IOWA STATE UNIVERSITY
Digital Repository

1981

Zinc and prostaglandin interrelationship in metabolism

Simin Meydani

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Dietetics and Clinical Nutrition Commons, Human and Clinical Nutrition Commons, and the Medical Nutrition Commons

INFORMATION TO USERS

8122541

MEYDANI, SIMIN

ZINC AND PROSTAGLANDIN INTERRELATIONSHIP IN METABOLISM

Iowa State University   PH.D.   1981

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

Zinc and prostaglandin

interrelationship in metabolism

by

Simin Meydani

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major: Nutrition

Approved:

Members of the Committee:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1981

TABLE OF CONTENTS

DEDICATION

TO MY PARENTS

INTRODUCTION

Since Prasad et al. (1) reported zinc deficiency in
Egypt and Iran, research interest in zinc metabolism and its
functions in the body has increased. The biochemical functions
in which zinc has been implicated as necessary include: 1)
enzymes and enzymatic function, 2) protein synthesis, and,
3) carbohydrate and lipid metabolism.

Zinc deficiency has been shown to affect almost all
stages of development from prenatal to adolescence (2, 3, 4)
in all species studied. Important features of zinc deficiency
include: 1) impaired food intake and growth retardation, 2)
alopecia and gross skin lesions, 3) impaired wound healing,
4) hypogonadism and suppression of the secondary sexual
characteristics in males, 5) infertility, prolonged labor
and parturition associated with excessive bleeding in the
female, and, 6) congenital malformation of the fetus. Abnormal
synthesis of nucleic acids seems to be the main mechanism
underlying manifestations of zinc deficiency (5).

Zinc is also necessary for normal cell-mediated and
humoral immune responses. The weight of lymphoid organs
(thymus, spleen and mesenteric lymph nodes) is reduced in
zinc deficiency (6). Depressed _in vivo_ and _in vitro_ immune
responses have been shown in animals (6, 7, 8) and human
beings (9, 10) in zinc deficiency compared to normal. Zinc

deficiency seems to affect cell-mediated immune response by the loss of the T-cell helper function.

One of the biological functions of zinc which is of particular interest to this study is its role in lipid metabolism and prostaglandin (PG) synthesis. There are great similarities between essential fatty acid deficiency and zinc deficiency symptoms such as growth retardation and skin lesions. Bettger et al. (11) showed that zinc deficiency accentuated signs of EFA deficiency. They suggested that zinc is involved in the metabolism of arachidonic acid and therefore can affect PG metabolism. Manifestation of zinc deficiency in acrodermatitis enteropathica (AE, a heredity disease with faulty zinc absorption) has been attributed to its effect on PG synthesis (12). Abnormal metabolism of essential fatty acids has been reported in AE patients (13, 14, 15).

O'Dell et al. (16) demonstrated that the effects of zinc deficiency in pregnant rats were similar to the effects of toxic doses of aspirin which inhibit PG synthesis. Horrobin and Cunnane (17) showed that the symptoms of zinc deficiency can be partly treated by administration of evening primrose oil (72% linoleic acid, C18:2n-6, and 10% gamma-linolenic acid, C18:3n-6). They suggest that zinc seems to be essential for the conversion of linoleic acid to gamma-linolenic acid,

and for the mobilization of dihomogammalinolenic acid (C20: 3n-6) from membrane stores to form the one series of PG (see figure 1).

With all the important roles of zinc in the biochemical functions of the body, very little is known about its absorption and the mechanism(s) involved in it. Different investigators (18, 19, 20) have been able to separate a zinc binding ligand (ZBL) from the rat intestinal mucosa and contents. The exact nature of the ZBL is not clear, and indeed, the literature is very contradictory about this subject. PGE (20, 21, 22) has been implicated as a possible ligand and there is direct evidence for (20, 21, 22, 23) and against (24, 25, 26) it. There are however, indirect indications that PG might be involved in zinc homeostasis. Aspirin is one of the most generally used drugs in the therapy of rheumatoid arthritis (RA) and zinc level is altered in RA patients (27, 28). In addition, aspirin is teratogenic in rats and Koshakji and Schulert (29) postulated that this teratogenic effect might be due to the insufficient availability of zinc to the fetus.

If PG are involved in zinc metabolism modifying their level in different organs by changing their percursors in the diet or using an inhibitor of PG synthesis like aspirin and indomethacin should affect the zinc status of the rat. By the same token, if zinc is involved in PG synthesis

elimination of zinc from the diet should change the synthetic potential and/or existing level of PG in the tissues.

The purpose of this study was to find: 1) what role, if any, PG might play in zinc absorption and biological functions; this includes measuring food intake and weight gain, zinc concentration in different tissues, serum alkaline phosphatase activity (a zinc metalloenzyme), and the <u>in vitro</u> immune response to mitogens under conditions of altered zinc and PG status in rats. 2) What might be the effect of zinc deficiency on PG synthesis as measured by PG level in different tissues of rats.

REVIEW OF LITERATURE

Biological Significance of Zinc

The essentiality of zinc for microorganisms has been
known for 100 years (30). Todd et al. (31) demonstrated in
1934 that zinc was needed for normal growth in rats. Only
in 1961, however, was it suspected that zinc deficiency
occurred in human beings. Subsequently, the presence of zinc
deficiency among Iranian and Egyptian villagers was discovered
and the clinical symptoms were described (32). Since then
research interest in zinc metabolism and its function in the
body has increased. The biochemical functions in which zinc
has been implicated as necessary include: 1) enzymes and
enzymatic function, 2) protein synthesis, and 3) carbohydrate
and lipid metabolism.

Since the identification of zinc as an essential
component of carbonic anhydrase (33), more than forty zinc
metalloenzymes have been recognized (34). Those identified
in mammalian tissues include alkaline phosphatase, carboxy-
peptidase and alcohol dehydrogenase. Important enzymes in
metabolism of nucleic acids are zinc dependent, such as
thymidine kinase and DNA and RNA polymerases. This critical
role of zinc in nucleic acid metabolism and protein synthesis
can explain the high requirement of zinc in young animals
and its importance for normal growth and development. Zinc

deficiency is shown to affect almost all stages of development including prenatal (2), postnatal, infancy and childhood (3), and adolescence (4) in all species studied.

Impaired food intake and growth retardation are prominent features of zinc deficiency and those effects probably arise from impaired DNA synthesis and cell division. Nutritional dwarfism and hypogonadism were the main clinical manifestations of zinc deficiency demonstrated in Iran and Egypt (4). The growth retardation effect of zinc deficiency is a combination of impaired appetite, food utilization, and normal taste sensation (hypogeusia). Hambidge et al. (35) discovered that poor growth and appetite, together with hypogeusia in young children in Denver, was associated with subnormal hair zinc level. Supplementation of children's diet with zinc corrected taste acuity, improved growth and increased hair zinc level.

One of the features of zinc deficiency which is seen in all species is hair loss (alopecia) and gross skin lesions. Acrodermatitis enteropathica (AE), a heredity disease which appears in early infancy and is characterized by pustular and eczematoid skin lesions, alopecia, and diarrhea is due to impaired zinc absorption and responds to zinc therapy (36). The skin lesions and impaired wound healing in zinc deficiency are attributed to the involvement of zinc in RNA and DNA synthesis, incorporation of sulfur amino acids into skin

proteins, and collagen synthesis. Reduced collagen synthesis and alterations in collagen cross-linking in skin of zinc deficient rats have been reported (37).

Zinc is necessary for spermatogenesis and the development of the primary and secondary sex organs in the male, and for all phases of the reproductive process in the female from estrus to parturition and lactation. Atrophic seminiferous tubules were observed in the earliest study of the histopathology of zinc deficiency in the rat (37). Hypogonadism, with suppression of the secondary sexual characteristics were among the first clinical features of zinc deficiency reported (1, 32). The effects of zinc deficiency in the female depend on the severity, timing, and duration of the deficiency. Hurley and Swenerton (38) showed that feeding a diet nearly free of zinc to female rats from weaning to maturity caused severe disruption of estrous cycles and in most cases no mating took place and the animals were infertile. When pregnancy occurred more than half of the implantation sites were resorbed. Surviving fetuses had less than half the normal body weight and nearly all showed gross congenital malformations which affected every organ system. The abnormalities seen were beyond that of food restriction.

The effect of severe maternal zinc deficiency is very rapid. Hurley and Shrader (39) showed that after only 3 days of maternal deprivation of dietary zinc, abnormal development

was seen in preimplantation embryos. Gallaher and Hurley
(40) in a recent publication demonstrated that the effect
seen is a direct result of too little zinc for proper cell
division and growth and not due to an indirect effect on
maternal metabolism. In their study they demonstrated that
zinc content of intrauterine fluid decreased significantly
after four days of zinc deprivation. In addition to effects
on the fetus, zinc deficiency has deleterious effects on
mothers (41). Zinc deficiency during pregnancy caused
difficulty of parturition, excessive bleeding and longer
total time of delivery. The behavior of mothers after birth
was also different; for example, they did not consume the
afterbirth and the offspring were randomly distributed about
the cage.

Zinc deficiency also impairs reproduction and normal
development of the fetus in non-human primates (42). The
relationship between zinc deficiency and congenital mal-
formation in human beings is not known. The only documented
congenital deformation in humans related to zinc deficiency
is that of Hambidge et al. (43) reporting an outcome of
pregnancy of two AE patients. One of them gave birth to an
achondroplastic dwarf, who died 1/2 hour after delivery.
After the mother started therapy with diiodohyoxyquin
(which increases zinc absorption), she gave birth to two
normal children. Jameson (44) states that there is a strong

association between complications of pregnancy and low levels of zinc during the first trimester and there seems to be a higher incidence of congenital malformations in the infants of women with lower plasma zinc levels during pregnancy.

One of the organ systems upon which zinc deficiency seems to have a detrimental effect is the central nervous system. Structural changes in the brain and organs associated with the central nervous system of fetus and offspring due to maternal zinc deficiency are well-documented (45, 46). Whether these structural changes have functional significance is not clear. Behavorial changes due to zinc deficiency has been reported however in rat and monkey (47).

The main mechanism underlying manifestations of zinc deficiency during development seems to be abnormal synthesis of nucleic acid (5). The impairment of nucleic acids causes alterations in differential rates of growth. These changes in normal growth rate lead to asynchrony in the development of tissues and organs (5). Zinc also is needed for normal skeletal development (37), and might have a role in pathogenesis of atherosclerosis. Recently it was shown (48) that zinc might be an important trace mineral in the process of post eruptive mineralization of the dental enamel and may reduce the susceptibility of teeth to caries. There is interesting evidence for involvement of zinc in some disease states like sickle cell anemia (49) and arthritis (50). Zinc is also necessary for normal cell-mediated and humoral immune

responses which will be discussed in more detail.

Role of zinc in the immune system

The recent interest in the effects of nutritional imbal-
ances on immunity functions has led to the study of immune
responses in a variety of deprivation states including zinc
deficiency. The first clue to the involvement of zinc in
immune response came from the work of Miller et al.(51) in 1968.
Their results indicated that zinc deficient animals had a
reduced weight gain, anorexia, and other biochemical and
physiological abnormalities. Of particular interest is their
observation that thymus and spleen weight were markedly de-
pressed. In addition, peripheral lymphocyte counts and serum
gamma globulin were reduced. Subsequently, decreased thymus
weight and atrophy of thymic tissue were noted in rats (6) and
mice (52). The study in mice is the first study that shows
functional impairment of the immune system in zinc deficiency.
In this study (52), it was shown that zinc deficient mice have
depressed antibody response to keyhole limpet hemocyanin-p-
azophenylarsonate antigen.

Subsequently other investigators have confirmed these
results. Frost et al.(6) showed that in zinc deficient
Blab/c mice the weights of lymphoid organs (thymus, spleen,
and mesenteric lymph node) were far more depressed than those
of non-lymphoid organs. They showed also that zinc deficient
animals had altered response to sheep erythrocytes as measured

by hemolytic plaque-forming cells. The overall response to this antigen was depressed and delayed and it lasted longer than that of control animals. The authors have attributed the decrease in lymphoid organ weights and depressed antibody response to an impairment of DNA synthesis in zinc deficient animals. Both production of new lymphoid cells and active proliferation of resting antigen sensitive cells require a normal and constant DNA synthesis which is impaired in zinc deficiency. In order for the B-cell to respond to sheep erythrocytes it needs a T-cell to perform a "helper" function. Frost et al. (6) suggested that in the zinc deficient animal there is a loss of T-cell helper function. T-cells also control the duration of response and have a suppressive function over the B-cell response. These investigators, therefore, concluded that the impaired and longer lasting response to antigen in zinc deficient animals is due to loss of T-cell helper function.

This conclusion was confirmed by other investigators (7). DePasquale-Jardieu and Fraker (53) showed that a significant part of loss of T-cell helper function was due to zinc deficiency itself. Further suppression was produced, however, due to hyperactivity of the adrenal cortex and concomitant rise of glucocorticoids in zinc deficiency. Glucocorticoids have been shown (54) to cause functional alterations of the thymus.

Beach et al. (55) studied the effect of postnatal zinc deficiency on the immune response of mice pups. They fed lactating mice from the day of parturition and their pups to 8 weeks of age, diets containing varying levels of zinc from 2.5 to 100 ppm. They observed severe growth retardation of lymphoid tissues, especially thymus. The magnitude of splenic and thymic hypogenesis was directly dependent upon the severity of the dietary zinc deficiency. There was also decreased splenic cellularity in the zinc deprived rats. Furthermore, at 4 weeks of age direct splenic plaque forming cell responses to sheep erythrocyte immunization were significantly diminished in severely and moderately zinc deprived rats during the postnatal period. In addition the serum immunoglobin profile of the zinc deficient mice was altered with decreased IgM, IgG and IgA.

These findings have great implications in studying the prevalance of infection and immune deficiency among malnourished children. Low levels of plasma zinc commonly observed in children with moderate to severe protein energy malnutrition might be the cause of defective cell mediated immunity often seen in these children. Therapeutic administration of zinc might be beneficial to such patients. Zinc deficiency can also occur in patients with small bowel malabsorption syndrome and those receiving total parenteral nutrition. These patients have increased incidence of sepsis that

might be due to impaired cellular immunity caused by zinc deficiency.

Abnormal cellular immune response is reported in AE patients (9). In infants with protein energy malnutrition and hypozincemia, it was found that atrophic thymus glands were restored to near normal with zinc therapy (56). Pekarek et al. (10) assessed the cellular immune response of a patient on total parenteral nutrition before and after zinc therapy. Blood analysis and anthropometric measurements indicated that the patient had low zinc and energy intake, but other nutrients such as vitamins A, B_2, C and folate were normal. Cellular immunity was measured by delayed skin reactivity to dinitrochlorobenzene and by in vitro lymphocyte transformation studies before and after zinc therapy. Both of these indices were abnormal before zinc therapy and returned to normal after 3 weeks of zinc therapy. Patients with Crohn's disease, who have low plasma zinc levels, demonstrated depression of T-lymphocytes (57). The above mentioned investigations suggest that adequate zinc nutriture is essential in humans just as it is in experimental animals for maintenance of certain aspects of cellular immunity.

Because of the key role that zinc metalloenzymes play in RNA and DNA synthesis the effect of this trace element on cellular immunity might be due to its control over replication of cells involved in the immune response. Gross

et al.(8) tested this hypothesis in rats. They first demonstrated that zinc deficiency in rats depresses the blastogenic response of lymphocytes from thymus, spleen and peripheral blood to phytohemaglutinin (PHA) and conconavalin A (ConA) and pokeweed mitogen (PWM). PHA and ConA are primarily T-cell mitogens and PWM is both a T- and B-cell mitogen. In a subsequent study (58) they tested whether the depressed responses to PHA and ConA were due to impaired DNA and RNA synthesis or due to the role that zinc plays as a functional component of plasma membrane. Zinc supplementation has an inhibitory effect on many macrophage functions including mobility (59), phagocytosis, oxygen consumption, and bactericidal activity (60). Zinc plays an important role in the stabilization of plasma membrane. Zinc deprivation might result in membrane labilization with subsequent changes in membrane receptor-site availability and function.

Chvapil et al.(60) showed that zinc-8-hydroxyquinolone which can not permeate the cell has a similar blastogenic effect to zinc. Gross et al.(58) used Levamisole (L-2,3,5,6-tetrahydrochloride-6-phenylimidazo (2,1-b) thiazole hydrochloride) to test the two possibilities. Levamisole improves reticuloendothelial cell function and phagocytosis and enhances responses to mitogens and antigens. They showed that addition of levamisole to cultures of zinc deprived spleen lymphocytes significantly improved their response to

PHA by 54%. The addition of levamisole to control lymphocyte cultures had no effect. Although levamisole's mode of action is not completely understood, it might promote the expression of T-cell receptors and inhibit the expression of C_3 receptors on B cells. Because levamisole does not affect DNA or RNA synthesis and metabolism, and it corrects the depressed blastogenic response of lymphocytes in zinc deprived rats, the authors have suggested that "the immunodepression in zinc deficiency is a combined result of the biochemical lesion and membrane alterations, with the DNA synthetic defect not being rate limiting".

The mechanism of zinc function in the immune system still remains unclear and needs further investigation. The above mentioned studies, however, elucidate the important role of zinc in the immune response and indicate that it can be used as a measurement of zinc status. Furthermore, in setting dietary requirements for zinc for animals and humans the total integrity of the immune system should be considered.

Role of zinc in lipid metabolism and PG synthesis

Zinc deficiency has been shown to cause changes in adipose tissue metabolism (61). Some of the changes can be accounted for by different patterns of food intake between zinc deficient and pair fed control groups. Quaterman and Florence (62), however, noted that there was lower plasma insulin concentration after administration of a dose of glucose in

zinc deficient animals even beyond the differences caused by food intake pattern. Lower plasma insulin level was followed by a transient rise in plasma free fatty acid (FFA) concentration. The rise in FFA was attributed to an increased degradation of reserve triglyceride.

Other investigators (63) also showed a rise in serum FFA in rats due to zinc deficiency. Sullivan et al. (64) recently reported that zinc deficiency enhanced lipid peroxidation in liver microsomes of rats. In that study, they compared the effect of zinc deficiency with that of ethanol on enhancement of lipid peroxidation. Cirrhotic patients have low serum zinc levels (65) and chronic alcoholism is associated with liver cirrhosis. Sullivan et al. (64) fed groups of rats a control or a zinc deficient diet and half of each group received 3.85 g/kg body weight (BW) of ethanol by stomach tube. Although they mentioned that there were pair fed groups for zinc deficient and ethanol fed groups, the results of the analysis for these groups were not shown. Their results showed that although zinc deficiency did not cause fatty liver as ethanol did, both groups had similar rises in hepatic phospholipids.

Zinc deficiency and not alcohol caused an increase in diene conjugation of microsomal lipids which is used as an indicator of in vivo lipid peroxidation. Both zinc deficient and ethanol fed groups showed increases in in vitro enzymatic and non-enzymatic lipid peroxidation activity as measured by generation of malondialdehyde. They (64) have postulated that

the mechanism of increased lipid peroxidation in zinc deficien-
cy might be an increase in microsomal phospholipids leading
to an increase in the level of unsaturated fatty acids.
Increased unsaturated fatty acids can furnish more substrate
for lipid peroxidation. Finally they have concluded that
zinc deficiency might play a role in the development of
alcoholic hepatitis.

Chvapil et al. (66) showed that pharmacological doses
of zinc (1000 ppm) decreased lipid peroxidation in whole
liver homogenates and in the liver microsomal fraction, but
not in other organs. This can be explained by the fact that
excess zinc accumulates in liver but not in other organs.
They also showed that the concentration of arachidonic acid
(C20:4n-6) in the total fatty acids of the liver was signif-
icantly higher in rats fed a high zinc diet than a
control diet and the concentration did not change after 60
minutes incubation at $37^{o}C$. In addition, the magnitude of
spontaneous hemolysis of erythrocytes was inhibited in rats
fed 1000 ppm zinc in comparison with 40 ppm zinc. They have
concluded, therefore, that dietary zinc controls lipid
peroxidation in liver and red blood cells. The dietary level
of zinc is about 30 ppm and although they mentioned that three
groups of animals were fed 0.5 ppm, 40 ppm, and 1000 ppm
zinc, they only showed the data for 40 ppm and 1000 ppm zinc.
One can suspect that perhaps there was not a significant

difference between 0.5 ppm and 40 ppm zinc fed animals.
Therefore, the physiological importance of their finding
remains questionable.

Koo and Turk (67) studied the effect of zinc deficiency
on intestinal transport of triglyceride (TG) and showed that
the rate of TG absorption decreased markedly in zinc deficient
rats, although the digestion of TG was not affected by zinc
deficiency. The only abnormality observed was in chylomicron
formation which resulted in accumulation of fat droplets with-
in mucosa. This can be explained by impairment of protein
synthesis in zinc deficiency.

There are great similarities between essential fatty
acid (EFA) deficiency and zinc deficiency symptoms. For
example, both deficiencies are accompanied by growth retarda-
tion, parakeratosis, lengthened gestation period, parturition
accompanied by excessive bleeding, and sterility of males.
These resemblances have suggested that there might be a
physiological interaction between the two. Bettger et al. (11)
looked at these interactions in the rat. They fed rats diets
containing low levels of zinc or EFA or both. A control group
with adequate zinc and EFA and pair fed groups also were tested.
They showed that low zinc status accentuated signs of EFA
deficiency including dermal lesions and growth rate but had
no effect on the fatty acid (FA) composition of plasma.
Analysis of FA in dorsal foot skin of the rats, however, showed

that the severe zinc deficiency significantly affected the FA composition of skin from both the adequate and the low EFA groups. There was a two fold increase in percentage of arachidonic acid. They suggested that zinc is involved in the metabolism of arachidonic acid, perhaps by decreasing its catabolism. This could indicate a change in PG metabolism. It is worth pointing out here that Ziboh and Hsia (68) showed that external application of PGE_2 cured the dermal lesions of EFA deficiency. If zinc is essential for the conversion of arachidonic acid to PG, similar pathology should result from zinc deficiency, EFA deficiency or inhibition of PG synthesis.

Later Bettger et al. (69) looked at interaction of zinc and polyunsaturated fatty acids (PUFA) in chicks. They did not find similar results. There was a negative correlation between PUFA and zinc deficiency and PUFA aggravated the signs of zinc deficiency. Although the authors stated that zinc deficiency caused an increase in arachidonic acid levels in dorsal foot skin of chicks, when the levels were compared to that of a pair fed control group, the result was not significant, i.e. the increase in C20:4n-6 is mainly due to food restriction. Although administration of indomethacin (an inhibitor of PG synthesis) improved the dermal lesions, topical application of PGE_2 and $PGF_{2\alpha}$ had no significant effect which is contrary to the results found by Ziboh and Hsia (68). The differences observed might be due to species

differences and different modes of PG metabolism and function.

It has been suggested (12) that the manifestation of zinc deficiency in AE patients is due to its effect on PG synthesis. Abnormal metabolism of EFA is reported in AE patients (13, 14, 15). Patients with AE respond to zinc therapy or administration of penicillamine. Penicillamine was shown to increase PGE_1 production (70). Clinical improvements may occur after supplementation of the diet with EFA even in the absence of zinc or diodoquin (13) which increases zinc absorption. This might indicate that at least part of the abnormalities in AE patients is due to altered EFA metabolism caused by zinc deficiency.

Manku and Horrobin (71) showed that in an isolated perfused rat mesenteric preparation, zinc is a powerful stimulator of the production of a substance which has the characteristics of PGE_1. They also postulated that zinc might be involved in the mobilization of dihomo-γ-linolenic acid (C20:3n-6) (72). Horrobin et al. (73) suggested that the function of zinc in treatment of acne is via stimulation of PG synthesis. O'Dell et al. (16) demonstrated that the effects of zinc deficiency on pregnant rats were similar to the effects of toxic doses of aspirin which inhibits PG synthesis. Both zinc deficiency and aspirin excess produced prolonged gestation, difficult parturition and lowering of blood pressure and body temperature.

Horrobin and Cunnane (17) proposed that zinc has two effects on EFA/PG metabolism. First, it seems to be essential for the activity of the delta-6-desaturase which converts linoleic acid to gamma-linolenic acid and, second, it seems to be required by the enzyme system which mobolizes C20:3n-6 from membrane stores in order to form the one series of PG (see Figure 1). Their hypothesis is based on a series of experiments which will be summarized below.

1) On the perfused mesenteric vascular bed of the rats, zinc stimulated PGE_1 production within minutes (72); therefore, they suggest that the effect was not dependent on new enzyme synthesis. The effect could be blocked completely by aspirin-like drugs which block conversion of both C20:3n-6 and C20:4n-6 to their respective PG. The effect could be imitated only by C20:3n-6 and PGE_1 and not by arachidonic acid or by two series PG. The zinc effect could also be blocked by lithium. Lithium seems to block conversion of stored C20:3n-6 to free C20:3n-6 but not the conversion of C20:3n-6 to PGE_1 (72).

2) Male Wistar rats were maintained on a zinc deficient diet for 5 weeks (74, 75) and compared with zinc deficient rats supplemented with a) evening primrose oil (EPO-72% C18: 2n-6 and 10% C18:3n-6), b) safflower oil (SFO-83% C18:2n-6), and c) olive oil (OLO 10% C18:2n-6). The oils were injected subcutaneously at 250 µl/day. The severe growth retardation, hunched posture, keratosis, and alopecia of the zinc deficient
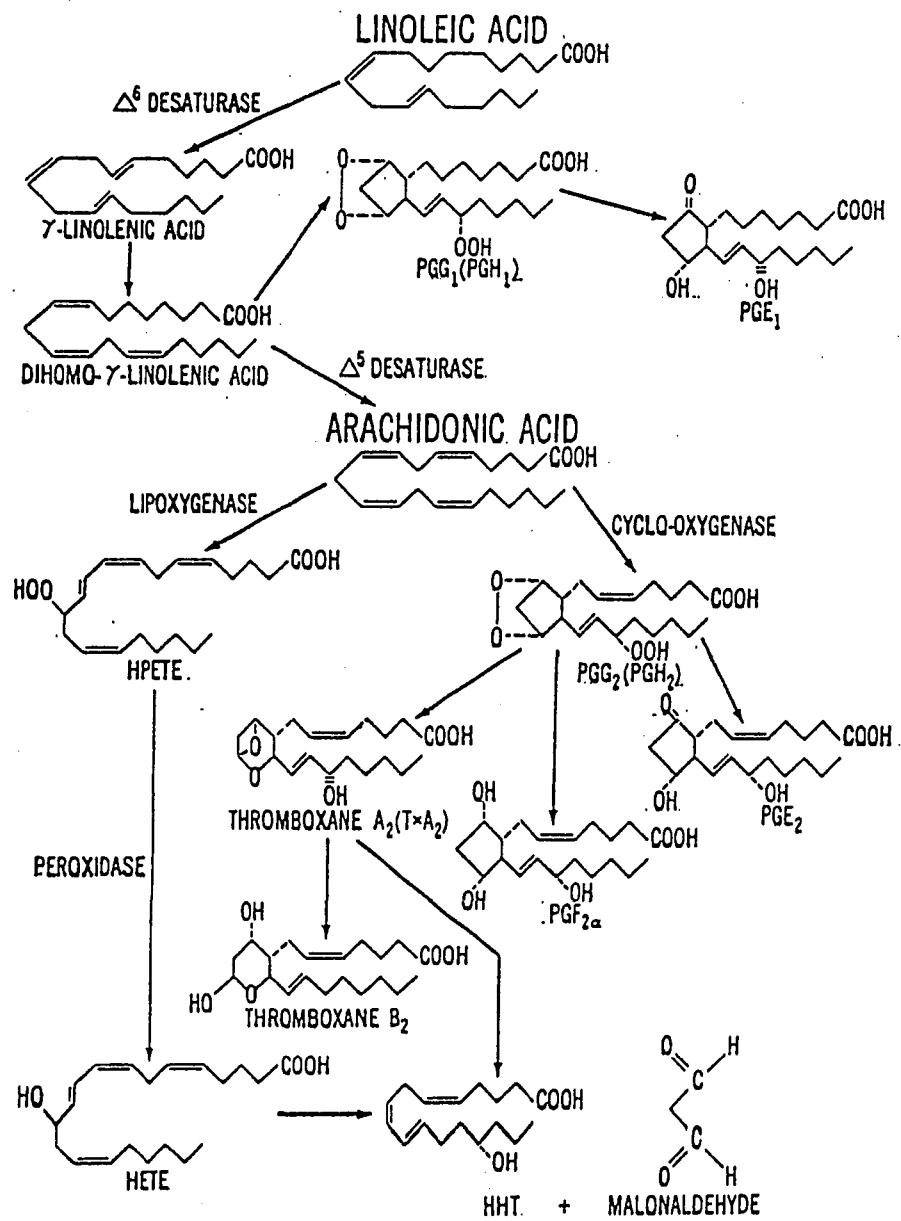
Figure 1: Biosynthetic pathway of PGE_1, E_2, $F_{2\alpha}$, and TXB_2

rats were not affected by OLO. SFO treated rats had improved growth and dermal condition. The growth improvements in EPO treated rats were three times those of SFO treated rats but dermal lesion improvement was similar. The EPO also increased organ weights (thymus, adrenal, and epididymal fat). The authors have attributed the difference seen to the presence of free C18:3n-6 in EPO and concluded that in zinc deficiency there is a block in C18:2n-6 desaturase which converts C18:2n-6 to C18:3n-6 which after elongation to 20C can be used for PG synthesis. Since safflower oil affected the dermal lesions similarly to EPO they suggested that the effect of C18:2n-6 in dermal lesions is not mainly via PG synthesis.

3) The same experiment was repeated in male rats (17). SFO had no effect in reversing the effects of zinc deficiency on overall growth or on the weights of any organ, whereas primrose oil produced a substantial improvement toward normality.

In summary, zinc seems to play regulatory roles in the metabolism of EFA and specifically in PG production. Zinc and EFA interaction may be of importance in a variety of clinical syndromes.

Zinc Absorption and Metabolism

In rats, zinc is mainly absorbed from the duodenum, ileum and jejunum. Homeostatic control of body zinc is partly regulated through zinc absorption (76). Zinc absorption is

affected by different factors including phytic acid, fiber, calcium, protein source and zinc itself (35).

The mechanism of zinc absorption is not completely understood. Different hypotheses have been proposed with scientific evidence for and against them. However, the general belief is that it is an active transport across the lumen and some sort of a ligand, the nature of which is very controversial, is involved. Suso and Edwards (77) were the first to propose that a low molecular weight zinc-binding ligand (LMW-ZBL) might be involved in transport of zinc. Hahan and Evans (18) separated a low molecular-weight [65]zinc-complex from the rat intestinal lumen and mucosa following oral administration of [65]Zn. Analysis of the [65]Zn-complex by thin layer chromatography (TLC) indicated that the complex had properties different from that of zinc salts and simple zinc-amino acid complexes. Subsequently Evans et al. (76) separated this ligand from pancreatic secretions of rat and dog and demonstrated that [65]Zn absorption was markedly diminished in rats in which the pancreatic duct was ligated.

Furthermore, the uptake of [65]Zn by epithelial cells from everted intestinal segments was markedly increased in the presence of a ZBL fraction from pancreatic secretions. The authors suggested that a low molecular weight ZBL from the pancreas may facilitate zinc uptake into intestinal cells. It was also shown that metal-free albumin might promote the

release of zinc from epithelial cells by interacting with
zinc-binding sites on the basolateral plasma membrane. On
the basis of these experiments Evans proposed a mechanism:
"The pancreas secretes a ligand into the duodenum where zinc
complexes with the molecule; complexed with the ligand, zinc
is transported through the microvillus and into the epithelial
cell 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. (19) reported the presence of a relatively
low molecular weight ZBL in human milk which was not found in
cow milk. The molecular weight was estimated by gel-filtration
to be 8700. They suggested that this ZBL is responsible for
the efficacy of human milk in treatment of patients with
acrodermatitis enteropathica (children with AE will only
develop signs of zinc deficiency if they are weaned from
breast milk and fed cow milk). This LMW-ZBL was absent
from the cow milk. They postulated that this ligand enhances
the absorption of zinc in the normal neonate and that the
beneficial properties of breast milk over cow milk for human
neonates is due to the presence of this ligand.

Duncan and Hurley (78) subsequently separated the same
ligand from rat milk. The interesting observation was that
in new-born rats up to the age of 18 days, zinc was mostly
associated with higher molecular weight ligand, but after 18

days zinc was associated mostly with a low molecular weight

fraction. This suggested that the presence of a ZBL in rat

milk prior to the development of the intestinal ZBL may

enhance intestinal absorption of this nutrient during this

period.

The exact nature of the ZBL is very controversial. Song

and Adham (20) purified the ZBL from rat intestine and demonstra-

ted that the ligand was similar to one of the prostaglandins.

They then showed that PGE_2 facilitates both zinc absorption

in vivo and zinc uptake into intestinal segments in vitro (23).

In a subsequent study (22), they showed that addition of 200 µg

PGE_2 to the mucosal medium increased transport of ^{65}Zn

across the everted jejunal sacs while $PGF_{2\alpha}$ decreased its

transport. In contrast, addition of $PGF_{2\alpha}$ to the serosal

medium increased the transport of ^{65}Zn from serosa to mucosa

and PGE_2 decreased it. Addition of 400 µg of PGE_2 or $PGF_{2\alpha}$

did not have an effect on histidine transport which was used

as a control molecule. Oral administration of PGE_2, with ^{65}Zn,

three hours before the animals were sacrificed, caused an

increase in zinc content of liver and pancreas. Further

administration of 3 mg indomethacin orally, which amounts

to almost 10 mg/kg BW and is a toxic dose, caused decreased

liver zinc. They have concluded that PGE_2 and $PGF_{2\alpha}$ with

their opposite effects on zinc absorption regulate transport

of zinc across the intestinal mucosa. Whether the same

conclusion can be reached under physiological conditions is not clear.

Sobocinski et al. (79) used doses of indomethacin between 1 and 10 mg/100 gm/BW injected subcutaneously. They injected fed or fasted animals 17 hours before killing them. Zinc was measured in plasma and liver. Metallothionein was also quantitated in liver. The single injection of indomethacin in fed animals produced, by 24 hours, decreased plasma zinc, increased liver zinc, liver metallothionein, and gastrointestinal lesions. The same dose in fasted animals did not cause a significant change in zinc homeostasis. Indomethacin in fasted animals does not cause enteropathy. The authors concluded that in their study the effect of indomethacin on zinc homeostasis is associated with drug-induced enteropathy. In contrast to Song and Adham (22), these investigators showed that subcutaneous injection of 10 mg/100 gm/BW of indomethacin 16 hours prior to administration of an oral dose of ^{65}Zn increased zinc absorption. Song and Adham (22) used fasted animals and administered indomethacin orally whereas Sobocinski et al. (79) used fed animals and indomethacin was injected, which might explain the discrepancy seen between these two studies.

Evans and Johnson (21) showed that subcutaneous injection of aspirin reduced zinc absorption from bovine milk. These investigators have also demonstrated that ultraviolet spectral

characteristics of a purified fraction of ZBL from porcine duodenum is similar to PGE_2. In addition, ^{14}C from labeled arachidonic acid given orally to rats appeared in the ZBL from intestine.

The proposition that ZBL is a PG has been challenged by other investigators. Indeed, Evans and Johnson (21) who had originally suggested that ZBL is a PG later stated (80) that PGE_2 is not a biologically important ZBL and that they were misled because of the anomalous behavior of labile zinc complexes in solvent systems unbuffered with zinc ion. In their previous works they used classical gel-filtration chromatography. Apparently the dextran gel binds zinc and therefore cannot be used to detect labile zinc binding ligands in biological fluids unless it is preequilibrated with buffer containing zinc. Equilibration with buffer containing zinc also prevented dissociation of metal-ligand complex which would occur otherwise.

Using the modified gel-filtration method Evans and Johnson (81) showed that the ligand is picolinic acid, a product of tryptophan metabolism. Subsequently, they fed rats diets containing 5% casein and 5% casein supplemented with either picolinic acid or tryptophan. Rats fed the low protein diet gained 170 ± 17 g in six weeks while rats fed the same diet supplemented with picolinic acid (0.2 mg per gram diet) gained 221 ± 8 g. The rats fed the unsupplemented

diet absorbed 133 ± 33 mg zinc per day while the supplemented rats absorbed 205 ± 26 mg zinc per day. The kidneys of the rats fed the supplemented diet had higher concentrations of zinc. The investigators further provided evidence that there was a linear relationship between the amount of pyridoxine HCl in the diet and the amount of zinc absorbed (82). Pyridoxal is needed as a cofactor in the pathway of tryptophan to picolinic acid.

Robertson et al. (83) showed that metabolism of tryptophan was impaired in children who were affected with AE. Evans (84) demonstrated that human milk had a much higher concentration of picolinic acid than cow milk. Furthermore, pancreatic extract that is used under the trade name of Viokase was shown to have a therapeutic effect on AE patients (85). This pancreatic extract was shown to have a high concentration of picolinic acid. Based on the above observations, Evans (84) proposed a new hypothesis; "The production of picolinic acid from tryptophan and the secretion of this ligand into the intestinal lumen may be the rate limiting step in the absorption of dietary zinc."

Interestingly, Lonnerdal et al. (24) at the same time proposed that the ZBL is citrate. They separated ZBL from human milk by means of gel-filtration, and ion-exchange chromatography. They treated their gels with sodium boro-hydride to reduce charged groups and were able to recover 98%

of zinc from the column. They confirmed their results with infrared spectroscopy and nuclear magnetic resonance. These authors then concluded that the therapeutic value of human milk for AE patients is due to the greater content of bio-available zinc-citrate in human milk than that of cow milk. They showed that zinc-picolinic acid complex in their system eluted at an entirely different position than did the ZBL of human milk. In addition, they were not able to find appreciable amounts of PGE_2 in the ZBL. There are some unexplained points in this study; for example, the concentration of citrate in cow milk is higher than in human milk. Furthermore, there is no citrate in rat milk due to the activity of an ATP-citrate lyase in rat mammary tissue (86). In addition, Casey et al. (87) demonstrated that addition of citric acid or picolinic acid to the diet of children with AE did not change the uptake of zinc in these patients as measured by zinc tolerance tests.

Cousins (88) disagrees in a number of points with the above reported conclusions. He believes that liver plays a key role in zinc metabolism. Richards and Cousins (25) showed that changing dietary zinc causes changes in liver zinc associated with a higher molecular weight ligand (about 9000 Dalton) which was later characterized as metallothionein. They proposed that in liver the protein acted as a temporary storage form which is found in appreciable amounts during

short-term elevations of intrahepatocyte zinc. These investigators then showed that zinc-metallothionein complex was also found in intestine. The amount of zinc associated with the protein was dependent on the dietary zinc. When protein synthesis was inhibited by use of actinomycin D, zinc accumulated in mucosal cells and excess zinc did not influence absorption (88). Smith et al. (89) using isolated, vascularly perfused rat intestine concluded that LMW-ZBL was not required for zinc absorption. In their system 3µM PGE_2 decreased zinc absorption from lumen into the intestinal cells. Aspirin at 150 mg/kg BW decreased transfer of infused ^{65}Zn to the vascular perfusate. At such a concentration of aspirin the effect might be due to other properties of aspirin than PG synthesis, especially since addition of PGE_2 decreased absorption rather than increasing it. They have thus proposed that "zinc absorption is regulated, at least in part, by the amount of zinc that is bound to the inducible intestinal zinc binding protein."

Subsequently, Cousins et al. (90) demonstrated that LMW-ZBL is actually a degradation product of metallothionein. They indicated that proteolysis is a major problem when working with preparations of intestinal cells. The zinc-protein complex is also sensitive to temperature. These authors suggested that the intestinal LMW-ZBL as separated by gel filtration chromatography is a product of the method of isolation. However, they do not rule out the possibility that one or more

LMW-ZBL might play a role in zinc absorption.

Panemangalore and Brady (91) also showed that zinc depletion decreased incorporation of zinc into metallothionein. When the rats were replete with zinc, the incorporation of the nutrient into metallothionein increased. Starcher et al. (92) also provided evidence that zinc absorption is directly proportional to intestinal metallothionein levels. In the sheep intestine, however, metallothionein was not responsive to dietary changes in zinc (93). Schricker and Forbes (26) showed that ZBL separated by them from intestinal mucosa using gel-chromatography behaved differently from PGE_2-zinc complex. Their preliminary characterization of the ligand led them to believe that it is a small peptide.

From the above described studies it is clear that the nature of ZBL is not unequivocally determined. In fact, the ZBL might actually be a heterogeneous group of compounds, each binding zinc under a particular set of conditions. Cousins et al. (90) demonstrated that when cytosolic zinc was increased by either oral administration of zinc or in vitro addition of Zn^{2+} an appreciable amount of zinc was associated with LMW-ZBL in contrast to the normal situation. Cousins and Smith showed (86) that in milk binding of zinc with low molecular weight ligand depends on zinc concentration.

Although the mechanism proposed by Cousins indicates that metallothionein has an important regulatory effect on zinc homeostasis, it does not clarify how zinc passes across

the luman wall into the mucosa. Presence of another zinc binding ligand, the nature of which is not clear, might play an important role.

As mentioned before, the role of PG in zinc absorption and/or metabolism is not clear yet. There are direct indications for (16, 20, 21, 22, 23) and against (24, 25, 26) it. There is, however, indirect evidence that indicates PG might be involved in zinc homeostasis. Aspirin is one of the most generally used drugs in the therapy of rhuematoid arthritis (RA) and zinc levels are altered in RA patients (94). Kennedy et al. (27) have demonstrated that plasma zinc concentrations in 47 males and 67 females with RA was lower than normal. Niedermeier and Griggs (28) also have shown that zinc sulphate therapy is beneficial in patients with RA. Koshakji and Schulert (29) showed that aspirin was teratogenic in rats. They postulated that this teratogenic effect might be due to the insufficient availability of Zn, Fe, and Mn to the fetus. Furthermore, Cunnane et al. (75) demonstrated that symptoms of zinc deficiency can be treated by administration of EFA which are precursors of PG synthesis.

Although PG might not be structural components of ZBL, there are strong indications that they are involved in the regulation of zinc homeostasis. Further investigations considering the interaction of these physiologically important compounds with zinc metabolism is highly desirable.

Role of PG in Cell-mediated Immunity

Prostaglandins are biologically active lipids synthesized from PUFA (figure 1) in the body and are found in almost all tissues. Since their discovery in the 1930s they have been implicated in the regulation of diverse biological functions such as adrenergic nerve transmission (95), blood pressure and blood flow (96), platelet aggregation (97), uterine and ovarian contraction (98), luteolysis (98), lipolytic processes (99), chronic inflammation (100), and cell-mediated immunity (101). Excellent reviews on the biological functions of PG are available (102, 103).

One of the biological systems in which PG play an important regulatory role is the immune system. PG have been shown to affect the secondary humoral and cell-mediated immune responses (104). The role of PG in chronic inflammation and the implication in the etiology of RA has been studied extensively (100). The function of PG in the immune system that is of interest to this study is its role in the control of cell-mediated immune response.

PGE_1 and PGE_2 were first considered in cell-mediated immunity because of the earlier discovery that cyclic adenosine monophosphate (cAMP) might be a second messenger in the activation of lymphocytes. Parker et al. (105) showed that addition of PGE_2 to human peripheral blood lymphocytes depressed cellular division induced by PHA. Stockman and Mumford (106) showed that addition of PGE_1, PGE_2, $PGF_{2\alpha}$ and

PGA_2 decreased the blastogenic response of human peripheral blood lymphocyte to PHA (primarily a T-cell mitogen). The blastogenic response to ConA (both B-cell and T-cell mitogen) was not as much affected. Furthermore the blastogenic response to PWM (a B-cell mitogen) was not affected. The author, therefore, concluded that PG primarily exert their effect on T-cells.

Schultz et al. (107) demonstrated that exogenously added PGE_1 and PGE_2, but not $PGF_{2\alpha}$, inhibited the tumoricidal activity of interferon-activated macrophages of mice. Since the activated macrophage releases high concentrations of PGE_2, the authors postulated that PGE_2 could act locally in negative feedback inhibition to limit cell activities. Subsequently, it was shown that mitogen and antigen stimulated cultures of human and mouse lymphocytes released PG into the media. This was confirmed by Webb and Nowowiejski (108).

Increase in PG synthesis due to antigen injection was also shown in vivo. Webb and Osheroff (109) showed that within 2 minutes following the intravenous injection of sheep blood cells (SRBC) there was a 20 to 80 fold increase in $PGF_{2\alpha}$ in the spleen. The authors pointed out that the increase of $PGF_{2\alpha}$ is dependent upon thymus-derived T-cells, since no such increase was observed in athymic mice. Ferber et al. (110) reported that phospholipids of stimulated lymphocytes had higher ratios of polyenoic acids (C18:2 +C20:4) to saturated fatty acids. Zimecki and Webb (111) showed that indomethacin,

an inhibitor of PG synthesis, enhanced the in vitro primary response to SRBC. Goodwin et al. (112) administered indomethacin to two patients with common variable immunodeficiency and measured the effect on depressed cellular immune response. They showed that the in vitro response of their lymphocytes to PHA increased during indomethacin treatment. In addition, after indomethacin injection the patients showed positive skin reactions to injected antigens.

The reports of effects of EFA on the immune system are controversial and depend on whether an in vivo or in vitro system is used (113). Dewille et al. (114) showed that EFA deficiency depressed humoral immunity in mice. An opposite effect of EFA was observed on cell-mediated immunity using in vitro binding of lymphocytes to SRBC (115). In addition, it is not clear whether the effect seen is due to a change in PG synthesis or is due to changes in membrane fluidity associated with EFA deficiency.

The mechanism of PG function in cell-mediated immunity is not completely understood. Most investigators believe, however, that the endogenous synthesis of PG following antigen or mitogen stimulation is a negative regulatory signal for the lymphocyte. Webb et al. (101) proposed the hypothetical model for a role of PG in cell-mediated immunity (figure 2). In this model, PG are part of a feedback inhibitory loop that controls the magnitude of response to mitogenic or antigenic signals. The impairment of this regulatory function has been

suggested (116) to play an important role in diseases with deficient T-cell function such as multiple sclerosis, Hodgkin's disease, Crohn's disease, and cardiomyopathy.
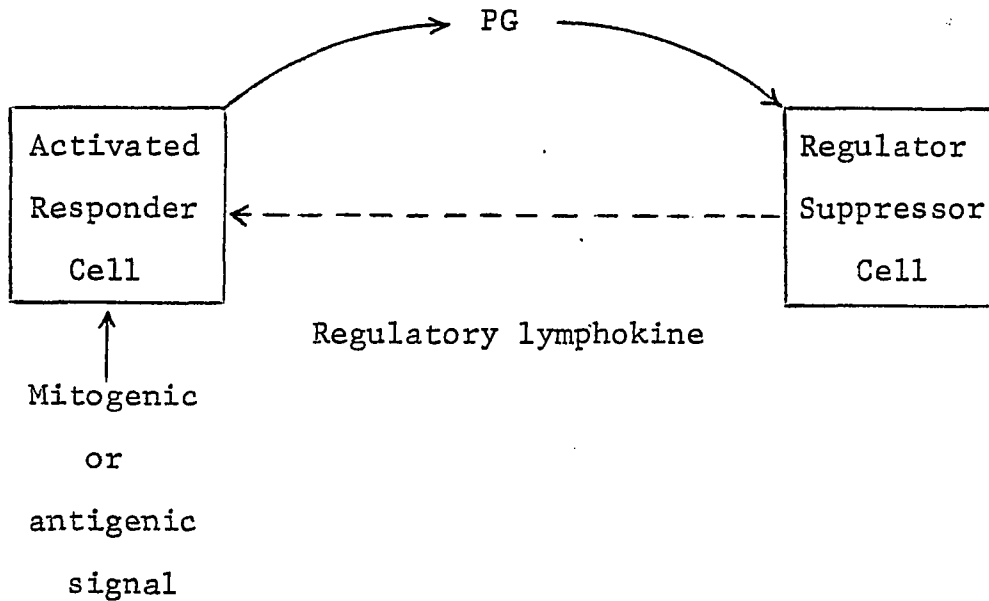
Figure 2: Role of PG in control of cell-mediated immunity. (Reference 101)

Conclusion

With all the important roles of zinc in the biochemical functions of the body, very little is known about its absorption and metabolism. Different investigators (18, 77, 78) have been able to separate a zinc binding ligand from the rat intestinal mucosa and contents. The exact nature of the ZBL is not clear and indeed the literature is very contradictory

about this subject. Prostaglandin E has been implicated as a
possible substance and there is some evidence in its support
(20, 21, 23). There is also indirect evidence (27, 28, 29)
that indicates PG might be involved in metabolism of zinc.
On the other hand, zinc seems to be involved in synthesis
and/or availability of precursors for biosynthesis of PG (17).
There are great similarities between clinical features of zinc
deficiency and EFA deficiency such as growth retardation,
impairment of normal sex organ function, parakeratosis, etc.
Acrodermatitis enteropathica, a hereditary disease with abnormal
zinc absorption, can be treated with administration of zinc
sulphate or EFA (13, 14).

Furthermore, administration of toxic doses of aspirin,
an inhibitor of PG synthesis, to pregnant rats resulted in
clinical abnormalities similar to those of zinc deficiency (16).
Although these studies strongly suggest that PG and zinc
interact with each other in some point of their metabolism,
it can not be accepted as a fact yet, the reason being mainly
that most of these studies were carried out under unphysiologi-
cal conditions. In addition, both zinc and PG have such a
wide variety of physiological functions that it is very hard to
attribute an effect seen to a specific biological function.

Nevertheless, because zinc and PG both play important roles in
normal body functions any information about their interaction
with each other can be very informative and interesting.

If PG are involved in zinc metabolism, modifying their level in different organs by changing their precursors in the diet or using an inhibitor of PG synthesis like aspirin or indomethacin should affect the zinc status. The correlation between dietary PUFA concentration and PG synthesis is documented (117, 118, 119) and aspirin and indomethacin are effective inhibitors of PG synthesis (120). By the same token, if zinc is involved in PG synthesis, elimination of zinc from the diet should change the synthetic potential and/or the existing level of PG in the tissues.

The purpose of this study was to find: 1) what role, if any, PG might play in zinc absorption and biological function. This included measuring zinc concentration in different tissues, serum alkaline phosphatase activity (a zinc metallo-enzyme), and _in vitro_ immune response to mitogens; 2) what might be the effect of zinc deficiency on PG synthesis which includes measuring PG in different tissues.

METHODS AND MATERIALS

The study included three experiments:

Experiment I   The purpose of this study was to determine the dose of aspirin or indomethacin which would inhibit prostaglandin (PG) synthesis, but would not have toxic effects.

Experiment II   This experiment was conducted to study the role of prostaglandins in zinc metabolism and the effect of zinc deficiency on PG synthesis.

Experiment III   This experiment was undertaken to study the effect of zinc deficiency and inhibition of PG synthesis on the blastogenic response of peripheral blood lymphocytes to PHA and ConA.

Animals and Care

In all the studies, weanling male rats of Wistar strain born and raised in the stock colony of the Iowa State University Food and Nutrition Laboratory were used. The rats were individually caged in stainless steel cages and maintained at $22^{\circ}C$ and 45-55% relative humidity. Acid washed glass food jars and polyethylene bottles with polyethylene stoppers also were used. The animals were randomly assigned to the experimental diets, which were provided fresh twice weekly. Diets and deionized water were provided ad libitum unless otherwise specified. All the utensils used in providing the

diets were either stainless steel or acid washed. The animals were weighed weekly.

Experiment I

In this experiment 18 weanling male rats were randomly assigned to one of the following groups.

Group A - was fed the control diet with the composition shown in Table 1. This diet is adequate in zinc and meets the requirement of rats according to the National Academy of Sciences (121). It is similar in gross composition to the average diet consumed in the U.S.

Group B - was fed the control diet with 50 mg/kg body weight (BW)/day of aspirin (2-hydroxybenzoic acid acetate 2-carboxyphenyl ester) mixed with the diet weekly.

Group C - was fed the control diet but received 50 mg/kg BW/day of aspirin (Sigma Company, St. Louis, MO) intra-peritoneally (IP). Aspirin was dissolved in sterile physiological saline.

Group D - was fed the control diet with 2.5 mg/kg BW/day of indomethacin (1-[P-chlorobenzoyl]-5-methoxy-2-methyl indole-3-acetic acid)(Sigma Company, St. Louis, MO) in the diet.

Group E - was fed the control diet with 5 mg/kg BW/day of indomethacin in the diet.

Group F - was fed the control diet but received 1 mg/kg BW/day of indomethacin IP. Indomethacin was dissolved in

Table 1: Composition of diets

Ingredient	% Weight
Egg white[a]	19.0
DL-Methionine[a]	0.4
Corn starch[b]	25.3
Sucrose[c]	25.2
Cellulose[a]	2.5
Zinc free salt mix[d,e]	4.5
Vitamin mix[f]	2.0
Fat (combination of beef tallow[g] and safflower oil[h] or safflower oil)	21.2
Total	100.0

[a]United States biochemical, Cleveland, Ohio.

[b]Argo brand from Iowa State University food service.

[c]Iowa State University food service.

[d]According to National Academy of Sciences (121); made with analytical grade reagents. For all except zinc deficient group zinc was added. Amount in 100 kg diet: Ca, 300 gm; chlorine, 60 gm; sodium, 251 gm; phosphorus 500 gm; selenium, 10 mg; potassium, 900 gm; iodine, 100 mg; manganese, 7 gm; magnesium, 30 gm; iron, 5 gm, chromium, 0.5 gm; copper, 1.5 gm; zinc, 3 gm.

[e]Salts used were: $CaCO_3$, 1500 gm; NaCl, 98.80 gm; K_2HPO_4, 2004 gm; Na_2HPO_4, 654.6 gm; $MnSO_4.H_2O$, 21.60 gm; MgO, 99.60 gm; $FeSO_4.7H_2O$, 24.90 gm; $CrCl_3.6H_2O$, 2.2 gm; $CuSO_4.5H_2O$, 5.90 gm; $ZnSO_4.H_2O$, 13.20 gm; Na_2SeO_3, 20,90 mg; KI, 131.0 mg.

[f]Amount in 100 kg diet: vitamin A acetate, 400,000 IU; vitamin D_2, 40,000 IU; Dl-α tocopherol acetate, 20,000 IU; menadione, 100 mg; vitamin B_{12}, 10 mg; biotin, 50 mg; folic acid, 200 mg; thiamin HCl, 500 mg; para-amino-benzoic acid, 20 g; ca pantothenate, 2 g; niacin, 3 g; pyrodoxin HCl, 1 g; riboflavin, 1 g; choline chloride, 400 g; ascorbic acid, 10 g; corn starch to 2 kg.

[g]Carriage House, Meat and Provisions Company, Ames, Iowa.

[h]Vegetable Oil Company, Richman, CA.

sterile 1 M phosphate buffer, pH 8. The animals were fed the experimental diets for 15 days.

Animals were weighed weekly and food intake was recorded.

Necropsy and sample collection

On the day of necropsy, food jars were taken out at 6 A. M. and returned to the cages at 8 A.M. to synchronize eating. The animals were anesthetized with ether and the following samples were collected.

1) Blood was collected from the exposed jugular vein. It was incubated for 10 minutes at $37^{\circ}C$ in a shaking water bath. After exactly 10 minutes 0.1 ml/ml of whole blood of 4.2 mM aspirin in 0.1 M potassium phosphate buffer (KPi), pH 7.4, was added to the blood. The serum was removed and immediately frozen and kept at $-20^{\circ}C$ for analysis of PG.

2) Approximately 0.5 gm liver samples were homogenized on ice with a polytron homogenizer with 10 volumes of 0.1 M KPi for 2 minutes. The homogenates were then incubated at $37^{\circ}C$ in a shaking water bath for 10 minutes. PG synthesis was stopped by addition of 0.5 volumes of 42 mM aspirin in 0.1 M KPi, pH 7.4. The samples were then frozen immediately.

Another 0.5 gm sample from each liver was homogenized in 10 volumes of 42 mM aspirin in 0.1 M KPi solution for 2 minutes. These samples were also frozen for PG analysis.

Experiment II

Seventy weanling male rats weighing about 50 gm were randomly assigned to the following groups:

Group A - was fed the control diet with the composition shown in Table 1. All the control diets were supplemented with 30 ppm zinc, added to the salt mix. Fat in the control diet was composed of 20% beef tallow and 1.2% safflower oil.

Group B - was fed a high polyunsaturated fatty acid (PUFA) diet. This diet was similar to the control diet except that 21.2% fat was provided from safflower oil.

Group C - was fed the control diet plus 2 mg/kg BW/day of indomethacin (this dose was selected based on the results of Experiment I).

Group D - was pair fed to Group C with the control diet.

Group E - was fed the control diet plus 50 mg/kg BW/day of aspirin. In order to get a homogeneous distribution of drugs in the diet, the drug dilution method was used (which involved mixing drug with a very small amount of diet and increasing the diet gradually). The drugs were added to the diets weekly and the amount added was increased with increase of body weight and food intake.

Group F - was fed a zinc deficient diet which was the control diet except that no zinc was added to the salt mix and extreme care was taken to avoid any zinc contamination (see animals and care). The analysis of the diet using a

dry ashing method (122) showed that the diet had less than
1 ppm zinc.

Group G - was pair fed to Group F with the control diet.

Food intakes of Group C and Group F were recorded
daily considering their waste. The average consumed by these
groups was fed to their corresponding pair fed groups. The
animals were maintained on the diets for 30 days.

Necropsy and sample collection

All animals were fasted four hours prior to anesthesia,
then were anesthetized with ether. Stainless steel surgery
equipment was used for all the biopsies.

The following samples were collected:

A. Blood was collected from the exposed jugular
vein in 10 ml polyethylene disposable syringes with stainless
steel needles for the following samples.

1. 2 ml for PG analysis. This sample was
processed as explained above.

2. 2 ml was delivered to acid washed poly-
ethylene tubes which contained 2 drops of heparin (Sigma
Company, St. Louis, MO)(1000 U/ml deionized water of Na
heparin). The blood was then centrifuged at 909 X g for
10 minutes. Plasma was separated and frozen for zinc analysis.

3. About 2 ml of blood was used to obtain serum
for alkaline phosphatase measurement.

B. Thymus was taken out and weighed.

C. Lung - two samples were taken from the lung.

1. Approximately 0.5 gm sample was weighed and homogenized with 10 volumes of 42 mM aspirin solution in 0.1 M KPi, pH 7.4. Homogenization was done on ice with a polytron homogenizer. Care was taken that all the samples were handled in exactly the same way. This sample was frozen on dry ice and saved for PG analysis.

2. Approximately 0.5 gm lung was weighed and homogenized with 10 volumes of 0.1 M KPi, pH 7.4 on ice. The homogenate was then incubated for 10 minutes in a $37^{o}C$ shaking waterbath. After 10 minutes the PG synthesis was stopped by addition of 0.5 volume of 42 mM aspirin solution in 0.1 M KPi buffer. This sample was frozen on dry ice and saved for PG analysis.

3. The rest of the lung was weighed and kept in polyethylene plastic bags for zinc analysis.

D. Liver - two samples were taken for PG analysis. The procedure and handling of samples were similar to that of the lung samples. The rest of the liver was also weighed and saved for zinc analysis.

E. The small intestine was excised from pylorus to cecum. The intestine was cleansed of excess blood with tissue. It was then cut into segments and the

contents flushed with a 0.85% NaCl solution. The volume was recorded. Zinc contamination was avoided by using deionized water in preparation of saline and wearing polyethylene gloves. The following samples were taken from the contents:

1. 2 ml was delivered to small tubes which contained 1 ml of 42 mM aspirin in 0.1 M KPi buffer for PG analysis.

2. 2 ml was delivered to acid washed polyethylene containers for zinc analysis.

F. Mucosa - pieces of intestine were then cut open and mucosa was scraped off with acid washed glass slides and weighed. The total mucosal collection was then homogenized in 10 volume of 0.1 M KPi buffer on ice. Two ml of homogenate was incubated for 10 minutes in a $37^{\circ}C$ shaking waterbath. At 10 minutes PG synthesis was stopped by addition of 1 ml of 42 mM aspirin in 0.1 M KPi buffer. One ml of homogenate was removed to an acid washed polyethylene container for zinc analysis before addition of aspirin.

All the samples were immediately frozen on dry ice and kept at $-20^{\circ}C$ for the appropriate analysis.

Experiment III

Twenty-four weanling male rats were randomly assigned to the following groups:

Group A - was fed the control diet.

Group B - was fed the high polyunsaturated fat diet.

Group C - was fed the control diet plus 2 mg/kg BW/day of indomethacin.

Group D - was fed the zinc deficient diet.

The composition of the diets was similar to that of Experiment II.

The animals were maintained on the diets for 30 days. Food intakes and weight gains were recorded.

Necropsy and sample collection

After four hours fasting the animals were anesthetized with ether, and blood was collected from the exposed jugular vein. A 2 ml sample was processed for PG analysis as explained before and 5 ml was delivered to a centrifuge tube containing 10 U/ml heparin (1000 U/ml Na heparin in water) under sterile conditions for a lymphocyte stimulation assay.

Analytical Methods

Prostaglandin analysis

Prostaglandins $F_{2\alpha}$, E_1, E_2, 6 keto $PGF_{1\alpha}$ (PGI_2), thromboxane B_2, and 13, 14, dihydro 15 keto prostaglandin $F_{2\alpha}$ were measured by radioimmunoassay as described by Hwang et al. (117) and McCosh et al. (1923). The method involved an

overnight precipitation of each PG specific antiserum with anti-rabbit gamma globulin. The PGE_1 antiserum had a cross reactivity of 15% with PGE_2 and the PGE_2 antiserum had a cross reactivity of 19% with PGE_1. The other PG antisera did not show cross reactivity with each other. Parallelism of each PG in each tissue was checked. Standards were a gift from the Upjohn Company (Kalamazoo, MI). Tritiated PGE_1, E_2, TXB_2, and $PGF_{2\alpha}$ were purchased from New England Nuclear (Boston, MA) and tritiated 13,14 dihydro 15 keto-$PGF_{2\alpha}$ and 6 keto $PGF_{1\alpha}$ were purchased from Amersham (Arlington Heights, IL).

Zinc analysis

Plasma was diluted 1 to 4 with deionized water and the diluted samples were directly analyzed with a Unicam SP 90 atomic absorption spectrometer and used in accordance with the manufacturer's operating manual. The wave length was set at 214 nm, the most sensitive absorption line for zinc. The air flow was 6 l/min and that of propane was 0.5 l/min.

All the tissue samples were digested in nitric acid using the method discussed by Slavin et al. (124). Standards were prepared by diluting stock atomic absorption zinc reference solution (Fisher Scientific Company, Fairlawn, NJ) in nitric acid and deionized water so that the standards and samples had similar water to nitric acid ratios. For plasma standards were diluted with deionized water only (125).

Alkaline phosphatase

Serum alkaline phosphatase activity was measured using
a Sigma kit (kit number 104LL). The 15 minute incubation
method was used and the direction in the accompanying manual
(126) was followed.

Lymphocyte stimulation with PHA and ConA

The method developed by Jackson and Laird (personal
communication, National Animal Disease Laboratory, Ames, IA)
was used. Heparinized blood was centrifuged at 815 X g for
40 minutes. The buffy coat was collected in 10 ml pipets
and suspended in approximately 10 ml Ca and Mg free Dulbecco
medium (phosphate buffered saline solution, pH 7.2 made at
the National Animal Disease Laboratory, Ames, IA) with
heparin (10 U/ml).

The suspension was centrifuged for 40 minutes at 815 X g.
Contaminating red blood cells were lysed with ammonium
chloride in 0.05 M tris buffer pH 7.2 with 10 U/ml heparin
(8.3 gm ammonium chloride/1). Cells were then washed with
medium containing heparin. The composition of the medium
was as below:

Values given for 100 ml of medium. RPMI 1640 (Grand
Island Biological Co., Grand Island, NY); 30 mM HEPES buffer
(Sigma Co., St. Louis, MO), 1.2 ml; 30 mM $NaHCO_3$ (Mallinckrodt,
St. Louis, MO), 4 ml; 1.6 mM L-glutamine (Grand Island Bio-
logical Co., Grand Island, NY), 0.8 ml; sterile water, 85 ml;

penicillin potassium G (Pfizer, New York, NY), 100 U; strep-

tomycin sulfate (Pfizer, New York, NY), 10 mg; gentocin

(Schering, Kenilworth, NJ), 15 mg.

After centrifugation the cells were resuspended in the

medium at a concentration of 4 x 10^6 cell/ml. Viability of

the cell suspension was determined using the trypan blue

exclusion method (127) and exceeded 90%. The procedure

usually yields about 70% lymphocytes. The stimulants, PHA

and ConA, affect T-cell lymphocytes specifically. The rela-

tive number of cells as possibly affected by treatment is

corrected for by use of the stimulation index which is a

ratio of stimulated to nonstimulated ^{3}H-thymidine incorpora-

tion.

Two T-cell mitogens, PHA and ConA, were used. PHA and

ConA dilutions were prepared in medium with 10% heat inacti-

vated fetal calf serum (prepared at National Animal Disease

Laboratory, Ames, IA). PHA (Burroughs-Wellcome, Greensburg,

NC) and ConA (Calbiochemicals, San Diego, CA) were used in

0.5 and 1 μg per well concentrations. Mitogens were added

to each well in 100 μl volumes. All concentrations were

tested in triplicate. Control cultures containing unstimu-

lated lymphocytes were also tested in triplicate.

Cultures were then incubated for 72 hours at 37°C in a

humidified atmosphere of air with 5% CO_2. At this time 1 μCi

of ^{3}H-thymidine (New England Nuclear, Boston, MA; Specific

activity 6.7 Ci/mmol) in 22 μl of medium was added to each well. The cultures were returned to incubators for 12 hours. The cells were then harvested with a micro-automated sample harvester (Otto Hiller Co., Madison, WI) onto a glass-fiber filter (0.5 x 8 inches) (Whatman, Inc., Clifton, NJ). Cell samples were placed in polyethylene miniscintillation vials (Isolab, Akron, OH). Four milliliters of scintillation fluid (Scintiverse, Fisher Scientific Co., Fairlawn, NJ) was added. The vials were counted in a Packard Tri-Carb C2425 liquid scintillation counter (Downers Grove, IL). Results were obtained as mean CPM, which were used to calculate the mean stimulation index (SI) for each mitogen concentration. The SI is the ratio of the mean CPM of stimulated cultures to those of unstimulated cultures.

Statistical analysis

Results of Experiment II were analyzed by analysis of variance (128) and means were compared by use of Duncan's multiple range test at a 5% level of probability (129). Group means are reported with pooled standard error of the means. Data in Experiment I and III were analyzed by Student's t-test. In addition, the results of Experiment II were also analyzed by Student's t-test. There was no difference between the results obtained by these two different statistical procedures unless otherwise mentioned.

RESULTS

Experiment I

This experiment was designed to determine a non-toxic dose of aspirin and indomethacin that would inhibit PG production. Rats in Group F (receiving 1 mg/kg BW/day of indomethacin by injection) died after 2 injections. The only pathological sign seen at autopsy was local tissue reaction at the injection site. The phosphate buffer which was used to dissolve indomethacin had a pH of 8 which could have been the cause of the local reaction. This pH was recommended by the researchers at the Merck Company (personal communication, Merck & Co. Inc., Rahway, NJ) to make the indomethacin soluble in the buffer. According to the correspondent at the Merck Company, they had used this method before with success. Rats in Group E (receiving 5 mg/kg BW/day indomethacin in the diet) died after 8 days with peritonitis which is one of the predominant signs of indomethacin toxicity. Group B (receiving 50 mg/kg BW/day of aspirin in the diet), and Group C (receiving 50 mg/kg BW/day aspirin intraperitoneally) had food intakes and weight gains similar to those of the control group (Table 2). The weight gains and food intakes of the rats in Group D (receiving 2.5 mg/kg BW/day of indomethacin in the diet) were slightly lower than those of the control group.

Table 2. The effect of prostaglandin synthesis inhibitors
on weight gain and food intake of rats. (mean ±
standard error of the mean, n=3)

Treatment	Weight gain	Food intake
	gm	gm
Control	62.00 ± 5.55	11.00 ± 0.25
Aspirin (50 mg/kg BW/day IP)	60.00 ± 0.01	9.50 ± 0.55
Aspirin (50 mg/kg BW/day PO)	62.00 ± 2.53	11.00 ± 0.15
Indomethacin (2.5 mg/kg BW/day PO)	49.00 ± 3.54	9.30 ± 0.71

$PGF_{2\alpha}$ was measured in the serum samples and the results indicated that all the treatments used (aspirin, injected or used in the diet, and indomethacin in the diet) caused inhibition of $PGF_{2\alpha}$ production by more than 50%. Based on the above results it was decided that 50 mg/kg BW/day of aspirin and 2 mg/kg BW/day of indomethacin in the diet are safe and effective doses. These doses inhibit PG production but do not interfere with the normal growth of the animals. In addition, they can be given in the diet the same as the other treatments. Therefore, those doses were used in Experiment II.

Experiment II

The purpose of this experiment was to study the effect
of changing PG level in vivo on the zinc status of the rats
and the effect of zinc deficiency on PG production. Table
3 shows the average food intakes and weight gains of the
animals receiving different treatments.

Table 3: The effect of zinc deficiency and prostaglandin
modifiers on food intake and weight gain of the
rats (means with the same letter are not signif-
icantly different. n=10 per treatment).

Treatment	Food intake	Weight gain
	gm	gm
Control	12.40^a	$161.90^{a,b}$
PUFA[1]	12.80^a	179.30^a
Indo.[2]	11.50^a	$170.00^{a,b}$
Asp.[3]	12.10^a	156.90^b
-Zinc	6.60^b	59.70^d
-Zinc PF[4]		88.90^c
Pooled SEM[5]	0.65	6.58

[1]Polyunsaturated fatty acid diet.

[2]2mg/kg BW/day indomethacin in the diet.

[3]50 mg/kg BW/day aspirin in the diet.

[4]Pair fed to zinc deficient rats with control diet.

[5]Standard error of the mean.

As can be seen in Table 3, aspirin, indomethacin and PUFA fed groups had similar food intakes and weight gains to those of the control group. Food intake and weight gain were reduced significantly in the zinc deficient group as expected. The zinc deficient pair fed group had reduced weight gain but not as much as the zinc deficient group. There were no differences in food intake and weight gain of the indomethacin pair fed group from those of the control or the indomethacin fed group and preliminary analyses of PG and zinc in their serum revealed that they were not different from the control group. Therefore, no further analyses were performed on this group.

Table 4: The effect of zinc deficiency and prostaglandin modifiers on organ weights of the rats (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	gut mucosa	liver	lung	tibia	thymus[1]
			gm		
Control	3.10^a	9.20^a	1.20^a	0.33^a	0.39^a
PUFA	$2.70^{a,c}$	10.30^a	1.40^a	0.35^a	
Indo.	$2.60^{a,b,c}$	10.20^a	1.40^a	0.34^a	0.46^a
Asp.	$2.70^{a,b}$	9.20^a	1.20^a	0.32^a	0.35^a
-Zinc	1.90^c	4.00^b	0.67^b	0.22^b	0.16^b
-Zinc PF	$2.20^{b,c}$	4.90^b	0.75^b	0.27^b	
Pooled SEM	0.23	0.38	0.01	0.02	0.05

[1]n=6.

Table 4 shows the organ weights of animals with different treatments. Both food restriction and zinc deficiency caused decreased weight of all organs studied. Organ weights of PUFA, indomethacin and aspirin treated animals were the same as those of controls. Although the organ weights of the zinc deficient animals were slightly lower than their pair fed group, the difference is not statistically significant. When the ratio of organ weight to the body weight was compared (Table 5) the zinc deficient group had a significantly higher organ weight ratio than other groups in all organs studied except for the thymus. There was no difference between the thymus weight to body weight ratio of zinc deficient animals and that of the control group.

Table 5: Effect of zinc deficiency and prostaglandin modifiers on the organ weight to body weight ratio of the rats (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	gut mucosa	liver	lung	tibia	thymus[1]
Control	$0.019^{b,c}$	0.057^b	0.008^b	0.0021^b	0.0025^a
PUFA	0.015^c	0.059^b	0.008^b	0.0019^b	
Indo.	0.016^c	0.059^b	0.008^b	0.0020^b	0.0026^a
Asp.	0.018^c	0.059^b	0.008^b	0.0022^b	0.0020^a
-Zinc	0.035^a	0.071^a	0.012^a	0.0040^a	0.0025^a
-Zinc PF	0.026^b	0.056^a	0.009^b	0.0032^a	
Pooled SEM	0.002	0.004	0.001	0.0003	0.0004

[1] n=6 per treatment.

As far as the general appearance of the animals, where the
zinc deficient animals were less active than controls and
had hair loss, the indomethacin and aspirin fed group were
healthy and normal and no difference could be detected between
them and the control group. The same was true for the PUFA
treatment.

Table 6 shows the zinc concentration in different organs.
The zinc concentration was significantly lower in the plasma,
gut contents and tibia of the zinc deficient group than those
of the control and the zinc deficient pair fed groups. Zinc
deficiency, indomethacin and aspirin treatments decreased
the zinc concentration in the mucosa, and PUFA diet increased
it, but the difference was not statistically significant
(control, 32.50; PUFA, 39.80; indomethacin, 26.90; aspirin,
27.40; zinc deficient, 18.70). Aspirin, indomethacin, and
PUFA treatments did not change the zinc concentration in any
other organ tested. Zinc concentrations in lung and liver
were not affected by the treatments.

Table 7 shows the results of alkaline phosphatase activity
in the serum. The student's t-test was used for analysis of
this parameter. Zinc deficiency decreased serum alkaline
phosphatase activity significantly ($P < 0.05$). Other treat-
ments did not have any effect on the activity of this
enzyme.

Prostaglandins $F_{2\alpha}$, E_2, E_1, 6 keto $F_{1\alpha}$ and 13,14dihydro-
15 keto prostaglandin $F_{2\alpha}$ (met $F_{2\alpha}$) were measured in serum,

Table 6: The effect of zinc deficiency and prostaglandin modifiers on zinc concentrations in plasma, gut contents, gut mucosa, liver, lung, and tibia of rats (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	Plasma	Gut content	Gut mucosa	Liver	Lung	Tibia
	μg/ml	μg	μg/gm	μg/gm	μg/gm	μg/gm
Control	$1.40^{a,b}$	45.50^{a}	$32.50^{a,b}$	29.00^{a}	$16.80^{b,c}$	150.30^{a}
PUFA	1.10^{b}	50.80^{a}	39.80^{a}	30.60^{a}	$20.80^{a,b}$	153.00^{a}
Indo.	$1.20^{a,b}$	49.30^{a}	$26.90^{a,b}$	29.70^{a}	$17.40^{b,c}$	160.80^{a}
Asp.	1.50^{a}	49.80^{a}	$27.40^{a,b}$	28.60^{a}	16.40^{c}	177.10^{a}
-Zinc	0.50^{c}	6.80^{b}	18.70^{b}	25.70^{a}	$19.60^{a,b,c}$	39.80^{b}
-Zinc PF	$1.30^{a,b}$	50.20^{a}	$31.30^{a,b}$	32.50^{a}	21.70^{a}	161.10^{a}
Pooled SEM	0.09	10.10	5.00	2.30	7.20	9.80

gut contents and mucosa, liver and lung. Thromboxane B_2 was also measured in serum and lung. Tables 8-14 show the PG levels in the different organs measured.

Table 7: The effect of zinc deficiency and prostaglandin modifiers on serum alkaline phosphatase activity of the rats (n=10 per treatment)

Treatment	Alkaline phosphatase[1]
	Sigma unit/ml
Control	9.79 ± 1.11
PUFA	6.83 ± 0.65
Indo.	7.89 ± 0.76
Asp.	11.19 ± 1.52
-Zinc	3.66 ± 0.33[2]
-Zinc PF	7.46 ± 1.07

[1]Mean ± standard error.

[2]Significantly different from the control, $P < 0.05$.

In serum, indomethacin and aspirin treatments inhibited synthesis of all PG tested more than 50% and almost 90% in the case of PGE_2, $PGF_{2\alpha}$, and TxB_2. Production of met $F_{2\alpha}$ was also inhibited significantly by indomethacin (Table 8). Zinc deficiency decreased PGE_2 (19.40 ng/ml for the control rats and 12.30 ng/ml for the zinc deficient rats) and $PGF_{2\alpha}$ (8.80

ng/ml for the control rat and 6.40 ng/ml for the zinc deficient rats) production slightly but the difference did not reach statistical significance.

Table 8: The effect of zinc deficiency and prostaglandin modifiers on concentration of different PG in the serum after 10 minutes incubation at 37°C in rats (means with the same letter are not statistically different) (n=10 per treatment).

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	TxB_2
			ng/ml		
Control	3.20^a	19.40^a	8.80^a	$0.19^{b,c}$	78.10^a
PUFA	3.40^a	$14.30^{b,c}$	$6.50^{a,b}$	0.28^b	76.37^a
Indo.	$1.50^{b,c}$	1.70^b	$1.70^{b,c}$	0.09^d	11.42^b
Asp.	1.00^c	0.90^b	1.00^c	$0.15^{c,d}$	3.71^b
-Zinc	$2.60^{a,b}$	$12.30^{a,b}$	$6.40^{a,b}$	0.40^a	70.60^a
-Zinc PF	3.20^a	17.70^a	$7.00^{a,b}$	0.20^b	119.22^a
Pooled SEM	0.47	4.62	1.60	0.03	20.76

The interesting observation was that zinc deficiency significantly increased production of met $F_{2\alpha}$. No increase in PG production was observed by increasing the PUFA content of the diet.

In the gut contents indomethacin and aspirin inhibited PGE_2 and PGI_2 synthesis (Table 9). The inhibition was not statistically significant for PGE_1, $PGF_{2\alpha}$, and met $F_{2\alpha}$.

Table 9: The effect of zinc deficiency and prostaglandin modifiers on the total amount of different PG in the gut contents of rats (means with the same letter are not significantly different)(n=10 per treatment)

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	PGI_2
			ng		
Control	$10.77^{a,b}$	63.38^a	115.16^a	4.54^a	52.00^a
PUFA	15.33^a	41.44^b	109.62^a	7.11^b	$35.00^{a,b}$
Indo.	$9.00^{a,b}$	41.60^b	$96.62^{a,b}$	4.75^a	17.14^c
Asp.	$8.88^{a,b}$	27.50^b	$103.00^{a,b}$	3.84^a	14.28^c
-Zinc	4.00^b	26.44^b	63.00^b	2.73^a	10.22^c
-Zinc PF	6.70^b	29.25^b	$93.16^{a,b}$	3.15^a	$23.00^{b,c}$
Pooled SEM	2.14	4.89	12.18	0.81	4.94

Zinc deficiency decreased PGE_1, PGE_2, $PGF_{2\alpha}$, and PGI_2 production. Food restriction (zinc deficient pair fed group) also decreased the concentration of the above mentioned PG, however, zinc deficiency seemed to have a greater effect than food restriction alone on $PGF_{2\alpha}$ and PGI_2 levels. The effect of PUFA on PG production was not statistically significant. There was a positive correlation between PGE_1 and zinc concentration in the gut content (correlation coefficient 0.35, $P<0.01$).

In gut mucosa, the effect of none of the treatments reached statistical significance, except for the inhibition of met $F_{2\alpha}$ production by indomethacin (Table 10). This is partly due to the large standard error. The variability among the samples within a treatment seen in mucosa might be due to the fact that PG production is very sensitive to trauma, and it is very hard to standardize trauma to the tissue during scraping of the gut to obtain mucosa.

In the liver samples, homogenized in 42 mM aspirin as rapidly as possible, indomethacin and aspirin treatments decreased PG synthesis. Zinc deficiency and food restriction both decreased synthesis of met $F_{2\alpha}$ significantly but food restriction was more effective than zinc deficiency. Synthesis of other PG were depressed but the differences did not reach statistical significance. Animals on high PUFA diet had lower levels of PGE_2 and met $F_{2\alpha}$ than controls. The in vitro production of PG was similarly affected, i.e., indomethacin and aspirin inhibited PG synthesis in liver homogenates incubated for 10 minutes prior to addition of aspirin. Zinc deficiency and food restriction also decreased PG synthesis, but the depression was only significant for PGE_1. Zinc deficiency itself, however, decreased formation of $F_{2\alpha}$ significantly.

Table 10: The effect of zinc deficiency and prostaglandin modifiers on the concentration of different PG in the gut mucosa of rats after 10 minutes incubation at $37^{\circ}C$ (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	PGI_2
			ng/gm		
Control	$27.11^{a,b}$	346.63^a	$626.90^{a,b}$	$39.42^{a,b}$	$133.71^{a,b}$
PUFA	18.00^b	236.22^a	$579.60^{a,b}$	$40.93^{a,b}$	$133.50^{a,b}$
Indo.	21.60^b	186.90^a	$595.00^{a,b}$	12.02^c	77.30^b
Asp.	18.60^b	230.44^a	464.13^b	$21.03^{b,c}$	65.83^b
-Zinc	$29.60^{a,b}$	351.00^a	889.33^a	$31.69^{b,c}$	166.30^a
-Zinc PF	36.90^a	274.80^a	885.88^a	63.44^a	164.30^a
Pooled SEM	4.07	65.59	109.57	8.39	29.35

Table 11: The effect of zinc deficiency and prostaglandin modifiers on the concentration of different PG in rat liver samples homogenized in 42 mM aspirin immediately (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	PGI_2
			ng/gm		
Control	38.78^a	94.22^a	130.11^a	10.06^a	$18.00^{a,b}$
PUFA	51.10^a	19.63^b	58.57^a	3.53^b	27.78^a
Indo.	37.50^a	$45.89^{a,b}$	33.78^a	1.93^b	$8.33^{b,c}$
Asp.	22.11^a	11.56^b	38.38^a	2.83^b	4.11^c
-Zinc	22.44^a	11.60^b	79.78^a	3.12^b	$12.22^{b,c}$
-Zinc PF	21.30^a	7.90^b	39.38^a	2.81^b	$6.67^{b,c}$
Pooled SEM	9.87	19.77	32.10	2.11	4.04

Table 12: The effect of zinc deficiency and prostaglandin modifiers on the concentration of different PG in rat liver homogenates after 10 minutes of incubation at $37^\circ C$ (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	PGI_2
		ng/gm			
Control	222.89^a	$953.44^{a,b}$	$726.11^{a,b,c}$	59.87^a	166.22^a
PUFA	243.78^a	1188.90^a	861.10^a	$20.75^{b,c}$	139.20^a
Indo.	94.33^b	374.60^c	386.00^c	15.61^c	103.80^a
Asp.	120.38^b	$698.43^{b,c}$	$496.75^{b,c}$	$23.38^{b,c}$	104.25^a
-Zinc	129.50^b	$677.11^{b,c}$	$694.33^{a,b,c}$	16.56^c	156.50^a
-Zinc PF	116.70^b	$549.11^{b,c}$	$747.10^{a,b}$	$42.90^{a,b}$	155.40^a
Pooled SEM	31.76	140.59	108.88	7.63	28.17

In vivo synthesis of PG in the lung was inhibited by aspirin and indomethacin (Table 13). Both inhibitors were more effective in inhibiting PGE_2 synthesis than other PG. Zinc deficiency and food restriction both decreased synthesis of PGE_2 significantly. PGE_1 synthesis was also reduced but did not reach statistical significance. Although not statistically significant, zinc deficiency seemed to be more effective in decreasing synthesis of PGE_1 than food restriction alone (7.86 ng/gm vs 13.56 ng/gm). Food restriction on the other hand was more effective in reducing met $F_{2\alpha}$ production than zinc deficiency. PUFA increased synthesis of PGI_2 significnatly. The in vitro synthesis of PG in the 10 minute incubation homogenates was inhibited by indomethacin and aspirin (Table 14). Aspirin was a more potent inhibitor here. Zinc deficiency increased production of met $F_{2\alpha}$ significantly.

In summary, indomethacin and aspirin inhibited PG production in all organs tested. The degree of inhibition at the dose used was slightly different among different PG and the organs tested. Production of met $F_{2\alpha}$ was also inhibited in most organs studied. PUFA increased synthesis of met $F_{2\alpha}$ in the gut contents, and that of PGI_2 in the lung samples homogenized in aspirin immediately. Zinc deficiency

Table 13: The effect of zinc deficiency and prostaglandin modifiers on the concentration of different PG in rat lung samples homogenized in 42 mM aspirin immediately (means with same letter are not significantly different) (n=10 per treatment)

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	PGI_2
			ng/gm		
Control	$25.43^{a,b}$	152.50^a	43.50^a	3.53^a	24.70^b
PUFA	44.50^a	61.00^b	39.50^a	$2.00^{a,b}$	59.78^a
Indo.	$16.00^{a,b}$	24.86^b	39.78^a	$2.32^{a,b}$	14.44^b
Asp.	$17.28^{a,b}$	23.00^b	23.00^a	1.00^b	12.00^b
-Zinc	7.86^b	46.80^b	24.14^a	$2.54^{a,b}$	22.33^b
-Zinc PF	13.56^b	49.44^b	20.00^a	1.58^b	5.94^b
Pooled SEM	8.60	9.62	7.16	0.49	5.94

Table 14: The effect of zinc deficiency and prostaglandin modifiers on the concentration of different PG in rat lung homogenates after 10 minutes incubation at 37°C (means with the same letter are not significantly different) (n=10 per treatment)

Treatment	PGE_1	PGE_2	$PGF_{2\alpha}$	Met $F_{2\alpha}$	PGI_2
			ng/gm		
Control	$215.75^{a,b}$	$709.00^{a,b}$	748.79^a	10.93^b	5852.70^a
PUFA	$213.50^{a,b}$	973.30^a	751.13^a	$16.34^{a,b}$	4959.30^a
Indo.	107.88^c	$570.56^{b,c}$	354.78^b	5.91^b	3575.80^b
Asp.	88.89^c	369.25^a	345.25^b	6.95^b	206.11^c
-Zinc	229.80^a	918.90^a	628.90^a	22.38^a	5770.50^a
-Zinc PF	166.90^b	959.90^a	$533.88^{a,b}$	$14.36^{a,b}$	5634.00^a
Pooled SEM	17.71	93.02	85.73	3.48	386.37

68

and food restriction significantly decreased PGE_2 synthesis in gut contents, and liver and lung samples homogenized in aspirin immediately, PGE_1 synthesis in incubated liver homogenate, $PGF_{2\alpha}$ and PGI_2 synthesis in gut content, and met $F_{2\alpha}$ production in liver homogenates. The synthesis of other PG in the different organs was also decreased but the differences did not reach statistical significance. Zinc deficiency significantly decreased synthesis of $PGF_{2\alpha}$ and PGI_2 in the gut contents more than food restriction. Zinc deficiency also increased production of met $F_{2\alpha}$ in serum, and lung homogenates incubated for 10 minutes. In addition, in the gut contents, there was a positive correlation between zinc and PGE_1 concentrations.

Experiment III

The purpose of this study was to investigate the effect of zinc deficiency and change in PG level on the blastogenic response of peripheral blood lymphocytes to T-cell mitogens (PHA and ConA). The data in this experiment were analyzed by the Student's t-test and results are reported as mean ± standard error of the mean.

Zinc deficient animals had decreased food intake and weight gain (Table 16) when compared to the control animals. The other treatments did not affect food intake or weight gain.

Table 15: The effect of zinc deficiency and PG modifiers
on food intake and weight gain of rats (mean
± SEM, n=6 per treatment)

Treatment	Food intake	Weight gain
	gm	
Control	14.23 ± 1.46	187.33 ± 13.75
PUFA	13.20 ± 0.58	217.00 ± 13.75
Indo.	13.62 ± 0.42	206.17 ± 15.42
-Zinc	10.00 ± 1.66[1]	99.17 ± 17.50[1]

[1]Significantly different from the control at $P<0.05$.

Table 16 shows the serum PGE_1 and plasma zinc concentration. As in Experiment II, zinc deficiency decreased plasma zinc concentration and the other treatments did not have any effect. Indomethacin decreased PGE_1 level significantly. The experimental conditions of Experiments II and III were similar and the results of food intake, weight gain, plasma zinc and serum PGE_1 in Experiment III agree with those of Experiment II. Therefore, it is assumed that the other parameters that were not measured in Experiment III behaved similarly to those of Experiment II.

Table 17 shows the blastogenic response of peripheral blood to two different concentrations of PHA and ConA. Zinc deficiency decreased the stimulation index (SI,

Table 16. The effect of zinc deficiency and PG modifiers
on plasma zinc and serum PGE_1 concentrations of
rats (mean ± SEM, n=6 per treatment)

Treatment	Zinc	PGE_1
	(µg/ml)	(ng/ml)
Control	1.89 ± 0.10	3.18 ± 0.95
PUFA	1.43 ± 0.06	2.23 ± 0.33
Indo.	1.63 ± 0.42	1.60 ± 0.25[1]
-Zinc	0.40 ± 0.10[1]	2.83 ± 0.46

[1]Significantly different from the control at $P < 0.05$.

ratio of the mean cpm of stimulated cultures to that of
unstimulated cultures) significantly at both concentrations
of PHA and ConA. Indomethacin, on the other hand, increased
the SI which is in agreement with the inhibitory effect of
PG on T-cell response to mitogens. The effect of PUFA was
not significantly different from that of the controls.

Table 17: The effect of zinc deficiency and prostaglandin modifiers on blastogenic response of peripheral blood to PHA and ConA (mean ± SEM, n=6 per treatment)

Treatment	PHA[1]		ConA[1]	
	5 µg/ml	10 µg/ml	5 µg/ml	10 µg/ml
Control	34.00 ± 10.89	60.25 ± 23.10	72.00 ± 29.27	51.25 ± 18.81
PUFA	24.27 ± 15.99	102.63 ± 73.92	150.58 ±106.26	133.33±102.33
Indo.	67.00 ± 45.19	258.00 ± 87.10^{2}	297.25 ± 146.43^{3}	251.50 ± 131.38^{3}
-Zinc	2.00 ± 0.57^{2}	7.40 ± 5.07^{4}	3.40 ± 1.32^{5}	2.80 ± 1.22^{2}

[1]Stimulation index ± standard error of a mean.

[2]Significantly different from control at $P<0.025$.

[3]Significantly different from control at $P<0.05$.

[4]Significantly different from control at $P<0.1$.

[5]Significantly different from control at $P<0.01$.

DISCUSSION

The Effect of PG Synthesis Inhibition on Zinc Status

Aspirin and indomethacin both decreased PG synthesis in all tissues studied. For most PG the inhibition was more than 50%. Increasing safflower oil from 1.2 to 21.2% did not change the PG concentration in any tissue in this experiment. This is in contrast to some previous studies (118, 119). The observed discrepancy might be explained by the difference in the feeding state of the rats. Mathias and Sullivan (personal communication, Department of Food Science and Nutrition, Colorado State University) showed that serum PG levels were higher in fasted rats than in fed rats but the PG concentrations in serum of fasted rats did not reflect differences in dietary linoleate. In addition, beef tallow with 0.4% calories as linoleate caused the biggest rist in serum PGE_1, E_2, $F_{2\alpha}$, and TxB_2 compared to higher-percentage-linoleate rats. In the presently reported experiment, the rats were fasted for four hours prior to necropsy which may explain why PG level did not show a change with increased linoleate in the diet.

Zinc deficiency in Experiments II and III decreased the food intake and weight gain of rats. Doses of indomethacin and aspirin that caused more than 50% inhibition of PG

synthesis, however, did not affect normal food intakes and weight gains of the rats. Whereas zinc deficient animals were less active than normal and lost hair, the rats fed indomethacin and aspirin were active and healthy; no difference in general appearance could be detected between them and the control animals. Zinc deficient animals had lower plasma and tibia zinc concentrations than the control and zinc deficient pair fed animals. No difference could be detected between plasma and tibia zinc concentrations of indomethacin and aspirin fed animals and that of control animals. Indomethacin and aspirin slightly decreased zinc concentrations in gut mucosa. The decrease was not, however, statistically significant.

Serum alkaline phosphatase activity was reduced by zinc deficiency. Indomethacin and aspirin did not have any effect on serum alkaline phosphatase activity. In addition, zinc deficiency and indomethacin had the opposite effect with regard to the blastogenic response of peripheral blood lympho-cytes to mitogens.

Song and Adham (22) showed that addition of 200 μg PGE_2 to the mucosal medium of everted jejunal sacs increased transport of ^{65}Zn while $PGF_{2\alpha}$ decreased its transport. The opposite effect was seen by addition of these PG to the sero-sal medium. They also showed that administration of 100 μg PGE_2 , with ^{65}Zn three hours before the animals were sacri-ficed caused an increase in zinc content of liver and pancreas.

Furthermore, oral administration of almost 10 mg/kg BW of indomethacin caused decreased liver zinc. The authors therefore suggested that PGE_2 and $PGF_{2\alpha}$ act as physiological regulators of intestinal zinc transport. We can not reach the same conclusion from our study because inhibition of PG synthesis more than 50% with two different PG inhibitors did not change the zinc status of the rats as measured by general appearance, weight gain, tissue zinc concentration, alkaline phosphatase activity, and immune response.

The dose of indomethacin used by Song and Adham (10 mg/kg BW) is a high dose. In our first experiment, rats receiving 5 mg/kg BW/day of indomethacin died within 8 days with severe peritonitis. Indomethacin, and for that matter aspirin, interfere with biological functions other than PG synthesis. It is worth noting that the dose required for PG inhibition is lower than that needed for other inhibitory functions (120). In addition, the total amount of PG in the gut contents measured by radioimmunoassay in this experiment was 63.38 ng for PGE_2, 115.16 ng for $PGF_{2\alpha}$, 10.77 ng for PGE_1, and 57.00 ng for PGI_2. The dose of PG administered in the study by Song and Adham (22) is 200 µg, almost 1000 times what is found under normal conditions in the gut contents. It will be very interesting to see whether similar results to those of Song and Adham can be obtained using more physiological doses of PG and their synthesis inhibitors.

Smith et al. (89) showed that in isolated vascularly

perfused rat intestine direct infusion of 5 μM PGE_2 (about 1500 μg PGE_2) into the lumen decreased both the cell uptake and plasma transfer phases of zinc absorption. In the same system luminal infusion of 150 mg/kg BW of aspirin did not affect the mucosal uptake of ^{65}Zn but decreased the transfer of ^{65}Zn from mucosa to the vascular supply. Sobocinski et al. (79) showed that subcutaneous injection of 10 mg/kg BW of indomethacin in fed rats increased zinc absorption. The authors attributed this effect to drug induced enteropathy. The doses of PG and indomethacin used by these investigators are also unphysiological; nevertheless, they emphasize the point that results obtained under unphysiological conditions should be interpreted with caution. The high doses might effect other cellular functions which indirectly influence zinc absorption. Song and Adham (22) in the above mentioned study showed that whereas administration of 200 μg PGE_2 increased zinc absorption, administration of 400 μg PGE_2 did not affect histidine absorption which was used as a control molecule. The authors concluded, therefore, that the effect of PGE_2 on zinc absorption is specific. It is not clear why 400 μg PGE_2 rather than 200 μg PGE_2 was used for histidine absorption. Furthermore, the effect of indomethacin on histidine absorption was not shown. It is also possible that the percent inhibition achieved in my experiment was not enough to show a deleterious

effect on zinc status. If PG are involved in zinc absorption, however, one would expect to see at least some effect by 50% inhibition of their synthesis.

The Effect of Zinc Deficiency on PG Synthesis

Horrobin and Cunnane (17) suggested that zinc is required for the activity of the delta-6-desaturase enzyme that converts linoleic acid to C18:3n-6; and for the mobilization of dihomo-γ-linoleic acid (C20:3n-6) which implies that zinc is needed for the generation of the one series of PG. Bettger et al. (11) suggested that zinc deficiency accentuates the signs of EFA deficiency and speculated that zinc is involved in the metabolism of arachidonic acid, perhaps decreasing its degradation. It has also been speculated (12) that the manifestation of zinc deficiency in AE patients is due to its effect on PG synthesis. O'Dell et al. (16) showed that toxic doses of aspirin produced pathological signs similar to those of zinc deficiency and therefore suggested that zinc deficiency might interfere with the production of PG.

Prostaglandins were not measured in any of the above mentioned studies and speculations were made based on indirect evidence. In addition, in the study by Horrobin and Cunnane (17) zinc deficient rats were injected with evening primrose oil (EPO) subcutaneously and noticed that the general condition of the rats and skin lesions improved but were not fully

recovered. They attributed the beneficial effect of EPO over safflower oil to its content of C18:3n-6 (precursor for PGE_1 synthesis). These investigators concluded therefore that the manifestations of zinc deficiency might be partly due to inhibition of PGE_1 synthesis and that PGE_1 synthesis is impaired in zinc deficiency. They did not look, however, at the effect of EPO injection on rats receiving zinc adequate diet but pair fed to zinc deficient animals.

It is possible that EPO will have similar beneficial effects over safflower oil in calorie restricted rats, i.e. the defect seen in enzyme delta-6-desaturase might be due to other factors than zinc deficiency.

The present study shows that in most organs studied and for most PG measured food restriction is as effective in reducing PG levels as zinc deficiency. There were, of course, some exceptions. For example, in the gut contents zinc deficiency was effective in reducing PGE_1, $PGF_{2\alpha}$, and PGI_2 levels. The difference was not significant by the Duncan multiple range test, but it was significant using the Student's t-test at the $P<0.05$ level of probability.

Zinc deficiency increased 13,14 dihydro 15 keto-prostaglandin $F_{2\alpha}$ (met $F_{2\alpha}$) in serum and in 10 minute incubated lung homogenates. An increase in met $F_{2\alpha}$ means that the PG was being catabolized faster and therefore, at any specific time less PG might be available to perform

normal biological functions. When there is an increase in met $F_{2\alpha}$ one might expect to see a decrease in $PGF_{2\alpha}$ level. Although the level of $PGF_{2\alpha}$ did not decrease significantly in serum and lung homogenates, it is not contradictory to an increase in met $F_{2\alpha}$, the reason being that in tissue incubation one is looking at the synthetic ability of the tissue and not the absolute level of PG. Therefore, although the synthetic ability of PG was not changed the PG was being degraded faster. The change in met $F_{2\alpha}$ suggests that zinc might be involved in control of PG level by changing its degradation rate.

Zinc, PG, and Immune Response

Zinc deficiency decreased the thymus weight. When the thymus weight to body weight ratio is considered, however, there was no difference between zinc deficient and control animals. Indomethacin did not cause any change in thymus weight.

The results of this study show that zinc and PG have opposite effects on blastogenic response of peripheral blood to T-cell mitogens. Rats with zinc deficiency had a decrease in the stimulation index, whereas those with PG deficiency (in rats treated with indomethacin) had an increase. PUFA did not change the stimulation index which is not surprising, considering that PG production was not

changed by PUFA in this study. This finding is important in interpreting the results of studies in which EFA is used as a PG modifier. An effect seen by EFA on immune response or any other physiological function can not be attributed precisely to PG unless they are measured in the system used. This might also explain the controversy surrounding the role of EFA in immune response (113).

To my knowledge this study is the first investigation on the effect of long term administration of low doses of indomethacin on the blastogenic response of lymphocytes. The results indicate that with administration of 2 mg/kg BW of indomethacin there is a three fold increase in the stimulation index. These results can have important implications in the management of patients who are under indomethacin treatment for long periods of time such as rheumatoid arthritic patients. Because of the pro-inflammatory role of PG, drugs that inhibit their synthesis such as aspirin and indomethacin are used in treatment of rheumatoid arthritis (RA). Production of IgG by the synovial B-cells and the subsequent formation of immunocomplexes is involved in the manifestation of RA (120). Antibody production by B-cells is controlled by T-cells. Indomethacin therefore might be beneficial in treatment of RA by control of antibody production in addition to the control of inflammation. Further investigations on the mechanism of therapeutic effect of indomethacin in RA might help in better understanding of the etiology of RA.

SUMMARY

Experiments were conducted to investigate the role of
prostaglandins (PG) in zinc absorption and biological functions
(food intake and weight gain, alkaline phosphatase activity,
T-cell-mediated immune response) and the role of zinc in PG
synthesis. PG levels were modified by either changing their
precursors (essential fatty acids) concentration in the diet
or administering an inhibitor of their synthesis, aspirin or
indomethacin. Zinc level was modified by controlling the
dietary concentration.

Male weanling rats were assigned randomly to one of the
following groups:

Group A - (control) was fed a semi-purified diet adequate in
zinc with composition similar to that of the average American
diet. This diet contained 20% by weight of beef tallow and
1.2% by weight of safflower oil.

Group B - (PUFA) was fed the control diet except that 21.2%
by weight of diet was composed of safflower oil (polyunsatu-
rated fat - PG precursors.

Group C - (-Zinc) was fed a zinc deficient diet which is the
control diet without supplementation of zinc. The level of
zinc in this diet was less than 1 ppm.

Group D - (Indo.) was fed the control diet with the exception
that 2 mg/kg body weight/day of indomethacin was added to
the diet.

Group E - (Asp.) similar to group D except that 50 mg/kg

body weight/day of aspirin was added to the diet.

Group F - (-Zinc PF) was pair fed to the zinc deficient group with the control diet.

Group G - (Indo. PG) was pair fed to the indomethacin treated group with the control diet.

The rats were fed the assigned diets for one month after which they were anesthetized with ether. Samples from blood, gut contents and mucosa, liver, lung, and tibia were collected for zinc, PG, and alkaline phosphatase measurements.

In another experiment weanling rats were fed one of the following diets; 1) control diet, 2) PUFA diet, 3) -Zinc diet, 4) Indo. diet for one month. The rats were then anesthetized with ether and blood was collected for assay of PG and zinc concentrations and for lymphocyte stimulations with T-cell mitogen assays.

The zinc deficient rats were less active, and had decreased food intake, weight gain, organ weight, plasma and tibia zinc concentrations and blastogenic response of lymphocytes to T-cell mitogens. There was no difference in food intake, weight gain and organ weights of rats treated with aspirin, indomethacin, or PUFA from that of control rats. Indomethacin, aspirin, and PUFA did not significantly change zinc concentrations in any of the tissues measured. Indomethacin and aspirin slightly decreased, and PUFA increased zinc concentration in the mucosa. The decrease,

however, did not reach statistical significant. Alkaline phosphatase activity was not altered by PG, indomethacin, aspirin, or PUFA. Indomethacin increased the blastogenic response of peripheral blood lymphocytes to T-cell mitogens. There was more than 50% inhibition of PG synthesis by indomethacin and aspirin. This inhibition of PG synthesis, however, did not affect the zinc status of the rats as measured by general appearance, food intake, weight gain, organ weight, zinc concentration in different organs, serum alkaline phosphatase activity, and cell mediated immune response to T-cell mitogens. Safflower oil did not affect either PG level or zinc status of the rats.

In most organs studied and for most PG measured both zinc deficiency and food restriction decreased PG level to the same extent. Zinc deficiency seemed to be more effective in reducing PGE_1, $PGF_{2\alpha}$, and PGI_2 level in the gut contents than food restriction alone. The interesting observation was that zinc deficiency increased metabolite of $PGF_{2\alpha}$ in serum and incubated lung homogenates, significantly. This rise in metabolite of $PGF_{2\alpha}$ was associated with a slight decrease in $PGF_{2\alpha}$ in serum and incubated lung homogenates. Lung is an important organ for degradation of prostaglandins. It is proposed that zinc might be involved in controlling tissue level of PG by changing their degradation rate.

REFERENCES

1. Prasad, A.S., J.A. Halsted, and M. Nadimi. 1961.
    Syndrome of iron deficiency anemia, heptosplenomegaly,
    hypogonadism, dwarfism, and geophagia. Am. J. Med.
    31:532-546.

2. Hurley, L.S. 1969. Zinc deficiency in developing rats.
    Am. J. Clin. Nutr. 22:1332-1339.

3. Hambidge, K.M. 1977. The role of zinc & other trace
    metals in pediatric nutrition & health. Pediatr.
    Clin. North Am. 24:95-106.

4. Halsted, J.A., H.A. Ronaghy, and P.A. Abadi. 1972.
    Zinc deficiency in man. Am. J. Med. 53:277-284.

5. Hurley, L.S. 1977. Zinc deficiency in prenatal &
    neonatal development. Pages 47-58 in G.J. Brewer and
    A.S. Prasad, eds. Zinc metabolism: Current aspects in
    health & disease. A.R. Liss Inc., New York, New York.

6. Frost, P., J.C. Chen, I. Rabbani, J. Smith, and A.S.
    Prasad. 1977. The effect of zinc deficiency on the
    immune response. Pages 143-150 in G.J. Brewer and A.S.
    Prasad, eds. Zinc metabolism: Current aspects in
    health & disease. A.R. Liss Inc., New York, New York.

7. Fraker, P.J., S.M. Hass, and R.W. Luecke. 1977. Effect
    of zinc deficiency on the immune response of the young
    adult A/J mouse. J. Nutr. 107:1889-1895.

8. Gross, R.L., O.L. Fong, and P.M. Newberne. 1979.
    I-Depressed immunological function in zinc deprived
    rats as measured by mitogen response of spleen, thymus,
    and peripheral blood. Am. J. Clin. Nutr. 32:1260-1266.

9. Ender, L., Z. Katona, and K. Gycirkovitz. 1975. Zinc
    deficiency in acrodermatitis enteropathica. Lancet 1:1196.

10. Pekarek, R.S., H.H. Sandstead, R.A. Jacob, and D.F.
    Barcome. 1979. Abnormal cellular immune responses
    during acquired zinc deficiency. Am. J. Clin. Nutr.
    32:1466-1471.

11. Bettger, W.J., P.G. Reeves, E.A. Moscatelli, G. Reynolds,
    and B.L. O'Dell. 1979. Interaction of zinc & essential
    fatty acids in the rat. J. Nutr. 109:480-488.

12. Evans, G.W., and P.E. Johnson. 1977. Defective prosta-
glandin synthesis in acrodermatitis enteropathica.
Lancet 1:52.

13. Cash, R., and C.K. Berger. 1969. Acrodermatitis enter-
opathica: Defective metabolism of unsaturated fatty
acids. J. Pediatr. 74:717-729.

14. White, H.B., and J.M. Montalvo. 1973. Serum fatty
acids before & after recovering from acrodermatitis
enteropathica: Comparison of an infant with her family.
J. Pediatr. 83:999-1006.

15. Neldner, K.H., L. Hagler, W.R. Wise, F.B. Stifel, E.G.
Lufkin, and R.H. Herman. 1974. Acrodermatitis entero-
pathica: A clinical & biochemical survey. Arch.
Dermatol. 110:711-721.

16. O'Dell, B.L., G. Reynolds, and P.G. Reeves. 1977.
Analogous effects of zinc deficiency and aspirin toxic-
ity in the gregnant rat. J. Nutr. 107:1222-1228.

17. Horrobin, D.F., and S.C. Cunnan. 1980. Interaction be-
tween zinc, essential fatty acids & prostaglandins: Rel-
evance to acrodermatitis enteropathica total parenteral
nutrition, the glucagonoma syndrome, diabetes, anorexia
nervosa & sickle cell anemia. Med. Hypotheses 6:277-296.

18. Hahan, C., and G.W. Evans. 1973. Identification of a
low molecular weight ^{65}Zn complex in rat intestine.
Proc. Soc. Exp. Biol. Med. 144:793-795.

19. Hurley, L.S., J.R. Duncan, M.V. Sloan, and C.D. Eckhert.
1977. Zinc binding ligands in milk & intestine: A role
in neonatal nutrition. Proc. Nat. Acad. Sci 74:3547.

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

21. Evans, G.W., and P.E. Johnson. 1978. Copper & zinc
binding ligands in the intestinal mucosa. Pages 98-105
in M. Krichgessner, ed. Trace element metabolism in
man and animals-3. Institut Für Ernährungsphysiologie
Technische Universität, München Fresising-Weihenstephen.

22. Song, M.K., and N.F. Adham. 1979. Evidence for an im-
portant role of prostaglandins E_2 and $F_{2\alpha}$ in the regu-
lation of zinc absorption in the rat. J. Nutr. 109:
2152-2159

23. Song, M.K. and N.F. Adham. 1977. Prostaglandin regulation of zinc absorption in rats. Fed. Proc. 36:1138.

24. Lonnerdal, B., A.G. Stanislowski, and L.S. Hurley. 1980. Isolation of a low molecular weight zinc binding ligand from human milk. J. Inorg. Biochem. 12:71-78.

25. Richards, M.P., and R.J. Cousins. 1976. Metallothionein & its relationship to the metabolism of dietary zinc in rats. J. Nutr. 106:1591-1599.

26. Schricker, B.R., and R.M. Forbes. 1978. Studies on the chemical nature of a low molecular weight zinc binding ligand in rat intestine. Nutr. Rep. Int. 18:159-166.

27. Kennedy, A.C., G.S. Fell, P.J. Rooney, W.H. Stevens, W.C. Dick, and W.W. Buchanan. 1975. Zinc: Its relationship to osteoporosis in rheumatoid arthritis. Scand. J. Rheumatol. 4:243-245.

28. Niedermeier, W., and J.H. Griggs. 1971. Trace metal composition of synovial fluid and blood serum of patients with rheumatoid arthritis. J. Chron. Dis. 23:527-536.

29. Koshakji, R.P., and A.R. Schulert. 1973. Biochemical mechanisms of salicylate teratology in the rat. Biochem. Pharmacol. 22:407-416.

30. Prasad, A.S. 1977. Nutritional aspects of zinc. Dietetic Currents 4:27-32.

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

32. Halsted, J.A. 1970. Human zinc deficiency. Trans. Am. Clin. & Climate Ass. 82:170-176.

33. Keilin, D., and T. Mann. 1939. Carbonic anhydrase. Nature 144:442-443.

34. Parisi, A.F., and B.L. Vallee. 1969. Zinc metalloenzymes: Characteristics & significance in biology & medicine. Am. J. Clin. Nutr. 22:1222-1239.

35. Hambidge, K.M., C. Hambidge, M. Jacobs, and J.D. Baum. 1972. Low level of zinc in hair, anorexia, poor growth, & hypogeusia in children. Pediatr. Res. 6:868-874.

36. Neldner, K.H., and K.M. Hambidge. 1975. Zinc therapy of acrodermatitis enteropathica. N. Engl. J. Med. 292:879-882.

37. Underwood, E.J. 1977. Trace elements in human & animal. 4th ed. Academic Press, New York.

38. Hurley, L.S., and H. Swenerton. 1966. Congenital malformations resulting from zinc deficiency in rats. Proc. Soc. Exp. Biol. Med. 123:692-696.

39. Hurley, L.S., and R.E. Shrader. 1975. Abnormal development of preimplantation rat eggs after 3 days of maternal dietary zinc deficiency. Nature 254:427-429.

40. Gallaher, D., and L.S. Hurley. 1980. Low zinc concentration in rat uterine fluid after four days of dietary zinc deficiency. J. Nutr. 110:591-593.

41. Apagar, J. 1968. Effect of zinc deficiency on parturition in the rat. Am. J. Physiol. 215:160-163.

42. Swenerton, H., and L.S. Hurley. 1980. Zinc deficiency in Rhesus & Bonnet monkeys including effects on reproduction. J. Nutr. 110:575-584.

43. Hambidge, K.M., K.H. Neldner, and P.A. Walravens. 1975. Zinc, AE, & Congenital malformations. Lancet 1:577-578.

44. Jameson, S. 1976. Effects of zinc deficiency in human reproduction. Acta Med. Scand. Suppl. 593:4-89.

45. Warkany, J., and H.G. Petering. 1972. Congenital malformations of the central nervous system in rats produced by maternal zinc deficiency. Teratology 5:319-334.

46. McKenzie, J.M., C.J. Fosmire, and H.H. Sandstead. 1975. Zinc deficiency during the latter third of pregnancy: Effects on fetal rat brain, liver,& placenta. J. Nutr. 105:1466-1470.

47. Sandstead, H.H., C.J. Fosmire, E.S. Halas, R.A. Jacob, P.A. Strobel, and E.O. Marks. 1976. Zinc deficiency: Effects on brain & behavior of rats & Rhesus monkeys. Teratology 16:229-234.

48. Fang, M.M., K.Y. Lei, and L.T. Kilgore. 1980. Effect of zinc deficiency on dental caries in rats. J. Nutr. 110:1032-1037.

49. Prasad, A.S., A. Abbasi, and J. Ortega. 1977. Zinc deficiency in man: Studies in sickle cell disease. Pages 211-237 in G.J. Brewer and A.S. Prasad, eds. Zinc metabolism: Current aspects in health & disease. A.R. Liss Inc., New York New York.

50. Simkin, P.A. 1977. Zinc sulphate in rheumatoid arthritis. Pages 343-353 in G.J. Brewer and A.S. Prasad, eds. Zinc metabolism: Current aspects in health & disease. A.R. Liss Inc., New York, New York.

51. Miller, E.R., R.W. Lueke, D.E. Ullrey, B.U. Baltzer, and J.A. Holfer. 1968. Biochemical, skeletal, and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95:273-286.

52. Haas, S., P. Fraker, and R.W. Lueke. 1976. The effect of zinc deficiency on the immune response of A/J mouse. Fed. Proc. 35:659.

53. DePasquale-Jardieu, P., and P.J. Fraker. 1979. The role of corticosterone in the loss in immune function in the zinc deficient A/J mouse. J. Nutr. 109:1847-1855.

54. Kinoshita, Y., S. Kymura, and M. Kukamizu. 1974. Cytolytic effect of glucocorticoids on the thymus-medullary lymphocytes incubated in vitro. Exp. Cell Res. 87:387-389.

55. Beach, R.S., M.E. Gershwin, R.K. Makishima, and L.S. Hurley. 1980. Impaired immunologic ontogeny in postnatal zinc deprivation. J. Nutr. 110:805-815.

56. Golden, M.H.N., A.A. Jackson, and B.E. Golden. 1977. Effect of zinc on thymus of recently malnourished children. Lancet 2:1057-1059.

57. Meyers, S., D.B. Sachar, R.N. Taub, and H.D. Janowitz. 1976. Anergry to dinitrochlorobenzene & depression of T-lymphocytes in Crohn's disease & ulcerative colitis. Gut 17:911-915.

58. Gross, R.L., N. Osdin, L. Fong, and P.M. Newberne. 1979. II- In vitro restoration by levamisole of mitogen responsiveness in zinc-deficient rats. Am. J. Clin. Nutr. 32:1267-1271.

59. Chvapil, M., C. F. Zukoski, B. G. Hotter, L. Stankova, D. Montgomery, E. C. Carlson, and J. C. Ludwig. Zinc and cells. Pages 269-281 in G. J. Brewer and A. S. Prasad, eds. Zinc metabolism: Current aspects in health and disease. A. R. Liss Inc., New York, New York.

60. Chvapil, M., J. Ryan, and Z. Brada. 1971. Effect of chelating agents and metals on the stability of liver lysosomes. Biochem. Pharmacol. 21:1097-1105.

61. Quaterman, J., C. F. Mills, and W. R. Humphries. 1966. The reduced secretion of, and sensitivity to insulin in zinc deficient rats. Biochem. Biophys. Res. Commun. 25:354-358.

62. Quaterman, J., and E. Florence. 1972. Observations on glucose tolerance and plasma levels of free fatty acids and insulin in the zinc deficient rat. J. Nutr. 28:75-79.

63. Roth, H. P., and M. Kirchgessner. 1977. Influence of zinc deficiency on lipid metabolism. Int. J. Vitam. Nutr. Res. 47:275-283.

64. Sullivan, J. F., M. M. Jetton, and H. K. Hahn. 1980. Enhanced lipid peroxidation in liver microsomes of zinc-deficient rats. Am. J. Clin. Nutr. 33:51-56.

65. Vallee, B. L., W. E. C. Wacker, A. F. Bartholomay, and E. D. Robin. 1956. Zinc metabolism in hepatic dysfunction. I-serum zinc concentration in Laennec's cirrhosis and their validation by sequential analysis. N. Engl. J. Med. 255:403-408.

66. Chvapil, M., Y. M. Peng, A. L. Ronson, and C. Zukoski. 1974. Effect of zinc on lipid peroxidation and metal content in some tissues of rats. J. Nutr. 104:434-443.

67. Koo, S. I., and D. E. Turk. 1977. Effect of zinc deficiency on intestinal transport of triglyceride in the rat. J. Nutr. 107:909-919.

68. Ziboh, V. A., and S. L. Hsia. 1972. Effects of PGE_2 on rat skin: Inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. Lipid Res. 13:458-467.

69. Bettger, W. J., P. G. Reeves, E. A. Moscatelli, J. E. Savage, and B. L. O'Dell. 1980. Interaction of zinc and polyunsaturated fatty acids in the chick. J. Nutr. 110:50-58.

70. Cunnane, S. C., H. Zimner, and D. F. Horrobin. 1979. Copper inhibits pressor responses to noradrenaline but not potassium. Interaction with PGE_1, E_2, I_2, and penicillamine. Can. J. Physiol. Pharmacol. 57:35-40.

71. Manku, M. S., and D. F. Horrobin. 1976. Chloroquin, quinine, procaine, quanidine, and clomipramine are prostaglandin agonists and antagonists. Prostaglandins 12:789-801.

72. Manku, M. S., D. F. Horrobin, M. Karmazyn, and S. C. Cunnane. 1979. Prolactin and zinc effects on rat vascular reactivity: Possible relationship to dihomo-gamma-linolenic acid and to prostaglandin synthesis. Endocrinology 104:741-779.

73. Horrobin, D. F., R. A. Karmali, M. Karmazyn, R. O. Morgan, and M.S. Manku. 1977. Zinc, acrodermatitis, acne, and prostaglandins. Br. Med. J. 2:190-191.

74. Cunnane, S. C., D. F. Horrobin, K. B. Ruf, and G. E. Sella. 1979. Essential fatty acid supplementation inhibits the effect of dietary zinc deficiency. Page 23 in International Prostaglandin Conference, Washington.

75. Cunnane, S. C., D. F. Horrobin, K. B. Ruf, and G. Sella. 1979. Prevention of dietary effects of zinc deficiency by administration of essential fatty acids. J. Physiol. 296:83P-84P.

76. Evans, G. W., C. I. Grace, and H. J. Votava. 1975. A proposed mechanism for zinc absorption in the rat. Am. J. Physiol. 228:501-505.

77. Suso, F. A., and H. M. Edwards. 1971. Binding capacity of intestinal mucosa and blood plasma for zinc. Proc. Soc. Exp. Biol. Med. 137:306-309.

78. Duncan, J. R., and L. S. Hurley. 1978. Intestinal absorption of zinc: A role for a zinc binding ligand in milk. Am. J. Physiol. 4:E556-E559.

79. Sobocinski, P. Z., G. L. Knutsen, W. J. Canterbury, and E. C. Haver. 1979. Altered zinc homeostasis and hepatic accumulation of metallothionein in indomethacin-induced enteropathy. Toxicol. Appl. Pharmacol. 50:557-564.

80. Evans, G. W., and P.E. Johnson. 1979. Detection of labile zinc-binding ligands in biological fluids by modified gel filtration chromatography. Anal. Chem. 51:839-843.

81. Evans, G. W., and E. C. Johnson. 1980. Zinc absorption in rats fed a low protein diet and a low protein diet supplemented with tryptophan or picolinic acid. J Nutr. 110:1076-1080.

82. Evans, G. W., and E. C. Johnson. 1979. Zinc absorption in rats fed varying levels of vitamin B_6 antagonist. Clin. Res. 27:682A.

83. Robertson, A. F., G. S. Schuerger, R. R. Brown, and W. B. Karp. 1973. Tryptophan metabolism in acrodermatitis enteropathica. J. Pediatr. 83:1012-1016.

84. Evans, G. W. 1980. Normal and abnormal zinc absorption in man and animals: The tryptophan connection. Nutr. Rev. 38:137-142.

85. Krieger, I., and G. W. Evans. 1980. A variant of acrodermatitis enteropathica without hypozincemia. Therapeutic effect of pancreatic enzyme production due to zinc binding ligand. J. Pediatr. 96:32-35.

86. Cousins, R. J., and K. T. Smith. 1980. Zinc binding properties of bovine and human milk in vitro: Influence of changes in Zinc content. Am. J. Clin. Nutr. 33:1083-1088.

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

88. Cousins, R. J. 1979. Regulation of zinc absorption: role of intracellular ligands. Am. J. Clin. Nutr. 32:339-346.

89. Smith, K. T., R. J. Cousins, B. L. Silbon, and M. L. Failla. 1978. Zinc absorption and metabolism by isolated, vascularly perfused rat intestine. J. Nutr. 108:1849-1857.

90. Cousins, R. J., K. T. Smith, M. L. Failla, and L. A. Markowitz. 1978. Origin of low molecular weight zinc binding complexes from rat intestine. Life Science 23:1819-1826.

91. Panemangalore, M., and F. O. Brady. 1979. The influence of zinc status on the levels of metallothionein in isolated perfused rat liver. J. Nutr. 109:1825-1835.

92. Starcher, B. C., J. C. Glauber, and J. G. Madaras. 1980. Zinc absorption and its relationship to intestinal metallothionein. J. Nutr. 110:1391-1398.

93. Saylor, W., and M. Leach. 1980. Copper and zinc binding proteins in sheep liver and intestine: Effects of dietary level of metals. J. Nutr. 110:460-469.

94. Simkin, P. A. 1976. Oral zinc sulfate in rheumatoid arthritis. Lancet 2:539-542.

95. Siggins, G., B. Hoffer, and F. Bloom. 1971. Prostaglandin norepinephrine interaction in brain: Microelectrophoretic and histochemical correlates. Ann. N.Y. Acad. Sci. 180: 302-323.

96. Lee, J., H. Kannegiesser, J. O'Toole, and E. Westura. 1971. Hypertension and the renomedullary prostaglandins: A human study of the hypertension effect of PGA. Ann. N.Y. Acad. Sci. 180:218-240.

97. Robinson, G., A. Arnold, B. Cole, and R. Herman. 1971. Effect of prostaglandins on function and cyclic AMP levels of human blood platelets. Ann. N.Y. Acad. Sci. 180:324-331.

98. Henman, J. W. 1972. Developing applications of prostaglandins in obstetrics and gynecology. Am. J. Obstet. Gynecol. 113:130-138.

99. Illiano, G., and P. Cuarecasos. 1971. Endogenous prostaglandins modulate lipolytic processes in adipose tissues. Nature (New Biol.) 234:72-74.

100. Bonta, I. L., and M. J. Parham. 1978. Prostaglandins and chronic inflammation. Biochem. Pharmacol. 27:1611-1623.

101. Webb, D. R., T. J. Rodgers, and I. Nowowiejski. 1979. Endogenous prostaglandin synthesis and the control of lymphocyte function. Ann. N.Y. Acad. Sci. 332:262-270.

102. Elattar, T. M. A. 1978. Prostaglandins: Physiology, biochemistry, pharmacology and clinical applications. J. Oral. Pathol. 7:175-207.

103. Ramwell, P. W. 1973. The prostaglandins. vol. 1, 2, 3. Plenum Press, New York.

104. Goodwin, J. S., D. S. Selinger, R. P. Messner, and W. P. Reed. 1978. Effect of indomethacin in vivo on humoral and cellular immunity in humans. Infect. Immun. 19:430-433.

105. Parker, C. W., T. J. Sullivan, and H. J. Wender. 1974. Role of PG in cell mediated immunity. P. Greengard and G. A. Robison, eds. Pages 1-80 in Advances in cylic nucleotide research. vol. 4. Raven Press, New York, N.Y.

106. Stockman, G. D., and D. M. Mumford. 1974. The effect of prostaglandins on the in vitro blastogenic response of human peripheral blood lymphocytes. Exp. Hematol. 2: 73-82.

107. Schultz, R. M., N. A. Pavlidis, W. A. Stylos, and M. A. Chirigos. 1978. Regulation of macrophage tumoricidal function: A role for prostaglandins of the E series. Science 202:320-321.

108. Webb, D. R., and I. Nowowiejski. 1978. Mitogen-induced changes in lymphocyte prostaglandin level: A signal for the induction of suppressor cell activity. Cell Immunol. 41:72-85.

109. Webb, D. R. and P. L. Osheroff. 1976. Antigen stimulation of prostaglandin synthesis and control of immune responses. Proc. Natl. Acad. Sci. USA 73:1300-1304.

110. Ferber, E., G. G. DePasquale, and K. Resch. 1975. Phospholipid metabolism of stimulated lymphocytes. Composition of phospholipid fatty acids. Biochim. Biophys. Acta 398:364-376.

111. Zimecki, M., D. R. Webb. 1976. The regualtion of the immune response to T-independent antigens by prosta-glandins and B cells. J. Immunol. 117:2158-2164.

112. Goodwin, J. S., A. D. Bankhurst, S. A. Murphy, D. S. Selinger, R. P. Messner, and R. C. Williams. 1978. Partial reversal of the cellular immune defect in common variable immunodeficiency with indomethacin. J. Clin. Lab. Immunol. 1:197-199.

113. Field, E. J. 1975. Unsaturated fatty acids and cellular immunity. Lancet 2:934.

114. Dewille, J. W., P. J. Fraker, D. R. Romsos. 1979. Effects of essential fatty acid deficiency, and various levels of dietary polyunsaturated fatty acids, on humoral immunity in mice. J. Nutr. 109:1018-1027.

115. Offner, H., J. Clausen. 1978. The enhancing effect of unsaturated fatty acids on E Rosette formation. Int. Arch. Allergy Appl. Immunol. 56:376-379.

116. Cunnane, S. C., M. S. Nanku, and D. F. Horrobin. 1979. The Pineal and regulation of fibrosis: Pinealectomy as a model of primary biliary cirrhosis: Roles of melatonin and prostaglandins in fibrosis and regulation of T-lymphocytes. Med. Hypotheses 5:403-414.

117. Hwang, D. H., M. M. Mathias, J. Dupont, and D. L. Meyer. 1976. Linoleate enrichment of diet and prostaglandin metabolism in rats. J. Nutr. 105:995-1002.

118. Meydani, S. N., M. M. Mathias, and C. L. Schatte. 1978. Dietary fat type and ambient oxygen tension influence pulmonary prostaglandin synthetic potential. Prostaglandin and Medicine 1:241-249.

119. Hassam, A. G., A. L. Willis, J. P. Denton. 1979. The effect of essential fatty acid deficient diet on the levels of prostaglandins and their fatty acid precursors in rabbit brain. Lipids 14:78-80.

120. Flower, R. J. 1974. Drugs which inhibit PG biosynthesis. Pharmacol. Rev. 26:33-67.

121. National Academy of Sciences. 1972. Nutrient requirements of laboratory animals. 2nd ed. NAS, Washington, D.C.

122. Stahr, H. M. 1977. Analytical toxicological method manual. The Iowa State University Press, Ames, Iowa. 47 pp.

123. McCosh, E. J., D. L. Meyer, and J. Dupont. 1976. Radioimmunoassay of prostaglandins E_1, E_2, $F_{2\alpha}$ in unextracted plasma, serum and myocardium. Prostaglandins 12:472-486.

124. Slavin, S. W., G. E. Peterson, and P. C. Lindhal. 1975. Determination of heavy metals in meats by atomic absorption spectroscopy. Atomic Absorption Newsletter 14:57-59.

125. Hackley, B. M., J. C. Smith, and J. A. Halsted. 1968. A simplified method for plasma zinc determination by atomic absorption spectrophotometry. Clin. Chem. 14:1-5.

126. Sigma technical bulletin No. 104. Sigma Chemical Co., St. Louis, Mo. September, 1979.

127. Verhaegen, H., D. Decree, W. DeCock, and F. Verbruggen. 1977. Restoration by levamisole of low enrossette forming cells in patients suffering from various disease. Clin. Exp. Immunol. 27:313-318.

128. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. 6th ed. The Iowa State University Press, Ames, Iowa.

129. Duncan, D. B. 1955. Multiple range and multiple F-test. Biometrics 11:1-6.

130. Fye, K. H., H. M. Moutsopoulos, and N. Talal. 1978. Rheumatic diseases. H. H. Fundenberg, D. P. Stites, J. I. Caldwell, and J. V. Wells eds. Pages 442-452 in Basic and clinical immunology. 2nd ed. Lange Medical Publications, Los Altos, Cal.

ACKNOWLEDGEMENTS