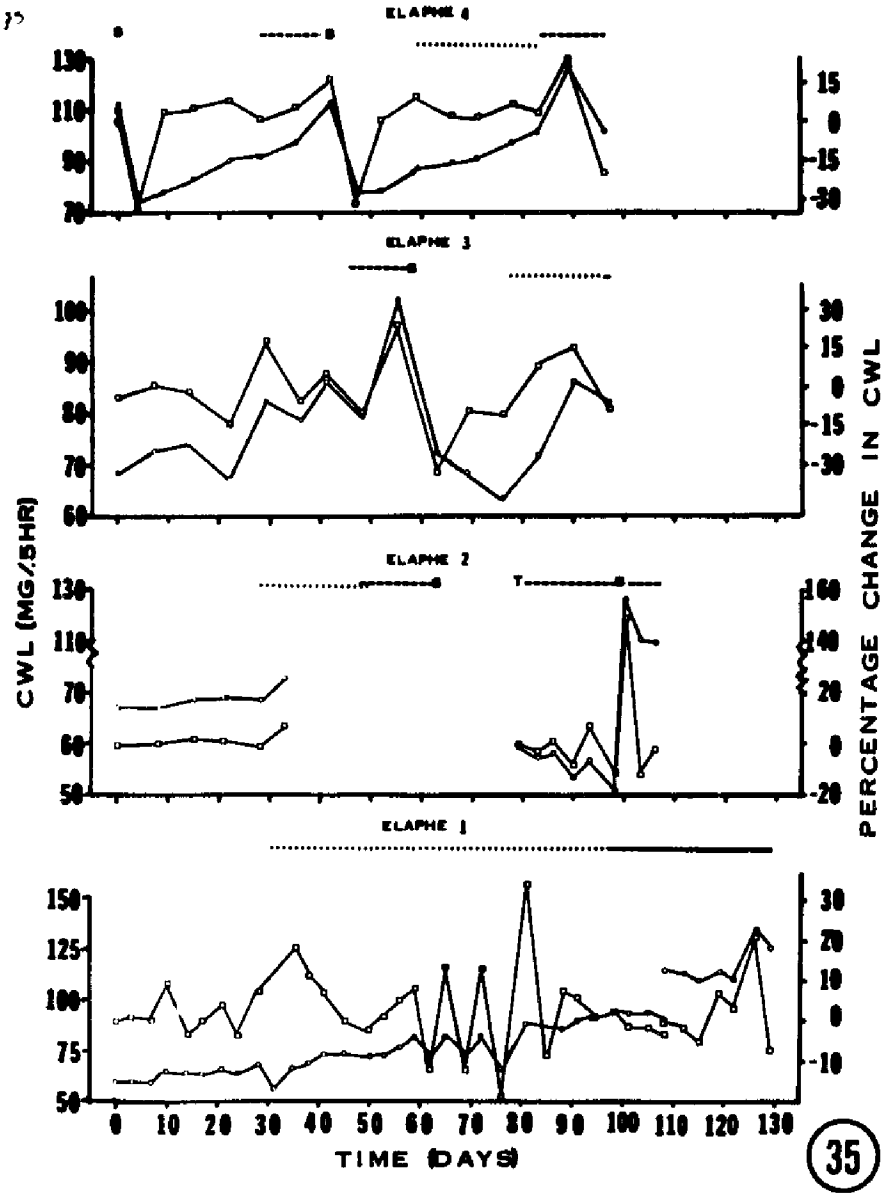
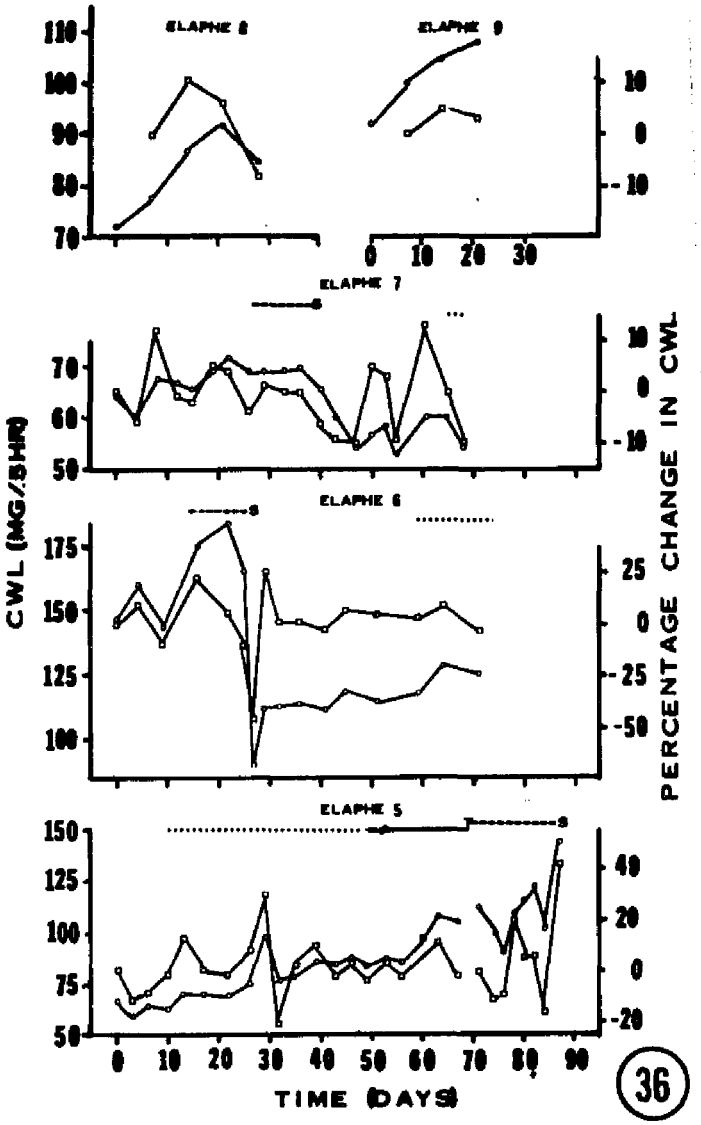

ELAPHE 4
ELAPHE 3
ELAPHE 2
ELAPHE 1
CWL (MG/.5HR)
PERCENTAGE CHANGE IN CWL
TIME (DAYS)

Figure 36. Absolute CWL (open circles) and percent daily changes in CWL (open squares) as a function of time since the initial measurement, for five Elaphe obsoleta quadrivittata (the dashed line indicates the time the epidermis was in the renewal phase, the dotted line indicates the time that the epidermis showed the "odd" resting condition, the solid line indicates the time in which both an "odd" resting condition and a "trauma" reaction were found, 'T' indicates the time of thyroidectomy, 'S' indicates the time of shedding).


ELAPHE 8
ELAPHE 9
ELAPHE 7
ELAPHE 6
ELAPHE 5
CWL (MG/8HR)
PERCENTAGE CHANGE IN CWL
TIME (DAYS)

od. This trend was atypical for this animal. For example, on day 78 the first run (126.5) had approximately the same value as the first run on day 29, but the subsequent runs (121.9 and 122.1) did not decrease. Thus the continuous decrease during day 29 was probably due to prolonged drying of a small amount of undetected urine (this animal's cloaca was not taped).

Often in rat snakes which showed a prolonged resting phase the successive values of CWL fluctuated, that is, increased and then decreased. This alternation can be seen in Elaphe #5 during the first twenty days and between days 40 and 60.

These animals showed some unusual patterns of epidermal activity by comparison with the normal shedding cycle. The biopsy on the sixth day (figure 37) showed a typical perfect resting condition (Maderson, Chiu, and Phillips, 1970b). On day 10 (figure 38) the germinal layer suggested renewed proliferative activity, but biopsies on days 13 and 17 (figures 39 and 40) showed maintenance of this "odd" condition, with no indication of the onset of the renewal phase. This "odd" condition was seen up to the time of thyroidectomy on day 69.

The overall change in CWL in Elaphe #1 (figure 35) measured at 200 cc/minute was: 0.29 mg/day or 0.98 mg/measurement. There was no definite trend in the pattern of fluctuations throughout the experiment. This animal had periods in which the overall rate of CWL was stable (days 10 to 30, 41 to 72, and 91 to 108) alternating with periods in which CWL generally increased (days 31 to 41, and 76 to 91). From day 108 on, the flow was changed to approximately 400 cc/minute. It was hoped that this change would reduce

Figure 37. Photomicrograph through the outer scale surface of Elaphe skin in the perfect resting condition. Arrows indicate pycnotic nuclei within the alpha layer, for other symbols see figure 1.

Figure 38. Photomicrograph through the outer scale surface of Elaphe skin in the "odd" resting condition. Although the germinal cells are active (columnar) there is no sign of a clear layer which characterizes early renewal (for symbols see figures 1 and 37).

Figure 39. Photomicrograph through the outer scale surface of Elaphe skin in the "odd" resting condition. This biopsy was taken several days after the one illustrated in figure 38, yet there is still no sign of generation formation (for symbols see figures 1 and 37).

Figure 40. Photomicrograph through the outer scale surface of Elaphe skin in the "odd" resting condition. This biopsy was taken several days after the one illustrated in figure 39. By this time the alpha layer is considerably thicker than normal (for symbols see figures 1 and 37).

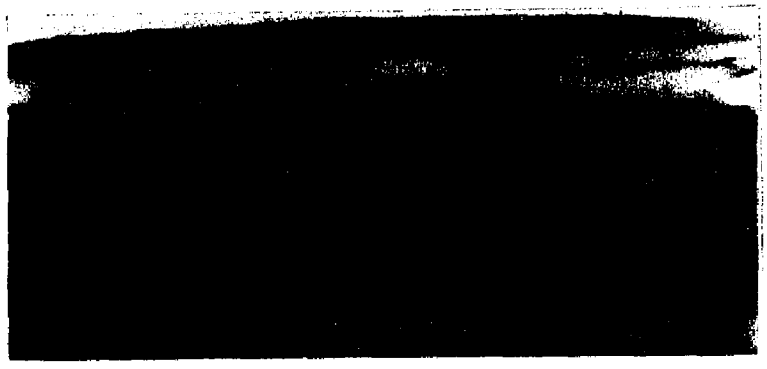
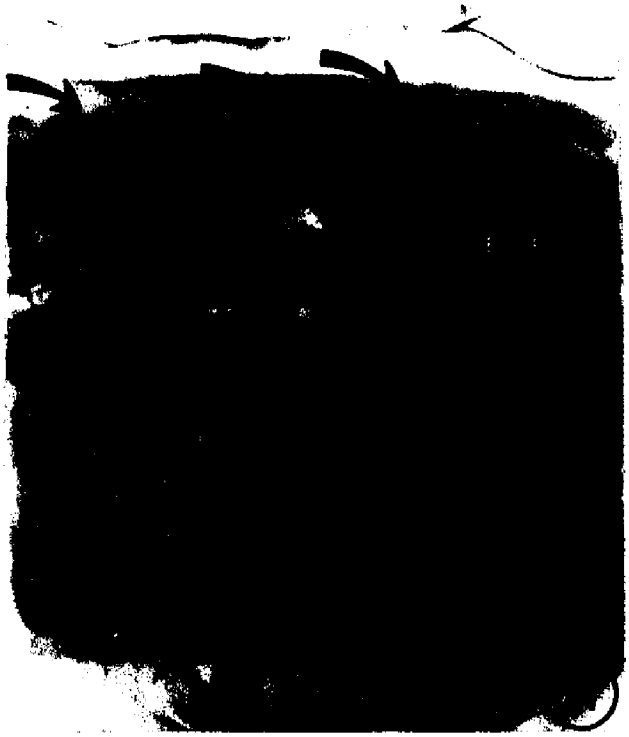


Figure 41. Photomicrograph through the outer scale surface of Elaphe skin in the "odd" resting condition showing a trauma reaction. The "odd" resting condition is much advanced compared to figure 40. There are many layers of flattened immature cells. Between the immature cells and the alpha layer are nests of migrant eosinophils (E) (for other symbols see figures 1 and 37).



these fluctuations. The rate of CWL was higher at 400 cc/minute, although the daily fluctuations still persisted.

On day 31 the epidermis showed the "odd" epidermal condition, which persisted until the end of the experiment without the animal entering a renewal phase. On day 98 the epidermis had a trauma reaction, this was found on all subsequent neck biopsies.

During the resting phase, Elaphe #7 (figure 36) did not show much variation between measurements and there was no trend for the rate of CWL to increase throughout the measuring period.

On day 65 there were definite signs of an "odd" resting histology, however, subsequent biopsies did not reveal any evidence of an epidermal trauma reaction.

The results for Elaphe #2 (figure 35) are divided into two parts. This animal was measured periodically over a thirty-three day period, not measured for thirty-seven days, then it was thyroidectomized and measured approximately thrice weekly thereafter. CWL during this animal's resting phase was remarkably constant. The biopsy taken on day fifteen showed that the germinal cells were dense and columnar, and the next biopsy on day 21 did not seem to be more advanced. However, on days 28 and 33 there were definite signs of an "odd" epidermal condition. This animal shed naturally on day 63, which means it probably entered a renewal phase on day 49, or sixteen days after the first series of runs was terminated.

After shedding on day 28, Elaphe #6 (figure 36) showed a fairly constant small rise in CWL of 0.32 mg/day or 1.5 mg/measurement. On day 59 its skin showed an "odd" resting condition which was maintained for the next two biopsies.

Elaphe #3 (figure 35) was fed the day after each weekly experiment. For the first 85 days of the experiment the animal was maintained at constant humidity, by keeping it on a mesh grid above water in a ten gallon tank. For the remainder of the experiment, ambient humidity was not controlled. Although CWL generally increased during the prolonged resting period, there were large fluctuations which did not show any particular pattern. No "odd" epidermal condition was observed during the initial prolonged resting period, but such did characterize the second, beginning eighteen days after shedding (the seventy-sixth day of the experiment), and lasting through the end of the experiment. A trauma reaction was seen in the last biopsy.

Elaphe #8 (figure 36) was held at high humidity and treated in the manner described for Elaphe #3, except that Elaphe #8's first measurement was performed at a low flow rate of 200 cc/minute. Elaphe #8 exhibited a fairly steep rise in CWL of: 0.44 mg/day or 2.48 mg/measurement, during its resting period. The neck biopsy taken on day 0 showed an "odd" resting histology, and from day 7 on all biopsies showed a trauma reaction.

Elaphe #9 (figure 36) was held at a constant low humidity by placing the animal in a ten gallon tank on a wire mesh support over Drierite. This animal was fed one day after each weekly measurement. Its first measurement was also performed at an air flow rate of approximately 200 cc/minute. The rise in CWL during the measuring period was: 0.76 mg/day or 4.0 mg/measurement. A biopsy taken on the day of the last measurement showed a possible "odd" resting condition.

Elaphe #4 (figure 35) was measured during two prolonged resting periods separating three natural renewal phases and sheds. During both prolonged resting periods there were sharp increases in CWL: for the first, 0.73 mg/day or 3.5 mg/measurement; and for the second, 0.59 mg/day or 3.05 mg/measurement. No biopsies were taken during the first cycle, however, the biopsy at day 59 showed an "odd" resting condition and by day 71 there were unmistakable signs of such. There were no microscopic indications of a trauma reaction in any of the biopsies of this animal. The last measurement (day 96) was made just after the animal died.

ii. CWL during the renewal phase

α. One measurement per cycle: Chamber determinations of CWL during the resting phase were made at a flow rate of 200 cc/min, for twelve Elaphe which were not previously stressed that cycle. Their mean rate of CWL (72.95 mg/0.5 hr) was not significantly different ($P > 0.05$; the Mann-Whitney U test was used for this and all subsequent statistical tests reported in this section) from similar measurements of seven rat snakes measured at 400 cc/minute (mean CWL 108.89 mg/0.5 hr). Therefore all the resting phase data were combined. Animals measured at the cloudy eye stage, or at the pre/post-shed did not differ significantly in weight (cloudy eye mean weight: 407.3 gms; pre/post-shed mean weight: 377.5 gms), nor in CWL ($0.1 < P < 0.02$, cloudy eye mean CWL: 141.5 mg/0.5 hour; pre/post-shed mean CWL: 110.7 mg/0.5 hour). Since the weights of the animals measured whilst in the resting phase (mean weight: 330 grams) were not significantly different ($0.4 < P < 0.8$) from those in the renewal phase (mean weight 390 grams), their absolute rates

of CWL were directly compared. CWL measured during the resting phase ($n = 19$, mean CWL 86.19 mg/0.5 hour) was significantly different ($0.002 < P < 0.01$) than CWL measured during the renewal phase ($n = 14$, mean CWL 123.92 mg/0.5 hour).

CWL for these and other rat snakes measured once per cycle was expressed in terms of surface area by using the regression equation: $SA = 12.5W^{0.67}$. These animals were divided into four groups depending on their epidermal condition (table 23). This table shows: 1) that there was a significant decrease in CWL from the post-shedding condition to the resting phase ($0.001 < P < 0.005$), 2) that CWL increased significantly from the resting phase to the renewal phase ($0.001 > P$), and 3) that CWL does not significantly change from the renewal phase to the pre-shed period ($0.1 < P$).

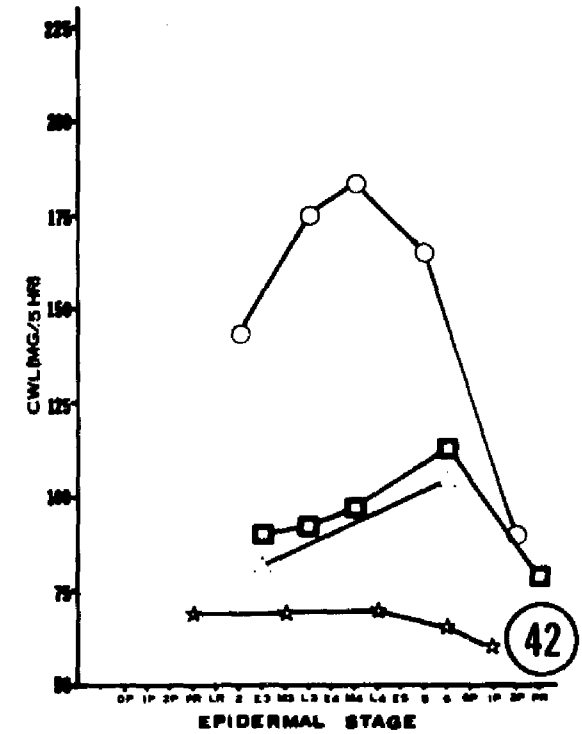
β. Several measurements per cycle: During chamber measurements four animals naturally entered the renewal phase. Most of the data for these animals support the general model (figure 42) and will not be further discussed. Elaphe #7 showed no increase in CWL during any part of the renewal phase, but did show a slight drop in CWL during the pre-shed period and a further drop after shedding. Elaphe #6 had an increase in CWL during the renewal phase, and a sharp drop during early stage 5, the stage in which a sharp increase was expected. However, this discrepancy can be explained. Although biopsies were not taken when this animal was measured, on the basis of the fact that this animal shed two days later, it may be estimated to have been in an early stage 5 at the time of the measurement. However, at the time of shedding its outer generation was dry and

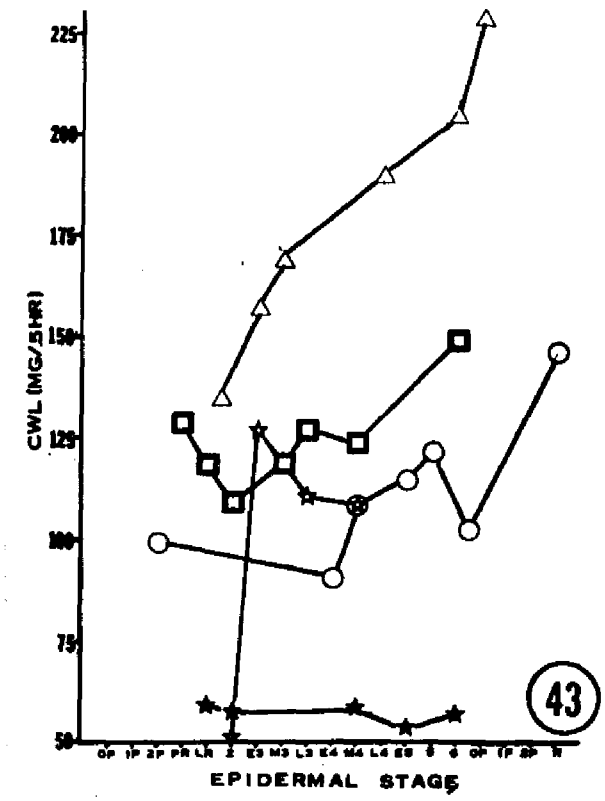
Table 23. CWL ($mg/cm^2/hr$) in Elaphe obsoleta quadrivittata, measured once per cycle.

Epidermal condition	N	Mean CWL	SD
Post-shed	9	0.158	0.0355
Rest	17	0.121	0.0230
Renewal	12	0.201	0.0690
Pre-shed	8	0.160	0.0480

Figure 42. The pattern of CWL during the shedding cycle of Elaphe #6 (open circles), Elaphe #4 (open squares), Elaphe #3 (open triangles) and Elaphe #7 (open stars, for other symbols see figure 15).

Figure 43. The pattern of CWL during the shedding cycle of Elaphe #5 (open circles), Elaphe #10 (open squares), Elaphe #11 (open triangles), and the shedding cycles of Elaphe #2 (shaded stars - first cycle, open stars - second cycle, for other symbols see figure 15).


CWL (MG/5 HR)
225
200
175
150
125
100
75
50
EPIDERMAL STAGE
42


CWL (MG/5HR)
225
200
175
150
125
100
75
50
EPIDERMAL STAGE
43

brittle, suggesting that the loss of the outer generation had been delayed. Thus the prorated scoring of an early stage 5 may have been a full stage off. As expected, this animal's subsequent measurement while it was in a post-shedding condition was substantially lower than the previous measurement.

Four animals were thyroidectomized so that more data could be obtained in a predictable manner on the relation between shedding and CWL in rat snakes. Several previous reports indicated that thyroidectomy only affects the length of the resting phase while the renewal phase is unaffected (Chiu and Lynn, 1970ab; Maderson, Chiu, and Phillips, 1970a). It was felt that this was a strong rationale for assuming that CWL of thyroidectomized animals during the renewal phase would resemble that of unoperated animals.

CWL increased during the renewal phase of Elaphe #5, #10, and #11 (figure 43). Elaphe #2's first cycle showed almost no change in CWL throughout the renewal phase, and during the second cycle CWL actually decreased.

Measurements were also recorded during the resting and renewal phases for other rat snakes not subjected to weekly measuring regimes. In general these results (table 24) support the data obtained by measuring an animal several times weekly. The unusually high value for Elaphe #5 at the end of the resting phase was probably due to the higher flow rate used for that measurement (255 cc/minute) compared to the flow rate used for its other runs conducted during the renewal phase (range: 192 to 212 cc/minute).

b. Constrictor constrictor

The relation between the shedding cycle and CWL was

Table 24. CWL (mg/0.5 hr) during the shedding cycle in irregularly measured Elaphe obsoleta quadrivittata.

Animal	First post-shed	First rest	Renewal	Pre-shed	Second post-shed	Second rest
Elaphe #5		99.4 (pR)[a]	137.2 (m4)	135.0	132.2 (Op)	
Elaphe #5		152.3 (1R)		130.0	119.0 (Op)	74.0 (pR)
Elaphe #1	134.1 (1p)	63.8 (pR)				
Elaphe #12	70.7 (Op)	74.0 (pR)	78.7 (e4)			
	69.7 (2p)					
Elaphe #13		86.5 (pR)	124.3 (e5)			
		87.6 (pR)				
Elaphe #14				155.3	137.1 (Op)	108.9 (pR)

a. Stage in the shedding cycle, see figure 15.

also investigated in two boa constrictors, for several cycles. Both boas were acclimated to 30°C and a twelve hour photoperiod for one and a half shedding cycles (two and a half months) prior to isolation. During the acclimation period they were fed weekly and had constant access to a small watering dish (which they could not fit into). Both boas were gaining weight and were judged to be in good condition prior to isolation. Each was transferred from a large holding cage in a control temperature room to a ten gallon tank (a wire support prevented their contact with any excrement) in a BOD box. Once isolated they did not have access to food, although water was supplied.

Animals were anesthetized with Nembutol (10 mg/kg of a 2.5% solution in isotonic saline) in combination with Valium (3.3 mg/kg of a 0.5% solution of injectable Valium), injected subcutaneously in the gular region. During the following hour the animals were checked to see if they would require an additional dose to keep them out for the duration of the experiment. Occasionally an animal showed enough activity to break the head-body seal. When this occurred it was removed and allowed at least three days (usually seven) to recover before the procedure was repeated. Their cloacas were not taped. The snakes were placed into the chamber by folding them back on themselves. The exhaust flowmeters were periodically checked for signs of respiration.

Figure 44 shows the results of three sets of runs following a one half hour pre-drying out period at 400 cc/minute. Frequently the rate of CWL kept decreasing past the amount of time that was available to measure that animal. The runs for each animal were

Figure 44. Three typical CWL runs for Constrictor constrictor, shown as a function of consecutive half hourly measurements (open circles - CC 2; shaded circles - CC 1, March 23 run; open triangles - CC 1, May 12 run). Probably it is only fortuitous that the value for the first interval for CC 2 coincides with the first interval for the May 12 run of CC 1.

CWL (MG/5HR)
TIME (1998 WATER DYNAMICS)
44

compared as paired observations using a Wilcoxon's signed rank test (table 25). Since there was a significant decrease through the first three runs, the third run was used for all comparisons.

The experimental protocol outlined above seemed to stress the animals and they did not shed normally. Usually the outer generation is quite moist when it is shed (table 16), and it does not stick to the underlying generation. During this experiment the snakes never shed properly, the outer generation seemed to dry out and remained stuck to the inner generation. If the snakes did not shed within several days after the clearing of the spectacle, it was obvious that they could no longer remove their old generation. It was manually removed by holding the snake under luke-warm water (approximately 30°C) for a few seconds, then peeling off some shed, and then the entire procedure was repeated. It is possible to remove the outer generation just after the spectacle clears, since that generation is always moist at this time. However, if this is done, the beta layer of the immature inner generation keratinizes abnormally. Therefore, the outer generation was not removed unless the animal showed evidence of a "retained shed".

Figures 45 and 46 clearly show that when the shed was retained the rate of CWL was always lower than it was earlier during the renewal phase of the shedding cycle. It is probably fortuitous that all of CC 1's retained shed values were so similar (figure 45). There was a general decrease in CWL during the resting phase and an increase during the renewal phase. Since the measurements were usually seven days apart only one measurement was taken during the middle of the renewal phase (usually when the eye was cloudy) in

Table 25. Mean CWL (mg/0.5 hr) for three sequential half hourly runs for two Constrictor constrictor.

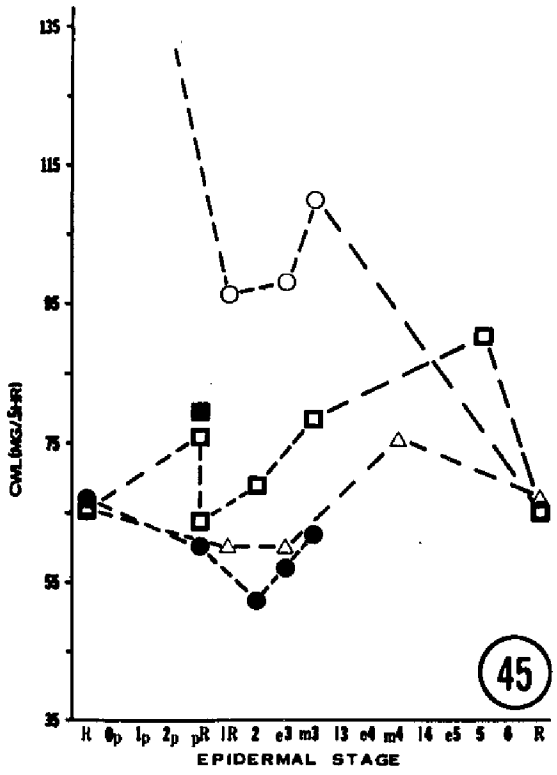
	First run		Second run		Third run
CC 1	88.30	a	77.95	b	75.93
CC 2	92.06	a	88.01	a	79.56

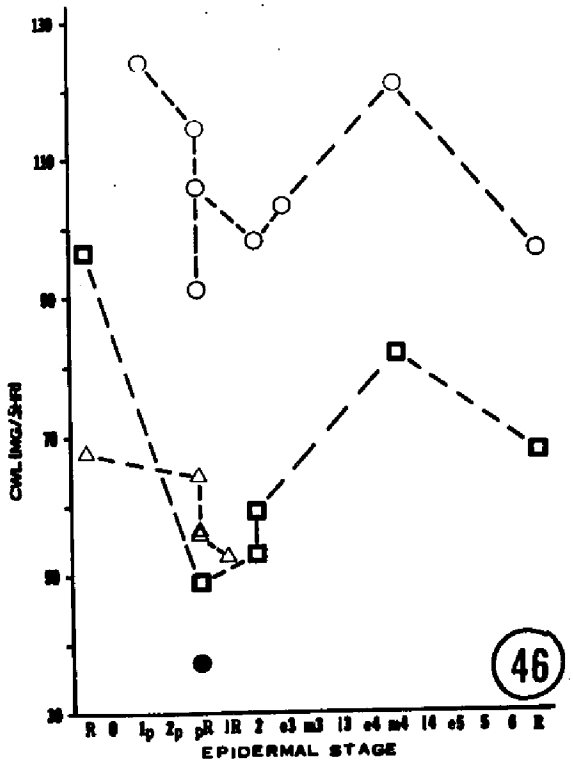
a. $P < 0.005$

b. $0.005 < P < 0.01$.

Figure 45. The pattern of CWL during the shedding cycles of CC 1 (open circles - first cycle, open squares - second cycle, open triangles - third cycle, shaded circles - fourth cycle, shaded square - fifth cycle, R - retained shed; for other symbols see figure 15).

Figure 46. The pattern of CWL during the shedding cycles of CC 2 (open circles - first cycle, open squares - second cycle, open triangles - third cycle, shaded circle - fourth cycle, R - retained shed; for other symbols see figure 15).


135
115
95
75
55
35
CWL (MG/2HR)
45
R θp 1p 2p pR IR 2 e3 m3 I3 e4 m4 I4 e5 5 6 R
EPIDERMAL STAGE


130
110
90
70
50
30
CWL (MG/2HR)
46
R θ 1p 2p pR IR 2 e3 m3 I3 e4 m4 I4 e5 5 6 R
EPIDERMAL STAGE

each cycle. Only during the second cycle of CC 1 were two measurements made during the renewal phase. In this case, CWL was higher during the early renewal phase than during the resting phase, and was higher still as the eye cleared at stage 5.

C. THE SHEDDING CYCLE AND CAPSULE DETERMINATIONS OF CWL

Animals measured with capsules were usually acclimated in the same manner as were members of the same species used in chamber studies.

1. During the Resting and Renewal Phases

a. Gekko gecko

The relationship between the shedding cycle and CWL was investigated for three large tokays (figure 47). Tokay H was measured for two sets of two consecutive cycles each. The two sets were separated by several cycles. Mid-sized capsules (#3) were used for all tokays, except for tokay H's cycles 3 and 4, for which large capsules (L) were employed.

During tokay H's cycles 1 and 3, the rate of CWL increased from the post-shed condition to the end of that cycle's renewal phase. In tokay H's second cycle, CWL was measured twice soon after shedding (0 and 1 day post). Tokay H's rate of CWL decreased between these two measurements. This basic relationship between the shedding cycle and CWL was also found for the other two tokays examined.

Table 26 presents a summary of capsule data on tokays measured once in a previously unstressed cycle, with a mid-sized capsule (#3). An analysis of variance was carried out on these

Figure 47. Capsule determinations of CWL during the shedding cycles of Tokay H (open circles - first cycle, mid-sized capsule; open squares - second cycle, mid-sized capsule; shaded squares - third cycle, large capsule; shaded triangle - fourth cycle, large capsule) and the shedding cycle of Tokay BB (open triangles, mid-sized capsule) and Tokay CC (shaded circles, mid-sized capsule, for other symbols see figure 15).

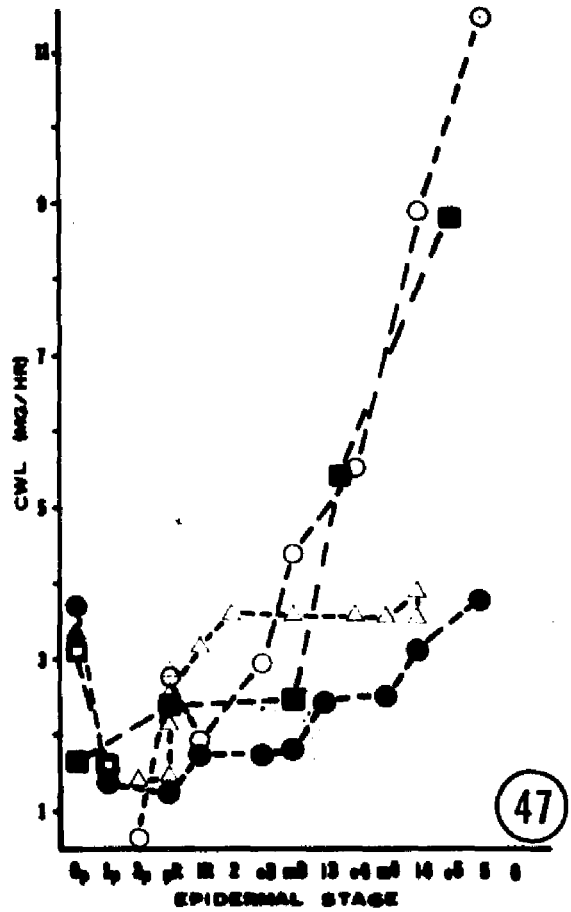

CWL (MG/HR)
EPIDERMAL STAGE
47

Table 26. Capsule determinations of CWL (mg/hr) during the shedding cycle, for Gekko measured only once per cycle.

Epidermal stage	N	Mean CWL	SD
Post-shed	12	1.48	0.82
Perfect rest through stage 2	13	1.46	0.61
Stage 3	9	1.38	0.54
Stage 4	14	1.48	0.72
Stage 5	5	2.10	1.82
Stage 6	5	2.80	2.38

data, and showed that the groups were not significantly different from each other. However, it should be noted that despite the large intragroup variability the mean group CWL conforms to the trend previously described.

b. Lacerta lepida

Capsule measurements were made on three Lacerta using a mid-sized capsule (#3). It was difficult to anesthetize these animals (with a safe dose) for long periods. Many runs were aborted due to slight animal movement, which not only broke the seal, but also spread vacuum grease onto the measuring surface.

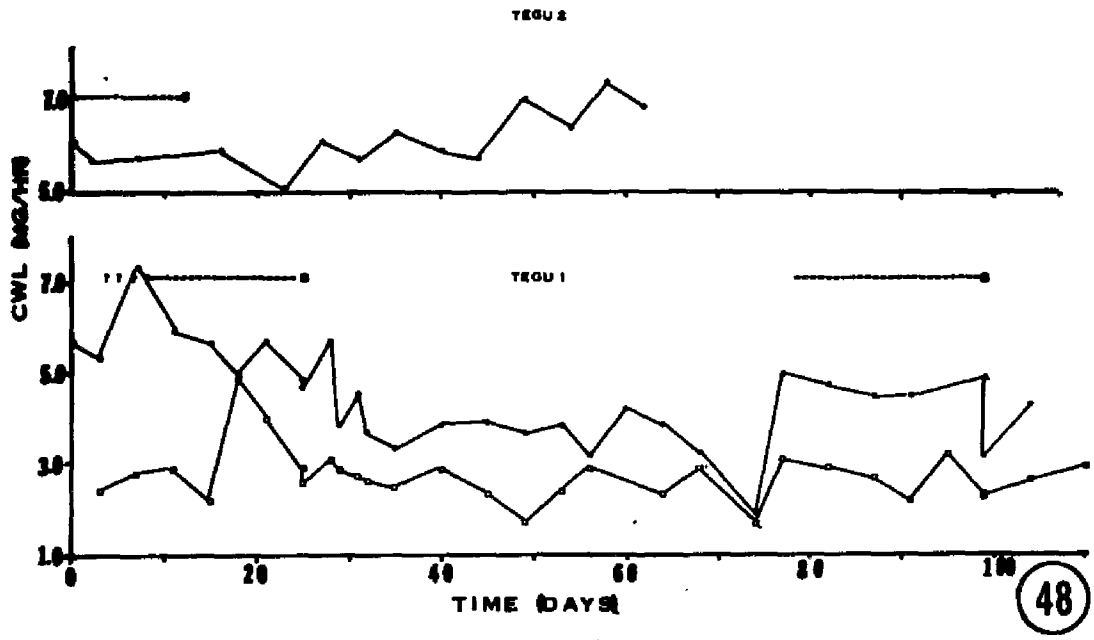
One cannot draw any specific conclusions from these data alone, although in some respects they supplement other data. In one Lacerta CWL was higher during the late renewal phase than during the resting phase (2.46 versus 0.84 and 1.56 mg/hour). In another Lacerta CWL was higher in the post-shed condition (2.37 mg/hour) than during the resting phase (1.43 to 2.4 mg/hour), and lowest during the early renewal phase (0.84 mg/hour).

c. Tupinambis nigropunctatus

Capsule measurements were also carried out on two black tegus, and in one of these animals, two body sites were examined (figure 48). The belly skin is the easiest area to measure since it is flat. Belly measurements could only be made routinely while the animal was upside-down, although it seemed possible that this might affect cutaneous blood flow and in other ways affect CWL. Therefore, CWL was also determined on the mid-back of the largest tegu.

Initially all biopsies were taken from the neck region. When it was realized (day 25, see figure 48) that epidermal activity

Figure 48. CWL as a function of time since the initial measurement for Tupinambis nigropunctatus (dashed line - time in which the epidermis was in the renewal phase, ???? - during this period it is not known if the epidermis was in the renewal phase, 'S' - shedding; for TT 1: open circles - belly measurements, open squares - back measurements).


TEGU 2
TEGU 1
CWL
TIME (DAYS)
0
20
40
60
80
100

was not synchronized throughout the tegu's body, separate biopsies were taken from the anterior back and belly.

The back skin on TT 1 clearly showed an increase in CWL on days 18 and 21, just before the first shed on day 25. However, CWL was lower during shedding than measurements taken several days prior to shedding. CWL decreased following shedding and was very variable during the subsequent resting phase. Back biopsies revealed cyclic melanocyte activity (Szabo et al., 1973). CWL from the back of TT 1 was high on day 77 when this animal was probably in a resting condition. CWL remained relatively high throughout the ensuing renewal phase, up to stage 5 on day 87. CWL then decreased for several days prior to shedding. Histological examination of back biopsies showed that the alpha layer of the inner epidermal generation began keratinizing several days before shedding.

The pattern of CWL during the shedding cycle for the belly skin of TT 1 resembled that described for its back skin, except that CWL did not increase prior to the first shed. TT 2 showed a modest increase in CWL during its renewal phase. After shedding its rate of CWL was very variable, but tended to increase throughout the ensuing renewal phase.

d. Elaphe obsoleta quadrivittata

Capsule measurements were also made on rat snakes. Because these animals had unpredictably long cycles, it was decided to measure their rate of CWL following thyroidectomy (see p. 136). Rat snakes are too slim-bodied to measure CWL with a moderately sized capsule in vivo. Therefore, large biopsies (60 mm square) were removed from these animals and sewn back onto the body after the de-

terminations were made. All measurements were made with large capsules on fresh material. Elaphe #15 was sham-thyroidectomized and was used in the same manner as the other snakes. Thus Elaphe #15 served as a control, since it remained in the perfect resting condition, to see if the skin deteriorated as the animal weakened following such trauma. The rate of CWL for Elaphe #15 did not increase with time (4.14, 5.46, 3.60, and 3.54 mg/hr, figure 49). Three other snakes clearly showed an increase during the renewal phase. CWL decreased in Elaphe #17 at stage 6. Histologically, this decrease was associated with the establishment of a keratinizing inner alpha layer. Elaphe #18 was allowed to recuperate after thyroidectomy until it was in a pre-shed condition: CWL was measured before and after shedding, and showed a sharp decrease following shedding.

e. Constrictor constrictor

Capsule measurements of CWL during the shedding cycle were also made on two boa constrictors in association with the cellophane stripping experiment (see table 34). In both cases CWL ($mg/cm^2/hr$) was higher when the animals were in the post-shed condition than during the resting or the early renewal phases. The last measurement of CWL in CC 2 was while the animal's epidermis was in a mid to late stage 4, and CWL at this time was higher than at any other time.

2. Measurements at the Pre-Shed and Post-Shed Conditions and on the Shed Outer Generation

Table 27 summarizes capsule determinations of CWL made on skin just before and just after shedding, and on the material that was shed (outer epidermal generation). Different animals were mea-

Figure 49. Capsule determinations of CWL during the shedding cycle of Elaphe #11 (open circles), Elaphe #15 (open squares), Elaphe #16 (open triangles), Elaphe #17 (shaded circles) and Elaphe #18 (shaded squares).

CWL(MG/HR)
EPIDERMAL STAGE
49

Table 27. CWL ($mg/cm^2/hr$) in squamates during the shedding period and on shed material-capsule determinations.

Species, #	Pre-shed	Post-shed	Shed Material	Region
Gekko 1	0.20	0.12	-	Belly
Gekko 2	0.20	0.32[a]	0.29	Belly
Gekko 3	-	0.18	0.38	Belly
Gekko 4	0.20	0.20	-	Belly
Gekko 5	0.17	0.18	0.31	Belly
Gekko 6	-	0.13	0.41	Belly
	-	-	0.17	Back
Gekko 6	0.11	0.09	0.13	Belly
Tupinambis 1	0.31	0.35	0.38	Back
	0.58	0.56	0.48	Belly
Tupinambis 1	0.27	0.28	0.19	Back
	0.58	0.38	0.26	Belly
Tupinambis 2	1.06	11.31	-	Belly
Tupinambis 4	-	-	5.90	Tail
Elaphe	0.79	-	0.57[b]	Belly
Constrictor	0.52	-	0.26	Neck

a. This piece of skin was excised and then measured in vitro, 0.27 $mg/cm^2/hr$.

b. Measured at 27°C.

sured with different sized capsules, therefore the data are presented in terms of surface area. Except where otherwise noted, all measurements were made at 30°C and during the pre-shed and post-shed periods were performed on living anesthetized animals. All measurements on shed material were performed *in vitro*. Since CWL is not significantly different when measured *in vivo* or *in vitro* (table 7) it is legitimate to directly compare values obtained by the two different procedures.

The data in table 27 would seem to suggest that the shed material is as impermeable to water as the whole integument. Shed material removed from the tail of TT 4 had a very high rate of CWL. This material had three small cracks in the hinge regions. The presumptive shed of TT 2 was removed while the epidermis of the animal was probably in a stage 5. Although the reported CWL values are only relative (these measurements were only preceded by a seven minute pre-drying period, instead of a twelve minute pre-drying period), the extremely high value probably reflects the immature status of the inner generation.

The measurement on material shed from a boa, was much lower than the *in vivo* measurement taken at late stage 4.

SECTION IV. STUDIES TO DETERMINE THE LOCATION OF THE PERMEABILITY BARRIER

A. COMPARTMENT STUDIES ON INFLATION AND FEEDING

The compartmentalized system was used in two indirect ways to test the relative permeability of the hinge region. CWL was measured

on dead animals "before" and "after" their lungs were inflated and ligated, and on live animals "before" and "after" a heavy feeding. Both techniques caused volume expansion and thus integumentary distension, by the unfolding of the hinge regions. Each animal's volume (weight or water displacement) was determined preceding, and subsequent to, the "after" series of measurements.

The animal's surface area was determined by substituting its weight (or volume) into a regression equation: $SA = 12.5W^{0.67}$ for snakes, and $SA = 10W^{0.67}$ for lizards.

For live or dead animals (table 28) the increase in surface area for lizards (mean 1.14, range 0.97 to 1.23) was generally smaller than that for snakes (mean 1.30, range 1.13 to 1.59). In both squamates, inflation caused a greater increase in surface area than feeding. In two cases (Elaphe #13 and tokay 4) there was evidence that air leaked out of the animal, since water displacement subsequent to the "after" runs was less than the displacement prior to the "after" runs.

If the increase in CWL was greater than the increase in surface area (that is, if the value in column 5 in table 28 is greater than 1.0) this would imply that hinges are more permeable than the outer scale surfaces. For only two of the fourteen rat snakes was the value in column 5 considerably greater than 1.0. Although fed snakes had values very close to 1.0 (mean 0.99, range 0.85 to 1.22) the value for inflated snakes was considerably less than 1.0 (mean 0.89, range 0.67 to 1.34). For the smaller sample of tokays, in general, fed or inflated animals did not show values considerably different than 1.0.

Table 28. The effect of skin distension on CWL as measured by the chamber technique for several rat snakes and tokays.

Species #	1 Before wt (gms)	2 Before CWL (mg/0.5 hr)	3 After CWL (mg/0.5 hr)	4 Increase in SA	5 3 2 X 4
Elaphe 1[f]	221	46.8	75.2	1.32	1.22
Elaphe 2[f]	169	51.1	58.2	1.31	0.87
Elaphe 3[f]	236	73.0	80.9	1.13	0.98
Elaphe 4[f]	297	65.8	80.8	1.21	1.01
Elaphe 5[f]	260	73.7	88.0	1.28	0.94
Elaphe 6[f]	206	59.5	77.1	1.31	0.99
Elaphe 7[f]	211	78.7	87.6	1.19	0.94
Elaphe 8[f]	374	172.0	175.6	1.21	0.85
Elaphe 9[f]	306	91.1	160.2	1.31	1.34
Elaphe 10[i]	350	115.4	102.7	1.34	0.67
Elaphe 11[i]	303	72.2	77.4	1.36	0.79
Elaphe 12[i]	528	140.2	147.3	1.31	0.80
Elaphe 13[i]	429	147.3	221.8	1.48	1.02
Elaphe 14[i]	366	136.0	158.2	1.59	0.73

Table 28. The effect of skin distension on CWL as measured by the chamber technique for several rat snakes and tokays.

(continued)

	1	2	3	4	5
		Before	After		
	Before	CWL	CWL	Increase	3
Species #	wt (gms)	(mg/0.5 hr)	(mg/0.5 hr)	in SA	2 X 4
Gekko 1[f]	110	29.1	34.5	1.08	1.10
Gekko 2[i]	105	15.0	18.1	1.15	1.05
Gekko 3[i]	65	9.6	13.8	1.20	1.20
Gekko 4[i]	125	37.9	36.6	0.97	0.99
Gekko 5[i]	99	32.2	42.5	1.23	1.07
Gekko 6[i]	80	16.0	16.9	1.20	0.88

f. Fed

i. Inflated

Tokays 3 and 6 were remeasured after deflation. In both cases this rate of CWL was about the same as the inflated rate (13.8 and 17.4, respectively).

Figure 50 shows the rate of CWL for the pre-drying run and the following two half hourly runs for several squamates, both before and after manipulation (feeding or inflation). Note, there is considerable variation in the pattern which defines these runs.

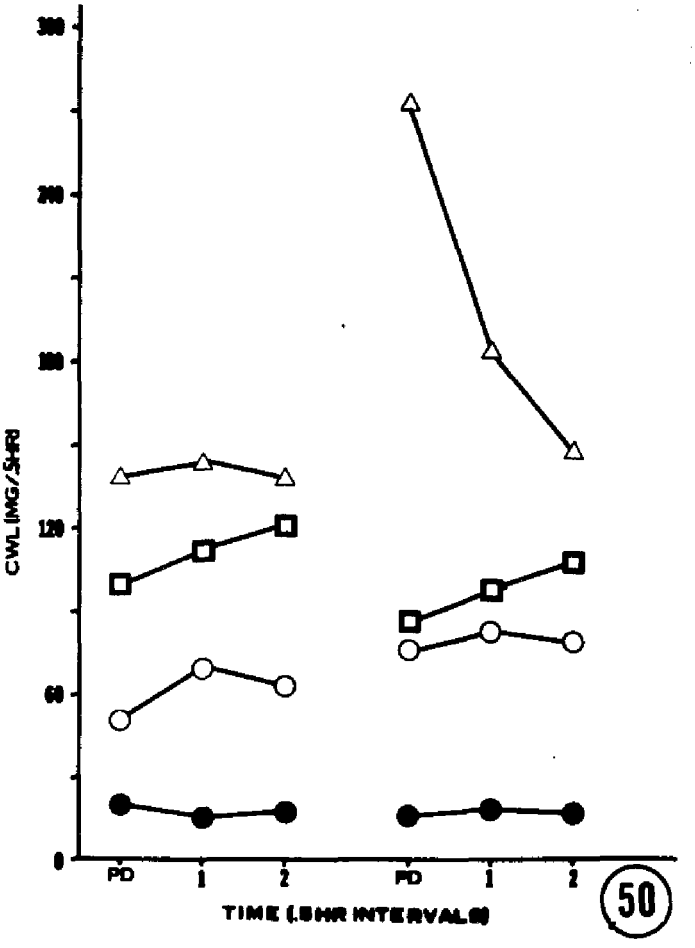
B. CAPSULE STUDIES ON INTEGUMENTARY DISTENSION

For capsule determinations (table 29), CWL was measured before and after the skin was distended. Squamate skin, especially that of snakes, can distend more horizontally (circumferentially) than lengthwise (antero-posteriorly), and it was difficult to mark snake skin accurately. Thus the increases in surface area reported in table 29 are only approximations. A measurement for a semi-aquatic turtle is included. This animal's skin was stretched maximally, however the new surface area was not measured. In all cases, although the surface area increased dramatically, CWL did not.

C. THE EFFECT OF AIR FLOW REVERSAL ON CWL

In chamber studies, the normal direction of air flow over the animal was posterior to anterior (see figure 5). The direction could be changed simply by connecting the body compartment's exhaust brass tube to the incoming air and allowing the brass inlet tube to act as the exhaust. No corresponding change was made to the head compartment's air flow. Generally, under normal circumstances, reversal of air flow over the body caused a decrease in CWL. Generally CWL was

Figure 50. CWL for the second half hourly pre-drying period (PD) and for two subsequent half hourly runs for several squamates (open circles - Elaphe #4, open squares - Elaphe #10, open triangles - Elaphe #12, and shaded circles - Tokay 6) both before and after skin distension.



50

Table 29. Skin distension and capsule determinations of CWL for several reptiles.

Species	1 CWL before (mg/hr)	2 CWL after (mg/hr)	3 Increase in SA	4 $\frac{2}{1 \times 3}$	5 $\frac{2 \times 3}{1}$
Elaphe A	0.50	0.51	3.6	0.28	3.67
Elaphe B	2.55	1.43	1.5	0.37	0.84
Elaphe C	1.51	1.00	2.7	0.25	1.79
Elaphe D	4.25	2.56	1.5	0.40	0.90
Elaphe E	6.60	5.16	1.4	0.57	1.09
Gekko	3.90	4.00	1.43	0.71	1.47
Pseudemys[a]	69.51	50.70	b	c	c

a. Inguinal skin

b. Not measured

c. Not applicable

higher at faster air flow rates (table 8). These two procedures were tried on inflated and fed snakes (table 30).

As expected, in almost all cases the rate of CWL during reverse flow, or at 200 cc/minute, was lower than with normal flow or at 1000 cc/minute. For Elaphe #3 the inflated normal flow (column 5) following the reverse flow was slightly lower than the preceding reverse flow (column 4).

D. THE DIFFERENTIAL PERMEABILITY OF THE HINGE REGION

Another attempt to determine the differential permeability of the hinge region was made by applying grease to various parts of the scale. Of course, this could only be done for exposed hinges, that is, on scales lacking significant overlap.

The restrained animal was first measured in the usual manner. The capsule was then removed, and stopcock grease was applied via a 20 gauge needle attached to a syringe. On larger animals, several applications of grease were necessary to fill the hinges. Covering the entire outer scale surface caused some of the grease to run into the hinge regions. Therefore, an attempt was made to put only as much grease on the outer scale surfaces as was applied to the hinges. Since the data for the black tegu are not very different than those for crocodilians, they were included.

Occluding the hinges with grease still allowed a mean transmission of 74.5% ($\pm$ 19.4, SD) of the control CWL (table 31).

E. EFFECTS OF CELLOPHANE STRIPPING

1. Inherent Problems in the Application of the Cellophane

Table 30. The effect of direction of air flow and flow rate on chamber determinations of CWL (mg/0.5 hr) in normal, inflated, and fed snakes.

	BEFORE			AFTER	
	1	2	3	4	5
Animal #	Normal	Reverse	Normal	Reverse	Normal
Elaphe 3[b]	PD 1/2 hr	PD 0 hr	PD 1/2 hr	PD 1/2 hr	PD 0 hr
		64.1			82.1
	74.0	69.2	148.7	72.7	74.8
	72.8	71.2	106.4	99.2	82.3
	73.1	70.1	80.9	73.9	79.0
Elaphe 7[b]	PD 1/2 hr	PD 0 hr	PD 1/2 hr	PD 0 hr	
		72.8		83.4	
	83.6	76.6	88.1	83.2	
	78.1	77.2	87.9	82.3	
	79.2	75.9	87.2	82.5	
Elaphe 9[a]	PD 1 hr	PD 0 hr		PD 1 hr	PD 0 hr
		171.68			285.00
		172.68			314.14
	183.74	169.70		317.88	326.64
	182.22			329.44	

Table 30. The effect of direction of air flow and flow rate on chamber determinations of CWL (mg/0.5 hr) in normal, inflated, and fed snakes.

(continued)

	BEFORE			AFTER	
	1	2	3	4	5
Animal #	Normal	Reverse	Normal	Reverse	Normal
	200 cc/min	1000 cc/min	200 cc/min	1000 cc/min	
Elaphe 4	PD 1 hr	PD 1/2 hr	PD 1 hr	PD 1/2 hr	
	50.3	65.7	75.9	86.8	
	68.8	67.8	82.2	87.0	
	62.8	70.0	79.4	101.0	

a. 200 cc/min

b. 400 cc/min

Table 31. The permeability of different parts of the scale to water for several reptiles.

Species #	Capsule size	Temp. °C	1	2	3
Caiman A	S	27	0.93	95.25	84.80
Caiman A	L	30	1.26	64.48[b]	
Caiman B	3	27	0.22	30.30[a]	
Caiman B	L	30	1.06	84.56[b]	73.83[c]
Crocodylus A	L	26	0.64	73.03	
Crocodylus B	L	25	0.84	65.25	
Crocodylus B	4	27	0.87	87.90	
Tupinambis 2	L	30	0.84	82.05	
Tupinambis 4	L	30	0.55	85.53	

1. Control CWL ($mg/cm^2/hr$)
2. Greased hinge CWL divided by control CWL, multiplied by 100
3. Greased OSS CWL divided by control CWL, multiplied by 100

a. Transverse hinge regions only

b. Horizontal and midventral hinge regions only

c. Only as much grease as on hinges

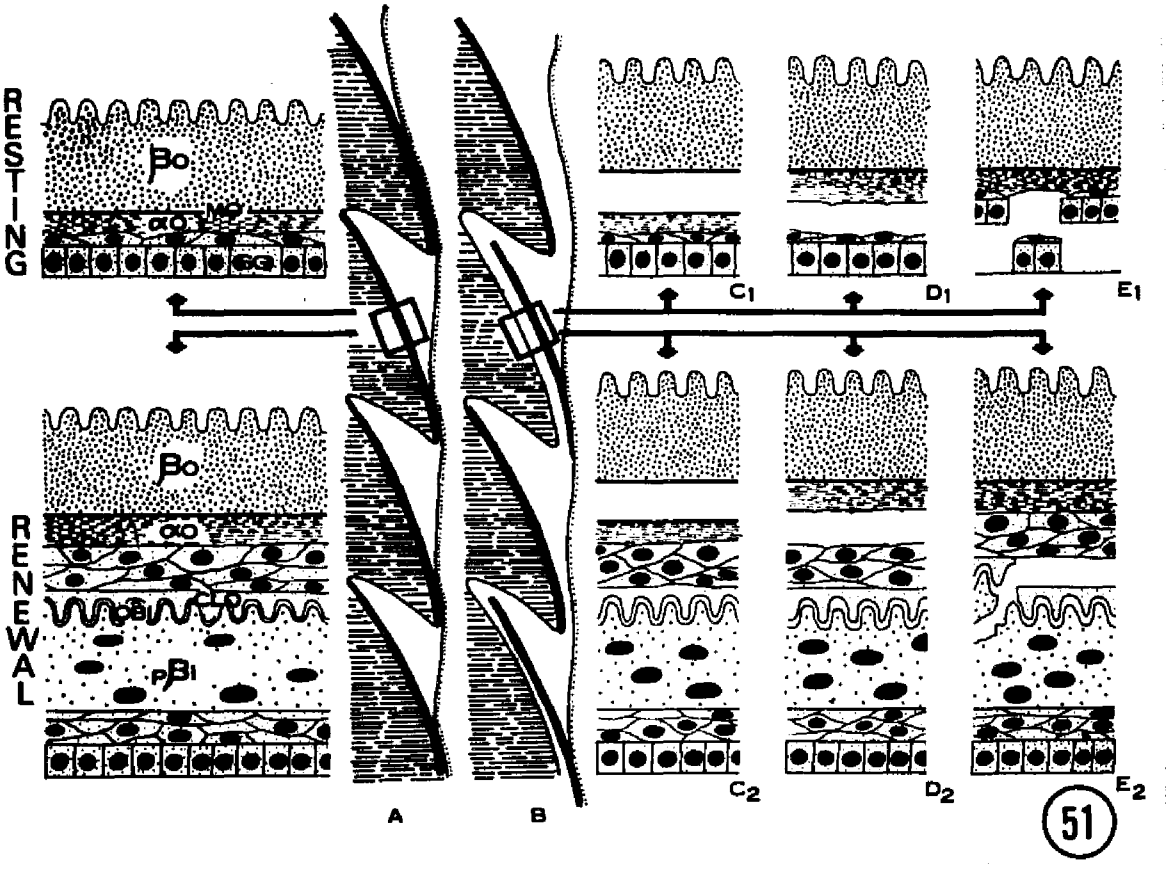
Stripping Technique to the Squamate Integument

The squamate integument is heterogeneous at two distinct levels - the gross and the microscopic - and this presents specific practical problems in executing and interpreting experiments using cellophane stripping. Certain aspects of the system are shown diagrammatically in figure 51, to illustrate these problems.

Since the squamate integument consists of overlapping scales, application of adhesive cellophane tape can only effect contact between the tape and a limited percentage of the total outer scale surfaces (figure 51A). Even over those regions where contact is effected, it may not adhere with equal strength. Therefore, when the tape is removed from the body surface (figure 51B), the epidermis of one scale might be completely unharmed, while that over adjacent units might be damaged. However, the degree of damage also varies within and between scales. In the results presented below, it must be emphasized that the description does not explain what happened over the entire area of skin stripped, but only the events over all or part of the outer scale surface of individual scales. It is not possible to quantify the variability of the effect, but an attempt is made to give some empirical estimate of the frequency with which any particular sequence of events was observed.

The stratified organization of the squamate epidermis suggests that there are a number of locations where a split could occur, and as will be detailed below, it seems that splits were obtained in every possible location, albeit some with much greater frequency than others. Figure 51 illustrates certain problems of interpretation which were encountered. Three examples of possible splitting

Figure 51. Diagrammatic representation of the possible effects of stripping the squamate epidermis during the resting and renewal phases. For an explanation see the text.


RESTING
RENEWAL
βo
αo
pβ1
C1
D1
E1
C2
D2
E2
A
B
51

locations are represented: first (figure 51, C_1 and C_2), removal of the beta layer; second (figure 51, D_1 and D_2), a split within the alpha layer so that everything above it was lost; and third (figure 51, E_1 and E_2), where the living epidermal cells or even the dermis, were exposed.

Identification of the location of the original splitting site in sequential biopsies was based on the identification of the most superficial corneous materials which were assumed to have remained after stripping. Sometimes none were observed (figure 51, E_1 and E_2), and very characteristic changes in the tissue accompany such conditions (p. 179). Stripping always had an "all or nothing" effect in removing the mature, syncytial beta layer from the posterior (exposed) outer scale surface. However, one cannot tell whether the split occurred between the beta and mesos layers, within the latter, or between the mesos and the alpha layers. Where only a small amount of the original alpha layer was detected, there was no way of knowing with certainty whether, on that scale, the split occurred between the mesos layer and a thin alpha layer, or within a slightly thicker alpha layer. The significance of these problems will be discussed.

Figure 51 shows the "resting phase splits" ($C_1 - E_1$) and "renewal phase splits" ($C_2 - E_2$). The variability in the splitting of the mature corneous layers was the same throughout the resting and early to mid renewal phases. During the late renewal phase, the entire outer epidermal generation was usually removed by stripping. When the strip exposed living cells (figure 51, E_1 and E_2), more cells remained above the stratum germinativum during the renewal

phase strips, and the presumptive fates of such cells are different than those of similarly located cells during the resting phase.

2. Macroscopic Observations on the Effects of Cellophane Stripping the Squamate Integument

The number of scales within the region to which tape was applied, which were affected by the process, was assessed by examining the sticky surface of the tape after removal. Removed beta layers were seen as shiny templates which adhered to the tape. Scales on an animal from which the beta layer (and perhaps other components, see below) were removed, had a shiny appearance, which was lost within twenty-four hours. "Good" stripping of snake integument, that is, experiments where repeated examinations of the tape suggest many scales were affected, seemed to produce a general anatomical picture of reduced scale overlap after several days. This impression was confirmed, and partially explained, by examination of individual scales under low power magnification. When corneous materials were removed with watchmaker's forceps, the scale surface buckled to a greater or lesser degree, and its shape changed. Similar buckling was observed in all squamates studied (figures 52, 60).

Observations on stripped regions for seven through fourteen days post-stripping suggested a dried up appearance in snakes, and some indications of flaking of superficial materials were seen in all squamates. Sometimes entire scales seemed to disappear, and this was associated with the induction of a "wound rejection reaction" (Maderson and Roth, 1972) by the stripping process (see p. 179).

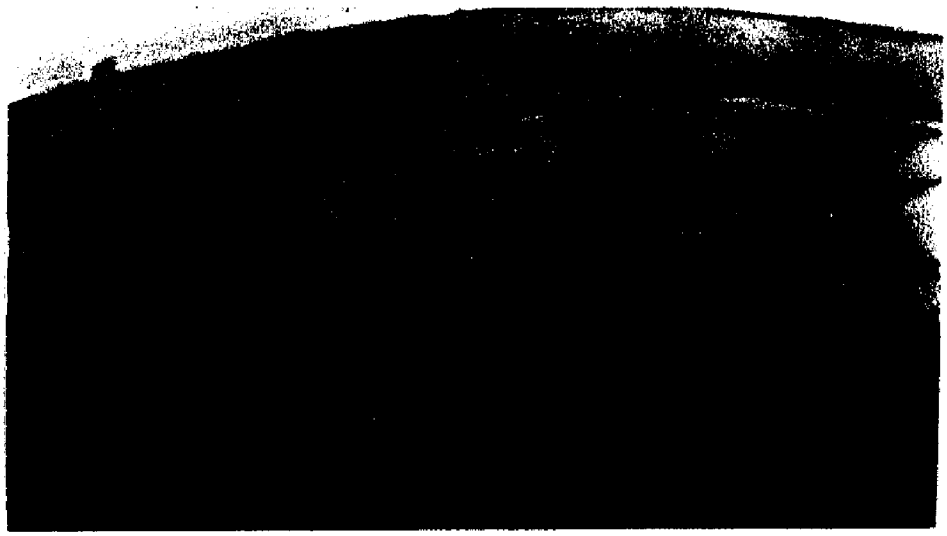
3. The Histological Effects Produced by Stripping the Squamate Integument During the Resting Phase

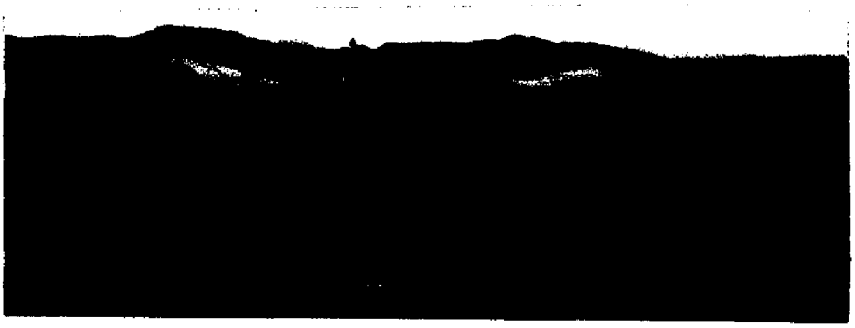
Figure 52. Photomicrograph through the outer scale surface of Lacerta lepida skin just after removal of the beta layer with watchmaker's forceps. The epidermis is buckled; that is, it is thrown into irregular folds (E - epidermis, D - dermis). (X 100).

Figure 53. Photomicrograph through the outer scale surface of Dipsosaurus dorsalis skin showing the untraumatized perfect resting condition (M - mesos layer; for other symbols see figure 1). (X 400).

Figure 54. Photomicrograph through the outer scale surface of Dipsosaurus dorsalis skin after application of cellophane tape during the perfect resting condition. Although the tape did not remove the beta layer, the epidermis is still atypical (see p. 176, for symbols see figures 1 and 53). (X 400).

Figure 55. Photomicrograph through the outer scale surface of Gekko gecko skin after cellophane stripping. The india ink (II) on top of the epidermis indicates to what level the epidermis was stripped (for other symbols see figure 1). (X 400).





Study of serial sections revealed that quite frequently individual scales within areas to which tape was applied showed no differences from unstripped control areas (figure 5). However occasionally, but particularly in experiments with D. dorsalis, while the corneous materials were still present in regions which were stripped, the living cells beneath showed differences from control regions. In contrast to the flattened germinal cells, and a single layer of suprabasal cells (the perfect resting condition: Maderson, Chui, and Phillips, 1970a) seen in control regions (figure 53), these regions showed a low columnar germinal layer, with two to three layers of polygonal suprabasal cells (figure 54), one to two days after tape was applied. Such results indicate that stripping affected the epidermis even when corneous materials were not removed.

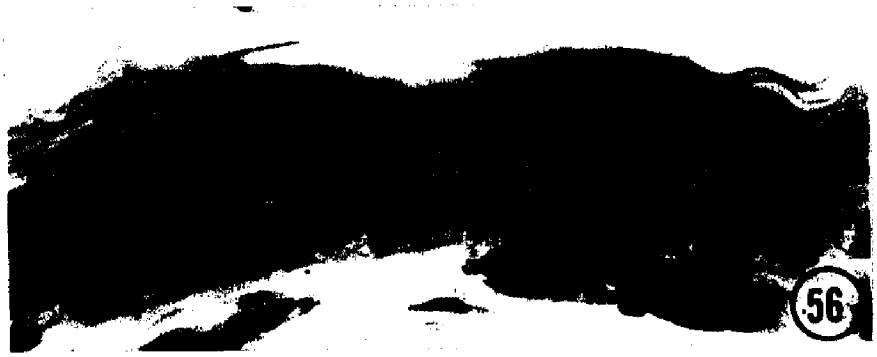
In the majority of cases, stripping removed the entire beta layer, the mesos layer, and perhaps much of the alpha layer (figure 55). Within one day, mitotic figures were frequently seen in the germinal layer, and often it appeared that the alpha tissue was thicker than in the post strip controls. This initial rapid restoration was probably brought about by the rapid maturation of those suprabasal cells present at the time of stripping. Over subsequent days, germinal proliferation continued, and although three to five layers of polygonal suprabasal cells were always present, the alpha tissue became steadily thicker with elapsed time (figure 56), indicating continuing contributions from suprabasal cells. The net result of this pattern of activity was the production of a lamellate, hyperplastic tissue, with the same histological characteristics as the normal alpha layer, essentially resembling the wound epithelium

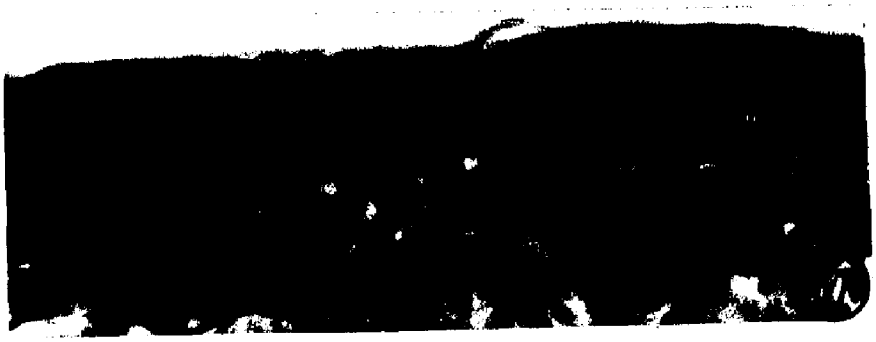
Figure 56. Photomicrograph through the outer scale surface of Gekko gecko skin (late stage 3) eight days after stripping in the post-shed condition. The alpha layer is hyperplastic and lamellate (for symbols see figure 3). (X 400).

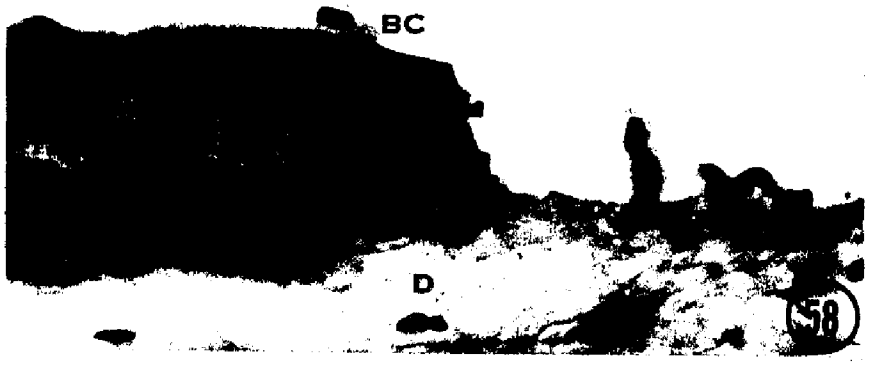
Figure 57. Photomicrograph through the outer scale surface of Gekko gecko skin three days after cellophane stripping. There is extensive acantholysis of the germinal nuclei, some spaces are marked (X; for other symbols see figure 1). (X 400).

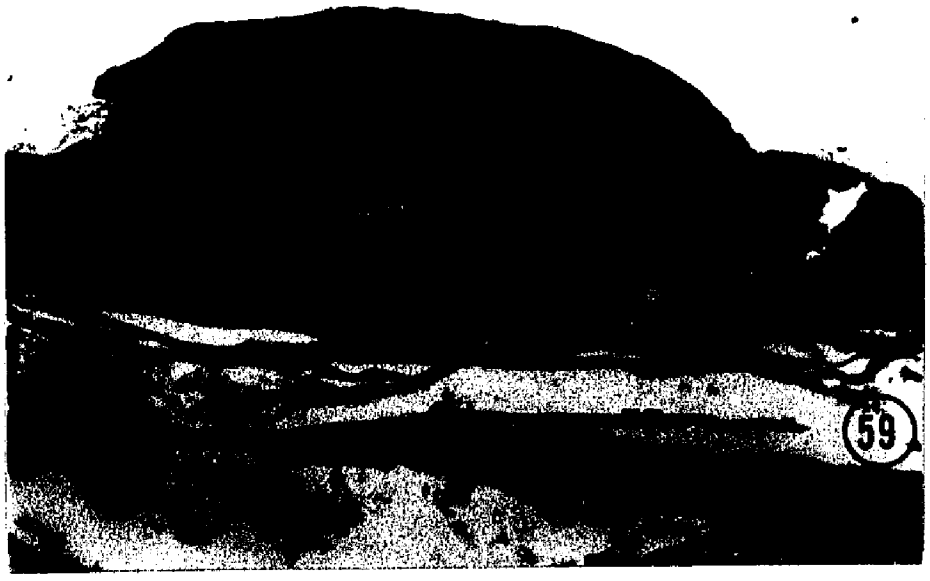
Figure 58. Photomicrograph through the outer scale surface of Gekko gecko skin just after stripping with cellophane tape (BC - blood cells; for other symbols see figure 1). (X 600).

Figure 59. Photomicrograph through the outer scale surface of Dipsosaurus dorsalis skin three days after cellophane stripping. The rejected material (RM) shows signs of the dermis (D). Epithelial migration has produced a new continuous epithelium (EP). (X 40).


56




BC
D
58


59

described by Maderson and Roth (1972).

In those experiments where daily biopsies following stripping overlapped the onset of the renewal phase, the affected area showed a typical clear layer/Oberhautchen complex (Maderson, 1966), beneath a hyperplastic alpha tissue, and differentiation of the inner epidermal generation continued normally. At the subsequent shed, the alpha tissue was removed from the body surface along with the outer generation of the non-stripped scales.

Occasionally, within a few daya after stripping there was patchy acantholysis of the germinal cells and/or the suprabasal cells (figure 57). When this was seen, subsequent biopsies often showed parakeratotic material on the innermost aspect of the remaining portion of the alpha layer. The wound epithelium produced in such areas over the next few days was somewhat looser in appearance, and showed a greater tendency to separate into lamellae, than did that described above.

Quite often, immediate post-strip biopsies showed complete absence of corneous materials, and disrupted living cells, even showing regions where the germinal layer was absent (figure 58). The state of the living cells was reminiscent of those in epidermal tissues eight to twelve hours after single incision wounds (Maderson and Roth, 1972). One and two day post-strip biopsies often showed complete absence of superficial corneous materials - thus implying an origin from the post-strip material just described - and there were dead and dying non-cornified cells at the surface. The dermis beneath showed signs of disruption exactly comparable to those described by Maderson and Roth (1972). This is to say, the extreme

damage inflicted upon the epidermis precipitated a "wound rejection reaction" of dermal materials. Sometimes two days, and always three days after stripping, biopsies showed pieces of rejected dermis at the surface, identified by melanophore remains (figure 59). Beneath this, epithelial immigration into the deeper dermis had taken place, and a wound epithelium showing alpha type corneous material was present.

Very occasionally in stripped lizard material, the hyperplastic alpha tissue, and the subjacent living cells contained various blood cells: such a condition was most common when the stripping damaged living cells. However, eosinophil immigration into the epidermal tissues always occurred in snakes (figure 60). This immigration began two to three days after stripping and continued for five to six days. The histological picture thus produced resembled that seen during the early renewal phase of the normal cycle (Maderson, 1965b), but differed in that the immigration here was over the distal two-thirds of the outer scale surface.

4. The Histological Effects Produced by Stripping the Squamate Integument During the Early Renewal Phase

In some cases where only the beta layer was removed, there were no detectable changes in the subjacent living cells. Under such circumstances, later biopsies showed no effects on the differentiation of either the innermost cellular components of the outer epidermal generation (lacunar and clear layers), nor on the Oberhautchen and beta cells beneath.

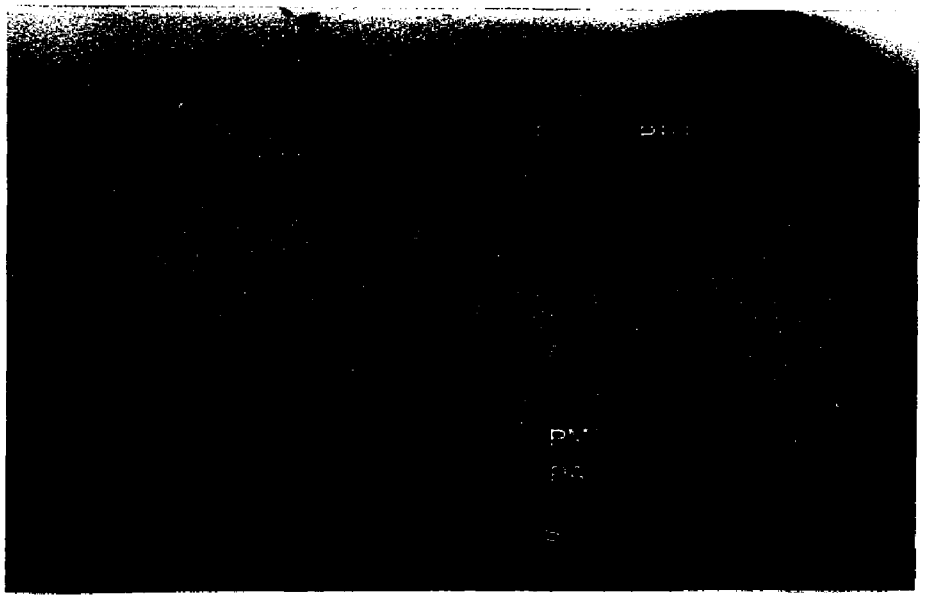
Where the strip removed corneous materials including all or part of the alpha layer, the net result was the production of a

Figure 60. Photomicrograph through the outer scale surface of Constrictor constrictor skin, two days after stripping the epidermis during the post-shed condition. The alpha layer is thick and there are many nests of migrant eosinophils (ES, for other symbols see figure 1).

Figure 61. Photomicrograph through the outer scale surface of Gekko gecko skin, six days after stripping the epidermis during early renewal. The metaplastic tissue (M) must have been derived from presumptive feather keratin synthesizing cells (for other symbols see figure 3).

Figure 62. Photomicrograph through the outer scale surface of Constrictor constrictor skin six days after stripping the skin during the mid renewal phase (mid stage 4). Probably the entire outer generation was removed from this region, also note the presumptive beta layer is warped (for symbols see figure 4).

Figure 63. Photomicrograph through the outer scale surface of Caiman sclerops skin. The cornified layer (C) is uniform and lamellate (for other symbols see figure 1).



hyperplastic alpha tissue, essentially comparable to that seen in a resting phase strip. However, such material was produced in quite a different manner. Following the removal of superficial corneous materials, depending on the exact stage of the renewal phase, the cells which were exposed from without inwards may be identified as follows: presumptive lacunar tissue (one to two layers in lizards, four to six layers in snakes), a single epithelium - the presumptive clear layer, another single epithelium - the presumptive Oberhautchen, and one or several layers of presumptive beta cells (Maderson, 1965b, 1966). Intense germinal activity would normally be seen during this part of the cycle. Examination of biopsies two to five days post-strip (figure 61) revealed hyperplastic alpha tissue which must have been derived by the maturation of the persumptive cell populations described above. Five to seven days after a stage 2 condition was recognizable, one would expect to see a stage four condition with readily recognizable Oberhautchen and differentiating beta cells. Such a condition was seen in control material, or over the scales which were not stripped. However, if the epidermis was stripped successfully during stage 2, five to seven days later the characteristic elements could not be recognized in the deep regions of the tissue. A metaplasia of the persumptive Oberhautchen and beta cells must therefore have occurred. If the strip was performed during stage 2 or early to mid stage 3, there was an eventual delayed differentiation of an inner epidermal generation. Stripping during late stage 3 through mid stage 4 did not give the affected epidermis enough time to "catch up", so that only hyperplastic alpha material was present in such regions by the time shedding occurred, and this

material often remained on the animal's body.

5. The Histological Effects Produced by Stripping the Squamate Integument During the Late Renewal Phase

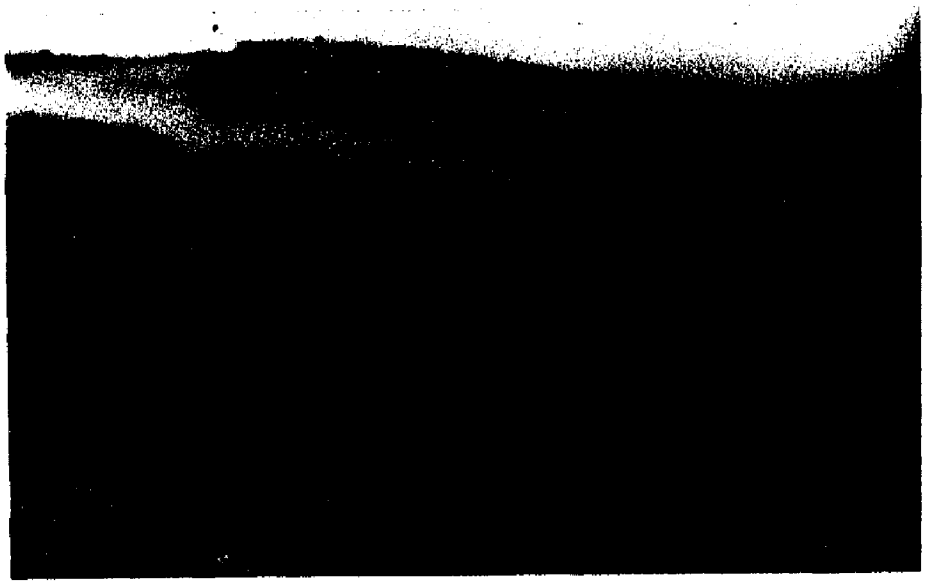
If the stripping was effected after mid stage 4, when the new Oberhautchen and beta cells were fairly well advanced in synthesizing their complement of feather protein precursors (Maderson et al., 1972) the following was seen: the exposed lacunar tissue and clear layer formed a minimal superficial alpha-like tissue, and the subjacent beta layer (with the Oberhautchen) continued its pattern of differentiation although some gross "warping" was seen (figure 62).

Stripping the epidermis during stage 5 or later, invariably removed the outer epidermal generation in toto, perhaps precociously disrupting the intercellular bonds between the clear layer and the Oberhautchen. Some "warping" of the new beta layer was seen, but no specific effect on germinal proliferation and differentiation were detectable in subsequent biopsies.

6. The Histological Effects Produced by Stripping the Caiman Integument

The corneous material of intact caiman epidermis was thick and lamellate, and laid above approximately six layers of rather flattened suprabasal cells (figure 63). Stripping reduced the thickness of the corneous region. By two days, the germinal cells were columnar, and there were more layers of polygonal suprabasal cells (figure 64). The living cells maintained this appearance for up to fourteen days after stripping, and there was a steady replacement of corneous materials whose histological appearance resembled the normal

Figure 64. Photomicrograph through the outer scale surface of Caiman sclerops skin, seven days after cellophane stripping the integument. The germinal cells are active, there are many layers of immature cells, and there is some sign of newly keratinized corneous material (NC), for other symbols see figures 1 and 63.



tissue.

7. Patterns of CWL in Reptiles Following Epidermal Stripping

Tables 32-34 and figures 65 and 66 document the effects of cellophane stripping of the corneous layers of the epidermis on CWL in a variety of reptiles, as measured by compartment and capsule techniques.

As has been described above, since the effects of stripping were unpredictable in terms of how many scales within a stripped area were affected, and to what degree, the absolute values are less important than the general trend which was observable throughout. There was a sharp increase in CWL immediately after stripping, and this effect was gradually ameliorated following the trauma.

The pattern of recovery in tokay 6 (table 33 and figure 65) and in body regions D of the two boas (table 34 and figure 66) was somewhat less dramatic than in other tokays, and in other regions of the snake's body, respectively. Although the two snakes were stripped lightly in those regions (that is, only ten applications were made, so that fewer scales were affected) the common denominator with tokay 6 (table 33 and figure 65) is that the stripping was affected during the renewal phase. For both snakes (table 34 and figure 66), the immediate post-strip CWL for skin regions A through C showed comparable values, and similar recovery patterns. However, the figures for region E for each animal are somewhat different, the value for boa 2 being nearly twice that for boa 1. Table 34 shows that for boa 1 the epidermal histology showed a mid stage 4, and for boa 2 a mid to late stage 4. However the sections also revealed that in the case of boa 2, the outer epidermal generation was re-

Table 32. The effects of stripping by cellophane tape on CWL (mg/0.5 hr) in two lizard species as measured by the chamber technique (pR - perfect resting condition, pS - post-shed condition, na - not applicable, length of cycle unknown, D - dead).

					Days post-strip										
Animal	A	B	C	D	2	4	5	6	7	8	9	11	13	14	16
Gekko A	pR	3	20.4	56.2	56.2		30.9				31.3				
Gekko B	pR	5	13.4	50.0	27.4		20.1		24.8		22.4	31.2		30.3	34.8
Gekko C	pS	1	20.7	105.5	36.1	21.7		14.8	D						
Gekko D	pR	10	19.3	47.2	30.5		23.7		20.0	D					
Dipsosaurus	pR	na	7.1	44.0	21.4				8.8			6.6	6.9		

A. Epidermal histology

B. Days post-shed

C. Pre-strip CWL

D. Post-strip CWL

Table 33. The effects of stripping by cellophane tape on CWL ($mg/cm^2/hr$) in lizards and a caiman as measured by the capsule technique (pS - post-shed condition, pR - perfect resting condition, e3 - early stage 3, S - shed, D - dead, na - not applicable - since caimans do not have a shedding cycle).

					Days post-strip												
Animal	A	B	C	D	1	2	3	4	5	6	7	8	9	10	11	13	15
Tokay 1[a]	pS	2	1.42	11.50	6.90		3.76	1.75	1.52	1.13	1.47			1.19	1.22	1.23	1.18
Tokay 2[a]	pS	1	0.23	7.63	6.02		2.10		1.02		0.84		0.78				
Tokay 3[a]	pS	2	0.17	6.79		3.14		1.46		0.56		0.84		0.41			
Tokay 4[a]	pR	5	0.23	7.85	2.90		1.64		0.94		0.66		0.70				
Tokay 5[a]	pR	5	0.15	5.31		2.08		0.66									
Tokay 6[a]	e3	13	0.28	6.33	6.27	5.73	4.84	3.50	3.03	3.11	3.28 S	1.13	1.01				
Tegu A[b]	pS	1	0.96	3.82	4.29	2.64	D										
Tegu B[b]	pR	6	0.43	2.93	3.33	3.04	2.98	2.54	1.66	1.19	1.07	0.99	0.83	0.64	0.59		
Caiman[b]	na	na	0.94	7.92	6.67	7.17	5.94	5.15	4.88	3.17	2.80	1.64	1.89	1.96	2.16		

Table 33. The effects of stripping by cellophane tape on CWL ($mg/cm^2/hr$) in lizards and a caiman as measured by the capsule technique (pS - post-shed condition, pR - perfect resting condition, e3 - early stage 3, S - shed, D - dead, na - not applicable - since caimans do not have a shedding cycle).

(continued)

A. Epidermal histology

B. Days post-shed

C. Pre-strip CWL

D. Post-strip CWL

a. Capsule size - 4.52 cm^2

b. Capsule size - 8.40 cm^2

Table 34. The effects of stripping by cellophane tape on weekly measurements of CWL ($mg/cm^2/hr$) in two boas as measured by the capsule technique (pS - post-shed condition, pR - perfect resting condition, 2 - stage 2, m4 - mid stage 4, m-14 - mid to late stage 4).

	Boa 1[a]					Boa 2[b]				
	Days post-shed					Days post-shed				
	0	7	14	21	28	0	7	14	21	28
Epidermal stage	pS	pR	pR	2	m4	pS	pR	pR	2	m-14
Skin region										
Control	0.43	0.26	0.37	0.21	0.21	0.48	0.38	0.09	0.32	0.52
Region A	5.36[c]	1.19	0.33	0.61	0.46	5.73[c]	1.11	0.71	0.77	0.33
Region B		5.37[c]	1.22	0.50			6.21[c]	0.65	0.36	0.44
Region C			5.99[c]	1.54	0.89			5.58[c]	0.48	0.49
Region D				2.73[d]	1.62				3.08[d]	1.88
Region E					6.82[c]					11.23[c]

a. Capsule size 8.40 cm^2

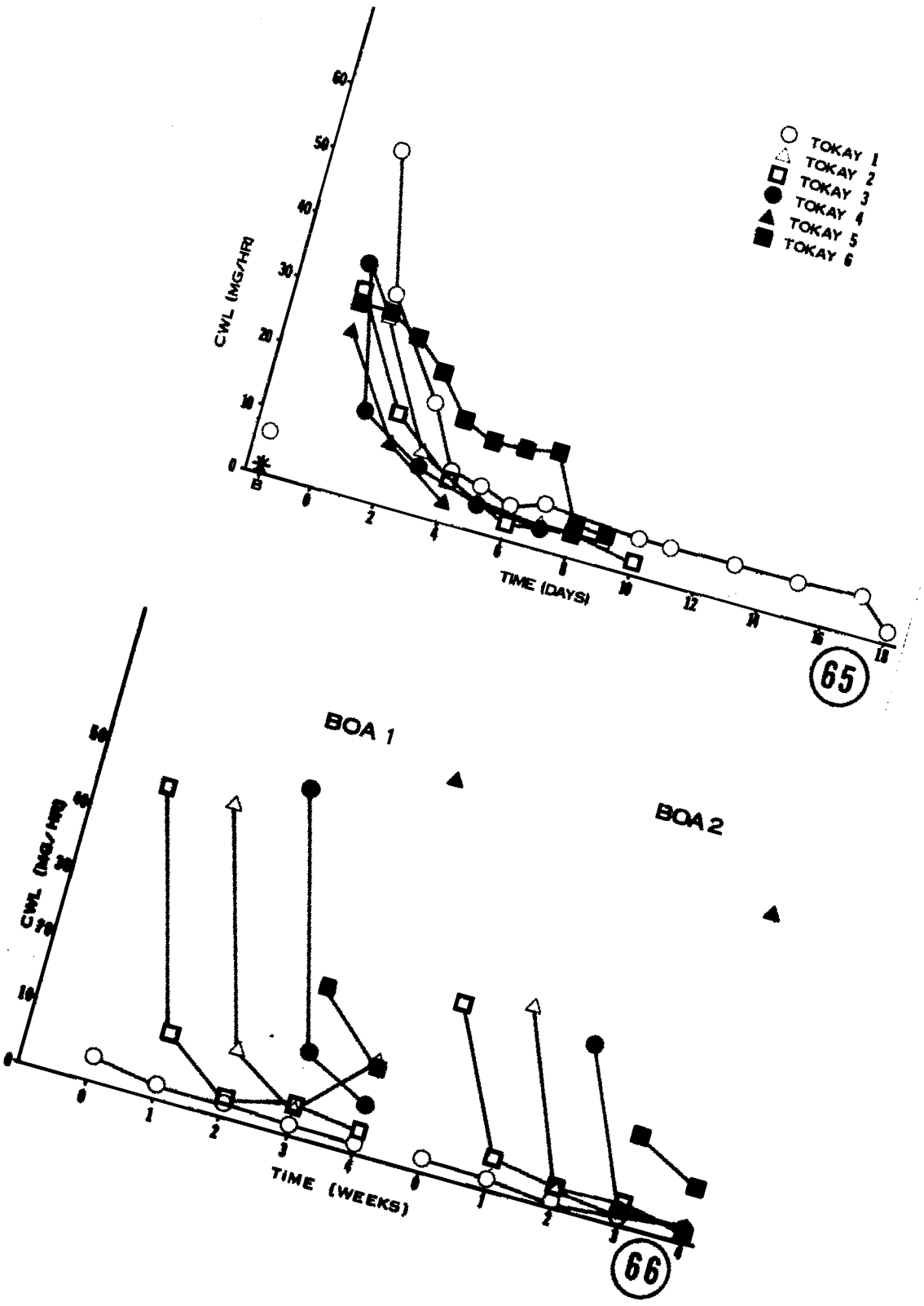
b. Capsule size 4.52 cm^2

c. Measurement immediately after stripping

d. Measurement immediately after a light stripping

Figure 65. CWL as a function of time since stripping for several tokays (B - before stripping, * - CWL value for tokays 2 to 6).

Figure 66. The effect of stripping and the pattern of regeneration of the barrier for two Constrictor constrictor (open circles - control region, open squares - region A, open triangles - region B, closed circles - region C, closed squares - region D, closed triangles - region E).


TOKAY 1
TOKAY 2
TOKAY 3
TOKAY 4
TOKAY 5
TOKAY 6
CWL (MG/HR)
TIME (DAYS)
65
BOA 1
BOA 2
CWL (MG/HR)
TIME (WEEKS)
66

moved from nearly all scales, but in boa 1, it was absent from only some scales.

SECTION VI. COMPARATIVE RATES OF CWL

A. CWL IN TETRAPODS

Table 35 presents results of CWL determinations using capsules for a variety of tetrapods not extensively studied as part of this thesis. These data can be compared to the relative rates of whole animal CWL reported in the literature (see table 1).

B. REGIONAL RATES OF CWL

On larger animals it was possible to measure CWL in more than one region. All data in table 36 are presented as paired observations, since the experimental protocol often differed between pairs.

Table 35. Capsule determinations of CWL ($mg/cm^2/hr$) for a variety of tetrapods.

Animal	Condition	N	Ambient temperature (°C)	Capsule size	Site	CWL	SD
Homo	in vivo	1	27	L	arm	1.08	
Rattus	in vitro	1	30	L	flank	0.65	
Iguana	in vivo	4	22-27	3	belly	0.20	0.10
Iguana	in vitro	1	25	3	belly	0.14	
Dipsosaurus	in vivo	3	30	3	belly	0.36	0.02
Caiman	in vivo	5	22-27	3 and 4	belly	0.57	0.27
Caiman	in vivo	4	30	L	belly	1.47	0.48
Caiman	in vitro	1	30	4	belly	3.63	
Crocodylus	in vivo	6	25-27	4 and L	belly	0.77	0.14
Geochelone	in vivo	1	30	L	plastron	0.57	
Pseudemys	in vitro	3	30	L	neck and inguinal	4.88	1.52
Rana	in vivo	1	28	4	belly	10.47	

Table 36. Regional CWL ($mg/cm^2/hr$) for various reptiles.

		Area 1		Area 2	
Species	Condition	Location	CWL	Location	CWL
Tupinambis	in vivo	ventral thigh	0.42	lower belly	0.67
Tupinambis	in vivo	dorsal thigh	0.57	dorsal calf	0.55
Tupinambis	in vivo	back	lower	belly	higher*
Elaphe	in vitro	back	0.71	belly	0.54
Elaphe	in vitro	back	0.42	belly	0.36
Crocodylus	in vivo	lower belly	1.49	lower jaw	0.91
Crocodylus	in vivo	upper belly	0.71	lower jaw	0.69
Pseudemys	in vitro	leg	6.04	neck	4.3

*see figure 48

DISCUSSION

SECTION I. GENERAL ASPECTS OF CUTANEOUS WATER LOSS

A. THEORETICAL ANALYSIS

In tetrapods the rate of CWL must either be evaporation or diffusion limited, so that whenever the rate of CWL is lower than the rate of evaporation from a similarly shaped free water surface, it must be diffusion limited. The rate of CWL for the reptiles used in this study was considerably lower than the rate of free water evaporation (also see Spotila and Berman, 1976).

According to Fick's analysis (see Scheuplein, 1972) the rate of diffusion (J, moles/cm^2hr) of a substance through a membrane of thickness δ (cm), is related to the concentration difference of the substance (ΔC, moles/cm^3) across the membrane by the equation:

$$J = \frac{D\Delta C}{\delta} , \qquad (1)$$

where D (cm^2/sec) is the diffusion coefficient (it characterized the particular substance and the membrane). If the membrane has an affinity for the diffusing substance, Fick's relationship becomes:

$$J = \frac{K_m D\Delta C}{\delta} , \qquad (2)$$

where K_m (a dimensionless ratio) is the solute membrane distribution coefficient (Scheuplein, 1972). Scheuplein combines constants to arrive at a permeability coefficient, K_p (cm/hr), so that:

$$J = K_p \Delta C. \qquad (3)$$

If the rate of diffusion is measured at a variety of concentration

gradients the constancy of K_p can be determined.

The total membrane resistance (r_t, where $r = k_p^{-1}$) of a complex sandwich type membrane, is equal to the sum of the resistances of each membrane in the series (that is the resistances are additive). The total resistance of a membrane complex with different parallel pathways (spotted or striped type membranes) is equal to the sum of the permeabilities of each pathway multiplied by its fractional area of the membrane (Scheuplein, 1972).

The tetrapod integument represents a complex sandwich type membrane, with four basic components in series which might affect the total membrane resistance: 1) the dermis, 2) the living epidermal layers, 3) the cornified epidermal layers, and 4) the vapor boundary layer separating the epidermis from the freely moving air of the environment. The first two components cannot limit water loss since they are moist (they would have to be partly dehydrated to have a significant barrier function); the fourth component cannot affect the rate of CWL since the phenomenon is neither evaporation limited nor very high (Machin, 1969; Spotila and Berman, 1976; Spruit, 1970). Logically therefore, the cornified epidermal layers must constitute the main permeability barrier, and this assumption is supported by the fact that their removal by cellophane stripping produces a greatly increased rate of CWL (p. 187).

Two further pieces of data must be considered with respect to the total membrane resistance. First, in a scaled integument there are parallel pathways represented by the outer scale surface and hinge regions. Second, in squamates, the cornified epidermal layers comprise two keratinaceous protein types in vertical series (alpha

and beta keratin).

It is obvious from Fick's Law that the rate of diffusion (CWL) is directly related to the concentration gradient. However, this concept can only be rigorously applied when the concentration gradient is small, although the general principle probably still applies in conditions where a large concentration gradient exists (Scheuplein, personal communication). Scheuplein and Blank (1971) estimate that for human skin at 90% ambient relative humidity (water vapor concentration 0.002 M), the concentration of water in the stratum corneum is 27 M so that the resulting concentration gradient would be approximately 27 M. If the ambient humidity is 50% (or 0.001 M) the concentration gradient is practically unaffected. However, a decrease in ambient humidity (from 90% RH to 50% RH) might dry the corneum and thus decrease the membrane's permeability coefficient (k_p). Therefore, it is possible for CWL to decrease in spite of an increase in the concentration gradient. Thus the lack of a linear relationship between CWL and ambient humidity is not surprising (tables 9 and 10). Since the ambient humidity in the chamber is inversely related to the air flow rate, neither is it surprising that no linear relationship exists between flow rate and CWL (table 8; Cohen, 1975). Similarly, temperature (a parameter not directly studied in this thesis) might affect CWL mainly by its action on the saturation deficit (p. 22) which would counter its effect on the water holding ability of the keratin and a direct Q_{10} effect on diffusion.

The foregoing theoretical analysis explains why the rates of CWL for anesthetized, dead and control animals were not significantly

different (table 7). Such conditions would not be expected to alter the permeability coefficient of an already dead (corneous) material nor the concentration gradient and therefore, should not affect CWL. This has been substantiated for human subjects (Scheuplein, 1972) and is the underlying rationale for the use of in vitro measuring systems. However, if the skin dries out, CWL sometimes increases dramatically, since complete dehydration probably disrupts the barrier. Most clinical investigations have found no relationship between normal CWL and blood flow (Scheuplein, 1972; however, see Hattingh, 1972c). Cohen (1975) found that the rate of water loss (EWL) in C. cerastes increased after the IP administration of a vasodilator. However, Cohen's paper does not preclude the possibility that the increase was due to increased activity of the subject causing water to be lost via non-cutaneous routes.

Hydrating tokay skin (by immersing the animal in water) during the renewal phase had no effect on the histology of the epidermis nor any significant effect on CWL (p. 68). If hydration was done after shedding an increase in the rate of CWL occurred. Histologically this increase was correlated with a swelling of the immature (keratinizing) and mature (keratinized) alpha tissues. Although the beta layer is mature at the time of shedding it is probable that this layer did not interfere with the movement of water (through or around it) into the immature alpha layer. Hydration of the tokay alpha layer increased the skin's permeability just as increased cutaneous permeability occurs when mammalian keratin is hydrated (Scheuplein, 1972). Scheuplein (1972) explains that there is a lower activation energy for water diffusion through hydrated kera-

tin than through dehydrated material. However, hydration did not affect CWL during the renewal phase, in spite of the fact that water was probably able to go through or around the beta layer, to the alpha layer. The explanation might be that water cannot enter alpha keratin (under the mild conditions used) if it is already cornified (to use an example, as if it were hardened cement), although it can enter if cellular dehydration is still occurring (moist cement). It is possible that more drastic treatment (for example, with a base; Spruit and Malten, 1971) of renewal material would cause CWL to increase. Preliminary results indicate that ambient acclimation humidity is in some way related to CWL. Hydrated tokays acclimated to low humidity had lower rates of CWL than hydrated tokays acclimated to high humidity (table 11). However, since food was usually withheld from all these tokays for several weeks, their low rate of CWL compared to animals whose food was withheld for 4-5 days, might reflect the former's poor nutritional status. Similar results were found for Lacerta (p. 84). One may speculate that keratinized tissues which mature under the conditions of low ambient humidity have improved barrier functions, a possibility with obvious ecological advantages, which deserves detailed investigation.

Although prolonged dehydration did not affect the rate of CWL in either tokays or lacertas, a single set of obervations indicates that it might in I. iguana (p. 83). The reason for this is not clear, although similar observations have been made for mammals (Haines and Shield, 1971; Haines et al., 1973, 1974; Sokolov, 1962).

While long term changes in a tokay's state of hydration did not seem to affect CWL, short term changes did have such an effect.

When a dehydrated tokay was allowed to drink, or forcibly given water, its awake rate of CWL increased immediately (approximately doubled). This elevated rate was also present a day later. The mechanism for this increase was not investigated nor can a reasonable hypothesis be offered. However, the increase did not occur in anesthetized animals, nor when a small volume of fluid was given. The increase was much greater than could be expected from a simple stretching effect (tables 28 and 29). It would seem that the stimulus must be relatively large and that an anesthetized animal may not sense the stimulus. It might seem that such results could be explained on the basis of gastrointestinal fluid uptake, which would result in a decrease in plasma osmolarity, being slower in anesthetized than in normal dehydrated animals. However, such an explanation is negated by the fact that the next day's rate of CWL was still lower for the anesthetized animals, although certainly enough time had elapsed to absorb the fluid. In addition, a reasonable change in plasma osmolarity would not have a measurable effect on the concentration gradient and thereby CWL.

Generally the rate of CWL decreased after the treatment (table 13) but increased by the next day (table 14). The decrease immediately after the treatment was small and it was probably due to a small amount of keratin dehydration following such prolonged measurements. The following day's enhanced rate of CWL may have been the result of cloacal keratin stripping, which occurred when the cloacal tape was removed after the first day (see page 187). The use of saline injections and hydrated animals did not elucidate the circumstances in which the response is to be expected, although it certain-

ly was most pronounced in awake, dehydrated animals given water.

B. THE MEASUREMENT OF CUTANEOUS WATER LOSS

One must ask why should CWL be measured? It is wrong to think that such studies give any real indication of an animal's "natural" water relations. Animals are rarely exposed for long periods to harsh desiccating conditions, such as those employed to study CWL. It is now realized that many reptiles are homeothermic ectotherms. Their temperature regulation, to a large extent, depends upon behavioral adaptations. Similarly it seems probable that reptiles are able to sense their water status and the environmental conditions which affect it, and would conceivably enter a burrow to escape desiccation, much as they would to escape hyperthermia. When the methodologies used to measure EWL and especially CWL are considered, it is likely that such studies have only marginal ecological applicability. Such ecological goals are best served by field studies, although it must be realized that at present no field method is available for compartmentalizing EWL. Investigations on CWL can only serve as studies on the comparative functional morphology of the integument. That is, one aspect of integumentary physiology, permeability, should be studied in different species and under different circumstances to understand that property. When this goal is appreciated the necessity of keeping a controlled measuring system becomes obvious.

The most finely controllable system used in this study was the *in vitro* capsule technique. However, many questions cannot be answered by using such a technique, and if the rate of CWL is low,

large pieces of skin must be used which are not always available. The *in vivo* capsule technique is not as controllable as the *in vitro* technique, but it is possible to investigate temporal changes in an individual, such as those occurring during the shedding cycle or after cellophane stripping. However, the results of the capsule technique are usually variable because the measuring error is large compared to the rate of CWL. Therefore, in most cases the capsule technique was used only to confirm chamber data. Chamber runs are not controllable to the extent that capsule ones are. Some important variables in the chamber technique include: postural changes, type and rate of air flow, the animal's surface area and the exposed surface area. Attempts were made to reduce variability in all aspects of this measuring system by carefully regulating possible sources of error, and these may now be discussed. Materials with low permeability to water vapor were used for the chamber, tubing, and connections. This reduced the possibility that changes in ambient humidity would be taken as changes in CWL. This consideration is especially important when the rate of CWL is low and the surface area of the materials is large. If measurements are made on an animal in an acrylic chamber (and/or with long pieces of connecting tubing) the results can be corrected by subtracting the background rate of water loss without the animal *in situ*. However, this increases the variability, and is not therefore the protocol of choice.

The collar used in this system was usually tight enough to cause some irritation, which was probably compounded by the heavy application of vacuum grease. However, it was only in this manner that the seal between head and body compartments could be checked,

without the rigorous checking procedure itself causing a leak. The appropriate air flow rate was selected by determining the near maximum flow rate which rarely disrupted the seal in a particular experiment.

Flowmeters were used as part of the inlet and exhaust system for both the head and body compartments. Inspection of the reading on these four flowmeters gave a good indication of a head-body compartment leak or a change in the output of the air source. However the flowmeter used in this study did not give an accurate indication of the flow rate, therefore a wet test meter was attached to the body compartment's exhaust. This allowed determination of the average flow rate for each half hourly interval.

The time needed for equilibration to occur must be empirically determined. Initially the moisture in the exhaust air derives from the chamber, and from water adsorbed onto the animal's surface (see Haines et al., 1973). Thus initial rates are indications of ambient humidity of the BOD box (where the chamber was kept) and of the vivarium. After this moisture is removed, the rate of moisture uptake by the exhaust Drierite tube should equal the rate of diffusion through the skin. The same system required different lengths of time to equilibrate for different species. Although at 400 cc/min the half hourly rate of moisture gain by the Drierite was constant for G. gecko and I. iguana (that is, the system was in equilibrium), this was not so for: T. nigropunctatus, L. lepida, D. dorsalis, and C. constrictor. Although there was a trend for rat snakes it was highly variable. This relationship is not size dependent nor related to the total rate of CWL. However, generally, the group which

took longer to equilibrate had lower surface specific rates of CWL (see table 37). Hydrated keratin is more permeable than dehydrated keratin (table 7; and see Scheuplein, 1972). Therefore, it is possible that the integuments of the animals which took longer to equilibrate were more sensitive to dehydration (possibly their keratin had a lower solvent-membrane partition coefficient) so that continuous exposure to the desiccating atmosphere caused a continuous decrease in CWL.

Consideration of various techniques to estimate the magnitude of the head non-integumentary water loss, all involved attempting to estimate head surface area, differential greasing, or other further traumatic treatments of living animals. It was decided that no such approaches were justified in light of the difficulties inherent in introducing further inadequately controllable variables.

The use of anesthetized animals reduced the chance that a head-body compartment leak would develop. Mildly anesthetized animals showed few body movements and generally, hung limply by their necks. Although this probably put extra strain on the neck, reproducible results were obtained. The main effect of animal activity was to expose the usually covered hinge regions. Although the hinge is probably as impermeable as the rest of the scale (see below), because the hinge is covered, pockets of high humidity build up in it. In a quiescent animal, its particular rate of CWL would represent the sum of CWL from the outer scale surfaces, plus that from the hinge regions. Moisture lost from the latter would have evaporated from a region of high humidity. If the animal becomes active, desiccating air would scrub the hinge regions, producing an increased rate

of CWL, and upon return to a quiescent state, CWL would diminish below the original resting rate as humidity builds up again in the hinges.

The cloaca was taped to reduce the possibility that an animal would urinate, and to facilitate detection of urination (by examining the tape) since the chamber was opaque. Cloacal taping is therefore useful when measuring an animal only once per cycle or possibly when measuring hydrated animals which frequently urinate. However, usually the cloacas of animals which are measured several times per cycle should not be taped. The probability of stripping keratin when the tape is removed is greater than the possibility of a small amount of urination going undetected.

C. THE EXPRESSION OF CUTANEOUS WATER LOSS

Since the amount of water lost from an animal increases with time, it is obvious that CWL must be expressed as a function of time. However, deciding whether CWL should also be expressed as a function of weight (that is, mg/gm/hr) or of surface area ($mg/cm^2/hr$) raises several questions. When an animal's weight changes, does its surface area also change? If the surface area of a scaled animal does increase, is this accomplished by an unfolding of the hinge regions or by a stretching of the integument? Are small changes in surface area associated with detectable changes in CWL? Does CWL expressed on the basis of weight or surface area have any meaning, if CWL does not increase linearly with weight or surface area?

Some insight into these questions can be gained by examining the data on the relationship between body weight and CWL (p. 62).

Each of eight tokays was measured at the same time of the shedding cycle for several months. In some cases during this period the animals gained weight, others lost weight, and in still others weight fluctuated with no clear overall trend. None of the individual regressions of body weight and CWL were significant (that is, there was no significant relationship between an animal's rate of CWL and its weight during that measurement). If short term weight changes in tokays were accompanied by surface area changes a consistent difference in CWL could not be detected. The regression coefficient for mean individual body weight and mean individual CWL was significantly different from 0 (see p. 62). This indicates that CWL as a function of weight or surface area is meaningful when mean values from different animals are compared, but not when two measurements on a single animal, or one measurement on each of two animals are compared. For tokays the group regression coefficient (0.707) was significantly different from 1.0, although not significantly different from 0.67 (the accepted surface area to weight regression coefficient). Thus the rate of CWL for tokays could be compared on the basis of surface area since the relationship between surface area and CWL is linear.

Generally, CWL decreased after stretching (table 29). If integumentary distension caused keratin to stretch, the thickness of the barrier would decrease, and an increase in CWL per unit surface area would result. If however, integumentary distension unfolds the hinge regions, then there is more integument (outer scale surfaces plus hinges) under the capsule before distension than after. Column 5 in table 29 gives the extrapolated rate of CWL for the entire

stretched region as a fraction of its original rate. Note that in rat snakes a small amount of distension (1.4-1.5 X) does not cause the total rate of water loss to change from its original level (0.84-1.09). Greater distension (2.7-3.6 X) resulted in a greater total rate of CWL (1.79 and 3.67 times its original rate). The skin from a single tokay was distended only moderately (compared to Elaphe), but since tokay skin is less elastic, a high rate of total water loss (1.47 X) would be expected. Hence this is additional evidence that the small amount of distension that might occur during daily weight fluctuations would have no detectable effect on CWL.

The regression coefficient for CWL and body weight for rat snakes (0.211) is not significantly different from 0 or 0.67. Since each of the twelve data points used for this regression were single observations, it is possible that many individual points were not representative of the animal's mean rate of CWL and mean body weight. This situation is reminiscent of the relationship between body weight and CWL for individual tokays.

The regression coefficient relating CWL to weight for I. iguana (0.893) is significantly different from 0.67. This could mean that as iguanas increase in size their surface area changes with the 0.893 power of body weight or that the rate of CWL per unit surface area increases. Although each of the ten data points used for this curve represents a mean of several measurements for an individual animal, they were usually obtained over a one to two week period rather than over several months. Hence, it is possible that the iguana curve is not as reliable as the tokay curve.

In conclusion, when comparing whole animal compartment values

taken on the same animal several weeks, or even months apart, there is nothing to be gained, with regard to reducing variability, by expressing the absolute value on the basis of weight or surface area. For this reason every effort was made in this study to use individual comparisons rather than group comparisons. When comparing intraspecific rates of water loss, surface area or weight can be used if the regression coefficient relating CWL to weight is close to 0.67 or 1.0, respectively. When comparing interspecific rates of CWL, all species must be shown to obey the same relationship between CWL and weight or surface area. If such a relationship has not been rigorously established, only the broadest generalizations can be made when comparing animals of different size. If interspecific studies must be made, it is best to compare capsule values.

D. COMPARISON OF CHAMBER AND CAPSULE DETERMINATIONS OF CWL

Table 37 presents a comparison of capsule and chamber values of CWL for some of the animals used in this study. The chamber values of CWL are expressed both as a function of weight (mg/gm/hr) and of surface area ($mg/cm^2/hr$), to allow a general comparison to the literature values (see table 1). The relationship between weight and surface area was determined by using a regression coefficient of 0.67; with a constant of 10 for the lizards and the caiman, and 12.5 for snakes. The mean weight used for *G. gecko*, *I. iguana*, and *E. o. quadrivittata* is the mean weight of the animals used to calculate their specific regressions.

Whole body weight was used to determine body compartment weight-specific water loss. Calculated whole body surface area was similar-

Table 37. The rate of CWL for animals used in this study - comparison of the *in vivo* and *in vitro* techniques.

	CAPSULE			CHAMBER				
Species	N	CWL ($mg/cm^2/hr$)	Condition	N	Mean Weight	CWL ($mg/gm/hr$)	CWL ($mg/cm^2/hr$)	Capsule ($mg/cm^2/hr$) / Chamber ($mg/cm^2/hr$)
Dipsosaurus	3	0.36	*in vivo*	16	43.0	0.16	0.058	6.2
Lacerta	6	0.26	*in vivo*	53	89.1	0.29	0.126	2.1
Iguana				a	287.7	0.816	0.529	
Tupinambis	7	0.57	*in vivo*	b	2000	0.141	0.173	3.29
Gekko	15	0.176	*in vivo*	a	52.4	0.631	0.233	0.73
Elaphe	8	0.50	*in vitro*	a	273.3	0.111	0.056	8.93
Constrictor	6	0.27	*in vivo*	19	1465	0.098	0.087	3.10
Geochelone	1	0.57	*in vivo*					
Pseudemys	3	4.88	*in vitro*					
Caiman	4	1.47	*in vivo*	1	361	1.197	0.83	1.77
Rana	1	10.47	*in vivo*					

a. From regression

b. A low value

ly used to determine surface-specific body compartment water loss. Therefore, in both cases real body CWL was underestimated. CWL could be expressed on the basis of weight or surface area within the body compartment. However, this would involve estimating head weight or surface area and subtracting these values from whole animal values. Such a correction would certainly be different for animals with relatively large heads (tokays) and relatively small heads (desert iguanas or snakes). It was felt that such additional estimations would not simplify a literature comparison.

The aquatic reptiles had high rates of CWL. Generally, capsule measurements resulted in higher area-specific rates of CWL than chamber measurements. This can be explained on the basis of the higher surface-specific flow of desiccating air for the capsule measurements, which would scrub more of the hinge region. The variation in the ratio of capsule to chamber determinations of surface-specific CWL would be related to complex interactions between: the real surface to weight relationship, the degree of scale overlap, the animal's activity during the measurement, and to positional effects. Of these possibilities the latter is probably the most important (Cohen, 1975; Krakauer, 1970; Spotila and Berman, 1976).

The low rate of CWL for T. nigropunctatus as determined by the chamber method is probably misleading, since this species was too large for the chamber. The low rate of CWL for mesic snakes might reflect the fact that very little of their body was exposed perpendicularly to the air flow.

SECTION II. THE SQUAMATE SHEDDING CYCLE AND PATTERNS OF CWL

A. GENERAL RELATIONSHIP

In spite of numerous anomalous observations a general relationship appears to exist between patterns of CWL and the various stages of the squamate shedding cycle. While this general relationship reflects more clearly the situation for those species where the data are most complete, most aspects of the general pattern were confirmed for all species studied, and no compelling contrary data were obtained. The general pattern will first be presented and anomalous data will be discussed for each species.

Generally, the rate of CWL is high after shedding, then decreases as the epidermis enters the perfect resting condition, remains constant while the epidermis is in this condition, but increases during the renewal phase and is high during shedding. These physiological changes may be correlated with changes in the alpha layer which is thought to be the main permeability barrier. Following shedding the alpha layer thickens and causes CWL to decrease. During the perfect resting phase the alpha layer is usually stable, as is CWL. Sometime during the renewal phase, the barrier begins to deteriorate and CWL increases. The exact time when this process of deterioration begins is not known for any of the animals studied, although it certainly varies interspecifically, and possibly intraspecifically. The rate of CWL begins to decrease as a new alpha layer matures.

The terms used to describe the shedding cycle in this thesis are based on histological criteria rather than the gross appearance of the animal. It is common knowledge that snakes go through periods of "glossy skin", "cloudy eye", "clear eye", and shedding, in each

cycle, and these gross changes may be correlated with the post-shedding phase, mid renewal, late renewal, and shedding histology, respectively. However, a critical morphological observation in this thesis is that the gross changes are often variable with respect to the precise histological conditions, although most of the histological stages show very little variability with respect to actual shedding (see also Maderson, 1966; Maderson, Chiu, and Phillips, 1970b). However, even the shedding event itself does not always correspond to a single defined histological condition, since in stressed animals, animals in poor health, frequent shedders, and sometimes in apparently normal animals, shedding will occur late with respect to the histological maturation of the inner generation. Thus, although there have been several recent studies on some aspect of cutaneous physiology with respect to the shedding cycle, none recorded precise histological observations, and therefore permit only the most general conclusions.

The pattern outlined above will next be examined for each squamate species studied, and an attempt will be made to resolve apparently anomalous data.

B. SPECIES RELATIONSHIPS

1. Gekko gecko

The enumerated Exceptions to the general pattern detailed on pages 93 to 94, may be grouped into several categories for discussion.

Many Exceptions (#s: 1, 5, 6, 7, 12, 13, and 14) did not show a decrease between the early post-shed resting condition (0p-2p)

and a later stage in the cycle. This may result from either a precocious or rapid development of the alpha layer, or less probably to a precocious increase in CWL during the early renewal phase. This situation occurred most frequently in animals with short cycles (mean cycle length, 17.2 days).

Other Exceptions (#s: 2, 4, 8, 15, 16, and 18) showed a continuing decrease from the post-shed condition to the next measurement. This was probably due to a retarded alpha keratinization process lasting several days after shedding. This occurred most frequently in animals with long cycles (mean cycle length, 20.8 days).

Several other Exceptions (#s: 3, 9, 11, and 17) showed a decrease late in the cycle: these were usually of small magnitude. This may result from minor differences in cloacal taping or variation in the amount of neck put into the head compartment.

Exception #10 showed a decrease between early stage 5 and stage 6. This may be due to a precocious development of the alpha layer prior to shedding.

Data from animals measured once per cycle (table 19) suggest that CWL does not increase during the early renewal phase. However, five of these animals were later measured several times per cycle and in general did show an increase during this period. To explain this paradox, the rationale for each of these experiments must be understood. The advantage of measuring animals only once per cycle is that the value obtained is free from consideration of any previous trauma. It might seem possible that during an animal's stay in the chamber, keratin would be rubbed off at the point where its neck contacts the neoprene membrane. The areas without a kera-

tinaceous covering would lose water at a faster rate, thus causing increased CWL the next time the animal is measured. This possibility is unlikely since unanesthetized animals were measured for long periods and they did not show any tendency for CWL to increase with time (figure 7).

Certain data indicate that lower rates of CWL were found in animals whose cloacas were not taped (p. 40), and such animals seem to have very little increase in CWL during the renewal phase (Tokay R, second cycle, figure 27). However, some taped animals (Tokay P, second cycle, figure 26, and Tokay Y, first cycle, figure 29) held in the same type of desiccating environment as Tokay R, also showed only a small increase in CWL during the renewal phase. Thus not only was CWL diminished in desiccating conditions, but also the pattern of change was minimized.

Often when tape was removed from the cloaca some of the underlying keratin (beta layer) was removed with it. This stripping affected the corneous barrier, and thus initiated a cellular hyperplastic response to restore the damaged barrier (pp. 173-184, and see below). For the next series of measurements, the cloaca must again be taped. If on this occasion the tape does not exactly cover the stripped region, an artificially high rate of CWL would be recorded. Also, since several days were allowed between measurements, variations in the rate of the barrier regeneration might affect this artifactual rate of CWL. The effect of cloacal stripping could not be too large however, since it did not obscure the decrease in CWL following shedding in non-taped animals (for example, Tokay R, second cycle, figure 27). However, the possibility exists that the in-

crease observed during the renewal phase in tokays might be due to the effects of stripping rather than to the renewal process itself. This hypothesis can be tested.

If the taping and regeneration hypothesis is correct, the closer together a pair of measurements are taken within a cycle (between stages pR and 14), the greater should the second value be, relative to the first. If the increase in CWL during the renewal phase is due to the renewal process itself, one would predict the reverse; since the farther apart a pair of measurements is, the more advanced the second measurement will be in the renewal phase.

Table 38 gives the ratio of the second measurement to the first, according to the number of days between the measurements, and the epidermal stage at the time of the first measurement. Based on the weighted average there does not seem to be any trend, although the smallest increase occurred when only one day separated the two measurements. When 8 to 10 days separated the two measurements, the second was 55% greater than the first (n = 3). The evidence in association with the effects of cloacal taping on snake water loss (see below) also indicate that the taping regeneration hypothesis cannot explain the bulk of the evidence that CWL increases during the renewal phase.

Although neck abrasion and/or cloacal taping may affect the rate of CWL, it is necessary to provide a different reason to explain most of the data concerning the renewal phase. Many attempts were made to measure tokays throughout a cycle without taping the cloaca, but only one was successful. In all other cases the animal always managed to urinate during the runs whether lightly anesthe-

Table 38. Ratio of paired measurements of CWL according to days between measurements and the stage at the time of the first measurement.

Initial epidermal stage	DAYS BETWEEN MEASUREMENTS 1	2	3	4	5	6	7
pR	1.04 (2)[a]	1.31 (2)	1.18 (4)	1.05 (4)	1.53 (1)	-	1.25 (6)
1R	-	-	-	1.11 (1)	-	-	-
2	-	1.12 (1)	1.06 (2)	1.04 (3)	-	-	1.05 (2)
e3	1.07 (1)	1.08 (3)	1.39 (4)	1.26 (3)	1.14 (1)	-	1.22 (4)
m3	-	1.34 (1)	0.87 (1)	-	1.14 (1)	-	-
13	-	1.27 (1)	1.21 (2)	1.35 (1)	-	-	-
e4	-	1.18 (3)	1.04 (1)	1.11 (1)	1.27 (1)	-	-
m4	-	-	1.23 (4)	-	-	-	-
weighted average	1.05	1.19	1.20	1.13	1.27	-	1.21

a. Sample size

tized or not.

If animals are measured only once per cycle, the trend for CWL must be reconstructed from individual cycles, in which case intercyclic variation would be an important factor. Thus during the course of the half year that the first series of measurements (once per cycle) were in progress, animal weight and ambient humidity changed. The coefficient of variation, considering only measurements taken four days after shedding, was often 25% (table 19). If the data for these measurements are expressed in terms of surface area, there is as much intercyclic variation as intraspecific variation, again indicating the heterogeneous nature of these data. Histological examination of biopsies from the eight animals failed to reveal consistent quantitative differences in the corneous tissues, therefore suggesting that the differences in CWL were due to qualitative differences in the barrier.

If CWL actually increases during the renewal phase, and the low values of CWL obtained during the renewal phase from animals measured once per cycle are fortuitous, one would not predict that all measurements taken during the early and mid renewal phases would be very close to the mean of the four day post-shed value. Also, these results are not in accord with a null hypothesis that there is no tendency for CWL to change from four days post-shed to a later stage in the renewal phase (13 decreases, 3 increases; $P = 0.025$). Another possibilty is that at four days post-shed the rate of CWL had not reached its nadir. However, even this explanation is unlikely since usually measurements of CWL taken two days post-shed have already leveled off. Also, the biopsies accompanying four day post-

shed measurements usually showed only a single layer of immature suprabasal cells.

Although the group comparisons (p. 94) confirm that CWL decreased from shedding until after the perfect resting phase, there was only an insignificant increase from the perfect resting phase to the early renewal phase. Similarly the group capsule data (table 26) did not allow resolution of the relation between CWL and this part of the shedding cycle. However, capsule data from individuals measured throughout the cycle (figure 47) invariably showed an increase throughout the renewal phase, although the rate of increase was quite variable. Unlike the chamber technique, with the capsule there is no reason to believe that the measurement process itself has a deleterious effect on the skin. These individual results showed that sometimes CWL was several fold higher in the late renewal phase than during the resting phase. It is interesting that the tokay which had the sharpest increase in CWL during the shedding cycle as measured with capsules also had a sharp increase in the rate of CWL during the renewal phase when measured with the chamber technique (figure 19). This seeming coincidence might indicate that some animals are more prone to dramatic changes in CWL during the renewal phase than others.

In summary, there being no reason to believe that changes in CWL which were recorded during the early renewal phase were technical artifacts and since the only data which do not fit this pattern are those obtained from individual animals measured only once per cycle for many cycles, it is concluded that the observed increase was real.

2. Other lizards

Data on CWL throughout the shedding cycle were obtained on three other lizard species: Iguana iguana, Lacerta lepida, and Tupinambis nigropunctatus. For these animals epidermal histogenesis was asynchronous over the body, so that some regions entered a given histological stage and eventually shed before other regions. Since CWL was measured from the body, the biopsies had to be taken from other regions. Therefore, an estimate of the asynchrony between the biopsy region, and that of the body, had to be made in order to permit any generalizations about CWL during the shedding cycle.

Iguanas and lacertas had longer cycles than tokays and their inner alpha layers began maturing before they shed. Therefore, CWL during the late renewal phase and shedding would not be expected to be very high because of precocious development of the new barrier (see below). Also, CWL would not be expected to decrease much after shedding, since these animals entered the perfect resting condition almost immediately after shedding. The situation for the tegu is more complicated mainly because of the paucity of renewal phase and shed biopsy material, the chromophobic nature of all keratinized layers, and the exaggerated thickness of the mesos layer. Generally, the mesos layer is not considered as an important barrier because of its thinness (see below), however, with the black tegu it might be important.

Although the iguana data seem to follow the general pattern, statistical analysis would not be meaningful because of the small sample size. The relationship is not clear with Lacerta since although two animals showed an increase during the renewal

phase, one did not. However, for most other aspects of the shedding cycle the Lacerta data seemed to follow the general pattern. Capsule data did not further elucidate the problem. The rate of CWL increased in the black tegu from the early renewal phase onwards, and then decreased after shedding. The capsule data indicated that CWL decreased after shedding, but because of the variability of the data, it is hard to tell if CWL increased during the renewal phase.

3. Elaphe obsoleta quadrivittata

a. CWL during the resting phase

The data on the rate of CWL during the prolonged resting period were highly variable. Essentially, several animals showed a fairly consistent increase (Elaphe #s: 1, 4, 5, 8, and 9), in others CWL remained fairly constant (Elaphe #s: 2, 6, and 7), while in others it showed such large fluctuations for all or part of the experimental period (Elaphe #s: 1, 3, and 5) that no discernible trend could be established. The actual technique of measurement could not explain these anomalies, therefore various husbandry procedures and possible biological variables have to be considered.

i. Relative humidity and the maintenance environment

Originally it was thought that habitat differences and erratic changes in ambient humidity might physically affect the cutaneous barrier and thus indirectly be the cause of the daily fluctuations in the rate of CWL. Therefore, three animals (Elaphe #s: 3, 8, and 9) were placed in a constant environment with respect to temperature and moisture, and measured frequently thereafter. Since Elaphe #3 showed marked daily fluctuations, probably larger in magnitude than those for any other experimental subject, maintenance

humidity is probably not a satisfactory explanation.

ii. Possible trauma caused by cloacal taping

The process of cloacal taping and retaping caused keratin to be stripped off (p. 216). Subsequent occasional exposure of this stripped region due to incomplete cloacal taping, would result in an artificially high rate of CWL, while occasional complete protection of the stripped region with tape would result in a sharp decrease in CWL compared to the previous measurements. In this context frequent measurements would mean frequent stripping, and in the long run a general increase in CWL during the prolonged resting period might be predicted. However, Elaphe #5 was not taped and, in general, showed an increase in CWL during its prolonged resting period, while Elaphe #s 6 and 7 were taped and did not show an increase. Animals which were measured frequently should show the sharpest increase in CWL, yet Elaphe #s 8 and 9 had the sharpest rise, and were only measured weekly. Finally, the last measurement of Elaphe #1 in this series (126.5 mg/0.5 hr) was considerably less than its first measurement after a 41 day rest (207.0 mg/0.5 hr). CWL increased during this period although the animal was not taped or stressed in the interim. Therefore, it seems that taping and other measuring stresses could not account for the increase in CWL during the prolonged resting period.

iii. Histological correlates of the prolonged resting phase

Biopsies taken from all animals studied during these prolonged resting phases revealed an eventual loss of the "perfect resting condition" histology. Biopsies showed an "odd" RS with

several layers of suprabasal cells, and a hyperkeratotic alpha layer containing many pycnotic nuclei. This was not the case in the boas which have a long (approximately 60 day) but regular cycle, nor in any lizard, even in those whose cycles are long and variable (for example, Dipsosaurus, Iguana, and Lacerta). It is known that many snakes shed only once or twice a year (Klauber, 1956), but it is not known if similar histological changes occur in these natural circumstances.

The onset of the appearance of this "odd" condition from the time of the last shed was variable [mean 21.3 $\pm$ 7.84 (S.D.) days, range 10-29 days]. There was no evidence explaining why it began at different times, nor was there any evidence which suggested what triggered the response. Elaphe #8 had this "odd" resting condition on the first day of measurement, so that it cannot be interpreted as a by-product of stress brought about by frequent measurement.

Since epidermal activity in snakes, even those which shed infrequently, is synchronous over the entire body, and since this "odd" resting condition did not seem to be a response to local trauma, it would seem that such a condition should also characterize the entire body epidermis. Normally trunk skin was not biopsied to assess the epidermal condition in chamber measurements. However, on two occasions, trunk necropsies were taken when the neck epidermis showed the "odd" resting condition. In both cases the trunk epidermis showed a typical perfect resting condition. However, in these cases (Elaphe #s 6 and 7) the "odd" histology had only recently appeared in the neck. Several trunk biopsies from snakes which were not part of this experiment, were taken a considerable time after a

shed. Often these biopsies showed a variant resting condition, in which the germinal cells were not very active, yet there were several suprabasal cell layers.

iv. Relation between dehydration during cornification and CWL during the prolonged resting phase

The possibility must be considered that the production of new cells throughout the prolonged resting phase resulted in an increased rate of water loss due to the moisture liberated when the newly formed cells dehydrate during their keratinization. Felsher and Rothman (1945) felt that this might explain the high rate of CWL in some hyperkeratotic diseases. There are two reasons to believe that this explanation could not be of major importance in these snakes. Firstly, this could only cause CWL to reach a new, higher level, but if the rate of keratinization remained constant, so should the amount of excess water lost from this source. Secondly, if the original barrier remained intact, increases in keratinization should not increase CWL. The tissues under the barrier (suprabasal living cells) are always in a water phase, and would therefore be wet from dermal fluid even without the increased keratinization.

Aside from the unknown factors which cause the "odd" resting condition to occur, there is another fundamental problem. Since this cellular activity causes the alpha layer to thicken, it should be associated with a decrease in the rate of CWL, not an increase. However, in snakes where this "odd" histology was found, the rate of CWL increased. Animals which had stable rates of CWL during part of their prolonged resting period (Elaphe #s 2 and 7), did not have the "odd" resting histology, but Elaphe #9 showed a

sharp increase in CWL although its epidermis showed a perfect resting condition.

v. CWL and alpha cracking during the prolonged resting phase in the rat snake

It is possible that the continuing production of new cells caused the overlying barrier to crack (see below for a further extension of this possibility). However, the continual cracking of the old barrier, together with the continual formation of a new barrier should not cause the rate of CWL to continuously increase, but rather it should quickly reach a new equilibrium. Thus it must be assumed that a deterioration of the original barrier is associated with the production of a new parakeratotic, inferior barrier. Such an assumption could also explain the daily fluctuations which were observed in most of the experimental animals. Since in snakes the normal epidermal population is synchronized, it is possible that the keratinization of the new inferior barrier, and the disruption of the outermost good barrier during the prolonged resting phase were synchronized. A low rate of CWL during the prolonged resting phase would indicate the synchronized keratinization of a new cornified layer, while a high rate of CWL would indicate the synchronized disruption of an old layer of the barrier. It was not possible to confirm the cracking histologically (see below).

Elaphe #1's high rate of CWL following a several week measuring hiatus can be explained by postulating that in the interim a new inferior corneous layer replaced the normal one. At the end of the regular series of measurements Elaphe #1 weighed 295 grams, and after six weeks of regular feeding its weight returned to

374 grams. This must mean that its surface area enlarged (see p. 207). A corneous parakeratotic layer is probably less elastic than the normal layer, and thus it is possible that it cracked, and the result was a dramatic increase in CWL. This may also explain why the rate of CWL for the three animals which were fed (Elaphe #s 3, 8, and 9) were so high and fluctuated so much.

vi. Cutaneous trauma pathology and CWL

All biopsies examined in association with chamber measurements were taken from the gular neck region. This region contains approximately 50 scales which could be biopsied. Therefore, eventually there were few scales not in immediate contact with a wounded region. In fact, it was only the last several biopsies of Elaphe #1 and Elaphe #5, and the last biopsy of Elaphe #3, which showed "trauma" reactions (figures 35 and 36). Can it be assumed that the entire gular skin showed a "trauma" response, or is it possible that the last biopsies were traumatized due to their juxtaposition to true wounds?

The second possibility seems unlikely for several reasons. Often a "trauma" reaction was found in the scale's center but not near its edges. Furthermore, if the presence of a trauma reaction was due to biopsying adjoining wounds, one might expect that some biopsies would show "trauma" and others not. As the number of regions biopsied increased, more biopsies should show "trauma", but one would expect to occasionally find normal material. This was not seen: once a "trauma" response was found, all subsequent biopsies of that snake's gular region showed the "trauma" reaction. Finally, Elaphe #8 showed a "trauma" reaction on day 7 (and thereafter) al-

though this was only the second gular biopsy. Thus it is probable that the entire gular epidermis produced a "trauma" response to some stimulus.

Does this imply that the measuring technique stressed the animal in such a way that it caused the entire epidermis to show a "trauma" response? A trunk biopsy of Elaphe #3 taken after the neck epidermis already showed a clear "trauma" reaction, showed a normal histology. Throughout the experiment, notes were kept on the physical condition of the experimental subjects. All animals which histologically showed a trauma reaction had external signs of an erythemic gular integument. This was never grossly observed for the trunk. Probably a combination of unusual posture, constriction of the gular skin by the neoprene membrane, and the constant application of grease, aggravated by the frequent biopsies, caused the head skin to show a "trauma" response.

In summary, it is hypothesized that the increase in CWL during the prolonged resting phase (which characterized these animals) was related to the production of a parakeratotic tissue with inferior barrier properties. The corneous layer over the trunk epidermis was atypical in that it possessed many pycnotic nuclei, although it probably was not characterized by the extreme cellular activity which characterized the gular integument. The gular "trauma" reaction is hypothesized to be a local response to unusual stimuli and unrelated to the measured rate of CWL.

b. CWL during the renewal phase

Taken as a whole, the rat snake chamber and capsule data strongly suggest that CWL decreased after shedding, and that CWL was

higher during the renewal phase than during the resting phase. Several sorts of protocols which involved frequent measurement of CWL in the same animal (normal or thyroidectomized), also indicated that CWL increased throughout the renewal phase. Unlike the situation for tokays, there is no evidence that CWL significantly increased during the early renewal phase. However, these patterns were not supported by group comparisons. Several of the seeming exceptions can be explained on the basis of a precocious development of the inner alpha layer or delayed shedding (see results), others are not so easily explained.

4. Constrictor constrictor

The data obtained by measuring boas with chambers and capsules are in accord with the general trend. The infrequent nature of their measurement makes it impossible to determine if CWL was high during the early renewal phase. These data also emphasized that during the retained shed condition, CWL was always low.

C. ANALYSIS

Several investigators measured EWL during shedding. It is presumed that in such circumstances the animal was able to remove its outer generation while in the chamber. If so, the increase in evaporative water loss that Bogert and Cowles (1947) found for an indigo snake, that Gans et al. (1968) found for L. doliata, and that Minnich (1970) found for Dipsosaurus dorsalis, might be due to the exposure of the inner aspect of the outer generation as it was everted during the process of shedding. However, this would not provide any information about the skin's permeability during shedding. When

Gans et al. (1968) removed the outer generation just before shedding, the rate of CWL was not high. Gans et al. (1968) incorrectly assumed that this indicated that the increase in CWL during shedding is due to increased activity involved in the removal of the outer generation.

When CWL is measured, the animal cannot shed normally since its head is in a fixed position. Therefore, it is possible that Claussen (1967) measured percutaneous water loss during shedding, although no mention was given as to how advanced the animal was in the process. Cohen (1975) measured EWL and CWL before, during, and after shedding, and his limited data are in general agreement with those presented here.

One aspect of the relationship between CWL and the shedding cycle remains to be clarified. Why does the rate of CWL increase during the renewal phase - a period during which there are no apparent morphological changes in the cornified layers of the outer epidermal generation? In order to appreciate this problem more fully, it is essential to determine the location of the normal squamate permeability barrier.

SECTION III. THE LOCATION OF THE PERMEABILITY BARRIER

A. INTRODUCTION

If an animal's epidermis consists of equal amounts of two different types of keratinaceous protein distributed in a striped, checkerboard or spotted pattern, and if the animal's rate of CWL is less than one half of that of a similarly shaped free water surface,

then it can be concluded that both keratins are a barrier to water loss. If the animal's rate of CWL is considerably less than this then both materials offer considerable resistance to the movement of water. A highly permeable material could occur on an animal with a low rate of CWL if it only composed a very small percentage of the total surface area. Thus in those non-lepidosaurian reptiles that have low rates of CWL, both alpha and beta keratin must be able to offer considerable resistance to the movement of water since each constitutes a large fraction of the animal's total body surface. Of course, this does not mean that one material is not a better barrier, in terms of resistance per unit thickness, than the other.

The relative permeability of alpha and beta keratin were tested by applying vacuum grease to different parts of the scale (table 30). Since covering the hinges with grease did not reduce CWL more than would be expected on the basis of its fractional surface area, consequently the hinges are not more permeable than are the outer scale surfaces. On one occasion when only the hinge regions were greased, CWL was reduced by less than 5%, while when only the outer scale surfaces were greased, CWL was reduced by less than 16%. Theoretically these percentages should total 100%, and thus this datum seems to indicate that grease is permeable (that is, it does not cut down CWL). However this series of experiments was performed with a small capsule, hence the absolute values are unreliable. In addition, since the grease tends to run away from the inner rim of the capsule, consequently a larger percentage of the greased integument loses its grease when the capsule is small.

Studies on the scaleless mutant snakes (Licht and Bennett,

1972; Bennett and Licht, 1975) have revealed rates of CWL equal to those of normal animals. Such mutants lack the thickened beta layer which designates a normal outer scale surface, but the alpha layer is of normal proportions. This indicates that the hinge region (where alpha keratin predominates) is not more permeable than the outer scale surface (where feather keratin predominates).

B. DYNAMIC ASPECTS OF THE HINGE AND CWL

Krakauer (1970) presented two pieces of evidence suggesting that the hinge regions and indirectly the alpha layer, are more permeable than the outer scale surface and therefore indirectly the beta layer. CWL was high after feeding (where the hinge regions are distended) and when snakes rested in a curled position (which exposes more hinge than when the animal is straight). A minor objection is that in both cases Krakauer compares distended hinge to a normal piece of integument. However, a major fault is that the system did not reach equilibrium. When the hinge is distended, moisture which was previously allowed to build up therein escapes, producing a high transitory rate of CWL, but this does not imply that the hinge is more permeable than the outer scale surface. In fact, Krakauer uses this argument to explain the seemingly high rate of CWL during activity. Elaphe #12 (figure 50) is an example of a non-equilibrated run, obtained in essentially the same manner as Krakauer's single observation. When Krakauer investigated postural changes, data points were obtained every two minutes, but from Lasiewski's formula (Lasiewski et al., 1966) one can determine that the lag between a change in CWL and an equilibrated reading would

take much longer to occur.

Several attempts were made to indirectly test the relative permeability of the hinge regions. A small amount of integumentary distension did not cause an increase in CWL (table 28). If the hinges were very permeable, a large increase in CWL should have occurred. The chamber results reported in table 27 are comparable to these capsule results. Total CWL decreased after inflation but not after feeding. The explanation for this discrepancy might be due to epidermal dehydration in the inflated dead animals.

CWL was lower when the air passed over the animal in a cephalocaudad direction rather than in the more frequently studied caudalcephalad direction. This is because the hinges are not so effectively scrubbed when air passes posteriad. In all cases CWL was higher after distension than before (although not per unit surface area, see table 27). If hinges are more permeable than outer scale surfaces, then the difference between normal flow (or 1000 cc/min) and reverse flow (or 200 cc/min) should be greater before than after distension. However, the difference was about the same both before and after distension. Finally, the two in vitro measurements made on black tegu skin with grease on the hinges indicated that squamates are similar to crocodilians in that the hinge is not very permeable.

C. THE SHEDDING CYCLE

1. Possible Resistance of Each of the Cell Layers of the Epidermal Generation

The unspecialized squamate epidermis may have six recognizable cell types which cornify over the outer scale surface. These

are from without inwards: the Oberhautchen, beta, mesos, alpha, lacunar and clear layers. If it is assumed that only keratinized materials in the epidermis can be a major barrier to CWL, then any or all of these layers must be considered.

The lacunar and clear layers may be disregarded since they are not present when CWL is lowest, during the perfect resting condition.

The Oberhautchen layer can probably be eliminated from consideration because it is very thin (a single cell layer thick, and resistance is directly related to thickness) and because its structure is obviously specialized for shedding (spinules and serrations, see Maderson, 1970).

The mesos layer keratinizes temporally after the beta layer but before the alpha layer. Its thickness varies greatly interspecifically (Maderson, Mayhew, and Sprague, 1970), being thin in many squamates, but thick in black tegus. Alexander and Parakkal (1969) suggested that this region is intermediate in structure between the beta and alpha layers, and that it might be a transitional region. This is doubtful since the ultrastructure of its constituent cells is the same whether they lie near the beta layer or near the alpha layer (Maderson et al., 1972). The mesos layer, like the alpha layer, is about equally thick throughout the entire epidermis. Maderson et al. (1972) stress that the cells of the mesos layer differ from those of the alpha layer in several respects, in terms of permeability the most important difference is the lack of abundant filaments. Since the material which offers resistance to the movement of water is a lipid-keratin matrix it would seem that without keratin filaments

this material could not be the barrier. Thus no function can be assigned to the mesos layer at present but its role as a permeability barrier is doubtful especially in those animals where it is very thin (e.g., tokays). Where it is thick, it might supplement the other keratin layers in some way.

The beta layer is rarely present to any degree in the hinge (Roth and Jones, 1967), but it is always thick on the outer scale surface. The inner beta layer begins keratinizing during the mid renewal phase (early stage 4) and the cells of this layer synchronously lose their nuclei in most squamates (but asynchronously in black tegus) during the late renewal phase (stage 5). This layer dehydrates (cornifies) during stage 6 of the shedding cycle. While Alexander and Parakkal (1969) stated that the beta layer does not cornify completely until several days after shedding, Maderson et al. (1972) and Roth and Jones (1970) did not observe any significant ultrastructural changes in the beta layer after shedding.

The alpha layer begins to be formed during the late renewal phase but usually does not show clear signs of keratinization until just before shedding. Maturation of the alpha layer is never synchronous and it is rarely completed until several days after shedding.

2. Evidence as to the Location of the Permeability Barrier Emanating from Studies on CWL Throughout the Shedding Cycle

The rate of CWL decreases for several days after shedding. During this same period, the alpha layer reaches maturity as a result of the continual production and differentiation of presumptive alpha cells. Occasionally in pathological conditions the outer gen-

eration is not shed at its usual time in relation to the maturation of the inner epidermal generation. When this occurs, the epidermis has a stage 6 histology in the sense that the outer generation remains in situ, but the inner generation matures to a "perfect resting condition", as the rate of CWL decreases. If the outer (old) generation is removed (or naturally sheds) CWL is not affected. Such observations stress the importance of the new alpha layer as the permeability barrier.

The outer generation is moist in those animals which remove it in one or a few large pieces. The outer generation is brittle in a delayed shed condition because the epidermal barrier formed under it, and prevented the underlying moisture (from the body tissues) replenishing that lost by evaporation.

With respect to the condition of the outer generation two extreme conditions can be envisaged.

At the time of shedding the outer generation is moist, indicating that the inner generation is immature. Therefore, CWL would be high both before shedding (when the old generation is "leaky" and the new generation is immature) and after shedding when the new generation is still immature. A high rate of CWL might not be disadvantageous under certain conditions; if water is plentiful, if the length of time the animal is incapacitated during shedding is reduced, or if some specialization (for example, climbing footpads) is dependent on the synchronous (on all four limbs) appearance of the new generation (Maderson, 1970).

The other extreme condition is that at the time of shedding the outer generation is dry, this means that the inner generation

started to mature well before the outer generation became "leaky". Thus the rate of CWL would be low and water would be economized. This strategy would seem to be appropriate in the reverse situations to those described above.

In tokays and some other squamates the permeability of the outer generation increases at about the time of shedding while that of the inner generation decreases until the inner generation is less permeable than the outer generation. Thereafter the outer generation loses more moisture than it receives through the inner generation, and consequently dehydrates. It is possible that dehydration of the constituent cells of the clear layer/Oberhautchen complex facilitates their eventual separation and brings about shedding (Maderson, 1970).

D. KERATIN HYDRATION

Experiments show (p. 68) that in tokays, the alpha layer swells when the animal is immersed in water within the first few days after shedding. Since all microscopic indications were that the beta layer was mature at the time of shedding, such experiments indicate that the water reached the alpha layer and hydrated it only if it was in the immature state. If water reached the alpha layer after shedding the beta layer was not an effective barrier. If water reaches the mature alpha layer several days after shedding, the rate of CWL and the alpha layer are not affected. Likewise the resistance of the human stratum corneum decreases when it is hydrated (Scheuplein, 1972).

E. STRIPPING STUDIES

1. Introduction

As shown in figure 67, the effects of cellophane stripping on CWL essentially resemble that which has been described in mammals. Immediately following stripping there is a sharp increase, and then CWL returns gradually to pre-trauma levels. When tokays are stripped during the resting phase the results agree most closely with data available for man (Eriksson and Lamke, 1971; see fig. 67), but if tokays are stripped during the renewal phase, the physiological effectiveness of the barrier is more slowly restored. The pattern for the caiman is rather erratic. Although at first sight the data for tegus seem erratic, in fact the curve describing restoration is approximately the same as that for tokays in the resting phase, though there is a lag period of about 3 days, during which the rate of CWL continues to increase after stripping. These apparent anomalies assist our interpretation of the extent to which the physiological data correlate with the morphological data, and thereby permit an identification of the normal barrier in reptile skin.

2. Caiman

In mammalian material, the stimulated germinal proliferation and subsequent differentiation of daughter cells accompanying restoration of the barrier, involves only the synthesis of alpha-type protein. Although there are some initial indications of parakeratosis, the restoration of the barrier in mammals seems to involve merely rapid production and differentiation of cells.

The caiman data essentially resemble the mammalian data except that the corneous cells of the outer scale surface synthesize

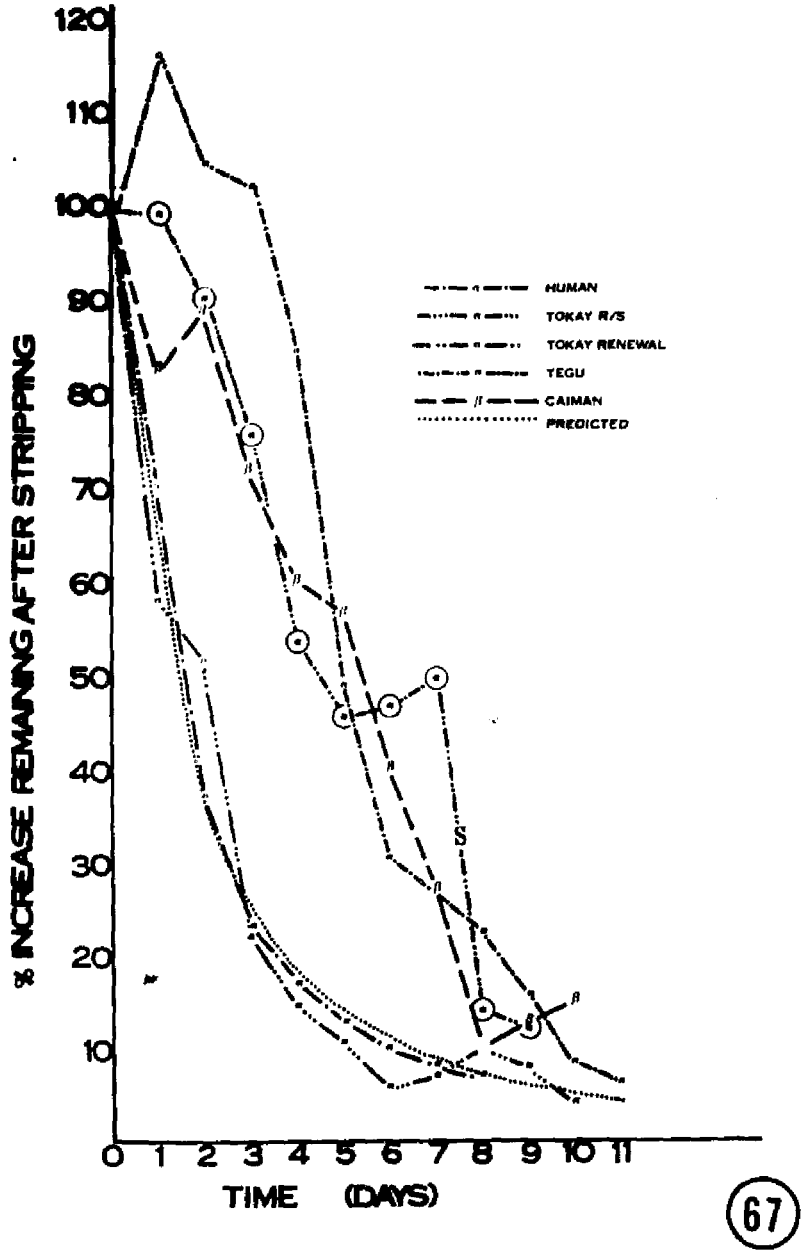

% INCREASE REMAINING AFTER STRIPPING
120
110
100
90
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9 10 11
TIME (DAYS)
HUMAN
TOKAY R/S
TOKAY RENEWAL
TEGU
CAIMAN
PREDICTED

Figure 67. The change in the percent increase in CWL remaining after stripping with time, for several experimental studies and a theoretical one.

feather-type fibrous proteins (Baden and Maderson, 1970). The thinning of the tissues by stripping apparently decreases the effectiveness of the barrier. The barrier is restored by an accelerated production and differentiation of cells whose histology resembles that of normal tissues. Therefore the conclusion can be reached that the feather-protein containing cells of the corneous tissues of caiman epidermis constitute the normal barrier against CWL in an exactly analogous fashion as do the alpha-protein containing cells of mammalian stratum corneum. The available morphological data are insufficient to explain the slower, somewhat erratic process of barrier restoration in caimans. Explanation must await information concerning the control of epidermal proliferation and differentiation in this reptile.

3. Squamates

In squamates, the important question is, to what extent do some or all of the various corneous tissues act as physiological barriers? In the light of the inherent variability of the stripping technique when applied to the squamate integument, and also certain morphological and physiological variants which have been reported here between individuals and species, we must approach an analysis of the present data with caution.

The rate of CWL is inversely proportional to the resistance the barrier offers to the passage of water (Scheuplein and Blank, 1971). Since the resistance of the barrier is directly related to its thickness, this relationship can be expressed as:

$$CWL \propto \frac{1}{T} \text{ or } CWL = \frac{K}{T} \qquad (4)$$

where K is the permeability constant of the barrier to water and T is thickness. If we assume that the barrier is homogeneous and a lamellate structure, and that stripping removes 95% of its thickness, the increased CWL after stripping is given by:

$$\frac{K}{0.05T} - \frac{K}{T} = \frac{19K}{T} \qquad (5)$$

Let us now assume that 2.5% of the original thickness is restored during the first 24 hrs post-strip. The percent increase in CWL remaining after 24 hrs is given by:

$$\frac{\frac{K}{0.075} - \frac{K}{T}}{\frac{19K}{T}} \times 100 \text{ or } \frac{12.3K}{T} \times \frac{T}{19K} \times 100 = 64.9\% \qquad (6)$$

Assuming that the rate of morphological restoration improves during the second day post-strip, and thereafter, so that 5% of the original thickness is restored per day, the percent of increased CWL remaining on any day is given by:

$$\frac{\frac{1}{0.025 + 0.05\,n} - 1}{19} \times 100 \qquad (7)$$

where n is the number of days after stripping.

The "predicted" line in fig. 67 is drawn through points obtained by the formula just described. The curve fits two sets of data almost perfectly: first, those describing the recovery of the tokay epidermis stripped during the resting phase, and second, some human data (Eriksson and Lamke, 1971). The rapidity of restoration of these tokays may be explained by the fact that this is the time in the cycle when such proliferation and differentiation as does take place is associated with the production and maturation of alpha-

protein containing cells (Chiu and Maderson, 1975). What then of the other squamate data in figure 67?

No explanation can be offered as to why CWL increases during the three days following stripping in black tegus, but the lag in restoration of physiological effectiveness of this system is correlated with a lag phase in cytological events not observed in other lizards. Tegus are unusual in that there is no response in the living epidermal cells until three days after stripping. However, the first decrease in CWL coincides with the first histological changes. Thereafter CWL continues to decrease, essentially following the same pattern as that for the resting phase tokays, as the alpha tissue becomes thicker. Thus while the lag in cytological response cannot be explained, it seems legitimate to emphasize that amelioration of the physiological trauma is associated with the maturation of alpha-protein containing cells.

Restoration of physiological effectiveness after stripping of the skin during the renewal phase is neither as dramatic nor as rapid, as that seen in the resting phase experiments. This can be explained as follows. The alpha-like tissue resulting from metaplasia is not as thick as that which is formed by resting phase animals. It appears that although the metaplasia does ameliorate the physiological trauma, it is imperfect. However, if we follow the pattern of restoration further, we see that eventually the animal sheds. Under normal circumstances there is an increase in CWL around the time of shedding (see p. 213) and this probably disguises the restoration to some extent. Once the shed has occurred, the next measurement is very much lower. Thus the animals, although much dif-

ferent than those stripped in the resting phase, underscore the relationship between alpha tissue and barrier effectiveness. The initial response to trauma is the production of alpha-like tissue by metaplasia of other presumptive cell types. Then, although delayed, differentiation of an inner epidermal generation occurs normally, and when this latter acquires its own, normal alpha layer, physiological effectiveness is finally restored.

The preponderance of evidence thus indicates that the alpha layer is the main permeability barrier in the squamate epidermis. However, if this is so, why does the rate of CWL increase dramatically after the beta layer is stripped off?

Following forceps or cellophane stripping, there is always a degree of buckling of the outer scale surface. This is interpreted as indicating that the beta layer has a dual mechanical function: not only does it protect the underlying tissues from abrasion, but it also facilitates the maintenance of scale shape. The forces against which the beta layer acts must have their origin in the complex arrangement of interlocking vertical and horizonal collagen struts and pillars which characterize the squamate dermis. When the beta layer is removed, the resultant disturbance of the equilibrium of forces within the scale causes a distortion of the outer scale surface. This distortion disrupts the architecture of the alpha layer, thus causing a sudden increase in CWL. The control mechanisms for cyclic cell proliferation and differentiation depend upon the existence of normal corneous tissues (Flaxman et al., 1968): when these are disturbed, germinal proliferation results. The daughter cells produced undergo their patterns of differentiation within a

new environment of mechanical tensions, and the resultant alpha-hyperplastic material consists of cells which provide a satisfactory barrier. The phyisological effectiveness of the epidermis is thereby replaced.

This interpretation does not remove the possibility that the beta layer plays a partial role in providing a barrier in normal epidermis. The hypothesis suggested above is inherently untestable since no form of microscopic examination could reveal "cracks" between mature alpha cells, although if breakage occurs, such might be visible with electron-microscopic study.

SECTION IV. CWL DURING THE RENEWAL PHASE

Throughout the thesis the importance of the keratinized layers as barriers to water loss has been stressed. Also, the thickness of the barrier has been stressed as the most important determinant of CWL, for example in explaining the rate of CWL after shedding, the rate of CWL during the retained shed condition, and the rate of return of the permeability barrier after stripping. However, during the renewal phase of the normal shedding cycle, the outer keratinized layers do not change their thickness, and yet the rate of CWL increases. How can this phenomenon be explained?

In the integuments of most amniotes the production of epidermal cells and their keratinization is a constant process. The distance between the point where a cell dies and where it loses its attachments to neighboring living cells is relatively short. In addition, the integuments of most amniotes lack scales. However, in lepido-

saurs, the topographic relationship between the mature cornified elements and the germinal population which exists throughout the resting phase, is disrupted during the renewal phase by the production of a new inner epidermal generation. Furthermore, lepidosaurs frequently have small, curved (granular) scales. As the renewal process occurs, either the keratinized layers must be pushed outwards, or the germinal population must be pushed inwards: the former possibility will be examined first.

The situation for the alpha layer will be examined first since most of the evidence obtained in this thesis indicates that this is the main permeability barrier in squamates. The alpha layer is to an extent extensible, the exact amount depending on the moisture content of the keratin. However, if a large critical stress is applied, this layer would crack or become so distorted that its barrier properties would be compromised. Before the effects of alpha cracking on the rate of CWL can be analyzed, the predicted increase in skin surface area during the renewal phase must be estimated. The surface area of the alpha layer will increase most during growth if the scale is spherical.

Tokay gular biopsies representing typical stages of the shedding cycle were examined under oil immersion to determine the thickness of the alpha layer (table 39). For the resting phase, and the first stages of the renewal phase, the thicknesses of the beta layer, the alpha layer, and the subjacent immature cells were determined. During the mid and late renewal phases, the cells proximal to the outer alpha layer can be subdivided into three groups: the cells between the inner beta layer and the outer alpha layer (including the outer

Table 39. The thickness of the epidermal layers (in microns) during the shedding cycle.

Stage	No.	Bo	Ao	Living layers	Between Ao and Bi	Bi	Below Bi
PS	8	5.1	2.0	14.6			
pR	10	5.1	2.2	11.7			
3	7	8.0	2.8	17.4			
14	13	5.8	3.7		8.1	14.7	13.0
5	6	6.0	4.0		3.3	10.7	20.5

lacunar tissue, the outer clear layer, and the inner Oberhautchen); the inner beta layer; and the epidermal cells beneath the inner beta layer (including the presumptive mesos and alpha cells, and the stratum germinativum).

Several scales were projected and the outline of those scales which most closely fitted the arc of a circle were traced. A chord connecting the ends of the arc was drawn, a right angle bisector was constructed, and extended until it intersected the arc. The radius was found by approximation (mean 899.14 microns, n = 7) with the aid of a compass. The surface area of the corresponding sphere was determined ($4\pi r^2$). Since the distance between the basement membrane and the outer alpha layer during the perfect resting condition is 11.7 microns, and during stage 4 it is 35.8 microns, the increase in the length of the radius which describes the scale is 24.1 microns. The surface area of the late renewal scale is therefore 105.4% as great as during the resting condition. Thus the alpha layer must enlarge 5.4%. The same general conclusion was found by examining the thickness of various epidermal layers for a single individual for a single shedding cycle (table 40). Since none of these scales is actually a sphere, these estimates of stretching are exaggerated maxima.

If the alpha layer cracks, 5.4% of the scale will not be covered by the main barrier. Could such a small percent increase account for a doubling of the rate of CWL which is often found during the renewal phase?

The permeability of a spotted membrane is equal to the permeability of each type of surface multiplied by its fractional area of

Table 40. The thickness of the epidermal layers (in microns) during the shedding cycle of a single tokay.

Days post-shed	Days pre-shed	Epidermal stage	Bo	Ao	Living layers	Between Ao and Bi	Bi	Below Bi
2	23	PS	7	1	21			
5	20	pR	5	2	15			
8	17	lR		2	20			
11	14	e3	4	1	19			
15	10	m3	5	2	25			
18	7	e4	4	2		8	8	11
21	4	l4	5	1		5	14	16
23	2	5	4		3[a]		5	18.5
25	0	6	6	1	17			

a. Thickness of Ao + living layers

the membrane, or,

$$k_p \text{ total} = \int A_{cr} k_{pcr} + \int A_{\alpha} k_{p\alpha} \quad (8)$$

$$= \frac{\int A_{cr} \text{ X } D_{cr}}{\delta} + \frac{\int A_{\alpha} \text{ X } D_{\alpha} \text{ X } k_m}{\delta} \quad (9)$$

where "alpha" represents the normal barrier, and "cr" the places where it is cracked (for other abbreviations see p. 197).

If $A_{cr} = 0.05$, then $A_{\alpha} = 0.95$,

if $\delta = 2.5 \text{ X } 10^{-4}$ cm,

if $D_{\alpha} = 10^{-10}$ cm^2/sec (a typical mammalian value, see Scheuplein and Blank, 1971),

if $D_{cr} = 0.257$ cm^2/sec (diffusion coefficient of water vapor in air, see Monteith, 1973),

and if $k_m = 2800$ (a typical mammalian value, see Scheuplein and Blank, 1971), then:

normal $k_p = 1.12 \text{ X } 10^{-2}$ cm/hr.

The permeability coefficient if 5% of the barrier is cracked is:

$k_{p\ (cr\ +\ \alpha)} = 5.14 \text{ X } 10^{1}$ cm/hr,

then the increase in CWL would be $4.59 \text{ X } 10^{3}$.

CWL does not increase 4000 times during the renewal phase, since these calculations are based on the assumption that scales are basically spherical, and probably because the alpha layer is not the only barrier. The important point is however that keratin is so impermeable, that if it is missing, even in only a few regions, CWL increases dramatically.

Although there is evidence that the clear layer cracks just before shedding (Maderson, Mayhew, and Sprague, 1970, fig. 8), there is no direct evidence that the alpha layer cracks. If we assume that

the putative cracks are small - possibly one micron or less, then they would have a dramatic effect on CWL. Scheuplein (personal communication) remarked that the hypothesized cracked alpha layer might be thought of as a piece of sintered glass.

Even if the postulated amount of stretching does not cause the alpha layer, and/or its constituent cells to crack, it might cause changes in their macromolecular organization (filament-lipid matrix) thus influencing their permeability coefficients. Such macromolecular changes could certainly account for the degree of increase in CWL which appears to characterize the renewal phase.

Another possibility is that during the renewal phase the germinal population might move inwards as new daughter cells are produced. This would mean that the increase in epidermal height during the renewal phase is accompanied by a decrease in the size of the dermis, either by condensation, reabsorption, cross-sliding (as in the muscle fiber), vasoconstriction, or moisture loss. A decrease in the size of the dermis is especially appealing if the beta layer acts as an unstretchable brace. The fact that the removal of the beta layer causes the scale to buckle indicates that normally the dermis would tend to contract and does so when the beta layer's hold on the scale is relaxed by some disturbance. Such a disturbance might be brought about by the production of new cells during the renewal phase which would allow the dermis to decrease in size.

Dermal resorption in association with shedding has been described by Zimmerman and Pope (1948) as an essential step in the formation of the rattle of rattlesnakes. Although they did not interpret the formation of the rattle with respect to epidermal cellular

dynamics during the shedding cycle, their illustrations indicate what probably happens. At the time of shedding the dermis of the old distal lobe of the end body (of the tail) is reabsorbed and its overlying beta layer shrinks as it cornifies. At the same time the elements of the outer generation begin to disintegrate, except for the beta layer which forms the rattle proper.

Reabsorption during the renewal process has also been advocated as the possible mechanism involved in the formation of the free margin of the scales in lizards and snakes (Jackson and Reno, 1975; Lillywhite and Maderson, 1968). In this case reabsorption would occur after the cells which make up the free margin were produced, but before they solidified, that is between stages 3 and 6.

If a small amount of reabsorption occurs during the renewal phase, then the dermis would have to expand just after shedding, otherwise after several shedding cycles the dermis would disappear. After shedding the cornified layers would solidify on the expanded dermal template. If the expansion occurred just before shedding, it might cause the "unzipping" of the clear layer/Oberhautchen complex (Maderson, 1966, 1970), and it would be the first mechanical event of shedding.

During a normal shedding cycle, dermal expansion does not necessarily imply dermal accretion as described by Zimmerman and Pope (1948) for rattle formation. Stretching might be accomplished by vasodilation. Although there have been no detailed studies relating cutaneous blood flow to shedding, the possibility has been raised by Bruner (1907), and the characteristic immigration of blood cells during the snake renewal phase (Maderson, 1965b) obviously involves the

vasculature in some way.

If dermal regression and expansion normally occur during the shedding cycle, then the formation of the free margin in some species, and the evolution of the mechanism permitting rattle formation in crotalids, would represent utilizations of a universal phenomenon in lepidosaurs.

Could changes in dermal structure during the renewal phase be the direct cause of the increase in CWL?

It is possible that the regression of the dermis might change the forces acting on the alpha layer (which is normally stabilized by the opposing actions of the beta layer and the dermis). Such a change in forces acting on the alpha layer might disrupt the barrier, and has been invoked as an interpretation of the sudden rise in CWL following removal of the beta layer (p. 244).

If there is dermal regression during the normal renewal phase, yet another explanation could be offered for the measured increase in CWL at this time. Although there is little doubt that CWL increases in tokays during the renewal phase (see pp. 214-220), this does not necessarily imply that the permeability of the integument changes uniformly. It is possible that merely handling the animal while it is in the renewal phase causes cracks to form in the outer generation: such cracks might not appear on all scales, they might be large or small, and they might be randomly distributed across the body surface. Such an explanation would be in accord with the data reported in table 27, which in general shows that shed material measured <u>in vitro</u> is not highly permeable, although it is occasionally more permeable than the inner generation. Such a hypothesis is sup-

ported by Dunson and Robinson's (1976) finding that the shed skin of sea snakes was sometimes very permeable, though otherwise impermeable to water. They feel that the shed material which was very permeable had holes in it. On all occasions when tegu shed material was measured in vitro, and was found to have cracks, a very high rate of CWL was recorded.

Finally, the observed increase in CWL during the renewal phase might be due to other factors. The data presented on page 40 and in tables 12 and 13 suggest that squamates can regulate their rate of CWL in some unknown fashion, and it must be recalled that debate still exists on this point in mammalian studies (see pp. 2-3).

BIBLIOGRAPHY

Ainsworth, M. (1960). Methods for measuring percutaneous absorption. J. Soc. Cosmet. Chem. 11: 69-78.

Alexander, N. J. (1970). Comparison of alpha and beta keratin in reptiles. Z. Zell. Mik. Anat. 110: 153-165.

Alexander, N. J., and P. F. Parakkal (1969). Formation of α and β type keratin in lizard epidermis during the molting cycle. Z. Zell. Mik. Anat. 101: 72-87.

Baden, H. P., and P. F. A. Maderson (1970). Morphological and biophysical identification of fibrous proteins in the amniote epidermis. J. Exper. Zool. 174: 225-232.

Baker, H.,and A. M. Kligman (1967). Measurement of transepidermal water loss by electrical hygrometry. Arch. Dermatol. 96: 441-452.

Bartek, M. J., J. A. LaBodde, and H. I. Maibach (1972). Skin permeablity in vivo: comparison in rat, rabbit, pig, and man. J. Investig. Dermatol. 58: 114-123.

Bartlett, P. N., and D. M. Gates (1967). The energy budget of a lizard on a tree trunk. Ecology 48: 315-322.

Baverstock, P. R. (1975). Effects of variations of growth rate on physiological parameters in the lizard Amphibolorus ornatus. Comp. Biochem. Physiol. 51A: 619-631.

Benedict, F. G. (1932). The physiology of large reptiles. Carnegie Inst. Wash. Pub. #425, 539 pp.

Bennett, A. F., and P. Licht (1975). Evaporative water loss in scaleless snakes. Comp. Biochem. Physiol. 52A: 213-215.

Bentley, P. J. (1971). Endocrines and Osmoregulation. Springer-Verlag, New York, 330 pp.

Bentley, P. J., and K. Schmidt-Nielsen (1966a). Cutaneous water loss in reptiles. Science 151: 1544-1549.

Bentley, P. J., and K. Schmidt-Nielsen (1966b). Cutaneous water loss in reptiles (a reply). Science 152: 1523.

Bernstein, M. H. (1969). Cutaneous and respiratory evaporation in painted quail, Excalfactoria chinensis. Amer. Zool. 9: 1099.

Bernstein, M. H. (1971a). Cutaneous and respiratory evaporation in painted quail, Excalfactoria chinensis during ontogeny of ther-

moregulation. Comp. Biochem Physiol. 38: 611-619.

Bernstein, M. H. (1971b). Cutaneous water loss in small birds. Condor 73: 468-469.

Bettley, F. R. (1970). The epidermal barrier and percutaneous absorption. In: Introduction to Biology of Skin. Ed. R. H. Champion, T. Gillman, A. J. Rook, and R. T. Sims, pp. 342-354.

Bettley, F. R., and K. A. Grice (1965). A method for measuring the transepidermal water loss, and a means of inactivating sweat glands. Brit. J. Dermatol. 77: 627-637.

Bogert, C. M., and R. B. Cowles (1947). Moisture loss in relation to habitat selection in some Floridian reptiles. Amer. Mus. Novit. 1358: 1-34.

Bouverot, P., G. Hildwein, and D. LeGuff (1974). Evaporative water loss, respiratory pattern, gas exchange and acid base balance during thermal panting in Peking ducks exposed to moderate heat. Respir. Physiol. 21: 255-269.

Bradshaw, S. D. (1970). Seasonal changes in the water and electrolyte metabolism of Amphibolorus lizards in the field. Comp. Biochem. Physiol. 36: 689-719.

Bradshaw, S. D., V. H. Shoemaker, and K. A. Nagy (1972). The role of adrenal corticosteroids in the regulation of kidney function in the desert lizard Dipsosaurus dorsalis. Comp. Biochem. Physiol. 43A: 621-635.

Bruner, H. L. (1907). On the cephalic veins and sinuses of reptiles, with description of a mechanism for raising the venous blood pressure in the head. Am. J. Anat. 7: 1-117.

Bryant, S. V., A. S. Breathnach, and A. D'A. Bellairs (1967). Ultrastructure of the epidermis of the lizard (Lacerta vivipara) at the resting stage of the sloughing cycle. J. Zool. (Lond.) 152: 209-219.

Burch, G. E., and T. Winsor (1946). Rate of insensible perspiration locally through living and dead human skin. Arch. Intern. Med. 74: 437-444.

Bustard, H. R., and P. F. A. Maderson (1965). The eating of shed epidermal material in squamate reptiles. Herpetologica 21: 306-308.

Cane, A. K., and R. I. C. Spearman (1967). A histolchemical study of keratinization in the domestic fowl (Gallus gallus). J. Zool. (Lond.) 153: 337-352.

Cena, K., and J. L. Monteith (1975). Transfer properties in animal coats. III. Water vapor diffusion. Proc. Roy. Soc. Lond. B 188: 413-423.

Chew, R. M. (1955). The skin and respiratory water loss of Peromyscus maniculatus sonoriensis. Ecology 36: 463-467.

Chew, R. M. (1961). Water metabolism of desert inhabiting vertebrates. Bio. Revs. 36: 1-31.

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

Chiu, K. W., and W. G. Lynn (1970a). The effect of ACTH on the sloughing frequency of normal and thyroidectomized Anolis carolinensis (iguanidae, lacertilia). Bio. Bull. 138: 129-137.

Chiu, K. W., and W. G. Lynn (1970b). The role of the thyroid in skin-shedding in the shovel-nosed snake, Chionactis occipitalis. Gen. Comp. Endo. 14: 467-474.

Chiu, K. W., and P. F. A. Maderson (1975). The microscopic anatomy of epidermal glands in two species of gekkonine lizards, with some observations on testicular activity. J. Morph. 147: 23-40.

Claussen, D. C. (1967). Studies of water loss in two species of lizard. Comp. Biochem. Physiol. 20: 115-129.

Cloudsley-Thompson, J. L. (1967). Water relations and diurnal rhythm of activity in the young Nile monitor Varanus niloticus (L.). Brit. J. Herpet. 3: 296-300.

Cloudsley-Thompson, J. L. (1969). Water relations of the young Nile crocodile. Brit. J. Herpet. 4: 107-112.

Cloudsley-Thompson, J. L. (1971). The Temperature and Water Relations of Reptiles. Merrow, England, 159 pp.

Cohen, A. C. (1975). Some factors affecting water economy in snakes. Comp. Biochem. Physiol. 51A: 361-368.

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

Crawford, E. C., and K. Schmidt-Nielsen (1967). Temperature regulation and evaporative cooling in the ostrich. Am. J. Physiol. 212: 347-353.

Dawson, T. J., and A. J. Hulbert (1970). Standard metabolism, body temperature, and surface areas of some Australian marsupials.

Am. J. Physiol. 218: 1233-1238.

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

Diefenbach, C. O. da C. (1973). Integumentary permeability to water in Caiman crocodilus and Crocodylus niloticus (crocodilia: reptilia). Physiol. Zool. 46: 72-78.

Dmi'el, R. (1972). Effect of activity and temperature on metabolism and water loss in snakes. Am. J. Physiol. 223: 510-516.

Dunson, W. A., and G. D. Robinson (1976). Sea snake skin: permeable to water but not to sodium. J. Comp. Physiol. 108B: 303-311.

Elick, G. E., and J. A. Selander (1972). Comparative water loss in relation to habitat selection in small colubrid snakes. Am. Mid. Nat. 88: 424-439.

Eriksson, G., and L. O. Lamke (1971). Regeneration of human epidermal surface and water barrier function after stripping. Acta Dermatoven. 51: 169-178.

Felsher, Z., and S. Rothman (1945). Insensible perspiration of skin in hyperkeratotic conditions. J. Investig. Dermatol. 6: 271-278.

Fishelson, L. (1973). Observations on skin structure and sloughing in the stonefish Synaniegh verlim and related fish species as a functional adaptation to their mode of life. Zeit. Zell. Mik. Anat. 140: 497-508.

Flaxman, B. A., P. F. A. Maderson, G. Szabo, and S. I. Roth (1968). Control of cell differentiation in lizard epidermis in vitro. Devel. Biol. 18: 354-374.

Fraser, R. D. B., T. P. MacRae, and G. E. Rogers (1972). Keratins: Their Composition, Structure and Biosynthesis. Charles C. Thomas, Springfield, Illinois, 304 pp.

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

George, J. C. (1947). A comparative study of the permeability to water of the skin of some representative vertebrates. J. Univ. Bombay 16: 28-32.

Goodman, A. B., and A. V. Wolf (1969). Insensible water loss from human skin as a function of ambient vapor concentration. J. Appl. Physiol. 26: 203-207.

Grice, K., H. Salter, M. Sharratt, and H. Baker (1971). Skin tem-

perature and transepidermal water loss. J. Investig. Dermatol. 57: 108-110.

Grice, K. H. Sattar, and H. Baker (1972). The effect of ambient humidity on transepidermal water loss. J. Investig. Dermatol. 58: 343-346.

Haines, H., and C. F. Shield (1971). Reduced evaporation in house mice (Mus musculus) acclimated to water restriction. Comp. Biochem. Physiol. 39A: 53-61.

Haines, H., C. Ciskowski, and V. Harms (1973). Acclimation to water restriction in the wild house mouse, Mus musculus. Physiol. Zool. 46: 110-128.

Haines, H., W. V. MacFarlane, C. Setchell, and B. Howard (1974). Water turnover and pulmocutaneous evaporation of Australian desert dasyurids and murids. Am. J. Physiol. 227: 958-963.

Hattingh, J. (1972a). A comparative study of transepidermal water loss through the skin of various animals. Comp. Biochem. Physiol. 43A: 715-718.

Hattingh, J. (1972b). The correlation between transepidermal water loss and the thickness of epidermal components. Comp. Biochem. Physiol. 43A: 719-722.

Hattingh, J. (1972c). The influence of blood flow on transepidermal water loss. Acta Dermatol. 53: 365-370.

Hattingh, J. (1973). The relationship between skin structure and transepidermal water loss. Comp. Biochem. Physiol. 45A: 685-688.

Hemmingsen, A. M. (1960). Energy metabolism related to body size and respiratory surfaces, and its evolution. Copenhagen Reports of the Steno Memorial Hospital and Nordisk Insulin Laboratorium 9: 7-109.

Horton, D. R. (1972). Lizard scales and adaptation. Sys. Zool. 21: 441-443.

Hudson, J. W., D. P. Deavers, and S. R. Bradley (1972). A comparative study of temperature regulation in ground squirrels with special reference to desert species. In: Comparative Physiology of Desert Animals. Ed. G. M. O. Maloiy, pp. 191-213.

Hulbert, A. J., and T. J. Dawson (1974). Water metabolism in perameloid marsupials from different environments. Comp. Biochem. Physiol. 47A: 617-633.

Hulbert, A. J., and R. W. Rose (1972). Does the devil sweat? Comp. Biochem. Physiol. 43A: 219-222.

Hurlbert, S. H. (1966). Cutaneous water loss in reptiles. Science 152: 1523.

Idson, B. (1973). Water and the skin. J. Soc. Cosmet. Chem. 24: 197-212.

Jackson, M. K., and H. W. Reno (1975). Comparative skin structure of some fossorial and subfossorial leptotyphloid and colubrid snakes. Herpetologica 31: 350-359.

Jelenko, C. (1967). Studies in burns 1. Water loss from the body surface. Ann. Surg. 165: 83-96.

Jenkins, H. L., and J. Treise (1969). An adhesive tape stripping technique for epidermal histology. J. Soc. Cosmet. Chem. 20: 451-466.

Johns[illegible], C., and S. Shuster (1969). The measurement of transepidermal water loss. Brit. J. Dermatol. 81, Supp. 4: 40-46.

Khalil, F., and G. Abdel-Messeih (1954). Water content of tissues of some desert reptiles and mammals. J. Exper. Zool. 125: 407-414.

Khalil, F., and G. Abdel-Messeih (1959a). Water, nitrogen and lipids content of tissues of Varanus griseus Daud. Z. Verg. Physiol. 42: 403-409.

Khalil, F., and C. Abdel-Messeih (1959b). The storage of extra water by various tissues of Varanus griseus Daud. Z. Verg. Physiol. 42: 415-421.

Khalil, F., and G. Abdel-Messeih (1961). The storage of extra water by various tissues of Uromastix aegyptia (Forskal). Z. Verg. Physiol. 45: 78-81.

Khalil, F., and G. Abdel-Messeih (1962). Tissue constituents of reptiles in relation to their mode of life 1. Water content. Comp. Biochem. Physiol. 5: 327-330.

Klauber, L. M. (1956). Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind. Univ. Calif. Press, Berkeley, 1476 pp.

Kleiber, M. (1961). The Fire of Life. Wiley, New York, 454 pp.

Kligman, A. M. (1964). The biology of the stratum corneum. In: The Epidermis. Ed. W. Montagna and W. Lobitz. pp. 387-433.

Krakauer, T. (1970). The ecological and physiological control of water loss in snakes. Dissertation, U. Fla., 56 pp.

Krakauer, T., C. Gans, and C. V. Pagnelli (1968). Ecological corre-

lation of water loss in burrowing reptiles. Nature 218: 659-660.

Kropach, C., and J. D. Soule (1973). An unusual association between an ectoproct and a sea snake. Herpetologica 29: 17-19.

Lamke, D. O., and B. Wedin (1971). Water evaporation from normal skin under different environmental conditions. Acta Dermatoven. 51: 111-119.

Lasiewski, R. C., A. L. Acosta, and M. H. Bernstein (1966a). Evaporative water loss in birds 1. Characteristics of the open flow method for determination and their relation to stimulates of thermoregulatory ability. Comp. Biochem. Physiol. 19: 445-457.

Lasiewski, R. C., A. L. Acosta, and M. G. Bernstein (1966b). Evaporative water loss in birds 2. A modified method for determination by direct weighing. Comp. Biochem. Physiol. 19: 459-470.

Lasiewski, R. C., M. H. Bernstein, and R. D. Ohmart (1972). Cutaneous water loss in the roadrunner and poorwill. Condor 73: 470-472.

Lee, P., and K. Schmidt-Nielsen (1971). Respiratory and cutaneous evaporation in the zebra finch: effect on water balance. Am. J. Physiol. 220: 1598-1605.

Licht, P., and A. F. Bennett (1972). A scaleless snake: tests of the role of reptilian scales in water loss and heat transfer. Copeia 1972: 702-707.

Lillywhite, H. B., and P. F. A. Maderson (1968). Histological changes in the epidermis of the subdigital lamellae of Anolis carolinensis during the shedding cycle. J. Morph. 125: 379-402.

Machin, J. (1969). Passive water movements through the skin of the toad Bufo marinus in air and in water. Am. J. Physiol. 216: 1562-1568.

MacMillan, R. E., and A. K. Lee (1967). Australian desert mice: independence of exogenous water. Science 158: 383-385.

Maderson, P. F. A. (1965a). The structure and development of the squamate epidermis. In: Biology of Skin and Hair Growth. Ed. A. G. Lyne and B. F. Short, pp. 129-153.

Maderson, P. F. A. (1965b). Histological changes in the epidermis of snakes during the sloughing cycle. J. Zool. (Lond.) 146: 98-113.

Maderson, P. F. A. (1966). Histological changes in the epidermis of the tokay (Gekko gecko) during the sloughing cycle. J.

Morph. 119: 39-50.

Maderson, P. F. A. (1968). Observations on the epidermis of the tuatara (Sphenodon punctatus). J. Anat. 103: 311-320.

Maderson, P. F. A. (1970). Lizard hands and lizard glands: models for evolutionary study. Forma et Functio 3: 179-204.

Maderson, P. F. A. (1972a). Why? When? and How?: some speculations on the evolution of the vertebrate integument. Amer. Zool. 12: 159-171.

Maderson, P. F. A. (1972b). On how an archosaurian scale might have given rise to an avian feather. Amer. Nat. 106: 424-428.

Maderson, P. F. A., and P. Licht (1967). Epidermal morphology and sloughing frequency in normal and prolactin treated Anolis carolinensis (iguanidae, lacertilia). J. Morph. 123: 157-172.

Maderson, P. F. A., and S. I. Roth (1972). A histological study of the early stages of wound healing in lizards in vivo and in vitro. J. Exper. Zool. 180: 175-186.

Maderson, P. F. A., K. W. Chiu, and J. G. Phillips (1970a). Endocrine-epidermal relationships in squamate reptiles. In: Hormones and the Environment. Mem. Soc. Endo. Ed. G. K. Benson and J. G. Phillips. 18: 259-284.

Maderson, P. F. A., K. W. Chiu, and J. G. Phillips (1970b). Changes in the epidermal histology during the sloughing cycle in the rat snake Ptyas kerrus Schegel, with correlated observations on the thyroid gland. Bio. Bull. 139: 304-312.

Maderson, P. F. A., W. W. Mayhew, and G. Sprague (1970). Observations on the epidermis of desert living iguanids. J. Morph. 130: 25-36.

Maderson, P. F. A., B. A. Flaxman, S. I. Roth, and G. Szabo (1972). Ultrastructural contributions to the identification of cell types in the lizard epidermal generation. J. Morph. 136: 191-210.

Mali, J. W. H. (1956). The transport of water through the human epidermis. J. Investig. Dermatol. 27: 451-469.

Malkinson, F. D. (1964). Permeability of the stratum corneum. In: The Epidermis. Ed. W. Montagna and W. Lobitz. pp. 435-452.

Matoltsy, A. G., and T. Huszar (1972). Keratinization of the reptilian epidermis: An ultrastructural study of turtle skin. J. Ultra. Res. 38: 87-101.

Matoltsy, A. G., A. Schrogger, and M. Matoltsy (1962). Observations

on the regenerating skin barrier. J. Investig. Dermatol. 38: 251-253.

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

Monteith, J. L. (1973). Principles of Environmental Physics. American Elsevier, New York, 241 pp.

Morgareidge, K. R., and H. T. Hammel (1975). Evaporative water loss in box turtles: effects of rostral brain stem and other temperatures. Science 187: 366-368.

Munsey, L. D. (1972). Water loss in five species of lizards. Comp. Biochem. Physiol. 43A: 781-794.

Neuman, C., A. E. Cohn, and G. E. Burch (1941). A quantitative method for the measurement of the rate of water loss from small areas, with results of finger tip, toe tip and postero-superior portion of the pinna of normal resting adults. Am. J. Physiol. 132: 748-756.

Norris, K. S. (1967). Color adaptation in desert reptiles and its thermal relationships. In: Lizard Ecology: A Symposium. Ed. W. W. Milstead. pp. 162-229.

Onken, H. D., and C. A. Moyer (1963). The water barrier in human epidermis. Arch. Dermatol. 87: 584-590.

Parakkal, P. F., and N. J. Alexander (1972). Keratinization, a Survey of Vertebrate Epithelia. Academic Press, New York, 59 pp.

Pettus, D. (1958). Water relationships in Natrix sipedon. Copeia 1958: 207-211.

Pinson, E. A. (1942). Evaporation from human skin with sweat glands inactivated. Am. J. Physiol. 137: 492-503.

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

Rieger, M. M., and D. E. Deem (1974). Skin moisturizers: methods for measuring water regain, mechanical properties, and transepidermal moisture loss of stratum corneum. J. Soc. Cosmet. Chem. 25: 239-252.

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

Rose, F. L. (1969). Desiccation rates and temperature relationships of Terrapene ornata following scute removal. Southwest Nat. 14: 67-72.

Roth, S. I., and W. A. Jones (1967). The ulstrastructure and enzymatic activity of the boa constrictor (Constrictor constrictor) skin during the resting phase. J. Ultra. Res. 18: 304-323.

Roth, S. I., and W. A. Jones (1970). The ultrastructure of epidermal maturation in the skin of the boa constrictor (Constrictor constrictor). J. Ultra. Res. 32: 69-93.

Ruibal, R. (1968). The ultrastructure of the surface of lizard scales. Copeia 1968: 698-703.

Scheuplein, R. J. (1972). Properties of the skin as a membrane. In: Advances in Biology of the Skin, Vol. 12: Pharmacology of the Skin. Ed. W. Montagna, E. J. Van Scott, and R. B. Stoughton. pp. 125-152.

Scheuplein, R. J., and I. H. Blank (1971). Permeability of skin. Physiol. Rev. 51: 702-747.

Schmid, W. D. (1972). Nocturnalism and variance in ambient vapor pressure of water. Physiol. Zool. 45: 302-309.

Schmidt-Nielsen, K. (1969). The neglected interface: the biology of water as a liquid gas interface. Quart. Rev. Biophys. 2: 283-304.

Schmidt-Nielsen, K. (1973). How Animals Work. Cambridge Univ. Press, London, 114 pp.

Schmidt-Nielsen, K., and P. J. Bentley (1966). Desert tortoise, Gopherus agassizii: cutaneous water loss. Nature 250: 489-490.

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

Shkolnik, A., and A. Borut (1969). Temperature and water relations in two species of spiny mice (Acomys). J. Mammal. 50: 245-255.

Smith, R. M., and R. Suthers (1969). Cutaneous water loss as a significant contribution to temperature regulation in heat stressed pigeons. Physiologist 12: 358.

Snyder, G. K. (1971). Adaptive value of a reduced respiratory metabolism in a lizard, a unique case. Respir. Physiol. 13: 90-101.

Sokal, R. R., and F. J. Rohlf (1973). Introduction to Biostatistics. W. H. Freeman, San Francisco, 368 pp.

Sokolov, W. (1962). Skin adaptations of some rodents to life in the desert. Nature 193: 823-825.

Soule, M. (1966). Trends in the insular radiation of a lizard. Amer. Nat. 100: 47-64.

Soule, M., and W. C. Kerfoot (1972). On the climatic determination of scale size in a lizard. Sys. Zool. 21: 97-105.

Spearman, R. I. C., and P. A. A. Riley (1969). Comparison of the epidermis and pigment cells of the crocodilia with those of two lizard species. Zool. J. Linn. Soc. 48: 453-466.

Spotila, J. R., and E. N. Berman (1976). Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. Comp. Biochem. Physiol. 55A: 407-411.

Spotila, J. R., O. H. Soule, and D. M. Gates (1972). Biophysics of alligator heat and climate spaces. Ecology 53: 1094-1102.

Spruit, D. (1970). The water barrier of stripped and normal skin. Dermatologica 141: 54-59.

Spruit, D. (1971). The diurnal variation of water vapor loss from the skin in relation to temperature. Brit. J. Dermatol. 84: 66-70.

Spruit, D., and H. E. Herweyer (1967). The ability of the skin to change its insensible perspiration. Dermatologica 134: 364-370.

Spruit, D., and K. E. Malten (1971). Water vapor loss and the skin barrier. Trans. St. John. Hosp. Dermatol. Soc. 57: 167-176.

Stettenheim, P. (1972). The integument of birds. In: Avian Biology, Vol. II. Ed. D. S. Farner and J. R. King. pp. 2-63.

Stewart, G. R., and R. S. Daniel (1972). Scales of the lizard Gekko gecko: surface structure examined with the scanning electron microscope. Copeia 1972: 252-257.

Stewart, G. R., and R. S. Daniel (1973). Scanning electron microscope of scales from different body regions of three lizard species. J. Morph. 139: 377-388.

Sweeney, T. M., and D. T. Downing (1970). The role of lipids in the epidermal barrier to water diffusion. J. Investig. Dermatol. 55: 135-140.

Szabo, G., P. F. A. Maderson, S. I. Roth, and R. M. Kostick (1973). Melanocyte activity in the epidermis of the boa constrictor (Constrictor constrictor) during the sloughing cycle. Anat. Rec. 176: 377-388.

Tercafs, R. R. (1963). Phenomenes de permeabilite au niveau de la peau des reptiles. Arch. Intern. Physiol. Biochem. 71: 318-320.

Tercafs, R. R., and E. Schoffeniels (1965). Phenomenes de permeabilite au niveau de la peau des reptiles. Ann. Soc. Roy. Zool. Bel. 96: 7-22.

Thiele, F. A. J., and K. G. van Senden (1966). Relationship between skin temperature and the insensible perspiration of the human skin. J. Investig. Dermatol. 47: 307-312.

Warburg, M. R. (1965). Studies on the water economy of some Australian frogs. Aus. J. Zool. 13: 317-330.

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

Weigand, D. A., and J. R. Gaylor (1973). Removal of stratum corneum in vivo: an improvement of the cellophane stripping technique. J. Investig. Dermatol. 60: 84-87.

Wills, J. T. (1972). Percutaneous absorption. In: Pharmacology of the Skin. Ed. W. Montagna, pp. 170-176.

Winston, P. W., and D. H. Bates (1960). Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237.

Yoshimura, H. (1964). Organ systems in adaptation: the skin. In: Handbook of Physiology, Section 4, pp. 109-131.

Zimmerman, A. A., and C. H. Pope (1948). Development and growth of the rattle of rattlesnakes. Fieldiana Zool. 32: 355-413.