VITAMIN A REQUIREMENTS OF YOUNG RED-EARED SLIDER TURTLES (PSEUDEMYS SCRIPTA ELEGANS)

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
MARILYN PATRICIA ANDERSON
1972

The vit

ackling re

udied. Tw

ttles eac

periment

itamin A l

separate

nd a 25 wa

yophilized

roup (I, I

f hog hear

clusive c

asal diet

f fresh ho

ed the bas

etinol pe

iets deve

keratinizi

ABSTRACT

VITAMIN A REQUIREMENTS OF YOUNG RED-EARED SLIDER TURTLES (*PSEUDEMYS SCRIPTA ELEGANS*)

By

Marilyn Patricia Anderson

The vitamin A requirement for growth and maintenance of health of hatchling red-eared slider turtles, *Pseudemys scripta elegans*, was studied. Two lots of 40 turtles each were divided into groups of 10 turtles each. One group in each lot was killed at the beginning of the experiment to serve as controls for normal histology and initial liver vitamin A levels. The remaining 3 groups in each lot were maintained in separate tanks of distilled water and were provided with a wood float and a 25 watt reflectorized lamp. The turtles were fed experimental, lyophilized diets based on the nutrient requirements of the chick. One group (I, Lot 1; IV, Lot 2) in each lot was fed the basal diet composed of hog heart supplemented with glucose, corn oil, minerals and vitamins exclusive of vitamin A. Two groups (II and III) in Lot 1 were fed the basal diet supplemented with 140 and 280 IU of retinyl palmitate per kg of fresh hog heart, respectively. Two groups (V and VI) in Lot 2 were fed the basal diet supplemented with 900 and 1800 IU of all-trans retinol per kg of fresh hog heart, respectively. All groups fed the diets developed similar clinical signs and lesions characterized by keratinizing squamous metaplasia of nasal, oral, tympanic, ocular and

ıary tract memb

ıchea, bronchi a

ous tissue wer

ıotic and mine

ver vitamin A l

ot 2 initial

pectively. Th

ot 1 were 2.9

min A values

g/gm, respe

ed slider turt

is possible th

lled water on

to vitamin A

urinary tract membranes. In contrast the mucous membranes of the trachea, bronchi and esophagus were normal. The pancreas, bones and nervous tissue were unaffected. Renal tubule epithelia were degenerate, necrotic and mineralized. Urinary tract calculi occurred frequently. Liver vitamin A levels at necropsy, in µg/gm of fresh liver, for Lot 1 and Lot 2 initial control groups (0 and VII) were 10.45 and 8.74, respectively. The liver vitamin A values for Groups I, II and III of Lot 1 were 2.91, 1.76 and 9.10 µg/gm, respectively. The liver vitamin A values for Groups IV, V and VI of Lot 2 were 1.92, 3.40 and 2.48 µg/gm, respectively. The livers of 6 older, wild-caught, red-eared slider turtles had an average of 19.55 µg of vitamin A per gram. It is possible that depletion of body ions and osmotic action of distilled water on some membranes initiated lesions which mimicked and/or led to vitamin A deficiency.

VITAMIN A REQUIREMENTS OF YOUNG RED-EARED SLIDER TURTLES (*PSEUDEMYS SCRIPTA ELEGANS*)

By

Marilyn Patricia Anderson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1972

I extend spe
ger, for his g
y academic progr
he Department of
he academic envi
ade this project
My sincere a
he Animal Husba
he execution of
ppreciated was
nd interest in
eptiles at the
I am also
me and to Dr.
nalysis of my
Thanks are
ly for guidanc

ACKNOWLEDGMENTS

I extend special thanks to my major professor, Dr. Vance L. Sanger, for his guidance and encouragement during this project and my academic program. I also thank Dr. C. C. Morrill, Chairman of the Department of Pathology, Michigan State University, for providing the academic environment, research facilities and scholarship that made this project possible.

My sincere appreciation is extended to Dr. Duane E. Ullrey of the Animal Husbandry Department for his enthusiasm and guidance during the execution of this project from its beginning to its end. Also appreciated was financial support from the Detroit Zoological Society and interest in the project by Mr. James Langhammer, Curator of Reptiles at the Detroit Zoo.

I am also indebted to the Animal Husbandry Department for computer time and to Dr. William Magee for his assistance in the statistical analysis of my data and its processing for computerized readout.

Thanks are also extended to Mrs. Rosemary Covert and Dr. William Ely for guidance in liver vitamin A analysis.

TABLE OF CONTENTS

Table

LIST OF TABLES

Table

4.1 Body measurem
1) or Day 191

4.1 Body measurem
turtle at Day

4.1 Mineral cont

LIST OF FIGURES

INTRODUCTION

The role of reptiles in research is steadily increasing, especially in areas of biochemistry and physiology. Also many varieties of reptiles are sold as pets which includes about 15,000,000 turtles annually in the United States (Anon., 1972). Zoological parks also find reptiles to be a great attraction to the visiting public.

The 2 major problems of reptilian adaptation to captivity are nutrition and environment. Most reptiles are known to be either omnivorous, carnivorous or herbivorous and a few qualitative nutrient requirements have been established empirically. However, quantitative nutrient requirements are not known for any reptile, nor is the role of trace elements known.

Statement of Problem

Pseudemys scripta elegans is the small red-eared slider turtle commonly sold in pet shops. Many of these turtles die within several months of purchase. They become anorectic, their eyelids swell shut and their shells become deformed. The problem appears to be nutritionally and/or environmentally related and is occasionally complicated by secondary infections. Dietary deficiency of the vitamins A and D is thought to be partly responsible for the ocular and shell lesions, respectively, since specific vitamin therapy improved the condition of many afflicted turtles (Elkan and Zwart, 1967; Wallach, 1971a).

Since the red-eared slider and its close relatives, *Chrysemys*, *Graptemys*, *Clemmys*, *Emys*, *Chinemys* and *Pseudomedusa* are not only kept

a pets b

pplicans

ative nu

The

nd the

nvles

ipart

th-eare

as pets but are also important as research animals and zoological park specimens, there is a need for establishing the qualitative and quantitative nutrient requirements of these turtles.

Objectives

The objectives of this study were to describe the clinical signs and the macroscopic and microscopic changes in vitamin A deficient turtles and to determine the quantitative vitamin A requirement for support of growth and maintenance of health of the recently-hatched red-eared slider turtle.

LITERATURE REVIEW

Vitamin A

A fat-soluble accessory factor (fat-soluble A or vitamin A) was found to be an essential nutrient in 1913 (McCollum and Davis, 1913). A comprehensive review of literature on vitamin A was made by Moore (1957). Sebrell and Harris (1967) and DeLuca and Suttie (1970) were editors of multiple author books in which the recent developments in vitamin A research were reviewed.

Vitamin A is a series of fat-soluble isomers. The all-*trans* retinol is the predominant naturally occurring and most biologically active form. Vitamin A is unstable to oxygen and light especially in the absence of antioxidants. Powdered vitamin A, in the palmitate and acetate ester forms, is readily available for commercial purposes. The powder may be stabilized and made water dispersible by the addition of a gelatin and sugar coating. Freezing also prolongs the storage life of vitamin A.

Physiology of Vitamin A

Absorption of vitamin A occurs in the small intestine. It is decreased in protein deficiency and it is facilitated by the presence of bile due to emulsifying effects and/or antioxidant properties (Bernhard, Ritzel and Steiner, 1954).

Storage of vitamin A is mainly in the liver (70 to 90%, Jones, 1965) in both hepatocytes and Kupffer cells, and in normal animals significant

concentrations of vitamin A are also found in the kidneys, lungs, blood and milk. Goodman (1970) found that retinol circulates in the plasma in association with a specific retinol transport protein complexed with a prealbumin protein.

Vitamin A is not excreted from the body of most animals, even with high levels of absorbed vitamin A, unless there is infection or chronic renal disease. However, depletion of liver vitamin A, especially with high stores, normally occurs at rates greater than that calculated for physiological needs while with low stores the rate of depletion is slow (Moore, 1957). In rats stress of cold temperature increases depletion of normal liver stores (Sundaresan, Winters, and Therriault, 1967) but not of low stores (Phillips, 1962) and dietary levels of vitamin E inversely modifies the biological utilization of vitamin A (Davies and Moore, 1941). Growth restriction from any cause decreases liver vitamin A depletion (Hayes, 1971; Johnson and Bauman, 1948).

The function of vitamin A, other than in vision, is unknown. Dingle (1961) and Roels (1967) suggest that vitamin A is a membrane active substance. Fell (1970) suggested that vitamin A promotes the fusion of lysosomal and cell wall membranes which enables release of proteolytic enzymes from the cell. One of these proteolytic enzymes, cathepsin, promotes the resorption of bone and cartilage matrices similar to that observed in clinical hypervitaminosis A. Amino-caproic acid, cortisol and antiserum to cathepsin D inhibits the action of vitamin A on *in vitro* cartilage matrix breakdown (Fell, 1970). Cortisol is thought to stabilize membranes and amino-caproic acid inhibits a lysosomal protease.

Parnell and Sherman (1962) found that different epithelial tissues have different threshold levels of response to vitamin A, the

gastrointestinal tract having one of the lowest and the skin the highest. Fell (1957) found that skin *in vitro* formed mucus-secreting cells under the influence of excess vitamin A.

Wolf and DeLuca (1970) said that cells of epithelia in general form mucus-secreting cells when vitamin A levels are adequate and form keratinized cells when vitamin A levels are inadequate. They hypothesize that vitamin A is involved in glycoprotein (mucus) synthesis which is mediated by a specific transfer ribonucleic acid (t-RNA) which in turn translates a specific messenger ribonucleic acid (m-RNA). When vitamin A is inadequate the t-RNA for glycoprotein synthesis is not produced which allows another t-RNA to form and translate its own m-RNA which in turn allows the formation of a different cell type, i.e., a keratinized cell forms from a mucus-secreting cell type. This biochemical explanation supports Hayes' suggestion that the switch of one cell type to another type depends upon the stage of differentiation of that cell (Hayes, 1971).

Thompson (1970) found that retinoic acid could functionally replace retinol except in vision and reproduction. He suggested that testicular androgen secretion was reduced in vitamin A deficiency because of reduced circulating gonadotropin without reduced production of gonadotropin by the pituitary. He also suggested that retinol acts directly upon the testicular germinal epithelium and that retinol or retinal but not retinoic acid maintains pregnancy by direct action on the uterus and/or by action of vitamin A on steroid biosynthesis.

Vitamin A Deficiency in Mammals and Birds

Lesions and clinical signs of vitamin A deficiency in mammals and birds are well documented (Moore, 1957; Sebrell and Harris, 1967; DeLuca and Suttie, 1970; Follis, 1958; Barnicot and Datta, 1956). The

main clinical signs and lesions include: 1) night blindness, 2) ataxia, 3) increased cerebrospinal fluid pressure, 4) xerophthalmia, 5) arrest of skeletal growth, 6) deafness (dogs) and 7) blindness (calves). The main histopathologic lesion is keratinizing squamous metaplasia of various ectodermal, mesodermal and endodermal epithelia and occurs in the following approximate order: 1) ducts of salivary and accessory oral and pharyngeal glands, 2) respiratory epithelium of the nares, trachea and bronchi, 3) conjunctiva, cornea and ducts of paraocular glands and 4) pancreatic ducts, enamel organ and esophageal gland ducts. Other microscopic lesions associated with vitamin A deficiency include: 1) atrophy of seminiferous tubules, 2) kidney tubule degeneration with or without squamous metaplasia and 3) formation of calculi in the urinary bladder and kidney tubules (Beaver, 1961; Elvehjem and Neu, 1932; VanLeersum, 1928).

Jungherr (1943) found in chickens that there was an inverse relationship between the level of dietary vitamin A and the severity of vitamin A deficiency lesions in nasal epithelia. He also found that 5-week-old chicks had low storage of vitamin A in the liver regardless of high, medium or low levels of dietary vitamin A whereas in 7-month-old chickens there was an approximate direct correlation of liver storage and dietary levels of vitamin A. He considered 15 IU of vitamin A/gm of liver as borderline between vitamin A deficiency and insufficiency in growing chicks and suggested that 250 to 350 IU of vitamin A/head/day in the diet be the minimum chick requirement up to 8 weeks of age.

Vitamin A Deficiency in Turtles

Elkan and Zwart (1967) reviewed the literature on a common ocular disease of turtles that is characterized by swollen eyes and blindness. They described the clinical signs and lesions in 32 turtles which had this ocular disease and concluded that the turtles were affected by a "metabolic disorder dominated by vitamin A deficiency." They saw keratinizing squamous metaplasia, accompanied by infiltration with eosinophilic granulocytes, in the lacrimal and harderian glands, conjunctiva, nictitating membrane, pancreatic ducts, kidney collecting tubules, ureters, urinary bladder and bile ducts. Corneas were hyperkeratotic and fatty degeneration of the liver occurred frequently. The livers of 2 affected turtles (*Graptemys sp.*) had 9 and 19 IU of vitamin A/gm, respectively. Other sporadic lesions seen were: 1) acute proliferative glomerulonephritis, 2) eosinophilic granulocytic infiltration of kidney interstitial tissue, 3) chronic nephritis, 4) acute eosinophilic granulocytic thyroiditis, 5) enlarged thyroid follicles with hyperplastic epithelium and 6) perivascular eosinophilic granulocytic infiltration in the liver. Some turtles could not dive due to uncontrolled buoyancy and many sought the highest and driest location in their environment. Some affected turtles recovered with oral vitamin A therapy. Elkan and Zwart suggested that the eosinophilic granulocytic infiltrations might be a peculiarity of chelonians in regard to vitamin A deficiency.

The liver vitamin A values Elkan and Zwart found were extremely low compared to values for other reptiles and amphibians which ranged from 35 to 8000 IU/gm of liver (Gillam, 1938).

Calcium and the Urinary Tract

Calcification of renal tubules and formation of calculi in the urinary tract are not only common with vitamin A deficiency but also with ion deficiencies. In chloride deficient rats calcium salts precipitate in convoluted and collecting tubules, often resulting in tubule obstruction (Follis, 1958). In magnesium deficient rats tubules and glomeruli degenerate and become calcified and calculi form in tubule lumens (Follis, 1958).

Smith and Williams (1971) stated that all renal stones contain a matrix of complex mucoproteins and that over 90% contain calcium. They also listed a few conditions which contribute to the formation of calcium-containing calculi sich as oliguria, urine stasis, alkaline urine, phosphaturia, increased calcium and/or ammonia excretion and foreign bodies. They also state that calcium content of urine increases with excessive dietary calcium and with metabolic acidosis (but not respiratory acidosis); excretion of calcium may be above or equal to normal in humans with calcium-containing calculi. Epstein (1971) said that: 1) excretion of calcium in urine depends directly upon glomerular filtration rate and/or blood calcium levels, 2) calcium and sodium reabsorption takes place in the proximal renal tubule and urinary calcium is directly influenced by the amount of urinary sodium, 3) after excessive dietary intake of calcium, urinary calcium loss may continue for several months after withdrawal of dietary calcium, 4) calcium will precipitate in dead or degenerate renal tissue and 5) lesions of hypercalcemia, from any cause, includes degeneration and necrosis of renal tubules, exclusive of proximal tubules, and calcified cellular debris which may form obstructing calculi in tubules.

Calcium and the Urinary Tract

Calcification of renal tubules and formation of calculi in the urinary tract are not only common with vitamin A deficiency but also with ion deficiencies. In chloride deficient rats calcium salts precipitate in convoluted and collecting tubules, often resulting in tubule obstruction (Follis, 1958). In magnesium deficient rats tubules and glomeruli degenerate and become calcified and calculi form in tubule lumens (Follis, 1958).

Smith and Williams (1971) stated that all renal stones contain a matrix of complex mucoproteins and that over 90% contain calcium. They also listed a few conditions which contribute to the formation of calcium-containing calculi sich as oliguria, urine stasis, alkaline urine, phosphaturia, increased calcium and/or ammonia excretion and foreign bodies. They also state that calcium content of urine increases with excessive dietary calcium and with metabolic acidosis (but not respiratory acidosis); excretion of calcium may be above or equal to normal in humans with calcium-containing calculi. Epstein (1971) said that: 1) excretion of calcium in urine depends directly upon glomerular filtration rate and/or blood calcium levels, 2) calcium and sodium reabsorption takes place in the proximal renal tubule and urinary calcium is directly influenced by the amount of urinary sodium, 3) after excessive dietary intake of calcium, urinary calcium loss may continue for several months after withdrawal of dietary calcium, 4) calcium will precipitate in dead or degenerate renal tissue and 5) lesions of hypercalcemia, from any cause, includes degeneration and necrosis of renal tubules, exclusive of proximal tubules, and calcified cellular debris which may form obstructing calculi in tubules.

Sodium Deficiency (Follis, 1958)

Corneal epithelium becomes hyperkeratotic in sodium deficient rats. Sodium deficient rats also have retarded growth and subsequent weight loss, swollen eyelids and hair loss. The swollen eyelids result from tarsal and meibomian gland duct blockage and cystic dilatation of acini which undergo squamous metaplasia.

Sodium depletion can be produced by excessive water intake, dietary restriction and inhibition of sodium reabsorption.

Reptilian Nutrition

Nutrition or poikilothermic animals is intimately related to environment. Ambient temperatures affect body temperature and thus *in vivo* enzymatic reaction rates. Energy requirements to maintain body temperature also depend on the ambient temperature. Seasonal influences include not only temperature changes but also daylight length-hormonal balances which in turn affects appetite and nutrient requirements for general daily activity, reproduction, hibernation and estivation. Even if the proper temperature, humidity, lighting and food are adequate, a reptile in captivity may still suffer from inadequate nutrition as a result of its peculiar behavior, anatomy and physiology (Wallach, 1971b; King, 1971). For example, an inferior individual may starve to death under the intimidation of a dominant one, especially if there is over-crowding or inadequate furniture such as perches or hiding places.

Wallach (1971b) and Marcus (1971) reviewed and described the environmental and nutritional and infectious diseases of reptiles, respectively. Some of the major nutritional problems are: 1) steatitis of vitamin E deficiency, 2) goiter of iodine deficiency, 3) skeletal diseases of calcium-phosphorus-vitamin D imbalance and 4) edema in

chelonians due to vitamin A deficiency. In all in-tances these disease-nutrient relationships are established on empirical bases only.

Turtle Anatomy and Physiology

Skeletal

Gans, Bellairs and Parsons (1969) edited a comprehensive review of reptilian osteology and dentition and chelonian shell formation. The cortex of chelonian long bones is mainly primary bone without haversian systems and near the epiphysis it is thin and of endosteal origin. The medullary space contains cancellous bone and hemopoietic tissue. The epiphyses do not form secondary centers of ossification in turtles. At the ends of long bones, unique to young turtles, crocodiles and birds, is a large mass (cone) of cartilage which becomes eroded from all sides, sometimes leaving temporary islands of cartilage in the medulla (Figure 10). The outer border of the epiphysis is composed of fibrocartilage becoming undifferentiated cartilage internally. Flattened cartilage cells in the growth zone form columns and hypertrophy; these columns may be poorly developed in young turtles. Irregular cavities are eroded into the cartilaginous masses at the ends of the long bones but once columns are formed the erosion is regular, following the columns. Some endochondral bone formation in turtles appears to involve a true metaplasia of cartilage to bone instead of osteoblastic bone formation after cartilage destruction.

Suzuki (1963) described the skeletal system of *Pseudemys scripta elegans* hatchlings (carapace length of 29 to 35 mm), juveniles and adults and correlated histologic differences with age, sex and seasons. He found that hatchling femurs had an ossified endosteal layer and a periosteal collar of osteoid whereas turtles a few months older had

femurs with both layers well calcified. At the ends of hatchling long bones was a large medullary cone of cartilage which was removed by the time the turtles attained a carapace length of 40 to 65 mm. He also found that calcification of cartilage and osteoid occurred primarily in periods of active growth which coincided with adequate food intake. Clark and Gibbons (1969) found a positive correlation between plastron length and calcium content of the plastron and carapace.

Sense Organs

Gans and Parsons (1970a) edited a comprehensive review of ocular, nasal and auditory structures in reptiles. The nasal vestibule in *Pseudemys* is lined by keratinized stratified squamous epithelium. The external nasal gland enters the dorsolateral wall of the posterior vestibulum. The nasal cavity proper has sensory olfactory epithelium posterodorsally, nonsensory respiratory epithelium anteroventrally and an area of sensory, perhaps vomeronasal, epithelium anteroventrally (McCotter, 1917). Bowman's glands, which occur only in the subepithelial sensory olfactory epithelium, are alveolar in form and lined by columnar or pyramidal cells which may become cuboidal in the ducts.

Turtles have a lacrimal gland temporally and a harderian gland nasally in the orbital cavity. With the light microscope these glands are indistinguishable except as to location (Elkan and Zwart, 1967). The optic nerve passes between these glands. In some species of turtles the lacrimal gland cell is almost identical to reptilian renal tubule cells, both microscopically and ultrastructurally (Cowan, 1971). Both glands empty into the base of the conjunctival sac through numerous ducts. There is no nasolacrimal canal.

The middle ear of *Pseudemys* is connected with the pharynx by a narrow eustachian tube which is lined by a mucous membrane. The tympanum is lined by a thin squamous epithelium and the tympanic cavity is lined with several types of epithelium varying from simple squamous to cuboidal with scattered mucus-secreting cells.

Cephalic Vasculature (Bruner, 1907)

There are extensive intracranial and extracranial venous sinuses which have numerous bidirectional anastomoses. Their function is thought to be equalization of blood pressure in the head region. The orbital sinus receives most of the blood from the anterior cephalic region and the internal jugular vein is its only outlet. The internal jugular vein which drains over 90% of the cephalic region is equipped with a special constrictor muscle which is responsible for the swell mechanism of cephalic skin molting.

Muscular tone keeps a turtle's eyelids open and prevents orbital sinus distention. Closing of the lids operates by localized increases in blood pressure.

The cerebrospinal and extracellular spaces in the brain of *Pseudemys* are described by Heisey (1970). The spaces are large compared to mammals.

Osmoregulation

Blood osmotic pressure is regulated primarily by the kidneys. Dantzler and Schmidt-Nielsen (1966) found that changes in the glomerular filtration rate of turtles varied only with the number of functioning nephrons which work to full capacity or not at all.

Extrarenal osmoregulation occurs in the mucosa of the pharynx and cloaca (Dunson, 1967a), in the urinary bladder and paired accessory

urinary bladders (Rosen, 1970; Steinmetz, 1967; Steinmetz, Omachi and Frazier, 1967) and in most reptiles in specialized salt secreting glands (lacrimal glands in turtles) located in the cephalic area (Dessauer, 1970; Cowan, 1969; Cowan, 1971).

Trobec and Stanley (1971) found that *Chrysemys* held in distilled water for 40 days were depleted of their sodium stores by 166 μeq/kg of body weight/day (or about 0.2%/day of their total sodium). They also found that turtles held in tap water for 48 days had a net loss of sodium without clinically apparent ill effects.

Natural History of *Pseudemys*

Cagle (1950) described in detail the life history of *Pseudemys scripta*. This turtle is found in quiet, shallow, fresh water where there is abundant sunlight and vegetation. Cagle found the turtles active between temperatures of 10 to 35 C. Egg laying occurs from April to July and hatching from July to September; some turtles overwinter in the nest. The yolk mass helps shape the carapace and plastron when it is drawn into the body shortly after hatching. Measurements of 86 hatchlings from Louisiana were:

Carapace lengty, mm	$\bar{x}$ = 32.46; range, 28.0-35.8
Plastron length, mm	$\bar{x}$ = 30.89; range, 27.1-33.8
Body weight, gm	$\bar{x}$ = 8.07; range, 5.4-10.0
Yolk mass, cm	1-3

No hatchlings started growing earlier than the following April whether or not they overwintered in the nest. In Illinois the growing season is from May to October and in Louisiana from April to November. Hatchlings approximately double their size in the first growing season then continue to grow at reduced rates in succeeding seasons (Carr, 1952).

Clark and Gibbons (1969) examined stomach contents in *Pseudemys scripta* and found the turtles to be omnivorous. They found a shift in hatchlings and juveniles during the growing season from a basically carnivorous diet to a more herbivorous diet, the latter typical of adults.

Body weight-carapace length relationships were published for 3 species of turtles. The following table summarizes the weight-length (W-L) formulas:

Table 1. Body weight-carapace length relationships in turtles

Genus	Formula	W units	L units	Authority
Chrysemys	$W = 4.4 \times 10^{-4} L^{2.79}$	gm	mm	Graham, 1971
Trionyx	$W = 0.1202 L^{2.95}$	gm	cm	Dunson, 1967b
Chelydra	$W = 1.6 \times 10^{-4} L^{3.06}$	lb	in	Lagler and Applegate, 1943
Chelydra	$W = 3.36 \times 10^{-4} L^{2.93}$	gm	cm	*

*From the data of Lagler and Applegate (1943) converted from the avoirdupois system to the metric system by Mosimann (see Dunson, 1967b).

MATERIALS AND METHODS

Animals

Eighty hatchling, red-eared slider turtles, *Pseudemys scripta elegans*, were acquired from a commercial dealer. Forty of these turtles (Lot 1, Groups 0, I, II and III) were received in May 1971 and may have been holdovers from the 1970 hatching season. Nine of these turtles had ulcers on the forefeet and/or tail, probably the result of cannibalism and/or trauma. One healthy turtle had a crimp in its shell at the plastron-bridge junction. The other 40 turtles (Lot 2, Groups IV, V, VI and VII) were received in August 1971; all had an egg tooth and 10 had some yolk material adherent to the plastron. The egg teeth were lost within 2 weeks indicating that all turtles in this lot were received within a few days of hatching. For the purpose of identification, the plastron of each turtle was photographed to record the unique pattern of spots and ocelli.

Group Assignment

Lot 1. The 9 turtles with skin ulcers and the 1 with the crimped shell were placed in Group 0. The remaining 30 turtles were ranked by weight. Each of the 3 heaviest turtles was randomly assigned to treatment Group I, II or III. Each turtle in succeeding groups of 3 was similarly assigned to one of the 3 treatment groups.

Lot 2. The 40 turtles were ranked by weight and each turtle in successive groups of 4 was randomly assigned to Group IV, V, VI or VII. Turtles in Groups IV, V and VI were in turn randomly assigned, by similar weight outcome groupings, to subgroup A or B.

Turtle Groups

Table 2. Turtle groups and dietary treatments

Lot	Group	Diet	Vitamin A Supplement*	Zinc Supplement**
1	0	none	none	none
1	I	Basal	0	0
1	II	Supplemented	140†	0
1	III	Supplemented	280†	0
2	IV A	Basal	0	0
2	IV B	Supplemented	0	100
2	V A	Supplemented	900††	0
2	V B	Supplemented	900††	100
2	VI A	Supplemented	1800††	0
2	VI B	Supplemented	1800††	100
2	VII	none	none	none

*IU (International Units) of vitamin A/kg of fresh hog heart.

**ppm (parts per million) of elemental zinc.

†Retinyl palmitate, gelatin coated.

††All-*trans* retinyl acetate.

Diets

The basal diet was based on the chick nutrient requirements (NAS, 1971). Table 3 lists the basal diet ingredients and formula.

Table 3. Formula for the basal diet*

Ingredient	Amount
Fresh ground hog heart, gm**	500
$MnSO_4 \cdot H_2O$, mg	19.1
KIO_3, mcg	38.0
Vitamin D_3, ICU	22.5
α-tocopheryl acetate, IU	4.5
Folic acid, mcg	135
L-ascorbic acid, mg	50
$Ca(H_2PO_4)_2 \cdot H_2O$, gm	2.78
$CaCO_3$, gm	5.84
Glucose monohydrate, gm	1.12
Corn oil, gm	2.25
Ethoxyquin (antioxidant), mg	13.5

*Contained 4.9 μg of vitamin A/gm (dry basis) after lyophilization.

**Contained 3.4 μg of vitamin A/gm (wet basis).

The dry ingredients were combined to form a premix for the basal diet. The premix for Lot 2 was equal to that of Lot 1 minus the KIO_3 which was later added to the hog heart as an aqueous solution (2 μg KIO_3/ml distilled water).

Hearts from 100-kg hogs were trimmed of fat, large blood vessels and coarse connective tissue. After at least 1 coarse and 2 fine mechanical

grindings, the hog hearts were placed in aluminum pans for hand mixing with premix (9.83 gm premix/500 gm fresh ground hog heart). The ethoxyquin was mixed with the corn oil and hand mixed with the heart.

When Lot 1 diets were prepared, a vitamin A premix was made by adding 140 mg of gelatin coated retinyl palmitate (500,000 USP units/gm) to 500 gm of glucose monohydrate. The following amounts of vitamin A premix and balancing glucose were added per 500 gm of hog heart:

Diet, group	I	II	III
Premix, gm	0	0.5	1
Glucose, gm	1	0.5	0

When Lot 2 diets were prepared all-*trans* retinyl acetate in cottonseed oil base (100 IU/mg) was added to the corn oil before adding the ethoxyquin. Zinc was added to subgroup B diets in an aqueous solution which was prepared by adding distilled water, qs to 1000 gm, to 4.40 gm of $ZnSO_4 \cdot 7H_2O$. The amount of zinc solution added to the hog heart (hh) was determined by the following formula: (hh,gm) x (0.20) x (100)/1000 = (solution, gm).

All diet ingredients were thoroughly hand mixed and then mechanically reground at least 8 times. The diets were frozen in 5- to 10-mm thick layers and lyophilized. The diets were stored at -70 C. Only 1- to 2-week quantities were kept at refrigeration temperatures for daily use.

Housing

Each group of turtles was housed in a separate 10-gallon aquarium* that was half filled with distilled water. Each tank was provided with

*Metaframe stainless steel aquarium, Metaframe Corporation, Maywood, N.J. 07607.

a 1 x 10 x 15 cm wood float, an 8-inch 25-watt lamp with reflector and a plastic water filter box containing nylon fiber and activated charcoal. The lights and air-driven water filters operated 24 hours a day. Room temperature was between 21 and 30 C.

Sanitation

Lot 1

Twice a week the aquariums and filter boxes were washed with detergent* and thoroughly rinsed under pressure with tap water. Immediately after the second semi-weekly wash the aquariums and filter boxes were soaked for 10 minutes in iodine solution** and thoroughly rinsed with tap water. Filter fiber was rinsed semi-weekly and changed bi-weekly. The wood floats were autoclaved weekly. The turtles were rinsed in iodine solution** before return to cleaned aquariums.

Lot 2

The aquariums and filter boxes were washed weekly with detergent* and thoroughly rinsed with tap water. Semi-weekly the aquariums were thoroughly rinsed with tap water. At weekly intervals the wood floats were autoclaved and the filter fiber changed. The turtles were rinsed weekly in iodine solution** before being returned to the cleaned aquariums.

---

*Pyroneg, Diversey Chemical Company, Chicago, Ill. 60606.

**Wescodyne, West Chemical Products, Inc., 42-16 West Street, Long Island, N.Y.

Parameters Monitored

Food Consumption

Each turtle group was fed in a separate 2-quart plastic box* containing enough distilled water to just cover the turtles. They were fed twice a day for 30 minutes at each feeding.

Twice a week afternoon food consumption determinations were run for each group. Approximately 0.5 gm of food was offered to each group. The uneaten food (orts) was filtered back using a 12-cm, #4 "Whatman" filter paper, a Buechner funnel and vacuum. The filters with orts were dried overnight at 54 C and reweighed. Food consumed = food offered (filter with orts - filter). Simultaneous wet and dry control determinations revealed a 30% weight loss of food offered resulting from leaching, drying and handling.

Body Weight

Each turtle was weighed twice a week in midafternoon in conjunction with the late afternoon food consumption determinations.

Body Length

Bi-weekly, in conjunction with body weight determinations, the plastron and carapace of each turtle was measured along the midline using a vernier caliper.

Clinical Signs

Turtles were observed at least twice daily and notes made describing behavior, clinical signs and gross lesions.

---

*Freezette Food Container (Style 159), Republic Molding Corp., Chicago, Ill. 40648.

Necropsies

Turtles in Groups 0 and VII were killed on Day 0 for their respective experimental lot and served as controls for normal histology and gross anatomy.

Turtles were killed by decapitation when body weight decreased and/or anorexia was evident. Fresh organ weights were recorded for heart, liver, kidneys (together) plastron and carapace. An attempt was made to remove the adrenal gland and gonad from each kidney before weighing. The following tissues were placed in neutral buffered 10% formalin until histologic examination was made: thyroid, heart, trachea, lungs, spleen, neck retractor muscles, kidneys, urinary bladders, esophagus, stomach, intestines, pancreas, piece of liver, right and/or left femur, fat bodies and the entire head with the dorsal meninges exposed. After fixation with formalin the heads were placed in decalcifying solution* for approximately 30 minutes and then returned to formalin after a tap water rinse. The plastron, carapace and remaining liver were frozen. Tissues were sectioned at 6 μ and stained with hematoxylin and eosin.

Liver Vitamin A Analysis

The method used for quantitating vitamin A levels in the liver utilized the Carr-Price reaction in which vitamin A in chloroform with antimony trichloride to form a transiently stable blue solution with absorption maximum at 620 mμ (Carr and Price, 1926). A homogenate of 1 part liver to 2 parts deionized distilled water was refluxed in 45 ml of alcoholic potassium hydroxide** for 20 minutes and then diluted to

---

*Cal-Ex, Fisher Scientific Company, Fair Lawn, N.J.

**9 ml of 11 N KOH plus 91 ml of absolute ethyl alcohol.

100 ml with deionized distilled water. Duplicate 30-ml aliquots were taken and extracted with petroleum ether.* Three- to five-milliliter aliquots of extract were read for OD at 440 mμ in a Coleman Jr. II spectrophotometer to check for carotene and then evaporated under 25 psi (1.75 kg/cm^2) vacuum at 55 C. A few samples with readings at 440 mμ were checked at various mμ settings before evaporation. The residue was suspended in 1 ml of chloroform after which 2 ml of antimony trichloride solution** were added. An immediate reading of the transiently stable OD at 620 mμ was made to determine vitamin A content (plus carotene if present). Micrograms of vitamin A in the evaporated extract was determined from a standard curve. Micrograms of vitamin A per gram of liver was mathematically determined.

---

*Skellysolve F, Skelly Oil Company, 605 West 47th Street, Kansas City, Mo.

**200 mg of $SbCl_3$ qs to 1 liter with chloroform.

RESULTS

Body Weight, Plastron and Carapace Lengths

These parameters for Lot 1 and Lot 2 turtles on Day 0 of the respective experiments are summarized in Table 4. The Day 0 body weight-carapace length relationship for Lot 1 turtles is $W = 0.567\ L^{2.13}$; for Lot 2 turtles, $W = 0.202\ L^{3.01}$. "W" is body weight in grams and "L" is carapace length in centimeters.

Table 5 summarizes changes in the above parameters in Lot 1 turtles between Day 0 and Day 76 of the experiment. There was a statistically significant difference ($P<0.05$) for each of the above parameters between Groups I and III but not between Groups I and II.

Table 6 summarizes changes in the above parameters in Lot 2 turtles between Day 0 and Day 191 of the experiment. There was no statistically significant difference ($P>0.05$) for each of the above parameters between any of the groups. Since there was no statistically significant difference ($P>0.05$) between the no-zinc and zinc (A and B) subgroups, the A and B data were combined in each group.

Organ and Body Weights at Necropsy

A correction for the age, in days, for each turtle was made in the statistical analysis of these parameters. Tables 7 and 8 summarize these data for Lot 1 and Lot 2, respectively.

Statistically there were high simple correlations between heart, liver and combined kidney weights and between body weight and weights of these organs.

Table 4. Day 0 body measurements of hatchling *Pseudemys scripta elegans*

Lot	Group	Number of Turtles	Body weight, gm		Plastron length, mm		Carapace length, mm	
			$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range
1	0	10	7.78	5.63 - 9.56	32.23	30.10 - 34.65	34.08	32.08 - 36.04
1	I	10	7.80	5.78 - 9.69	32.10	29.50 - 34.25	34.12	30.29 - 36.83
1	II	10	7.73	5.22 - 9.83	32.08	28.12 - 34.25	33.96	29.50 - 35.84
1	III	10	7.77	5.89 - 9.30	32.25	30.49 - 35.24	33.98	32.08 - 36.43
2	IV	10	8.56	6.91 - 9.96	32.33	30.10 - 35.24	34.97	32.87 - 36.83
2	V	10	8.72	7.73 - 10.37	32.55	30.89 - 34.45	34.97	29.46 - 36.83
2	VI	10	8.66	7.66 - 9.95	32.23	31.48 - 34.25	34.37	29.46 - 37.62
2	VII	10	8.64	7.52 - 9.89	32.00	29.50 - 34.25	34.71	32.08 - 37.42
1	all	40	7.77	5.22 - 9.83	32.16	28.12 - 35.24	34.01	30.29 - 36.83
2	all	40	8.64	6.91 - 10.37	32.29	29.50 - 35.24	34.90	32.08 - 37.62

Table 5. Shell length and body weight changes in Lot 1 turtles from Day 0 to Day 76

Group	I	II	III	
Number of Turtles	6	9	7	
Vitamin A/kg wet diet	0	140	280	± SE
Body weight (Day 0), gm	8.01	8.01	8.39	0.38
Body weight (Day 76) gm	7.86	8.82	12.01*	1.06
Body weight gain, mg/day	- 2.0	10.7	47.6*	11.2
Plastron length (Day 0), mm	32.27	32.30	32.92	0.52
Plastron length (Day 76), mm	33.03	33.40	35.95**	0.92
Plastron length gain, μ/day	10.0	14.5	39.9*	7.4
Carapace length (Day 0), mm	34.25	34.34	34.76	0.46
Carapace length (Day 76), mm	34.98	35.64	38.58**	1.14
Carapace length gain, μ/day	9.6	17.1	50.3*	10.8

*Significantly greater than the least 2 values ($P<.05$).

**Significantly greater than the least value ($P<.05$).

Table 7. Shell length, organ and body weights of Lot 1 turtles at necropsy

Group	I	II	III	
Number of turtles	10	10	10	
Vitamin A, IU/kg wet diet	0	140	280	± SE
Body weight, gm	7.57	6.93*	9.64	0.72
Plastron weight, mg	608	500	802**	62
Carapace weight, mg	1220	1029	1704**	160
Liver weight, mg	436	363	638**	67
Heart weight, mg	33	28	44**	0.4
Kidney weight, mg	48	44	72**	0.7
Plastron length, mm	33.10	31.90*	34.86	0.81
Carapace length, mm	35.01	33.73	37.48	1.00

*† Significantly less than the greatest value ($P<.05$).

**† Significantly greater than the least 2 values ($P<.05$).

† Correction for differences in the age of turtles was made.

Table 8. Shell length, organ and body weights of Lot 2 turtles at necropsy

Group*	IV	V	VI	
Number of turtles	10	10	10	
Vitamin A IU/kg wet diet	0	900	1800	± SE
Body weight, gm	10.80	11.61	13.44	0.78
Plastron weight, mg	828	929	1023	67
Carapace weight, mg	1733	1996	2356	194
Liver weight, mg	841	825	1071	74
Heart weight, mg	36	44	51	4
Kidney weight, mg	145	137	163	10
Plastron length, mm	33.48	35.11	35.11	0.75
Carapace length, mm	36.26	37.57	38.76	0.91

*No significant differences between the groups (P>.05); correction for differences in the age of turtles was made.

Table 9 is a summary of organ weights as a percent of body weight at necropsy. These data were not statistically analyzed.

Only Lot 2, Group VII, turtles had large yolk sacs in the abdominal cavity at the time of necropsy. The dimensions of these yolk sacs were:

Diameter $\bar{x}$ = 5.60 mm; range = 3.76-7.13 mm

Length $\bar{x}$ = 11.27 mm; range = 6.73-18.61 mm

Food Consumption

Tables 10 and 11 summarize afternoon food consumption of Lot 1 and Lot 2, respectively, expressed as: 1) mg of food consumed/gm of turtle weight and 2) mg of food consumed/turtle. Values for food consumed were reduced by 30% before calculating the above data to correct for loss of food offered due to leaching, drying and handling.

Tissue Vitamin A Levels at Necropsy

Livers from all Lot 1 and Lot 2 turtles were analyzed for vitamin A and carotene. In addition livers from 6 wild-caught red-eared slider turtles (4 juvenile females and 2 mature males) were analyzed for vitamin A and carotene; the kidneys from 3 were also analyzed. Table 12 summarizes liver weights and liver vitamin A levels in the experimental and wild turtles. All livers and kidneys examined were negative for carotene. No correction for age differences of the turtles was made. Table 13 summarizes the liver and kidney vitamin A data from the 6 wild turtles. Table 14 (A) summarizes liver vitamin A levels in Lot 1 turtles after statistical correction for age differences of the turtles. Table 14 (B) summarizes liver vitamin A levels in Lot 2 turtles without correction for age differences.

A few of the green leg-pit fat bodies from 1 turtle (Lot 2, Group V) were analyzed for carotene and vitamin A. The fat was negative for

Table 9. Organ weight as percent of body weight at necropsy, Lot 1 and Lot 2 turtles

Lot	Group	No. of Turtles	Liver		Heart		Kidneys		Plastron		Carapace		Plastron + Carapace	
			$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range
1	all	39	5.1	1.8-9.4	.40	.23-.62	.62	.29-1.22	7.8	5.7- 9.8	16.0	11.6-20.8	23.8	17.3-30.0
1	0	9	3.8	1.8-6.4	.31	.23-.42	.39	.29-0.51	8.1	5.7- 9.8	16.3	11.6-20.5	24.4	17.3-30.0
1	I,II, III	30	5.6	2.2-9.4	.43	.27-.62	.69	.34-1.22	7.6	5.9- 9.3	15.9	12.4-20.8	23.6	18.8-29.0
2	all	40	5.6	3.1-8.4	.41	.26-.63	.48	.20-1.28	7.8	5.6-10.7	18.0	11.2-22.3	25.9	16.9-32.9
2	VII	10	3.9	3.1-4.6	.30	.26-.38	.31	.20-0.41	7.4	6.8- 8.2	17.4	16.2-18.6	24.8	23.4-26.7
2	IV,V, VI	30	6.1	4.2-8.4	.45	.28-.63	.54	.31-1.28	8.0	5.6-10.7	18.2	11.2-22.3	26.2	16.9-32.9

Table 10. Food consumption at the p.m. feeding, Lot 1 turtles

Month	Group I $\bar{x}$	Group I range	Group II $\bar{x}$	Group II range	Group III $\bar{x}$	Group III range
			A. mg of food/gm of turtle weight			
June	3.2	2.1-4.1	2.8	1.6-3.6	3.1	1.1-4.6
July	2.2	0.3-3.6	1.3	0.0-4.4	1.9	0.1-4.8
August	2.4	1.6-3.5	1.2	0.5-1.9	1.0	0.0-1.9
September	1.6	0.4-3.4	1.4	0.0-4.0	1.0	0.6-1.9
October	-	-	3.2	1.7-4.5	-	-
November	-	-	3.9	2.6-6.5	-	-
			B. mg of food/turtle			
June	24.5	16.0-31.0	21.9	12.2-27.9	24.5	8.6-37.4
July	17.5	2.2-28.7	11.0	0.0-35.5	18.9	1.3-43.7
August	19.0	12.8-28.3	10.9	4.5-17.7	12.0	0.0-24.5
September	12.4	2.8-25.9	12.6	0.0-32.2	14.7	8.6-27.6
October	-	-	26.1	14.2-37.4	-	-
November	-	-	31.0	20.3-57.3	-	-

Table 11. Food consumption at the p.m. feeding, Lot 2 turtles

Month	Group IV $\bar{x}$	Group IV range	Group V $\bar{x}$	Group V range	Group VI $\bar{x}$	Group VI range
			A. mg of food/gm of turtle weight			
September	0.9	0.0-2.1	1.2	0.0-2.5	1.0	0.1-2.0
October	1.2	0.2-2.5	0.8	0.3-2.1	1.0	0.0-2.2
November	0.9	0.3-2.1	1.0	0.0-2.3	0.8	0.1-1.5
December	0.6	0.0-1.4	0.6	0.0-1.8	0.6	0.3-1.0
January	0.3	0.0-0.7	0.5	0.0-1.3	0.5	0.0-1.2
February	0.6	0.1-2.0	0.2	0.0-1.0	0.4	0.0-1.4
March	0.4	0.0-1.2	0.2	0.0-0.3	0.2	0.0-0.8
April	0.1	0.0-0.6	1.6	0.0-4.6	0.4	0.0-1.7
			B. mg of food per turtle			
September	8.1	0.0-17.8	10.4	0.0-21.5	8.6	0.9-17.4
October	10.6	1.9-22.4	5.4	1.9-12.2	8.8	0.0-19.0
November	8.3	2.8-19.0	9.6	0.0-22.8	7.8	0.5-13.9
December	5.1	0.1-11.4	5.6	0.0-18.1	5.6	2.9-9.9
January	2.7	0.0- 5.9	4.8	0.0-13.7	5.0	0.0-12.7
February	5.1	0.8-16.9	2.4	0.0-10.4	4.1	0.0-14.0
March	3.9	0.0-10.8	1.9	0.0- 8.6	2.2	0.0- 8.8
April	1.0	0.0- 3.3	13.5	0.0-38.5	4.8	0.0-19.6

Table 12. Liver weights and liver vitamin A levels at necropsy in Lot 1, Lot 2 and 6 wild red-eared slider turtles*

Group	Number of Turtles	Liver weight, gm $\bar{x}$	Liver weight, gm range	µg vitamin A/gm liver $\bar{x}$	µg vitamin A/gm liver range
0	10	0.301	0.122-0.579	10.45	0.36-61.45
I	10	0.395	0.120-0.688	2.91†	0.44-10.10
II	10	0.466	0.144-0.924	1.76†	0.42- 2.98
III	10	0.575	0.126-1.229	9.10†φ	0.43-34.00
IV	10	0.552	0.322-1.074	1.92††	0.00- 3.00
V	10	0.540	0.293-1.047	3.40††	1.10- 4.35
VI	10	0.752	0.425-1.310	2.48††	0.54- 4.78
VII	10	0.335	0.227-0.389	8.74	4.01-13.54
wild**	6	3.278	2.059-4.582	19.55	1.70-39.94

*Uncorrected for age differences of turtles.

**4 juvenile females and 2 mature males.

†No significant differences ($P>.05$).

††No significant differences ($P>.05$).

φ3 turtles died early.

Table 13. Liver and kidney vitamin A levels in 4 juvenile female and 2 mature male red-eared slider turtles*

Sex	Liver		Kidneys	
	weight, gm	µg of vitamin A/gm	weight, gm	µg of vitamin A/gm
F	1.62	14.30	0.14	9.32
F	1.37	1.70	0.30	1.10
F	0.46	39.94	-	-
F	0.87	8.19	-	-
M	1.31	14.50	0.32	5.28
M	0.57	38.67	-	-
$\bar{x}$	1.03	19.55	0.25	5.23

*Negative for carotene.

Table 1

Vitami

IU/kg

-g/gm

IU/gm

Vita

IU/k

-g/

IU/

di
th

Table 14. Liver vitamin A levels at necropsy*

A. Lot 1††

Vitamin A	Group 0	Group I**	Group II**	Group III**	
IU/kg of wet diet	-	0	140	280	± SE†
µg/gm of fresh liver	7.83	3.45	3.47	8.65	3.46
IU/gm of fresh liver	26.1	11.5	11.6	28.8	11.5

B. Lot 2††

Vitamin A	Group IV**	Group V**	Group VI**	Group VII	
IU/kg of wet diet	0	900	1800	-	± SE†
µg/gm of fresh liver	1.92	3.40	2.48	8.74	0.44
IU/gm of fresh liver	6.4	11.3	8.3	29.1	1.47

*No carotene present.

**No significant difference ($P>.05$); Lot 1 - corrected for age differences of the turtles; Lot 2 - uncorrected for age differences of the turtles.

†Lot 1 - excludes Group 0; Lot 2 - excludes Group VII.

††Ten turtles per group.

carotene but had 8.33 µg of vitamin A/gm. The liver from this turtle had 0.75 µg of vitamin A/gm. The weight of fat analyzed was 0.11 gm; the total liver weight was 1.074 gm.

At the time of necropsy, yolk sacs from Lot 2, Group VII, turtles were mistakenly placed in formalin (10%, neutral, buffered). They were stored in the formalin for 11 months at room temperature which varied from 21 to 40 C. Two of these yolk sacs were negative for carotene, although they were yellow in color, but had 6.02 µg and 2.54 µg of vitamin A/gm, respectively.

Clinical Signs and Gross Lesions

Groups I through VI had similar clinical signs and gross lesions characterized by severe palpebral distention with fluid, dyspnea, and anorexia, with weight loss and lethargy.

Palpebral distention was the most constant lesion, usually bilateral and usually the first sign of disease. A few turtles appeared to have exophthalmos (Figure 1). Many eyelids became baggy when the distention subsided and often exposed dry, granular conjunctivae, corneas and nictitating membranes (Figure 2). The xerophthalmia was seen as early as 2 days and as late as 3 weeks after onset of palpebral distention. However, 1 Group II turtle with swollen palpebra, which continued to eat, had glistening corneas and normal conjunctiva when killed after 40 days.

Weight loss, from decreasing appetite, followed onset of palpebral distention by a few days to 1 week. Anorexia was confirmed by the seventh to twenty-first day when turtles had drastic weight loss and/or defecated only mucus with or without bile. Affected turtles that continued to eat grabbed at the floating food after bumping into it with

Figure 1. Turtle with exophthalmos caused by periocular lymphatic distention and distended by keratinized debris. x 4.

Figure 2. Turtle with dry conjunctiva and protruding nictitating membrane. x 4.

their heads.
noses near t
ments often
the food. A
gas-filled
lower intes

Dyspne
ing, snicki
pumping. D
of yellow
turtles ha
Mouth brea
the surfac
the jaw (i
along the

Anore
day and ni
caused th
Many dysp
tended to

Seve
one side
to the su

Post
15 (A),
tissues

their heads. Some nudged the food with their nose and/or held their noses near the food before grabbing it for ingestion. Grabbing movements often fell short of the food or were aimed off to the side of the food. At necropsy anorectic turtles frequently had empty and/or gas-filled intestines. Some had only a few pellets of bile in the lower intestine.

Dyspnea was characterized by sneezing, gasping, whistling, squeaking, snicking and mouth breathing assisted by vigorous abdominal muscle pumping. Dyspnea was due to laryngeal obstruction with soft plaques of yellow material and/or to obstruction of the external nares. Many turtles had bulging snouts in the area of the external nasal glands. Mouth breathing turtles had small, firm, yellow plugs of material on the surface of the tongue (in lingual glands), at the dorsal angle of the jaw (in dorsal buccal glands) and on the floor of the oral cavity along the tongue (in sublingual glands).

Anorectic turtles became lethargic, tended to remain on the float day and night and did not respond rapidly, if at all, to stimuli that caused the other turtles to flee the float for safety of the water. Many dyspneic turtles spent normal amounts of time underwater but they tended to surface frequently and noisily.

Several turtles could not regulate their buoyancy. A few listed to one side or head down. Others could not stay submerged without bobbing to the surface after paddling movements ceased.

Postmortem Examination

Postmortem findings in Groups I through VI were equivalent. Tables 15 (A), (B) and 16 summarize the incidence of lesions in the various tissues from Lot 1 and Lot 2 turtles. All further remarks on postmortem

Table 15. I

Group	
Epithelia	
Lacrimal gla	
Harderian gl	
Conjunctiva	
Cornea*	
Eustachian t	
Dorsal bucca	
Sublingual g	
Lingual glan	
Buccal mucos	
Larynx	
Trachea	
Bronchi	
External na	
Nasal reces	
Respiratory	
Olfactory n	
Esophagus	
Pancreatic	
Urinary bla	
Accessory u	

Group	
Epithelia	
Lacrimal gl	
Harderian g	
Conjunctiva	
Cornea*	
Eustachian	
Dorsal bucc	
Sublingual	
Lingual gla	
Buccal mucc	

Table 15. Incidence of squamous metaplasia

Group	A. Lot 1 0	I	II	III
Epithelia				
Lacrimal gland	0/10	7/9	5/10	6/10
Harderian gland	0/10	5/8	3/9	5/8
Conjunctiva	0/10	7/8	7/8	6/9
Cornea*	0/10	7/7	5/9	6/7
Eustachian tube	0/10	6/9	4/10	6/9
Dorsal buccal glands	0/9	7/8	2/6	4/5
Sublingual glands	0/9	6/6	4/7	5/6
Lingual glands	0/10	4/6	3/5	5/6
Buccal mucosa	0/10	8/9	6/9	5/8
Larynx	0/8	3/6	3/7	4/6
Trachea	0/7	0/9	0/5	0/7
Bronchi	0/8	0/10	0/7	0/9
External nasal gland	0/3	3/5	2/7	1/4
Nasal recess	0/10	2/6	4/8	2/6
Respiratory nasal mucosa	0/10	6/7	5/10	6/10
Olfactory nasal mucosa	0/10	0/6	1/6	1/10
Esophagus	0/4	0/8	0/4	0/5
Pancreatic ducts	0/5	0/7	0/5	0/8
Urinary bladder	0/3	2/7	0/6	2/5
Accessory urinary bladders	0/6	1/5	0/4	1/5

Group	B. Lot 2 IV	V	VI	VII
Epithelia				
Lacrimal gland	4/9	7/8	6/10	0/8
Harderian gland	3/5	4/6	-	0/4
Conjunctiva	10/10	9/10	10/10	0/10
Cornea*	3/7	7/8	7/9	0/10
Eustachian tube	8/9	9/10	9/10	0/10
Dorsal buccal glands	4/5	4/4	7/7	0/1
Sublingual glands	4/5	1/1	5/6	-
Lingual glands	6/8	6/7	7/8	0/4
Buccal mucosa	10/10	9/10	8/10	0/10

Table 15 (co

Group
Larynx
Trachea
Bronchi
External na
Nasal reces
Respiratory
Olfactory n
Esophagus
Pancreatic
Urinary bla
Accessory u

* Hy

** Fo

Table 15 (cont'd.)

B. Lot 2

Group	IV	V	VI	VII
Larynx	6/6	5/6	9/9	0/7
Trachea	0/8	0/5	0/9	0/8
Bronchi	0/8	1/10**	0/8	0/9
External nasal gland	0/2	4/4	2/4	0/1
Nasal recess	2/2	2/2	-	0/4
Respiratory nasal mucosa	8/8	8/9	7/8	0/9
Olfactory nasal mucosa	0/6	4/4	0/1	0/3
Esophagus	0/6	0/5	0/5	0/5
Pancreatic ducts	0/4	0/5	0/8	0/3
Urinary bladder	1/9	2/9	1/7	0/5
Accessory urinary bladders	2/4	1/2	0/6	0/5

*Hyperkeratosis.

**Focal, associated with focal pneumonia.

Table 16.

Lesion
Renal mine
Swollen pa
Urolithia

Table 16. Incidence of lesions other than squamous metaplasia in Lot 1 and Lot 2 turtles

Lesion	Group	Lot 1				Lot 2			
		0	I	II	III	IV	V	VI	VII
Renal mineralization		0/1	5/5	7/7	6/6	7/9	8/9	4/10	0/3
Swollen palpebra		0/10	8/10	7/10	7/10	7/10	7/10	8/10	0/10
Urolithiasis		0/10	3/10	3/10	3/10	0/10	4/10	0/10	0/10

examination are v

otherwise.

No lesions

trachea, pancrea

cranium, femur,

Kidneys

The kidney

disease had de

proximal tubul

proximal, dist

and collectin

glomeruli wer

Proximal

tion, but wit

The bodies w

and stained

clear halo a

Cells

pyknotic nu

fragmented

material si

clumps of

metaplasia

Some

ing squamo

mineralize

from an as

examination are valid for only Groups I through VI unless indicated otherwise.

No lesions were observed in any of the following tissues: esophagus, trachea, pancreatic ducts, optic nerve, retina, brain, peripheral nerves, cranium, femur, thyroid, neck retractor muscles and stomach.

Kidneys

The kidneys from turtles that had the previously described clinical disease had degenerative changes characterized by cloudy swelling of proximal tubule cells (Figure 3) and atrophy and mineralization of proximal, distal and collecting tubule cells (Figure 4). Some distal and collecting tubule epithelia had squamous metaplasia (Figure 4). The glomeruli were normal.

Proximal tubule cells in many kidneys with little or no mineralization, but with cloudy swelling, had distinct intracytoplasmic bodies. The bodies were small, round, homogeneous to concentrically laminated and stained magenta. These bodies were frequently surrounded by a clear halo and were solitary or multiple per cell (Figure 3).

Cells of tubules undergoing mineralization had deeply stained walls, pyknotic nuclei and lightly stained cytoplasm. Many similar cells were fragmented and collapsed. Others formed rings of amorphous, clumped material similar to that described by VanLeersum in rats (1928). Solid clumps of mineralized material (calculi) filled many tubules. Squamous metaplasia of tubule epithelium was seen in a few turtles in these areas.

Some renal pelvises and their respective ureters had extreme keratinizing squamous metaplasia which was always associated with presence of mineralized material in the lumen, with or without inflammatory cells from an ascending inflammation.

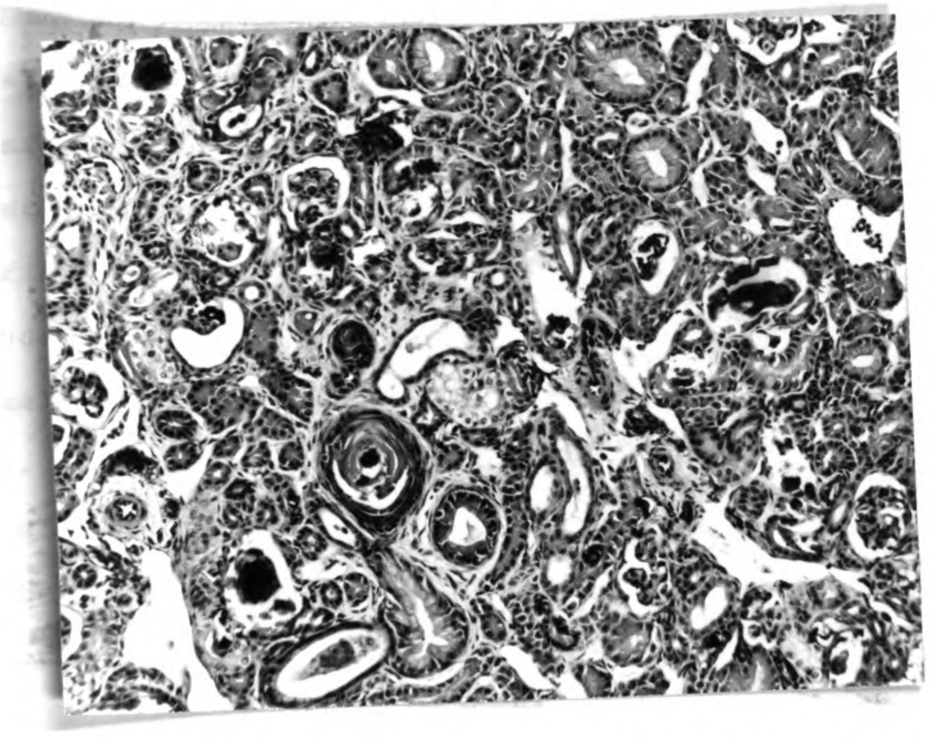

Figure 3. Cloudy swelling of the epithelium in the proximal convoluted tubule with unidentified single and multiple cytoplasmic inclusions. H & E stain; x 560.

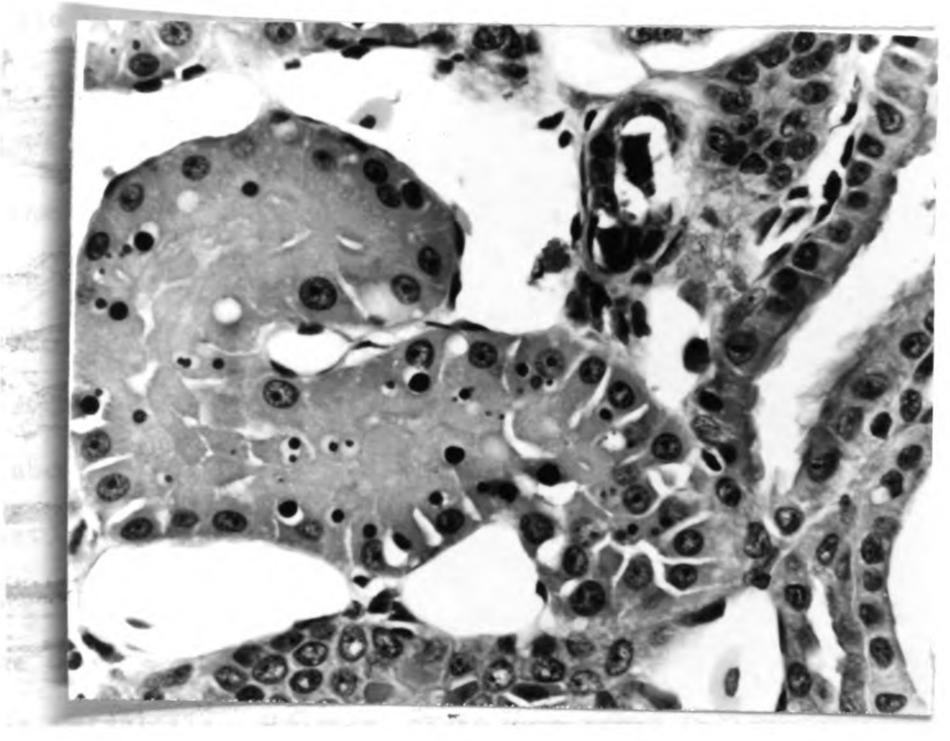

Figure 4. Calcification and squamous metaplasia of renal tubules in the absence of inflammation. H & E stain; x 140.

Many seve

mineralized tu

some affected

interstitial

specifically

had moderate

proximal tub

tissue. On

tration of

moderate mi

observation

Urinary Bl

Each

One calcul

a side an

smooth.

Three mor

Three oth

are show

Mic

accessor

6) and a

metaplas

pseudome

Th

squamou

and occ

Many severely affected kidneys, those with large numbers of mineralized tubules, had no evidence of inflammation (Figure 4). In some affected kidneys there were a few eosinophilic granulocytes in interstitial and subcapsular areas, but they did not appear to be specifically associated with affected tubules. Other affected kidneys had moderate numbers of eosinophilic granulocytes in the base of proximal tubule cells and/or within the interstitial and subcapsular tissue. One clinically normal turtle (in Group VI A) had heavy infiltration of eosinophilic granulocytes throughout the kidney as well as moderate mineralization of proximal tubules. Kidney lesions preceded observation of squamous metaplasia in the lower urinary tract.

Urinary Bladder and Accessory Urinary Bladders

Each of 7 turtles had a large calculus in the urinary bladder. One calculus was yellow and triangular in shape and measured 5 mm on a side and was 2 mm thick. Its surface was finely granular, almost smooth. The other 6 calculi were yellow, rough and 2 to 4 mm in diameter. Three more turtles had small, green-yellow grit in the urinary bladder. Three others had similar grit in the ureters. Representative calculi are shown in Figure 5.

Microscopically about one-fourth of the urinary bladders and/or accessory urinary bladders had keratinizing squamous metaplasia (Figure 6) and about 50% of these were accompanied by calculi. Grossly the metaplastic epithelia appeared as thick, soft, yellow mucosal pseudomembranes.

The bladder walls were never inflamed regardless of the degree of squamous metaplasia. However, there were mixed inflammatory cell types and occasionally bacteria among the keratinized and mineralized debris

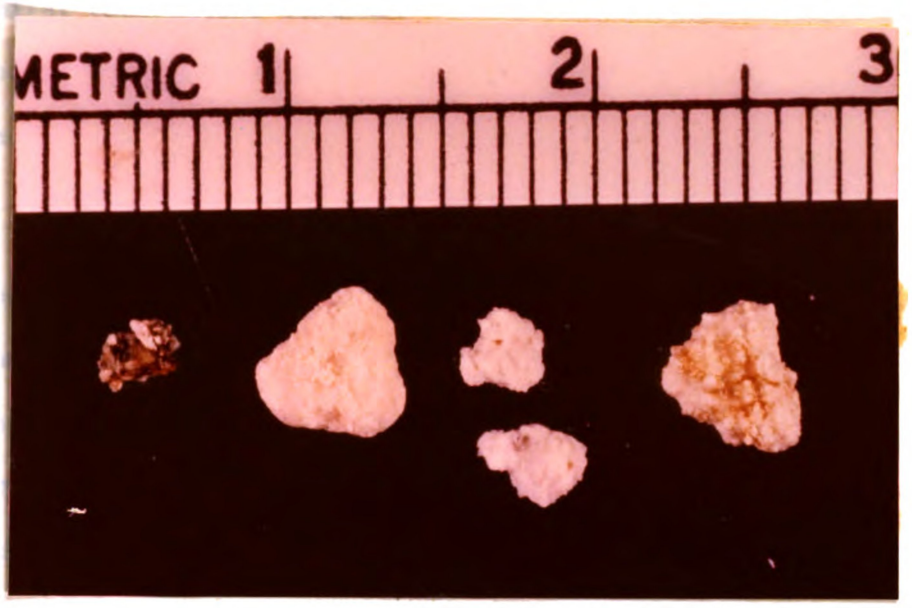

Figure 5. Calculi from the urinary bladder.

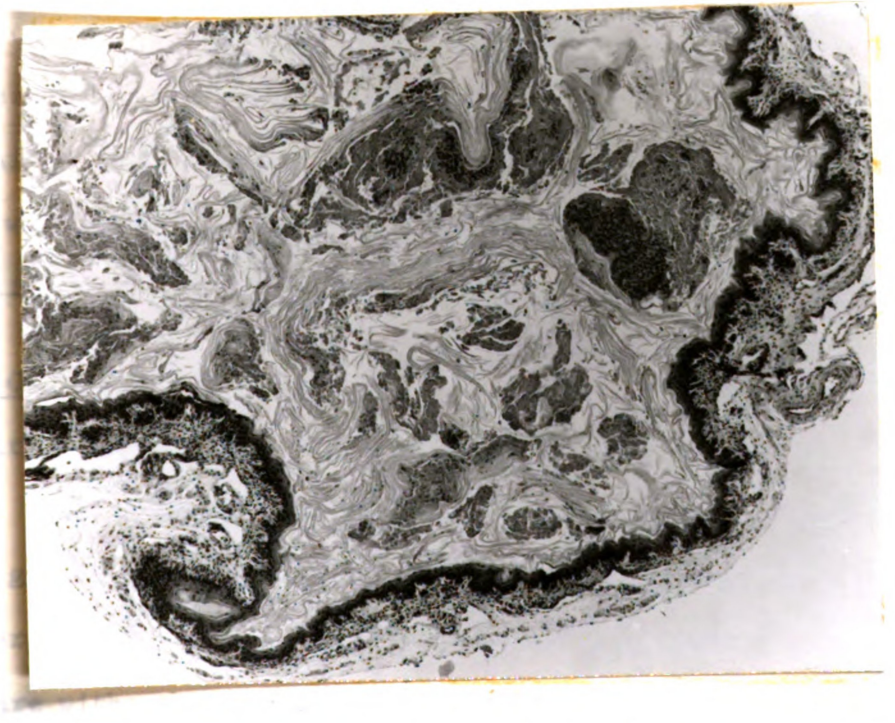

Figure 6. Squamous metaplasia of the urinary bladder mucosa. The lumen is filled with keratinized debris and masses of desquamated, degenerating epithelial cells. H & E stain; x 55.

found in t

had simila

Focal

was freque

Larynx

More

of squamo

plaques.

the laryn

along th

and mixe

Trachea

Th

normal

focal c

pneumon

tended

epithe

Bucca

gener

bucca

of s

stru

resu

found in the lumens of the bladders (Figure 6). Ureters and cloacas had similar concomitant lesions.

Focal to diffuse squamous metaplasia of cloacal and penile mucosas was frequent, with or without the presence of bladder lesions.

Larynx

More than 50% of the laryngeal mucosas examined had varying degrees of squamous metaplasia (Figure 7) which grossly appeared as yellow plaques. The lesions never extended beyond the anterior portion of the laryngeal tube and were most severe on the pharyngeal surface and along the fissure of the epiglottis. Occasionally fibrinous exudate and mixed types of inflammatory cells covered the affected mucosa.

Trachea and Bronchi

The mucosa of these 2 structures had adequate mucus secretion and normal columnar to cuboidal cells with abundant cilia. There was no focal or diffuse squamous metaplasia except in 1 turtle which had focal pneumonia. Several bronchi and bronchioles in this turtle were distended with inflammatory cells and lined by a thin stratified squamous epithelium; other portions of the lung were normal.

Buccal Cavity

With few exceptions the turtles had squamous metaplasia of the general buccal mucosa, lingual glands, sublingual glands and dorsal buccal glands. The ducts of the glands were affected first. Many glands of severely affected turtles had squamous metaplasia of the entire structure without inflammation. Atrophy of the acini soon followed the resulting accumulation of secretion, cellular debris and/or inflammatory

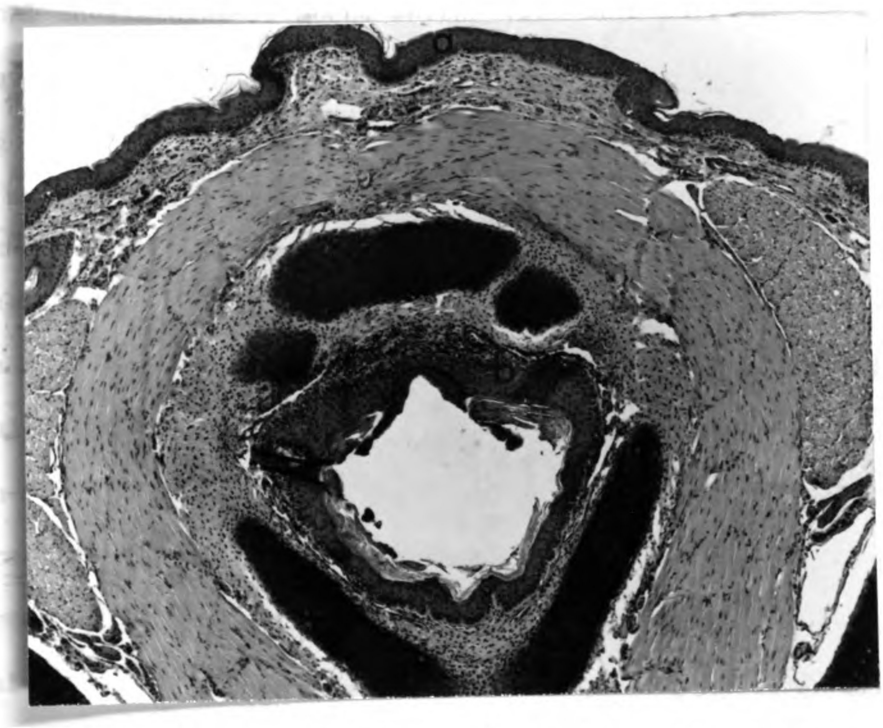

Figure 7. Cross section of the larynx in which squamous metaplasia of the pharyngeal (a) and laryngeal (b) membranes is evident. H & E stain; x 55.

cells. Unless the glands were ruptured, no inflammation of the gland wall of its surrounding tissue was seen.

Tympanic Cavity

Over two-thirds of the turtles had keratinizing squamous metaplasia or hyperkeratosis of the tympanic cavity mucosa. Mildly affected turtles had focal undermining of mucus epithelial cells by squamous cells. More severely affected turtles had thick layers of keratinized material filling the tympanic cavity (Figure 8).

Nasal Cavity

The nasal vestibulum was hyperkeratotic in dyspneic turtles. Most turtles had varied degrees of keratinizing squamous metaplasia of the external nasal glands, ventral and lateral nasal cavity (respiratory and non-olfactory sensory epithelia) and/or internal choanal mucosa. The dorsal epithelium of the nasal cavity (olfactory sensory epithelium) and Bowman's glands were unaffected. Turtles with bulging snouts had external nasal glands that were distended with layers of keratinized material. Less severely affected glands had squamous metaplasia only in the ducts.

In the nasal cavity proper the first structures to have squamous metaplasia were ridges and protruding structures. There was little if any inflammation of the epithelia. Some cavities and acini of the external nasal gland contained mixed types of inflammatory cells in debris and/or serofibrinous exudate.

Ocular Area

The swollen palpebra seen grossly were due to distended lymphatics without interstitial edema (Figure 9).

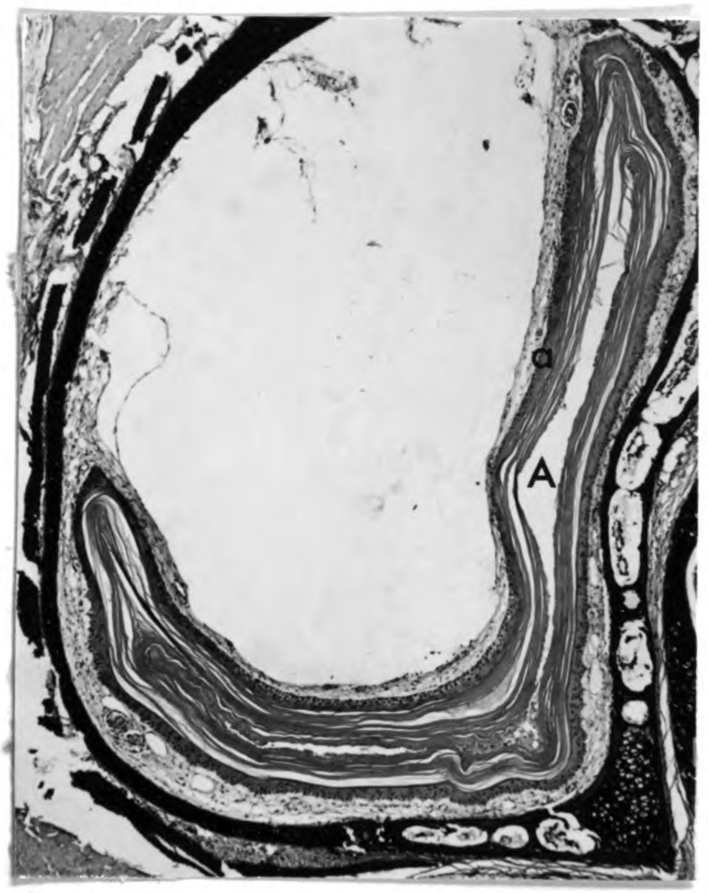

Figure 8. Cross section of the tympanic cavity (A) in which the lining (a) has undergone keratinizing squamous metaplasia and has been detached by lymphatic distention. H & E stain; x 55.

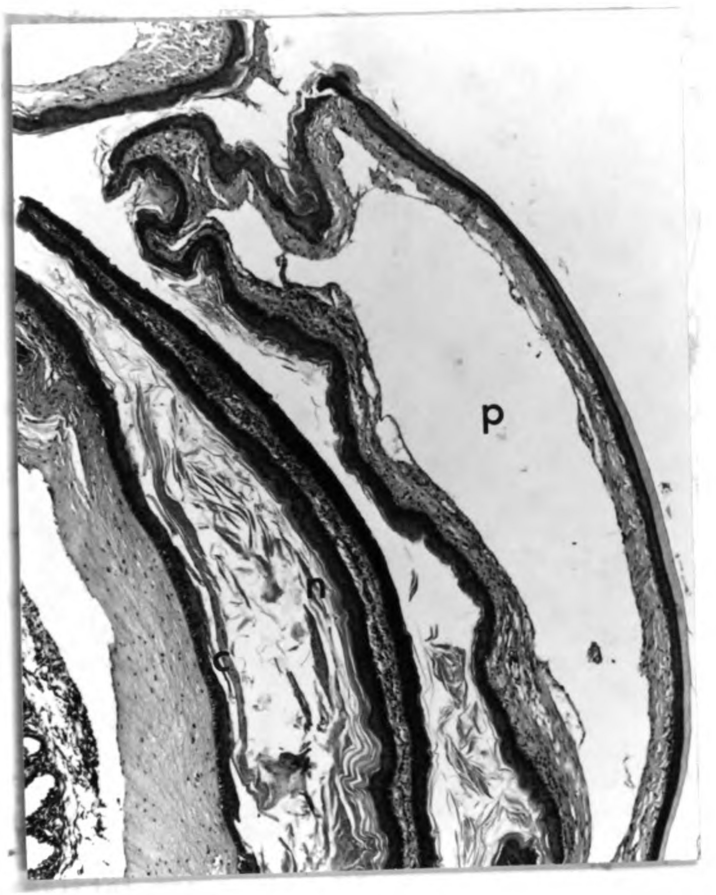

Figure 9. Cross section through the eye. Notice the lymphatic distention of the palpebrum (p), hyperkeratosis of the cornea (c) and keratinizing squamous metaplasia of the nictitating membrane (n) and palpebral conjunctiva. H & E stain; x 55.

With few exceptions all turtles had squamous metaplasia of the conjunctiva and nictitating membrane mucosa (Figure 9). Over two-thirds of the turtles had hyperkeratosis of the cornea (Figure 9) without evidence of primary inflammation or edema.

More than 50% of the turtles had some degree of squamous metaplasia of lacrimal and/or harderian glands. The lesions started in the ducts of the glands and the deep acinar epithelia remained normal, atrophied or underwent squamous metaplasia. Secretions, sometimes mixed with eosinophilic granulocytes, tended to accumulate in the acini. Severely affected glands had large cavities lined with stratified squamous epithelium and packed with layers of keratinized material. No inflammatory cells were seen within the gland walls or interstitially unless glands had ruptured, in which case a granulomatous inflammation with eosinophilic granulocytes was seen.

Lungs

Seven of 60 turtles (3 in Lot 1 and 4 in Lot 2) had pneumonia. One had interstitial pneumonia with infiltration of eosinophilic granulocytes. The others had bronchopneumonias with eosinophilic granulocytes and serofibrinous exudate. Bronchial epithelium was normal except in 1 turtle that had focal squamous metaplasia secondary to inflammation. Only 1 turtle had a buoyancy difficulty clinically. The others were lethargic and stayed out of the water.

Two turtles had a large, encapsulated granuloma with a caseous center, on the anterior pleura. No etiologic agent was seen.

Miscellaneous Observations

Three turtles in Lot 1 had trematode ova in 1 or more of these tissues: lung, nasal respiratory submucosa, pancreas, spleen, liver,

wall of th

walls. T

surroundi

Sev

accumula

other p

unknown

A

due to

varyin

appar

of eo

unkno

in t

leg

at

or

reg

wall of the urinary bladder, esophageal wall, intestinal and gastric walls. The only host reaction to the ova was 1 layer of macrophages surrounding each ovum.

Several pancreases had slight periductular and/or interstitial accumulations of eosinophilic granulocytes for unknown reasons. Several other pancreases had focal vacuolar degeneration of exocrine cells, for unknown reasons.

About 10% of the livers had diffuse fatty degeneration, probably due to fat mobilization secondary to anorexia. All livers contained varying amounts of melanin-like pigment in the Kupffer cells (this apparently is normal in turtles). A few livers had slight infiltration of eosinophilic granulocytes in sinusoidal and periductular areas, for unknown reasons.

Anorectic turtles had serous atrophy of fat bodies. Fat bodies in the anterior leg pits were affected before those in the posterior leg pits.

Femurs of all turtles were normal. The cartilaginous mass (cone) at the ends of the medullary cavity (Figure 10) tended to be smaller or totally reabsorbed in the proximal end in turtles that lived longest, regardless of their treatment group.

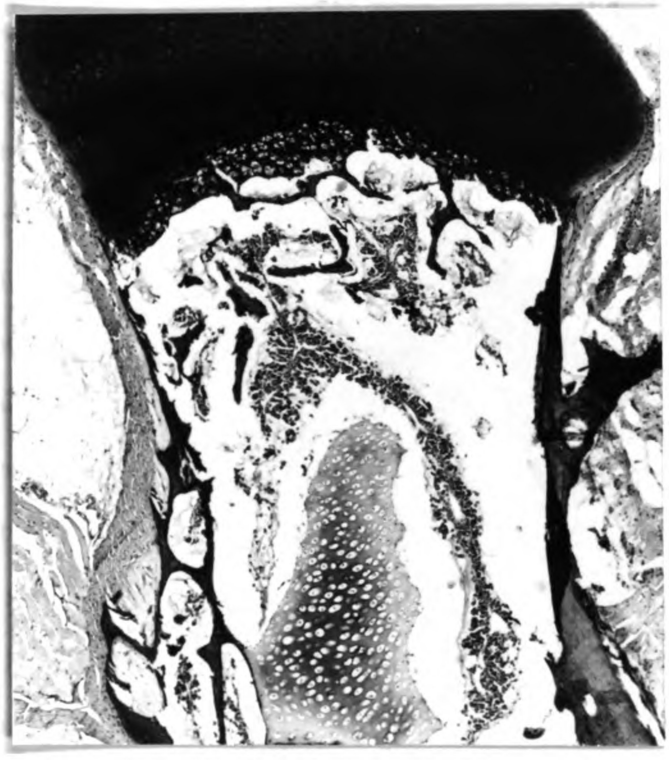

Figure 10. Partial resorption of the cartilaginous cone in the medullary cavity of the femur indicating normal bone development. H & E stain; x 55.

DISCUSSION

When the clinical signs and lesions in Lot 1 turtles appeared to be equivalent in all dietary treatment groups, a second experiment, with Lot 2 turtles, was conducted. The form of vitamin A for Lot 2 diets was oil-based all-*trans* retinol to insure a more diffuse and even distribution in the food mixture than was possible with the gelatin-coated retinyl palmitate used for Lot 1 diets. Half of the turtles in each Lot 2 treatment group were supplemented with zinc in the diet to rule out the possibility of parakeratosis of zinc deficiency due to high dietary calcium. The zinc supplement had no influence on the disease. Although direct comparison of Lot 1 and Lot 2 results is not valid because of seasonal differences and differences in ages of the turtles, the disease syndrome was equivalent in both lots of turtles. Lot 1 had earlier onset of the disease (by 2 months), perhaps due to overwintering utilization of body stores and/or to being on experiment during the season when rapid growth commences in nature.

The disease syndrome observed in these turtles is consistent with vitamin A deficiency in turtles as described by Elkan and Zwart (1967). The lesions are also consistent with those in vitamin A-deficient birds and mammals as summarized by Moore (1957), Sebrell and Harris (1967) and Follis (1958). The corneal hyperkeratosis and squamous metaplasia of epithelia in the urinary tract, the oral, nasal and tympanic cavities and the ocular structures are typical of vitamin A-deficient animals. The distention of palpebral and periorbital lymphatics might be a

manifestation of equalization of pressure in the cephalic area. Since the turtles have extensive bidirectional vascular anastomoses, any increase in cerebrospinal fluid pressure might be diverted to the less confined structures, the palpebra. This might also account for the absence of clinically apparent blindness, ataxia or other central nervous system disturbance associated with increased cerebrospinal fluid pressure.

Changes in the lacrimal and harderian glands did not contribute greatly to the palpebral swelling but did contribute to exophthalmos. Lymphatic and venous-sinus distention in the periorbital areas and accumulation of keratinized material in the conjunctival sac also contributed to exophthalmos.

The role of the thyroid appeared insignificant. In contrast to Elkan and Zwart (1967) there were no inflammatory, hyperplastic or degenerative lesions seen in any of the thyroids examined microscopically (6/30 in Lot 1 and 13/30 in Lot 2). No gross abnormalities of the thyroid were seen in any of the turtles.

Elkan and Zwart (1967) did not mention calculi in the urinary tract of their turtles or mineralization of kidney tubules. These lesions were frequent in the turtles in this study. Similar lesions were reported in birds (Elvehjem and Neu, 1932) and mammals (Beaver, 1961; VanLeersum, 1928) that were deficient in vitamin A.

In contrast to Elkan and Zwart (1967) the turtles in this study had very little squamous metaplasia within the kidney, glomeruli were normal and there was no evidence of chronic interstitial nephritis. Some turtles had a few eosinophilic granulocytes (EG) at the base of renal tubule cells, a lesion also reported by Elkan and Zwart (1967). In addition the turtles had kidneys with extensive degeneration of

proximal tubule cells characterized by cloudy swelling and the presence of homogeneous to laminated intracytoplasmic bodies. The lower tubules were either normal or had become severely mineralized. Few intermediate stages were seen. Squamous metaplasia was only observed in a few kidneys. These had extensive mineralization of tubules and intratubular calculi.

Elkan and Zwart (1967) did not mention findings in the nasal and oral cavities, trachea and bronchi or esophagus. The turtles in this study had extensive squamous metaplasia of nasal and oral epithelia but the other 3 tissues were normal. In contrast to Elkan and Zwart these turtles did not have squamous metaplasia of pancreatic and bile ducts nor extensive perivascular infiltration of the liver by EG. However, the livers had fatty degeneration, probably due to fat mobilization accompanied by anorexia. Compared to Elkan and Zwart, infiltration of EG in areas of squamous metaplasia was less frequent. The EG were either present for no apparent reason, because of focal infection or because keratinized material entered subepithelial tissues. The turtles frequently rubbed their swollen or dry eyes, which may have ruptured affected paraocular glands.

The lesions seen in the turtles of this study and the lesions reported by Elkan and Zwart (1967) appear similar but may not be entirely comparable. Their turtles came from many private sources with no standardization of age or diet.

With 4 exceptions, the vitamin A liver levels in the 86 *Pseudemys scripta elegans* turtles examined in this study were low like those reported by Elkan and Zwart (1967) for 2 turtles (2.7 and 5.7 μg of vitamin A/gm of liver). One Group 0, one Group III and 2 wild turtles had the following respective fresh liver vitamin A values: 61, 34,

39 and 40 µg/gm. The rest of the turtles had from 0 to 20 µg/gm with most having below 10. In contrast Gillam (1937) found the following liver vitamin A values in 3 other species of reptiles: 2500 and 650 µg/gm in 2 giant monitor lizards (zoo specimens), 860 µg/gm in a 25-foot python (zoo specimen) and 35 µg/gm in an alligator. He also found 10 µg/gm in a frog's liver.

The average liver vitamin A values in Groups 0 and VII were high compared to their respective dietary treatment groups. It appears that turtles in Groups I through VI depleted their liver vitamin A stores regardless of the level of dietary vitamin A supplementation. Perhaps they were unable to ingest sufficient quantities of the diet and/or to absorb the vitamin A. Any decrease in vitamin A absorption was not due to insufficient bile since bile secretion was abundant even in turtles which were anorectic. Renal lesions might have contributed to loss of vitamin A by leakage through injured tubules. Perhaps young turtles are similar to chicks, which Jungherr (1943) found incapable of vitamin A storage until they attained an age of about 2 months.

There is an alternate hypothesis to explain the observed lesions. Since the turtles were on a relatively high calcium diet there might have been a persistent hypercalciuria. Secondly, since the turtles were maintained in distilled water, ion depletion of the body could have occurred similar to that reported by Trobec and Stanley (1971). Ion depletion, especially of chloride, could produce renal tubule degeneration (Follis, 1958) and with hypercalciuric calcification of the tubules could occur thus initiating a vicious cycle of ion loss and tubule degeneration. A possible change from a normal isotonic or hypotonic urine to an abnormally hypertonic urine could have stimulated squamous metaplasia of the lower urinary tract epithelia. Calcified

tubule cells would slough and serve as nidi for calculus formation which in turn would be an irritatnt to epithelia. These changes would facilitate urine stasis and produce a favorable environment for bacteria. Ascent of bacteria to the kidney could account for the infiltration of eosinophilic granulocytes into the renal tubule cells and interstitium.

Since the cloaca and urinary bladders participate in ion conservation squamous metaplasia could result in further ion depletion and acid-base imbalance. With a need for conservation of chloride, gastric hydrochloric acid secretion might be curtailed initiating anorexia. Similarly, with need for salt conservation the lacrimal gland might curtail sodium chloride secretion. With concomitant osmotic action of distilled water on the eye and lack of hypertonic secretion from the lacrimal gland, the ocular structures would undergo squamous metaplasia and/or hyperkeratosis. Osmotic action of distilled water might also account for the squamous metaplasia of the nasal, oral and otic cavity membranes. Loss of pharyngeal ion conservation mechanisms might also contribute to ion depletion.

This second hypothesis is supported by the fact that renal lesions preceded lower urinary tract lesions and that those structures exposed to osmotic action of distilled water were most severely affected.

The second hypothesis to explain the disease syndrome of the turtles in this study appears most correct. If the disease was primary vitamin A deficiency changes would be expected in additional tissues such as the trachea, bronchi, pancreatic ducts and bones. No significant lesions were seen in any of these tissues. Lack of esophageal lesions might be explained on anatomic grounds. In contrast to mammals, the esophagus does not contain accessory tubuloacinar glands and the

epithelium resembles that of the trachea. It has ciliated, pseudostratified columnar epithelium that contains abundant periodic acid-Schiff positive cytoplasmic granules. Perhaps the physiological needs of vitamin A by the esophageal, tracheal and bronchial epithelium were minimal compared to other epithelia experiencing osmotic action of distilled water and thus would be the last to undergo squamous metaplasia. The lack of bone lesions might be due to the slow rate of growth in turtles and the seasonal and individual variation. For example, after 8 months 1 turtle in Group VI gained 3% of its original length whereas another turtle in the same group gained 20%. A Group III turtle gained 24% of its original length in 3 months. It appears that osmotic action of distilled water on some membranes resulted in lesions that mimicked and/or led to vitamin A deficiency.

SUMMARY

Forty red-eared slider turtles, *Pseudemys scripta elegans*, in each of 2 lots were used in an attempt to determine the vitamin A requirement for growth and maintenance of health of captive turtles. Thirty turtles in each lot were fed diets composed of ground hog heart supplemented with corn oil, glucose, minerals and vitamins. The remaining 10 turtles in each lot were killed on Day 0 of their respective experiment to serve as controls for normal histology, gross anatomy and initial liver vitamin A stores.

Three equal groups of turtles in each lot were kept in separate tanks of distilled water and provided with a wood float, a reflectorized lamp and an air-driven water filter containing activated charcoal and nylon fiber. The turtles were fed twice a day for 30 minutes at each feeding. Lot 1 turtles were fed similar diets containing either no vitamin A supplement or 140 or 280 IU of vitamin A/kg of fresh hog heart. Lot 2 turtles were fed similar diets containing either no vitamin A supplement or 900 or 1800 IU of vitamin A/kg of fresh hog heart.

All turtles fed the diets had similar signs and lesions characterized by swollen eyelids, dyspnea, anorexia, lethargy, weight loss and keratinizing squamous metaplasia of nasal, oral, tympanic, ocular and urinary tract membranes. Urinary calculi occurred frequently. Renal lesions were primarily degeneration of proximal tubule cells and mineralization of distal tubules and collecting ducts. In contrast the trachea, bronchi, esophagus, pancreatic ducts, bones and nervous tissue were normal.

Liver vitamin A values at necropsy in Lot 1 and Lot 2 standard groups were 10.45 and 8.74 μg of vitamin A/gm of fresh liver, respectively. Liver vitamin A values in the Lot 1 and Lot 2 groups fed diets without vitamin A supplementation were 2.91 and 1.92 IU/gm, respectively. Liver values were 1.76 and 9.10 IU/gm for Lot 1 groups fed the diets supplemented with 140 and 280 IU/kg, respectively. Liver values were 3.40 and 2.48 IU/gm for Lot 2 groups fed the diets supplemented with 900 and 1800 IU/kg, respectively.

It appeared that depletion of body ions and osmotic action of distilled water on some membranes initiated lesions that mimicked and/or led to vitamin A deficiency.

BIBLIOGRAPHY

BIBLIOGRAPHY

Anon.: Prohibition on importation of pet turtles. J.A.V.M.A., 160, (1972): 1482-1483.

Barnicot, N. A., and Datta, S. P.: Vitamin A and bone. In *The Biochemistry and Physiology of Bone*. G. H. Bourne (ed.), Academic Press, New York, 1956: 507-538.

Beaver, D. L.: Vitamin A deficiency in the germ-free rat. Am. J. Path., 38, (1961): 335-357.

Bernhard, K., Ritzel, G., and Steiner, K. U.: Helv. Chim Acta, 37, (1954): 306. Cited in *Vitamin A*, by Moore, 1957, p. 194.

Bruner, H. L.: The cephalic veins and sinuses of reptiles. Am. J. Anat., 7, (1907): 1-117.

Cagle, F. R.: The life history of the slider turtle, *Pseudemys scripta troosti* (Holbrook). Ecological Monographs, 20(1), (1950): 31-54.

Carr, A.: *Handbook of Turtles. The Turtles of the United States, Canada, and Baja, California.* Cornell Univ. Press, Ithaca, New York, 1952.

Carr, F. H., and Price, E. A.: Colour reactions attributed to vitamin A. Biochem. J., 20, (1926): 497-501.

Clark, D. B., and Gibbons, J. W.: Dietary shift in the turtle *Pseudemys scripta* (Schoepff) from youth to maturity. Copeia, (1969): 704-706.

Cowan, F. B.: Gross and microscopic anatomy of the orbital glands of *Malaclemys* and other Emydine turtles. Can. J. Zool., 47, (1969): 723-729.

Cowan, F. B.: The ultrastructure of the lacrymal 'salt' gland and the harderian gland in the Euryhaline *Malaclemys* and some closely related Stenohaline Emydines. Can. J. Zool., 49, (1971): 691-697.

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

Davies, A. W., and Moore, T.: Interaction of vitamins A and E. Nature, 147, (1941): 794-796.

DeLuca, H. F., and Suttie, J. W.: *The Fat-Soluble Vitamins*. The Univ. of Wisconsin Press, Madison, 1970.

Dessauer, H. C.: Blood chemistry of reptiles: physiological and evolutionary aspects. In *Biology of the Reptilia*, Vol. 3. C. Gans and T. S. Parsons (ed.), Academic Press, New York, 1970b: 1-72.

Dingle, J. T.: Studies on the mode of action of excess vitamin A. III. Release of a bound protease by the action of vitamin A. Biochem. J., 79, (1961): 509-512.

Dunson, W. A.: Sodium fluxes in fresh-water turtles. J. Exp. Zool., 165, (1967a): 171-182.

Dunson, W. A.: Relationship between length and weight in the spiney softshell turtle. Copeia, (1967b): 483-485.

Elkan, E., and Zwart, P.: The ocular disease of young terrapins caused by vitamin A deficiency. Path. Vet., 4(3), (1967): 201-222.

Elvehjem, C. A., and Neu, V. F.: Studies in vitamin A avitaminosis in the chick. J. Biol. Chem., 97, (1932): 71-82.

Epstein, F. H.: Calcium nephropathy. In *Diseases of the Kidney*, 2nd ed., Vol. II, M. B. Strauss and L. G. Welt (ed.), Little, Brown and Co., Boston, 1971: 903-931.

Fell, H. B.: The effect of excess vitamin A on cultures of embryonic chicken skin explanted at different stages of differentiation. Proc. Roy. Soc. (Biol.), 146, (1957): 242-256.

Fell, H. B.: The direct action of vitamin A on skeletal tissue *in vitro*. In *The Fat-Soluble Vitamins*. H. F. DeLuca and J. W. Suttie (ed.), The Univ. of Wisconsin Press, Madison, 1970: 187-202.

Follis, R. H., Jr.: *Deficiency Disease*. Charles C. Thomas, Publishers, Springfield, Ill., 1958.

Gans, C., Bellairs, A. d'A., and Parsons, T. S.: *Biology of the Reptilia*, Vol. I. Morphology A. Academic Press, New York, 1969.

Gans, C., and Parsons, T. S.: *Biology of the Reptilia*, Vol. 2. Morphology B. Academic Press, New York, 1970a.

Gans, C., and Parsons, T. S.: *Biology of the Reptilia*, Vol. 3. Morphology C. Academic Press, New York, 1970b.

Gillam, A. E.: The vitamin A and A_2 contents of mammalian and other animal livers. Biochem. J., 32, (1938): 1496-1500.

Goodman, DeW. S.: Retinol transport in human plasma. In *The Fat-Soluble Vitamins*. H. F. DeLuca and J. W. Suttie (ed.), The Univ. of Wisconsin Press, Madison, 1970: 203-212.

Graham, T. E.: Growth rate of the red-bellied turtle, *Chrysemys rubriventris*, at Plymouth, Massachusetts. Copeia, (1971): 353-356.

Hayes, K. C.: On the pathophysiology of vitamin A deficiency. Nutrition Review, 29(1), (1971): 3-6.

Heisey, S. R.: Cerebrospinal and extracellular fluid spaces in turtle brain. Am. J. Physiol., 219, (1970): 1564-1567.

Johnson, R. M., and Bauman, C. A.: Relative significance of growth and metabolic rate upon the utilization of vitamin A by the rat. J. Nutr., 35, (1948): 703-715.

Jones, L. M.: Fat-soluble vitamins. In *Veterinary Pharmacology and Therapeutics*, 3rd ed., L. M. Jones (ed.), Iowa State Univ. Press, Ames, Iowa, 1965: 872-902.

Jungherr, E.: Nasal histopathology and liver storage in subtotal vitamin A deficiency of chickens. Bull. No. 250. Storrs Agricultural Experiment Station (Conn.), 1943.

King, F. W.: Housing, sanitation, and nutrition of reptiles. J.A.V.M.A., 159(11), (1971): 1612-1615.

Lagler, K. F., and Applegate, V. C.: Relationship betweeen the length and weight in the snapping turtle *Chelydra serpentina* Linnaeus. Am. Nat., 77, (1943): 476-478.

Marcus, L. C.: Infectious disease of reptiles. J.A.V.M.A., 159(11), (1971): 1626-1631.

McCollum, E. V., and Davis, M.: The necessity of certain lipins in the diet during growth. J. Biol. Chem., 15, (1913): 167-175.

McCotter, R. E.: The vomero-nasal apparatus in *Chrysemys punctata* and *Rana catesbiana*. Anat. Rec., 13, (1917); 51-67.

Moore, T.: *Vitamin A*. Elsevier Publishing Co., New York, 1957.

National Academy of Sciences: Nutrient requirements of poultry, 6th revised ed. National Research Council, Washington, D.C., 1971.

Parnell, J. P., and Sherman, B. S.: Effect of vitamin A on keratinization in the A-deficient rat. In *Fundamentals of Keratinization*. E. O. Butcher and R. F. Sognnaes (ed.), Publication No. 70. Am. Assn. for the Advancement of Science, Washington, D.C., 1962: 113.

Phillips, W. E. J.: Low-temperature environmental stress and the metabolism of vitamin A in the rat. Can. J. Biochem. Physiol., 40, (1962): 491-499.

Roels, O. A.: Biochemical systems. D. Effect on membranes. In *The Vitamins*, Vol. 1. W. H. Sebrell, Jr., and R. S. Harris (ed.), Academic Press, New York, 1967: 193-209.

Rosen, S.: The turtle bladder. I. Morphological studies under varying conditions of fixation. Exp. Molec. Path., 12, (1970): 286-296.

Sebrell, W. H., and Harris, R. S.: *The Vitamins*, Vol. 1. Academic Press, New York, 1967.

Smith, L. H., Jr., and Williams, H. E.: Kidney stones. In *Diseases of the Kidney*, 2nd ed., Vol. II. M. B. Strauss and L. G. Welt (ed.), Little, Brown and Co., Boston, 1971: 973-996.

Steinmetz, P. R.: Characteristics of hydrogen ion transport in urinary bladder of water turtle. J. Clin. Invest., 46(10), (1967): 1531-1540.

Steinmetz, P. R., Omachi, R. S., and Frazier, H. S.: Independence of hydrogen ion secretion and transport of other electrolytes in turtle bladder. J. Clin. Invest., 46(10), (1967): 1541-1548.

Sundaresan, P. R., Winters, V. G., and Therriault, D. G.: Effect of low environmental temperature on the metabolism of vitamin A (retinol) in the rat. J. Nutr., 92, (1967): 474-478.

Suzuki, H. K.: Studies on the osseous system of the slider turtle. Ann. N.Y. Acad. Sci., 109, (1963): 351-410.

Thompson, J. N.: The role of vitamin A in reproduction. In *The Fat-Soluble Vitamins*. H. F. DeLuca and J. W. Suttie (ed.), The Univ. of Wisconsin Press, Madison, 1970: 267-281.

Trobec, T. N., and Stanley, J. G.: Uptake of ions and water by the painted turtle, *Chrysemys picta*. Copeia, (1971): 537-542.

VanLeersum, E. C.: Vitamin A deficiency and calcification of the epithelium of the kidney. J. Biol. Chem., 79, (1928): 461-464.

Wallach, J. D.: Medical care of reptiles. J.A.V.M.A., 155(7), (1971a): 1017-1034.

Wallach, J. D.: Environmental and nutritional diseases of captive reptiles. J.A.V.M.A., 159(11), (1971b): 1632-1643.

Wolf, G., and DeLuca, L.: Recent studies on some metabolic functions of vitamin A. In *The Fat-Soluble Vitamins*. H. F. DeLuca and J. W. Suttie (ed.), The Univ. of Wisconsin Press, Madison, 1970: 257-265.

APPENDICES

APPENDIX A

BODY MEASUREMENTS OF EACH TURTLE AT DAY 0 AND DAY 76 (LOT 1) OR DAY 191 (LOT 2)

Table A-1. Body measurements of each turtle at Day 0 and Day 76 (Lot 1) or Day 191 (Lot 2)

Lot	Group	Turtle No.	Day of Necropsy (Day-N)	Body weight, gm		Plastron Length 1/128 inch		Carapace Length 1/128 inch	
				Day 0	Day 76	Day 0	Day 76	Day 0	Day 76
1	0	3-82	0	9.56	-	175	-	180	-
1	0	3-83	0	9.10	-	164	-	174	-
1	0	3-84	0	8.93	-	166	-	175	-
1	0	3-85	0	8.85	-	172	-	182	-
1	0	3-86	0	7.53	-	166	-	167	-
1	0	3-87	0	7.50	-	163	-	176	-
1	0	3-14	0	7.02	-	162	-	172	-
1	0	3-15	0	6.95	-	153	-	162	-
1	0	3-16	0	6.75	-	155	-	165	-
1	0	3-17	0	5.63	-	152	-	168	-
1	I	3-52	105	9.69	9.92	172	177	180	185
1	I	3-53	38	8.84	-	173	-	186	-
1	I	3-54	130	8.68	7.46	169	169	179	179
1	I	3-55	93	8.44	8.06	170	173	175	179
1	I	3-56	72	8.04	-	165	-	179	-
1	I	3-57	130	7.66	7.23	161	162	168	168
1	I	3-58	73	7.32	-	156	-	167	-
1	I	3-59	84	6.92	5.19	150	150	163	159
1	I	3-60	114	6.67	9.31	156	170	173	190
1	I	3-61	28	5.78	-	149	-	153	-
1	II	3-62	130	9.83	12.24	164	178	181	197
1	II	3-63	119	9.11	11.21	173	184	180	198
1	II	3-64	104	8.69	10.65	169	178	177	189
1	II	3-65	119	8.29	8.54	160	161	176	177
1	II	3-66	186	8.26	10.70	167	175	174	187
1	II	3-67	99	7.59	6.63	165	165	173	171
1	II	3-68	105	7.07	5.99	161	160	173	169
1	II	3-69	182	6.83	6.95	155	156	164	163
1	II	3-70	104	6.43	6.50	154	161	163	169
1	II	3-71	39	5.22	-	142	-	149	-

Table A-1 (cont'd.)

Lot	Group	Turtle No.	Day of Necropsy (Day-N)	Body weight, gm		Plastron Length 1/128 inch		Carapace Length 1/128 inch	
				Day 0	Day 76	Day 0	Day 76	Day 0	Day 76
1	III	3-72	105	9.30	13.30	172	188	184	205
1	III	3-73	114	9.24	18.71	178	211	183	228
1	III	3-74	86	8.66	9.41	161	168	176	182
1	III	3-75	104	8.27	10.25	166	175	172	183
1	III	3-76	130	8.23	14.89	163	187	175	209
1	III	3-77	104	8.00	11.12	166	184	173	192
1	III	3-78	103	7.02	6.38	158	158	166	165
1	III	3-79	18	6.70	-	154	-	163	-
1	III	3-80	31	6.38	-	156	-	162	-
1	III	3-81	25	5.89	-	155	-	162	-
					Day 191		Day 191		Day 191
2	IV A	5-34	245	9.96	10.18	178	183	182	186
2	IV B	5-35	217	9.40	9.66	166	169	186	191
2	IV A	5-36	163	9.01	-	172	-	178	-
2	IV B	5-37	245	8.94	8.37	168	169	183	183
2	IV A	5-38	245	8.70	9.00	164	169	177	182
2	IV B	5-39	152	8.29	-	154	-	166	-
2	IV B	5-40	141	8.25	-	166	-	180	-
2	IV A	5-41	87	8.15	-	159	-	174	-
2	IV B	5-42	221	8.02	7.59	154	154	172	173
2	IV A	5-43	217	6.91	7.25	152	157	168	177
2	V A	5-44	206	10.37	12.20	169	189	186	209
2	V B	5-45	60	9.80	-	165	-	179	-
2	V A	5-46	245	9.10	8.62	174	175	186	189
2	V B	5-47	181	8.84	-	164	-	176	-
2	V B	5-48	197	8.60	8.50	167	175	175	183
2	V A	5-49	223	8.47	10.10	162	170	172	182
2	V A	5-50	152	8.19	-	165	-	176	-
2	V B	5-51	201	8.09	14.62	156	215	169	222
2	V A	5-52	209	7.99	8.86	160	169	176	188
2	V B	5-53	209	7.73	7.40	162	166	171	177

Table A-1 (cont'd.)

Lot	Group	Turtle No.	Day of Necropsy (Day-N)	Body weight, gm		Plastron Length 1/128 inch		Carapace Length 1/128 inch	
				Day 0	Day 76	Day 0	Day 76	Day 0	Day 76
					Day 191		Day 191		Day 191
2	VI B	5-54	245	9.95	14.18	170	187	187	216
2	VI A	5-55	246	9.68	11.10	173	181	190	202
2	VI B	5-56	212	9.13	9.48	163	165	173	178
2	VI A	5-57	246	8.96	13.63	164	184	183	208
2	VI A	5-58	216	8.51	13.05	161	188	175	205
2	VI B	5-59	245	8.42	9.75	159	170	173	186
2	VI A	5-60	216	8.19	8.37	160	164	172	179
2	VI B	5-61	216	8.15	9.71	160	169	172	186
2	VI B	5-62	245	7.99	13.40	159	184	169	202
2	VI A	5-63	246	7.66	8.31	159	164	171	179
2	VII	5-64	0	7.52	-	152	-	162	-
2	VII	5-65	0	8.80	-	171	-	189	-
2	VII	5-66	0	8.26	-	161	-	171	-
2	VII	5-67	0	8.15	-	157	-	178	-
2	VII	5-68	0	9.89	-	165	-	179	-
2	VII	5-69	0	9.39	-	173	-	183	-
2	VII	5-70	0	8.90	-	151	-	170	-
2	VII	5-71	0	9.04	-	167	-	179	-
2	VII	5-72	0	8.40	-	170	-	180	-
2	VII	5-73	0	8.02	-	149	-	162	-

APPENDIX B

BODY MEASUREMENTS AND LIVER VITAMIN A VALUES OF EACH TURTLE AT DAY-N (DAY OF NECROPSY)

Table B-1. Body measurements and liver vitamin A values of each turtle at Day-N (day of necropsy)

Group	Turtle No.	Day-N	Plastron Length 1/128"	Carapace Length 1/128"	Body Wt. gm	Plastron Wt. gm	Carapace Wt. gm	Heart Wt. gm	2 Kidney Wt. gm	Liver Wt. gm	µg Vit. A/gm Fresh Liver
0	3-82	0	175	180	9.088	0.816	1.667	0.025	0.046	0.579	10.042
0	3-83	0	164	174	8.631	0.685	1.462	0.020	0.041	0.400	8.212
0	3-84	0	166	175	8.249	0.783	1.688	0.027	0.032	0.294	2.304
0	3-85	0	172	182	8.663	0.645	1.566	0.029	0.026	0.479	2.698
0	3-86	0	166	167	7.491	0.692	1.267	0.021	0.024	0.276	9.053
0	3-87	0	163	176	6.882	0.678	1.082	0.024	0.030	0.214	61.454
0	3-14	0	162	172	7.016	0.495	0.985	-	-	0.194	7.653
0	3-15	0	153	162	6.875	0.525	1.030	0.021	0.020	0.236	1.773
0	3-16	0	155	165	6.758	0.386	0.781	0.018	0.024	0.122	0.356
0	3-17	0	152	168	5.876	0.468	0.931	0.025	0.028	0.207	0.926
I	3-52	105	177	184	9.048	0.685	1.535	0.046	0.058	0.601	1.135
I	3-53	38	174	185	7.821	0.637	1.122	0.036	0.056	0.399	0.742
I	3-54	130	170	178	7.469	0.619	1.158	0.039	0.051	0.482	2.747
I	3-55	93	173	177	7.516	0.575	1.134	0.030	0.040	0.408	1.278
I	3-56	72	172	185	8.772	0.752	1.618	0.032	0.058	0.488	1.458
I	3-57	130	162	167	7.284	0.587	1.080	0.026	0.034	0.688	0.438
I	3-58	73	156	164	6.105	0.414	0.864	0.022	0.043	0.191	3.768
I	3-59	84	151	159	5.268	0.441	0.811	0.024	0.034	0.191	2.424
I	3-60	114	170	189	7.805	0.723	1.475	0.039	0.059	0.378	10.100
I	3-61	28	144	152	5.130	0.349	0.689	0.019	0.029	0.120	5.000
II	3-62	130	175	193	11.156	0.740	2.050	0.044	0.046	0.924	1.782
II	3-63	119	184	200	9.660	0.770	1.695	0.041	0.090	0.692	0.755
II	3-64	104	178	189	10.208	0.866	1.702	0.040	0.035	0.560	0.422
II	3-65	119	161	176	7.936	0.626	1.145	0.035	0.034	0.482	1.569
II	3-66	186	174	188	9.170	0.652	1.327	0.037	0.063	0.500	2.714
II	3-67	99	164	171	7.171	0.477	0.937	0.030	0.047	0.407	2.203
II	3-68	105	161	170	6.325	0.476	0.919	0.027	0.057	0.324	1.234
II	3-69	182	156	163	6.110	0.455	0.758	0.024	0.031	0.402	2.976
II	3-70	104	159	168	6.019	0.439	0.994	0.030	0.034	0.227	2.624
II	3-71	39	144	144	4.429	0.260	0.572	0.021	0.054	0.144	1.334

Table B-1 (cont'd.)

Group	Turtle No.	Day-N	Plastron Length 1/128"	Carapace Length 1/128"	Body Wt. gm	Plastron Wt. gm	Carapace Wt. gm	Heart Wt. gm	2 Kidney Wt. gm	Liver Wt. gm	µg Vit. A/gm Fresh Liver
III	3-72	105	189	207	11.964	0.949	2.128	0.064	0.079	0.792	33.999
III	3-73	114	213	231	16.568	1.433	3.002	0.075	0.152	1.229	0.430
III	3-74	86	162	182	9.177	0.740	1.652	0.057	0.046	0.578	1.714
III	3-75	104	175	184	9.009	0.720	1.467	0.044	0.070	0.684	0.968
III	3-76	130	190	212	13.266	1.087	2.764	0.053	0.088	1.005	1.282
III	3-77	104	183	192	9.707	0.842	1.819	0.048	0.060	0.646	3.040
III	3-78	103	157	165	6.530	0.537	0.933	0.025	0.080	0.366	1.052
III	3-79	18	153	160	5.845	0.427	0.846	0.016	0.031	0.126	11.905
III	3-80	31	154	159	4.329	0.388	0.660	0.015	0.028	0.139	19.608
III	3-81	25	152	160	4.719	0.440	0.684	0.018	0.051	0.189	16.970
IV A	5-34	245	183	187	9.985	0.802	1.779	0.043	0.041	0.623	2.686
IV B	5-35	217	169	190	9.332	0.711	1.576	0.041	0.041	0.450	0.000
IV A	5-36	163	172	178	9.368	0.745	1.563	0.035	0.040	0.715	2.632
IV B	5-37	245	169	182	8.064	0.541	1.283	0.044	0.037	0.436	1.882
IV A	5-38	245	169	184	9.060	0.697	1.525	0.037	0.038	0.618	3.004
IV B	5-39	152	159	171	7.278	0.601	1.181	0.030	0.032	0.325	2.778
IV B	5-40	141	171	184	6.970	0.580	1.253	0.035	0.046	0.322	2.916
IV A	5-41	87	184	204	13.005	1.130	2.709	0.042	0.166	1.074	0.748
IV B	5-42	221	154	172	6.701	0.539	1.078	0.027	0.057	0.561	1.158
IV A	5-43	217	157	175	7.170	0.514	1.272	0.025	0.034	0.393	1.436
V A	5-44	206	189	207	14.981	1.081	2.716	0.090	0.102	1.047	1.101
V B	5-45	60	165	174	9.378	0.528	1.055	0.026	0.029	0.429	6.150
V A	5-46	245	176	188	8.138	0.641	1.460	0.032	0.033	0.346	4.348
V B	5-47	181	174	188	8.655	0.924	1.926	0.035	0.046	0.483	3.283
V B	5-48	197	173	182	8.218	0.705	1.621	0.037	0.038	0.475	2.778
V A	5-49	223	170	180	9.605	0.873	1.760	0.040	0.042	0.649	3.455
V A	5-50	152	187	200	9.223	0.875	2.055	0.053	0.049	0.644	5.172
V B	5-51	201	201	213	12.777	1.158	2.724	0.057	0.068	0.696	1.144
V A	5-52	209	169	186	7.448	0.574	1.510	0.032	0.023	0.334	3.166
V B	5-53	209	165	175	6.934	0.530	1.048	0.036	0.037	0.293	3.431

Table B-1 (cont'd.)

Group	Turtle No.	Day-N	Plastron Length 1/128"	Carapace Length 1/128"	Body Wt. gm	Plastron Wt. gm	Carapace Wt. gm	Heart Wt. gm	2 Kidney Wt. gm	Liver Wt. gm	µg Vit. A/gm Fresh Liver
VI B	5-54	245	187	216	14.100	1.020	2.773	0.049	0.055	0.980	0.544
VI A	5-55	246	181	201	10.428	0.867	2.116	0.055	0.063	0.662	2.724
VI B	5-56	212	165	184	9.003	0.692	1.495	0.044	0.067	0.726	2.551
VI A	5-57	246	201	228	16.025	1.280	3.392	0.079	0.143	1.310	1.258
VI A	5-58	216	186	201	12.451	0.995	2.694	0.039	0.056	0.722	4.784
VI B	5-59	245	170	184	10.087	0.762	1.716	0.047	0.039	0.560	4.815
VI A	5-60	216	164	177	7.765	0.687	1.423	0.039	0.041	0.425	1.593
VI B	5-61	216	169	184	10.494	0.782	1.726	0.066	0.063	0.700	0.694
VI B	5-62	245	184	202	12.803	0.949	2.556	0.058	0.064	0.917	3.047
VI A	5-63	246	164	178	8.075	0.637	1.337	0.040	0.031	0.520	2.778
VII	5-64	0	152	162	7.377	0.530	1.194	0.025	0.015	0.227	8.974
VII	5-65	0	171	189	8.929	0.605	1.504	0.023	0.037	0.316	13.377
VII	5-66	0	161	171	8.252	0.619	1.349	0.024	0.027	0.381	7.870
VII	5-67	0	157	178	8.053	0.658	1.435	0.021	0.031	0.336	9.792
VII	5-68	0	165	179	9.904	0.722	1.712	0.026	0.032	0.360	7.532
VII	5-69	0	173	183	9.680	0.674	1.748	0.026	0.025	0.347	7.812
VII	5-70	0	151	170	8.640	0.693	1.562	0.030	0.025	0.389	4.012
VII	5-71	0	167	179	8.983	0.613	1.640	0.029	0.027	0.364	7.372
VII	5-72	0	170	180	8.182	0.665	1.523	0.021	0.020	0.278	7.083
VII	5-73	0	149	162	7.876	0.561	1.284	0.025	0.026	0.351	13.541
Adult (Sex)											
F	6-19	10-4-71	355	386	69.87	5.890	14.636	0.212	0.186	2.059	14.30
F	6-20	10-4-71	449	481	134.46	13.265	25.609	0.303	0.269	3.488	1.70
F	6-21	10-3-71	463	489	142.44	14.609	30.788	0.337	0.361	3.054	39.94
F	6-22	10-3-71	444	501	146.10	12.669	31.642	0.493	0.436	3.142	8.19
M	6-23	10-3-71	488	539	188.55	13.602	32.915	0.409	0.415	3.345	14.50
M	6-24	10-3-71	519	554	184.68	18.802	41.942	0.431	0.494	4.582	38.67

APPENDIX C

MINERAL CONTENT OF LOT 1, LOT 2, AND ADULT TURTLE SHELLS

Table C-1. Mineral content of Lot 1, Lot 2, and adult turtle shells

Turtle No.	Dry, fat-free Wt.,gm	Shell Ash gm	Calcium ppm of ash	Calcium % of ash	Magnesium ppm of ash	Magnesium % of ash	Phosphorus ppm of ash	Phosphorus % of ash
3-82	.6714	.3822	488.93	12.79	7.24	0.19	201.15	5.26
3-83	.6480	.1240	510.65	41.18	7.27	0.59	193.1	15.57
3-84	.7428	.1122	487.58	43.46	7.74	0.69	206.55	18.41
3-85	.8341	.1826	795.30	43.55	5.42	0.30	179.78	9.84
3-86	.5475	.0726	331.99	45.73	4.33	0.60	144.50	19.90
3-87	.5519	.0879	389.65	44.33	6.49	0.74	167.86	19.10
3-14	.4281	.0752	296.59	39.44	4.33	0.58	144.98	19.28
3-15	.4575	.0717	289.34	40.35	5.45	0.76	143.04	19.95
3-16	.3321	.0579	251.85	43.50	4.92	0.85	125.60	21.69
3-17	.3740	.0614	256.51	41.78	4.42	0.72	123.66	20.14
3-52	.8341	.1826	407.87	22.34	7.97	0.44	324.96	17.80
3-53	.6073	.1376	586.52	42.62	6.92	0.50	212.44	15.44
3-54	.5991	.1384	614.88	44.43	6.11	0.44	264.4	19.10
3-55	.5552	.0846	407.87	46.39	4.84	0.57	161.52	19.09
3-56	.8612	.1677	709.55	42.31	9.22	0.55	320.43	19.11
3-57	.7181	.1872	845.61	45.17	11.07	0.59	337.74	18.04
3-58	.3814	.0839	341.52	40.70	4.24	0.50	472.58	56.33
3-59	.4705	.1200	573.72	47.81	8.67	0.72	261.66	21.81
3-60	.8691	.1709	730.55	42.75	5.76	0.34	255.15	14.93
3-61	1.6189	.3803	1729.12	45.47	22.99	0.60	633.91	16.69
3-62	1.1510	.2519	1074.44	42.65	14.30	0.57	470.14	18.66
3-63	1.0429	.1958	910.9	46.52	8.73	0.44	354.76	18.12
3-64	.9930	.2025	950.43	46.96	9.14	0.45	401.09	19.81
3-65	.7573	.2206	601.97	27.29	8.19	0.37	250.99	11.38
3-66	.7093	.1920	849.05	44.22	12.96	0.68	301.44	15.70
3-67	.4957	.0944	423.72	44.88	4.42	0.47	172.25	18.25
3-68	.4875	.1337	592.42	44.31	6.93	0.52	343.8	18.24
3-69	.4890	.1175	507.77	43.21	7.39	0.63	215.40	18.33
3-70	.4969	.0820	348.70	42.52	4.24	0.52	161.79	19.73
3-71	.2573	.0462	209.61	45.37	1.54	0.33	96.65	20.92

Table C-1 (cont'd.)

Turtle No.	Dry, fat-free Wt.,gm	Shell Ash gm	Calcium		Magnesium		Phosphorus	
			ppm of ash	% of ash	ppm of ash	% of ash	ppm of ash	% of ash
3-72	1.1182	.2041	856.75	41.98	13.70	0.67	308.84	15.13
3-73	1.6798	.2840	1150.33	40.50	17.76	0.62	478.71	16.86
3-74	.8971	.1888	840.03	44.49	10.78	0.57	338.58	17.93
3-75	.9100	.1921	823.42	42.86	5.42	0.28	365.92	19.05
3-76	1.5180	.2866	1278.5	44.61	15.49	0.54	483.14	16.86
3-77	1.1433	.2423	983.52	40.59	12.10	0.50	407.75	18.28
3-78	.4270	.1031	404.99	39.28	5.65	0.55	158.70	15.39
3-79	.4792	.1292	599.40	46.39	9.14	0.71	224.5	17.38
3-80	.3330	.0870	389.65	44.79	5.26	0.60	136.51	15.69
3-81	.3271	.0610	250.17	41.01	4.96	0.81	126.56	20.75
5-34	1.1172	.2629	1342.66	51.07	18.76	0.71	158.6	6.03
5-35	1.8426	.1978	854.86	43.22	10.55	0.53	297.03	15.02
5-36	.9344	.1945	887.64	45.64	9.53	0.49	355.77	18.29
5-37	.6498	.0886	404.21	45.62	8.00	0.90	178.11	20.10
5-38	.9935	.2235	999.90	44.74	12.26	0.55	373.55	16.71
5-39	.6423	.1201	331.99	27.64	4.33	0.36	220.33	18.35
5-40	.7342	.1690	733.51	43.40	9.14	0.54	309.87	18.34
5-41	1.4535	.2891	250.17	8.65	18.02	0.62	538.12	18.61
5-42	.6855	.1507	656.0	43.53	7.76	0.51	265.39	17.61
5-43	.6905	.1495	637.2	42.62	7.28	0.49	223.62	14.96
5-44	.3165	.0776	324.14	41.77	4.43	0.57	151.79	19.56
5-45	.4847	.0742	307.58	41.45	4.74	0.64	144.01	19.41
5-46	.9160	.2186	988.12	45.20	11.49	0.52	384.24	17.58
5-47	1.2418	.2698	1240.50	45.98	13.04	0.48	441.82	16.38
5-48	.9496	.2274	854.87	37.59	13.89	0.61	351.22	15.45
5-49	1.1306	.3200	1548.7	48.40	16.29	0.51	603.89	18.87
5-50	1.2134	.2232	1026.80	46.00	11.08	0.50	320.89	14.38
5-51	1.6763	.1954	823.42	42.14	13.60	0.70	296.54	15.18
5-52	.7980	.2040	899.39	44.09	9.02	0.44	313.83	15.38
5-53	.5276	.1020	349.3	34.24	4.74	0.46	202.31	19.83

Table C-1 (cont'd.)

Turtle No.	Dry, fat-free Wt.,gm	Shell Ash gm	Calcium ppm of ash	Calcium % of ash	Magnesium ppm of ash	Magnesium % of ash	Phosphorus ppm of ash	Phosphorus % of ash
5-54	1.4547	.2609	1156.59	44.33	15.96	0.61	434.98	16.67
5-55	1.1603	.2006	886.62	44.20	12.81	0.64	327.53	16.33
5-56	.8484	.1283	503.70	39.26	7.36	0.57	199.77	15.57
5-57	1.8389	.4302	1989.31	46.24	26.29	0.61	748.27	17.39
5-58	1.4748	.3008	1366.06	45.41	13.21	0.44	511.86	17.02
5-59	1.0122	.2498	1113.50	44.58	12.23	0.49	454.60	18.20
5-60	.9810	.1632	718.34	44.02	9.50	0.58	328.73	20.14
5-61	.9513	.1789	755.08	42.21	11.65	0.65	334.04	18.67
5-62	1.1883	.1634	697.08	42.66	8.74	0.53	290.61	17.79
5-63	.8578	.2267	1038.19	45.80	13.20	0.58	459.78	20.28
5-64	.5031	.0605	242.02	40.00	4.33	0.73	114.00	18.84
5-65	.5532	.0508	190.82	37.56	4.27	0.84	107.25	21.11
5-66	.5737	.0551	220.39	40.00	4.62	0.84	118.82	21.57
5-67	.5347	.0642	276.57	45.07	4.71	0.73	135.74	21.14
5-68	.6467	.0764	307.58	40.26	6.58	0.86	154.22	20.19
5-69	.6490	.0777	303.51	39.06	6.96	0.90	161.79	20.82
5-70	.5073	.0581	185.47	31.92	3.83	0.66	122.69	21.12
5-71	.6075	.0643	254.19	39.53	6.02	0.94	137.71	21.42
5-72	.5737	.0640	284.63	47.44	5.73	0.90	100.91	15.77
5-73	.5313	.0551	228.61	41.49	6.11	1.11	122.70	22.27
6-19	.3396	.2045	950.40	46.47	11.84	0.58	392.92	19.21
6-20	.3883	.2352	1144.16	48.65	15.86	0.67	476.40	20.26
6-21	.5451	.3429	1557.91	45.43	22.22	0.65	635.52	18.53
6-22								
6-23	.4882	.3017	1552.60	51.46	15.22	0.50	547.91	18.16
6-24	.6209	.3841	1762.41	45.88	20.49	0.53	668.665	17.41

VITA

Marilyn P. Anderson was born in Detroit, Michigan, on June 26, 1944. She received her primary and secondary education in the public schools of Detroit and Pontiac, Michigan, and Arlington, Virginia. She graduated from Pontiac Northern High School in 1962. She attended Michigan State University receiving a BS in 1966 and a DVM in 1967.

In 1968 she entered the Peace Corps, serving in Brazil until 1970 as veterinary advisor to ACARES, the agricultural extension agency in the State of Espírito Santo.

In 1971 she reentered Michigan State University to pursue a Master of Science degree in veterinary pathology.