1982

Effect of zinc on protein-energy malnutrition

Ebenezer Asibey-Berko
Iowa State University

Asibey-Berko, Ebenezer

EFFECT OF ZINC ON PROTEIN-ENERGY MALNUTRITION

Iowa State University PH.D. 1982

Effect of zinc on protein-energy malnutrition

by

Ebenezer Asibey-Berko

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Nutrition

Approved:

Members of the Committee:

Signature was redacted for privacy.

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1982

TABLE OF CONTENTS

	Page
DEDICATION	x
INTRODUCTION	1
REVIEW OF LITERATURE	2
Brief History of Zinc	2
Brief History of Protein-Energy Malnutrition	2
Zinc and Protein in Diets	4
Biological Significance of Zinc	6
Some Roles of Zinc and Protein	8
Zinc Absorption and Its Mechanism	12
PEM Symptoms Vs. Zinc-Deficiency Symptoms	14
Conclusion	15
MATERIALS AND METHODS	17
Care of Animals	17
Experiment I	20
Experiment II	21
Experiment III	22
Experiment IV	22
Depletion-Repletion	22
Analytical Procedures	23
Dietary zinc	23
Plasma zinc, copper and iron	24
Plasma alkaline phosphatase	24
Plasma proteins	25
Tissue moisture	25
Tissue zinc, copper, iron and sodium	25

Liver protein 26
Urine creatinine 26
Statistical analyses 26

RESULTS 27

Experiment I 27

Development of kwashiorkor symptoms 27

Experiment II 61

Eye disorders 61

Experiment III 62

Experiment IV 72

DISCUSSION 100

The Rat Model for PEM 100

Liver Pathology and Alteration 102

Diminution of liver weight 102
Elevated liver minerals 103
Elevated liver moisture 104
Reduced liver protein concentration 105

Mineral Interrelationships 105

Zinc and iron 105
Zinc and sodium 106
Zinc and copper 107

Possible Dietary Zinc Toxicity in PEM 107

Some Specific Contributions of Dietary Protein and Zinc to Kwashiorkor 113

Loss of appetite and growth failure 113
Edema and liver moisture 113
Brain disorders 114
Alkaline phosphatase 115
Mortality rates 115

SUMMARY 116

BIBLIOGRAPHY 119

ACKNOWLEDGMENTS 128

LIST OF TABLES

		Page
Table 1.	Identical symptoms of PEM and zinc deficiency	15
Table 2.	Design of dietary treatments	18
Table 3.	Composition of diets	19
Table 4.	Cumulative food intake of rats (Experiment I: depletion)	28
Table 5.	Body weight of rats (Experiment I: depletion)	30
Table 6.	Body length and urine creatinine of rats after 35 days on diet (Experiment I: depletion)	34
Table 7.	Fresh organ weights and organ/body weight ratios of rats at death (Experiment I: depletion)	36
Table 8.	Plasma concentration of alkaline phosphatase, zinc and copper of rats after 2 days on diets (Experiment I: depletion)	39
Table 9.	Concentration of alkaline phosphatase, zinc, proteins and copper of rats after 9 days on diet (Experiment I: depletion)	41
Table 10.	Plasma alkaline phosphatase, zinc, copper and proteins of rats after 84 days (Experiment I: depletion)	45
Table 11.	Liver protein and copper of rats at death (Experiment I: depletion)	47
Table 12.	Liver zinc and iron of rats at death (Experiment I: depletion)	50
Table 13.	Liver correlation coefficients (r-values) from rats. Only values > 0.5 are reported (except for liver protein). Level of significance in all cases = 0.0001 (in range = 36-41. Experiment I: depletion)	55
Table 14.	Muscle moisture; number of days on diet before death; muscle zinc and iron of rats at death (Experiment I: depletion)	58
Table 15.	Plasma proteins after 5 weeks on diet (Experiment III: depletion)	63

Table 16. Body weight, muscle zinc, iron, moisture and sodium after 5 weeks on diets (Experiment III: depletion) 64

Table 17. Liver weight, moisture, zinc, and iron after 5 weeks on diet (Experiment III: depletion) 67

Table 18. Liver weight, moisture and sodium after 5 weeks on diet (Experiment III: depletion) 69

Table 19. Mid-brain weight, moisture, zinc and iron after 5 weeks on diet (Experiment III: depletion) 70

Table 20. Mid-brain weight, moisture, sodium and copper after 5 weeks on diet (Experiment III: depletion) 73

Table 21. Liver correlation coefficients (r-values) for rats. Unreported values are < 0.5 or insignificant (Experiment III: depletion) 75

Table 22. Liver moisture, muscle moisture, days on diet before mortality and days on diet before appearance of edema (Experiment II: depletion) 76

Table 23. Rate of death of rats on low protein in response to different levels of dietary zinc (Experiment II: depletion) 78

Table 24. Body weight data of rats during repletion (Experiment IV: repletion) 79

Table 25. Plasma zinc, iron and proteins of rats after 4 weeks depletion (Experiment IV: repletion study) 81

Table 26. Plasma zinc, iron, and proteins of rats after 7 days repletion with control (HPHZ) diet (Experiment IV: repletion) 83

Table 27. Plasma zinc, iron and proteins of rats after 14 days repletion with control (HPHZ) diet (Experiment IV: repletion) 84

Table 28. Plasma zinc, iron and proteins of rats after 21 days repletion with control (HPHZ) diet (Experiment IV: repletion) 85

Table 29. Liver iron and zinc of rats after 4 weeks on depletion diet (Experiment IV) 86

Table 30. Liver zinc and iron of rats after 7 days repletion (HPHZ) diet (Experiment IV) 87

Table 31. Liver zinc and iron of rats after 14 days repletion with control (HPHZ) diet (Experiment IV) 88

Table 32. Liver zinc and iron of rats after 21 days repletion with control (HPHZ) diet (Experiment IV) 89

Table 33. Liver sodium and protein of rats after 4 weeks on depletion diet (Experiment IV) 93

Table 34. Liver sodium and protein of rats after 7 days repletion with control (HPHZ) diet (Experiment IV) 94

Table 35. Liver sodium and protein of rats after 14 days repletion with control (HPHZ) diet (Experiment IV) 95

Table 36. Liver sodium and protein of rats after 21 days repletion with control (HPHZ) diet (Experiment IV) 96

LIST OF FIGURES

		Page
Figure 1.	Cumulative food intake. Dietary zinc effect on food intake was not significant until after 2 weeks depletion	29
Figure 2.	Growth curves. Only dietary protein affected body weight changes significantly in first week of feeding (Table 5)	31
Figure 3.	(A) Treatment 2 (LPHZ) showed significantly earlier mortality than treatment 1 (LPLZ) in Experiment I. (B-D) Significantly different values for high-protein rats (Table 6)	35
Figure 4.	Plasma alkaline phosphatase on days 2 and 9 for LP rats and days 2, 9 and 84 for HP rats. Decreasing levels with age confirm literature (Tables 8-10)	40
Figure 5.	Significantly different plasma protein levels in A, C and D for low-protein rats and in B for high-protein rats (Table 9)	43
Figure 6.	Liver proteins. Differences significant only for HP rats. In A, B and D (Table 11)	49
Figure 7.	Liver zinc. Significantly different values in A and B for LP rats and in C for HP rats (Table 12)	52
Figure 8.	Liver iron. Significantly different for LP rats in A and B. Significantly different for HP rats in A (Table 12)	53
Figure 9.	Regression relationships between body-weight, total liver zinc and other liver parameters (Table 13)	57
Figure 10.	Muscle zinc and iron. Significantly different wet and dry tissue zinc values for LP rats. Significantly different wet-tissue zinc values for HP rats. No significant muscle iron differences (Table 14)	60
Figure 11.	Shows effect of dietary zinc on survival rate during PEM. Experiment I: Deaths started 2 weeks earlier (significant: $p \leq 0.01$) in LPHZ group. Experiment II: Deaths started 1 week earlier in the same group but not significant statistically (Tables 14 and 22)	77

Figure 12. Rats previously on LPLZ diet gained weight faster during repletion than those previously on LPHZ diets (Table 24) 80

Figure 13. Graph suggesting a dilution of liver zinc towards normal levels (in LP rats) as fresh liver tissue forms during repletion. Rate of dietary zinc uptake into liver lags behind tissue biosynthesis during repletion (Tables 29-32) 90

Figure 14. Increasing total liver zinc with repletion and growth. PEM rats previously on LPHZ diet lag behind PEM rats previously on LPLZ diet (Tables 33-36) 97

Figure 15. Increasing total liver protein with repletion and growth in all groups. Rats previously on LPHZ diet were behind LPLZ rats after 3 weeks (Tables 33-36) 98

Figure 16. Rats previously on LPHZ diets show better rate of liver protein biosynthesis than LPLZ rats after 3 weeks (Tables 33-36). Figure 15 suggests that total liver weight was still lower at this time in LPHZ group than LPLZ group 99

Figure 17. Toxicity of 30 ppm dietary zinc in PEM rats would suggest drastically reduced therapeutic margin for dietary zinc in PEM. Control rats are known to show no toxic effects on diets with up to 7000 ppm dietary zinc 111

DEDICATION

To My Wife and Parents

INTRODUCTION

Remarkable advances have been made in trace mineral research, especially in the last decade (1). A number of elements, including zinc, have been found to be essential to man (2). Knowledge of interrelationships between the actions of several nutrients in the body is growing. Zinc and copper, for example, compete for uptake in the intestinal lumen. This is due to similarity of their atomic orbitals. Selenium and mercury interrelate through direct chemical reaction. Phosphate (HPO_4^{-2}), arsenate (AsO_4^{-2}) and vanadate (VO_4^{-2}) compete in the same way because of similarity of molecular orbitals (3). For totally different reasons, some nutrients exert very similar biological effects. The antioxidant effects of selenium and vitamin E are examples (4). Skin pathology produced by deficiency of essential fatty acids or zinc is another. There is also a whole range of very similar clinical symptoms which both protein-energy malnutrition (PEM) and zinc deficiencies share (5).

The need to identify the exact roles of dietary zinc and protein in PEM has become increasingly necessary. Clinical findings are showing the presence of zinc deficiency in many cases of PEM (6, 7, 8). Thus, there is the possibility that the PEM syndrome may be an overlapping of the deficiency symptoms of both protein and zinc.

REVIEW OF LITERATURE

Brief History of Zinc

Zinc was first recognized by Raulin in 1869 to be essential for Aspergillus niger. Somer and Lipman, in 1926, determined that it is essential for higher plants. Later, it was found to be essential for many experimental animals, including the rat (9). Its deficiency in man was first suspected in 1961 and established in 1963 (10). Prasad and Oberleas (1) emphasizes the great importance of zinc by pointing out that its metabolic roles are so numerous "that biochemistry may have to be rewritten around this one element."

Brief History of Protein-Energy Malnutrition

The term "protein-calorie malnutrition" (PCM) was first introduced by Jelliffe (11) in 1959 to cover not only marasmus and kwashiorkor but also their mild subclinical stages. Some prefer the synonym "protein-energy malnutrition" (PEM) to "protein-calorie malnutrition." The preference is meant to emphasize that insufficient dietary energy leads to the metabolic energy deficiency of the syndrome and that the calorie is only the most customary measure of this energy.

The newer term - "energy-protein malnutrition" (EPM) is coming into greater use (12). This is to emphasize the unconfirmed suspicion that marasmus is the more predominant form of protein-energy malnutrition rather than kwashiorkor. While an overall lack of food energy causes marasmus, kwashiorkor appears to be caused by a deficiency of

dietary protein. A definition of the three basic forms of PEM - marasmus, kwashiorkor, marasmic-kwashiorkor - has been discussed by Waterlow (13). Edema and severe dermatosis are the distinguishing features of kwashiorkor. Extreme weight loss or growth failure typifies marasmus.

Malnutrition has been known for centuries in many parts of the world. Commonly, it was in the form of marasmus in North America and Europe. Marasmus occurred elsewhere, too (14). Detailed study of PEM began in the 1920s. It, however, took the appointment of the first female medical officer (Cecily Williams) to Gold Coast (now Ghana) to give a detailed description of the disease in 1932 (12, 14). The disease she described was the kwashiorkor form of PEM. "Kwashiorkor" was the name of the edemic form of PEM in Gold Coast. For a time, some suspected it was "infantile pellagra" resulting from niacin deficiency. Later, Trowell (cited by Darby, 15) noticed unusually low plasma albumin levels associated with it. Helen McKay suggested an association with dietary amino acid or protein deficiency.

Cecily Williams admits not being the first to have described kwashiorkor. Her work opened the door, however, to serious work on PEM.

In 1906, Czeny and Keller (Germany) called the syndrome "Mehlnachschwaden." In 1908, Correa (Mexico) called it "Celebrilla." In 1924, Phillip and Proctor (Kenya) called it "Oedema and Ascariasis." In 1926, Normet and Kerandel (Indochina) called it "Bouffisure d'Annan." In 1927, Frontali (Italy) called it "Starchy Food Dystrophy."

Numerically, PEM is adjudged the major cause of illness in the world today (16). Efforts in the last twenty years have helped to abruptly reduce mortalities from PEM. Unfortunately, mortality rates began increasing again in the 1970s, possibly because of rising food shortages and costs.

Zinc and Protein in Diets

Studies (17) show that not all dietary zinc may be available for absorption. It is less available in plant products than animal products. In plant products, it may be bound by fiber, phytate, calcium, starch or wheat proteins. Calcium does not inhibit zinc absorption in man but does in other animals (6). Contrary to popular thought, it is fiber and not phytate that binds more zinc (1). Fiber may be beneficial to health (18), but excesses, by causing mineral losses, can be detrimental to health. The optimum dietary level and type for health needs to be determined.

Zinc availability is improved by dietary cysteine, histidine, ethylenediaminetetraacetate (19) and high dietary proteins (20). This might imply the likely involvement of dietary amino acids in the mechanism of zinc absorption. Protein deprivation can, on the other hand, cause negative zinc balance (21). This might explain the incidence of zinc deficiency in cases of PEM in Egypt, South Africa, and India (6, 7, 8). Oberleas and Prasad (22) have suggested zinc-supplementation of high-protein vegetable mixtures formulated to treat kwashiorkor.

Zinc intake should come from a balanced diet with adequate animal proteins. Meat, liver, eggs and seafood (especially oysters) are good sources of available zinc. Next follow milk and whole-grain products - whole wheat, rye bread, oatmeal, whole corn. Zinc content of most municipal water is negligible (23). Some of the best sources of animal protein (meat, liver, eggs, seafood and milk) are the best sources of available zinc.

A zinc intake of 15 mg/day is recommended for adults, with an extra 5 mg/day in pregnancy and 25 mg/day total for lactation (23). For preadolescent children, a dietary allowance of 10 mg/day is recommended. Newborns have a negative zinc balance and declining tissue stores. There is uncertainty about the safety of interfering with this negative balance in newborns. An allowance of 3 mg/day for the first 6 months of life is recommended for them (23).

Estimates of protein needs of man come under occasional review by international groups of experts (24). Daily recommended intakes are 0.8 gm/kg body weight for adults and 2.4 gm/kg for infants dropping to 1.4 gm/kg by one year of age. Increased intakes are recommended for pregnancy and higher intakes for pregnant teenage girls for their own growth as well as the baby's. These recommendations are valid only when energy needs are met. Insufficient energy intake will cause degradation of some of the recommended protein intake for energy (24). There is information about the percentage of each essential amino acid that a good quality protein should have and also the percentages required by various age groups (23).

Biological Significance of Zinc

In 1956, "Present Knowledge in Nutrition" cited less than 5 known zinc-metalloenzymes. Today, about 80 zinc metalloenzymes are known (25). Some occur in plants, rats, yeasts, bacteria, man and other life forms. There are also nonenzyme proteins that bind zinc in the body. Metallothionein, transferrin and nucleoproteins bind about 2% of plasma zinc. Cysteine and histidine bind about 2%. Alpha$_2$-macroglobulins firmly bind about 30%. Plasma albumin loosely binds about 66%.

Some well-known zinc-metalloenzymes are carbonic anhydrase, aldolase, lactic dehydrogenase, malic dehydrogenase, and alcohol dehydrogenase (e.g., retinol dehydrogenase). Others are glutamate dehydrogenase, pancreatic carboxypeptidases A and B, NADH diaphorase, alkaline phosphatases, thymidine kinase, RNA polymerase, DNA polymerase and pyridoxal phosphokinase (26). These enzymes do not respond to zinc-deficiency to the same extent in all tissues. The tissues in which they are most affected in deficiency are serum, bone, pancreas, and intestinal mucosa (27). Within any one of these tissues, the different enzymes also show different sensitivities to zinc status. Some lose activity quickly while others do so only in severe zinc deficiency. There are differences in the zinc-ligand affinities of the various metalloenzymes that account for this. Those (e.g., alkaline phosphatase) that bind zinc loosely, quickly lose it during deficiency. Others like the dehydrogenases bind it tightly and lose activity only in severe deficiency. Those enzymes with low zinc affinity tend to have high turnover of the enzyme protein in the tissues and vice versa.

Serum alkaline phosphatase loses about 48% of its activity in rats after four days on a zinc-deficient diet. Within 3 days of zinc supplementation, the activity returns to normal. This reduction in activity occurs before loss of appetite, reduction in growth or appearance of skin lesions. This enzyme and carboxypeptidase A are the ones that first respond to zinc-deficiency in man (28). Pancreatic carboxypeptidases A and B are important in protein digestion. The level of A is consistently depressed in zinc deficiency. Its activity in animals can fall 24% after 2 days on zinc-deficient diet, but this does not seem to affect protein digestion or digestibility significantly (29).

Carbonic anhydrase activity is most detectable in the blood, stomach and intestines. Blood activity falls in zinc deficiency. After about 30 days however, the activity may be found the same as for the controls. This is caused by increased erythrocyte count in the deficient rats. Erythrocytes are rich in carbonic anhydrase. It is best then to express this enzyme's activity/unit of erythrocytes. A reduction in its activity can then be shown at any stage of zinc deficiency. It is suggested in these studies that enzyme activities should be expressed per gram of protein (1).

No significant changes have been seen in most of the dehydrogenases in zinc-deficiency. Some studies seem to suggest a slight increase in activity in some of them. The exception is retinol dehydrogenase. Altogether, alkaline phosphatase and carboxypeptidase A, which lose activity before any clinical symptoms appear, are suspected to be involved in

starting clinical symptoms of zinc-deficiency. The mechanism is not clear.

Some Roles of Zinc and Protein

Both zinc and protein deficiencies can separately impair vision. They seem to involve a common mechanism. Both deficiencies suppress protein synthesis. Retinol-binding protein concentration is decreased in the liver because of low protein synthesis. Vitamin A accumulates in liver for lack of retinol-binding protein (RBP) to mobilize it. The failure to move Vitamin A from liver reduces supplies of it to the eyes and can cause xerophthalmia (30a). Xerophthalmia literally means "dry eye." Secretions of the goblet cells of the conjunctiva dry up, leading to discontinuity of the fluid films usually present over the surface of the conjunctiva and cornea. This drying may be caused by various clinical conditions. Vitamin A deficiency is the commonest cause (30b, 30c). When zinc fails to reach retinol dehydrogenase in zinc deficiency it causes degeneration of the cones and rods. Only severe zinc deficiency is reported to have this effect. The result is night blindness (31a, 31b). Retinol dehydrogenase is needed to change retinol to retinaldehyde for the cones and rods. Levels of serum zinc correlate with levels of serum retinol-binding protein and total serum protein. However, data on the correlation between plasma Vitamin A and zinc is conflicting (30c). Plasma Vitamin A is usually reduced in zinc-deficient animals. It is also reduced in animals on restricted food intake even when dietary Vitamin A and zinc are adequate. This

suggests the possibility that on a zinc-deficient diet, the lowered plasma Vitamin A may be due to the decreased food intake that accompanies zinc-deficiency rather than to zinc-deficiency per se. More research is needed to clarify this. Like alkaline phosphatase, it is suspected that a determination of serum retinol-binding protein may be a good indication of zinc-deficiency (32). Research is needed to decide which of the two indices may be more sensitive.

The most crucial biological function of zinc seems linked with DNA and RNA syntheses and cell division (33). These involve the activities of thymidine kinase, RNA polymerase and DNA polymerase. Ribonuclease is also affected. At plasma zinc concentrations below 10^{-4} M, ribonuclease is activated and breaks down RNA. It has been shown in malnourished boys in Iran (34) that there is poor growth if dietary protein is adequate and zinc is deficient.

In zinc deficiency, DNA synthesis decreases as a result of inability to incorporate thymidine into DNA (35). In one study, progressive fall of thymidine incorporation into DNA of liver, kidney and spleen occurred within five days on zinc-deficient diet. Thymidine kinase is required for incorporation of thymidine into DNA (33).

In PEM, there are not enough dietary amino acids for protein biosynthesis. In zinc deficiency, if amino acids are available, they can still not be used for protein biosynthesis. This explains the great similarity of symptoms produced by two deficiencies.

Zinc affects protein utilization. Protein digestibility is not altered significantly despite the fall in carboxypeptidase A activity

(29). Net protein utilization (NPU), however, decreases in zinc deficiency. Urinary excretion of N and S also increase because of increased degradation of the sulfur amino acids (cysteine and methionine). This leads to an overall reduction of the tissue proteins of zinc-deficient rats (36, 37). An abnormal pattern of plasma proteins also results (38). In zinc-deficiency, methionine and cysteine cannot be incorporated into body proteins (39). They are degraded and lost via urine, mainly as taurine (40). Cysteine is involved in forming a collagen precursor. Collagen is involved in wound-healing. Keratin, an albuminoid protein of skin is also rich in cysteine (or cystine). About four times as much cysteine goes to the skin and hair of control rats as in zinc-deficient rats. This disorder of sulfur-amino acid metabolism seems to contribute to the development of skin lesions and hair disorders in zinc deficiency. It also may explain the role of zinc in wound-healing (41).

Zinc is involved also with some hormones. Crystalline insulin contains 0.5% zinc. Insulin contains two polypeptide chains and a total of 51 amino acids in the two (42). Zinc deficiency does not affect its activity significantly (1). There is, however, a decreased activity of insulin in PEM - this being a specific effect of protein deficiency (16, 43). Plasma insulin levels decrease in PEM. Glucose tolerance is impaired also. The severe loss of potassium which accompanies PEM is considered responsible for the lowered plasma insulin concentration and activity. Potassium is important

in the control of insulin secretion from the beta-cells of the pancreas (16).

Growth hormone cannot induce growth in zinc deficiency. There may be adequate dietary protein and high growth hormone concentrations, but no growth occurs if zinc is deficient. Human growth hormone contains 240 amino acid residues (44). Its plasma concentration increases in PEM. It cannot stimulate growth but does stimulate lipolysis in PEM. Its concentration is inversely related to the concentration of plasma albumin in PEM (16).

Cortisol levels also increase in PEM because of stress from infections as well as the overall decrease in catabolism.

Zinc deficiency causes hypogonadism in male animals. This is not due to insufficient sex hormones. Though serum lutenizing hormone (LH) concentrations fall slightly, pituitary concentrations are unchanged. The serum fall is due to reduction in food intake rather than to zinc deficiency (45). Testes of male rats atrophy in zinc deficiency (1). Zinc is needed for spermatogenesis in males. The effect on females depends on degree of severity, timing and duration of deficiency. The estrous cycle may be severely disrupted in the female. There may be no mating. If pregnancy occurs, fetal malformation (i.e., teratogenesis) may occur (46). Teratogenicity from zinc deficiency has not been documented in humans.

Unlike in zinc deficiency, in PEM, sex hormone lutenizing hormone and testosterone) concentrations are low. Low sex hormone makes sexual maturation impossible (47). This, together with the

disruption of spermatogenesis during zinc deficiency and an overlapping of the two disorders will cause double suppression of sexual maturation in young males.

Aldosterone is a mineralocorticoid hormone produced under control of the renin-angiotensin hormone system. It is produced by the adrenal cortex and promotes Na^+ absorption and K^+ excretion in the kidneys, sweat glands, salivary glands, intestinal mucosa, urinary bladder, etc. It thus helps regulate water and electrolyte balance (48). Its elevated level in plasma in kwashiorkor probably contributes to the high water and Na^+ retention and, perhaps, edema of kwashiorkor (49).

Zinc plays an important role in the immune system (36, 50). Zinc deficiency causes reduced thymus and spleen weights. Peripheral lymphocyte counts and serum gamma-globulins are also lowered, causing reduction in synthesis of immunoglobulins or antibodies. Zinc deficiency thus causes a suppression of immune response.

On the other hand, prostaglandin synthesis increases in zinc deficiency (51, 52). There is increased lipolysis. There also may be an increase in microsomal phospholipids, leading to an increase in levels of unsaturated fatty acids. Increased polyunsaturated fatty acids furnish substrate for lipid peroxidation as well as for prostaglandin synthesis.

Zinc Absorption and Its Mechanism

Intestinal zinc absorption decreases with age both in man and the rat. Most absorption occurs in the small intestine — especially mid-jejunum. Only minimal amounts are absorbed in the cecum, stomach, and colon (20). Only about 5-15% of the total dietary zinc is absorbed in man (53). Figures vary. Absorbed zinc is taken up by different tissues at different rates. Peak uptake in the rat occurs in the liver and pancreas. 3-4 hours after ingestion of food. Zinc accumulation is slow in the blood cells, femur and muscles. The majority of the zinc ultimately is deposited in the muscle, bone, and skin of the rat. In PEM, plasma but not erythrocyte zinc is lowered (8). Individual cells with the highest zinc concentrations are those of liver, pancreas, kidney, heart, pituitary, adrenals, prostate gland and leucocytes. Erythrocytes contain 6-8 times more than plasma. Erythrocytes are rich in carbonic anhydrase, a zinc-metalloenzyme.

Hansen and Lehman (54) have reported a drastic fall in liver zinc in PEM. Zinc is known to have a protective effect on the liver, partly because of its inhibition of lipid peroxidation in cell membranes. In stress, body zinc is redistributed. In some inflammations, zinc moves from the plasma and accumulates in the liver. In PEM, zinc moves into the edema fluid. With normal zinc intake, healthy animals have low urinary zinc excretion and high fecal zinc excretion. Fecal zinc is a combination of unabsorbed dietary zinc and endogenous zinc secreted from the vascular spaces into the intestines. In man, as much as 18% of intravenous ^{65}zinc may be excreted into the intestine (53). Aging

may increase this endogenous zinc loss. The small intestine is the main site of return of endogenous zinc into the gastrointestinal tract (20). Some endogenous zinc is also lost via pancreatic and biliary excretions, e.g., as part of pancreatic carboxypeptidases. Endogenous zinc loss may be twice as much as urinary zinc excretion. Loss via urine can be enhanced by excess dietary histidine. This urinary zinc loss leads to lowered blood zinc. Small amounts of zinc are also lost from nails and falling hair.

The mechanism of zinc absorption is still unclear but various hypotheses have been advanced. There is scientific evidence for and against each of them. It is accepted generally that the mechanism involves active transport from the lumen of the gastrointestinal tract (GIT). A ligand is believed to be secreted into the lumen, which binds dietary zinc for absorption. After extensive study, Evans et al. (55) proposed the following mechanism: "The pancreas secretes a ligand into the duodenum where zinc complexes with it. Thus complexed with the ligand, the zinc gets transported through the microvilli into the epithelial cells where the metal is transferred to binding sites on the basolateral plasma membrane. Metal-free albumin interacts with the plasma membrane and removes zinc from the receptor sites."

Hurley et al. (56a, 56b) reported finding such a zinc-binding ligand in human and rat milk and its absence from cow's milk. It is a low molecular-weight ligand that workers have conflictingly identified as prostaglandin (57), picolinic acid (58), citrate (59) and a metallothionein degradation product (60). Evans and Johnson, early among those thinking it was prostaglandin, later identified it as picolinic

acid. Lonnerdal et al. (59) think it is citrate. They found the zinc-binding function associated with citrate rather than with picolinic acid in chromatographic studies. The problem with the citrate hypothesis is that while the zinc-binding ligand (ZBL) shows high activity in human milk and little activity in cow's milk, citrate does the opposite. Also, there is known to be ZBL activity in rat milk while citrate has no activity there. In acrodermatitis enteropatica (AE) an infant disease caused by zinc malabsorption, the ligand is thought to be produced in insufficient amounts. Evans et al. observed correction of this disorder when human milk rather than cow's milk was given to children with the disease. Human milk is rich in picolinic acid.

Casey et al. (61) demonstrated, however, that when zinc tolerance tests are used, no picolinic acid uptake by AE patients is seen. They finally concluded that ZBL is a degradation product of metallothionein. Some metallothionein is located in intestinal mucosa cells. It has been demonstrated that zinc absorption is directly proportional to intestinal metallothionein levels.

Recent work by Hurley and Lonnerdal (56b) suggests strongly that the ZBL may be citrate.

PEM Symptoms Vs. Zinc-Deficiency Symptoms

A great number of the symptoms of PEM also occur during zinc deficiency (7) (see Table 1).

Some symptoms unique to PEM are irritability, fatty liver and occasional diarrhea. Those unique to zinc deficiency are geophagia,

Table 1. Identical symptoms of PEM and zinc deficiency

PEM	Zinc-deficiency
Growth failure	Growth failure
Lethargy	Lethargy
Loss of appetite	Loss of appetite
Skin defects	Skin defects
Enlarged liver	Enlarged liver
Hair loss and abnormalities	Hair loss and abnormalities
Hyperammonemia	Hyperammonemia
Impaired mental function	Learning disabilities in rats
Vitamin A accumulation in liver	Vitamin A accumulation in liver

loss of taste acuity (i.e., hypogeusia) and hypogonadism.

In an experimental production of edema, Kohman (62) explained that rats with edema consistently show reduced testicle size. It is possible that this is an effect of underlying zinc deficiency.

Conclusion

Treatment of clinical PEM involves dietary therapy. In a study of kwashiorkor in Egypt, Sandstead et al. (6) found that at clinical recovery, the patients' plasma zinc was still below normal (90 ± 10 µg/dl) (1). In similar studies by others (8), plasma zinc rose to normal after three days of diet therapy. What were the differences between the two treatments? The latter study did not last long enough to

see the full effect of the early restoration of plasma zinc level on recovery. The authors point out, however, that zinc content of their therapeutic diet provided only (3.4 mg zinc/day) a fraction of the recommended dietary allowance 10.0 mg/day for children (23). It is known that inadequate dietary zinc during therapy, can slow down the rate of recovery (63). It can particularly slow down "catch-up growth" during recovery (34). Some clinicians consider a child recovered and ready for discharge when he reaches a normal weight-for-height. The normal weight-for-height is the 50 percentile weight for a given height and age, according to the Boston Standards (13).

I hoped through this study to be able to examine some questions more closely. What will happen to PEM development if the full requirement of zinc is being supplied? What contributions do dietary protein and zinc make to the PEM syndrome? To what extent does low dietary protein affect dietary zinc absorption?

With PEM being numerically the major cause of illness in the world (16), the effects of all factors that affect it need to be examined.

MATERIALS AND METHODS

Four experiments were carried out: three depletion experiments followed by a depletion-repletion study.

Care of Animals

In the first three experiments, rats were ordered from Biolab[1]. Rats for the 4th experiment were obtained from Quality Laboratory Animals[2] because of some problems Biolab was having at the time.

The rats were received at the age of 3 weeks — just after weaning. All were males of the Wistar strain. They usually had a weight distribution range of about 40-60 g. They were kept individually in stainless steel wire mesh cages. Since the cages were in galvanized steel racks, plexiglass sheets were inserted between their tops and the horizontal partitions of the racks. That way, the rats could not gnaw at the rack and obtain zinc.

All glassware, drinking bottles, food jars, mortars and pestles used were acid-washed with 2N HCl. Nitric acid was avoided for washing because of its ability to bind zinc firmly to glass (64). Because of this, blank solutions became an absolute necessity when nitric acid had to be used in a perchloric acid wet-ashing procedure (65). Food and deionized water were provided ad libitum.

In addition to assigning a specific number to each cage, each

---

[1]Biolab Corp., Box 8586, 5228 Centerville Road, St. Paul, Minnesota 55110.

[2]Quality Laboratory Animals, Box 4220, Madison, Wisconsin 53711.

treatment was identified with a specific color code. Ventilation in the animal room provided 5-6 changes of air per hour to remove moisture, bacteria and dust. Temperature was maintained at 77-78°F and relative humidity at 45-55%. A cycle of "lights-off" from noon to midnight and "lights-on" from midnight until noon was maintained. That way, most handling could be kept within the morning hours with lights-on. The noise level in the room was kept at minimum. Cages and accessories were changed once a week. Until drinking bottles and food jars were replaced with fresh ones, care was taken to avoid interchanging them. The environment was kept clean.

Compositions of diets used in these experiments are shown in Table 3. Zinc levels of various batches of diet (prior to zinc-supplementation) ranged from 0.40-0.95 ppm. Dietary zinc analyses included a perchloric acid digestion (65) and analysis with an atomic absorption spectrophotometer.

All the experiments, except Experiment II followed a 2 x 2 factorial design. This meant four different dietary treatments — two low protein diets (LPLZ and LPHZ) and two high protein diets (HPLZ and HPHZ).

Table 2. Design of dietary treatments

Treatment	Zinc (mg/kg)	Protein (%)	Abbreviation
1	6	0.4	LPLZ
2	30	0.4	LPHZ
3	6	20	HPLZ
4	30	20	HPHZ

Table 3. Composition of diets

Ingredients	Low protein basal diet[a] % weight	High protein basal diet[a] % weight
Egg albumin[b]	0.4	20.0
Corn starch[c]	84.1	64.5
Corn oil[d]	9.0	9.0
Water-soluble vitamin premix[e]	1.0	1.0
Fat-soluble vitamins[e] (in oil)	1.0	1.0
Zinc-free mineral mix[f]	4.5	4.5
Total	100.0	100.0

[a]To each of these, zinc is later added as $ZnSO_4 \cdot 7H_2O$ at two different levels (30 ppm and 6 ppm) to make a zinc-adequate and a moderately zinc-deficient diet.

[b]Spray-dried. United States Biochemicals.

[c]Teklad Test Diets. Zinc-free. Division of ARS/Sprague Dawley.

[d]ICN Pharmaceuticals, Life Sciences Division, Cleveland, Ohio.

[e]Based on National Academy of Sciences nutrient requirement of the rat with additional allowances. Used analytical grade reagents only. Amount on 100 kg diet: riboflavin, 1 gm; thiamine·HCl, 0.5 gm; pyridoxine·HCl, 2.8 gm; Calcium pantothenate, 7.2 gm; vitamin B_{12}, 2.0 mg; choline chloride, 90 gm; folic acid, 0.5 gm; biotin, 1.0 gm; inositol, 40 gm; retinol acetate, 72 mg; vitamin D_2 (ergocalciferol), 150,000 IU; dL-α-tocopherol acetate, 14,000 mg.

[f]Based on National Academy of Sciences nutrient requirements of the rat with additional allowances. Used analytical reagents only. Amount in 100 kg diet: calcium, 600 gm; chlorine, 60 gm; sodium, 252 gm; phosphorus, 500 gm; selenium, 10 mg; potassium, 897 gm; iodine, 100 mg; manganese, 7 gm; magnesium, 60 gm; iron, 5 gm; chromium, 500 mg; copper, 1.5 gm.

The low protein and high protein diets are isocaloric. Each diet provides about 4317 calories/kg.

For normal weight gain in human infants, 5.5% of total dietary energy must come from protein (66). For rapid "catch-up" growth during recovery from PEM, the protein:energy ratio must be about 0.1, i.e., protein must provide about ten percent (10%) of the total dietary energy (63, 66). In our experimental diets, protein provided 0.4% and 19% of the total dietary energy of our low protein and high protein diets, respectively. These meant P:E ratios of 0.004 and 0.19, respectively.

Experiment I

The objectives of this experiment were to find out how long it would take to induce kwashiorkor using the low protein diets to check effects of different dietary zinc concentrations on PEM development, and to follow the entire course of the disease until death.

A 2 x 2 factorial design was used. Forty-three male weanling rats were randomly assigned to the four diets so that there were at least 9 rats/treatment. The experiment lasted 84 days (i.e., 12 weeks). Blood samples were collected on the 2nd, 9th and 84th days of the study from the tail tip. These went into heparinized containers for plasma preparation. Plasma was stored at -20°C.

Weekly weights and food intakes were recorded. Observations on general behavior and appearance of edema were made.

Urine samples were collected on days 35 to 38 of the study — for 72 hours exactly. Anodized aluminum gauze replaced galvanized gauze

in the funnels for urine collection. Hydrochloric acid (2 ml 1N HCl/ bottle) served as a preservative. Total volumes were measured and samples frozen and later freeze-dried.

Body lengths of all rats involved in the urine collection were measured from the nose tip to the base of the tail.

Any deaths among the rats on the various treatments were recorded. Blood collection at the end of the study was by heart-puncture. Gastrocnemius muscles, liver, spleen and both kidneys were removed, blotted and weighed just after death. They were wrapped in aluminum foil, immediately frozen in liquid nitrogen and stored at -20°C in polyethylene bags.

Experiment II

The purpose of this study was to find the effects of dietary zinc on survival rate during PEM.

Only the low protein diets — LPLZ (6 ppm zinc) and LPHZ (30 ppm zinc) were used in this study. Thirty male weanling wistar rats were assigned to each treatment. Experimental feeding lasted 12 weeks. Observations on general behavior, skin, eye and edema changes were recorded. Liver and Gastrocnemius muscles were removed from dead rats. They were immediately blotted, weighed, frozen in liquid nitrogen and stored at -20°C in polyethylene bags.

Experiment III

The purpose of this study was to check the reproducibility of some of the findings of Experiment I and to also look at new parameters.

A 2 x 2 factorial design involving all four treatments was used. Forty-eight weanling male wistar rats were randomly assigned such that there were 12 rats/treatment. Body weight data were collected at the beginning and end of the study.

For necropsy, rats were anesthetized with ether and blood was collected by cardiac puncture and placed in heparinized containers. Plasma was stored at -20°C. Gastrocnemius muscles, mid-brain and liver were collected, blotted and weighed. They were wrapped with aluminum foil, quickly frozen in liquid nitrogen and stored at -20°C in polyethylene bags.

Experiment IV

This experiment was undertaken to find the effects of zinc status during depletion on subsequent rate of recovery from PEM. The depletion part also offered an opportunity to recheck some of the findings of Experiments I and III.

Depletion-Repletion

The 2 x 2 factorial design was used again. A total of 96 rats were randomly assigned with 24 rats/treatment and weighed weekly throughout the study.

After four weeks, all rats were fed the HPHZ (control) diet, except the 24 rats that were to be killed that day.

The depleted animals were anesthetized with ether before sample collection. Blood was collected by heart puncture and put into heparinized containers for plasma preparation.

The liver was perfused in each animal as part of this study. This was to check whether perfusion would significantly change the pattern of results obtained in Experiments I and III for the liver - especially iron concentration. To perfuse, water was injected via the hepatic portal vein into the liver and out via the vena cava. This resulted in a pale brownish liver, cleared of blood. Livers were then removed, blotted, wrapped in aluminum foil and immediately frozen in liquid nitrogen. They were weighed and stored at -20°C.

The remaining rats on control diet were weighed each week. Batches of 24 rats (6/treatment) were sacrificed at the end of the 5th, 6th and 7th weeks of study. Blood and liver samples were collected and processed as before for storage at -20°C.

Analytical Procedures

Dietary zinc

Digestion of diet was done with a perchloric acid-nitric acid procedure (67). Samples were made up to volume with deionized water and analyzed with an atomic absorption spectrophotometer.

Plasma zinc, copper and iron

Plasma samples were diluted 1:4 with deionized water to get a density approximately equal to those of the aqueous standards (68). A Unicam SP.90 atomic absorption spectrophotometer was used. Diluted samples were aspirated directly into the flame. Instructions in the manufacturer's manual (69) were closely followed. Zinc, copper and iron were analyzed using a flame of either air:acetylene (5 liters: 1 liter)/min or air:propane (5 liters: 0.5 liters/min.). Absorption wavelengths were 214.8, 324.8, 248.3 mm, respectively. These were the most sensitive absorption lines (70, 71).

Since plasma copper was not concentrated by extraction, (70), a scale expansion procedure for the SP.90 was used to increase the sensitivity of measurement. A 1000 µg/ml zinc standard from Fisher[1] was used for the zinc analyses. Iron wire was dissolved in 1:1 HNO_3 and made to volume with deionized water for the 1 mg/ml iron standard. Copper metal was dissolved in a minimum volume of conc. HNO_3 and diluted with 1% HNO_3 to volume to give 1 mg/ml standard. Working standards were made from this stock.

Plasma alkaline phosphatase

A 37°C, fifteen-minute incubation method described by Wooton (72), was used. It involves a potassium ferricyanide reagent and spectrophotometric reading at 510 nm.

---

[1]Fisher Scientific Company, Fairlawn, New Jersey, USA.

Plasma proteins

Total protein determinations were done with the Biuret method of Weichselbaum (73, 74). Albumin determination followed the procedure of Dumas (73), involving bromcresol green. Using literature concentrations of fibrin in rat plasma (75), calculations of per cent globulin were made.

Tissue moisture

Liver, muscle and mid-brain moisture determinations were made in a vacuum oven following a procedure in the A.O.A.C.[1] manual (76).

Tissue zinc, copper, iron and sodium

All tissue samples were digested in a 1:1 dilution of nitric acid according to the method of Slavin et al. (77). Stock standards were processed in a manner similar to that for plasma. Enough deionized water was used until the same nitric acid/water ratios were obtained as in the digested samples. Emission spectrophotometry (68), rather than absorption spectrophotometry, was used for sodium analyses. No light source lamp was needed. Only air:acetylene (5 litres:1 liter/min.) was used. The wavelength was 560 mm. Generally, a higher level of digest dilution was needed to get samples within the linear range of the instrument.

---

[1]Association of Official Analytical Chemists.

Liver protein

Liver protein determinations were done with a fast semi-automated microkjeldahl procedure (78). It allowed a maximum of nine samples to be run simultaneously in duplicates.

Urine creatinine

Previously freeze-dried urine samples, representing 72 hours urine excretions, were dissolved in deionized water. To get reasonably high creatinine concentrations, the minimum volume of water possible was used. Creatinine determinations were done with a method described by Wooton (72).

Statistical analyses

Results of each experiment were analyzed with the General Linear Model (GLM) regression procedure used in the SAS computer program system (79). Mean values were further compared by the PROC TTEST (i.e., t-test) procedure of the SAS system (80). Correlation analyses were also done using the same program system.

RESULTS

Experiment I

The experiment was designed to find out how long it would take to induce kwashiorkor, using the low-protein diets. It was also to check the effect of different dietary zinc concentrations on PEM development and to follow the entire course of the disease until death.

Development of kwashiorkor symptoms

Lower food and water intakes (Table 4 and Figure 1) became apparent among the low dietary protein rats (LPLZ and LPHZ) before the end of the first week of feeding. By the end of that week, the food intake of the high protein rats (HPLZ and HPHZ) was significantly higher ($p \leq 0.05$) than that of the low protein rats. There was a nonsignificant, but slightly better food intake by the LPLZ rats compared with the LPHZ rats until the 3rd week when a reversal occurred.

Body-weight changes followed the food-intake patterns closely. Body weights of both low-protein groups fell continuously until an approximate minimum of 31.0 gm per animal had been reached on the 5th week (Table 5 and Figure 2). There was a slightly faster weight loss by the LPHZ as compared to LPLZ group. In the 3rd week, LPLZ body weights began falling fast until they matched the LPHZ weights.

By the end of the first week, the high protein rats (which had been gaining weight) were significantly ($p \leq 0.05$) heavier than the low protein rats. By the 6th week, HPHZ and HPLZ rats, respectively, weighed about 10 and 7 times more than the LP rats.

Table 4. Cumulative food intake of rats (Experiment I: depletion)

Dietary treatment	# of rats	Week 1	Week 2	Week 3	Week 6
LPLZ − 1	4	28.2 ± 1.7 gm[a]	50.6 ± 3.6	71.0 ± 6.3	124.6 ± 13.6
LPLZ − 2	5	27.7 ± 2.3 gm	49.8 ± 4.3	73.8 ± 5.7	142.3 ± 12.1
HPLZ − 3	7	59.4 ± 3.0	119.0 ± 5.9	181.1 ± 8.5	421.4 ± 25.2
Control (HPHZ) − 4	8	62.5 ± 3.2	141.6 ± 7.3	234.8 ± 10.7	540.1 ± 21.1
T-test comparison between					
LP treatments		NS	NS	NS	NS
HP treatments		NS	*	**	**
Significant dietary effect ($P \leq 0.05$)[b]		Pr only	Pr only	Pr (major effect) Z Pr x Z	Pr (major effect) Z Pr x Z

[a]Mean ± SEM (No. of rats).

[b]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

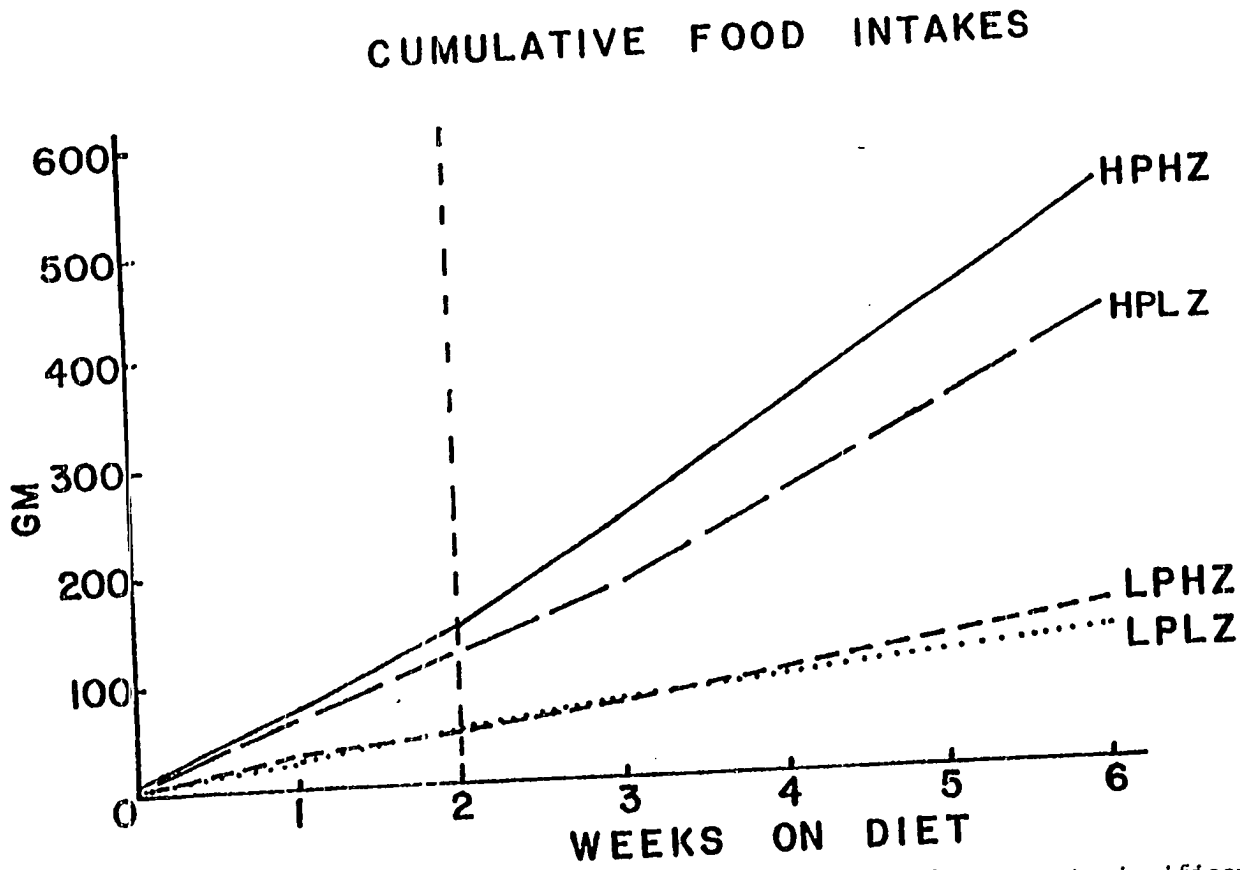


Figure 1. Cumulative food intake. Dietary zinc effect on food intake was not significant until after 2 weeks depletion

Table 5. Body weight of rats (Experiment I: depletion)

Dietary treatments	1st week 0 day	1st week 2 days	2nd week (9 days)	3rd week (16 days)	At death (45-84 days)
LPLZ – 1	52.8 ± 4.4[a] (9)	56.1 ± 2.0 (9)	46.7 ± 2.1 (9)	41.5 ± 1.7 (9)	31.8 ± 1.0 (9)
LPHZ – 2	53.4 ± 4.2 (8)	48.5 ± 2.6 (8)	43.4 ± 2.7 (8)	36.3 ± 1.8 (8)	31.0 ± 2.3 (8)
HPLZ – 3	56.0 ± 3.6 (12)	68.9 ± 2.6 (12)	99.6 ± 4.0 (12)	141.6 ± 9.6 (12)	316.7 ± 10.8 (12)
Control (HPHZ) – 4	53.3 ± 2.9 (10)	69.3 ± 3.7 (10)	115.4 ± 7.0 (10)	187.9 ± 10.5 (10)	382.4 ± 18.8 (10)
T-test comparison between					
LP treatments	–[b]	*	NS	NS	NS
HP treatments	–	NS	NS	**	**
Significant dietary effects ($P \leq 0.05$)[c]		Pr only	Pr Z[d]	Pr (major effect) Z Pr x Z	Pr (major effect) Pr x Z

[a] Mean ± SEM (No. of rats).

[b] No data.

[c] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

[d] Level of significance = 0.0686.

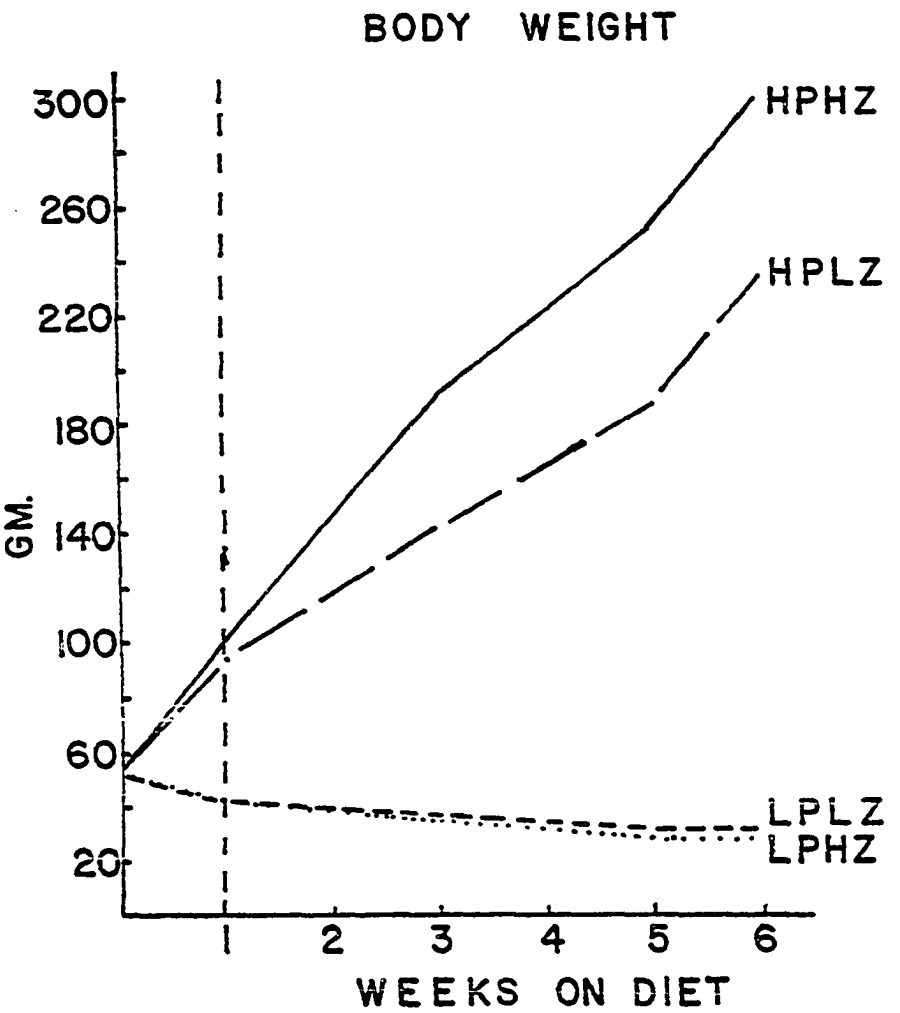


Figure 2. Growth curves. Only dietary protein affected body weight changes significantly in first week of feeding (Table 5)

Tables 4 and 5 indicate that the major cause of loss of body weight and loss of appetite in the protein-deficient groups was the lack of dietary protein. For the first two weeks, no effects of dietary zinc deficiency were seen at all. It was in the 3rd week, presumably because of depletion of body zinc stores, that dietary zinc effects began to show. This led immediately to quick loss of food intake and loss of body weight by the LPLZ rats. HPLZ rats also gained weight at a significantly slower rate than the control rats.

A severe reduction in physical activity among both groups of low-protein rats was seen in the second week. They did not resist any attempt to be picked up from their cages.

In the 3rd week, pronounced hair changes were noticeable among the low protein rats. Beginning with LPHZ rats, hair was lost around head and eyes. Body hair took on a rough appearance. By the 4th week, there was total hair loss on tails of most low dietary protein rats.

Edema appeared in the 5th week. First among the LPLZ rats and a few days later, among the LPHZ rats. The sequence was repeated in Experiments II and III. Statistically (Table 22), the difference in day of onset was not significant. The edema was characterized by a bloated appearance, followed by localized fluid accumulation at the chest, sometimes spreading down between the fore legs. Swelling of dorsal surfaces of fore feet was noticed in Experiment I. Sometimes slight increases in body weight accompanied the edema. The edema sometimes diminished or disappeared altogether and reappeared a few days later.

of dermatoses among the low protein rats were about 20%. Most dermatosis occurred on the dorsal surfaces of the fore paws but a few had it on the tails. Some few severe cases of peeling of large areas of inner sides of the fore legs and on the hind legs up towards the thighs were seen. One case of skin peeling on the abdomen was seen. It was impossible to decide which low-protein group had more skin disorders.

Severe cases of upward curvature of the spine occurred in both low protein groups, after approximately 5 weeks. The same observation was made in Experiment II. In depletion studies III and IV, this was not seen.

Some eye disorders observed by others (30a, 31a) were also seen in this experiment and systematically checked in Experiment II. The disorders were accompanied by keratinization (31b).

Urine creatinine excretion is known to reflect overall body musculature (81). The data in Table 6 suggest that the PEM (i.e., low dietary protein) rats excreted significantly ($p \leq 0.05$) less urinary creatinine than the high protein rats. The high protein rats on low zinc (HPLZ) excreted significantly ($p \leq 0.01$) less creatinine than the controls (HPHZ). Among the PEM rats, LPHZ excreted numerically (but not statistically significantly) lower amounts of creatinine. Dietary protein, zinc and their interactions show equal effects on the creatinine excretion at this 7th week of study. The low-protein rats had significantly lower body length and creatinine/body length ratios. Dietary protein effects are the major causative factor.

Body, liver, spleen and kidney weights were significantly lower among the PEM (i.e., LPLZ and LPHZ) rats (Table 7). There was also a significant ($p \leq 0.01$) difference between the body, liver and kidney

Table 6. Body length and urine creatinine of rats after 35 days on diet (Experiment I: depletion)

Dietary treatment	Urine creatinine (mg/dl)	Body length (cm)	$\frac{\text{Creatinine}}{\text{Body length}}$ ratio
LPLZ − 1	1.4 ± 0.13[a] (9)	13.7 ± 0.21[b] (9)	0.07 ± 0.01 (9)
LPHZ − 2	0.68 ± 0.09 (9)	13.2 ± 0.25 (9)	0.05 ± 0.01 (9)
HPLZ − 3	5.32 ± 0.41 (12)	20.5 ± 0.23 (12)	0.26 ± 0.02 (12)
Control (HPHZ) − 4	9.35 ± 0.74 (13)	22.4 ± 0.31 (13)	0.41 ± 0.03 (13)
T-test comparison between			
LP treatments	NS	NS	NS
HP treatments	**	**	**
Significant dietary effects ($P \leq 0.05$)[c]	Equal effects of Pr, Z, Pr x Z	Pr, Pr x Z, Z Major effects	Pr, Pr x Z, Z Major effects

[a]Mean ± SEM (No. of rats).

[b]Distance from tip of nose to base of tail. Tail tips excluded for this study because of necrosis.

[c]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

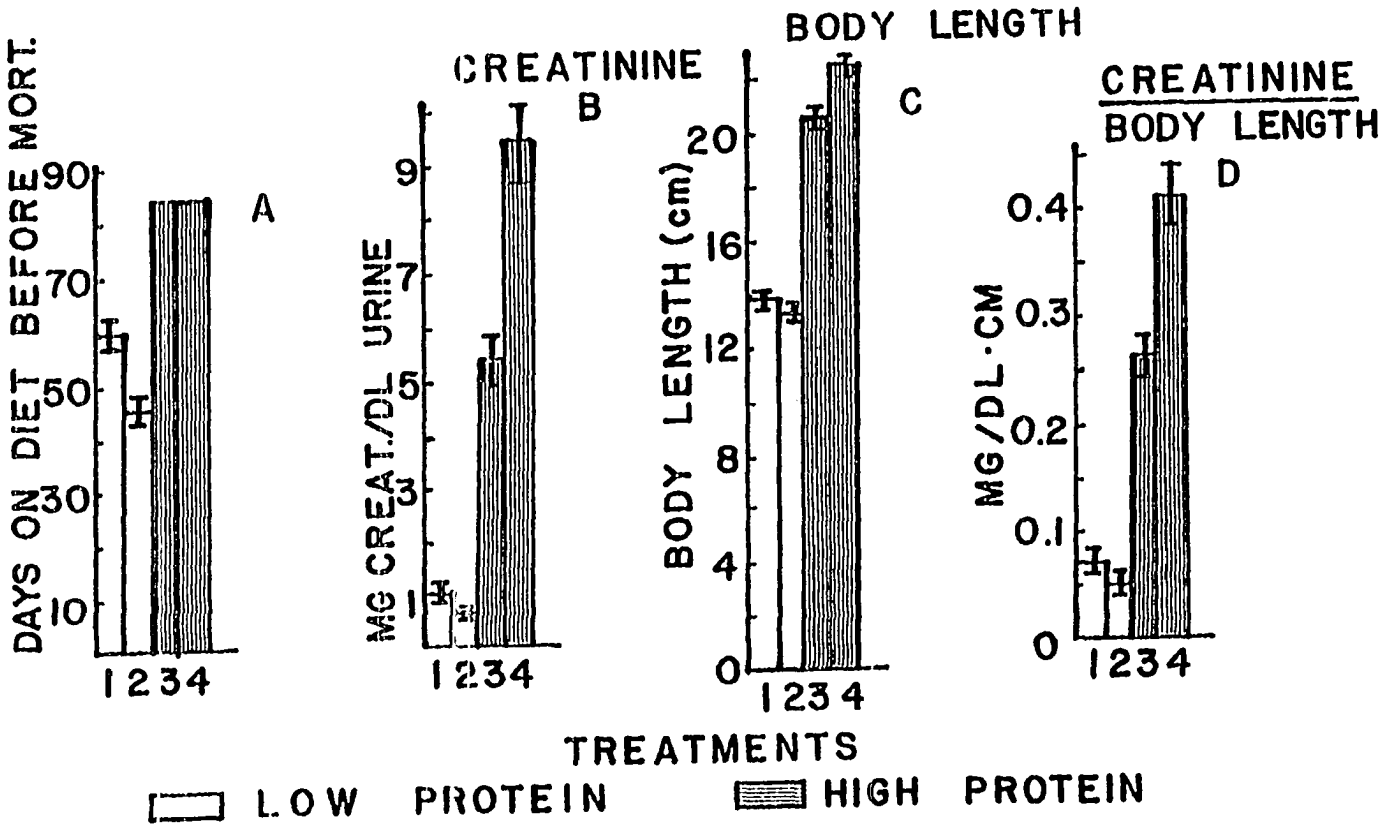


Figure 3. (A) Treatment 2 (LPHZ) showed significantly earlier mortality than treatment 1 (LPLZ) in Experiment 1. (B-D) Significantly different values for high-protein rates (Table 6)

Table 7. Fresh organ weights and organ/body weight ratios of rats at death (Experiment I: depletion)

Dietary treatments	Body weight (gm)	Liver	
		Weight (gm)	Liver / Body wt. ratio
LPLZ − 1	31.8 ± 1.0[a] (9)	0.93 ± 0.04 (9)	0.03 ± 0.00 (9)
LPHZ − 2	31.0 ± 22.3 (8)	0.95 ± 0.10 (7)	0.03 ± 0.00 (7)
HPLZ − 3	316.7 ± 10.8 (12)	10.0 ± 0.62 (12)	0.03 ± 0.00 (12)
Control (HPHZ) − 4	386.5 ± 17.9 (13)	13.4 ± 0.41 (13)	0.04 ± 0.00 (13)
T-test comparison between			
LP treatments	NS	NS	NS
HP treatments	**	**	NS
Significant dietary effects ($P \leq 0.05$)[b]	Pr (Major effect) Z Pr x Z	Pr (Major effect) Z Pr x Z	Pr only

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Spleen		Kidney	
Weight (gm)	Spleen / Body wt. ratio	Weight (gm)	Kidney / Body wt. ratio
0.02 ± 0.01 (9)	0.0007 (9)	0.44 ± 0.02 (9)	0.014 ± 0.000 (9)
0.04 ± 0.01 (5)	0.0013 (5)	0.46 ± 0.04 (5)	0.014 ± 0.000 (5)
0.56 ± 0.04 (12)	0.0018 (12)	2.13 ± 0.07 (12)	0.007 ± 0.000 (12)
0.63 ± 0.03 (12)	0.0016 (12)	2.73 ± 0.10 (12)	0.007 ± 0.000 (12)
NS NS	NS NS	NS **	NS NS
Pr only	Pr (Major effect) Pr x Z	Pr (Maior effect) Z Pr x Z	Pr only

Skin disorders were noticed. Most incidences of dyspigmentation were noticed on the tails. Lesions on tails were also seen. While liver/body weight, and spleen/body weight ratios were significantly ($p \leq 0.05$) lower in the PEM rats, their kidney/body weight ratios were significantly higher than in the high protein rats. The differences in organ weights and organ/body weight ratios are mainly an effect of dietary protein level.

After 2 days on diet, there was a slightly numerically higher (NS)[1] plasma alkaline phosphatase for the high-protein rats. After 9 days (Table 9), there was still no significant difference between the low protein rats and high protein rats. The rats on high zinc (regardless of dietary protein), however, did not show significantly higher alkaline phosphatase. In Table 10, after 84 days, there was still no significant difference between alkaline phosphatase levels of the high protein rats. Level of zinc deficiency (6 ppm) induced in these studies was not severe. For both alkaline phosphatase and copper (Table 8), plasma levels were not significantly different between any of the four treatments. HPLZ rats were the first to show significantly depressed plasma zinc concentration. After 9 days (Table 9), plasma zinc had fallen significantly in all groups except the control (i.e., HPHZ). The PEM rats fed high zinc (LPHZ) had significantly ($p \leq 0.01$) higher levels than LPLZ. The controls had significantly ($p \leq 0.01$) higher levels than HPLZ. Dietary protein, zinc and Pr x Z interactions equally affect the plasma zinc concentrations.

---

[1] Not significant.

Table 8. Plasma concentration of alkaline phosphatase, zinc and copper of rats after 2 days on diets (Experiment I: depletion)

Dietary treatment	Alkaline phosphatase (KAU%)[a]	Zinc (μg/ml)	Copper (μg/ml)
LPLZ – 1	48.7 ± 7.9[b] (9)	2.52 ± 0.14 (5)	–[c]
LPHZ – 2	46.3 ± 8.1 (8)	2.03 ± 0.21 (7)	1.58 ± 0.07 (4)
HPLZ – 3	54.7 ± 4.5 (12)	1.37 ± 0.09 (12)	1.63 ± 0.09 (10)
Control (HPHZ) – 4	54.2 ± 7.6 (10)	2.50 ± 0.21 (9)	1.58 ± 0.08 (10)
T-test comparison between			
LP treatments	NS	NS	–[c]
HP treatments	NS	**	NS
Significant dietary effects ($P \leq 0.05$)[d]	No sig. Pr or Z effects	Pr x Z	No sig. Pr or Z effects

[a] King Armstrong Units/100 ml plasma.

[b] Mean ± SEM (No. of rats).

[c] No data.

[d] Pr = dietary zinc; Z = dietary zinc; Pr x Z = interaction.

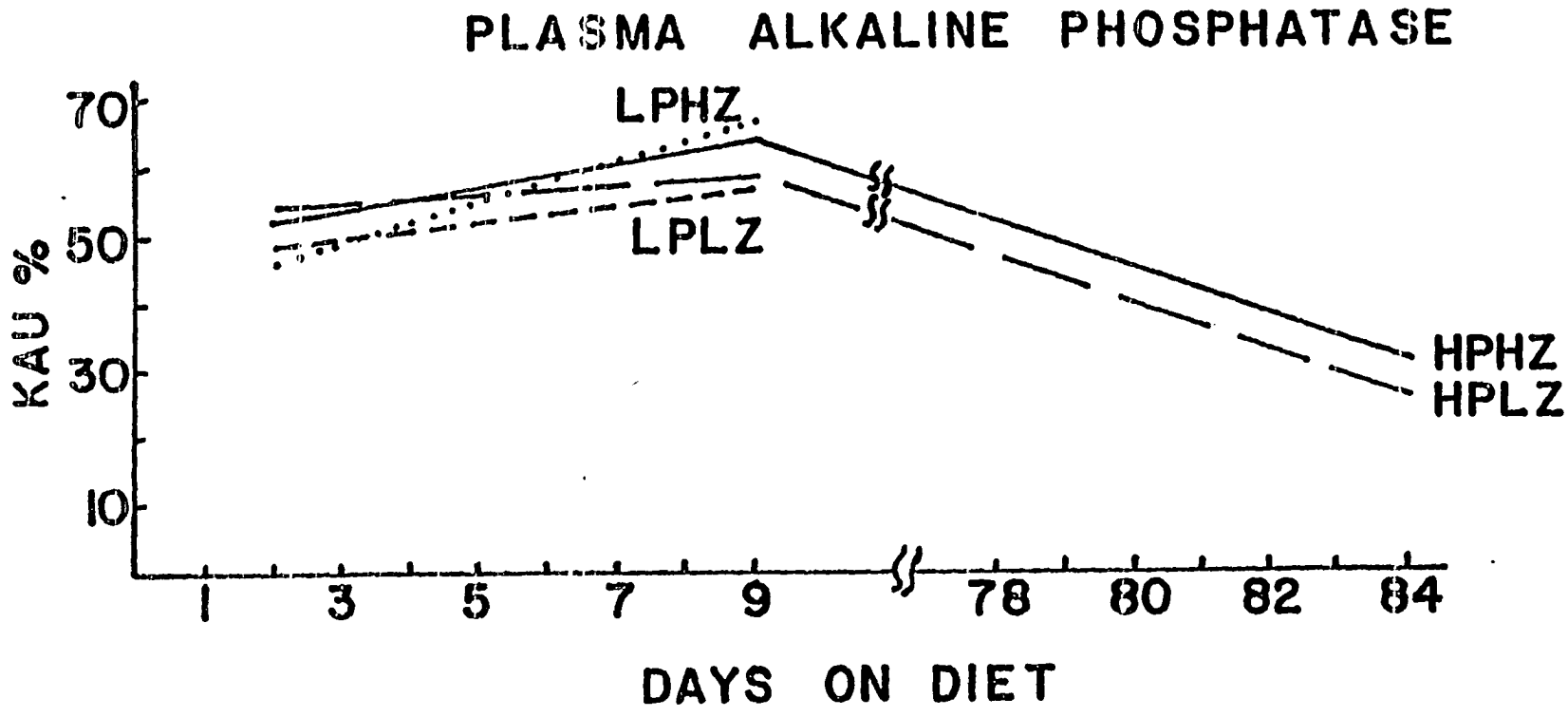


Figure 4. Plasma alkaline phosphatase on days 2 and 9 for LP rats and days 2, 9 and 84 for HP rats. Decreasing levels with age confirm literature (Tables 8-10)

Table 9. Plasma concentration of alkaline phosphatase, zinc, proteins and copper of rats after 9 days on diet (Experiment I: depletion)

Dietary treatment	Alkaline phosphatase (KAU%)	Zinc (μg/ml)
LPLZ − 1	56.8 ± 3.8[a] (9)	0.84 ± 0.05 (9)
LPHZ − 2	66.5 ± 6.6 (9)	1.34 ± 0.14 (9)
HPLZ − 3	54.6 ± 3.9 (12)	1.0 ± 0.04 (12)
Control (HPHZ) − 4	61.5 ± 6.9 (13)	2.66 ± 0.15 (13)
T-test comparison between		
LP treatments	NS	**
HP treatments	**	**
Significant dietary effects ($P \leq 0.05$)[b]	No sig. Pr or Z effects	Pr Z Pr x Z

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

[c] No data.

Proteins (gm/dl)				Copper (μg/ml)
Total	Albumin	Globulin	A/G ratio	
5.82 ± 0.18 (9)	2.87 ± 0.09 (9)	2.71 ± 0.20 (9)	1.11 ± 0.09 (9)	–[c]
4.74 ± 0.29 (9)	2.75 ± 0.21 (9)	1.80 ± 0.10 (9)	1.55 ± 0.12 (9)	–
6.42 ± 0.15 (12)	3.96 ± 0.09 (12)	2.20 ± 0.12 (12)	1.86 ± 0.12 (12)	1.24 ± 0.06 (12)
6.35 ± 0.12 (13)	3.86 ± 0.12 (13)	2.30 ± 0.14 (13)	1.84 ± 0.18 (13)	1.24 ± 0.06 (13)
**	NS	**	**	NS
NS	*	NS	NS	NS
Pr (Major effect)	Pr only	Z	Pr only	No sig. Pr or Z effects
Z		Pr (Major effect)		

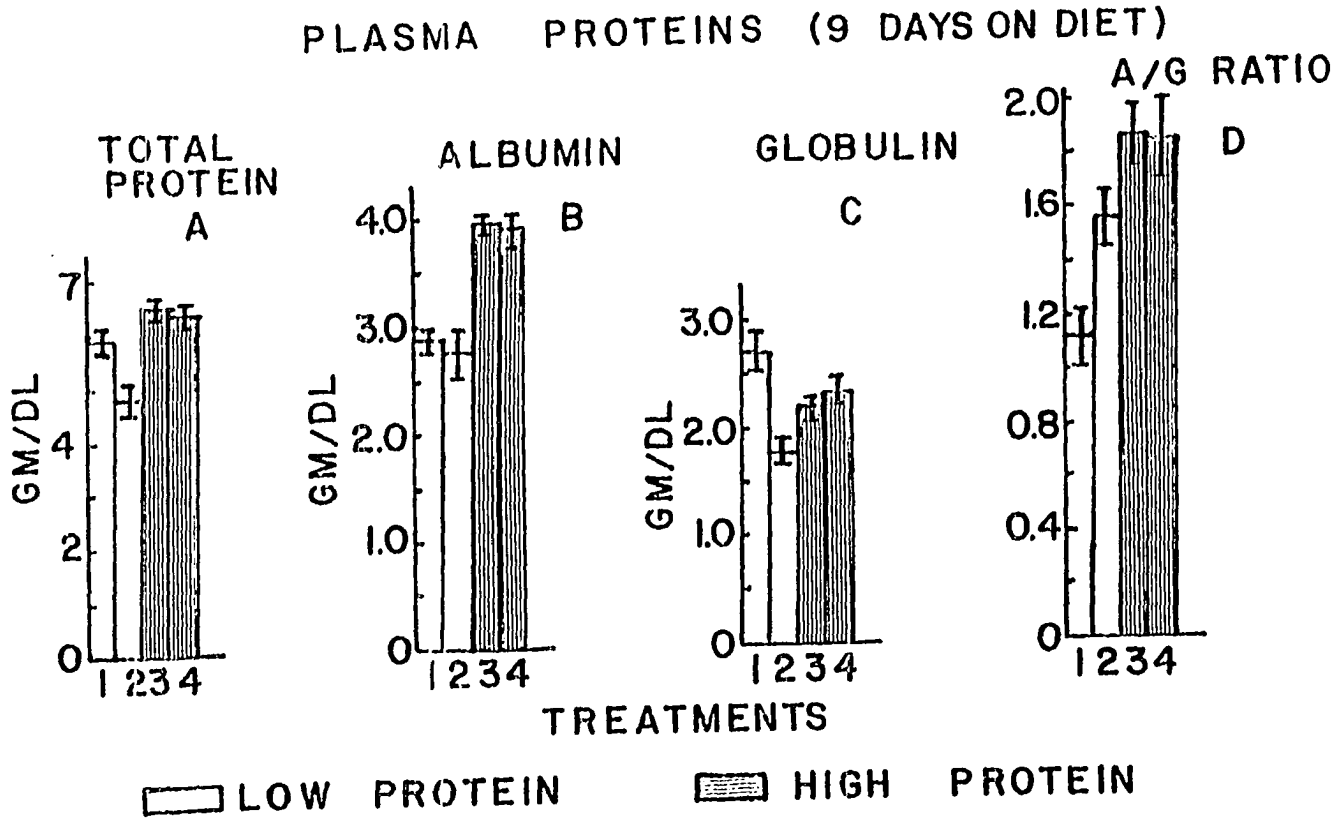


Figure 5. Significantly different plasma protein levels in A, C, and D for low protein rats and in B for high-protein rats (Table 9)

High dietary protein rats had significantly higher plasma protein contents than the PEM rats. Main effects here are dietary protein. In the case of plasma globulin, there is a Pr x Z main effect. The LPHZ rats had a significantly ($p \leq 0.01$) lower plasma total protein and globulin and also lower (NS) albumin than the LPLZ rats. The A/G (Albumin/Globulin) ratio had the same pattern between them.

Again, no dietary effects were seen on plasma copper either on the 9th day (Table 9) or 84th day (Table 10) of the study.

A different picture emerges for copper in the liver (Table 11). Dietary effects were seen on the liver copper concentrations as compared with plasma (Tables 8, 9, 10). The LPLZ rats had significantly higher ($p \leq 0.05$) concentrations than the LPHZ rats. Since the LPLZ rats had significantly lower liver weights, their total liver copper concentrations were significantly lower than for the high dietary protein rats.

There was a significantly higher concentration of moisture in the livers of the PEM rats. This condition seems to be due mainly to differences in dietary protein.

On a wet weight basis, livers of PEM rats had significantly lower protein levels (gm/100 gm). A check on Experiment IV (Table 33), showed a dietary protein effect also on a dry weight basis.

Just as in the case of copper (Table 11), the PEM rats had significantly higher liver zinc and iron concentrations (µg/gm).

Regardless of dietary protein levels, the rats fed low zinc had significantly ($p \leq 0.05$) higher liver iron concentrations. For the rats fed high protein, this picture was repeated in Experiment III

Table 10. Plasma alkaline phosphatase, zinc, copper and proteins of rats after 84 days (Experiment I: depletion)

Dietary treatment	Alkaline phosphatase (KAU%)	Zinc (μg/ml)	Copper (μg/ml)
HPLZ − 3	26.2 ± 2.1[a] (12)	1.46 ± 0.11 (12)	1.38 ± 0.05 (12)
Control (HPHZ) − 4	30.2 ± 1.8 (13)	2.69 ± 0.11 (13)	1.47 ± 0.09 (13)
T-test comparison between HP treatments	NS	**	NS
Significant dietary effects ($P \leq 0.05$)[b]	No sig. Pr or Z effects	Z only	No sig. Pr or Z effects

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc.

Proteins (gm/dl)			
Total	Albumin	Globulin	A/G ratio
7.97 ± 0.16 (12)	4.30 ± 0.13 (12)	3.35 ± 0.15 (12)	1.32 ± 0.08 (12)
8.89 ± 0.30 (13)	4.55 ± 0.11 (13)	3.99 ± 0.30 (13)	1.20 ± 0.08 (13)
*	NS	NS	NS
Z	No sig. Z effect	No sig. Z effect	No sig. Z effect

Table 11. Liver protein and copper of rats at death (Experiment I: depletion)

Dietary treatment	Wet liver weight (gm)	% liver moisture[a]
LPLZ - 1	0.93 ± 0.04[b] (9)	80.9 ± 1.8 (11)
LPHZ - 2	0.95 ± 0.11 (7)	80.1 ± 0.9 (10)
HPLZ - 3	10.1 ± 0.6 (12)	67.5 ± 1.4 (12)
Control (HPHZ) - 4	13.4 ± 0.4 (12)	65.8 ± 1.4 (12)
T-test comparison between		
LP treatments	NS	NS
HP treatments	NS	NS
Significant dietary effects ($P \leq 0.05$)[c]	Pr (Major effect) Z Pr x Z	Pr only

[a]Taken from Experiment II for LP treatments; from Experiment I for HP treatments.

[b]Mean ± SEM (No. of rats).

[c]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Protein			Copper		
Dry liver (gm/100 gm)	Wet liver (gm/100 gm)	Total liver (gm)	Dry liver (µg/gm)	Wet liver (µg/gm)	Total liver (µg)
52.6 ± 3.0 (9)	10.0 ± 0.6 (9)	0.094 ± 0.002 (9)	34.4 ± 3.5 (9)	6.58 ± 0.66 (9)	6.16 ± 0.78 (9)
44.2 ± 4.4 (7)	8.8 ± 0.9 (7)	0.084 ± 0.014 (7)	24.4 ± 2.2 (6)	4.85 ± 0.43 (6)	4.75 ± 0.33 (6)
52.3 ± 0.6 (12)	17.0 ± 0.2 (12)	1.723 ± 0.111 (12)	14.6 ± 0.6 (12)	4.73 ± 0.18 (12)	47.36 ± 2.91 (12)
48.5 ± 0.8 (12)	16.6 ± 0.3 (12)	2.240 ± 0.090 (12)	16.0 ± 1.4 (12)	5.46 ± 0.47 (12)	73.41 ± 6.82 (12)
NS **	NS NA	NS **	** NS	NS NS	NS **
Z only	Pr only	Pr Pr x Z	Pr (Major effect) Z Pr x Z	Pr x Z	Pr (Major effect) Z Pr x Z

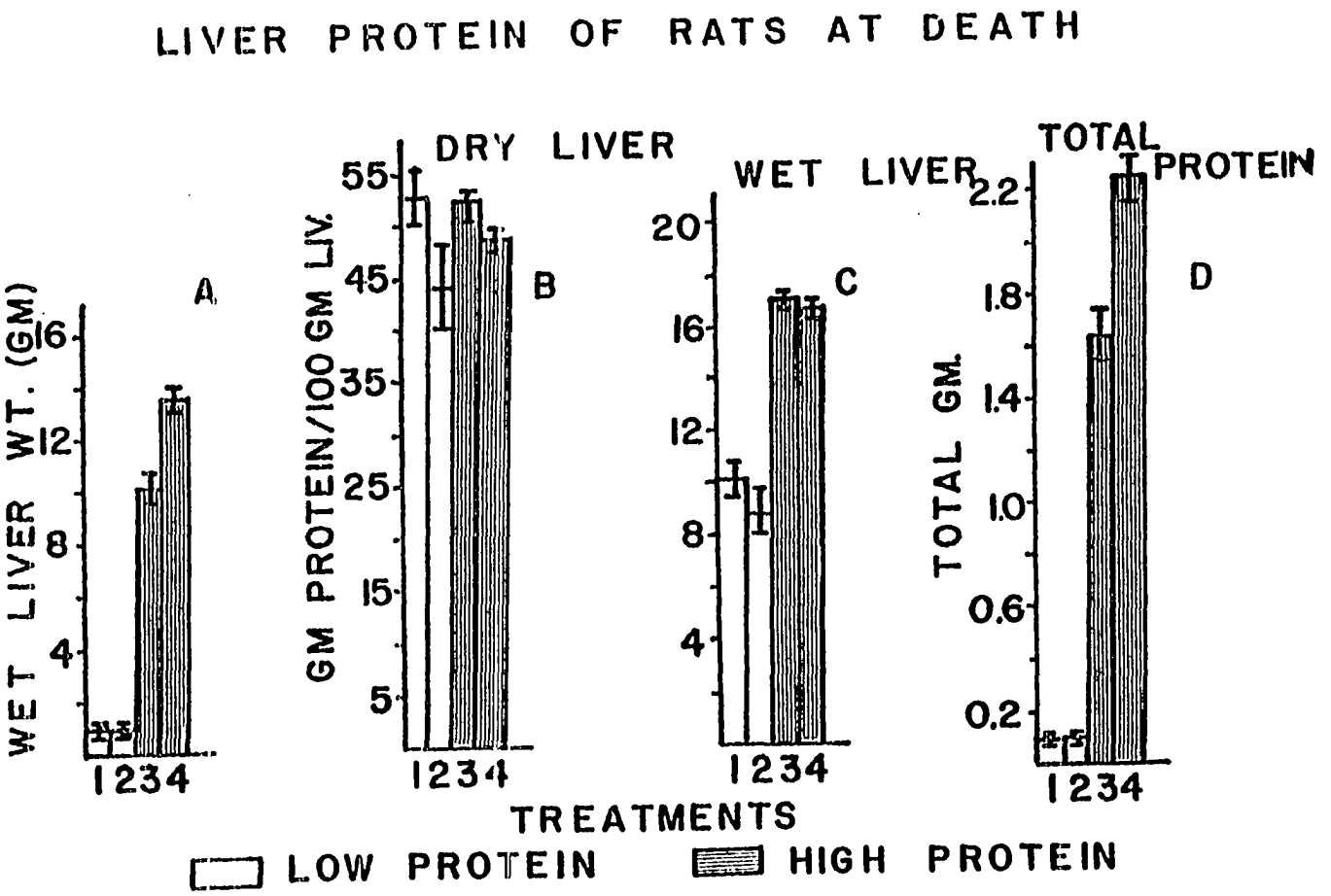


Figure 6. Liver proteins. Differences significant only for HP rats. In A, B and D (Table 11)

Table 12. Liver zinc and iron of rats at death (Experiment II: depletion)

Dietary treatments	Wet liver weight (gm)	% liver[a] moisture
LPLZ - 1	0.93 ± 0.04[b] (9)	80.9 ± 0.9 (11)
LPHZ - 2	0.95 ± 0.11 (7)	80.1 ± 0.9 (10)
HPLZ - 3	10.1 ± 0.6 (12)	67.5 ± 1.4 (12)
Control (HPHZ) - 4	13.5 ± 0.4 (12)	65.8 ± 1.4 (13)
T-test comparison between		
LP treatments	NS	NS
HP treatments	**	NS
Significant dietary effects ($P \leq 0.05$)[c]	Pr (Major effect) Z Pr x Z	Pr only

[a]Data for LP treatments from Experiment II; HP treatment data from Experiment I.

[b]Mean ± SEM (No. of rats).

[c]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Protein			Copper		
Dry liver (µm/100 gm)	Wet liver (µm/100 gm)	Total liver (µm)	Dry liver (µg/gm)	Wet liver (µg/gm)	Total liver (µg)
211.5±20.0 (9)	40.4±3.8 (9)	38.0±4.8 (9)	2240±242 (9)	427.9±46.2 (9)	398.0±46,9 (9)
135.1±12.0 (6)	26.9±2.4 (6)	26.0±0.8 (6)	1358±103.9 (5)	272.7±17.2 (6)	271.2±21.5 (6)
87±1.4 (12)	28.3±0.44 (12)	284.8±16.3 (12)	382.5±26.5 (12)	124.4±8.6 (12)	1229.8±78.5 (12)
85.6±2.3 (12)	29.3±0.9 (12)	394.0±15.1 (12)	306.7±17.0 (12)	104.2±5.8 (12)	1401.2±79.3 (12)
*	*	NS	*	*	NS
NS	NS	**	*	NS	NS
Pr (Major effect)	Pr	Pr (Major effect)	Pr (Major effect)	Pr (Major effect)	Pr (Major effect)
Z	Z	Z	Z	Z	Z
Pr x Z	Pr (Major effect)	Pr x Z	pr x Z	Pr x Z	Pr x Z

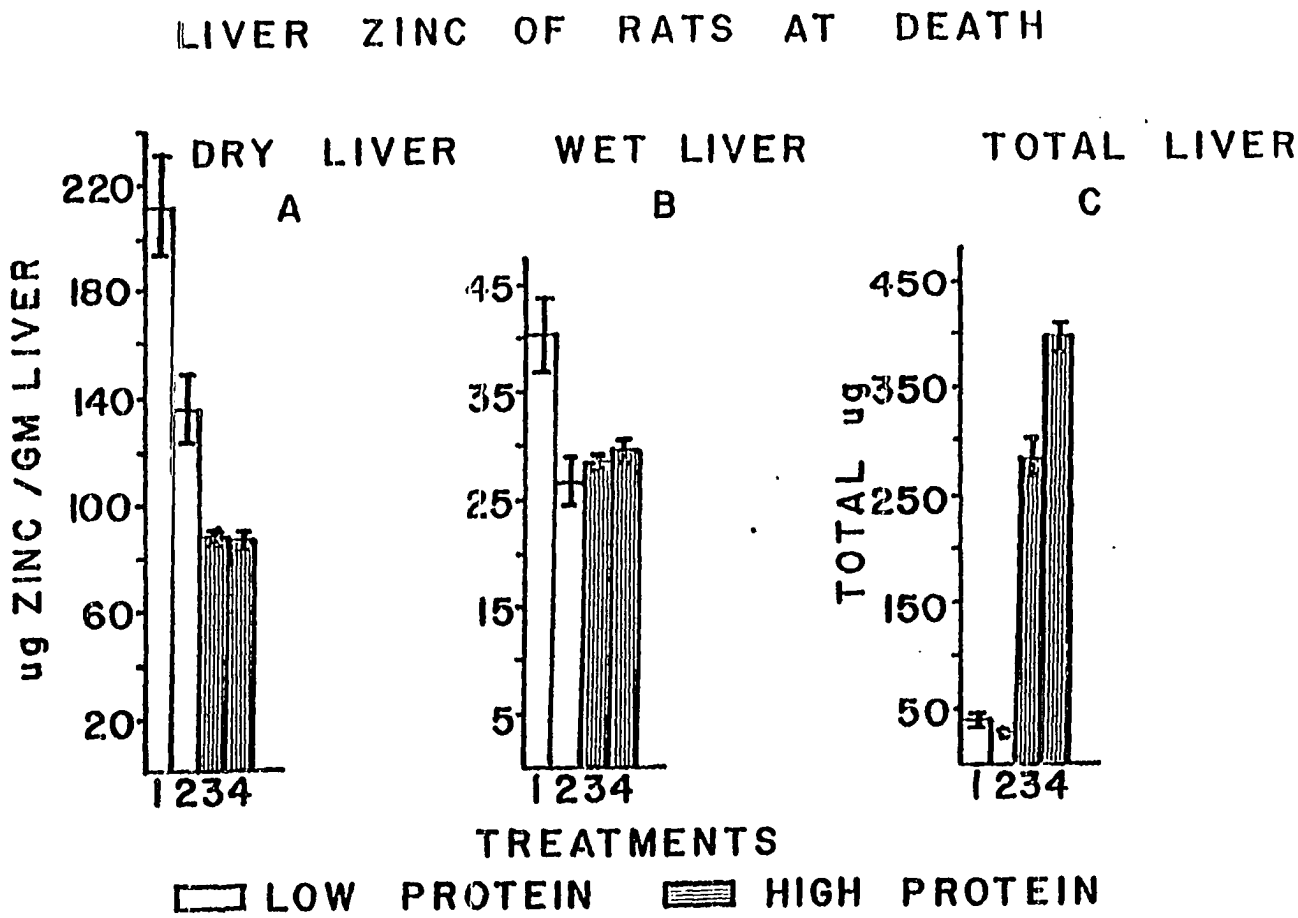


Figure 7. Liver zinc. Significantly different values in A and B for LP rats and in C for HP rats (Table 12)

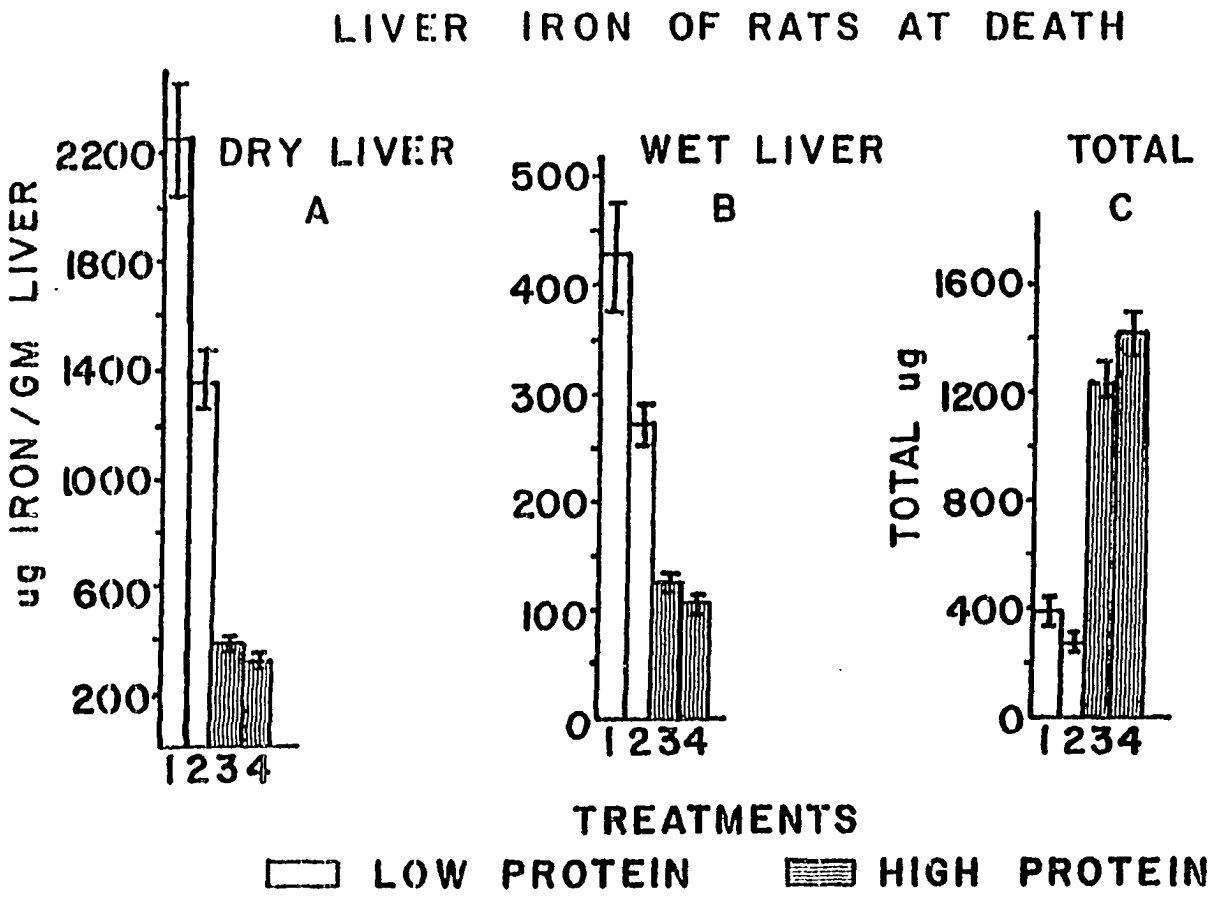


Figure 8. Liver iron. Significantly different for LP rats in A and B. Significantly different for HP rats in A (Table 12)

(Tables 16, 17, 19) for muscle, liver and brain. It was confirmed again in Experiment IV (Table 29) where perfused livers were examined. Dietary protein effects were mainly responsible for the differences in liver iron and zinc. Dietary zinc and Pr x Z effects were present but secondary.

The concentrations (µg/gm) of minerals - zinc, copper and iron - in the liver showed significant negative correlations (-0.74, -0.71, -0.84, respectively) with body weight. They show a similar relationship (-0.77, -0.75, -0.87 for zinc, copper, iron, respectively) with dietary protein. The picture is the same for their relationship with liver protein. Iron showed the highest negative correlation in these instances. Iron did the same in Experiment III (Table 21).

Concentrations (µg/gm) of the minerals also correlated negatively with their respective total liver concentrations. Thus, for µg/gm mineral vs. total µg mineral, r is -0.69, -0.63, -0.64 for zinc, copper and iron, respectively.

From the weeks 6-8, about 70% of the PEM rats (LPLZ and LPHZ combined) died. No deaths occurred among high protein rats. This was due apparently to dietary protein differences. Zinc did have some secondary effect in causing significantly ($p \leq 0.01$) earlier deaths among the PEM rats fed high dietary zinc (LPHZ). On the average, the LPHZ rats died two weeks earlier than the LPLZ rats. By the 84th day of the study (12 weeks) no death had occurred among the rats fed high protein regardless of the dietary zinc level. The zinc deficiency induced in this study (6 ppm dietary zinc) was moderate.

Table 13. Liver correlation coefficients (r-values) from rats. Only values > 0.5 are reported (except for liver protein). Level of significance in all cases = 0.0001 (in range = 36-41. Experiment I: depletion)

Variable	Body wt.	Wet liver wt.	Zinc		Copper	
			D[a]	W[b]	D	W
Dietary protein	0.96	0.94	-0.77		-0.75	
Dietary zinc	–	–	–	–	–	–
Body weight	–	–	-0.74	–	-0.71	–
Wet liver weight	–	–	-0.74	–	-0.70	–
Zinc (dry)	–	–	–	–	0.93	0.63
Zinc (wet)	–	–	–	–	–	0.75
Copper (dry)	–	–	–	–	–	–
Copper (wet)	–	–	–	–	–	–
Iron (dry)	–	–	–	–	–	–
Iron (wet)	–	–	–	–	–	–
Total zinc	–	–	–	–	–	–

[a]D = dry liver.

[b]W = wet liver.

[c]Not significant.

Iron		Protein (gm/100 gm)		Total zinc	Total copper	Total iron	Total (gm)
D	W	D	W				
-0.87	-0.83	0.11[c]	0.93	0.93	0.84	0.90	0.94
–	–	–	–	–	–	–	–
-0.84	-0.81	0.09	0.90	0.90	0.88	0.90	0.98
-0.83	-0.81	–	0.88	0.98	0.91	0.92	1.00
0.87	0.86	–	-0.64	-0.69	-0.63	-0.64	-0.74
0.62	0.63	–	–	–	–	–	–
0.81	0.79	–	-0.66	-0.65	–	-0.63	-0.71
–	–	–	–	–	–	–	–
–	–	–	-0.72	-0.81	-0.74	-0.70	-0.83
–	–	–	-0.66	–	-0.71	–	-0.80
–	-0.78	–	0.87	–	–	0.91	0.98

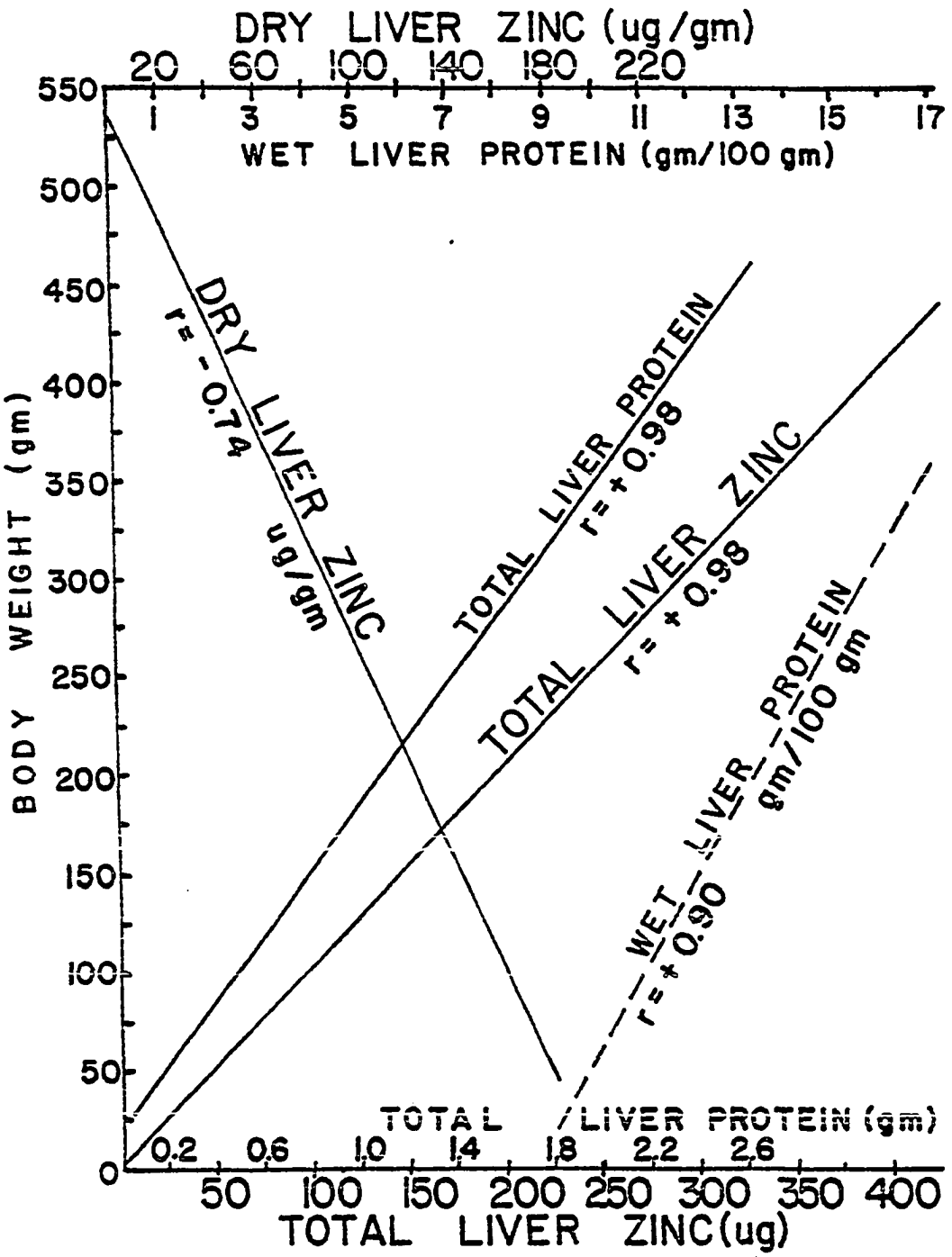


Figure 9. Regression relationships between body-weight, total zinc and other liver parameters (Table 13)

Table 14. Muscle moisture; number of days on diet before death; muscle zinc and iron of rats at death (Experiment I: depletion)

Dietary treatment	% muscle[a] moisture	No. of days before death
LPLZ - 1	71.7 ± 1.5[b] (9)	59.8 ± 2.6 (9)
LPHZ - 2	75.0 ± 1.3 (7)	45.5 ± 2.3 (8)
HPLZ - 3	71.5 ± 0.7 (10)	No deaths (12)
HPHZ - 4	71.4 ± 0.6 (9)	No deaths (12)
T-test comparison between		
LP treatments	NS	**
HP treatments	NS	NS
Significant dietary effects ($P \leq 0.05$)[c]	No sig. dietary effects	Pr (Major effect) Z Pr x Z

[a]Data from Experiment II for LP and Experiment I for HP.

[b]Mean ± SEM (No. of rats).

[c]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Zinc		Iron	
Dry muscle (g/gm)	Wet muscle (g/gm)	Dry muscle (g/gm)	Wet muscle (g/gm)
66.0 ± 16.3 (9)	48.6 ± 4.8 (9)	337.9 ± 21.3 (8)	99.0 ± 6.2 (8)
26.4 ± 14.3 (7)	32.7 ± 3.7 (7)	332.3 ± 38.3 (7)	86.1 ± 10.1 (7)
53.0 ± 1.5 (12)	15.2 ± 0.4 (12)	121.5 ± 29 (12)	34.5 ± 0.8 (12)
63.0 ± 3.6 (10)	18.0 ± 1.0 (10)	123.4 ± 2.6 (9)	35.6 ± 0.7 (9)
NS *	* *	NS NS	NS NS
Pr (Major effect) Pr x Z	Pr (Major effect) Z Pr x Z	Pr only	Pr only

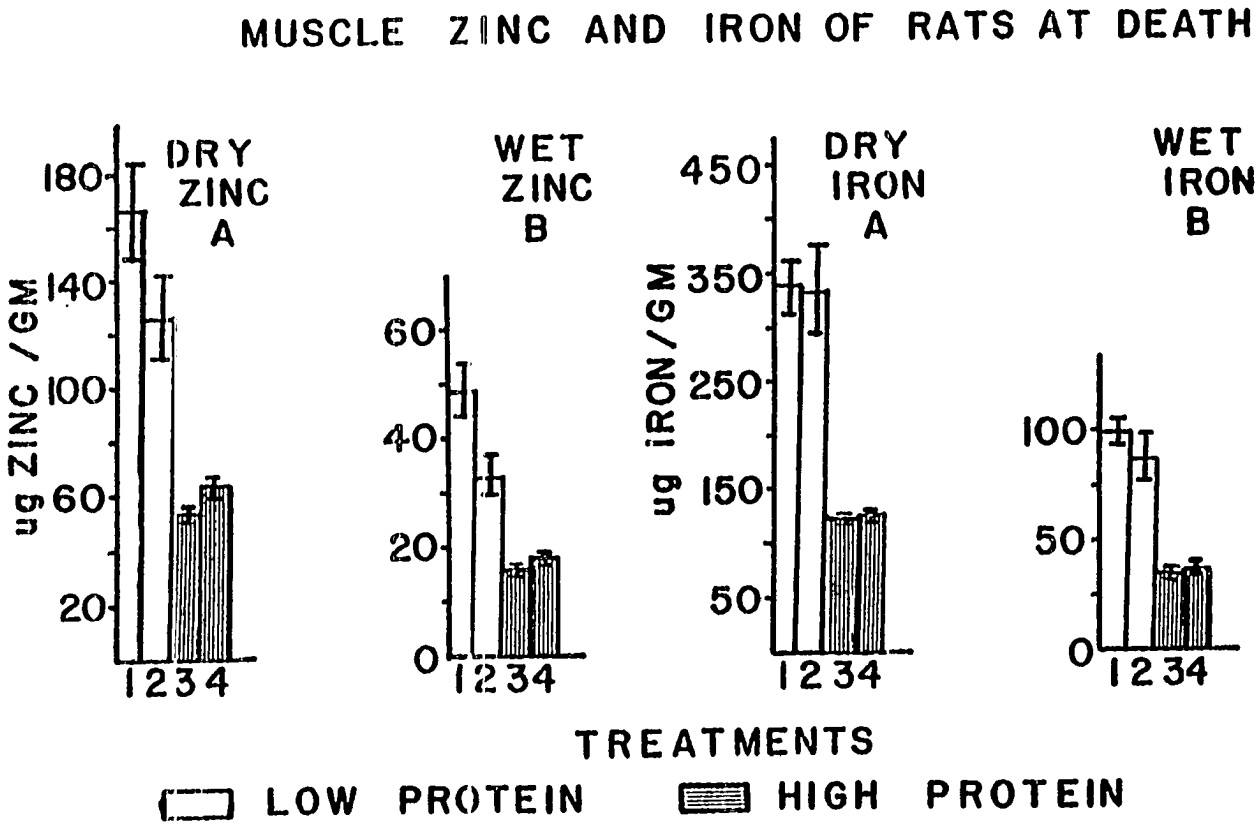


Figure 10. Muscle zinc and iron. Significantly different wet and dry tissue zinc values for LP rats. Significantly different wet-tissue zinc values for HP rats. No significant muscle iron differences (Table 14)

Muscle moisture, on the average, was the same in all treatments. No significant dietary effects occurred.

Significantly higher muscle zinc and iron concentrations occurred among the PEM rats compared to all others. In adequate dietary protein groups, muscle zinc concentration was significantly ($p \leq 0.05$) lower in the rats on low zinc.

Experiment II

This study was undertaken to find the effects of dietary zinc on survival rate during PEM. The course of development of PEM in this study was generally the same as for Experiment I. Only low protein diets (LPLZ and LPHZ) were used.

Eye disorders

Eye disorders occurred among both groups of PEM rats. Histologically, keratinization of the corneal and conjunctival epithelium is characteristic of xerophthalmia. Xerophthalmia is caused primarily by hypovitaminosis A. A degree of protein malnutrition is believed to be present (as in the case of our LP rats) when ocular symptoms develop (31b). In the presence of severe protein deficiency, xerophthalmia can be followed by keratomalacia. This is a severe ulceration of the cornea. If our LP rats had low plasma vitamin A - a possibility which wasn't checked - then the right conditions were present for keratomalacia. Fifty per cent of the LPLZ rats developed some eye disorder during the study. Nine

(i.e., 30%) of the 30 LPLZ rats had a total closure of one or both eyes at the end of the study.

Those fed high zinc had a lower incidence of eye disorders. The disorders were less serious in the LPHZ group. Altogether, 3 out of the 30 rats had a total closure of one eye or both at the end of the study.

Between the two PEM groups (Tables 22-23) death began one week earlier among the LPHZ rats. This time difference was not statistically significant. Deaths started 2 weeks earlier (Table 14) among the same LPHZ group in Study I. That was statistically significant.

Liver and muscle moisture were not significantly different between the low protein groups.

Experiment III

This study was conducted for exactly five weeks to check some of the findings of Experiment I. The brain, which was not looked at in Experiment I, was examined in this study. Following a report (82) of an effect of zinc on sodium transport in leucocytes during PEM, tissue sodium was also examined in this study.

As in Experiment I (Table 9), the PEM rats (Table 15) had significantly lower plasma proteins. In this as in the other two studies (Tables 9 and 25), dietary zinc had no significant effect on plasma albumin. Dietary zinc in all cases had a significant secondary effect on plasma globulin concentrations.

Table 15. Plasma proteins after 5 weeks on diet (Experiment III: depletion)

Dietary treatment	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A/G ratio
LPLZ – 1	2.34 ± 0.17[a] (10)	1.37 ± 0.13 (10)	0.87 ± 0.23 (10)	5.9 ± 2.3 (10)
LPHZ – 2	1.84 ± 0.16 (9)	1.29 ± 0.12 (9)	0.47 ± 0.11 (9)	3.6 ± 1.4 (8)
HPLZ – 3	4.77 ± 0.27 (9)	3.02 ± 0.07 (9)	1.56 ± 0.29 (9)	3.0 ± 0.8 (9)
Control (HPHZ) – 4	4.21 ± 0.12 (11)	3.00 ± 0.06 (11)	1.02 ± 0.13 (11)	3.6 ± 0.5 (11)
T-test comparison between				
• LP treatments	S	NS	NS	NS
• HP treatments	NS	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[b]	Pr (Major effect) Z	Pr only	Pr (Major effect) Z	No sig. dietary effects

[a]Mean ± SEM (No. of rats).

[b]Pr = dietary protein; Z = dietary zinc.

Table 16. Body weight, muscle zinc, iron, moisture and sodium after 5 weeks on diets (Experiment III: depletion)

Dietary treatments	Body wt. (gm)	% muscle H_2O	Zinc	
			Dry muscle (μg/gm)	Wet muscle (μg/gm)
LPLZ − 1	32.0 ± 0.3[a] (6)	71.7 ± 1.5 (10)	80.5 ± 3.3 (12)	23.6 ± 1.0 (12)
LPHZ − 2	32.3 ± 1.0 (6)	75.0 ± 1.3 (7)	86.6 ± 5.6 (12)	22.4 ± 1.4 (12)
HPLZ − 3	124.5 ± 7.9 (6)	71.5 ± 0.7 (10)	58.5 ± 3.3 (12)	16.7 ± 0.9 (12)
Control (HPHZ) − 4	208.2 ± 7.9 (6)	71.4 ± 0.6 (9)	57.7 ± 2.4 (12)	16.4 ± 0.7 (12)
T-test comparison between				
• LP treatments	NS	NS	NS	NS
• HP treatments	**	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[c]	Equal effects of Pr Z Pr x Z	Pr only	Pr only	Pr only

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Iron		Sodium	
Dry muscle (μg/gm)	Wet muscle (μg/gm)	Dry muscle (μg/gm)	Wet muscle (μg/gm)
210.0 ± 12.3 (12)	61.6 ± 3.6 (12)	2.44 ± 0.10 (12)	0.71 ± 0.03 (12)
199.2 ± 10.1 (12)	51.6 ± 2.6 (12)	2.57 ± 0.19 (12)	0.67 ± 0.05 (12)
123.5 ± 6.4 (12)	35.2 ± 1.8 (12)	1.59 ± 0.06 (12)	0.45 ± 0.02 (12)
111.8 ± 5.4 (12)	32.0 ± 1.5 (12)	1.39 ± 0.04 (12)	0.40 ± 0.01 (12)
NS	*	NS	NS
NS	NS	*	*
Pr only	Pr (Major effect) Z	Pr only	Pr only

As in Experiment I (Table 9) and Experiment IV (Table 25), PEM rats fed high zinc had the lowest plasma protein concentrations. No significant differenqes occurred between plasma protein concentration of control and the HPLZ rats.

Table 16 corresponds with Table 14 of Experiment I. The same muscle moisture patterns in Table 14 can be seen here. Confirming Experiment I, zinc and iron concentrations of muscle were significantly ($p \leq 0.05$) higher in the PEM rats. The highest muscle iron concentrations occurred with the rats fed low dietary zinc, reflecting trends seen in Tables 12 and 14 for liver and muscle. The same pattern occurred in the brain (Table 19).

There were significantly higher muscle sodium concentrations among the PEM rats (Table 12). In the presence of adequate dietary protein, rats fed low dietary zinc had the highest muscle sodium concentrations. The same occurred for liver (Table 18) and brain (Table 19). This was further confirmed in Experiment IV (Table 33). A similar relationship between sodium and zinc occurred with iron and zinc.

Most of the data (Table 19) and in Table 18 have been referred to in connection with Tables 12 and 16.

The PEM rats (LPLZ and LPHZ) had significantly ($p \leq 0.05$) lower mid-brain weights than those fed high protein diets. While control total liver weights in Experiments I, III and IV (Tables 12, 18, 33) were 5-13 fold higher than livers of PEM rats, mid-brain weights in Table 15 are less than 1.5 fold bigger in controls than in PEM rats, suggesting much faster

Table 17. Liver weight, moisture, zinc, and iron after 5 weeks on diet (Experiment III: depletion)

Dietary treatments	Wet liver weight (gm)	% liver moisture
LPLZ - 1	1.57 ± 0.08[a] (12)	80.9 ± 0.9 (11)
LPHZ - 2	1.45 ± 0.06 (12)	80.1 ± 0.9 (10)
HPLZ - 3	4.51 ± 0.24 (12)	67.5 ± 1.4 (12)
Control (HPHZ) - 4	7.60 ± 0.49 (12)	65.8 ± 1.4 (13)
T-test comparison between		
LP treatments	NS	NS
HP treatments	**	NS
Significant dietary effects ($P \leq 0.05$)[b]	Equal effects of Pr Z Pr x Z	Pr only

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein, z = dietary zinc; Pr x Z = Interaction.

Zinc			Iron		
Dry liver (µg/gm)	Wet liver (µg/gm)	Total liver (µg)	Dry liver (µg/gm)	Wet liver (µg/gm)	Total liver (µg)
139.8 ± 10.3 ($\bar{12}$)	26.7 ± 2.0 ($\bar{12}$)	43.0 ± 4.7 ($\bar{12}$)	1.09 ± 0.10 ($\bar{8}$)	0.21 ± 0.02 ($\bar{8}$)	0.31 ± 0.02 ($\bar{8}$)
136.2 ± 9.1 ($\bar{12}$)	27.1 ± 1.8 ($\bar{12}$)	40.1 ± 4.2 ($\bar{12}$)	1.06 ± 0.05 ($\bar{10}$)	0.21 ± 0.01 ($\bar{10}$)	0.30 ± 0.02 ($\bar{10}$)
86.2 ± 4.0 ($\bar{12}$)	28.0 ± 1.3 ($\bar{12}$)	125.5 ± 6.1 ($\bar{12}$)	0.23 ± 0.03 ($\bar{9}$)	0.08 ± 0.01 ($\bar{9}$)	0.35 ± 0.05 ($\bar{9}$)
100.7 ± 6.0 ($\bar{12}$)	34.4 ± 2.0 ($\bar{12}$)	268.1 ± 28.4 ($\bar{12}$)	0.16 ± 0.01 ($\bar{11}$)	0.05 ± 0.0 ($\bar{11}$)	0.40 ± 0.02 ($\bar{11}$)
NS NS	NS *	NS **	NS *	NS *	NS NS
Pr only	Pr only	Equal effects of Pr Z Pr x Z	Pr only	Pr only	Pr only

Table 18. Liver weight, moisture and sodium after 5 weeks on diet (Experiment III: depletion)

Dietary treatments	Wet liver weight (gm)	% liver[a] moisture	Sodium		
			Dry liver (mg/gm)	Wet liver (mg/gm)	Total liver (mg)
LPLZ − 1	1.57 ± 0.08[b] (12)	80.9 ± 1.8 (11)	5.17 ± 0.45 (8)	0.99 ± 0.09 (8)	1.67 ± 0.14 (8)
LPHZ − 2	1.45 ± 0.06 (12)	80.1 ± 0.09 (10)	4.59 ± 0.31 (7)	0.91 ± 0.06 (7)	1.46 ± 0.10 (7)
HPLZ − 3	4.51 ± 0.24 (12)	67.5 ± 1.4 (12)	2.16 ± 0.07 (11)	0.70 ± 0.02 (11)	3.15 ± 0.22 (11)
Control (HPHZ) − 4	7.60 ± 0.49 (12)	65.8 ± 1.4 (13)	1.93 ± 0.05 (12)	0.66 ± 0.02 (12)	5.05 ± 0.40 (12)
T-test comparison between					
• LP treatments	NS	NS	NS	NS	NS
• HP treatments	**	NS	**	NS	**
Significant dietary effects ($P \leq 0.05$)[c]	Equal effects of Pr, Z, Pr x Z	Pr only	Pr only	Pr only	Pr (Major effect), Z, Pr x Z

[a] LP data from Experiment II; HP data from Experiment I.

[b] Mean ± SEM (No. of rats).

[c] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Table 19. Mid-brain weight, moisture, zinc and iron after 5 weeks on diet (Experiment III: depletion)

Dietary treatments	Wet mid-brain weight (gm)	% brain moisture
LPLZ - 1	1.08 ± 0.04[a] (11)	81.0 ± 0.68 (6)
LPHZ - 2	1.06 ± 0.03 (11)	79.7 ± 0.62 (6)
HPLZ - 3	1.21 ± 0.04 (12)	80.7 ± 0.99 (6)
Control (HPHZ) - 4	1.37 ± 0.04 (12)	80.3 ± 0.80 (6)
T-test comparison between		
LP treatments	NS	NS
HP treatments	*	NS
Significant dietary effects ($P \leq 0.05$)	Pr (Major effect) Pr x Z	No sig. Pr or Z effects

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Zinc			Iron		
Dry mid-brain (µg/gm)	Wet mid-brain (µg/gm)	Total mid-brain (µg)	Dry mid-brain (µg/gm)	Wet mid-brain (µg/gm)	Total mid-brain (µg)
80.5 ± 1.9 ($1\bar{2}$)	15.3 ± 0.4 ($1\bar{2}$)	16.5 ± 0.51 ($1\bar{1}$)	121.0 ± 10.8 ($1\bar{2}$)	23.0 ± 2.1 ($1\bar{2}$)	24.6 ± 2.2 ($1\bar{1}$)
78.4 ± 1.6 ($1\bar{2}$)	15.9 ± 0.3 ($1\bar{2}$)	16.8 ± 0.36 ($1\bar{1}$)	101.4 ± 9.0 ($1\bar{2}$)	20.6 ± 1.8 ($1\bar{2}$)	21.9 ± 2.4 ($1\bar{1}$)
78.5 ± 1.4 ($1\bar{2}$)	15.1 ± 0.8 ($1\bar{2}$)	18.4 ± 0.64 ($1\bar{2}$)	124.4 ± 5.8 ($1\bar{2}$)	24.0 ± 1.1 ($1\bar{2}$)	29.4 ± 2.1 ($1\bar{2}$)
82.1 ± 1.7 ($1\bar{1}$)	16.2 ± 0.3 ($1\bar{1}$)	22.1 ± 0.90 ($1\bar{1}$)	87.1 ± 8.3 ($1\bar{1}$)	17.2 ± 1.6 ($1\bar{1}$)	23.0 ± 1.9 ($1\bar{1}$)
NS NS	NS *	NS **	NS **	NS **	NS *
No sig. Pr or Z effects	Z only	Pr (Major effect) Z Pr x Z	Z only	Z only	Z only

much faster brain growth than liver growth with attainment of maximum brain weight close to the weaning time.

Brain moisture (as in the muscle) is the same in all treatments, with no significant dietary effects on it. More direct main effects of dietary zinc were seen in brain than in either the liver or muscle. Levels of wet mid-grain zinc and all mid-brain iron concentrations responded only to dietary zinc. Compared with liver and muscle, much less increase in brain zinc and iron concentrations occurred. On dry weight basis, there were no significant differences between zinc concentrations of PEM and nonPEM rats. As mentioned in connection with Tables 12 and 14, significantly higher brain iron occurs among the rats fed low dietary zinc when dietary protein is adequate.

Total brain sodium (Table 20) was significantly lower in PEM rats (LPLZ and LPHZ) compared with the nonPEM rats. In the brain, sodium concentration (µg/gm dry wt.) is affected only by dietary zinc. As mentioned for muscle and liver (Tables 16 and 18), the higher sodium concentrations were in the brains of rats on low dietary zinc.

Similar to plasma (Tables 8, 9, and 10) but differing from the liver (Table 11), brain copper levels (Table 20) were independent of dietary protein or zinc. No significant group differences occurred.

Experiment IV

This study was undertaken to investigate the effects of zinc-status in PEM on the rate of recovery from the disease. The initial

Table 20. Mid-brain weight, moisture, sodium and copper after 5 weeks on diet (Experiment III: depletion)

Dietary treatments	Wet mid-brain weight (gm)	% brain moisture
LPLZ - 1	1.08 ± 0.04[a] (11)	81.0 ± 0.68 (6)
LPHZ - 2	1.06 ± 0.03 (11)	79.7 ± 0.62 (6)
HPLZ - 3	1.21 ± 0.04 (12)	80.7 ± 0.99 (8)
Control (HPHZ) - 4	1.37 ± 0.04 (12)	80.3 ± 0.80 (6)
T-test comparison between		
LP treatments	NS	NS
HP treatments	*	NS
Significant dietary effects ($P \leq 0.05$)[b]	Pr (Major effect) Pr x Z	No sig. Pr or Z effects

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Sodium			Copper		
Dry mid-brain (mg/g)	Wet mid-brain (mg/g)	Total mid-brain (mg)	Dry mid-brain (µg/gm)	Wet mid-brain (µg/gm)	Total mid-brain (µg)
7.08 ± 0.13 ($1\bar{2}$)	1.34 ± 0.03 ($1\bar{2}$)	1.44 ± 0.04 ($1\bar{1}$)	12.29 ± 0.63 ($1\bar{2}$)	-	2.57 ± 0.20 ($1\bar{1}$)
6.72 ± 0.20 ($1\bar{2}$)	1.36 ± 0.04 ($1\bar{2}$)	1.44 ± 0.44 ($1\bar{1}$)	11.55 ± 0.83 ($1\bar{2}$)	-	2.47 ± 0.19 ($1\bar{1}$)
7.09 ± 0.13 ($1\bar{2}$)	1.37 ± 0.03 ($1\bar{2}$)	1.66 ± 0.05 ($1\bar{2}$)	10.96 ± 0.55 ($1\bar{2}$)	-	2.57 ± 0.16 ($1\bar{2}$)
6.64 ± 0.09 ($1\bar{1}$)	1.31 ± 0.02 ($1\bar{1}$)	1.78 ± 0.07 ($1\bar{1}$)	11.77 ± 0.77 ($1\bar{1}$)	-	3.15 ± 0.21 ($1\bar{1}$)
NS	NS	NS	NS	-	NS
*	NS	NS	NS	-	*
Z only	No sig. Pr or Z effects	Pr only	No sig. Pr or Z effects	-	No sig. Pr or Z effects

Table 21. Liver correlation coefficients (r-values) for rats. Unreported values are < 0.5 or insignificant (Experiment III: depletion)

Variables		Body wt.	Wet liver wt.	Zinc (μg/gm)		Iron (μg/gm)		Sodium (mg/gm)		Total zinc	Total iron[a]	Total sodium
				D[f]	W[f]	D	W	D	W			
Dietary protein		0.90 (24)[c]	0.85 (48)	-0.65 (48)	—	-0.94 (38)	-0.92 (38)	-0.90 (38)	-0.70 (38)	0.74 (48)	0.39[b] (38)	0.75 (38)
Dietary zinc		—	—	—	—	—	—	—	—	—	—	—
Body wt.		—	—	-0.69 (24)	—	-0.92 (18)	-0.91 (18)	-0.78 (19)	-0.54 (19)	0.90 (24)	0.34[d] (18)	0.93 (19)
Wet liver wt.		—	—	-0.43[e] (48)	—	-0.85 (38)	-0.85 (38)	-0.75 (38)	-0.59 (38)	0.96 (48)	0.51 (38)	0.97 (38)
Zinc (μg/gm)	D	—	—	—	—	0.60 (38)	—	0.73 (38)	—	-0.23 (48)	-0.05 (38)	-0.44 (38)
	W	—	—	—	—	—	—	—	—	—	—	—
Iron (μg/gm)	D	—	—	—	—	—	—	0.86 (28)	—	-0.75 (38)	-0.24 (38)	-0.76 (28)
	W	—	—	—	—	—	—	0.83 (28)	—	—	—	—
Sodium (mg/gm)	D	—	—	—	—	—	—	—	—	-0.63 (38)	-0.25 (28)	-0.63 (38)
	W	—	—	—	—	—	—	—	—	—	—	—

[a] Poor overall correlations; r < 0.5 in all cases.

[b] Level of significance = 0.0162.

[c] Value of n.

[d] Not significant.

[e] Significant level = 0.0026.

[f] D = dry liver; W = wet liver.

Table 22. Liver moisture, muscle moisture, days on diet before mortality and days on diet before appearance of edema (Experiment II: depletion)

	Days on diet before mortality	Days on diet before edema	% liver moisture	% muscle moisture
LPLZ	56.41 ± 1.97[a] (22)	34.05 ± 1.13 (21)	81.8 ± 1.7 (10)	71.7 ± 1.5 (10)
LPHZ	54.26 ± 2.38 (23)	34.76 ± 1.42 (21)	80.6 ± 1.1 (7)	75.0 ± 1.3 (7)
T-test comparison between • LP rats	NS	NS	NS	NS
Significant dietary effect ($P^b \leq 0.05$)[c]	No sig. Z effect	No sig. Z effect	No sig. Z effect	No sig. Z effect

[a] Mean ± SEM (No. of rats).

[b] Probability.

[c] Z = dietary zinc.

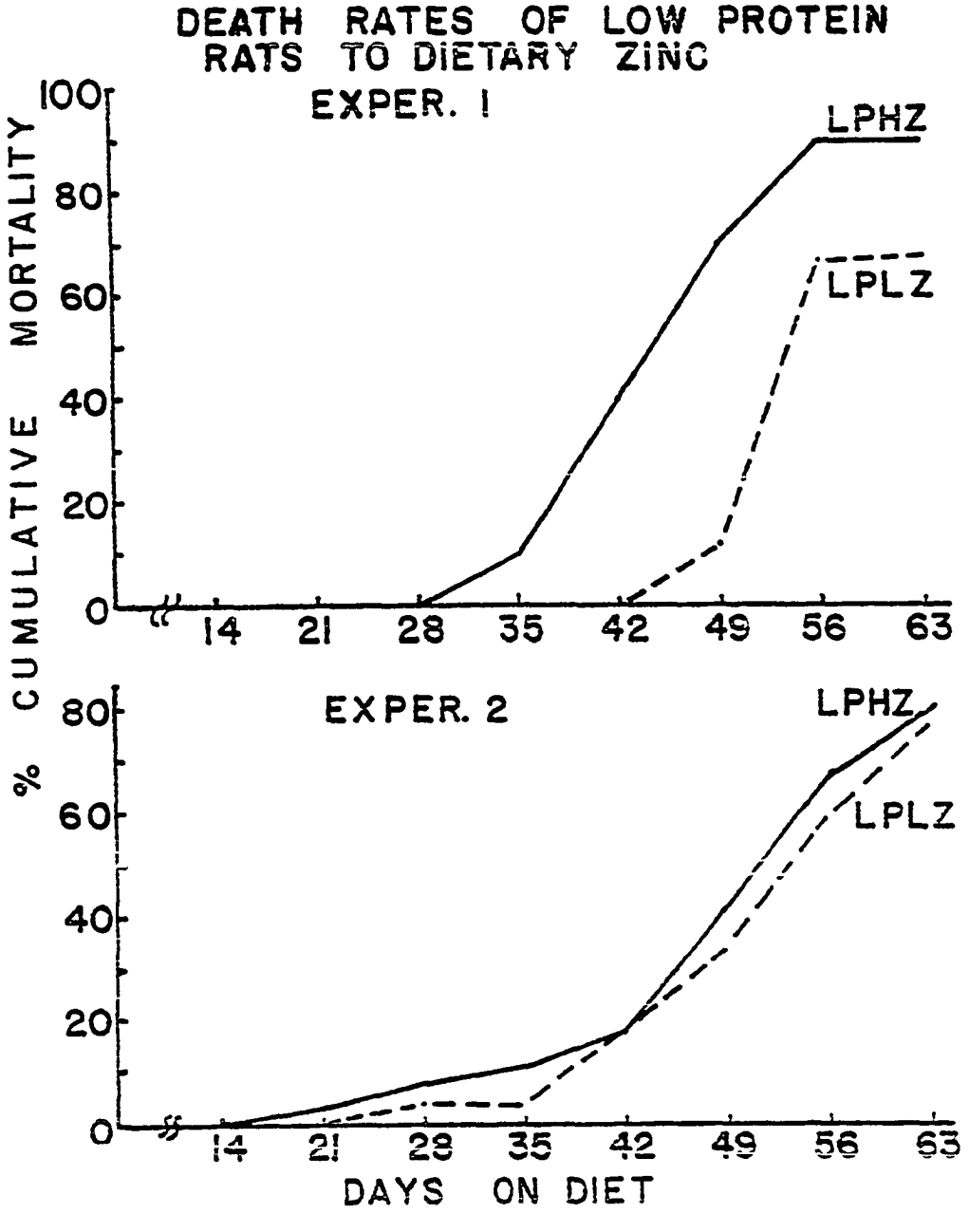


Figure 11. Shows effect of dietary zinc on survival rate during PEM. Experiment I: Deaths started 2 weeks earlier (significant: $p \leq 0.01$) in LPHZ group. Experiment II: Deaths started 1 week earlier in the same group but not significant statistically (Tables 14 and 22)

Table 23. Rate of death of rats on low protein in response to different levels of dietary zinc (Experiment II: depletion)

	Days on diet	7	14	21	28	35	42	49	56	63	
LPLZ (27)	No. dead	—	—	—	1	0	4	4	7	5	Total = 21
	% cumulative mortality	—	—	—	3.7	3.7	18.7	33.3	59.3	77.8	77.8
LPHZ (28)	No. dead	—	—	1	1	1	2	7	7	3	Total = 22
	% cumulative mortality	—	—	3.6	7.1	10.7	17.9	42.9	67.9	78.6	78.6

Table 24. Body weight data of rats during repletion (Experiment IV: repletion)

Depletion diets	0 day repletion (end of 4 weeks depletion)	7 days repletion	14 days repletion	21 days repletion
LPLZ — 1	36.9 ± 1.1[a] (6)	72.6 ± 3.2 (6)	112.8 ± 4.04 (6)	151.2 ± 4.93 (5)
LPHZ — 2	37.5 ± 1.6 (6)	63.9 ± 3.2 (6)	117.5 ± 8.04 (6)	119.8 ± 13.0 (6)
HPLZ — 3	190.1 ± 8.3 (6)	237.9 ± 9.9 (6)	258.2 ± 11.77 (6)	248.3 ± 21.4 (6)
Control (HPHZ) — 4	197.7 ± 12.1 (6)	239.8 ± 12.0 (6)	253.5 ± 17.95 (6)	299.3 ± 8.3 (6)
T-test comparison between				
• LP treatments	NS	NS	NS	NS
• HP treatments	NS	NS	NS	NS
Significant dietary effects ($P^b \leq 0.05$)[c]	Pr only	Pr only	Pr only	Pr (Major effect) Pr x Z

[a]Mean ± SEM (No. of rats).

[b]Probability.

[c]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

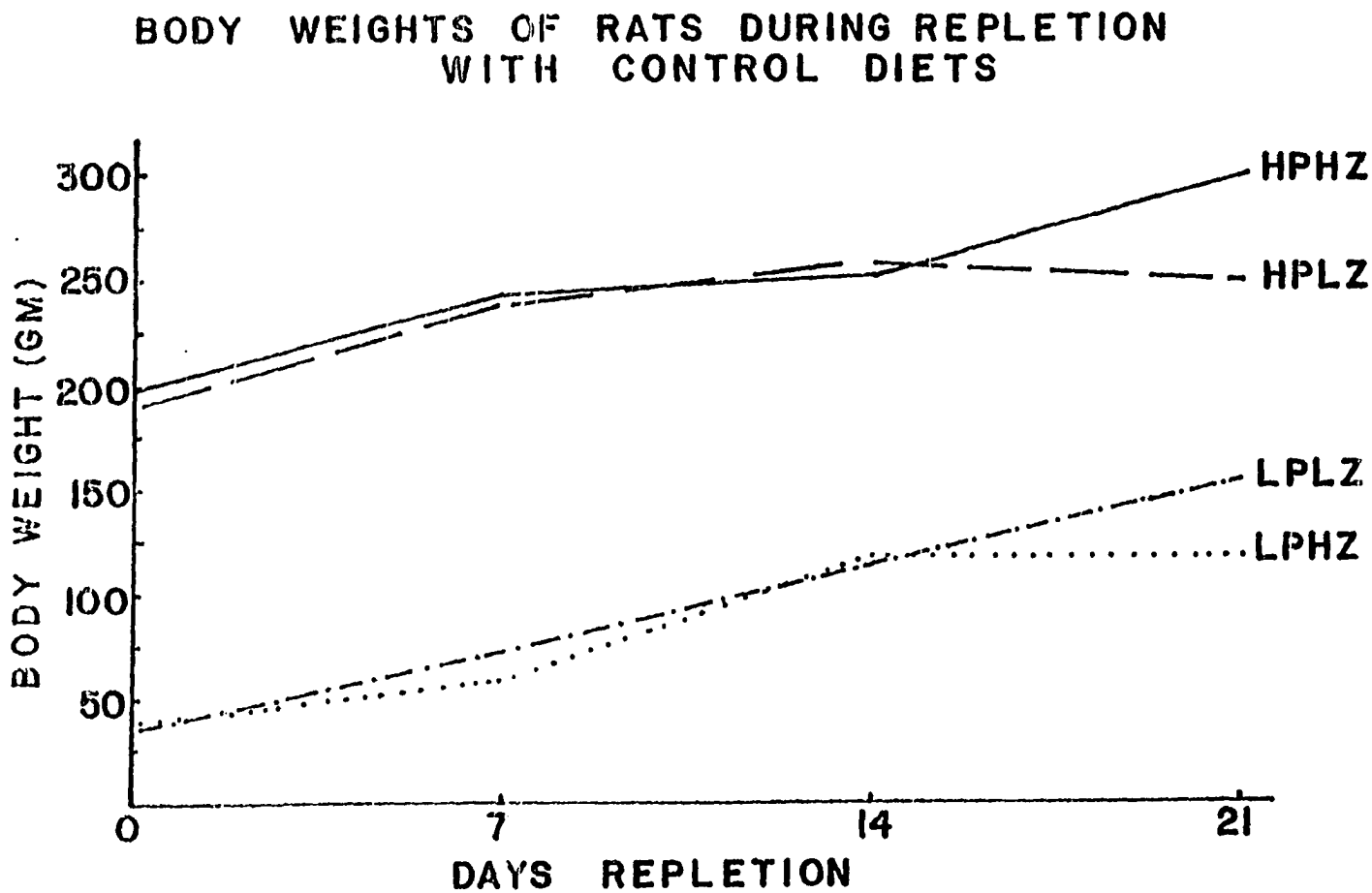


Figure 12. Rats previously on LPLZ diet gained weight faster during repletion than those previously on LPHZ diets (Table 24)

Table 25. Plasma zinc, iron and proteins of rats after 4 weeks depletion (Experiment IV: repletion study)

Depletion diets	Zinc (μg/ml)	Iron (μg/ml)	Proteins (gm/dl)			
			Total	Albumin	Globulin	A/G ratio
LPLZ – 1	0.84 ± 0.06[a] (6)	3.96 ± 1.37 (4)	2.37 ± 0.05 (6)	1.27 ± 0.06 (6)	1.05 ± 0.09 (6)	1.29 ± 0.18 (6)
LPHZ – 2	0.94 ± 0.05 (6)	6.60 ± 0.46 (6)	2.19 ± 0.10 (6)	1.13 ± 0.08 (6)	0.98 ± 0.04 (6)	1.15 ± 0.08 (6)
HPLZ – 3	0.98 ± 0.12 (4)	5.71 ± 0.29 (5)	4.14 ± 0.07 (6)	2.27 ± 0.03 (6)	1.71 ± 0.05 (6)	1.33 ± 0.04 (6)
Control (HPHZ) – 4	1.80 ± 0.09 (6)	6.45 ± 0.37 (6)	4.47 ± 0.09 (6)	2.32 ± 0.08 (6)	1.97 ± 0.09 (6)	1.19 ± 0.09 (6)
T-test comparison between						
• LP treatments	NS	NS	NS	NS	NS	NS
• HP treatments	**	NS	*	NS	*	NS
Significant dietary effects ($P \leq 0.05$)	Pr (Major effect) Z Pr x Z	Z only	Pr (Major effect) Pr x Z	Pr only	Pr (Major effect) Pr x Z	No sig. Pr or Z effects

[a]Mean ± SEM (No. of rats).

[b]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

depletion also offered an extra opportunity to recheck some of the findings of Experiments I and III.

There was a faster increase in body weight for the LPLZ rats than LPHZ rats during 3 weeks repletion (Table 24 and Figure 12). Only significant effects of dietary protein were seen in the first two weeks of repletion. A significant Pr x Z interaction occurred in the 3rd week of repletion.

Plasma zinc and protein concentrations here (Table 25) reflect the same patterns seen in the depletion studies of Experiments I and III (Tables 9 and 15). Plasma proteins were lowest in the LPHZ rats. LPHZ rats had a slightly higher (NS) plasma zinc concentration. As in Experiment I (Table 9), the plasma zinc differences were due to the effects of both dietary protein and zinc.

Regardless of protein status, rats fed high dietary zinc had significantly ($p \leq 0.05$) higher plasma iron concentrations. The highest plasma iron levels occurred in the LPHZ rats. This is the direct opposite of the effects in tissues. This is the only depletion study in which plasma iron was assayed.

After 3 weeks repletion (Table 28) plasma zinc levels of rats that had lower zinc status in PEM, had reached normal concentrations. This was significantly higher than that of the LPHZ rats. Plasma protein concentrations of both groups of PEM rats had doubled during repletion. There were no significant differences between LPLZ and LPHZ rats with regard to plasma protein levels. Both PEM groups had attained normal

Table 26. Plasma zinc, iron, and proteins of rats after 7 days repletion with control (HPHZ) diet (Experiment IV: repletion)

Depletion diets	Zinc (μg/ml)	Iron (μg/ml)	Proteins (gm/dl)			
			Total	Albumin	Globulin	A/G ratio
LPLZ – 1	1.95 ± 0.17[a] (6)	5.42 ± 0.62 (6)	3.64 ± 0.06 (6)	2.41 ± 0.06 (6)	1.09 ± 0.03 (6)	2.23 ± 0.10 (6)
LPHZ – 2	1.77 ± 0.12 (6)	5.53 ± 0.37 (5)	4.01 ± 0.26 (6)	2.52 ± 0.11 (6)	1.33 ± 0.15 (6)	1.97 ± 0.16 (6)
HPLZ – 3	2.36 ± 0.22 (6)	5.77 ± 0.45 (6)	4.97 ± 0.22 (6)	2.87 ± 0.14 (6)	1.90 ± 0.07 (6)	1.51 ± 0.03 (6)
Control (HPHZ) – 4	2.07 ± 0.17 (5)	6.40 ± 0.49 (6)	4.53 ± 0.30 (6)	2.69 ± 0.15 (6)	1.66 ± 0.15 (6)	1.67 ± 0.09 (6)
T-test comparison between						
• LP treatments	NS	NS	**	NS	NS	NS
• HP treatments	NS	NS	NS	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[b]	Small but insignificant Pr effect only	No sig. Pr or Z effects	Pr only	Pr only	Pr (Major effect) Pr x Z	Pr only

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Table 27. Plasma zinc, iron and proteins of rats after 14 days repletion with control (HPHZ) diet (Experiment IV: repletion)

Depletion diets	Zinc (μg/ml)	Iron (μg/ml)	Proteins (gm/dl)			
			Total	Albumin	Globulin	A/G ratio
LPLZ — 1	1.78 ± 0.07[a] (6)	4.82 ± 0.22 (5)	4.16 ± 0.08 (6)	2.11 ± 0.04 (6)	1.89 ± 0.06 (6)	1.12 ± 0.04 (6)
LPHZ — 2	1.69 ± 0.17 (6)	5.62 ± 0.41 (6)	3.81 ± 0.13 (6)	1.94 ± 0.06 (6)	1.73 ± 0.08 (6)	1.13 ± 0.04 (6)
HPLZ — 3	2.15 ± 0.13 (6)	4.20 ± 0.38 (6)	4.61 ± 0.17 (6)	2.17 ± 0.04 (6)	2.25 ± 0.13 (6)	0.98 ± 0.06 (6)
Control (HPHZ) — 4	2.16 ± 0.12 (6)	4.29 ± 0.42 (6)	4.35 ± 0.06	2.17 ± 0.03 (6)	2.01 ± 0.06 (6)	1.09 ± 0.04 (6)
T-test comparison between						
• LP treatments	NS	NS	NS	*	NS	NS
• HP treatments	NS	NS	NS	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[b]	Pr only	Pr only	Pr (Major effect) Z	Pr (Major effect) Z	Pr (Major effect) Z	Small insig. Pr effect

[a] Mean ± SEM (No. of rats).

[b] Pr = dietary protein; Z = dietary zinc.

Table 28. Plasma zinc, iron and proteins of rats after 21 days repletion with control (HPHZ) diet (Experiment IV: repletion)

Depletion diets	Zinc (μg/ml)	Iron (μg/ml)	Proteins (gm/dl)			
			Total	Albumin	Globulin	A/G ratio
LPLZ – 1	2.14 ± 0.16[a] (5)	4.07 ± 0.25 (4)	4.26 ± 0.13 (5)	2.14 ± 0.06 (5)	1.94 ± 0.10 (5)	1.12 ± 0.06 (5)
LPHZ – 2	1.56 ± 0.09 (5)	4.88 ± 0.27 (4)	4.22 ± 0.23 (6)	2.15 ± 0.10 (6)	1.90 ± 0.13 (6)	1.14 ± 0.03 (6)
HPLZ – 3	1.56 ± 0.15 (5)	5.77 ± 0.60 (4)	4.65 ± 0.15 (6)	2.13 ± 0.05 (6)	2.33 ± 0.14 (6)	0.93 ± 0.06 (6)
Control (HPHZ) – 4	2.11 ± 0.17 (5)	4.30 ± 0.42 (5)	4.82 ± 0.12 (6)	2.36 ± 0.05 (6)	2.66 ± 0.34 (6)	0.95 ± 0.10 (6)
T-test comparison between						
• LP treatments	*	NS	NS	NS	NS	NS
• HP treatments	*	NS	NS	*	NS	NS
Significant dietary effects ($P \leq 0.05$)[b]	Pr x Z	Pr x Z	Pr only	No sig. Pr or Z effects	Pr only	Pr only

[a]Mean ± SEM (No. of rats).

[b]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Table 29. Liver iron and zinc of rats after 4 weeks on depletion diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver moisture[a]	Iron		Zinc	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (μg/gm)	Total liver (μg)
LPLZ – 1	1.2 ± 0.1[b] (6)	82.7 ± 0.97 (6)	913.7 ± 173.5 (6)	205.5 ± 49.1 (6)	111.3 ± 21.5 (6)	25.0 ± 6.0 (6)
LPHZ – 2	1.6 ± 0.2 (6)	87.0 ± 1.6 (6)	793.4 ± 128.6 (6)	155.7 ± 29.1 (6)	138.7 ± 21.0 (6)	27.0 ± 4.9 (6)
HPLZ – 3	9.3 ± 0.8 (6)	82.4 ± 0.65 (6)	131.1 ± 7.0 (6)	213.5 ± 19.0 (6)	72.8 ± 1.9 (6)	119.9 ± 11.6 (6)
Control (HPHZ) – 4	10.2 ± 1.2 (5)	83.9 ± 1.06 (6)	102.4 ± 3.2 (6)	164.5 ± 19.5 (5)	66.0 ± 3.5 (5)	108.8 ± 16.4 (5)
T-test comparison between						
• LP treatments	NS	–[c]	NS	NS	NS	NS
• HP treatments	NS	–	**	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[d]	Pr only	–	Pr only	No sig. effect of Pr or Z	Pr only	Pr only

[a]Post water-perfusion.

[b]Mean ± SEM (No. of rats).

[c]No data.

[d]Pr = dietary protein; Z = dietary zinc.

Table 30. Liver zinc and iron of rats after 7 days repletion (HPHZ) diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Zinc		Iron	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (μg/gm)	Total liver (μg)
LPLZ − 1	3.1 ± 0.25[b] (6)	79.4 ± 0.58 (6)	87.0 ± 3.0 (6)	55.4 ± 5.4 (6)	265.0 ± 16.0 (6)	166.0 ± 12.9 (6)
LPHZ − 2	2.8 ± 0.28 (6)	78.7 ± 1.29 (6)	89.4 ± 3.2 (6)	52.2 ± 5.6 (6)	239.7 ± 19.8 (6)	134.8 ± 7.6 (6)
HPLZ − 3	8.6 ± 0.45 (6)	78.9 ± 0.82 (6)	77.8 ± 3.5 (6)	140.7 ± 10.3 (6)	215.1 ± 8.6 (6)	388.7 ± 25.8 (6)
Control (HPHZ) − 4	8.9 ± 0.56 (6)	79.6 ± 0.80 (6)	71.1 ± 2.8 (6)	129.1 ± 11.1 (6)	195.6 ± 7.6 (6)	354.5 ± 28.9 (6)
T-test comparison between						
• LP treatments	NS	–[c]	NS	NS	NS	NS
• HP treatments	NS	–	NS	NS	NS	NS
Significant dietary effects ($P^{d} \leq 0.05$)[e]	Pr only	–	Pr only	Pr only	Pr only	Pr only

[a]Post-water perfusion.

[b]Mean ± SEM (No. of rats).

[c]No data.

[d]Probability.

[e]Pr = dietary protein.

Table 31. Liver zinc and iron of rats after 14 days repletion with control (HPHZ) diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Zinc		Iron	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (μg/gm)	Total liver (μg)
LPLZ – 1	6.31 ± 0.62[b] (6)	82.4 ± 1.3 (6)	83.3 ± 6.9 (6)	91.1 ± 13.1 (6)	246.3 ± 22.4 (6)	267.4 ± 33.7 (6)
LPHZ – 2	7.30 ± 1.14 (5)	83.0 ± 1.5 (6)	77.0 ± 3.4 (6)	88.1 ± 7.2 (5)	274.2 ± 20.7 (6)	317.5 ± 45.5 (5)
HPLZ – 3	16.3 ± 1.69 (6)	84.8 ± 0.7 (6)	77.0 ± 3.6 (6)	186.0 ± 12.0 (6)	295.4 ± 14.4 (6)	712.5 ± 43.0 (6)
Control (HPHZ) – 4	15.7 ± 1.49 (6)	84.1 ± 0.6 (6)	77.4 ± 4.3 (6)	192.8 ± 22.7 (6)	241.8 ± 14.9 (6)	593.8 ± 52.8 (6)
T-test comparison between						
• LP treatments	NS	–[c]	NS	NS	NS	NS
• HP treatments	NS	–	NS	NS	*	NS
Significant dietary effects ($P \leq 0.05$)[d]	Pr only	–	No sig. dietary effect of Pr or Z	Pr only	Pr x Z	Pr only

[a]Post-water perfusion.

[b]Mean ± SEM (No. of rats).

[c]No data.

[d]Pr = dietary protein; Z = dietary zinc; Pr x Z = interaction.

Table 32. Liver zinc and iron of rats after 21 days repletion with control (HPHZ) diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Zinc		Iron	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (μg/gm)	Total liver (μg)
LPLZ – 1	8.14 ± 0.49[b] (5)	82.4 ± 0.7 (5)	77.1 ± 1.12 (5)	109.4 ± 4.2 (5)	193.1 ± 12.9 (5)	273.9 ± 20.1 (5)
LPHZ – 2	5.32 ± 0.18 (5)	82.1 ± 1.4 (6)	79.7 ± 2.5 (6)	81.5 ± 3.4 (5)	291.0 ± 47.1 (6)	246.1 ± 16.8 (5)
HPLZ – 3	11.9 ± 1.68 (5)	81.0 ± 2.1 (6)	63.7 ± 2.2 (6)	153.0 ± 32.3 (5)	224.0 ± 20.3 (6)	507.3 ± 100.8 (5)
Control (HPHZ) – 4	12.4 ± 0.98 (6)	80.1 ± 1.3 (6)	71.5 ± 4.4 (6)	171.1 ± 9.5 (6)	202.1 ± 16.4 (6)	487.4 ± 46.2 (6)
T-test comparison between						
• LP treatments	**	–[c]	NS	**	NS	NS
• HP treatments	NS	–	NS	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[d]	Pr only	–	Pr only	Pr only	No sig. effect of Pr or Z	Pr only

[a]Post water-perfusion.

[b]Mean ± SEM (No. of rats).

[c]No data.

[d]Pr = dietary protein; z = dietary zinc.

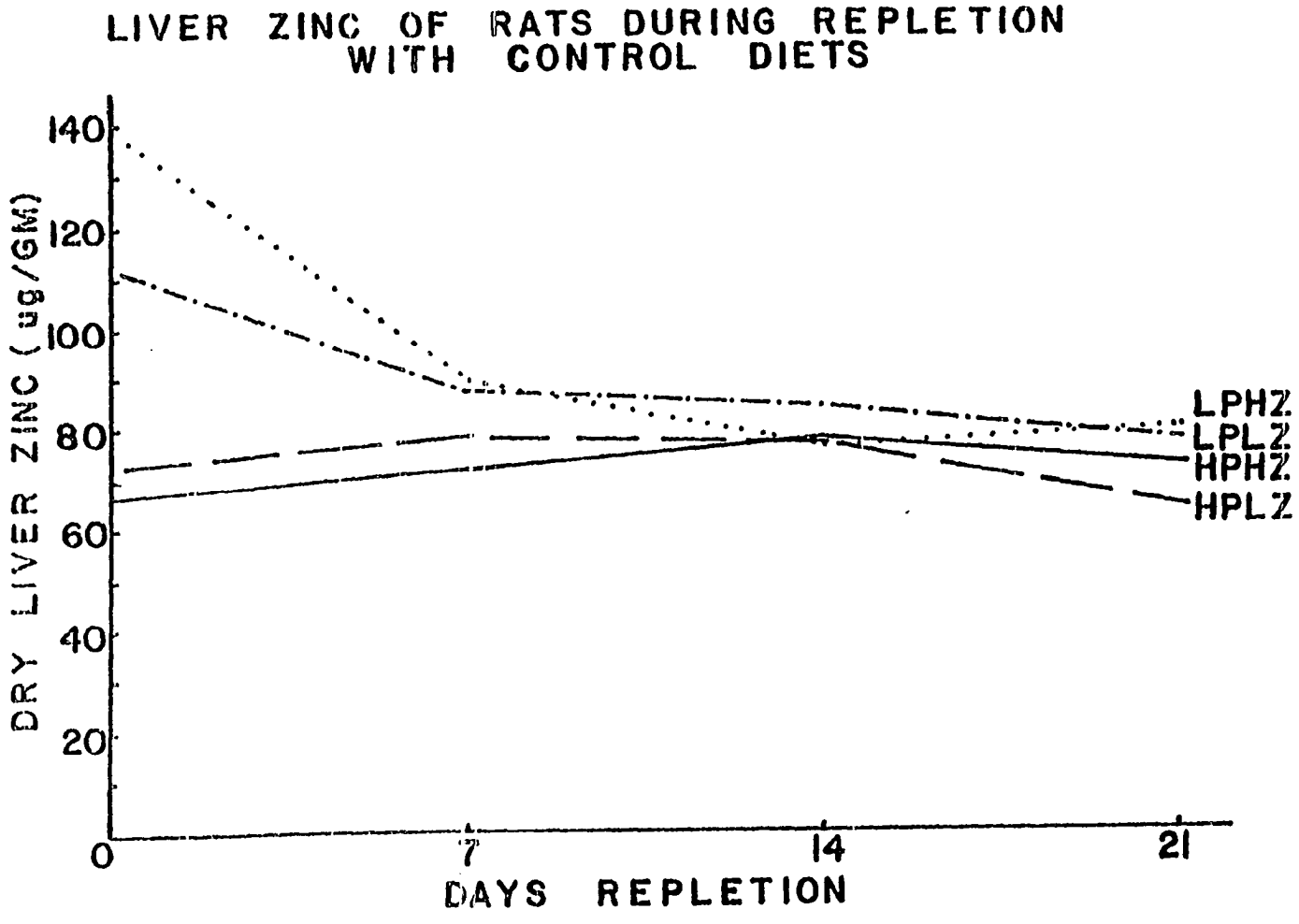


Figure 13. Graph suggesting a dilution of liver zinc towards normal levels in LP rats as fresh liver (tissue forms) during repletion. Rate of dietary zinc uptake into liver lags behind tissue biosynthesis during repletion (Tables 29-32)

plasma albumin concentrations while recovery of plasma globulin still lagged behind.

The picture for liver iron and zinc at the end of depletion (Table 29) was similar to what was seen in Experiment I (Table 12) and Experiment III (Table 17). Since these livers were perfused, the per cent liver moisture here does not represent the true composition of nonperfused liver (Tables 11, 12, Experiment I). Liver perfusion in this study was to check whether elimination of residual liver blood would change the general pattern of higher minerals (Fe^{++}, Zn^{++}, Na^{+}) concentrations in the PEM rats.

After 3 weeks repletion (Table 32), liver weights, zinc and iron of PEM rats had not reached control levels yet. Main effects were due to dietary protein. Among the PEM rats. those that had higher zinc status during PEM still had significantly ($p \leq 0.01$) lower liver weights. They also had slightly higher concentrations of liver zinc and iron. The total liver zinc of the LPHZ rats was significantly lower than that of the LPLZ rats.

The depletion data for sodium and protein in Table 33 have been discussed in connection with data from Experiments I and III. There were significantly higher liver sodium concentrations (µg/gm) in PEM rats and significantly lower liver protein in PEM rats. The difference between this and Experiment I (Table 11) and Experiment III (Table 18) is that no zinc effects occurred (Table 25). The only effects were from dietary protein.

After 3 weeks repletion (Table 36), liver protein concentrations (g/100 gm) were at control levels in all groups as indicated by the disappearance of significant dietary protein or zinc effects. Highest liver protein concentration (g/100 gm) was seen in the LPHZ livers. Total liver protein was significantly lower in the livers of the recovering PEM rats. This may be because of the lower liver weights of the recovering PEM rats.

Altogether, there were not many significant dietary zinc effects in the liver analyses of Experiment IV. The effects still showed up in plasma analyses, mostly as secondary effects to those of dietary protein.

There also seemed to be a general decrease in dietary effects on plasma and tissue minerals (Tables 28, 29, 32, and 36) with increasing repletion.

Table 33. Liver sodium and protein of rats after 4 weeks on depletion diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Sodium		Protein	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (gm/100 gm)	Total liver (gm)
LPLZ – 1	1.2 ± 0.1[b] (6)	82.7 ± 0.97 (6)	3410.8 ± 644.4 (6)	773.5 ± 184.6 (6)	62.1 ± 0.76 (6)	0.13 ± 0.01 (6)
LPHZ – 2	1.6 ± 0.2 (6)	87.0 ± 1.6 (6)	3208.4 ± 500.6 (6)	632.8 ± 116.5 (6)	60.5 ± 2.21 (5)	0.11 ± 0.01 (5)
HPLZ – 3	9.3 ± 0.8 (6)	82.4 ± 0.65 (6)	1675.2 ± 106.5 (6)	2719.3 ± 213.6 (6)	69.1 ± 0.70 (5)	1.17 ± 0.11 (5)
Control (HPHZ) – 4	10.2 ± 1.2 (5)	83.9 ± 1.06 (6)	1452.7 ± 128.3 (6)	2352.3 ± 313.9 (5)	68.1 ± 2.28 (6)	1.13 ± 0.16 (5)
T-test comparison between						
• LP treatments	NS	–[c]	NS	NS	NS	NS
• HP treatments	NS	–	NS	NS	NS	NS
Significant dietary effects ($P^d \leq 0.05$)[e]	Pr only	–	Pr only	Pr only	Pr only	Pr only

[a]Post-water perfusion.

[b]Mean ± SEM (No. of rats).

[c]No data.

[d]Probability.

[e]Pr = dietary protein.

Table 34. Liver sodium and protein of rats after 7 days repletion with control (HPHZ) diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Sodium		Protein	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (gm/100 gm)	Total liver (gm)
LPLZ – 1	3.1 ± 0.25[b] (6)	79.4 ± 0.58 (6)	2615.7 ± 177.0 (6)	1645.6 ± 149.7 (6)	62.9 ± 1.72 (6)	0.40 ± 0.04 (6)
LPHZ – 2	2.8 ± 0.28 (6)	78.7 ± 1.29 (6)	2784.8 ± 180.5 (6)	1587.2 ± 126.4 (6)	63.9 ± 2.07 (5)	0.40 ± 0.03 (5)
HPLZ – 3	8.6 ± 0.45 (6)	78.9 ± 0.82 (6)	2049.6 ± 104.8 (6)	3685.6 ± 220.0 (6)	62.6 ± 1.54 (5)	1.14 ± 0.05 (5)
Control (HPHZ) – 4	8.9 ± 0.56 (6)	79.6 ± 0.80 (6)	2168.7 ± 30.6 (6)	3929.6 ± 281.3 (6)	64.5 ± 1.76 (6)	1.17 ± 0.09 (6)
T-test comparison between						
• LP treatments	NS	–[c]	NS	NS	NS	NS
• HP treatments	NS	–	NS	NS	NS	NS
Significant dietary effects ($P^d \leq 0.05$)[e]	Pr only	–	Pr only	Pr only	No dietary effect of Pr or Z	Pr only

[a] Post-water perfusion.

[b] Mean ± SEM (No. of rats).

[c] No data.

[d] Probability.

[e] Pr = dietary protein; Z = dietary zinc.

Table 35. Liver sodium and protein of rats after 14 days repletion with control (HPHZ) diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Sodium		Protein	
			Dry liver (μg/gm)	Total liver (μg)	Dry liver (gm/100 gm)	Total liver (gm)
LPLZ − 1	6.31 ± 0.62[b] (6)	82.4 ± 1.3 (6)	2542.0 ± 175.4 (6)	2789.0 ± 374.5 (6)	62.2 ± 1.64 (6)	0.71 ± 0.06 (6)
LPHZ − 2	7.30 ± 1.14 (5)	83.0 ± 1.5 (6)	2242.3 ± 110.4 (6)	2647.5 ± 346.1 (5)	65.6 ± 0.85 (6)	0.74 ± 0.06 (5)
HPLZ − 3	16.3 ± 1.69 (6)	84.8 ± 0.7 (6)	2124.3 ± 135.8 (6)	5222.1 ± 595.8 (6)	63.9 ± 1.78 (6)	1.55 ± 0.11 (6)
Control (HPHZ) − 4	15.7 ± 1.49 (6)	84.1 ± 0.6 (6)	2316.5 ± 169.9 (6)	5652.2 ± 494.8 (6)	62.4 ± 2.41 (6)	1.53 ± 0.11 (6)
T-test comparison between						
• LP treatments	NS	–[c]	NS	NS	NS	NS
• HP treatments	NS	–	NS	NS	NS	NS
Significant dietary effects ($p^{d} \leq 0.05$)[e]	Pr only	–	No sig. effect of Pr or Z	Pr only	No sig. effect of Pr or Z	Pr only

[a]Post water-perfusion.

[b]Mean ± SEM (No. of rats).

[c]No data.

[d]Probability.

[e]Pr = dietary protein; Z = dietary zinc.

Table 36. Liver sodium and protein of rats after 21 days repletion with control (HPHZ) diet (Experiment IV)

Depletion diets	Wet liver weight (gm)	% liver[a] moisture	Sodium		Protein	
			Dry liver (µg/gm)	Total liver (µg)	Dry liver (gm/100 gm)	Total liver (gm)
LPLZ – 1	8.14 ± 0.49[b] (5)	82.4 ± 0.7 (5)	1990.2 ± 106.7 (5)	2817.3 ± 147.8 (5)	61.9 ± 1.58 (5)	0.88 ± 0.05 (5)
LPHZ – 2	5.32 ± 0.18 (5)	82.1 ± 1.4 (6)	2144.2 ± 108.2 (6)	2089.8 ± 50.8 (5)	69.1 ± 1.89 (6)	0.68 ± 0.04 (5)
HPLZ – 3	11.9 ± 1.68 (5)	81.0 ± 2.1 (6)	1968.1 ± 183.4 (6)	5138.6 ± 1205.2 (5)	64.5 ± 2.33 (6)	1.49 ± 0.25 (5)
Control (HPHZ) – 4	12.4 ± 0.98 (6)	80.1 ± 1.3 (6)	1954.6 ± 208.0 (6)	4675.7 ± 478.6 (6)	64.3 ± 3.1 (6)	1.54 ± 0.06 (6)
T-test comparison between						
• LP treatments	**	–[c]	NS	**	*	*
• HP treatments	NS	–	NS	NS	NS	NS
Significant dietary effects ($P \leq 0.05$)[d]	Pr only	–	No sig. effect of Pr or Z	Pr only	No sig. effect of Pr or Z	Pr only

[a] Post water-perfusion.

[b] Mean ± SEM (No. of rats).

[c] No data.

[d] Pr = dietary protein; Z = dietary zinc.

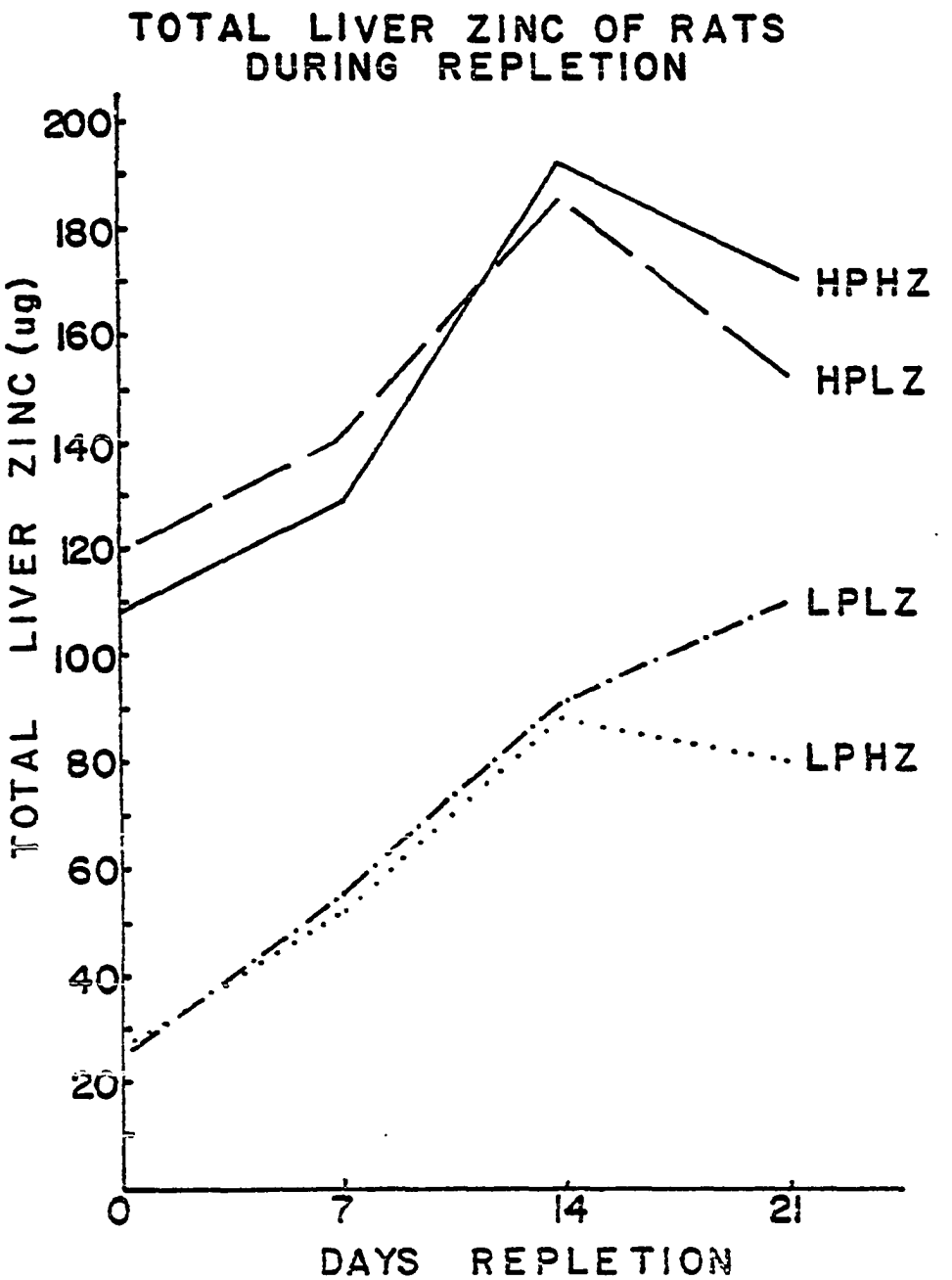


Figure 14. Increasing total liver zinc with repletion and growth. PEM rats previously on LPHZ diet lag behind PEM rats previously on LPLZ diet (Tables 33-36)

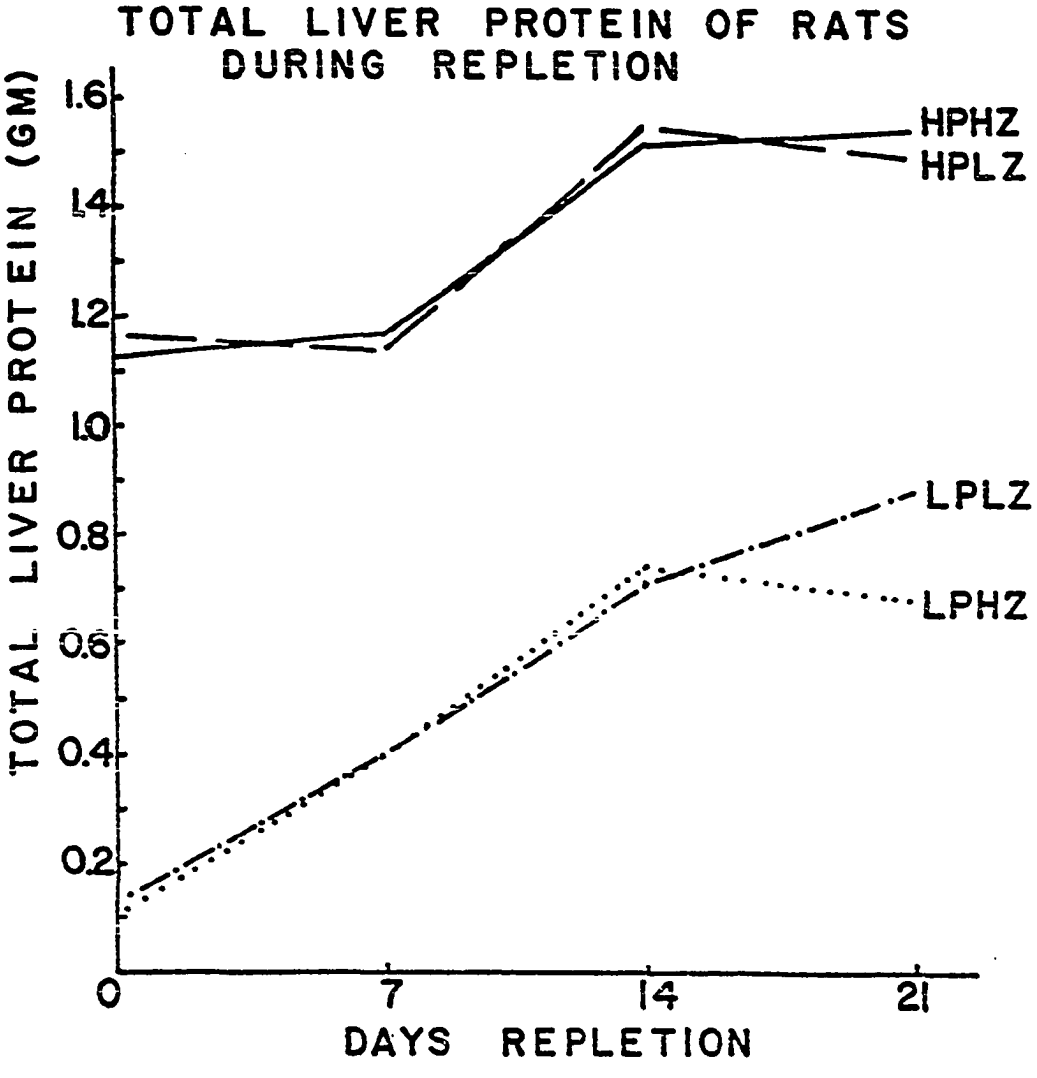


Figure 15. Increasing total liver protein with repletion and growth in all groups. Rats previously on LPHZ diet were behind LPLZ rats after 3 weeks (Tables 33-36)

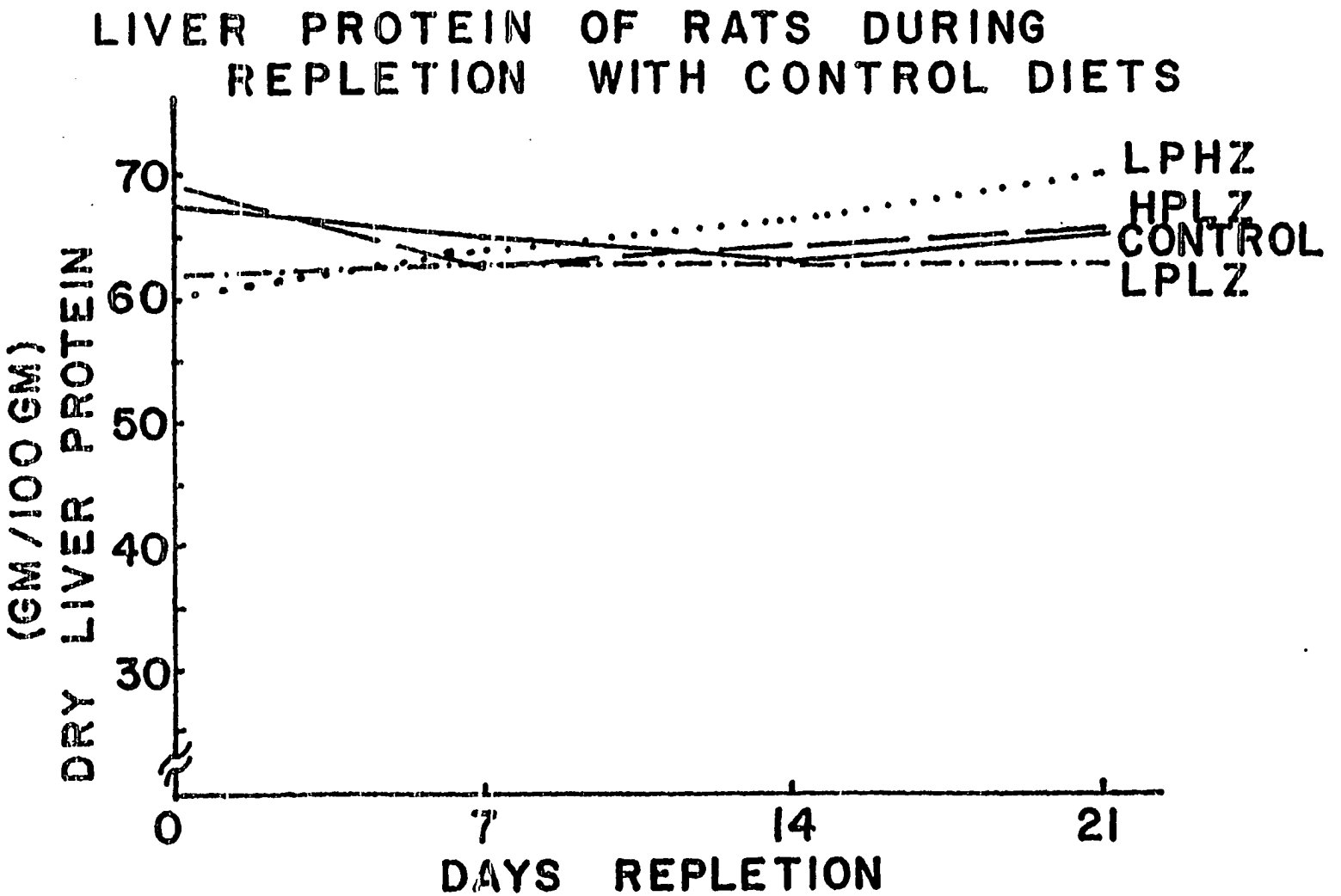


Figure 16. Rats previously on LPHZ diets show better rate of liver protein biosynthesis than LPLZ rats after 3 weeks (Tables 33-36). Figure 15 suggests that total liver weight was still lower at this time in LPHZ group than LPLZ group

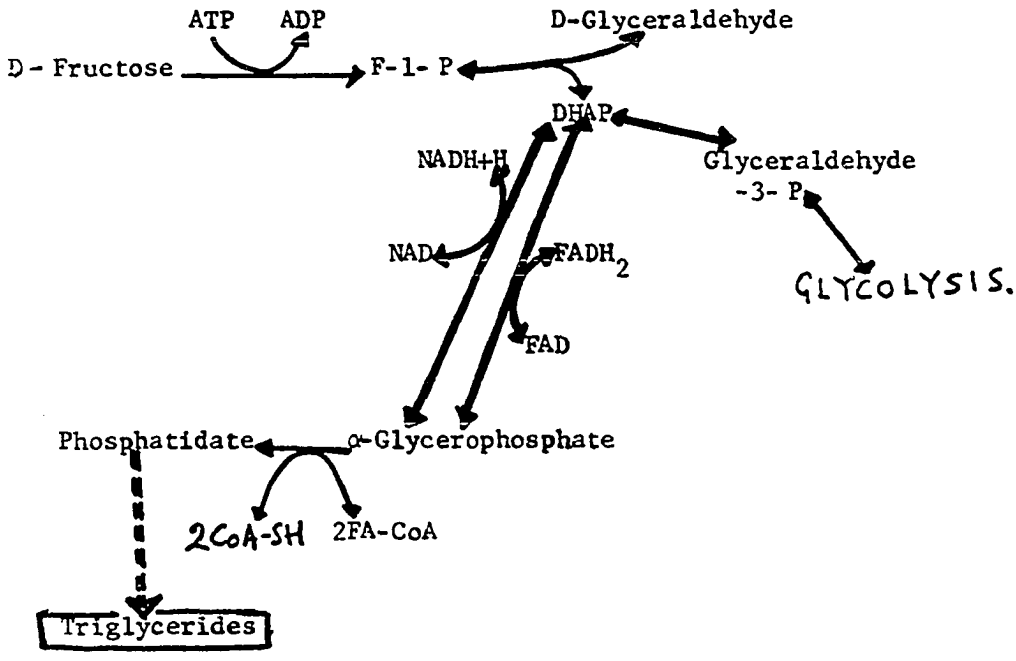
DISCUSSION

The Rat Model for PEM

The results of depleting rats fed the diets (Table 1) used in this study indicate a successful induction of clinical PEM in our Wistar rats. Loss of appetite, loss of body weight, edema, hair disorders, dermatosis, lethargy, severe hypoalbuminemia, low plasma A/G ratios (Tables 6 and 22) are documented symptoms of kwashiorkor (5, 83, 84, 85).

A striking feature of this study is the rapidity with which PEM developed. Edozien, using 0.5% lactalbumin found that rats took 3.5 months to develop kwashiorkor (85). Feeding egg albumin - a higher quality protein - at 0.4%, we expected kwashiorkor later than the 4 weeks within which it occurred. The biological values of egg ablumin and lactalbumin are 97% and 84%, respectively (86). The rapid development of edema may be due to our use of younger, newly-weaned (45 gm average weight) rats. Edozien used older (100-130 gm) rats. This might suggest a more rapid development of PEM in younger human infants. Next to edema, some consider fatty liver the second major difference between kwashiorkor and marasmus (87). It does not occur in all cases of kwashiorkor (88) and it did not occur in our rats. Surcrose-containing protein-deficient diets usually induce kwashiorkor with fatty liver (83, 85). Hypoalbuminemia and other kwashiorkor symptoms are produced along with it. Fructose, from the sucrose, is considered mainly responsible for the fatty livers (89). Liver has a glucokinase but no hexokinase for production of fructose-6-phosphate. Therefore, a direct link with the glycolytic pathway is missing. Instead,

fructose undergoes a series of reactions in the liver that stimulate hepatic triglyceride synthesis:



There is reduced protein biosynthesis in the liver in kwashiorkor (24, 90, 91). The triglyceride cannot be incorporated into chylomicrons and so is deposited in the liver. It also might be possible that an infant developing kwashiorkor on a cassava-rich diet can develop fatty liver. It should not be because of stimulation of hepatic triglyceride synthesis but because of a direct breakdown in the mechanism for making chylomicrons (i.e., lipoproteins) (92). By Waterlow's classification (13), subjects weighing less than 60% of the standard weight-for-age and showing edema, have marasmic-kwashiorkor. Taking our control rat weights as "standard weight-for-age," both groups of our PEM rats (LPLZ and LPHZ) may more correctly be described (Table 2) as marasmic-kwashiorkor models. Also,

the fluctuating nature of the edema in some rats of this study will perhaps identify more easily with the marasmic-kwashiorkor syndrome.

The occurrence of serious eye disorders in 30% of the LPLZ and only 10% of the LPHZ groups, suggest the possibility that xerophthalmia in kwashiorkor occurs where serious zinc deficiency is also present. This is speculative because Vitamin A status was not determined in these experiments.

Liver Pathology and Alteration

The following differences were noted between livers of the PEM and nonPEM rats:

1) Significantly elevated liver moisture among PEM rats (Table 8).
2) Significantly elevated liver concentrations of Cu^{++}, Zn^{++}, Fe^{++}, Na^{+} (Tables 8, 9 and 12) in PEM.
3) Significantly depressed liver protein concentrations in PEM (Tables 8 and 25).
4) In comparison with the brain or kidney, the liver is more severely diminished in weight in PEM (Tables 4, 13, and 26).

Diminution of liver weight

Among the organs examined, the spleen seemed the most retarded in growth, followed by the liver, the kidney and the brain in that order (Tables 4 and 15). The lower effect of protein depletion on brain weight suggests that most of its growth occurred in the pre-weaning period before

the rats entered the experiment. Liver and spleen were, however, growing very actively at the beginning of the experiment and were very dependent on dietary protein. Literature reports rapid brain growth (1-2 mg wt. increase/min.) at birth (16).

An interesting observation is the liver/body weight ratio (Table 4) which is about 0.03-0.04 regardless of dietary protein or zinc treatment. This suggests a very close positive correlation between body weight and liver weight. It might be possible to derive a good prediction equation liqking body weight to wet liver weight.

Elevated liver minerals

McLaren (93) points out that as a rule, essential elements such as zinc, iron, copper, sodium, etc. tend to accumulate in liver during physiological disorders. Nonessential elements tend to accumulate in the kidney and bone. Compared with controls, the PEM rats (75) showed about 20% more liver moisture for 100 gm liver. They showed 700-800% more iron/gm dry liver (Table 26); 170-210% more zinc per gm dry liver and 220-223% more sodium/gm dry liver (Table 33). Quantitatively, the sodium increases (expressed in µg). The ratios of Zn:Fe:Na per gm dry liver in controls (Tables 13 and 14) were 5:7:88 as compared with 2:18:80 in PEM rats. Absolute amounts of these elements in PEM rat livers were higher as shown.

Increases in liver zinc and iorn appear to be due to unavailability of adequate protein to bind and transport them out. About 66% of all zinc is transported bound to ablumin, 30% to alpha$_2$-macroglobulins, some to transferrin, nucleoproteins, metallothionen, cystine and histridine. Iron

is transported by transferrin. Its release from the liver also requires the enzyme xanthine oxidase (25) - a protein. Because of depressed hepatic protein biosynthesis in PEM (91, 92, 94), these elements cannot be moved out of the liver. Overal rates of protein turn over in children decrease by about 40% in PEM (90). Our correlation data (Table 9B), show strong negative correlations ($p \leq 0.0001$) between liver protein concentration (gm/100 gm wet liver) and liver zinc, copper and iron concentrations (µg/gm). The same is true for sodium. This could mean that increased protein biosynthesis in the liver leads to increased removal of Zn, Fe, Cu and Na^+ from the liver. A factor that may be more responsible for the increased sodium concentration is increased plasma aldosterone concentration in kwashiorkor (16, 93, 95). While aldosterone secretion remains unchanged, its catabolism is lowered in kwashiorkor because of disorders of the liver. This will increase K^+ excretion and Na^+ retention.

Elevated liver moisture

Retention of sodium in liver, because of the aldosterone effect, also will cause an influx of water becuase of osmotic pressure elevation by sodium. Breakdown of cytosolic protein and removal of the amino acids in PEM will reduce cytosolic colloid osmotic pressure. This is apparently insufficient to reverse water influx due to the increased osmotic pressure caused by sodium. The increased liver moisture possibly contributes to the hepatomegaly (84) in kwashiorkor. The sodium retention due to aldosterone perhaps contributes significantly to edema. A combination of hypoalbuminemia (Tables 6, 11 and 21) and sodium retention in the extracellular fluids may completely account for the edema (96).

Reduced liver protein concentration

PEM rats had significantly reduced liver protein concentrations (Tables 8 and 25). There are several literature reports of serious decrease in hepatic protein biosynthesis in PEM (91, 92, 94). Plasma albumin synthesis occurs in the liver. A reduction in hepatic protein biosynthesis, therefore, leads to hypoalbuminemia. With an adequate protein diet, the liver synthesizes and exports about 30% of its own protein mass daily in rats (24, 97). In marasmus, muscle breakdown provides amino acids to liver for synthesizing export proteins (plasma proteins, etc). In kwashiorkor, an adequate supply of amino acids to liver via muscle breakdown is prevented because of a lowered plasma cortisol/insulin ratio. In kwashiorkor, the liver breaks down its own proteins — mainly cytosolic proteins like enzymes (24, 90) — to obtain amino acids to continue synthetizing export proteins. Our data (Table 8) showing severe liver protein decreases on a wet-weight basis, as compared with dry-weight basis, reflects both the lowered cytosolic protein content as well as elevated moisture in PEM livers. In a direct measurement, Atshushi et al. (91) demonstrated about 38% decrease in rate of protein synthesis in rat liver after 10 days of dietary protein depletion.

Mineral Interrelationships

Zinc and iron

In all three experiments (I, III and IV) in which tissue analyses were done, low dietary zinc caused significantly increased tissue iron concentrations. This increase was seen for liver (Tables 9, 13, and

and 23); for muscle (Tables 10 and 12); for brain (Table 15). Settlemire and Matrone (98) proposed that decrease in tissue iron at high dietary zinc results from inhibition of iron incorporation into ferritin, its increased release or both. Kang et al. (99) showed, as we have done also in this study, that this effect of dietary zinc on iron does not occur only at the toxic zinc levels reported by Settlemire and Matrone but also at subtoxic or normal dietary amounts of zinc. Because both zinc and iron bind to transferrin (100), high dietary zinc should reduce iron intake to liver because of competition for binding with transferrin at the serosal side of the intestinal mucosa.

Zinc and sodium

A relationship similar to that of zinc and iron was noted between zinc and sodium in all three tissues. Low dietary zinc produced high sodium concentrations and high dietary zinc caused low tissue sodium concentrations. This is seen in muscle (in Table 12), liver (Tables 14 and 25), and brain (Table 16). Possibly, a similar relationship will exist between iron and sodium. Apart from one study (82) reporting an effect of dietary zinc on leucocyte sodium transport, no literature has been seen on this zinc-sodium relationship. We have confirmed that in addition to the leucocyte, this relationship exists in other tissues, i.e., liver, muscle and brain. This occurs not only in PEM but also in protein-adequate rats as well (Tables 14 and 16). Patrick et al. showed that sodium transport out of the leucocyte was stimulated by increased dietary zinc. The same mechanism may be responsible for the results we saw in liver, brain and muscle — i.e., effects of zinc on the

sodium pump. Given the stimulatory effect of zinc on EFA and triglyceride synthesis (51, 52, 101), it is possible that low zinc causes disintegration of cell membranes and inactivation of the sodium pump in the process. Sodium will consequently accumulate within cells. Elevation of dietary zinc may correct the disorder and enable the sodium pump to function properly. Some researchers (96, 102, 103) suspect a breakdown of the sodium pump as contributing to Na^+ accumulation in ECF in kwashiorkor – leading to edema. The detailed role of aldosterone here needs, however, to be clarified.

Zinc and copper

No significant relationship was seen between dietary zinc and tissue copper (Tables 5, 6, 8, and 16). Kang et al. (99) made the same observation about hepatic copper.

Possible Dietary Zinc Toxicity in PEM

Comparisons here are mainly between the two groups of rats on low dietary protein. One group was fed deficient dietary zinc (LPLZ) and the other on moderately high dietary zinc (LPHZ). Both developed PEM but had some differences.

At the beginning of protein-depletion, decreased food intake first occurred in (Table 1) the LPHZ group. This continued until the 3rd week when the cumulative food intake of the LPLZ rats fell behind the LPHZ rats. The LPLZ rats may have become depleted of zinc by the 3rd week and suffered further loss of appetite. These numerical

differences, though not quite significant statistically, were observed again in subsequent studies.

Reflecting the food intake data, the LPHZ rats lost weight faster (Table 2) in the first three weeks than the LPLZ rats. After 2 days of depletion, the LPHZ rats weighed significantly ($p \leq 0.05$) less than the LPLZ rats. Between the 3rd week and the time of mortality, LPLZ rats lost enough weight to catch up with the LPHZ rats. Urine creatinine (Table 3) was also much lower among LPHZ rats, suggesting less musculature.

Significantly lower ($p \leq 0.01$) plasma protein concentration occurred in the LPHZ rats (Table 6) in Experiment I. Similar trends occurred in Experiment III (Table 11) and Experiment IV (Table 21).

LPHZ rats had lower liver protein concentrations (gm/100 gm) than the LPLZ rats (Table 8). Though not statistically different, the same trend showed up again in Experiment IV (Table 30). From the end of depletion (Table 21) through the period of repletion (Tables 26, 27, and 28) in Experiment IV, plasma iron and other parameters were measured each week. For most of that time, the LPHZ rats had higher plasma iron levels than either LPLZ or the control (HPHZ). For PEM animals, high plasma iron levels may be more deleterious. Much of their iron, not being bound to transferrin, is free and promotes bacterial multiplication and, therefore, infection (16). Because the LPHZ rats already had significantly lower plasma globulin concentrations (Table 6), their immune response to infection should be very weak; antibody concentrations (immunoglobulins) should be low. Earlier mortality should occur in this group, in comparison with the LPLZ group, during PEM. The failure for a significant difference to

occur between LPLZ and LPHZ plasma iron levels (Table 21) after depletion, may be due to the small number of measurements (n - 4) in the LPLZ group. The standard error of the mean in this group was high because the number was small. A closer examination of plasma iron levels coupled with bacterial counts in the LPLZ and LPHZ groups would be useful in future studies.

Mortalities occurred significantly ($p \leq 0.01$) earlier by 2 weeks in the LPHZ rats in Experiment I (Table 10). Considering a 3-year life-span for the rat and a 50-year life expectancy for a developing country with much PEM, the mortality results will suggest 8-month earlier mortality (on the average) for a kwashiorkor child if he should ingest even moderately high dietary zinc than if he did not. In Experiment II, deaths again appeared to start earlier among the LPHZ rats (Table 18 and Figure 11) but differences between the mean lengths of time before mortality were not significant.

With both groups of PEM rats eating the same high-protein diet of the controls for repletion, the rats previously on LPLZ diet recovered faster than those originally on LPHZ diet. Possibly the disorders caused by ingesting moderately high zinc in PEM made recovery slower for the LPHZ group. After 3 weeks of repletion, the previous LPHZ group compared with their previous LPLZ counterparts as follows:

1) Significantly lower ($p \leq 0.01$) total liver zinc and lower total liver iron (Table 24).

2) Significantly ($p \leq 0.01$) lower total liver sodium (Table 26) and significantly ($p \leq 0.01$) lower total liver protein.

3) Twenty per cent lower body weights than (Table 20) after 3 weeks repletion. This weight-for-age measurement is one of the criteria for classifying PEM in children (13). Also, weight-for-height is frequently used (62). A child with kwashiorkor is considered ready for clinical discharge when he attains normal weight-for-height.

The diets used for the repletion phase (i.e., control diets during depletion) satisfied conditions needed for rapid "catch-up" growth in kwashiorkor patients: (A) at least 10% of the total dietary energy should be from dietary protein (63, 66); protein provided 19% of the total energy of the HPHZ repletion diet (i.e., P:E ratio = 0.19); and (B) the diet was more than sufficient in vitamins, minerals and fats.

The consistently lower plasma protein concentrations of the LPHZ group, their lower liver protein and iron concentrations, the slight but consistently lower body weight after depletion, their earlier onset of mortality during depletion, higher plasma iron and slower recovery during repletion, all suggest a possible dietary zinc toxicity.

Cousins (104) explained that high dietary zinc induces metallothionein synthesis in the intestinal mucosa as well as in hepatocytes. This may be one important difference between our two PEM groups. Induction of thionein protein first involves nucleic acid biosynthesis. In addition to this being an energy requiring process, purine synthesis requires extensive amino acid usage; glutamate, glycine, aspartate and glutamine are all required. This means a diversion of energy and amino acids into nucleic acids and metallothionein in the LPHZ rats. This could possibly contribute to the significantly lower plasma protein

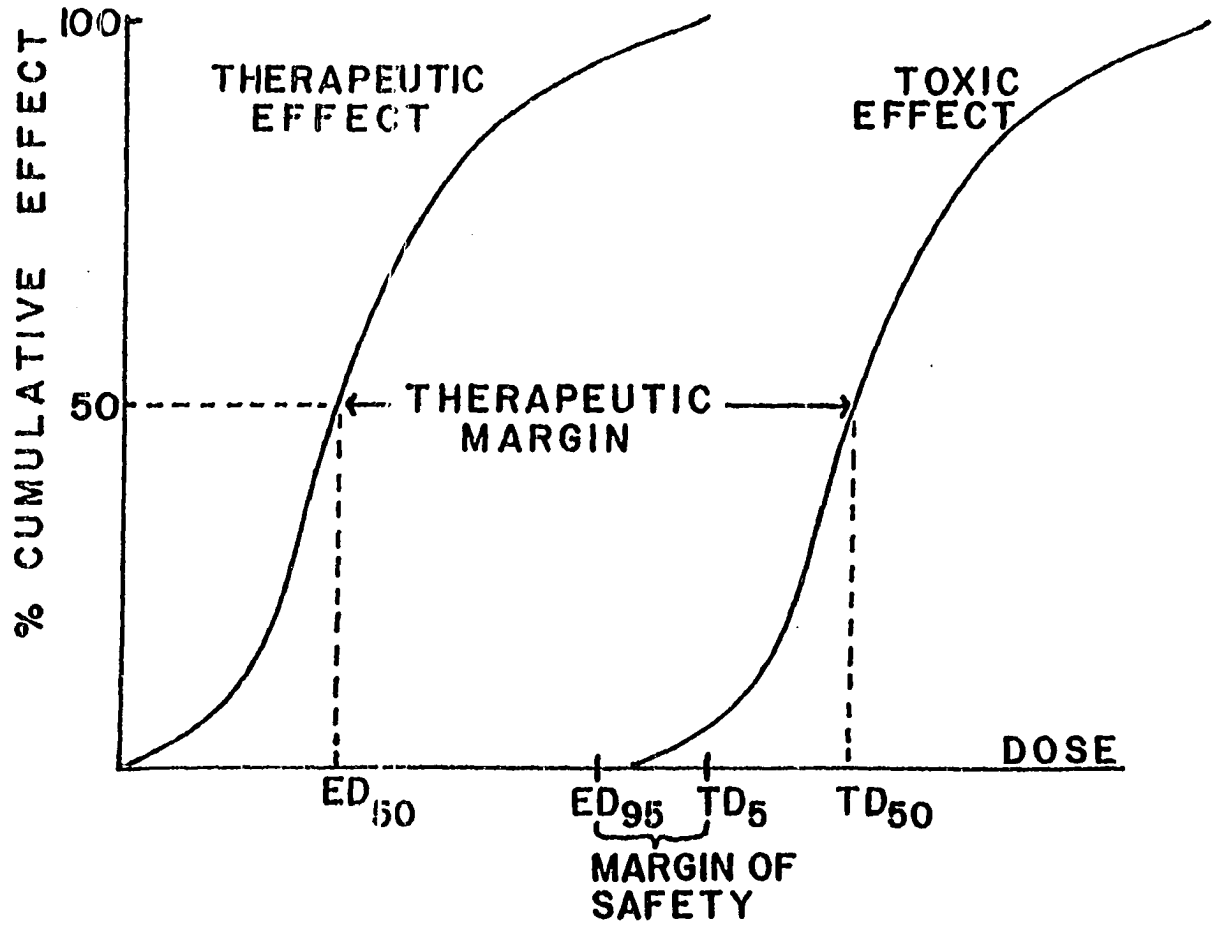


Figure 17. Toxicity of 30 ppm dietary zinc in PEM rats would suggest drastically reduced therapeutic margin for dietary zinc in PEM. Control rats are known to show no toxic effects on up to 7000 ppm dietary zinc.

concentrations of the LPHZ rats, compared with the LPLZ rats. This might also contribute to a lower body weight and lower overall musculature (as measured by urine creatinine) in the LPHZ rats. It may be worth comparing intestinal and hepatic nucleic acid concentrations in the LPLZ and LPHZ groups in the future.

In relationship to this possible dietary zinc toxicity to the PEM rat at a moderate level of 30 ppm, Smith and Larson (103) noted zinc toxicity in young healthy rats at 7000 ppm diet. At 10,000 ppm, 75% of their rats died in 3-5 weeks. The young rats fed adequate dietary protein also showed anemia and subnormal growth. We did not check for anemia, but subnormal growth (lower body weight; lower overall musculature) were present in the LPHZ rats.

Zinc toxicity at 30 ppm dietary zinc indicates that there is a drastically reduced tolerance to excess dietary zinc in the rat with kwashioror. In toxicological terms, there is a drastically reduced therapeutic range (105) for dietary zinc in the PEM rat. The toxic effect curve (Figure 17) is shifted far to the left with a decrease in the therapeutic margin.

In health, the rat requires 12 ppm dietary zinc. When consuming a soy protein based diet, this increases to 18 ppm (106) because of unavailability of zinc bound by phytate. The 30 ppm in our diet thus represents a very moderate excess above the requirement.

Since iron uptake into tissues is hindered by high dietary zinc (98), hemopoiesis should decrease leading to anemia as reported by Smith and Larson (103).

Some Specific Contributions of Dietary Protein and Zinc to Kwashiorkor

Loss of appetite and growth failure

For the first 2 weeks of the study, the significantly lower food intake and body weights of the rats on low protein diets were due solely to effects of dietary protein. This can be seen in Tables 1 and 2. Thus, the kwashiorkor syndrome is initiated specifically by dietary protein deficiency. Dietary zinc effects developed after 2 weeks. By this time, if the diet was zinc-deficient, tissue zinc was depleted, causing further loss of appetite and body weight. When dietary zinc was low, the zinc deficiency effects on body weight were secondary to the protein effects.

The same was true for the decreased body lengths (Table 3) and low organ weights (Tables 4, 15, and 23). Whereas adequate dietary zinc (12 ppm) might not produce these adverse effects of 30 ppm zinc, all excess levels may be toxic to some extent.

Edema and liver moisture

There is no significant dietary zinc contribution to either edema (Table 18) or moisture increases in the liver. Dietary protein deficiency (as seen in Table 13) is the sole cause of these symptoms. In kwashiorkor, hypoalbuminemia and sodium retention in the extracellular fluid (ECF) have been reported jointly responsible for edema (82, 96). Our study shows that both hypoalbuminemia (Tables 6, 11, and 21) and tissue sodium levels (Tables 12, 14, and 25) are dependent on dietary protein concentrations where water accumulation occurs.

Significant dietary zinc effects on tissue sodium occur in the brain (Table 16), but no moisture accumulation occurs there (Table 15). Body edema and high moisture in PEM rat livers are solely due to the effects of dietary protein deficiency.

Brain disorders

Brain disorders occur in kwashiorkor as well as in dietary zinc deficiency (1, 5, 95, 107, 108, 109). Irreversible brain damage may occur if kwashiorkor occurs before the usual weaning time (16, 108). Apart from this, repair can slowly occur. Bartel et al. (110) report that even 12 years after clinical kwashiorkor, development of the brain may not have been complete. There might still be a higher level of slow brain-wave activity than normal. It appears from a 15-year follow-up study that former kwashiorkor patients have close to normal mental performance at that time (111).

Our study suggests more effect of dietary zinc on the brain than on other tissues examined. Brain iron levels (Table 15) were directly dependent on dietary zinc. Brain zinc (µg/gm wet tissue) and sodium (µg/gm) dry tissue concentrations were solely influenced by dietary zinc levels. The data in Table 16 suggest that a zinc-deficiency state, whether in PEM or not, will increase sodium (mg/gm dry weight) concentration in the brain. Physiologically, if sodium should accumulate intraneurally and cannot be actively pumped out (31a, 82) nerve cells will be depolarized and not carry normal impulses. A refractory state results. This might slow down generation of nerve impulses and contribute to the physiological disorders of kwashiorkor and zinc deficiency.

Alkaline phosphatase

Plasma alkaline phosphatase activities (Figure 4) followed the pattern reported in literature (112, 113). The highest activities occur in young animals with rapidly growing bones. With age, plasma activities of this enzyme fall. Neither dietary protein nor the moderate dietary zinc deficiency significantly affected plasma alkaline phosphatase activities (Tables 5, 6 and 7). This casts doubt on the sensitivity of plasma alkaline phosphatase as a tool for measuring zinc status (28).

Mortality rates

Mortality rates (Tables 10 and 18) were affected by dietary protein, zinc and protein-zinc interactions. The largest effects were from protein deficiency.

In summary, kwashiorkor is a protein-deficiency syndrome; only protein deficiency initiates it. If accompanied by moderate zinc deficiency (as seems to be a usual human circumstance), mortality would be due mainly to the dietary protein deficiency. Dietary zinc can, however, aggravate protein deficiency disease. Moderate zinc excesses can cause significant decreases in plasma proteins (especially globulins). This excess may slow down overall protein biosynthesis, elevate plasma iron and promote earlier mortality. Zinc deficiency can significantly increase brain sodium levels with possible adverse effects on the central nervous system. Zinc deficiency also contributes to further decrease in appetite and loss of body weight. Dietary zinc levels near the normal requirement may be safer than even moderately excessive ones.

SUMMARY

Four dietary treatments of rats with different levels of protein and zinc were used:

(a) LPLZ — low dietary protein (0.4%) and moderately low zinc (6 ppm).

(b) LPHZ — low dietary protein and moderately high (30 ppm) zinc.

(c) HPLZ — high dietary protein (20%) and moderately low zinc.

(d) HPHZ — 20% protein and 30 ppm zinc for controls.

Loss of appetite, body weight, development of edema, lethargy, hair disorders, skin dermatosis, severe hypoalbuminemia occurred in the rats fed the low-protein diets. These symptoms of kwashiorkor occurred within 4 weeks.

The loss of appetite and body weight were initiated by protein deficiency only. When moderate dietary zinc deficiency was present further loss of appetite and body weight occurred a bit later.

Decreases in total plasma protein levels were contributed to significantly by dietary protein and zinc deficiencies, as well as their interactions. The primary cause was dietary protein deficiency. Albumin levels were affected only by dietary protein while globulin levels were affected by both dietary protein and zinc.

Dietary zinc deficiency made no significant contribution to edema or elevated liver moisture observed in rats with PEM. Only dietary protein effects were responsible for these conditions. No moisture increase was seen in the brain with any dietary treatments. Muscle had

small increases in moisture content. The livers had elevated zinc, iron, sodium and copper concentrations. The Na^+ elevation may have substantially contributed to elevated moisture of tissues in the rats with PEM.

Main effects of dietary zinc were primarily in the brain. While Na^+ elevation in the liver was a main effect of dietary protein deficiency, increased sodium in the brain was due to dietary zinc deficiency. Even more strongly than with sodium, brain iron changes were controlled entirely by effects of zinc deficiency. Plasma iron concentrations of both PEM and nonPEM rats were also controlled by effects of dietary zinc. It is proposed that intraneural accumulation of sodium, accompanying suppression of sodium-pump activity should be carefully checked in kwashiorkor as well as in zinc-deficiency.

A zinc-sodium relationship, similar to the well-documented zinc-iron relationship was noted in all the three tissues studied, liver, muscle, brain. Low dietary zinc caused elevated tissue sodium whereas high dietary zinc caused decreased tissue sodium. This confirms the work of Patrick et al. (82) who reported this relationship in leucocytes of children with PEM. They attributed this effect to a decrease in sodium-pump activity in zinc deficiency and increase with adequate dietary zinc. We saw the relationship both in the PEM and nonPEM condition.

The results in this study also suggest increased toxicity of excess dietary zinc in animals with PEM. While dietary zinc toxicities have been reported in normal animals with 7000 ppm, there seem to be toxicity symptoms in the PEM rat at 30 ppm dietary zinc. This is only

a very small excess above the 12 ppm dietary zinc requirements for the rat. Marginal toxicity symptoms of 30 ppm zinc were seen in LPHZ rats as:

1) significantly lowered plasma protein concentrations;
2) elevated plasma iron concentrations. Lowered liver protein and body weights;
3) significantly earlier mortality in one study and (not significant but) earlier mortality in another study;
4) slower rate of recovery during repletion. This was shown by lower body weight at end of repletion and significantly lower liver zinc, sodium and protein.

BIBLIOGRAPHY

1. Prasad, A. S., and Oberleas, D., Eds. 1976. Trace elements in human health and disease. Vol. I. Academic Press, New York.

2. Agglelt, P. J. 1980. Animal models for study of trace metal requirements. Proc. Nutr. Soc. 39: 241.

3. Hill, C. H., and G. Matrone. 1969. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. Fed. Proc. 29: 1474-1481.

4. Burk, R. F. 1976. Selenium. Pages 100 and 313 in Present Knowledge in Nutrition. 4th edition. Nutrition Foundation, Inc., New York.

5. Davidson, S., Passmore, R., Brock, J. S., and Truswell, A. S. 1975. Protein-energy malnutrition. Pages 302-317 in Human Nutrition and Dietetics. 6th edition. Churchill Livingstone, London.

6. Sandstead, H. H., Shukry, A. S., Prasad, A. S., Gabr, M., Hefney, A. E., Mokhtar, N., and Darby, W. J. 1965. Kwashiorkor in Egypt. Clinical and biochemical studies with special reference to plasma zinc and serum lactic dehydrogenase. Am. J. Clin. Nutr. 17: 15-26.

7. Smit, Z. M., and Pretorius, P. J. 1964. Studies in metabolism of zinc. Part 2: Serum zinc levels and urinary zinc excretions in South African Bantu kwashiorkor patients. J. Trop. Pediatr. 9: 105-112.

8. Kumar, S., and Rao, K. S. J. 1973. Plasma and erythrocyte zinc levels in protein-calorie malnutrition. Nutr. Metab. 15: 364-371.

9. Todd, W. R., Elvehjem, C. A., and Hart, E. B. 1934. Zinc in the nutrition of the rat. Am. J. Physiol. 107: 146-156.

10. Prasad, A. S., Schulert, A. R., Miale, A., Farid, Z., and Sandstead, H. H. 1963. Zinc and iron deficiencies in male subjects with dwarfism without ancyclostomiasis, schistosomiasis or severe anaemia. Am. J. Clin. Nutr. 12: 437-444.

11. Jelliffe, D. B. 1959. Protein-calorie malnutrition in tropical pre-school children. J. Pediatr. 54: 227.

12. McLaren, D. S. 1974. The great protein fiasco. The Lancet 2: 93.

13. Waterlow, J. C. 1972. Classification and definition of protein-calorie malnutrition. Br. Med. J. 3: 566.

14. Williams, C. D. 1975. On the fiasco. The Lancet 1: 793.

15. Darby, W. J. 1973. Cecily Williams. Nutr. Rev. 3: 331.

16. Present knowledge in Nutrition. 4th edition. Nutrition Foundation, Inc., New York.

17. O'Dell, B. L., Burpo, C. E., and Savage, J. E. 1972. Evaluation of zinc availability in foodstuffs of plant and animal proteins. J. Nutr. 192: 653.

18. Burkitt, D. P., Walter, A. R. P., and Painter, N. S. 1972. Effect of dietary fiber on stools and transit times and its role in the causation of disease. The Lancet 2: 1408-1411.

19. Hoekstra, W. G. 1967. Pages 141-146 in Present Knowledge in Nutrition. 3rd edition. The Nutrition Foundation, Inc., New York.

20. Methfessel, A. H., and Spencer, H. 1973. Zinc metabolism. (I) Intestinal absorption of zinc. (II) Secretion of zinc into the intestine. J. Appl. Physiol. 34: 58-67.

21. Campen, D. V., and House, W. A. 1974. Effect of a low protein diet on retention of an oral dose of ^{65}Zn and tissue concentrations of Zn, Fe and Cu, in rats. J. Nutr. 104: 89-90.

22. Oberleas , D., and Prasad, A. S. 1969. Growth as affected by Zn and protein nutrition. Am. J. Clin. Nutr. 22: 1304.

23. Recommended dietary allowances. 8th edition. 1974. Nat. Acad. Sci., Washington, D.C.

24. Marilyn, C. C., and Munro, H. N. 1976. Protein. Page 43 in Present Knowledge in Nutrition. 4th edition. Nutrition Foundation, Inc., New York.

25. Goodhart, R. S., and Shils, M. E. 1976. Modern nutrition in health and disease. 5th edition. Lea and Febiger, Philadelphia.

26. Anon. 1977. Zinc and polymorphonuclear leukocyte function. Nut. Rev. 35 (10): 266

27. Prasad, A. S. , and Oberleas, D. 1971. Changes in activities of zinc-dependent enzymes in zinc-deficient tissues of rats. J. Appl. Physiol. 31: 842-846.

28. Arakwa, T., Tamura, T. Igarshi, Y., Susuki, H., and Sandstead, 1976. Zinc deficiency in two infants during total parenteral alimentation for diarrhea. Am. J. Clin. Nutr. 29: 197-204.

29. Somers, M., and Underwood, E. J. 1969. Studies of zinc nutrition in sheep. (II) Influence of zinc deficiency in ram labbs upon the digestibility of the dry matter and the utilization of the nitrogen and sulfur of the diet. Aust. J. Agric. Res. 20: 899-903.

30a. Oomen, H. A. P. C. 1976. Vitamin A deficiency, xerophthalmia and blindness. Page 73 in Present Knowledge in Nutrition. 4th edition. Nutrition Foundation, Inc., New York.

30b. WHO, Geneva, 1976.

30c. Cecil Smith J. 1980. The Vitamin A-Zinc Connection: A review. Page 62 in Micronutrient interactions: Vitamins, minerals and hazardous elements. Vol. 355. Academy of Sciences, New York.

31a. Guyton, A. C. 1976. Textbook of medical physiology. 5th edition. Saunders, Philadelphia, Pa.

31b. Blindness, Jerusalem, 25th - 27th Aug. 1971.

32. Smith, J. C., McDaniel, E. G., Fan, F. E., and Halsted, J. A. 1973. Zinc: A trace element essential in Vitamin A metabolism. Science 181: 954-955.

33. Prasad, A. S., and Oberleas, D. 1974. Thymidine Kinase and incorporation of thymidine into DNA in zinc-deficient tissues. J. Lab. Clin. Med. 83: 634-639.

34. Ronaghy, H. A., Reinhold, J. G., Mahloudji, M., and Gharami. M. 1974. Zinc supplementation of malnourished school boys in Iran: Increased growth and other effects. Am. J. Clin. Nutr. 27: 112-121.

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

36. Miller, E. R., Leucke, R. W., Ullrey, D. E., Baltser, B. V., Bradley, B. L., and Hoefer, J. A. 1968. Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278-286.

37. MaCapinlac, M. P., Pearson, W. N., Barney, G. H., and Darby, W. J. 1968. Protein and nucleic acid metabolism in the testes of zinc-deficient rats. J. Nutr. 95: 569-577.

38. Fox, M. R. S., and Harrison, B. N. 1965. Effects of zinc deficiency on plasma proteins of Japanese quail. J. Nutr. 86: 89-92.

39. Anthony, W. L., Woosley, R. L., and Hsu, J. M. 1971. Urinary excretion of radiosulfur following taurine ^{35}S injection in zinc-deficient rats. Proc. Soc. Exp. Biol. Med. 138: 989-992.

40. Thuer, R. C., and Hoekstra, W. G. 1966. Oxidation of ^{14}C-labelled carbohydrate, fat and amino acid substances by zinc-deficient rats. J. Nutr. 89: 448-454.

41. Pories, W. J., and Strain, W. H. 1966. Zinc and wound-healing. Pages 378-394 in Zinc Metabolism. Thomas, Springfield, Illinois.

42. Mahler, H. R., and Cordes, E. H. 1967. Biological chemistry. International edition. Harper and Row, New York.

43. Whitehead, R. G., and Lunn, P. G. 1979. Endocrines in P.E.M. Proc. Nutr. Soc. 38: 69

44. Harper, H. A. 1965. Review of physiological chemistry. 10th edition. Lange Medical Publications, Los Altos, Calif.

45. Gombe, S., Apgar, J., and Hansen, W. 1973. Effect of zinc deficiency and restricted food intake on plasma and pituitary LH and hypothalamic LRF in female rats. Biol. Reprod. 9: 415-419.

46. Hurley, L. S., and Swenerton, H. 1966. Congenital malformations resulting from zinc deficiency in rats. Proc. Soc. Exp. Biol. Med. 123: 692.

47. Herbert, D. C. 1980. Growth patterns and hormonal profile on male rats with PCM. Anatomical Records 197: 339.

48. Metzler, D. E. 1977. Biochemistry. The chemical reactions of living cells. Iowa State University Press, Ames, Iowa.

49. Anon. 1974. Aldosterone secretion in infantile malnutrition. Nutr. Rev. 32 (10): 296

50. Frost, P., Chen, J. C., Rabbani, I., Smith, J., and Prasad, A. S. 1977. The effect of zinc-deficiency on the immune response. Page 143 in Zinc Metabolism: Current Aspects in Health and Disease. A. R. Liss, Inc., New York.

51. Quaterman, J., and Florence, E. 1972. Observations on glucose tolerance and plasma levels of free fatty acids and insulin in zinc-deficient rats. J. Nutr. 28: 75.

52. Roth, H. P., and Kirchgessner, M. 1977. Influence of zinc deficiency on lipid metabolism. Int. J. Vitam. Nutr. Res. 47: 275.

53. Spencer, H., Rosoff, B., Feldstein, A., Cohn, S. H., and Gusmano, E. 1965. Metabolism of zinc-65 in man. Radiat. Res. 24: 432.

54. Hansen, J. D. L., and Lehman , B. H. 1969. Serum zinc and copper concentrations in children with protein-calorie malnutrition. S. Afr. Med. J. 43: 1248-1251.

55. Evans, G. W., Grace, C. I., and Votava, H. J. 1975. A proposed mechanism for zinc absorption in the rat. Am. J. Physiol. 228: 501.

56a. Hurley, L. S., Duncan, J. R., Sloan, M. V., Edkhart, C. D. 1977. Zinc-binding ligands in milk and intestines. Proc. Nat. Acad. Sci. 74: 3547.

56b. Hurley, L. S., Lonnerdal, B. 1982. Zinc Binding in Human Milk: Citrate Versus Picolinate. Nutr. Rev. 40: 65.

57. Song, M. K., and Adham, N. F. 1976. Possible role for a protaglandin-like substance in zinc absorption. Fed. Proc. 35: 1667.

58. Evans, G. W., and Johnson, E. C. 1980. Zinc absorption in rats fed a low-protein diet and a low protein diet supplemented with tryptophan or picolinic acid. J. Nutr. 110: 1076.

59. Lonnerdal, B., Stanislowski, and Hurley, L. S. 1980. Isolation of a low M. Wt. zinc-binding ligand from human milk. J. Inorg. Biochem. 12: 71.

60. Cousins, R. J. K., Smith, K. T., Failla, M. L., and Markowitz, L. A. 1978. Origin of low molecular weight zinc-binding ligand complexes from rat intestines. Life Science 23: 1819.

61. Casey, C. E., Walravens, P. A., and Hambidge, K. M. 1980. Variation in zinc absorption. Fed. Proc. 39: 651.

62. Kohman, E. 1920. The experimental production of edema as related to protein deficiency. Am. J. Physiol. 51, No. 2: 378.

63. Ashworth, A. 1979. Progress in the treatment of protein-energy malnutrition. Proc. Nutr. Soc. 38: 89.

64. Sandell, E. B. 1950. Colorimetric determination of traces of metals. 2nd edition. Interscience Publishers, London.

65. Kagi, F. 1949. Das Mineralisieren von Milch and Milchprodukten nach der Perchlorsalt-petersaure-Methode. XIIth Intern. Dairy Congr. 2, Sect. 2: 706-713. Stockholm.

66. Anon. 1980. Protein-energy intake and weight-gain. Nutr. Rev. 38 (1): 13.

67. Gorsach, T. T. 1970. Selected decomposition procedures. Page 136 in The Destruction of Organic Matter. Pergamon Press, Oxford.

68. Dreosti, I. E., and Quicke, G. V. 1968. Blood copper as an indicator of copper status with a note on serum protein and leucocyte counts in copper-deificient rats. Br. J. Nutr. 22: 1.

69. Manual for Model SP-90 Unicam Spectrophotometer. Unicam Instruments Ltd., Cambridge, England.

70. Pinta, M. 1971. Atomic absorption spectrometry. Adam Hilger Ltd., London, England.

71. Slavin, M. 1978. Atomic absorption spectroscopy. 2nd edition. Wiley, New York.

72. Wooton, I. D. P. 1964. Microanalysis in medical biochemistry. 4th edition. Churchill, London.

73. White, W. L., Erickson, M. M., and Stevens, S. C. 1976. Chemistry for the clinical laboratory. 4th edition. Mosby Co., St. Louis.

74. Weichselbaum, T. E. 1946. An accurate and rapid method for determination of proteins in small amounts of blood, serum and plasma. Am. J. Clin. Pathol. 16 (10): 40.

75. Long, C., editor. 1961. Biochemists' Handbook. D. Van Nostrand Company, Inc., Princeton, N. J.

76. Meat and Meat Products. 11th edition. A. O. A. C., Arlington, Va.

77. Slavin, S. W., Peterson, G. E., and Lindhal, P. C. 1975. Determination of heavy metals in meats by atomic absorption spectrscopy. Atomic Absorption Newsletter 14 (3): 57.

78. Joslyn, M. A. 1970. Methods in food analyses. 2nd edition. Academic Press, New York.

79. Barr, A. J., Goodnight, J. H., Sall, J. P., and Helwig, J. T. 1976. A user's guide to SAS 76. Sparks, Raleigh, N. C.

80. Snedecor, G. W., and Cochran, W. G. 1967. Statistical methods. 6th edition. Iowa State University Press, Ames, Iowa.

81. Oser, B. L. 1965. Hawk's physiological chemistry. 14th edition. McGraw-Hill Book Co., Inc., New York.

82. Patrick, J., Golden, E. B., and Golden, H. N. 1980. Leucocyte sodium transport and dietary zinc in protein energy malnutrition. Am. J. Clin. Nutr. 33: 617-620.

83. Whitehead, R. G. 1980. Animal models for study of PEM. Proc. Nutr. Soc. 39: 227.

84. Newmann, C. G., Swendseid, M. E., Jacob, M., Stiehm, R. E., and Dirige, O. V. 1979. Biochemical evidence of thiamin deficiency in young Ghanaian children. Am. J. Clin. Nutr. 32: 99-104.

85. Edozien, J. C. 1968. Experimental Kwashiorkor and marasmus. Nature 220: 917.

86. Albanese, A. A. 1959. Protein and amino acid nutrition. Academic Press, New York.

87. Anon. 1976. Protein deprivation, refeeding and ammonia intoxication. Nutr. Rev. 34: 52.

88. Olson, R. E., Editor. 1975. Protein-calorie malnutrition. Academic Press, New York.

89. Patrick, . S., and Mackay, A. M. 1973. Experimental protein-energy malnutrition in baby baboons. (2) Liver pathology. Br. J. Nutr. 30: 171.

90. Millward, D. J. 1979. Protein deficiency, starvation and protein metabolism. Proc. Nutr. Soc. 38: 77.

91. Atshushi, S., Katsuhiko, N., and Yasuo, N. 1979. Effect of protein depletion on the rate of protein synthesis in the rat liver. Biochimica et Biophysica Acta 561: 475.

92. Ononogbu, I. C. 1980. The toxicity of cassava. IBIS, Sept. 1981 X.

93. McLaren, D. S. 1981. Bioaccumulation of elements. 7th Wellcome Visiting Professor Series. Iowa State University, Ames, Iowa, June 1-5.

94. Janet, B., Ezra, S., O'Neill, M., Herbert, N., Sebeck, B., and Shamberger, J. 1979. Effect of protein depletion and repletion on liver structures, nitrogen content and serum proteins. Annals of Surgery August 1979: 144.

95. Anon. 1974. Aldosterone secretion in infantile malnutrition. Nutr. Rev. 32 (10); 296.

96. Patrick, J. 1979. Oedema in PEM - Role of the sodium pump. Proc. Nutr. Soc. 38: 61.

97. Pike, E., and Brown, H. 1967. Nutrition: An integrated approach. John Wiley and Sons, Inc., New York.

98. Settlemire, C. T., and Matrone, G. 1967. In vivo interference of zinc with ferritin iron in the rat. J. Nutr. 70: 514.

99. Kang, H. K., Harvey, W. P., Valentine, J. L., and Swendseid, M. E. 1977. Zinc, iron, copper, magnesium concentrations in tissues of rats fed various amounts of zinc. Clin. Chem. 23 (10): 1834.

100. Anon. 1973. The role of transferrin in iron absorption. Nutr. Rev. 31: 131.

101. Sullivan, J. F., Jetton, M. M., and Hahn, H. K. 1980. Enhanced lipid peroxidation in liver microsomes of zinc-deficient rats. Am. J. Clin. Nutr. 33: 51.

102. Coward, W. A. 1979. Pathogenesis of edema in kwashiorkor - Plasma proteins. Proc. Nutr. Soc. 38: 51.

103. Smith, S. E., and Larson, E. J. 1946. Zinc toxicity in rats. Antagonistic effects of copper in the liver. J. Biol. Chem. 163: 29.

104. Cousins, R. J. 1979. Regulatory aspects of zinc metabolism in liver and intestines. Nutr. Rev. 37 (4): 97.

105. Ariens, E. J., Simonis, A. M., and Offermeir, J. 1976. Dose-response relations. Page 129 in Introduction to General Toxicology. Revised printing. Academic Press, New York, San Francisco.

106. NAS, Washington, D. C.

107. Anon. 1973. Present knowledge of relationship of nutrition to brain development and behavior. Nutr. Rev. 31 (8); 242.

108. De Silva, C. C., and Baptist, N. G. 1969. Tropical nutritional disorders of infants and children. Charles Thomas, Publisher, Springfield, Illinois.

109. Klavins, J. V., Kinney, T. D., and Kaufman, N. 1962. The influence of dietary protein on iron absorption. Br. J. Exp. Pathol. 43: 172.

110. Bartel, P. R., Freiman, I., Rosen, E. U., and Geefhysen, J. 1979. Long-term effects of kwashiorkor on the electroencephalogram. Am. J. Clin. Nutr. 32: 753.

111. Moodie, A. D., Bowie, M. D., Maun, M. D., and Hansen, J. D. L. 1980. A prospective 15-year follow-up study of kwashiorkor patients. S. Afr. Med. J. 58: 671.

112. Heaton, F. W. 1965. Effect of magnesium deficiency on plasma alkaline phosphatase activity. Nature 207: 1292.

113. Loveless, B. W., and Heaton, F. W. 1976. Changes in alkaline phosphatase and inorganic pyrophosphatase activities of rat tissues during magnesium deficiency. The importance of controlling feeding pattern. Br. J. Nutr. 36: 487.

ACKNOWLEDGMENTS

I wish to thank major professor, John Hathcock, and the chair of the department, Dr. Dupont, who helped me in many, many ways through my program. Between them, the World Food Institute and the Ghana Food Research Institute, my Ph.D. program was made possible.

Thanks also to Drs. Serfass and Mark Love for direct involvement with my research in many ways. Dr. Serfass' assistance with the atomic absorption spectrophotometry was invaluable. I received useful direction for the tissue moisture work from Dr. Love.

I must also thank Professor James Olson and Donald Hotchkiss for both serving on my committee and giving counsel when I needed it.

Cynthia Shriver put much time into my final rat study. Jean Stewart helped with some analytical procedures, while Kathy Morrison and Dr. Hotchkiss helped with my statistical analyses. I thank them.

Thanks finally to God for protection and help through the years of academic study here.