ABSTRACT

THE PATHOLOGY OF ZINC DEFICIENCY IN RATS

by Delbert L. Whitenack

The gross and microscopic pathology was determined in rats fed a zinc-deficient diet. Control rats were fed a diet containing 50 p.p.m. zinc and deficient rats were fed a diet containing 0.9 p.p.m. zinc for 6 weeks.

The first gross signs in deficient rats were decreased appetite and retarded growth rate. By the third week there were erythema of skin and generalized thinning, roughening, and loss of luster of hair coat in all deficient rats. Lesions were more severe in male rats; these animals had additional lesions of bilateral alopecia of the front paws, ventral thorax and abdomen, back, and submandibular and ventral cervical regions. The male rats also had cutaneous seborrhea in the areas of alopecia. Gross changes became progressively more severe throughout the experiment.

Histopathologic changes were confined to stratified squamous epithelium of the tongue, esophagus and skin; the dermis; and the epithelium of the seminiferous tubules of the testes. The changes in the stratified squamous epithelium were characterized by an increase in number of basal cells, acanthosis, and incomplete keratinization. The changes in the dermis were increased numbers of mononuclear cells and hypertrophic sebaceous glands. The primary change in epithelial cells of germinal epithelia of the seminiferous tubules of the testes was incomplete maturation. All gross and microscopic changes, with the exception of seborrhea, were manifestations of zinc deficiency.

THE PATHOLOGY OF ZINC DEFICIENCY IN RATS

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A THESIS

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

MASTER OF SCIENCE

Department of Pathology

1968

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. Kenneth K. Keahey, major professor, and Dr. C. K. Whitehair for their counsel and guidance in the conduct of this experiment and for their assistance in the preparation of this manuscript.

Sincere thanks ~~is~~ are expressed to Dr. R. W. Luecke, Biochemistry Department, for his generous cooperation in the use of experimental animals.

The author wishes to express his appreciation to all members of the Department of Pathology for technical and professional aid and guidance.

The writer especially wishes to acknowledge his gratitude to his wife, Gwen, whose sacrifices and encouragement made this study possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

A major emphasis in recent years has been given to the importance of zinc in naturally occurring health problems in man and animals. There are numerous reports on the interrelationships between zinc and other minerals and the availability of zinc from different protein sources. Much attention has been given a disease, parakeratosis, a zinc deficiency in swine. The pathology of zinc deficiency has primarily been described from clinical cases. There is limited information on the pathology of experimentally produced zinc deficiency. Knowledge of the mode of action of zinc in metabolism is confined to enzymes which have been isolated and identified as metalloenzymes. Thus, it appears that zinc has multiple functions in protein synthesis at the molecular level. The objective of this research was to determine the gross and microscopic pathology in rats fed a deficient diet of known zinc composition.

REVIEW OF LITERATURE

Conclusive evidence that zinc is essential for normal growth and development of mice was given in 1934 (Todd et al.). On a synthetic diet low in zinc the rate of growth of mice was accelerated by the addition of zinc salts. A year later, Stirn et al. (1935) observed that growth was retarded in rats on a zinc-deficient diet. The importance of zinc as a dietary requirement did not receive much additional attention until 1955, when Tucker and Salmon, at the Alabama Experiment Station, noted that zinc would cure or prevent swine parakeratosis, a serious problem in swine raising operations. This latter work precipitated a number of reports on the importance of zinc in animal nutrition. In recent years a zinc deficiency has been identified with an important clinical problem in man associated with dwarfism and hypogonadism (Prasad et al., 1963).

This review pertains primarily to the gross and microscopic lesions of zinc deficiency in rats.

Feeding Experiments

Day and McCollum (1940) formulated a ration which was extremely low in zinc. Control animals received .15 mg. of zinc daily. When young rats were placed on a zinc-deficient diet, they ceased gaining weight in 2 to 3 weeks. Thinning of the hair became apparent after the third week and was followed by alopecia. Millar et al. (1958) reported that weanling rats fed a zinc-deficient diet for 8 weeks had marked

retardation in body growth and had depressed growth and development of the following organs: testis, epididymis, accessory sex organs, and pituitary gland. In many cases there was severe atrophy of testicular germinal epithelia. All the observed changes produced by zinc deficiency, except the testicular atrophy, were reversed when adequate zinc was added to the diet.

The histological changes in zinc-deficient rats were studied by Follis et al. (1941). Microscopically there was hyperkeratinization and acanthosis of the epidermis. Accompanying these epidermal changes were atrophy of hair follicles and hypertrophy of sebaceous glands. Alterations in the esophagus consisted of an increase in thickness of the epithelial lining with an appearance of large, partially keratinized cells on the surface.

The early work in the mouse and rat indicated that the zinc requirement was less than 5 mg./kg. of diet.

Swine Parakeratosis

In 1953 Kernkamp and Ferrin reported a dermatosis of swine that was characterized by hard, dry, crusted proliferation of the superficial layer of the epidermis. Microscopically the encrusted masses were composed of cornified epithelium and collections of keratin and debris. There was an increase in number of basal cells and a degree of acanthosis which increased with the duration of the lesion. Keratinization was incomplete and corneal cells were larger than normal and retained their nuclei. Hair follicles were partially or wholly atrophic, and sebaceous glands were hypertrophic. The changes in the epithelium of the tongue and esophagus consisted of an increased number of basal cells and a

thickened stratum corneum. The retained squamous cells were large and had persistent nuclei.

A zinc deficiency was first recognized in pigs as a disease entity in 1942. Prior cases of this skin disease were apparently observed but were mis-diagnosed as sarcoptic mange. Zinc deficiencies occurred in pigs that were usually in dry lot and were not found in pigs that had access to good pasture. This condition was named parakeratosis. In 1955 Tucker and Salmon elucidated the essential role of zinc in prevention and cure of parakeratosis.

Zinc Deficiency in the Chick

Following discovery of the essential role of zinc in nutrition of the pig, investigations were extended to the chick (O'Dell *et al.*, 1958). Zinc deficiency in the growing chick resulted in decreased growth rate, shortening and thickening of the long bones, and a tendency for the hock joints to enlarge. There was poor feather development and scaling of the skin, particularly on the feet. Microscopically, there were hyperkeratosis and acanthosis of the skin and parakeratosis of the epithelium of the esophagus. The minimum requirement for zinc in the chick was suggested as 35 p.p.m.

Zinc Deficiency in Man

A primary zinc deficiency has not been described in man. Prasad *et al.* (1963) studied zinc metabolism in patients with a syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. Iron deficiency anemia and hepatosplenomegaly were most often the result of metazoan or protozoan infestations. The zinc content of plasma, red blood cells, and hair was consistently lower in dwarfs when compared with

normal Egyptian subjects. Significant increased plasma zinc turnover rate supported the concept of a zinc deficiency. A lower rate of excretion of zinc in urine and feces indicated that there was conservation of zinc. The external genitalia were remarkably small, and there was absence of facial, pubic, or axillary hair. The skin was roughened and hyperpigmented. The diet of dwarfs consisted of bread made from wheat and corn flour, beans, and occasionally white cheese.

Role of Zinc as an Enzyme Activator and Its Dietary Interrelationships

The mechanisms by which zinc functions in growth are not fully understood. Zinc functions as a catalyst and has an integral part of several metalloenzymes. In the zinc-deficient state, a decrease in the activity of various zinc-dependent enzymes may account for the observed clinical manifestations (Prasad, 1967).

There are several factors involved which affect the availability of dietary zinc. Stevenson and Earle (1956), Luecke *et al*. (1957), Forbes (1960), and Hoekstra (1964) reported that increased dietary calcium raised the dietary zinc requirement. Recently, Heth and Hoekstra (1965) have indicated that dietary calcium interferes with intestinal absorption of zinc.

Another and more important factor involved in the availability of dietary zinc is the protein source. Certain proteins, especially soybean meal, appear to contain zinc in a form which makes it unavailable to animals. Recent work (Forbes and Yohe, 1960; O'Dell and Savage, 1960; Oberleas *et al*., 1962; Likuski and Forbes, 1965) indicates that the presence of phytic acid in soybeans renders the zinc unavailable for absorption.

MATERIALS AND METHODS

This investigation was in cooperation with the Department of Biochemistry, Michigan State University. The experimental animals used were rats fed a purified zinc-deficient diet to determine the effect of zinc deficiency on growth, liver alcohol dehydrogenase, and intestinal alkaline phosphatase. The diet, which contained 0.9 p.p.m. zinc, was formulated by the Department of Biochemistry (Table 1).

Since zinc is ubiquitous in nature, the rats were maintained in stainless steel cages. The rubber stoppers of the water bottles were separated from the rats by a stainless steel plate. Deionized distilled water and feed were offered ad libitum. Control animals received the same synthetic diet supplemented with 50 p.p.m. zinc.

Five male and 5 female deficient rats and 5 control male and female rats were euthanatized with ether on the 43rd day of the experiment. The following tissues were taken for histopathologic examination: tongue; proximal, middle, and distal esophagus; adrenal glands; testes; ovaries; pancreas; eyes; liver; spleen; kidneys; brain; lung; thyroid; and skin from the dorsal shoulder, lumbar area, ventral abdomen, feet, and mandible. These tissues were fixed in 10% buffered formalin, trimmed, and processed in an Autotechnicon* and embedded in Paraplast.** Sections were cut at

*Technicon Company, Chauncey, N. Y.

**Aloe Scientific Division of Brunswick, St. Louis, Mo.

Table 1. Composition of zinc-deficient diet

	Gm./kg.
Glucose monohydrate[1]	577.9
Egg white solids (spray dried)[2]	200.0
Corn oil	100.0
Cellulose[3]	30.0
Salt mix[4]	37.0
Vitamin-glucose mix[5]	50.0
Vitamin A and D concentrate[6]	5.0
Alpha-tocopherol	0.1

[1]Cerelose, Corn Products Co., Argo, Ill.

[2]General Biochemicals, Inc., Chagrin Falls, Ohio.

[3]Solka Floc, Brown Co., Berlin, New Hamp.

[4]Phillips, P. H., and Hart, E. B. J. Biol. Chem., 109: 657. 1935. Reagent grade salts were used and $ZnCl_2$ was omitted.

[5]Composition similar to that used by Forbes, R. M., and Yohe, M. J. Nutr., 70: 53. 1960.

[6]Vitamin A and D concentrate: 2000 I.U. vitamin A and 250 I.U. vitamin D_2 per gram.

6 microns and stained with hematoxylin and eosin. Sections of the testes were stained with Oil Red O for determination of fat content.

RESULTS

Signs

Growth rate was one of the first and most marked signs of zinc deficiency. Initial weights of the rats averaged 47 Gm. Final weights of deficient rats averaged 82 Gm. and control rats 192 Gm. Total weight of diet consumed by deficient rats averaged 263 Gm. and control rats 492 Gm. Control rats made consistent, steady weight gains throughout the experiment. Deficient rats made consistent weight gains throughout most of the experiment but at a slower rate. Male deficient rats had slight weight loss during the final days of the experiment. Although the rats on the zinc-deficient diet had an interest in food, there was marked depression of food intake.

Gross Pathology

The initial change in all rats on the zinc-deficient diet was a generalized thinning, roughening, and loss of luster of hair coat with erythema by the third week of the experiment.

Lesions were more severe in deficient male rats, and these animals had additional lesions of bilateral alopecia of the front paws, ventral thorax and abdomen, and submandibular and ventral cervical regions. The deficient male rats also had cutaneous seborrhea in these areas of alopecia. There was concomitant bilateral alopecia on the dorsal surface of the body. The skin of these alopecic areas was roughened and scaly (Figures 1, 2, and 3). The gross pathology in female rats on the zinc-

Figure 1. Control rat (left) fed a 50 p.p.m. zinc-supplemented diet. Female (middle) and male (right) rats fed a zinc-deficient diet (0.9 p.p.m.).

Figure 2. Male rat fed a zinc-deficient diet (0.9 p.p.m.). Note alopecia and exudation of ventral surface.

Figure 3. Male rat fed a zinc-deficient diet (0.9 p.p.m.). Note dorsal alopecia, rough hair coat, and exudation.

Figure 4. Female rat fed a zinc-deficient diet (0.9 p.p.m.). Note moderate roughening of hair coat.

deficient diet was primarily growth retardation, rough hair coat, and a slight scaliness of the skin in the dorsal cervical area (Figures 1 and 4).

Microscopic Pathology

The stratified squamous epithelium of the esophagus of rats fed the 50 p.p.m. zinc-supplemented diet was characterized by (1) a basal stratum consisting of a single layer of columnar cells, (2) stratum spinosum consisting of 2 to 3 layers of cells with indistinct cell borders and large oval nuclei, (3) stratum granulosum consisting of a single layer of cells containing cytoplasmic granules and flattened nuclei, and (4) stratum corneum consisting of cornified cells devoid of nuclei (Figure 5).

The columnar cells of the basal stratum of the esophageal epithelium of rats fed 0.9 p.p.m. zinc had an irregular arrangement, were increased in number, and contained cytoplasmic vacuoles. The stratum spinosum had 8 to 10 layers of cells with indistinct and irregular cell borders. These cells contained many cytoplasmic vacuoles and some nuclei were pyknotic. The stratum granulosum was indistinct. The stratum corneum was greatly thickened by many cell layers of partially keratinized cells. Cell borders were discernible and cell size was irregular. The cytoplasm was homogeneous and cells had persistent pyknotic nuclei (Figure 6). Similar changes were observed in the epithelium of proximal, middle, and distal esophagus and the posterior region of the tongue (Figure 7).

Control animals had normal stratified squamous epithelium of the skin (Figure 8). Microscopic lesions in the skin of rats fed a zinc-deficient diet were confined to the back, shoulders, ventral abdomen and thorax, submandibular area, and front paws. The changes in the epidermis

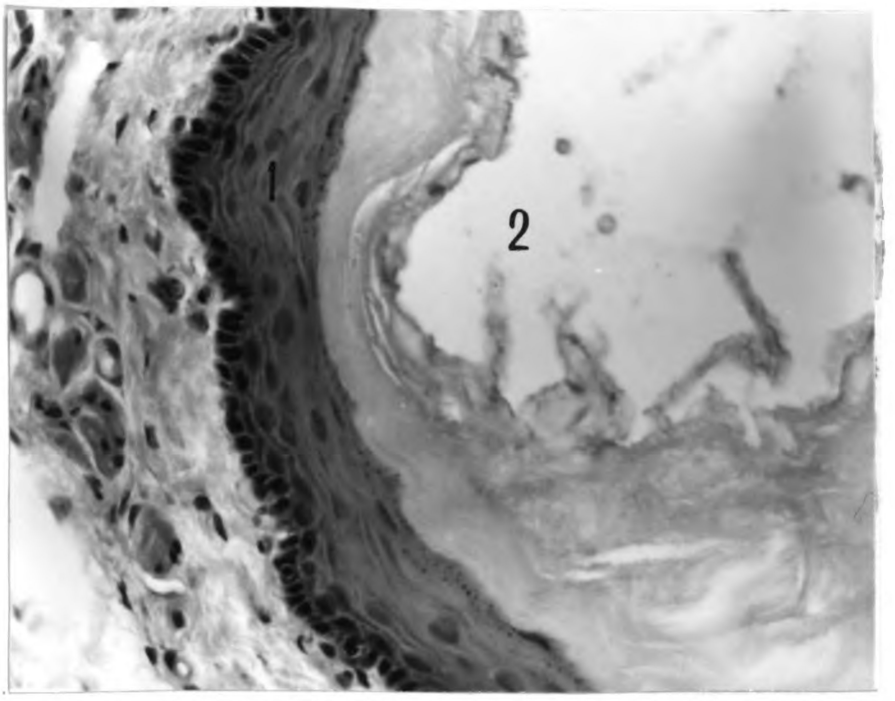

Figure 5. Esophagus from a zinc-supplemented rat (50 p.p.m. in diet). Stratified squamous epithelium (1). Lumen of esophagus (2). Hematoxylin and eosin. x 425.

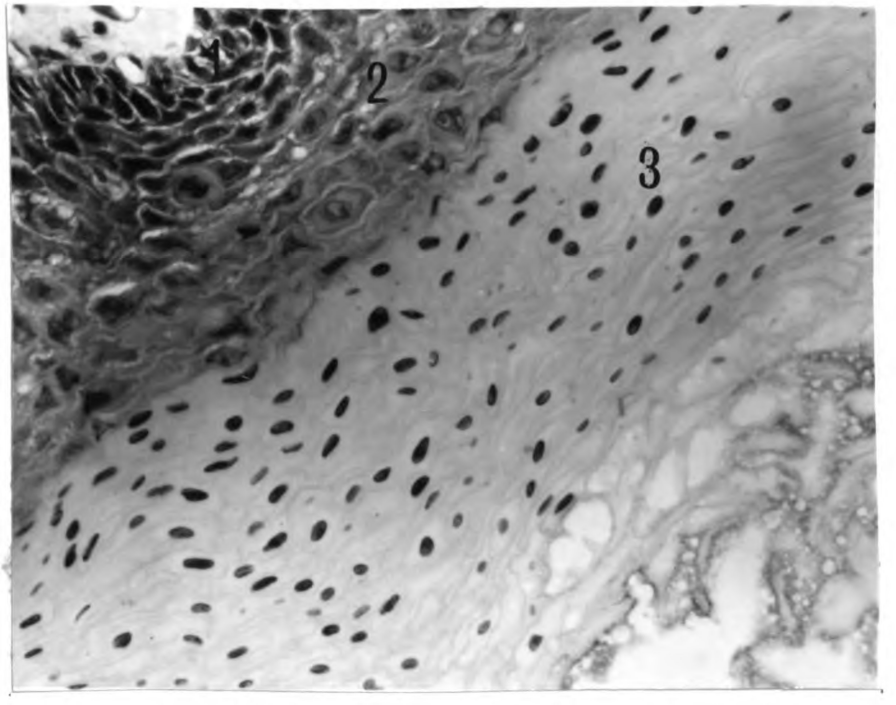

Figure 6. Esophagus from a zinc-deficient rat (0.9 p.p.m. in diet). Basal cells more numerous (1). Prickle cell layer 8 to 10 cells thick (2). Parakeratosis, note pyknotic nuclei (3). Hematoxylin and eosin. x 425.

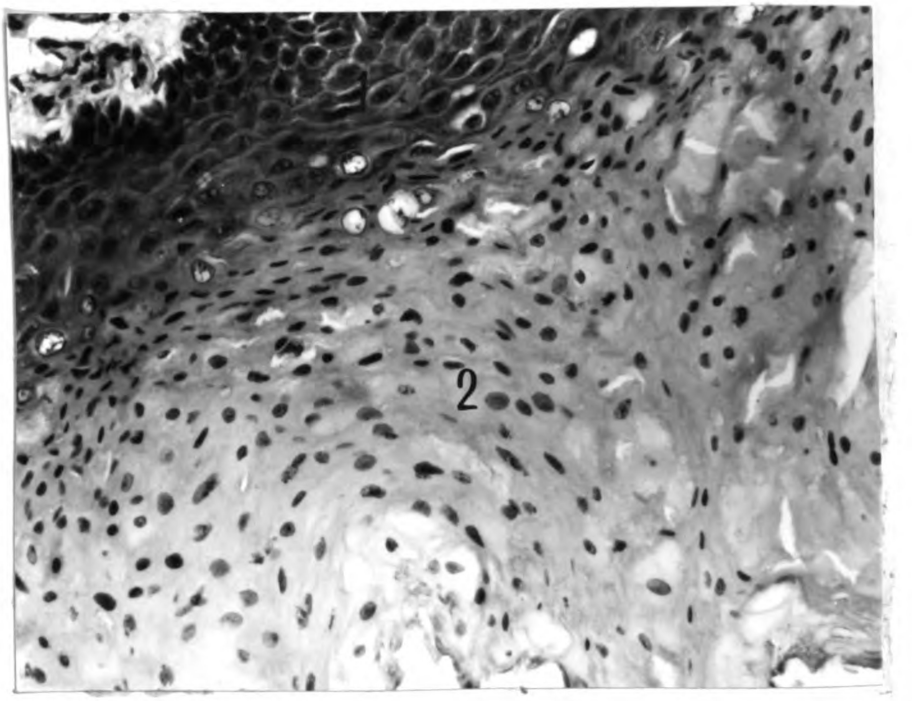

Figure 7. Tongue from a zinc-deficient rat (0.9 p.p.m. in diet). Increased thickness of cell layers of stratified squamous epithelium (1). Partially keratinized cells (2). Hematoxylin and eosin. x 325.

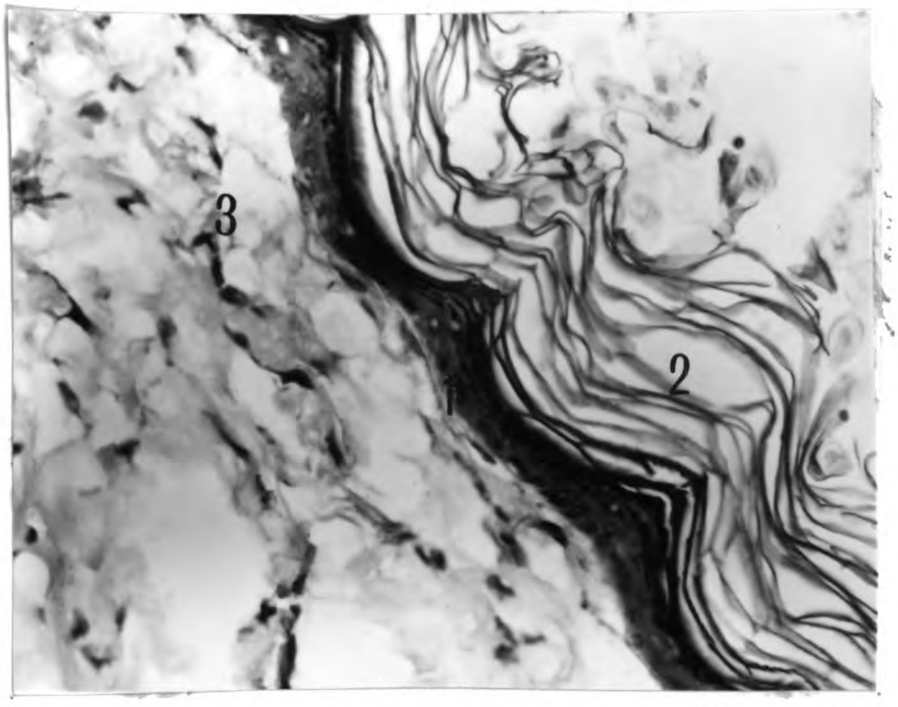

Figure 8. Skin from a zinc-supplemented rat (50 p.p.m. in diet). Stratified squamous epithelium (1). Cornified layer (2). Dermis (3). Hematoxylin and eosin. x 325.

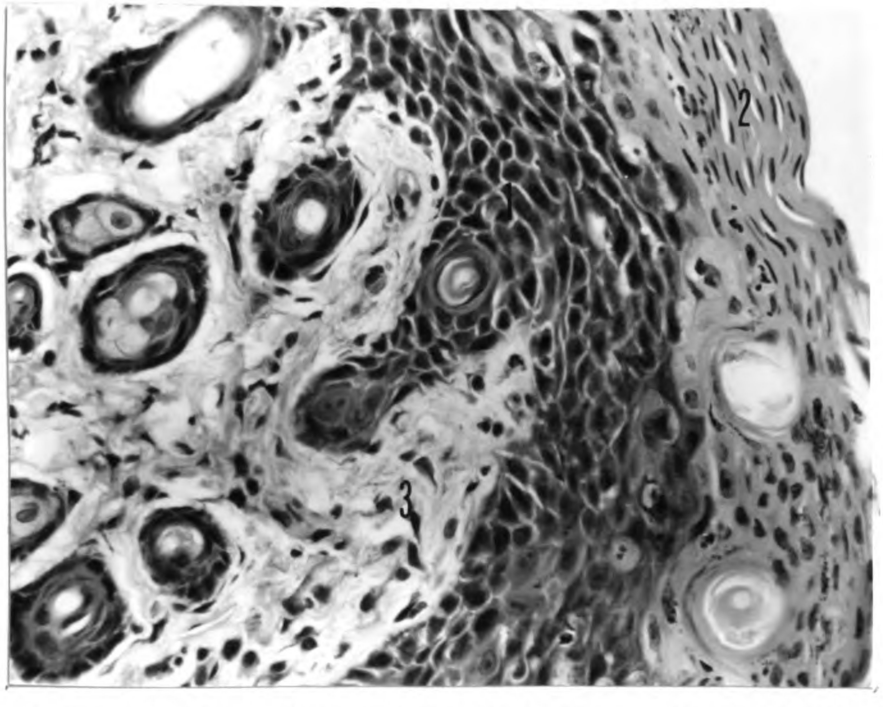

Figure 9. Skin from a zinc-deficient male rat (0.9 p.p.m. in diet). Acanthosis (1). Parakeratosis (2). Increased number of mononuclear cells in dermis (3). Hematoxylin and eosin. x 325.

were increased numbers of basal cells, increased numbers and size of prickle cells, and increased thickness of the stratum corneum. Most of the keratinized cells of the stratum corneum had retained, pyknotic nuclei. Within or on the surface of the keratinized layer were many inflammatory foci which contained bacterial colonies, neutrophils, and necrotic debris. These focal lesions were most prominent in the skin of the abdomen, cervical area, and front paws.

The dermis had increased numbers of mononuclear cells and hypertrophic sebaceous glands (Figure 9).

The germinal epithelia of the seminiferous tubules of the gonads in male rats fed the zinc-deficient diet were incompletely developed, reflecting almost complete aspermatogenesis. The tubules contained abundant spermatogonia but were devoid of spermatids and spermatozoa (Figure 10). The seminiferous tubules of the gonads of control rats contained all stages of spermatogenesis (Figure 11).

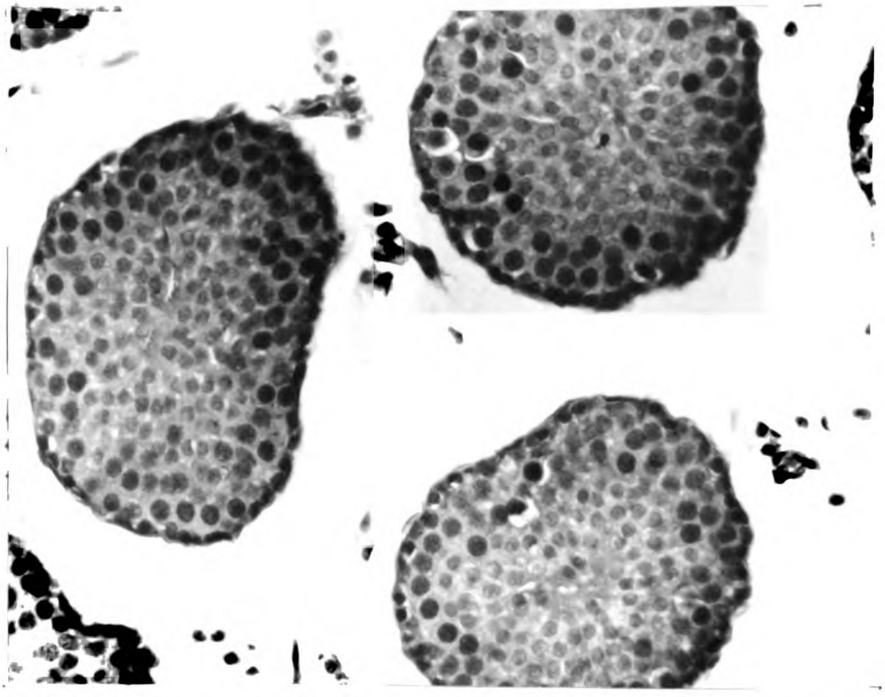

Figure 10. Testis from a zinc-deficient rat (0.9 p.p.m. in diet). Aspermatogenesis in seminiferous tubules. Hematoxylin and eosin. x 325.

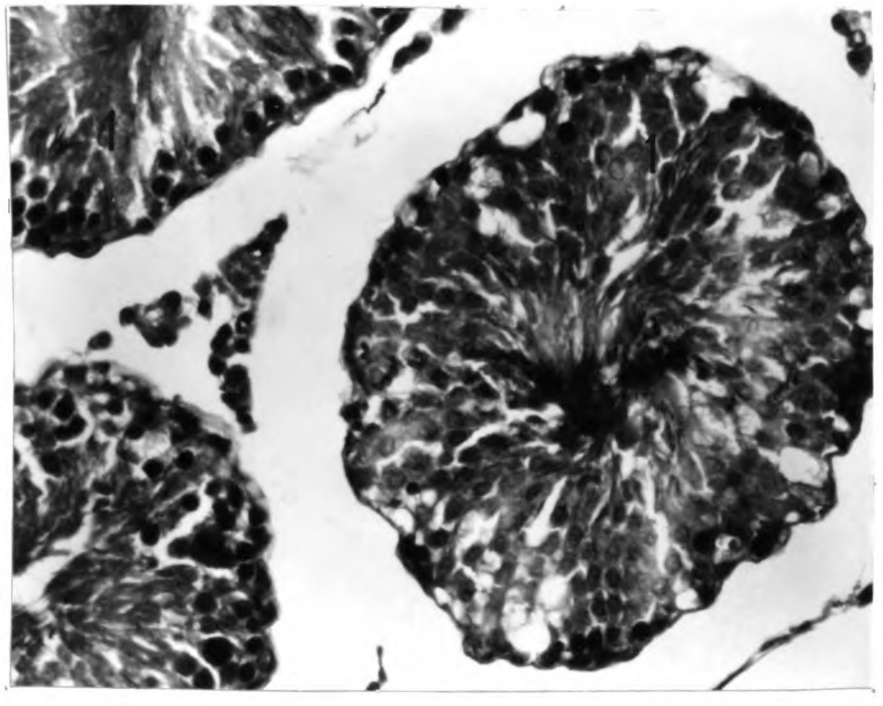

Figure 11. Testis from a zinc-supplemented rat (50 p.p.m. in diet). Spermatogenesis in seminiferous tubules. Hematoxylin and eosin. x 387.5.

DISCUSSION

In distribution, the pathologic changes in rats fed the zinc-deficient diet were limited to the testes, tongue, esophagus, and skin. This was in agreement with Follis (1941).

The most obvious changes in zinc-deficient rats were reduced growth rate and reduced food consumption. These were in agreement with the findings of other workers (Day and McCollum, 1940; Millar *et al.*, 1958). Pathologic changes were more severe in male deficient rats than in females. Changes such as loss of weight toward the end of the experiment and regional alopecia were indications of a greater requirement for zinc in the male.

The seborrhea and hypertrophy of sebaceous glands of the dermis in the male deficient rats are in agreement with the observations of Follis (1941). Seborrhea has been reported by Follis (1958) as a sign of biotin deficiency. Recent work by Luecke *et al.* (1968) strongly indicates that an increase of the level of biotin from 0.2 mg./kg. of diet to 4 mg./kg. will eliminate seborrhea. By extrapolation, it was thought that biotin deficiency may have been precipitated by the use of spray-dried egg-white solid in the experimental deficient diet. The foci of inflammation in the corneum of the skin were probably a result of secondary bacterial invaders. Histopathologically these lesions were identical with those observed in focal *Staphylococcus aureus* dermatitis.

The seminiferous tubules of control rats contained all stages of spermatogenesis, while in zinc-deficient rats only primitive germinal epithelia were in evidence. Two possible explanations are: incomplete

spermatogenesis as a result of zinc deficiency, or incomplete spermatogenesis because of sexual immaturity. Luecke (1967) fed zinc-deficient rats of comparable age and under identical experimental conditions a diet containing an adequate quantity of zinc for a period of 2 weeks. The seminiferous tubules of the testes in these rats had all stages of spermatogenesis. Conversely, Millar et al. (1958) reported depressed development of the testes with severe atrophy of testicular germinal epithelia. Normal spermatogenesis was not observed when these deficient animals were fed a diet containing adequate zinc.

Histopathologic changes of zinc deficiency in this experiment were limited to the stratified squamous epithelium of the skin, tongue, and esophagus and epithelial cells lining the seminiferous tubules of the testes. These changes are consistent with the results of Follis (1941). It is known that zinc plays an important role in metabolism as a component of several metalloenzymes: other functions have not been determined. Metalloenzymes are widely distributed in animal tissues and are not limited to epithelia. Additional research may help to explain the interrelationships of lesions produced in zinc deficiency.

SUMMARY

The gross and microscopic pathology was determined in rats fed a zinc-deficient diet. Control rats were fed a diet containing 50 p.p.m. zinc and deficient rats were fed a diet containing 0.9 p.p.m. zinc for 6 weeks.

The first gross signs in deficient rats were decreased appetite and retarded growth rate. By the third week there were erythema of skin and generalized thinning, roughening, and loss of luster of hair coat in all deficient rats. Lesions were more severe in male rats; these animals had additional lesions of bilateral alopecia of the front paws, ventral thorax and abdomen, back, and submandibular and ventral cervical regions. The male rats also had cutaneous seborrhea in the areas of alopecia. Gross changes became progressively more severe throughout the experiment.

Histopathologic changes were confined to stratified squamous epithelium of the tongue, esophagus, and skin; the dermis; and epithelium of the seminiferous tubules of the testes. The changes in the stratified squamous epithelium were characterized by an increase in number of basal cells, acanthosis, and incomplete keratinization. The changes in the dermis were increased numbers of mononuclear cells and hypertrophic sebaceous glands. The primary change in epithelial cells of germinal epithelia of the seminiferous tubules of the testes was incomplete maturation. All gross and microscopic changes, with the exception of seborrhea, were manifestations of zinc deficiency.

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VITA

The author was born in Mooreland, Oklahoma, on February 23, 1930. He received his primary and secondary education in the public schools of Mooreland, Oklahoma. After graduation from high school in 1948, he continued his education at Oklahoma State University, Stillwater, Oklahoma. He received a B.S. degree in June, 1954, with a major in agricultural education.

From August, 1954, to August, 1960, he was with the Technical Cooperative Administration Contract, Oklahoma State University, as instructor of animal science in the Imperial Ethiopian College of Agricultural and Mechanical Arts located in Dire Dawa, Ethiopia.

He entered the School of Veterinary Medicine, Oklahoma State University in September, 1961, and received his D.V.M. degree in May, 1965. Following graduation, he entered the graduate school of Michigan State University, majoring in pathology. His current appointment as instructor in pathology was September 1, 1966.

The author married Gwen A. Blakesley in 1950. They have two children: Kim, 10, and Jill, 6.