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Title: HISTOLOGIC EFFECTS OF CHRONIC SELENIUM DEFICIENCY IN SELECTED TISSUES OF LABORATORY RATS

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The selenium-responsive syndromes and the discovery of Factor 3-selenium focused attention on selenium in metabolism. Two hypotheses have been advanced to explain the metabolic effect of selenium: 1) substitution or sparing of alpha tocopherol, and 2) a primary role in metabolism. The first hypothesis is supported by Scott and Desai, who found that selenium and alpha tocopherol produced similar responses in certain deficiency diseases. The earlier inability to produce lesions of a selenium deficiency in the rat in the presence of ample vitamin E has caused the validity of the second hypothesis to be questioned. This obstacle to identifying the metabolic role of selenium was overcome when Weswig and Whanger succeeded in developing a selenium-deficient rat.

Selenium-deficient rats were obtained from the colony of Weswig and Whanger and observed until deficiency lesions appeared. At this time they were divided randomly into selenium deficient and

supplemented groups. The deficient group was maintained on the low selenium rations and the supplemented group was fed this diet with 100 ppb selenium added as sodium selenite. The rats were necropsied at 80, 147 and 221 days of age. Body, heart, liver and genital weights were recorded and tissues were selected for histological and histochemical studies.

Signs in the selenium deficient rats were poor growth, alopecia and sterility. The histopathologic lesions of the skin, liver, genitalia, and musculoskeletal system are described. The pathologic changes resembled those found in exudative diathesis of chicks, unthriftiness in lambs and calves, and infertility in ewes. Liver necrosis and myopathy were not observed.

Histochemical changes included a reduction in the number of sulfhydryl reactive sites in the liver, testicle, muscle and back skin. Morphological changes were directly related to endothelial degeneration and hypoplasia. Correlation of clinical lesions and cellular changes indicated that selenium deficiency in the rat altered protein production and physiology of the vasculature.

Histologic Effects of Chronic Selenium Deficiency in Selected Tissues of Laboratory Rats

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INTRODUCTION

Selenium toxicity in animals has been known for centuries (15). The study of selenium as a nutritional element was initiated by the discovery that Factor 3-selenium prevented dietary liver necrosis in rats. Various workers found that selenium had a therapeutic action on white muscle disease in lambs and calves (18, 20), exudative diathesis in chicks (22, 30) and liver necrosis in pigs (27, p. 235).

The actions of vitamin E and selenium have been confused because of the similarity of the therapeutic effects of the compounds in certain diseases and in some geographical areas. In some areas or disease conditions one compound has been effective but not the other. The interrelationship of vitamin E and selenium has been studied because of the concomitant occurrence of vitamin E-responsive and selenium responsive diseases (18, 19).

Theories of the action of selenium have included: 1) substitution or sparing of alpha tocopherol (30), 2) substitution for sulfur in biologically active compounds (31) and 3) an apparent relationship due to the similarity of specific deficiency diseases from simultaneous dietary deficiencies (22). The inability to produce lesions of selenium deficiency in rats in the presence of ample alpha tocopherol has

prevented the separation of the effects of selenium and vitamin E deficiencies.

Doctors P. H. Weswig and P. D. Whanger have developed a selenium-deficient rat on a vitamin E supplemented torula yeast ration (38). The ration contains 18 ppb selenium and 60 ppm vitamin E. Second generation rats on this ration have effects of selenium deficiency which do not respond to vitamin E therapy. Lesions observed in these rats could be prevented by the addition of 40 ppb selenium to the ration. Rats that developed deficiency lesions were cured in 30 days by the addition of 80 ppb selenium to the ration.

This study was to ascertain some of the morphologic and histochemical aspects of the lesions present in the selenium deficient rat of Weswig and Whanger. Observations were related to several hypotheses of the metabolic role of selenium.

REVIEW OF SELENIUM EFFECTS IN RATS AND DOMESTIC ANIMALS

Selenium toxicity has been recognized in the United States since 1860 when Madison reported a fatal disease of horses grazing on seleniferous plants near Fort Randall, Nebraska (15). Both acute and chronic syndromes have been described in horses and cattle grazing on seleniferous plants (7, 32). The acute form, termed "blind staggers," is characterized by congestion and diffuse hemorrhage in the lungs and intestinal tract; focal necrosis of the liver; nephritis; and splenic congestion. The chronic form, or "alkali disease," is characterized by atrophy of the heart and liver, loss of hair and hooves, and erosion of the joint surfaces of the long bones.

Inorganic compounds of selenium also produce acute and chronic forms of poisoning. The acute syndrome in livestock is similar to the "blind staggers" syndrome of organic selenium poisoning. Magg and Glenn studied this form of selenosis in cattle and sheep (15). They fed sodium selenite to cattle at levels of 0.25 mg and 0.40 mg per pound of body weight per week for 19 weeks. Toxic signs, including skeletal muscle trembling, appeared when blood levels of selenium were greater than 3.0 ppm. Morphologic changes were rumenitis, abomasitis and enterocolitis. Two steers developed polioencephalomalacia and became comatose. The lethal oral dose

for sheep was 36.5 mg sodium selenate per head per day for 80 days. Lesions in sheep affected with acute selenosis included myocarditis, pneumonia, splenitis and lymphadenitis (15).

Harr et al. reported that in rats lesions from chronic dietary exposure to sodium selenite or selenate included myocarditis, toxic hepatitis, biliary and pancreatic duct inflammation, bronchitis, and renal cortical nephritis (10). The dietary threshold level of inorganic selenium toxicity was 0.25 ppm. Lesions in the rat from acute selenosis were hepatitis and myositis. The "blind staggers" syndrome did not occur in their rats. The "staggers" syndrome has not been produced in laboratory animals by feeding either sodium selenite or seleniferous plants (15).

Nelson et al. (21) and Volgarev and Tscherkes (37) reported the development of hepatic neoplasms in rats fed seleniferous grain or mixed selenide. Harr et al. were not able to reproduce these results in studies of rats fed inorganic selenium (selenite or selenate) throughout their life spans (10).

The lesions of selenium-responsive natural diseases vary widely in different species. Focal liver necrosis is characteristic in rats and swine (27, pp. 235, 255). Cardiac and skeletal myopathies occur in calves, lambs and ewes (11, 20). Chicks develop extensive hemorrhage and edema (22, 30). Gizzard edema and cardiac and skeletal myopathies occur in poults (22, 30). Hartley and Grant

reported selenium-responsive infertility and unthriftiness in ewes, and unthriftiness in lambs and calves in New Zealand and Australia (11). Oksanen and others reported that nutritional muscular dystrophy of chicks and swine in the Scandinavian countries responded better to alpha tocopherol than to selenium (23; 27, pp. 236, 261; 30).

The parallel response of vitamin E and selenium was first studied by Schwarz and Foltz in Factor 3 (34). The inability to demonstrate selenium deficiency effects in the presence of adequate vitamin E suggested that selenium replaced or spared vitamin E and was not an essential nutritional requirement (30). Desai and Scott supported this hypothesis by finding that selenium increased the retention of d-alpha tocopherol (6).

Selenium compounds prevented exudative diathesis in vitamin E deficient chicks (30) and liver necrosis in vitamin E deficient rats (29). Orstadius, Nordstrom and Lannek demonstrated a synergistic effect of selenium and alpha tocopherol in muscular dystrophy and, less conclusively, in hepatosis dietetica of swine (25). Selenium therapy was successful in correcting kwashiorkor anemia and improving growth rate in a limited number of infants which did not respond to vitamin E supplementation (12).

Muth et al. (20) and Hartley and Grant (11) reported natural selenium-responsive deficiency syndromes in animals fed diets that

contained adequate vitamin E. Muth et al. found a direct correlation between the selenium content of the alfalfa hay fed to sheep and the incidence of white muscle disease, but not between total tocopherol levels in the hay or blood plasma and the incidence of white muscle disease (20). Hartley and Grant were able to prevent the delayed form of white muscle disease and reduce the losses of lambs from the congenital form of the disease with selenium supplementation (11). Selenium levels were low in tissues of animals with white muscle disease and were low to normal in animals affected with selenium-responsive unthriftiness.

The tocopherol levels of new born lambs were lower than levels in adults, regardless of the presence or absence of white muscle disease. Alpha tocopherol supplementation had no effect on the congenital forms of white muscle disease and had a marginal effect on the severity of selenium-responsive unthriftiness (11, 20). Conversely, Sondergaard reported that selenium had no effect on some of the diseases associated with avitaminosis E (34).

Selenium and vitamin E responsive syndromes form two groups of diseases that are not mutually excludable by clinical or therapeutic responses. Research has not adequately defined the relative metabolic roles of the two agents (28, 35).

There are several possible metabolic roles for selenium. It could function as a primary lipid antioxidant by decreasing formation

of free radicals from decomposing lipid and nonlipid peroxides (35). Selenium could substitute for vitamin E in some metabolic transformations because of their similar action in hydrogen-ion transfer (13, p. 418). It could act synergistically with alpha tocopherol in the metabolism of peroxides (35). Compounds of selenium can also act as antioxidants by maintaining and protecting the sulfhydryl groups (35). Inorganic selenium is believed to be metabolized along the same enzyme systems as sulfur and the analogs of the two are interchangeable in metabolism (27, p. 334; 31). Their activity includes transport across the cell membrane and enzymatic conversion into amino acids. Selenium analogs may also function in protein and/or enzyme synthesis (31).

McConnell (16) and Allaway et al. (1) reported that selenium can be retained by animals over long periods. McConnell recovered radioactive selenium from the milk of a dog 278 days after injection (16). Allaway et al. found that ewes that had been fed low (10 ppb) selenium diets for one year were still able to transfer an adequate level of selenium to their lambs (1). Weswig and Whanger found that adult rats fed a selenium deficient ration (18 ppb) transferred sufficient selenium to their pups to permit normal growth and development (38). The second generation pups on this diet were clinically abnormal.

Selenium crosses the placental membranes of the rat, sheep, dog and mouse (39). The concentration of selenium in fetal blood is

lower than in the dam's blood and is directly dependent upon the amount of selenium available to the dam (4). The concentration of selenium in the blood of nursing lambs approximated the selenium levels in the blood of the dam. Thus, the milk appeared to be the major source of selenium for the lamb (24).

Weswig and Whanger developed a selenium-deficient rat on a ration that was supplemented with vitamin E (38). Weaned female OSU-brown rats were placed on a torula yeast ration containing 18 ppb selenium (Table 1). They were given demineralized distilled water ad libitum in glass bottles. These selenium-depleted females and their pups were maintained on the selenium deficient ration. The first generation rats were clinically normal. The second generation pups grew slowly, had alopecia and were sterile.

Table 1. Composition of Selenium-Deficient Ration

Torula yeast	30.0%
Salts (Hubbell-Mendel-Wakeman)[a]	5.0%
Sucrose	51.5%
Corn Oil	5.0%
Cellulose[b]	7.5%
B-vitamin Mix[c]	1.0%
Vitamin A	10 mg/kg
Vitamin D	100 mg/kg
Vitamin E	60 mg/kg

[a] Nutritional Biochemical Corp., Ohio.

[b] B. W. Solka Floc

[c] Prepared by mixing the following amounts in grams of vitamins with sufficient corn starch to give a final weight of 1000 grams: thiamine hydrochloride 0.4, riboflavin 0.8, pyrodoxine hydrochloride 0.5, D-calcium pantothenate 4.0, inositol 20, menadione 0.4, folic acid 0.4, niacin 4.0, choline dihydrogen citrate 4.24, and vitamin B_{12} 0.02.

The ration contained 18 ppb selenium as determined by fluorometric analysis.

METHODS AND PROCEDURES

Husbandry

Twenty-four selenium-deficient rats from the colony of Weswig and Whanger were observed until 50 days of age. All of the rats had signs of selenium deficiency and were randomly divided into selenium-deficient and selenium-supplemented groups. Four other rats were maintained on a commercial ration. The deficient groups were maintained on the basal diet containing 18 ppb selenium. The supplemented groups were fed the basal ration with 100 ppb added selenium in the form of sodium selenite. All groups received distilled, demineralized water ad libitum in glass bottles. The rats were housed in suspended wire cages for the duration of the experiment.

The feeding regimen and the age of necropsy for the experimental groups are summarized in Table 2. The selenium-deficient and the selenium-supplemented groups were necropsied at 80, 147 and 221 days of age. The rats fed the commercial ration were necropsied at 65 days of age.

Table 2. Experimental Design

Group Number	Diet	Age at Necropsy (days)
1	Basal ration	80, 147 and 221
2	Basal ration + 100 ppb selenium	80, 147 and 221
3	Commercial ration	65

Necropsy Procedures

Rats were killed by CO_2 inhalation. Body, liver, ovary and testicle weights were recorded. Portions of skin from the back (thoracic region) and the nasal tactile areas, cardiac and skeletal muscle, liver, uterus, ovary, testicle and the proximal epiphyseal plate of the femur were fixed in 10% formalin. The bone sections were decalcified in 5% formic acid and dehydrated in dioxane (3, p. 10). The other tissues were dehydrated in xylene and alcohol (3, p. 8).

Histological Procedures

Tissues were embedded in paraffin, cut at 6 μ, and stained with hematoxylin and eosin stains. Skin, liver and genital sections were stained for nucleic acid activity with toluidine blue O, and with methyl green (36, p. 264). Cardiac and skeletal muscle sections were stained by the Von Kossa technic for deposited calcium and with Gomori's one-step trichrome stain to demonstrate striations (3, p. 66). Sulfhydryl reactive sites in skin, liver, muscle and genitalia were demonstrated by Deguchi's (5) modification of Gomori's nitro-blue tetrazolium method.

To facilitate the evaluation of sulfhydryl staining, high contrast 35 mm color projection slides magnified 1000 times were made from the nitro-blue tetrazolium stained tissues. Slides of similar areas

in tissues from selenium-deficient and selenium-supplemented rats were projected alternately, compared, and ranked as to the degree of difference between selenium deficient and supplemented groups.

The number of hair follicles per cm in the tactile and back skins was determined in 10 areas of each slide. The number of epidermal and hepatic cells in a 1 mm length of skin surface or hepatic cord was determined (Table 3). The differences between observations in comparable rats from each treatment group were analyzed by multiple-regression procedures.

Table 3. Summary of Weight and Histologic Differences.

	Average Difference Selenium - Non-selenium		Without Selenium		Percent Difference With Selenium	t-value	Degrees of Freedom (n - 1)	Significance
Body Weight	48	gm.	171	gm.	28%	3.096	8	1%
Heart Weight	0.13	gm.	0.65	gm.	20%	2.571	3	5%
Liver Weight	0.63	gm.	9.95	gm.	6%	69.284	6	0.5%
Testicle Weight	0.13	gm.	0.72	gm.	18%	30.88	3	0.5%
Back Skin	17.3	cells/mm.	129.56		13%	3.444	7	1%
Back Skin	8.83	/10 fields	29.67		30%	3.094	5	2.5%
Tactile Skin	12.623	cells/mm.	137		9%	4.1999	7	0.5%
Tactile Skin	0.67	/10 fields	43		2%	0.0835	5	N.S.
Liver	0.37	cells/mm.	19.1		2%	0.0637	7	N.S.

RESULTS

Clinical Observations

Rats in each of the groups were alert and active. Those fed the commercial ration were larger than rats fed either the deficient or supplemented rations. Rats that received selenium supplementation were 28% larger than those on deficient rations (Table 3).

Rats on the deficient rations were sterile. Those on the supplemented and commercial rations had normal sized litters. Alopecia persisted in the rats fed the deficient rations. The hair coat of the rats on the supplemented diet became normal.

Skin

The loss of hair was from alopecia rather than focal debridement. Haris were morphologically normal and the shafts were not broken. There was no hyperkeratosis, discoloration or exfoliation.

The skin from the back of the deficient rats was edematous. The dermal layer contained fewer capillaries and arterioles than were present in the skin of rats from the other groups. The endothelium of these vessels was thickened, and nuclei of the endothelial cells were

swollen or pyknotic. Follicular cells were degenerate and their cytoplasm contained hydropic vacuoles.

The germinal layer of the back skin of the deficient rats contained 13% fewer cells than the comparable tissue in selenium supplemented rats (Table 3). The number of hair follicles and sebaceous glands was reduced in the deficient rats.

The tactile skin of the rats on the deficient ration was congested. The number of cells in the germinal layer of the epidermis and the number of hair follicles in the tactile skin of the deficient rats were 9% and 2% less than in comparable tissue from the supplemented rats.

The nucleic acid content of the epidermal cells, hair follicles, sebaceous glands and vascular endothelium of the skin was determined by comparison of the toluidine blue O and methyl green stains in tissues from rats on both the deficient and supplemented rations. The amount of deoxyribonucleic acid was the same in both groups. There was less ribonucleic acid in the epidermal cells, hair follicles, sebaceous glands and blood vessels of the back skin of the deficient rats than in the supplemented rats. The amount of ribonucleic acid in the cells of the tactile skin was the same in both groups. The number of sulfhydryl reaction sites in the blood vessels and the hair follicles of the back skin of deficient rats was less than in tissue from supplemented rats (Table 4). There was no difference in the number of sulfydryl sites in the tactile skin of the two groups.

Table 4. Difference Between the Sulfhydryl Groups in Various Tissues from Selenium Deficient and Supplemented Rats.

Tissue	Se Deficient (18 ppb)	Se Supplemented (100 ppb)
Liver	-	+++
Testicle	-	++
Muscle	-	+
Back skin-epi.	-	(±)
Back skin-hair	-	(+)
Tactile skin	-	-
Tactile hair	-	-
Uterus	-	-
Ovary	-	-

Liver

Livers from all of the rats were normal in color and texture. Those from rats on the deficient rations were 6% smaller than those from the supplemented rats (Table 3). The number of hepatic cells/mm of liver cord was the same in both groups (Table 3).

Livers from rats in the deficient group were congested and the hepatocytes varied in size and nuclear detail. Double nucleated cells were common in the deficient rats, but not in the supplemented animals. Hepatic cords and sinusoids of the deficient rats were tortuous and constricted. Kupfer's cells were prominent and the chromatin of the bile duct cells was marginated.

The liver of one of the selenium supplemented rats contained an area of focal hyperplasia. This focus contained numerous double nucleated and hyperplastic hepatocytes and proliferated bile ducts.

The amount of ribonucleic or deoxyribonucleic acid in the hepatic cells was the same in both groups. The number of stainable sulfhydryl groups in the livers of deficient animals was less than in the supplemented groups (Table 4).

Genitalia

Testicles from selenium deficient rats were edematous and weighed 18% less than those from supplemented rats. Seminiferous

tubules of the deficient rats did not contain normal numbers of mature sperm. There were few spermatogonia or primary and secondary spermatocytes in some tubules, and in others the genital tissues were necrotic. The endothelium of the testicular blood vessels was thickened and the nuclei of the endothelial cells were swollen. Degeneration of the seminiferous tubules was directly related to the severity of vascular degeneration.

The ribonucleic and deoxyribonucleic acid content of the germinal and endothelial cells was the same in both the supplemented and deficient groups. The number of sulfhydryl reactive sites in the spermatogonia and endothelial cells of the testicles was less in the deficient rats than in rats from the supplemented groups.

Ovaries of the selenium deficient rats contained numerous primordial follicles, but few developing or mature follicles. Mature follicles were cystic or did not contain an ovum. Follicular cells were normal and corpora lutea and atretic follicles were found in most of the tissue sections. There was a large necrotic area in the ovary of one 80-day-old deficient rat. Oogenesis was normal in rats from the supplemented or commercial rations. There were the same number of sulfhydryl reactive sites in the ovaries of the deficient and supplemented rats.

Endometria from deficient rats were infiltrated with large numbers of eosinophils. The columnar cells and endometrial glands

were vacuolated and degenerate. The same number of sulfhydryl-reactive sites was present in the endometria of rats from all the groups.

Musculoskeletal

The epiphyseal plates and adjacent hemopoietic elements of the femurs from all the rats were normal. The hearts of selenium-deficient rats weighed 20% less than those from the supplemented groups (Table 3). The endothelium of the vessels was degenerate and the muscle fibers were separated by edema. There were fewer sulfhydryl-reactive sites in the skeletal muscle fibers of the selenium-deficient rats than in muscle from the selenium-supplemented rats (Table 4). The location of the sulfhydryl-reactive sites corresponded to the site of primary calcium deposition reported in white muscle disease. Calcium was not observed in Von Kossa-stained sections of striated or cardiac muscle.

DISCUSSION

The reported causes of nutritional muscular dystrophy in calves, lambs and swine are unsaturated fatty acids, low vitamin E levels, selenium deficiency and combinations of these factors (23). Lanneck and Orstadius incriminated unsaturated fatty acids as the cause of hepatic necrosis in swine (14). Selenium, or vitamin E therapy, alone or in combination, has been effective in some of these conditions but not in others. Similar therapeutic response does not mean that the pathogenesis of the diseases or their lesions are the same, or that the mode of action of the two compounds is similar.

Muth et al. (20) and Hartley and Grant (11) found that a specific myopathy in lambs and calves, white muscle disease, could be prevented by selenium supplementation but not by administration of alpha tocopherol. The lesion of white muscle disease is primary calcification or necrosis of the cardiac and skeletal muscle with secondary degeneration of the muscle fiber. The calcium is deposited adjacent to the Z-line of the muscle fiber. The lesion is associated with elevated levels of serum glutamic oxaloacetic transaminase (SGOT).

Selenium-deficient rats did not have either muscular

calcification or elevated SGOT levels (17). The muscles were edematous and the number of sulfhydryl-reactive sites was less than in selenium-supplemented rats. Sulfhydryl-reactive sites were adjacent to the Z-line of the muscle fiber.

Wright and Bell (40) found that swine absorbed 85% of the selenium in rations that contained 0.35 ppm selenium, and that absorption of selenium by sheep was related to the formation of elemental selenium from selenites and selenium analogs of amino acids. One-half of the ingested selenium was excreted in the feces. The difference in muscle lesions between ruminants and rats may result from either a species difference in selenium metabolism or from a difference in the absorption rate of selenium.

The primary lesion in selenium-deficient rats was endothelial degeneration. This was related to the anti-inflammatory action of selenium reported by Roberts (26). The testes were the most severely altered tissue examined and the most dependent upon adequate blood supply. The vasculature of the testicle was markedly abnormal as indicated by morphologic and histochemical changes. Other genital organs were also sensitive to selenium metabolism.

Endothelial degeneration in the ovary and uterus was severe enough to damage the tissue. Histamine released from the injured cells may have initiated the infiltration of eosinophils into the endometrium (33, p. 266).

Genital effects followed the principle of organ response demonstrated by Gunn and Gould (9). They were able to prevent the effect of cadmium toxicosis in the testicle by selenium therapy. The vascular system of the testicle was the primary site of injury by cadmium and was the area protected by selenium. Gunn and Gould's observations and the lesions present in selenium-deficient rats indicate that selenium has an effect on the vascular endothelium (9).

Morphologic and histochemical changes in the skin were also related to vascular lesions. Lesions in back skin of deficient rats were multiple foci of hypoplasia. They were associated with edema and decreased numbers of capillaries and arterioles. Since selenium supplementation corrected the alopecia of selenium-deficient rats, hair papillae were quiescent rather than necrotic. The more vascular nasal-tactile skin was less affected by selenium deficiency than the back skin.

Endothelial degeneration in the skeletal muscle caused edema similar to the lesions of exudative diathesis in chicks (34). Myopathy did not occur in this experiment. Godwin and Fraser reported skeletal muscle degeneration and calcification in rats on a low selenium ration (8). Electrocardiograms were abnormal although lesions were not present in the cardiac muscle. Godwin and Fraser's (8) ration was low in both vitamin E and sulfur and contained 20 to 30 ppb selenium, whereas Weswig and Whanger's (38) ration was

supplemented with adequate vitamin E (Table).

The focal hepatic necrosis described by Schwarz and Foltz (29) and by Godwin and Fraser (8) did not occur in the deficient rats in this experiment. The torula yeast rations used in both studies were not supplemented with vitamin E and this, rather than selenium deficiency, may have caused the hepatic necrosis.

Selenium supplementation increased the body weight and rate of gain of selenium-deficient rats in this study. This observation confirms the findings of Weswig and Whanger (38) relative to the role of vitamin E in rat growth. The increase in growth rate and the improvement of hair coat resembled the selenium-responsive unthriftiness syndrome of lambs and calves described by Hartley and Grant (11).

The physical appearance of selenium-deficient immature rats was similar to the hypopituitary syndrome described by Smith and Jones (33, p. 965). However, the characteristic metaphyseal lesions of hypopituitarism were absent (2, p. 1239). If selenium affects the pituitary-endocrine system, it apparently is not associated with the growth hormone. A selenium-pituitary relationship may control vascularization through the thyroid and adrenal glands.

Histochemical observations included a decrease of ribonucleic acid and sulfhydryl reactive sites in some of the tissues from deficient

rats. The number of sulfhydryl-reactive sites was decreased in the endothelium, liver, testicle and muscle of the deficient rats. These tissues were reported by Thompson to contain large amounts of sulfhydryl groups (36, pp. 283-284). The decrease in sulfhydryl-reactive sites coincided with morphologic changes. Gunn and Gould demonstrated that selenium is closely associated with sulfhydryl groups (9). Tappel and his associates indicated that selenium may protect sulfhydryl enzymes from oxidative inactivation (35).

Observations from this experiment support the hypothesis that cellular growth and division are associated with selenium metabolism. In the selenium-deficient rats there was almost complete cessation of spermatogenesis after the secondary division. Hepatocytes were of various sizes, and excessive number of multinucleated cells were present. These changes were similar to lesions produced by pyrrozolidine alkaloids. Hypoplastic epidermal cells in the back skin of selenium-deficient rats contained small amounts of ribonucleic acid. Sulfhydryl-reactive sites were not present adjacent to the Z-line of the striated muscle of selenium-deficient rats. The normal number of sites was present in the muscle of selenium-supplemented rats. This area of the muscle fiber is the site of primary calcification in white muscle disease (18).

CONCLUSION

Selenium deficiency in rats fed a torula yeast ration with vitamin E supplementation was characterized by poor growth, alopecia and sterility. Pathology was similar to that found in the selenium-responsive syndromes of exudative diathesis in chicks, unthriftiness in lambs and calves and infertility in ewes. The rats did not have liver necrosis or myopathy. The primary lesion was vascular failure associated with failure of spermatogenesis, muscular edema, epidermal hypoplasia and hepatic toxicity.

Selenium deficiency reduced the growth of rats and the weight of the body, heart, liver and testicle. The size of the epidermal cells and the number of hair follicles in the back skin were also reduced. Histochemical effects included reduction in the number of sulfhydryl-reactive sites in the liver, testicle, muscle and back skin. The amount of ribonucleic acid in the epidermal cells of the back skin was also reduced.

Correlation of morphologic, histochemical and clinical lesions indicated that selenium deficiency in the rat altered the production of protein and the physiology of the vasculature. Lesions of selenium deficiency and histochemical effects were associated with endothelial degeneration and hypoplasia.

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