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**Factors influencing the skin shedding phenomenon in the garter
snake *Thamnophis sirtalis***

Contard, Paul Christopher, Ph.D.

City University of New York, 1987

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FACTORS INFLUENCING THE SKIN SHEDDING
PHENOMENON IN THE GARTER SNAKE THAMNOPHIS SIRTALIS

by

Paul Contard

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.

1987

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Paul Christopher Contard

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

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Abstract

FACTORS INFLUENCING SKIN SHEDDING IN GARTER SNAKES

(THAMNOPHIS SIRTALIS)

by

Paul Contard

Advisor: Professor Paul F. A. Maderson

The skin shedding phenomenon in garter snakes (Thamnophis sirtalis) was studied. The actual "shedding" of the outer epidermal generation is preceded by the completion of a newly synthesized inner generation (the renewal phase). Onset of the epidermal renewal phase is marked by the appearance of a dullness over the animal's body, particularly the spectacle of the eye (the so-called cloudy eye phase). Epidermal biopsies were removed to determine the stage of the shedding cycle and for ultrastructural analysis. Both endogenous (ie. thyroid hormone) and exogenous (ie. environmental temperature and ambient relative humidity) factors were investigated utilizing both intact and thyroidectomized animals.

Exposure to constant high ambient temperature induced a single shed in a large majority of intact garter snakes. Repeat sheds occurred only in a few animals infected with skin fungus. A majority of intact animals shed when transferred directly to high constant temperature (28 oC) after having spent six, ten, or fourteen weeks at very low constant temperature (3 oC) .

Synchrony of onset of the renewal phase was observed among these animals.

Maintenance of intact animals under conditions of constant low relative humidity and high temperature (28 °C) also induced sheds in most experimental animals. A moderate degree of synchrony of onset of the renewal phase was observed in this group. Feeding appeared to have no effect on skin shedding in any of animals.

Renewal phases occurred in all thyroidectomized garter snakes maintained at temperatures of 21 °C and higher. The number of post-thyroidectomy renewal phases varied from 1 to 3. These animals appeared to lose the ability to actually "shed". While some continued to shed following the first two renewal phases, none of the animals did so following the third renewal phase. The outer epidermal generations appeared to stack one on top of the other. Thyroidectomized animals lost weight rapidly and died when original weight decreased by 20+%. Feeding appeared to have no effect on skin renewal, shedding, or viability in these animals.

Ultrastructural analysis of skin biopsies removed from these snakes revealed morphological defects in the organelles responsible for epidermal lipid synthesis (ie. mesosomes).

A new hypothesis is proposed suggesting an interaction between these endogenous and exogenous factors in the skin shedding phenomenon. Roles of epidermal lipid metabolism and cutaneous water loss are addressed.

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INTRODUCTION

SECTION I. ANATOMY OF THE VERTEBRATE EPIDERMIS

A. SQUAMATES

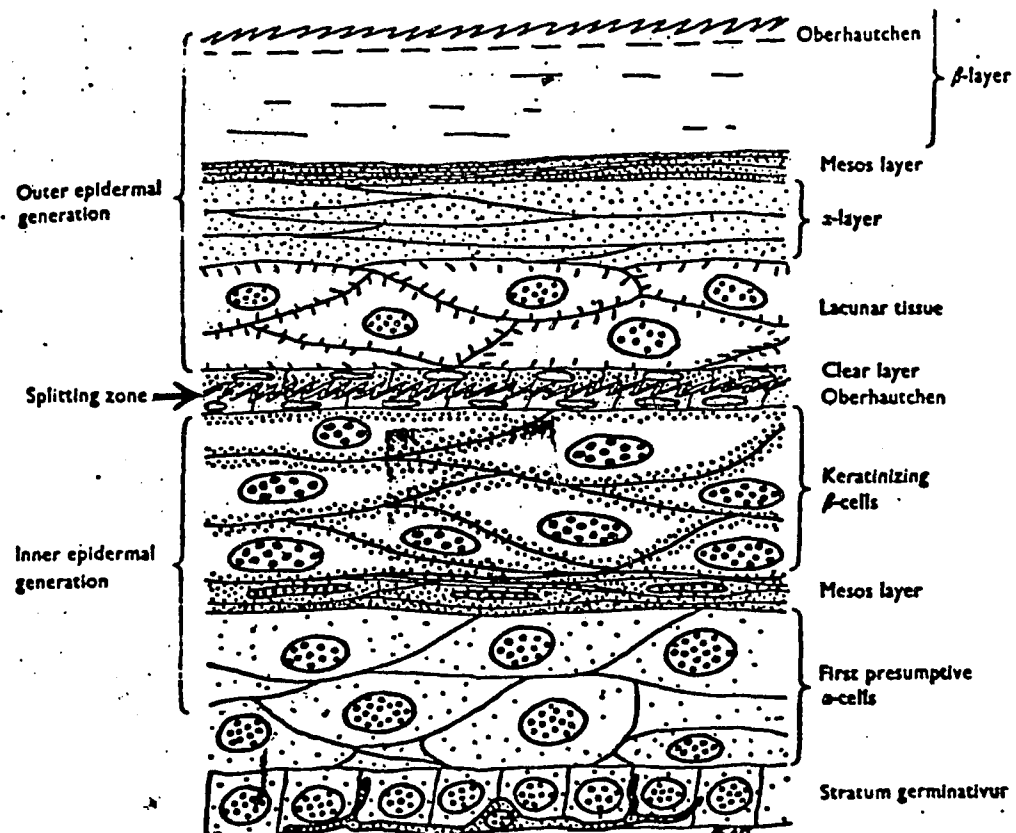
The squamate integument consists of a series of elevated scales continuous with one another at hinge regions. Each scale has an outer and an inner surface, the latter overlying the former in the contiguous series. (Maderson, 1965).

Squamate epidermis differs from that of mammals in that the latter undergoes continuous desquamation of dead, hardened stratum corneum cells, whereas the former demonstrates a periodic shedding of distinct epidermal generations (Maderson, 1965, 1966). The entire outer generation is removed as one piece (Maderson et al, 1970b).

Several layers of living and keratinized tissues, which derive from a stratum germinativum, characterize the epidermis (fig. 1). These layers, which constitute an epidermal generation, include, from without inward, the Oberhautchen, beta-layer, mesos layer, alpha layer, lacunar tissue, and clear layer. Periodically, the stratum germinativum shows increased levels of cell proliferation leading to the formation of an immature inner epidermal generation. The fully mature outer generation is then sloughed off.

During the renewal phase, just prior to shedding, a sequence of

FIGURE 1 Diagrammatic representation of squamate epidermis approximately 4-8 days prior to shedding. The outer generation is shed when the innermost cells (the clear layer) keratinize. At this time, the Oberhautchen, beta, mesos, and a small part of the alpha layer of the new inner generation have also matured. Figure is taken from Maderson (1967).



1

differentiative changes takes place in the epidermis. These changes have been described in detail at both light and electron microscopic levels (Alexander and Parakkal, 1969; Bryant et al, 1967; Chui and Maderson, 1975; Landman, 1979; Maderson, 1965, 1966, 1985; Maderson et al, 1972; Maderson et al, 1970a; Maderson et al 1970b; Maderson and Licht, 1967; Roth and Jones, 1967, 1970). The shedding cycle has been divided into six arbitrary steps in order to facilitate description (Maderson, 1965) (figs. 2 and 3). In the post-shedding condition, the incomplete outer generation contains an Oberhautchen, beta layer, mesos layer, some alpha tissue, and several layers of immature cells, lying above the stratum germinativum. At this time, the three outermost layers are fully mature, but the alpha layer is not. Within a few days after shedding, the immature cells above the stratum germinativum mature and are incorporated into the alpha layer. Upon full maturation of this layer, the germinal population becomes mitotically quiescent. This is referred to as the "perfect resting condition" (Maderson et al, 1970a).

Animals which shed infrequently demonstrate a prolonged perfect resting condition, during which time, the epidermis contains only a single generation. Germinal activity recommences in the late resting stage thus apparently determining the lacunar tissue and clear layer. The cells of the Oberhautchen and beta layers also begin to differentiate.. These cells of these layers synthesize the beta or feather type keratin and will synchronously lose their

FIGURE 2 Schematic representation of histogenesis of snake epidermis throughout a single renewal cycle. A shed is followed by a resting stage which is further subdivided into post-shedding, perfect resting, and completion periods depending on the state of maturation of the outer generation. The renewal phase, the period between the end of the resting stage and the next shed, is divided into five arbitrary stages which describe the degree of maturation of the inner epidermal generation. The abbreviations are as follows. The various components of the outer epidermal generation (OG) are, from without inwards, the Oberhautchen (Obo), the beta layer (β_o), the mesos layer (m), the alpha layer (α_o), the lacunar tissue (lto), and the clear layer (clo). The layers of the inner epidermal generation (IG) include the Oberhautchen (Obi), the beta layer (β_i), the mesos layer (m) and the alpha layer (α_i). Undifferentiated living cells (LLC) may be found above the stratum germinativum (sg). (Figure taken from Maderson, 1984, p. 114).

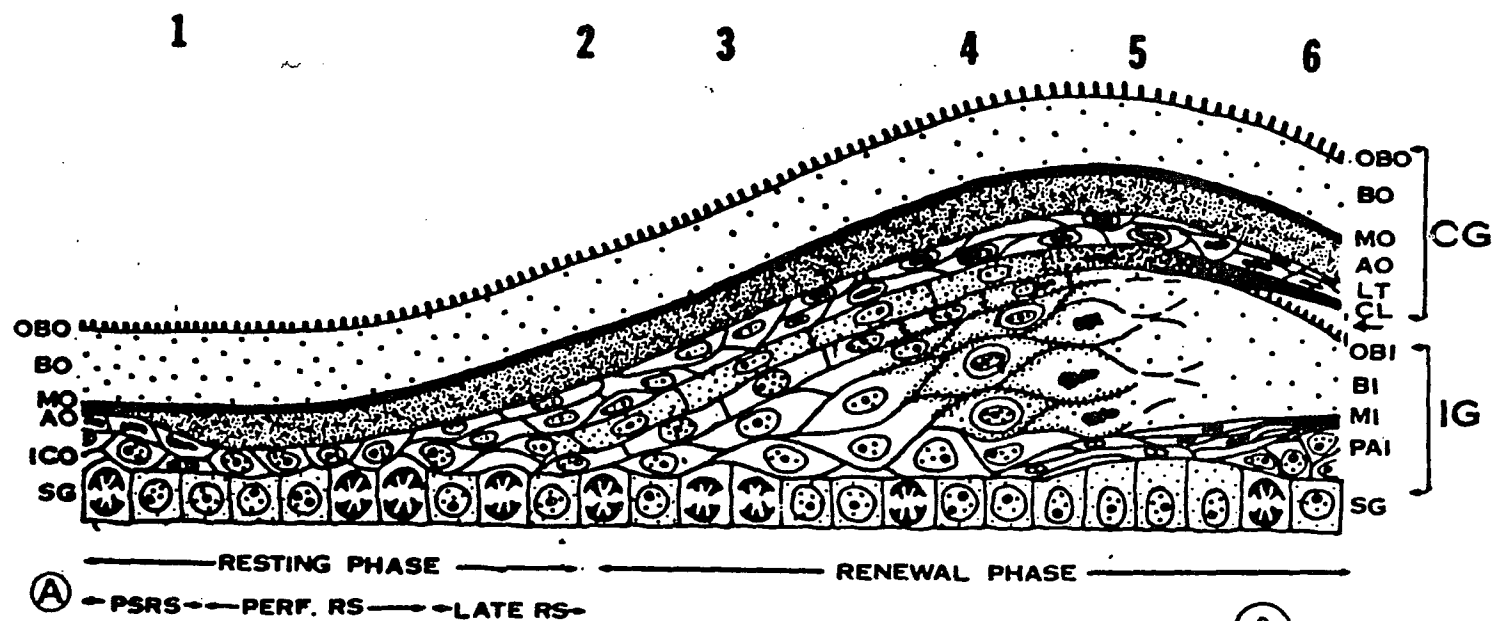


FIGURE 3 A schematic representation of cyclic activity in the epidermis of a snake through part of its life. The renewal phase is divided into five arbitrary stages (2 - 6) depending upon the degree of maturation of the inner epidermal generation. This concludes with a shed (S). A shed is followed by a resting phase (RS) which is further subdivided into post-shedding (PS. RS.), perfect (PERF. RS.), and late (L. RS.) resting phases. The late resting phase is followed by the onset of another renewal phase.



membranes a few days prior to shedding. At this time, the beta layer is separated from the basal layer by several layers of living cells. These cells will eventually differentiate into the mesos layer and will synthesize primarily the alpha-type keratin (Maderson et al, 1972). It is uncertain, however, as to whether the mesos cells synthesize alpha keratin exclusively. Glycolipids and phospho- lipids have also been found throughout this layer (Landman, 1979).

Among the various data which are available, concerning our knowledge of the hormonal control of squamate skin shedding (Maderson, 1985, pp.543-555), is the repeated demonstration that hypophysectomy and/or thyroidectomy of a variety of snakes, including Thamnophis, will result in those animals undergoing repeated renewal phases (Chui and Lynn, 1970; Chui et al, 1983). Restoration to renewal patterns found in intact animals is achieved in thyroidectomized snakes by injection of exogenous iodinated thyronines (thyroxine, triiodothyronine, and diiodothyronine). This cannot, however, be achieved by the administration of exogenous L-thyronine, diiodotyrosine, or moniodotyrosine (Maderson and Chiu, 1975).

During the renewal phase of snakes, conspicuous changes occur in the appearance of the integument, especially over the eye (Maderson, 1965). During stage three of the shedding cycle, the skin exhibits a slight over-all dulling and the spectacle of the eye becomes slightly cloudy. During stage

four, the eye is completely opaque, but it becomes clear again while in stage five. Although this phenomenon is poorly understood, it can be used as an indicator to the histological condition of the epidermis. Zucker (1977) has found similar, but more subtle, changes in the integument of several lizards.

B. MAMMALS

The vertebrate integument has an outer, stratified, squamous epidermis separated by a basement membrane from the connective tissue of the inner dermis. In mass, the integument is the largest organ of the body. Its primary function is to serve as a physiological barrier between the internal and external environments of the organism.

The epidermis of mammals is the best known of the amniote systems and it can be subdivided into four regions. The deepest is the stratum germinativum. The germinal cells, cuboidal or columnar in shape, lie on the basement membrane. Daughter cells, produced by mitotic division, undergo a maturation process (keratinization) and eventually replace the cornified units lost by insensible desquamation at the body surface. When the new cells emerge from the basal layer, they take up new positions in the stratum spinosum which consists of several layers of polygonal-shaped cells. The name, "stratum spinosum", refers to the light microscopic appearance of the many desmosomes which appear as fine spine-like processes across small

spaces between adjacent cells

Immediately above the stratum spinosum is the stratum granulosum, which is two to four cells thick. The cytoplasm of the flattened, nucleated cells is characterized by the presence of basophilic keratohyalin granules. The stratum granulosum cells mark the transition to the fully mature stratum corneum. This consists of fifteen to twenty layers of thin, flattened cells (Holbrook and Oddland, 1974) and is approximately 0.5 microns thick in most cases (Plewig and Marples, 1970). The cells in this region become filled with an alpha-keratin matrix and die when the nuclei and all other cytoplasmic organelles disappear.

During keratinization, a thick marginal band appears just beneath the plasma membrane. This creates the thickened envelope of the cornified cell. Each fully mature keratinocyte in the stratum corneum is bound by a plasma membrane which appears to be intimately attached to the internal, dense marginal band, forming a complex twenty nanometers in total thickness. The interstices of the cells are filled by a dense amorphous mass which appears dark in heavy metal stained transmission electron microscopic sections. This material is thought to be comprised partly of the filament-aggregating stratum corneum basic protein called filaggrin, whose phosphorylated precursor is a histidine rich protein derived from the keratohyalin granules (Dale et al, 1980 a,b). Enclosed within this dense continuum is an array of

electron-lucent filaments. These filaments are believed to be polymers of alpha keratin (Dale et al, 1978).

The complex of filaments within the amorphous matrix makes the epidermis mechanically elastic. However, it is the marginal band which is cystine rich and stabilized by gamma-glutamyl lysine cross links (Rice and Green, 1977), which makes the keratinized cell resistant to mechanical and chemical destruction.

Broad sheets of lipids occur in the intercellular spaces of the inner-most cells of the stratum corneum (Elias, 1981). These lipid leaflets appear to play a role in the prevention of cutaneous water loss (Elias and Brown, 1978). They break down as the outermost layers of the stratum corneum desquamate.

SECTION II. EPIDERMAL BIOCHEMISTRY: PROTEIN

A. SQUAMATES

X-ray diffraction studies have revealed that squamate epidermis synthesizes both alpha and beta type keratin (Baden and Maderson, 1970). The latter is a major constituent of the oberhautchen and beta layers. The former has been found in all other layers of the squamate epidermis (Maderson, 1985; Maderson et al, 1972).

X-ray diffraction studies have yielded important clues regarding the

structure of beta keratin. When fibers of alpha keratin are subjected to moist heat, they can be stretched to almost double their original length. In this stretched condition, they yield x-ray diffraction patterns which resemble that of beta keratin (Lehninger, 1976). It has been theorized (Pauling and Corey, 1953) that the transition from the alpha to the beta keratin structure, when hair or wool is steamed, is caused by the thermal breakage of interchain hydrogen bonds which normally stabilize the alpha helix. Breakage of these bonds allows for the consequent stretching of the relatively tight alpha helix into a more extended, zig-zag conformation characteristic of beta keratin.

Polypeptide chains in the beta conformation are arranged in pleated sheets which are cross-linked by interchain hydrogen bonds (Lehninger, 1976). This structure is highly stable due to the fact that all of the peptide linkages participate in this cross-linking. The side chains lie above and below the zig-zagging plane of the pleated sheet.

There are two important differences between alpha and beta keratin. Firstly, the R groups of alpha keratin are bulkier and more highly charged than those of beta keratin. Secondly, in alpha keratin, all of the polypeptide chains are parallel; ie. they run in the same N-terminal to C-terminal direction. On the other hand, adjacent polypeptide chains in beta keratin are anti-parallel; ie. one chain will run in an N-terminal to C-terminal direction while an

adjacent chain will run in a C-terminal to N-terminal direction (Lehninger, 1976).

B. MAMMALS

All eukaryotic cells possess a complex intracellular cytoskeleton which consists of 1) actin-containing microfilaments (approximately 7 nm in diameter); 2) Tubulin-containing microtubules (approximately 20-25 nm in diameter); and 3) Intermediate filaments (approximately 7-10 nm in diameter) (Goldman et al, 1979). It is the intermediate filaments which vary the most from species to species (and tissue to tissue), in terms of chemistry and properties, and which are most abundant in mammalian and squamate keratinocytes. The inner, living cells synthesize and retain these filaments. Eventually, they constitute up to two thirds of the mass of the terminally differentiated keratinocytes (Lazarides, 1980).

Originally, all of the filamentous elements were referred to as tonofilaments. However, this term is both misleading and a misnomer (Mercer et al, 1964). They are now referred to as keratin filaments (Fraser et al, 1972). In recent years, a great deal of information contributing to our understanding of keratin filaments, has been accumulated.

The keratin filaments in mammalian epidermis first appear as small, wavy, electron lucent elements in transmission electron micrographs. As

cellular differentiation continues, the elements enlarge and elongate, eventually extending across the cell to form complex, intra-cellular networks which interconnect the innermost plaques of the desmosomal junctions of the cell membranes (Brody, 1960). In longitudinal section, the filaments appear to be unbranched with little ultrastructural detail. In cross section, however, they appear as dark staining circles with centers and peripheries of reduced electron density. This suggests a tubular structure (Matoltsy and Parakkal, 1965). In terminally differentiated corneous cells, the filaments also appear tubular, but their diameters are usually slightly larger and this may be due to an association with lipids (Matoltsy and Parakkal, 1965; Odland, 1964). Fillagrin is a cystine-free matrix protein of epidermal cells (Steinert et al, 1981). In vitro, it has been shown to aggregate epidermal keratin filaments into ordered macrofibrils which display a typical keratin pattern when viewed in cross section (Dale et al, 1978, 1980b; Delap et al, 1980).

Mammalian keratin proteins first occur in living cells in their reduced (thiol) forms. However, these are oxidized to disulfide bonds and the resulting filaments become highly insoluble. As recent studies have indicated that a majority of these disulfide bonds are intra-chain, not cross-linking (Baden et al, 1976; Steinert, 1978), the insolubility of the epidermal protein(s) is seemingly not due to an abundance of disulfide cross-links. There is speculation that the thickened cellular envelope and/or several other

types of covalent links may be responsible for this insolubility (Abernathy et al, 1977; Harding and Rogers, 1971; Matoltsy, 1976; Steinert, 1975; Steinert and Idler, 1979).

The insolubility of keratin has made extraction of these proteins very difficult. Early attempts involved the use of degradation solvents such as sodium hydroxide (Matoltsy and Odland, 1955; Rothberg, 1960) and formic acid (O'Donnell, 1977). Ureathiol solvents have also been used (Steinert, 1975). This procedure disrupts the cell membrane and releases keratin filaments in the form of their constituent polypeptide subunits.

Recent studies have provided detailed information on the chemical properties of individually purified mammalian keratin filaments (Fuchs and Green, 1978; Huang et al, 1975; Lee et al, 1975; Steinert and Idler, 1975; Steinert et al, 1980b; Steinert and Idler, 1979). When isolated mammalian keratin filaments are mixed in vitro with rat or mouse fillagrin, they become aggregated into large, insoluble fibrous structures (Dale et al, 1978, 1980a; Steinert et al, 1980a, 1981). The size and shape of these fibrous structures depend on both the total concentration of filaments and fillagrin mixed, and on the relative amounts mixed (Dale et al, 1980b).

The stoichiometry of the in vitro assemblages of these mammalian keratin filaments suggests that the insoluble fibrous structures formed have a three-chained composition (Baynes et al, 1978; Skerrow, 1974). All

mammalian filaments examined thus far have yielded an alpha type x-ray diffraction pattern (Bailey et al, 1943; Fraser et al, 1972) More recent work involving the isolation of partially digested keratin filaments has lead to the formulation of a molecular model for this type of keratin. In this model, three subunits are aligned such that the adjacent alpha helical regions form three chained coiled- coils (Pauling and Corey, 1953). These coils are interdispersed by region of non- alpha helix (Crewther et al, 1976; Crewther et al, 1978; Dobb et al, 1973; Doolittle et al, 1978; Lotay and Speakman, 1977; Parry, 1980; Parry et al, 1977; Skerrow et al, 1973; Steinert, 1978a,b; Steinert et al 1980c).

Section III. EPIDERMAL BIOCHEMISTRY: LIPIDS

A. SQUAMATE EPIDERMIS

Much less is known about the lipid components of squamate epidermis. However, preliminary studies on the lipids of snake epidermis have revealed considerable differences from those in mammals. In particular, glycosylceramides are present in major amounts in the keratinized tissues of reptiles (Birkby et al, 1982). The identities of many squamate epidermal lipids have yet to be determined.

In addition to polar lipids, snake epidermis also synthesizes a large amount of non-polar lipids. Ahern and Downing (1974) examined the

composition of these lipids in some detail in the Florida Indigo snake, Drymarchon corais. They reported that these non-polar lipids constituted about 8% of the dry weight of cast skins, comprising methyl ketones (20%), free secondary alcohols (15%), free primary alcohols (30%), free cholesterol (15%), free fatty acids (5%), and hydrocarbons (5%). The free fatty acids present consisted mainly of palmitic, stearic, oleic and linoleic acids. A remarkable feature of the lipids of this snake skin is the presence of such high proportions of long chain methyl ketones. Although methyl ketones are wide-spread in nature, their occurrence previously had been limited to plants, insects, and micro-organisms (Forneg and Markovitz, 1971).

Transmission electron microscopy has confirmed the notion that a majority of the lipid constituents of the squamate epidermis are synthesized in organelles called mesos granules, located in the mesos layer (Landman, 1979). This topic is dealt with in greater detail in Section V of the Introduction and in the Discussion section of this thesis.

B. MAMMALIAN EPIDERMIS

Epidermal basal cells have a complex lipid composition (Yardley, 1983). The lipid content of basal cells differs markedly from that of stratum corneum cells, emphasizing one of the dramatic changes which characterize the process of keratinization. Kooyman (1932) was the first to report this

after he had discovered that there was a much lower concentration of phospholipids in corneous cells than in those of the subjacent living cells. Long (1970) cut cow snout epidermis into six horizontal slices and reported that the amount of phospholipid decreased dramatically in the most superficial stratum corneum. This decrease in phospholipids was accompanied by an increase in the concentration of triacylglycerol and fatty acid.

The lipid content of pig epidermis has been examined in great detail (Gray et al, 1978, 1980, 1982). From these studies, it was concluded that while phospholipids disappear from corneous cells, the content of ceramide, cholesterol, and fatty acids increases markedly. It is believed that this change in lipid composition in keratinizing cells results both from a rearrangement of fatty acids liberated by catabolic processes and by dé novo synthesis of cholesterol and ceramide (Gray et al, 1982).

The specific amounts and types of each of these lipids have been reported in a variety of mammals. Human epidermis contains large amounts of the phospholipids sphingomyelin and phosphatidylcholine (Gray and Yardley, 1975; Tsambaos et al, 1977; Tsambaos and Mahrle, 1979). Ceramides (Nicolaidis, 1965) have been identified as a major component of epidermal lipids. The least polar of the epidermal ceramides, 1-(3'-o-acyl)-beta-glucosyl ceramide, has been implicated as being involved

in the water barrier properties of the epidermis (Gray et al, 1978).

Other lipids isolated from mammalian epidermis include gangliosides (Gray and Yardly, 1975), sterols (Gray et al, 1978, 1980, 1982) and free fatty acids (Gray et al, 1982).

SECTION IV. CUTANEOUS WATER LOSS

It was long assumed that the integument of amniote vertebrates was "waterproof." However, it is now known that cutaneous water loss (CWL) contributes significantly to total evaporative water loss (see review, Lillywhite and Maderson, 1982). Thus, one major function of the epidermis is to limit the amount of body water lost to the environment.

Lipids have been demonstrated to play a role in the permeability barrier of mammalian skin. It has long been known that a diet deficient in essential fatty acids, in particular linoleic, results in scaly skin with a greatly reduced permeability barrier (Elias and Brown, 1978; Elias et al, 1980; Elias and Friend, 1975; Elias et al, 1977; Elias and Nemaric, 1977; Hartop and Prottey, 1976; Menton, 1968, 1970; Prottey, 1976, 1977).

In recent years, investigators have attempted to answer the question as to what part, or parts, of the squamate epidermis provides the primary barrier to CWL. Jelenko and Ginsburg (1971) demonstrated that water transmission through the skin was directly correlated to the content of "water holding"

lipids in poikilotherm integuments. Maderson et al (1978) examined the relationship of integumentary permeability to the structural features of the epidermis of several squamate species, by stripping epidermal layers with cellophane tape. It was discovered that removal of the outer beta layer had little effect on the rate of CWL. However, water loss increased significantly when stripping removed the mesos and alpha layers. These studies did not, however, determine which cellular and chemical component(s), within the mesos and alpha layers, is (are) actually involved in the barrier function.

Roberts and Lillywhite (1980) showed that extraction of epidermal lipids dramatically increases evaporative water loss from *in vitro* preparations of shed snake skins. However, when protein constituents were disrupted, only a slight increase in CWL was observed. Histochemical (Roberts and Lillywhite, 1980) and transmission electron microscopic (Landman, 1979) studies have indicated that extracellular epidermal lipids are located primarily within the mesos layer and probably constitute the principal barrier to water loss across the squamate epidermis. Although the alpha layer resembles the mammalian stratum corneum, lipids are absent therefrom and are confined to the mesos layer (Lillywhite and Maderson, 1982).

Roberts and Lillywhite (1983) examined shed epidermal generations of eleven species of snake from varying habitats. They showed that shed skins from organisms exhibiting the lowest permeability of untreated

epidermis, showed the highest permeability following lipid extraction. They also demonstrated that the quantity of lipid removed correlated with the habitat aridity of the snakes. Untreated skins, on the other hand, were at least 97-72% more effective as barriers to CWL.

Dunson and Freda (1985) demonstrated that when shed skins from the amphibious snake Agkistrodon piscivorus were subjected to lipid extraction, an increase in water permeability resulted. Similar findings in an arboreal snake (Opheodrys aestivus) and a semi-aquatic snake (Nerodia rhombifera) were reported by Baeyens and Roundtree (1983). Lamellar bodies have been demonstrated to be responsible for the production of extracellular epidermal lipids in mammals (Menton 1970). Mice fed a diet deficient in essential fatty acids have lamellar bodies in their epidermis, which although are secreted in normal quantities, are largely empty. As a result of this, the intercellular spaces of the stratum granulosum lack the lamellar pattern seen in control animals (Menton 1970; Elias and Brown, 1978). Lamellar granules almost identical to those found in mammals have been found in the epidermis of birds and reptiles (Landmann, 1980).

SECTION V. LAMELLAR GRANULES

The intercellular spaces in part or parts of the horny tissues of the epidermis of birds, reptiles and mammals contain multimembranous sheets

(Landmann, 1980). These sheets appear to arise as the result of discharge from organelles called lamellar granules (Landmann, 1980). They have also been referred to as membrane coating granules (Matoltsy and Parakkal, 1965), Odland bodies (Wilgram, 1965), keratinosomes (Weinstock and Wilgram, 1970), cementosomes (Hashimoto, 1971), lamellar bodies (Elias and Friend, 1975), and mesos granules (Landmann, 1979). Such organelles were first described by Selby (1957) and Odland (1960) in mammalian epidermis. Roth and Jones (1970) and Landmann (1979, 1980) demonstrated the existence of these structures in non-mammalian epidermis. Landmann (1980) emphasizes that morphologically, lamellar granules in the epidermis of all three classes of amniote vertebrates are almost identical, and that no such organelles exist in the epidermis of amphibians or fish.

Typically, lamellar granules have an internal lamellate structure of alternating major and minor dark bands separated by light lines. Each granule is surrounded by a trilaminar membrane (Landmann, 1980). In squamate epidermis, these organelles have been shown to originate in the mesos layer during stage four of the renewal phase (see fig. 1) (Roth and Jones, 1970; Landmann, 1979). In mammalian epidermis, they arise in the cells of the stratum granulosum (Elias and Friend, 1975). The membranes of these organelles fuse with the cell membranes of the keratinocytes and their contents are released, via exocytosis, leading to the formation of the

multiple membranous sheets in the intercellular spaces of the horny layer (Breathnach et al, 1973; Elias and Friend, 1975; Lavker, 1976; Matoltsy, 1976; Matoltsy and Bednarz, 1975). It is believed that these intercellular sheets are responsible for the formation of the barrier to cutaneous water loss (Elias and Brown, 1978; Landmann, 1980; Matoltsy and Bednarz, 1975).

It is generally accepted that the contents of the mesos granules consist of glycosphingolipids and phospholipids (Elias et al, 1979). There is a structural similarity between lamellar granules and synthetic phospholipid preparations (Frithiaf and Wersoll, 1965). Olah and Rohlech (1966) and Elias et al (1977) showed that the lamellate contents could be extracted from the granules using various lipid solvents. Hashimoto (1971) and Wolff and Wolff-Shreiner (1976) showed that phospholopase C digested the contents of lamellar granules and this was confirmed by Landmann (1980). A carbohydrate moiety has also been identified. Whether it is a glycolipid (Gray and Yardly, 1975; Elias et al, 1977a; Elias et al, 1979), or a glycoprotein (Hayward and Hackerman, 1973; Hayward, 1979) has yet to be determined.

Wertz and Downing (1982) have investigated the assembly of these granules in pig epidermis. They suggested that it might be promoted by recently identified 1-(3'-o-acyl)-beta-D-glucosyl-N-(w-hydroxyacyl) sphingosines which have 30 and 32 carbon hydroxy acids as amides and linoleic acid esterified to glucose. Such a role for the molecules

may explain the effects of essential fatty acid deficiency in mice wherein lamellar granules fail to assemble and the barrier to water diffusion is lost (Elias and Friend, 1975) (see Discussion, Section VI).

SECTION VI. STATEMENT OF PURPOSE

There exist three major unanswered questions regarding skin shedding in squamate reptiles. They may be summerized as follows:

- 1) What is its biological role? A question for ecologists.
- 2) How does it relate to water metabolism? A question for physiologists.
- 3) What controls cyclic epidermal cell proliferation and differentiation?
A question for developmental biologists.

We propose that all three questions are inter-related and demand an interdisciplinary approach. In this thesis, we have taken such an approach, attempting to demonstrate the correlation between environmental (ie. ambient temperature and relative humidity) and internal (ie. thyroid hormone) factors. Let us first discuss the experimental protocol used to demonstrate a relationship between skin shedding in garter snakes and environmental factors.

Data collected from the earliest studies cited in this thesis suggested that it was possible to induce sheds in intact animals by exposing them to

constant high temperature (ie. 28 oC). A series of subsequent experiments, involving the exposure of groups of intact animals to a variety of constant ambient temperatures, was then conducted with similar results. This led us to believe that cutaneous water loss may play a role in initiating the renewal phase. We attempted to demonstrate this relationship by exposing groups of intact garter snakes to conditions of high temperature and low relative humidity. Once again, we were able to induce renewal phases and sheds in these groups of animals.

Next, we sought to elucidate the effects of thyroid hormone(s) on the shedding phenomenon. It has been demonstrated that thyroidectomy of any snake, at any time, will lead to the onset of a series of renewal phases (see review, Maderson, 1984). It was this relationship which led us to conduct a series of experiments involving the exposure of thyroidectomized garter snakes to a variety of constant temperature regimes. Data collected from these studies indicated that all thyroidectomized animals entered repeated renewal phases when maintained at constant temperatures of 21 oC and higher. Skin biopsies were removed from both intact and thyroidectomized snakes. These samples were prepared for both light and electron microscopic analysis to determine whether or not any observable morphologic changes occurred as a result of thyroidectomy.

Thus, the protocol presented in the materials and methods section of this

thesis represents an attempt on our part to examine the effects of both environmental and internal factors on skin shedding in garter snakes. It is our belief that this is the most effective way to approach this problem.

MATERIALS AND METHODS

SECTION I. GENERAL MATERIALS

The animals used in this study were adult Garter snakes (Thamnophis sirtalis). All animals were purchased commercially at various times of the year; the major shipments in Fall and Winter involved animals from central Canada. They were weighed at the beginning of each experiment, exposed to 12/12 hour light/dark photoperiods, given water ad libitum, checked daily for cloudy eyes and starved throughout the experiments unless otherwise indicated.

SECTION II. LIGHT MICROSCOPY

All tissue samples prepared for light microscopy were fixed in Bouin's solution for twenty four hours, dehydrated in graded ethanol, and embedded in paraplast. Seven micron serial sections were cut on an American Optical 820 microtome, mounted on glass slides, and stained with hematoxylin and eosin.

SECTION III. TRANSMISSION ELECTRON MICROSCOPY

All scale samples prepared for ultrastructural analysis were cut sagittally, fixed for 24 hours in paraformaldehyde-glutaraldehyde according to Karnovsky (1965), and post-fixed in ferrocyanide-osmium tetroxide

(Karnovsky, 1971) for three hours. One half of each scale was dehydrated in graded solutions of ethanol, the other half in graded solutions of acetone. All samples were embedded in Spurr's resin (Spurr, 1969). Thick (1.0 micron) survey sections were cut using a glass knife, stained with toluidine blue, and examined under the light microscope to determine the stage of the shedding cycle represented.

Thin sections (0.1 microns) were cut with a Dupont diamond knife on a Porter Blum MT-2B ultramicrotome, and mounted on naked 75 by 300 mesh copper grids. Each grid was stained with uranyl acetate (ref.) and lead citrate (Reynolds, 1963) for periods of fifteen and twenty minutes respectively. The grids were then examined in a Phillips EM 300 transmission electron microscope.

SECTION IV. RATIONALE FOR CHOICES OF TEMPERATURE REGIMES

Intact and thyroidectomized animals were maintained under a number of different ambient temperatures. These included room temperature (circa 24.5 oC), 28 oC, 21 oC, 17 oC, 8 oC, and 3 oC. The rationale for choosing these temperature regimes was as follows.

Constant 28 oC was chosen because it represented the highest constant temperature at which both intact and thyroidectomized animals could survive. Viability of the animals was substantially decreased when they were exposed

to constant temperatures higher than 28 oC.

Constant 21 oC was chosen since this is the lowest temperature at which all thyroidectomized animals will enter one or more renewal phase following surgery.

Constant 17 oC was chosen because it represented the temperature below which no thyroidectomized animals entered the renewal phase.

Constant 3 oC was chosen since it approximated the temperature at which the animals hibernated.

Constant 8 oC was chosen because it represented a low temperature intermediate between 17 oC and 3 oC.

When no particular constant temperature regime was indicated, animals were maintained at room temperature which varied daily from 21.5 oC to 24.5 oC.

SECTION V. STUDIES ON INTACT SNAKES

A. HIGH TEMPERATURE INDUCED SHEDS

1. Experiment A-1: Maintenance of Intact Garter Snakes at Constant 28 oC

Eight animals were purchased in October, 1979, immediately placed at 28 oC, and weighed at ten day intervals. Skin biopsies were taken from each animal, starting on the fortieth day of the experiment and every fifth day

thereafter, and prepared for light microscopic examination.

2. Experiment A-2: Transferring Intact Garter Snakes From 28 oC to 17 oC Following a Shed at 28 oC

Thirty animals were purchased in September, 1981, weighed, and divided into three groups of ten animals each as follows:

- Group 1 (experimental); placed at 28 oC
- Group 2 (control); placed at 28 oC.
- Group 3 (control); placed at 17 oC.

All of the animals were weighed at ten day intervals and identified by scale color patterns, weight, and tags made of masking tape.

Immediately after shedding, each animal from Group 1 was transferred to 17 oC for a period of 21 days, then transferred back to 28 oC. Animals in Groups 2 and 3 were kept under original conditions throughout the experiment.

3. Experiment A-3: Transferring Intact Garter Snakes From 28 oC to 17 oC or 8 oC Following a Shed at 28 oC

Fifty animals were purchased in September, 1982, weighed, and divided into four groups as follows.

- Group 1 (experimental): twenty animals placed at 28 oC

- Group 2 (control): ten animals placed at 28 oC.
- Group 3 (control): ten animals placed at 17 oC.
- Group 4 (control): ten animals placed at 8 oC.

Animals were identified as described above (see experiment A 2).

Upon shedding, animals in Group 1 were transferred to either 17 oC or 8 oC on an alternating basis (ie. the first animal to shed was transferred to 17 oC; the second to 8 oC; the third to 17 oC etc.).

These Group 1 animals were kept at 17 oC or 8 oC for a period of 21 days; after which time they were returned to 28 oC. Animals in Groups 2, 3, and 4 were maintained under original conditions throughout the experiment.

4. Experiment A-4: Maintenance of Intact Garter Snakes at 3 oC for Six, Ten, or Fourteen Weeks Prior to Transfer to 28 oC.

Thirty animals were purchased in October, 1983, immediately placed at 3 oC, and were kept in the dark.

After a period of six weeks, ten animals were removed, weighed, placed at 28 oC in a tank containing a large trough of water, and exposed to 12 hour light/12 hour dark photoperiods. They were observed daily for cloudy eyes and/or shedding, and weighed at five day intervals. When the last animal in the group had shed, all of the animals in that group were transferred back to 3 oC and maintained under original conditions for a period of six weeks. After

this time, the animals were transferred back to 28 oC and maintained and observed as described above.

After a period of ten weeks (from the start of the experiment) a second group of ten animals was removed from 3 oC and placed at 28 oC as described above. These animals were checked daily for cloudy eyes and/or shedding and weighed at ten day intervals.

After a period of fourteen weeks (from the start of the experiment) a third group of ten animals was removed from 3 oC and treated as described above.

SECTION VI. STUDIES ON THYROIDECTOMIZED GARTER SNAKES

A. THYROIDECTOMY PROCEDURES

1. Anesthesia

Animals were weighed and kept at room temperature for a period of twenty four hours. Each animal was injected with nembutal (sodium pentobarbital) subcutaneously at a concentration of 3.0 mg nembutal/ 100 grams body weight. Following the injections, the animals were placed at 3 oC for twenty four hours prior to surgery.

2. Surgery

Each animal was removed from 3 oC, restrained, and placed under a dissecting microscope. The ventral surface was palpated to locate the heart and the region was marked. An initial median ventral incision was made

through the skin with a sterile #11 scalpel blade approximately 3mm anterior to the location of the heart. This incision was elongated using sterile McClure iris scissors. In some cases, the incision was held open with hemostats.

All connective tissues were trimmed away to allow access to the thymus gland, a large yellow amorphous mass. Probing of the thymus gland was carried out using sterile watchmaker's forceps. The thyroid gland was usually located at the most anterior aspect of the ascending aorta. It could be identified easily by its oval shape, deep red color, and size (approximately 3mm in length).

Once located, the thyroid gland was pulled upward using watchmaker's forceps. Care was taken not to sever any major blood vessels, especially the two thyroid arteries which enter the gland from either side. While the gland was held with a pair of watchmaker forceps, the thyroid arteries were cauterized with the tip of a pointed probe heated to red heat in a bunsen burner flame. The gland was placed in a vial of Bouin's solution, then fixed and stained for histological examination. The incision was sutured using surgical silk and the animal was placed at 21 °C. After a recovery time of about 24-48 hours, the animals were placed under experimental conditions.

3. Sham Surgery

In the case of sham operations, the above procedure was carried out

with the exception of the actual excision of the thyroid gland and cauterization of the thyroid arteries.

B. THYROIDECTOMY EXPERIMENTS

1. Experiment B-1: Maintenance of Thyroidectomized Garter Snakes at Constant 28 oC, 21 oC or 10 oC.

Nineteen animals were purchased in September, 1979. They were weighed, thyroidectomized, placed at 21 oC and checked daily for cloudy eyes. Following the completion of the first post-surgical cloudy eye phase, each animal was placed into one of three groups as follows:

- Group 1: seven animals maintained at 21 oC.
- Group 2: six animals maintained at 28 oC.
- Group 3: six animals maintained at 10 oC.

Skin biopsies were taken from each animal, starting with the first day of the first cloudy eye phase, and every other day thereafter for the duration of the experiment. The tissue samples were prepared for light microscopic examination. The animals were weighed at ten day intervals.

2. Experiment B-2: Maintenance of Thyroidectomized Garter Snakes at 10 oC Followed by Transfer to 15 oC or 28 oC

Fourteen animals were purchased in October, 1979. They were weighed,

thyroidectomized, placed at 10 oC and checked daily for cloudy eyes and/ or shedding. After a period of forty six days, the animals were divided into two groups as follows:

- Group 1: seven animals transferred to 28 oC.

- Group 2: seven animals transferred to 15 oC.

Group 1 animals remained under these conditions for the duration of the experiment. The four surviving animals in group 2 were transferred to 28 oC after having spent forty eight days at 15 oC.

Skin biopsies were taken from each animal, starting with day forty one of the experiment, and every fifth day thereafter. The tissue samples were prepared for light microscopic examination. Each animal was weighed at ten day intervals throughout the experiment.

3. Experiment B-3: Maintenance of Intact and Thyroidectomized Garter Snakes at Constant 21 oC

Twenty animals were purchased in June, 1982, weighed, and divided into two groups as follows:

- Group 1: ten intact animals placed at 21 oC.

- Group 2: ten thyroidectomized animals placed at 21 oC.

All animals were weighed at ten day intervals.

C. THYROIDECTOMY AND FEEDING EXPERIMENTS

1. Experiment C-1: Fed Intact and Thyroidectomized Garter

Snakes Maintained at Room Temperature

Fifteen animals were purchased in June, 1983, weighed, and divided into three groups of five animals as follows:

- Group 1:Thyroidectomized
- Group 2: Sham
- Group 3: Intact

All three groups were maintained at room temperature (circa 24.5 C), and exposed to variable photoperiods. Earth worms were weighed and offered to each animal individually and animals were weighed at ten day intervals.

2. Experiment C-2: Comparisons of the Effects of Feeding and

Starvation in Intact, Sham, and Thyroidectomized Garter Snakes

Thirty animals were purchased in August, 1983, weighed, and divided into six groups of five animals as follows:

- Group 1a:(Experimental) intact animals.
- Group 1b:(Experimental) sham animals.
- Group 1c:(Experimental) thyroidectomized animals.
- Group 2a:(Control) intact animals.
- Group 2b:(Control) sham animals.

-Group 2c:(Control) thyroidectomized animals.

All six groups were maintained at 21 oC. The control groups (2a, 2b and 2c) were starved throughout the experiment. The experimental groups (1a,1b and 1c) were fed earthworms ad libitum. All animals were weighed at ten day intervals.

3. Experiment C-3: Comparisons of the Effects of Feeding and

Starvation in Intact and Thyroidectomized Garter Snakes

Maintained at Constant 28 oC Following Six Weeks at Either 3 oC

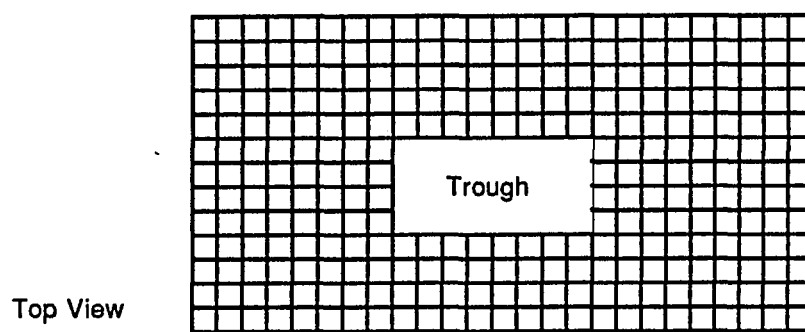
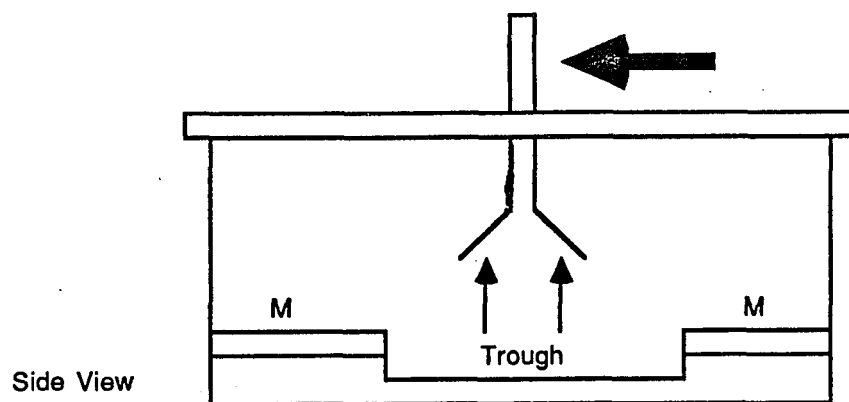
or Room Temperature

Thirty animals were purchased in September, 1983, and stored at either room temperature (circa 24.5 oC) or 3 oC for a period of six weeks prior to the start of the experiment. During this time, the animals kept at room temperature were fed earthworms ad libitum while the animals kept at 3 oC were starved. Both groups were given water ad libitum.

At the end of the six week period, two groups of fifteen animals were established of which four had been kept at 3 oC, the rest at room temperature. Both groups were placed at 28 oC in specially designed tanks (fig. 4).

Animals in group 1 were fed a steady diet of earthworms and goldfish from the outset of the experiment. Animals in group 2 were starved throughout the experiment. All animals were weighed at ten day intervals and checked daily for cloudy eyes and/ or shedding. Ventral scales of each animal

Figure 4 Diagrammatic representation of specialized holding tanks. The floor of the tank is covered by a plastic mesh (M) which allows for drainage of water and waste products. In the middle of the mesh is a large trough filled with water. The animals are free to either lay on the mesh or immerse themselves completely in water. An outlet (small arrows) attached to suction tubing (large arrow) removes excess moisture from the tank.



were clipped in order to facilitate identification.

As animals in each group shed, they were removed on an alternating basis (ie. the first, third, fifth, etc. animal in each group to shed), thyroidectomized, and returned to their original tanks. The animals were then treated as they were prior to surgery.

SECTION VII. EFFECTS OF RELATIVE HUMIDITY ON SKIN SHEDDING IN GARTER SNAKES

A. CONTROLLED RELATIVE HUMIDITY CHAMBERS

Chambers designed to maintain constant relative humidity were constructed (see fig. 5) . These chambers contained a vent on one side, to which a vacuum hose could be attached, and an inlet on the other side, to which a canister of drierite (calcium sulfate) could be attached.

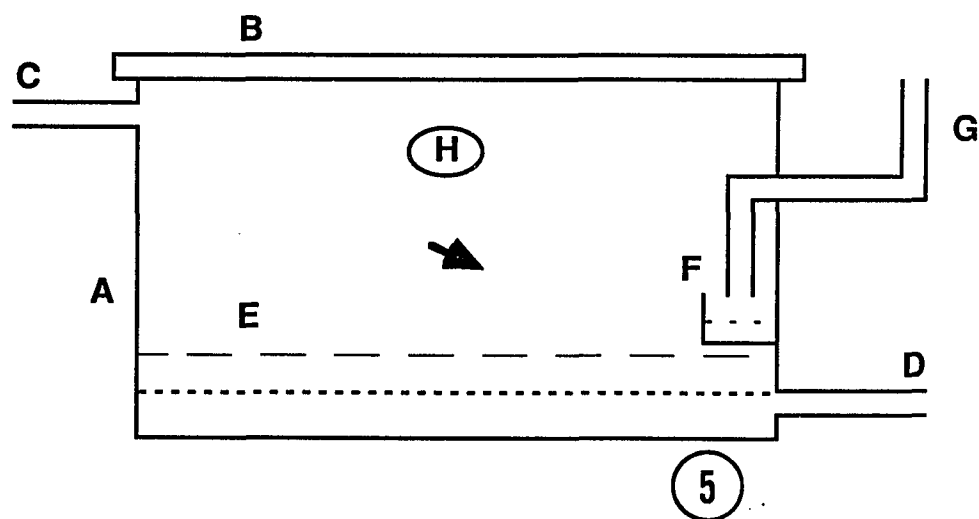
B. CONTROLLED RELATIVE HUMIDITY EXPERIMENTS

1. Experiment D-1: Effects of Controlled Relative Humidity on Intact Garter Snakes Maintained at Constant 28 oC

Thirty animals were purchased in November, 1984, weighed and divided into three groups. The three groups of animals were placed into the controlled relative humidity environments as follows:

-Group 1: no vacuum and no drierite.

Figure 5 Relative humidity chambers used to determine the effects of relative humidity on skin shedding in Garter snakes. The animals are placed in a glass container (A), the hinged lid of which (B) seals tightly with gaskets. Air, dehydrated by passage through drierite columns, is pulled through the inlet tube (C) as the exhaust tube (D) at the extreme other end of the container, is connected to a vacuum pump. The animals lie on a plastic mesh screen (E) which permits rapid evaporation of urine and/or feces. A water dish (F) is close to the exhaust tube and is replenished twice daily through the tube (G). The relative humidity inside the chamber can be read off the hygrometer (H).



-Group 2: vacuum only.

-Group 3: vacuum and drierite.

All three groups were maintained at 28 oC and weighed at ten day intervals. The experiment was run until all of the animals in a particular group shed.

2. Experiment D-2: Effects of Controlled Relative Humidity on Animals

Maintained at Constant 21 oC

This experiment was carried in exactly the same manner as experiment D 1 with two exceptions. First, this experiment was run during the months of January and February, 1985. Second, the three groups were maintained at 21 oC instead of 28 oC.

SECTION VIII. ULTRASTRUCTURAL EXAMINATION OF THE EFFECTS OF THYROIDECTOMY ON EPIDERMAL DIFFERENTIATION IN GARTER SNAKES

Ten animals were purchased in May, 1982, weighed, and placed at 28 oC. Each animal was biopsied daily from the onset of the first cloudy eye phase through its conclusion.

After having shed, each animal was thyroidectomized, returned to 28 oC, exposed to 12 hour light/ 12 hour dark photoperiods, and given water ad libitum. Skin samples were removed daily for the duration of each subsequent cloudy phase and prepared for transmission electron microscopy.

RESULTS

SECTION I. HIGH TEMPERATURE INDUCED SHEDS IN INTACT SNAKES

A. Experiment A-1: MAINTENANCE OF INTACT SNAKES AT CONSTANT

28 oC

1. Shedding Summaries (Table 1)

Of the eight animals placed at 28 oC, five shed. Only four cloudy eye phases were observed but a fifth shed skin was found in the cage. The animals entered the cloudy eye phase within one day of one another. The average number of days spent in the cloudy eye phase by those four animals was 3.25 days.

2. Weight Relationships(Table 2)

As the animals were starved throughout the experiment, for the most part, all showed weight losses over the ten day intervals between weighings. However, some animals did demonstrate stable weights over periods of time and one was observed to have gained weight initially, probably due to water consumption.

The percent of original weight at death ranged from 43.8% to 70.0%, with an average of about 62.2%.

3. Light Microscopic Analysis

Skin biopsies were taken from each animal at day forty of the

Table 1. Shedding summary for intact Garter snakes placed at constant 28 oC.

Animal #	Sex	Temp.	No. of Sheds	No. of Days Prior To Cloudy Eyes	No. of Days Spent in Cloudy Eyes	No. of Days Alive
1	M	28 oC	1	18	3	62
2	M	28 oC	1	17	2	55
3	F	28 oC	0	-	-	57
4	F	28 oC	1	17	4	68
5	M	28 oC	1	17	4	74
6	M	28 oC	1	*	-	59
7	F	28 oC	0	-	-	53
8	M	28 oC	0	-	-	60

Note: * indicates that this animal shed, but no cloudy eye phase was observed.

TABLE 2. Weight relationships among intact garter snakes maintained at constant 28 oC

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90
1	39	94.8	94.8	79.5	74.3	71.8	<u>66.7</u>			
2	27	107.4	92.6	77.8	66.7	62.9	<u>55.5</u>			
3	32	96.9	90.7	87.5	81.2	81.2	<u>78.1</u>			
4	44	93.2	90.9	79.6	75.0	70.5	65.9	<u>56.8</u>		
5	66	96.9	90.9	80.3	77.3	77.3	75.8	<u>66.7</u>		
6	32	90.6	87.5	84.4	71.8	68.7	<u>43.8</u>			
7	20	100.0	90.0	80.0	75.0	75.0	<u>70.0</u>			
8	20	100.0	90.0	90.0	80.0	80.0	<u>60.0</u>			

Note: The weight at the beginning of the experiment is given in grams on day 1: subsequent weights are expressed as percentages of original weight. The percent of original weight at death is underlined. This format will be followed for all subsequent weight relationship tables.

experiment and every five days thereafter. By the time the first biopsy was removed, all five animals had already shed. Sections showed the perfect resting stage in the epidermis in all animals (figs. 6 and 7).

B. Experiment A-2: TRANSFERRING INTACT SNAKES FROM 28 oC TO 17 oC FOLLOWING A SHED AT 28 oC.

1. Shedding Summaries (Tables 3, 4, and 5)

Eighteen of the twenty animals placed at 28 oC entered a cloudy eye phase and shed. However, the synchrony among animals regarding onset of cloudy eyes, observed in experiment A-1, was absent in this study. The number of days prior to the onset of cloudy eyes ranged from 1 to 53. The amount of time spent in cloudy eye phase was again fairly constant; ranging from 2 to 3 days with an average of approximately 3 days. Of the eighteen animals which shed, seven were male and eleven were female. The two animals which failed to shed were both males. None of the control animals maintained throughout at 17 oC entered the cloudy eye phase.

Of the ten animals transferred from 28 oC to 17 oC, only one entered a second cloudy eye phase. It was later discovered that this animal was suffering from a fungal infection of the skin.

2. Weight Relationships (Tables 6, 7, and 8)

All of the animals were starved throughout the experiment

Table 3. Skin shedding summaries for intact animals originally placed at 28 oC then transferred to 17 oC following a shed.

Animal #	Sex	Primary Temp.	Secondary Temp.	No.CE Phases at Primary Temp.	No. CE Phases at Secondary Temp.	No. Days Prior to First CE Phase	No.Days Spent in CE Phase	No. Days Alive
1	M	28 oC	17 oC	1	0	2	3	34
2	F	28 oC	17 oC	1	0	24	2	60
3	F	28 oC	17 oC	1	0	24	2	42
4	F	28 oC	17 oC	2*	0	53	3	71
5	M	28 oC	17 oC	1	0	9	3	59
6	M	28 oC	17 oC	0	0	-	-	13
7	F	28 oC	17 oC	1	0	34	2	69
8	M	28 oC	17 oC	1	0	10	3	75
9	F	28 oC	17 oC	1	0	49	2	54
10	F	28 oC	17 oC	1	0	1	2	73

Note: (*) denotes that this animal was found to be suffering from a fungal infection of the skin.

TABLE 4. Skin shedding summaries for intact Garter snakes maintained at constant 28 oC.

Animal #	Sex	Temp.	No. of CE Phases	No. Days Prior to Onset of CE Phase	No. Days Spent in CE Phase	No. Days Alive
11	F	28 oC	1	15	5	69
12	F	28 oC	1	3	3	24
13	M	28 oC	1	18	3	25
14	M	28 oC	1	12	2	79
15	F	28 oC	1	2	5	77
16	M	28 oC	0	-	-	68
17	M	28 oC	1	21	3	74
18	M	28 oC	1	23	3	54
19	F	28 oC	1	28	3	45
20	F	28 oC	1	9	3	61

TABLE 5 Skin shedding summaries for intact Garter snakes maintained at constant 17 oC.

Animal #	Sex	Temp.	No. of CE Phases	No. Days Prior to Onset of CE Phase	No. Days Spent in CE Phase	No. Days Alive
21	M	17 oC	0	-	-	65
22	M	17 oC	0	-	-	68
23	M	17 oC	0	-	-	73
24	F	17 oC	0	-	-	50
25	F	17 oC	0	-	-	62
26	M	17 oC	0	-	-	71
27	M	17 oC	0	-	-	68
28	F	17 oC	0	-	-	59
29	F	17 oC	0	-	-	63
30	M	17 oC	0	-	-	67

Table 6. Weight relationships among intact Garter snakes originally placed at 28 oC then transferred to 17 oC following a shed.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80
1	44	93.2	81.8	84.1	68.2				
2	47	104.2	108.5	104.2	97.9	95.7			
3	48	97.9	100.0	91.6	85.4	81.2			
4	59	96.6	96.6	91.5	83.0	77.9	66.1	55.9	
5	56	100.0	96.4	96.4	83.9	80.3	66.1		
6	59	89.9							
7	60	98.3	96.7	91.2	86.5	79.6	74.5	69.3	
8	60	101.6	105.0	103.3	101.6	98.3	85.0	71.6	68.3
9	76	100.0	102.6	98.7	88.2	82.8	77.6		
10	68	94.1	91.1	91.1	83.8	64.7	64.7	64.7	

Note: For format, see Table 2.

Table 7. Weight relationships among intact Garter snakes maintained at constant 28 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80
11	74	102.7	102.7	102.7	101.3	101.3	91.8	<u>83.7</u>	
12	79	94.9	91.1	<u>86.0</u>					
13	86	102.3	102.3	<u>101.1</u>					
14	90	100.0	97.7	96.6	91.1	84.4	74.4	<u>67.7</u>	
15	50	100.0	90.0	88.0	86.0	83.1	80.0	<u>74.0</u>	
16	65	103.1	103.1	96.9	96.9	95.4	92.3	<u>87.6</u>	
17	43	95.3	93.0	88.3	81.4	<u>74.4</u>			
18	78	97.4	97.4	98.7	96.1	91.0	<u>84.6</u>		
19	102	99.0	98.0	98.0	94.1	<u>85.2</u>			
20	59	100.0	96.7	94.2	91.5	88.8	86.3	84.7	81.3

Note: For format, see Table 2.

Table 8. Weight relationships among intact Garter snakes maintained at constant 17 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80
21	72	97.2	91.6	90.2	87.5	79.1	<u>75.0</u>		
22	80	102.5	102.5	97.5	91.2	91.2	90.0	<u>85.0</u>	
23	53	105.6	107.5	98.1	90.5	83.0	71.6	67.9	<u>60.3</u>
24	59	100.0	100.0	93.2	88.1	<u>79.6</u>			
25	65	100.0	100.0	98.4	90.7	89.8	<u>79.6</u>		
26	41	97.5	97.5	97.5	92.6	90.2	85.3	<u>78.0</u>	
27	39	97.4	100.0	97.0	93.6	89.7	82.1	<u>76.9</u>	
28	82	100.0	98.7	97.5	97.5	95.1	<u>89.0</u>		
29	78	98.7	96.5	94.1	90.6	86.0	<u>84.6</u>		
30	71	100.0	100.0	98.6	98.6	95.7	91.5	<u>85.9</u>	

Note: For format, see Table 2.

and for the most part, they showed a steady decline in weight. There were instances, as in experiment A-1, where animals were either stable in weight, or actually gained weight over a period of time; this is attributed to water consumption.

Of the three groups studied, the animals maintained at constant 17 oC appeared to be the healthiest. The number of days alive recorded for this group ranged from 62 to 73 days with an average of 64.6 days. The number of days alive for the group maintained at 28 oC ranged from 24 to 79 days with an average of 57.6 days. The animals which were transferred from 28 oC to 17 oC showed a range of 34 to 75 days with an average of 59.6 days. One animal in this group died on the thirteenth day of the experiment and was never transferred.

C. Experiment A-3: TRANSFERRING INTACT SNAKES FROM 28 oC TO
EITHER 8 oC OR 17 oC FOLLOWING A SHED AT 28 oC

1. Shedding Summaries (Tables 9, 10, 11, and 12)

Twenty seven of the thirty animals placed at 28 oC entered a cloudy eye phase and shed their skins. Two animals (one male and one female) failed to enter a cloudy eye phase, and one animal escaped ten days after the start of the experiment. Of the twenty seven animals which entered a cloudy eye phase and shed, fourteen were male and thirteen were female.

Table 9. Shedding summaries for intact Garter snakes originally placed at 28 oC, transferred to 17 oC or 8 oC for a period of 21 days following a shed, then returned to 28 oC.

Animal #	Sex	Primary Temp.	Secondary Temp.	No. CE Phases at Primary Temp.	No. CE Phases at Secondary Temp.	No. Days Prior to First CE Phase	No. Days Spent in CE Phase	No. Days Alive
1	M	28 oC	17 oC	1	0	34	3	120*
2	F	28 oC	17 oC	1	0	51	3	98
3	M	28 oC	17 oC	1	0	33	5	120*
4	F	28 oC	17 oC	1	0	30	4	120*
5	F	28 oC	17 oC	1	0	27	2	100
6	F	28 oC	17 oC	1	0	26	3	79
7	F	28 oC	17 oC	1	0	28	5	120*
8	M	28 oC	17 oC	1	0	31	3	64
9	M	28 oC	17 oC	1	0	26	2	88
10	M	28 oC	8 oC	1	0	33	3	93
11	F	28 oC	8 oC	1	0	35	4	120*
12	F	28 oC	8 oC	1	0	58	2	120*
13	F	28 oC	8 oC	1	0	21	3	79
14	M	28 oC	8 oC	1	0	42	4	89
15	M	28 oC	8 oC	1	0	31	3	88
16	M	28 oC	8 oC	1	0	24	3	97
17	M	28 oC	8 oC	1	0	31	4	96
18	F	28 oC	8 oC	1	0	29	4	120*
19	F	28 oC	8 oC	1	0	38	4	77
20		F	28 oC	-----ESCAPED-----				

Note: The experiment was conducted for a total of 120 days. (*) indicates that the animal was still alive at the conclusion of the study.

Table 10. Shedding summaries for intact Garter snakes maintained at constant 28 oC.

Animal #	Sex	Temp.	No. of CE Phases	No. Days Prior to Onset of CE Phase	No. Days Spent in CE Phase	No. Days Alive at 28 oC
21	M	28 oC	1	23	3	61
22	M	28 oC	1	44	3	52
23	F	28 oC	1	39	4	73
24	F	28 oC	0	-	-	88
25	F	28 oC	1	22	5	120
26	F	28 oC	1	19	3	51
27	M	28 oC	1	35	3	49
28	M	28 oC	1	55	4	50
29	M	28 oC	1	31	2	61
30	F	28 oC	0	-	-	73

TABLE 11 Shedding summaries for intact Garter snakes maintained at constant 17 oC.

Animal #	Sex	Temp.	No. of CE Phases	No. Days Alive at 17 oC
31	F	17 oC	0	89
32	F	17 oC	0	94
33	M	17 oC	0	83
34	F	17 oC	0	120
35	M	17 oC	0	72
36	F	17 oC	0	59
37	F	17 oC	0	78
38	F	17 oC	0	75
39	M	17 oC	0	88
40	M	17 oC	0	104

TABLE 12 Shedding summaries for intact Garter Snakes maintained at constant 8 oC.

Animal #	Sex	Temp.	No. Of CE Phases	No. Days Alive at 8 oC
41	M	8 oC	0	115
42	F	8 oC	0	120
43	F	8 oC	0	120
44	M	8 oC	0	120
45	M	8 oC	0	103
46	M	8 oC	0	59
47	F	8 oC	0	120
48	M	8 oC	0	79
49	M	8 oC	0	81
50	M	8 oC	0	120

Although most animals placed at 28 oC did enter a cloudy eye phase and shed, there was no synchronization between individuals. The amount of time prior to the onset of cloudy eyes ranged from 19 to 58 days. However, the duration of the cloudy eye phases remained short; ranging from 2 to 5 days with an average of 3 days. None of the animals originally placed at 28 oC entered a second cloudy eye phase. This included animals which were 1) maintained at 28 oC throughout the experiment; 2) transferred to 17 oC or 8 oC, following an initial shed, then returned 28 oC after a period of twenty one days.

Of the ten animals originally maintained at 17 oC, none entered a cloudy eye phase, nor did any of these animals shed. The same was true of the ten animals originally maintained at 8 oC.

2. Weight Relationships (Tables 13, 14, 15, and 16)

a. General comments on weight relationships and viability

In all four groups, some animals were either stable in weight or gained weight at the start of the experiment. As no animals were fed during the experiment, these results are attributed to water consumption. The experiment lasted for a total of 120 days. The phrase "number of days alive" refers to the time from the start of the experiment.

b. Comparisons among groups

Experimental Group 1a (Table 13): The animals (# 1-9) in this

Table 13. Weight relationships among intact Garter snakes originally maintained at 28 oC, until they shed, transferred to 17 oC or 8 oC for a period of 21 days, then transferred back to 28 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
1	19	94.7	94.7	94.7	94.7	89.4	89.4	84.2	78.9	68.4	63.2	52.6	52.6*
2	28	96.4	96.4	92.8	85.7	85.7	82.1	82.1	75.0	68.2			
3	48	97.9	93.7	93.7	93.7	91.7	85.4	83.3	83.3	83.3	81.3	77.1	77.1*
4	66	100.0	98.4	96.9	95.4	95.4	93.9	92.4	89.4	87.9	84.8	83.3	83.3*
5	23	95.6	95.6	86.9	86.9	86.9	82.6	78.2	65.2	56.5			
6	37	94.6	94.6	91.8	89.2	89.2	86.5	78.4	70.3				
7	56	100.0	98.2	98.2	94.7	94.7	92.8	92.8	91.1	87.5	87.5	83.9	76.7*
8	14	92.8	92.8	85.7	85.7	85.7	78.6	64.3					
9	20	100.0	100.0	95.0	90.0	90.0	90.0	85.0	75.0	65.0			
10	17	100.0	94.1	88.2	88.2	88.2	88.2	82.3	70.5	64.5			
11	35	97.2	97.2	91.4	88.6	88.6	88.6	88.6	82.8	80.0	74.1	67.1	60.0*
12	44	100.0	95.5	93.2	93.2	90.5	90.5	88.4	87.6	87.6	84.1	79.5	70.4*
13	17	94.1	88.2	88.2	88.2	88.2	82.3	78.4	70.5				
14	14	100.0	101.3	101.3	95.6	91.2	91.2	86.1	78.3	69.6			
15	36	102.7	100.0	97.2	97.2	92.4	92.4	86.9	83.3	75.0			
16	17	100.0	100.0	100.0	100.0	94.1	94.1	94.1	82.3	78.5	70.5	66.7	
17	31	103.2	100.0	100.0	100.0	96.7	93.1	87.1	80.6	80.6	72.1		
18	56	98.2	96.4	96.4	96.4	91.3	89.2	89.2	80.3	80.3	80.3	76.7	75.0*
19	96	98.9	97.9	94.7	94.7	94.7	92.7	84.3	79.1				
20	33	-----ESCAPED-----											

Note: For format, see Table 2. The experiment was carried out for a period of 120 days.

(*) indicates that the animal was still alive at the conclusion of the experiment.

Table 14. Weight relationships among intact Garter snakes maintained at constant 28 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
21	32	100.0	96.8	87.5	78.1	71.8	68.7						
22	57	96.5	92.9	92.9	87.7	82.4							
23	39	100.0	97.4	97.4	92.3	89.7	79.5	74.3					
24	41	100.0	97.5	97.5	95.1	90.2	85.3	82.9	75.6	70.7			
25	63	100.0	100.0	98.4	95.2	92.1	92.1	90.1	90.1	88.8	85.7	82.1	80.9*
26	74	98.6	95.9	93.2	87.8	82.4							
27	28	96.4	85.7	85.7	76.1	71.4							
28	17	94.1	88.2	88.2	82.3	76.5	64.7						
29	21	100.0	100.0	90.4	85.7	76.1	66.7						
30	17	100.0	95.7	91.5	87.2	87.2	80.8	76.6					

Note: For format see tables 2 and 13.

Table 15. Weight relationships among intact Garter snakes maintained at constant 17 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
31	59	100.0	96.6	93.2	91.5	86.4	86.4	84.7	81.3	76.2			
32	68	100.0	98.5	95.6	92.7	91.3	88.4	84.1	82.6	78.2			
33	18	94.4	94.4	88.2	88.2	76.4	70.5	70.5	64.7				
34	87	98.8	98.8	98.8	96.5	95.4	95.4	95.4	93.1	93.1	91.9	90.8	88.5*
35	43	100.0	95.3	93.1	93.1	88.7	83.7	79.0					
36	75	100.0	98.6	94.6	94.6	93.3	92.0						
37	93	100.0	100.0	98.9	96.7	96.7	91.2	88.5	86.2				
38	41	100.0	100.0	97.5	97.5	95.1	95.1	92.6					
39	38	94.7	94.7	94.7	94.7	92.1	92.1	89.4	86.8	86.8			
40	27	96.2	96.2	96.2	88.8	88.8	88.8	85.1	85.1	81.4	81.4	70.3	

Note: For format see Tables 2 and 13.

Table 16. Weight relationships among intact Garter snakes maintained at constant 8 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
41	17	100.0	100.0	98.5	97.6	94.1	94.1	94.1	94.1	88.2	82.3	82.3	76.4*
42	63	100.0	98.1	98.1	98.1	98.1	96.8	96.8	95.2	95.2	93.6	91.5	90.5*
43	47	100.0	100.0	97.8	95.7	95.7	95.7	93.6	91.4	91.4	91.4	91.4	89.3*
44	36	100.0	100.0	98.7	98.7	97.2	97.2	97.2	94.4	94.4	94.4	91.6	88.9*
45	28	96.4	96.4	92.8	89.2	89.2	89.2	85.7	85.7	82.1	82.1		
46	37	97.2	94.6	94.6	94.6	91.8	89.1						
47	43	100.0	100.0	100.0	97.7	97.7	97.7	97.7	97.7	97.7	95.3	95.3	93.0*
48	21	100.0	100.0	100.0	95.2	85.7	85.7	85.7	85.7	80.9			
49	16	93.7	93.7	93.7	93.7	93.7	87.5	81.2	81.2	81.2			
50	29	95.8	95.8	95.8	95.8	95.8	91.6	87.5	87.5	87.5	83.3	83.3	79.2*

Note: For format see Table 2 and 13.

group were transferred from 28 oC to 17 oC following a shed, then returned to 28 oC after a period of 21 days. All nine animals showed a steady decline in weight throughout the course of the experiment.

Four of the nine animals in this group (two males, two females) were still alive at the conclusion of the experiment. The percent of original weight for these animals at the conclusion of the experiment ranged from 52.6% to 76.7% with an average of 72.4%.

Five of the animals in this group (two males, three females) died during the course of the experiment. The number of days alive for these animals ranged from 64-100 with an average of 85.8 days. The percent of original weight at death ranged from 56.6% to 70.3% with an average of 64.9%.

Experimental Group 1b (Table 13): These animals (# 10-19) were originally maintained at 28 oC then transferred to 8 oC following a shed. They were returned to 28 oC after a period of 21 days. All ten animals showed a steady decline in weight over the course of the experiment. Three animals (all females) were still alive at the conclusion of the experiment. The percent of original weight for these animals at that time ranged from 60.0% to 75.0% with an average of 68.5%.

Seven animals (five males, two females) died during the course of the experiment. The number of days alive for this group ranged from 77 to 97 with an average of 88.4 days. The percent of original weight at death ranged

from 64.5% to 79.1% with an average of 71.1%.

Control Group Group 2 (Table 14): All ten animals maintained at constant 28 oC showed a steady decline in weight over the course of the experiment. One animal (female) was alive at the conclusion of the experiment. The percent of original weight for this animal at that time was 80.9%.

The remaining nine animals (five males, four females) died during the course of the experiment. The number of days alive ranged from 50 to 88 with an average of 62 days. The percent of original weight at death ranged from 64.7% to 82.4% with an average of 73.0%.

Control Group 3 (Table 15): All ten animals maintained at constant 17 oC showed a steady decline in weight over the course of the experiment. One animal (female) in this group was alive at the conclusion of the experiment and its percent of original weight at that time was 88.5%.

The remaining nine animals (four males, five females) were alive for periods ranging from 59 to 104 days with an average of 82.4 days. The percent of original weight at death ranged from 64.7% to 86.8% with an average of 80.7%.

Control Group 4 (Table 16): All ten animals maintained at constant 8 oC showed a steady decline in weight over the course of the experiment. However, the weight loss was not as extensive as in the previous

groups. Six of the ten animals (three males, three females) were alive at the conclusion of the experiment. The percent of original weight for these animals at that time ranged from 76.4% to 93.0% with an average of 86.2%.

The four animals which died during the course of the experiment (all males) lasted for periods ranging from 59 to 103 days with an average of 80.5 days. The percent of original weight at death ranged from 80.9% to 89.1% with an average of 83.3%.

D. Experiment A-4: MAINTENANCE OF INTACT SNAKES AT 3 oC FOR SIX, TEN, OR FOURTEEN WEEKS PRIOR TO TRANSFER TO 28 oC.

1. Shedding Summaries

Group 1 (Tables 17 and 18): Nine of ten animals (four males, five females) survived transfer to 28 oC after having spent 3 weeks at 3 oC (Table 17). All nine entered a cloudy eye phase following the transfer. The number of days prior to the onset of the cloudy eye phase ranged from 8 to 19 with an average of 11.3 days. The length of the cloudy eye phases ranged from 3 to 4 days. All nine animals were returned to 3 oC after the last animal in the group had shed.

Upon a second exposure to 28 oC, after six additional weeks at 3 oC, three of the nine animals (one male, two females) died (Table 18). Of the six surviving animals (three males, three females), all entered a cloudy eye phase

Table 17. Shedding summaries for intact Garter snakes maintained at 3 oC for six weeks prior to being transferred to 28 oC.

Animal #	No. of Weeks at 3 oC	No. of Sheds at 3 oC	No. of Sheds at 28 oC	No. of Days Prior to the onset of CE Phase at 28 oC	No. of Days in the CE Phase at 28 oC
1	6	0	1	19	3
2	6	0	*	-	-
3	6	0	1	10	4
4	6	0	1	9	4
5	6	0	1	12	3
6	6	0	1	8	3
7	6	0	1	12	3
8	6	0	1	12	4
9	6	0	1	12	4
10	6	0	1	8	3

Note: * indicates that the animal died prior to entering the cloudy eye phase.

Table 18. Shedding summaries among intact Garter snakes upon a second six week exposure at 3 oC prior to transfer to 28 oC.

Animal #	No. of Weeks at 3 oC (Upon Second Exp.)	No. of Sheds at 3 oC	No. of Sheds at 28 oC	No. of Days Prior to the onset of CE Phase at 28 oC	No. of Days in the CE Phase at 28 oC
1	6	0	1	15	3
2	*	-	-	-	-
3	6	0	**	-	-
4	6	0	1	9	4
5	6	0	1	8	5
6	6	0	1	14	2
7	6	0	**	-	-
8	6	0	1	12	4
9	6	0	**	-	-
10	6	0	1	8	5

Note: * indicates that the animal died during the first exposure to 3 oC. ** indicates that these animals died during the second exposure to 3 oC.

and shed. The number of days prior to the onset of the cloudy eye phase ranged from 8 to 15 with an average of 11 days. The lengths of these cloudy eye phases ranged from 2 to 5 days with an average of 3.8 days.

No animals entered a cloudy eye phase at 3 oC.

Group 2 (Table 19): Nine of ten animals (three males , six females) survived being transferred to 28 C after spending 10 weeks at 3 oC. All nine entered a cloudy eye phase within 9 to 15 days, following the transfer, with an average of 9.5 days. The length of time spent in the cloudy eye phase ranged from 3 to 5 days with an average of 3.2 days. No animals entered a cloudy eye phase at 3 C.

Group 3 (Table 20): Seven of ten animals (four males, three females) survived being transferred to 28 oC after spending 14 weeks at 3 oC. Of those seven, all entered a cloudy eye phase, following the transfer, within 8 to 15 days, with an average of 10.8 days. The length of the cloudy eye phases ranged from 3 to 5 days with an average of 3.8 days. No animals entered a cloudy eye phase at 3 oC.

2. Weight Relationships

a. General comments

None of the animals were fed during the course of the experiment and the isolated instances of weight gains are attributed to water consumption.

Table 19. Shedding summaries among intact Garter snakes maintained at 3 oC for ten weeks prior to transfer to 28 oC.

Animal #	No. of Weeks at 3 oC	No. of Sheds at 3 oC	No. of Sheds at 28 oC	No. of Days Prior to the onset of CE Phase at 28 oC	No. of Days in the CE Phase at 28 oC
11	10	0	1	10	4
12	10	0	1	12	3
13	10	0	1	12	4
14	10	0	1	8	4
15	10	0	1	15	3
16	10	0	1	8	3
17	10	0	1	12	5
18	10	0	*	-	-
19	10	0	1	9	4
20	10	0	1	11	3

Note: * indicates that the animal died prior to entering a cloudy eye phase

Table 20. Shedding summaries for intact Garter snakes maintained at 3 oC for fourteen weeks
prior to transfer to 28 oC.

Animal #	No. of Weeks at 3 oC	No. of Sheds at 3 oC	No. of Sheds at 28 oC	No. of Days Prior to the onset of CE Phase at 28 oC	No. of Days in the CE Phase at 28 oC
21	14	0	1	15	4
22	14	0	1	12	4
23	14	0	1	11	3
24	14	0	*	-	-
25	14	0	1	9	4
26	14	0	1	11	4
27	14	0	1	8	3
28	14	0	*	-	-
29	14	0	*	-	-
30	14	0	1	10	5

Note: * indicates that the animal died prior to entering a cloudy eye phase.

b. Comparisons among groups

Group 1 (Tables 21a and 21b): All ten animals (six males, four females) lost weight during the first six week exposure to 3 oC (Table 21a). The percent of original weight at the end of this period ranged from 85.7% to 95.3% with an average of 90.9%. One animal (male) died four days after transfer to 28 oC. The percent of original weight for this animal at death was 88.9%. The remaining nine animals (five males, four females) showed a decline in weight during the three week exposure to 28 oC. The percent of original weight at the end of this period ranged from 73.7% to 81.4% with an average of 78.0%.

Only six of the remaining nine animals survived a second six week exposure to 3 oC (Table 21b). Of the three animals (one male, two females) which died, the percent of original weight (ie. percent of weight from the start of the second exposure to 3 oC) at death ranged from 85.7% to 95.8% with an average of 92.0%.

For the six surviving animals (four males, two females) the percent of original weight at the end of the six week exposure to 3 oC ranged from 82.4% to 95.6% with an average of 89.6%. Each of these animals continued to lose weight during the three week exposure to 28 oC. At the end of this time, the percent of original weight ranged from 70.6% to 88.0% with an average of 79.4%.

Table 21a. Weight relationships among intact Garter snakes maintained at 3 oC for six weeks prior to transfer to 28 oC.

Animal		Weight (gm)												
#	Sex	Day 1 (3 oC)	7	14	21	28	35	42	Day 1 (28 oC)	5	10	15	20	
1	M	32	96.8	96.8	93.7	93.7	90.6	90.6	90.6	87.5	81.3	18.1	75.0	
2	M	27	103.7	96.3	96.3	92.5	92.5	88.9	88.9	88.9				
3	F	46	95.6	95.6	91.3	91.3	91.3	91.3	91.3	86.9	84.7	82.6	80.4	
4	M	21	100.0	95.2	95.2	95.2	90.4	85.7	85.7	85.7	80.9	80.9	76.2	
5	F	52	100.0	100.0	96.1	96.1	96.1	94.2	94.2	92.3	92.3	88.5	86.5	
6	F	39	97.4	94.8	92.3	92.3	92.3	92.3	92.3	89.7	87.2	82.1	79.5	
7	F	31	100.0	100.0	96.8	96.8	93.5	93.5	93.5	87.1	83.8	77.4	77.4	
8	M	25	96.0	92.0	92.0	92.0	88.0	88.0	88.0	84.0	80.0	76.0	72.0	
9	M	19	100.0	94.7	94.7	94.7	89.5	89.5	89.5	84.2	84.2	73.7	73.7	
10	M	43	102.3	97.7	97.7	95.3	95.3	95.3	95.3	90.7	86.0	83.7	81.4	

Note: For format, see Tables 2 and 13.

Table 21 b. Weight relationships among intact Garter snakes upon a second six week exposure to 3 oC followed by transfer to 28 oC.

Animal #	Sex	Weight (gm.) Day 1 (3 oC)	7	14	21	28	35	42	Day 1 (28 oC)	5	10	15	20
1	M	24	100.0	100.0	95.8	95.8	95.8	87.5	87.5	87.5	83.3	83.3	79.1*
3	F	37	97.3	94.6	94.6	94.6							
4	M	16	100.0	93.7	93.7	87.5	87.5	87.5	87.5	81.2	81.2	75.0	75.0*
5	F	45	100.0	97.8	97.8	95.6	95.6	95.6	95.6	93.3	93.3	91.1	88.0*
6	F	31	100.0	96.7	96.7	96.7	96.7	93.5	93.5	93.5	90.3	83.9	80.6*
7	F	24	100.0	95.8									
8	M	17	100.0	94.1	94.1	94.1	88.2	82.3	88.3	76.5	76.5	70.6	70.6*
9	M	14	100.0	92.8	92.8	85.7							
10	M	35	100.0	97.1	97.1	94.3	94.3	91.4	91.4	91.4	88.6	85.7	82.8*

Note: For format, see Tables 2 and 13.

Group 2 (Table 22): All ten animals (three male, seven female) survived the ten week exposure to 3 oC. At the end of this time, the percent of original weight ranged from 83.9% to 95.2% with an average of 90.6%.

One animal (female) died three days after transfer to 28 oC. The percent of original weight of this animal at death was 92.1%. The remaining nine animals survived the three week exposure to 28 oC. The percent of original weight of these animals at the end of this time ranged from 74.2% to 88.2% with an average of 79.9%.

Group 3 (Table 23): All ten animals (five males, five females) survived the fourteen week exposure to 3 oC. At the end of this period of time, the percent of original weight ranged from 80.0% to 92.7% with an average of 87.5%.

Three animals (one male, two females) died shortly after transfer to 28 oC. The percent of original weight at death for these animals ranged from 80.9% to 92.7% with an average of 88.5%.

The remaining seven animals (four males, three females) survived a three week exposure to 28 oC. The percent of original weight at the end of this period of time ranged from 71.4% to 85.0% with an average of 77.6%.

Section II: SKIN SHEDDING AND THYROIDECTOMY IN GARTER SNAKES

A. EXPERIMENT B-1: MAINTENANCE OF THYROIDECTOMIZED SNAKES

Table 22. Weight relationships among intact Garter snakes maintained at 3 oC for ten weeks prior to transfer to 28 oC.

Animal	Sex	Weight (gm)		Day 1									
		Day 1 (3 oC)	7	21	35	49	63	70	(28 oC)	5	10	15	20
11	F	68	100.0	98.5	98.5	94.1	92.6	92.6	92.6	92.6	89.7	89.7	88.2
12	F	34	97.0	97.0	94.1	94.1	91.2	91.2	91.2	82.4	79.4	76.5	76.5
13	M	27	100.0	100.0	96.3	92.6	92.6	92.6	92.6	85.2	81.5	81.5	77.8
14	F	57	98.2	98.2	96.5	96.5	94.7	91.2	91.2	89.5	89.5	87.7	85.9
15	F	49	102.0	95.9	95.9	95.9	93.8	91.8	91.8	87.7	83.7	79.6	79.6
16	F	71	100.0	98.5	97.2	95.8	92.9	91.6	91.6	91.6	88.7	87.3	83.1
17	M	29	100.0	93.1	89.6	89.6	86.2	86.2	86.2	82.7	79.3	79.3	75.8
18	F	63	98.4	98.4	95.2	95.2	95.2	92.1	92.1				
19	F	40	100.0	97.5	95.0	92.5	87.5	87.5	87.5	85.2	82.4	80.0	77.5
20	M	31	100.0	96.8	96.8	90.3	83.8	83.8	83.8	80.6	80.6	77.4	74.2

Note: For format, see Tables 2 and 13

Table 23. Weight relationships among intact Garter snakes maintained at 3 oC for fourteen weeks prior to transfer to 28 oC.

Animal		Weight (gm.)	Day 1											
#	Sex	Day 1(3 oC)	14	28	42	56	70	84	98	(28 oC)	5	10	15	20
21	M	23	95.6	95.6	91.3	87.0	87.0	84.6	84.6	84.6	82.4	80.8	78.7	76.5
22	M	47	97.8	95.7	95.7	91.5	91.5	98.4	87.2	87.2	87.2	84.9	82.7	78.4
23	F	69	98.6	97.1	94.7	93.2	92.7	91.3	89.7	89.7	86.4	81.3	79.0	76.5
24	F	62	100.0	98.3	95.4	92.5	92.5	91.0	91.0	91.0				
25	M	28	96.4	94.5	92.8	92.8	89.2	89.2	87.6	87.6	84.1	80.0	75.8	70.5
26	F	49	97.9	95.9	93.8	93.8	91.3	89.7	89.7	89.7	85.6	83.6	78.9	74.2
27	M	33	96.4	93.9	92.0	91.9	90.7	89.5	88.4	88.4	84.5	81.2	76.5	68.4
28	M	21	98.2	95.2	95.2	92.3	90.2	88.7	86.5	86.5	83.4			
29	F	55	97.1	98.1	96.3	94.2	93.1	89.6	87.5	87.5				
30	F	67	98.5	98.5	96.7	95.4	90.1	88.7	86.0	86.0	83.2	79.5	75.4	67.3

Note: For format, see Tables 2 and 13.

Table 23a Summary of data for high temperature induced shed experiments on intact Garter snakes.

Exp. #	# of Animals	Prim. Temp	Second. Temp	# of Animals in CE Phase Prim./Sec Temp	Aver # CE Phases per Animal Prim./Sec Temp	Aver # Days Prior to CEs Prim./Sec. Temp	Aver.# Days Alive	Aver % Wt. at Death
A-1	8	28 oC	-	4/-	1/-	17/-	61.0	62.2
A-2a	10	28 oC	17 oC	9/0	1/0	30/0	55.0	73.6
A-2b	10	28 oC	-	9/-	1/-	14.6/-	57.6	80.5
A-2c	10	17 oC	-	0/-	0/-	0/-	64.6	79.3
A-3a	9	28 oC	17 oC	9/0	1/0	31.8/0	101.0	64.9
A-3b	10	28 oC	8 oC	10/0	1/0	31.2/0	97.9	71.1
A-3c	10	28 oC	-	8/-	1/-	33.5/-	60.8	73.0
A-3d	10	17 oC	-	0/-	0/-	0/-	79.7	80.7
A-3e	10	10 oC	8 oC	0/-	0/-	0/-	91.7	83.3

Table 23b Summary of data collected on intact animals maintained at 3 oC for 6, 10 or 14 weeks
prior to transfer to 28 oC.

Exp. #	# of Animals	# Weeks at 3 oC	# Animals to Shed at 3 oC	# Animals to Shed at 28 oC	Aver. # Days Prior to Shed at 28 oC	Aver. % Original Wt. at Death
A-4a	10	6	0	9	11.3	92.0
A-4b	10	10	0	9	10.8	88.9
A-4c	10	14	0	7	11.0	87.3

AT CONSTANT 28 oC, 21 oC, OR 10 oC.

1. Shedding Summaries

a. General comments

While all nineteen of the thyroidectomized animals entered a cloudy eye phase, not all of these animals actually shed their outer epidermal generations. The appearance of the first post-surgical cloudy eye phase was highly synchronized among these animals; ranging from 14 to 17 days. The lengths of the first post-surgical cloudy eye phases ranged from 6 to 8 days with an average of 6.8 days.

b. Comparisons among groups

Group 1 (Table 24): Of the seven animals which remained at 21 oC, two were males and five were females. Three of those animals (all females) underwent two additional cloudy eye phases while the remaining four animals (two female and two males) underwent one additional cloudy eye phase. The amount of time between cloudy eye phases ranged from 8 to 17 days with an average of 13.4 days. The number of days spent in the cloudy eye phase (subsequent to the first cloudy eye phase) ranged from 5 to 10 with an average of 8.5 days. In only three instances did an animal actually shed following a second cloudy eye phase. In the rest of the animals, the outer generation(s) remained attached to the body. No animals shed following a third cloudy eye phase.

Table 24. Shedding summaries for thyroidectomized Garter snakes maintained at constant 21 oC.

Animal #	Sex	No. of CE Phases	No. Days Prior to CE Phase	No. Days Alive Following Thyroidectomy
			No. Days in CE Phase	
1	F	3	14/8 , 13/10 , 13/9	51
2	F	3	14/7 , 8/12, 8/7	50
3	M	2	16/5 , 15/9	60
4	M	2	15/6 , 14/9	56
5	F	2	15/6 , 17/8	58
6	F	3	15/6 , 15/9 , 11/8	72
7	F	3	17/7 , 10/8 , 13/7	67

Group 2 (Table 25): Of the six animals transferred to 28 oC, following a renewal phase at 21 oC, three were male and three were female. Three of these animals (two female, one male) underwent two additional cloudy eye phases, two (both male) underwent three additional cloudy eye phases, and one (female) underwent four additional cloudy eye phases. The number of days between cloudy eye phases, at 28 oC, ranged from 4 to 7 with an average of 6.1 days. The length of time spent in the renewal phase ranged from 3 to 7 days with an average of 4.5 days. In only two cases was the skin actually shed following a second cloudy eye phase. In the rest of the animals, the old outer generations stacked, one on top of the other, on the body surface. No animals shed following a third cloudy eye phase.

Group 3 (Table 26): None of the animals placed at 10 oC, following a cloudy eye phase at 21 oC, underwent any additional cloudy eye phases.

2. Weight Relationships

a. General Comments

All of the animals lost weight and eventually died. Once again, there were instances where animals either gained weight or maintained a constant weight over a period of time. This has been attributed to water consumption. Overall, the number of days alive ranged from a minimum of 38 to a maximum of 120.

Table 25. Shedding summaries for thyroidectomized Garter snakes maintained at 21 oC then
to 28 oC following a cloudy eye phase.

Animal #	Sex	No. of CE Phase at 21 oC	No. of Days Prior to Onset of CE Phase	No. of CE Phases at 28 oC	No. of Days Prior to Onset of CE Phase	No. of Days Alive Following Tx
			No. of Days Spent in CE Phase at 21 oC		No. of Days Spent in CE Phase at 28 oC	
8	M	1	15/7	3	8/5 , 5/6 , 6/9	52
9	F	1	15/7	2	9/4 , 8/3	52
10	M	1	16/8	2	6/5 , 6/6	68
11	F	1	16/7	2	8/5 , 7/5	55
12	M	1	17/7	3	6/4 , 6/3 , 6/3	51
13	M	1	17/7	4	4/4 , 5/5 , 4/4 , 5/7	64

Table 26. Shedding summaries for thyroidectomized Garter snakes maintained at 21 oC then transferred to 10 oC following a cloudy eye phase.

Animal #	Sex	No. of CE Phases at 21 oC	No. of Days Prior to Onset of CE Phase <u>No. of Days Spent in</u> CE Phase at 21 oC	No. of CE Phases at 10 oC	No. of Days Alive Following Thyroidectomy
14	F	1	15/8	0	38
15	M	1	17/6	0	94
16	M	1	17/6	0	115
17	F	1	17/7	0	90
18	F	1	15/8	0	120
19	M	1	15/9	0	116

b. Comparisons among groups

Group 1 (Table 27): The number of days alive for these animals ranged from 51 to 72 with an average of 59.1 days. The percent of original weight at death ranged from 50.0 % to 86.2 % with an average of 71.2%.

Group 2 (Table 28): These six animals remained alive from 51 to 68 days with an average of 57.0 days. The percent of original weight at death for these animals ranged from 54.5% to 81.8% with an average of 69.5%.

Group 3 (Table 29): The number of days that these animals remained alived ranged from a minimum of 38 to a maximum of 120. The average number of days alive was 95.5 days. The percent of original weight at death ranged from 60.0% to 84.8% with an average of 73.4%.

3. Light Microscopic Analysis

Serial biopsies were taken from each of the animals at five day intervals. Biopsies taken during the first cloudy eye phase, from animals maintained at 28 oC and 21 oC, revealed histologic changes identical to those described by Maderson (1965) (Fig. 8, 9, 10, 11, 12, 13). Although not evident in the micrographs, biopsies taken from thyroidectomized animals seemed to come apart during sectioning making microtomy difficult. This worsened with subsequent renewal phases suggesting that cell adhesion may have been disrupted. (see Dicussion section V C)

Biopsies removed from animals maintained at constant 10 oC reveal that

Table 27. Weight relationships among thyroidectomized Garter snakes maintained at constant 21 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
1	82	96.3	90.2	89.0	89.0	83.5	81.7						
2	94	100.0	100.0	95.7	88.3	86.2							
3	44	95.4	88.6	86.1	75.0								
4	24	95.8	91.6	87.5	75.0								
5	19	100.0	100.0	89.5	81.1	73.6							
6	28	100.0	96.4	96.4	89.3	84.2	86.8	61.2					
7	18	100.0	100.0	88.9	88.9	75.0	55.0	50.0					

Note: For format, see Tables 2 and 13.

Table 28. Weight relationships among thyroidectomized Garter snakes maintained at 21 oC then transferred to 28 oC following a cloudy eye phase.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
8	88	97.7	96.6	94.3	88.6	81.8							
						—							
9	101	97.0	97.0	93.1	88.2	77.1							
						—							
10	53	94.3	94.3	90.5	83.0	73.6							
						—							
11	114	98.2	96.5	93.8	85.9	71.9	61.4	58.7					
								—					
12	35	100.0	94.3	88.6	82.8	71.2							
						—							
13	33	101.6	100.0	93.9	81.8	78.7	54.5						
							—						

Note: For format, see Tables 2 and 13.

Table 29. Weight relationships among thyroidectomized Garter snakes maintained at 21 oC then transferred to 10 oC following a cloudy eye phase.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
14	24	100.0	91.2	86.2									
15	25	100.0	100.0	92.0	88.0	84.0	80.0						
16	22	100.0	100.0	95.4	90.9	86.7	82.3	82.3	81.8	79.0	75.6	71.2	
17	36	100.0	97.2	91.7	88.8	86.1	80.5	80.5	77.8	68.9			
18	33	100.0	96.7	93.4	91.0	87.8	84.8	84.8	84.8	81.7	79.6	77.1	75.4
19	20	101.7	100.0	95.0	91.5	91.0	85.0	80.0	80.0	80.0	78.5	71.8	63.4

Note: For format, see Tables 2 and 13.

these animals appear to be in the perfect resting condition during the period of time that they are at this temperature (fig. 14, 15, 16, 17).

B. Experiment B-2: MAINTENANCE OF THYROIDECTOMIZED SNAKES AT 10 oC FOLLOWED BY TRANSFER TO 15 oC OR 28 oC.

1. Shedding Summaries (Tables 30 and 31)

Of the fourteen thyroidectomized animals placed at 10 oC for a period of 46 days, none entered a cloudy eye phase. Group 1 animals, transferred to 28 oC did, however enter at least one additional cloudy eye phase at that temperature. In fact, two of these animals (both males) entered one subsequent cloudy eye phase, one animal (male) entered two additional cloudy eye phases, and four animals (two males, two females) underwent three subsequent cloudy eye phases. Only three animals actually shed following the first cloudy eye phase at 28 C. None of the animals shed following any of the other cloudy eye phases.

The amount of time prior to onset of cloudy eyes, following transfer to 28 oC, ranged from 7 to 8 days. Thus, a high degree of synchrony was observed. The number of days between cloudy eye phases ranged from 4 to 10 with an average of 5.7 days. The amount of time spent in the cloudy eye phase ranged from 4 to 10 days with an average of 5.1 days.

The animals in group 2 were transferred to 15 oC after having spent 46

Table 30. Shedding summaries for thyroidectomized Garter snakes maintained at 10 oC for 46 days then transferred to 28 oC.

Animal #	Sex	No. of CE Phases at 10 oC	No. of CE Phases at 28 oC	No. of Days Prior to Onset of CE Phase No. of Days Spent in CE Phase at 28 oC	No. of Days Alive Following Thyroidectomy
1	F	0	3	7/4 , 6/5 , 8/4	86
2	F	0	3	8/5 , 6/6 , 6/5	86
3	M	0	3	7/4 , 4/4 , 4/4	79
4	M	0	3	7/4 , 4/4 , 4/10	86
5	M	0	1	8/7	62
6	M	0	1	7/6	60
7	M	0	2	8/4 , 10/6	72

Table 31. Shedding summaries for thyroidectomized Garter snakes maintained at 10 oC for 46 days, transferred to 15 oC for 48 days, then placed at 28 oC for the remainder of the experiment.

Animal #	Sex	No. of CE Phases at 10 oC	No. of CE Phases at 15 oC	No. of CE Phases at 28 oC	No. of Days Prior to Onset of CE Phase	No. of Days Alive Following Tx
					No. of Days Spent in CE Phase at 28 oC	
8	F	0	0	1	3/2	99
9	F	0	0	1	2/6	109
10	M	0	0	1	2/4	99
11	F	0	0	1	2/3	99
12	F	0	0	•	—	81
13	M	0	0	1	2/5	99
14	M	0	0	1	3/4	105

days at 10 oC, but none entered a cloudy eye phase at this secondary temperature (Table 31). After a period of 48 days at 15 oC, the six surviving animals were placed at 28 oC. All six entered a cloudy eye phase within 3 days of transfer. The amount of time spent in the cloudy eye phase ranged from 2 days to 6 days with an average of 4 days. These animals died shortly after the conclusion of the first cloudy eye phase.

2. Weight Relationships (Tables 32 and 33)

All of the animals steadily lost weight and eventually died. In this experiment, however, the weight loss appeared to be slow at first, accelerating just prior to death. Among the seven animals transferred to 28 oC, five showed a weight loss of 30.0% or more at the time of death (Table 32). The average percent of original weight at death was 60.9%. However, two animals showed only modest weight losses at the time of death: One animal was at 92.6% and 96.0% of the original weight respectively. The animals in this group remained alive under experimental conditions for periods ranging from 60 to 86 days with an average of 75.8 days.

Of the six animals placed at 15 oC for 48 days (following 46 days at 10 C), then at 28 oC, all showed weight losses of 23% or more at death with an average of 27.6% (Table 33). One animal, originally in this group, died prior to being placed at 28 oC. The animals in this group remained alive for periods ranging from 81 to 109 days with an average of 96.7 days.

Table 32. Weight relationships among thyroidectomized Garter snakes maintained at 10 oC for 46 days then transferred to 28 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
1	59	98.3	97.6	94.1	93.2	86.4	81.3	74.5	69.4	61.0			
2	57	100.0	100.0	96.4	96.4	96.4	94.7	91.2	85.9	77.1	50.8		
3	32	96.8	96.8	90.8	84.3	81.2	75.0	62.5					
4	30	93.3	93.3	93.3	93.3	90.0	83.3	73.3	70.0	60.0			
5	27	103.0	100.0	96.3	92.6	92.6	89.0						
6	25	100.0	96.0	92.0	92.0	91.0	87.3						
7	20	100.0	95.0	95.0	90.0	85.0	81.0	70.0					

Note: For format, see Tables 2 and 13.

Table 33. Weight relationships among thyroidectomized Garter snakes maintained at 10 oC for 46 days, transferred to 15 oC for 48 days, then placed at 28 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
8	69	95.6	92.7	88.4	86.9	84.1	83.5	81.1	78.0	78.2	72.4		
9	63	101.3	98.4	95.2	95.2	93.6	90.4	85.7	80.9	82.5	74.6		
10	25	101.8	100.0	96.0	92.3	89.0	84.0	80.0	72.0	76.0	68.0		
11	65	98.5	92.3	92.3	92.3	90.7	87.6	84.6	81.5	80.0	76.9		
12	44	100.0	95.5	95.5	95.4	90.9	84.0	77.2	70.5				
13	25	104.0	96.0	92.0	88.0	88.0	84.0	80.0	80.0	72.0			
14	17	105.8	100.0	94.1	94.1	88.2	82.3	76.4	82.3	76.4	76.4	70.6	

Note: For format, see Tables 2 and 13.

3. Light Microscopic Analysis

Serial biopsies were removed from each animal at five day intervals.

As was the case in experiment B-1, the skin samples showed a perfect resting condition epidermis for the entire period of time spent by the animals at 10 oC and 15 oC (fig. 18, 19, 20, 21). Skin samples taken from animals transferred to 28 oC showed an epidermis entering the renewal phase within one week of transfer (fig. 22, 23, 24, 25).

C. Experiment B-3: MAINTENANCE OF INTACT AND THYROIDECTOMIZED SNAKES AT CONSTANT 21 oC.

1. Shedding Summaries (Tables 34 and 35)

Of the ten intact animals placed at 21 oC, two (one male, one female) entered a single cloudy eye phase while eight (four males, four females) failed to enter a cloudy eye phase at all (Table 34). One animal remained in the cloudy phase for a period of 4 days, while the other showed the condition for 5 days.

On the other hand, of the ten thyroidectomized animals to be placed at 21 oC, all entered at least one cloudy eye phase (Table 35). One female showed a single post-surgical cloudy eye phase. One male entered two cloudy eye phases. Seven animals (five males, two females) showed three cloudy eye phases while one female showed four. The length of time in the cloudy

Table 34. Shedding summaries for intact Garter snakes maintained at constant 21 °C.

Animal #	Sex	No. of CE Phases at 21 °C	No. of Days in CE Phase	No. of Days Alive
1	M	0	-	82
2	F	0	-	73
3	F	1	4	75
4	F	0	-	61
5	F	0	-	58
6	M	0	-	68
7	M	0	-	79
8	M	0	-	43
9	M	1	5	52
10	F	0	-	72

TABLE 35 Shedding summaries for thyroidectomized Garter snakes maintained at constant 21 °C.

Animal #	Sex	No. of CE Phases	No. of Days Spent in CE Phase	No. of Days Alive
11	M	3	7, 5, 7	51
12	M	3	9, 7, 7	54
13	F	3	9, 9, 10	49
14	M	3	7, 8, 5	57
15	F	3	6, 5, 8	61
16	F	4	10, 9, 10, 3*	68
17	M	3	8, 8, 7	57
18	M	3	7, 9, 8	59
19	F	1	6	37
20	M	2	8, 8	45

Note: * indicates that this animal died while in a cloudy eye phase.

eye phase ranged from 5 to 10 days with an average of 7.7 days.

2. Weight Relationships (Tables 36 and 37)

The number of days that the intact animals remained alive ranged from 43 to 79 with an average of 66.3 days. The thyroidectomized animals survived for periods ranging from 37 days to 68 days with an average of 53.8 days.

As noted in previous experiments, all of the animals under study lost weight and eventually died. This was true of both the intact and thyroidectomized animals. The percent of original weight at death, among the intact animals, ranged from 79.1% to 93.6% with an average of 83.8%. In the case of the thyroidectomized animals, the percent of original weight at death ranged from 58.8% to 83.8% with an average of 74.7%.

Section III: THE EFFECTS OF THYROIDECTOMY AND FEEDING ON SKIN SHEDDING IN GARTER SNAKES.

A. Experiment C-1: FED INTACT AND THYROIDECTOMIZED GARTER SNAKES MAINTAINED AT ROOM TEMPERATURE

1. Feeding Results

Each of the fifteen animals fed when offered food. Initially, the amounts of food consumed were approximately the same in all three groups. Although animals in Groups 3 (intacts) and 2 (shams) fed throughout the

Table 36. Weight relationships among intact Garter snakes maintained at constant 21 °C.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90
1	25	100.0	96.0	92.0	92.0	84.0	80.0	84.0	79.0	
2	37	97.3	97.3	91.9	89.2	83.7	78.4			
3	33	96.9	96.9	93.9	93.9	87.8	87.8	84.8	81.8	
4	48	97.9	93.7	93.7	93.7	89.6	87.5			
5	72	100.0	101.3	100.0	97.2	94.4	93.0			
6	63	98.4	96.8	96.8	96.8	93.6	93.6			
7	30	100.0	100.0	93.3	86.7	86.7	86.7	83.3	80.0	
8	17	100.0	94.1	88.2	88.2					
9	18	100.0	100.0	94.4	89.9	77.8				
10	22	100.0	100.0	94.5	86.4	81.2	79.1			

Note: For format, see Tables 2 and 13.

Table 37. Weight relationships among thyroidectomized Garter snakes maintained at constant 21 oC.

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90
11	29	100.0	101.4	96.6	93.1	82.7				
						—				
12	25	96.0	84.0	79.0	76.0	64.0				
						—				
13	47	97.8	93.6	89.4	87.2	82.9				
						—				
14	17	100.0	94.1	94.1	82.3	76.5	64.7	58.8		
								—		
15	63	100.0	100.0	93.1	90.4	88.9	85.7	80.9		
								—		
16	68	98.5	98.5	95.5	94.1	94.1	92.6	86.7	83.8	
									—	
17	26	96.1	96.1	88.5	84.6	80.7	76.9	65.3		
								—		
18	33	100.0	100.0	96.9	93.9	90.9	84.8	78.7		
								—		
19	31	100.0	93.5	87.0	80.6					
					—					
20	29	96.5	93.1	89.6	82.7	68.9				
						—				

Note: For format, use 2 and 13.

Table 37a Summary of data collected from thyroidectomized Garter snakes maintained at various temperature regimes.

Exp. #	# of Animals (Tx or Intact)	Prim. Temp	Second. Temp	# of Animals in CE Phase Prim./Sec Temp	Aver. # CE Phases per Animal Prim./Sec Temp	Aver. # Days Prior to CEs Prim./Sec. Temp	Aver.# Days Alive	Aver % Wt. at Death
B-1a	7 (Tx)	21 oC	-	7/-	2.7/-	13.5/-	59.1	71.2
B-1b	6 (Tx)	21 oC	28 oC	6/6	1/2.7	16/6.2	57.0	69.5
B-1c	6 (Tx)	21 oC	10 oC	6/0	1/0	16/0	95.5	73.4
B-2a	7 (Tx)	10 oC	28 oC	0/7	0/2.3	0/6.5	75.8	61.0
B-2b	7 (Tx)	15 oC	28 oC	0/6	0/1	0/2.2	96.7	72.4
B-3a	10 (Int)	21 oC	-		1/-	14.5/-	66.3	83.8
B-3b	10 (Tx)	21 oC	-	10	2.8/-	11.3/-	53.8	74.7

duration of the experiment, this was not the case with the thyroidectomized animals in Group 1. Following surgery, all five animals fed through the first cloudy eye phase. Upon completion of the first cloudy eye phase, only the two animals which actually shed continued to feed. This feeding pattern continued until the completion of the second cloudy eye phase at which time, these animals also failed to shed and stopped feeding. The three animals which failed to shed, following the first cloudy eye phase, discontinued feeding at that point.

2. Shedding Summaries (Table 38)

Of the five intact animals in Group 3 two females entered a single cloudy eye phase and shed. The amount of time prior to the onset of the cloudy eyes was 29 days for one animal and 47 days for the second animal. One animal spent 3 days in the cloudy eye phase, the other 2 days. The other three animals in the group (two males, one female) failed to enter the cloudy eye phase.

Two of the five sham animals (one male, one female) in Group 2 entered a single cloudy eye phase and shed. The number of days prior to the onset of cloudy eyes was 23 for one animal and 32 for the second animal. The amount of time spent in cloudy eye phase was 4 days and 5 days respectively. None of the other animals in the group (two males, one female) entered the cloudy eye phase.

TABLE 38 Shedding frequencies among thyroidectomized (Tx'ed), sham, and intact Garter snakes which had been fed and maintained at room temperature (circa 24.5 oC).

Animal #	Sex	Tx'ed/Sham/Intact	No. of CE Phases	No. of Days in CE Phase	No. of Days Alive From Start of Experiment
1	M	Tx'ed	3	4, 6, 5	53
2	M	Tx'ed	2	7, 4	63
3	F	Tx'ed	3	5, 6, 4	59
4	F	Tx'ed	3	5, 5, 7	45
5	F	Tx'ed	3	5, 8, 6	47
6	M	Sham	0	-	110
7	M	Sham	0	-	52
8	M	Sham	1	4	93
9	F	Sham	1	5	95
10	F	Sham	0	-	81
11	F	Intact	1	3	93
12	M	Intact	0	-	71
13	F	Intact	0	-	120*
14	F	Intact	1	2	120*
15	M	Intact	0	-	120*

Note: The experiment was carried out for a total of 120 days. * indicates that the animal was still alive at the conclusion of the experiment.

All five of the thyroidectomized animals (two males, three females) in Group 1 entered at least two cloudy eye phases during the course of the experiment. One animal showed two cloudy eye phases while the other four showed three subsequent cloudy eye phases. The amount of time prior to the onset of the first cloudy eye phase among these animals ranged from 12 to 15 days. The amount of time spent in the cloudy eye phase among the animals ranged from 4 to 7 days with an average of 5.5 days. Only two of the five animals actually shed following the first cloudy eye phase. The remaining three animals did not shed following the first cloudy eye phase. None of the five animals shed following any subsequent cloudy eye phases.

3. Weight Relationships (Table 39)

The number of days alive among the intact animals in Group 3 ranged from 71 to more than 120 (the experiment was terminated after a period of 120 days when three of the animals were still alive). Of the two animals (one male, one female) which did die, the percent of original weight at death was 93.9% and 82.3% respectively.

The sham animals in Group 2 remained alive for periods ranging from 52 to 110 days with an average of 86.2 days. The percent of original weight at death ranged from 86.9% to 97.7% with an average of 93.8%.

The five thyroidectomized animals in Group 1 remained alive for periods ranging from 47 to 63 days with an average of 53.4 days. At death,

Table 39. Weight relationships among thyroidectomized, sham, and intact Garter snakes which had been fed and maintained at room temperature (circa 24.5 oC).

Animal #	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
1	33	100.0	96.9	93.9	81.8	72.7							
2	21	100.0	100.0	90.5	85.7	76.2							
3	45	102.0	100.0	97.8	93.3	91.1	80.0						
4	68	100.0	100.0	101.5	98.5	94.1	77.9						
5	47	100.0	97.8	95.7	87.2	80.5							
6	55	100.0	100.0	101.8	101.8	100.0	100.0	98.1	96.4	92.7	92.7	92.7	
7	23	100.0	95.6	86.9	91.3	86.9							
8	41	100.0	102.4	104.8	100.0	100.0	100.0	97.5	97.5	95.1			
9	61	103.2	100.0	98.4	95.1	95.1	95.1	95.1	93.4	93.4			
10	75	100.0	100.0	97.3	97.3	97.3	98.6	96.0	96.0				
11	82	100.0	98.8	98.8	100.0	97.6	96.3	95.0	93.9	93.9			
12	17	100.0	100.0	94.1	88.2	88.2	88.2	82.3					
13	58	100.0	100.0	98.3	95.5	94.8	94.8	93.0	93.1	89.6	89.6	87.9	87.9*
14	81	101.4	103.7	102.5	102.5	102.5	101.2	101.2	98.8	98.7	100.0	98.7	97.6*
15	45	100.0	102.2	102.2	100.0	97.8	97.8	95.5	95.5	91.1	91.1	93.3	91.9*

Note: For format, see Tables 2 and 13. Animals 1-5 are intact, 6-10 are sham, and 11-15 are thyroidectomized.

the percent of original weight ranged from 66.7% to 80.8% with an average of 75.6%.

B. Experiment C-2: COMPARISONS OF THE EFFECTS OF FEEDING AND STARVATION IN INTACT, SHAM, AND THYROIDECTOMIZED GARTER SNAKES MAINTAINED AT CONSTANT 21 °C

1. Feeding Results

The results were very similar to those reported in experiment C-1.

All fifteen animals in the experimental (fed) group ate initially. Animals in Groups 1 (intacts) and 2 (shams) fed for the duration of the experiment. All five thyroidectomized animals in Group 3 fed through the first cloudy eye phase, when three shed. These three animals continued to feed through the conclusion of the second cloudy eye phase, but none shed at which point feeding ceased. The two animals which failed to shed, following the first cloudy eye phase, stopped feeding at that time.

2. Shedding Summaries

Experimental Group 1a (Intact) (Table 40): Of the five animals in this group, two (one male, one female) experienced a cloudy eye phase and shed during the course of the experiment. The amount of time prior to the onset of the cloudy eye phase was 50 days in one and 64 days in the other. In both cases, the cloudy eye phase lasted for a period of 3 days. None of the

Table 40. Shedding summaries among intact, sham, and thyroidectomized (Txed) Garter snakes fed and maintained at room temperature (circa 24.5 °C).

Animal #	Sex	Intact/Sham/Txed	No. of CE Phases	No. of Days Between CE Phases No. Days Spent in CE Phase	No. Days Alive From the Start of the Exp.
1	F	Intact	0		120*
2	M	Intact	1	50/3	120*
3	F	Intact	1	64/3	106
4	F	Intact	0	-	99
5	M	Intact	0	-	120*
6	M	Sham	0	— ESCAPED —	
7	F	Sham	0	-	101
8	M	Sham	0	-	120
9	F	Sham	0	-	98
10	M	Sham	0	-	115
11	F	Txed	4	9/12, 10/8, 10/9, 11/8	52
12	F	Txed	4	9/11, 10/8, 11/9, 12/8	69
13	F	Txed	4	9/12, 10/8, 14/9, 13/8	108
14	M	Txed	4	9/10, 14/8, 10/9, 13/8	64
15	F	Txed	3	10/9, 13/7, 12/9	72

Note: All animals were fed a diet of earth worms. The experiment was carried out for a period of 120 days.

* indicates that the animal was still alive at the conclusion of the experiment.

remaining three animals entered a cloudy eye phase.

Experimental Group 1b (Sham) (Table 40): Of the four animals in this group (one escaped at the beginning of the experiment), none entered a cloudy eye phase.

Experimental Group 1c (Thyroidectomized) (Table 40): The animals (one male and four females) in this group entered the cloudy eye phase following surgery. One animal experienced post-surgical cloudy eye phases on three occasions while the remaining four each underwent four post-surgical cloudy eye phases. As stated previously, only three of these five animals actually shed following the first cloudy eye phase. The remaining two animals failed to shed. None of the animals shed following the second renewal phase nor did any shed following subsequent cloudy eye phases.

The amount of time prior to the onset of the first cloudy eye phase was 9 days in four of the animals and 10 days in the fifth. The number of days between cloudy eye phases ranged from 10 to 14 with an average of 11.4 days. The amount of time spent in the cloudy eye phase ranged from 7 to 12 days with an average of 9.1 days.

Control Group2a (Intact) (Table 41): One female entered a cloudy phase and shed during the course of the experiment: it took 77 days to enter the cloudy eye phase which lasted 5 days. None of the remaining animals (three males, one female) showed cloudy eyes.

Table 41. Shedding summaries among intact, sham, and thyroidectomized (Tx'ed) Garter snakes maintained at constant room temperature (circa 24.5 °C).

Animal #	Sex	Intact/Sham/Tx'ed	No. of CE Phases	No. of Days Between CE Phases		No. Days Alive From Start of Exp.
				No. Days Spent in CE Phase		
16	F	Intact	0	-		110
17	M	Intact	0	-		120
18	M	Intact	0	-		120
19	F	Intact	1	77/5		98
20	M	Intact	0	-		85
21	F	Sham	1	72/5		81
22	F	Sham	1	69/3		92
23	F	Sham	1	72/4		85
24	M	Sham	0	-		91
25	M	Sham	0	-		94
26	F	Tx'ed	3	11/11, 12/7, 13/8		66
27	M	Tx'ed	4	11/13, 13/9, 12/7, 11/9		51
28	M	Tx'ed	3	11/13, 11/7, 12/4		97
29	F	Tx'ed	3	11/13, 10/9, 11/7		67
30	F	Tx'ed	4	11/11, 12/9, 11/9, 11/6		71

Note: All animals were starved throughout the experiment

Control Group 2 b (Sham) (Table 41): Three of the four animals in this group (all female) entered a cloudy eye phase and shed during the experiment. The amount of time prior to the onset of the cloudy eye phase was 69 days for one animal and 72 days for the two remaining animals. One animal showed a cloudy eye condition for 3 days, a second animal showed it for 4 days, the third for 5 days. Two males failed to enter the cloudy eye phase.

Control Group 2 c (Thyroidectomized) (Table 41): All five of the animals in this group (two males, three females) underwent several cloudy eye phases following surgery. Three animals showed three post-surgical cloudy eye phases, two showed four. Of the five animals, only two actually shed following the first cloudy eye phase. None of the animals shed following the second cloudy eye phase nor did any shed following subsequent cloudy eye phases.

The amount of time following surgery, prior to the onset of the first cloudy eye phase, was 11 days for all five animals. The number of days between subsequent cloudy eye phases ranged from 10 to 13 with an average of 11.6 days.

The number of days spent in the cloudy eye phase ranged from 7 to 13 with an average of 9.2 days.

3. Weight Relationships

Experimental Group 1 a (Intact) (Table 42): Only two of the five

Table 42. Weight relationships among intact, sham, and thyroidectomized Garter snakes fed and maintained at room temperature (circa 24.5 oC).

Animal #	Intact Sham Tx'ed	Weight (gm.) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
1	Intact	68	100.0	97.1	95.6	94.1	91.2	94.1	94.1	91.2	88.2	85.3	85.3	85.3*
2	Intact	28	96.4	92.8	89.3	85.7	82.1	85.7	78.5	75.0	78.5	78.5	75.0	75.0*
3	Intact	61	101.6	95.1	90.1	90.1	88.5	90.1	86.8	83.6	80.3	78.7	77.1	
4	Intact	78	101.3	100.0	96.2	96.2	93.6	91.1	89.7	88.5	85.8	83.3		
5	Intact	21	100.0	100.0	95.2	90.5	90.9	90.4	85.7	85.7	80.9	80.9	76.2	76.2*
6	Sham	49		97.9	93.8	-----ESCAPED-----								
7	Sham	56	96.4	96.4	92.8	91.1	89.3	87.5	89.3	87.5	82.1	78.6		
8	Sham	23	104.3	100.0	91.3	87.6	86.9	86.9	82.6	82.6	86.9	86.9	82.6	82.6*
9	Sham	72	98.6	98.6	94.4	90.3	93.1	91.6	93.1	94.4	90.3	90.3		
10	Sham	33	100.0	93.9	84.8	75.7	75.7	75.7	81.8	81.8	81.8	81.0	81.8	78.7*
11	Tx'ed	68	100.0	100.0	95.5	86.7	88.2	85.3						
12	Tx'ed	48	102.1	91.7	85.4	81.3	77.1	75.0	64.6					
13	Tx'ed	78	100.0	100.0	94.8	94.8	88.5	87.2	84.6	80.7	79.5	74.3	71.7	
14	Tx'ed	33	103.0	103.0	96.9	87.8	90.9	84.8	72.7					
15	Tx'ed	60	98.3	96.7	88.3	86.7	81.7	76.7	68.3					

Note: For format, see Tables 2 and 13. Animals were fed a diet of earth worms.

animals in this group died during the course of the experiment which lasted for a period of 120 days. Under experimental conditions, the animals in this group remained alive for an average of 113 days.

Despite being fed, all animals showed a steady weight loss throughout the experiment. Of the two females in this group which died during the course of the experiment, one was at 77.1% of its original weight at death, the other 83.3%. Of the three animals which remained alive, the percent of original weight, at the conclusion of the study, was 85.3%, 75.0% and 76.2%.

Experimental Group 1b (Sham) (Table 42): Two of the four animals (both female) in this group (one escaped) died during the course of the experiment. Under experimental conditions, the animals in this group remained alive for an average of 109.8 days.

Once again, all of the animals in this group exhibited a steady decline in weight despite being fed. The percent of original weight at death for the two animals which died was 78.6% for one animal and 90.3% for the other. Of the two animals which remained alive, the percent of original weight at the conclusion of the experiment was 82.6% and 78.7%.

Experimental Group 1c (Thyroidectomized) (Table 42): Of the five animals in this group, all died before the experiment ended. The average number of days alive ranged from 52 to 108 with an average of 73 days.

As noted for groups 1a and 1b all of the animals showed a loss in weight

throughout the experiment. However, the weight loss observed here appeared to be more extreme. Here, the percent of original weight at death ranged from 64.6% to 85.3% with an average of 72.1%.

Control Group 2a (Intact) (Table 43): Three of the five animals (one male, two females) in this group died during the course of the experiment. The animals of this group survived for an average of 106.6 days under experimental conditions.

Once again, all of the animals showed a steady decline in weight. For the three animals which did die prior to the conclusion of the study, the percent of original weight at death was 79.0%, 85.8% and 76.0%. Of the animals which remained alive, the percent of original weight at the conclusion of the experiment was 85.0% and 72.0%.

Control Group 2b (Sham) (Table 43): All five animals died before the experiment ended. The number of days alive ranged from 81 to 94 with an average of 88.6 days.

The weight loss observed in previous studies was also seen here. The percent of original weight at death ranged from 74.1% to 88.6% with an average of 84.0%.

Control Group 2c (Thyroidectomized) (Table 43): As in the previous group, all animals in this group died prior to the conclusion of the experiment. The number of days alive ranged from 51 to 97 with an average of 70.4 days.

Table 43. Weight relationships among intact, sham, and thyroidectomized Garter snakes starved and maintained at room temperature (circa 24.5 oC).

Animal #	Intact Sham Tx'ed	Weight (gm.) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
16	Intact	53	98.1	96.2	96.2	92.4	92.4	94.3	90.3	88.6	86.7	86.7	79.0	
17	Intact	40	102.5	100.0	97.5	95.0	92.5	92.5	92.5	90.0	87.5	85.0	85.0	85.0*
18	Intact	44	100.0	90.0	84.1	84.5	81.8	81.0	79.5	78.1	77.2	75.0	72.3	69.0*
19	Intact	78	101.2	97.4	94.8	92.3	91.0	91.0	91.0	89.7	88.4	85.8		
20	Intact	25	96.0	93.4	93.0	93.0	92.0	88.0	88.0	84.0	76.0			
21	Sham	61	100.0	93.4	93.4	90.2	91.8	90.2	88.5	86.8				
22	Sham	104	98.1	95.1	92.3	91.3	92.3	91.3	91.3	89.4	86.5			
23	Sham	43	102.3	95.3	93.0	90.6	89.0	89.0	88.4	86.0	82.7			
24	Sham	35	100.0	97.8	97.1	94.3	95.1	91.4	91.4	86.5	83.8			
25	Sham	27	96.2	92.4	88.8	88.8	85.6	81.5	80.0	77.8	74.1			
26	Tx'ed	52	98.0	92.3	86.5	84.3	80.7	78.8	76.0					
27	Tx'ed	77	100.0	101.0	98.7	94.8	90.9	87.0						
28	Tx'ed	41	98.7	97.6	82.9	81.0	78.0	75.6	78.0	73.1	65.8			
29	Tx'ed	99	97.0	93.9	92.9	84.9	82.6	80.9	78.0					
30	Tx'ed	59	98.3	91.5	86.4	84.7	81.3	79.7	72.6					

Note: For format, see Tables 2 and 13.

The percent of original weight at death ranged from 65.8% to 87.0% with an average of 77.7%.

C. Experiment C-3: COMPARISONS OF THE EFFECTS OF FEEDING
AND STARVATION IN THYROIDECTOMIZED GARTER SNAKES
MAINTAINED AT CONSTANT 28 °C FOLLOWING SIX WEEKS
AT EITHER 3 °C OR ROOM TEMPERATURE.

1. Feeding Results

a. General comments

All twenty four intact Garter snakes maintained at room temperature (RT) fed when offered food. No food was offered to animals maintained at 3 °C.

b. Comparisons among groups

Group 1 (Experimental): All eleven intact animals, originally maintained at RT for six weeks, and all four animals originally maintained at 3 °C for six weeks, fed when transferred to 28 °C. The intact animals continued to feed throughout the course of the experiment. All eight of the thyroidectomized (Tx'ed) animals fed following surgery. Seven of the eight animals shed following a cloudy eye phase. These animals continued to feed. The animal which did not shed following the first cloudy eye phase stopped feeding at that time. Three of the seven animals, which shed following the

first cloudy eye phase, shed following a second eye phase. These animals continued to feed until the end of the third cloudy eye phase at which time they did not shed. The four animals which failed to shed after the second cloudy eye phase stopped feeding at that time.

Group 2 (Control): These fifteen animals were not fed for the duration of the experiment (120 days).

2. Shedding Summaries

a. General comments

None of the twenty four animals, maintained at RT for six weeks, shed during this period of time. The same was true of the six animals maintained at 3 oC for six weeks.

b. Comparisons among groups

Group 1 (Fed) (Tables 44 and 46): Nine out of ten of the animals (six males, four females), originally maintained at room temperature, entered a cloudy eye phase and shed following transfer to 28 oC. The number of days prior to the onset of the first cloudy eye phase ranged from 10 to 39 with an average of 19 days. The number of days spent in the cloudy eye phase ranged from 4 to 7 with an average of 5.7 days.

All four of the animals (one male, three females) , maintained at 3 oC, showed cloudy eyes and shed following transfer to 28 oC. The number of days prior to the first cloudy eye phase ranged from 15 to 25

days with an average of 19.5 days. The number of days spent in the cloudy eye phase ranged from 4 to 7 with an average of 5.5 days.

Of the seven intact animals, none entered a second cloudy eye phase during the course of the experiment (120 days). All seven thyroidectomized animals did enter additional cloudy eye phases following surgery. Four animals entered three additional cloudy eye phases and three entered two additional cloudy eye phases. The number of days prior to the onset of the first post-surgical cloudy eye phase ranged from 11 to 13 with an average of 11.8 days. The number of days spent in the cloudy eye phase ranged from 6 to 13 with an average of 8.8 days.

Group 2 (Starved) (Tables 45 and 47): All eleven animals (three males, eight females), originally maintained at RT (circa 24.5 oC) for six weeks, shed following transfer to 28 oC. The number of days prior to the onset of cloudy eyes ranged from 10 to 43 with an average of 15.5 days. The number of days spent in the cloudy eye phase ranged from 4 to 7 with an average of 5.2 days.

All four animals, maintained originally at 3 oC, entered a cloudy eye phase and shed following transfer to 28 oC. The number of days prior to the onset of cloudy eyes ranged from 15 to 18 with an average of 16.5 days.

Of the eight intact animals, none entered a second cloudy eye phase at 28 oC (120 days). All seven thyroidectomized animals did enter at least one

Table 44. Shedding summaries among fed intact Garter snakes maintained at either room temperature (circa 24.5 oC) or 3 oC for six weeks prior to transfer to 28 oC.

Animal #	Sex	Original Temp.	No. of Sheds at Original Temp.	No. of Sheds at 28 oC	No. of Days Prior to First CE Phase	No. of Days in CE Phase
1	M	RT	0	1	10	4
2	F	RT	0	1	10	6
3	M	RT	0	1	15	6
4	F	RT	0	1	10	4
5	M	RT	0	1	27	4
6	M	RT	0	1	11	7
7	M	RT	0	1	14	5
8	F	RT	0	0	-	-
9	F	RT	0	1	21	5
10	F	RT	0	1	39	4
11	M	RT	0	1	14	6
12	F	3 oC	0	1	35	7
13	F	3 oC	0	1	15	4
14	F	3 oC	0	1	15	5
15	M	3 oC	0	1	23	6

Note: Animals maintained at room temperature for six weeks prior to transfer to 28 oC were fed earth worms over that period of time while those animals maintained at 3 oC were starved. All animals were fed after being placed at 28 oC.

Table 45. Shedding frequencies among starved intact Garter snakes maintained at either room temperature (circa 24.5 oC) or 3 oC for six weeks prior to transfer to 28 oC.

Animal #	Sex	Original Temp.	No. of Sheds at Original Temp.	No. of Sheds at 28 oC	No. of Days Prior to First CE Phase	No. of Days in CE Phase
16	F	RT	0	1	10	5
17	F	RT	0	1	11	6
18	F	RT	0	1	10	5
19	M	RT	0	1	10	6
20	F	RT	0	1	12	7
21	M	RT	0	1	16	5
22	F	RT	0	1	16	5
23	M	RT	0	1	43	5
24	F	RT	0	1	10	5
25	F	RT	0	1	12	4
26	F	RT	0	1	12	7
27	M	3 oC	0	1	17	6
28	F	3 oC	0	1	16	7
29	F	3 oC	0	1	18	5
30	M	3 oC	0	1	15	4

Note: Those animals maintained at room temperature were fed while the animals kept at 3 oC were starved.

None of the animals in this group were fed following transfer to 28 oC.

Table 46. Frequency of cloudy eye phase among fed intact and thyroidectomized Garter snakes maintained at 28 oC following six weeks at either room temperature (circa 24.5 oC) or 3 oC.

Animal #	Tx'ed/Intact	No. of Subsequent CE Phases at 28 oC.	No. of Days Between CE Phases	No. of Days in CE Phase	No. of Days Alive Following Transfer to 28 oC	No Of Days Alive Following Tx
1	Tx'ed	3	11, 17, 15	13, 7, 9	84	74
2	Intact	0	-	-	78	-
3	Intact	0	-	-	68	-
4	Tx'ed	2	13, 11	9, 8	51	41
5	Tx'ed	3	11, 14, 13	8, 9, 10	93	63
6	Intact	0	-	-	120*	-
7	Tx'ed	2	12, 16	10, 8	73	59
8	Intact	0	-	-	97	-
9	Intact	0	-	-	120*	-
10	Tx'ed	3	12, 14, 16	9, 7, 7	107	68
11	Tx'ed	2	13, 14	11, 10	64	50
12	Tx'ed	3	11, 14, 15	6, 8, 8	93	58
13	Intact	0	-	-	96	-
14	Intact	0	-	-	93	-
15	Intact	0	-	-	120*	-

Note: * indicates that the animal was still alive at the conclusion of the experiment.

Table 47. Frequency of cloudy eye phase among starved intact, and thyroidectomized Garter snakes maintained at 28 oC following six weeks at either room temperature (circa 24.5 oC) or 3 oC.

Animal #	Tx'ed/Intact	No. of Subsequent CE Phases at 28 oC.	No. of Days Between CE Phases	No. of Days in CE Phase	No. of Days Alive Following Transfer to 28 C	No. of Days Alive Following Tx
16	Intact	1	53	3	120*	74
17	Tx'ed	3	12, 14, 14	9, 7, 8	67	-
18	Intact	1	71	2	120*	-
19	Intact	1	67	4	120*	-
20	Tx'ed	2	11, 13	7, 7	59	38
21	Tx'ed	3	14, 11, 10	8, 8, 7	80	59
22	Intact	1	59	3	120*	-
23	Intact	1	43	2	93	-
24	Tx'ed	3	10, 13, 10	7, 7, 6**	68	53
25	Intact	1	73	4	120*	-
26	Intact	0	-	-	120*	-
27	Intact	1	68	5	97	-
28	Tx'ed	1	11	8	52	-
29	Tx'ed	2	10, 11	9, 2**	51	28
30	Tx'ed	1	14, 12	8, 6**	59	40

Note: * indicates that the animal was still alive at the conclusion of the experiment. ** indicates that the animal died while in the cloudy eye condition.

Table 48. Weight relationships among fed intact and thyroidectomized Garter snakes maintained at 28 oC after six weeks at either room temperature (circa 24.5 oC) or 3 oC.

Animal #	Tx/Int.	Original Temp.	Weight (gm) Day 1 (28 oC)	Day Txed at 28 oC	10	30	50	60	70	80	90	100	110	120
1	Tx	RT	45	14	98.7	87.6	77.8	72.7	69.7					
2	Int.	RT	68	-	100.0	95.6	88.1	83.2	80.3	78.5				
3	Int.	RT	43	-	100.0	95.3	91.4	89.6	84.3					
4	Tx	RT	79	14	100.0	89.2	79.3							
5	Tx	RT	36	31	100.0	97.2	88.1	84.1	81.3	78.8	73.6	70.8		
6	Int.	RT	44	-	98.4	100.0	98.7	98.7	96.5	97.0	97.0	95.6	94.0	95.6*
7	TX	RT	41	19	100.0	90.5	85.3	81.6	78.5	74.7				
8	Int.	RT	110	-	100.0	101.8	100.0	99.1	98.1	98.1	95.4	94.6		
9	Int.	RT	102	-	98.7	ESCAPED								
10	Tx	RT	72	44	100.0	98.7	96.4	94.4	88.2	83.5	80.7	76.2	72.1	
11	Tx	RT	51	20	98.0	94.3	87.2	83.1	76.7					
12	Tx	3 oC	63	32	96.8	95.2	86.7	83.4	79.6	74.2	72.8	69.5		
13	Int.	3 oC	92	-	100.0	101.7	97.8	96.7	96.7	92.4	91.5	89.5		
14	Int.	3 oC	112	-	98.9	97.8	96.7	92.3	92.3	89.0	88.7			
15	Int.	3 oC	61	-	100.0	98.4	96.9	95.1	90.2	90.2	90.2	89.5	89.5	87.5*

Note: For format, see Tables 2 and 13. Animals were thyroidectomized on an alternating basis following a shed at 28 oC.

additional cloudy eye phase at 28 oC. Three animals entered three additional cloudy eye phases, three animals entered two additional cloudy eye phases, and one animal entered one additional cloudy eye phase. The number of days between cloudy eye phases ranged from 10 to 14 with an average of 11.9 days. The number of days spent in a complete cloudy eye phase ranged from 7 to 9 with an average of 7.7 days. Three animals died while in the cloudy eye phase.

3. Weight Relationships

a. General Comments

The twenty four Garter snakes, originally maintained at RT for six weeks, were fed earth worms and goldfish ad libitum. At the end of this period of time, the percent of original weight of these animals ranged from 94.7% to 86.0% with an average of 91.2%.

The percent of original weight at the end of six weeks of the eight unfed animals maintained at 3 oC ranged from 92.0% to 87.4% with an average of 90.3%.

b. Comparisons among groups

Group 1 (Fed) (Tables 46 and 48): Of the fourteen animals in this group (one animal escaped eight days after transfer to 28 oC), six remained intact throughout the experiment. Five of the seven animals died prior to the end of the experiment. The number of days alive at 28 oC ranged

from 68 to 97 with an average of 86.4 days. The percent of original weight at death ranged from 78.5% to 94.6% with an average of 87.2%. Two animals were alive at the conclusion of the experiment (120 days). The percent of original weight of these animals were 95.6% and 87.6%.

The remaining seven animals were thyroidectomized following a shed at 28 oC. All of these animals died during the course of the experiment. The number of days alive at 28 oC following thyroidectomy ranged from 41 to 74 with an average of 59 days. The percent of original weight at death ranged from 69.5% to 79.3% with an average of 73.2%.

Group 2 (Starved) (Tables 47 and 49): Of the fifteen animals in this group, eight remained intact throughout the course of the experiment. Four of these animals died prior to the conclusion of the experiment. The number of days alive at 28 oC ranged from 70 to 98 with an average of 89.5 days. The percent of original weight at death ranged from 70.9% to 81.0% with an average of 74.9%. Four intact animals were alive at the conclusion of the experiment. The percent of original weight ranged from 68.7% to 71.6% with an average of 70.7%.

The remaining seven animals were thyroidectomized following a shed at 28 oC. All of these animals died prior to the conclusion of the experiment. The number of days alive at 28 oC following thyroidectomy ranged from 29 to 74 with an average of 48.7 days. The percent of original weight at death

Table 49. Weight relationships among starved intact and thyroidectomized (Txed) Garter snakes maintained at 28 oC following six weeks at either room temperature (circa 24.5 oC) or 3 oC.

Animal #	Tx/Int.	Original Temp.	Weight (gm) Day 1 (28 oC)	Day Tx'ed at 28 oC	10	30	50	60	70	80	90	100	110	120
16	Int.	RT	63	-	100.0	94.2	89.5	86.3	82.1	78.2	76.4	71.1	70.5	68.7
17	Tx	RT	65	17	98.5	94.3	87.3	82.4	78.5	74.1	70.2			
18	Int.	RT	96	-	100.0	94.9	87.6	84.3	81.2	78.9	77.3	75.2	73.1	71.5*
19	Int.	RT	78	-	97.4	91.0	86.5	84.2	81.0					
20	Tx	RT	28	19	98.6	89.3	81.2	75.0						
21	Tx	RT	31	21	98.9	94.7	82.2	79.3	74.8					
22	Int.	RT	85	-	100.0	95.8	87.3	86.5	84.1	80.0	78.3	76.5	74.2	71.6*
23	Int.	RT	68	-	100.0	94.3	86.3	84.1	82.0	79.4	76.7	73.4		
24	Tx	RT	75	15	96.7	88.3	81.2	74.3	71.0					
25	Int.	RT	93	-	100.0	94.7	84.5	82.1	81.0	78.5	74.3			
26	Int.	RT	82	-	100.0	98.7	92.1	88.7	84.5	82.3	79.8	76.5	74.3	71.0*
27	Int.	3 oC	44	-	96.8	89.6	82.6	80.1	79.2	77.1	74.6	70.9		
28	Tx	3 oC	59	23	97.8	89.4	80.7							
29	Tx	3 oC	114	23	94.2	87.6	77.1							
30	Tx	3 oC	31	19	98.1	87.6	79.3	74.5						

Note: For format, see Tables 2 and 13. Animals were thyroidectomized on an alternating basis after having shed at 28 oC.

Table 49a Summary of data collected from starved and fed intact Garter snakes maintained at room temperature.

Exp. #	# Animals	Fed Starved	Tx Sham Intact	# Animals to Enter CE Phase	Average # CE Phases per Animal	Average # Days Alive	Average % of Original Wt at Death
C-1a	5	Fed	Tx	5	3.5	73	72.5
C-1b	5	Fed	Sham	0	0	108.5	84.5
C-1c	5	Fed	Intact	2	1	113.0	80.2
C-2a	5	Starved	Tx	5	3.4	70.4	75.9
C-2b	5	Starved	Sham	3	1	88.6	82.8
C-2c	5	Starved	Intact	1	1	106.6	80.3

Table 49b Summary of data collected from fed and starved intact Garter snakes maintained at 3 oC or room temperature for six weeks prior to transfer to 28 oC.

Exp #	# Animals	Fed/starved	Original Temp.	# Sheds at Original temp	# of Animals to Shed at 28 oC	Average # Days Prior to First CE Phase
C-3a	11	Starved	RT	0	10	17.1
C-3b	4	Starved	3 oC	0	4	22.0
C-3c	11	Fed	RT	0	11	14.7
C-3d	4	Fed	3 oC	0	4	16.8

Table 49c Summary of data from fed and starved intact and thyroidectomized garter snakes maintained at either room temperature or 3 oC for six weeks prior to transfer to 28 oC.

Exp #	# of Animals	Tx/ Intact	Fed/Starved	Original Temp	# Animals to Enter CE Phase at 28 oC	Aver # CE Phases/ Animal at 28 oC	Aver # Days Prior to CE Phase	Aver # Days Alive	% Orig Wt at Death
C-3e	5	Int	Fed	RT	0	0	0	96.6	87.7
C-3f	6	Tx	Fed	RT	6	2.5	12.0	78.7	73.8
C-3g	2	Int	Fed	3 oC	0	0	0	103.0	88.7
C-3h	2	Tx	Fed	3 oC	2	3.0	13.0	93.0	73.5
C-3i	7	Int	Starved	RT	6	1	52.0	115.0	76.2
C-3j	4	Tx	Starved	RT	4	2.8	11.7	68.5	72.8
C-3k	2	Int	Starved	3 oC	2	1	39.5	74.5	78.9
C-3l	2	Tx	Starved	3 oC	2	2	12.0	55.0	72.7

ranged from 70.2% to 80.7% with an average of 74.7%.

Section IV: EFFECTS OF RELATIVE HUMIDITY ON SKIN SHEDDING IN INTACT GARTER SNAKES

A. Experiment D-1: EFFECTS OF CONTROLLED RELATIVE HUMIDITY ON INTACT GARTER SNAKES MAINTAINED AT CONSTANT 28 °C.

1. Relative Humidity Measurements

Relative humidity within the chambers was monitored constantly using hygrometers. The relative humidity within the control chambers (no vacuum, no drierite) ranged from 70% to 85%. Relative humidity within the chambers attached to a vacuum line ranged from 60 to 74%. Relative humidity within the drierite chambers ranged from 10 to 20%.

2. Shedding Summaries

Group 1 (Control) (Table 50): Eight of ten animals (five males, three females) in this group, maintained at constant 28 °C, entered a cloudy eye phase and shed. The number of days prior to the onset of the cloudy eye condition ranged from 14 to 67 with an average of 35.2 days. The number of days spent in cloudy eye phase ranged from 2 to 5 with an average of 3.9 days. None of the animals entered a second cloudy eye phase at 28 °C.

Group 2 (Vacuum) (Table 52): Nine of ten animals (five males, four females) in this group, maintained at constant 28 °C with a vacuum line

Table 50. Shedding summaries among intact Garter snakes maintained at constant 28 oC.

Animal #	Sex	No. of CE Phases at 28 oC	No. of Days Prior to Onset of CE Phase	No. of Days Spent in CE Phase	No. of Additional CE Phases at 28 oC	No. of Days Alive at 28 oC
1	M	1	14	3	0	77
2	M	1	67	4	0	59
3	F	0	-	-	0	63
4	F	1	31	5	0	76
5	F	1	23	4	0	110
6	M	1	29	3	0	60
7	M	0	-	-	-	79
8	M	1	49	2	0	54
9	M	1	52	3	0	77
10	F	1	17	4	0	120

Table 51. Weight relationships among intact Garter snakes maintained at constant 28 oC.

Animal #	Sex	Weight (gm)	10	20	30	40	50	60	70	80	90	100	110	120
1	M	41	92.7	89.5	83.6	75.1	72.5	68.7	64.3	59.6				
2	M	39	96.8	93.2	89.5	84.3	80.7	76.8						
3	F	63	93.2	84.1	77.3	71.4	68.9	63.7	56.1					
4	F	47	97.0	86.6	84.1	77.9	71.0	64.5	59.6	51.7				
5	F	63	92.4	90.4	84.6	81.1	78.7	71.4	68.8	61.2	55.6	53.2	49.6	
6	M	20	101.2	93.6	81.4	76.5	69.7	63.5						
7	M	32	93.2	88.8	81.4	76.6	68.9	64.4	59.7	56.6				
8	M	17	98.4	93.6	89.7	84.3	74.4	70.1						
9	M	27	97.5	92.3	85.7	83.1	79.6	74.5	69.3	61.0				
10	F	103	94.7	90.5	88.5	86.1	85.0	83.6	80.0	76.4	73.8	71.0	68.6	65.1*

Note: For format, see Tables 2 and 13.

Table 52. Shedding summaries for intact Garter snakes maintained at 28 oC in chambers to which vacuum lines were attached in order to remove excess moisture.

Animal #	Sex	No. of CE Phases at 28 oC	No. of Days Prior to Onset of CE Phase	No. of Days Spent in CE Phase	No. of Subsequent CE Phases at 28 oC	No. of Days Alive at 28 oC
11		1	31	3	0	65
12		1	14	3	0	80
13		1	37	3	0	63
14		1	33	4	0	108
15		1	52	3	0	57
16		1	42	4	0	77
17		1	23	4	-	29**
18		1	32	3	0	60
19		0	-	-	-	87
20		1	21	3	0	74

Note: ** indicates an animal which died as result of getting its head caught in the vacuum tubing.

attached to the chamber to remove excess moisture, entered a cloudy eye phase and shed. The number of days prior to the onset of the cloudy eye condition ranged from 14 to 52 with an average of 31.6 days. The number of days spent in the cloudy eye phase ranged from 3 to 4 with an average of 3.3 days. None of the animals entered a second cloudy eye phase at 28 oC.

Group 3 (Drierite)(Table 54): These animals were maintained at 28 oC in chambers where the air was dried by drierite. All fifteen animals (eight males, seven females) in this group entered a cloudy eye phase and shed. The number of days prior to the onset of the cloudy eye condition ranged from 10 to 24 with an average of 15.2 days. This range is much narrower than those reported in groups 1 and 2. The number of days spent in the cloudy eye phase ranged from 2 to 5 with an average of 3.1 days. Only one animal in the group entered a second cloudy eye phase at 28 oC. It was later discovered that this animal was suffering from fungal infection of the skin.

3. Weight Relationships

a. General Comments

None of the animals were fed for the duration of the experiment and all animals lost weight. In a few cases animals gained weight initially, probably due to water consumption.

b. Comparisons among groups

Group 1(Control) (Table 51): Nine of ten animals (six males, three

Table 53. Weight relationships among intact Garter snakes maintained at 28 oC in chambers to which a vacuum line was attached to remove excess moisture.

Animal #	Sex	Weight (gm)	10	20	30	40	50	60	70	80	90	100	110	120
11	M	27	98.7	93.2	84.6	80.0	75.3	69.7	61.3					
12	F	39	92.0	88.6	83.5	78.6	77.5	76.1	74.2	71.0				
13	F	48	98.0	87.5	81.4	72.3	64.5	53.6	55.7					
14	M	41	93.3	89.5	86.1	84.5	80.0	76.3	72.6	69.7	66.4	60.8	59.7	
15	M	17	98.6	92.1	89.5	81.3	77.4	73.8						
16	F	59	96.6	89.9	84.3	78.4	73.5	67.6	59.9	54.7				
17	M	27	96.3	91.0	84.6**									
18	M	35	93.3	88.9	84.2	78.0	73.1	68.8						
19	M	41	90.0	84.3	82.1	77.6	69.0	61.4	54.6	53.2	49.1			
20	F	73	95.4	84.9	81.0	76.5	69.7	64.0	59.2	53.7				

Note: For format, use Tables 2 and 13. ** indicates that this animal died as a result of getting its head caught in the vacuum tubing.

Table 54. Shedding frequencies for intact Garter snakes maintained at 28 oC and constant low relative humidity.

Animal #	Sex	No. of CE Phases at 28 oC	No. of Days Prior to Onset of CE Phase	No. of Days Spent in CE Phase	No. of Subsequent CE Phases at 28 oC	No. of Days Alive at 28 oC
21	F	1	14	3	0	84
22	F	1	10	3	0	78
23	M	1	15	3	0	60
24	M	1	14	4	0	63
25	M	1	13	4	0	58
26	F	1	19	3	0	107
27	F	1	21	2	0	93
28	M	1	14	5	0	69
29	M	1	13	3	0	87
30	M	1	12	3	0	50
31	M	1	24	4	0	55
32	F	1	12	3	0	69
33	F	1	16	3	0	86
34	M	1	19	3	1*	77
35	F	1	13	3	0	104

Note: Air was dried over drierite before entering the chamber. Relative humidity within the chambers averaged about 10%. * indicates that animal was suffering from a skin fungal infection.

females) in this group died before the experiment ended (120 days). The number of days alive at 28 °C ranged from 54 to 110 with an average of 72.8 days. The percent of original weight at death ranged from 49.6% to 76.8% with an average of 60.3%. One animal (female) was alive at the conclusion of the experiment. The percent of original weight for this animal at that time was 65.1%.

Group 2 (Vacuum) (Table 53): All ten animals (six males, four females) in this group died before the experiment ended. The number of days alive at 28 °C ranged from 57 to 108 with an average of 74.5 days. The percent of original weight at death ranged from 49.1% to 73.4% with an average of 60.9%. One animal (no. 17) died 29 days after the start of the experiment as a result of getting its head stuck in the vacuum line and its weight data were excluded from the calculations.

Group 3 (Drierite) (Table 55): All fifteen animals (eight males, seven females) died before the experiment ended. The number of days alive at 28 °C ranged from 50 to 107 with an average of 76.1 days. The percent of original weight at death ranged from 49.5% to 77.0% with an average of 60.1%.

B. Experiment D-2 EFFECTS OF CONTROLLED RELATIVE HUMIDITY ON SNAKES MAINTAINED AT CONSTANT 21 °C.

1. Relative Humidity Measurements

The relative humidity in the control chamber (no vacuum, no drierite)

Table 55. Weight relationships among intact garter snakes maintained at 28 oC and constant low relative humidity.

Animal #	Sex	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
21	F	47	101.3	98.7	96.4	89.6	84.0	77.9	74.5	72.3	68.2			
22	F	44	98.8	91.0	86.5	84.3	79.6	74.5	72.0	71.2				
23	M	37	91.2	84.7	76.5	68.7	59.5	53.1						
24	M	31	93.4	85.6	98.4	71.0	68.5	61.2	58.7					
25	M	27	97.0	89.4	84.6	81.1	79.3	77.0						
26	F	57	96.5	91.9	84.2	81.5	77.6	71.3	64.5	61.1	58.7	56.6	51.0	
27	F	63	98.7	94.5	93.2	88.9	86.6	80.0	78.8	73.4	69.9	63.6		
28	M	19	92.0	87.9	81.4	76.5	68.9	63.5	57.1					
29	M	27	94.7	86.5	80.1	76.6	69.9	63.8	60.4	57.2	51.1			
30	M	29	98.0	89.5	81.6	74.5	64.7							
31	M	38	93.1	87.7	80.0	78.6	76.3	73.3						
32	F	61	97.7	89.0	81.4	74.3	66.7	59.8	52.3					
33	F	69	98.5	87.6	82.1	76.5	71.4	66.7	61.3	58.4	54.0			
34	M	21	91.0	85.6	81.4	73.0	68.2	59.7	54.3	49.5				
35	F	33	93.3	90.7	86.5	81.4	76.7	69.1	65.6	59.4	55.0	50.0		

Note: For format, see Tables 2 and 13. Air was dried over drierite before entering the chamber.
Relative humidity within the chambers averaged about 10%.

ranged from 70% to 80%. The relative humidity within the vacuum chamber ranged from 55% to 65%. The relative humidity within the drierite chamber ranged from 10% to 20%.

2. Shedding Summaries

Group 4 (Control) (Table 56): Two of the ten animals (both females) in this group entered a cloudy eye phase and shed. One animal entered the cloudy eye phase 29 days after the start of the experiment, the second after 45 days. The animals spent 4 and 5 days respectively in the cloudy eye phase. Neither entered a second cloudy eye phase during the experiment.

Eight of ten animals (four males, four females) failed to enter even a single cloudy eye phase during the course of the experiment (120 days).

Group 2 (Vacuum) (Table 58): Only one (female) of ten animals in this group entered a cloudy eye phase 35 days after the start of the experiment. It spent 3 days in the cloudy eye phase and then shed, but it did not enter a second cloudy eye phase at 21 oC.

Nine of ten animals (five males, four females) failed to enter even a single cloudy eye phase during the course of the experiment (120 days).

Group 3 (Drierite) (Table 60): Three of fifteen animals (two males, one female) in this group entered a cloudy eye phase and shed. The number of days prior to the onset of cloudy eyes were 37, 16 and 48 with an average of 33.7 days. These three animals spent 3, 5 and 3 days respectively,

Table 56. Shedding summaries for intact Garter snakes maintained at constant 21 oC.

Animal #	Sex	No. of CE Phases at 21 oC	No. of Days Prior to Onset of CE Phase	No. of Days Spent in CE Phase	No. of Subsequent CE Phases at 21 oC	No. of Days Alive at 21 oC
1	F	1	45	5	-	78
2	F	0	-	-	-	50
3	F	0	-	-	-	65
4	M	0	-	-	-	77
5	M	0	-	-	-	120
6	F	0	-	-	-	96
7	F	1	29	4	0	120
8	M	0	-	-	-	60
9	F	0	-	-	-	120
10	M	0	-	-	-	58

Table 57. Weight relationships among intact Garter snakes maintained at constant 21 oC.

Animal #	Sex	Weight (gm)	Day 10	20	30	40	50	60	70	80	90	100	110	120
1	F	93	96.7	89.9	86.6	82.3	79.0	73.4	69.5	66.4				
2	F	82	98.5	91.6	86.0	83.1	80.0							
3	F	90	100.0	95.5	87.6	84.3	82.1	80.5	80.5	77.3				
4	M	21	97.7	94.1	89.5	84.6	82.1	80.0	78.6	75.9				
5	M	47	101.7	98.5	96.8	91.4	89.1	86.0	84.3	81.6	78.9	75.0	72.4	70.5*
6	F	105	97.7	94.5	87.1	84.6	77.7	75.1	73.2	70.0	67.3	66.8		
7	F	92	102.7	98.6	94.3	91.1	89.1	88.6	85.6	82.1	78.4	77.1	76.3	74.6*
8	M	31	96.6	91.3	88.4	86.5	83.4	80.4						
9	F	150	100.0	97.5	93.6	89.6	84.2	84.2	82.0	80.0	76.9	74.2	71.0	70.4*
10	M	47	98.7	92.3	89.5	86.9	81.4	78.6						

Note: For format, see Tables 2 and 13.

Table 58. Shedding frequencies among intact Garter snakes maintained at 21 °C in chambers to which a vacuum line was attached in order to remove excess moisture.

Animal #	Sex	No. of CE Phases at 21 °C	No. of Days Prior to Onset of CE Phase	No. of Days Spent in CE Phase	No. of Subsequent CE Phases at 21 °C	No. of Days Alive at 21 °C
11	F	0	-	-	-	68
12	M	0	-	-	-	120
13	F	1	35	3	0	60
14	F	0	-	-	-	120
15	F	0	-	-	-	70
16	M	0	-	-	-	58
17	M	0	-	-	-	120
18	F	0	-	-	-	120
19	M	0	-	-	-	77
20	M	0	-	-	-	90

Table 59. Weight relationships among intact Garter snakes maintained at 21 oC in chambers to which a vacuum line was attached in order to remove excess moisture.

Animal #	Sex	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
11	F	75	97.0	91.5	88.7	84.3	80.0	75.6	71.4					
12	M	53	100.0	89.5	84.2	79.5	74.6	68.8						
13	F	129	101.1	98.7	94.6	89.5	84.5	81.0	79.6	74.3	73.0	71.4	69.1	65.4*
14	F	86	103.5	98.0	94.0	89.6	88.0	86.7	84.6	82.3	78.8	76.5	74.1	71.1*
15	F	80	98.8	91.4	87.5	85.7	82.3	77.4	74.1					
16	M	51	93.4	89.5	84.6	77.5	75.4	73.2						
17	M	36	102.0	98.7	94.1	92.6	88.0	86.2	84.3	82.6	81.0	78.7	74.0	70.0*
18	F	112	100.0	98.4	94.3	91.6	89.2	86.7	84.6	82.0	79.5	76.4	74.3	69.4*
19	M	23	94.2	91.0	87.6	84.3	80.8	76.3	74.5	71.5				
20	M	29	98.7	92.4	88.7	86.3	81.4	78.9	76.7	72.3	69.6			

Note: For format, see Tables 2 and 13.

Table 60. Shedding summaries among intact Garter snakes maintained at 21 oC and constant low relative humidity.

Animal #.	Sex	No. of CE Phases at 21 oC	No. of Days Prior to Onset of CE Phase	No. of Days Spent in CE Phase	No. of Subsequent CE Phases at 21 oC	No. of Days Alive at 21 oC
21	M	0	-	-	-	61
22	F	0	-	-	-	82
23	F	0	-	-	-	79
24	F	0	-	-	-	65
25	F	1	37	3	0	94
26	F	0	-	-	-	120
27	F	0	-	-	-	52
28	M	1	16	5	-	73
29	M	0	-	-	-	115
30	M	0	-	-	-	120
31	M	0	-	-	-	120
32	F	0	-	-	-	108
33	F	0	-	-	-	115
34	F	0	-	-	-	105
35	M	1	48	3	0	97

Note: Air was dried over drierite before entering the chamber. Relative humidity within the chamber averaged between 10-20%.

in the cloudy eye phase, with an average of 3.6 days.

Twelve of fifteen animals (four males, eight females) failed to enter even a single cloudy eye phase during the course of the experiment (120 days).

3. Weight Relationships

a. General comments

All animals were starved and all steadily lost weight throughout the course of the experiment. In some cases, animals gained weight initially, probably due to water consumption.

b. Comparisons among groups

Group 1 (Control) (Table 57): Seven of ten animals (2 males, 5 females) died before the experiment ended. The number of days alive at 21°C ranged from 50 to 96 with an average of 69.1 days. The percent of original weight at death ranged from 66.4% to 80.4% with an average of 75.3%.

Three animals (2 males, 1 female) survived through the conclusion of the experiment (120 days). The percent of original weight for these animals ranged from 73.4% to 74.6% with an average of 72.8%.

Group 2 (Vacuum) (Table 59): Six of ten animals (4 males, 2 females) died before the experiment ended. The number of days alive at 21°C ranged from 58 to 90 with an average of 70.5 days. The percent of original weight at death ranged from 63.5% to 74.1% with an average of 70.1%.

Four animals (1 male, 3 females) survived through the conclusion of the

experiment (120 days). The percent of original weight at this time ranged from 65.4% to 71.1% with an average of 68.9%.

Group 3 (Drierite) (Table 61): Twelve of fifteen animals (5 males, 7 females) died before the experiment ended. The number of days alive at 21 °C ranged from 52 to 115 with an average of 83.8 days. The percent of original weight at death ranged from 61.2% to 81.6% with an average of 72.8%.

Three animals (1 male, 2 females) survived through the conclusion of the experiment (120 days). The percent of original weight at that time ranged from 68.0% to 72.3% with an average of 70.6%.

Section V: EFFECTS OF THYROIDECTOMY ON THE ULTRASTRUCTURE OF THE EPIDERMIS OF GARTER SNAKES

Ultrastructural analysis of serial biopsies taken from thyroidectomized Garter snakes revealed defects which appear to be restricted to the lamellar granules of the mesos layer. In control intact animals, these organelles begin to form at about stage 4 of the renewal phase. Thick (1 micron) sections were used to determine the stage of the renewal phase of individual biopsies. Ultrastructurally, the lamellar granules consist of assemblies of alternating light and dark bands surrounded by a bilaminar membrane (fig. 26 and 27). In biopsies taken during the first post thyroidectomy renewal phase, a number of organelles fail to organize into the lamellar pattern. The disruption of the

Table 61. Weight relationships among intact Garter snakes maintained at 21 oC and constant low relative humidity.

Animal #	Sex	Weight (gm) Day 1	10	20	30	40	50	60	70	80	90	100	110	120
21	M	55	93.7	88.9	86.5	81.3	78.4	75.0						
22	F	68	94.3	89.7	84.0	80.1	76.5	69.8	66.7	61.2				
23	F	108	100.0	96.8	92.5	89.1	86.3	82.6	76.0	73.9				
24	F	68	98.7	94.1	89.0	86.5	82.3	78.2	74.1					
25	F	74	100.0	100.0	98.0	96.7	91.2	88.9	84.5	81.2	78.3			
26	F	76	101.2	98.7	98.7	94.6	91.9	87.3	85.3	81.4	78.2	76.5	73.0	69.6*
27	F	81	96.3	89.2	84.3	82.0	81.6							
28	M	29	98.8	94.3	89.6	84.4	79.0	75.7	71.2					
29	M	37	102.3	100.0	98.6	94.1	91.0	88.4	86.9	81.5	78.0	75.0	71.2	
30	M	39	100.0	96.7	93.2	89.0	87.4	83.5	81.4	77.6	71.2	69.5		
31	M	48	101.7	100.0	98.3	96.5	94.6	91.7	87.3	85.2	81.4	78.7	75.0	72.3*
32	F	116	97.6	93.4	87.4	86.3	85.7	84.0	83.2	82.1	80.0	76.3	73.2	
33	F	128	102.0	100.0	98.5	91.2	87.3	85.4	83.0	80.5	78.5	76.1	74.1	
34	F	81	97.3	94.5	90.0	87.6	84.5	82.1	78.7	76.5	74.3	72.1	70.0	68.0*
35	M	27	101.1	98.0	96.8	93.7	89.1	86.9	81.4	76.7	73.1	70.8		

Note: For format, see Tables 2 and 13. Air was dried over drierite before entering the chamber.
Relative humidity within the chambers averaged between 10 - 20%.

Table 61a Summary of data collected from relative humidity experiments on intact Garter snakes.

Exp #	# of Animals	Temp.	Type of Humidity Chamber*	# of Animals Which Shed	Aver # of Sheds per Animal	Aver # Days Prior to CE Phase	Aver # Days Alive	Aver % Original Wt at Death
D-1a	10	28 oC	-	8	1	35.2	77.5	60.6
D-1b	10	28 oC	Vac	9	1	31.7	74.6	60.9
D-1c	15	28 oC	LH	15	1	15.3	76.0	59.6
D-2a	10	21 oC	-	2	1	37.0	84.4	75.1
D-2b	10	21 oC	Vac	1	1	35.0	90.3	71.4
D-2c	15	21 oC	LH	3	1	33.7	93.7	72.8

* Vac = Chambers attached to vacuum lines.
 LH = Low relative humidity chambers.

lamellar pattern worsened with subsequent cloudy eye phases until a majority of the membrane coating granules appear filled with amorphous material. The surrounding membrane was intact (fig. 28-31).

DISCUSSION

SECTION I: INTRODUCTION

Squamate reptiles periodically shed their outer epidermal generation following the differentiation of a new inner epidermal generation (Maderson, 1985). In this thesis, we have attempted to at least partially answer the question as to why this phenomenon occurs. Let us start by asking the question "why would an animal need to form a new epidermis."

Every day wear and tear has a cumulative affect on the epidermis; feeding, locomotion, etc. all impart physical damage. After a period of time, it might cease to function as efficiently as it should. The organism must find a way to initiate the differentiation of a new epidermis, one which would meet the functional demands of every day life. How could this be accomplished? One possibility is the existance of a biological clock which, at certain intervals, would stimulate the onset of a renewal phase. A second possibility for an animal being able to form a new epidermis could be that the animal is confronted by some environmental stress with which the present epidermis can not cope. Mechanisms could exist which could a) detect the fact that the epidermis was not functioning effectively and, b) initiate the onset of the renewal phase, overriding the epidermal "clock". Failure to do so could result in the death of the animal.

These two different scenarios would explain a stimulated shed, but the underlying reason for both is the same, ie. the maintenance of an epidermis competent to meet environmental challenges at all times. In the first case, the animal copes with normal wear and tear via biological clocks which would periodically initiate skin renewal. There would be no a priori reason to assume that among two or more animals the clocks would be synchronized. Therefore, in any group of intact snakes maintained under constant "non-stressful" conditions, no predictable pattern of shedding occurrence would be observed. In the second model, if a group of intact garter snakes was exposed to environmental conditions which similarly stressed the epidermis of all the animals, one would predict that all of the animals in the group would enter the cloudy eye phase at approximately the same time. Thus, in subsequent sections of this discussion, we have used the synchrony of onset of cloudy eye phases within a group of animals as one parameter to estimate the effects of any given environmental stress on the epidermis. The more obvious the synchrony of onset of the renewal phase the greater the effect of any given environmental stress on the epidermis.

SECTION II: THE SKIN AS A PERMEABILITY BARRIER

So far we have discussed the functions of the epidermis in general terms. One important function of the epidermis of terrestrial vertebrates is

protection against the loss of water. The squamate epidermis is a passive membrane barrier to cutaneous water loss (CWL). As such, Fick's Law of Diffusion (Maderson et al, 1978) can be applied, leading to the following biological relationship:

Rate of CWL=Permeability Constant (PC)/ Barrier Thickness (BT)

The first variable, PC, represents the innate characteristics of the barrier which contribute to the prevention of water diffusion (ie. the presence of hydrophobic substances). Thus, the permeability constant is directly proportional to the rate of CWL. On the other hand, barrier thickness is inversely proportional to the rate of CWL. It is the interaction of the two variables, PC and BT, which determines the effectiveness of the barrier. Lillywhite and Maderson (1982: p. 402) state that "two sympatric species could show identical mean values for CWL with one having a very thick mesos layer with a poor PC and the other having a very thin mesos layer and an exceptionally good PC." Thus, CWL could change substantially in a constant environment if either PC and/or BT were changed; or, conversely environmental stress (ie. a drop in relative humidity) while PC and BT remained constant (Maderson, 1985). We now propose that it is an increase in the rate of CWL which initiates the onset of a renewal phase. We have

examined the effects of factors which might increase the rate of cutaneous water loss in Garter snakes. We will examine first the effects of constant high temperature on skin shedding patterns in groups of intact snakes.

SECTION III: HIGH TEMPERATURE INDUCED SHEDS IN INTACT SNAKES

A. ANIMALS MAINTAINED AT CONSTANT 28 oC

Intact garter snakes, maintained at constant 28 oC, undergo a single renewal phase, shed, and appear to remain healthy, for the duration of the experiment (see Results, Section I). Animals maintained at constant 17 oC or 8 oC do not enter the renewal phase, and this is not apparently related to age, sex, or feeding vs. starvation.

In order to explain this result, one must first consider the effects of a sudden increase in ambient temperature on the animals. First, the snakes will experience greater stress with respect to CWL. Second, they will become more active. Under the changed conditions, the mesos barrier could become less effective due either to instability of the intermolecular lipid-lipid, and /or lipid-protein bonds, and/ or actual physical damage to the tissue. These, in turn, would have a direct effect on the permeability constant of the epidermis, since Roberts and Lillywhite (1980) demonstrated the importance of the mesos lipids in contributing to the permeability constant. If one now considers Fick's Law of diffusion, it can be shown that

even a small change in PC has the potential of increasing trans-epidermal diffusion rates by a substantial amount (Maderson, 1984). An increase in CWL could serve as a signal initiating the onset of a renewal phase. Maderson (1984) proposed that an increase in water flux across the barrier could serve as the initiating signal. An increase in CWL would lead to a concomitant increase of blood osmolarity. Such an increase in blood osmolarity could then be monitored by the hypothalamus which in turn could serve to remove an inhibition on the germinal cells. The epidermis enters a renewal phase and a mesos layer capable of meeting the newly imposed environmental demands is produced.

In Section I of this discussion, we emphasized the use of synchrony of onset of cloudy eye phases as a useful parameter in evaluating the degree of stress that various environmental factors may impose on the epidermis. In these studies, a relative lack of synchrony was reported for most of the experiments. We use the term "relative" because, although the number of days prior to the onset of cloudy eye phases varied widely from one animal to another within groups, 90% of all animals maintained at constant 28 oC did enter a cloudy eye phase within the 120 days that the experiment was carried out. This response was less synchronized than those reported for groups of animals maintained under conditions of low relative humidity and high temperature, and for groups of animals transferred to 28 oC following

exposures of six weeks or more at 3 oC (see Results section I , Discussion section III). However, it does represent a much greater degree of synchrony than that observed in groups of animals maintained at a constant temperature of 21 oC (see Results, section I). What does this mean, and how does it relate to the proposal that increased cutaneous water loss is responsible for the initiation of a renewal phase?

The relative degree of synchrony indicates that exposure of Garter snakes to constant 28 oC does stress the epidermis to some extent. Apparently, however, different degrees of stress are experienced by different animals. How can we explain this? We must keep two things in mind. First, because the animals were purchased commercially, we knew nothing about the environmental conditions under which the original epidermis was laid down. Second, an animal will produce an epidermis capable of dealing with a specific environmental stress. That is, those animals which had originally laid down an epidermis under more stressful conditions (ie., higher ambient temperature, lower relative humidity, etc.) will possess an epidermis which is more capable of dealing with the newly imposed stress. This would be the case as long as the imposed stress does not exceed the degree of stress under which the epidermis was laid down. Stated in terms of Fick's Law of Diffusion, these animals would have an epidermis possessing a permeability constant which was more effective than those animals whose epidermis was

laid down under less stressful conditions.

While 80-90% of the animals maintained at a constant high temperature eventually shed, none underwent a second cycle during the experiments lasting approximately 120 days (see tables 3, 4, 9, 10). This was true of animals kept at constant 28 oC throughout the study, and for animals which were transferred to a lower temperature (17 oC or 8 oC) for a period of 21 days following a shed at 28 oC and then returned to 28 oC. This same rationale can be used to explain the fact that chronologic consistency has rarely been recorded for any species of snake (Maderson, 1985, p. 122). If an animal exposed to a given environmental stress responds by forming a mesos layer capable of dealing with that stress, there would be no reason for that animal to undergo further skin renewal as long as the environment, and the new mesos layer, remain constant. Either normal wear and tear will eventually damage the mesos layer to the point where it is no longer capable of effectively preventing trans-epidermal water loss, or the epidermal "clock" would, in turn, signal the onset of yet another renewal phase. In confirmation of previous reports (see references in Maderson, 1984) we found that fungal infections produce frequent, repeated shedding in intact Garter snakes. This phenomenon can best be explained by assuming that these infections physically damage the mesos layer to the point where an increase in CWL is experienced and a renewal phase is precipitated.

B. ANIMALS MAINTAINED AT 3 °C FOR SIX, TEN, OR FOURTEEN WEEKS
PRIOR TO TRANSFER TO CONSTANT 28 °C.

When intact Garter snakes are maintained at constant 3 °C for periods of six weeks or more, they do not enter a cloudy eye phase over that period of time. However, they will enter a cloudy eye phase and shed when transferred to constant 28 °C (see Results, experiment A-4). The onset of the cloudy eye condition was synchronized between animals in each group. What is the significance of this, and how do these results relate to the concept that the renewal phase might be precipitated by an increase in cutaneous water loss?

As mentioned in section I of this discussion, synchrony of onset of the cloudy eye phase has been used as a parameter in determining the degree of stress placed on the epidermis of Garter snakes by various environmental conditions. Since the synchrony observed in this experiment is very pronounced, transfer of intact Garter snakes from constant 3 °C to constant 28 °C apparently results in a great deal of stress being placed on the epidermis of these animals. Let us examine the possible nature of this stress.

One possibility is that the mesos integrity may be compromised at 3 °C. Under these conditions, a deterioration of their molecular integrity would result in a defective water barrier. However, the very low ambient

temperature would either not stress the epidermis to the point where an increase in CWL would occur, or it would lower the animal's overall metabolism to the point where germinal cell proliferation is slowed considerably despite some increase in the rate of CWL. Transfer to 28 oC would either sufficiently stress the epidermis leading to an increase in CWL, and/ or increase the rate of germinal proliferation, resulting in the onset of the cloudy eye condition.

Secondly, a sudden transfer of animals from 3 oC to 28 oC results in an increase in activity among those animals. This increase in activity could lead to physical damage to the mesos lipids and/or tissues resulting in a defective water barrier and an increase in CWL. This increase in CWL would then serve to initiate a renewal phase and a subsequent shed.

Thirdly, long term maintenance at 3 oC may serve not only to decrease activity among the animals, but may also be a dehydrating environment. Under such conditions, tissue water may slowly decrease, leading to a "shrinking" of the scales. Such a shrinkage could serve to physically disrupt the mesos tissue, leading to a less effective barrier to CWL. On exposure to 28 oC, a sudden increase in CWL would be experienced by all of the animals, leading to the synchronous onset of the cloudy eye condition.

We can not say for sure which of these possibilities actually explains the results reported here. Only an epidermal lipid analysis at 3 oC can verify

whether or not these lipids are unstable at that temperature. Also, although we do know that a dramatic increase in activity does occur among these animals when transferred from 3 oC to 28 oC, limitations of present technology may make it impossible to directly demonstrate damage to the mesos lipids as a result of this increased activity. Such damage may involve molecular deterioration of the lipids or disruption of lipid-protein bonds.

SECTION IV. LOW RELATIVE HUMIDITY INDUCED SHEDS IN INTACT GARTER SNAKES

The data presented here suggest a strong correlation between low relative humidity and/or high temperature regimes and onset of the renewal/shedding cycle (see Results, experiment D-1). Animals maintained at constant low relative humidity (10-20%) and high temperature (28 oC) will enter a cloudy eye phase, within 23 days from the onset of an experiment, and shed. A great deal of synchrony with regard to the onset of the cloudy eye condition, exists among animals in a given group.

What is the significance of this? Once again, we must keep in mind that synchrony of onset of the cloudy eye condition among the animals of a given group serves to indicate the degree of stress placed on the epidermis by given environmental factors. In this case, the combination of high temperature and low relative humidity appears to stress the epidermis to the

point where it can no longer cope with the environmental conditions. This, in turn, results in the over riding of the "clock" leading to the onset of the renewal phase.

These results are consistent with those reported in studies of a variety of arthropods (see Discussion, Section VIa) and with the proposed hypothesis that the rate of CWL plays an important role in inducing the onset of a renewal phase. An environmental regime of high temperature and low relative humidity would serve to stress the epidermis leading to an increase in CWL. A new epidermis is then produced with mesos lipids capable of meeting the newly imposed environmental demands.

Control experiments were designed in which control animals were exposed to exactly the same conditions as experimental animals except for the ambient relative humidity (see Material and Methods, experiment D-1). Experimental animals were maintained at a constant relative humidity of 10-20% while control animals were maintained at a relative humidity of greater than 70%. While the experimental groups demonstrated a very pronounced degree of synchrony of onset of cloudy eye conditions, this degree of synchrony was absent in control animals. These animals entered the cloudy eye phase with the same degree of synchrony as the rest of our animals maintained at constant 28 oC (see Results section IV, Discussion section III).

However, is low relative humidity by itself enough to initiate the onset

of the cloudy eye condition? Apparently, the answer to this question is negative. Only two of fifteen animals maintained at 21 °C and an average relative humidity of 10-20%, entered a cloudy eye phase and shed. These results were no different than those reported in the control groups (see Results, experiment D-2) which, as in the previous experiment, were exposed to exactly the same environmental conditions as were experimental animals with the exception of the ambient relative humidity. These animals were maintained at a relative humidity which exceeded 70%.

The results reported here suggest that a combination of high temperature and low relative humidity will induce a synchronous onset of cloudy eyes among a group of inactive Garter snakes. But why is a combination of these two factors necessary? Why is low relative humidity alone apparently insufficient? To answer this question, let us consider all the effects of low and high temperatures on the animals.

Animals maintained at 21 °C are much less active than those at 28 °C. Perhaps the increase in activity, at 28 °C, serves to physically disrupt the mesos tissue. Since the lipids of the mesos tissue serve as the barrier to CWL (Landmann 1980; Roberts and Lillywhite, 1980), their disruption, along with the increased environmental stress imposed by the low ambient humidity, leads to an increase in CWL. This initiates a renewal phase during which mesos lipids capable of dealing with this greater environmental stress

are synthesized.

SECTION V. STUDIES ON THYROIDECTOMIZED GARTER SNAKES

A. EFFECTS OF THYROIDECTOMY ON SHEDDING FREQUENCY IN GARTER SNAKES

A major problem in our understanding of the control of periodic squamate skin shedding concerns the role of thyroid hormones (see review Maderson, 1985: pp. 543-555). A model has been proposed to explain some of the relevant data in the context of somatic effects of cutaneous water loss (Maderson, 1984, 1985).

Maderson (1984) makes two assertions. Firstly, that thyroid hormones are related in some way to shedding frequency and secondly that skin function can be analysed via Fick's Law of Diffusion. Let us begin by discussing whether or not these assertions are substantiated by the data presented in this thesis.

According to the data presented here, the first assertion is indeed substantiated. Thyroidectomy of any snake, at any time in its life, will bring about a series of 2 - 5 continuous cloudy eye conditions at temperatures of 21 oC and higher. The duration of the cloudy eye phase in thyroidectomized animals appears to be, on average, several days longer than in control animals. Eventually, the animals lose a threshold amount of weight and die

(see Results, section II). Individual animals always enter the first cloudy eye phase 14 - 16 days post thyroidectomy. This demonstration of 100% synchrony implies that thyroidectomy may produce some sort of molecular and/or metabolic degradation in the skin. Cycles of proliferation continue until the death of the animal. This is not affected by age, sex, or feeding vs. starvation (see Results, section III). Since these effects can be prevented by post-thyroidectomy injection of exogenous iodinated thyronines Chui et al (1983) suggested the possibility that thyroid secretion(s) or their metabolite(s) inhibit proliferation of the cells of the stratum germinativum.

What then is the nature of the relationship between thyroid hormones and the control of shedding frequency? How does a lack of thyroid hormones relate to the general hypothesis that initiation of a renewal phase in Garter snakes is brought about by an increase in CWL? Gupta et al (1975) suggest that thyroid hormones play a significant role in the control of lipid metabolism in snakes. If this is the case, then repeated onset of the renewal phase may result from an increase in CWL which, in turn, derives from disturbances of epidermal lipid metabolism. This now brings us to Maderson's second assertion, that is that the skin may be analysed by Fick's Law of Diffusion. Lipid disturbances would have a direct effect on the permeability constant of the integument, since mesos lipids appear to contribute to the permeability barrier (Landmann, 1979, 1980; Roberts and Lillywhite, 1980).

Thus, the integrity of the barrier mechanism to CWL would be compromised, resulting in a less efficient barrier. But how could the animal cope with this increase in CWL? Three possibilities exist.

First, there might be an immediate response. That is, the animal would simply seek water. In fact, we have observed that thyroidectomized Garter snakes maintained at constant 28 °C splash the water out of their dishes and onto the floor of their container. This behavior was not observed in intact animals.

Secondly, the thyroidectomized animal may attempt a short term solution by adding more more zonulae occludentes to the epidermis since Landmann et al (1981) reported the contribution of these structures to the permeability barrier of the grass snake. However, if overall lipid metabolism is affected by a lack of thyroid hormones, then one would expect that membrane lipid metabolism would also be affected. Thus, the additional tight junctions may prove ineffective since these structures are dependent upon membrane lipids.

A third, long term response would be the laying down of a new barrier. However, due to the lipid defects associated with the absence of thyroid hormones, it is an ineffective epidermis, one whose mesos lipids cannot cope with environmental stress. As a result, the cycle starts over again. Put another way, the animal "knows" what to do, it just can't do it correctly. We

note that while thyroidectomized Garter snakes enter several cloudy eye phases, few actually "shed" more than once, in fact, some animals fail to "shed" at all (see Results, experiments B1- B3). This results in a stacking of outer generations, one on top of the other. Attempts to remove these inhibited sheds result in destruction of the underlying inner generation, implying some sort of structural insufficiency in this layer. Biopsies sectioned for light microscopy reveal that the overall integrity of the skin is affected by a lack of thyroid hormones (see Results section V).

If we accept the premise that the epidermis represents a passive membrane barrier to CWL, and, as such, Fick's Law is applicable, then the rate of CWL is inversely proportional to the thickness of the epidermis: $CWL = \text{Permeability Constant (PC)} / \text{Barrier Thickness (BT)}$. Removal of the thyroid gland results in defective epidermal lipids and hence, a defective barrier to CWL (as a result of changing PC). The only way in which the organism can attempt to normalize CWL is by increasing the thickness of the epidermis. This increased thickness, however, proves ineffective resulting in the induction of subsequent renewal phases.

The foregoing sequence of events could explain the initiation of all renewal phases subsequent to the initial post-surgical renewal phase. What, however, can explain the onset of the first renewal phase? In keeping with the theory that increased rates of CWL serve as inducers of the renewal

phase, one must examine the immediate effects that thyroidectomy will have on the barrier to CWL. Surgery per se can not be significant as sham animals do not enter the cloudy eye phase with any greater frequency than do intact controls (see Results, section II). Three possible explanations are consistent with the proposed theory concerning increased rates of CWL.

First, Landmann et al (1981) reported that two morphologically and topographically distinct permeability barriers exist in the epidermis of the grass snake. They include tight junctions, in the form of continuous zonulae occludentes which interconnect the apico-lateral plasma membranes of the upper-most living cells, and the intercellular lipid sheets in the overlying mesos layer. Tight junctions are structures of the cell membrane. Cell membranes are dynamic, the protein and lipid components are constantly undergoing replacement (Singer and Nicolson, 1972). If thyroidectomy leads to a disruption of overall lipid metabolism, then one would expect cell membranes to be adversely affected leading to the formation of defective tight junctions. Thus, one possible explanation for the initiation of the first post-thyroidectomy renewal phase is the formation of "leaky" tight junctions due to abnormal lipogenesis in the absence of thyroid hormone. This could occur almost as soon as circulating titers of thyroid hormone reached some critical concentration.

A second possibility is related to the integrity of intercellular lipid

sheets located in the overlying mesos layer. These sheets are formed intracellularly in the lamellar granules (Landmann, 1980). We have demonstrated that these cellular organelles are defective in the epidermis of thyroidectomized garter snakes (see Results, section V). Although the synthesis of defective mesos lipid sheets could not explain the first post-thyroidectomy renewal phase (since presumably the lipid sheets would have already been laid down prior to thyroidectomy) the possibility exists that thyroid hormone is necessary for the maintenance of these lipids. This being the case, a drop in circulating titers of thyroid hormone, following removal of the thyroid gland, would lead to faulty maintenance of the mesos lipids giving rise to a defective barrier to CWL leading to the initiation of a renewal phase.

A third possibility is that thyroid hormones may play a role in directly inhibiting the cells of the stratum germinativum (Chui et al, 1983). Thus , removal of the source of thyroid hormone lifts this inhibition, allowing the cells to proliferate..

A combination of these three possibilities may exist. This is, of course, all conjecture. To date, there is no direct evidence to support these proposals, however, studies on human dermatopathologic conditions are consistent with these suggestions (see this Discussion, section VI C). An analysis of the effect of thyroid hormone on the cells of the stratum

germinativum, maintenance of tight junctions, and maintenance of mesos lipids, is needed.

Chui et al (1983) reported that injection of exogenous iodinated thyronines restored normal shedding frequencies in the thyroidectomized garter snakes to some extent. This restoration was not, however, complete. How can this be explained?

Let us begin by considering the possibility that thyroid hormone receptors (ie. on the plasma membrane and/or within the cytoplasm) are associated with germinal cells. Perhaps the integrity and/or number of these receptors requires the presence of thyroid hormones. Thus, absence of appropriate hormones in the thyroidectomized garter snakes may cause defects in these receptors and injection of exogenous thyronines would have less of an effect than anticipated.

These speculations are amenable to experimental verification. First, thyroid hormone receptors must be shown to exist in the stratum germinativum. Second, direct effects on the numbers and/or binding abilities of these receptors by the absence or presence of thyroid hormone must be demonstrated.

The involvement of thyroid hormone in lipid metabolism does not appear to be unique to snakes. Faas and Carter (1982) reported that in thyroidectomized rats, changes in fatty acid desaturation and microsomal

lipid fatty acid composition were observed. They reported a 43% decrease in linoleate desaturation in the thyroidectomized animals. Increased proportions of linoleate and decreased arachidonate were reported to be the major changes in microsomal fatty acid composition. These findings were consistent with those reported by Hoch et al (1980).

B. EFFECTS OF THYROIDECTOMY ON VIABILITY

Removal of the thyroid gland decreases viability in garter snakes. After approximately two weeks post-surgery, animals begin losing weight rapidly and soon die (see table 42). Non-fed, intact, control animals, on the other hand, lose weight much less rapidly when exposed to similar environmental conditions (see table 43).

Feeding appears to have little to do with this phenomenon. Thyroidectomized garter snakes will feed as long as they continue to "shed". Only when the outer generations are not lost from the body do the animals stop feeding. Thus, cessation of feeding appears to be the result of the restrictions placed on the animals movements resulting from a stacking of unshed outer generations. Drastic weight loss and eventual death is still observed in the animals which fed up to the third cloudy eye phase (see table 43).

What then is responsible for the diminished viability of

thyroidectomized snakes? Once again a relation to CWL is indicated. This is suggested by the evidence presented by Chui (1982) indicating that the thyroid hormone has little effect on oxidative metabolism. The animals continue to lose water trans epidermally as incompetent inner generations continue to be produced. When a critical weight loss has occurred (circa 25% of original weight), the animals die (see Tables 27 and 28).

C. HISTOLOGIC AND ULTRASTRUCTURAL CHANGES IN THE EPIDERMIS OF THYROIDECTOMIZED GARTER SNAKES

Morphologic changes characterise the epidermis of thyroidectomized Garter snakes. Histologic examination of serial biopsies show that general tissue organization becomes progressively worse with each new generation and imply defects of cellular adhesion and arrangement. Such effects can be explained if we accept Gupta et al's (1975) assertion that thyroid hormone regulates all aspects of lipid metabolism in snakes, including the phospholipid constituents of cell membranes. Such changes would be consistent with Elias' (1983) report that abnormal desquamation in human pathologies may be due to abnormal lipogenesis in the epidermis.

Ultrastructural analysis of serial biopsies, taken from thyroidectomized snakes reveal defects which appear to be restricted to the lamellar granules of the mesos layer. In intact control animals these

organelles begin to form at about stage 4 of the renewal phase.

Morphologically, they consist of alternating light and dark bands surrounded by a trilaminar membrane (see fig. 27). As stated before, these organelles are believed to be responsible for the synthesis of the barrier to CWL (Landmann, 1979, 1980).

In biopsies taken during the first post thyroidectomy renewal phase, a number of the organelles fail to organize into the lamellar pattern (see fig. 29). This disruption of the lamellar pattern worsens with subsequent cloudy eye phases until a majority of the granules appear to be filled with an amorphous material (see fig. 31). Such defective lamellar granules would presumably form a defective epidermal barrier to CWL. This increase in trans-epidermal diffusion of water would then serve as the signal initiating subsequent renewal phases.

An explanation for the lack of lamellae in the lamellar granules of thyroidectomized Garter snake epidermis can be derived from an analysis of a model for the formation of lamellar granules (Wertz and Downing, 1982).

Assembly of the lamellar granules appears to take place in the endoplasmic reticulum (ER) (Wolff and Wolff-Shreiner, 1976). Fatty acids, with chain lengths of 16 carbon atoms, are synthesized in the cytoplasm and embedded into the membrane of the ER. Growth of these chains continues until they extend across the entire lipid region of the ER membrane. At a

length of 30 carbon atoms, the methyl groups of the fatty acid chain emerges into the lumen of the ER and is hydroxylated by a microsomal enzyme oxidase (Fulco, 1974). Incorporation of the hydroxy acid into acylglucosylceramide, also synthesized in the ER, then takes place (Wertz and Downing, 1982). Attachment of linoleic acid to the glucose portion would provide attraction for adjacent folds of membrane. This results in the observed stacking of disk shaped sections of ER membranes (Wolff and Wolff-Shreiner, 1976).

Wertz and Downing (1982) and Wertz et al (1984) have proposed that the sphingosine, acylglucosylceramide, is essential for the formation and stability of the lamellar granules. It is possible that hypothyroidism may effect the synthesis or stability of this moiety, preventing the formation of the lamellar pattern in the lamellar granules. Another possibility is that thyroidectomy may affect the metabolism of linoleic acid which appears to be necessary for the normal formation of lamellar granules (Wertz and Downing, 1982). Thyroidectomy does appear to affect the metabolism of this moiety in rats (Faas and Carter, 1982). These might explain the ultrastructural observations on thyroidectomized snake epidermis reported in this thesis (see Results, Section V). Confirmation of this hypothesis would require further biochemical examination of the epidermal lipids of both intact and thyroidectomized Garter snakes.

SECTION VI. SUPPORTING EVIDENCE IN SPECIES OTHER THAN SNAKES

A. ARTHROPODS

Data derived from studies of arthropods support the hypothesis that germinal cells respond to increased trans-epidermal diffusion of water. The cuticle represents a major barrier to water loss in scorpions (Hadley, 1970, 1974). When cuticular lipid compositions of xeric and non-xeric species of scorpion were studied, biochemical differences were shown to exist. The cuticular lipids of the xeric species were shown to consist primarily of unbranched, saturated hydrocarbons while those of the non-xeric species comprised chiefly unsaturated, branched hydrocarbons of shorter length (Hadley and Jackson, 1977).

A similar situation has been reported in a species of tenebrionid beetle (Hadley, 1977a). The lipid composition of the cuticle depends on the time of year at which the cuticle is analyzed. Winter beetles, acclimated to 35 oC for a period of five to ten weeks, and beetles studied during the summer exhibited higher quantities of cuticular hydrocarbons than did control winter beetles. The cuticular lipids of summer, and temperature-acclimated beetles collected during the winter, consisted primarily of long chained, unbranched hydrocarbons. On the other hand, not only were fewer hydrocarbons present in control winter beetles, but those that were present were mostly branched, unsaturated, and of shorter chain length. When Hadley (1977b) examined the cuticular transpiration and transition temperature of four species of

tenebrionid beetle, he discovered that the effective barrier to water flux appeared to be largely attributable to the presence of cuticular hydrocarbons. Thus, "summer" and "winter" beetles would have different PC's attributable to the presence of different types of lipids.

The cuticular permeability of two Arizonian vejovic scorpions was examined with respect to the temperature/relative humidity regimes of the habitats in which they were collected (Hadley, 1977b). Hadley concluded that cuticular permeability can be altered to meet environmental requirements and that in scorpions, predictable changes in cuticular lipid composition are at least partly responsible. Toolson and Hadley (1977) examined seasonal changes in scorpion cuticle permeability and lipid composition. They reported that cuticular lipids play an important role in the water economy of arthropods and that acclimatory changes in the cuticular lipids are important in the adaptation of arthropods to changing environments.

Similar relationships between cuticular permeability and lipid composition have been demonstrated in the black widow spider (Hadley, 1978), desert cicada (Hadley, 1979), desert tenebrionid beetle (Hadley et al, 1980), and the epigean lycosid spider (Hadley et al, 1981). Quinlan and Hadley (1983) concluded that cuticular lipids may be of importance for the markedly low cuticular permeability exhibited by the semi-arid terrestrial isopod Venezillo arizonicus.

B. MICE

The effects of essential fatty acid (EFA) deficiency on the permeability of epidermis in mice has been examined (Menton, 1968, 1970; Elias and Brown, 1978). When animals are denied essential fatty acids in their diet, the epidermis becomes more permeable to water soluble tracers. Elias and Brown (1978) demonstrated that this increase in epidermal permeability was accompanied by abnormalities in lamellar body elaboration and deposition. Normal numbers of lamellar bodies were present. However, the internal lamellae of the lamellar bodies decreased in comparison to controls. Furthermore, the contents of the lamellar bodies, when secreted into the intercellular space, failed to coalesce into the broad, lipid-rich sheets that filled the interstices of the stratum corneum of controls. Similar ultrastructural findings in thyroidectomized Garter snakes are reported here (see Results, section V).

C. PATHOLOGIC CONDITIONS IN HUMANS

A considerable body of data indicates that some scaling skin diseases may be due to disturbed epidermal lipid metabolism (Elias and Brown, 1978). Various types of hyperkeratotic skin changes appear to be related to the administration of hypocholesterolemic drugs (Elias, 1981a; Elias 1981b;

Yardley and Summerly, 1981). Abnormal epidermal lipid metabolism has also been implicated in some of the more common dermatoses such as psoriasis (Gara et al, 1964), atopic dermatitis (Mustakallio et al, 1967), and X-linked ichthyosis in which the enzyme steroid sulfatase is absent in all tissues of the body leading to a deficiency in sterol synthesis (Shapiro et al, 1978). Squire et al (1978) examined skin lesions from patients with Kyrle-Flegel disease. Electron microscopic examination of these lesions revealed defective lamellar granules lacking the lamellar pattern. These granules appeared to be filled with an amorphous material. When skin samples from adjacent, non-lesion regions were examined, normal lamellar granules, with an internal lamellate pattern, were found. In many of the scaling dermatoses, excessive transepidermal water loss also occurs (Grice and Bettley, 1967; Grice et al, 1975).

Rosenberg et al (1986) reported that scaly ichthyosis, resulting from altered epidermal lipogenesis, accompanied hypothyroidism in rats. The most significant decreases in lipogenesis were in the neutral lipids, particularly the sterol and sterol-ester moieties. Total cholesterol pool size was also reduced in thyroidectomized epidermis. Similar decreases in sterol biosynthesis were also reported in human keratinocytes cultivated in medium devoid of thyroid hormone. They concluded that altered keratinocyte sterologogenesis may be a major pathologic factor in the origin of scaly

epidermis in hypothyroid humans.

SECTION VII CONCLUDING COMMENTS

In this thesis, we have presented a morphological hypothesis to explain skin shedding in Garter snakes. The major points of the hypothesis are as follows:

- 1). The skin of snakes, like that of other amniotes, provides a passive barrier to insensible CWL. This barrier depends upon intra- and extracellular lipids in the corneous tissues which have a permability constant, PC. In snakes, the lipids reside predominantly in the mesos layer (Landman, 1980; Roberts and Lillywhite, 1983).
- 2). The mesos layer in snakes consists of up to 15 layers of extremely flattened, interdigitating cells, between which there are deposits of lipid lamellae. The mechanism whereby these lamellae are attached to the membranes of the mature cells is still the subject of debate (Wertz and Downing, 1982).
- 3). Deformations can be tolerated by the tissue, the cells, and the lipid lamellae during normal feeding and locomotion.

4). Excess environmental stress and/or disruption of the tissue organization, be it via mechanical means or thyroidectomy, will result in a decrease in the PC of the mesos lipids. This, in turn, results in an increase in CWL which serves to change the body osmolality. This change in body osmolality is monitored by the hypothalamus and inhibition of germinal proliferation is removed.

5). The animal sheds. In intact animals this results in the formation of a new epidermis with an improved PC capable of dealing with environmental stress. In thyroidectomized Garter snakes, defective mesos lipids are formed with a low PC. This results in chronic CWL which continues to stimulate germinal proliferation via the hypothalamic feedback mechanism.

The importance of the epidermis as a barrier to CWL cannot be understated. A clear correlation exists between water loss in reptiles and the aridity of the habitat. Species of reptiles from more desiccating habitats have lower rates of CWL than those species from more mesic habitats (Bently and Schmidt-Neilson, 1966; Dial and Fitzpatrick, 1982). Baeyens and Roundtree (1983) compared evaporative water loss and epidermal permeability in an arboreal snake (Opheodrys aestivus) and a semi-aquatic

snake (Nerodia rhombifera). They reported that rates of water loss were significantly higher in the semi-aquatic species and that shed epidermis in N. rhombifera was much more permeable to water than that of the arboreal species. The permeability of shed epidermis increased in both species following lipid extraction. However, a greater increase occurred in the shed epidermis of O. aestivus. They concluded that epidermal lipid content may be of particular significance in the adaptation of O. aestivus to an arboreal environment.

Kabayoshi et al (1983) studied evaporative water loss in Anolis carolinensis under conditions of low relative humidity. They reported a 50% reduction in transepidermal water loss, in animals acclimated in dry air (< 30% relative humidity) for more than seven days, when compared to animals acclimated to moist air (> 95% relative humidity). They concluded that A. carolinensis has the ability to improve its water balancing state, under desiccating conditions, by decreasing CWL. However, they fail to offer an explanation as to how this is accomplished nor is any mention made of skin shedding frequencies in these animals.

We believe that skin shedding serves as a means by which Garter snakes can correct sustained increases in CWL. The data presented in this thesis, albeit circumstantial, strongly support this notion. This proposal is also supported by the data presented for arachnids, fatty acid deficient mice,

and pathologic skin conditions in humans (see Discussion, Section VI).

It is apparent that if this theory is to be directly tested, two things will be needed. To begin with, a technique for constantly monitoring CWL, without undue stress to the animals, must be developed. At the present time, such technology does not exist.

Secondly, a more reliable and detailed analysis of epidermal lipids must be carried out. Presently, the literature regarding epidermal lipids in squamates has, at best, been inconsistent. This may in part be explained by the association that exists between epidermal lipids and proteins. Complete epidermal lipid extraction can only take place by disruption of epidermal keratin. Such disruption may, in turn, lead to destruction of the lipid moieties.

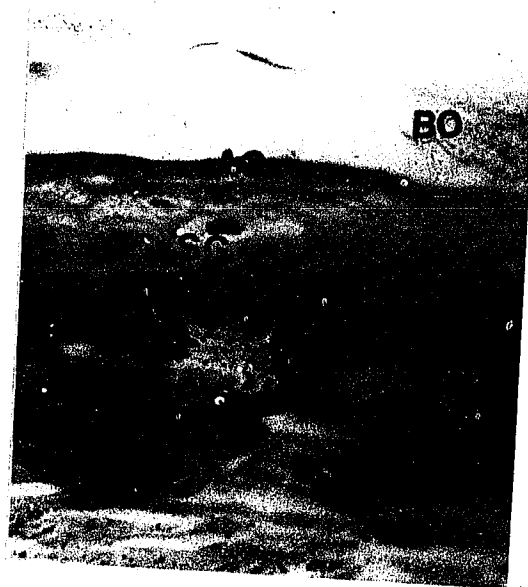
Appendix I
(Micrographs)

Key to Abbreviations

AI Alpha layer of inner epidermal generation
AO Alpha layer of outer epidermal generation
BI Beta layer of inner epidermal generation
BO Beta layer of outer epidermal generation
MI Mesos layer of inner epidermal generation
MO Mesos layer of outer epidermal generation
CLO Clear layer of outer epidermal generation
IG Inner epidermal generation
LTO Lacunar tissue associated with the outer epidermal generation
OBI Oberhautchen of inner epidermal generation
OBO Oberhautchen of outer epidermal generation
OG Outer epidermal generation
PIG Presumptive inner epidermal generation
PMI Presumptive mesos layer of inner epidermal generation
PAI Presumptive alpha layer of inner epidermal generation
PBI Presumptive beta layer of inner epidermal generation
PCLO Presumptive clear layer of outer epidermal generation
PLTO Presumptive lacunar layer of outer epidermal generation
POBI Presumptive Oberhautchen layer of inner epidermal generation
SG Stratum germinativum

Figure 6 Photomicrograph of intact garter snake epidermis, from an animal which did not shed at constant 28 oC, depicting the resting condition. (x 600).

Figure 7 Photomicrograph of intact garter snake epidermis, from an animal which did shed at constant 28 oC, depicting the resting condition. In this, and many other photographs, the beta layer of the outer generation is absent as it is lost during processing (x 600).



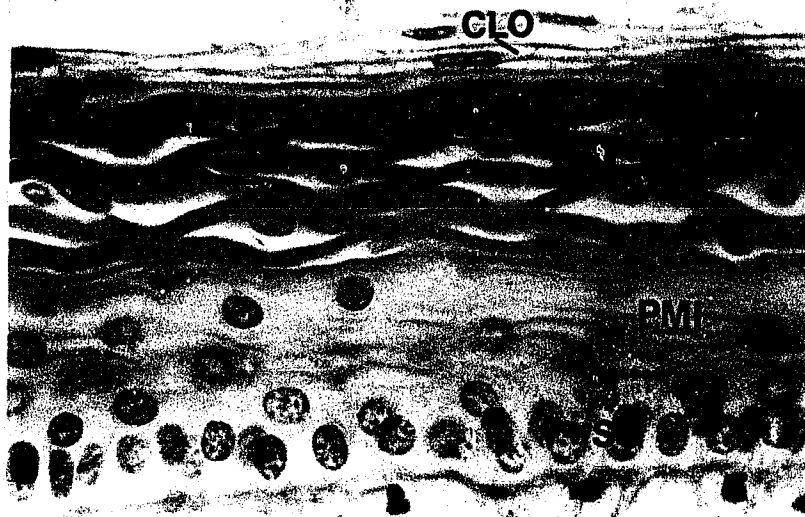
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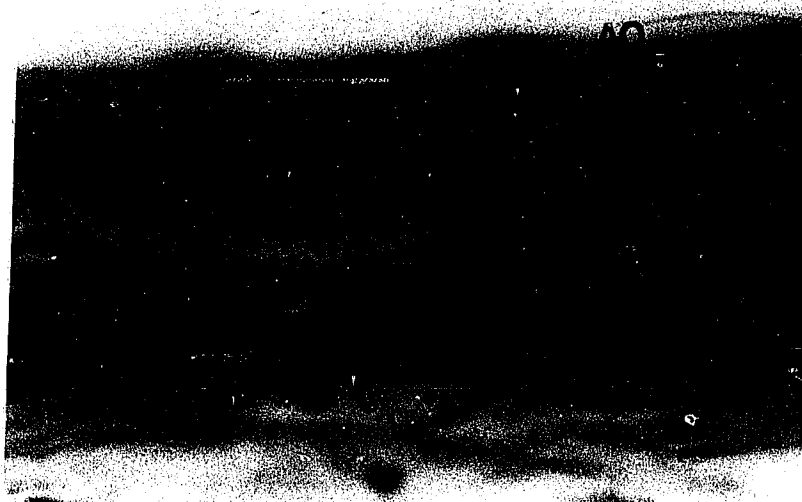
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Figure 8 Photomicrograph of thyroidectomized garter snake epidermis, during the first post-surgical renewal phase at 28 oC, depicting a mid to late stage 4 condition. Note that most of the outer generation has been lost during histological preparation. (x 600)

Figure 9 Photomicrograph of thyroidectomized garter snake epidermis, during the first post-surgical renewal phase at 21 oC, depicting a mid to late 3 condition. The outer generation has been lost during histologic preparation. (x 600)



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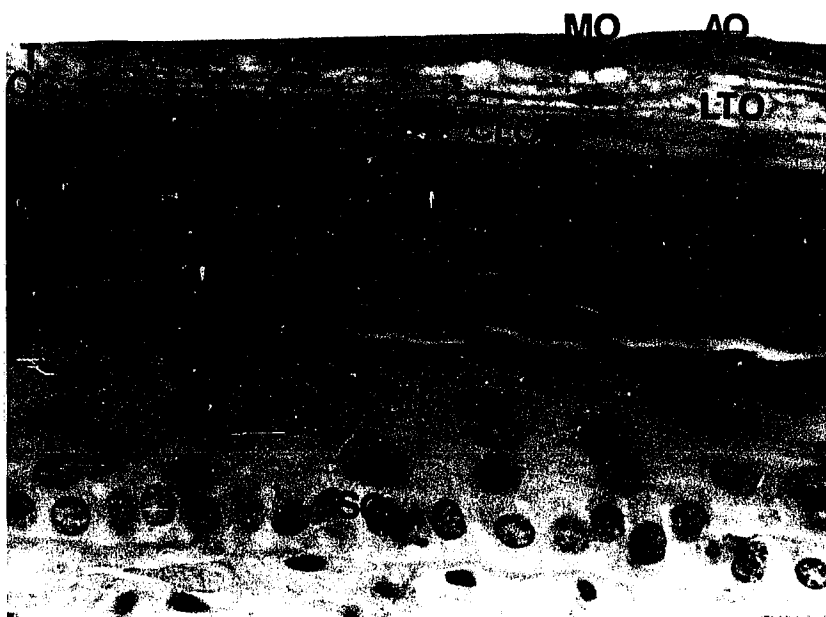
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Figure10 Photomicrograph of thyroidectomized garter snake epidermis, during the second post-surgical renewal phase at 28 oC, a mid stage 4 condition. The outer generation has been lost ~~in~~ histologic preparation. (x 600).

Figure11 Photomicrograph of thyroidectomized garter snake epidermis, during the second post-surgical renewal phase at 21 oC, depicting late stage 4. (x 600).



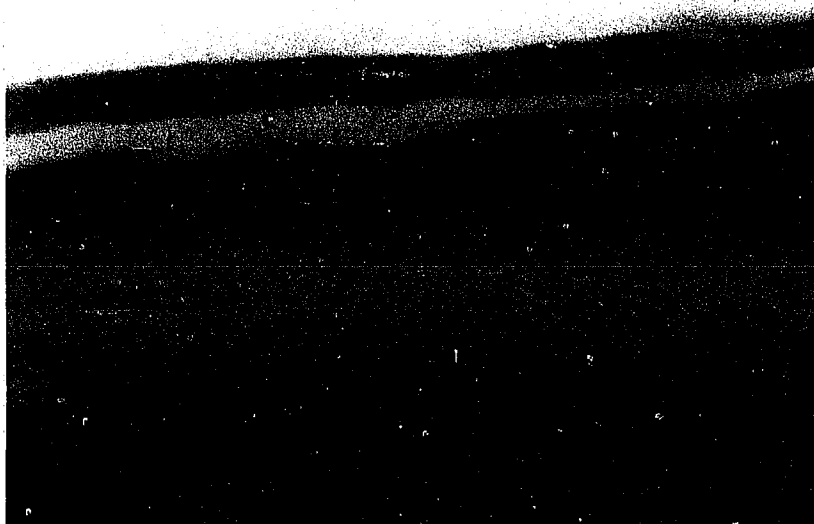
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Figure 12 Photomicrograph of thyroidectomized garter snake epidermis, during the third post-surgical renewal phase at 28 oC, depicting late stage 4 to early 5. The outer generation has been lost during histologic preparation. (x 600)

Figure 13 Photomicrograph of thyroidectomized garter snake epidermis, during the third post-thyroidectomized renewal phase at 21 oC, depicting mid to late stage 4. The outer generation has been lost during histologic preparation. (x 600)



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Figure 14 Photomicrograph of thyroidectomized garter snake epidermis, from an animal which spent 65 days at 10 oC, depicting the resting condition. (x 600)

Figure 15 Photomicrograph of thyroidectomized garter snake epidermis, from an animal which spent 85 days at constant 10 oC, depicting the resting condition. (x 600)



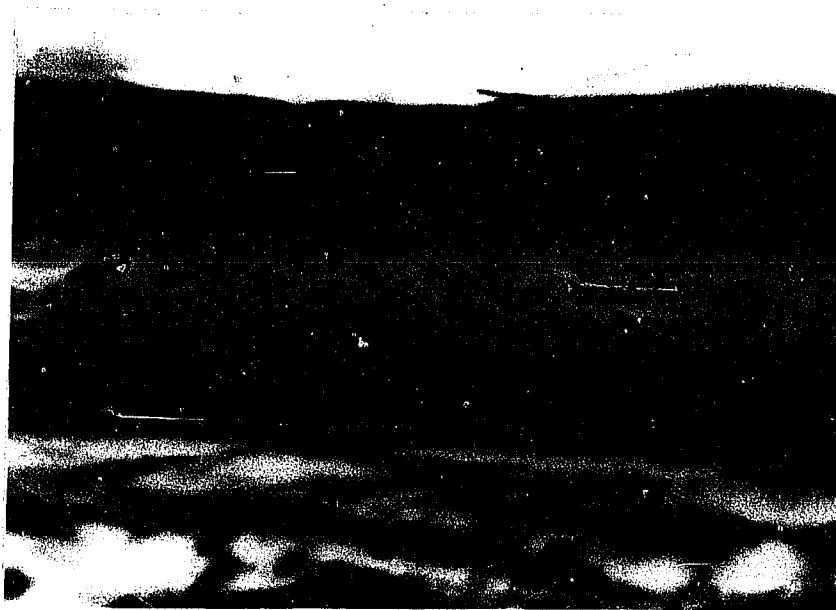
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Figure 16 Photomicrograph of thyroidectomized garter snake epidermis, from an animal which spent 65 days at constant 10 oC, depicting the resting condition. (x 600)

Figure 17 Photomicrograph of thyroidectomized garter snake epidermis, from an animal which spent 85 days at constant 10 oC, depicting the resting condition. (x 600)



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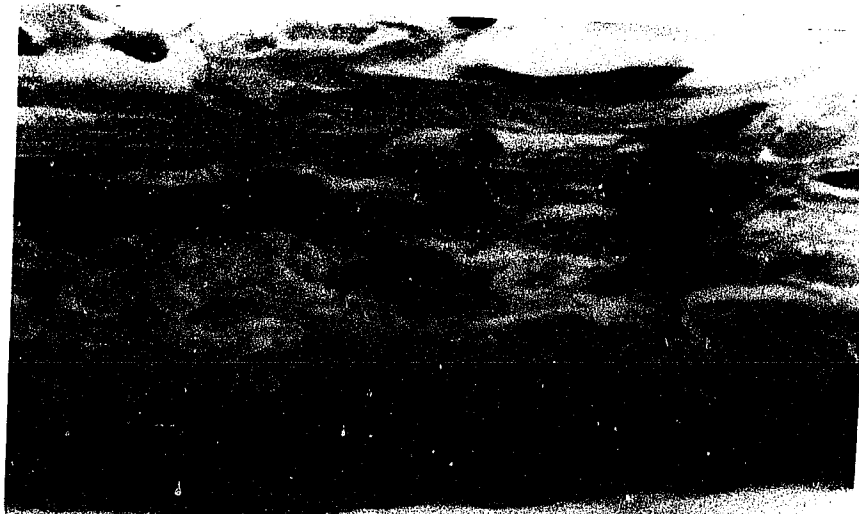
Figure 18 Photomicrograph of thyroidectomized garter snake epidermis, from an animal which spent 40 days at constant 10 oC, depicting the resting condition. (x 600)

Figure 19 Photomicrograph of thyroidectomized garter snake epidermis, from the same animal represented in fig. 18, after having spent 46 consecutive days at 10 oC followed by 39 consecutive days at 15 oC. The late resting condition is depicted. (x 600)

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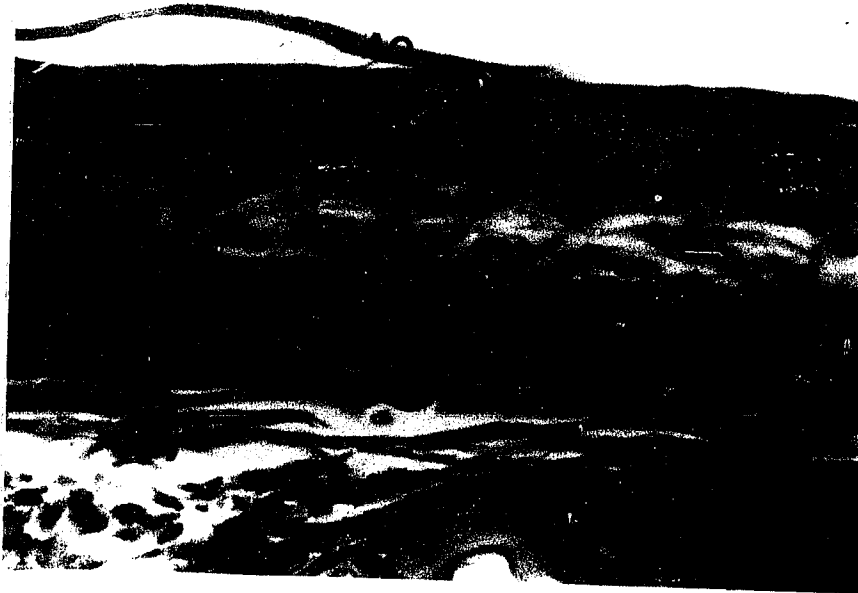
Figure 20 Photomicrograph of thyroidectomized garter snake epidermis, from an animal which spent 40 days at constant 10 oC, depicting the resting condition. (x 600)

Figure 21 Photomicrograph of thyroidectomized garter snake epidermis, from the same animal represented in fig. 20, after having spent 46 consecutive days at 10 oC followed by 39 consecutive days at 15 oC. The late resting stage is depicted. (x 600)

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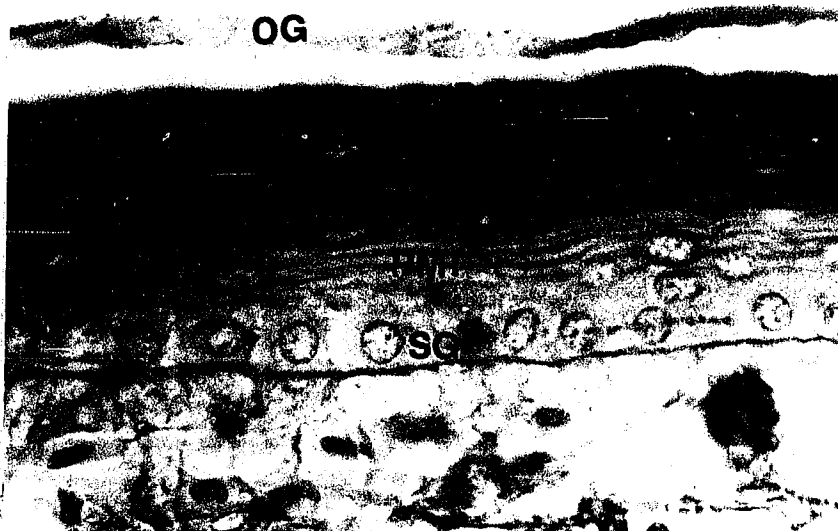
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Figure 22 Photomicrograph of thyroidectomized snake epidermis, from an animal at consecutive day number 40 at constant 10 oC, depicting the resting condition. (x 600)

Figure 23 Photomicrograph of thyroidectomized garter snake epidermis, from the same animal represented in fig. 22, at day number 9 at 28 oC after having spent 46 consecutive days at 10 oC. Late stage 4 is depicted. The outer generation has been removed during histologic preparation. (x 600)



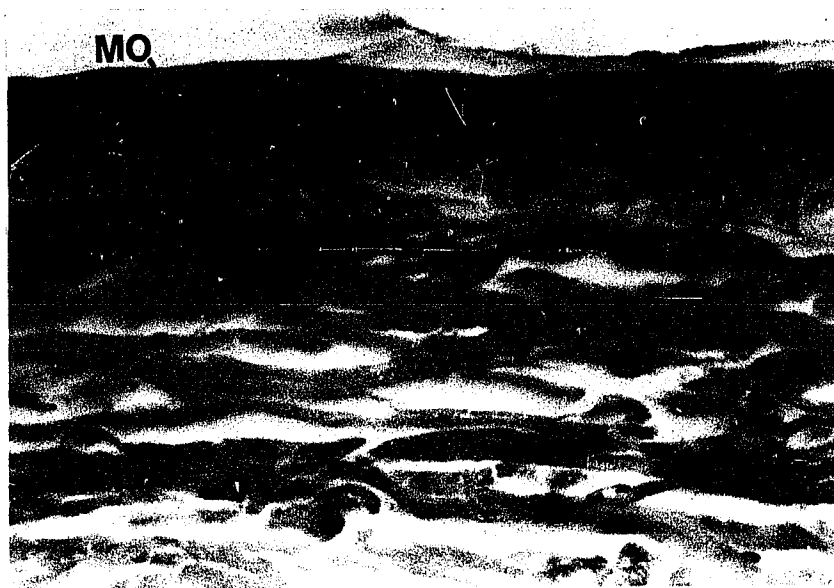
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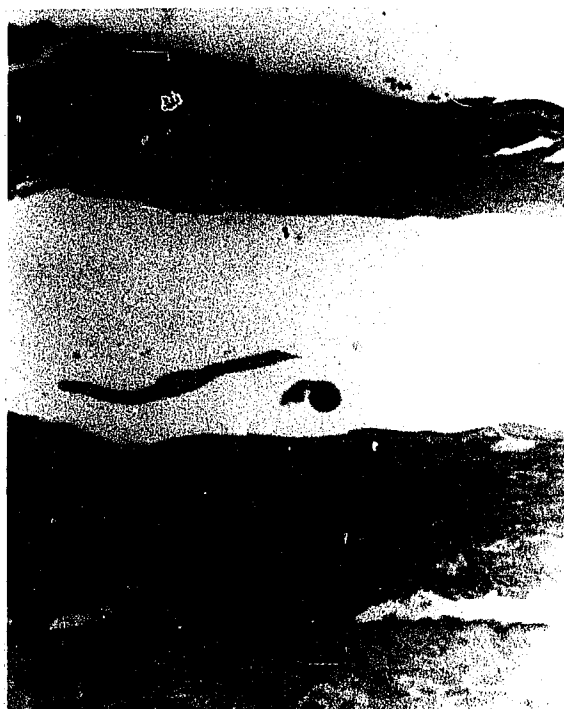
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Figure 24 Photomicrograph of thyroidectomized garter snake epidermis, on consecutive day number 40 at 10 oC, depicting the resting condition. (x 600)

Figure 25 Photomicrograph of thyroidectomized garter snake epidermis, from the same animal represented in fig. 24, on day 9 at 28 oC after having spent 46 consecutive days at 10 oC. Stage 4 of the renewal phase is depicted. (x 600)



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Figure 26 Photomicrograph of intact garter snake epidermis depicting stage 4 of the renewal phase. (x 600)

Figure 27 Electron micrograph of an intracellular mesos granule during stage 4 of the renewal phase of the same skin sample as in figure 26. Note the precisely stacked lipid lamellae (LL) lying within the membrane bounded (broad arrow) organelle. (x 200,000)



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Figure 28 Photomicrograph of thyroidectomized garter snake epidermis, during the first post-surgical renewal phase, depicting late stage 4. (x 600)

Figure 29 Electromicrograph of an intracellular lamellar granule, from the same tissue sample depicted in fig. 28, during late stage 4 of the first post-surgical renewal phase. Some organized lipid lamellae (LL) are still visible but many have been replaced by an amorphous material (X). The membrane surrounding the organelle is intact (arrow). (x 200,000).



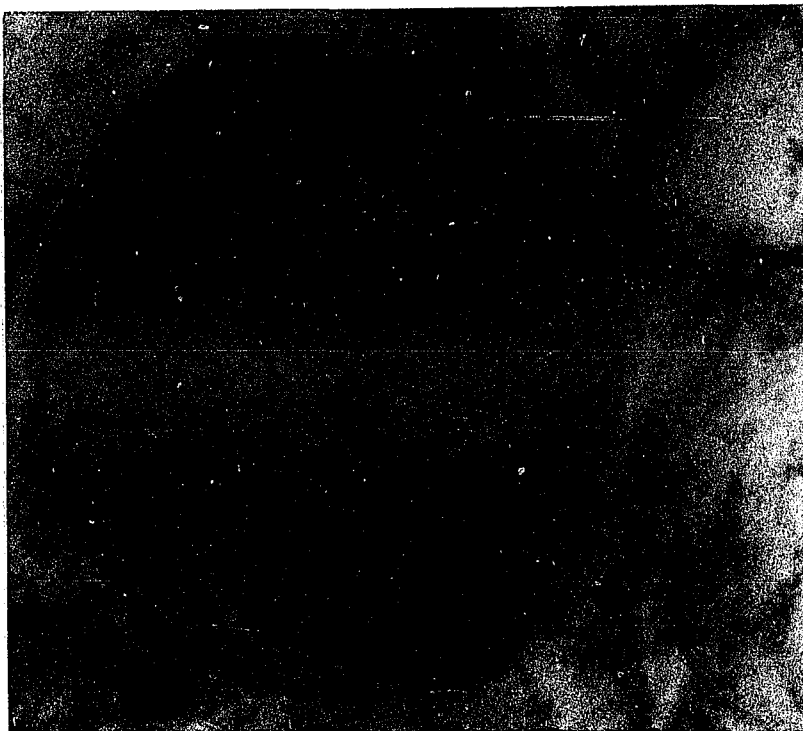
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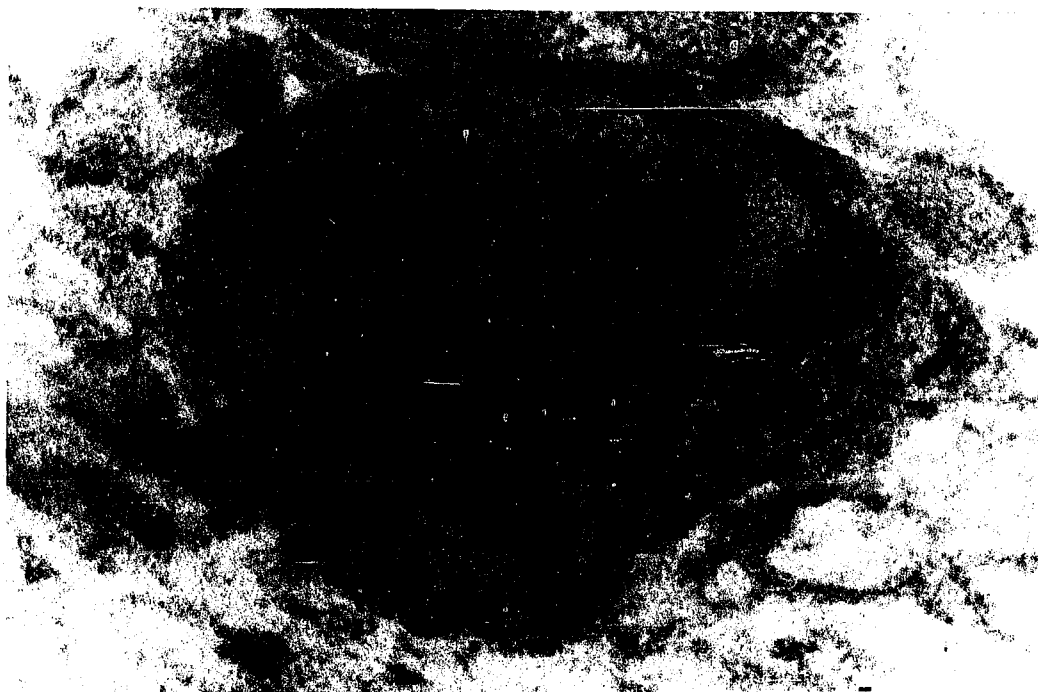
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FIGURE 30 Electron micrograph of an intracellular lamellar granule, from a thyroidectomized garter snake, during stage 4 of the second post-surgical renewal phase. By this time, only a few poorly organized lipid lamellae (LL) are present, most having been replaced by an amorphous material (X). The membrane surrounding the organelle is still intact (arrow). (x 200,000)

Figure 31 Electron micrograph of an intracellular lamellar granule, from the epidermis of a thyroidectomized garter snake, during stage 4 of the third post-surgical renewal phase. Note that no stacks of lipid lamellae are seen, and their place is taken by an amorphous material (X). The membrane surrounding the organelle is still intact (arrow). (x 200,000)



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