THE EFFECT OF NON-WEIGHT-BEARING EXERCISE AND PROTEIN INTAKE DURING PREGNANCY ON MATERNAL AND FETAL ZINC CONTENT IN THE SPRAGUE-DAWLEY RAT

by

Rebecca Ann Asente

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Human Nutrition and Foods

APPROVED:

L. J. Taper, Chairman

S. J. Ritchey

R. A. Teekell

July, 1985

Blacksburg, Virginia

THE EFFECT OF NON-WEIGHT-BEARING EXERCISE AND PROTEIN INTAKE DURING PREGNANCY ON MATERNAL AND FETAL ZINC CONTENT IN THE SPRAGUE-DAWLEY RAT

by

Rebecca A. Asente

(ABSTRACT)

To study the effect of exercise and protein intake during pregnancy on maternal and fetal zinc status in the rat, one hundred and seventy-nine pregnant Sprague-Dawley rats were divided into four groups: sedentary-standard protein diet, sedentary-high protein diet, exercising-standard protein diet and exercising-high protein diet. The standard protein diet contained 7.22% protein, while the high protein diet contained 24.77% protein; all other nutrients were supplied in amounts required for normal parturition of the laboratory rat. After aclimatization, the exercising dams, regardless of diet, were made to swim continuously for one and one-half hours/day until sacrifice. The four major groups were further subdivided into 28 groups, designated by three-day intervals according to gestational day--days 3, 6, 9, 12, 15, 18, and 21. Uterine tissues were retained for zinc content analysis; fetal and placental tissues were separated from uterine tissue for days 15 through 21 only. The concentration of uterine zinc was affected solely by gestation; absolute placental tissue zinc values were lowest in the sedentary-high and exercising-low protein groups, while the exercising-high protein group possessed the greatest zinc value. No significant difference was detected in fetal zinc concentrations. Fetal tissue from exercising dams weighed significantly less ($p<0.05$) than fetal tissue from the sedentary dams; and sedentary-high protein dams pro-

duced significantly more ($p<0.05$) fetuses than the exercising-high protein dams. Both protein intake and exercising during pregnancy significantly affect normal parturition and zinc metabolism in the rat.

ACKNOWLEDGEMENTS

The idea for this study was originally conceived by my partner, Sharon Cameron, without whose help this study could not have been completed. The impetus behind my involvement and accomplishments is, however, due to a number of people.

I wish, first of all, to recognize my advisory committee: Dr. L.J. Taper, director, for her helpful comments on my various attempts to combine the vast amount of materials that were necessary to confront; Dean S.J. Ritchey and Dean R.A. Teekell for their poignant and timely recommendations on the final draft; overall, for their expertise in the science of nutrition concerning pregnancy and trace mineral metabolism.

I also wish to extend my gratitude to Dr. K.H. Hinklemann, who, on such short notice, provided pertinent recommendations concerning computer and statistical analysis.

Deep appreciation is extended to Leslie K. Reynolds, for her invaluable friendship and technical assistance throughout the study. Likewise, Jelpi P. Picou, Jr. is to be recognized for his editorial assistance through pages and pages of chaff in search of the wheat.

And most importantly, I wish to express sincere appreciation to my parents, James and Mary F. Asente for their financial, spiritual and emotional support throughout my graduate work.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

In the past decade, physical education and activity have grown in popularity, and just recently exercise during pregnancy has likewise received popular attention. While it is generally accepted that the status of certain trace minerals, one being zinc, is affected during pregnancy, the ultimate effect of exercise during pregnancy on zinc status is currently unknown.

Both pregnancy and exercise training alter the body's normal physiological homeostasis. During gestation the pregnant woman's physiological adjustments include: plasma volume expansion; a dilution of certain blood nutrients and components in relation to plasma volume; an increased heart rate and stroke volume resulting in greater cardiac output; a redistribution of blood flow increasing vascularity in the uterus, kidneys, skin, breast and gastrointestinal tract; and an increase in the woman's ventilation rate via an increase in tidal volume, thus maximizing gaseous exchange (1).

The physiological adjustments associated with physical activity and athletics are similar to those observed during pregnancy. The physically active body displays cardiorespiratory changes such as blood and hemoglobin expansion, although the increase in hemoglobin is not directly linear to the blood volume expansion. There is also an increased efficiency in gaseous exchange; but such changes are related to exercise frequency, duration, intensity and hereditary factors (2). Complications arise when an attempt is made to account for individual differences associated with gestational adaptation and when trying to deduce nutrient status as related to the effects of exercise training.

Zinc is an essential trace element (3) involved in numerous metabolic pathways. One known function of zinc is its vital role in nucleic acid synthesis. Exercise is a protein catabolic stage with anabolism of the torn protein tissue occurring during resting phases. Thus, zinc is most likely involved in tissue development associated with training.

Dietary protein, both type and amount, affects zinc nutriture. Zinc deficiency has been correlated with insufficient dietary protein intake; and a disproportionately high intake of protein can elevate zinc requirements.

At this time, the hypozincemia frequently observed in pregnant women has not been conclusively explained by trace mineral investigators. Data exists, though, which reveals that zinc supplementation may be ineffectual in altering hypozincemia during gestation in humans and that supplementation may actually harm appropriate maternal-fetal growth and development. The recommended dietary allowance of zinc for pregnant women is 20mg/day. This requirement satisfies both the woman's normal zinc needs (15mg/day) and a liberal 5mg/day to assure adequate absorption and utilization for normal placental and fetal tissue growth (3).

Pregnancy increases the demand for protein. Exercise in a previously untrained pregnant female may further increase this demand. Variable protein intakes and physical exertion by an untrained pregnant female may affect mineral status, specifically zinc, thereby possibly harming placental-fetal growth.

The overall goal of this project is to evaluate the effect of mild aerobic exercise (vs inactivity) and protein intake on trace mineral nutriture in

pregnant Sprague-Dawley rats. The specific objectives are: to determine maternal and fetal zinc status, thereby evaluating maternal-fetal transfer of zinc.

CHAPTER II

REVIEW OF LITERATURE

ZINC

Characteristics and Properties of Zinc

Zinc is a bluish-white, relatively soft metal with the atomic number 30, an atomic weight of 65.37 and a density slightly less than iron. It is divalent in all of its compounds and is a composite of five stable isotopes: zinc-64, -66, -67, -68, and -70 with their abundance being 48.86%, 27.62%, 4.12%, 18.71%, and 0.69%, respectively. As yet, six radioisotopes of zinc have been identified--zinc-62, -63, -65, -69, -72, and -73. The most commonly used radioisotopes are zinc-65, with a half-live of 244 days, and zinc-69, with a half-life of 14 hours for its isomer and 58 minutes for zinc-69 itself. Zinc sulfate ($ZnSO_4$), acetate [$Zn(C_2H_3O_2)_2$] and oxide (ZnO) are those inorganic zinc compounds most frequently used in nutrition studies (4).

Zinc in the Human Body

Zinc is present in every human tissue and tissue fluid with concentrations varying as to site. Approximately 90% of total body zinc content is located in muscle and bone, with the highest concentration of zinc being in the tissue of the reproductive tract. Hypothetically, there is approximately 2.3g of zinc in a 70kg man, making zinc the most prevalent trace element in human tissue (4).

Natural Food Sources of Zinc

Foods high in protein provide the most substantial amount of zinc in the diet (4, 5). Brewer's yeast, seafood, and red meats provide highly bioavailable zinc to the body, while such foods as spinach and soybeans (6) contain dietary components which may bind with zinc, thus lowering its bioavailability in the gut. The specific zinc content of certain common foods in the American diet is listed in Table 1. It is also estimated from various studies that drinking water composes approximately 4.3% of the average food-zinc intake in the United States (4).

Zinc and Other Dietary Components

Phytate--Phytic acid (inositolhexaphosphoric acid) or phytate in seeds when consumed regularly can result in zinc deficiency (4, 7). Foods containing soy protein, other seed meals, mustard or rapeseed and/or rice bran, which is approximately 5% phytate, create a need for zinc supplementation because phytate complexes with zinc, thus decreasing zinc's bioavailability (4).

The protein source, whether animal or vegetable, may also affect mineral metabolism. Davis et al. (6) gave chicks a diet with soybean as the protein component (25% soybean). The deficiency symptoms, which consequently developed, related to an interference in normal mineral utilization. The authors cited the possibility that phytic acid, present in many plant foods and known to interfere with the absorption of calcium, iron and zinc, may be the interfering agent. By feeding rats diets varying in zinc, phytate and calcium content, Morris and Ellis (7) found that calcium furthers the phytate effect of lowering zinc's bioavailability.

Table 1. Zinc Content of Selected Foods (4)

Food	Zinc (mg/100g-3.5oz)
Oysters	100
Roast beef	4.0-8.0
Chicken, dark meat white meat	3.0 1.0
Fish (perch)	2.5
Liver	4.0
Egg	1.5 (0.7mg/egg)
Nuts	3.0
Wheat germ	13.0
Whole wheat bread	3.0 (1mg/slice)
White bread	1.0
Legumes	1.0-5.0
Milk	0.3/100ml (1.4mg/0.47 liter)

Calcium--Excessive amounts of calcium in the diets of rats have resulted in lowering the absorption of zinc (7, 8). There was a greater loss of zinc-65, resulting from a decrease in zinc-65 absorption and a lowered rate of bdy zinc-65 turnover in rats fed diets with excess amounts of calcium (8).

Copper--Zinc and copper are inversely related (5, 9-11). Excessive dietary zinc intake results in increased fecal copper losses (11).

Zinc Requirement

Man--The current recommended dietary allowance (RDA) for zinc is 15mg of zinc per day for adults. This RDA is based on human metabolic studies utilizing mixed diets resulting in an overall positive zinc balance with intakes of 12.5mg/day (12). Radioisotope studies revealed a 6mg/day turnover of body zinc. The RDA of 15mg of zinc/day allows for approximately 40% dietary zinc absorption. This is based on the assumption that a mixed diet with animal products is being consumed; diets high in vegetable content result in even less dietary zinc availablilty and absorption (3).

Rat--Laboratory rats require zinc in their diet for proper growth and development (13). Assuming the animal is living in a zinc-free environment and is fed a diet containing casein as the protein source, the rat, for maximal weight gain, requires 12mcg of zinc/kg body weight (14).

Zinc Absorption and Homeostasis

Nonpregnant State--As stated above, there are certain components of

food which may render dietary zinc unavailable for absorption because of the formation of insoluble complexes. Dietary calcium, phytate, fiber, and the practice of geophagia are known to make zinc unavailable for absorption, while EDTA (ethylenediaminetetraacetate) and alcohol result in hyperzincuria. Excessive amounts of cadmium, lead, mercury, arsenic, and/or copper at the cellular level are known to displace zinc via competition at some receptor or binding sites (15). Various disease states, such as liver disease (cirrhosis), gastrointestinal disorders (malabsorption syndromes), diabetes, collagen diseases, parasitic infections, and genetic disorders (Table 2), are frequently associated with zinc deficiency resulting from inadequate absorption and/or utilization of absorbed zinc (16, 17).

The absorption and homeostatic mechanisms associated with zinc nutriture are becoming better defined with the use of radio- or stable isotopes in both animal models and human subjects.

Cotzias et al. (18) in 1962 concluded zinc metabolism to be subject to both positive and negative feedback occurring at the gastrointestinal tract site according to body zinc content. The actual mechanisms involved in body zinc turnover, and thus "positive and negative feedback," are now believed to be known.

Utilizing zinc-69 in human subjects, Foster et al. (19) developed a kinetic model of zinc metabolism in man. In 17 subjects, 40% of ingested zinc was absorbed either directly or within 4 hours. Zinc in the portal circulation was absorbed by the liver with 67% efficiency, and it took an average of 5 days for zinc exchange with red blood cells. This model only accounted for 10% of total body zinc which is rapidly mobilized when

Table 2. Causes of Zinc Deficiency in Human Subjects (16)

Dietary	Excessive intake of phytate, fiber, polyphosphates, clay and laundry starch, and alcohol
Malabsorption	Pancreatic insufficiency, steatorrhea, gastrectomy, intestinal mucosal disease
Cirrhosis of the liver	
Renal disease	Nephrotic syndrome, renal tubular disease
Renal Dialysis	
Chronically debilitating diseases	Neoplastic diseases, chronic infection
Psoriasis (skin loss)	
Burns	
Parasitic infections (chronic blood loss)	
Iatrogenic	Penicillamine therapy, total parenteral nutrition, surgical trauma
Genetic	Sickle cell disease, acrodermatitis enteropathica
Pregnancy	

cellular requirements arise. The remaining 90% of tissue body zinc was not accounted for.

Evans et al. (20, 21) and Evans (22) have developed an experimental model explaining the controlling factors of zinc absorption and, therefore, zinc homeostasis. Initially, based on studies of zinc loading in zinc deficient rats, Evans and coworkers (20) believed the regulation of zinc nutriture to be partially dependent on the amount of zinc present in the intestine and the amount of plasma zinc present. By isolating a low molecular weight ligand from the pancreas of rats and the pancreatic secretion from a dog, Evans and associates (21) developed a systematic model defining the absorption process and controlling factors of zinc absorption and homeostasis; this model was further developed by Evans (22).

The model, much more complex than was originally assumed (Figure 1), involves pancreatic secretions containing a low molecular weight zinc-binding ligand present in the lumen of the intestines. The ligand has been isolated from rat intestine in two separate investigations. Cousins and coworkers (23) concluded the ligand to be a degradation product from metallothionein and other zinc-binding proteins from the intestine. While Evans et al. (24) concluded, after purification and characterization, that pyridine-2-carboxylic acid or picolinic acid (PA) is the zinc-binding ligand. When feeding PA to weanling rats, the rats absorbed 90% of zinc intake, while rats not fed PA absorbed only 50% of zinc intake.

Evans (22) proposed that the ligand binds dietary zinc and enables zinc absorption through the intestinal microvilli into the intestinal epithelial cells to the basolateral plasma membrane. The intestinal plasma membrane possesses zinc receptor sites specific for apotransferrin and/or

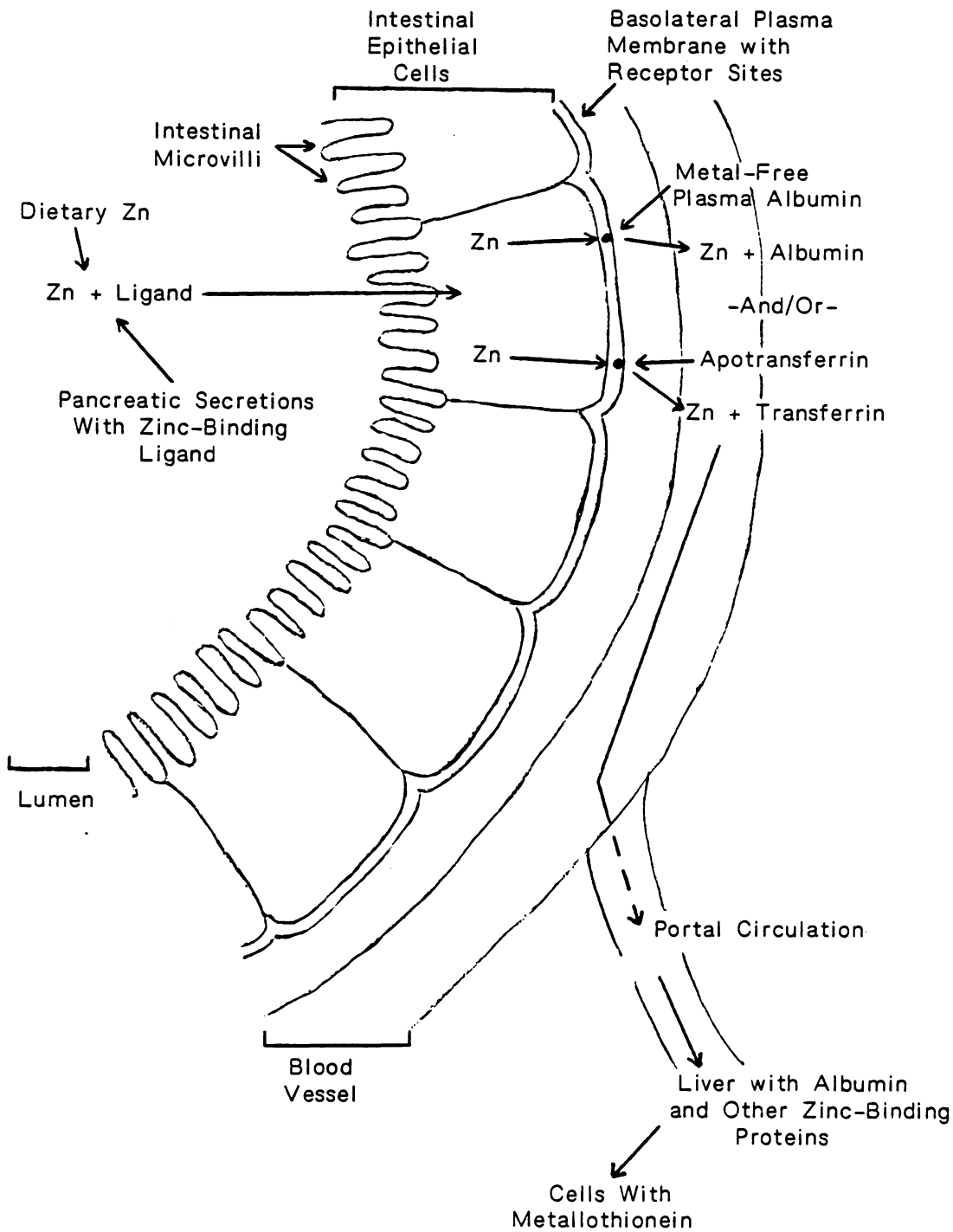

Figure 1. Zinc Absorption and Homeostasis

plasma albumin uptake. The transferrin/zinc complex is circulated through the portal blood to the liver where zinc is released for uptake by albumin and other zinc-binding proteins. Thus the controlling factors include, zinc bioavailability, the production of pancreatic zinc-binding ligand, and the quantity of metal-free plasma albumin and apotransferrin integral in zinc transfer.

Another important component in zinc nutriture not addressed by Evans is the presence of metallothionein. Metallothionein, located within the cell, according to Richards and Cousins (25) binds with absorbed zinc, where zinc is sequestered until it is required for cellular activity. Plasma zinc concentrations control metallothionein synthesis at the transcriptional level of protein synthesis. As plasma zinc increases, hepatic metallothionein synthesis is stimulated and so, in turn, zinc storage for cellular activity is created.

Pregnant State--Davies and Williams (26) concluded, from tracing the metabolic route of zinc-65 in pregnant rat carcasses and their fetal tissue, that both maternal and fetal zinc demands are met by an increased absorption and retention of zinc in the maternal system. By the twenty-first day of gestation, zinc absorption, via the duodenum, doubled in the dams. In the pregnant rats, the retention of absorbed zinc was found to be significantly greater during the latter half of pregnancy when compared to the first half (26).

Again utilizing zinc-65, Feaster and colleagues (27) concluded that the placental transfer of zinc freely occurs throughout gestation. Fetal body content (absolute concentrations) of both zinc-65 and total zinc

consistently increased in all stages of gestation and peaked at term (27).

Swanson et al. (28) reported apparent absorption of zinc-70 in pregnant women to be similar to nonpregnant women. It was estimated that the pregnant women absorbed 25% of the 16mg zinc/day intake resulting in an actual absorption of 4mg of zinc/day. Daily zinc retention was figured to be within 1.0mg zinc/day after accounting for excreting, secretion and sweat loss of zinc.

Zinc Excretion and Homeostasis

From research involving zinc-65 turnover in mice, Cotzias and associates (18) concluded fecal loss of zinc to be the major excretory route with urinary losses being negligable. Urinary zinc excretion remains fairly stable while fecal loss is related to dietary intake (29). In a metabolic balance study of pregnant women, fecal zinc excretion comprised 95% of total zinc excretion, while urinary zinc comprised only 5%. Pregnant women when compared to nonpregnant women, excreated more zinc via the urinary route (30). Swanson and King (30) cited that the physiological phenomenon of renal spill-over of nutrients associated with pregnancy could be a logical explanation for the discrepancy.

Hess and colleagues (31) revealed sweat loss of zinc to compose 50 to 66% of zinc excretion; fecal loss, including endogenous loss, was calculated as 30%; and urinary loss from 6 to 25% of zinc excretion. In fact, dermal zinc loss was calculated to be approximately 16 times greater in women between luteal and menstrual phases. The authors cited other research explaining a correlation between dermal zinc loss and an hormonal

(estrogen and progesterone) surge during this cycle, causing a greater than normal loss of zinc via the sebaceous glands. Ritchey and Taper (29) and Jameson (32) point to the fact, though, that zinc loss is in relation to many factors such as, climate, dietary habits and intake, physiological state, stress (exercise), pathological insult and more.

The homeostatic mechanism controlling zinc retention and excretion may involve a decrease in urinary, fecal and endogenous cellular (renal tubule, pancreatic, and intestinal mucosal cells) excretion when albumin and amino acid bound serum zinc fall below normal, thereby reducing zinc uptake. Subsequently a reduction in zinc sweat loss may reflect another homeostatic response to zinc deficiency (32).

Zinc Transport

In the blood, a circadian cycle has been detected concerning zinc concentrations in adults. Albumin, a macromolecular zinc ligand, transports approximately 43% of serum zinc. Zinc is loosely bound to one of the many histidine moieties comprising albumin, which is usually unsaturated with zinc. The micromolecular zinc ligands, histidine and cysteine, were found to be unique in their ability to cross the blood-brain barrier easily; porphyrin-bound zinc accounts for only a small portion of the micromolecular zinc ligands. About a third of blood zinc is incorporated by α_2-macroglobulin, another macromolecular zinc ligand; the incorporation and subsequent metabolism occurs solely in the liver tissue. Amino acid and albumin bound zinc make-up the diffusible, freely exchangable zinc, while zinc ligands with a molecular weight greater than 50,000 comprise the non-diffusible zinc

portion (4).

Zimmerman and associates (33) formulated a zinc transport system occurring during pregnancy (Figure 2). Three pools of available zinc exist --α_2-macroglobulin comprising 32% of total zinc, zinc loosely bound to albumin comprising 66% of total zinc, and amino acid bound (histidine and cysteine) zinc comprising 2 to 5% of total zinc. The α_2-macroglobulin ligand is an intracellular mediator, transporting or retrieving zinc for complex metabolic demands; albumin-bound zinc is principally transported for rapid exchange to major tissue sites.

During the last trimester of pregnancy, Zimmerman and associates (33) found, in human subjects, that serum albumin zinc concentrations were similar in maternal and fetal compartments, but the amount of zinc bound to albumin in the fetal compartment was two times greater than in the maternal system. The amount of α_2-macroglobulin ligand was below fetal levels in maternal serum, but the concentration of zinc bound to this ligand was greatest in the maternal system.

Zinc and Metabolism

Nonpregnant State--The development of zinc deficiency states in animals and diseases in humans associated with zinc deficiency have provided information regarding zinc metabolism.

Protein metabolism was found to be altered in zinc deficient rats. Theur and Hoekstra (34) revealed that zinc deficiency is correlated with an increase in leucine and lysine oxidation, whereas negliable effects or no effect was found on carbohydrate and fat metabolism. Zinc administration

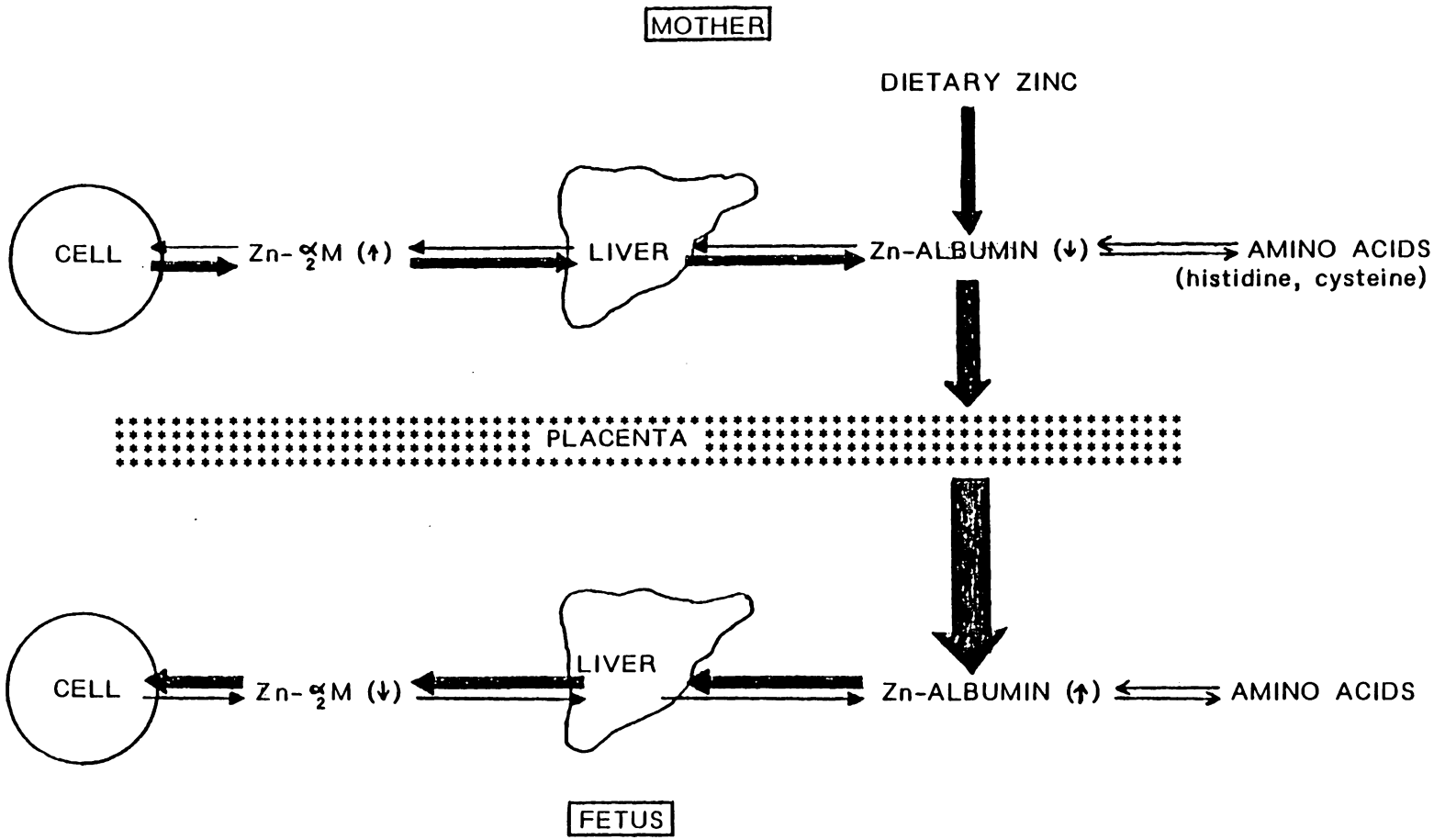

Figure 2. Hypothetical scheme for serum zinc transport in mother and fetus by albumin and α_2-macroglobulin in normal pregnancy. An increased (↑) or decreased (↓) zinc-protein fraction in mother or fetus relative to the other fraction may reflect increased fetal cellular zinc uptake, diminished zinc binding by maternal serum albumin, or other factors (33).

(injections of 100mcg) was followed by a reversal of lysine oxidation. Significant impairment of liver and kidney protein synthesis was also related to zinc deficiency by Hsu and colleagues (35). In zinc deficient rats, the tracing of C-14 labeled dl-methionine showed that after 4 to 8 hours from injection there was no difference in pancreatic protein synthesis, but there was a significant reduction in the incorporation of protein into liver and kidney tissue. This is of concern when considering that cystine (an amino acid metabolized from methionine) is integral in hair and skin biochemistry. Hsu and Anthony (36) observed significant increases in the urinary excretion of total nitrogen, urea and uric acid from zinc deficient rats when compared to control values. Protein catabolism is thus associated with zinc deficiency; such results are striking when remembering that zinc absorption, and therefore metabolism, is dependent on the synthesis of proteins--the low molecular zinc-binding ligand and plasma albumin.

Because zinc deficiency has been associated with dermatitis in pigs, Thompson et al. (37) isolated skin acid mucopolysaccharides from zinc deficient pigs. The pigs displayed a reduction in galatosamine and hexosamine and an elevation, although not significant, in hyaluronic acid. This may reveal a relationship between connective tissue metabolism and zinc.

Meydani and Dupont (38) have correlated zinc in the regulation of prostaglandin (PG) metabolism. Enzymes involved in PG synthesis are known to require zinc. Zinc deficient rats possessed lower PGE_1, $PGF_{2\alpha}$ and Met I, a metabolite of PGI_2, in the gut when compared to control values; no change was found in the mucosal concentrations in the gastrointestinal tract. The authors speculated that the symptoms of acrodermatitis enteropathica (AE) may be related to abnormal PG metabolism due to zinc defi-

ciency since PG's are cytoprotective, antisecretory agents normally present in the gut. AE is a rare, autosomal disease manifested by dermatitis, skin lesions, gastrointestinal disturbances, stomatitis, alopecia and failure-to-thrive. The exact etiology is unknown; but it is postulated that such manifestations could be due to essential fatty acid deficiency, an absence of intestinal mucosal enzymes or an inborn error of metabolism, specifically a defect in the normal production of the low molecular weight zinc-binding ligand necessary in zinc absorption (39, 40). These manifestations may be more closely related to an impairment of protein metabolism, explained earlier, as associated with zinc deficiency.

Alterations in PG synthesis found with zinc deficiency may be more significant when referring to Crohn's disease; a disease characterized by regional enteritis or inflammation of the intestinal mucosa resulting in anorexia, probable alterations in zinc absorption and an increase in gastrointestinal zinc loss. Patients with Crohn's disease have been found to be zinc deficient when measuring plasma and hair zinc. Solomons and associates (41) believe the problem to be in the area of plasma zinc and albumin binding resulting in deficient zinc concentrations. Zinc sulfate administration has reversed growth and sexual retardation and improved the gastrointestinal symptoms of one patient with regional enteritis and zinc deficiency (42). These improvements may be related to the role of zinc in PG synthesis, protein synthesis and, therefore, tissue healing (as related to intestinal mucosa) and growth.

The role of zinc in normal growth and development has been the explanation as to why infants may be born with spina bifida cystica. Compared to controls, the mothers of infants born with spina bifida possessed, through-

out gestation, significantly higher hair zinc levels. The average birth weight and length of infants with spina bifida were also significantly below average values for normal infants (43).

Pregnant State--In a study performed in 1971, Henkin et al. (44) found at term concentrations of total zinc in maternal serum to be below non-pregnant normal values. Absolute levels of free zinc, though, remained constant in maternal serum. This was attributed to a decrease in quantity or availability of zinc-binding protein and/or an alteration in zinc-binding capacity. It was concluded that alterations in zinc metabolism occur in the maternal system and not in the fetal system. Fetal serum zinc levels (bound and free) were not found to differ significantly from adult, non-pregnant concentrations.

Hypoalbuminaemia within the maternal system may be one of the causes of maternal hypozincemia. Giroux and associates (45) revealed, from an examination of the serum of pregnant women compared, in vitro, to serum of nonpregnant women, that maternal serum albumin has a lower affinity for zinc. This may explain why pregnant women, when supplemented with a readily available form of zinc, maintain lower average serum zinc concentrations when compared to nonpregnant values (30).

In a more recent and extensive investigation, Zimmerman et al. (33) found total protein, albumin and α_2-macroglobulin levels to fall by 16 to 24% in the serum of pregnant women from the first to the third trimester of pregnancy. There was an overall increase in the above mentioned serum protein components in umbilical serum. This may be due to both plasma expansion normally occurring throuhgout gestation creating a dilution effect

and an increase in placental-fetal zinc uptake.

Zinc Biochemistry

Nonpregnant State--The biochemical roles of zinc have also been defined from observations during the development of zinc deficiency states. The major function of zinc is in the synthesis of nucleic acids and, therefore, protein metabolism. Zinc has also been found to be necessary for the activation of various other enzymes unassociated with nucleic acid synthesis.

Zinc deficiency in Euglena gracilis produced an initial increase in protein synthesis and, thereafter, a decrease in the net rate of RNA synthesis. Zinc deficiency causes altered protein synthesis resulting in growth failure of the organism. Schneider and Price (46) stated, in 1962, that the actual mechanism affected during zinc deficiency could cause a reduction of RNA in the ribosome-microsome cellular fraction which causes a reduction in protein synthesis. Various studies have long since extended and strengthened the knowledge pertaining to zinc's role in protein synthesis.

Ribonuclease activity is believed to be controlled by zinc; Somers and Underwood (47) found significant reductions of RNA, DNA and protein nitrogen and significant increases in non-protein-nitrogen concentrations and ribonuclease activity in zinc deficient rats. These concentrations and the ribonuclease activity are indicative of protein catabolism. Overall growth and testicular development in the zinc deficient rats was also found to be significantly immature when compared to control rats.

Zinc was concluded to be a metalloenzyme integral in Escherichia coli DNA-dependent RNA polymerase (48); zinc is important as a catalyst

for this enzyme or in the maintenance of the polymerase's structural integrity. Sandstead and Rinaldi (49) showed that zinc deficient rats develop an impairment of *in vivo* DNA synthesis in liver parenchymal cells.

Human lymphocytes are known to possess an optimal zinc concentration in order to stimulate lymphocyte DNA synthesis--in fact the addition of zinc beyond 0.1mM reduces DNA synthesis (50). Auld et al. (51) believe zinc and the leukemic process to be linked. The difference between normal and leukemic leukocytes lies in the faulty activity of reverse transcriptase (specifically avian myeloblastosis virus RNA-dependent DNA polymerase); zinc is necessary for the formation of DNA from RNA templates.

Zinc's role in nucleic acid and protein metabolism has been further supported by studies utilizing radioactive thymidine. Thymidine's incorporation into protein tissue is dependent upon thymidine kinase activity--an enzyme necessary for DNA synthesis. Rat liver cells (52), rat connective tissue (specifically polyribosomes integral in collagen synthesis) (53), and other various rat organs form zinc deficient rats (54) have been found to significantly reduce thymidine incorporation into DNA.

A variety of other enzymes are known to be affected by zinc deficiency. Isolated soybean protein with an added chelating agent, when fed to rats, resulted in zinc deficiency. The deficiency is characterized by a reduction in lactic dehydrogenase activity indicative of energy metabolism alteration (55).

Zinc with ATP can either activate or inhibit phosphofructokinase activity, depending on the mineral's concentration (56). This enzyme is necessary for glycolysis, specifically in the metabolic step involving lactate

production from glucose-6-phosphate.

A deficiency in zinc is often correlated with mood and visual disturbances (57); this is probably due to the role of zinc in retinol alcohol dehydrogenase activity. Morrison and colleagues (58) administered zinc and vitamin A to cirrhotic patients resulting in a reversal of abnormal dark adaptation previously diagnosed in these patients. All of the patients possessed below normal serum zinc concentrations previous to zinc administration. Zinc enhances the activity of the dehydrogenase enzyme, thereby allowing retinaldehyde (essential in rhodopsin formation) to form from retinol.

Huber and Gershoff (59) concluded, after measuring the activity of several enzymes in zinc deficient rats, that the effect of zinc deficiency may be tissue-site specific. In zinc deficient rats, carbonic anhydrase activity was significantly reduced in the stomach and pituitary; lactic dehydrogenase was significantly reduced in the heart, kidneys and gastrocnemius muscle; and, alkaline phosphatase activity was significantly reduced in the duodenum, kidneys, stomach and serum of the deficient rats.

In all of the above mentioned studies, when feeding rats zinc deficient diets, the investigators did control for an inanition effect--meaning that the enzymatic alterations are due to zinc deficiency.

Pregnant State--Growth retardation during gestation resulted from zinc deficiency in pregnant rats. The livers from the dams displayed a reduction of the incorporation of radioisotopically labeled thymidine into liver DNA and significant decreases in liver DNA, RNA, protein and lipid concentrations (60).

Specific organs were affected, as measured by lactic dehydrogenase and succinic dehydrogenase, in zinc deficient dams. Lactic dehydrogenase (an enzyme involved in glycolysis--the reduction of pyruvate to lactate) activity was not altered in maternal liver, heart, and brain and fetal liver and heart, but the enzyme's activity increased in maternal serum. Succinate dehydrogenase (a flavoprotein integral in the citric acid cycle) activity increased in maternal and fetal heart tissue and was not affected in maternal and fetal liver tissue (61, 62). Both enzymes are indicative of carbohydrate and energy metabolism.

Zinc is not only an integral component of various enzymes involved in protein, carbohydrate and energy metabolism but also provides a necessary component of amniotic fluid. Zinc is a naturally occurring inhibitory component associated with a low molecular weight peptide in amniotic fluid. When Escherichia coli was added in vitro to amniotic fluid, the addition of zinc was found to inhibit the bacteria's growth (63). Scane and Hawkins (64) believe bound zinc to be a necessary element present in the antibacterial components of amniotic fluid. The antibacterial activity of a high molecular weight β-lysin and a low molecular weight antibacterial peptide (<1000 Daltons), when exposed in vitro to Bacillus subtilis, is related to the concentration of bound zinc available. Further research is needed to delineate the specific antibacterial role of zinc in amniotic fluid.

Zinc Deficiency

Nonpregnant State--Zinc is recognized as an essential trace element for

man and various animals (3), with the magnitude of its involvement in various biochemical pathways escalating as further research in conducted.

Bacteria and animal models revealed the specific role of zinc in biochemistry and metabolism via the development of a deficiency state. In vitro studies involving the development of zinc deficiency in the bacteria, Euglena gracilis (46) and Escherichia coli (48) have shown zinc deficiency to alter protein synthesis and metabolism. Cultures of zinc deficient avian myeloblastosis virus (51) reveal abnormal DNA formation from RNA templates.

Protein catabolism and defective protein synthesis (specifically the nucleic acids--RNA and DNA) have been produced in rats fed diets deficient in zinc. Protein catabolism, detected by an abnormally high urinary excretion of total nitrogen, urea and uric acid, has been proven to result from zinc deficiency in the rat (36). Defective protein synthesis is also evident from research detecting an excessive oxidation of specific amino acids (34), a reduction in ^{14}C-labeled methionine incorporation into kidney and liver tissue (35), and a reduction of cystine-^{35}S incorporation into skin protein (65) in zinc deficient rats. Zinc deficiency in rats is also associated with a significant reduction in RNA, DNA and protein nitrogen concentrations and an abnormal elevation of non-protein-nitrogen concentrations and ribonuclease activity indicative of protein catabolism and defective protein synthesis (47). Rat liver parenchymal cells display a reduction of DNA synthesis traced via radioactive thymidine tissue incorporation (49, 52). Somers and Underwood (47) found that rat testicular tissue not only becomes immature in weight and development but also dis-

plays protein catabolism and abnormal synthesis when the rats were fed zinc deficient diets.

Fernandez-Madrid et al. (53) and Thompson et al. (37) have correlated zinc deficiency with protein synthesis and connective tissue development in studies involving rats and piglets. Alterations in skin acid mucopolysaccharides in zinc deficient piglets were observed (37); while a significant reduction of total collagen, noncollagenous protein and a reduction in the number of large collagen synthesis polyribosomes were found in zinc deficient rats (53). Histological findings from zinc deficient rats reveal abnormally situated epithelial cells lining the papilla of the tongue, a loss of basal cell polarity, smaller than normal taste buds, and parakeratosis of overlying epithelial cells of the tongue (66). Such cellular alterations of the tongue tissue may be the cause of hypogeusia frequently associated with zinc deficiency (67, 68).

Fraker and associates (69) reported a decrease in host resistance to infection, i.e. an altered immune system, in zinc deficient mice. Zinc deficient rats have also revealed abnormally low amounts of several gastrointestinal prostaglandins (38).

Prasad and colleagues (70) initially described human case studies involving zinc deficiency in young Iranian adult males. The clinical signs of zinc deficiency were dwarfism, testicular atrophy, skeletal retardation, alterations in serum alkaline phosphatase and, in two case studies, night blindness. Populations living in the geographical areas of Iran, Egypt, Turkey, Morocco, Tunisia, Portugal and Panama have been found to have a greater incidence of zinc deficiency features than other areas. Halsted

and associates (71) attributed this to: 1) the decreased bioavailability of dietary zinc, due to low animal protein intake and great quantities of cereals included in the diets; 2) an increase in zinc loss via sweat and/or due to intestinal parasitic infections; and, 3) zinc loss due to various infections and disease states. All of the clinical features associated with zinc deficiency in man can be accounted for when remembering the essential role of zinc in nucleic acid synthesis and various essential enzymatic activities involved in energy, carbohydrate and protein metabolism.

Sensory alterations involving taste acuity and night blindness have also been correlated with zinc deficiency in man. Abnormal dark adaptation in cirrhotic patients was reversed with zinc administration (58). Zinc sulfate administration also corrected abnormalities associated with taste acuity or hypogeusia in a single blind study involving 103 subjects (68).

The ravaging effects of both Acrodermatitis Enteropathica and Crohn's disease are also attributed to a zinc deficiency state (40, 42). Zinc administration has alleviated many, if not all, of the symptoms associated with both disease conditions.

Pregnant State--Apgar (72) concluded, from an extensive study utilizing pregnant rats fed diets supplying 0 to 0.9ppm zinc, that 0.6 to 0.9ppm of zinc is required during the last four days of gestation for normal parturition. The last days of gestation were found to be the most critical period for zinc administration in the prevention of stressful parturition in the rat (73).

Swenerton and Hurley, in two studies (55, 74), correlated reproductive failure, manifested as continual anestrus in female weanling rats and

cessation of menstrual cycle in female rhesus and bonnet monkeys, with zinc deficiency. Apgar (75) also related zinc deficiency to a significant reduction in mating activity in female rats, with a significant number of zinc deficient dams unable to maintain pregnancy to term. Zinc deficiency in dams has also resulted in an abnormal loss of weight, lower food intake and, therefore, lower protein intake throughout gestation (76, 77) and below normal plasma zinc at parturition (76) when compared to control values. Golub and associates (78) found zinc deficient dams (4ppm zinc) to display classic zinc deficiency signs by the third trimester, including dermatitis, anorexia, abnormally low plasma zinc concentrations and below normal serum alkaline phosphatase activity; the associated decrease in food intake was believed to exacerbate the clinical signs of zinc deficiency.

A high incidence of congenital malformations has also been associated with maternal zinc deficiency in dams receiving approximately 0.3 to 0.5ppm zinc (79). Moderate zinc deficiency (0.8 to 2.0ppm zinc) during days 3 to 15 of gestation resulted in severe fetal anomalities (80). Cox et al. (61), though, found no fetal malformations in 16 and 22 day old fetuses from zinc deficient dams (0.75ppm zinc). Fetal malformations resulted from transitory--days 6 to 14 of gestation--maternal zinc deficiency (0.4±0.1ppm zinc) with a high incidence of stillbirths resulting and a poor pup survival rate (81). McKenzie et al. (60) produced congenital malformations in fetuses, manifested by retarded intrauterine growth, specifically concerning brain size and liver development, from dam zinc deficiency ($<$1mg zinc) in the third trimester of gestation. Zinc deficiency throughout the total 21 days (0.3 to 0.5ppm zinc) of gestation in rats produced

a 41% rate of fetal resorption, a 90% rate of fetal gross malformations and fullterm pups weighing half the weight of normal pups (82). Hurley and colleagues (82) related fetal malformations according to the gestational period of zinc deficiency and the growth stage of the fetus.

Pregnant primates (Macaca mulatta), fed 4ppm zinc beginning at conception, had a greater incidence of stillbirths, abortions and delivery complications, although no fetal malformations were found. Male neonates from the zinc deficient dams weighed significantly less than female neonates from the same colony (83).

Apgar (84) explained the complications observed during gestation in zinc deficient dams and fetuses as a response to maternal zinc stores being inadequate to meet gestational needs. Hurley and Swenerton (85) and Hurley and associates (82) believe the complications are due to the inability of the maternal compartment to mobilize sufficient zinc stores to meet normal fetal growth demands. Bone and liver tissue zinc concentrations from zinc deficient dams were found to be very similar to values retrieved from nonpregnant rats fed the same zinc deficient diet (82).

Zinc Toxicity

Pregnant State--Excessive dietary zinc during gestation is associated with various mineral tissue alterations in pregnant rats and fetuses (86). Maternal liver, kidneys, brain and thymus increased in zinc quantity; liver copper levels decreased; calcium levels increased in heart and brain tissues, while it decreased in kidney tissues; and, magnesium was reduced in spleen and kidney tissues compared to control values when the dams were fed a diet

with excessive amounts of zinc (0.4%). The fetal tissue when compared to controls from these dams contained: an elevation of total body and liver zinc; a fall in total body and liver copper; a decrease in iron levels in total body tissue; an increase in both calcium and magnesium in the fetal liver tissue; and an increase in magnesium in total body tissue. It was hypothesized that zinc and the other minerals measured share an antagonistic relationship. Excessive zinc in the body may shorten the lifespan of red blood cells, thereby increasing iron's turnover rate and/or alter the storage of iron in transferrin. Copper absorption is impaired on the intestinal level by excessive dietary zinc. Zinc may alter calcium's normal incorporation into bone and depress the absorption and utilization of magnesium (86).

Ahokas and colleagues (87) found, when feeding dams diets containing various levels of cadmium and zinc (5.0ppm/ml water), that maternal food intake, fetal weight, fetal DNA, and protein/DNA ratio were elevated to control values. Cadmium and zinc are known to share a competitive relationship at metalloenzyme binding sites. Excessive zinc is, therefore, thought to protect against cadmium toxicity.

Pregnant Human Zinc Status

The recommended dietary allowance (RDA) for zinc intake by pregnant women is 20mg/day. This is based on the reasoning described earlier (see Zinc Requirement section) with the additional 5mg/day required for normal growth and development of placental and fetal tissue (0.75mg zinc/day) and an additional liberal safety factor since the absorption

and utilization of zinc is known to be affected by many components (3). Intakes less than the RDA for zinc were found in pregnant subjects by various researchers (30, 88-93). A steady decline of plasma and/or serum zinc concentration [Breskin et al., (94) indicated that serum zinc concentrations are normally approximately 16% greater than plasma zinc concentrations] has been correlated with the developing stages of pregnancy (93-97). No change in plasma zinc concentrations in pregnant women has been found by Bogden et al. (98). Mukherjee and associates (97) detected a pattern in maternal plasma zinc levels--maternal plasma zinc steadily falls until the 7th month of pregnancy and then stabilizes or plateaus by the 8th and 9th months; the concentrations sharply increase after delivery. A study of zinc-70 utilization revealed that pregnant women possess 19 to 27% less serum zinc than do nonpregnant women (28).

Many researchers have not correlated dietary zinc with the changes in serum or plasma zinc throughout pregnancy (30, 88, 90, 93, 94), believing the RDA for zinc to be too high and that hypozincemia observed in pregnancy could possibly be a normal physiological phenomenon. Yet, dietary zinc levels have been shown by some researchers, to affect serum or plasma zinc levels throughout gestation (99, 100).

Hambidge and Droegemueller (95) associated the fall in zinc concentrations during pregnancy with the elevation in estrogen naturally occurring as pregnancy progresses. Low serum zinc values have been found in oral contraceptive users (95). Tuttle and associates (93) correlated the hypozincemia with elevations in total plasma volume, which is a normal physiological response during gestation. The greater plasma volume could be

creating a dilution effect, thereby giving an illusion that the pregnant woman possesses below normal zinc concentrations. But Bogden et al. (98), since they found no change in zinc concentrations during pregnancy, believe the hypozincemia seen in some pregnancies is due to the activity of antagonistic metals. Swanson and King (30) recommend a reevaluation of current zinc standards in judging zinc values of pregnant women. Breskin and associate (94) point to the fact that an excessive intake and/or absorption of zinc can create an antagonistic effect upon other important vitamins and minerals also necessary for proper maternal-fetal growth and development. No significant difference has been found in infant birth weights and Apgar scores when compared to zinc concentration (88, 89, 93, 94). Although, in those women who spontaneously aborted their fetuses, serum zinc concentrations were significantly lower than women with normal gestations (94). Mukherjee and colleagues (97) associated fetal-maternal complications and fetal distress with low plasma zinc and albumin levels; they concluded that maternal plasma zinc concentrations during the 2nd trimester of pregnancy are inversely correlated with fetal weight.

Low concentrations of zinc have been associated with various adverse effects, such as, decreased bactericidal properties of amniotic fluid (101). Laitinen et al. (102), when analyzing the amniotic fluid (AF) of 129 women, found a slight decrease in AF zinc from 1.2mcM/L at 15 to 19 gestational weeks to 1.0mcM/L at 26 to 40 gestational weeks. A fetal malformation was correlated with an excessive AF zinc concentration of 9.0mcM/L, and one spontaneous abortion with no malformations was correlated with an AF zinc concentration of 3.6mcM/L. The authors believe AF zinc con-

centrations to be a good indicator of pregnancy outcome.

Parallel to the Laitinen et al. conclusions, Zimmerman and associates (33) detected abnormally high fetal zinc concentrations--77% greater when compared to control values--in 11 out of 12 fetuses born with neural tube defects. Two anencephalic fetuses possessed amniotic fluid zinc concentrations two times the normal amount which reflects a possible increase in urinary zinc from these fetuses. Zimmerman et al. (33) concluded such anomalities to be due to an endogenous metabolic defect rather than dietary zinc intake.

Zinc-Protein Interrelationship

Nonpregnant State--The amount and type of protein has been known to affect zinc nutriture (103). VanCampen and House (104) concluded that a low protein diet consumed by male rats resulted in a significant decrease in oral zinc-65 (9 or 38ppm) absorption and an increase in endogenous excretion of zinc. The rats consuming the low protein diets (5% casein) had significantly lower plasma, liver and small intestine zinc concentrations.

Evans and Johnson (105) also found total daily zinc absorption to significantly decrease when male rats were fed a low protein diet (5% casein). Hardie-Muncy and Rasmussen (106), though, did not find a low protein/zinc correlation. They concluded that the low protein diet had no effect on zinc concentrations at the protein percents tested--either 10% casein or 10% soybean.

An excess of protein is another factor to consider when investigating mineral metabolism. Colin et al. (107) found urinary zinc concentrations

to be significantly greater in women fed a high protein diet versus a moderate protein diet (15.2g N/day vs 7.9g N/day, respectively), while zinc retention did not differ according to protein level. The authors concluded that the amount of protein ingested may not have been high enough to affect zinc retention. Greger and Snedeker (108) also correlated urinary zinc excretion and protein intake. Urinary zinc excretion was significantly greater in males fed high protein diets (24.1g/day).

In two experiments, Sandstead and colleagues (109, 110) calculated zinc requirements via balance determination from excreta; the requirement for zinc increased as protein intake increased. A disproportionate intake of protein adversely affects zinc nutriture.

Pregnant State--When considering zinc status, protein intake must be taken into account; this is especially important when dealing with the dietary intake of low-income pregnant women (99). Mittal et al. (90) found a significant correlation between protein/energy intake and zinc status. Hunt et al. (88) also found a significant correlation between protein and zinc intake. Zinc is abundant and most bioavailable in foods of animal origin (88). The consumption of animal proteins must increase or zinc supplementation may be required in order for pregnant women to receive adequate zinc intakes according to the RDA recommendations--this may be unrealistic, especially concerning the low-income population.

Focusing on protein type, animal or vegetable, King et al. (111) concluded that plasma zinc concentrations are affected to a greater extent by pregnancy than by protein source. Pregnant women, when compared to nonpregnant women, possessed lower plasma zinc concentrations (approx-

imately 20% lower), no matter the protein source, even though the pregnant women consumed approximately two times the amount of zinc than the nonpregnant women (111).

Apgar (112) found a significantly higher incidence of death by the end of gestation (23rd day) in protein and zinc deficient dams (5% egg white with <1mcg zinc/g). Those dams who were protein deficient but supplemented with zinc (100ppm zinc) delivered a normal size litter with no apparent difficulties.

Protein

Protein Requirement

Man--There is a continual need for protein by the human body since protein is continually being lost via urine, feces, sweat, other secretions and excretions, sloughed skin, hair, and nails in the form of urea, creatinine, uric acid and nitrogen. The recommended dietary allowance for protein is 0.8g/day/kg body weight. This requirement is based on: 1) the minimum requirement for the maintenance of a nitrogen equilibrium; 2) adjustment for poor utilization and quality of protein; and, 3) meeting specific metabolic needs--growth, pregnancy, etc.--which would increase the grams of protein required per day per kg body weight. All of this allows for individual variation including stage of growth and development.

Rats--In rats, Nelson and Evans (115) concluded that a 5% protein diet (as casein) is minimal to allow for reproduction; for optimal performance, though, 15 to 20% protein is required. Ideally, a supply of 12% net protein

received during gestation will result in normal rat parturition.

Excessive Protein

Both Anderson and associates (116) and Musten et al. (117) concluded that rats possess the ability to adapt to differing levels of protein intake. The homeostatic mechanism involves the maintenance of amino acid metabolism; serine-threonine dehydrogenase activity was found to rise with increasing protein intake. As dietary protein is elevated, amino acid absorption increases and so plasma amino acid concentrations rise; this results in a short term reduction of food intake, and amino acid catabolism occurs, detected by measuring serine-threonine dehydrogenase activity. The enzyme's activity is proportional to protein intake, until it reaches its maximum threshold (116).

Peng and colleagues (118) measured L-methionine-U-^{14}C equilibrium and food intake in male rats. Rats consuming only 2% protein (casein) significantly lowered their food intake, while rats fed 20, 40, or 60% protein consumed more food than control rats. Food intake and weight gain was significantly greater in rats supplied a 10% casein diet. The same rats (10% casein group) were found to achieve an equilibrium between catabolism and anabolism of L-methionine-U-^{14}C; they also expired more CO_2 than the rats consuming the 2% protein diet. This discrepancy may be due to a conservation of amino acids by the rats consuming the 2% protein diet.

Diets providing an excessive amount of protein have been found to have adverse effects on both calcium and phosphorus retention in both male and female subjects (119, 120). Li and Anderson (121) found male rats

to significantly decrease their food intake when consuming a high protein diet (70% casein) deficient in the electrolytes potassium, sodium and chloride. This effect was reversed with the supplementation of potassium and sodium. The authors concluded that both electrolytes alter renal capacity resulting in a greater ability to handle ammonia. These experiments illustrate the symbiotic relationship between protein and minerals.

Protein and Pregnancy

During pregnancy there is a greater requirement for protein because of the vast metabolic changes occurring, such as the expansion of blood volume, uterus, and breasts, and the increased use of amino acids for fetal and placental protein synthesis (3). The Food and Nutrition Board (3) cites that, initially, the added protein requirement during pregnancy is +10g protein/day, derived from various balance studies. But, because of the uncertainty existing as to individual variation, +30g protein/day is recommended. Naismith (122), though, concluded that there is no physiological basis for extra protein during pregnancy; the maternal system possesses the ability to adapt to differences in protein intake. This adaptation mechanism is referred to as the biphasic nature of protein metabolism during pregnancy. Protein catabolizing enzymes within the liver were found to reduce activity in early pregnancy; Naismith explains that this allows for protein storage in the maternal system for later fetal anabolism to meet protein requirements. Progesterone, excreted in greatest amounts during the first half of pregnancy, may suppress the secretion of protein catabolizing enzymes from the adrenal cortex (122).

Dams were supplemented with adequate protein in early gestation and then given protein deficient diets, during days 6 through 10 of gestation; fetal development was then compared to fetuses born from zinc deficient dams (receiving a zinc deficient diet throughout gestation). No difference was found in litter size, but fetal birth weight, total body protein and DNA, and brain weight, protein and DNA content were significantly greater in the protein supplemented group. Naismith and Morgan pointed to the fact that the protein deficiency in the earlier protein supplemented group occurred during days 6 through 10 when fetal growth is less rapid. The authors explained that the last week of gestation is when the greatest neuronal growth occurrs, yet brain weight, protein and DNA content were higher than controls--concluding that protein metabolism must be biphasic in nature during pregnancy (123).

Marino and colleagues, in a more recent investigation (124), found nitrogen retention in pregnant women to be greatest during the last quarter of gestation. The subjects were supplied the RDA for protein and other nutrients while participating in the metabolic balance study. No significant differences were detected in fecal or integumental nitrogen losses between women in their fourth quarter of pregnancy, second quarter of pregnancy and/or nonpregnant controls. Women in their fourth quarter of pregnancy excreted significantly less total urinary nitrogen and showed an average nitrogen retention of 2.2g N/day. The nitrogen retention of women in their second quarter of pregnancy resembled that of the controls--0.7 and 0.6g N retention, respectively. Marino and associates do not agree with Naismith; they did not find that protein was reserved in the early part of pregnancy for later gestational needs (124). Rats and humans,

though, could possess protein responses during gestation unique to their own specie.

King et al. (125), testing the nitrogen retention of pregnant adolescents, illustrated the uncertainty existing when referring to nutrient, specifically protein, absorption. With a nitrogen intake of 9.3 to 20.0g/day (90% animal origin), there was only 30% retention of nitrogen, indicating poor nitrogen utilization by these pregnant adolescents. Ashe et al. (126) found pregnant women, receiving 58.3 to 62.8g protein/day (9.2g to 10.1g N/day, respectively), to be consuming approximately 75% of their protein requirement. Both factors, percentage of protein intake and protein utilization, must be considered if proper growth and development is to occur during pregnancy.

Exercise

Exercise and Zinc

Serum zinc concentrations were found to be significantly greater in nonrunners versus trained runners (94±12 vs 76±13mcg/dl, respectively), (127). Dressendorfer and Sockolov (127) attribute the difference in zinc concentrations to either sweat loss or a redistribution of zinc stores in the athlete. The conclusions of Oberleas et al. (128) agree with the previous results--preseason male wrestlers had below normal erythrocyte zinc values (data not given) with variable plasma and urin zinc values. In the late and after season periods zinc concentrations were below normal in the erythrocyte and plasma parameters (128).

Krotkiewski et al. (129), in light of below normal zinc concentrations in trained athletes, concluded that zinc is probably more involved in anaerobic exercise where there is an increase in lactate production (see Zinc Biochemistry section). Isometric endurance in women significantly increased after 14 days of exercise and a 135mg zinc/day supplement. This may be attributed to zinc's role in lactate dehydrogenase activity or glucose metabolism (129), while Lukaski et al. (130) found maximal exercise performed by trained and untrained males to have no adverse effect on mineral concentrations.

Zinc mobilization during exercise, though, was concluded to be impaired in zinc deficient males. Male subjects, when receiving 3.6mg of zinc/day for 120 days, displayed a fall in blood zinc levels when comparing pre- and post-exercise concentrations. Plasma zinc concentrations rose after maximal exercise, when the same men were administered 33.6mg of zinc/day during a 30 day supplement period. Lukaski and colleagues (131) theorized that the stress of exercise causes mobilization of stored zinc to the liver for utilization. The fasting plasma zinc reflects the amount of circulating zinc available for metabolically active tissues; this was found to drop after maximal exercise in zinc deficient male subjects.

Exercise and Protein

Acute exercise, performed by sedentary women ingesting approximately 1.1g protein/day/kg body weight (RDA, 0.8g protein/day/kg body weight), was found to have no effect on myofibrillar protein catabolism (132). Rennie and associates (133) concluded the exercise period to be a total

body protein catabolic phase followed by an anabolic period after exertion. Labeled carbon with leucine (approximately 40g protein/m^2) was ingested by four male subjects, and nitrogen and CO_2 production was measured--exercise was associated with an increase in amino acid catabolism.

Exercise and Pregnancy

The Food and Nutrition Board (3) cites heavy losses of nitrogen from the skin, via sweat, during vigorous activity. This loss of nitrogen may in turn be affecting the metabolism of other nutrients, i.e. minerals with sweat as one excretory pathway, specifically zinc. Conclusive data is lacking as to the effect of exercise or stress during pregnancy. Information concerning both maternal and fetal responses, physically and biochemically, is needed, especially since physical activity and employment during pregnancy are gaining attention and acceptance.

It is currently known from human studies that the maternal response to exercise is manifested by an increase in the ventilation rate (134, 136), greater cardiac output (136-139), a decrease in plasma glucose concentration [although no change has also been found (137, 138)], and an increase in plasma norepinephrine, epinephrine and glucagon concentrations (137, 138). Fetal responses to exercise are contradictory including, an increase in fetal catecholamine concentrations resulting in fetal activity (137), an increase in fetal breathing movements (140), and an increase in fetal heart rate (141); all of these effects are transitory and return to baseline values within approximately thirty minutes following maternal exercise. The implications of such effects are not clearly understood, but it is postulated

that uteroplacental insufficiency may be the result of the above mentioned maternal exercise effects (142).

Nagy and King (143) found that pregnant women during their third trimester voluntarily lowered their daily activity rate, but that their total energy expenditure was significantly higher than in nonpregnant controls. It is not known as to what extent the alteration in basal energy expenditure has on the above mentioned effects of exercise.

Animal studies have furthered the confusion of the maternal-fetal responses to maternal exercise. Overall fetal weight has been found to be slightly lower in fetuses from exercising mice and guinea pigs when compared to fetuses from non-exercising pregnant animals (143-146). Placental morphological studies have proven that there is a decrease in placental weight and, therefore, placental exchange surface area resulting in a decrease in fetal blood volume and maternal and fetal-placental blood flow (144, 146, 147); this, in turn, may have adverse effects on gaseous and nutrient exchange.

Maternal weight loss has been correlated with increasing exercising demands with a subsequent increase in food intake (144-146).

No significant changes have been related to exercise during pregnancy and fetal outcome in humans. Erdelyi (148), Collings et al. (149) and Pomerance et al. (135) found labor and delivery, infant birth weight and length, infant head circumference, and Apgar score to be similar when comparing those women who exercised and those who did not during pregnancy. Berkowitz and associates (150), though, found a trend of decreased preterm delivery risk in those pregnant women who exercised mildly during pregnancy, even though they had never exercised previously.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Female Sprague-Dawley rats (from the Charles River Breeding Laboratory, Wilmington, MA), approximately 2 months old, were randomly housed in single suspended wire-bottom, stainless steel cages and fed ad libitum a stock diet (Purina Stock Diet #5001, Ralston Purina Co., St. Louis, MO) and distilled water. The rats were individually housed in a temperature controlled room (approximately 22°C) with a 12-hour light-dark cycle. Approximately two days were given for environmental adjustment, and during this time the rats were earmarked with eartags for identification. Body weights were recorded every day between 9 and 11 a.m. All rats were given three days to aclimatize (Appendix I) to swimming in 38 to 40°C water approximately one-half foot deep (10 to 12 rats/tub) for 5 to 10 minutes. Swimming is an aerobic, non-weight-bearing activity which has been utilized with rats as an exercise mode in a variety of past investigations (151-155).

After the aclimatization period, the rats were randomly designated as either sedentary (n=88) or exercising (n=91) (Figure 3). When body weight attained approximately 180g, the female rats were individually housed in a staggered fashion with male Sprague-Dawley rats in a separate mating room. Mating was confirmed by the presence of copulation plugs, which were detected and recorded at approximately 8 a.m. every morning.

At this time, designated day 1 of gestation, the mated rat was then

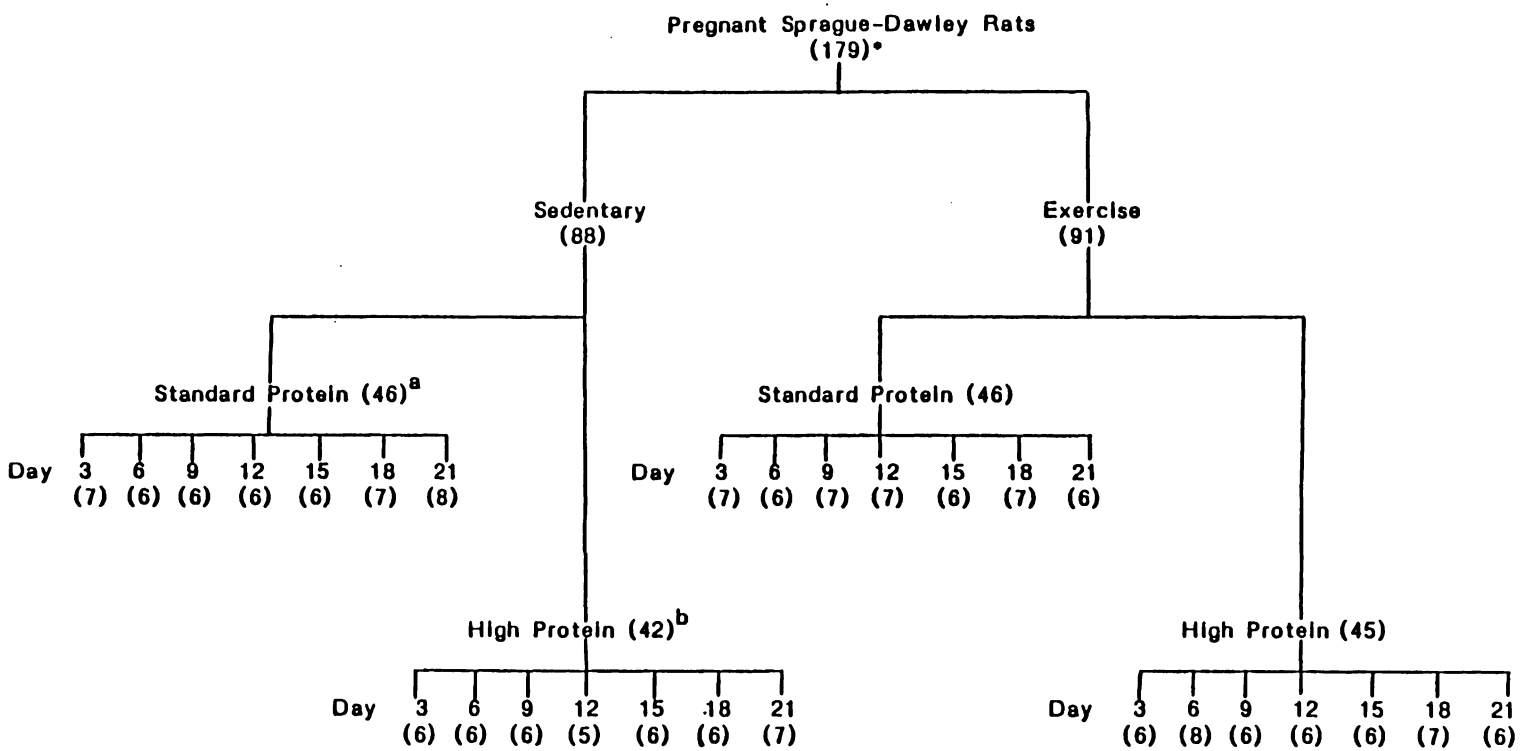

Figure 3. Experimental Design

*Values within parentheses indicate no. of animals/group

[a]7.22% Protein

[b]24.77% Protein

randomly assigned to either a standard (sedentary, n=46; exercise, n=46) or high (sedentary, n=42; exercise, n=45) protein diet and supplied deionized water in plastic bottles (Figure 3). Daily food intake was calculated by weighting each individual feedcup (with food, if present) every 3 days and subtracting this amount from the total amount of food plus feedcup given previously. The weight of the feedcup was then subtracted from the value, thus rendering the true three-day food intake. These values per rat were then added together and divided by the corresponding gestational days. Any spillage of food was weighed, recorded and then subtracted from daily intake values. The total weight gained per rat was calculated by figuring the difference in weight from the first day of gestation until the day of sacrifice. The composition of the experimental diets is shown in Table 3. The standard protein diet contained 7.22% protein, while the high protein diet contained 24.77% protein derived via the Kjeldahl method (Appendix II). The standard protein diet supplied the minimum amount of protein necessary for normal rat parturition (115); the high protein diet supplied approximately twice the protein recommended for optimal performance in the pregnant laboratory rat (115). The zinc content obtained via atomic absorption spectrophotometry (Perkin-Elmer, Model 503, Norwalk, CT) of the standard protein diet was 0.0335 mg zinc/g food and the high protein diet was 0.0372 mg zinc/g food.

The mated exercise group was then made to swim continously in 38 to 40°C water approximately 3 to 4 feet deep (Appendix I) for one and one-half hours each day until sacrifice. As the exercise group was swimming, both food and water were removed from an equal number of

Table 3. Composition of Experimental Diets

Dietary Component	Standard Protein (g/100g)	High Protein (g/100g)
Casein[1]	7.99	30.17
Cornstarch	41.47	30.38
Dextrose	41.47	30.38
Fat[2]	4.99	4.99
Methionine[3]	0.10	0.10
Vitamins[4]	0.99	0.99
Minerals[5]	2.99	2.99

[1]Casein. Vitamin Free. Nutritional Biochemicals Corp., Cleveland, OH.

[2]Crisco Vegetable Oil. Proctor and Gamble, Cincinnati, OH.

[3]dl Methionine. Nutritional Biochemicals Corp., Cleveland, OH.

[4]AIN Vitamin Mixture 76. Nutritional Biochemicals Corp., Cleveland, OH.

[5]AIN Mineral Mixture 76. Nutritional Biochemicals Corp., Cleveland, OH.

rats in the sedentary group.

Sacrifice of Animals and Tissue Analysis

Pregnant rats were sacrificed at three-day intervals (Figure 3). On each day of sacrifice, the rats were weighed and food was removed at approximately 9 a.m. every morning. The rats within the exercise group were exercised before being sacrificed.

The pregnant rats were anesthetized via carbon dioxide or chloroform, and then blood was drawn by heart puncture (Becton-Dickinson vacutainers #6527, suitable for trace element determinations). Immediately after sacrifice, the heart, liver (after perfusion with 8.7% saline solution), kidneys, right tibia, right gastrocnemius muscle, pancreas, spleen, and uterus (placenta and fetal tissue for days 15 through 21 rats only) were removed, weighed (tissue wet-weight), and frozen. For purposes of this experiment, uterine, fetal, and placental tissues were used.

Before tissue analysis, the organs were freeze-dried and weighed again (tissue dry-weight). The tissue to be analyzed was ground into an homogenous mass and approximately 50 mg weighed in duplicate for wet-ashing (Appendix III). Diluted samples were then analyzed for zinc content by atomic absorption spectrophotometry (Perkin-Elmer, Model 503, Norwalk, CT), zinc content was then calculated (Appendix IV).

Statistical Analysis

Analysis of Covariance under the General Linear Models statistical

procedure (156, 157) was used to adjust for age differences of the rats (range--39 to 77 days of age). This statistical procedure was also used to examine daily food intake and total weight gain of the pregnant rats.

A Three-Way Factorial Analysis of Variance under the General Linear Models statistical procedure (156, 157) was performed for comparison of tissue weights and zinc concentrations in uterine, placental and fetal tissues.

When a significant F value was found, Least Squares Means Multiple comparison tests were executed on all parameters measured. The Least Squares Means analysis was used rather than Duncan's Multiple Range analysis since the number of observations per cell were unequal. The least squares procedure calculates proportional averages adjusted to the number of observations per cell. Thus, missing observations are accounted for in this statistical procedure, and the observations present can be utilized with results being statistically reliable. The level of significance was set, a priori, at $p < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Age

No significant difference ($p < 0.05$) was found between the ages of the pregnant rats (mean±SD, 56±15; range--39 to 77 days of age); the rats of different ages were evenly distributed among the groups. Thus, it is assumed, in all of the following analyses of the various parameters measured, that age did not significantly affect the results.

Daily Food Intake

The average daily food intake of the animals is provided in Table 4. The Analysis of Covariance procedure revealed statistical differences in daily intake due to three factors: exercise level, diet and day of gestation.

Exercising dams consumed significantly less ($p < 0.01$) food per day than did the sedentary dams, 15.1g and 16.2g, respectively. Those dams administered the high protein diet also consumed significantly less ($p < 0.01$) food (15.2g/day) than did the dams provided the standard protein diet (16.3g/day). The pregnant rats sacrificed on gestational days 3 and 6 consumed significantly less (day3--$p < 0.01$; day 6--$p < 0.05$) food when compared to dams sacrificed on all other days.

Hill and associates (151), while forcibly swimming rats for two hours, 5 days/week, concluded that exercising rats lower their food intake when

Table 4. Average Daily Intake by Dams[1]

Treatment	Daily Intake (g)
Exercise Level[a]:	
Sedentary (88)*	16.2±0.2
Exercise (91)	15.1±0.2
Diet[b]:	
Standard (92)	16.3±0.2
High (87)	15.2±0.2
Day:	
3 (26)	9.9±0.5[c]
6 (28)	14.8±0.6[d]
9 (25)	16.5±0.6[e]
12 (24)	16.7±0.5[e]
15 (24)	16.6±0.5[e]
18 (27)	17.2±0.7[e]
21 (27)	17.8±0.7[e]

[1]Values shown are group mean±SEM

[a]Differ significantly ($p < 0.01$)

*N, average values are given for all animals within designated groups

[b]Differ significantly ($p < 0.01$)

[c-e]Different superscripts indicate values which are significantly different ($p < 0.05$)

compared to sedentary controls. This same effect on food intake was also found by LeBlanc and colleaques (153). In a different investigation, Hill et al. (155) found exercising and sedentary rats to have equivalent food intakes. Walberg and associates (152), though, found the opposite effect on food intake; exercising obese and lean Zucker rats consumed more food than controls.

Musten and colleaques (117) believe that rats possess an internal mechanism which regulates protein intake, thus caloric intake. The rat will increase or decrease food intake in proportion to the dietary protein content of a diet supplied (117). Rats, when consuming diets containing 20, 40, or 60% casein, displayed a lower food intake when compared to rats consuming diets of 2 or 10% casein. This illustrates Peng and associate's (118) conclusion that an inverse relationship exists between food intake and dietary protein percent.

The food intake by the dams that were sacrificed on days 3 and 6, in this experiment, correlated with lower growth needs until day 9 when growth requirements by the maternal-fetal compartments escalate. Rapid fetal growth occurs after approximately the tenth day of gestation in the rat (123); it was at approximately this time that the dams in this experiment began to increase their average food intake.

Total Weight Gain

The average total weight gained by the animals is shown in Table 5. Tables 6 and 7 provide an extensive statistical tabulation of the exercise level-day and diet-day interactions.

Table 5. Average Total Weight Gain by Dams[1]

Treatment	Total Gain (g)
Exercise Level [a]:	
Sedentary (88)*	64.3±1.5
Exercise (91)	46.8±1.5
Diet[b]:	
Standard (92)	50.2±1.5
High (87)	61.0±1.5
Day:	
3 (26)	0.05±3.6[c]
6 (28)	17.0±3.9[d]
9 (25)	30.1±3.6[e]
12 (24)	55.0±3.3[f]
15 (24)	62.1±3.3[f]
18 (27)	93.4±4.2[g]
21 (27)	131.4±4.4[h]
Exercise Level-Diet:	
Sedentary-Standard (46)	55.9±2.1[i]
Sedentary-High (42)	72.8±2.2[j]
Exercise-Standard (46)	44.4±2.1[k]
Exercise-High (45)	49.3±2.1[k]

[1] Values shown are group mean±SEM

[a] Differ significantly ($p < 0.01$)

* N, average values are given for all animals within designated groups

[b] Differ significantly ($p < 0.01$)

[c-h] Different superscripts indicate values which are significantly different ($p < 0.01$)

[i-k] Different superscripts indicate values which are significantly different ($p < 0.05$)

Table 6. Average Total Weight Gain, Exercise Level-Day Interaction, by Dams[1]

Treatment	Total Gain (g)
Sedentary:	
3 (13)*	1.9±4.5[a]
6 (12)	23.0±5.0[b]
9 (12)	35.6±4.7[c]
12 (11)	60.3±4.5[hi]
15 (12)	63.0±4.9[deh]
18 (13)	112.7±5.0[f]
21 (15)	154.0±5.0[g]
Exercise:	
3 (13)	-1.8±4.5[ab]
6 (14)	10.9±4.7[a]
9 (13)	24.6±4.5[bc]
12 (13)	49.7±4.3[deh]
15 (12)	61.2±4.2[deh]
18 (14)	74.2±5.1[di]
21 (12)	108.8±5.2[f]

[1]Values shown are group mean±SEM

*N, average values are given for all animals within designated groups

[a-i]Different superscripts indicate values which are significantly different ($p < 0.05$)

Table 7. Average Total Weight Gain Diet-Day Interaction, by Dams[1]

Treatment	Total Gain (g)
Standard:	
3 (14)*	-1.9±4.3[a]
6 (12)	13.2±4.8[b]
9 (13)	30.4±4.5[c]
12 (13)	56.9±4.2[d]
15 (12)	52.8±4.4[d]
18 (14)	80.8±4.9[e]
21 (14)	118.9±5.1[f]
High:	
3 (12)	2.0±4.6[a]
6 (14)	20.7±4.9[bc]
9 (12)	29.8±4.7[c]
12 (11)	53.1±4.6[d]
15 (12)	71.4±4.5[e]
18 (13)	106.1±5.2[g]
21 (13)	143.9±5.3[h]

[1]Values shown are group mean±SEM

*N, average values are given for all animals within designated groups

[a-h]Different superscripts indicate values which are significantly different ($p < 0.05$)

The Analysis of Covariance procedure indicated a significant effect ($p < 0.05$) of exercise, diet and stage of gestation on weight gain. The interaction between exercise and diet, and diet and day also significantly affected ($p < 0.05$) weight gain.

Sedentary dams gained significantly more weight (64.3g) than did the exercising dams (46.8g) during the course of the study. The total weight gained by the sedentary pregnant rats, combined over dietary treatments and gestation, was significantly greater than that of the exercising dams ($p < 0.01$).

Weight gain was found to differ significantly ($p < 0.01$) between the two dietary treatments. Those dams on the standard protein diet gained significantly less weight (50.2g) when compared to dams consuming the high protein diet (61.0g).

Total weight gain differed significantly according to gestational day in ascending order; the actual figures and levels of significance are shown in Table 5.

The sedentary pregnant rats gained significantly more weight throughout gestation when compared to exercising pregnant rats consuming either diet (Table 5). Sedentary dams consuming the high protein diet gained significantly more weight ($p < 0.01$) than the dams in the three other groups.

The interaction between gestational day and exercise or diet significantly affected the total amount of weight gained. The amount of weight gained by the dams was directly proportional to the stage of gestation--as gestation progressed, the amount of weight gained increased. Dams within the exercising or standard protein diet treatments gained less weight

than those rats within the sedentary or high protein diet treatments. The actual values, both average weight gain (mean±SEM) and least squares means comparisons, are provided in Tables 6 and 7.

Exercising rats have been found to gain less weight than sedentary controls (151-153). Nelson and associates (146), utilizing analysis of covariance to adjust for possible differences in fetal weight and maternal preconceptual weight, concluded that pregnant guinea pigs, when exercised, gain significantly less weight than their sedentary counterparts. Exercise and weight gain are inversely related. Food intake was not measured by Nelson and associates.

Rats consuming a 2, 40 or 60% casein diet gained significantly less weight than rats supplied a 10 or 20% casein diet (118). Musten et al. (117) fed weanling rats a 5, 10, 20, 40, 50, 60 or 70% casein diet and consluded that as dietary protein percent increases, total weight gain significantly increases. Weanling rats fed a 20% casein diet gained five times the weight when compared to weanling rats fed a 5% casein diet (117).

As the gestational day advances, maternal-fetal nutrient requirements escalate as growth occurs more rapidly (158); thus, it follows that this should result in a total weight increase. When referring to the interaction of gestation with exercise level or dietary treatment, exercising dams again gained significantly less weight than sedentary dams; dams consuming the standard protein diet also gained significantly less weight than those dams consuming the high protein diet. These interaction results correlate with the previously mentioned effects of exercising and dietary protein content.

Uterine Zinc Concentrations and Tissue Weights

The average uterine zinc concentrations of the dams are shown in Table 8. The concentration of zinc within the uterine tissue of the dams was found to be affected solely by gestation. The Analysis of Covariance procedure showed a significant difference ($p < 0.05$) in uterine zinc content due to gestational day.

Uterine tissue from dams sacrificed on days 3, 6 and 9 possessed significantly less ($p < 0.05$) zinc than did uterine tissue from dams sacrificed on day 18. Uterine tissue zinc content from dams sacrificed on day 3 also differed significantly from dams sacrificed on gestational day 21 ($p < 0.05$). The values of uterine zinc retrieved according to gestation falls significantly from days 3 to 9; thereafter, the zinc concentration rises and plateaus from days 12 to 15 and increases by days 18 and 21.

Average uterine tissue dry- and wet-weights are shown in Table 9. Initially, the uterine tissue, while wet, weighed significantly more ($p < 0.05$) from dams consuming the high protein diet; but when comparing wet and dry weights it can be seen that the uterine tissue from the high protein group contained significantly more moisture than did the tissue from the standard protein group.

As would be expected, the uterine tissue weights, both wet and dry, increased as gestation progressed. The uterine compartment increases in size to accommodate the developing fetal tissue during the progression of gestation.

Cox et al. (61) and Davies and Williams (26) concluded that fetal

Table 8. Average Uterine Zinc Concentrations[1]

Treatment	Uterine Zinc (mg/g wet-weight)	Absolute Zinc (mg/organ wet-weight)
Exercise Level:		
Sedentary (88)*	0.08±0.004	0.17
Exercise (91)	0.08±0.004	0.17
Diet:		
Standard (92)	0.09±0.004	0.17
High (87)	0.08±0.004	0.17
Day:		
3 (24)	0.07±0.01[a]	0.03
6 (19)	0.08±0.01[ab]	0.04
9 (14)	0.07±0.11[ab]	0.09
12 (21)	0.09±0.01[a]	0.25
15 (20)	0.09±0.01[a]	0.19
18 (26)	0.10±0.01[c]	0.35
21 (23)	0.10±0.01[b]	0.32
Exercise Level-Diet:		
Sedentary-		
Standard (46)	0.09±0.05	0.17
High (42)	0.07±0.01	0.17
Exercise-		
Standard (46)	0.09±0.01	0.17
High (45)	0.08±0.01	0.16

[1]Values shown are group mean±SEM

*N, average values are given for all animals within designated groups

[a-c]Different superscripts indicate values which are significantly different ($p < 0.05$)

Table 9. Average Uterine Tissue Weights[1]

Treatment	Wet (g)	Dry (g)
Exercise Level:		
Sedentary (88)*	2.14±0.08	0.46±0.02
Exercise (91)	2.00±0.07	0.46±0.02
Diet:		
Standard (92)	1.96±0.07[a]	0.45±0.02
High (87)	2.18±0.08[b]	0.45±0.02
Day:		
3 (26)	0.48±0.12[c]	0.19±0.03[i]
6 (28)	0.53±0.13[d]	0.13±0.04[i]
9 (25)	1.38±0.19[e]	0.26±0.05[i]
12 (24)	2.88±0.13[f]	0.42±0.04[j]
15 (24)	2.22±0.13[g]	0.51±0.04[j]
18 (27)	3.48±0.12[h]	0.81±0.03[k]
21 (27)	3.50±0.12[h]	0.89±0.03[k]
Exercise Level-Diet:		
Sedentary-		
Standard (46)	1.94±0.09	0.44±0.03
High (42)	2.34±0.13	0.47±0.03
Exercise-		
Standard (46)	1.97±0.09	0.46±0.03
High (45)	2.02±0.10	0.43±0.03

[1] Values shown are group mean±SEM

* N, average values are given for all animals within designated groups

[a-b] Different superscripts indicate values which are significantly different ($p < 0.05$)

[c-h] Different superscripts indicate values which are significantly different ($p < 0.01$)

[i-k] Different superscripts indicate values which are significantly different ($p < 0.01$)

growth of the rat occurs most rapidly beginning on gestational day 17 until birth. Maternal absorption of zinc, in the rat, has been found to double by the 21st day of gestation due to greater fetal demands (26).

In this experiment, as the uterine tissue grew to accommodate the growing fetal tissue, uterine zinc concentrations also rose. It may be possible that the dams were beginning to mobilize either their zinc stores or recently absorbed dietary zinc for placental transfer to the fetal tissue. A decline in uterine zinc, though, would be expected if normal homeostatic mechanisms associated with zinc nutriture were operating correctly. Since fetal growth is most rapid beginning on day 17 of gestation, the expected pattern of utering zinc would be a sharp decline in uterine zinc, indicating zinc mobilization and transfer from the placenta to the fetal tissue.

As can be seen from the data in Table 8, this did not occur. Both uterine mg zinc/g wet tissue weight and absolute zinc concentrations did fall, although the difference was insignificant from day 18 to 21. Such a decline could indicate the beginning of zinc mobilization to the fetal tissue.

Placental Zinc concentrations and Tissue Weights

The average placental zinc concentrations of the dams are shown in Table 10. The Analysis of Covariance procedure indicated a significant effect due to gestation and the interaction between exercise level and diet on placental zinc content.

In this experiment, placental zinc concentration steadily declined from gestational day 15 to 21 until it reached a low of 0.07mg zinc/g

Table 10. Average Placental Zinc Concentrations[1]

Treatment	Placental Zinc (mg/g wet-weight)	Absolute Zinc (mg/organ wet-weight)
Exercise Level:		
Sedentary (34)*	0.08±0.01	0.44
Exercise (35)	0.09±0.01	0.46
Diet:		
Standard (36)	0.08±0.01	0.44
High (33)	0.08±0.01	0.47
Day:		
15 (22)	0.09±0.01[a]	0.23
18 (22)	0.08±0.01[b]	0.47
21 (25)	0.07±0.01[b]	0.59
Exercise Level-Diet:		
Sedentary-Standard (20)	0.09±0.01[c]	0.45
Sedentary-High (14)	0.08±0.01[c]	0.43
Exercise-Standard (16)	0.08±0.01[d]	0.43
Exercise-High (19)	0.10±0.01[d]	0.51
Exercise Level-Day:		
Sedentary-		
15 (9)	0.08±0.01	0.18
18 (11)	0.08±0.01	0.50
21 (12)	0.07±0.01	0.63
Exercise-		
15 (10)	0.11±0.01	0.29
18 (12)	0.09±0.01	0.43
21 (11)	0.07±0.01	0.56

[1] Values shown are group mean±SEM

* N, average values are given for all animals within designated groups

[a-b] Differ significantly ($p < 0.05$)

[c-d] Differ significantly ($p < 0.01$)

wet weight. The placental zinc concentration from dams sacrificed on day 21 is similar to placental zinc concentration measured by McKenzie et al. (60). McKenzie et al. (60) found that pair-fed controls (pair-fed to dams made zinc deficient during the latter third of gestation) possessed 0.065mg zinc/g dry weight, while ad libitum-fed control dams possessed 0.064mg zinc/g dry weight.

Placental tissue from dams within the sedentary-high protein diet group contained an average of 0.07mg zinc/g wet weight; this is significantly lower ($p < 0.01$) than the average of 0.1mg zinc/g wet weight within the placental tissue of exercising-high protein diet group. This relationship still exists when absolute zinc values are compared.

The placental tissue weights , both wet and dry, are presented in Table 11. When comparing placental tissue weights between the standard protein groups and the high protein groups, the dams consuming the high protein diet were able to produce larger placentas--this difference is significant ($p < 0.05$) when referring to dry tissue weight. A high protein diet during gestation supplies the maternal compartment with substantially more amino acids, DNA and RNA available for greater placental cellular growth, therefore more efficient nutrient transfer (1, 123). Mayel-Afshar and Grimble (159) found placental tissue from protein deficient pregnant rats to weigh less and contain less protein tissue when compared to controls. Placental tissue from the protein deficient dams on gestational day 17 contained 76% protein and on day 21 only 64% protein when compared to controls. Maternal undernutrition is correlated with placental growth retardation, resulting in an overall decline in placental transport

Table 11. Average Placental Weights[1]

Treatment	Wet (g)	Dry (g)
Exercise Level:		
Sedentary (34)*	5.83±0.22	1.02±0.04
Exercise (35)	5.24±0.22	0.95±0.05
Diet:		
Standard (36)	5.30±0.22	0.92±0.04[a]
High (33)	5.77±0.22	1.05±0.05[b]
Day[c]:		
15 (22)	2.55±0.28	0.54±0.06
18 (22)	5.61±0.27	1.00±0.05
21 (25)	8.44±0.27	1.43±0.05
Exercise Level-Diet:		
Sedentary-Standard (20)	5.23±0.30[d]	0.92±0.06
Sedentary-High (14)	6.43±0.32[e]	1.12±0.06
Exercise-Standard (16)	5.37±0.32[d]	0.92±0.06
Exercise-High (19)	5.11±0.32[d]	0.97±0.06
Exercise Level-Day:		
Sedentary-		
15 (9)	2.35±0.40	0.45±0.08[f]
18 (11)	6.24±0.38	1.13±0.08[g]
21 (12)	8.90±0.36	1.49±0.07[h]
Exercise-		
15 (10)	2.75±0.40	0.63±0.08[f]
18 (12)	4.99±0.37	0.85±0.08[i]
21 (11)	7.98±0.40	1.36±0.08[i]

[1] Values shown are group mean±SEM

* N, average values are given for all animals within designated groups

[a-b] Different superscripts indicate values which are significantly different ($p < 0.05$)

[c] Differ significantly ($p < 0.01$) within both wet and dry weight categories

[d-e] Different superscripts indicate values which are significantly different ($p < 0.05$)

[f-i] Different superscripts indicate values which are significantly different ($p < 0.05$)

of nutrients to the fetal tissue (160). Protein deficiency could, therefore, adversely affect protein synthesis and cellular growth producing inferior placentas.

As would be expected, the placental tissue grew significantly more as gestation progressed. In this experiment, the decline of placental zinc concentrations from days 15 to 21 is due to the dilution of zinc during rapid placental tissue growth; this effect is evident when comparing the absolute placental zinc concentrations. The absolute zinc concentration per organ actually rose as gestation progressed. This effect is still observed when comparing dry tissue weights between the exercise level-day interaction, although the exercising dams produced significantly less placental tissue than the sedentary dams (Table 11).

As the weight of the placental tissue increases, so does the surface area and diffusing capacity. In exercising pregnant guinea pigs, Nelson et al. (146) and Smith et al. (144) identified placental weight as a function of exercise. In both studies, the average placental weight from guinea pigs forcibly exercised for one hour/day weighed significantly less than control, sedentary values. In the present study, both placental wet and dry tissue weights were greater in the sedentary group when compared to the exercising group, yet the difference was statistically insignificant. Sedentary dams consuming the high protein diet had significantly greater average placental wet weights ($p < 0.01$) when compared to the average placental wet weights from the exercising dams supplied the high protein diet. This difference became insignificant when comparing dry tissue weights.

Fetal Zinc Concentrations and Tissue Weights

The average fetal zinc concentrations from the dams are shown in Table 12. No difference in fetal zinc concentration was found within or between treatments.

The absolute fetal zinc values, according to gestational day, are similar to values measured by Hurley and Swenerton (85) in zinc-supplemented dams. Hurley and Swenerton (85) measured fetal zinc values that rose with the advancement of gestation. Feaster and associates (27) found fetal zinc concentrations (mcg/g) to decline steadily from day 15 to 21 of gestation. Fetal zinc concentrations (mcg/g) were also found to decline from day 16 to 22 in fetuses from dams fed control, basal diets by Cox and associates (61).

Hurley and Swenerton (85) stated that the greatest rate of accumulation of zinc (absolute concentrations) in the fetal tissue of the rat occurs over the last days of gestation; Feaster and associates (27) concluded that, as fetal tissue develops, a subsequent fall in fetal zinc concentration (mcg/g) occurs. Cox et al. (61) also correlated significant increases in fetal weight during gestation with a subsequent fall in fetal zinc concentrations (mcg/g). In the present experiment, absolute fetal zinc values increased as gestation progressed; this is in agreement with the conclusions of Hurley and Swenerton (85).

The average fetal tissue weights, both wet and dry, are given in Table 13. Fetal tissue from the exercising dams weighed significantly less ($p<0.05$) than fetal tissue from the sedentary dams. As gestation progressed, fetal

Table 12. Average Fetal Zinc Concentrations[1]

Treatment	Fetal Zinc (mg/g wet-weight)	Absolute Zinc (mg/organ wet-weight)
Exercise Level:*		
Sedentary (37)	0.14±0.02	3.25
Exercise (36)	0.12±0.20	2.35
Diet:		
Standard (37)	0.12±0.02	2.39
High (35)	0.15±0.02	3.20
Day:		
15 (23)	0.11±0.03	0.31
18 (26)	0.16±0.02	2.26
21 (24)	0.13±0.02	5.93
Exercise Level-Diet:		
Sedentary-		
Standard (20)	0.12±0.03	2.51
High (16)	0.16±0.03	4.09
Exercise-		
Standard (17)	0.11±0.03	2.28
High (19)	0.13±0.03	2.41

[1] Values shown are group mean±SEM

* N, average values are given for all animals within designated groups

Table 13. Average Fetal Tissue Weights[1]

Treatment	Wet (g)	Dry (g)
Exercise Level:		
Sedentary (37)*	23.07±1.20	2.82±0.15
Exercise (36)	19.13±1.23	2.23±0.16
Diet:		
Standard (37)	20.45±1.20	2.50±0.15
High (35)	21.75±1.23	2.56±0.16
Day[b]:		
15 (23)	2.91±1.54	0.42±0.20
18 (26)	14.06±1.45	1.45±0.19
21 (24)	46.33±1.46	5.71±0.19
Exercise Level-Diet:		
Sedentary-		
Standard (20)	20.89±1.65	2.59±0.21
High (16)	25.25±1.73	3.06±0.22
Exercise-		
Standard (17)	20.00±1.73	2.41±0.22
High (19)	18.25±1.73	2.05±0.22

[1]Values shown are group mean±SEM

[a]Differ significantly ($p < 0.05$) within both wet and dry weight categories

*N, average values are given for all animals within designated groups

[b]Differ significantly ($p < 0.01$) within both wet and dry weight categories

tissue weights became significantly heavier ($p < 0.01$). The association between fetal weight and gestation has been illustrated in various experiments in both control and zinc-deficient dams (61, 73, 85). Fetal tissue weight increases as the fetal tissue cells progress through the hyperplastic and hypertrophic cellular growth stages (1).

Although the differences are statistically insignificant, the fetal zinc concentrations, when observing exercise level, diet and exercise level and diet interaction, correlate with placental wet weights. Sedentary dams consuming the high protein diet possessed greater placental weights and greater fetal zinc concentrations. This is in agreement with the conclusions of Nelson et al. (146), Smith et al. (144) and Mayel-Afshar and Grimble (159); placental weight has been found to be proportional to exercise time (144, 146) and dietary protein content (159). The interaction of inactivity with a high protein diet allowed for greater placental tissue growth and vascularity, therefore greater zinc transfer to the fetal tissue.

Fetal Number

The average number of fetuses are shown in Table 14. the Analysis of Covariance procedure revealed that the number of fetuses (gestational days 15 through 21 only) was significantly affected by the interaction of exercise level and diet only ($p < 0.01$).

Dams within the sedentary-standard protein group produced significantly fewer ($p < 0.05$) fetuses than dams within the sedentary-high protein group, 12.0 fetuses and 13.6 fetuses, respectively. Dams within the exercising-high protein diet group produced significantly fewer ($p < 0.01$) fetuses

Table 14. Average Fetal Number[1]

Exercise Level-Diet	Fetal Number
Sedentary-Standard (20)*	12.0±0.5[a]
Sedentary-High (16)	13.6±0.5[b]
Exercise-Standard (17)	13.0±0.5[ab]
Exercise-High (19)	11.8±0.5[a]

[1]Values shown are group mean±SEM

*N, average values are given for all animals within designated groups

[a-b]Different superscripts indicate values which are significantly different ($p < 0.05$)

than the dams within the sedentary-high protein group, 11.8 fetuses versus 13.6 fetuses, respectively. The least number of fetuses were found within the exercising-high protein group; the greatest number of fetuses were found within the sedentary-high protein group.

Both Smith and associates (144) and Nelson and associates (146) correlated a decrease in the number of fetuses per guinea pig with an increase in exercise time; such a relationship was not measured in this experiment. The sedentary-high protein dams produced the greatest number of fetuses, while the exercising-standard protein dams produced a similar number of fetuses. When comparing fetal number between exercising and sedentary dams, dams from both treatments produced an average of approximately 12 fetuses. This same effect on fetal number was found when comparing the two dietary groups; the dams from both dietary treatments, stand or high protein, produced an average of approximately 12 fetuses. This is in agreement with the conclusions of Mayel-Afshar and Grimble (159). Mayel-Afshar and Grimble (159) found pregnant rats to produce an equivalent amount of fetuses regardless of dietary protein content. Protein deficiency has been correlated, though, with fetal tissue containing only 78% of the protein content when compared to controls (159).

The results concerning average fetal number per group, in this experiment, must be related solely to an exercise-dietary protein interaction. Further research in this area, involving pregnancy, exercise and various dietary protein levels, may delineate the discrepancy concerning fetal number found in this experiment. The interaction of the stress of

exercise plus the lower food intake observed in the dams consuming the high protein diet may have caused the exercising-high protein group to produce less fetuses.

CHAPTER V

SUMMARY AND CONCLUSION

The present experiment was conducted to measure the effect of exercise and dietary protein on maternal and fetal zinc status; daily food intake and total weight gain per dam were also measured. One hundred and seventy-nine pregnant Sprague-Dawley rats were divided into two groups: sedentary and exercise. These two groups were further subdivided into four groups: sedentary-standard protein diet, sedentary-high protein diet, exercising-standard protein diet and exercising-high protein diet. The standard protein diet contained 7.22% protein, while the high protein diet contained 24.77% protein. All other nutrients were supplied in amounts required for normal development of the pregnant laboratory rat. After aclimatization, the exercising dams, regardless of diet, were made to swim continuously for one and one-half hours each day until sacrifice. The four major groups were still further subdivided into twenty-eight groups designated by three-day intervals according to gestational day--days 3, 6, 9, 12, 15, 18 and 21 of gestation. Tissues retained for zinc content analysis included uterine, fetal and placental tissues. Fetal and placental tissues were separated from uterine tissue for days 15 through 21 only. The effects of exercise, dietary protein, gestational day and all interactions between the main effects were determined.

The average total weight gained by the dams correlated with daily intake when comparing exercise level and gestational day. The exercising dams consumed significantly less food and gained significantly less weight

than the sedentary dams. As gestation progressed, all of the dams increased their average daily food intake and gained significantly more weight when compared to the earlier time period. The dams consuming the high protein diet gained more weight but consumed less food; dams consuming the standard protein diet gained less weight but consumed more food. These findings are in agreement with other investigations (117, 118).

The concentration of uterine zinc was affected solely by gestation; as gestation progressed a greater concentration (both mg zinc/g tissue wet-weight and absolute values) of zinc was measured in the uterine tissue. Neither exercise or dietary protein significantly affected uterine zinc concentrations.

Placental zinc content was found to decrease while placental tissue weight increased with the advancement of gestation. This effect was found to be due to the dilution of zinc concentrations as the tissue grew; the absolute concentration of zinc rose as gestation progressed. The sedentary-high protein group possessed the lowest placental zinc concentrations while having the greatest placental tissue weights. The exercising-high protein group possessed the greatest placental zinc concentrations while having the lowest placental tissue weights. When comparing absolute zinc values in the placental tissue, the sedentary-high protein and exercising-standard protein groups possessed the lowest zinc concentrations; the exercising-high protein group possessed the greatest zinc concentration.

No significant difference was detected in fetal zinc concentrations, but fetal weight differences were found. As gestation progressed, fetal weight increased. the fetal tissue from exercising dams was found to

weigh significantly less than fetal tissue from the sedentary dams. The sedentary-high protein group produced the greatest number of fetuses, while the exercising-high protein group produced the least.

In summary, as gestation progressed in all of the dams, there was a significant increase in daily food intake and total weight gain. Uterine zinc concentrations rose while uterine tissue weights, both wet and dry, increased. Absolute placental and fetal zinc concentrations rose while placental and fetal tissue weights, both wet and dry, increased.

The exercising dams were found to ingest less food, gain less weight, and possess fetal tissue weights significantly below fetal tissue from sedentary dams. It appears that the exercising dams had lower absolute uterine, greater absolute placental, and lower absolute fetal zinc concentrations when compared to the sedentary dams. These effects are probably due to the exercising dams possessing lower placental tissue weights, therefore reducing uteroplacental zinc transfer to the fetal tissue.

Sedentary dams consuming the high protein diet appeared to have a lower average absolute placental zinc concentration when compared to the exercising-high protein group. The sedentary-high protein group possessed the greatest average placental wet-weight, the greatest absolute fetal zinc concentration (although statistically insignificant), and produced the greatest number of fetuses. The high protein diet allowed for optimum placental protein synthesis and cellular growth resulting in the more efficient nutrient transfer to the fetal tissue for development. The interaction of inactivity with a high protein diet allowed for greater placental tissue growth and vascularity, therefore greater zinc transfer to the fetal

tissue.

Exercising during gestation decreases placental blood flow, adversely affecting nutrient and gaseous exchange concerning both amino acids and zinc metabolism. Amino acids and zinc, along with a variety of other nutrients, are vital in protein synthesis, thus cellular and tissue development. During exercise the reduction in nutrient transfer from the placental to the fetal tissue results in inferior placental and fetal development. The stress of exercise was not imposed upon the sedentary-high protein dams, thus allowing for proper placental blood flow. These dams had ample zinc and dietary protein for placental transfer and cellular development of fetal tissue.

Zinc deficiency was not imposed on any of the dams in this experiment, therefore, enough zinc was available for protein synthesis. These dietary zinc levels were such that uterine zinc concentrations did not sharply decline as gestation progressed.

Apparent from this experiment is that both exercise and dietary protein content do affect maternal and placental-fetal development; the answer as to exactly how the development is effected needs to be further investigated. Future investigations could include a greater variety of diets containing greater protein than the high protein diet utilized in this experiment. The pregnant animals could also be subjected to various levels of exercise while on the various diets. Studies utilizing such experimental techniques as radio- or stable isotopes, and morphological and/or stereological methods of analysis could detect, more precisely, the physiological alterations due to exercise and dietary protein during gestation. After

further investigation, more precise conclusions as to dietary protein content and exercise duration, frequency and intensity can be made concerning exercise and protein intake during pregnancy.

Zinc is clearly an essential trace mineral; it is vital in the protein synthesis required for proper maternal and placental-fetal development. The role of caloric intake, vitamins and other minerals, as well as their interactions, also need to be investigated in relation to both pregnancy and exercise.

LITERATURE CITED

1. Committee on Maternal Nutrition, Food and Nutrition Board. Maternal nutrition and the course of pregnancy. Washington, D.C.: National Academy of Sciences, 1970.

2. Fox EL, Mathews DK. The physiological basis of physical education and athletics. Philadelphia, PN: Saunders College Publishing, 1981.

3. Committee on Dietary Allowances, Food and Nutrition Board. Recommended Dietary Allowances. Washington, D.C.: National Academy of Sciences, 1980.

4. Committee on Medical and Biologic Effects of Environmental Pollutants, Subcommittee on Zinc. Zinc. Baltimore, MD.: University Park Press, 1979.

5. Halsted JA, Smith JC, Irwin MI. A conspectus of research on zinc requirements of man. J Nutr 1974; 104: 345-378.

6. Davis PN, Norris LC, Kratzer FH. Interference of soybean proteins with the utilization of trace minerals. J Nutr 1962; 77: 217-223.

7. Morris ER, Ellis R. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J Nutr 1980; 110: 1037-1045.

8. Heth DA, Hoekstra WG. Zinc-65 absorption and turnover in rats. J Nutr 1965; 85: 367-374.

9. Murthy L, Klevay LM, Petering HG. Interrelationships of zinc and copper nutriture in the rat. J Nutr 1974; 104: 1458-1465.

10. Greger JL, Zaikis SC, Abernathy RP, Bennett OA, Huffman J. Zinc, nitrogen, copper, iron, and manganese balance in adolescent females fed two levels of zinc. J Nutr 1978; 108: 1449-1456.

11. Taper LJ, Hinners ML, Ritchey SJ. Effects of zinc intake on copper balance in adult females. Am J Clin Nutr 1980; 33: 1077-1082.

12. Spencer H, Osis D, Kramer L, Norris C. Intake, excretion, and retention of zinc in man. In: Trace Elements in Human Health and Disease, edited by AS Prasad. 1976, vol. 1, pp. 345-361.

13. Todd WR, Elvehjem CA, Hart EB. Zinc in the nutrition of the rat. Am J Physiol 1934; 107: 146-156.

14. Forbes RM, Yohe M. Zinc requirement and balance studies with the rat. J Nutr 1960; 70: 53-57.

15. Oberleas D, Harland BF. Nutritional agents which affect metabolic zinc status. In: Progress in Clinical and Biological Research, edited by GJ Brewer, AS Prasad. 1977, vol. 14, pp. 11-24.

16. Prasad AS. Zinc in Human Nutrition. Boca Raton, FL: CRC Press, Inc. 1979.

17. Hambidge KM, Neldner KH, Walravens PA, Weston WL, Silverman A, Sabol JL, Brown RM. Zinc and acrodermatitis enteropathica. In: Zinc and Copper in Clinical Medicine, edited by KM Hambidge, BL Nichols,Jr. 1978, vol. 2, pp. 81-98.

18. Cotzias GC, Borg DC, Selleck B. Specificity of zinc pathway through the body: turnover of Zn-65 in the mouse. Am J Physiol 1962; 202: 359-363.

19. Foster D, Aamodt R, Henkin R, Berman M. Zinc metabolism in man: a kinetic model based on in vivo studies. Clin Res 1978; 26: 497a.

20. Evans GW, Grace CI, Hahn C. Homeostatic regulation of zinc absorption in the rat. Proc Soc Exp Biol Med 1973; 143: 723-725.

21. Evans GW, Grace CJ, Votava HJ. A proposed mechanism for zinc absorption in the rat. 1975; 228: 501-505.

22. Evans GW. Transferrin function in zinc absorption and transport. 1976; 151: 775-778.

23. Cousins RJ. Smith KT, Failla ML, Markowitz LA. Origin of low molecular weight zinc-binding complexes from rat intestine. Life Sci 1978; 23: 1819-1826.

24. Evans GW, Johnson PE, Johnson EC. Purification and characterization of a zinc-binding ligand in rat intestine. J Nutr 1979; 109 (a): xxxii.

25. Richards MP, Cousins RJ. Mammalian zinc homeostasis: requirement for RNA and metallothionein synthesis. Biochem Biophys Res Commun 1975; 64: 1215-1223.

26. Davies NT, Williams RB. The effect of pregnancy and lactation on the absorption of zinc and lysine by the rat duodenum in situ. Br J Nutr 1977; 38: 417-423.

27. Feaster JP, Hansard SL, McCall JT, Davis GK. Absorption, deposition, and placental transfer of zinc-65 in the rat. Am J Physiol 1955; 181: 287-290.

28. Swanson CA, Turnlund JR, King JC. Effect of dietary zinc sources and pregnancy on zinc utilization in adult women fed controlled diets. J Nutr 1983; 113: 2557-2567.

29. Ritchey SJ, Taper LJ. Utilization of zinc by humans. In: Nutritional Bioavailability of Zinc, edited by GE Inglett. 1983, pp. 107-114.

30. Swanson CA, King JC. Zinc utilization in pregnant and nonpregnant women fed controlled diets providing the zinc RDA. J Nutr 1982; 112: 697-707.

31. Hess FM, King JC, Margen S. Zinc excretion in young women on low zinc intakes and oral contraceptive agents. J Nutr 1977; 107: 1610-1620.

32. Jameson S. Zinc and pregnancy. In: Zinc in the Environment, edited by JO Nriagu. 1980, pp. 183-197.

33. Zimmerman AW, Dunham BS, Nochimson DJ, Kaplan BM, Clive JM, Kunkel SL. Zinc transport in pregnancy. Am J Obstet Gynecol 1984; 149: 523-529.

34. Theuer RC, Hoekstra WG. Oxidation of 14-C-labeled carbohydrate, fat and amino acid substrates by zinc-deficient rats. J Nutr 1966; 89: 448-454.

35. Hsu JM, Anthony WL, Buchanan PJ. Zinc deficiency and incorporation of 14-C-labeled methionine into tissue proteins in rats. J Nutr 1967; 99: 425-432.

36. Hsu JM, Anthony WL. Effect of zinc deficiency on urinary excretion of nitrogenous compounds and liver amino acid-catabolizing enzymes in rats. J Nutr 1975; 105: 26-31.

37. Thompson RW, Gilbreath RL, Bielk F. Alterations of porcine skin acid mucopolysaccaharides in zinc deficiency. J Nutr 1975; 105: 154-160.

38. Meydani SN, Dupont J. Effect of zinc deficiency on prostaglandin synthesis in different organs of the rat. J Nutr 1982; 112: 1098-1104.

39. Verburg DJ, Burd LI, Hoxtell EO, Merrill LK. Acrodermatitis enteropathica and pregnancy. Obstet Gynecol 1974; 44: 233-237.

40. Successful outcome of two pregnancies in a patient with congenital acrodermatitis enteropathica. Nutr Rev 1982; 40: 78-80.

41. Solomons NW, Rosenberg IH, Sandstead HH, Vo-Khactu KP. Zinc deficiency in Crohn's disease. Digestion 1977; 16: 87-95.

42. Sandstead HH. Zinc nutrition in the United States. Am J Clin Nutr 1973; 26: 1251-1260.

43. Bergmann KE, Makosch G, Tews KH. Abnormalities of hair zinc concentration in mothers of newborn infants with spina bifida. Am J Clin Nutr 1980; 33: 2145-2150.

44. Henkin RI, Marshall JR, Meret S. Maternal-fetal metabolism of copper and zinc at term. Am J Obstet Gynecol 1971;110: 131-134.

45. Giroux E, Schechter PJ, Schoun J. Diminished albumin binding of zinc in serum of pregnant women. Clin Sci Molec Med 1976; 51: 545-549.

46. Schneider E, Price CA. Decreased ribonucleic acid levels: a possible cause of growth inhibition in zinc deficiency. Biochim Biophys Acta 1962; 55: 406-408.

47. Somers M, Underwood EJ. Ribonuclease activity and nucleic acid and protein metabolism in the testes of zinc-deficient rats. Aust J Biol Sci 1969; 22: 1277-1282.

48. Scrutton MC, Wu CW, Goldthwait DA. The presence and possible role of zinc in RNA polymerase obtained from Escherichia coli. Proc Nat Acad Sci 1971; 68: 2497-2501.

49. Sandstead HH, Rinaldi RA. Impairment of deoxyribonucleic acid synthesis by dietary zinc deficiency in the rat. J Cell Physiol 1969; 73: 81-84.

50. Berger NA, Skinner SAM. Characterization of lymphocyte transformation induced by zinc ions. J Cell Biol 1974; 61: 45-55.

51. Auld DS, Kawaguchi H, Livingston DM, Vallee BL. RNA-dependent DNA polymerase (reverse transcriptase) from avian myeloblastosis virus: a zinc metalloenzyme. Proc Nat Acad Sci 1974; 71: 2091-2095.

52. Fujioka M, Lieberman I. A zinc requirement for synthesis of deoxyribonucleic acid by rat liver. J Biol Chem 1964; 239: 1164-1167.

53. Fernandez-Madrid F, Prasad AS, Oberleas D. Effect of zinc deficiency on nucleic acids, collagen, and noncollagenous protein of the connective tissue. J Lab Clin Med 1973; 182: 951-961.

54. Prasad AS, Oberleas D. Thymidine kinase activity and incorporation of thymidine into DNA in zinc-deficient tissue. J Lab Clin Med 1974; 83: 634-639.

55. Swenerton H, Hurley LS. Severe zinc deficiency in male and female rats. J Nutr 1968; 95: 8-18.

56. Tamaki N, Ikeda T, Funatsuka A. Zinc as activating cation for muscle glycolysis. J Nutr Sct Vitaminol 1983; 29: 655-662.

57. Moynahan EJ. Letter. Lancet 1976; 1(7950): 91.

58. Morrison SA, Russell RM, Carney EA, Oaks EV. Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. Am J Clin Nutr 1978; 31: 276-281.

59. Huber AM, Gershoff SN. Effects of dietary zinc on zinc enzymes in the rat. J Nutr 1973; 103: 1175-1181.

60. McKenzie JM, Fosmire GJ, Sandstead HH. Zinc deficiency during the latter third of pregnancy: effects on fetal rat brain, liver, and placenta. J Nutr 1975; 105: 1466-1475.

61. Cox DH, Chu RC, Schlicker SA. Zinc deficiency in the maternal rat during gestation, and zinc, iron, copper, and calcium content and enzyme activity in maternal and fetal tissues. J Nutr 1968; 98: 449-458.

62. Lehninger AL. Principles of Biochemistry. New York, NY: Worth Publishers, Inc., 1982.

63. Schlievert P, Johnson W, Galask RP. Bacterial growth inhibition by amniotic fluid. Am J Obstet Gynecol 1976; 125: 906-910.

64. Scane TMN, Hawkins DF. Antibacterial activity in human amniotic fluid: relationship to zinc and phosphate. Br J Obstet Gynaecol 1984; 91: 342-348.

65. Hsu JM, Anthony WL. Impairment of cystine-^{35}S incorporation into skin protein by zinc-deficient rats. J Nutr 1971; 101: 445-452.

66. Catalanotto FA, Nanda R. The effects of administration of a zinc deficient diet on taste acuity and tongue epithelium. J Oral Pathol 1977; 6: 211-220.

67. Catalanotto FA, Lacy P. Effects of a zinc deficient diet upon fluid intake in the rat. J Nutr 1977; 107: 436.

68. Schechter PJ, Friedewald WT, Bronzert DA, Raff MS, Henkin. Idiopathic hypogeusia: a description of the syndrome and a single blind study with zinc sulfate. In: International Review of Neurobiology, edited by C Pfeiffer. 1972, suppl 1, pp. 125-140.

69. Fraker PJ, Caruso R, Kierszenbaum F. Alteration of the immune and nutritional status of mice by synergy between zinc deficiency and infection with Trypanosoma cruzi. J Nutr 1982; 112: 1224-1229.

70. Prasad AS, Halsted JA, Nadimi M. Syndrome of iron deficiency anemis, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med 1961; 31: 532-546.

71. Halsted JA, Ronaghy HA, Abadi M, Haghshenass M, Amirhakemi GH, Barakat RM, Reinhold JG. Zinc deficiency in man: the Shiraz experiment. Am J Med 1972; 53: 277-284.

72. Apgar J. Zinc requirement for normal parturition in rats. Nutr Repts Int 1976; 13: 281-286.

73. Apgar J. Effect of zinc repletion late in gestation on parturition in the zinc-deficient rat. J Nutr 1973; 103: 973-981.

74. Swenerton H, Hurley LS. Zinc deficiency in rhesus and bonnet monkeys, including effects on reproduction. J Nutr 1980; 110: 575-583.

75. Apgar J. Effect of zinc deficiency on maintenance of pregnancy in the rat. J Nutr 1970; 100: 470-476.

76. Halas ES, Wallwork JC, Sandstead HH. Mild zinc deficiency and undernutrition during the prenatal and postnatal periods in rats: effects on weight, food consumption, and brain catecholamine concentrations. J Nutr 1982; 112: 542-551.

77. Fosmire GJ, Greeley S, Sandstead HH. Maternal and fetal response to various suboptimal levels of zinc intake during gestation in the rat. J Nutr 1977; 107: 1543-1550.

78. Golub MS, Gershwin ME, Hurley LS, Baly DL, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys. Am J Clin Nutr 1984; 39: 265-280.

79. Diamond I, Hurley LS. Histopathology of zinc-deficient fetal rats. J Nutr 1970; 100: 325-329.

80. Hickory W, Nanda R, Catalanotto FA. Fetal skeletal malformations associated with moderate zinc deficiency during pregnancy. J Nutr 1979; 109: 883-891.

81. Hurley LS, Mutch PB. Prenatal and postnatal development after transitory gestational zinc deficiency in rats. J Nutr 1973; 103: 649-656.

82. Hurley LS, Gowan J, Swenerton H. Teratogenic effects of short-term and transitory zinc deficiency in rats. Teratol 1971; 4: 199-204.

83. Golub MS, Gershwin ME, Hurley LS, Baly DL, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys. Am J Clin Nutr 1984; 39: 879-887.

84. Apgar J. Effect of zinc deficiency on parturition in the rat. Am J Physiol 1968; 215: 160-163.

85. Hurley LS, Swenerton H. Lack of mobilization of bone and liver zinc under teratogenic conditions of zinc deficiency in rats. J Nutr 1971; 101: 597- 604.

86. Cox DH, Schlicker SA, Chu RC. Excess dietary zinc for the maternal rat, and zinc, iron, copper, calcium, and magnesium content and enzyme activity in maternal and fetal tissues. J Nutr 1969; 98: 459-466.

87. Ahokas RA, Dilts PV, LaHaye EB. Cadmium-induced fetal growth retardation: Protective effect of excess dietary zinc. Am J Obstet Gynecol 1980; 136: 216-221.

88. Hunt IF, Murphy NJ, Gomez J, Smith JC. Dietary zinc intake of low-income pregnant women of Mexican descent. Am J Clin Nutr 1979; 32: 1511-1518.

89. Hunt IF, Murphy NJ, Cleaver AE, Faraji B, Swendseid ME, Coulson AH, Clark VA, Browdy BL, Cabalum MT, Smith JC. Zinc supplementation during pregnancy: effects on selected blood constituents and on progress and outcome of pregnancy in low-income women of Mexican descent. Am J Clin Nutr 1984; 40: 508-521.

90. Mittal R, Bhargawa U, Anand S, Vir SC. Zinc status of Indian pregnant women of low-income group and its effect on the outcome of pregnancy. Ind J Nutr Dietet 1982; 19: 117-124.

91. Oliva J. Copper and zinc retention in pregnant women. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA, 1981.

92. Moser PB, Reynolds RD. Dietary zinc intake and zinc concentrations of plasma, erythrocytes, and breast milk in antepartum and postpartum lactating and nonlactating women: a longitudinal study. Am J Clin Nutr 1983; 38: 101-108.

93. Tuttle S, Aggett PJ, Campbell D, MacGillivray I. Zinc and copper nutrition in human pregnancy: a longitudinal study in normal primigravidae and in premigravidae at risk of delivering a growth retarded baby. Am J Clin Nutr 1985; 41: 1032-1041.

94. Breskin MW, Worthington-Roberts BS, Knopp RH, Brown Z, Plovie B, Mottet NK, Mills JL. First trimester serum zinc concentrations in human pregnancy. Am J Clin Nutr 1983; 38: 943-953.

95. Hambidge KM, Droegemueller W. Changes in plasma and hair concentrations of zinc, copper, chromium, and manganese during pregnancy. Obstet Gynecol 1974; 44: 666-672.

96. Vir SC, Love AHG, Thompson W. Zinc concentration in hair and serum of pregnant women in Belfast. Am J Clin Nutr 1981; 34: 2800-2807.

97. Mukherjee MD, Sandstead HH, Ratnaparkhi MV, Johnson LK, Milne DB, Stelling HP. Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. Am J Clin Nutr 1984; 40: 496-507.

98. Bogden JD, Thind IS, Kemp FW, Caterini H. Plasma concentrations of calcium, chromium, copper, iron, magnesium, and zinc in maternal and cord blood and their relationship to birth weight. J Lab Clin Med 1978; 92: 455-462.

99. Sarram M, Younessi M, Khorvash P, Kfoury GA, Reinhold JG. Zinc nutrition in human pregnancy in Fars Province, Iran. Am J Clin Nutr 1969; 22: 726-732.

100. Cavdar AO, Babacan E, Arcasoy A, Ertem U. Effect of nutrition on serum zinc concentration during pregnancy in Turkish women. Am J Clin Nutr 1980; 33: 542-544.

101. Tafari N, Ross SM, Naeye RL, Galask RP, Zaar B. Failure of bacterial growth inhibition by amniotic fluid. Am J Obstet Gynecol 1977; 128: 187-189.

102. Laitinen R, Siimes ASI, Vuori E, Salmela SS. Amniotic fluid zinc in risk pregnancies. Biolog Trace Elem Res 1984; 6: 415-421.

103. Ritchey SJ. Interrelationships among protein, zinc, and copper in human nutrition. Cer Chem 1981; 58: 18-21.

104. VanCampen D, House WA. Effect of a low protein diet on retention of an oral dose of zinc-65 and on tissue concentrations of zinc, iron, and copper in rats. J Nutr 1974; 104: 84-90.

105. Evans GW, Johnson EC. Zinc absorption in rats fed a low-protein diet and a low-protein diet supplemented with tryptophan or picolinic acid. J Nutr 1980; 110: 1076-1080.

106. Hardie-Muncy DA, Rasmussen AI. Interrelationships between zinc and protein level and source in weanling rats. J Nutr 1979; 109: 321-329.

107. Colin MA, Taper LJ, Ritchey SJ. Effect of dietary zinc and protein levels on the utilization of zinc and copper by adult females. J Nutr 1983; 113: 1480-1488.

108. Greger JL, Snedeker SM. Effect of dietary protein and phosphorus levels on the utilization of zinc, copper and manganese by adult males. J Nutr 1980; 110: 2243-2253.

109. Sandstead H, Jacob R, Logan G, Munoz J, Klevay L. Effect of dietary protein on requirements for zinc and calcium. Clin Res 1978; 26: 701A.

110. Sandstead H, Klevay L, Jacob R, Munoz J, Johnson L, Dintzis F. Mineral requirements: influence of fiber and protein. Am J Clin Nutr 1979; 32: 933A.

111. King JC, Stein T, Doyle M. Effect of vegetarianism on the zinc status of pregnant women. Am J Clin Nutr 1981; 34: 1049-1055.

112. Apgar J. Effects of some nutritional deficiencies on parturition in rats. J Nutr 1975; 105: 1553-1561.

113. Calloway DH, Margen S. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. J Nutr 1971; 101: 205-216.

114. Scrimshaw NS Hussein MA, Murray E, Rand WM, Young VR. Protein requirements of man: variations in obligatory urinary and fecal nitrogen losses in young men. J Nutr 1972; 102: 1595-1604.

115. Nelson MM, Evans HM. Relation of dietary protein levels to reproduction in the rat. J Nutr 1953; 51: 71-84.

116. Anderson HL, Benevenga NJ, Harper AE. Associations among food and protein intake, serine dehydratase, and plasma amino acids. Am J Physiol 1968; 214: 1008-1013.

117. Musten B, Peace D, Anderson CH. Food intake regulation in the weanling rat: self-selection of protein and energy. J Nutr 1974; 104: 563-572.

118. Peng YS, Meliza LL, Vavich MG, Kemmerer AR. Changes in food intake and nitrogen metabolism of rats while adapting to a low or high protein diet. J Nutr 1974; 104: 1008-1017.

119. Anand CR, Linkswiler HM. Effect of protein intake on calcium balance of young men given 500mg calcium daily. J Nutr 1974; 104: 695-700.

120. Hegsted M, Linkswiler HM. Long-term effects of level of protein intake on calcium metabolism in young adult women. J Nutr 1981; 111: 244-251.

121. Li ETS, Anderson GH. Dietary minerals modify the food intake suppressing effects of high casein diets fed to rats. J Nutr 1982; 112: 717-721.

122. Naismith DJ. Adaptations in the metabolism of protein during pregnancy and their nutritional implications. Nutr Rept Int 1973; 7: 383-390.

123. Naismith DJ, Morgan BLG. The biphasic nature of protein metabolism during pregnancy in the rat. Br J Nutr 1976; 36: 563-566.

124. Marino DD, Costa FM, King JC. Nitrogen retention of healthy women in early and late pregnancy. Fed Proc 1982; 41: 285.

125. King JC, Calloway DH, Margen S. Nitrogen retention, total body potassium-40 and weight gain in teenage pregnant girls. J Nutr 1973; 103: 772-785.

126. Ashe JR, Schofield FA, Gram MR. The retention of calcium, iron, phosphorus, and magnesium during pregnancy: the adequacy of prenatal diets with and without supplementation. Am J Clin Nutr 1979; 32: 286-291.

127. Dressendorfer RH, Sockolov R. Hypozincemia in runners. Phys Sport Med 1980; 8: 97-100.

128. Oberleas D, White RC, Hurley RS, Prasad AS. Evidences for alterations of zinc and magnesium in conditioned athletes. Fed Proc 1972; 31: 668.

129. Krotkiewski I, Gudmundsson M, Backstrom P, Mandroukas K. Zinc and muscle strength and endurance. Acta Physiol Scand 1982; 116: 309-311.

130. Lukaski HC, Bolonchuk WW, Klevay LM, Milne DB, Sandstead HH. Maximal oxygen consumption as related to magnesium, copper, and zinc nutriture. Am J Clin Nutr 1983; 37: 407-415.

131. Lukaski HC, Bolonchuk WW, Klevay LM, Milne DB, Sandstead HH. Changes in plasma zinc content after exercise in men fed a low-zinc diet. Am J Physiol 1984; 247: E88-E93.

132. Plante RI, Houston ME. Exercise and protein catabolism in women. Ann Nutr Metab 1984; 28: 123-129.

133. Rennie MJ, Edwards RHT, Krywawych S, Davies CTM, Halliday D, Waterlow JC, Millward DJ. Effect of exercise on protein turnover in man. Clin Sci 1981; 61: 627-639.

134. Jopke T. Pregnancy: A time to exercise judgment. Phys Sport Med 1983; 11: 139-148.

135. Pomerance JJ, Gluck L, Lynch VA. Physical fitness in pregnancy: its effect on pregnancy outcome. Am J Obstet Gynecol 1974; 119: 867-876.

136. Hutchinson PL, Cureton KJ, Sparling PB. Metabolic and circulatory responses to running during pregnancy. Phys Sportsmed 1981; 9: 55-61.

137. Platt LD, Artal R, Semel J, Sipos L, Kammula RK. Exercise in pregnancy. Am J Obstet Gynecol 1983; 147: 487-491.

138. Artal R, Platt LD, Sperling M, Kammula RK, Jilek J, Nakamura R. Exercise in pregnancy. Am J Obstet Gynecol 1981; 140: 123-127.

139. Pijpers L, Wladimiroff JW, McGhie J. Effect of short-term maternal exercise on maternal and fetal cardiovascular dynamics. Br J Obstet Gynaecol 1984; 91: 1081-1086.

140. Marsal K, Lofgren O, Gennser G. Fetal breathing movements and maternal exercise. Acta Obstet Gynecol Scand 1979; 58: 197-201.

141. Hauth JC, Gilstrap LC, Widmer K. Fetal heart rate reactivity before and after maternal jogging during the third trimester. Am J Obstet Gynecol 1982; 142: 545-547.

142. Pomerance JJ, Gluck L, Lynch V. Maternal exercise as a screening test for uteroplacental insufficiency. Obstet Gynecol 1974; 44: 383-387.

143. Nagy LE, King JC. Energy expenditure of healthy women during early and late pregnancy. Fed Proc 1982; 41: 285.

144. Smith AD, Gilbert RD, Lammers RJ, Longo LD. Placental exchange area in guinea pigs following long-term maternal exercise: a stereological analysis. J Dev Physiol 1983; 5: 11-21.

145. Terada M. Effect of physical activity before pregnancy on fetuses of mice exercised forcibly during pregnancy. Terat 1974; 10: 141-144.

146. Nelson PS, Gilbert RD, Longo LD. Fetal growth and placental diffusing capacity in guinea pigs following long-term maternal exercise. J Dev Physiol 1983; 5: 1-10.

147. Clapp JF. Acute exercise stress in the pregnant ewe. Am J Obstet Gynecol 1980; 136: 489-494.

148. Erdelyi GJ. Gynecological survey of female athletes. J Sport Med Phys Fitness 1962; 2: 174-179.

149. Collings CA, Curet LB, Mullin JP. Maternal and fetal responses to a maternal aerobic exercise program. Am J Obstet Gynecol 1983; 145: 702-707.

150. Berkowitz GS, Kelsey JL, Holford TR, Berkowitz RL. Physical activity and the risk of spontaneous preterm delivery. J Reprod Med 1983; 28: 581-588.

151. Hill J, Davis J, Tagliaferro A. Aerobic exercise, dietary obesity and metabolism in young rats. Fed Proc 1981; 40: 904.

152. Walberg JL, Mole PA, Stern JS. Effect of swim training on development of obesity in the genetically obese rat. Am J Physiol 1982; 242: R204-R211.

153. LeBlanc J, Dussault J, Lupien D, Richard D. Effect of diet and exercise on norepinephrine-induced thermogenesis in male and female rats. J Appl Physiol: Respirat Environ Exercise Physiol 1982; 52: 556-561.

154. Walberg JL, Greenwood MRC, Stern JS. Lipoprotein lipase activity and lipolysis after swim training in obese Zucker rats. Am J Physiol 1983; 245: R706-R712.

155. Hill JO, Davis JR, Tagliaferro AR. Effects of diet and exercise training on thermogenesis in adult female rats. Physiol Behav 1983; 31: 133-135.

156. SAS Institute, Inc. SAS User's Guide: Statistics, 1982 edition. Cary, NC: SAS Institute, Inc, 1982.

157. SAS Institute, Inc. SAS User's Guide: Basics, 1982 edition. Cary, NC: SAS Institute, Inc., 1982.

158. National Academy of Science, National Research Council. Nutrient Requirements of Laboratory Animals. #10, 1966.

159. Mayel-Afshar S, Grimble RF. Changes in protein turnover during gestation in the foetuses, placentas, liver, muscle and whole body of rats given a low-protein diet. Biochim Biophys Acta 1983; 156: 182-190.

160. Winick M. Nutrition, intrauterine growth retardation and the placenta. Trophoblast Res 1983; 1: 7-14.

161. Rama Sastry RV, Barnwell SL, Moore RD. Factors affecting the uptake of α-amino acids by human placental villus: acetylcholine, phospholipid methylation, calcium and cytoskeletal organization. Trophoblast Res 1983; 1: 81-100.

APPENDICES

Appendix I

Aclimatization, Exercise Equipment and Procedure

Equipment:

1 Celsius Thermometer

2 62.2 X 92.7 X 71.1cm Plastic Tubs

3 Standard Plastic Large Trash Barrels

3 Heat Lamps

1 Electric Fan

Approximately 12 Standard White Towels

Procedure:

1. Warm tap water until approximately 38 to 40°C.
2. Fill tubs with 38 to 40°C water until approximately 3 to 4 feet deep. To aclimatize fill tub approximately one-half foot deep.
3. Place 6 to 12 rats/tub in water. For aclimatization, place 10 to 12 rats/tub in water.
4. Time the swimming process for one and one-half hours while watching the rats swim continuously. During the aclimatization period, allow the rats to walk in the water for 5 to 10 minutes.
5. After swimming, place 6 to 8 rats into each trash barrel lined with towels.
6. Turn on heat lamp situated above each trash barrel.
7. Allow the rats to dry completely--at times hand drying with a towel may be necessary.
8. Place rats back into their proper cages.

Appendix II

Analysis of Nitrogen Content in Experimental Diets (Kjeldahl)

Procedure:

1. Weigh out 1.2-1.5g sample (in duplicate) and place in Kjeldahl flasks.
2. Turn on Kjeldahl hood.

Digestion:

1. Add 10g Na_2SO_4-$CuSO_4$ mixture and 4 glass beads to each flask.
2. Prepare two blanks.
3. Pour 25ml of concentrated H_2SO_4 down the side of each flask (slowly turn the flask as you pour).
4. Swirl flask gently to mix.
5. Place labeled flask on burners.
6. Turn heat onto "2."
7. Heat on this setting until the samples stop frothing and begin to clear or are less black in color. During this time, turn flasks frequently to rinse down carbonaceous material.
8. Turn heat onto "4." Heat until samples turn clear green in color.
9. Turn heat up to "7" and heat for 30 minutes.
10. Turn heat off and allow the samples to cool on the heating apparatus until vapors are no longer apparent.
11. Once cooled, move the flasks to a holding rack and stopper them tightly. Allow the samples to cool completely.
12. Add 250ml of distilled water to each sample. Add slowly at first, mixing during the addition. Re-stopper the flask.

Distillation:

1. In a 500ml Erlenmeyer flask mix 25ml of 4% boric acid and 25ml of H_2O.
2. Add 4 to 5 drops of methyl-red/bromcresol green indicator.
3. Place Erlenmeyer flasks on the bottom shelf of the distillation apparatus.
4. Turn on the water to the condensor. Place on "manual" and check that the cold water is flowing.
5. Turn the burners onto "2."
6. Swirl each digestion flask to mix contents.
7. To each Kjeldahl flask add 1/16tsp of granular zinc and then immediately add 70-85ml of 50% NaOH.
8. Connect the flask to the distillation rack. Swirl slowly and then vigorously to mix the contents of the flask. If the mixture does not turn blue, the acid was not neutralized--more NaOH should be added to the remaining samples.
9. Turn the heat up to "5" immediately.
10. Place the Erlenmeyer flask under the distillation rack and insert the delivery tube under the surface of the liquid.
11. Distill until about 150ml are in the collection flasks. Then lower the flask so that the tube is out of the liquid.
12. Distill to 225ml total.
13. Turn heat off and allow dripping to finish.

Titration:

1. Titrate each sample with 0.1N HCL.
2. Titrate the blanks first and titrate them until they turn a peach color.
3. Titrate all of the samples until the color matches the endpoint of the blank.

Calculations: (One equivalent of HCL reacts quantitatively with one equivalent of N as ammonium borate.)

1. Normality of Acid X 14.00 = mg of N equivalent to 1ml of acid

2. Normality of Acid X 14.00= C factor

3. Total N= (ml HCL used to titrate - ml of acid to titrate blank) x C factor

4. $\frac{\text{Total N}}{\text{sample weight (g)}}$ = mg N/g

5. mg N/g X 6.25 X 0.1= % Protein

Appendix III

Nitric-Perchloric Wet Acid Oxidation

Procedure:

1. Weigh 50mg of sample into 50ml clean (acid washed) beakers and cover with watchglasses.

2. Prepare two beakers for blanks.

3. Add 3.0ml of redistilled nitric acid and allow to sit for approximately 12 hours.

4. Turn on perchloric hood and hotplate to 150°F.

5. Add 1.5ml of 70% perchloric acid and heat at 150°F until sample dissolves and foaming ceases.

6. Increase temperature slowly to a maximum of 325°F and allow samples to reflux in acid until clear.

7. Remove watchglasses and dry down to a white ash. If charring occurs, add nitric acid dropwise, after the beaker is cooled, and allow to reflux with watchglass on.

8. When samples have dried to a white ash, dissolve in 10ml of 10% HCL (from 6M Ultrapure HCL).

9. Cover beakers containing ash and 10% HCL with parafilm and allow to sit at least 2 hours before emptying into labelled polypropylene tubes. Stopper tubes tightly and refrigerate.

10. Make appropriate mineral standards.

11. Shake samples well and allow them to reach room temperature.

12. Samples are now ready to be read on the Perkin-Elmer Atomic Absorption Spectrophotometer (Model 503).

Appendix IV

Calculation of Tissue Zinc Content*

Procedure:

1. After obtaining absorption readings for the standards (0.1, 0.5, 1.0, 3.0, 5.0 PPM zinc), blanks, and tissue samples, calculate the average of four absorption readings per sample.

2. Perform Linear Regression, calculating the standards first and then the blanks.

3. Calculate the correlation value.

4. Calculate the concentration of zinc from the average absorption reading for each sample.

5. Multiply the concentration value by the dilution factor (10) for each sample.

6. Divide this number by the corresponding sample weight and derive the PPM of zinc for each sample.

7. Calculate the average value of the duplicate zinc PPM values.

8. Calculate the percent error by:

$$\frac{\text{large PPM value - small PPM value}}{\text{small PPM value}} \times 100 = \%\ \text{Error}$$

9. To derive the amount of zinc in each gram of wet tissue, multiply the PPM of zinc for each average sample value by 100 and this gives mg of zinc per gram wet tissue weight.

10. To derive the absolute value of zinc (mg/organ) in each organ, multiply the total average organ wet tissue weight (g) by the mg of zinc per gram wet tissue weight.

*SHARP Scientific calculator, EL-512

The vita has been removed from the scanned document