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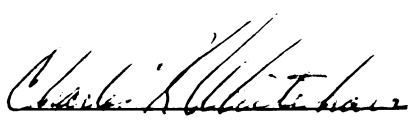
THE INFLUENCE OF A VITAMIN A DEFICIENCY ON *TRYPANOSOMA LEWISI* AND *PSEUDOMONAS AERUGINOSA* INFECTIONS IN THE RAT

presented by

Francisco Benedito Rangel Filho

has been accepted towards fulfillment
of the requirements for
DOCTOR OF PHILOSOPHY
_____ degree in _____

Department of Pathology



Major professor

Date 10/28/80

O-7639

# THE INFLUENCE OF A VITAMIN A DEFICIENCY ON *TRYPANOSOMA LEWISI* AND *PSEUDOMONAS AERUGINOSA* INFECTIONS IN THE RAT

By

Francisco Benedito Rangel Filho

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Pathology

1980

ABSTRACT

THE INFLUENCE OF A VITAMIN A DEFICIENCY ON *TRYPANOSOMA LEWISI* AND *PSEUDOMONAS AERUGINOSA* INFECTIONS IN THE RAT

By

Francisco Benedito Rangel Filho

Experiments were conducted to determine the role and interrelationship of a vitamin A deficiency and infections due to *Trypanosoma lewisi* and *Pseudomonas aeruginosa* in the young rat. The rats were from dams fed a semipurified vitamin A-deficient diet during pregnancy and lactation. After weaning the rats were fed the vitamin A-deficient diet until clinical signs of vitamin A deficiency occurred. When clinical signs of vitamin A deficiency appeared, the rats were fed 1, 5, or 50 IU of retinol/kg ration and either exposed or unexposed to *T. lewisi*. A spontaneous infection with *P. aeruginosa* occurred when clinical signs of vitamin A deficiency appeared.

Vitamin A deficiency was characterized by retarded growth, incoordination, rough haircoat, xerophthalmia, low liver and serum vitamin A values, metaplasia of epithelial cells of the respiratory tract, and intrafollicular keratinization of the thyroid gland. *Trypanosoma lewisi* infection was characterized by anemia, parasitemia, anemia, splenomegaly, splenitis, hepatic and renal infarcts, and glomerulonephritis. Splenic lesions were more severe in rats fed the vitamin A-deficient diet. Hepatitis and nephritis were present in

rats fed the vitamin A-deficient diet and were associated with both *T. lewisi* and *P. aeruginosa*. A spontaneous *P. aeruginosa* infection was closely associated with and enhanced by vitamin A deficiency.

A vitamin A deficiency enhances the incidence and severity of *T. lewisi* and *P. aeruginosa* infection in rats.

DEDICATION

TO MY WIFE, WILMA, AND MY DAUGHTERS,

MORAIMA AND AYMARA

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. C. K. Whitehair, chairman of his guidance committee, for assistance and help throughout this research and in preparation of this dissertation.

The author also wishes to thank Dr. G. R. Carter for his continued friendship, help, encouragement, and guidance during the tenure of his public health training at Michigan State University, and for the use of his laboratory facilities.

Appreciation is expressed to Dr. H. W. Cox for his generous assistance, cooperation, and counsel on the *Trypanosoma lewisi* aspects of this research and for the use of his laboratory facilities.

Sincere appreciation is extended to Dr. H. D. Stowe for providing the guidance, cooperation, and facilities in his laboratory and in the Food Science building to conduct the nutritional aspects of this research.

To Dr. R. F. Langham, the author is grateful for his teaching in basic pathology and for technical assistance during this research.

To Dr. S. D. Sleight, the author extends appreciation for his helpful suggestions during the preparation of this dissertation.

Sincere appreciation is extended to all faculty and staff members, especially in the Department of Pathology, at Michigan State University for their friendly assistance, companionship and hospitality during the author's tenure at Michigan State University.

The author also thanks Drs. A. P. Telles, R. Miranda, S. L. Stockham, T. Mullaney, and Linda J. Stegherr and Barbara Goelling for their cooperation in this research.

Thanks are expressed to the Brazilian Government, who provided through the Universidade Federal Ruaral do Rio de Janeiro and the program for Superior Education in Agriculture (PEAS) the financial support for this research training program. Appreciation is also expressed to Dr. J. M. Hunter, Director of the Latin American Studies Center, and his associates, especially Mrs. June E. Mills, for their assistance during the author's tenure at Michigan State University. The author wishes to thank Ms. Janice Fuller for typing this dissertation.

Finally, the author wishes to express his gratitude and appreciation primarily to his mother, Mrs. Virgolina T. Rangel, with whom he learned to live, to read, and to write and, secondly, to his wife, Wilma, and daughters, Moraima and Aymara, whose sacrifice, encouragement, and love made this research training program possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

# INTRODUCTION

The production of sufficient food that is safe and of adequate quality to supply the world's rapidly growing population represents a major challenge to science. The relationship between famine and pestilence has been observed since the beginning of civilization. Egyptians, Greeks, Romans, and Arabians referred to these disasters in an old manuscript in the first century of the Christian Era (Coutinho, 1966). Recently, many workers have indicated that resistance to parasitic and microbial infections is decreased as a result of nutritional deficiencies (Scrimshaw et al., 1968).

In many areas of the world, especially in tropical areas, including Brazil, parasitic diseases are difficult to treat and control. Among these parasitoses, the trypanosomes are particularly important in man and animals. In the African and American continents, approximately 70,000,000 people have been exposed to the risk of trypanosomiasis (WHO Technical Report Series No. 635, 1979).

Since the observations first published by McCollum and Davis (1913) and Osborne and Mendel (1913), the vitamins have been studied intensively and their importance in the maintenance of health and growth for man and animals has been repeatedly confirmed. Among the vitamins, vitamin A has occupied an important position, protecting the epithelial cells of the respiratory, alimentary, and genito-urinary tracts, the cutaneous tissue, hematopoietic system, central nervous system, and eyes

against several pathologic effects. Additionally, immunocompetence can be decreased significantly in vitamin A deficiency, as reported by Chandra and Newberne (1977).

Rats have been found to be particularly suitable for nutritional studies involving the vitamins. Because *Trypanosome lewisi* is known to produce anemia, splenomegaly and glomerulonephritis in the rat (Thoongsuwan and Cox, 1978), it was decided that an experimental *T. lewisi* infection in rats might serve as a model to study the effect of levels of vitamin A on the course of trypanosomal infection. Such a study was believed to have possible implications for animal and human health, particularly where vitamin A deficiencies and parasitic diseases coexist.

*Pseudomonas aeruginosa* is a frequent cause of hospital-acquired infection, and its importance has increased with reports of its resistance to many antibiotics. The few antibiotics that are effective are rather toxic and must be given parenterally (Moffet, 1980). *Pseudomonas aeruginosa* is widespread in nature and is physiologically versatile (Franklin and Franklin, 1971). It has been reported in wound infections in various animal species and has been associated with bovine mastitis, abortion in the cow and mare, atrophic rhinitis in swine, and otorrhea in dogs and cats (Carter, 1979). It produces complications and deaths in hospitalized patients, especially in infants and elderly people, and in those with serious underlying diseases of a hematologic, metabolic, or malignant nature and in individuals with severe burns (Moffet, 1980).

# LITERATURE REVIEW

A voluminous amount of information is available on vitamin A. This literature review will pertain to (1) general properties of vitamin A, (2) interactions of vitamin A and infection, (3) host-parasite relationships of *Trypanosoma lewisi*, and (4) the role of *Pseudomonas aeruginosa* in disease.

## Vitamin A

### History and Nature

Hopkins (1906) reported that rats fed a diet composed of pure protein, carbohydrate, fat, and the known necessary minerals failed to survive. Hopkins (1912) later observed that after adding small amounts of dried vegetables or an alcohol extract of milk solids to the diet, the rats grew and lived. On the other hand, when the diet was supplemented with mineral ash from milk or vegetables, the animals died. He concluded that the "accessory food factors" were organic in nature.

Funk (1912) proposed that these unknown organic compounds be called "vitamines" (*vita* means life and *amine* was added because the substances he was studying were amines). Drummund (1920) concluded that all life-promoting factors were not "amines", and he proposed that the final "e" should be dropped, resulting in the name "vitamin", which has remained to the present.

McCollum and Davis (1913) and Osborne and Mendel (1913), working independently, reported that there was an unidentified substance in fatty foods which was essential for growth and reproduction in rats. McCollum and Davis proposed the name of "fat-soluble A" factor. From that time until the present, research on vitamin A has progressed rapidly, and the chemical structure, metabolism, lesions of deficiency and toxicity are well known. Remaining to be elucidated are specific metabolic pathways and functions.

McCollum and Simmonds (1917) reported that "fat-soluble A" factors added to a deficient diet would restore and maintain the growth of the rats and prevent xerophthalmia. Fredericia and Holm (1925) observed that vitamin A would also cure night blindness.

A growth-promoting substance in plant extracts reported by Steenbock et al. (1921) provided the bases for understanding the role of the provitamin A-carotene.

Crystalline vitamin A was obtained from fish livers by Holmes and Corbett (1937), and Wald (1935, 1936) reported the isolation of the chromophore from bleached retinas. Morton and Goodwin (1944) proved that the chromophore was of retinal origin. The total synthesis of crystalline vitamin A was achieved by Isler et al. (1947), and the first total synthesis of B-carotene was by Milas et al. (1950).

Vitamin A is a pale yellow crystal. Chemically it is a fat-soluble, long chain alcohol which has a number of isometric forms. The most active and the most commonly found form in mammalian tissues is the trans-vitamin A. The alcohol form is retinol, the aldehyde form is retinal, and the acid form is retinoic acid. The provitamin A carotenoids are found in vegetables.

Vitamin A and the provitamin A carotenoids are comparatively stable to alkali and heat under ordinary cooking temperatures. They are easily oxidized and rapidly destroyed on exposure to acid and to ultraviolet light (Chaney, Ross and Witschi, 1979).

Metabolism and Requirements

The metabolism and function of vitamin A in man and animals have frequently been reviewed. At the present time it is accepted that vitamin A is present in animal products and commercially supplemented foodstuffs as a palmitate ester. In the small intestine this form of vitamin A is hydrolyzed to the free vitamin A alcohol by pancreatic juice and bile salts. The retinol form of vitamin A resulting from this biochemical reaction is absorbed. Under normal conditions, 90% of the ingested vitamin A is absorbed. B-carotene is solubilized less well than vitamin A by surface active agents, and about 70% of that ingested is absorbed. The efficiency of the intestinal absorption decreases as the intake increases, so any condition that upsets intestinal functions can also affect the absorption of vitamin A (WHO Technical Report No. 590, 1976).

In the epithelial cell of the gut, retinaldehyde is reduced to retinol (Fidge, 1968), which is transported via the lymph to the blood stream and stored for the most part as palmitate in the Kupffer cells of the liver (Wake, 1971).

The retinol esters are hydrolyzed to retinol, forming a "retinol-binding protein" complex which is synthesized in the liver during the mobilization of vitamin A and is then released into the blood plasma (Kanai et al., 1968).

In vitamin A deficiency, the release of "retinol-binding" protein is inhibited and the concentration of apo-"retinol-binding" protein in the liver rises, while plasma retinol and retinol-binding protein levels decrease (WHO Technical Report No. 590, 1976). On the other hand, retinol-binding protein synthesis is depressed in protein deficiency and consequently the steady state levels of retinol-binding protein and of retinol in the plasma are reduced (Glover, 1973).

Free retinol, but not the retinol-binding protein complex, is membrane-active and causes the rapid release of bound hydrolases from lysosomal particles, leaving the vitamin A-treated lysosomes intact (Dingle, 1972). Only one function of vitamin A has been well defined biochemically, viz., its interactions with various opsins of the retina to form visual pigments (WHO Technical Report No. 590, 1976).

Vitamin A seems to control in some way the differentiation of epithelial tissues, particularly of the skin, trachea, salivary glands, testes, and goblet cells of the gut. It also has an important role in the eyes and vision and in the immune response (Scrimshaw, Taylor and Gordon, 1968; Chandra and Newberne, 1977; Hodges, 1979; Hodges, 1980).

The daily requirements for vitamin A in man depend upon age and sex. Women during pregnancy or lactation have a higher requirement than those who are not pregnant or lactating. The requirement for the former varies from 800 to 1200 µg/day. Adult men usually require 1,000 µg/day; however, children of both sexes from under 1 year to 12 years old require from 400 to 800 µg/day. Ideally, boys should have 1,000 µg/day and girls 800 µg/day (Marks, 1975). However, Hodges (1980) agreed with Sauberlich et al. (1974) that the true requirement for vitamin A has not yet been firmly established. They recommended the daily allowance for adults of 800 to 1000 Retinol Equivalents (a Retinol Equivalent is

equal to 1.0 μg of retinol, or 6.0 μg of B-carotene; for practical purposes, 1 Retinol Equivalent ≅ 5 IU) and recommended a slightly higher daily allowance for lactating women.

Vitamin A exists only in animals, where it is found free and as esters of higher fatty acids. However, the carotenoids, or vitamin A precursors, are widely distributed in vegetables and are a part of the yellow and orange pigments of most fruits and vegetables (Marks, 1975). Thus, the domestic herbivora use carotene to supply their vitamin A requirement (Maynard et al., 1979).

Carotene from late-cut grass is not utilized as efficiently as that of early-cut grass. The carotene of silage may not be as effective as the carotene of hay. The species of plants and their dry-matter content may also influence carotene utilization (Maynard et al., 1979).

The lack of vitamin A in the diet is the simplest and most easily understood cause of vitamin A deficiency. However, other and more usual causes include: (1) a poor quality or inadequate volume of food consumed, producing simple starvation; (2) interference with intake arising from anorexia, mechanical obstruction, or dental disease; (3) interference with absorption of nutrients due to lack of digestive secretions as a result of pancreatic or hepatic diseases or hypermotility of the intestinal tract; (4) interference with storage, transport, or utilization of vitamin A as in hepatic, thyroid, or kidney diseases or in hypoproteinemia; and (5) increased requirements associated with pregnancy, lactation, or hyperparathyroidism. These factors have been indicated by Follis (1958), Smith and Goodman (1971), DeLuca (1978), and Maynard et al. (1979).

Pathology

Effects of hypo- and hypervitamonisis A on man and animals have been observed. Conclusions have been reported relating to the eys and vision, growth, epithelial integrity, mucus secretion, anemia, and miscellaneous disorders, such as increased cerebrospinal fluid pressure, altered bone development, and teratologic effects (Bessey and Wolbach, 1939; Scrimshaw et al., 1968; Chandra and Newberne, 1977; DeLuca, 1978; Hodges, 1979; Mejia, 1979; Hodges, 1980).

Eyes and Vision. In man, especially in infants, as in animals, vitamin A deficiency most commonly affects the eyes and vision. The eyes are affected by impairment of dark adaptation resulting in nyctalopia, or night blindness, and by corneal destructive lesions that may produce permanent blindness or severe impairment of vision (WHO Technical Report No. 590, 1976; Maynard et al., 1979).

Night blindness, associated with vitamin A deficiency, results from the failure of the rod vision cycle, as reported by Holmes (1925), Schmidt (1941), and Mitchell (1967). The photoreceptor action and its relation to vitamin A was first established by Wald (1943) and later by Morton (1969). Anderson and Hart (1943) suggested that the vacuoles seen between the rods may contain some metabolic products of the cycle of vision, which without a normal amount of vitamin A cannot be utilized. At the same time, Johnson (1943) reported that degeneration of the retina was histologically demonstrable.

As part of the widespread epithelial keratinization in vitamin A deficient rats, Wolbach and Howe (1925) described severe corneal keratinization. The blockage of lacrimal ducts with keratinized debris

and consequent dryness of the eyes (xerophthalmia) were described by Follis (1958). Keratinization of the cornea as a primary epithelial lesion of vitamin A deficiency was reported by Pirie and Overall (1972), who also detected epithelial alterations in the lens.

The blindness in vitamin A-deficient cattle was concluded by Moore et al. (1935) to result from destruction of the optic nerve in the optic foramen. Hayes et al. (1968) thought the optic nerve lesions were a consequence of increased cerebrospinal fluid pressure and altered remodeling of the sphenoid bone.

Growth. Mellanby (1944) reported that vitamin A and not vitamin D was probably responsible for growth. This observation was made during nutritional investigations relating bone growth and the nervous system. However, it was suggested that the coexistent anorexia with vitamin A deficiency or coexisting infections were responsible for the impairment of the normal growth (Hazzard et al., 1962). Evidence that the vitamin A requirement of the growing animal is related to body weight was provided by Paul and Paul (1966). Weight loss and death due to vitamin A deficiency were attributed to infection by Bieri (1969). Hayes (1971) clarified and established that the decreased growth rate in vitamin A deficiency occurred before the loss of appetite or decreased mitotic rate. Thus, there was something more fundamental than anorexia responsible for weight loss. Using germfree rats, Rogers et al. (1971) reported the cessation of the growth to be due to temporary vitamin A deficiency. There was a resumption of growth in germfree rats after additional vitamin A was included in the ration.

Vitamin A utilization in animals was directly related to the rate of growth, i.e., efficient utilization was associated with rapid growth (Corey and Hayes, 1972).

Zile et al. (1979) hypothesized that vitamin A stimulates growth by a direct role in cell replication in addition to or instead of stimulating the differentiation of epithelial and bone cells.

Epithelial Integrity. Vitamin A has long been known for its role in maintaining the integrity of the skin and other epithelial structures (Olson, 1972). Hodges (1980) concluded that metaplasia, or keratinization of epithelial tissue, a common feature of vitamin A deficiency, might be expected to render the organism more vulnerable to environmental toxins and carcinogens and may predispose the epithelium to neoplastic changes.

In early research, Wolbach and Howe (1925) assumed that keratinization of the epithelial surfaces and the squamous metaplasia could be considered pathognomonic lesions of vitamin A deficiency. Parnell and Sherman (1962) considered that the more severe the deficiency of vitamin A, the more severe were the keratinization and metaplastic processes of the epithelia. It has been speculated that vitamin A is required for the basal epithelial cell to differentiate into ciliated and mucus-secreting cells (Hayes, 1971). However, Fell and Mellanby (1953) worked with embryonic chick ectoderm cultivated *in vitro* in a medium containing excess vitamin A and observed that high amounts of vitamin A completely suppressed keratinization and caused the ectoderm to differentiate into mucus-secreting, often ciliated epithelia similar to normal nasal mucosa. Working with adult guinea pigs, Barnett and Szabo (1973) observed similar results.

The variation in intensity and distribution of the keratinization and squamous metaplasia in vitamin A deficiency have varied from species to species. Wolbach and Howe (1925) observed that many epithelial

surfaces such as those of the cornea, urinary and respiratory tracts, and salivary and pancreatic ducts promptly became metaplastic in vitamin A-deficient rats. Nielsen et al. (1966) observed a similar effect in the parotid ducts of the cow, and Langham et al. (1941) described metaplasia in the bovine urinary tract.

Urinary calculi in animals were associated with vitamin A deficiency and consequent keratinization and infection (McCollum et al., 1939; Schmidt, 1941). Tvedten et al. (1973) frequently observed urethral obstruction with absence of calculi in vitamin A-deficient rats. Zile et al. (1972) reported observations on calcium levels and urolith formation. They noted that there was a decreased urinary calcium excretion without alteration in the serum calcium levels. They thought that this decreased urinary calcium excretion may have contributed to urolith formation. However, Jawet et al. (1943) studied a large number of human patients with urinary calculi and did not find evidence of hypovitaminosis A.

Mucus Secretion. As was indicated by Nielsen et al. (1966), squamous metaplasia due to vitamin A deficiency could be detected by decreased amounts of mucus, as indicated by decreased amounts of periodic acid-Schiff-positive material in the epithelium in early stages of deficiency. DeLuca and Wolf (1970) observed a reduction in goblet cells in the small intestine of vitamin A-deficient rats. The formation of mucus in cell cultures of ectoderm was observed by Fell and Mellanby (1953) in the presence of excess vitamin A.

Becking (1973) observed decreased hepatic enzyme activities in vitamin A deficiency; however, Roels (1969) associated this problem with anorexia and impaired protein synthesis. Steward et al. (1969) reported

that the biological structure of membranes should have vitamin A as an integral part. Investigations by Mack (1975) revealed that vitamin A concentration was higher in membranes than in homogenates of cells. Weissman et al. (1963), working with excess vitamin A, and Roels et al. (1969a), working with vitamin A deficiency, both concluded that cellular membranes were weakened. A micelle theory was proposed by Lucy (1969) to try to explain the involvement of vitamin A in the fusion and rearrangement of membranes and the possibility of the communication of substances between the extracellular and cellular compartments.

Other nutrients may alter the availability and/or the utilization of vitamin A. The rate of utilization of vitamin A is much more rapid in vitamin E-deprived animals than in animals supplemented with vitamin E (Hebert and Morgan, 1953). Dicks et al. (1959) reported that the lesions produced in vitamin A-deficient rats were delayed in the rats supplemented with vitamin E. On the other hand, Rousseau et al. (1973) did not note any effects in the vitamin A-deficient calf when vitamin E was added to the diet. Duncan and Hurley (1978) reported a significant interaction between vitamin A and zinc in regard to the number of implantation sites and the proportion of fetuses malformed in rats.

Vitamin A Deficiency and Anemia. In an investigation on the relation of anemia to vitamin A deficiency, Kossler et al. (1976) concluded that blood regeneration cannot take place without vitamin A. The addition of vitamin A to a diet depleted of vitamin A brought about rapid formation of new blood cells in animals.

A number of papers making reference to anemia associated with vitamin A deficiency in man and animals have been published. Recently, Majia et al. (1977) and Hodges (1978) implied that there were indications

that vitamin A may be essential for hematopoiesis and that the effect of vitamin A may not be related directly to hemoglobin but to the availability of iron for synthesis of heme protein. Hodges (1978) observed that this anemia did not respond to medicinal iron, but it later responded to resupplementation with vitamin A. Mejia (1979) studied anemia in vitamin A-deficient rats and observed that immature rats that develop vitamin A deficiency quickly may not have evidence of anemia, probably because of the diminution of plasma volume with resultant hemoconcentration.

Miscellaneous. Several signs have been directly correlated with vitamin A deficiency in calves and the increased cerebrospinal fluid pressure. These include incoordination and syncope (Moore and Sykes, 1940) and convulsions (Mitchell, 1967). The brain volume per unit of live body weight of control calves compared to vitamin A-deficient calves was not significantly different. However, the volume of the cranial vault per unit of live weight of vitamin A-deficient calves was significantly smaller than the control calves (Gallina et al., 1970). Blakemore et al. (1957) concluded that the increased cerebrospinal fluid pressure was caused by constriction of the brain by the skull.

Edema and atrophy of the testes of vitamin A-deficient rats were reported by Wolbach and Howe (1925), and cessation of spermatogenesis and decreased libido in a vitamin A-deficient ram were noted by Dutt (1959).

Several teratologic effects have been reported, including microphthalmia in pigs characterized by small lens and retinal rosettes (Paulludan, 1961). An extra ear and toes, hydrocephalus, and cleft

palate were reported in rats (Warkani and Scraffenberger, 1944).

The pathology of hypervitaminosis A has been reviewed by Moore (1957). In human beings, especially in children, there have been published reports of hypervitaminosis A occurring after prolonged high intake. Skin changes (dry, rough skin), hepatomegaly and painful, swollen joints were present, and they disappeared on withholding the vitamin (Marks, 1975).

## Vitamin A-Infection Interrelationships

### General Considerations

An interrelationship between hypovitaminosis A and infection has been emphasized for many years, and numerous scientific investigations have demonstrated that a deficiency of this vitamin impairs, directly or indirectly, the immune system.

One of the earliest reports with adequate controls was by Blackberg (1928), who injected killed typhoid bacilli or small doses of live bacilli into rats deficient in vitamins A, D and the B-complex. He observed measurably lower titers of agglutinin and bacteriolysin, compared with values in control rats. McLaren et al. (1965) found, even in the absence of eye lesions, significantly lower levels of serum vitamin A in children who had died of protein-energy malnutrition than in children dying of other causes. He also noted low carotenoid levels in those who survived.

Guggenheim and Beuchler (1947) reported that interference with the integrity of the epithelium, particularly secretory epithelium, had an adverse effect on natural resistance to infection in animals.

Scrimshaw et al. (1968) concluded, after reviewing over 50 investigations of vitamin A-deficient animals, that both the frequency and

severity of bacterial, viral and protozoal infections were increased as a result of vitamin A deficiency.

Bang et al. (1972) and Bang et al. (1973), working with Newcastle disease virus, observed a rapid loss of lymphocytes from the thymus and bursa in vitamin A-deficient chicks. Cohen and Ellis (1974) found that rats fed a vitamin A-deficient diet had atrophy of the thymus and spleen and a poor response to diphtheria and tetanus toxoids compared to the response of control rats. In the same report, these authors stated that pretreatment of mice with large doses of vitamin A had a protective effect against later experimental bacterial infection.

Scrimshaw et al. (1968), in a special and comprehensive review on nutrition and infection, concluded that animals were more affected by disease when they were in a nutritionally deficient state (classified as a synergistic effect of malnutrition). Examples of less severe infections in nutritionally deficient animals were also observed (classified as antagonistic). Scrimshaw (1975) reported that tuberculosis was more frequently observed in vitamin A-deficient individuals and that there was a much higher frequency of complications such as bronchitis, otitis media, urinary tract infections, and bronchopneumonia. Conjunctival and corneal lesions of onchocerciasis were more common and severe in individuals on a low dietary intake of vitamin A.

The reports by Wissler (1947), Crane (1965), Newberne et al. (1968), Bang et al. (1972) and Chandra and Newberne (1977) emphasized the important role of vitamin A in the immune response. Vitamin A-deficient chicks had decreased lymphocytes and plasma cell populations in the upper respiratory tract and a failure of replacement of damaged epithelial cells. These modifications as well as the lymphocytic alterations in

the bursa of Fabricius were believed to predispose vitamin A-deficient chicks to Newcastle disease virus (Bang et al., 1972).

Hodges (1978) reported that a low concentration of vitamin A in plasma may predispose children to acute infectious diseases. He also theorized that inadequate vitamin A may be an important cause of blindness in children in Indonesia and other countries where a deficiency is frequently observed.

Parasitic infections have been investigated in relation to vitamin A status. *Schistosoma mansoni* parasites were observed by Krakower et al. (1940) to be destroyed in the liver and lungs of rats adequately supplemented with vitamin A. Yaeger and Miller (1963) reported that vitamin A-deficient rats were more susceptible to *Trypanosoma cruzi* infections and to bacterial infections than controls.

Indications that infection may precipitate a vitamin A deficiency in marginally deficient persons were described by Scrimshaw et al. (1968) and in animals by Newberne et al. (1968). In the latter report, when rats were fed a marginal amount of vitamin A and were infected with *Salmonella typhimurium*, they had signs of vitamin A deficiency.

The decreased resistance to infection is related to the effect on the integrity of the epithelial tissues as well as to alterations in the immune system. Both effects allow the penetration of pathogens through the vitamin A-deficient epithelial tissue (Scrimshaw et al., 1959).

The ciliated respiratory tract that is changed to nonciliated squamous epithelium in vitamin A-deficient animals may be a factor that will predispose animals to respiratory bacterial infection, according to Wolbach (1954), or mycoplasmal infection, as mentioned by

Tvedten et al. (1973). On the other hand, vitamin A absorption by the intestinal wall during intestinal infection may be impaired and predispose individuals to other infections, as observed by Sirakumar and Reddy (1971) and Kouwenhoven and Vander Horst (1972).

The phagocytic action of leukocytes is decreased in hypovitaminosis A in man and animals, allowing greater penetration and multiplication of pathogens into tissue (Scrimshaw et al., 1959).

Vitamin A-deficient animals are observed to be more susceptible than normal animals to the effects of bacterial toxins such as those of diphtheria, tetanus and klebsiellosis (Scrimshaw et al., 1959) or to aflatoxins (Reddy et al., 1973).

## *Trypanosoma lewisi*

### History

It is believed that Chaussat in France (1850) was the first to see *Trypanosoma lewisi* in a blood sample from black rats (*Rattus rattus*), although at that time he thought they were nematode larvae. Lewis (1878, 1879) reported a flagellate in blood samples from Indian rats. It was placed in the genus *Herpetomonas* by Kent (1880) under the name *Herpetomonas lewisi*. However, Laveran and Mesnil (1901) placed this parasite in the genus *Trypanosoma* after demonstrating all the essential characteristics of that genus. They named it *Trypanosoma lewisi*, and it is known up to the present time as the rat-trypanosome.

### Morphology

The morphological aspects of *T. lewisi* have been thoroughly studied by Minchin (1909), Taliaferro (1926), Hoare (1938), and Davis (1952). These authors described the trypomastigote blood stream forms (the

developmental stage of trypanosomes and allied flagellates) as a lanceolate shape with an elongated and flattened blade body-shape which was elliptic or oval in transverse section while its end tapered to a point. The body had a characteristic curve and was drawn out to a point at the posterior end. An undulating membrane with an attached marginal flagellum extended along the full length of the body. The free part of the flagellum was well developed and corresponded to the anterior portion of the parasite. The free flagellum measured around 7.2 to 7.8 μm, and the total length of the trypanosome varied from 21 to 36.5 μm. The breadth of the body was 1.5 to 2.2 μm. The nucleus was anterior to the middle of the body, and the kinetoplast was large and located some distance from the posterior tip of the body (Hoare, 1972).

Life Cycle

The metacyclic trypanosomes from feces of fleas enter the blood stream of the rat and transform to trypomastigotes in 4 to 6 days. Reproduction begins by multiple fission in which division is incomplete so that the newly formed individual remains attached by the posterior tip of the body, forming rosettes of trypomastigotes. When division is completed, they detach from each other and repeat the process. Eventually, division ceases and the rosettes disappear by day 8 to 9. These trypomastigotes are infective to rat fleas (***Nosopsyllus fasciatus***) when ingested with blood. The trypanosomes in the blood gradually decline in number, generally disappearing in 3 to 4 weeks. However, the time may be as short as 3 weeks or as long as 8 weeks.

In order to continue development, the trypanosomes must undergo a period of cyclic development in the rat flea to produce metacyclic

forms infective to rats. Within 6 hours after entering a flea's stomach, the trypomastigotes penetrate the epithelial cell and fold to form a U-shaped structure whose arms fuse, producing a pear-shaped body. As these bodies increase in size, the kinetoplast and nucleus divide. New flagella are formed from axones arising from daughter kinetoplasts, while the original axoneme and its flagellum persist. The flagella are scattered over the surface of the pyriform bodies, which produce 8 to 10 nuclei and many kinetoplasts. Division in epithelial cells terminates within 18 hours or it may continue 4 to 5 days.

When the infected cells rupture, the newly formed trypomastigotes enter the lumen of the stomach. They may penetrate other cells and divide again. Eventually, the small trypomastigotes migrate to the hindgut and rectum, where they transform into epimastigotes, which undergo another period of multiplication, producing various forms. The result of this period of multiplication is the production of many small metacyclic trypanosomes in the rectum. These are the infective stages voided in the feces of feeding fleas. Rats attempting to remove the biting fleas lick the area and, in the process, swallow the feces containing the metacyclic trypanosomes, which initiate infection in susceptible animals. Description of these steps was obtained from the classical literature and from the observations of Hoare (1972).

*Trypanosoma lewisi* in the posterior portion of the gut of the insect is characteristic of a group of trypanosomes, including *Trypanosoma melophagium* of sheep which is found in sheep ticks, *T. theileri* of cattle in tabanids, and *T. cruzi* of man and other animals in triatomid bugs. This "posterior station" type of development is believed to be the more primitive as compared with "anterior station" type of development of salivarian types of African trypanosomes.

Clinical transmission of salivarian trypanosomes is by *Glossina* spp (*Diptera, Muscidae:* tsetse flies). The ingestion of the trypanosomes from the mammalian host may (1) establish in the mouth parts only, multiplying as blastocrithidial forms and producing infective trypanosomes (subgenus *Duttonella*); (2) establish in the midgut first, multiplying as trypanosomes, then in the mouth parts in the blastocrithidial form and then producing infective trypanosomes as occur in the subgenus *Nannomonas*; or (3) finally, the third subgenera *Pycnomonas* and *Trypanozoon*, which have similar development as observed in the subgenus *Duttonella*, except that the blastocrithidial multiplication and infective trypanosome development is in the salivary glands (Weinman and Ristic, 1968).

Transmission in the salivaria is less "efficient" than in the stercoraria, as much smaller proportions of the vectors ingesting trypanosomes ultimately develop metacyclic trypanosomes (Weinman and Ristic, 1968).

The relative ease with which *T. lewisi* can be maintained in the laboratory in albino rats, with periodic blood transfers to new rats, has provided investigators with a good experimental model. As a result, much has been learned about the physiology of these trypanosomes.

## Pathology and Host-Parasite Relations

*Trypanosoma lewisi,* which is a natural parasite of rats, utilizes the rat flea, *Nosopsyllus fasciatus,* in temperate areas and *Xenopsylla cheopis* in tropical and subtropical areas as intermediate hosts and biological vectors (Faust, Beaver and Jung, 1975; Hoare, 1972; Ferrant and Jenkin, 1979). While *T. lewisi* is relatively harmless, it may

become pathogenic to its natural host either spontaneously or after "reinforcement" by rapid rat passages (Lavier, 1943).

This form of trypanosomiasis is a widely distributed parasitic infection of rats, and it has been studied critically by many researchers with a view to understanding other trypanosomiases affecting man and animals.

The features of the transmission of the trypanosomes by vectors to mammalian hosts have had a particular influence in their success as causes of infection (Hoare, 1972). He pointed out that fecal contamination, as observed in the stercoraria group, is an uncertain method of transmission, because the chances of infections are related to the infective droppings being deposited by the vectors on some vulnerable part of the host's body, such as the mucous membrane or abraded skin, through which the infective trypanosome forms must penetrate by their own efforts. This situation, on the other hand, is compensated for by the high infection rate in the vectors and the fact that these biological vectors are ectoparasites living temporarily or almost permanently on the body of the host, like fleas on rats or sheep-keds on sheep (Hoare, 1972).

The establishment of the infection in the host is influenced in general by the capacity of the parasite to be infective to one species, as with monoxenous parasites such as *T. lewisi*-like trypanosomes. The oligoxenous organisms can parasitize in several related groups of hosts, as does *T. theileri* in wild and domestic Bovidae, *T. vivax* in ruminants, *T. suis* and *T. simiae* in Suidae, and *T. equiperdum* in Equidae. The polyxenous parasites are infective for various unrelated mammals, among which are *T. congolense, T. brucei, T. evansi,* and *T.*

*cruzi*. *Trypanosoma rangeli*, a nonpathogenic species, can be infective for several unrelated species (Hoare, 1972).

The pathogenicity of *T. lewisi* was first observed under laboratory conditions by Roudsky (1911), who increased virulence of a strain by rapid passages in rats. After these passages it was possible to transmit the organism to mice and other rodents. Biot and Richard (1912) were successful in producing infection with *T. lewisi* in jerboas and dormice. Guinea pigs were successfully infected by Coventry (1929). When normal rat serum was administered to mice daily, Lincecome (1960) was successful in maintaining *T. lewisi* in mice for several years. After numerous serial passages, the virulence was so increased that it could kill this heterologous host. It was of interest that this parasite had not lost its infectivity for the normal rat host. These observations were confirmed by Muhlpfordt (1968), who in 1969 infected gerbils (*Meriones unguiculatus*) with *T. lewisi* without supplemental rat serum. The effect of the rat serum was apparently related to a specific growth factor required by *T. lewisi* (Lincecome, 1961).

Fugiwaro and Suzuki (1967) reported spontaneous arthritis associated with *T. lewisi* in 4- to 5-week-old albino laboratory rats. No organisms were detected in a culture of exudate collected from the affected joints. However, innumerable trypanosomes were observed in a smear prepared from the joint exudate. They observed that hindpaws were more often affected and the tibiotarsal articulations were most severely affected.

Thoongsuwan and Cox (1978) demonstrated that *T. lewisi* produced anemia, splenomegaly, and glomerulonephritis associated with autoantibody in laboratory rats.

Only one human infection has been attributed to *T. lewisi*. It occurred in a 4-month-old Malayan child who suffered from fever for 5 days and had numerous trypanosomes in his blood. These trypanosomes were indistinguishable from *T. lewisi*. They disappeared from the blood after the fever abated. The rats infesting the child's home were harboring *T. lewisi*. However, an attempt to infect rats from samples from the child failed (Johnson, 1933). There has been some speculation about this case, and Brumpt (1949) suggested that the trypanosome might have been a simian parasite that had temporarily established itself in man. Weinman (1970), who reported unidentified trypanosomes in Malayan macaques, suggested that human beings in Asia might harbor cryptic infections with one of them (thus reviving Brumpt's theory). However, Zeledon (1954) conceived that the accidental infection of the child in question might have been favored by a vitamin deficiency or other factors. That the *T. lewisi*-like parasite might have been *T. rangeli* was suggested by Baker (1970). However, *T. rangeli* is known only in South America.

Weinman and Ristic (1968) affirmed that among both stercoraria and salivaria there were also nonpathogenic parasites. In stercoraria *T. cruzi* is an important pathogen of man in the New World. No other species of stercoraria are really of economic significance. *Trypanosoma theileri* has occasionally appeared as a cause of an acute disease, as in the course of immunization of cattle against rinderpest with materials that probably contain trypanosomes (Hornby, 1952). Salivarian infections in wild Bovidae are also, typically, nonpathogenic as far as is known (Lumsden, 1962). On the other hand, the pathogenic effect of salivarian trypanosomes on cattle in Africa is seen as a devastating effect, of quite extraordinary importance, in large areas of Africa to

all livestock except poultry (Hornby, 1952). Wilson et al. (1963) estimate that the size of the area in Africa virtually devoid of cattle as a result of this pathogen is approximately 10.4 x $10^6$ $km^2$. Considering that most of the area concerned is of average fertility and estimating its carrying capacity at 12 head of cattle per $km^2$, they calculated that it could support 125 x $10^6$ head of cattle, or 11 x $10^6$ *more* than the 1962 estimate of the *total* cattle population of Africa (114 x $10^6$ animals).

The infection in different areas varies according to the strain of parasite and the resistance of the host. The virulence of a given strain appears to be a stable property. In some localities the prevalent disease was severe and of short duration, while in others it ran a chronic course (Hornby, 1952). In Nigeria, the infection in Zebu cattle has resulted in 100% mortality 3 to 4 months after an exposure to tsetse flies (Unsworth, 1953), while in Venezuela outbreaks of acute infection with 30% mortality were more common (Kubes, 1944). However, in some cases (e.g., the Congo [van Hoof et al., 1947]), the disease in calves may be subacute for 2 months, after which the trypanosomes disappear from the blood and the cattle recover spontaneously.

*Trypanosoma vivax* was infective to rats and rabbits when the animals were administered whole sheep blood or normal serum within 24 hours after inoculation of the trypanosomes (Desowitz and Watson, 1952). Unsworth (1953) got adaptation of *T. vivax* in the rat after 37 subpassages, and Taylor (1968) had adaptation success with mice. Desowitz (1963) reported that the factor in the blood supplement which facilitates infectivity of rodents with *T. vivax* was a serum protein fraction

which protects the trypanosomes against the antibodies elaborated by the rat.

## *Pseudomonas aeruginosa*

### History

From the classical and contemporaneous literature on ***Pseudomonas aeruginosa***, we find that the generic name was created in 1894 by Migula for rod-shaped, gram-negative, nonsporeforming, polarly-flagellated bacteria (Palleroni, 1978). The 1920's were particularly important in the history of the genus of ***Pseudomonas*** because of the research of den Dooren de Jong (1926, in Palleroni, 1978). His research dealt with the microbiological process of mineralization of organic matter in soil, i.e., participation of the microorganisms in the transformation of organic matter into carbon dioxide. The latter then became available for new cycles of transformation in biological systems. He also reported the formidable nutritional versatility of ***Pseudomonas*** strains.

The genus ***Pseudomonas*** was listed in the first edition of ***Bergey's Manual of Determinative Bacteriology*** (Palleroni, 1978). Jessen (1965), in research on ***P. aeruginosa***, considered some of the difficulties of outlining a rational taxonomic system for the genus. Presently the genus is composed of many species, which are defined mainly on the basis of growth, biochemical characteristics, and production of pigments.

The production of colored pigments by the ***Pseudomonas*** organism has received much attention (Moffet, 1980). However, many permanently non-pigmented species are now included in this genus. Fluorescence associated with pigment production was a characteristic of strains which were recognized by Krassilnikov (1959) in his study of more than 200 species.

At the present time, considerable expertise and laboratory facilities are required for the precise identification of all species belonging to this genus.

Morphology and Bacteriology

*Pseudomonas aeruginosa* is a gram-negative, aerobic rod which is catalase positive and splits sugars by oxidation. The rods vary in size from 0.5-1.0 to 1.5-4.0 μm, and they are motile by means of a simple polar flagellum (monotrichous) or by multiple polar flagella (multitrichous) (Franklin and Franklin, 1971; Carter, 1979; Moffet, 1980). They are not fastidious in their growth requirements, growing usually abundantly on blood-agar, tryptose agar, or trypticase soy-agar and less complex media (Carter, 1979). The colonial and morphological aspects on agar plates are rough, smooth or mucoid. Freshly isolated *P. aeruginosa* will vary from rough to smooth, and some cultures from lesions of patients with cystic fibrosis usually produce mucoid type colonies (Franklin and Franklin, 1971).

Three species of this genus, *P. aeruginosa, P. pseudomallei,* and *P. mallei,* are considered important pathogens for man and animals (Carter, 1979). *Pseudomonas pseudomallei* is the etiological agent of melioidosis, which is a disease of man and animals that varies from a benign pulmonary form to a systemic form with visceral abscesses and a terminal septicemia. This disease occurs in the southwest Pacific area in a number of countries, including Burma, Thailand, Vietnam, Indonesia and Australia. Actually, some cases have been observed in the United States, in individuals who emigrated from these areas, and also in soldiers who returned from Vietnam. *Pseudomonas mallei,* the cause of glanders of the Equidae and man, is encountered

mainly in remote areas of Asia and Africa. Its growth is stimulated by the addition of glycerine to the media, so glycerol is sometimes used (Franklin and Franklin, 1971; Carter, 1979).

Pathology

*Pseudomonas aeruginosa* is considered to have very little invasive power, except under exceptional circumstances (Jawetz, 1952). *Pseudomonas aeruginosa* was instilled into the ears of volunteers without giving rise to clinical infection (Perry and Nichols, 1969). Buck and Cooke (1969) administered *P. aeruginosa* in milk to 3 normal volunteers without causing clinical symptoms. Intact corneal epithelium also appears to be highly resistant to *P. aeruginosa*, according to Vaughan (1955). He pointed out that when the organism gained effective entrance through an injury to the corneal epithelium it would cause severe and permanent damage. Even minute abrasions, such as those due to the improper use of contact lenses in human beings, can result in *P. aeruginosa* infection (Gerke and Maglioco, 1971). *Pseudomonas aeruginosa* injected intraocularly established an infection in the rabbit and caused panophthalmitis (Riegelman, Vaughan and Okumoto, 1957; Crompton, Anderson and Kennare, 1962). Rogers (1960) named *P. aeruginosa* as being the most dangerous cross-infecting pathogen in a large children's hospital.

Carter (1979) referred to *P. aeruginosa* as responsible for wound infections, "bull nose" and atrophic rhinitis in swine, mastitis in cattle, abortions in cows and mares, otorrhea in dogs and cats, and septicemia in poultry. Recently, Moffet (1980) mentioned that *P. aeruginosa* caused otitis externa, pneumonia, urinary tract infection and osteomyelitis in humans. At the same time, he referred to

vasculitis and deep subcutaneous abscesses occurring as a result of the use of poor sterilization technique. Dorff et al. (1971) observed skin lesions in humans, which were the first manifestation of a papular rash, followed by vesicles, then black or purple ulcerations. The condition was called ecthyma gangrenosum.

Epidemiology

*Pseudomonas aeruginosa* is widely distributed in nature, especially in moist environments. It is physiologically versatile in that it can survive and multiply in a variety of contaminated materials. This is made possible by its ability to metabolize a variety of carbon compounds and to utilize ammonium compounds as a source of nitrogen (Franklin and Franklin, 1971). Multiplication occurs over a range of 10 to 43 C, with an optimum growth temperature of 37 C (Stanier et al., 1966). These properties are of great epidemiological significance, because they contribute not only to survival of the organism in hospital environments but also permit proliferation to occur. *Pseudomonas aeruginosa* is reported to be resistant to several widely used disinfectants and preservatives. It will not only survive but also actually multiply in some concentrations of quaternary ammonium compounds, especially if they contain organic matter (Adair et al., 1968). Benzalkonium chloride and its derivatives are among the disinfectants that are ineffective against *Pseudomonas* spp (Adair et al., 1968; Hardy et al., 1970). Other disinfectants such as cetrimide (Lowburry, 1951), chlorhexidine (Moore and Forman, 1966; Rogers, 1960), hexachlorophene (Ayliffe et al., 1969), and chlorxylenol have also been found to be ineffective under usual conditions (Lowburry, 1951; Rogers, 1960). The resistance of *P. aeruginosa* to many broad-spectrum antibiotics

tends to favor its growth in patients treated for other infections. Consequently, patients are superinfected by *Pseudomonas* strains either from endogenous or exogenous sources (Franklin and Franklin, 1971).

On the other hand, *P. aeruginosa* infections are uncommon in healthy persons with intact body defense mechanisms. Infants, the elderly, and those patients with serious underlying diseases, especially of metabolic, hematologic, or malignant etiology, are especially susceptible (Freid and Voist, 1968; McCabe and Jackson, 1962; Altemeier et al., 1966). *Pseudomonas* spp may be isolated in urinary and respiratory tract infections and from infected wounds. Prolonged catheterization of the urethra, intravenous techniques, tracheostomies, and respiratory supportive procedures are reported by Sootter et al. (1966) and Tabot (1969) to predispose to *P. aeruginosa* infection.

Treatment involving corticosteroids, antimetabolites or immunosuppressive compounds is also significant in predisposing patients to gram-negative infections, including *P. aeruginosa* (Altemeier et al., 1966; Forner et al., 1958).

Severe burns are often colonized by *P. aeruginosa* soon after the patient is hospitalized, and a bacteremia is usually observed about 5 days after the burn, when the fluid in the tissues is absorbed into the circulation (Rabin et al., 1961; Edmonds et al., 1972).

*Pseudomonas aeruginosa* may be involved in osteomyelitis, in puncture wounds in the foot, in hematogenous infections of a vertebra or clavicle, and complications related to heroin addiction (Lewis et al., 1972). Swimmer's rash associated with heated swimming pools is most frequently attributed to *P. aeruginosa* and is characterized by itching and occasional pustules (Washburn et al., 1976).

*Pseudomonas aeruginosa* has been isolated from several different sources in hospitals, including water faucets (Shooter et al., 1969), bottled medicines and lotions (Lowbury, 1951; Shooter et al., 1969), cold foods (Shooter et al., 1969), brushes (Ayliffe et al., 1969), oral thermometers (Johanson et al., 1969), liquid soaps, antiseptic creams, ward utensils, sinks (Ayliffe et al., 1969), and saline and distilled water (Lowbury, 1951). All these sources have a common property, viz., a moist environment, which appears to be important for the multiplication of the *P. aeruginosa*.

In a retrospective investigation of *P. aeruginosa* infections conducted in a general hospital in Brazil during the years 1966, 1968, and 1969, contamination with this organism in the oxygen humidifying bottle used in surgery and the dextrose solutions used in infant feeding was disclosed. That many hospitals have similar problems with *P. aeruginosa* is evident from the number of publications reviewed by Thomas et al. (1975).

## Summary

The literature provides evidence that vitamin A is an important nutrient throughout the world because of its role in maintaining optimum health of man and animals and becuase of rather limited amounts in natural foods. It has a specific role in maintaining the integrity of epithelial tissues and in resistance to infection. Only limited information is available delineating the role of vitamins in resistance to infectious diseases. Additional information on how vitamin A provides resistance to diseases would have beneficial results in improving the health of human beings and animals.

There is ample evidence that the bacterium *Pseudomonas aeruginosa* is the cause of a number of important diseases in man and animals. The protozoan *Trypanosoma lewisi* is a common parasite of rats. The pathogenicity of both these organisms is increased when individuals or animals are stressed in various ways, including a deficiency of vitamin A. Additional research on the role of vitamin A in the pathogenesis of *T. lewisi* and *P. aeruginosa* infections would aid in our understanding of these and similar infections and ultimately lead to improvement in the health of man and animals.

# OBJECTIVES

The objectives of this research were:

1. To determine the influence of vitamin A deficiency on the susceptibility of the rat to *Trypanosoma lewisi* infection.

2. To evaluate the lesions of *T. lewisi* as influenced by vitamin A deficiency.

3. To observe the influence of feeding different amounts of vitamin A to rats on susceptibility to a spontaneous exposure of *Pseudomonas aeruginosa*.

4. To provide specific material to improve my basic training in pathology so as to strengthen my future research in public health in Brazil.

# MATERIALS AND METHODS

Two experiments to evaluate the effects of dietary vitamin A levels on parasitic (*Trypanosoma lewisi*) and bacterial (*Pseudomonas aeruginosa*) infections were conducted using a total of 152 young, male, Sprague-Dawley strain rats. These experiments were carried out in 2 different locations in 1979 but under the same general experimental conditions, the first in facilities of the Food Science Department and the second in Building 5 of the Veterinary Research Farm.

## Experimental Design

### Production of Vitamin A-Deficient Young, Male Rats for Experiment 1

Twenty female, Sprague-Dawley strain rats in the first day of pregnancy and certified free of *Haemobartonella muris* were purchased from a commercial source.[a] The mean body weight was 261.00 ± 20.73 g (SEM). These rats were housed in individual cages in facilities of the Food Science Department, where the temperature was 20 to 30 C and the humidity 50 to 60%. Light was provided for 12 hours each day. These female rats were fed a semipurified vitamin A-deficient diet

[a]Spartan Research Animals, Inc., Haslett, MI.

diet containing all required nutrients for the rat except vitamin A (Table 1). Food and water were available *ad libitum*.

The baby rats born of the dams fed the vitamin A-deficient diet during pregnancy were weighed at 1 day of age and at weekly intervals until the end of the experiment. At weaning, 84 of 105 young, male rats were selected by a randomized process, placed in individual cages, and fed the same vitamin A-deficient diet and under the same conditions as their mothers until clinical signs of vitamin A deficiency were observed.

At approximately 6 weeks of age, clinical signs of vitamin A deficiency were present in these 84 male rats. The clinical signs included retarded growth, rough haircoat and weakness. At this same time, an infection with *P. aeruginosa* spontaneously occurred in these vitamin A-deficient rats. There was no evidence of this infection in any other rats in the facilities where approximately 10 other nutritional experiments were being done. The *P. aeruginosa* infection was limited to the vitamin A-deficient rats. At first, the rats were treated orally with tetracycline, and there was no improvement during a 3-day period. This was followed by injections of gentamicin, and rats continued to die during a 10-day period. The subcutaneous injection of a calculated requirement for 1-day dose of retinol (0.5 IU/100 gm of rat body weight) stopped the losses within 4 to 5 days. In order to maintain the rats on the deficient vitamin A diet from this time until the end of the experiment, 1 group (17 rats) was fed 1 IU of vitamin A (retinol)/kg of ration. A second group (17 rats) was fed 5 IU of vitamin A (retinol)/kg of ration, which was considered to satisfy the requirements for vitamin A, and a third group (17 rats) was fed 50 IU of vitamin A (retinol)/kg of ration. Rats were fed

Table 1. Percentage composition of vitamin A-deficient, normal, and supplemental semipurified diets

| | Deficient Diet | Normal Diet | Supplemented Diet |
|---|---|---|---|
| Casein (vitamin free) | 29.6 | 29.6 | 29.6 |
| Sucrose | 57.5 | 57.5 | 57.5 |
| Corn oil | 7.5 | 7.5 | 7.5 |
| Mineral mix* | 4.0 | 4.0 | 4.0 |
| Vitamin mix** | 1.0 | 1.0 | 1.0 |
| Methionine | 0.4 | 0.4 | 0.4 |
| Vitamin A IU as retinol/kg diet | 0 | 5 | 50 |

*Mineral mix used was Hegsted Salt Mix No. 4.

**Vitamin mix used was Vitamin Diet Fortification (less vitamin A) by ICN Nutritional Biochemicals, 26201 Miles Road, Cleveland, OH 44128.

their respective amounts of vitamin A for 2 weeks prior to exposure to *T. lewisi*. An additional group of 6 rats from Building 5, Veterinary Research Farm, that were of the same strain and age but had been fed a commercial ration and had no history of *P. aeruginosa*, were included as an additional set of controls. They were fed the ration containing 5 IU of vitamin A (retinol)/kg ration. Table 2 summarizes the experimental design.

*Trypanosoma lewisi* Experimental Infection

The *T. lewisi* strain was obtained from Dr. Herbert W. Cox, Michigan State University, who organized and supervised the infection procedures. The design of the experiment was a 3 x 2 factorial experiment, as given in Table 2. Rats in the infected groups were each inoculated intraperitoneally with 1 ml of solution containing $10^6$ trypanosomes obtained from the blood of previously infected rats, as described earlier (Cox, 1964). The inoculation day was considered day 0 of the experiment.

Necropsy and Pathologic Examination

The schedule for necropsies of *T. lewisi*-infected and control rats is indicated in Table 2. Just before necropsy, blood samples for serum vitamin A and hematologic examinations were obtained by severing the end of the tail or by cardiac puncture under ether anesthesia. The rats were then killed by exposure to chloroform.

Following systematic examination of organs of each carcass for gross abnormalities, the spleen and liver were collected and weighed individually on a top-loading balance.[b] Portions of spleen, liver,

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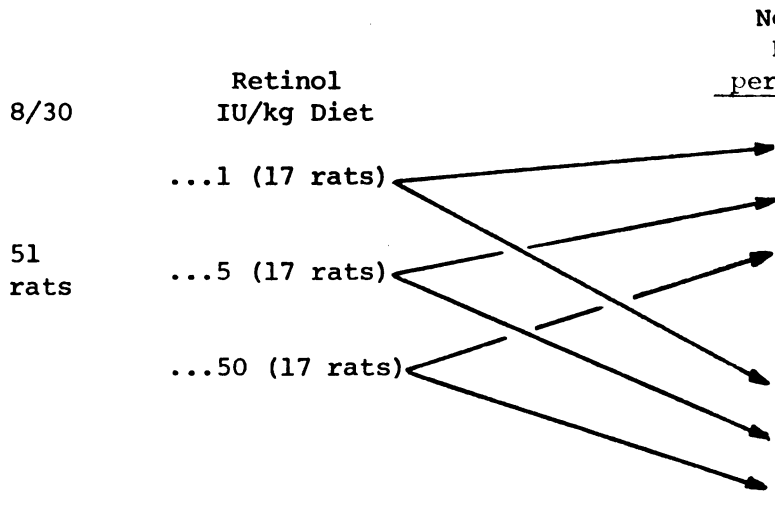
[b]Mettler Top-Loading Balance-P163, Mettler Instruments Corp., Princeton, NJ.

Table 2. General design for Experiment 1 and necropsy schedule

A. Schedule of Dietary Treatments

| 5/17 | 6/6 | | 6/28 | 7/17-8/16 | 8/17-8/30 | 8/30 RETINOL |
|---|---|---|---|---|---|---|
| Gestation Vit. A Def. Diet 20 Females | Birth | Nursing Period Vit.A Def.Diet | Weaned 105 Males Selected 84 | Clinical Vit.A Signs & Spontaneous *P. aeruginosa* | Selection of 51 Male Rats from 84 & Acclimatization to the 3 Different Vit.A Diets | 1 IU/kg diet (17 rats)<br>5 IU/kg diet (17 rats)<br>50 IU/kg diet (17 rats) |

B. Exposure to *Trypanosoma lewisi* and time and number of rats at necropsy




| No. of Rats per Group | Time of Necropsy: Noninfected 8/30(0) | 9/9(10th) | 9/22(22nd) |
|---|---|---|---|
| 6 | 2 | 1 | 3 |
| 6 | 2 | 1 | 3 |
| 6 | 2 | 1 | 3 |
| 6* | 3 | 0 | 3 |
| | Infected with *T. lewisi* | | |
| 11 | 0 | 3 | 8 |
| 11 | 0 | 3 | 8 |
| 11 | 0 | 3 | 8 |

*Controls from Building 5, not previously exposed to *P. aeruginosa*; all others were fed 5 IU vitamin A (retinol)/kg diet.

trachea, parotid salivary gland, pancreas, kidneys, urinary bladder, skin, bone marrow, adrenals, and both eyes were taken and fixed in 10% buffered formalin.

### Histopathologic Procedure

The tissues were embedded in paraffin, cut into 6 μ sections, and stained with hematoxylin and eosin stains for routine histologic examination. The oil red O stain for fat and the periodic acid-Schiff stain for glycogen were used on sections of liver (Luna, 1968).

### Erythrocytes and *T. lewisi* Counts

Trypanosomal parasitemia and anemia in the rats were determined by microscopic examination of blood drawn from the cut tip of the tail at 2-day intervals during the 22-day period on the same 3 rats in each treatment group. The blood was diluted in Sahli hemacytometer pipettes with Hayem's fluid. Erythrocyte and trypanosome counts were made with a Neubauer hemacytometer counting chamber. At the same time, samples were collected into heparinized microtubes for packed cell volume (PCV) and hemoglobin determinations.

## Vitamin A Analysis

### Liver Samples

Two 1 gm samples of liver were obtained from each rat. One sample was used for vitamin A analysis and the other was used to determine the dry weight.

Extraction and Assay Procedure. One gram of liver sample was homogenized in distilled water so that a total volume of 5 ml was

obtained.[c] A 0.5 ml aliquot was pipetted from this homogenized mixture into a 20 x 125 mm screw-cap tube (with teflon liner in cap) and extracted with 4.5 ml hexane for 5 minutes on a vortex mixer. Tubes were stoppered and centrifuged at 1000 x g for 10 minutes. The supernatant was then filtered through a 0.45 millipore filter. After filtering, 100 µl of the hexane extract was placed on a microporosil column and chromatographed with 60:40 hexane:chloroform solvent at 2.5 ml per minute in an isocratic system. Fluorometric detection of vitamin A forms was accomplished using 330 and 470 nm for the extraction and emission wavelengths, respectively.

Dry Weight Procedure. One gram of liver sample was weighed in an aluminum weighing pan and placed to dry at 56 C in an oven for 48 hours. After drying, the sample was weighed.

Serum Samples

One milliliter of the serum collected at necropsy was mixed with 1 ml of 100% ethanol on a vortex for 5 seconds. Two milliliters of hexane were then added and mixed on the vortex for 1 minute. Centrifugation at 1000 x g for 10 minutes followed, and the supernatant was filtered through a 0.45 millipore filter. The sera were assayed in the same manner as the liver samples.

Production of Vitamin A-Deficient Young, Male Rats for Experiment 2

For Experiment 2, to confirm the role of *P. aeruginosa* in vitamin A deficiency, 62 male, weanling rats were selected from females fed

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[c]Polytron, Kinematica Gm BH, Type PT 10/35, Brinkmann Instruments, Westbury, NY 11590.

the vitamin A-deficient diet during pregnancy and maintained the same as in Experiment 1.

After weaning, the 62 rats were divided into 2 groups of 31 each and were assigned "noninfected" and "infected" designations. Each group was then subdivided into 4 groups of 12, 7, 6, and 6 rats. They were fed the semipurified vitamin A-deficient diet containing 0, 1, 5, and 50 IU of vitamin A (retinol)/kg of diet, respectively. The experimental design is summarized in Table 3. More rats were included on zero amounts of vitamin A because of the higher expected death losses in this group. These rats were housed in groups of 6 or 7 in plastic cages with wire-mesh tops. Food and water were available *ad libitum*, and the environmental conditions were in general the same as those of Experiment 1.

*Pseudomonas aeruginosa* Infection

The *P. aeruginosa* culture for exposure was supplied by Dr. G. R. Carter. It was to be instilled in the nostril of the rats when signs of vitamin A deficiency were observed. However, approximately at the same time that vitamin A clinical signs were observed (Weakness, rough haircoat, porphyrin accumulation around eyes) during the 7th week of age (as in Experiment 1), *P. aeruginosa* infection spontaneously occurred in all vitamin A-depleted rats. Therefore, it was not necessary to instill the *P. aeruginosa* culture that had been prepared. The infection did not affect rats fed higher levels of vitamin A or other rats in the room.

Detailed records were maintained on incidence, duration of illness, morbidity and mortality. Tissues for examination and other procedures were as in Experiment 1.

Table 3. Design of Experiment 2: rats fed different amounts of vitamin A and exposed to *P. aeruginosa*

| | Vitamin A (IU/kg) | | | |
|---|---|---|---|---|
| | 0 | 1 | 5 | 50 |
| Noninfected | 12 | 7 | 6 | 6 |
| Infected | 12 | 7 | 6 | 6 |

## Statistical Analysis

The data collected were analyzed following orientation by Professor Cress. The Statistical Package for Social Sciences (SPSS-Northern University) at the Michigan State University Computer Center was used.

# RESULTS

## Experiment 1 - Vitamin A-Deficient Rats Infected with *Trypanosoma lewisi*

### Clinical Signs

Dams fed the vitamin A-deficient diet, starting on the first day of pregnancy, did not have clinical signs of vitamin A deficiency during gestation or lactation. In the young rats, the earliest clinical signs of vitamin A deficiency appeared when they were 51 days old. The signs of vitamin A deficiency included porphyrin accumulation around the eyes (Figure 1), xerophthalmia and corneal ulceration (Figure 2). In addition, there was a rough haircoat (Figure 3), weakness and incoordination (Figure 4). Sneezing was the first sign of *Pseudomonas aeruginosa* infection and was observed at the same time as signs of vitamin A deficiency. Despite treatment of each affected rat, 33 of the 84 rats selected for this research died. The 51 vitamin A-deficient rats that remained were considered in satisfactory condition to be used. After the *P. aeruginosa* infection subsided, all rats were fed a diet containing 1 IU (International Unit) of vitamin A (retinol)/kg of ration.

After exposure to *T. lewisi*, inactivity and inappetence were more prevalent among infected rats than among rats that were noninfected.

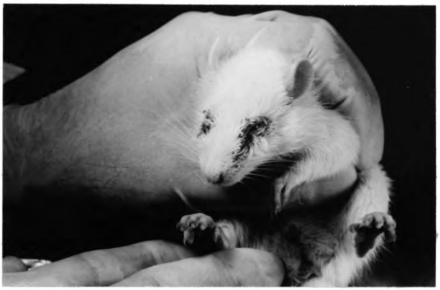

Figure 1. Photograph of 51-day-old rat which was fed a vitamin A-deficient diet since birth. Notice porphyrin accumulation around the eyes.

Figure 2. Photograph of a 57-day-old rat which was fed a vitamin A-deficient diet since birth. Notice xerophthalmia and corneal ulceration.

Figure 3. Photograph of a 51-day-old rat which was fed a vitamin A-deficient diet since birth. Notice rough haircoat.

Figure 4. Photograph of a 58-day-old rat which was fed a vitamin A-deficient diet since birth. Notice crossing of front legs as evidence of incoordination.

Growth

The growth rate of rats born of dams fed the vitamin A-deficient diet was compared with data for normal rats.[d] Rats fed a natural diet under commercial conditions weighed 0.8 gm more at birth than the rats born to dams fed the vitamin A-deficient diet. At weaning, the weight difference was 13.8 gm in favor of rats fed the natural diet. The greatest weight difference was at 6 to 9 weeks of age, which coincided with the time of *P. aeruginosa* infection. By 12 weeks of age, the rats fed the commercial diet were 89.0 gm heavier than those born to dams fed the vitamin A-deficient diet (Figure 5).

Laboratory Findings

Microbiologic Findings. *Pseudomonas aeruginosa* was isolated from rats which were necropsied during the infection with *P. aeruginosa*. Antibiotic sensitivity testing indicated that gentamicin was the appropriate antibiotic for this particular infection. However, treatment did not result in noticeable clinical improvement. Previously, tetracycline had also failed to give a response. However, after just 2 days of vitamin A administration, clinical signs diminished, and death losses started to decrease.

Data on mortality related to infection with *P. aeruginosa* are summarized in the Appendix (Table A1). The small amount of vitamin A seemed to prevent further losses due to *P. aeruginosa*.

Hematologic Findings. The results of erythrocyte counts from blood samples collected from rats fed different amounts of vitamin A

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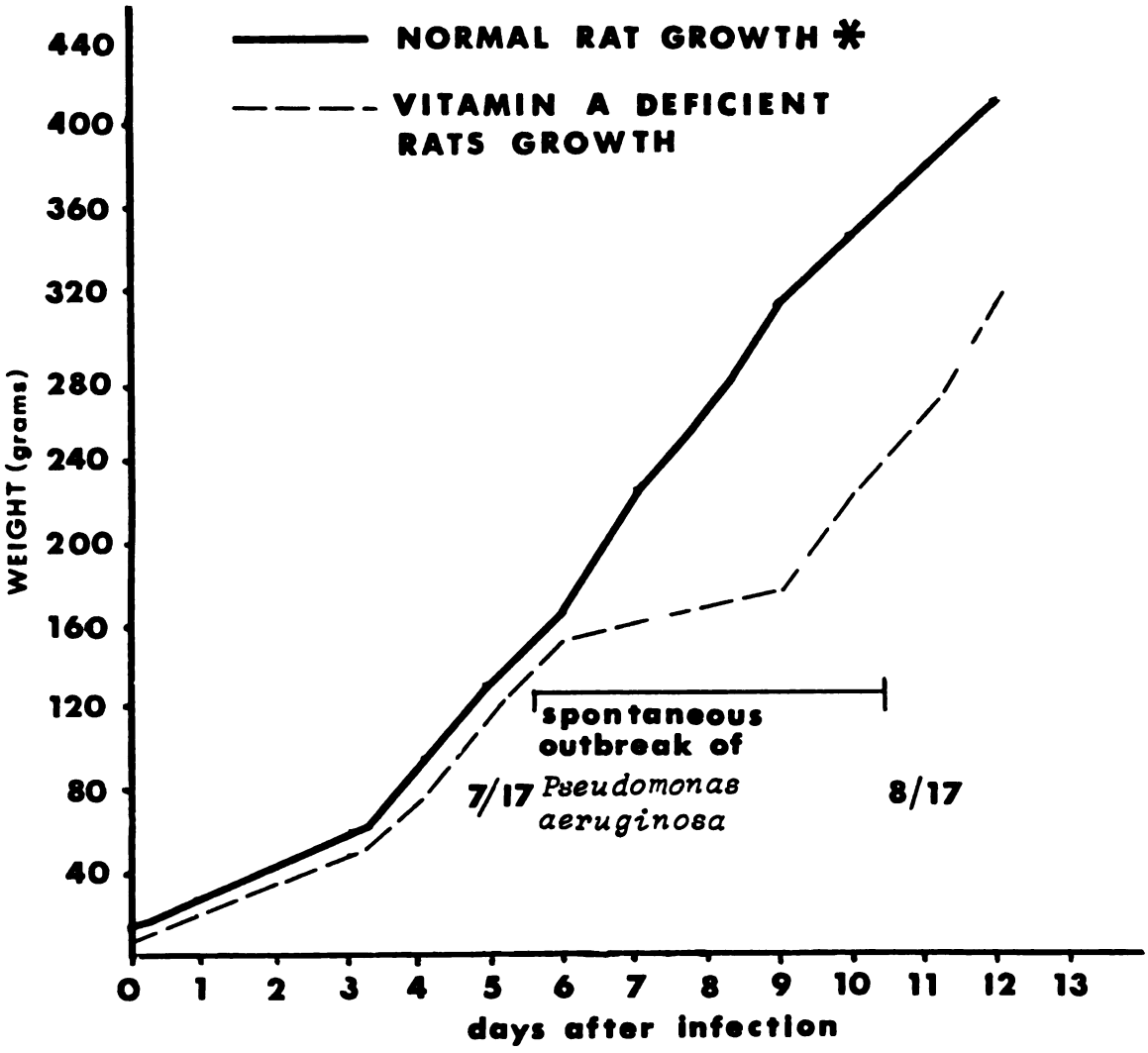
[d]Data for normal rats provided by Spartan Research Animals, Inc., Haslett, MI.

FIGURE 5. GROWTH RATES OF RATS FED CONVENTIONAL DIETS IN COMPARISON TO GROWTH RATES OF RATS FED VITAMIN A DEFICIENT DIETS




*DATA PROVIDED BY SPARTAN RESEARCH ANIMALS, INC., HASLETT, MICHIGAN.

(retinol)/kg of ration did not indicate any significant differences when compared with erythrocyte counts of rats fed a commercial diet and which did not have *P. aeruginosa* (Appendix, Table A2). Significant differences were not apparent on results of determination of PCV or hemoglobin concentration (Appendix, Table A3). On the other hand, all rats fed 1, 5, or 50 IU of vitamin A (retinol)/kg of ration and infected with *T. lewisi* had a significant decrease in erythrocyte counts after the 4th day of infection, when trypanosomes were observed in the blood smear. There was a suggestion that the increased amounts of vitamin A in the rats' diets delayed the onset of the anemia (Figure 6). Noticeable decrease in erythrocyte numbers was on the 6th day of infection in rats fed 1 IU of vitamin A (retinol)/kg of ration. The decrease was on the 7th and 8th days of infection, respectively, in rats fed 5 or 50 IU of vitamin A (retinol)/kg of ration. Packed cell volume and hemoglobin concentration of blood samples collected on days 0, 5, 6, 7, 17 and 21 were not consistent with the erythrocyte counts (Appendix, Table A3).

Trypanosome counts were done concurrently with erythrocyte counts in the infected rats (Figure 7). The parasites were present in the blood of all infected rats on the 4th day of infection (Figure 8). The peak of parasitemia was on the 8th day of infection. The decrease in parasitemia was gradual in all rats. However, on days 18 and 20, no trypanosomes were detected in blood samples of rats fed 5 and 50 IU of vitamin A (retinol)/kg of ration. Rats fed 1 IU of vitamin A (retinol)/kg of ration still had parasites in blood samples on the 22nd day of infection. Figure 8 summarizes these observations. The parasitemia was in inverse relationship to the erythrocyte count.

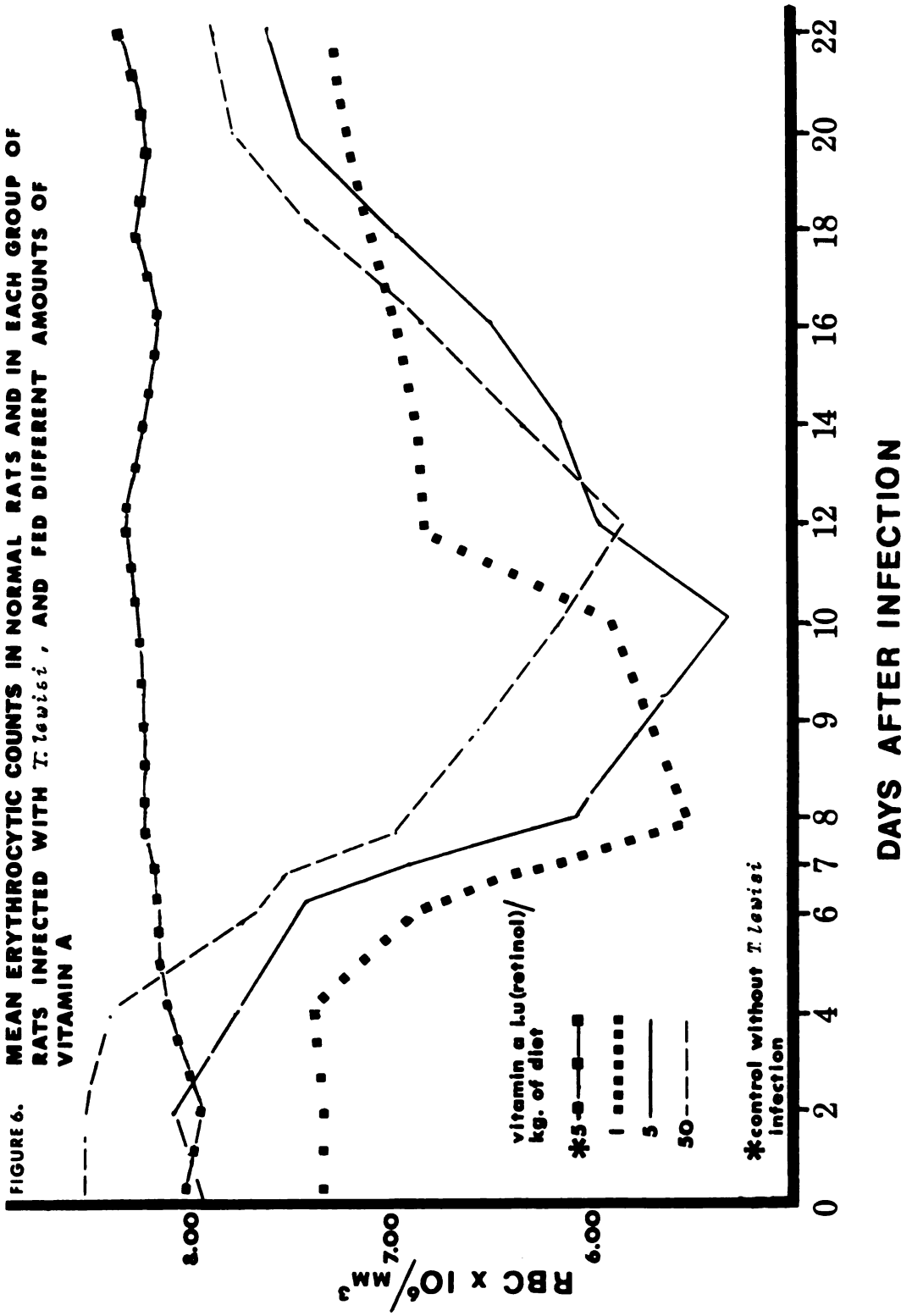


FIGURE 6. MEAN ERYTHROCYTIC COUNTS IN NORMAL RATS AND IN EACH GROUP OF RATS INFECTED WITH T. lewisi, AND FED DIFFERENT AMOUNTS OF VITAMIN A
RBC x 10⁶/mm³
8.00
7.00
6.00
0
2
4
6
7
8
9
10
12
14
16
18
20
22
DAYS AFTER INFECTION
vitamin a I.u.(retinol)/ kg. of diet
*5
1
5
50
*control without T. lewisi infection

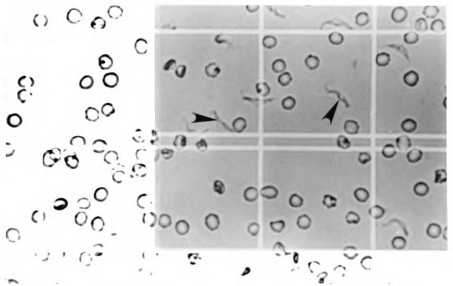
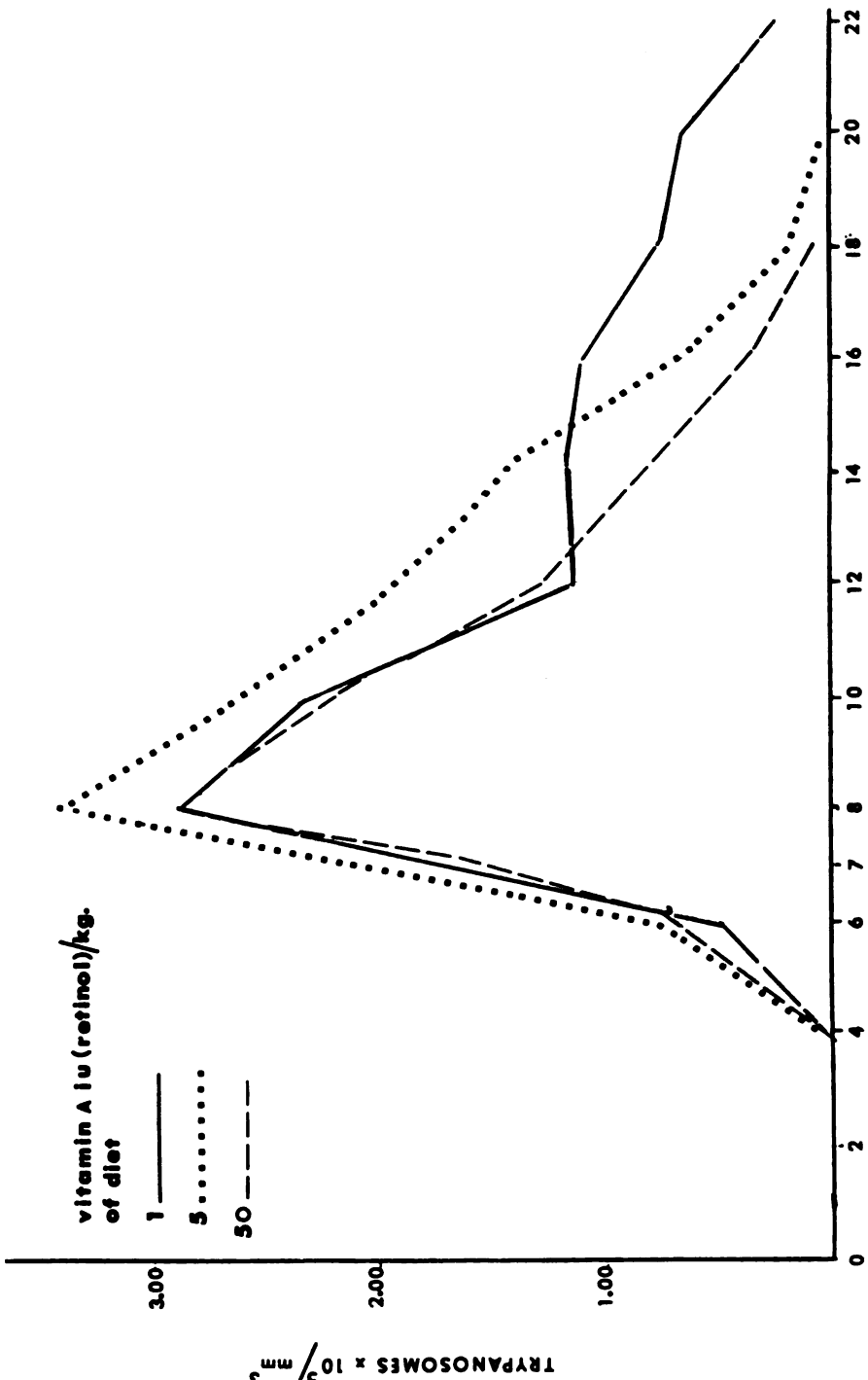


Figure 7. Photomicrograph of *Trypanosoma lewisi* (arrows) and erythrocytes of diluted blood in hemacytometer. X400.

FIGURE 8. THE COURSE OF PARASITEMIA FOLLOWING *T.lewisi* INFECTION IN RATS FED DIFFERENT AMOUNTS OF VITAMIN A.



Serum Vitamin A Analysis. Serum samples collected on days 0, 10, and 22 were analyzed for vitamin A (Table 4). The results of the analyses indicated that in the infected rats fed with 1 IU of vitamin A (retinol)/kg of ration the serum vitamin A was decreased in the acute phase (10th day) and in the 3rd week of infection when compared with noninfected rats fed the same diet. When the infected rats were fed 5 or 50 IU of vitamin A (retinol)/kg of diet, the results were inconsistent. However, the vitamin A values in these 2 groups were 3 to 4 times the values obtained in the rats fed 1 IU of vitamin A (retinol);kg of diet.

Liver Vitamin A Analysis. The results of the vitamin A analysis of liver samples are shown (Table 5). When infected rats were fed 1 IU of vitamin A (retinol)/kg of ration, the liver vitamin A level was decreased by the end of the 3rd week postinfection. However, in the rats fed 5 and 50 IU of vitamin A (retinol)/kg of diet, there was no reduction in vitamin A levels between the acute phase (10th day of infection) and at the end of the 3rd week of infection. In general, the vitamin A content of the liver in rats in various treatments was correlated with the amount in the diet and length of time the diet was fed.

Organ Weights. The weight of the liver and the spleen was expressed in percent of body weight. The results are summarized in Tables 6 and 7.

Hepatomegaly was present in all groups of rats infected with *T. lewisi* during the acute phase of infection and at all 3 levels of vitamin A supplementation. The results were significant ($p<0.05$) when the F-test was applied. However, the results of the data obtained at

Table 4. Serum vitamin A values at days 0, 10 and 22 for noninfected rats and rats infected with *Trypanosoma lewisi*

| Treatments | Days Postinfection | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | | Day 10 | | Day 22 | |
| Vitamin A IU/ kg of ration | Non-infected | Infected | Non-infected | Infected | Non-infected | Infected |
| 1 | 289.50± 21.50(2)[a] | --- | * | 178.67± 29.97(3) | 234.00± 31.50(3) | 169.38± 13.01(8) |
| 5 | 631.00± 66.00(2) | --- | 776.00 (1) | 523.67± 78.79(3) | 550.00± 49.33(3) | 539.88± 35.01(8) |
| 50 | 805.00± 26.00(2) | --- | 922.00 (1) | 707.00± 6.00(3) | 603.67± 56.38(3) | 677.00± 64.62(8) |

Values are expressed in ng/ml ($\bar{X}$ ± SEM).

[a]Figures in parentheses denote the number of samples.

*Animal died during the bleeding.

Table 5. Liver vitamin A values at days 0, 10 and 22 for noninfected rats and rats infected with *Trypanosoma lewisi*

| Treatments | Days Postinfection | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | | Day 10 | | Day 22 | |
| Vitamin A IU/ kg of ration | Non-infected | Infected | Non-infected | Infected | Non-infected | Infected |
| 1 | 3.05± 0.35(2)[a] | --- | 11.9(1) | 3.40± 0.50(3) | 3.00± 1.57(3) | 2.83± 0.64(8) |
| 5 | 10.15± 3.55(2) | --- | 9.0(1) | 4.23± 0.58(3) | 5.77± 0.49(3) | 6.81± 1.25(8) |
| 50 | 97.00± 29.0(2) | --- | 105.7 (1) | 118.70± 4.60(3) | 173.87± 49.45(3) | 129.63± 17.65(8) |

Values are expressed in µg/ml ($\bar{X}$ ± SEM).

[a]Figures in parentheses denote the number of samples.

Table 6. Summary of liver weight of noninfected and *T. lewisi*-infected rats fed different amounts of vitamin A

| Treatments | Days Postinfection | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | | Day 10 | | Day 22 | |
| Vitamin A IU/ kg of ration | Non-infected | Infected | Non-infected | Infected | Non-infected | Infected |
| 1 | 5.09(2)[a] | --- | 4.70(1) | 5.50(3)[b] | 3.85(3) | 4.76(8) |
| 5 | 4.41(2) | --- | 5.15(1) | 6.01(3)[b] | 3.66(3) | 4.54(8) |
| 50 | 4.97(2) | --- | 4.71(1) | 6.12(3)[b] | 3.95(3) | 4.24(8) |

Values are expressed as percent of body weight.

[a]Figures in parentheses denote the number of samples.

[b]Different ($p<0.05$) when compared with liver weight of noninfected rats at days 0 and 10 (F-test).

Table 7. Summary of spleen weight of noninfected and *T. lewisi*-infected rats fed different amounts of vitamin A

| Treatments | Days Postinfection | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | | Day 10 | | Day 22 | |
| Vitamin A IU/ kg of ration | Non-infected | Infected | Non-infected | Infected | Non-infected | Infected |
| 1 | 0.317(2)[a] | --- | 0.314(1) | 1.98(3)[b] | 0.295(3) | 1.706(3) |
| 5 | 0.334(2) | --- | 0.292(1) | 1.260(3)[b] | 0.294(3) | 1.092(3) |
| 50 | 0.353(2) | --- | 0.306(1) | 1.155(3)[b] | 0.295(3) | 1.062(3) |

Values are expressed as percent of body weight.

[a]Figures in parentheses denote the number of samples.

[b]Different ($p<0.01$) when compared with spleen weight of noninfected rats at days 0 and 10 (F-test).

the end of the 3rd week postinfection were not significant. The vitamin A supplementation apparently did not have an effect on the liver size during the infection.

Splenomegaly was particularly prominent in the infected rats fed 1 IU of vitamin A (retinol)/kg of ration in comparison to controls during the acute phase and in the 3rd week of infection. Rats fed 5 or 50 IU of vitamin A (retinol)/kg of diet also had splenomegaly. These results were significant ($p<0.01$) when an analysis of variance was performed using the F-test.

## Pathologic Findings

The more prominent lesions associated with specific factors and then those for a combination of factors will be given.

Gross Lesions. On gross examination, the consistent lesions associated with vitamin A deficiency were porphyrin accumulation around the eyes (Figure 1), xerophthalmia and corneal ulceration (Figure 2). The main gross lesion associated with *T. lewisi* infection was splenomegaly. This was especially prominent in the rats fed 1 IU of vitamin A (retinol)/kg of ration (Figure 9).

## Histopathologic Findings

Vitamin A. A variety of lesions were present in the rats fed the vitamin A-deficient ration (1 IU of vitamin A (retinol)/kg of diet). These lesions were not present in rats fed the larger amounts of vitamin A, and they appeared regardless of whether rats were infected or not infected with *T. lewisi* or *P. aeruginosa*. Squamous metaplasia of ciliated columnar epithelium lining the trachea was a constant lesion.

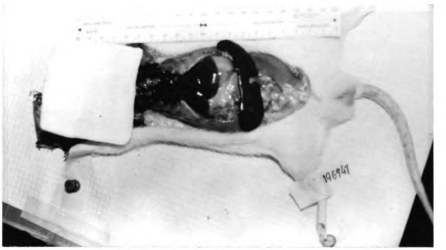


Figure 9. Photograph of abdominal cavity of a rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice prominent splenomegaly.

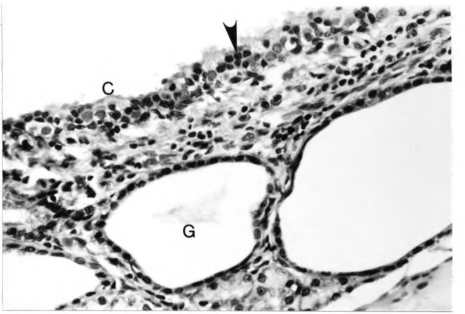



Figure 10. Photomicrograph of trachea of rat fed 1 IU of vitamin A (retinol)/kg of diet. Notice infiltration of inflammatory cells into the epithelium (arrow), loss of cilia (C), and cystic glands (G). Hematoxylin and eosin; X400.

In most sections, cilia or goblet cells could not be found and there was infiltration of inflammatory cells (Figure 10). Foci of basophilic mononuclear cells were in the tracheal lamina propria and the tracheal glands appeared dilated and in some areas had a cystic appearance. There was intrafollicular keratinization in the thyroid gland (Figure 11). There was a decreased number of apocrine glands and hair follicles in the skin of rats fed vitamin A-deficient diets in comparison with the skin of rats fed 5 or 50 IU of vitamin A (retinol)/kg of diet.

*Trypanosoma lewisi*. Rats exposed to *T. lewisi*, especially those fed the vitamin A-deficient diet, had prominent splenomegaly. There was a total disorganization of white pulp and no clear malpighian corpuscles when compared with the spleen from rats fed the required or 10X amounts of vitamin A. In some areas, there were foci of proteinaceous material containing fibrin, erythrocytes mixed with lymphocytes and plasma cells. Erythrophagocytosis by macrophages was evident (Figure 12). In rats infected with *T. lewisi* and fed the vitamin A requirements of 5 IU of vitamin A (retinol)/kg of diet, the lesions were similar in appearance. In rats infected with *T. lewisi* and fed 50 IU of vitamin A (retinol)/kg of ration, the lesions in the spleen were very mild. In general, splenomegaly was more apparent in *T. lewisi*-infected rats than in nonexposed rats.

Lesions Associated with Vitamin A Deficiency and *T. lewisi* and *P. aeruginosa* Infections. In the liver of rats fed the vitamin A-deficient ration (1 IU of vitamin A (retinol)/kg of diet) and previously exposed to *P. aeruginosa*, there were lesions in the periportal space

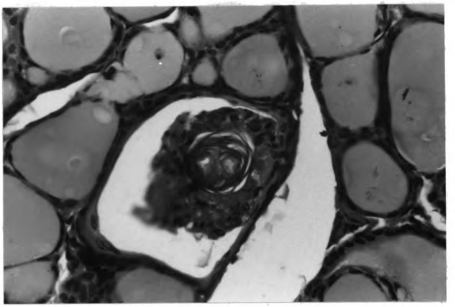


Figure 11. Photomicrograph to illustrate intrafollicular keratinization of thyroid gland of rat fed 1 IU of vitamin A (retinol)/kg of diet. Hematoxylin and eosin; X400.

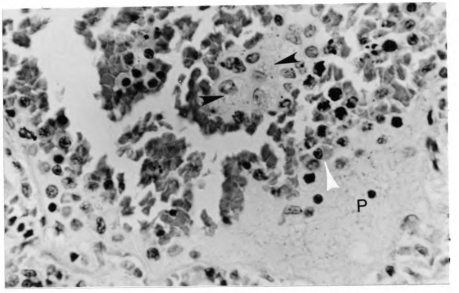



Figure 12. Photomicrograph of spleen of rat fed 5 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice proteinaceous material (P), plasma cells (white arrow), and erythrophagocytosis of macrophages. Hematoxylin and eosin; X640.

characterized by the presence of edema, lymphocytes and neutrophils mixed with erythrocytes and cellular debris as evidence of vasculitis (Figure 13). Also, in several areas there was pyknosis and karyolysis of hepatocyte nuclei and bile duct hyperplasia (Figure 14). Macrophages filled with green pigments were seen in these areas, and some areas had bile duct proliferation. There were extensive areas of necrosis of different sizes scattered throughout the liver (Figure 15).

Rats fed the required and 10X amounts of vitamin A and not infected with *T. lewisi* had hepatocytoplasmic vacuolization in perilobular areas. Frozen sections stained with the periodic acid-Schiff stain were negative for glycogen, and oil red O stains were negative for lipid. However, rats infected with *T. lewisi* had blood vessels with an endothelial disruption and an accumulation of proteinaceous material and trypanosomes (Figure 16). In the acute phase (10th day postinfection), the rats had a mild proliferation of mononuclear cells in the periportal spaces (Figure 17). These changes were more prominent at the end of the 3rd week of infection (Figure 18). Large veins had vacuolated and swollen endothelial cells and contained trypanosomes. There were infarcted areas in the livers of *T. lewisi*-infected rats, and they were more extensive in the rats fed the vitamin A-deficient diet (Figure 19).

The kidneys of vitamin A-deficient rats (not infected with *T. lewisi*) had cloudy swelling, pyknosis and hydropic degeneration of the epithelium of proximal convoluted tubules (Figure 20). Proteinaceous material was present in some tubules. In some areas, the tubules had lost their normal architecture, and in others they were dilated. The glomeruli appeared to be hyperemic. No inflammatory cells were found in the areas of tubular necrosis.

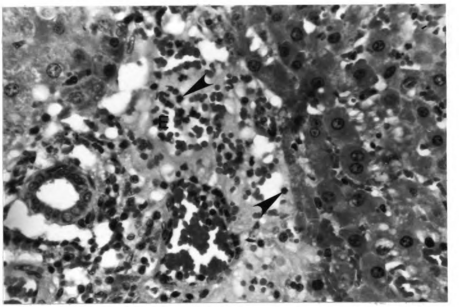


Figure 13. Photomicrograph of liver from rat fed 1 IU of vitamin A (retinol)/kg of diet. Notice edematous appearing periportal space with erythrocytes (E) and neutrophils (arrows), as evidence of vasculitis. Hematoxylin and eosin; X400.

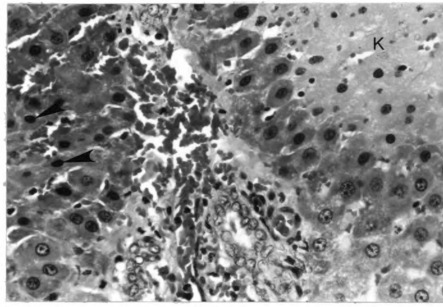



Figure 14. Photomicrograph of liver of rat fed 1 IU of vitamin A (retinol)/kg of diet. Notice pyknosis (arrows) and karyolysis (K) of hepatocytes. Hematoxylin and eosin; X400.

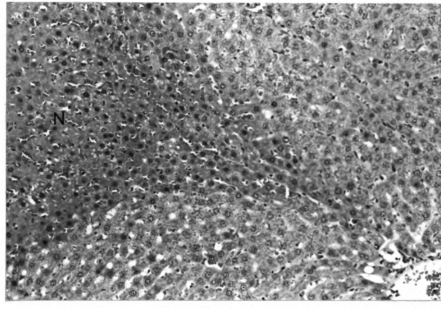



Figure 15. Photomicrograph of liver of rat fed 1 IU of vitamin A (retinol)/kg of diet. Extensive areas of necrosis were present (N). Hematoxylin and eosin; X160.

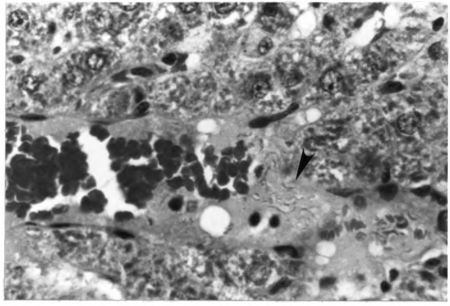


Figure 16. Photomicrograph of liver of rat fed 50 IU of vitamin A (retinol)/kg of diet. Notice the endothelial disruption of the blood vessel. Proteinaceous material is mixed with trypanosomes (arrow). Hematoxylin and eosin; X400.

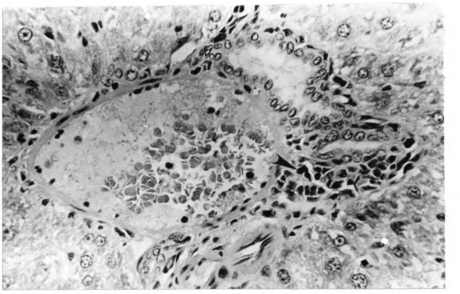


Figure 17. Photomicrograph of liver of rat fed 1 IU of vitamin A (retinol)/kg of diet, infected with *T. lewisi* and killed at 10 days. Notice mild proliferation of mononuclear cells (arrow) in the periportal space. Hematoxylin and eosin; X400.

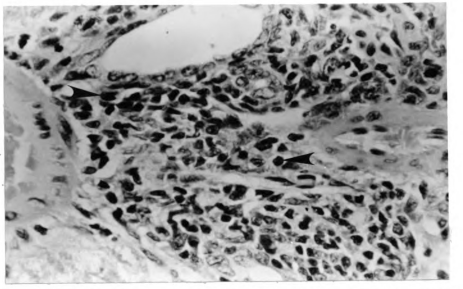


Figure 18. Photomicrograph of liver of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice prominent infiltration of mononuclear cells (arrows) in the periportal space. This was at the end of the 3rd week of infection. Hematoxylin and eosin; X640.

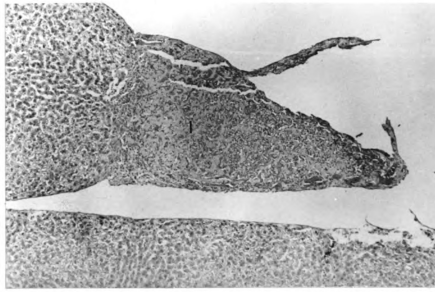
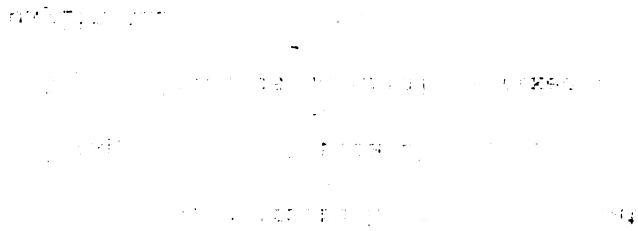



Figure 19. Photomicrograph of liver of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice extensive area of infarction (I). Hematoxylin and eosin; X64.



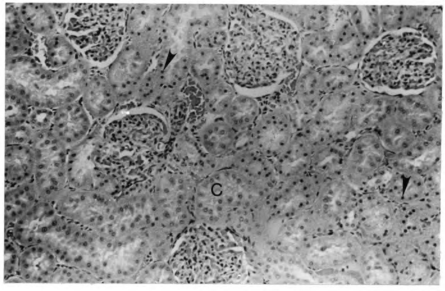



Figure 20. Photomicrograph of kidney of rat fed 1 IU of vitamin A (retinol)/kg of diet. Notice cloudy swelling (C), pyknosis (arrows), and hyperemia of glomerular tufts. Hematoxylin and eosin; X160.

The histologic sections of kidneys from rats fed 5 IU of vitamin A (retinol)/kg of ration, other than foci of pyknosis, were considered within normal limits. Some hyperemia in the tubules and glomeruli were observed. The rats fed 10X the normal required amount of vitamin A had pyknosis and hyperemia in the tubules in the cortex and medulla. Many tubules contained hyalin material and some resembled thyroid follicles (Figure 21).

Rats fed the vitamin A-deficient diet and infected with *T. lewisi* had focal areas of mineralization in the kidney (Figure 22). The glomeruli were hyperemic, and in some instances hypercellularity was a feature. Adhesions between the visceral and parietal portions of Bowman's capsule occurred (Figure 23). There were small areas of infarction (Figure 24) with glomerular destruction. Many trypanosomes were present in the lumen of blood vessels, especially in the large veins. In some areas near glomeruli, there were small cysts which contained trypanosomes. Pyknotic tubular cells were seen in several areas.

Sections of kidneys from rats infected with *T. lewisi* and fed 5 or 50 IU of vitamin A (retinol)/kg of ration had lesions milder than the lesions in rats fed the deficient diet (Figure 25).

The pancreatic cells and islets of Langerhans appeared to be normal in all groups of rats, whether exposed to *T. lewisi* infection or not. However, a heavy infiltration of eosinophils was around blood vessels containing *T. lewisi* (Figure 26). These vessels had vacuolated and swollen endothelial cells (Figure 27) and subendothelial edema. On the other hand, rats fed 50 IU of vitamin A (retinol)/kg, when infected with *T. lewisi*, had keratinization of the pancreatic ducts (Figure 28).

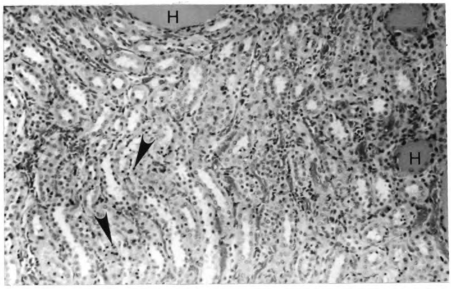



Figure 21. Photomicrograph of kidney of rat fed 50 IU of vitamin A (retinol)/kg of diet. Notice pyknosis (arrows) of tubular cells and foci of hyalin material (H) resembling thyroid follicles. Hematoxylin and eosin; X160.

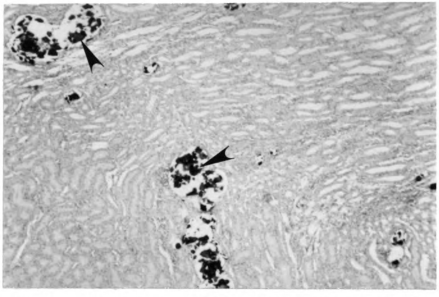


Figure 22. Photomicrograph of kidney of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice foci of mineralization (arrows). Hematoxylin and eosin; X64.

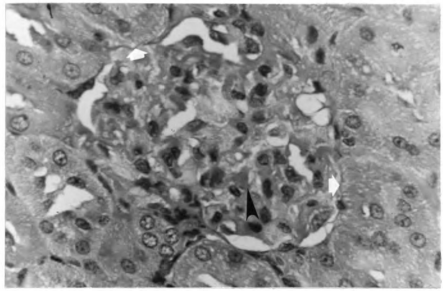


Figure 23. Photomicrograph of kidney of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice decreased subcapsular space, some points of adherence (white arrows), and hyperemia (black arrow). Hematoxylin and eosin; X400.

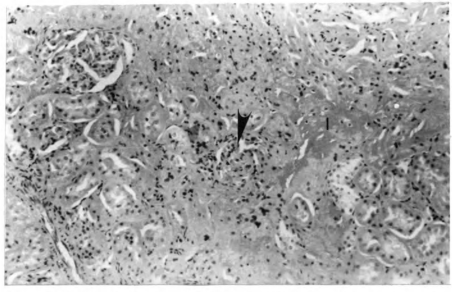


Figure 24. Photomicrograph of kidney of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice infarcted area (I) and glomerular destruction (arrow). Hematoxylin and eosin; X160.

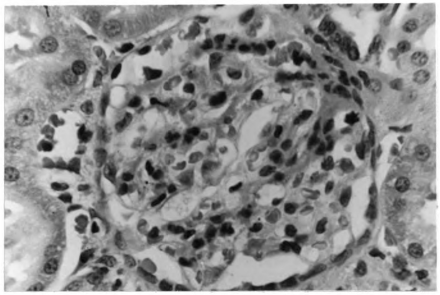


Figure 25. Photomicrograph of kidney of rat fed 5 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Lesions are similar but less severe than in Figure 23. Hematoxylin and eosin; X400.

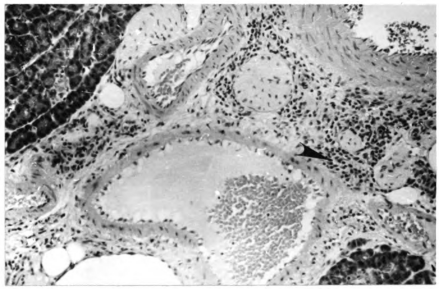


Figure 26. Photomicrograph of pancreas of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Extensive eosinophilic infiltration (arrow) around blood vessels. Hematoxylin and eosin; X160.

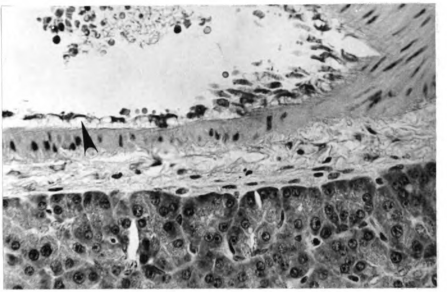


Figure 27. Photomicrograph of cross section of blood vessel of pancreas from rat fed 5 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice the vacuolated and swollen endothelial cells (arrow). Hematoxylin and eosin; X640.

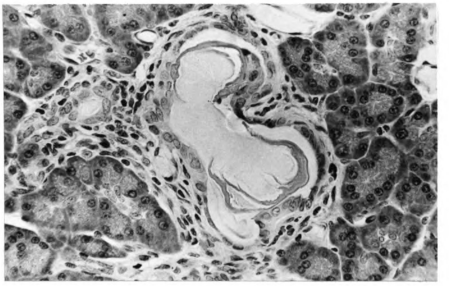


Figure 28. Photomicrograph of pancreas of rat fed 50 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice the keratinization of pancreatic duct. Hematoxylin and eosin; X400.

The skin sections from rats fed the vitamin A-deficient diet and infected with *T. lewisi* were heavily keratinized and bacterial colonies were present (Figure 29). This was absent in sections of skin from rats fed 5 or 50 IU of vitamin A (retinol)/kg of diet and also infected with *T. lewisi* (Figures 30 and 31).

## Experiment 2 - Spontaneous *Pseudomonas aeruginosa* Infection in Vitamin A-Deficient Rats

The results of Experiment 2 on determining the interrelationship of a vitamin A deficiency and *Pseudomonas aeruginosa* infection were in general very similar to results of these 2 factors in Experiment 1.

The clinical signs of vitamin A deficiency first appeared at approximately 6 weeks of age in rats fed the vitamin A-deficient ration (not supplemented with vitamin A) or the ration supplemented with 1 IU of vitamin A (retinol)/kg of ration. The clinical signs included porphyrin accumulation around the eyes, xerophthalmia, corneal ulceration, rough haircoat and weakness. At the same time as clinical signs of vitamin A deficiency appeared, a spontaneous outbreak of *P. aeruginosa* occurred in the rats in both groups.

The *P. aeruginosa* infection was severe in the rats fed the vitamin A-deficient diets. The rats fed 5 IU of vitamin A (retinol)/kg of diet were slightly affected. The infection did not involve rats fed 50 IU of vitamin A (retinol)/kg of diet or other rats maintained in the same room.

Morbidity was high (100%) in all 24 rats fed the vitamin A-deficient diet. All rats died during the 17-day period. In the 14 rats fed 1 IU of vitamin A (retinol)/kg of diet, 5 rats died during this same time period. One rat died in the 12 rats fed 5 IU of vitamin A

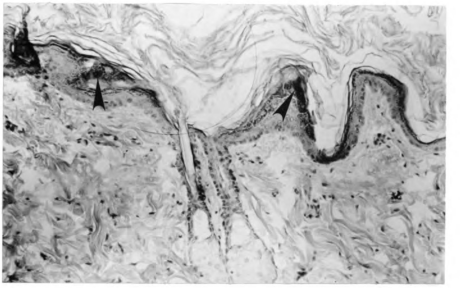


Figure 29. Photomicrograph of skin of rat fed 1 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Notice keratinization and bacterial colonies (arrows). Hematoxylin and eosin; X160.

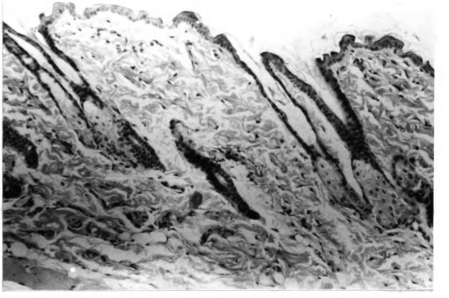


Figure 30. Photomicrograph of skin of rat fed 5 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Normal appearance. Hematoxylin and eosin; X160.

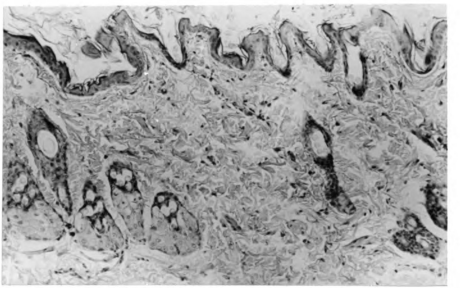


Figure 31. Photomicrograph of skin of rat fed 50 IU of vitamin A (retinol)/kg of diet and infected with *T. lewisi*. Increased number of apocrine glands in comparison to Figures 29 and 30. Hematoxylin and eosin; X160.

(retinol)/kg of ration. All 12 rats fed 50 IU of vitamin A (retinol)/kg of diet survived.

The duration of the infection varied between 3 and 8 days. The rats fed the basal vitamin A-deficient diet had the shortest period of infection and rats fed 5 IU of vitamin A (retinol)/kg of diet had the longest.

Mortality data following *P. aeruginosa* infection are summarized in the Appendix (Table A4).

# DISCUSSION

The purpose of the research conducted for this dissertation was to obtain additional information on the interrelationship of specific causes of public health problems. Vitamin A deficiency was selected as the nutritional factor because a great volume of experimental information was already available, a deficiency is a practical problem in man and animals, the research could be conducted using the rat as a model, and limited research has been done evaluating the interrelationship of vitamin A and infection.

*Trypanosoma lewisi* was selected because considerable information was also available on the pathogenesis of trypanosomiasis. Trypanosomiasis is an important problem in public health, and the infection could also be produced in the rat. In addition, only limited information was available as to the role of nutrition, such as a vitamin A deficiency, on the susceptibility to and recovery from trypanosomiasis. It was anticipated that evaluating the interrelationship of these 2 factors would be a challenge for research. The addition of a third factor, the *Pseudomonas aeruginosa* infection, was certainly not planned. While this infection did complicate a more precise interpretation of the interrelationship between a vitamin A deficiency and *T. lewisi* infection, it did demonstrate that public health problems can have a number of modifying factors that are very important for consideration in control and prevention.

Spontaneous *P. aeruginosa* infection in vitamin A deficiency deserves additional research. *Pseudomonas aeruginosa* infection has not been emphasized previously in vitamin A deficiency studies, yet it occurred in 2 different environments in this research. It was also a complication in Dr. Tvedten's previous vitamin A deficiency studies in rats. The 3 factors, a nutritional deficiency, a parasitic infection, and a bacterial infection, will be discussed. In general, the rat was a suitable experimental model on which to conduct this research on all 3 causative factors of disease.

## Vitamin A Deficiency

It was anticipated that the vitamin A-deficient rats that were to be exposed to *T. lewisi* at 8 to 10 weeks of age would have a more chronic vitamin A deficiency. These rats were born to mothers that were fed a vitamin A-deficient diet during pregnancy and lactation, and the young rats were fed the same deficient diet after weaning. This procedure produced a more acute deficiency than had been planned, and many of these young rats died with complications of *P. aeruginosa* infection. It was necessary, therefore, to supplement the deficient ration with 1 IU Of vitamin A (retinol)/kg of diet to keep them alive and to produce a more chronic deficiency for later exposure to *T. lewisi* infection.

The clinical signs and lesions associated with feeding the vitamin A-deficient ration were in close agreement with results of previous reported studies on vitamin A deficiency in the rat (Moore, 1957; DeLuca, 1978; Maynard et al., 1979; Hodges, 1980). The manifestations of vitamin A deficiency included poor growth, eye lesions, incoordination, skin lesions, metaplasia of the epithelium of the trachea, and

keratinization of the pancreatic ducts and thyroid follicles. These lesions were rather routinely present in rats fed the basal ration or the basal ration supplemented with 1 IU of vitamin A. They were not present in the rats fed either 5 or 50 IU of vitamin A (retinol)/kg of diet.

The degenerative changes in the liver and kidneys of rats were also associated more with a vitamin A deficiency than with other factors, as these lesions were absent or minimal in rats fed 5 or 50 IU of vitamin A. These lesions have been previously mentioned (Raica et al., 1969) for vitamin A deficiency in the germfree rat. In a general way, the liver and kidney lesions seemed to be due to both vitamin A deficiency and *P. aeruginosa* infection. In previous studies on vitamin A deficiency in rats, a role for *P. aeruginosa* was not clearly established (Tvedten, 1973). In previous research using germfree, vitamin A-deficient rats, kidney and liver lesions were mentioned, although they were not as severe as noted in this research. Therefore, the kidney and liver lesions that were present were probably due to both vitamin A deficiency and *P. aeruginosa* infection.

## *Trypanosoma lewisi* Infection

The gross and microscopic changes in the spleen were closely associated with *T. lewisi* infection. These lesions were not present in rats not exposed to *T. lewisi*. The lesions associated with *T. lewisi* were splenomegaly, splenitis, infarcts in the liver and kidney, and glomerulonephritis. In general, the splenic lesions were severe in infected rats fed the vitamin A-deficient diets. In rats fed 5 or 50 IU of vitamin A, splenic lesions were prominent but not as severe. Therefore, vitamin A (and also *P. aeruginosa*) appears to have a

modifying effect on *T. lewisi* involvement of the spleen. Kidney lesions also seemed to be more severe in *T. lewisi*-infected, vitamin A-deficient rats than in infected vitamin A-supplemented rats.

## *Pseudomonas aeruginosa* Infection

In both experiments, vitamin A deficiency appeared to increase the susceptibility to *P. aeruginosa* infection. This had not been anticipated. While it had the disadvantage of complicating the vitamin A-*T. lewisi* interrelationships, it had an advantage of supplying new information and an emphasis on this pathogen in vitamin A deficiency. Many previous researchers (Scrimshaw et al., 1968; Chandra and Newberne, 1977) alluded to a direct or indirect anti-infective property of vitamin A. However, this has never been firmly established (Hodges, 1980), perhaps for several reasons. One is that the role of microorganisms in a vitamin A deficiency has not been considered. A second reason is that until the advent of germfree procedures it was difficult to proceed with experimental techniques to clearly identify the role of microorganisms. Two reports are available in the literature (Rogers, 1971; Raica, 1969) on vitamin A deficiency in germfree rats. In both reports the clinical signs and lesions are different from the signs and lesions observed in this research. In this research, as in the research by Rogers et al. (1971) at the National Institutes of Health (NIH), conventional vitamin A-deficient rats died in 23 to 54 days. In contrast, when the NIH workers maintained littermate rats germfree and fed the same ration, they survived for as long as 272 days. These workers at NIH concluded that "early death in conventional (vitamin A) deficient rats must be a consequence of bacterial infection."

In mice, Cohen and Elis (1974) reported that vitamin A protected against a challenge of *P. aeruginosa* while controls developed a bacteremia. They concluded that vitamin A induces a nonspecific resistance to infection.

Since both vitamin A deficiency and *P. aeruginosa* infection are important public health problems, the implications of these 2 factors are an important area for future research.

## Application of Results

This research provided valuable experience for the author. The specific role and interrelationship of causative factors in disease were informative. The work illustrated the importance of and need for cooperation by individuals, disciplines and laboratories in pursuing major problems in medicine and public health. This interdisciplinary approach to solving disease problems by nutritionists, microbiologists, parasitologists, and pathologists would result in improved health of man and animals.

## SUMMARY

Experiments were conducted to determine the role and interrelationship of a vitamin A deficiency and infections due to *Trypanosoma lewisi* and *Pseudomonas aeruginosa* in the young rat. The rats were from dams fed a semipurified vitamin A-deficient diet during pregnancy and lactation. After weaning the rats were fed the vitamin A-deficient diet until clinical signs of vitamin A deficiency occurred. When clinical signs of vitamin A deficiency appeared, the rats were fed 1, 5, or 50 IU of retinol/kg ration and either exposed or unexposed to *T. lewisi*. A spontaneous infection with *P. aeruginosa* occurred when clinical signs of vitamin A deficiency appeared.

Vitamin A deficiency was characterized by retarded growth, incoordination, rough haircoat, xerophthalmia, low liver and serum vitamin A values, metaplasia of epithelial cells of the respiratory tract, and intrafollicular keratinization of the thyroid gland. *Trypanosoma lewisi* infection was characterized by anemia, parasitemia, anemia, splenomegaly, splenitis, hepatic and renal infarcts, and glomerulonephritis. Splenic lesions were more severe in rats fed the vitamin A-deficient diet. Hepatitis and nephritis were present in rats fed the vitamin A-deficient diet and were associated with both *T. lewisi* and *P. aeruginosa*. A spontaneous *P. aeruginosa* infection was closely associated with and enhanced by vitamin A deficiency.

A vitamin A deficiency enhances the incidence and severity of *T. lewisi* and *P. aeruginosa* infection in rats.

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# APPENDIX

Table A1. Mortality of rats fed vitamin A-deficient diet during spontaneous *Pseudomonas aeruginosa* outbreak, Experiment 1

| Date | No. Deaths |
|---|---|
| 7/26 | 1 |
| 7/27 | 3 |
| 7/28 | 2 |
| 7/29 | 5 |
| 7/30 | 3 |
| 7/31 | 3 |
| 8/01 | 1 |
| 8/02 | 2 |
| 8/03 | 1 |
| 8/04 | 4 |
| 8/05 | 2 |
| 8/06 | 1 |
| 8/07 | 1 |
| 8/08 | 1 |
| 8/09 | 2 |
| 8/10 | 1 |

Table A2. Erythrocyte counts from blood samples collected from rats fed different amounts of vitamin A (retinol)/kg of diet

| Vitamin A (retinol)/ kg of diet (IU) | Rat No. | | Erythrocyte Count 8/30 | 9/01 | 9/03 | 9/05 | 9/06 | 9/07 | 9/09 | 9/11 | 9/13 | 9/15 | 9/17 | 9/19 | 9/21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5* | 1 | | 7.61 | 7.99 | 7.42 | 7.83 | 8.11 | 7.91 | 8.17 | 7.27 | 8.00 | 7.84 | 8.37 | 8.17 | 8.30 |
| | 2 | | 7.59 | 7.63 | 7.17 | 7.88 | 8.04 | 8.15 | 8.29 | 8.27 | 7.97 | 8.03 | 7.91 | 8.15 | 8.06 |
| | 3 | | 7.85 | 7.74 | 7.37 | 7.65 | 8.07 | 7.52 | 7.94 | 8.34 | 8.13 | 8.58 | 8.32 | 8.04 | 8.21 |
| | | $\bar{X}$ | 7.68 | 7.78 | 7.32 | 7.78 | 8.07 | 7.86 | 8.13 | 7.96 | 8.03 | 8.15 | 8.20 | 8.12 | 8.19 |
| | | S | 14.5 | 18.4 | 13.2 | 12.1 | 3.5 | 31.8 | 17.8 | 59.9 | 8.5 | 38.4 | 25.2 | 6.9 | 12.1 |
| 1 | 4 | | 6.34 | 6.47 | 6.74 | 6.91 | 7.08 | 7.24 | 7.29 | 7.89 | 7.67 | 7.48 | 7.68 | 7.84 | 8.19 |
| | 5 | | 7.18 | 7.10 | 7.28 | 7.11 | 7.55 | 7.18 | 7.42 | 7.36 | 7.85 | 7.63 | 7.59 | 7.21 | 7.59 |
| | 6 | | 7.03 | 7.16 | 7.13 | 7.59 | 7.29 | 7.39 | 7.69 | 7.81 | 7.99 | 7.87 | 8.07 | 7.72 | 7.41 |
| | | $\bar{X}$ | 6.85 | 6.91 | 7.05 | 7.20 | 7.30 | 7.27 | 7.46 | 7.68 | 7.83 | 7.66 | 7.78 | 7.59 | 7.73 |
| | | S | 44.8 | 38.2 | 27.9 | 34.9 | 23.5 | 10.8 | 20.4 | 28.6 | 11.7 | 19.7 | 26.6 | 33.4 | 40.8 |
| 5 | 7 | | 8.05 | 8.35 | 8.19 | 8.44 | 8.16 | 8.21 | 8.19 | 7.88 | 7.80 | 7.91 | 8.36 | 8.08 | 8.36 |
| | 8 | | 7.87 | 7.50 | 7.89 | 7.91 | 7.81 | 7.93 | 8.01 | 8.51 | 8.42 | 8.04 | 8.37 | 8.12 | 8.03 |
| | 9 | | 8.09 | 7.98 | 8.13 | 7.84 | 8.28 | 8.22 | 8.15 | 8.29 | 8.26 | 8.32 | 7.93 | 8.28 | 8.45 |
| | | $\bar{X}$ | 8.00 | 7.94 | 8.07 | 8.06 | 8.08 | 8.12 | 8.11 | 8.22 | 8.16 | 8.09 | 8.22 | 8.16 | 8.28 |
| | | S | 11.7 | 42.6 | 15.9 | 32.8 | 24.4 | 16.5 | 9.5 | 32.0 | 32.2 | 20.9 | 25.1 | 10.5 | 22.1 |
| 50 | 10 | | 8.58 | 8.38 | 8.24 | 8.41 | 8.56 | 8.44 | 8.37 | 8.69 | 8.19 | 8.42 | 8.28 | 8.48 | 8.49 |
| | 11 | | 8.72 | 8.14 | 8.54 | 8.29 | 8.41 | 8.15 | 8.33 | 8.99 | 8.56 | 8.32 | 8.73 | 8.52 | 8.25 |
| | 12 | | 8.38 | 8.42 | 8.27 | 8.20 | 8.54 | 8.31 | 8.29 | 8.06 | 8.66 | 8.35 | 8.46 | 8.65 | 8.58 |
| | | $\bar{X}$ | 8.56 | 8.31 | 8.35 | 8.30 | 8.50 | 8.30 | 8.33 | 8.58 | 8.47 | 8.36 | 8.49 | 8.55 | 8.44 |
| | | S | 17.1 | 15.1 | 16.5 | 10.5 | 8.1 | 14.5 | 4.0 | 47.5 | 24.8 | 5.1 | 22.6 | 8.9 | 17.1 |

*Rats from Building 5, Veterinary Research Farm, not exposed to *P. aeruginosa* infection.

Table A3. Results of determination of hemoglobin concentration at days 0, 6, 7, 8, 18 and 22 for non-infected rats and rats infected with *Trypanosoma lewisi*

| | Vit. A (IU) diet | 8/30 | | | 9/5 | | | 9/6 | | | 9/7 | | | 9/17 | | | 9/21 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MCV | MCHC | MCH | MCV | MCHC | MCH | MCV | MCHC | MCH | MCV | MCHC | MCH | MCV | MCHC | MCH | MCV | MCHC | MCH |
| Control* | 5 | 68.1 | 33 | 22 | 65.7 | 34 | 22 | 65.1 | 33 | 21 | 65.1 | 33 | 21 | 61.2 | 33 | 20 | 63.2 | 33 | 21 |
| Non-infected | 1 | 81.8 | 32 | 26 | 76.4 | 35 | 27 | 74.1 | 35 | 26 | 74.3 | 33 | 25 | 77.4 | 32 | 24 | 75.4 | 33 | 25 |
| | 5 | 67.5 | 31 | 21 | 69.5 | 33 | 23 | 67.1 | 34 | 23 | 68.1 | 33 | 23 | 69.7 | 33 | 22 | 71.3 | 36 | 25 |
| | 50 | 68.3 | 32 | 22 | 70.8 | 32 | 22 | 66.8 | 33 | 22 | 67.9 | 35 | 23 | 71.5 | 32 | 23 | 72.3 | 32 | 23 |
| Infected | 1 | 74.0 | 31 | 23 | 72.8 | 34 | 25 | 84.1 | 33 | 28 | 96.9 | 31 | 30 | 81.8 | 31 | 25 | 82.3 | 32 | 27 |
| | 5 | 69.8 | 31 | 21 | 72.0 | 34 | 25 | 77.2 | 34 | 26 | 82.2 | 36 | 30 | 79.8 | 32 | 26 | 76.6 | 33 | 25 |
| | 50 | 70.1 | 31 | 21 | 73.4 | 31.6 | 23 | 77.5 | 33 | 25 | 75.4 | 33 | 25 | 78.8 | 30 | 24 | 77.3 | 32 | 25 |

*Control rats produced in Barn 5.

Table A4. Mortality of rats fed vitamin A-deficient diets during spontaneous *Pseudomonas aeruginosa* outbreak, Experiment 2

| Date | No. Deaths |
|---|---|
| 11/26 | 1 |
| 11/27 | 1 |
| 11/28 | 2 |
| 11/29 | 2 |
| 11/30 | 4 |
| 12/01 | 2 |
| 12/02 | 3 |
| 12/03 | 3 |
| 12/04 | 2 |
| 12/05 | 2 |
| 12/06 | 1 |
| 12/07 | 2 |
| 12/08 | 1 |
| 12/09 | 1 |
| 12/10 | 1 |
| 12/11 | 2 |
| 12/12 | 1 |

# VITA

# VITA

The author was born in Cambaiba, a small district of the Rio de Janeiro State, Brazil, on May 14, 1936.

He received his elementary education with his mother, who was a teacher and the Principal at the Grupo Escolar de Cambaiba. In 1950 he entered the Liceu "Nilo Peçanha", Niterói, Rio de Janeiro State, and completed his high school education in 1957. In 1957 he entered the Escola Tecnica "Ildefonso Simoes Lopes", Rio de Janeiro State, where he graduated in 1959 with a Diploma in Technical Agriculture. In 1962 he was admitted to the College of Veterinary Medicine, Universidade Federal Fluminense, Niterói, Rio de Janeiro State, where he graduated in 1966 with the degree Doctor of Veterinary Medicine.

The author practiced veterinary medicine on a livestock breeding farm of the Ministry of Agriculture, Ponta Grossa, Paraná State. In 1967 he was accepted into the Graduate School of Public Health at the National School of Public Health, Rio de Janeiro.

After obtaining a Master of Science degree in 1969, the author joined the faculty in the Department of Epidemiology and Public Health at the Institute of Veterinary, Universidade Federal Rural do Rio de Janeiro. He was elected as Chairman of the Department in 1975. He is currently on leave and will return to this position in November 1980.

The author was selected for the Programa de Ensino Agricola Superior (PEAS-Program) at Michigan State University in 1976 to pursue a Doctor of Philosophy degree in pathology.

The author is married to Wilma Alves Correa Rangel, and they have two daughters, Moraima and Aymara.