ABSTRACT

TORULOPSIS GLABRATA NEPHRITIS AND
VITAMIN A DEFICIENCY IN DOGS

By

Harold W. Tvedten

The pathogenesis of *Torulopsis glabrata* infection in the dog, the clinical signs and lesions of vitamin A deficiency, and the interrelationships of the nutritional status and resistance to infection were studied in 28 dogs. Two experiments utilized dexamethasone in various dosages to reduce the resistance of 5 dogs to the yeast infection. The other 23 dogs were used in 2 experiments with vitamin A and exposure to *T. glabrata* as treatment factors.

Torulopsis glabrata caused an interstitial nephritis in 9 of 17 exposed dogs. The yeast was usually found in the renal tubules which were surrounded by plasma cells, histiocytes and neutrophils. Some *T. glabrata* cells were in the interstitial areas where they were usually phagocytized. Pyelonephritis, cystitis and prostatitis occurred in 4 dogs with secondary bacterial invasion by *Escherichia coli* or *Staphylococcus aureus*. The *T. glabrata* was relatively nonpathogenic and the urinary infections appeared to be healing by 4 weeks after exposure.

A palatable ration was formulated which resulted in clinical signs of vitamin A deficiency after feeding for 7 to 13 weeks. The neurologic signs included head tilt, circling and loss of balance in 5 of 12 dogs fed the ration without vitamin A supplementation. Papilledema was clinically detected in 2 vitamin A-deprived dogs. At necropsy, calculi were detected in the urinary bladder of a vitamin A-deprived dog and epithelial defects were found on the tongue of another dog deprived of vitamin A.

The frequency and severity of the nephritis was greater in the vitamin A-deprived dogs. The increased severity was partially due to secondary bacterial invasion. The total leukocyte and neutrophil response to intravenous exposure was smaller in the vitamin A-deprived dogs and, on the basis of skin tests with antigens of *T. glabrata* and *C. albicans*, the vitamin A-deprived dogs appeared to have impaired immediate and delayed hypersensitivity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Appendix Page

INTRODUCTION

This research was on the relationship of nutrition and infection and was essentially a continuation of previous research on the influence of vitamins A and E on *Mycoplasma pulmonis* pneumonia in rats (Tvedten *et al.*, 1973). Rats deficient in vitamin A, vitamin E or both were more susceptible to the pneumonia than rats supplemented with vitamins A and E. The National Institutes of Health made funds available for a similar project in a larger species.

Torulopsis glabrata was chosen as the infectious agent for several reasons. The medical professions have become increasingly aware of the pathogenic role of the yeast in debilitated human patients. However, to our knowledge *T. glabrata* infection had not been reported in the dog, so basic information on the pathogenesis and lesions of the disease could be determined.

Vitamin A deficiency was chosen since it predisposed spontaneous, ascending urinary tract infections in rats (Tvedten *et al.*, 1973) as well as predisposing many other infections in other species (Scrimshaw, 1966). Moreover, the research on vitamin A deficiency in the dog was done in the 1940s and earlier with experimental rations unacceptable by today's standards. Some of this earlier

research had coexisting deficiencies of thiamine, iron and copper and may have had deficiencies of other nutrients such as vitamin E.

The dog was chosen as the experimental animal because a large percentage of the veterinary profession has been concerned with the treatment of this animal, and the dog is large enough to allow repeated sampling of blood, urine and other substances.

In summary, this research would supply basic information on the pathogenesis of a new disease in the dog and previous reports on the effects of vitamin A deficiency in the dog could be confirmed utilizing a currently acceptable vitamin A-deficient ration. Information gained on the relationship between *T. glabrata* and vitamin A deficiency would be of biomedical interest as well as public health significance.

OBJECTIVES

The objectives of this research were:

1. To describe the pathogenesis and lesions of *T. glabrata* infection in the dog.

2. To evaluate the nature of vitamin A deficiency in the dog and compare the clinical signs and lesions to those previously reported.

3. To determine if there is an interrelationship of *T. glabrata* infection and vitamin A deficiency in the dog.

4. To provide basic biomedical information on a yeast infection which has caused health problems in human patients.

LITERATURE REVIEW

This literature review will be divided into three parts: (1) *Torulopsis glabrata*--history, characteristics, pathogenicity and experimental studies; (2) yeast infections and resistance to them; and (3) vitamin A deficiency in general, in the dog and in relation to disease and immunity.

Torulopsis glabrata

History

Anderson (1917) originally named the yeast *Cryptococcus glabrata* after isolating it from human feces. Lodder and DeVries (1938) later put the yeast into the genus *Torulopsis*. *Torulopsis glabrata* was assumed to be found only in man (Lodder and Kreger-VanRij, 1952) until it was isolated from cattle, goats, swine and horses (Van Uden *et al.*, 1958; Van Uden, 1960), pigeons and doves (Kocan and Hasencleaver, 1972), rats and lambs (White *et al.*, 1972), soil (Cooke, 1961) and creek and pond waters and their sediments (Hasencleaver and Mitchell, 1962).

Characteristics

Torulopsis glabrata belongs to the family *Cryptococcaceae* and is closely related to *Cryptococcus* and *Candida* (Baker, 1971). It has antigens in common with *Candida* but also has its own specific

antigens allowing rapid serologic identification (Hasencleaver and Mitchell, 1960; Tsuchiya *et al.*, 1961). *Torulopsis glabrata* reproduces by budding, but does not form pseudohyphae, germ tubes, ascospores or urease like *Candida* (Baker, 1971; Strate, 1973). *Torulopsis glabrata* does not have a thick capsule like *Cryptococcus* (Grimley *et al.*, 1965).

Torulopsis glabrata is very sensitive to pH and only grows in acid medium (pH < 6.5) (Edebo and Spetz, 1965). Its colonies are tiny, white, raised and nonhemolytic on blood agar and remain small despite prolonged incubation (Marks and O'Toole, 1970). *Torulopsis glabrata* is the only yeast that ferments only dextrose and trehalose (Marks and O'Toole, 1970). It is widely distributed in nature and is saprophytic in many animals and in man (Marks and O'Toole, 1970).

Pathogenicity

Marks *et al.* (1970) reviewed 130 isolations of *T. glabrata* from 37 human patients in a general hospital. This represented about 1% of all specimens submitted for mycologic study. *Torulopsis glabrata* was judged pathogenic if: (1) tissue invasion was demonstrated histologically; (2) fungemia was demonstrated on 2 separate days; (3) *T. glabrata* was repeatedly isolated in large numbers from multiple sites and the patient died; or (4) there was persistent urine colonization demonstrated by culturing of a catheterized urine sample. The yeast had a pathogenic role in 11 of these 37 patients. Ten of the 11 patients had major underlying illnesses and had been treated with antibiotics, steroids or other

immunosuppressive drugs. Four patients with *T. glabrata* fungemia died after a febrile illness and hypotension. Six patients had histologic evidence of tissue invasion. Marks *et al.* (1970) thus categorized *T. glabrata* as an opportunistic pathogen in the altered human host.

Several others have reported on the pathogenic role of *T. glabrata* in human patients. Edebo and Spetz (1965) indicated that *T. glabrata* was a persistent inhabitant of the urinary tract, especially of diabetic patients. Other conditions reported were: in-dwelling intravenous catheter contamination (Rose and Heckman, 1971; Rodrigues *et al.*, 1971), meningoencephalitis (Wurzel *et al.*, 1972), septicemia (Minkowitz *et al.*, 1963), endocarditis (Lees *et al.*, 1971), and pulmonary infection (Strate, 1973; Oldfield *et al.*, 1968). Human patients have seldom recovered from severe *T. glabrata* infections, and this may be due to the seriousness of coexisting problems which allowed the fungemia to begin (Strate, 1973). These are but a sampling of many reports of torulopsosis in human patients.

Two clinical diseases in animals have been reported. *Torulopsis glabrata* was associated with inebriation in newborn animals and abortion of a bovine fetus. Inebriation due to ethanol intoxication was reported in pigs fed a 25% glucose diet (Bell *et al.*, 1950; Becker *et al.*, 1954), young calves fed milk replacers containing hydrolyzed starch (Abe *et al.*, 1971) and newborn lambs fed glucose in fat-free milk (Cunningham *et al.*, 1955). Abe *et al.* (1971) had cultured and counted unidentified yeasts from the feces of the calves. White *et al.* (1972) experimentally reproduced

ethanol intoxication in newborn lambs and, to a lesser extent, in newborn pigs by feeding glucose in fat-free milk. Naturally occurring *T. glabrata* in concentrations up to 10^6 viable cells/ml were found in stomach contents of the lambs and pigs. These yeast populations produced up to 500 mg of ethanol/100 ml of stomach contents of the lambs and pigs. This resulted in high ethanol levels in the plasma of lambs and obvious signs of intoxication. The pigs fed glucose in fat-free milk had ethanol produced in the stomach but did not have high plasma ethanol levels nor clinical intoxication like the lambs (White *et al.*, 1972). *Torulopsis glabrata* isolated from the stomachs of lambs, sheep, rats, calves and pigs also produced high levels of ethanol from glucose *in vitro* (White *et al.*, 1972).

An aborted bovine fetus of 6 months' gestation had many *T. glabrata* cells in its bronchioles and occasionally in its alveoli (Kirkbride *et al.*, 1972). The placenta was inflamed, necrotic and contained macrophages which had PAS-positive bodies similar to *T. glabrata*. The abortion was attributed to the *T. glabrata* placentitis.

The workers who had reported the bovine abortion determined the pathogenicity of *T. glabrata* in pregnant mice (Knudtson *et al.*, 1973). When *T. glabrata* from the bovine fetus was injected intravenously into female mice prior to insemination, it remained viable in the kidneys for 63 days but did not cause abortion. When *T. glabrata* was injected intravenously into mice pregnant for about 14 days, a mild placentitis and subsequent abortion occurred.

However, no transplacental transmission of *T. glabrata* to the fetuses could be demonstrated as in the aborted bovine fetus.

Torulopsis glabrata was isolated from placental tissues in 2 of 47 women with spontaneous abortions (Bartizal *et al.*, 1973). Several other organisms were also isolated from the other patients and, since no organism was consistently found, the various isolates were considered to be part of the normal flora of the women's genital tract and not a cause of abortion as in the mice or the cow.

Experimental Studies

Experimental induction of torulopsosis has been attempted in animals such as rabbits, guinea pigs, rats and mice with limited success (Benham, 1935; Lodder and DeVries, 1938; Lopez, 1952; Hasencleaver and Mitchell, 1962).

Lopez (1952) attempted to find the most susceptible laboratory animal and the most efficient means of inoculation. He exposed rabbits, guinea pigs, rats and white mice and found the mice to be most susceptible. Intraperitoneal and intravenous inoculations gave the most consistent results. Intraperitoneal inoculation produced a multifocal nodular peritonitis and intravenous inoculation caused a multifocal nephritis.

Hasencleaver and Mitchell (1962) reported that *T. glabrata* would remain viable in the tissues of the mouse for 8 to 10 weeks but would not develop a progressive disease unless the mouse was physiologically altered. The resistance of the mouse to infection was altered by cortisone administration, irradiation or inducing diabetes with alloxon. Moreover, the growth of *T.*

glabrata and severity of the disease in the mice was directly proportional to the dose of cortisone. Hasencleaver and Mitchell (1962) performed quantitative cultures of various organs and determined that *T. glabrata* had a predilection for the kidney after intravenous administration in the mouse.

Intravenous administration of *T. glabrata* to rabbits was without effect (Benham, 1935). Wildfeuer (1972) exposed rabbits and mice intravenously to 14 yeasts. *Torulopsis glabrata* and several *Candida* species were pathogenic for mice, but only *C. albicans* was pathogenic for the rabbits. Intracardial administration of *T. glabrata* to rats caused an adhesive pleuritis, from which *T. glabrata* was isolated (Lodder and DeVries, 1938).

Histopathologic studies were important to interpret the damage done by the yeast since the *T. glabrata* remained viable in various organs for 8 to 10 weeks postinoculation and could be reisolated from normal appearing mice (Hasencleaver and Mitchell, 1962). Lopez (1962) reported phagocytosis of *T. glabrata* by the macrophages in a multifocal granulomatous peritonitis and nephritis. He illustrated the morphologic similarity of *T. glabrata* and *Histoplasma capsulatum* while inside macrophages. *Candida albicans* also had a predilection for the kidney but caused microabscesses instead of reticuloendothelial proliferation (Lopez, 1952). Phagocytosis of *T. glabrata* by macrophages has also been reported in human tissues (Minkowitz *et al.*, 1963).

Resistance to Yeast Infections

Some references will be made on immunity to various mycoses, but most of this section will be devoted to *Candida albicans*. *Candida albicans* has been the topic of extensive immunologic study, unlike *T. glabrata*, but information from *C. albicans* research should be fairly applicable to *T. glabrata* due to their similarity. Resistance to a yeast or other organism may be divided into 3 parts: (1) the physical barrier of the skin and mucous membranes, (2) phagocytosis, and (3) immunity and other factors.

Physical Barrier

The skin and mucous membranes mechanically exclude foreign organisms from the interior of the body which maintains an environment favorable to cellular growth. The exterior of the physical barrier is made unfavorable for cellular growth by secreting long chain fatty acids, gastric acid or lysozyme, which inhibit the growth of organisms such as *Candida* or *Torulopsis*. Parts of the respiratory tract secrete mucus and possess cilia which flush away possible pathogens. Altered permeability of mucous membranes to pathogenic organisms will be discussed in the section on vitamin A deficiency.

Phagocytosis

Phagocytosis has been a major defense mechanism of higher animals to remove organisms which have penetrated the external physical barrier. Stanley and Hurley (1969) studied the fate of *C. albicans* in cell cultures of mouse peritoneal macrophages. The

C. albicans was actively phagocytized but multiplied within the macrophages and the more virulent strains produced filaments which ruptured the macrophages. Macrophage phagocytosis was more effective against *T. glabrata* in that *T. glabrata*'s growth was inhibited in cell cultures of mouse peritoneal macrophages (Howard and Otto, 1966). Macrophages can be "activated" by the T lymphocytes of cellular immunity to develop a far greater capacity to kill ingested yeast such as *Cryptococcus* than normally (Anonymous, 1973; Eisen, 1974).

The effectiveness of neutrophilic phagocytosis depends on the size of the organism (Davies and Denning, 1972). Neutrophils phagocytized and killed blastospores and propagules of *C. albicans* with a mean pseudohyphal length of 12 μ. When the mean pseudohyphal length was 70 μ, leukocytes clustered around propagules and inhibited their growth. Propagules with pseudohyphal lengths greater than 200 μ were killed with increasing difficulty as the pseudohyphae lengthened (Davies and Denning, 1972). *Torulopsis glabrata*'s cell wall components induced *in vitro* chemotaxis of neutrophils but were less chemotactic for neutrophils than cell wall components of *C. albicans* (Denning and Davies, 1973).

Immunity

The mechanisms of immunity to mycoses have been only briefly studied, but functional cellular immunity was shown to be important in resistance to yeast infections (Kong and Levine, 1967). Indirect evidence of this was the increased incidence of fungal infections in situations in which patients had impaired cellular

immunity such as congenital thymic aplasia or hypoplasia, "Swiss type" agammaglobulinemia, Hodgkin's disease or as a result of treatment with corticosteroids or cytotoxic drugs (Chilgren *et al.*, 1967; Anonymous, 1973).

Patients with chronic candidiasis often had no skin reaction to *Candida* antigens, failed to be actively sensitized to 2,4,-dinitrofluorobenzene or had negative reactions to other tests of cellular immunity such as lymphocyte transformation, production of macrophage inhibition factor or delayed skin reaction to ubiquitous antigens (Chilgren *et al.*, 1967; Buck and Hasencleaver, 1963). Congenital thymic aplasia and Swiss type agammaglobulinemia have been frequently associated with mucocutaneous candidiasis in man (Chilgren *et al.*, 1967), and thymic hypoplasia was implicated as the predisposing cause of disseminated histoplasmosis in a dog (Patnaik *et al.*, 1974). Hodgkin's disease has been associated with a generalized failure of cell mediated immunity and increased susceptibility to cryptococcal meningitis (Anonymous, 1973).

Several cases of prolonged mucocutaneous candidiasis in human patients with defective cellular immunity which had been refractile to medical treatment were successfully treated with lymphocytes or lymphocyte transfer factor from normal people (Rocklin *et al.*, 1970; Pabst and Swanson, 1972). Lymphocyte transfer factor can transfer cellular immunity from one person to another (Pabst and Swanson, 1972). Cellular responses in which granulomas were formed have been associated with stronger immunity to a mycotic infection (Kong and Levine, 1967).

Conversely, humoral immunity has been unimpaired in fungal infections (Buck and Hasencleaver, 1963; Anonymous, 1973; Sinski *et al.*, 1963). Antibodies have diagnostic significance but questionable protective effect (Kong and Levine, 1967). Buck and Hasencleaver (1963) reported a negative correlation between skin tests and agglutination titers in 300 women examined for vaginitis. The agglutination titers were fairly indicative of candidiasis but the lowest percentage of skin reactions were in the group of women with candidiasis and the highest percentage in the group of women without vulvovaginitis. The magnitude of the antibody titer corresponded to the severity of coccidioidomycosis in dogs and monkeys (Sinski *et al.*, 1963). Resistance to *Coccidioides immitis* in mice could not be transferred by serum (humoral immunity), but moderate success was obtained by transferring splenic cells (cellular immunity) from resistant to susceptible mice (Kong and Levine, 1967).

Humoral antibodies have even protected *Candida* from a body's other defenses (Smith and Louria, 1972). Smith and Louria (1972) isolated a macroglobulin of fast β mobility which caused clumping of *C. albicans* in 98% of adults (21 to 50 years old) (Louria *et al.*, 1972). This clumping factor was nonimmunologic but was inhibited by an IgG antibody specific for *C. albicans* in the sera of patients with candidiasis (Chilgren *et al.*, 1968; Louria *et al.*, 1972). As the agglutinating and precipitating antibody titer of rabbits increased the clumping ability decreased (Smith and Louria, 1972). Antibodies probably interfered with clumping

by competing successfully for binding sites on the yeast. Clumping activity was also reduced in the sera of patients with renal disease, hepatic disease, diabetes, carcinoma or leukemia, and characteristically absent in patients with candidiasis (Louria *et al.*, 1972).

Taschdjian *et al.* (1972) postulated that different antibodies were formed in systemic versus mucocutaneous candidiasis. Agglutination and complement fixation antibodies apparently form in response to cell wall antigens in mucocutaneous candidiasis while in systemic candidiasis the yeast ruptures and precipitating antibodies form against cytoplasmic antigens. However, Chilgren *et al.* (1968) reported precipitating antibodies in 3 patients with chronic mucocutaneous candidiasis. Also, Kong and Levine (1967) reported the antigenic portion of *Coccidioides immitis* and *Histoplasma capsulatum* in immune patients was the cell wall fragments and not their protoplasm.

Vitamin A Deficiency

General

A deficiency of vitamin A has been associated with a wide, complex variety of disorders in several different organ systems. Disturbances of vision, such as night blindness and xerophthalmia, occurred frequently in animals eating diets containing inadequate vitamin A (Moore, 1960). Body growth in general was depressed (Rogers *et al.*, 1971), and bone growth or bone remodeling in particular was abnormal (Mellanby, 1944). Abnormal bone formation

has contributed to neurologic disorders (Wolbach and Bessey, 1941). The epithelial surfaces underwent metaplasia when the availability of vitamin A significantly varied (Wolbach and Howe, 1925). The epithelial metaplasia was reflected in altered mucus or keratin formation (DeLuca and Wolf, 1970), by urinary calculus formation (McCollum *et al.*, 1939), or by decreased cerebrospinal fluid (CSF) absorption (Calhoun *et al.*, 1967). Adequate vitamin A was also required for normal male and female reproductive activity (Granquad *et al.*, 1969) as well as normal differentiation of the embryo (Thompson, 1969).

The clinical appearance of vitamin A deficiency in a particular animal may include any combination of these disorders and the manifestation of vitamin A deficiency has been both species specific and related to the severity and duration of the deficiency (McCollum *et al.*, 1939). For example, different epithelia underwent squamous metaplasia as the severity of vitamin A deficiency intensified (Parnell and Sherman, 1962). Loss of mucus-secreting goblet cells in the gastrointestinal tract occurred earliest in a deficiency. Later the columnar epithelium of the trachea, then the cornea, and finally, at the most severe stage of vitamin A deficiency, the epidermis became more keratinized. Similarly the parotid duct of the cow became increasingly more metaplastic and keratotic as vitamin A became less available (Nielsen *et al.*, 1966). The duct of the epithelium near its orifice underwent metaplasia first and then, as the deficiency progressed, the metaplasia continued up the duct towards the gland and, finally, the ducts within the parotid gland underwent metaplasia.

An example of species specificity is that xerophthalmia has been a frequent lesion of vitamin A deficiency in rats and man (Wolbach and Howe, 1925; Tvedten *et al.*, 1973; Oomen, 1969), while it has not been a common lesion in other animals such as the dog, pig or guinea pig (Crimm and Short, 1937; Moore, 1960; McCollum *et al.*, 1939). Conversely, the bone lesions of deficient dogs and calves were uncommon in rats or man (Moore, 1960). Pigs and poultry were the most susceptible of the common farm animals to vitamin A deficiency in general, with cattle next, while sheep and horses were relatively resistant (Moore, 1960). Other examples of variations of the manifestations of vitamin A deficiency will be described in the specific sections on the various lesions of vitamin A deficiency. A review of research on vitamin A deficiency in the dog as well as the influence of vitamin A on disease and immunity will also be discussed in later sections.

Vision

The visual deficits of vitamin A deficiency vary from total blindness to night blindness or xerophthalmia. Total blindness in deficient cattle has resulted from destruction of the optic nerve in the optic foramen (Moore *et al.*, 1935). The optic nerve lesion was thought to be a result of increased CSF pressure and altered remodeling of the sphenoid bone (Hayes *et al.*, 1968). Total blindness has also resulted from congenital malformations of the eyes in rats (Warkany and Schraffenberger, 1944) or pigs (Goodwin and Jennings, 1958; Palludan, 1961) from vitamin A-deficient dams.

Vitamin A has a photoreceptor action in the visual cycle (Wald, 1943; Morton, 1969) and night blindness has resulted from insufficient vitamin A for the cycle (Holm, 1925; Mitchell, 1967; Schmidt, 1941). Histologically demonstrable degeneration of the retina has been described (Johnson, 1943), and Anderson and Hart (1943) speculated that the vacuoles observed between the rods may contain metabolites of the visual cycle which cannot be utilized without adequate vitamin A.

Heavy keratinization of the cornea (xerophthalmia) has been described in vitamin A-deficient rats as part of widespread epithelial keratinization (Wolbach and Howe, 1925). Follis (1958) speculated xerophthalmia may also be due to blockage of lacrimal ducts with keratinized debris and subsequent dryness of the eye. However, Pirie and Overall (1972), who detected changes in the lens epithelium which were not influenced by the presence or absence of tears, speculated that corneal keratinization was a primary epithelial lesion of vitamin A deficiency.

Growth

A fat soluble nutrient in the ether extract of butter and eggs was needed in the diet of rats to maintain their growth (Osborne and Mendel, 1913; McCollum and Davis, 1913). Both vitamins A and D were present in the crude extracts, but vitamin A was probably the main growth promoting factor (Mellanby, 1944). Decreased growth has been attributed to the consistent anorexia with vitamin A deficiency (Hazzard *et al.*, 1962) or to coexisting infections (Mellanby, 1944). However, a decreased growth rate

occurred before loss of appetite or decreased mitotic rate so it may have had a more fundamental cause than anorexia, which undoubtedly contributed to weight loss (Hayes, 1971). Infection has not been the primary cause of cessation of growth, since growth stopped in germfree rats deficient in vitamin A. However, infection has secondarily sped weight loss and death (Bieri *et al.*, 1969). Germfree rats which had ceased growth because of vitamin A deficiency temporarily resumed growth after receiving additional vitamin A (Rogers *et al.*, 1971).

Reproduction

Among the lesions of vitamin A deficiency in rats first reported by Wolbach and Howe (1925) were edema and atrophy of the testes. Dutt (1959) described degenerative changes of ram testes with cessation of spermatogenesis and decreased libido. Thompson *et al.* (1969) demonstrated retinoic acid was able to maintain reproductive ability in chickens. The decreased size of gonads in vitamin A-deficient animals may be due to decreased biosynthesis of steroid hormones (Grangaud *et al.*, 1969).

Teratogenic Effects

The pigs born of sows fed vitamin A-deficient rations for long periods of time had a variety of malformations (Palludan, 1961; Goodwin and Jennings, 1958). The most consistent malformation was microphthalmia with retinal rosettes and small irregular lenses in 88 of 91 pigs (Palludan, 1961). Other malformations included hydrocephalus, cysts in the liver and kidney, cleft

palate, contracted limbs, extra toes or ears, and cardiac abnormalities. Malformed eyes also were reported in rats from vitamin A-deficient dams (Warkany and Schraffenberger, 1944).

Hydrocephalus was produced in rabbits by depriving their dams of vitamin A (Harrington and Newberne, 1970). Mellanby (1944) speculated that hydrocephalus occurred because the vitamin A deficiency caused increased intracranial pressure which occluded the CSF outflow tract. The increased intracranial pressure may also have been responsible for cerebellar herniation reported in pigs (Gitter, 1962) and lions (Tuch and Pohlenz, 1973). Excess vitamin A also caused malformed fetuses (Seidler, 1971).

Bone Growth

One of the earliest associations of vitamin A deficiency and abnormal bone growth was when Moore *et al.* (1934) discovered constriction of the optic nerve at the optic foramen of vitamin A-deficient calves. Mellanby (1938 and 1943) reported similar lesions in the optic, auditory and trigeminal cranial nerves in vitamin A-deficient dogs. He postulated an overgrowth of the bony labyrinth compressed and destroyed the vestibular and cochlear divisions of the eighth cranial nerve. This bony overgrowth of the labyrinth also occurred in guinea pigs and rats (Wolbach and Bessey, 1941). Wolbach (1947) postulated altered endochondral growth was responsible for decreased growth; however, by labeling growing bone with dyes at various times Gallina *et al.* (1970) demonstrated that increased osteoblastic activity and normal osteoclastic activity were responsible for altered bone growth.

The epiphyseal plates were histologically the same in the vitamin A-deficient and control calves (Gallina *et al.*, 1970). Abnormal tooth growth allowed excess wear and irregular shapes in vitamin A-deficient rats (Raica *et al.*, 1969). The ameloblasts underwent metaplasia resulting in the formation of hypoplastic enamel (McCollum *et al.*, 1939). The alkaline phosphatase of serum and bone was decreased in vitamin A-deficient rats (Zile *et al.*, 1973).

Constrictive lesions of the central nervous system occurred in deficient animals because the CNS grew at a normal rate and was trapped within a relatively slower growing and abnormal skeletal system (Wolbach and Bessey, 1941; Wolbach, 1947). This included the previously mentioned damage to cranial nerves II and VIII. Other examples were herniation of the cerebellum out the foramen magnum, multiple herniations of the cerebellum and cerebrum into the venous sinuses, and herniation of spinal roots into the dorsal root ganglion (Wolbach and Bessey, 1941; Tuch and Pohlenz, 1973). In addition, the gyri of the cerebellum have been flattened and the spinal cord has been compressed and deformed (Lazar *et al.*, 1971), the pituitary gland has become cystic (Spratling *et al.*, 1965; Madsen *et al.*, 1942; Mellanby, 1944) or internal hydrocephalus has developed (Mellanby, 1944).

Cerebrospinal Fluid Pressure

An increase in CSF pressure was an initial sign of vitamin A deficiency in the dog, calf, chick, rabbit, rat, pig, lamb, and human being (Moore and Sykes, 1940; Calhoun *et al.*, 1967; Corey and Hayes, 1972), while excess vitamin A decreased CSF pressure in

calves, pigs and dogs (Eaton, 1969). The increased CSF pressure was detected by direct measurement with a manometer or more easily estimated in the calf by observing the retina for papilledema. Eaton (1969), a member of the group at Storrs, Connecticut, who have done much of the recent work on vitamin A deficiency, stated that papilledema was fairly indicative of increased CSF pressure in the calf, but no fundic changes were present in dogs with increased CSF pressures (Moore and Sykes, 1940). Increased CSF pressure was so consistent in vitamin A deficiency of calves that it was used to establish minimum requirements of vitamin A (Eaton *et al.*, 1972).

The increased CSF pressure occurred concurrently with incoordination and syncope (Moore and Sykes, 1940), nervousness (Schmidt, 1941) and convulsions (Dutt and Vasudevan, 1962; Mitchell, 1967) and was probably the cause of the convulsions (Sprattling *et al.*, 1965). The increased CSF pressure and the relatively slow growth of the skeletal system probably acted in concert to create lesions such as hydrocephalus in the nervous system (Sprattling *et al.*, 1965). Hayes *et al.* (1968) postulated that increased CSF pressure contributed to the optic nerve lesion by compressing dural blood vessels and causing ischemia. The ischemia increased fibrosis and thickening of the dura with the greatest thickness over the altered bony contour about the optic foramen.

Blakemore *et al.* (1957) postulated that the increased CSF pressure was due to constriction of the brain by the skull. The

brain volume per unit of live body weight in vitamin A-deficient and normal calves was not significantly different but the volume of the cranial valut per unit of live weight was significantly smaller in vitamin A-deficient calves than in controls (Gallina *et al.*, 1970). By measuring inulin clearance with ventriculo-cisternal perfusion techniques Calhoun *et al.* (1967), Gallina *et al.* (1970) and Frier *et al.* (1974) have demonstrated decreased bulk CSF absorption while synthesis was unaffected. An ultra-structural study of the arachnoid granulations, site of bulk absorption of CSF, did not demonstrate a mechanism of how CSF absorption was decreased, but Hayes *et al.* (1971) did speculate that vitamin A deficiency altered the differentiation of the arachnoid cells.

Mikkilineni *et al.* (1973) reported that sodium and ash content of the CSF tended to be higher in calves fed the ration with the lowest level of vitamin A. However, Hazzard *et al.* (1962) could not detect a difference in the osmolarity which might have contributed to increased CSF pressure. Thus, decreased CSF absorption and confinement of the brain in a relatively smaller skull may both contribute to increased intracranial pressure (Eaton, 1969).

Epithelial Integrity

A pathognomonic lesion of vitamin A deficiency is widespread squamous metaplasia and keratinization of epithelial surfaces (Wolbach and Howe, 1925). The metaplasia is related to the availability of vitamin A with different epithelia becoming metaplastic

and more keratinized as the deficiency becomes more severe (Parnell and Sherman, 1962). Hayes (1971), in a review article, speculated that vitamin A is required for basal epithelial cells to differentiate into ciliated, mucus-secreting cells and, without vitamin A, the basal cells differentiate into keratin producing, stratified squamous cells. Excess vitamin A, conversely, can divert ectoderm, normally keratinizing squamous epithelium into mucus-secreting, ciliated epithelium (Fell and Mellanby, 1953; Barnett and Szabo, 1973).

The degree and distribution of squamous metaplasia and keratinization of vitamin A deficiency has varied from species to species. In the rat many epithelial surfaces such as respiratory, urinary, corneal, pancreatic and in the salivary ducts quickly become metaplastic (Wolbach and Howe, 1925), while in the cow frequently only the parotid duct (Nielsen *et al.*, 1966) and occasionally the urinary tract (Langham *et al.*, 1941) became metaplastic.

Keratinization and infection of the urinary tract have predisposed vitamin A-deficient animals to the formation of urinary calculi (Schmidt, 1941; McCollum *et al.*, 1939). Urethral obstruction, without calculi, also was more frequent in vitamin A-deficient rams (Schmidt, 1941) and rats (Tvedten *et al.*, 1973) than normal control animals. Decreased urinary calcium excretion occurred in vitamin A deficiency, although serum calcium levels were unchanged, and may have contributed to urolith formation (Zile *et al.*, 1972). However, vitamin A deficiency has been but one of many causes of uroliths and no evidence of vitamin A

deficiency was found in a survey with large numbers of human patients with urolithiasis (Jewett *et al.*, 1943).

Mucus Secretion

One of the earliest ways to detect squamous metaplasia in vitamin A deficiency was by decreased amounts of mucus as indicated by decreased amounts of PAS positive material in the epithelium (Nielsen *et al.*, 1966). Vitamin A deficiency also caused a marked decrease in goblet cells in the small intestine of the rat (DeLuca and Wolf, 1970), while an excess of vitamin A resulted in the formation of mucus in cell cultures of ectoderm (Fell and Mellanby, 1953).

Vitamin A deficiency created a defect in the pH 5 fraction which stops glycoprotein synthesis and thus mucus formation (DeLuca and Wolf, 1969 and 1970). The pH 5 fraction was one of 3 fractions (the other 2 are ribosomes and cell sap), usually derived from whole cells. Fractionating cells allowed localization of biochemical processes in protein synthesis. The pH 5 fraction contained activating enzymes and t-RNA. The biochemical step of incorporation of D-glucosamine into a fucose containing glycopeptide was markedly decreased in cell fractions from vitamin A-deficient animals (DeLuca *et al.*, 1968; DeLuca and Wolf, 1969 and 1970). By means of an indirect immunofluorescence technique, fucose-containing glycopeptide was only detected in the goblet cells of rat small intestine (DeLuca *et al.*, 1971). Thus the biochemical lesion of decreased fucose-containing

glycopeptide corresponded with the histologic lesion of decreased goblet cells in the small intestine.

Other biochemical defects have been described but the exact metabolic action of vitamin A has not been elucidated except in the visual cycle (Roels, 1969). Hepatic enzyme activities were decreased in vitamin A deficiency (Becking, 1973), but this may have been related to anorexia and impaired protein synthesis (Roels, 1969). The synthesis of nuclear RNA and thus formation of corticosterone from cholesterol was decreased in vitamin A deficiency (Johnson *et al.*, 1968 and 1969), although Dvorak (1973) could not substantiate this by differences in plasma 17 hydroxy-corticosteroids or by their production in the adrenal gland.

Vitamin A may be an integral part of the structure of biologic membranes (Seward *et al.*, 1969). More vitamin A has been found in membranes than homogenates of cells (Mack *et al.*, 1972). Excess (Weissmann *et al.*, 1963) or insufficient vitamin A (Roels *et al.*, 1969) weakened membranes. Hypervitaminosis A allowed the release of lysosomal enzymes (Weissmann *et al.*, 1963) which broke down the extracellular matrix around cartilage cells (Dingle, 1961). Lucy (1969) proposed a micelle theory on how vitamin A may be involved in the fusion and rearrangement of membranes allowing communication of substances between various cellular and extracellular compartments.

Vitamin A utilization and availability have been influenced by other nutrients. Vitamin E had a sparing effect on vitamin A (Dicks *et al.*, 1959). Although Rousseau *et al.* (1973) reported vitamin E had no effect on lesions of vitamin A deficiency in

the calf, Tvedten *et al.* (1973) reported the onset of vitamin A deficiency lesions in the rat were delayed by vitamin E supplementation. This emphasizes the need of properly balanced experimental rations in research on the role of vitamin A in various animals.

Vitamin A Deficiency in the Dog

The research I found on vitamin A deficiency in the dog was reported during the late 1930s and early 1940s and might have been of questionable value in light of current nutritional requirements. Most investigators did not use trace mineralized salt, so deficiencies of copper or iron may have been responsible for the anemia Crim and Short (1937) described in their dogs, since supplementation of iron and copper to dogs on a similar diet cured similar anemia (Russel and Morris, 1939).

Dried yeast and irradiated dry yeast (vitamin D source) were the only vitamin supplements, so coexisting deficiencies of other vitamins may have been present in some of the early research. Russel and Morris (1939) reversed a marked weight loss in their dogs by supplementing thiamine 4 weeks into their experiment; thus their dried yeast did not supply all of the B-complex vitamin requirement. Vitamin E was required by dogs (Brinkhous and Warner, 1941; Hayes *et al.*, 1969 and 1970), but this vitamin was not added to these earlier rations, which may account for some reports of muscle weakness (Frohring, 1937b).

Several workers reported lesions due to infection as part of the hypovitaminosis A syndrome. Steenbock *et al.* (1921) described what may have been a purulent conjunctivitis as xerophthalmia in

3 of 5 dogs, and these dogs also had an acute fatal pneumonia which they compared to the pneumonia of vitamin A deficiency in rats. Steenbock *et al.* (1921) were probably describing a respiratory infection such as canine distemper in dogs, as did Green and Mellanby (1928). Crimm and Short (1937) reported pneumonitis and a left shift in the white blood cell count, as did Russel and Morris (1939), which probably indicated an infectious process.

Other changes reported in dogs included hepatic vitamin A analyses and the description of lesions likely to be primary hypovitaminosis A lesions. Anorexia and weight loss were consistent findings in the dog (Frohring, 1937a,b; Russel and Morris, 1939). Nervousness and running in circles in their cages occurred in vitamin A-deprived dogs and was not cured by additional vitamin B-complex, although treatment with carotene in oil preceded a return to normal behavior (Frohring, 1937b). [The dog effectively converts carotene to vitamin A (Bradfield and Smith, 1938).] Smith (1942) reported all 18 fox pups fed a vitamin A-deficient diet had nervous derangement with head tilts and loss of balance. He also described myelin degeneration of the spinal cord and VIII cranial nerve and 2 of the foxes had papilledema. Cerebrospinal fluid pressure was increased in dogs with vitamin A deficiency (Moore and Sykes, 1940).

Morris and Russel (1939) assumed squamous metaplasia and keratinization of epithelia occurred in the dog as had been described in the rat (Wolbach and Howe, 1925), but they did not perform a histologic study. Smith (1942) reported stratification and keratinization of the epithelium of the cornea (xerophthalmia),

trachea, bronchi, renal pelvis, urinary bladder and vagina in foxes after 18 to 27 weeks of eating a vitamin A-deficient diet. The other lesions reported in vitamin A-deficient dogs were not convincing enough to review.

Vitamin A and Infection

Infections in vitamin A-deficient animals have been frequent and have often obscured the primary lesions of the deficiency (Moore, 1960). Vitamin A deficiency and spontaneous respiratory infections were almost synonymous in the rat, and early researchers described the infections as part of the hypovitaminosis A syndrome (Wolbach and Howe, 1925; Daniels *et al.*, 1923). The respiratory lesions in rats were probably due to *Mycoplasma pulmonis* which more readily infects vitamin A-deficient rats (Tvedten *et al.*, 1973). Green and Mellanby (1928) labeled vitamin A as an anti-infective agent because of increased bronchopneumonia in dogs deficient in fat soluble vitamins.

Scrimshaw (1966) reviewed reports on the relation of nutrition and infection which are too numerous to describe individually in this literature review. He divided the reports on the basis of the disease being more severe in the deficient animal (synergistic), less severe in the deficient animal (antagonistic) or no effect of the deficiency was noted on the severity of the disease. All 30 of the reports of vitamin A deficiency and bacterial or rickettsial infection had synergism between the deficiency and the infection. There were 13 reports on research on helminth infestations in vitamin A-deprived animals. Eleven

papers reported synergism between the severity of the disease and the deficiency while 2 papers that reported the vitamin A deficiency had no effect on the infestation. Three of 4 investigations on viral infection reported synergism, while 1 investigation reported no effect. The only report of antagonism was in a protozoan infection. Four of the other 5 studies on protozoan diseases had synergistic results, and the last investigation reported no effect. In general, malnutrition was more frequently antagonistic with viral diseases and blood protozoans since these agents required a healthy cell in which to live and multiply. Malnutrition and infections of bacteria, rickettsia and helminths were usually synergistic because of reduced resistance of the host (Scrimshaw *et al.*, 1959). However, vitamin A deficiency appeared in general to be synergistic with all classes of infectious agents (Herrick, 1972; Scrimshaw, 1966).

The mechanisms of decreased resistance to infection included epithelial integrity as well as defective immunity which will be discussed in a separate section. The epithelial metaplasia of hypovitaminosis A favored penetration of infectious agents such as *Salmonella* or poliomyelitis virus (Scrimshaw *et al.*, 1959). After localized destruction of mucociliated epithelium by viral infections, the epithelium was replaced by keratinized squamous cells (Bang and Bang, 1969). Loss of mucociliary clearance in the respiratory tract may have predisposed animals to bacterial infections (Wolbach, 1954), including mycoplasma (Tvedten *et al.*, 1973).

Intestinal absorption of vitamin A during infection was decreased, which intensified a deficiency of vitamin A which may have initially predisposed people to the infection (Kouwenhoven and Van der Houst, 1972; Sirakumar and Reddy, 1971). Disease also has reduced preexisting stores of vitamin A. For example, plasma vitamin A levels were decreased in patients with fever and the excretion of vitamin A in the urine was increased in patients with fever or chronic nephritis (Moore, 1960).

The phagocytic activity of leukocytes in vitamin A-deficient animals and man has been decreased, allowing infectious agents which had penetrated the body's external barrier to multiply more freely (Scrimshaw *et al.*, 1959). The acid phosphtase content of neutrophils and macrophages and thus their effectiveness was markedly lowered (Janoff and McCluskey, 1962). Nonspecific bactericidal substances were less active in peritoneal fluid of vitamin A-deficient rats, and in children with xerophthalmia the amount of lysozyme in their tears was increased by treatment with cod liver oil (Scrimshaw, 1966). Animals with hypovitaminosis A were also more susceptible to the effects of aflatoxin (Reddy *et al.*, 1973) and bacterial toxins such as tetanus, diphtheria or *Klebsiella* toxins (Scrimshaw *et al.*, 1959).

Vitamin A and Immunity

In review articles Scrimshaw (1959) and Herrick (1972) reported that vitamin A-deficient animals had a lowered antibody response to naturally occurring disease. Experimentally vitamin A-deficient animals given antigens such as red blood cells had a

smaller antibody response than vitamin A-supplemented animals (Axelrod, 1971; Jurin and Tannock, 1972). Vitamin A acted similarly to an adjuvant in increasing antibody titer to parenterally injected antigens (Dresser, 1968). When vitamin A alone was injected into footpads, it also acted like an adjuvant in increasing the size, cellularity and amount of blast transformation in the draining popliteal lymph nodes (Taub *et al.*, 1970).

Bang *et al.* (1972, 1973) have demonstrated a depression in the numbers of lymphocytes in vitamin A-deficient chickens. Similarly, Wolbach and Howe (1925) reported atrophy of the thymus and lymph nodes in vitamin A-deficient rats. This reflects on the animals' immunologic capacity since lymphocytes are the source of cellular and humoral immunity (Glick *et al.*, 1956; Eisen, 1974). When vitamin A deficiency and Newcastle disease virus infection were combined, a more severe lymphocyte depletion occurred than due to either separately (Bang *et al.*, 1973).

This review of the literature indicated no information existed on the pathogenesis or lesions of *T. glabrata* in the dog, and the information on vitamin A deficiency in the dog might have been of questionable value in light of current knowledge on the nutritional requirements of dogs. Thus the research undertaken in this dissertation would contribute new and useful information to basic and applied biomedical science.

MATERIALS AND METHODS

Four experiments will be described. The initial experiments were to infect 4 of 5 dogs with *T. glabrata* using the anti-inflammatory steroid dexamethasone[a] to reduce resistance to infection. Hereafter these were called s-experiments. Later research (hereafter called A-experiments) involved feeding a vitamin A-deficient ration to 23 dogs (12 not supplemented with vitamin A) and exposing 13 of them to *T. glabrata*. The signs and lesions of vitamin A deficiency, *T. glabrata* infection and the relationship of *T. glabrata* infection and vitamin A deficiency in the dog were determined.

Experimental Design

Initial Production of *T. glabrata* Infection in the Dog

The objective of this research (s-experiments) was to produce *T. glabrata* infection in the dog and to determine the pathogenesis of the disease. The information was required for the design of the later A-experiments with the yeast infection in vitamin A-deprived dogs. Information from the s-experiments was also used to help diagnose a canine case of systemic *Pneumocystis carinii*

[a]Azium, Shering Corporation, Chicago, Illinois.

infection (Tvedten *et al.*, 1974) which initially was suspected to have been torulopsosis.

The dogs were housed in steel cages with a wire mesh floor. During the day, they were free to run together in a room with a concrete floor. The room and cages were cleaned twice a day. All dogs were initially vaccinated for canine distemper and hepatitis.

The amounts of dexamethasone and yeast given to 5 adult, mixed breed dogs[a] and the time allowed between exposure and necropsy are given (Table 1). The dexamethasone was injected subcutaneously,

Table 1. Design of experiments with *T. glabrata* and dexamethasone

Wt. of dog (kg)	Sex	Dexamethasone (mg) per kg body wt.	Total amount of yeast	Days between exposure and necropsy
1) 5.34	M	1.00	1.74×10^9 (1)*	38
2) 5.34	M	0.375	1.06×10^8 (2)*	88
3) 6.08	M	0.00 (control)	1.06×10^8 (2)*	88
4) 7.44	F	0.269	0 (control)	88
5) 5.57	F	0.18	2.13×10^9 (1)	48

*Number of times exposed.

daily from 1 day pre-exposure until necropsy. An initial trial was conducted on Number 1 before the other 4 dogs were used. The

[a]Hodgin's Kennel, Howell, Michigan.

middle 3 dogs (2, 3, 4) were treated as a group, beginning on the same day, and killed on the 88th day. The fifth dog was exposed 40 days later than the middle 3 dogs but a necropsy was performed on the same day as the other dogs.

The *T. glabrata* was maintained on Sabouraud's dextrose agar (Figure 1) and transferred to fresh slants just prior to exposing the dogs. After 3 days' incubation at 37 C the rapidly dividing yeasts were harvested into sterile saline, counted in a hemacytometer and a measured amount (Table 1) injected intravenously. Because prolonged cultivation of *T. glabrata* on artificial media reportedly causes loss of virulence (Lodder and Kreger-Van Rij, 1938), the strain of yeast[a] was serially passed through 3 groups of rats by intraperitoneal injection in an attempt to maintain virulence for use in 2 of the dogs. After each group of rats were euthanatized the yeast was isolated and identified on the basis of sugar fermentation.

Vitamin A Deficiency Experiments

Twenty-three dogs were used in experiments on vitamin A deficiency and *T. glabrata* infection (Table 2). Selection of dogs for the various treatments was based on a table of random numbers. Seven dogs (trial I) were a litter of mongrel dogs raised at our laboratory, and 16 (trial II) were uniform, inbred, male Beagle dogs.[b] They were weaned at 4 to 5 weeks of age, and fed the

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[a] *Torulopsis glabrata*, culture number 098779, isolated from the urine of a human patient, was supplied by Dr. E. S. Beneke.

[b] Laboratory Research Enterprises, Inc., Kalamazoo, Michigan.

Figure 1. *Torulopsis glabrata* colonies appear as small to medium, white colonies on Sabouraud's agar.

Table 2. Design of experiments with *T. glabrata* and vitamin A deficiency

Nutritional status	No. of dogs exposed to *T. glabrata*	No. of dogs not exposed to *T. glabrata*
	Trial I	
Vitamin A-supplemented	2*	1
Vitamin A-deficient	3*	1
	Trial II	
Vitamin A-supplemented	4**	4
Vitamin A-deficient	4**	4

*Exposed intravenously to 1.5×10^9 *T. glabrata* cells.

**Exposed intravenously to 6×10^{10} *T. glabrata* cells.

vitamin A-deficient ration. They were exposed to *T. glabrata* at 19 weeks of age (trial I) and at 17 weeks of age (trial II). Necropsies were performed on dogs in trial I at 28 or 32 days after intravenous exposure and on dogs in trial II at 25, 26 or 27 days after intravenous exposure. Necropsies were performed on these dogs earlier postexposure since the 4 dogs in the s-experiments killed later than 38 days postexposure did not have detectable yeast in their kidneys.

The vitamin A-supplemented dogs were given 2100 IU of vitamin A/kg body weight/wk orally[a] or subcutaneously.[b] This amount was

[a]Vitamin A Capsules, Dewey Product Co., Grand Rapids, Michigan.

[b]Rocavit A, Hoffman-LaRoche, Inc., Nutley, New Jersey.

the suggested requirement for growth and maintenance (Morris, 1968). Trial I dogs were fed free choice. In trial II dogs were fed free choice for the first 5 weeks and then all dogs were fed the same amount of food per day for the final 10 weeks since the vitamin A-deprived dogs were eating less food. Housing was the same as in the s-experiments, except that the vitamin A-supplemented dogs (exposed or unexposed) were kept in one room and the vitamin A-deprived dogs were kept in another room.

The handling of the yeast for exposing the dogs was similar to that in the s-experiments, except the original stock culture was used for exposing the dogs in trial I and an isolate from the urine of a dog in trial I was used for exposing the dogs in trial II.

Rations

s-Experiments

The 5 dogs in the s-experiments were fed a commercial dry type dog food.[a] The dry kibble and separate fresh water were available free choice.

Vitamin A-deficient ration. The vitamin A-deficient ration (Table 3) was formulated by Professor D. E. Ullrey[b] to conform to NRC requirements. The ration was similar to that of Russell and Morris (1939). Dextrose replaced cane sugar to avoid diarrhea. Trace mineralized salt and vitamins (except vitamin A) in amounts

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[a]Ken-L-Ration, Quaker Oats Co., Chicago, Illinois.

[b]Animal Husbandry Department, Michigan State University, East Lansing, Michigan.

Table 3. Vitamin A-deficient ration (25% protein)

Ingredients	Percent
Cooked rolled oats[a]	60.0
Vitamin-free casein[b]	12.0
Meat meal[c]	10.0
Dextrose[d]	10.0
Lard[e]	5.0
Cottonseed and soybean oil[f]	1.0
Trace mineralized salt[g]	1.0
Dicalcium phosphate[h]	0.3
Calcium carbonate[i]	0.2
Vitamin premix[j]	0.5

[a]Baby Flakes, National Oat Co., Cedar Rapids, Iowa; or Quaker Quick Oats, Chicago, Illinois.

[b]Vitamin-Free Casein, Nutritional Biochemicals Co., Cleveland, Ohio.

[c]Big Chief Meat Meal, Badger By-Products Co., Milwaukee, Wisconsin.

[d]Cerelose, CPC International, Glenwood Cliffs, New Jersey.

[e]Shortnin', Peet Packing Co., Chesaning, Michigan.

[f]Wesson Oil, J. Hunt-Wesson Foods, Inc., Fullerton, California.

[g]Trace Mineralized Salt, Hardy Salt Co., St. Louis, Missouri.

[h]Dicalcium Phosphate, Occidental Chemical Co., Houston, Texas.

[i]Calcium Carbonate, Calcium Carbonate Co., Quincy, Illinois.

[j]Vitamin Premix:

Nutrient	Amount per kilogram
Vitamin D, IU	500.00
Vitamin E, IU	50.000
Choline, mg	1,200.000
Ascorbic acid, mg	1,000.000
Niacin, mg	11.400
Pantothenic acid, mg	3.400
Riboflavin, mg	2.200
Menadione, mg	2.000
Thiamine, mg	1.000
Pyridoxine, mg	1.000
Folic acid, mg	0.180
Vitamin B_{12}, mg	0.022

in excess of NRC were supplemented. Rolled oats previously cooked for higher starch digestibility comprised 60% of the diet. Cottonseed and soybean oils were used since they were essentially devoid of vitamin A (Mellanby, 1920; Corey and Hayes, 1972). Ascorbic acid was added to acidify the urine since *T. glabrata* only grows in an acidic environment (Edebo and Spetz, 1965).

The ration contained less than 220 IU of vitamin A per kg and less than 110 IU of vitamin A in the form of carotene per kg.[a] The chemical analysis of the ration was 90.98% dry matter and, on an air dried basis, it contained 25.25% crude protein, 11.37% ether extract, 5.76% ash, 1.35% calcium, 1.11% phosphorus and 6.70% cell wall constituents.[b]

The ration was palatable to the young dogs but had to be fed either dry or very wet. A moderate amount of water made the ration pasty, and it stuck to the roofs of the dogs' mouths. Moistening was only required during the first 2 weeks. The amount consumed by the dogs was measured before each feeding twice a day.

Serologic Tests

Agglutination Test

Preparation of the antigen. Hasencleaver and Mitchell's (1959) method was used. *Torulopsis glabrata* was cultivated on 4%

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[a]Warf Institute, Inc., Madison, Wisconsin.

[b]Animal Husbandry Department, Michigan State University, East Lansing, Michigan.

glucose, 1% neopeptone agar[a] (pH 6.8) slants. The yeast was harvested into 0.9% NaCl solution after 72 hours of incubation at 37 C. The yeast was killed by heating for 6 to 7 hours at 65 C in a water bath. The suspensions were then washed 3 times in 0.9% NaCl phosphate buffered solution and diluted to 3×10^6 cells/ml.

Agglutination procedure. The sera to be tested were diluted in twofold amounts. One half milliliter of the diluted serum was added to .5 ml of the antigenic suspension. The serum dilution in the first tube was 1:30 to avoid nonspecific reactions. The sera were heated at 56 C to decrease nonspecific reactions (Hasencleaver and Mitchell, 1959).

The numbers of yeast cells in the suspensions were determined by direct count in a hemacytometer and diluted to 3×10^6 cells/ml. The titer was read as the highest serum dilution causing definite aggregation of the yeast cells. Saline replaced test sera in one tube containing the yeast suspension as a control for each determination.

Migration Inhibition Test

Preparation of the antigen. Budtz-Jorgensen (1972) reported that a cell suspension of *C. albicans* was a better antigen in the capillary tube migration inhibition test than a saline extract of disintegrated candida cells. A final concentration of 10^7 candida cells per milliliter in the culture chambers was optimal.

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[a]Sabouraud's Agar, Difco Laboratories, Detroit, Michigan.

Therefore, a cell suspension of *T. glabrata* cells was used as an antigen in our MIF test. *Torulopsis glabrata* cells were prepared as for the agglutination test and were resuspended to give a concentration of 3×10^5 cells/ml as determined by a hemacytometer count. Since *T. glabrata* and *C. albicans* share common antigens (Hasencleaver and Mitchell, 1960; Tsuchiya *et al.*, 1961), a commercial preparation of *C. albicans* antigens at a 1:100 dilution[a] was also used in the MIF test.

Migration inhibition test procedure. This was a modification of the MIF test developed by Dr. V. Mallmann.[b] Blood was collected in a 10 ml syringe containing 1 ml of sodium citrate anticoagulant. Tubes of the blood were centrifuged for 15 minutes at 2300 rpm. The buffy coat was carefully pipetted and dispensed into a screw cap tube. Four blood micropipettes were filled with the buffy coat and one end was plugged with sterile paraffin. These micropipettes were centrifuged for 10 minutes at 2000 rpm.

The spot test version of the MIF test was continued by scoring the pipettes at the RBC/WBC interphase with a diamond pen and then breaking it. Cells from the 4 micropipettes were withdrawn from the WBC end into a .5 ml syringe containing 0.3 ml of M 199 solution. The cells and M 199 media were mixed well. Four drops of the mixture were placed on the floor of a small petri dish. The cells were allowed to attach at room temperature for 3 to 5

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[a]Dermatophyte "O", Hollister Stier, Downer's Grove, Illinois.

[b]Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan.

minutes. If longer amounts of time were allowed, more than one layer of cells attached to the floor of the dish.

The 4 spots were carefully rinsed with 2 changes of BSS. The spots were measured with a microscope equipped with an eyepiece micrometer. Media with or without the antigen were added and the petri dishes were incubated for 24 hours at 37 C. The spots were again measured with the dish in the same position and the amount of migration or inhibition of migration was noted.

Clinical Pathologic Tests

A hematologic examination, urinalysis and serum protein electrophoresis were performed at weekly intervals.

Urinalysis

Urine was collected weekly into sterile plastic syringes by means of sterile catheters to permit culturing of the sample. The color and turbidity of the sample were recorded, and the urine specific gravity was determined by the refractive index as measured by a refractometer.[a] Blood, ketone, glucose, pH and protein values were determined.[b] A positive protein reaction was confirmed by the sulfosalicylic acid method.[c]

Ten milliliters of urine were centrifuged at 1000 rpm for 5 minutes to obtain a urine sediment. The sediment was stained with new methylene blue and the microscopic examination recorded.

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[a]Goldberg Refractometer, American Optical Co., Buffalo, N.Y.

[b]Labstix, Ames Co., Elkhart, Indiana.

[c]Bumin Test, Ames Co., Elkhart, Indiana.

Hematologic Examination

Blood was collected weekly from jugular vein using minimal restraint. EDTA was the anticoagulant. A blood smear was made, stained with Wright's stain and examined microscopically. Micro-hematocrit tubes[a] were used to determine the packed cell volume and hemoglobin was determined by the standard cyanmethemoglobin method as described by Benjamin (1969), except that commercial reagents were used.[b] The number of white blood cells per milliliter were counted in a hemacytometer.[c]

Serum Protein Electrophoresis

The serum proteins of weekly serum samples were separated, stained and quantitated on cellulose acetate strips according to the "Zip Zone" electrophoresis method.[d] The stained strips were analyzed on a Densicord electrophoresis densitometer 552.[e]

Blood Urea Nitrogen

The amount of blood urea nitrogen in serum samples was determined by the Beckman BUN analyzer.[f]

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[a]Blue-Tip, Sherwood Medical Industries, Inc., St. Louis, Missouri.

[b]Hycel Cyanmethemoglobin Reagent and Standard, Hycel, Inc., Houston, Texas.

[c]Improved Neubauer, Scientific Products, Inc., St. Louis, Missouri.

[d]Titan III - XW, Helena Laboratories, Beaumont, Texas.

[e]Photovolt Corporation, New York, N.Y.

[f]Beckman Instruments, Inc., Fullerton, California.

Other Tests

Vitamin A Analysis

Bi-weekly plasma vitamin A levels were determined by Neeld and Pearson's (1963) method. The first samples were taken when the dogs were 8 to 9 weeks old. Hepatic vitamin A levels were determined on samples of liver collected at necropsy. The following modifications of Neeld and Pearson's method were made for hepatic samples: (a) aqueous hepatic homogenates were prepared in a tissue grinder,[a] (b) the homogenates were saponified for 10 minutes at 40 C with an alkaline solution of 50% KOH and 10% ascorbic acid, and (c) a measured amount of saponified aqueous homogenate was substituted for serum.

Renal Biopsies

Two exposed dogs in the s-experiments were anesthetized, and a 2 x 1 x 1 cm wedge of a kidney was removed through a flank incision at 10 and 70 days after exposure to *T. glabrata*. The kidney capsule was closed with a horizontal mattress suture.

Skin Tests

Skin tests were administered to dogs in the A-experiments 2 days prior to necropsy. An intradermal injection of .1 ml of a heat-killed suspension of *T. glabrata* cells (3×10^6) was given into 1 ear. A 1:10 dilution of soluble *Candida albicans* antigen[b]

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[a] Model 106, Talboys Engineering Corp., Emerson, N.J.

[b] Dermatophyte "O", Holister Stier, Downer's Grove, Illinois.

was injected intradermally into the other ear. The ears were examined for redness and swelling at 1, 6, 24 and 48 hours after the injection. The skin test site was collected at necropsy for histologic examination.

CSF Pressure Determinations

The CSF pressure was determined on anesthetized dogs on the day they were necropsied. A 20-gauge, 5-inch spinal needle was inserted through the dorsal midline of the neck into the cisterna magna. The stylet was removed and the needle was connected to a manometer when clear CSF dripped from the spinal needle. The maximum manometer reading was recorded.

Microbiology

Urine samples were aseptically collected after exposure to the yeast. Two drops of the urine were used to inoculate Sabouraud's agar plates. After 48 hours' incubation at 37 C the number of colonies were recorded. Samples of the colonies were suspended in a drop of lactophenol cotton blue on a glass microscope slide, covered with a coverslip and examined microscopically. The shape of the organism was recorded and, if yeast cells were found, they were identified by their staining, shape and biochemical reactions. The urine samples were collected at weekly intervals after exposure of the dogs to *T. glabrata*.

Samples of kidney, urinary bladder and spleen were aseptically collected at necropsy and used to inoculate Sabouraud's agar and blood agar plates. Yeast and bacteria isolated at the end of 24- to 48-hour incubation at 37 C were identified by staining, shape

and biochemical reactions. *Torulopsis glabrata* is the only yeast to ferment glucose, trehalose and no other sugar (Edebo and Spetz, 1965).

Euthanasia and Pathologic Examination

The dogs were humanely killed with sodium pentobarbital injected intravenously. A systemic gross examination of the organs was made. Tissues were fixed in 10% neutral formalin. The tissues were sectioned at 6 microns thickness and stained with hematoxylin and eosin or other special stains (Luna, 1968).

Renal samples from dog number 1 in the s-experiments were fixed in 3% glutaraldehyde for transmission electron microscopic examination.

RESULTS

General

All dogs maintained a healthy, normal appearance and a friendly nature. Neither the vitamin A deficiency nor the yeast infection was allowed to progress to a point which appeared painful to the dogs.

Clinical Signs

Mild but obvious central nervous system signs appeared in 5 of 12 dogs after eating the vitamin A-deficient ration for 7 to 13 weeks. Affected dogs developed a head tilt (Figure 2), would circle in the direction of the head tilt, and would occasionally "star-gaze" by staring at the ceiling. They appeared less aware of their surroundings and would swing their heads past a subject when turning to look at that subject. A short convulsion with running movements was observed in 1 dog. Another dog with the neurologic signs appeared to have impaired vision since he would occasionally walk into objects. He and a second dog had papilledema diagnosed on ophthalmic examination at 21 weeks of age.

The dogs also had impaired balance. When they were picked up, they twisted their bodies in an abnormal manner. Unaffected dogs, in comparison, did not struggle when picked up and always maintained their center of gravity such that if dropped they would

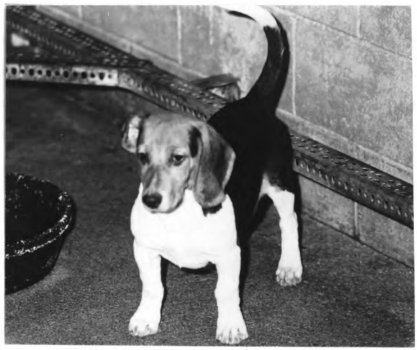

Figure 2a. Vitamin A-supplemented dog with a normal erect stance.

Figure 2b. Vitamin A-deprived dog with characteristic head tilt.

land on their feet. The lack of balance, the head tilt, and the circling in the direction of the head tilt clinically suggested a vestibular problem. Besides the neurologic signs, no other clinical signs were attributed to vitamin A deficiency and the dogs outwardly appeared normal.

Exposure to the yeast was not associated with any consistent signs. Neither fever during the first week after exposure nor anorexia was detected, and only 1 exposed, vitamin A-deprived dog had temporary (4 days) hematuria and polyuria.

A startling but temporary reaction occurred after intravenous injection of the yeast suspension. Most of the dogs, 2 to 3 minutes after the injection, either vomited, defecated, urinated, and/or fainted. If they fainted, they were conscious in 3 minutes and standing by 5 minutes. By 10 minutes postinjection, they appeared normal.

The vitamin A-deprived dogs appeared to have duller hair coats and were more nervous. They were harder to handle during specimen collection since some seemed hyperkinetic and refused to hold still.

Growth and Appetite

When the vitamin A-deprived dogs in trial I were fed free choice for the full 16 weeks with no attempt to pair-feed them, the vitamin A-deprived dogs ate on an average 15.7% less food than the vitamin A-supplemented dogs, and the differences between their mean weights increased each week to a maximum of 10% by the end of the experiment (Appendix A). This weight difference was

greater than in trial II (Appendix B). However, the vitamin A-deprived dogs increased their average weight from 758 gm at 4 weeks of age to 4330 gm at 21 weeks of age.

The growth rate of the other dogs fed the vitamin A-deficient ration was also uniform and good. The vitamin A-supplemented dogs in trial II increased their average initial weight of 1744 gm to 8792 gm in 14 weeks. The vitamin A-deprived dogs in trial II never weighed less than 92.5% of the supplemented dogs and averaged 96% of the weight of the supplemented dogs. At the end of the experiment the deprived dogs weighed 98% as much as the supplemented dogs. Thus weight changes were not greatly different in trial II.

The vitamin A-deprived dogs in trial II were also relatively less hungry than the vitamin A-supplemented dogs. The vitamin A-deprived dogs' feed consumption during the first 5 weeks of free choice feeding decreased to 87.1% of that consumed by supplemented dogs. For the last 10 weeks of the experiment each group of 8 dogs were offered 2400 gm of ration per day and the feed consumption of the deprived dogs slowly increased until both the vitamin A-deprived and -supplemented dogs ate the same amount (Appendix C). At the time of exposure to *T. glabrata* the deprived dogs were eating 96.7% as much as the supplemented dogs. However, the deprived dogs still ate more slowly and had food in their dishes later in the day than the supplemented dogs.

Laboratory Findings

Urinalysis

All dogs eating the experimental ration consistently had a urine pH of 5 to 6. Many oval budding yeast were easily observed in the urinary sediment of samples collected at 25 to 38 days postexposure from the dog receiving the highest amount of dexamethasone. Only occasional yeast were detected in the urinary sediments of other exposed dogs, and urinary culture was a more sensitive means of detecting urinary shedding of the yeast. Over 100 to 200 bacteria per microscopic field at 450 power were present in the urinary sediments of dogs with bacterial infections of the urinary tract (described under microbiologic or histologic findings).

Urine specific gravities of 1.010 $\pm$ 5 occurred in 7 dogs. Six of these dogs had many colonies of bacteria or yeast isolated from samples of their urine or organs. Five of the 6 dogs had gross lesions of the urinary tract, and the sixth dog was the only dog in the A-experiments to have a positive sulfosalicylic acid test for albumin in the urine and clinical signs of polyuria and hematuria. Only 1 dog with low urine specific gravity did not have evidence of urinary infection. Low urine specific gravity, polyuria and polydipsia in the s-experiments occurred after the initiation of dexamethasone treatment and were associated with the effect of the steroid.

Hematologic Findings

Anemia was not detected in the vitamin A-deprived dogs. The mean packed cell volume of all trial I dogs in the A-experiments increased from 29% at 8 weeks of age to 35.25% at 17 weeks of age without regard to vitamin status (Appendix D). Similarly, the mean packed cell volume of the trial II dogs in the A-experiment rose from 24.4% at 7 weeks of age to 38.5% at 20 weeks of age without any apparent influence of vitamin A status (Appendix E).

Leukocyte numbers in apparently normal dogs at 8 to 16 weeks of age (pre-exposure) were variable with a wide range (6800 to 21,000 WBC/mm^3). The white blood cell count averaged 13,483/mm^3 with a mean of 63% neutrophils, 30% lymphocytes, 5% monocytes and 2% eosinophils. Although the wide range in absolute numbers of leukocytes in normal dogs before exposure overlapped the leukocytic response to the yeast, general trends were noted when the weekly hematologic results of individual groups of dogs were analyzed (Table 4). A response was detected 1 day postexposure (Appendix F). The white blood cell count of exposed dogs in trial II increased (17.9% - vitamin A-supplemented, 6.1% - vitamin A-deprived), while the mean white blood cell count of unexposed dogs decreased (7.0% - vitamin A-supplemented, 32.8% - vitamin A-deprived) when compared with the mean of 3 weekly determinations made during the month preceding exposure to *T. glabrata*. A relative and absolute neutrophilia accounted for most of the increase since the neutrophil count in exposed dogs increased (68.2% - vitamin A-supplemented, 27.8% - vitamin

Table 4. Percentage change in mean leukocyte response to *T. glabrata* by trial II dogs when compared with leukocyte levels 1 month pre-exposure

Parameter	Treatment			
	+A -yeast (4 dogs)	+A +yeast (4 dogs)	-A -yeast (4 dogs)	-A +yeast (4 dogs)
1) WBC count*	-7.0	+17.9	-32.8	+6.1
2) neutrophil count*	-14.8	+68.2	-15.7	+27.8
3) WBC count**	-16.2	-7.58	-17.6	+3.8
4) neutrophil count**	-15.7	-3.4	-4.0	+9.6

*Determined 1 day after exposure to *T. glabrata*.

**Determined during the second to fourth week after exposure to *T. glabrata*.

A-deprived), while the neutrophil numbers decreased in unexposed dogs (14.8% - vitamin A-supplemented, 15.7% - vitamin A-deprived). Leukocyte changes during the next 3 weekly determinations were essentially the same in the groups except the vitamin A-deprived, exposed dogs had an increase in mean total white blood cell count (3.8%) and neutrophil numbers (9.6%), while the white blood count in the other 3 groups decreased from 7.6 to 17.6% and the neutrophil numbers dropped from 3.4 to 15.7% (Appendix G).

Leukocytic response to *T. glabrata* exposure in the s- experiments was difficult to interpret because only 5 dogs were used, the pre-exposure values were variable, and there was a strong

influence on the hemogram by factors such as dexamethasone treatment, renal biopsies, and the pregnancy of 1 dog (which resulted in the litter of 7 dogs used in trial I of the A-experiments). Dexamethasone injections increased the percentage of neutrophils from 65.5% to 86.5%, and renal biopsies increased the percentage of neutrophils from 61 to 80%. Dexamethasone injections caused a neutrophilia, monocytosis, eosinopenia, and lymphopenia.

Serum Protein Electrophoresis

A pronounced change in the electrophoretic pattern occurred in the dogs given dexamethasone. The α_2 peak was markedly elevated. The exposed dog given the highest amount of dexamethasone had 33% of his serum protein in the α_2 peak (2.25 gm, while the total serum protein was 6.8 gm/100 ml serum) (Appendix H).

Three dogs were treated as a group in the s-experiments and their changes in the α_2 portion of the electrophoretic pattern are listed (Table 5). Since the 2 dogs receiving dexamethasone had 51.3% or 45.1% increases in their α_2 protein value, while the dog given *T. glabrata* had a decrease in his α_2 value, it appeared that the dexamethasone was the factor elevating the α_2 fraction of the serum proteins. By including the large α_2 value of the dog given 1 mg dexamethasone/day with the 3 dogs in Table 5, it appeared that the increase in α_2 proteins was dose dependent on the amount of dexamethasone/kg administered.

The electrophoretic patterns of the trial I dogs in the A-experiments did not appear to have consistent changes due to an experimental factor but there was a consistent decrease in the

Table 5. Average changes in α_2 globulin values in weekly serum samples in 3 adult dogs

Time period	No. of samples	+Y-S* gm/100 ml	+Y-S* %	+Y+S* gm/100 ml	+Y+S* %	-Y+S* gm/100 ml	-Y+S* %
1) before dexamethasone	2	.655	10.2	1.02	15.6	1.09	14.8
2) after dexamethasone and exposure	8	.419	6.3	1.79	23.6	1.52	21.2
3) % change		-38.2		+51.3		+45.1	

*+Y = exposure intravenously to *T. glabrata*.

-Y = no exposure.

+S = receiving dexamethasone (0.375 and 0.269 mg/kg, respectively, for +Y+S and -Y+S).

-S = no steroid.

**% = % total serum proteins.

albumin/globulin (A/G) ratio in the exposed dogs after exposure when compared with unexposed dogs during the same time period (Appendix I). The mean A/G ratio of their unexposed counterparts increased from 1.30 to 1.40 during the same time period. Individual dogs had about the same total amount of protein for each of the 10 weekly tests, although different dogs had different characteristic total serum protein values from 6.1 to 6.7 gm/100 ml.

The mean A/G ratio in the group of exposed, vitamin A-deprived dogs in trial II of the A-experiments also decreased

after exposure to *T. glabrata* (Appendix J). The mean A/G ratio was 1.22, 2 weeks prior to exposure, and decreased to a mean of 1.05 during the 3 weeks after exposure. The mean A/G ratio of the other 3 groups (+A-Y, +A+Y and -A-Y) increased slightly or stayed the same (1.34 to 1.34, 1.09 to 1.17 and 1.15 to 1.24, respectively) during the same time period. The total amount of protein in the dogs' serum in all 4 groups was about the same on a given week but rose from a mean of 5.1 gm/100 ml at 15 weeks of age to a mean of 6.6 at 19.5 weeks of age.

Blood Urea Nitrogen

Blood urea nitrogen levels in 3 exposed dogs in the s-experiments were 7, 16 and 10 mg/100 ml and, in the unexposed, dexamethasone-treated dog, was 23 mg/100 ml.

Cerebrospinal Fluid Pressure

The average CSF pressure in 3 vitamin A-deprived dogs of trial I at 80 mm (60, 80 and 100 mm) was higher than the 2 vitamin A-supplemented littermates at 54 mm (42 and 66) (Appendix K). However, the average CSF pressure of 6 vitamin A-supplemented dogs (121.7 mm) and 8 vitamin A-deprived dogs (124.8 mm) in trial II was approximately the same.

The 2 highest CSF pressures (222 and 175 mm) and the 3 lowest CSF pressures (65, 66 and 96 mm) in trial II were in vitamin A-deprived dogs (Appendix K). The 2 vitamin A-deprived dogs in trial I with obvious nervous disorders had the 2 highest CSF pressures (80 and 100 mm). The 3 vitamin A-deprived dogs with neurologic disorders in trial II had high and low cerebrospinal

pressures (175, 144 and 66). However, CSF dripped rapidly from the spinal needle before the manometer could be attached in the dog with 66 mm of pressure, suggesting increased pressure.

The 3 vitamin A-deprived dogs in trial II with neurologic disorders were examined by an ophthalmologist.[a] The 2 dogs with CSF pressures of 175 and 144 mm had papilledema. The vitamin A-deprived dog with 222 mm pressure did not receive an ophthalmic examination since it did not have apparent neurologic disorders.

Vitamin A Status

The mean level of vitamin A in the plasma of the 12-week-old vitamin A-deprived dogs in trial I had decreased to 5.5 µg/ml after they had eaten the experimental ration for 7 weeks (Appendix L). This coincided with the first signs of neurologic disorders. The mean level of vitamin A in the plasma of the 13-week-old vitamin A-deprived dogs in trial II had decreased to 6.15 µg/100 ml after they had consumed the experimental ration for 8 weeks. The first signs of neurologic disorders were observed 1 week later.

The plasma vitamin A levels in the 8 vitamin A-deprived dogs of trial II slowly declined from 18 µg/100 ml at 9 weeks of age to 3 µg/100 ml at 18.5 weeks of age (Appendix M). The 8 vitamin A-supplemented dogs had variable plasma vitamin A levels which were highest just after the weekly or bi-weekly administration of vitamin A. The 8 vitamin A-supplemented dogs averaged 46 µg vitamin A/100 ml of plasma.

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[a]Dr. Gary Blanchard, Department of Small Animal Surgery and Medicine, Michigan State University, East Lansing, Michigan 48824.

Plasma vitamin A levels were determined on 18 samples from 4 of the adult dogs fed a commercial dog food in the s-experiments for comparison. The mean plasma vitamin A level of these samples was 84.76 µg/100 ml.

The mean value of vitamin A in hepatic samples of vitamin A-supplemented dogs was 90.51 µg/gm (trial I) and 74.79 µg/gm (trial II). In the vitamin A-deprived dogs the mean hepatic vitamin A levels were 21.40 µg/gm (trial I) and 6.24 µg/gm (trial II). One unexposed vitamin A-supplemented dog (trial II) had a low hepatic vitamin A value (9.89 µg/gm) which was unexplained.

Microbiologic Findings

All 12 vitamin A-deprived dogs had bacteria isolated from their urine samples, and 9 of these had significant numbers of bacteria (>10 colonies) on at least 2 consecutive weeks. Six of the 11 vitamin A-supplemented dogs had bacteria isolated from their urine samples, but only 1 of these dogs had large growths of bacteria on 2 consecutive weeks.

Torulopsis glabrata was isolated in moderate to large numbers from cultures of the organs of urogenital tract of the dog given the highest dosage of dexamethasone and 2 of the exposed, vitamin A-deprived dogs. Significant numbers of bacteria were isolated from cultures of the kidney and bladder of 5 of the 7 exposed, vitamin A-deprived dogs, while in the other 3 categories of the A-experiments only 1 dog in the vitamin A-supplemented, unexposed category had an isolate with many colonies of bacteria. This dog had hemolytic *Escherichia coli* isolated from the bladder.

Hemolytic *E. coli* were also identified from the other cultures of organs from the vitamin A-deprived, exposed dogs, except for cultures from 1 dog from which *Staphylococcus aureus* was identified.

Immunologic Findings

Earlier research on the use of the migration inhibition test in dogs indicated inhibition of leukocyte migration over 35% was positive (Legendre, 1974). Eighty-two percent of the dogs in the A-experiments had positive reactions to *T. glabrata* antigen and 74% had positive reactions to *C. albicans* antigen, regardless of whether they were exposed to *T. glabrata* or not. The positive reactions occurred between 5 and 26 days postexposure. Fifty-five percent of the dogs with positive reactions were positive to *T. glabrata* antigen even before they were exposed. Similarly, 41% of the dogs with positive reactions had greater than 35% inhibition of migration in the presence of *C. albicans* antigen before they were given the *T. glabrata* suspension intravenously.

Treatment groups could not be differentiated on the basis of agglutination titers during the 3 weekly tests after exposure of the dogs to *T. glabrata*. The agglutination titers ranged between 30 and 480 but appeared to have a random pattern without regard to treatment.

The skin reactions are summarized (Table 6). The most frequent reaction was to have swelling and redness both at 1 or 6 hours postinjection (immediate sensitivity) and at 24 or 48 hours (delayed sensitivity). All animals with immediate skin reactions

Table 6. Skin test results of the 16 dogs in trial II

Antigen	Supplemented* unexposed**	Supplemented* exposed**	Deprived unexposed**	Deprived* exposed**
C. albicans	2-D, 2-B	2-D, 2-B	1-D, 2-B, 1-N	4-B
T. glabrata	1-D, 3-B	1-D, 3-B	1-B, 3-N	1-D, 3-N

*Vitamin A status.

**Exposure intravenously to *T. glabrata*.

I = immediate, D = delayed, B = both, N = none.

also had delayed skin reactions, although 8 tests had only the delayed skin reaction. Seven skin tests had no gross reaction. Six of these were with the *T. glabrata* antigen and 1 was with *C. albicans* antigen. All 7 of the skin tests with no reaction were in vitamin A-deprived dogs. The *C. albicans* antigen consistently produced a more severe gross skin reaction.

Histologically, the sites of the skin tests had infiltration, primarily of mononuclear cells, around the adnexae, blood vessels, or in cords of inflammatory cells between the horizontal fibers of connective tissue. The inflammatory reactions were mild to moderate in severity and did not cause much distortion of the skin's architecture. Acanthosis and mild edema were often present.

The histologic lesions were comparable in severity to those noted grossly. Five of the 7 tests grossly reported as no reaction had a mild infiltration primarily of mononuclear cells. Although primarily a mononuclear infiltrate, purulent inflammation was found

areas with necrosis and edema. Only 2 dogs had more neutrophils than mononuclear cells, and they did not have over 60% neutrophils. One of these dogs was vitamin A-supplemented and exposed, and the other was vitamin A-deprived and exposed.

Pathologic Findings

The most severe infection with *T. glabrata* was in the first dog of the s-experiments which was given 1 mg of dexamethasone per kg/wt. In this dog several large colonies of the yeast developed within the renal tubules (Figure 3). The yeast cells were oval and often had a single bud. They were less frequent in the interstitial areas of the kidneys (Figure 4), and most of these appeared to be phagocytized. However, frequently occurring Russell's bodies (Figure 5) in plasma cells could easily be mistaken on PAS-stained sections for phagocytized yeast. The renal tubules were surrounded by a large and relatively pure population of plasma cells which grossly appeared as white streaks from the renal capsule into the renal medulla (Figure 6). About 2 to 3% of the kidney was grossly affected. Yeast were also in the lower urinary tract and prostate gland but did not stimulate any inflammation.

No yeast were found in renal sections of the other 3 exposed dogs of the s-experiments collected at 48 or 88 days after exposure. The dog killed at 48 days, only 10 days later than the severely affected dog, had renal inflammation most similar to the plasma cell interstitial nephritis just described, and it appeared the nephritis was almost healed.

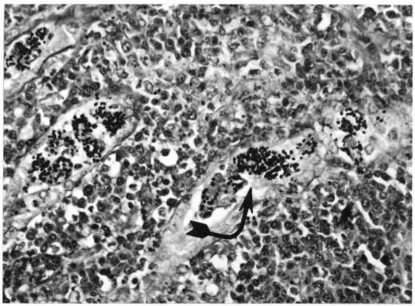

Figure 3. Inflamed kidney tissues of a dog treated with dexamethasone and exposed to *T. glabrata*. Large groups of *T. glabrata* are in the renal tubules (arrow). The inflammatory reaction around the renal tubules contains many plasma cells. PAS stain; x 125.

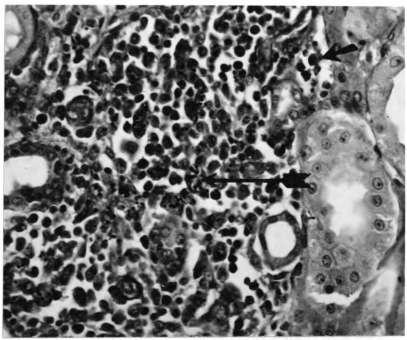

Figure 4. Interstitial inflammatory reaction to *T. glabrata* in the kidneys of the dog in Figure 3. The yeast are in interstitial areas, where they appear to be phagocytized (long arrows). Several plasma cells are present (short arrows). PAS stain; x 125.

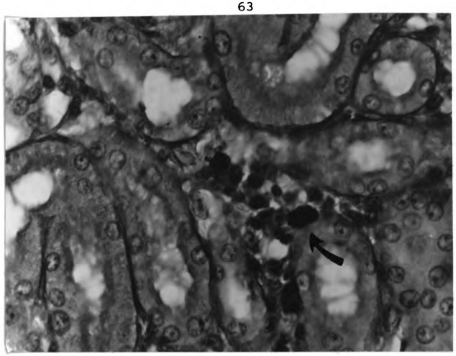

Figure 5. Section of kidney from a vitamin A-deprived dog infected with *T. glabrata*. A small group of plasma cells are situated between some renal tubules. Two plasma cells are so filled with Russell's bodies that they appear as dark areas (arrow). PAS stain; x 540.

Figure 6. Cross sections of a kidney histologically illustrated in Figures 3 and 4 infected with *T. glabrata*. The inflammatory reactions appear as white streaks (arrow) extending from the renal capsule into the renal medulla.

The main findings in the A-experiments also involved the urinary tract structures as summarized (Table 7). A subacute interstitial nephritis occurred in 8 of the dogs exposed to *T. glabrata*.

Table 7. Urinary tract findings in the dogs in the A-experiments

Treatment group	No. of dogs	Gross nephritis	Microscopic nephritis	LUGT inflammation[a]	Microscopic yeast[b]	Cultured yeast	Cultured bacteria[c]
+A-Y[d]	5	0	1[e]	1	0	0	2
+A+Y	6	2	2	0	1	3	1
-A-Y	5	0	0	0	0	0	3
-A+Y	7	5	6	4	4	5	7

[a]Microscopic evidence of inflammation in the lower urogenital tract.

[b]Yeast-like structures found microscopically in kidney sections.

[c]Organism cultured in appreciable number from urine or organs.

[d]+A = vitamin A supplemented, -A = vitamin A deprived, +Y = exposed to *T. glabrata* intravenously, -Y = not exposed.

[e]A mild inflammation of the renal pelvis due to *E. coli*.

One dog had a mild pyelonephritis not similar to the interstitial nephritis of *T. glabrata*. Of these 8 dogs with interstitial nephritis, only 2 were supplemented with vitamin A. Five of the 8 dogs in the A-experiments had yeast histologically detected in low numbers in their kidney sections (Figure 7).

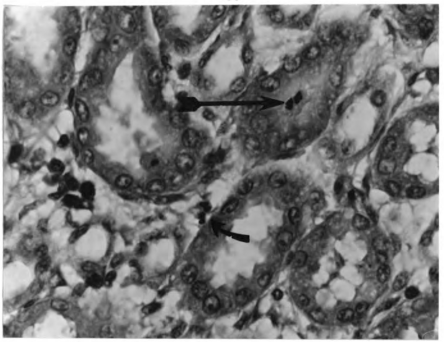

Figure 7. Section of kidney from a vitamin A-deprived dog exposed to *T. glabrata*. A small group of yeast are in a renal tubule (long arrow) and a few yeast cells appear phagocytized in the interstitial tissues (short arrow). Gram's stain; x 540.

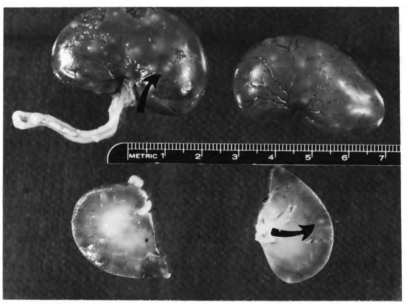

Figure 8. The kidneys of a vitamin A-deprived dog exposed to *T. glabrata*. Notice the many white foci on the surface of the kidneys and in the renal cortex (arrows).

The interstitial inflammatory pattern of the nephritis of the 8 exposed dogs in the A-experiment also appeared grossly as streaks from the renal capsule to the renal medulla. On the surface of the kidney these streaks appeared as white foci (Figure 8). In 2 of the exposed, vitamin A-deprived dogs with secondary *E. coli* infection the renal lesions were larger and more hemorrhagic (Figure 9). The average number of gross lesions per kidney was larger in the vitamin A-deprived dogs (50, 15, 8, 5, 4, 2, 0) than the vitamin A-supplemented dogs (3, 1). The vitamin A-deprived dog with only 2 renal lesions per kidney also had a fluid-filled abscess containing several compartments adjacent to the jugular vein from which a pure culture of *T. glabrata* was isolated (Figure 10). Histologically, the abscess was lined by a thin granulomatous layer and a thick fibrous layer.

The interstitial areas were crowded with a mixed population of neutrophils, histiocytes and plasma cells, often containing Russell's bodies. The concentration of any particular type of inflammatory cell varied in different parts of the lesions. Frequently neutrophils predominated near the center of lesions and the mononuclear cells predominated near the edges of the lesions. Small areas of inflammation were usually mononuclear consisting primarily of plasma cells. Occasionally 1 or 2 small necrotic areas of pink hyaline material were in the center of the lesions of a few of the dogs. The characteristic lesion would be termed a subacute interstitial nephritis. Four of the exposed dogs also having bacterial infections had enough inflammation around the renal pelvis to also be classified as pyelonephritis.

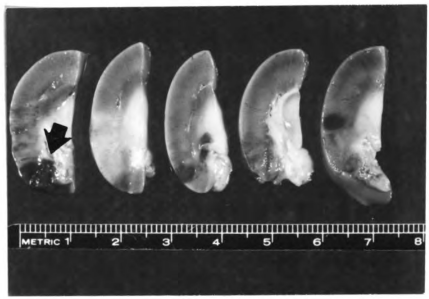

Figure 9. Cross sections of a kidney from a vitamin A-deprived dog exposed to *T. glabrata*. Notice the hemorrhagic focus (arrow) in the kidney sections. Hemorrhage was only present in 2 dogs with secondary *E. coli* infections.

Figure 10. A vitamin A-deprived dog in trial I which was exposed to *T. glabrata*. Notice the dark exudate on the fur of the neck. An abscess along the left jugular vein had ruptured and a pure culture of *T. glabrata* was isolated from the exudate.

Inflammatory lesions occurred in the lower urogenital tracts of 4 exposed, vitamin A-deprived dogs and 1 unexposed, vitamin A-supplemented dog. All dogs with lower urinary tract lesions or pyelonephritis had large numbers of bacteria isolated from cultures of the bladder or prostate gland. However, the 4 dogs with pure infections of *T. glabrata* had no detectable inflammatory lesions in the lower urinary tract. The lesions and number of cases of the lower urinary tract included ureteritis (2) (Figure 11), prostatitis (2), cystitis (3) (Figure 12) and cystitis with urinary calculi (1) (Figure 13a,b). The vitamin A-deprived dog with the urinary calculi also had a subacute purulent prostatitis with a mixed infection of *T. glabrata* and a coagulase-positive *Staphylococcus aureus*.

One vitamin A-supplemented dog not exposed to *T. glabrata* merited individual description. It was the only unexposed dog to have urinary tract lesions. It had subacute inflammation of the urinary bladder, which partially involved the prostate gland, ureters and renal pelvis of the kidneys. *Escherichia coli* was isolated in high numbers from cultures of the urinary bladder and only 2 colonies were isolated from cultures of the kidney. Although it had a mild pyelonephritis, it did not have an interstitial nephritis such as in the dogs exposed to *T. glabrata*. The dog also had a large thrombus which occluded a vessel in a pulmonary lymph node and caused a grossly apparent area of necrosis. Histologically this necrotic area was infiltrated with many neutrophils.

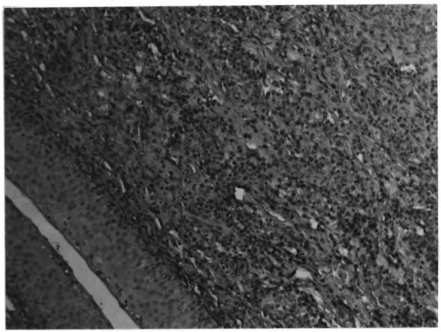

Figure 11. A greatly thickened ureter from an exposed, vitamin A-deprived dog with *E. coli* isolated from the lower urinary tract. The connective tissue around the epithelium is diffusely and heavily infiltrated with mononuclear cells, including many plasma cells (arrows). H&E stain; x 125.

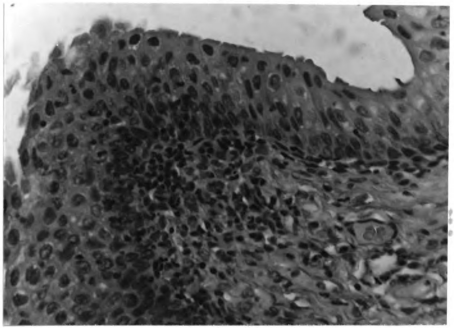

Figure 12. Urinary bladder of an exposed, vitamin A-deprived dog with cystitis. *E. coli* was isolated from the urinary bladder. Note the accumulation of mononuclear cells and neutrophils under the epithelium (arrow). H&E stain; x 312.

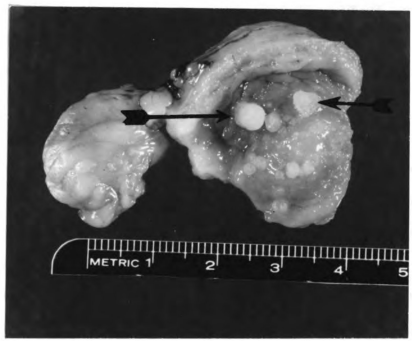

Figure 13a. The urinary bladder of a vitamin A-deprived dog exposed to *T. glabrata*. *S. aureus* was isolated from the bladder. Note the urinary calculi (arrows). The structure to the left is fat.

Figure 13b. Urinary calculi from the urinary bladder in Figure 13a.

The rats, in which *T. glabrata* had been injected intraperitoneally for maintenance of *T. glabrata* for later use in 2 of the dogs in the s-experiments, had a multifocal chronic granulomatous peritonitis. Small white granulomas, 1 to 2 mm in diameter, occurred in the omentum, on the stomach, and on the surface of the liver, especially on the side opposite the diaphragm (Figure 14). These granulomas had a central necrotic area containing yeast, a middle layer of macrophages and giant cells, and an outer layer of fibrous connective tissue. A more diffuse granulomatous reaction was also observed at the injection site, in which macrophages and granulation tissue infiltrated the muscular wall of the abdomen with yeast, necrosis, fibrin and neutrophils near the peritoneal cavity.

Epithelial defects occurred on the tongue of the dog that had the earliest neurologic signs of vitamin A deficiency. Grossly they appeared as several small areas (.4 to 1.2 mm in diameter) on the dorsal surface of the tongue (Figure 15) in which the papillae were shortened and the surface appeared smoother and pinker than the normal appearing, rough, white background (Figures 16 and 17).

Other Pathologic Findings

There were several incidental findings in the dogs that appeared unrelated to *T. glabrata* infection or vitamin A deficiency. All but 3 of the Beagle dogs in trial II had mild inflammatory lesions in the lungs. Histologically, they consisted of cuffs of mononuclear cells around small bronchioles and small blood vessels. Occasionally the epithelium of affected bronchioles was distorted by mononuclear cells and some neutrophils. These lesions were

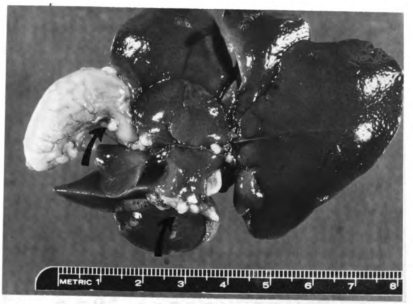

Figure 14. Liver and stomach of a rat exposed intraperitoneally to *T. glabrata*. Note the multiple white granulomas (arrows).

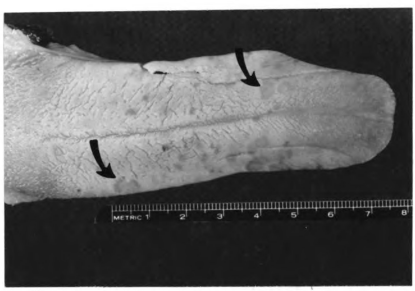

Figure 15. The tongue of the unexposed dog that had the earliest neurologic signs of vitamin A deficiency. Note the many smooth spots (arrows) on the dorsal surface.

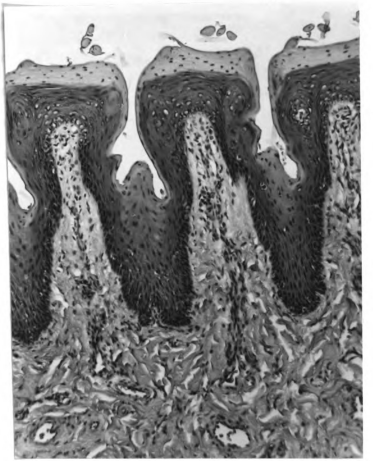

Figure 16. Normal appearing papillae on the unaffected dorsal surface of the tongue illustrated in Figure 15. H&E stain; x 125.

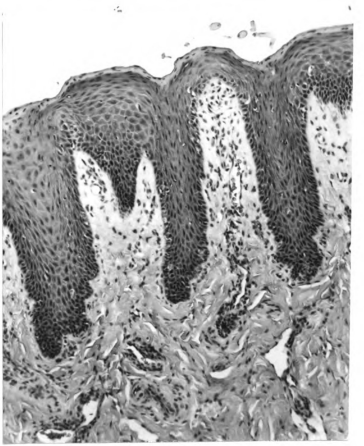

Figure 17. Area of the tongue illustrated in Figure 15 which appeared smooth and darker. No papillae were found. The stratum spinosum was thickened in these areas and the stratum corneum was thin in comparison to Figure 16. H&E stain; x 125.

mild and focal. Four of the dogs on gross examination also had red spots (1-2 mm in diameter) on the pleura which may have corresponded to the microscopic lesions. The histologic lesions occurred in all 4 categories of dogs in approximately equal numbers. One dog with a localized area of subacute inflammation and 4 other dogs picked at random had histiocytes filled with oil red O-positive lipid in lung sections. Inhalation of the powdery ration may have been the cause of the inflammatory lesions.

The left mandibular lymph node on gross examination of the Beagle dogs was partially green. Green pigment was also found histologically in some R-E cells primarily in the medulla of the lymph nodes. The left ear was the site of the dog's green tattoo, and the green pigment in the lymph nodes was probably tattoo ink.

One vitamin A-supplemented dog had a semicircular depression on the left side of the cerebellum. A small clear fluid-filled cyst in the meninges had fit into the depression. The cyst was probably congenital and had no apparent effect on the dog.

The 2 dogs given dexamethasone for 88 days had severe bilateral adrenal atrophy. The dog receiving the steroid daily for 48 days had atrophy of only 1 of its adrenal glands, and the dog receiving dexamethasone for only 38 days had histologically normal adrenals. The zona fasciculata and reticularis were greatly reduced while the zona glomerulosa was about normal thickness. The adrenal atrophy was probably secondary to the prolonged steroid treatment.

Ultrastructural Findings

Renal lesions from the severely infected dog in the s-experiments were examined by transmission electron microscopy since no published ultrastructural photographs of *T. glabrata* were found. The yeast was found in renal tubules. It was oval and had a thick cell wall (Figure 18). The internal structure was disorganized. The lack of internal structure may have been due to poor penetration of fixative or a cutting artifact due to the thick cell wall. Many plasma cells about the tubules had dilated endoplasmic reticulum containing dense homogeneous material interpreted to be Russell's bodies (Figure 19).

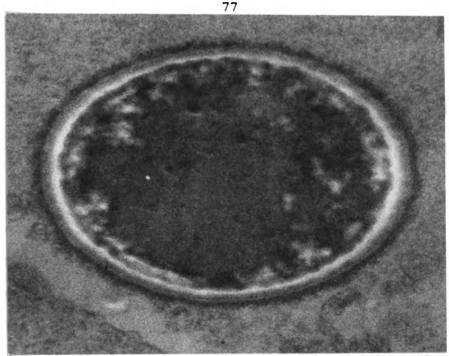

Figure 18. Electron micrograph of *T. glabrata* in the renal tubules of an exposed dog treated with dexamethasone. The yeast has a thick cell wall and indistinct internal detail. Lead citrate and uranyl acetate stain; magnification approximately 2.5 x 10^5.

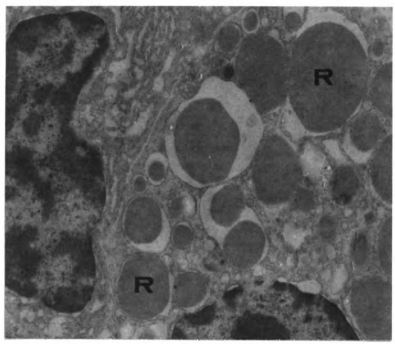

Figure 19. Electron micrograph of 2 plasma cells near the yeast in Figure 18. The cell on the lower right has dilated endoplasmic reticulum containing homogeneous Russell's bodies (R). Lead citrate and uranyl acetate stain; magnification approximately 1 x 10^4.

DISCUSSION

General

In general, the objectives of this research were accomplished. The pathogenesis and lesions of *T. glabrata* infection in the dog were determined. A vitamin A-deficient ration was developed that was palatable to 4- or 5-week-old and older dogs, and that depleted those dogs of most of their vitamin A stores by 23 weeks of age. The ration provided all nutrients (except vitamin A) currently known to be required by dogs. Neurologic dysfunction was confirmed to be an early sign of vitamin A deficiency and lesions suggestive of vitamin A deficiency were detected. And, vitamin A-deprived dogs were more susceptible to *T. glabrata* infection and secondary bacterial infections of the urinary tract.

Pathogenesis of *T. glabrata* Infection in the Dog

In this research the primary site of *T. glabrata* infection was the kidney. Nine of the 17 dogs exposed intravenously to the yeast had gross or histologic evidence of a multifocal interstitial nephritis. No other organ except the urogenital tract had lesions attributed to *T. glabrata* infection. This affinity of was similar to that reported in mice, rats and man (Hasencleaver and Mitchell, 1962; Lopez, 1952; Edebo and Spetz, 1965).

Other routes of exposure may have caused localized infection. Intraperitoneal exposure in mice resulted in a multifocal peritonitis while intravenous exposure caused a multifocal nephritis (Lopez, 1952). The abscess in 1 dog along the left jugular vein was at the site of venipuncture and probably was initiated by accidental injection of some of the yeast suspension outside of the vein. Intravenous exposure of the dogs was utilized since most dogs would probably be exposed via in-dwelling intravenous catheters as reported in human patients (Rose and Heckman, 1971; Rodrigues *et al.*, 1971).

The inflammatory reaction to the yeast was variable, but most affected dogs had a large focus of interstitial infiltration of plasma cells, histiocytes and neutrophils around renal tubules. This was in contrast to the reticuloendothelial proliferation reported in the kidney of mice (Lopez, 1952). The plasma cells with Russell's bodies implied antibody production was occurring, and the histiocytes and neutrophils appeared to have phagocytized yeast in the interstitial areas. The question of whether the antibodies were acting as opsonins to increase susceptibility to phagocytosis or in another function would be worth further research.

The dog with the severe nephritis which had received the highest level dexamethasone had a purely mononuclear reaction primarily consisting of plasma cells. Small foci in the kidneys of other dogs also were purely mononuclear. Marks *et al.* (1970) reported that the inflammatory response in human patients was

purely mononuclear but this was disputed by Strate (1973), who reported a neutrophilic response in the people he examined. The classical granulomas containing *T. glabrata* and necrotic debris in the peritoneal cavities of our rats were similar to those reported in mice experimentally exposed to *T. glabrata* (Lopez, 1952). The abscess along the jugular vein of 1 exposed, vitamin A-deprived dog appeared chronic and granulomatous in that it was a multicompartmentalized, fluid-filled structure with thick walls of dense connective tissue and only a thin layer of granulomatous inflammation.

The *T. glabrata* cells were in highest numbers within the renal tubules. When yeast appeared in the interstitial areas of the kidneys most appeared to be phagocytized. Usually the yeast has been phagocytized in the tissues of man and mice with *T. glabrata* (Minkowitz *et al.*, 1963; Lopez, 1952). However, Strate (1973) reported the yeast was entirely extracellular in tissue sections of infected human patients he examined. Care must be taken before recognizing phagocytosis on PAS-stained sections since Russell's bodies in plasma cells resemble phagocytized yeast in macrophages; however, *T. glabrata* stained Gram-positive while Russell's bodies did not.

When inflammation occurred in the lower urinary tract in 4 vitamin A-deprived dogs exposed to *T. glabrata* there was also bacterial infection due to *E. coli* or *S. aureus*. The inflammatory lesions included pyelonephritis, ureteritis, cystitis and prostatitis characterized by mononuclear infiltrates under the

transitional epithelium. Since lower urinary tract lesions also occurred in one unexposed dog with *E. coli* infection and no lower urinary tract lesions were detected in 4 dogs with pure *T. glabrata* infections, they probably were due to the secondary bacterial infection rather than *T. glabrata* infection.

Exposure to *T. glabrata* elicited a leukocytosis consisting primarily of a neutrophilia 1 day later. However, a wide range of WBC numbers (6800 to 21,000/mm^3) was detected in our dogs before exposure and was similar to the range reported for normal dogs under 1 year of age (7900 to 25,000/mm^3) (Rich, 1974). This wide range overlapped the response to *T. glabrata*. The neutrophilic response was not unique to *T. glabrata* but common to other infectious processes (Bentinck-Smith, 1969; Benjamin, 1969). The neutrophilia due to surgical trauma or increased adrenocortical steroids has been described (Benjamin, 1969).

Similarly the decreased A/G ratio was a nonspecific response to infection (Benjamin, 1969). Total serum protein values reportedly increase with age due to increased globulins (Salatka, 1971). The total serum protein values did increase with age in dogs in trial II but did not increase for some reason in dogs in trial I.

The cause of the severe reaction which occurred 3 minutes after intravenous injection of the yeast suspension was not investigated. A toxic product may have been produced by the yeast or the dogs may have had an anaphylactic reaction to antigens in the suspension.

Torulopsis glabrata was relatively nonpathogenic and probably would be a self-limiting infection in normal dogs. Appetite and body temperature were apparently unaffected by the yeast and no dogs died of the infection even though their resistance was reduced by dexamethasone or vitamin A deficiency.

Renal function was not impaired enough to elevate the blood urea nitrogen above normal in those dogs tested. The ability to concentrate urine may have been diminished. Seven dogs had urine specific gravities approximately that of a protein-free filtrate of serum (1.010 to 1.012), which is consistent with chronic interstitial nephritis (Wilkinson, 1969), and 6 of the dogs had urinary tract disease. However, water deprivation was not performed to check the dogs' capacity to concentrate urine.

None of the unexposed dogs had any evidence of *T. glabrata* infection even though they shared the same run area and the same feed dishes with infected dogs shedding *T. glabrata* in their urine for at least 4 weeks. This suggested the yeast was not easily transmissible as a detectable disease even to vitamin A-deprived dogs or dogs treated with dexamethasone. Of the 7 exposed dogs which were not treated with dexamethasone or deprived of vitamin A, only 2 had lesions in their kidneys. These were mild infections with an average of 2 small foci per kidney.

From research on *T. glabrata* in mice (Hasencleaver and Mitchell, 1962), it was suspected that *T. glabrata* infection in dogs would persist up to 8 weeks. However, by 4 weeks postexposure the infection appeared to be resolving since few yeast were detected in kidney sections of dogs. The results of the urine

cultures also suggest the renal infections of *T. glabrata* were healing by 4 weeks postexposure. The largest number of yeast were shed during the first 3 weeks and amount of yeast tended to decrease with time after exposure. Only the dog in the s- experiments, that had large numbers of yeast in kidney sections collected at 38 days postexposure, apparently had received too much dexamethasone to resist the infection.

Hasencleaver and Mitchell (1962) reported similar results in mice in that progressive infection only occurred in physiologically altered mice. The severity of the infection in their mice was closely dependent on the amount of cortisone acetate. Steroids such as cortisone and dexamethasone reduce resistance to infection in several ways. They are lympholytic, they inhibit macrophage phagocytosis, they decrease circulating antibody levels, they depress hemolytic complement and its attachment to the cell surface, they prevent lysosomes from attaching to phagocytic vacuoles, and they inhibit NADH oxidase which is required for hydrogen peroxide production in leukocytes (Zurier and Weissmann, 1973).

In summary, *T. glabrata* was a renal pathogen which produced a mild, self-limiting disease in normal or vitamin A-deprived dogs. The yeast appeared to be a serious problem only in 1 dog which was given a very large amount of dexamethasone.

Vitamin A Deficiency

The experimental ration utilized in this research was well accepted by the dogs as a weaning and growing ration. It was palatable, economical, easily handled, and resulted in a good rate

of growth. The ingredients were easily obtained and stored. It probably should be fed wet to avoid mild inhalation pneumonia. The levels of vitamin A detected in the plasma samples, the hepatic samples and the ration itself indicated the ration effectively depleted the dogs of vitamin A. The ration provided all other nutrients currently known to be required by the dog. Vitamin E was added to the ration, in contrast to some previously reported rations used in vitamin A deficiency studies in the dog. The onset of vitamin A deficiency signs would probably be delayed compared with previous rations due to the sparing effect of vitamin E on vitamin A (Tvedten *et al.*, 1973; Dicks *et al.*, 1959).

Since oatmeal and other common ingredients composed the bulk of this ration, it was apparent that similar mixtures could be used by dog owners to wean and grow their dogs. Thus, vitamin A deficiency could be an occasional clinical problem encountered by the practicing veterinarian.

The neurologic signs of vitamin A were important since these are more likely to be observed in a veterinary practice than the extreme emaciation reported in earlier articles (Frohring, 1937b; Russel and Morris, 1939). A head tilt, loss of balance, and circling in a 17-week-old puppy due to vitamin A deficiency would be difficult to differentiate from middle ear infections. Impaired vision and convulsions observed in the vitamin A-deprived dogs could be confused with canine distemper. The nervousness in our vitamin A-deprived dogs could be attributed to a poor disposition rather than a nutritional problem in the field. These neurologic

signs were previously reported in foxes (Smith, 1942) and dogs (Frohring, 1937b). The signs were probably due to overgrowth of bone around the cochlear and vestibular divisions of the VIII cranial nerve as reported in dogs (Mellanby, 1938). All these neurologic disorders of vitamin A deficiency occurred in normal appearing, active, friendly dogs. Thus, although vitamin A deficiency in dogs is unlikely with the availability of commercial, balanced rations, it is possible and should be included in the differential diagnosis of neurologic problems as it has been in bovine neurologic disease (Loew, 1975).

No previous reports of papilledema due to vitamin A deficiency in dogs were found, although it was diagnosed in 2 vitamin A-deprived dogs by means of an indirect ophthalmoscope in this research. Papilledema had been reported in 2 vitamin A-deficient foxes (Smith, 1942) and frequently in vitamin A-deficient cattle (Eaton, 1969). The histologic changes in optic papillae of these 2 dogs were suggestive of edema but could not be definitely differentiated from artifacts of fixation.

One vitamin A-deprived dog had triple phosphate calculi in his bladder. Calculi have been reported to be more frequent in vitamin A-deficient animals due to keratinization of the urinary tract and increased susceptibility to infections (Schmidt, 1941; McCollum *et al.*, 1939). However, there are several other causes of urinary calculi (Jewett *et al.*, 1943).

Increased CSF pressure has been reported in vitamin A-deficient dogs (Moore and Sykes, 1940) and was reported to be the

earliest and a consistent change of vitamin A deficiency in cattle (Eaton *et al.*, 1972). Although very high CSF pressures (175, 222) were detected in 2 vitamin A-deprived dogs, the average CSF pressure of the vitamin A-deprived dogs did not appear significantly higher than the vitamin A-supplemented dogs. Some of the low CSF pressures in vitamin A-deprived dogs were in dogs smaller than average. Their size may have influenced their CSF pressures. Although the same positioning of the dog was attempted during measurement of the CSF pressure, an error in the CSF pressure values may have occurred by varying the degree of flexion of the neck. Excessive flexing of the neck could elevate the CSF pressure by 15%.

No definite squamous metaplasia was detected in the vitamin A-deprived dogs in these experiments. Crimm and Short (1936) reported early metaplasia of the bronchiolar epithelium only after the dogs had been fed a vitamin A-deficient diet for 1 year. Even then there was no xerophthalmia, although the livers were depleted of vitamin A. Pneumonitis was also present in the dogs with bronchiolar metaplasia. Inflammation can cause squamous metaplasia of bronchiolar epithelium in rats (Lindsey *et al.*, 1971), so the metaplasia reported by Crimm and Short (1936) may not have been due to vitamin A deficiency. Morris and Russel (1939) speculated that squamous metaplasia due to vitamin A deficiency occurs in the dog but did not have histologic evidence in the dog.

Histologic evidence of xerophthalmia was reported in 3 foxes fed a vitamin A-deficient diet for 18, 25 and 27 weeks (Smith, 1942). These foxes also had stratification and keratinization

of the epithelium of the trachea, bronchi, renal pelvis, urinary bladder and vagina. Perhaps prolonged feeding of the ration in this research project would initiate metaplastic changes of the epithelium in dogs characteristic of vitamin A deficiency in other species (Wolbach and Howe, 1925).

The epithelial defects of the tongue occurred in a vitamin A-deprived dog but seemed the opposite type of change one would expect with vitamin A deficiency. Vitamin A deficiency has caused increased keratinization (Wolbach and Howe, 1925) and the defects in this dog had less keratinization than adjacent normal areas. The areas were less specialized since they apparently had lost the ability to form papillae.

Anemia was not detected, and the range of packed cell volumes (24.4% to 38.5%) was similar to that reported for dogs under 1 year of age (24.0% to 37.5%) (Rich, 1974). The anemia reported in vitamin A-deficient dogs (Crimm and Short, 1936) was probably due to a deficiency of iron and copper (Russel and Morris, 1938).

Interrelationship of *T. glabrata* and Vitamin A Deficiency

Vitamin A deficiency appeared to make the dogs more susceptible to *T. glabrata* infection as it had with many other species and diseases mentioned in the literature review. The frequency and severity of urinary tract lesions in dogs exposed to *T. glabrata* was greater in the vitamin A-deprived dogs than the vitamin A-supplemented dogs. A major reason for the increased severity of renal lesions was increased susceptibility to secondary bacterial invasion of the urinary tract. Five of these dogs had coexisting

bacterial infections while none of the exposed, vitamin A-supplemented dogs had bacteria isolated from their kidneys in appreciable numbers. Increased susceptibility to ascending urinary tract infection was also reported in vitamin A-deficient rats (Tvedten *et al.*, 1973).

The vitamin A-deprived dogs had a smaller, initial, total leukocyte and neutrophil response to exposure with *T. glabrata* than did the vitamin A-supplemented dogs. This may have allowed more viable yeast cells to reach and colonize the kidney. A mild leukocytosis and neutrophilia persisted 3 weeks longer in the vitamin A-deprived dogs suggesting the infection lasted longer in the vitamin A-deprived dogs. Also, the 7 skin tests with no reaction were only in vitamin A-deprived dogs, so they may have had impaired immediate and delayed hypersensitivity reactions.

The MIF test is a way of quantitating cellular immunity (Eisen, 1974) but was not useful in this research. The positive reactions of dogs before exposure reflect the inaccuracy of the MIF test in dogs. The canine leukocytes migrated rather poorly and increased the diameter of the spot only about 5% during the 24-hour migration. The increase in diameter was the primary measurement in the test. Slower migration in the influence of a specific antigen gave even a smaller change to estimate microscopically and probably resulted in considerable error. The amount of leukocyte migration was much greater in other species, such as the bovine, so that larger, more easily measured changes in the diameter of the spot of leukocytes could be measured. This would result in more reliable results.

Since no epithelial changes were detected histologically, epithelial integrity may have been intact. However, more subtle biochemical defects such as decreased mucus secretion in vitamin A deficiency (DeLuca and Wolf, 1970) were not analyzed microscopically.

SUMMARY

The pathogenesis of *Torulopsis glabrata* infection in the dog, the clinical signs and lesions of vitamin A deficiency, and the interrelationships of the nutritional status and resistance to infection were studied in 28 dogs. Two experiments utilized dexamethasone in various dosages to reduce the resistance of 5 dogs to the yeast infection. The other 23 dogs were used in 2 experiments with vitamin A and exposure to *T. glabrata* as treatment factors.

Torulopsis glabrata caused an interstitial nephritis in 9 of 17 exposed dogs. The yeast was usually found in the renal tubules which were surrounded by plasma cells, histiocytes and neutrophils. Some *T. glabrata* cells were in the interstitial areas where they were usually phagocytized. Pyelonephritis, cystitis and prostatitis occurred in 4 dogs with secondary bacterial invasion by *Escherichia coli* or *Staphylococcus aureus*. The *T. glabrata* was relatively nonpathogenic and the urinary infections appeared to be healing by 4 weeks after exposure.

A palatable ration was formulated which resulted in clinical signs of vitamin A deficiency after feeding for 7 to 13 weeks. The neurologic signs included head tilt, circling and loss of balance in 5 of 12 dogs fed the ration without vitamin A

supplementation. Papilledema was clinically detected in 2 vitamin A-deprived dogs. At necropsy, calculi were detected in the urinary bladder of a vitamin A-deprived dog and epithelial defects were found on the tongue of another dog deprived of vitamin A.

The frequency and severity of the nephritis was greater in the vitamin A-deprived dogs. The increased severity was partially due to secondary bacterial invasion. The total leukocyte and neutrophil response to intravenous exposure was smaller in the vitamin A-deprived dogs and, on the basis of skin tests with antigens of *T. glabrata* and *C. albicans*, the vitamin A-deprived dogs appeared to have impaired immediate and delayed hypersensitivity.

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VITA

VITA

A. Personal Data

1. Born: Milwaukee, Wisconsin, October 21, 1946

2. Married: Wife - Gretchen Flo, D.V.M., M.S. (Associate Professor of Orthopedics and Neurology, Michigan State University)

B. Educational History

1. B.S. with high distinction in experimental biology, University of Michigan, 1968
2. B.S. with high honor in veterinary science, Michigan State University, 1970
3. D.V.M., Michigan State University, 1971
4. M.S. in veterinary pathology, Michigan State University, 1973

C. Professional Experience

1. Small animal practice, Ann Arbor, Michigan, June 1971 to January 1972
2. Diagnostic and teaching rotation (full time), Michigan State University Veterinary Diagnostic Laboratory, June to September 1973

D. Publications

1. Published
   a. Tvedten, H. W., Whitehair, C. K., and Langham, R. F.: Influence of Vitamins A and E on Gnotobiotic and Conventionally Maintained Rats Exposed to *Mycoplasma pulmonis*. J.A.V.M.A., 163, (1973): 605-612.
   b. Tvedten, H. W.: Fatal Volvulus in a Puppy (A Case Report). V.S./S.A.C., 68, (1973): 506-510.
   c. Tvedten, H. W., and Keahey, K. K.: Esophago-Tracheal Fistulation After Esophageal Stenosis in a Horse. V.M./S.A.C., 69, (July, 1974): 868-869.
   d. Tvedten, H. W., and Langham, R. F.: Trophoblastic Emboli in a Chinchilla. J.A.V.M.A., 165, (1974): 828-829.

e. Tvedten, H. W., and Trapp, A. L.: Myopathy in Three Dogs. V.M./S.A.C., 70, (1975): 63-66.
f. Tvedten, H. W., Langham, R. F., and Beneke, E. S.: Systemic *Pneumocystis carinii* Nephritis in a Dog. J.A.A.H.A., 10, (1974): 592-594.
g. Tvedten, H. W.: Renal Secondary Hyperparathyroidism in a One Year Old Dog. V.M./S.A.C., 70, (March, 1975): 320-321.

2. Submitted
   a. Flo, G. L., and Tvedten, H. W.: Cervical Calcinosis Circumscripta in Three Related Great Dane Dogs. J.A.A.H.A.

3. In Preparation
   a. Tvedten, H. W., Whitehair, C. K., Langham, R. F., Carter, G. R., and Beneke, E. S.: *Torulopsis glabrata* Nephritis and Vitamin A Deficiency in Dogs. J.A.V.M.A.
   b. Tvedten, H. W., Carrig, C., and Flo, G. L.: Influence of Diet on the Formation of Hip Dysplasia.
   c. Tvedten, H. W., Whitehair, C. K., and Langham, R. F.: Influence of a High Protein Diet on Renal Structure.

E. Professional Organizations

1. American Veterinary Medical Association
2. Michigan Veterinary Medical Association
3. Mid-State Veterinary Medical Association (Vice President)
4. Zeta Chapter, Phi Zeta
5. American Animal Hospital Association

F. Professional Accomplishments

1. NIH Postdoctoral Fellowship (1973 and 1974)
2. Upjohn Postdoctoral Fellowship (1972)
3. MVMA award (highest scholastic standing in the veterinary class of June 1971)
4. Phi Zeta honor society, Michigan State University
5. MSU Veterinary Faculty Award
6. MSU Pharmacology Award
7. Phi Kappa Phi honor society, University of Michigan
8. University of Michigan General Scholarship Award

APPENDICES

Appendix A. Average body weights of the dogs in trial I which were fed free choice

Age (wks)	Vitamin A-supplemented (gm)	Vitamin A-deprived (gm)	Vitamin A-deprived (*)
4	752	758	101
5	962	1,029	107
6	1,250	1,235	99
7	1,428	1,415	99
8	1,683	1,660	99
9	1,917	1,879	98
10	2,270	2,261	100
11	2,697	2,559	95
12**	2,990	2,829	95
13	3,258	3,059	94
14	3,592	3,377	94
15	3,900	3,634	93
16	4,017	3,650	91
17	4,267	3,899	91
18†	4,255	3,987	94
19	4,425	4,081	92
20	4,705	4,394	93
21	4,828	4,330	90

*Expressed as a percent of the body weight of the vitamin A-supplemented dogs.

**First neurologic signs of vitamin A deficiency.

†Intravenous exposure of 5 of the 7 dogs to *T. glabrata*.

Appendix B. Average body weights of the dogs in trial II

Age (wks)	Vitamin A-supplemented (gm)	Vitamin A-deprived (gm)	Vitamin A-deprived (*)
6	1,744	1,787	102
7	2,217	2,218	100
8	3,076	2,882	94
9	3,552	3,308	93
10**	4,106	3,822	93
11	4,774	4,488	94
12	5,416	5,009	92
13	5,800	5,531	95
14†	6,397	6,097	95
15††	7,087	6,833	96
16	7,370	7,059	96
17	7,881	7,697	98
18	8,061	7,805	97
19	8,446	8,254	98

*Expressed as a percent of the body weight of the vitamin A-supplemented dogs.

**Stopped feeding free choice, each group of 8 dogs were offered 2400 gm of the ration per day.

†First neurologic signs of vitamin A deficiency.

††One-half of the dogs were exposed intravenously to *T. glabrata*.

Appendix C. Average amount of food consumed per day by each of the 2 groups of dogs in trial II

Age (wks)	Vitamin A-supplemented (gm)	Vitamin A-deprived (gm)	Vitamin A-deprived (*)
6	1,261	1,249	99
7	1,671	1,595	96
8	1,771	1,639	92
9	1,842	1,809	98
10**	2,311	2,014	87
11	2,314	2,039	88
12	2,329	2,221	95
13	2,357	2,336	99
14	2,400	2,279	95
15	2,400	2,296	96
16	2,400	2,321	97
17	2,400	2,342	98
18	2,400	2,393	100
19	2,443	2,443	100

*Expressed as a percent of the amount consumed by the vitamin A-supplemented dogs.

**Each group offered 2400 gm/day.

Appendix D. Average packed cell volumes (%) of blood samples from dogs in trial I

Age (wks)	Vitamin A-supplemented	Vitamin A-deprived
7	29.0	29.2
9	27.7	28.2
10	29.5	31.2
11	30.0	30.7
12	30.3	31.7
13	32.5	31.8
14	32.7	32.0
16	35.3	34.0
17	35.5	35.5

Appendix E. Average packed cell volume (%) of blood samples from dogs in trial II

Age (wks)	Vitamin A-supplemented		Vitamin A-deprived	
	Exposed*	Unexposed	Exposed	Unexposed
7	24.5	24.2	n.a.	n.a.
8	n.a.	n.a.	24.7	24.5
9	25.8	26.0	n.a.	n.a.
10	n.a.	n.a.	24.5	24.9
11	28.2	28.2	n.a.	n.a.
12	n.a.	n.a.	28.0	28.2
13	30.5	30.2	31.0	30.0
17	34.0	33.2	32.2	32.0
19	34.5	36.2	30.2	31.5
20	39.0	39.9	37.5	38.8

*Exposed intravenously to *T. glabrata*.

n.a. - not applicable.

Appendix F. Average leukocyte changes in blood samples taken from dogs in trial II one day after exposure to *T. glabrata*

Dog	Before exposure*		After exposure		% change**	
	Total leukocytes (no./mm^3)	Neutrophils (no./mm^3)	Total leukocytes (no./mm^3)	Neutrophils (no./mm^3)	Total leukocytes	Neutrophils
	Exposed to *T. glabrata*; supplemented with vitamin A					
CN	14,633	8,900	16,400	14,500	+12.1	+62.9
DM	11,833	6,676	10,000	7,500	-10.0	+10.8
DN	10,833	6,133	15,100	13,300	+39.4	+116.9
DF	11,833	7,333	15,400	13,350	+30.1	+82.1
	Exposed to *T. glabrata*; not supplemented with vitamin A					
CG	13,167	8,400	14,000	11,350	+6.3	+35.1
DC	14,833	9,600	15,300	11,300	+3.1	+17.7
DE	18,000	10,567	16,500	11,100	-8.3	+5.0
DD	15,000	9,467	18,500	14,500	+23.3	+53.3
	Not exposed to *T. glabrata*; supplemented with vitamin A					
BZ	12,967	7,133	12,500	5,600	-3.6	-21.5
CF	15,333	9,433	12,000	8,200	-21.7	-13.1
CH	14,100	9,283	16,000	8,800	+13.5	-5.2
CO	15,867	11,000	13,300	8,850	=16.2	-19.5
	Not exposed to *T. glabrata*; not supplemented with vitamin A					
AP	12,767	7,134	13,200	9,300	+3.4	+30.4
AR	11,600	7,200	10,800	6,800	-69.0	-5.6
BX	18,400	10,633	10,100	5,700	-45.1	-46.4
CA	17,567	10,400	14,000	6,100	-20.3	-41.3

*Average of 3 samples taken during the month before exposure.

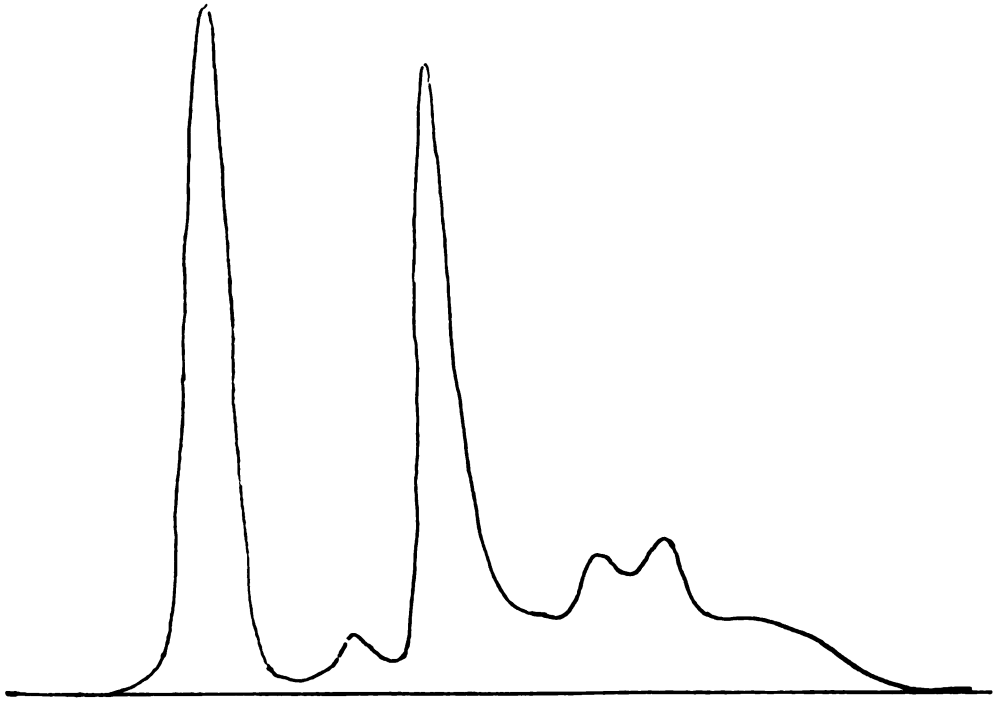
**Percent change in leukocyte numbers 1 day after exposure compared to the 3 samples before exposure.

Appendix G. Average leukocyte changes in blood samples taken from dogs in trial II during the second to fourth week after exposure to *T. glabrata*

Dog	Before exposure*		After exposure		% change**	
	Total leukocytes (no./mm^3)	Neutrophils (no./mm^3)	Total leukocytes (no./mm^3)	Neutrophils (no./mm^3)	Total leukocytes	Neutrophils
	Exposed to *T. glabrata*; supplemented with vitamin A					
CN	14,633	8,900	13,000	7,733	-11.2	-13.1
DM	11,833	6,767	8,833	4,867	-25.4	-28.1
DN	10,833	6,133	12,033	7,967	+11.1	+29.9
DF	11,833	7,333	11,267	7,167	-4.8	-2.3
	Exposed to *T. glabrata*; not supplemented with vitamin A					
CG	13,167	8,400	14,683	9,550	+11.5	+13.7
DC	14,833	9,600	16,500	11,700	+11.2	+21.9
DE	18,000	10,567	14,933	9,333	-11.7	-11.7
DD	15,000	9,467	15,667	10,833	+4.4	+14.4
	Not exposed to *T. glabrata*; supplemented with vitamin A					
BZ	12,967	7,133	11,733	6,533	9.5	8.4
CF	15,333	9,433	13,633	9,033	-11.1	-4.2
CH	14,100	9,283	12,050	8,100	-14.5	-12.7
CO	15,867	11,000	11,167	6,883	-29.6	-37.4
	Not exposed to *T. glabrata*; not supplemented with vitamin A					
AP	12,767	7,134	12,533	9,400	-1.8	+31.8
AR	11,600	7,200	10,500	7,400	-9.5	+2.8
BX	18,400	10,633	10,867	6,967	-40.9	-34.5
CA	17,567	10,400	14,333	8,700	-18.4	-16.3

*Average of 3 samples taken during the month before exposure.

**Percent change during the second to fourth week after exposure compared to 3 samples taken during the month before exposure.



Appendix H. Electrophoretic pattern of the serum proteins from the dog given 1 mg dexamethasone/kg body wt/day. The total serum protein value was 6.8 gm/100 ml and the α_2 portion accounted for 33% of that amount.

Appendix I. Albumin/globulin ratios in serum collected from dogs in trial I

Age (wks)	+A+Y*		+A-Y*	-A+Y*			-A-Y*
	BH	BE	FM	TK	TN	BB	BW
10	1.58	1.13	1.18	1.74	1.79	1.36	1.54
11	1.31	1.54	1.44	1.74	1.68	2.14	1.54
12	1.39	1.20	1.26	1.74	1.58	1.87	1.26
13	1.91	1.54	1.77	1.86	1.06	1.45	2.05
14	1.16	1.45	1.03	1.34	1.48	1.54	1.10
16	1.58	1.45	1.44	1.25	1.68	1.28	1.77
18	1.16	1.28	1.18	1.52	1.39	1.45	1.26
---**							
19	0.97	0.74	1.03	0.91	1.03	1.06	1.65
21	1.09	1.17	1.61	1.52	0.97	1.10	1.58
22	1.03	0.94	1.50	0.83	0.91	1.19	1.46

*+A = vitamin A-supplemented; -A = vitamin A-deprived; +Y = exposed to *T. glabrata*; -Y = not exposed to *T. glabrata*.

**Time when 5 of the dogs were exposed intravenously to *T. glabrata*.

Appendix J. Albumin/globulin ratios in serum collected from dogs in trial II

Dog	2 weeks before exposure*	1 week after exposure*	2 weeks after exposure*	3 weeks after exposure*
	Supplemented with vitamin A; not exposed to *T. glabrata*			
BZ	1.36	1.65	1.24	1.71
CF	1.17	1.62	1.15	0.91
CH	1.45	1.62	1.41	1.29
CO	1.38	1.26	1.15	1.03
	Supplemented with vitamin A; exposed to *T. glabrata*			
CN	0.86	1.15	1.12	1.16
DM	1.17	1.28	1.10	1.50
DN	1.08	1.12	1.14	1.09
DF	1.27	1.16	1.00	1.22
	Not supplemented with vitamin A; not exposed to *T. glabrata*			
AP	1.36	1.12	1.29	1.48
AR	0.86	1.24	1.55	1.03
BX	1.29	1.00	1.19	1.46
CA	1.08	1.00	1.57	0.92
	Not supplemented with vitamin A; exposed to *T. glabrata*			
CG	1.16	1.04	1.00	0.95
DC	1.27	1.00	1.07	1.38
DD	1.00	1.09	1.00	0.94
DE	1.43	1.03	1.07	0.97

*Exposed intravenously to *T. glabrata*.

Appendix K. Cerebrospinal fluid pressures of the dogs in trials I and II

Group	CSF pressure
Trial I	
Vitamin A-deprived	60, 80, 100, n.a.
Vitamin A-supplemented	42, 66, n.a.
Trial II	
Vitamin A-deprived	89, 106, 106, n.a. 124, 135, 170, n.a.
Vitamin A-supplemented	65, 66, 96, 110, 120, 144, 175, 222

n.a. = not available.

Appendix L. Plasma vitamin A levels of dogs in trial I which were first fed the vitamin A-deficient ration at 4 weeks of age

Age (wks)	Vitamin A-supplemented*				Vitamin A-deprived				
	BH	BE	FM	Average**	TK	TN	BB	BW	Average**
8	79.2	74.3	100	84.6	17.1	19.6	26.9	29.3	23.2
9	39.1	62.6	51.4	51.0	19.6	14.7	29.3	17.1	20.2
12†	4.9	22.0	31.8	19.6	4.9	4.9	0	12.2	5.5
18	53.8	17.1	9.8	26.9	4.9	4.9	4.9	12.2	6.7
19	14.7	22.0	40.1	25.6	1.0	7.3	2.4	7.3	4.5
20	39.1	25.4	46.5	37.0	15.6	24.9	7.3	13.7	15.4
21	26.9	45.5	7.4	26.6	23.5	26.9	32.0	17.1	24.9
22	33.3	39.7	29.3	34.1	7.3	9.8	23.5	21.0	15.4

*Given vitamin A subcutaneously.

**Average of the 3 or 4 plasma vitamin A values from dogs in that category for that weekly sampling.

†First neurologic signs of vitamin A deficiency.

Appendix M. Plasma vitamin A levels of dogs in trial II which were first fed the vitamin A-deficient ration at 4 weeks of age

Dog	9*	11.5*	13*	14.5*	17.5*	18.5*	Average**
Vitamin A-supplemented†							
BZ	46.5	117.4	22.0	80.7	19.6	95.8	63.7
CF	27.4	75.8	12.2	31.8	18.6	54.8	36.8
CH	17.6	70.9	8.3	23.5	24.5	46.5	31.9
CO	34.2	72.4	21.0	12.5	23.5	83.1	41.1
CN	29.3	83.1	7.3	31.8	22.0	48.9	37.1
DM	46.5	117.6	19.6	29.6	14.7	117.4	66.1
DN	26.9	68.5	8.3	36.7	26.9	49.9	36.2
DF	37.6	92.9	22.0	36.7	22.0	118.7	55.0
Avg.**	33.2	90.7	15.1	35.4	21.5	76.9	46.0
Vitamin A-deprived							
AP	35.2	29.3	13.0	10.8	4.9	0.5	15.6
AR	49.0	10.8	8.3	10.8	3.9	2.5	14.2
BX	7.3	9.8	4.9	7.3	7.3	4.9	6.9
CA	16.6	13.7	2.4	6.4	4.9	1.0	7.5
CG	19.6	12.7	4.9	3.9	2.4	1.0	7.4
DC	2.4	2.5	3.4	2.5	4.6	7.4	3.8
DD	4.9	14.7	4.9	5.9	5.9	2.4	6.4
DE	8.8	9.8	7.3	5.9	4.9	2.9	6.6
Avg.**	18.0	12.9	6.2	6.7	4.8	2.8	8.6

*Age of the dog in weeks.

**Average of that column.

†Supplemented with vitamin A orally.

Appendix N. Hepatic vitamin A values (µg vitamin A/gm liver) in trial I in which necropsies were performed when the dogs were 23 weeks of age

Dog	Vitamin A-supplemented	Vitamin A-deprived
TK	n.a.	22.5
BB	n.a.	38.2
TN	n.a.	0.0
BW	n.a.	24.9
BH	89.9	n.a.
BE	45.2	n.a.
FM	136.5	n.a.
Average*	90.5	21.4

*Average of that column.

n.a. - not applicable.

Appendix O. Hepatic vitamin A values (μg vitamin A/gm liver) of dogs in trial II in which necropsies were performed when the dogs were 21 weeks of age

+A-Y*	+A+Y*	-A-Y*	-A+Y*
52.6	123.5	0.0	2.3
120.5	65.1	0.0	2.3
90.3	47.4	15.9	6.8
9.9	89.2	11.2	11.4
		Average**	
68.3	81.3	6.8	5.7

*+A = vitamin A-supplemented; -A = vitamin A-deprived; +Y = exposed to *T. glabrata*; -Y = not exposed to *T. glabrata*.

**Average of the values from the 4 dogs in each group.