INFLUENCE OF ZINC DEFICIENCY ON GROWTH AND SEXUAL DEVELOPMENT IN RATS

by

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ABSTRACT

The processes of somatic growth and sexual maturation and activity have been investigated in the light of the metabolic model of nutritional zinc deficiency, in female rats.

Zinc deficiency is a dysmetabolic nutritional condition encountered in nature. Its consequences, mirrored by the model adopted, are growth arrest or delay and lack of sexual maturation in the young. Zinc deficiency in adult female rats inhibits, at least in part, sexual functions.

Pharmacologic induction of precocious sexual maturation meets with success only at the end-organ (i.e. ovary, uterus) level. The observable pattern of growth arrest is paralleled by low levels of growth hormone, insulin and thyroxine, as determined.

The absence of sexual maturation and activity are paralleled by low levels of FSH, LH, PRL and steroid sex hormones, as determined.

The results obtained from the hormonal determinations done indicate that zinc deficiency affects significantly the pituitary-end-organ axis, being causal to dwarfism and hypogonadism.

RESUME

Les processus de l'accroissement somatique et de la maturation et activité sexuelle ont été étudiés dans l'optique de la carence metabolique nutritionnelle du zinc, dans le rat femelle.

La carence en zinc ets une condition nutritionnelle dysmetabolique rencontrée dans la nature. Ses consequences, refletées par le model adopté, y sont l'arrêt de la croissance corporelle et le délai on manque de développement sexuel dans les jeunes femelles. Chez les adultes, la carence en zinc inhibe, au moins partiellement, les fonctions sexuelles.

L'induction pharmacologique de la puberté précoce ne porte que sur les organes périphériques (i.e. l'ovaire, l'uterus) mais n'influence pas le niveau hypophysaire.

Les déterminations obtenues de l'hormone somatotrophe (GH), de l'insuline et de la thyroxine reflètent les consequences observables de l'arrêt de la croissance. L'absence de la puberté est accompagnée par une baisse des gonadotropines, de la prolactine et des hormones steroides sexuelles.

L'ensemble des resultats des dosages hormonaux est indicatif du fait que la carence en zinc est nuisible au fonctionnement de l'axe pituitaire - organes sexuels, étant compatible avec le nanisme et l'hypogonadisme.

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ACTH	- adreno-cortico-tropic hormone
B	- corticosterone
DNA	- desoxyribonucleic acid
E_2	- estradiol-17β
E.B.	- estradiol benzoate
E.F.A.	- essential fatty acids. PRIMROSE OIL containing mixture of essential fatty acids.
E.F.A treatment	- 0.2 ml EFA/s.c. three times a week
FSH	- follicule stimulating hormone
GH	- growth hormone
LH	- luteotrophic hormone
LHRH	- luteinizing hormone releasing hormone
MSH	- melanocyte stimulating hormone
P_4	- progesterone
Placebo	- olive oil, used as placebo treatment in lieu of E.F.A. treatment
PRL	- prolactin
PTH	- parathyroid hormone
Purina	- purina pellets fed, ad libitum
RNA	- ribonucleic acid
Zn	- zinc
Zn↓	- zinc deficient - i.e. fed on a diet containing < 3 ppm zinc
Zn↑	- zinc supplemented, pair-fed to the Zn↓ group - In Experiment VI, Zn↑ signifies pair-fed - 20% - In Experiment VII, Zn↑ signifies ad libitum zinc supplemented feeding.

PART I

1. ZINC IN BIOLOGY

Zinc is an ubiquitous element, abundantly distributed in the crust of this planet. All forms of life contain it, albeit in trace amounts. It can be toxic to very few species, most others deriving significant benefits from its existence and chemo-electric properties. In natural conditions of zinc deficiency, plants and animals do not thrive and grow. Fruit growth is impeded and so is animal sexual maturation.

Directly or via a number of zinc containing enzymes or hormones, zinc affects several aspects of animal metabolism. Deficiency of it in the animal species studied, including man, is detrimental to growth, sexual maturation, collagen synthesis and wound healing, skin or fur and the senses of smell, taste and sight, to count but some of the effects. Replacement of zinc in the environment and diet may counter or defend against such effects.

The field of study of the role of zinc in metabolism is still in its incipient phase. It is hoped that future endeavors will bring about a clearer understanding of the role of this essential trace element in metabolism.

PART I

1.2. Zinc Metabolism

Occurrence

Zinc represents 4×10^{-3} of the earth's substance and is the 25th most common element. It is found in several ores in combination with other metals, in most soils and in all plants. Animals obtain it from plants and water (74,81).

Content and distribution of zinc in the human body:

The studies done to date indicate that the zinc content/70 kg human weight varies between 1.4 - 2.6 g (19). It is distributed as follows:

Tissue	Content (mgs)
muscle	1,420.0
bone	660.0
liver	40.5
G.I. tract	30.0
skin	25.2
kidney	19.8
brain	18.0
lung	16.6
heart	8.7
blood	6.0
bladder	4.4
spleen	3.8
aorta	2.6
prostate	1.7
testes	0.8
thyroid	0.4
ovary	0.3

Whole blood contains 700 to 1000 µg Zn/dl of which the red blood cells contain about 85%, the white blood cells about 12% and the serum about three per cent. The serum zinc exhibits a circadian variation

with the minimal values at 6 a.m. and the peak at 10 p.m. (81)

Intake and Bioavailability

Zinc is obtained mainly from meats and water. Although all vegetables contain zinc, the actual bioavailable quantity is very low due to the high amount of fiber, phytate (chelators) and calcium (competitor). An intake of 11 - 55 mg of zinc per day is recommended, according to age, sex and metabolic necessity (75).

Intestinal absorption and blood transport

A zinc-binding ligand fraction has been identified from the pancreatic secretions of the rat and the dog (39,80). It functions as to facilitate zinc uptake and absorption. This binding protein "travels "to the intestinal microvilli of the duodenal and jejunal membrane mainly, where, it is postulated, ligates the zinc ions it finds at specific receptor sites. The zinc-ligand complex "travels" to the basolateral plasma membrane. This membrane, it is postulated, contains zinc receptor sites which function to transfer zinc to the plasma albumin (17). Albumin removes the ligand-made available zinc from the intestinal epithelial cell membrane. It appears that the zinc-albumin concentration in the blood has a role in the homeostatic regulation of the zinc requirement, uptake and intestinal absorption, since it was found that the zinc concentration absorbed is inversely proportional to the plasma zinc concentration. In the blood, zinc is found bound to albumin (~ 45%) to α-macroglobulin (~ 35%) to ceruloplasmin and other globulins (~ 4%), to histidine, cysteine,porphyrins and in free form (< 1%) (71). It is removed readily by the tissues, the rate of removal being proportional to the relative level of specific organ requirement and deficiency (4). The mechanism of cellular zinc absorption is obscure. However it is known that the intracellular zinc uptake is a

function of competitive inhibition by calcium, iron, copper, and cadmium. Once taken up by certain organs (e.g. bone, hair), zince is not readily exchangeable with the plasma. It is probable that the specific sites of Zn uptake (which are the first to rapidly reduce the activity in the early stages of zinc deficiency)must contain zinc in a form freely exchangeable with the metal in circulation (i.e. the animals respond within 5-6 days to a suboptimal zinc supply when actually most tissues contain an appreciable zinc concentration) (79).

Excretion

Excretion of zinc is mainly via the feces (~ 96%) and varies with the intake. In general it is between 5-10 mg/24 hrs. About 80% of the fecal excreted zinc originates from the ingested zinc and the rest derives from the gastrointestinal tract and the bile. About 30% zinc reabsorption is thought to occur in the fraction secreted in the intestinal lumen (14). Urinary excretion which is about 500 µg/24 hrs and which represents about 1% of the total excretion is a rather constant feature and in non-pathological conditions is independent of the intake. In the urine, zinc is bound mainly to porphyrins and different amino acids. A significant variable in zinc secretion, in humans at least, is the amount of sweat loss (i.e. dependency on environmental temperature and physical activity). Sweating, which averages 115 µg zinc/ml could account for 3-12% of the total zinc excreted/24 hrs (16).

Zinc and metabolic components

Intracellularly zinc is associated structurally and functionally with metalloenzymes (over 80 discovered to date), proteins, ADP, DNA, RNA, copro- and uro-porphyrins and hormones. Examples of zinc metalloenzymes are: alcohol dehydrogenase, aldolase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase A and B, glutamic dehydrogenase, lactate dehydrogenase,

amino peptidase, etc. (38,57). Examples of zinc activated matalloenzymes are: arginase, enolase, lecithinase, histadine deaminase, leucyl-glycine dipeptidase, etc. (80). Zinc associated proteins are collagen, zinc-2-glycoprotein, metallothionein and leucocyte metalloproteins (51). Zinc is actively involved in nucleic acid synthesis and transcription for protein synthesis (1), (18), (33). Zinc complexes with ADP (it reduces the conversion of ATP to c-AMP in lipocytes) (110). Insulin is a zinc hormone. It is released from the proinsulin form as a six insulin molecules bound to 3 zinc molecules structure. Zinc is paramount for its transport, proper function and normal rate of catabolism. (3)

PART II

1. The Brain

The zinc content of the brain as a whole is about 13mg/g wet weight, less than that of the whole body (33µg/g wet weight) but considerably higher than that of the blood (1µg/g wet weight). It should be mentioned that the zinc concentration in the brain is the highest of all essential trace metals. Only the electrolytes (Na, K and Mg) are more prevalent than zinc in the brain. All brain structures contain zinc. Grey matter and the cortex as a whole contain significantly higher concentrations than white matter and the medulla. Brain regions such as the hippocampus, the hypothalamus, the pineal, the striatim, the cerebellum (the mossy fibres only), and the pituitary are among the highest in the body with respect to zinc content/structure (15).

The role of zinc in the brain as a whole or in different brain structures has been studied little as yet. It is known that a decrease of zinc concentration from the brain is associated with certain neurologic symptoms (i.e. ataxia, anorexia, dysosmia, dysgeusia, lack of libido, etc.) (26,27). The retina of the carnivorous animals contains a high zinc concentration. The metal is functionally and structurally associated with the retinal reductase and carbonic anhydrase enzymes, essential in the visual process. Zinc and Vitamin A deficiency are associated with nyctalopia (69). The olfactory lobe and the taste buds have a high zinc concentration. Zinc deficiency is associated with hyposmia, dysosmia or anosmia and hypogeusia, dysgeusia and ageusia (27). Zinc deficiency is associated with severe CNS congenital malformations in birds, animals, and possibly humans (13,18,32,35,68,73,75).

II. The Hypothalamus

The zinc concentration in the hypothalamus is probably the highest in the body (~0.066% of dry weight) for any particular organ. Zinc deficiency is associated with various degrees of neuroendocrine dysfunction or hypofunction. In view of the analytical difficulties regarding both the hypothalamic releasing factors and hormones and those of zinc determination, a precise, one-to-one functional relationship of the hypothalamic-related zinc metabolism awaits elucidation in the future. The only hypothalamic hormone studied in relation to zinc deficiency was LHRH. No change in the hypothalamic LHRH concentration was determined in that condition (42). However, data accumulated indicate, at least conjuncturally, that the hypothalamic function is disrupted in conditions of zinc deficiency. The latter condition is associated with anorexia, growth disruption, non-development or arrest of sexual maturation and/or activity, disruption of the maternal nesting instinct, to mention but the most striking features of (?) hypothalamus related zinc deficiency symptoms.

III. The Pituitary

The study of the role of zinc in pituitary function began in 1938. In several experiments pharmacologic stimulation of endocrine activity via treatment with pituitary hormones (FSH,LH, GH,TSH,ACTH) was significantly enhanced by the addition of zinc salts to the injectable hormonal preparation (65,66).

Several authors studied the relationships between the hypophysis and zinc metabolism. Parameters such as pituitary size and weight, hypophysectomy, hypophysectomy followed by pharmacologic stimulation with several hormones, zinc deficiency, pair-weighing and pair-feeding were used, varied and tentatively interpreted.

Millar et al studied the effects of testosterone and gonadotropin injections on the sex organ development of zinc deficient male rats (51). They found that in zinc deficient male weanling rats, the pituitary size and weight were significantly decreased when compared with the appropriate controls. In a weight pairing experiment (i.e. the weight-pair peers received sufficient zinc but only so much food as to gain the weight gained by their zinc deficient peers), the pituitary size was lower than normal in the zinc supplemented group. On refeeding that group the pituitary weight increased to normal values. On "refeeding" the zinc deficient rats with an appropriate amount of zinc the pituitary weight also approached the normal level. The conclusions were that the retarded growth of the sex glands and the body may have been due to the reduction of the pituitary output and thus may have been due to the inanition of the zinc deficiency (51).

Rudzig et al studied the effects of hypophysectomy and ACTH on zinc metabolism in the sex glands and adrenals of the male rat. They found that hypophysectomy had no effect on the zinc concentration in the whole blood.

They found also that five days post-hypophysectomy the Zn^{65} uptake was lowered in the dorsolateral prostate. If ACTH was given after hypophysectomy the Zn^{65} uptake in the dorsolateral prostate returned to approximately normal values. Millar et al studied the effects of dietary zinc deficiency on the reproductive system of male rats. They found that in the zinc deficient male rats the pituitary glands - stained with a glyco-protein stain - showed more cells considered to be gonadotrophs and thyrotrophs as compared to the cells of the appropriate controls. The pituitary weight in conditions of zinc deficiency was 5.8 ± 0.42 mg. If 200 mg of zinc per day were given to the pair-fed animals, the pituitary size increased to 6.4 ± 30 mg. If 200 mg of zinc per day and food ad libitum were offered to the male rats the pituitary weight increased 10.4 ± 0.62 mg (46) (53).

Ibrahim et al studied the effects of starvation on pituitary and serum follicle stimulating hormone and luteinizing hormone following ovariectomy in the rat. They found that the effects of starvation upon ovariectomized rats were that the pituitary LH values were increased significantly in the starved ovariectomized females as compared to those of the well-fed ovariectomized peers. Their conclusion was that the gonadotrophin was not affected by the starvation in the ovariectomized animal (61).

III a

Growth Hormone and Growth

Growth arrest is a constant characteristic of nutritional zinc deficiency of whatever etiology. This event can be easily reproduced in experimental laboratory work and has been identified as one of the components of the human syndrome originally described by Halstead, Prasad et al in 1961. The relationship between growth hormone and zinc metabolism has been the subject of research of several studies. Henkin et al studied the changes in zinc concentration in the plasma and urine in acromegaly Patients with acromegaly have a relatively low serum zinc concentration and an increased level of zincuria. Treatment of the disease is followed by a normalization of the serum and urine zinc status. The converse zinc status is associated with patients suffering from GH deficiency. Treatment with GH is followed by a normalization of the serum and urinary zinc values (23, 28, 65).

Macapinlac et al studied the effects of growth hormone on the growth pattern of zinc deficient rats. They found that there was no improvement in weight or size gain in the zinc deficient animals when given injections of bovine growth hormone over a period of time (48)

Ku et al studied nucleic acids and protein metabolism in zinc deficient pigs. These authors found that if growth hormone was given to zinc deficient pigs there was no improvement in growth and food intake influence on the serum zinc level, serum alkaline phosphatase concentration and parakeratotic lesions if the latter had already occurred (40)

Prasad studied the effect of growth hormone and zinc intake in hypophysectomized rats. When the hypophysectomized animals were given growth hormone and zinc they responded by a significant increase in growth irrespective of their zinc status. The authors concluded from the

experiment that the effects of growth hormone and zinc addition are additive but apparently independent of each other (58).

Sandstead et al found that in human zinc deficiency cases (40 boys) there was growth failure not related to thyroid deficiency. Ronaghy studied zinc deficiency effects in human females and reported two cases characterized by dwarfism, hypogonadism, sideroachrestic anemia and geophagia. The anemia responded to iron supplementation; the physical growth and sexual development responded to a well-balanced diet and the appropriate addition of zinc salt for three to six months. There was some growth under the dietary increased conditions but no sexual development unless zinc was added to the diet ($ZnSO_4.7H_2O$, 200 mgs ÷ t.i.d.) (65).

Prasad et al studied a syndrome of sideroachrestic anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia in Iranian boys. They found that therapy with zinc,added to a normo-caloric diet, resulted in increased somatic size in general and also of increased growth of the external genitalia and the appearance of secondary sex characteristics (59).

Coble et al studied endocrine functions in boys with retarded growth, delayed sexual maturation and zinc deficiency. This study was done on Egyptian boys who showed a severe zinc deficiency in the serum, a delay in pubertal development and low testosterone and LH levels. The authors' conclusion was that zinc deficiency disturbed primarily the pituitary function as evidenced by the hormonal output (9).

III b

ACTH

The serum zinc concentration exhibits a circadian rhythm similar, (albeit 4-5 hrs delayed) to that of the ACTH stimulated adrenal hormones. Addison's disease, due to hypopituitarism, is accompanied by an increase in serum zinc concentration, decreased zincuria and increased zinc content in several tissues (27).

Rudzik et al studied the effects of hypophysectomy and ACTH on zinc metabolism in the adrenals of the male rat. They found that when ACTH was given by chronic injection, there was a significant decrease in the whole blood zinc concentration in both intact and hypophysectomized male rats. The adrenal post-hypophysectomy effect was that after five days there was a significant decrease in zinc concentration in the gland. If ACTH was given to these animals there was also a decrease in zinc concentration in the adrenal. If intact normal animals were injected with ACTH there was a significant decrease in the adrenal zinc concentration (64).

Reeves et al studied the response of serum corticosterone to ACTH and stress in the zinc deficient rat. They found that in male rats who were stressed by hypertension, the plasma ACTH and zinc concentrations were higher than normal. Their observation was that the zinc level mimmicked the ACTH level. In their experiment the data obtained indicated that the basal serum corticosterone was not influenced by zinc deficiency. If ACTH was given, the corticosterone output increased in both the zinc deficient and intact male rats. These authors conducted also an *in vitro* ACTH experiment. They gave ACTH and zinc to adrenal tissue in incubation medium and noted an increase of corticosteroid production. If to that complex a zinc chelator was added in the incubation medium, the corticosteroid production decreased. If, to the same material

(+ zinc chelator) a zinc concentration in excess was added, the corticosteroid concentration increased back to the original values. Their conclusion was that zinc is needed for ACTH activity unto the stimulation of corticosteroid production (62).

Sandstead et al studied the human zinc deficiency effects and responses to treatment in 40 boys, 12-20 years old. They noted a decreased pituitary ACTH reserve and release. They also noted that more than half the zinc deficient boys responded with an abnormal delay in urinary output of 17-hydroxycorticosteroids after ACTH injection. After zinc treatment and adequate nutrition the adrenal cortex response of these boys redressed to almost normal (65).

Homonnai et al studied corticotropin and zinc phosphate and hydroxide effects. They showed that in in vitro experiments with human cell cultures the addition of zinc salts augmented the potency of corticotrophin upon the cellular function (30).

III c. TSH

Sandstead et al found in their field study on human zinc deficiency and endocrine manifestations that in 40 adolescent boys zinc deficient with growth failure and other symptoms there was no hypothyroidism but only some iodine deficiency, not nutritionally derived (65).

III d. PROLACTIN

Homonnai et al studied prolactin and zinc in the human ejaculate. They considered the hypothesis that prolactin and androgen regulate the human prostatic activity. In their study it was hypothesized that prolactin and zinc are related to prostatic function, prolactin as a regulator and zinc perhaps as a secretion product. The concentrations of both elements in the seminal fluid are positively correlated. The measurements of zinc concentration in seminal fluid in normospermic, normoandrogenic males could be used as an index of prolactin level and secretory activity of the prostate (30).

III e. The Gonadotrophins

Several investigators studied the relationships of gonadotrophins and zinc deficiency in human males and in animals, chiefly - rats of both sexes. Millar et al studied the effects of gonadotrophin injections in zinc deficient male rats. The data obtained show the following: (a) spermatogenesis is arrested in zinc deficient male rats, (b) there is a delayed maturation and/or atrophy of the germinal epithelium while (c) there is a significant deficiency of zinc in the testis, epididymis and dorso-lateral prostate. The authors found that zinc supplementation reversed most of the above findings with the possible exception of the atrophy ofthe germinal epithelium. In a second experiment they injected gonadotrophins and noted a marked increase in the rate of growth and development of the immature testis. No reversion was noted in the locations where atrophy had already occurred. The investigators' conclusion was that the retarded development of the genitalia was due to the reduced output of gonadotrophins and it was partially caused by the inanition found to exist in the condition of zinc deficiency (53).

Lei et al studied the function of the pituitary-gonadal axis in zinc deficient rats. They found that 5 minutes after LHRH (2mg) per rat was injected to zinc deficient male rats there was an increase in LH and FSH plasma concentration as compared to controls.After 3 hours there was an increase of plasma testosterone concentration. The authors' conclusions were that: (1) zinc affects specifically the testes, (2) gonadal function in conditions of zinc deficiency suffer via an alteration of testicular steroidgenesis (3) there may have been a decrease in androgen catabolism in conditions of zinc deficiency and (4) the augmented response to LH could have been due to a change in hypothalamic sensitivity.(42).

Millar et al studied the effect of testosterone and gonadotrophin injections on the sex organ development of the zinc deficient male rat. The authors found that in zinc deficient male rats there was a decrement in growth and in the size and weight of the sex organs. When the animals were injected with gonadotrophin and/or testosterone their whole body and especially the sex organs increased in size to approximately normal values but the zinc concentration remained low in all sex organs. The authors' conclusions were that the growth decrease was due to the inanition caused by the zinc deficient diet. That resulted in a decreased gonadotrophin output and a consequent fall in androgen production. (*NB. No actual measurements were done on the hormonal output (53).

Gombe et al studied the effects of zinc deficiency and restricted food intake on plasma and pituitary LH and hypothalamic LRF in female rats. They found that in these animals there was a significant growth decrease accompanied by a decrease in the zinc concentration in the plasma, low plasma LH values but normal LHRH values in the hypothalamus (20).

Smith et al conducted clinical field studies on 28 Indian malnourished adult men. They found that in conditions of malnourishment these people had a subnormal plasma testosterone. When they injected the subjects with hCG there was an increment albeit subnormal of the plasma testosterone. This finding was noted both at the beginning of the study and after the subjects were appropriately fed. In the same conditions of malnutrition, FSH and LH plasma concentrations were high. After appropriate feeding and/or hCG injection the FSH and LH concentrations decreased to approximately normal levels. The authors considered that the protein and caloric malnutrition effects were functional hypogonadism, including Leydig cell function, i.e. subnormal testosterone levels (318 ± 109 ng/dl) and

pituitary production increment of LH and FSH. The LH values were 22.5 ± 17.8 mIU/ ml vs a normal value of 10 ± 4.5 mIU/ml. The FSH values were 17.8 ± 14.1 mIU/ml vs a normal 13.5 ± 6 mIU/ml. After the hCG injections the plasma concentration decreased to 14.2 ± 9 mIU/ml, LH decreased to 14.7 ± 2 mIU/ml and the testosterone concentration increased to 553 ± 11.6 ng/dl (normal 582 ± 158 ng/dl). After refeeding the plasma testosterone levels returned to normal values. The authors' conclusions were that hCG helped to reduce the plasma LH and FSH concentrations but could not stimulate the testosterone production back to normal levels. That event occurred only after the hCG treatment was complemented by an adequate nutrition for a significant period of time (69).

FSH

Smith et al studied the pituitary gonadal axis in 28 Indian men with protein calorie malnutrition. They found that in those people the plasma FSH values were higher than normal at the beginning of the study, i.e. FSH 17.8 ± 14.1 mIU/ml. If HCG 4,000 international units i.m. were injected for three days the plasma FSH values decreased to 14.2 ± 9 mIU/ml. Refeeding for a period of time and hCG treatment had about the same effects. It should be stated that Indian normal men have FSH values of 13.5 ± 6 mIU/ml while their U.S. counterparts have values of 7.14 ± 1.9 mIU/ml (69).

Lei et al studied the function of the pituitary-gonadal axis in zinc deficient male rats. They found that if LHRH was given to these animals, after 5 minutes the FSH values were higher than those of the control counterparts. They also found that the baseline FSH values were much higher in the zinc deficient male rats than in the control animals (42).

LH

Smith et al found in their studies on 28 Indian protein-calorie malnourished men that the pituitary production of LH was increased and the LH plasma concentration was 22.5 ± 17.8 mIU/ml. If hCG injections were used as treatment for three days the LH values decreased to 14.7 ± 7.7 mIU/ml. Refeeding produced about the same results over a long period of time as the hCG treatment. The normal Indian men LH plasma values are about 10.0 ± 4.5 mIU/ml, while the U.S. normal LH plasma values for men are 10.9 ± 4 mIU/ml (69).

Lei et al studied the function of the pituitary-gonadal axis in zinc deficient male rats. They found that if those animals were injected with LHRH, after 5 minutes the LH plasma values became significantly higher than in the control animals. Apparently the LH plasma baseline level in zinc deficient females is lower than that of the normal control (42).

Gombe et al studied the effects of zinc deficiency and reduced food intake on plasma and pituitary LH and hypothalamic LRF in female rats. They found that the plasma LH concentration in the zinc deficient female rat was generally lower than that of the normal controls (20).

III f

The Pancreas

The pancreas, and specifically the islets of Langerhans have a high zinc content. As stated earlier, insulin exits from the pancreas in a form of 6 insulin molecules: 3 zinc molecules. The hormone can have its biologic actions independent of the zinc status. The role of zinc in insulin metabolism is that of "breaking" the rate of activity and of catabolism. As a result hypoglycemia can be prolonged (3).

With regards to the zinc status in patients with diabetes mellitus the plasma zinc concentration is usually higher than normal (in the fasting state) and zincuria is higher than normal irrespective of the level of proteinuria. The pancreatic zinc content in diabetes mellitus appears to be significantly lower than normal. The glucose tolerance curve has been found abnormal in almost 100% of cases of 40 adolescents suffering from nutritionally conditioned zinc deficiency (3) (65)

III g

The Parathyroid

It appears that zincuria is at least in part a function of circulating PTH concentration. This observation derives from clinical studies of hyperparathyroid patients.

Hyperparathyroidism, albeit associated with normo-zincemia, is also associated with hyperzincuria. If the parathormone levels are returned to normal - e.g. post partial parathyroidectomy, zincuria returns to normal values as well. The parathyroid gland probably influences the plasma and urine zinc concentrations via its activity on bone metabolism. As described earlier, bone contains a large zinc concentration. As such, in hyperparathyroidism normozincemia is maintained via the zinc release from bone matrix. By the same token, the ensuing aminoaciduria may be conducive to the hyperzincuria observed in this condition.(37, 47).

IV

The Adrenal

Rudzik et al studied the effects of adrenalectomy and cortisone on zinc metabolism in the sex glands and adrenal of the male rat. These authors found that a post adrenalectomy effect in male rats was a decrease in the zinc concentration in blood and in the dorsolateral prostate and testis. If the adrenalectomized animals were given cortisone for 14 days the zinc concentration in those tissues was returned to about normal levels. The adrenal glands contain a relatively large zinc concentration. This concentration increases to twice the normal values after 14 days of cortisone administration. The effect of adrenalectomy on the dorsolateral prostate (i.e. increase in the concentration of zinc) is due mainly to the increase in weight of the glands and not to an actual decrease of the zinc concentration. It is suggested that the dorsolateral prostate depends upon normal adrenal activity for normal function. The conclusions of the authors were that as there was a highly significant increase in zinc concentration in intact animals which were treated with cortisone for 14 days it followed that there was a functional relationship between adrenal activity and zinc concentration in the gland (64).

Ludwig et al studied the effects of hypophysectomy and ACTH on zinc metabolism in the adrenals of the male rat. They found that hypophysectomy was followed after about 5 days by a significant zinc concentration decrease in the adrenal gland. The adrenal weight decreased significantly in about 19 days and at that period of time the actual zinc concentration was found to be increased because of the decrease in the size and weight of the glands. Chronic ACTH treatment in intact animals brought about a significant zinc concentration decrease in the adrenal glands. In female rats, kept on a zinc deficient diet, there was a decrease of cholesterol

concentration in the adrenals in the post partum period. It is suggested that the delivery process is more traumatic in the depleted female rats than in the normal controls fed an appropriate amount of zinc (46).

Corticosteroids

Rudzig et al studied the effects of adrenalectomy and cortisone on zinc metabolism of the sex glands and adrenal of the male rat. They found that the zinc concentration in the blood, dorsolateral prostate and testis was significantly lower a few days after adrenalectomy. If the same rats were given cortisone treatment for 14 days there was a recovery to about normal levels of zinc concentration and Zn^{65} uptake. If only one large dose of cortisone, i.e. 25 mg instead of 12.5 mg, is given to intact animals, the zinc concentration in plasma is reduced to the level of that in the adrenalectomy situation (46).

Reeves et al studied the response of serum corticosteroids to ACTH in the zinc deficient rats. They found that zinc deficiency does not influence the basal serum corticosteroid production, i.e. the adrenal responds normally in this situation to ACTH by increasing corticosteroid production. These authors did an in vitro experiment on adrenal tissue by giving ACTH and zinc to the tissue in culture. The results indicated that there was an increase in corticosteroid production. By giving also a zinc chelator there was a decrease in corticosteroid production.Administrating zinc in excess of the chelator there was an increase in the corticosteroid production. It was concluded that ACTH needs zinc in order to activate corticosteroid production in the adrenal tissue in vitro. In an experiment done on female rats by the same authors parturition was rendered more stressful by zinc deficiency and induction of a catheter in the jugular vein of these rats. It was found that in the zinc deficiency case the serum corticosteroid level was about 50% that of controls. If the same zinc deficient gestant rat was given zinc on day 19 of gestation the corticosteroid level returned to approximately

75% normal (62).

Cox et al studied the mechanisms of hormonal stimulation of zinc uptake in human cell cultures. They studied specifically hormone cell interactions and characteristics of zinc accumulation. In these in vitro experiments done with human cell cultures, when glucocorticosteroids were added to the cell culture medium, there was a significant increase of zinc uptake by the cell culture (12).

V. MALE GONADAL FUNCTION

Conditioned nutritional zinc deficiency impedes gonadal and somatic development and function. It is calculated that at least 3% of the male population of Iran (military conscription rejects) are hypogonadal dwarfs. The same situation may well prevail in other countries, although no statistics are available. Hypogonadism induced by zinc deficiency may not be caused specifically by nutritional deficiency but by any dysmetabolic condition conducive to a negative zinc balance. One such condition is sickle cell anemia.

Prasad et al studied the effects of zinc deficiency in man, within the framework of sickle cell disease on one hand and nutritional hypozincemia on the other. The authors found that the patients affected with sickle cell anemia and the zinc deficient hypogonadal dwarfs have a number of common symptoms. These are (1) delayed onset of puberty and hypogonadism (2) a significant decrease of facial, pubic and axillary hair (3) short stature and low body weight (4) rough skin and poor appetite (5) delayed healing of leg or other ulcers (6) plasma, erythrocyte and leukocyte zinc concentrations, significantly lower in the sickle cell disease patients and (7) zincuria significantly higher than normal in the same patients (60,61).

The Male Genital Tract

Bertrand et al were the first to study, in 1921, the possible effects of zinc upon reproductive physiology. These authors found that there was a high quantity of zinc in the genital tract, especially that of the male rat. Further study of the relationships, structural and functional, between zinc and the male genital tract had to await the advent of modern trace metal analytic technology. It is by now an established fact that the mammalian male genital tract has a high zinc

content and that the ejaculate contains a high concentration of zinc (6).

The Testis

Millar et al studied the effects of testosterone and gonadotropin injections on the gonadal development in zinc deficient male rats. Dietary zinc deficiency in male rats is directly related to a reduction in size and weight of the testis, delayed maturation or atrophy of the germinal epithelium, arrested spermatogenesis and a significantly lower zinc concentration in the organ. If zinc is replaced in the diet, all the changes with the exception of the testicular and tubular atrophy are reversed. If irreparable testicular damage has occurred the testis and the epididymus do not regain normal zinc concentrations after zinc addition to the diet. In pair-feeding (zinc supplemented weight-pairs) the peers had lower than normal testicular size but no functional abnormality. After food addition the testes of these animals regained normal size. The authors accredited the severe functional testicular damage to the zinc deficiency per se while the reduced size was thought to be due to the combined effects of zinc deficiency and inanition. If zinc deficient male rats are treated with gonadotropin and testosterone the treatment produces a marked proliferation of testicular and interstitial cells and growth of accessory glands. This treatment produces also an increase in the rate of growth and development of the immature testis. However, the gonadotropins could not prevent the atrophy of the seminiferous tubules that occurred as a result of zinc deficiency. The conclusion of these authors was that dietary zinc deficiency brought about a lower pituitary gonadotropin production and/or release and therefore a significantly decreased androgen production. As to the endogenous gonadotropin production reduction, that is considered to be the cause of the decrease in growth

and rate of maturation of the testis. Zinc deficiency by itself was considered to bring about the tubular atrophy. A relatively large zinc concentration in the diet is needed by the testis only after the sperm production has already started (53).

Rudzik et al studied the effects of adrenalectomy and cortisone treatment on zinc metabolism in the male rat gonad. Adrenalectomy in these animals is followed by a significant decrease in zinc concentration of the testis. Adrenalectomy and cortisone treatment for 14 days results in a recovery to normal zinc concentration and uptake of Zn^{65} in the testis (64).

Millar et al mapped out some effects of dietary zinc deficiency on the reproductive system of male rats. They measured the testicular weight in three groups of animals, i.e. zinc deficient, weight-paired zinc deficient supplemented by $\frac{200}{1}$ µgZn/day and ad libitum rats fed on the same diet as the previous group. The testicular weights were 573 ± 44.9 mg, 1251 ± 41.4 mg, and 1483 ± 180.5 mg in the three groups described. The epididymal weights were 159 ± 8.6 mg, 321 ± 17.9 mg, and 373 ± 40.1 mg in the zinc deficient group, weight-paired zinc supplemented group and ad libitum groups respectively (52).

Swenerton et al studied the effects of severe zinc deficiency in male and female rats. In the male rats the authors found testicular atrophy and spermatogenic arrest (72).

Lei et al studied the function of the pituitary gonadal axis in zinc deficient rats. When zinc deficient male rats were injected with LHRH, after 5 minutes there was a significant increase in plasma concentrations of LH and FSH, however after about 3 hours the serum testosterone concentration was noticed to be still significantly lower than in

the normal controls. The authors hypothesized that there was a specific effect of zinc on the testis and that gonadal function in the zinc deficient animals was affected through some alteration of testicular steroidogenesis. They also hypothesized that perhaps the androgen catabolism was higher in the zinc deficient animals and that the increased response of LH release could be due to a change in the hypothalamic sensitivity to a steroid inhibition or a decreased negative feedback. Since the testosterone response was significantly diminished in the zinc deficient rats after LHRH injection, it followed that the testicular response was lower than normal because of a diminshed responsiveness of the zinc deficient testis to LH stimulation. The baseline FSH values were observed to be much higher in the zinc deficient male rats. It was considered that the testicular failure with respect to spermatogenesis was the result of zinc deficiency (42).

Millar et al studied the effects of testosterone and gonadotropin injections on the sexual function of the zinc deficient male rats. They found (like other authors) that zinc deficiency is accompanied by a retarded testicular growth. If these animals were injected with gonadotropin and testosterone, the size of the testis returned to normal values but the zinc concentration remained low. They considered that the lack of testicular growth was a result of inanition, which by itself is a result of zinc deficiency. This inanition resulted in reduced gonadotropin output and a consequent fall in androgen production. NB. No actual measurements of hormonal levels were performed in this experiment. (53).

Sandstead et al studied endocrine manifestations of human zinc

deficiency in human males. They found that in 40 cases studied (12-20 year old boys) there was a significant incidence of hypogonadism. This hypogonadism was considered to be, according to these and other experimental studies, due mainly to the zinc deficiency in the diet (65).

The Epididymus

Millar et al studied the effects of testosterone and gonadotropin injection on the sex organ development of zinc deficient male rats. They found that the zinc deficient male rats had a significantly lower epididymal size and weight than the normal controls. If the testis was already atrophied as a result of the zinc deficiency the addition of zinc to the diet brought about epididymal intake of zinc. However the epididymus did not regain its normal size. Pair-fed peers had smaller than normal epididymi but these structures could have been brought back to normal sizes and weights by adequate nutrition. The size and weight of the epididymi of the zinc deficient male rats could have been brought back to normal values by gonadotropin and/or testosterone treatment (52).

Millar et al studied the effects of dietary zinc deficiency on the reproductive system of the male rat. They found that the epididymi of the zinc deficient animals weighed 159 ± 8.6 mg. The epididymi of the zinc deficient males, pair-weighed, to whom 200 mg of zinc was added daily,weighed 321 ± 17.9 mg and the epididyma of the ad libitum animals weighed 373 ± 40.1 mg (53).

The Prostate

Millar et al studied the effects of testosterone and gonadotrophin injections of the sex organ development of zinc deficient male rats. They found that the dorso-lateral and ventral prostate of zinc deficient male rats showed a significant decrease in zinc concentration. They

considered that the reduced size of the prostate was due to the combined effects of inanition and zinc deficiency, as the pair-fed controls also had a prostate size reduction. When zinc was added to the diet the prostate returned to almost normal values. In an experiment in which zinc deficient male rats were treated with gonadotropin and testosterone the prostatic growth was observed but the zinc concentration in the organ remained low. The authors concluded that zinc accumulation was not essential to growth and development of dorso-lateral prostate although normally zinc accumulated rapidly in this gland. The authors concluded that the retarded growth of the dorso-lateral prostate was due mainly to a reduction in the output of pituitary gonadotropin as a result of inanition (52).

Rudzik et al studied the effects of adrenalectomy and cortisone on zinc metabolism in the sex glands. They found that adrenalectomy in zinc deficient male rats was followed by a significant reduction in zinc concentration in the dorso-lateral prostate. They noted that the decrease in zinc concentration in the dorso-lateral prostate was due mainly to an increase in the weight of the gland following adrenalectomy and not due to an actual decrease in the zinc concentration in the gland. The authors considered that the functional capacity of the dorso-lateral prostate was severely affected by adrenalectomy. If cortisone treatment was given during the post-adrenalectomy period for a few days, the zinc concentration returned to normal values. It was suggested that the dorso-lateral prostate is dependent upon normal adrenal activity for normal function (64).

Millar et al studied the effects of dietary zinc deficiency on the reproductive system of male rats. They measured the dorso-lateral prostate weight in zinc deficient, zinc supplemented pair-fed and ad libitum

rats was significantly decreased and spermatogenesis was arrested. The authors suggested that the small size of the testis, as found, could also have been due to the inherent inanition of zinc deficiency. In the experimental animals, spermatogenesis could not be maintained or tubular atrophy prevented in the absence of a normal concentration of zinc. Also the decrease in growth and rate of maturation of the testis was considered to be due to endogenous gonadotropin deficiency, i.e. not specifically and directly due to zinc deficiency. A relatively large zinc concentration is needed by the testis only after the sperm produced is in the final stages of maturation. The only specific effect of dietary zinc deficiency on the male rat reproductive system, as suggested by these authors, is to arrest spermatogenesis and to cause atrophy of the germinal epithelium (53).

Rudzik et al studied the effects of adrenalectomy and cortisone on zinc metabolism in the sex glands of the male rats. They found that the functional capacity of the dorso-lateral prostate was severely diminished by adrenalectomy. That functional capacity could have been brought back to normal values with a treatment of cortisone for 14 days. It was inferred that the prostatic fluid volume of the adrenalectomized animals was significantly decreased and as such detrimental to the ejaculate (64).

Millar et al studied the effects of dietary zinc deficiency on the reproductive capacity of male rats. They found that the testis atrophied (atrophy of the germinal epithelium) and spermatogenesis was arrested or non-existent (52).

Homonnai et al studied prolactin and zinc in the human ejaculate. In men followed in an infertility clinic the prolactin and zinc concentrations in the seminal fluid varied in a directly proportional way with the sperm count and mobility, the latter arbitrarily divided into a few

groups of sperm concentration. They suggested from the clinical study that an indexing of the zinc concentration, prolactin concentration and sperm count and mobility on a larger scale could be used to determine, clinically, fertility problems in men (30).

Marmor et al studied semen zinc levels in infertile and post-vasectomy patients and in patients with prostatitis. These authors found no positive correlation between zinc concentration and sperm count morphology and mortility, but they found that the sperm count varied proportionally with the zinc concentration in the seminal fluid (50).

Castration

Gunn et al studied the effect of androgen upon castration in relation to Zn^{65} uptake in rats. They found that castration was followed by a considerable decrease in blood Zn^{65} uptake. If androgens were given to these animals, the treatment prevented the zinc uptake decreases. If estrogen in high quantities was given it had an analogous action. In intact animals both hormones normally prevent Zn^{65} uptake (21).

VI. FEMALE GONADAL FUNCTION

The Ovary

Ibrahim et al studied the effects of starvation on pituitary and serum follicle stimulating hormone and luteinizing hormone following ovariectomy in the rat. They found that the pituitary LH was higher in the starved ovariectomized rats than in the well-fed ovariectomized animals. The authors' conclusion was that the pituitary was able to synthesize gonadotropins in spite of lower food intake (36).

Ovulation

Swenerton et al studied the effects of severe zinc deficiency upon male and female rats. One of the findings was that the zinc deficient females did not reach sexual maturation (i.e. the vagina did not open) and they were in continuous anestrous (72).

Pregnancy

Apgar studied the effects of zinc deficiency on the maintenance of pregnancy in the rat. The author found that if the animals were kept during gestation for two weeks on a zinc deficient diet there followed a difficult parturition and a reduced number of fetuses. Also some zinc deficient rats were unable to become pregnant. All animals were unable to carry pregnancy to term. The author found that, if she injected the animals with progesterone and estrone from gestation days 4-21, all of the rats were pregnant by day 8 and about half of them maintained pregnancy to term (2).

Apgar studied the effects of zinc deficiency on the maintenance of pregnancy in the rat. She found that zinc deficient female rats fed on zinc deficiency during gestation had a difficult parturition or or an actual inability to carry to term. If the same zinc deficient

females were given estrone from days 4-21 of gestation, all the females were pregnant by day 8 and maintained pregnancy to term and had a normal parturition (21).

Estrogen

Gunn et al studied the correlation between castration and Zn^{65} uptake in rats. They found that estrogen injection at a high dosage could prevent the observed decrease of Zn^{65} uptake in the body after castration. Otherwise, in the intact body, estrogen could cause actually an inhibition of Zn^{65} uptake (21).

Smith et al studied the pituitary-gonadal axis in men with protein-caloric malnutrition. They found that in 28 Indian men who suffered from protein calorie malnutrition the estradiol level at the beginning of the experiment was 16.8 ± 13.4 pg/ml (i.e. significantly lower than normal) while after the patients were nourished for an appropriate amount of time the estradiol level increased to 31.0 ± 21.1 pg/ml. The estrone level in those two circumstances was 56.7 ± 54.8 mIU/ml and 41.1 ± 80.4 mIU/ml respectively (69).

Hartoma studied the estrous cycle of zinc deficient female rats. He observed that in zinc deficient female rats there was a prolonged effect of estrogen. The author's experimental evidence pointed to the fact that zinc deficiency acts primarily as an inhibitor of steroid synthesis on one side and of metabolism on the other. (23).

PART III

Experimental Induction of Zinc Deficiency in Rats

Zinc deficiency can be induced in rats or in any other animals by eliminating this trace metal from food, water, cages and as far as it is possible, from the environment. The complexity and the difficulty of zinc elimination procedures are prime delaying factors in the development of this experimental field. The ubiquity of this trace metal and the difficulty of eliminating it and it alone are most significant problems that have confronted researchers for over 30 years.

I hold it fair to say that if it were not for the above problems, and the technical complexity of accurate determinations of zinc concentrations in various biologic materials, the field of zinc research would be quite far from the incipient stage at which it struggles presently.

At the present state of the art it is possible to house animals in suspended stainless steel or polypropylene cages and feed them on a special diet (zinc content less than 3 ppm) and demineralized water (zinc concentration equals 0.05 ppm). Care is taken to assure continually the least possible amount of zinc contamination. Several researchers in the field have established that the minimum zinc concentration requirement of rats is about 15 ppm zinc per day. Less zinc intake is suboptimal. The deficiency symptoms are inversely proportional to the suboptimal zinc concentration available. These facts have been confirmed during the period of investigation. The known zinc concentration available in our experimental conditions was of the order of 2 - 3 ppm range. The experimental animals were housed in animal rooms (on separate racks) together with other animals. (Animal rooms: McIntyre Animal Room and the Animal Room of the Research Section of the Women's Pavilion, Royal Victoria Hospital). The environmental zinc contamination must have

74,81.

been at a minimal level since the symptoms expressed by the animals were similar to those described by other researchers for animals allowed a zinc intake of less than 3 ppm/day. When 21 day old rats are subjected to such conditions of zinc deficiency, after 8-10 days, one can determine a decrease in the food requirement. Almost concomitant with that, hence hardly as a result of it, it is possible to determine the appearance of alopecia, periungueal, perioral and periocular keratosis and hemorrhage. Arrest of growth becomes apparent after about 10 days on the zinc deficient regimen and is determined to be significantly so after about 14 days. After 21 days of zinc deficient regimen, 42 day old rats appear dwarfed (size about that of 30 day old normal controls), with significant alopecia (spotty to almost complete) with perioral, periocular, periauricular and periungueal hyperkeratosis and/or hemorrhage. They are more "irritable" as determined repeatedly by the lack of ease of handling (as compared to the controls) and the biting reaction. The sexual maturation of Sprague-Dawley females normally occurs between 35-38 days of age and is evidenced by the opening of the vaginal orifice and estrus (as evidenced by the vaginal smear). In no case during our investigations was there such evidence of sexual maturation in the zinc deficient females. In one experiment, 6 females were observed until the age of 61 days. No vaginal opening was determined. Our observations confirmed those of Hurley and Swenerton.[72] Studies done by these investigators and others showed that in addition to the already described symptoms, the animals had esophageal and intestinal epithelial hyperkeratosis, gastrointestinal hemorrhage, weak collagen structure, etc. Several investigators determined reduced levels of zinc dependent enzymes.[73] Hormonal changes were observed in a number of experiments.[81] The present series of investigation is the first one aimed to determine in a concerted fashion the hormonal profile of several parameters in this condition.

The metabolic models of nutritional and environmental zinc deficiency were applied to the study of the sexual development and reproductive activity in female rats. A number of developmental and functional parameters were taken in consideration and investigated within the framework of anatomical pathology and hormonal analysis. In a "functional" order, the parameters studied were:

a. mating
b. pregnancy
c. delivery
d. lactation
e. weaning
f. growth
g. sexual maturation (i.e. "puberty")
h. pharmacologic induction of premature sexual maturation (i.e. via PMS and E_2-P_4)
i. the adult state in conditions of E.F.A. treatment.

As described in the introductory section, the subjects a - f were studied from different points of view by other investigators. The results of the present investigation generally confirm the results obtained previously either individually or groupwise. The present results are however, the only ones that encompass the field of female rat reproductive physiology as a whole, i.e., investigated by one experimenter with one type of nutritional environmental conditions, using four generations of one strain of animals (Sprague-Dawley), etc.

Although this field has been studied for the past 19 years, the lack of standardized procedure by the several experimenters involved hindered rather than helped the development of the field and a clear understanding of the effects of zinc deficiency upon the physiology of reproduction. The same criticism can be applied within defined limits, to the subject of the hormonal profile, as affected by this dysmetabolic, albeit conditioned physiologic state. We attempted to

investigate during the present study, the effects of zinc deficiency upon the gonadotrophs, prolactin, insulin, growth hormone, thyroxine, progesterone, estrogen, corticosterone as well as enzymes, tryglycerides, etc (as affordable by the SMA-24 procedure). The study is continuing along, i.e., studies of glucagon, somatostatin, prostaglandins, and TSH.

The attempts of pharmacological induction of precocious puberty via PMS and estradiol-17β-progesterone on one hand and of sexual maturation in general via EFA injections are original and novel to this field (subjects h - i). The question of the specificity of effect of zinc deficiency (i.e. what is affected directly and what as a "by-product") has been foremost on our minds. At the present moment, as reviewed in the Discussion section we could give a partial answer to it, the completion of which awaits our and perhaps others' future investigations.

b. Living Quarters and Protection Against Environmental Zinc Contamination

The animals in all cases were housed for the duration of the experiments in two types of cages. One type consisted of standard size suspended stainless steel cages. The materials with which the animals came in contact were stainless steel from the cage and the water bottle extension and glass from the food jars. The other type consisted of polypropylene cages with stainless steel tops. The materials with which the animals came in contact were: glass from the food jars; stainless steel from the cage tops and the glass bottle extension; betta-chips from the material that bedded the cages; the polypropylene material of the cage walls. The animals were housed in groups consisting of 2, 4, 5 or 6 animals, depending on the size and age of the animal. As far as the pregnant rats were concerned, they were housed as 2 per cage until day 18 or 19 of pregnancy, after which they were placed as 1 per cage until delivery and henceforth to the end of the lactation period and weaning. The only time the animals were handled during the experiments was related to the weighing procedure. The material with which they came into contact at that time was the stainless steel bowl of the scale. No differences occurred in size, weight, weight gain, appetite, alopecia size and development, hemorrhages or dermatosis development of the animals housed in the two types of cages. As stated earlier, the zinc concentration in the food and water was repeatedly measured and found to be less than 2 ppm. As to possible and unavoidable sources of zinc contamination, these were: zinc in the food dust from other cages and floating in the air, zinc in the hair dust and other protein material floating in the air, zinc on the walls of the newly washed cages, zinc in the betta chips and other sources unaccounted for. The animal sizes

at the end of all the experiments were similar to those found by other researchers in this field. The animal size reflected the daily zinc intake of 3 ppm or less (32, 72).

c. The Nutrition

FOOD COMPOSITION:

CORN STARCH	31.2%
SUCROSE	31.0%
EGG WHITE SOLIDS	20.0%
CORN OIL	10.0%
MINERAL MIX(1)	4.0%
CELLULOSE POWDER	3.0%
VITAMIN PREMIX (2)	0.5%
CHOLINE CHLORIDE	0.3%

(1) MINERAL MIX

Calcium Carbonate	2.1%
Calcium Phosphate	73.5%
Cupric Citrate	0.046%
Ferric Citrate	0.558%
Magnesium Oxide	2.5%
Magnesium Citrate	0.835%
Potassium Iodide	0.00072%
Potassium Phosphate	8.10%
Potassium Sulfate	6.8%
Sodium Chloride	3.06%
Sodium Phosphate	2.14%
Citric Acid	0.227%

(2) VITAMINS. Yield/Kg diet

Vitamin A	30,000 IU
Vitamin D_2	4,000 IU
Vitamin E	100 IU
Vitamin K	2 mg
Vitamin B_{12}	0.02 mg
Thiamine	20.58 mg
Riboflavin	20.0 mg
Niacinamide	100 mg
Calcium Pentothenate	60 mg
Pyridoxine	10 mg
Folic Acid	0.5 mg
D - Biotin	1.0 mg
i - Inositol	333 mg

WATER: Deionized water ([Zn] < 0.05 ppm)
Total Zinc Concentration < 2 ppm

The food consumption in the Zinc deficient group ranged between 5-35 g/day depending upon the size of the animals.

d. The Analytic Method of Zinc Determination

At the present time there are several methods of zinc determination. Examples of such analytic techniques are: neutron activation analysis, x-ray fluoresence spectrometry, optical emission spectroscopy, mass spectrometry, voltammetry and atomic absorption spectrometry.

The last named technique (A.A.S.) is sensitive, relatively rapid and quite specific for metals such as zinc. The principle underlying AAS is the measurement of light absorbance of an atomic vapor sample at a fixed, specific lambda (λ). The wave length (λ) of zinc has been determined to be 213.9 nm. A light emitter from the atomic absorption spectrometer, containing zinc in one electrode (i.e., a "zinc lamp") is used to transmit the light spectrum of the metal. A monochromator in the light path isolates the 213.9 nm. The amount of spectrum absorption reflects the concentration of atoms in the (vaporized) sample to be measured. The "vaporization" of the sample to the atomic state is done via a flame method or via vaporization on a graphite rod or furnace.

The atomic absorption spectrometer used for the zinc concentration determinations during the present investigation is a Perkin Elmer Model 260. The biologic materials measured were: serum, different organs (e.g. pineal, hippocampus, hypothalamus, hypophysis, liver, adrenal, ovary, testis, whole body (pups)) and feces, food and water samples. The solid materials were "digested" in hydrochloric acid before the metal determination. The zinc determination procedure was accomplished at the Montreal Research Institute courtesy of the collatoration of Dr. D. Horrobin and Mr. S. Cunnane.

e. Methodology of FSH, LH and PRL Determinations

Radioimmunoassay (RIA) of LH, FSH and PRL

The principle of RIA for these hormones is based on the random competition of radioactively labeled or nonlabeled FSH or LH and PRL. The hormones are prepared in buffer for a limited number of antigenic sites present on rabbit anti-FSH, anti-PRL or anti-LH antibodies. The antibodies bind the radioactive label in a manner inversely proportional to the concentration of unlabeled hormone in the sample. The free hormone is separated from the bound form by precipitation of the rabbit anti-FSH, anti-LH and anti-PRL with sheep anti-rabbit serum.

The apparatus used consisted of: Micromedic automated pipetting station (Model 25000), equipped with two Micromedic dispensing pumps (200 µl and 50 µl); continuous pipetting outfit (1 ml, B-D Cornwall); automatic pipet (5 ml, Hamilton); centrifuge; suction apparatus; beakers for diluted ^{125}I-FSH, ^{125}I-LH, ^{125}I-PRL, antibody, 1% BSA-PBS-2% NRS, pipets, glass tubes (Kimbell).

The reagents included: 1% BSA-PBS, 2% NRS for diluting antibody; tracer diluent; rabbit anti-FSH and anti-LH, anti-PRL sera; labeled FSH LH and PRL; sheep anti-rabbit (second antibody), diluted in PBS and EDTA (0.15M).

Procedure

Blood was collected by decapitation from the experimental animals, kept at 4^{o}C and centrifuged at 2500 rpm. for 20 mins. The serum was separated and collected in 13 x 100 glass tubes using Pasteur pipets; it was stored at -20^{o}C until it was assayed. The standard diluent was

prepared (rabbit anti-FSH, anti-LH and anti-PRL) antibody and sheep anti-rabbit serum antibody and the standard RIA procedure carried out during 4 days. The standard curve - linearized dose response curve - permits the calculation of the samples by covariance analysis. The values obtained were expressed as mIU/ml of sample. Intra- and interassay variations were accounted for and treated accordingly.

The rat serum FSH, LH and PRL concentrations were determinedby the RIA procedure in the Gamma Laboratory of the Research Unit, Women's Pavilion, Dept. of Obs. Gyn, Royal Victoria Hospital under the guidance of Dr. R. Benveniste and Dr. G. Hall and the technical concourse of Mrs. L. Carter and Mr. W. Mastromatteo.

RADIOIMMUNOASSAY (RIA) OF STEROIDS EXTRACTED FROM RAT PLASMA, ADRENALS AND OVARIES

The hormones measured from the sources mentioned above were estradiol-17β , progesterone and corticosterone. Antisera were obtained from commercial sources and specificity was tested. Apart from the corticosterone antiserum which cross-reacted with progesterone to the extent of 52%, all other antisera employed were specific to the steroid allowing accurate measurement without prior chromatographic purification. When necessary corticosterone was separated from progesterone on LH-20 columns. The eluting solvents were dichloromethane:methanol mixed in the proportion of (98:2).

EXTRACTION OF PLASMA, OVARIES AND ADRENALS

Either ovaries or adrenals were homogenized in distilled water and extracted with different solvents depending on the steroid of interest. For progesterone, the extracting solvent was petroleum ether; corticosterone was extracted with dichloromethane and estradiol-17β with di-ethyl-ether. Plasma samples were extracted in a similar manner.

RIA PROCEDURES

Tracer doses of the steroids (about 5000 dpm) purchased from NEN were incubated with the specific antisera, known amounts of steroids (to generate the standard curve) or unknowns (samples). Incubation time was 2hrs to overnight in 0.05 molar phosphate

buffer. The total assay volume was 0.6 mls. Bound steroid was separated from the free form by dextran-coated charcoal solution prepared in the buffer mentioned above.

The standard curve generated is plotted on a logit-log graph paper (linearized dose response curve) permitting the quantification of hormonal concentration in samples by covariance analysis. Values obtained were expressed as picograms/ml or picograms/ovary or adrenal.

The above procedures and tests were performed in the Beta Laboratory, Research Unit, Women's Pavilion, R.V.H. under the direction of Dr. C.St. G. Hall and the technical assistance of Ms. G. Dulac and Ms. C. Morin.

Adequate care was taken to reduce to a minimum the intra- and inter-assay variability.

PART IV

EXPERIMENTAL DESIGN

Experiment I. Growth in Zinc Deficiency

1. Figure 1, (page 60) 10, 21 day old Sprague-Dawley half sibling female rats were placed at random into two groups of 5 and kept for 21 days in suspended stainless steel cages and fed with food and water containing less than 3 ppm zinc (see appendix 1 for the food and water contents). During the duration of the investigation, the animals were weighed 9 times. This pilot study was an investigation of the growth pattern in pair-feeding conditions. Pair-feeding, as defined in our investigative procedure, signifies providing the experimental group and the control group with the same total quantity of food and water for the duration of the experiment. In this metabolic condition, the experimental animals show a unique pattern of anorexia in eating habits (described in the Results section). As such, the control group had the food jars refilled only when refilling was necessary for the experimental group. Both groups received the same amount of food, as depicted in figure 5, experiment 2. At the termination of the experiment, the animals were sacrificed by decapitation and the weights of the ovaries and the adrenals were determined (Table 1).

EXPERIMENTAL DESIGN

Experiment II: Growth in Zinc Deficiency

In this experiment 38, 21 day old siblings or half-siblings Sprague Dawley female rats were randomly distributed as follows:

1. 12 females were placed on a zinc deficient diet
2. 12 females were pair-fed to the above
3. 9 females were placed on a zinc deficient diet (in the Women's Pavilion Animal Room, R.V.H.).
4. 5 females were fed ad libitum on Purina pellets and tap water.

The animals were weighed nine times during the experiment. The duration of the experiment was 21 days. At its termination, blood was obtained from all animals (sacrificed by decapitation). Hormonal determinations were carried out, as depicted in Scattergrams I - III. The serum and fecal zinc concentrations were determined, as shown in Table II. In this experiment, three elements were added with regards to the growth pattern.

1) an ad libitum fed group (fed with Purina food and tap water). 2) the effect of two environments (re: the environmental zinc contamination). The zinc deficient group was divided in two subgroups: one of these was kept in the Animal Room, Women's Pavilion, R.V.H., and the other subgroup was kept at the McIntyre Building Animal Room, where the whole experiment was conducted. 3) accurate measurements of food and water intake, wastage, urine and fecal output were conducted. These are depicted in figure 5.

EXPERIMENTAL DESIGN

Experiment III: Pregnancy and Lactation in Conditions of Zn Deficiency

Experiment III was conducted on the subject of the effects of zinc deficiency during pregnancy and lactation. Nine 180 gram siblings, 60 days old Sprague Dawley female rats were mated with 3 sibling males. The pregnant dams were placed at random in 3 groups: 1) 4 as a zinc deficient group; 2) 4 as a zinc supplemented, pair-fed to the first group and 3) 1 placed on ad libitum Purina pellets and tap water regimen. The animals were maintained on the feeding regimens throughout the gestation and lactation periods. They were weighed 18 times during the duration of the experiment. All the animals carried the pregnancy to "term" (i.e. for 21-22 days). Thirty-five pups were born alive to the zinc deficient mothers; 39 pups were born alive to the zinc supplemented mothers; and 12 pups were born alive to the ad libitum fed mothers. Among the zinc deficient and zinc supplemented groups, 4 pups died in the first week in each group. None of the pups of the ad libitum fed mother died. At weaning, the mothers were sacrificed. A "laparotomy" was performed. Ovaries and uteri were dissected and weighed. The serum was used for progesterone and corticosterone studies. The data re: the growth and development of the pups are considered in detail in Experiment IV. Figures 3, 4 and 5 present the data re: growth and weight gain during pregnancy and lactation and pup development during lactation and 8 days post weaning.

Table II presents the hormonal profiles studied in the serum of the post-lactation mature females (i.e. FSH, LH, and E_2).

EXPERIMENTAL DESIGN

Experiment IV

Pup Growth in Conditions of Zinc Deficiency During Gestation, Lactation and Post Weaning Period.

As described in Experiment III, 9 sibling Sprague Dawley females, of average weight 180 ± 3 grams, were mated with 3 sibling Sprague Dawley males in the same period of time. When pregnancy was ascertained they were placed at random in 3 groups: 4 zinc deficient; 4 zinc supplemented; 1 Purina rat. All rats carried the pregnancy for 21-22 days and delivered. 35 pups were born from the zinc deficient dams; 39 pups from the zinc supplemented dams; and 12 pups from the Purina fed dam. During the first week of life 4 pups died in the zinc deficient group and 4 in the zinc supplemented group. The lactating mothers continued to receive the same regimen as during gestation. On day 7 of life the pups were weighed. From day 7 to day 21 (weaning day) the pups were weighed (within 30 minutes) 11 times. Figure 4 presents the weights and the rate of growth in the pups of the 3 groups, irrespective of pup-sex. All mothers had milk and all pups sucked and were visibly eager to suck. A determination of the milk production and pup weight change after feeding has been attempted. Irregular patterns were noted in all cases and no significant differences could be detected. (It is possible that the "Archimedes" method was too crude for the accurate measurement intended and as such no conclusion on the subject of milk production and food intake is suggested).

EXPERIMENTAL DESIGN

Experiment V: Pharmacological Induction of Precocious Sexual Maturation In Zinc Deficient Female Rats.

Part of the raison d'être of Experiment IV was the obtention of "pure", i.e. during fetal development, pre-weaning and post-weaning development of zinc deficient, zinc supplemented (pair-fed) and ad libitum, Purina fed siblings or half sibling female rats. 19 zinc deficient females, 23 zinc supplemented females, and 9 Purina females were used in the presently discussed experiment. On day 21 the females were divided from the male pups. There was no significant difference in weight between the two groups at that age.

Two experiments on the possibility of induction of precocious sexual maturation in the zinc deficient females thus obtained, were performed (82). 1) Pregnant Mare Serum Gonadotrophin (PMSG) priming. 28 females were used. They were divided as follows: 11 zinc deficient; 9 zinc supplemented, pair-fed; 8 Purina, ad libitum fed. On day 26 of life at 10 a.m., 5 zinc deficient, 5 zinc supplemented and 4 Purina females were injected with 20 I.U. PMSG/s.c. Six zinc deficient, 4 zinc supplemented and 4 Purina females were used as controls. On day 28 of life at 5 p.m., 54 hrs after the PMSG injection, 3 zinc deficient, 3 zinc supplemented and 3 Purina treated animals were sacrificed by decapitation. At the same time 2 of each control group were sacrificed. Serum was obtained and prepared for LH and progesterone analysis. On day 29 at 10 a.m., 72 hrs after the PMSG injection, the remainder of the treated and control animals were sacrificed and serum was obtained for the hormonal analysis. Uteri and ovaries were dissected under the microscope and weighed. Ova were searched for and counted in the fallopian canals at the 72 hrs post-PMSG period. The growth pattern of the females of all groups is depicted

on figure 4. The uterine weights are shown on Histogram I. Table V presents the statistical data i.e.: the uterine and ovarian weights and the number of ova observed 72 hrs after the PMSG injections. Table VI presents the statistical data i.e.: the LH and progesterone surges following the PMSG treatment.

2) Estradiol - 17β - progesterone stimulation of the precocious sexual maturation inducement of the zinc deficient female rats (82). 20 animals were used in this experiment. They were distributed as follows: 5 zinc deficient and 7 zinc supplemented to undergo the hormonal treatment and 4 animals respectively to serve as controls. On day 28 of life, at 12 hrs, the 5 zinc deficient and 7 zinc supplemented animals were injected with 10μg E_2/s.c.* On day 30 at 12 hrs the same animals were injected with 1mg progesterone/s.c.* At 5 p.m. the same day, all animals were sacrificed. Serum was obtained for LH and progesterone studies. The uteri were excised and weighed. Tables V and VI present the data i.e.: the uterine weights and the LH and progesterone surges. Histogram I depicts the uterine weights of the primed and the control animals.

Histogram II - depicts the body weights of the experimental animals

Histogram III - depicts the ovarian and uterine weights of the above

Histogram IV - depicts the LH and Progesterone determinations done during the experiment.

*NB. E.B. 10 μg/0.2 ml sesame oil s.c.

Prog. 1mg/0.2 ml [illegible]

EXPERIMENTAL DESIGN

Experiment VI: Growth and Sexual Maturation in Conditions of Zinc Deficiency

Sixty-nine, siblings or half siblings, 21 day old Sprague Dawley rats were used in this experiment. They were divided randomly in 6 groups. Twenty-five were assigned to a group that were to be sacrificed at the age of 35 days. Forty-four were assigned to a group to be sacrificed at the age of 42 days. The first group ("the 35 days-old") were subdivided as follows: 9 zinc deficient females: 9 zinc supplemented females: 7 Purina ad libitum females. The group to be sacrificed at the age of 42 days was divided as follows; 11 zinc deficient females; 26 zinc supplemented females and 7 Purina ad libitum females. In this particular experiment zinc supplemented signifies a restricted form of pair-feeding. The food provided to the zinc supplemented group was less in quantity by about 20% than that of the zinc deficient peers; 25 ppm zinc were provided in the demineralized water. As such, an intermediary stage between pair-feeding and weight-pairing was sought and achieved. The stress of that reduced availability of food took its toll: 7 of the "35 days-old" group and 2 of the "42 day-old" group died. At 35 and 42 days of age the animals were sacrificed. The pituitaries and ovaries were dissected out, weighed, homogenized and prepared for study of hormonal content. The serum obtained was prepared for the study of the hormonal content, of the zinc concentration and of biochemical tests (SMA 24, Biochemical Laboratory, R.V.H.). Pancreases were dissected out and prepared for the determination of somatostatin, glucagon and insulin.

The following hormones were assesses:

- Growth hormone serum content: Courtesy of Dr. H. Friesen, University of Manitoba, Department of Physiology, Protein and Polypeptide Laboratory.

- LH, FSH, PRL, pituitary and serum content: Courtesy of our Gamma Laboratory, Department of Obstetrics-Gynecology R.V.H.
- Ovarian progesterone, estrogen and corticosterone; serum progesterone and corticosterone: Courtesy of our Beta Laboratory, Department of Obstetrics-Gynecology R.V.H.
- Insulin concentration in the serum. Somatostatin, insulin and glucagon, pancreatic content: Courtesy of Dr. Yogesh Patel, Diabetes Mellitus Laboratory, R.V.H.
- The serum concentration was assessed: Courtesy of Dr. D. Horrobin and Mr. S.C. Cunnane, Montreal Research Institute.

The results obtained are tabulated as follow:

1. Table VII and Histogram 6 represent the body weight and the pituitary and ovarian weights of the rats at the age of 35 and 42 days.
2. Table VIII represents the serum and pituitary FSH content
3. Histogram 7 and Table IX represent the serum and pituitary LH content.
4. Histogram 8 and Table X represent the serum and pituitary PRL content.
5. Histogram 9 and Table XI represent the serum GH content.
6. Table XII and Histogram 10 represent the data re: the serum and ovarian progesterone content.
7. Table XIII represents the ovarian estrogen and serum corticosterone content.

8. Table XV represents the zinc concentration in serum, feces, pancreas and liver.
9. Table XVI presents a statistical summary of most of the pituitary and serum hormones assessed.
10. Table XIV presents the relevant statistical results obtained from the SMA-24 Biochemical tests done.
11. Table XVII presents the data re: the pancreatic concentrations of insulin and somatostatin from the 42 day old group.

EXPERIMENTAL DESIGN

Experiment VII. Effects of Relative Zinc Deficiency Regimen and Essential Fatty Acids Treatment on Growth and Sexual Maturation

An experiment was conducted at variance with the previous ones described. Previously the zinc deficient regimen meant an zinc availability of < 3ppm in the food and water. Zinc supplementation, pair-feeding, in experiment VI, (pair-feeding minus about 20%) was replaced in this experiment by zinc supplementation (~ 25 ppm Zinc) in water and zinc deficient food ad libitum.

The "zinc" parameter in the present experiment was assessed in the form of three groups. These were: one "relative zinc deficiency" group - i.e. zinc availability = zinc less than 14 ppm and more than 10 ppm; one zinc supplemented (Zn = ~ 25 ppm), ad libitum fed group; and one, Purina ad libitum fed group.

The introduction of the "essential fatty acid" parameter has been considered on the basis of the results of two pilot experiments, not previously described. Those results indicated clearly that the addition (by injection) of E.F.A. to the zinc deficient animals was followed by an increase in the serum zinc concentration, an increase in body weight superior by far to the caloric input of the E.F.A. intake, an amelioration of the dermal effects (alopecia, keratosis) and of the sexual activity (pregnancy, lactation). Forty-nine, 21 day old siblings or half siblings, Sprague Dawley female rats were divided randomly into 7 groups as follows: 10 zinc deficient; 4 zinc deficient + Placebo injection (i.e. olive oil); 7 zinc deficient + E.F.A.; 7 zinc supplemented ad libitum; 7 zinc supplemented + E.F.A. ad libitum; 7 Purina; 7 Purina + E.F.A. ad libitum. They were kept on the respective diets for 35 days. The E.F.A. or Placebo

(olive oil) injections were given as follows: 0.5 ml E.F.A. (or olive oil)/s.c. on alternate days, for the duration of the experiment. On day 56 of life the animals were sacrificed. Pituitaries, ovaries and adrenals were obtained.

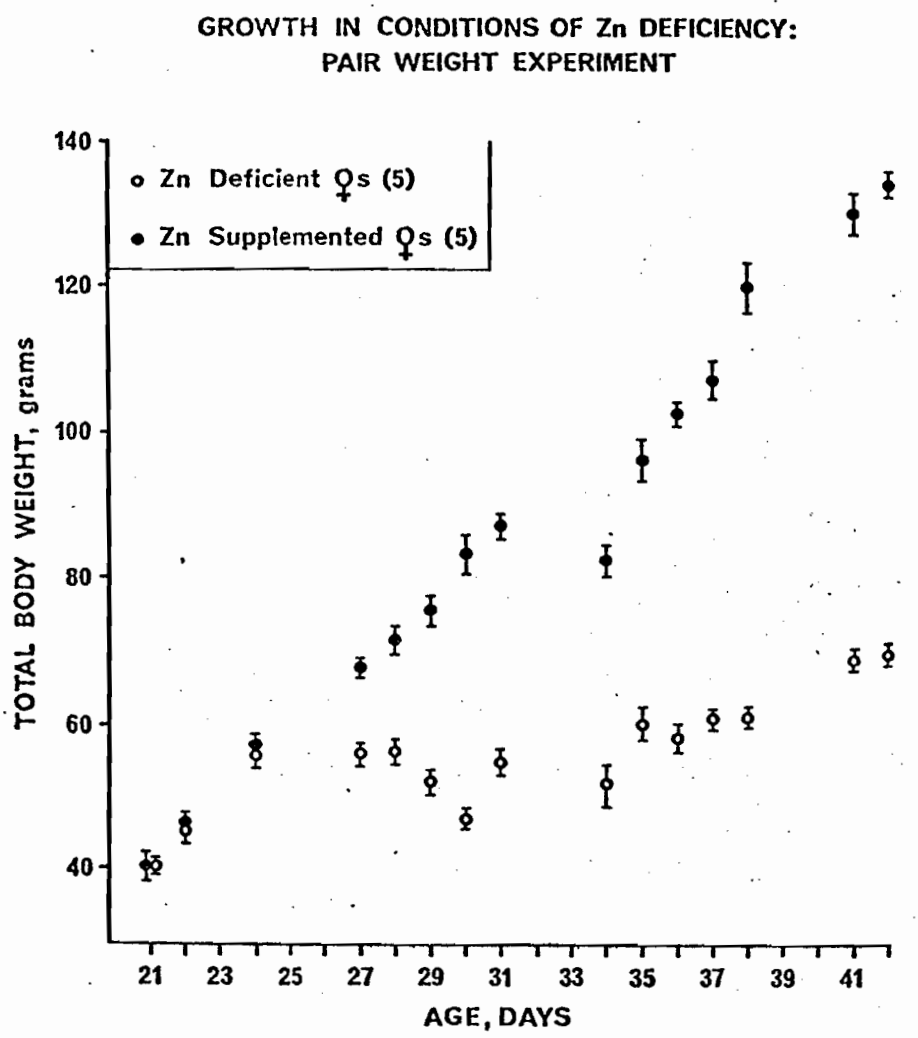
The data obtained are tabulated in Tables XVIII, XIX, XX, XXI, XXII, XXIII and Histogram 13.

Results

Experiment 1, figure 1, table 1.

The growth pattern in experiment 1 (figure 1, page 60) confirmed the results obtained from previous investigators. Such is the growth pattern, i.e., plateauing after about 14 days, in conditions of nutritional and environmental zinc availability of less than 3 ppm. Alopecia, parakeratosis, perioral, perinasal, periocular, periauricular keratosis and/or hemorrhage were evident on the experimental animals at the termination of the experiment, in confirmation of previous data. The 5 control females underwent sexual maturation (as evidenced by the opening of the vaginal orifice) between the days 36 - 38 of life. No vaginal opening could be visualized in the zinc deficient group. The ovarian and adrenal weights depicted in Table 1 indicate that both organs were of a significantly larger size in the control group as a whole.

Exp. I Fig. 1


GROWTH IN CONDITIONS OF Zn DEFICIENCY:
PAIR WEIGHT EXPERIMENT
o Zn Deficient ♀s (5)
• Zn Supplemented ♀s (5)
TOTAL BODY WEIGHT, grams
140
120
100
80
60
40
21
23
25
27
29
31
33
35
37
39
41
AGE, DAYS

Exp I

Table I

OVARIAN AND ADRENAL WTS. AT AGE 42 DAYS

		OVS (mg)	ADRS (mg)
Zn↓	N	10	10
	MEAN± SEM	44 ± 3	28 ± 2
	p	—	—
Zn↑	N	10	10
	MEAN± SEM	77 ± 4	65 ± 2
	p	< 0.0001	< 0.0001

RESULTS

Experiment II

In terms of growth the results confirmed the data obtained from Experiment I. The weights of the 3 groups at the termination of the experiment were:

1. Zinc deficient (21 animals). Mean weight = 58 grams
2. Zinc supplemented, pair-fed (12 animals). Mean weight = 110 grams
3. Ad libitum, Purina fed (5 animals). Mean weight = 145 grams

No significant difference was determined between the zinc deficient groups kept in the two locations as the mean weight for each group was around 58 grams.

A plateau in growth was evident after about 2 weeks of zinc deficiency treatment. No significant gain in weight and size occurred during the following and last week of the experiment. The ad libitum fed group grew significantly larger (i.e. mean weight = 145 grams) than the pair-fed zinc supplemented group (mean weight = 110 grams). The size and weight difference became evident after 7 days. After the second week the ad libitum fed group exhibited the spurt in growth that is associated normally with sexual maturation. No such spurt was evident in the zinc supplemented pair-fed (to the zinc deficient group) animals.

Alopecia, keratosis and hemorrhages were noted on all zinc deficient animals, as described earlier. Sexual maturation (i.e. vaginal opening) occurred between the 35th and 37th day of life in the ad libitum fed group (N = 5) and between the 37th to the 40th day of life in the zinc supplemented, pair-fed group (N = 12). Sexual maturation did not occur in any of the zinc deficient females (N = 21) by the termination of the experiment (day 42 of life). It should be stressed perhaps that all the animals were siblings or

half-siblings (same father, sister-mothers) and were initially distributed at random among the 3 groups.

As expected theoretically from the lack of observable sexual maturation, the serum progesterone values were significantly lower in the zinc deficient group than in the zinc supplement and the ad libitum fed groups i.e., $p<0.000($ and $p<0.002$ respectively. No significant difference in serum progesterone concentration was determined between the two latter groups (Scattergram 2). Since it was suggested theoretically that the zinc deficient group progesterone was mainly of adrenal origin, serum corticosterone was determined in order to provide an assessment of adrenal function in these conditions. The results, as indicated in Scattergram 2, show that serum corticosterone is significantly lower in the zinc deficient group than in the ad libitum controls ($p<0.01$). The serum corticosterone in the pair-fed zinc supplemented group was also significantly lower than in the ad libitum fed group ($p<0.02$), but was not significantly different from the zinc deficient group.

It would have perhaps been expected that the stress of zinc deficiency (in the zinc deficient-feeding group) and the stress of lack of nutrition and hunger (in the pair-fed, zinc supplemented group) would have been evidenced by a high serum corticosterone level, but, without exception, such a finding has not been obtained.

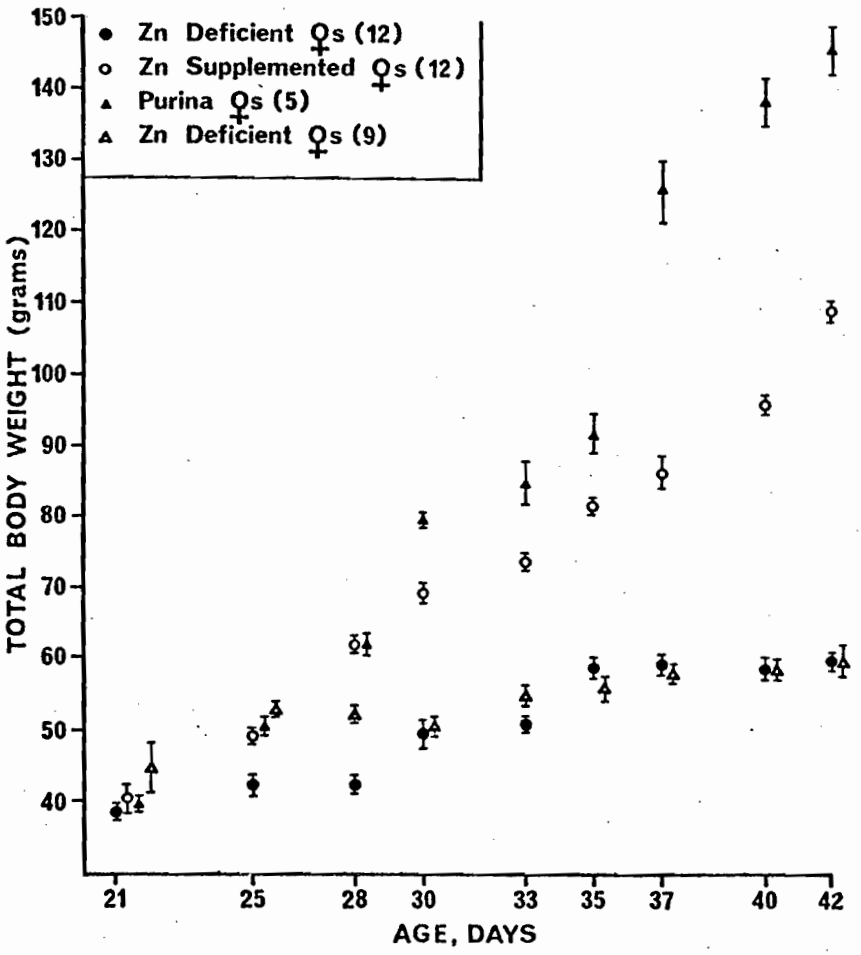
As shown in Scattergram I, the serum prolactin concentration has been evaluated. No previous investigation has been done in association with zinc deficiency and its influence on prolactin production level. The results obtained indicate a significant reduction of the serum prolactin levels in the zinc deficient group as compared to the zinc supplemented and the ad libitum fed groups ($p<0.008$ and $p<0.001$ respectively). Confirmation of these findings

has since been obtained twice, in two different experiments described later in this section. As indicated in Scattergram 4, the serum T_4 and T_3 were evaluated. Thyroxine was found to be significantly lower in the zinc deficient group than in the zinc supplemented, pair-fed group ($p<0.05$). No significant difference was determined with regards to the T_3 levels ($p<0.1$).

The zinc deficient animals exhibit a pattern of anorexia, lack of growth and sexual maturation, and epithelial pathology. It was considered necessary to investigate the effect of zinc deficiency on carbohydrate metabolism. As depicted on Scattergram 3, serum insulin and glucose levels were investigated. With regards to the glycemia, no significant differences were noted among the three groups. The insulinemia, however, was significantly lower in the zinc deficient group than in the zinc supplemented and the ad libitum fed groups ($p<0.05$ and $p<0.001$, respectively). Insulinemia was determined to also be significantly lower in the zinc supplemented group when compared to the ad libitum controls ($p<0.05$).

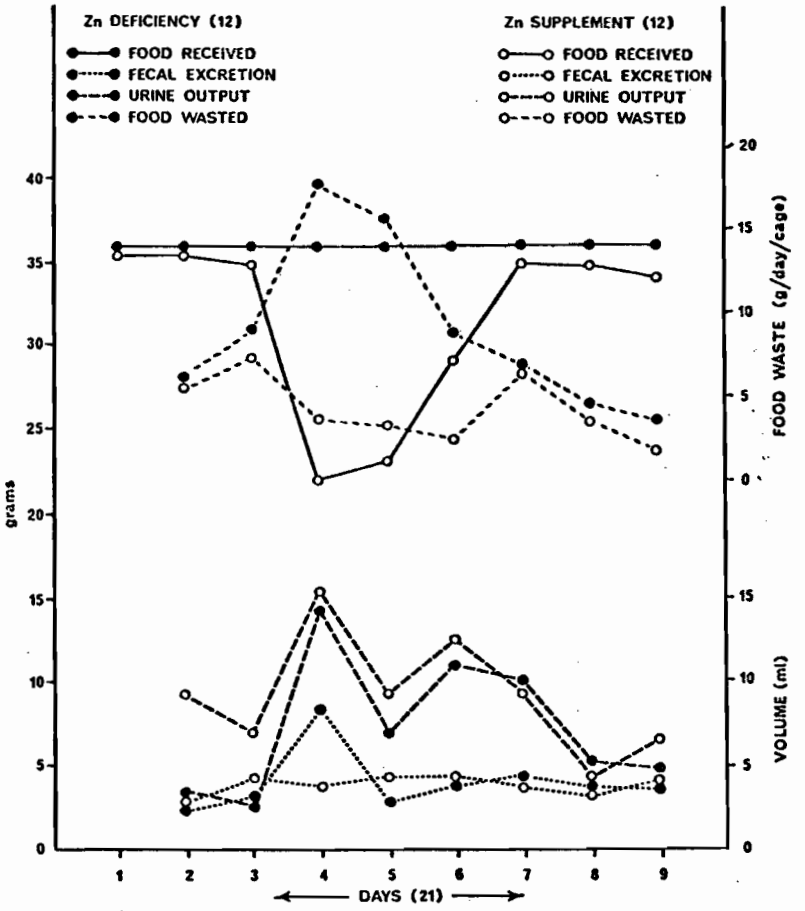
The serum and fecal zinc concentration were determined and tabulated in Table XV. The zinc deficient group exhibited a slight hypozincemia (normozincemia $\tilde{=}$ 90 - 250 mg/dl, according to the present standards). The other two groups exhibited a normozincemia pattern, the upper range of which was noted in the zinc supplemented group. Similar findings were determined in the fecal zinc concentration. The extremely low zinc concentration in the fecal pellets from the zinc deficient group are probably more representative of the intestinal epithelium turnover than of food and water intake and lack of absorption. The high values noted in the feces of the zinc supplemented group, on the other hand, are probably in the main the consequence of a high zinc intake in the drinking water ($\tilde{2}5$ ppm) and the equilibrium with the plasma zinc levels.

GROWTH IN ZINC DEFICIENCY



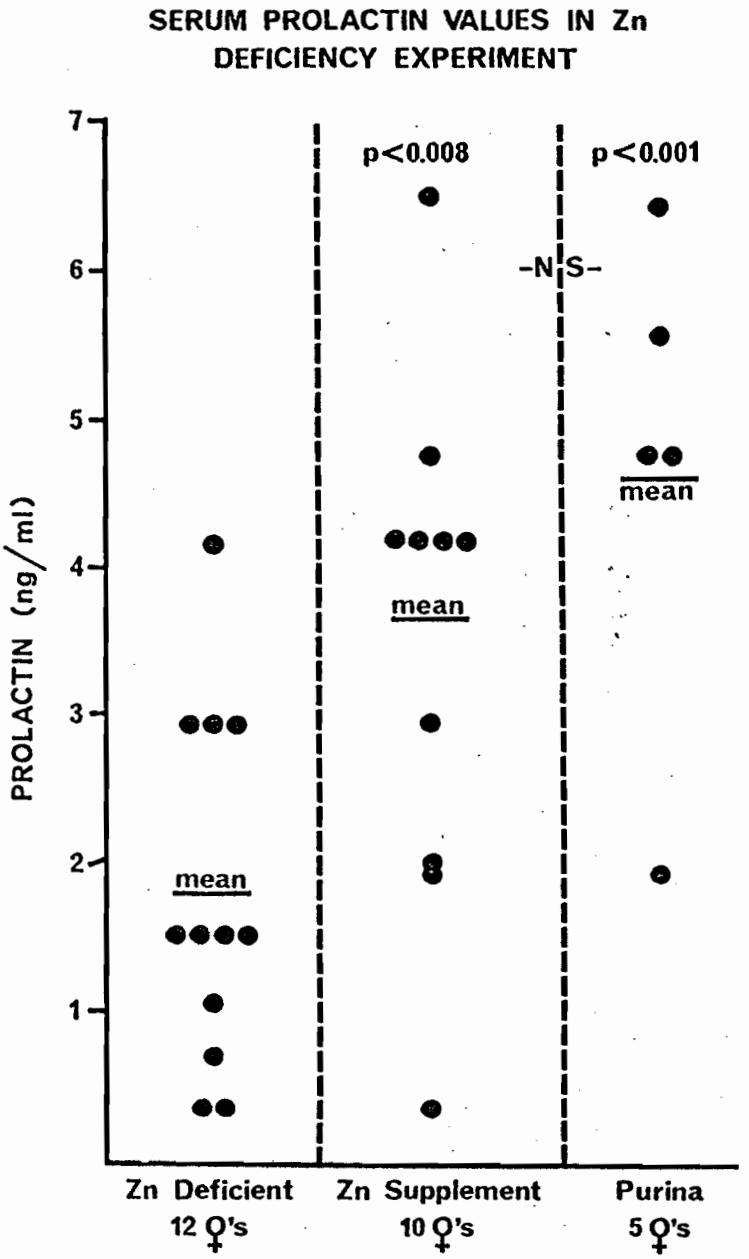
Exp II Fig. 2

FOOD INTAKE AND URINARY AND FECAL EXCRETION IN Zn DEFICIENCY EXPERIMENT



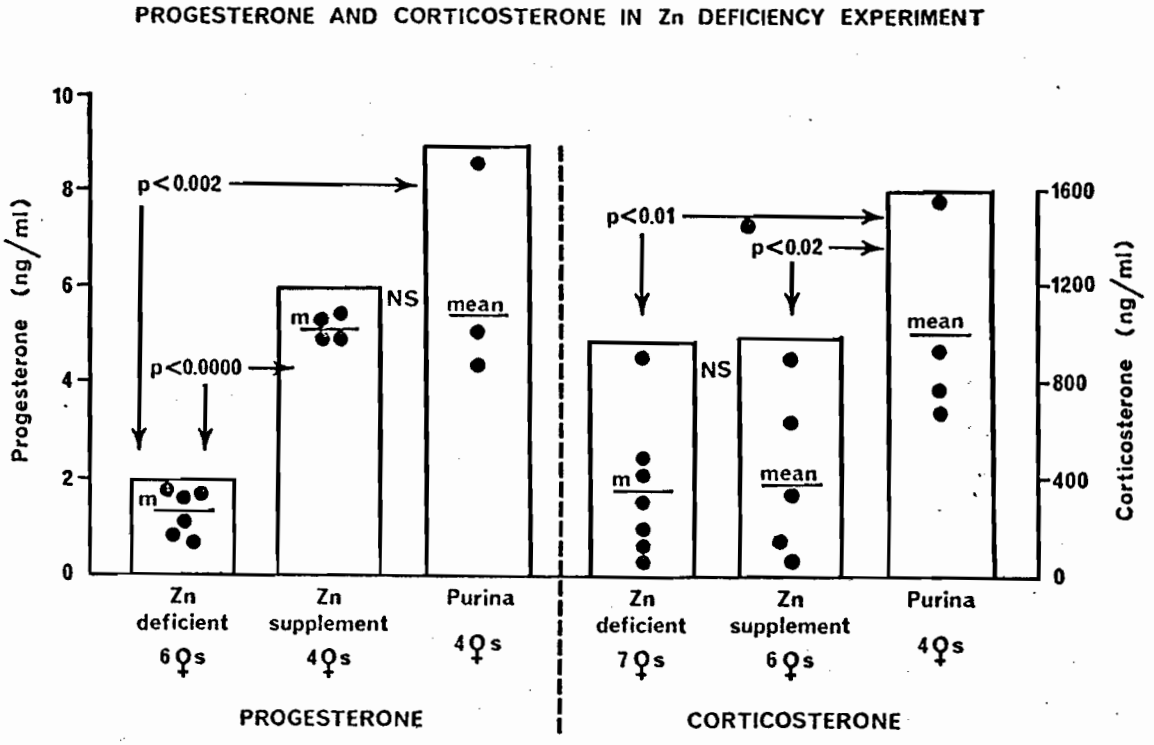
Exp. II

Scattergram 1



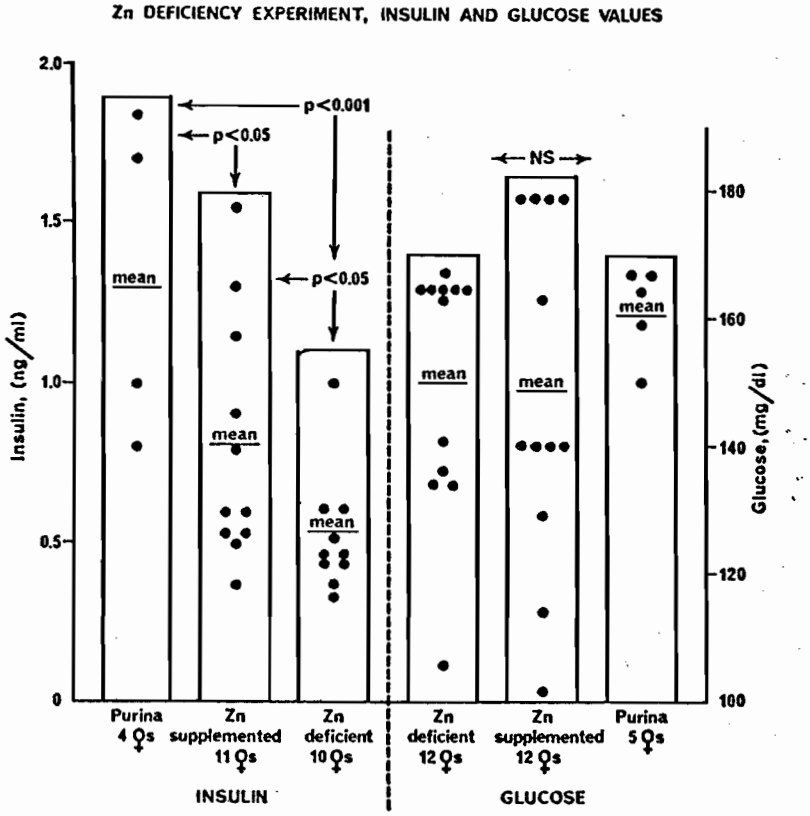
Exp. II

Scattergram 2

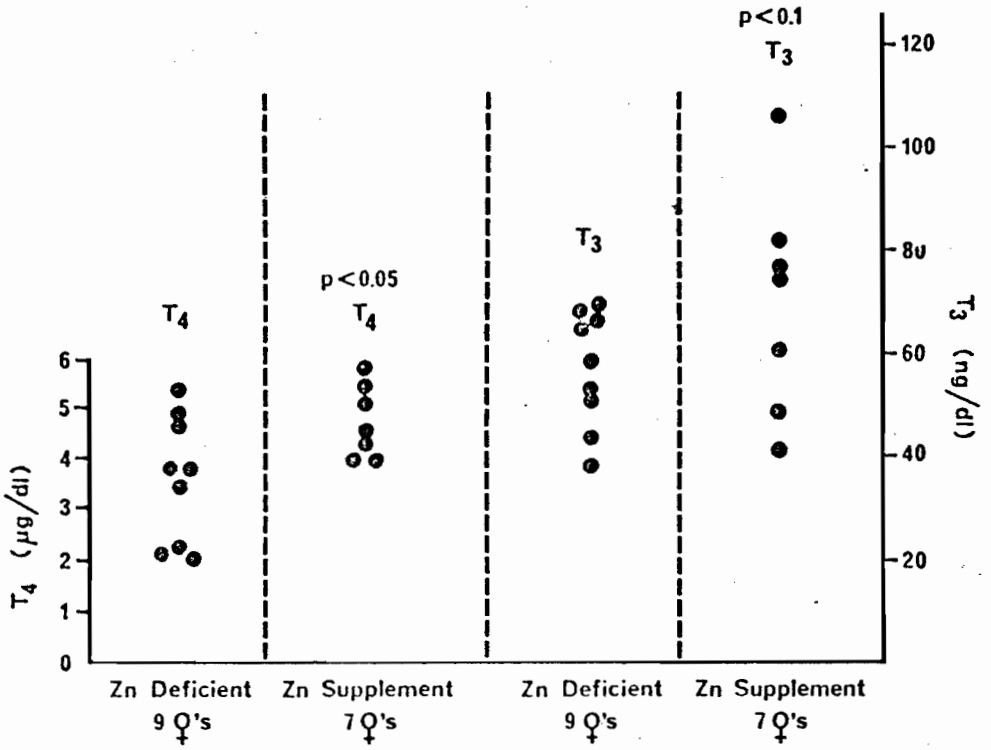


Exp. II

Scattergram 3



SERUM T_4 AND T_3 VALUES IN Zn DEFICIENCY EXPERIMENT



Exp II. Scattergram 4.

RESULTS

Experiment III

The data presented in figure 3 indicated that both the zinc supplemented and zinc deficient gestant females gained weigth during pregnancy. The weight gain of the zinc supplemented group was significantly higher than that of the zinc deficient group (i.e. by ~ 60 grams) by day 19 of pregnancy. The data, not depicted on figure 3, re: the weight gain pattern of the ad libitum fed gestant female indicates a more "obtuse" angle of gain. By day 19 (last weight determination before deliver) she weighed 235 grams. Weight changes of ± 30 grams were determined during the lactation period, on the day of weaning she weighed 260 grams.

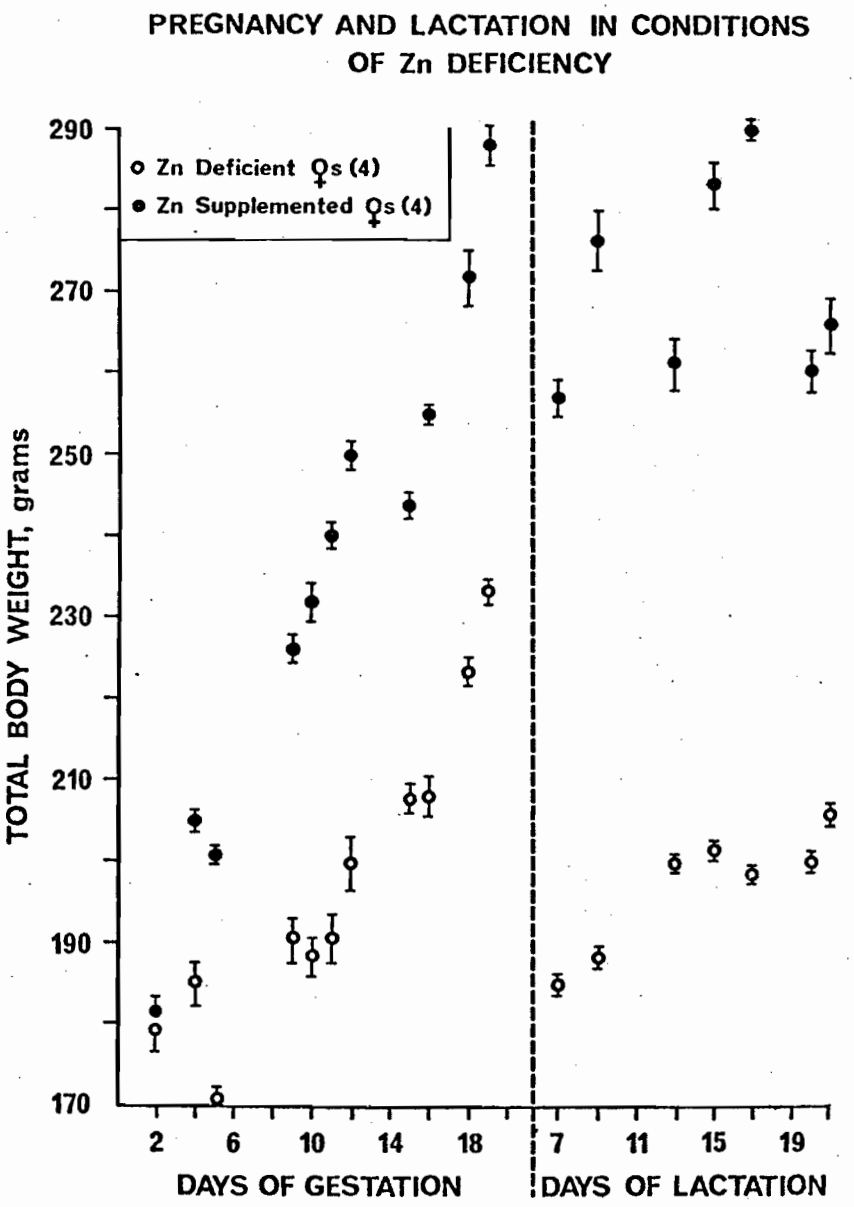
As noted from figure 3, the weights of the zinc deficient and zinc supplemented mothers were found to be decreased by the 7th day of lactation as compared to day 19 of gestation. It is of interest to note that in the case of the zinc deficient group the body weight was quite similar to that at the beginning of gestation: i.e. no net weight gain in spite of the passage of one month of life. On the other hand the body weight of the zinc supplemented mothers on day 7 of lactation was similar to that of day 16 of gestation (~ 255 grams). Thereafter the body weight pattern of the zinc supplemented mothers followed a scattered and irregular pattern , 270 ± 20 grams. That of the zinc deficient group followed a much more regular pattern, increasing to about 205 grams (interestingly similar to that at day 16 of gestation).

In general, in all 3 groups the body weight was determined to vary ± 20 grams during one day. It is considered that the lactation activity could account for the large changes in weight. It is clear, however, that

the animals were weighed within an interval of 15 minutes at 10 a.m. each time.

As compared to the ad libitum and the zinc supplemented mothers, the zinc deficient mothers showed less care for the pups (i.e. a degree of "carelessness", lack of cleanliness and preening, etc.). Notwithstanding the fact that the zinc deficient mothers were siblings, the pups of one exhibited complete alopecia while the others presented various degrees of fur growth patterns. By the last week of lactation the zinc deficient mothers exhibited various degrees of alopecia, keratosis (including corneal keretosis) and especially perinasal and perivaginal hemorrhage. The body sizes of the zinc supplemented dams were about 10% higher and that of the Purina-fed dam about 15 % higher than those of the zinc deficient dams. On the day of weaning, at the dissection, the abdominal fat and especially the periuterine fat were practically non-existent in the zinc deficient dams, while the same adipose tissue did not appear significantly decreased from the non-gestant state in the zinc supplemented or Purina-fed females. When the blood (obtained by decapitation) was centrifuged, a hematocrit of < 20 was strikingly evident in the Zinc deficient group as compared to controls. Nonspecifically localized necrotic or gangrenous spots (~ 2-4 mm in diameter) were noted on the serosa (? entire wall?) of the small intestine of two out of the four zinc deficient dams. No such finding was noted on the colon or any other abdominal location. No such finding was noted on any of the other females.

Table 2 presents the data obtained from the determinations of serum FSH, LH and estradiol-17β . The FSH and LH concentrations of the Purina and Zn supplemented dams were nonsignificantly higher than those from the Zn deficient groups. The estradiol levels were higher, but not significantly so, in the Zn deficient group than in the other groups.


PREGNANCY AND LACTATION IN CONDITIONS OF Zn DEFICIENCY
o Zn Deficient ♀s (4)
• Zn Supplemented ♀s (4)
TOTAL BODY WEIGHT, grams
290
270
250
230
210
190
170
2
6
10
14
18
7
11
15
19
DAYS OF GESTATION
DAYS OF LACTATION

Exp III Fig. 3

Exp III Table II

SERUM HORMONAL LEVELS IN POST-LACTATION DAMS

		FSH (ng/ml)	LH (ng/ml)	E_2 (ng/ml)
Zn↓	N	4	4	4
	MEAN±SEM	94.9 ± 10.3	6.9 ± 5.2	95.8 ± 39.5
	P	—	—	—
Zn↑	N	4	4	4
	MEAN±SEM	117.3 ± 48	11.9 ± 9.1	44.9 ± 45.1
	P	NS	NS	NS
PURINA	N	1	1	1
	MEAN±SEM	219 ± 0	12.8 ± 0	17.7 ± 0
	P	—	—	—

RESULTS

Experiment IV:

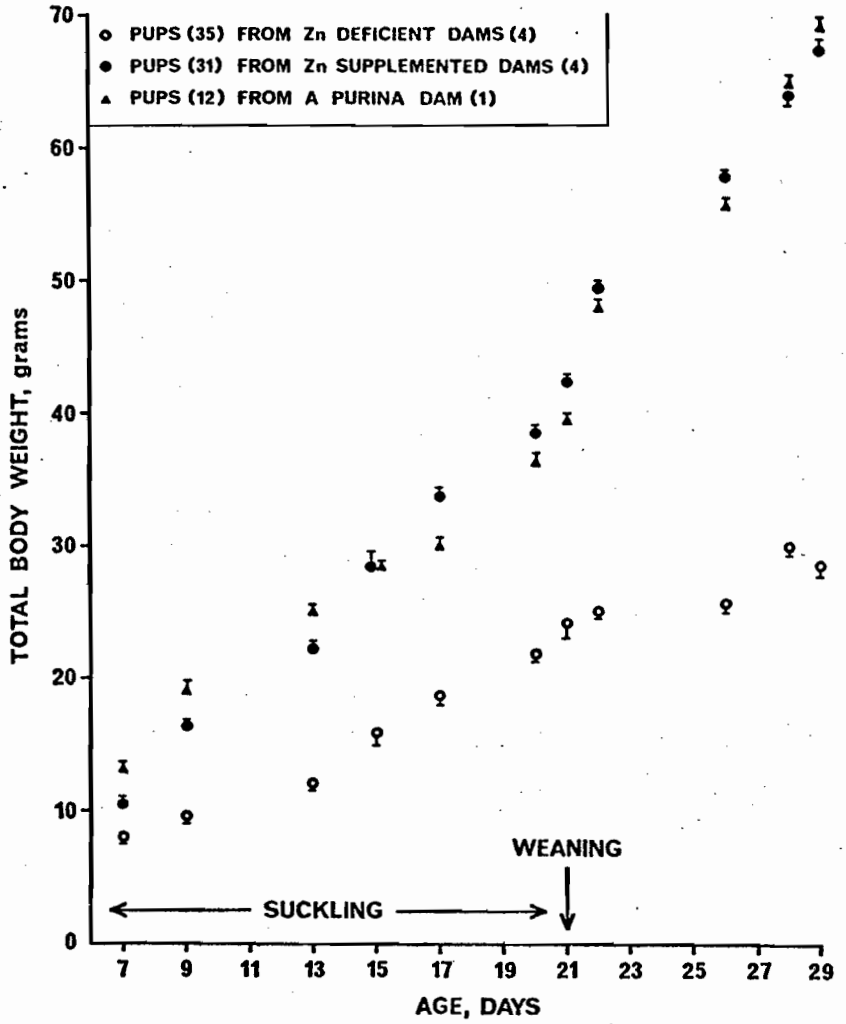
The furs of the Zn deficient group started developing only after about 14 days and by weaning time (21 days) about 30% of the groups were more evidently pink (no fur or little of it) than white (covered with fur). Perioral, periocular and periungueal keretosis and hemorrhage were noted during the last week of lactation in the zinc deficient group. Albeit in other respects "normal", the zinc deficient pups were less clean, no preening behavior was noted and were more difficult to handle (irrascible) than their peers.

No differences in the patterns of growth, development or behavior were noted in the pups from the zinc supplemented or Purina fed mothers. As indicated in figure 4 no significant difference in growth occurred between the zinc supplemented and Purina group although the mothers in the former group were fed less (pair-fed to the zinc deficient group) and were eager to eat whenever food was offered. On the other hand the zinc deficient group pups grew significantly less and by weaning time they weighed only about 50% of the zinc supplemented or Purina peers. It should perhaps be stressed that the mothers in the zinc supplemented and zinc deficient groups ate equal amounts of food (~ 35 grams/day) and the food quantity given was modeled according to the need of the zinc deficient group animals.

On day 21 the pups were placed on zinc deficient, zinc supplemented and Purina diets as were their respective mothers. As indicated again in figure 4 there was little growth in the zinc deficient group weanlings between the days 21 - 29 of life. By the end of the experiment a plateau seemed to be reached. The rate of growth of the zinc supplemented and Purina groups was evident and rather similar in pattern. The zinc

deficient pups were consuming about 8g/per day of food. The same amount was provided to the zinc supplemented group. Lack of proper absorption and, in retrospect, the influence of zinc deficiency on the hormonal output (vis GH, Insulin, T_4) have accounted for the lack of growth.

PUP GROWTH IN CONDITIONS OF Zn DEFICIENCY DURING GESTATION, LACTATION AND POST-WEANING PERIOD



Exp IV Fig. 4

RESULTS

Experiment V:

PMSG and estrogen-progesterone are two pharmacologic methods of induction of precocious sexual maturation of post-weaning female rats. In both cases the treatment is normally followed by an endogenous LH surge and ensuing progesterone surge. 54 hrs after the PMSG injection the endogenous LH concentration has been observed to peak. 72 hrs after the injection, ova are usually observable in the fallopian tubes, as the "crowning" of the precocious sexual maturation induction. The estrogen-progesterone method of induction of precocious puberty is usually evidenced by an LH peak 5 hrs after the P_4 injection.(82) These were the first experiments on the subject of the inducement of precocious sexual maturation in the condition of zinc deficiency. The results obtained indicate a partial response of the sexual axis in the zinc deficient females.

1) Uterine hypertrophy was evident and the figures shown in Histogram I and Table IV present the data clearly in this light. However, the zinc deficient females were only about 50% the size of the zinc supplemented and Purina peers. The hypertrophy of the uteri of the zinc deficient treated animals to only about 50 % of that of the peers would seem to be proportional to that expected according to the whole body size. By the same token, however, 20 I.U. of PMSG injected in bodies half the size of others, should have a much large effect on the former than on the latter. It is felt that no statistical manipulation of the data at the present stage of the investigation would enable us to obtain an unequivocal answer. It is concluded therefore only that the uteri of both groups underwent hypertrophy as a result of the PMSG treatment.

As indicated in Table IV a number of 34 ova, 48 ova and questionably 4 ova were detected at the 72 hrs period in the fallopian tubes of the

Purina, zinc supplemented and zinc deficient females. The questionable 4 ova were detected all in one tube (only) of 1 out of the 3 females - i.e. ?4 ova in one out of six fallopian tubes. The ova from the peer groups were detected in all fallopian tubes examined. Table VI points to the fact that no LH surge and no serum LH could be noted with the RIA method used, in the case of the Zinc deficient groups in both the PMSG and the E_2 - P_4 experiments. Serum LH peaks in the control animals of all groups could not be noted either, as expected on the basis of the age of the animals. LH responses were conspicuous in the zinc supplemented and the Purina groups in the PMSG experiment at both the 54 hrs and 72 hrs periods. The actual 54 hrs peaks theoretically expected could not be confirmed in this experiment, being missed probably by a margin of 2 - 3 hrs.

An LH response was evident also in the zinc supplemented group in the E_2 - P_4 experiment. The serum progesterone values determined in the PMSG experiment indicate that in the case of the treated animals, PMSG stimulated ovarian progesterone production did occur. Although higher progesterone values have been determined, they do not appear statistically significant. The P_4 values datermined in the case of the control animals are probably representative of adrenal P_4 production. Again, perhaps due to the animal, pituitary and adrenal size, the P_4 appears in lower serum concentrations in the case of the zinc deficient animals. Interesting indices of the zinc deficient influences on hepatic function and metabolism are obtained from the values derived from the E_2 - P_4 experiment, as shown in Table VI. According to the experimental design, the treatment groups were injected with P_4 only 5 hrs before sacrifice. The inexistence of an LH surge combined with the very large P_4 serum values obtained in the case of the

zinc deficient animals are indicative of 1) results = a mixture of endogenous and as yet non-catabolized exogenous progesterone and 2) a relative inability of the zinc deficient liver to catabolize the exogenous hormone in five hrs. Regarding the endogenous P_4 production, the data obtained from the control animals indicate that it is lower in the zinc deficient animals than in their peers.

To summarize the results of this double experiment, the zinc deficient animals respond in part to the pharmacologic attempt at inducing precocious sexual maturation. Thus, the uteri and the ovaries hypertrophy and, albeit questionably, ovulation may occur. Endogenous progesterone production is enhanced (the PMSG experiment). The catabolism of the exogenous (and endogenous) progesterone is delayed (the E_2 - P_4 experiment). No LH in the serum or LH surge could be determined in any of the zinc deficient animals stimulated either by PMSG or E_2 - P_4. It is felt that the reduced body size of the zinc deficient animals does not preclude the priming effects of the two pharmacologic means in that PMSG and E_2 - P_4 experiments have been carried out successfully in female rats 22 - 24 days old (i.e. not significantly larger but much younger in proportion when compared to the zinc deficient group used in this experiment).

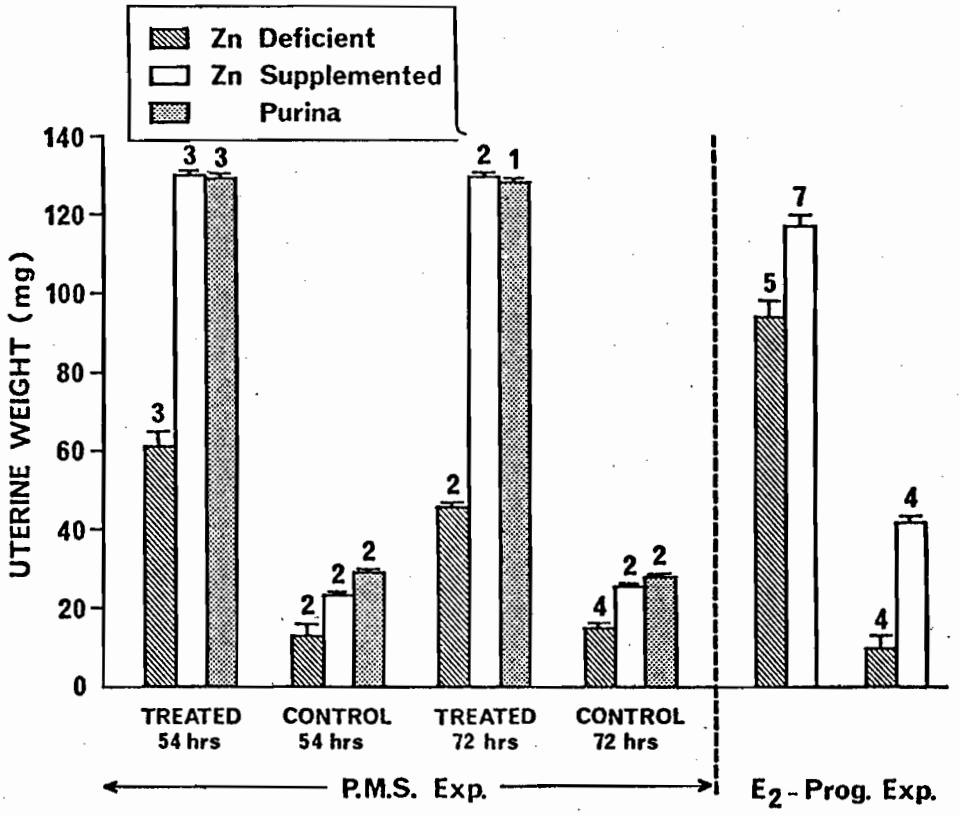
Table III and Histogram II present the data re: the body weights of the groups at the ages 26 and 30 days.

Table IV and Histogram III present the data re: the uterine and ovarian weights determined in the PMSG experiment.

Table V and Histogram 1 present the data re: the uterine weights in the E_2-P_4 experiment.

Table VI and Histogram 4 present the data re: the LH and Progesterone determinations in the PMSG and Estrogen-Progesterone experiments.

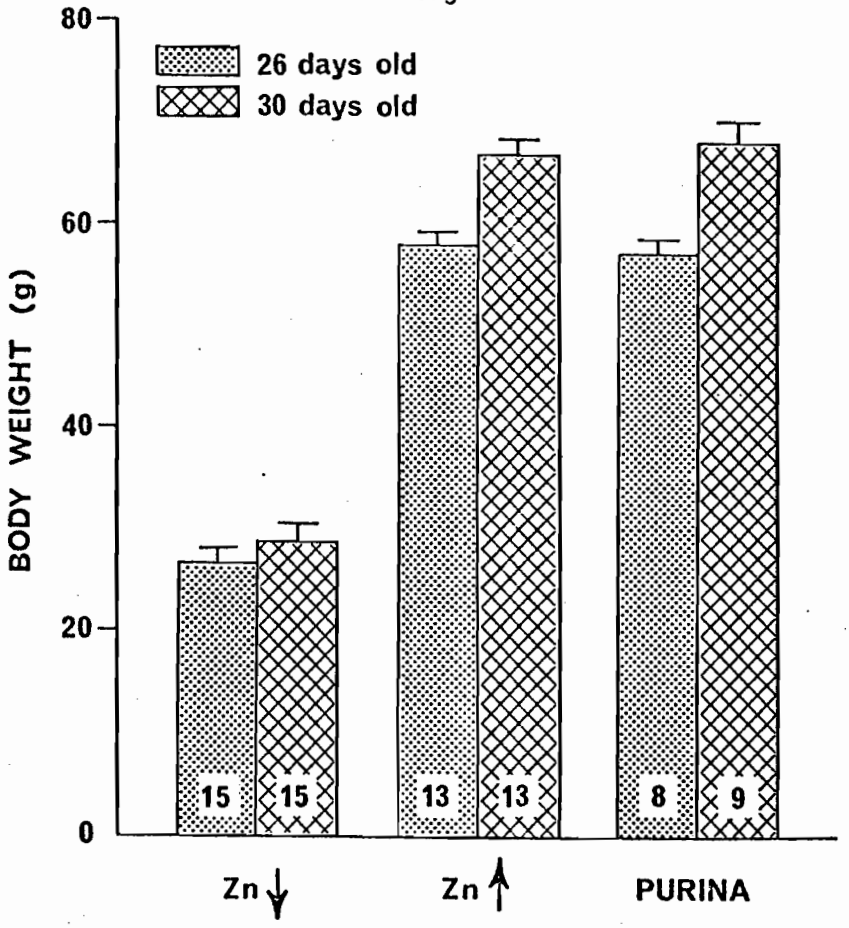
P.M.S. and E_2-PROG. EXP.: UTERINE WEIGHTS



Exp V Histogram I

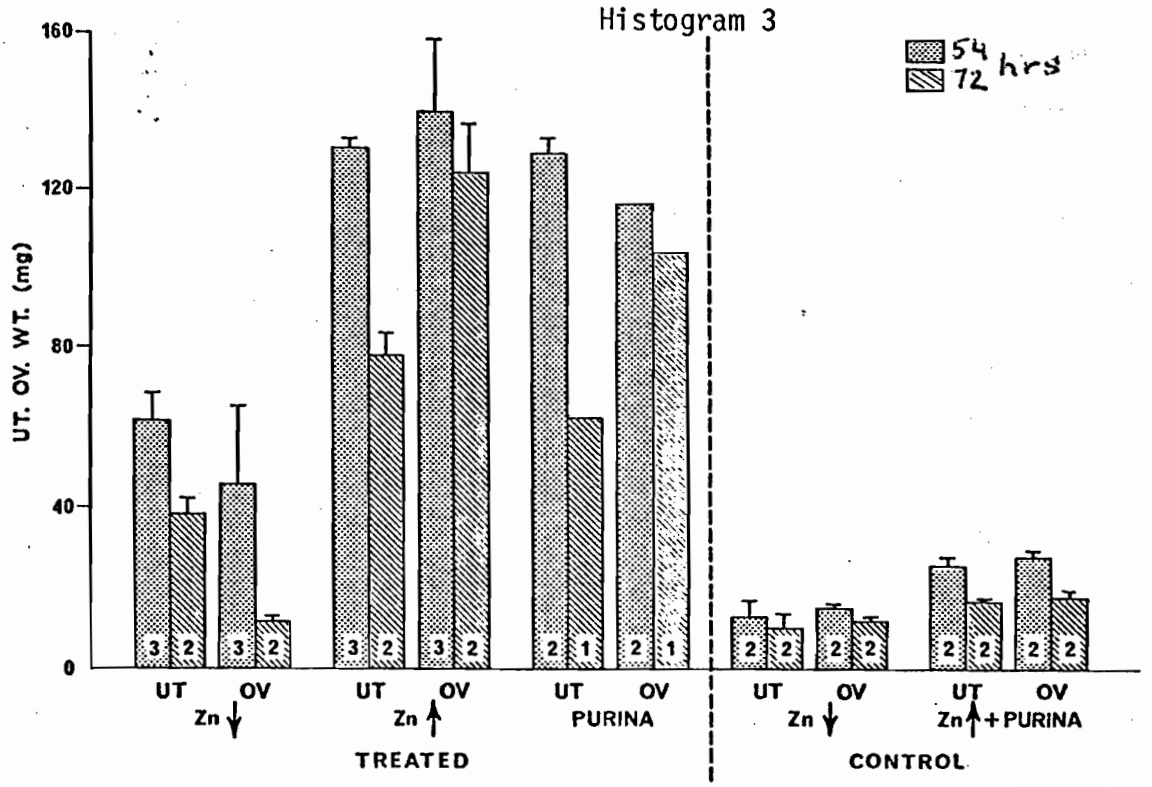
Exp. V. PMSG - E_2-P_4 Exp.

Histogram 2



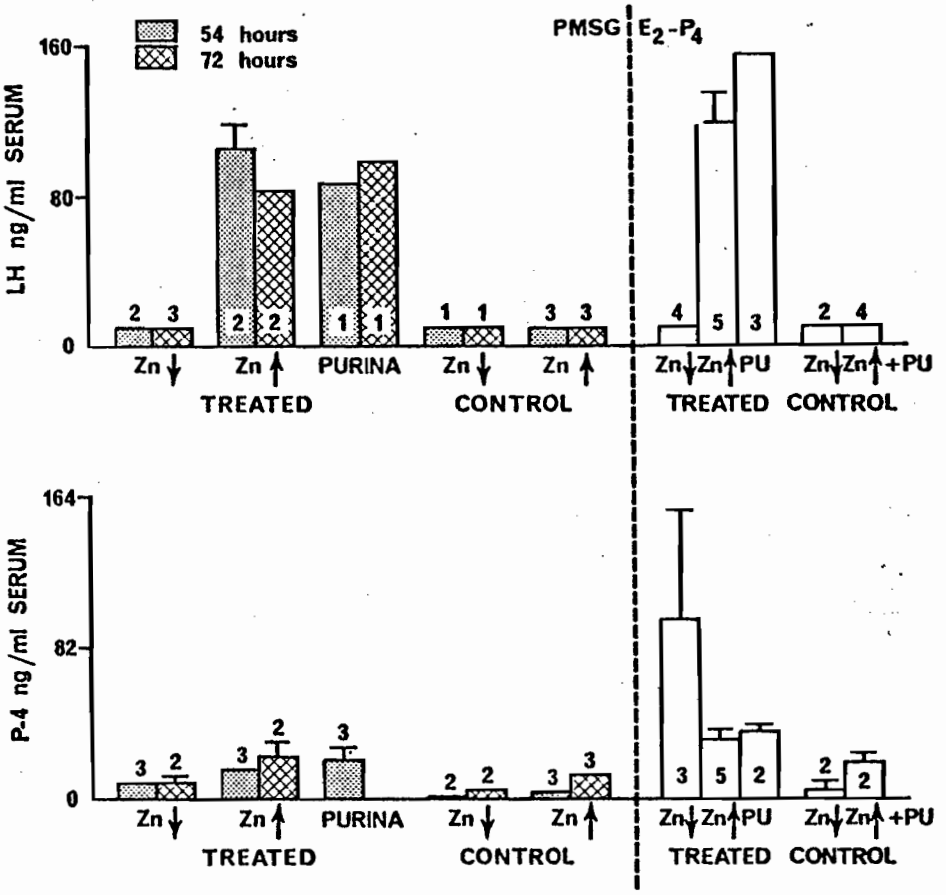
Body Weights of the Experimental Animals

Histogram 3



Exp. V. PMSG - E_2-P_4 Exp.

Histogram 4



LH and P_4 Stimulation by PMSG and E_2-P_4 Treatment

Exp V Table III

PMSG AND E_2-P_4 EXPERIMENTS. BODY WEIGHTS (gms)

	day 26	Zn↓	Zn↑	Purina
PMSG	N	15	13	8
	MEAN ±SEM	26.8 ± 1.2	57.7 ± 1.2	56.8 ± 1.4
	P	→	<0.0000	<0.0000
	day 29-30	Zn↓	Zn↑	Purina
E_2 | P_4	N	15	13	9
	MEAN± SEM	28.9 ± 1.6	66.7 ± 1.3	67.9 ± 1.8
	P	→	<0.00001	<0.00001

Exp V Table IV

UTERUS AND OVARY WEIGHTS (mgs)

PMSG. EXP.

		UTERUS (mg)		OVARY (mg)		OVA (No.)
Treated		54hrs	72hrs	54hrs	72hrs	72hrs
Zn↓	N	3	2	3	2	?4
	mean± SEM	62.0±6.4	46.0±19	38.7±3.4	11.5±1.5	
	P	—	—	—	—	
Zn↑	N	3	2	3	2	48
	mean± SEM	131.0±2.6	140.0±18	78.0±5.5	124.5±12.5	
	P	<0.0006	NS	< 0.003	< 0.01	
PURINA	N	2	1	2	1	34
	mean± SEM	129.5±3.5	116.3±0	62.5±4.5	103.8±0	
	P	<0.004	—	<0.02	—	
Controls						
Zn↓	N	2	2	2	2	0
	mean± SEM	13.0±4.0	15.0±1.0	10.5±3.5	12.0±1.0	
	P	—	—	—	—	
Zn+PURINA	N	2	2	2	2	0
	mean± SEM	25.5±2.5	27.5±1.5	17.0±1.0	17.5±1.5	
	P	NS	<0.02	NS	NS	

Exp V Table V

ESTROGEN-PROGESTERONE EXP. UTERINE WEIGHTS (mgs)

	Zn ↓			Zn ↑		Purina
	Treated		Control	Treated	Control - Zn↑ + Purina	Treated
N	3		2	5	2	1
Mean ± SEM	94.7 ± 5.2	10.5	± 6.5	113.2 ±4.1	45 ± 2.0	143.0 ± 0
P	—		—	0.03	0.03	——

Exp. V Table VI

LH AND P_4 SERUM CONCENTRATIONS: PMSG AND $E_2 - P_4$ EXP.

		PMGS				$E_2 - P_4$	
		54 hrs		72 hrs		LH	P_4
		LH	P_4	LH	P_4		
Zn↓	N	2	3	3	2	4	3
	MEAN± SEM	10(0)	8983.3 208.8	10(0)	8850± 3250	10(0)	96,667 ± 59,697
	P	—	—	—	—	—	—
Zn↑	N	2	3	2	2	2	5
	MEAN± SEM	105.4± 12.4	16400± 3172	84±0	23000± 8000	118±16	32400±5526
	P	—	NS	—	NS	—	NS
PURINA	N	1	3	1	—	3	2
	MEAN± SEM	87±0	22100± 5109	98±0	—	154.27±0	37500±2800
	P	—	NS	—	—	—	NS
Controls							
Zn↓	N	1	2	1	2	2	2
	MEAN± SEM	10(0)	820± 170	10(0)	4950± 50	10(0)	4550± 3150
	P	—	—	—	—	—	—
Zn↑+ Purina	N	3	1	3	1	4	2
	MEAN± SEM	10(0)	3440± 0	10(0)	13400± 0	10(0)	19750 ± 2850
	P	—	—	—	—	—	NS

EXPERIMENT VI. RESULTS

Table VII and Histogram VI present the data re: the body, pituitary and ovarian weights of the experimental animals.

Table VIII presents the statistical data re: the pituitary and serum FSH concentrations determined.

Table IX and Histogram VII present the data re: the pituitary and serum LH concentrations determined.

Table X and Histogram VIII present the data re: the pituitary and serum prolactin concentrations determined.

Table XI and Histogram IX present the data re: the growth hormone serum concentrations in the experimental animals.

Table XII and Histogram X present the data re: the ovarian and serum progesterone concentrations determined.

Table XIII presents the estradiol-17β ovarian concentrations and the corticosterone serum concentrations determined.

Table XV presents the data re: the Zn concentrations in serum, feces, pancreas and liver.

Table XVI depicts a statistical summary of most data accrued.

A widespread hormonal and biochemical profile of zinc deficiency during the post-weaning period of growth and sexual maturation has been attempted. It is the first experiment in the field of zinc deficiency studies where serum and pituitary GH concentrations have been assessed. It has long been known that growth is significantly stunted in rats after 10 - 14 days of zinc deficient regimen. The previous experiments described and the results obtained amply confirm this finding. Up to the present time it was not known if GH was affected in zinc deficiency. The results obtained in this experiment indicate that at age 35 days the serum GH concentration is not significantly affected by zinc deficiency

(i.e. 2 weeks of zinc deficient regimen). At 42 days of age, however, the serum GH concentration of both the zinc supplemented and Purina groups was significantly higher that that of the zinc deficient group. Were the serum GH concentration to vary according to food intake, it would have been lower in the zinc supplemented group, which was clearly not the case.

By day 42 of age all the zinc supplemented and Purina females had the vagina open. No zinc deficient female had the vagina open (i.e. sexually mature). No significant difference has been determined for the pituitary LH concentration. The serum LH concentration at 35 days of age is nonsignificantly different in all three groups. At age 42 days, no significant difference appears between the zinc deficient and the zinc supplemented groups, however the Purina group had a significantly higher serum LH concentration. With regards to the FSH, at age 35 days, no significant pituitary content difference has been determined. However, at age 42 days both the zinc supplemented and Purina groups exhibited a significantly higher pituitary FSH content. Interestingly, the serum FSH concentration did not appear to vary within all the groups assessed at both ages. While it is easy to conclude that there is a definite relationship between zinc deficiency and PRL, the results obtained in this experiment are not easy to assess. In the pituitary, the PRL concentration at age 35 days appears significantly higher in the zinc supplemented group, but not so in the Purina group relative to the zinc deficient group. At age 42, the Purina group PRL is significantly higher than that of both other groups, while there is no difference between the zinc deficient group and the zinc supplemented one. In the serum, at age 35 days the Purina group exhibits a significantly higher PRL concentration

than that of the others. At age 42 days the zinc supplemented group PRL is significantly higher that that of the zinc deficient group; no significant difference appears between the PRL concentration in the zinc supplemented and the Purina groups. The actual [PRL]'s in the Purina and the Zn deficient groups were 45 ng/ml and 1 ng/ml. The t-test however shows no significant differences.

The ovarian progesterone is not different among the three groups at the age of 35 days. At the age of 42 days the ovarian P_4 concentration is significantly higher than that in the other two groups. The serum P_4 concentration is similar at the age of 35 days between the zinc deficient and the zinc supplemented groups. However, the P_4 concentration is higher in the zinc deficient group than in the Purina group. No differences were determined in the serum P_4 concentration at the age of 42 days. Serum corticosterone concentration appears higher in the zinc deficient group at both 35 and 42 days of age than that of the Purina group and higher than that of the Zn supplemented group at age 35 days.

Table XIV presents a statistical summary of relevant data from the SMA-24 biochemical tests. In view of the fact that most data did not differ significantly among the groups concerned, no specific tabulation of the values involved was deemed necessary, although the figures are available.

The most significant finding regards Alkaline Phosphatase - the only zinc-enzyme studied in this experiment. Zinc deficiency affects the serum level of this enzyme. Alkaline Phosphatase is significantly lower in the Zn deficient group compared to the Purina group two weeks and three weeks after the implementation of the Zn regimen. No such difference is noted with regards to the Zn supplemented (pair-fed-20%) at the age 42 days. The reason may be the non specific consequence of malnutrition in the latter

group. Calcium is significantly reduced in the Zn deficient group at the age 35 days, however no significant differences are encountered at the age 42 days among the three groups.

Notwithstanding the differences in protein intake, the albumin level is rather similar in the three groups. So is uric acid, which points to the fact that protein metabolism is not significantly affected in this experimental model. The nonsignificantly different results with re: SGPT, point to the fact that the hepatic function is not affected in this condition.

The results re: the CO_2 total indicate that the only significant difference occurred at the age of 42 days between the Zn deficient and Zn supplemented groups (i.e. the Zn deficient group showed a slightly acid pH tendency). The glycemia was similar in all the cases studied, reflecting again the phenomenon of normoglycemia in Zn deficiency, associated with hypoinsulinemia.

TableXVII presents the data re: the pancreatic concentrations of insulin and somatostatin, from 42 day old animals. The amount of protein/gm of pancreatic tissue was similar in the Purina and zinc supplemented groups notwithstanding the difference in body size and weight. The protein/gm of tissue in the zinc deficient group was only about 60% of the above. The pancreatic content of insulin and somatostatin did not differ between the Purina and zinc deficient groups. Both insulin and somatostatin were found to be significantly lower in the zinc supplemented group. These results could be interpreted at the present state of knowledge of the relationships of zinc metabolism and pancreatic insulin (? and somatostatin) secretion as follows:

1) Zinc is required for proper pancreatic structure.

2.

a. Since carboxypeptidase B, the enzyme that cleaves the insulin moiety from the proinsulin molecule is a zinc enzyme and

b. Since insulin is secreted from the pancreas in the form of 6 insulin molecules:3 zinc molecules, it follows that in the condition of zinc deficiency the proinsulin may not be cleaved at a high rate and not sufficient insulin may be secreted from the pancreas, thus accounting for the high "insulin" level found in the pancreatic tissue in the zinc deficient animals. The high zinc intake in the zinc supplemented group may stimulate the activity of the proinsulin cleaving enzyme, thus accounting for the difference found between the zinc supplemented and Purina groups.

Further study is required for the comprehension of the zinc-somatostatin relationship and no explanation can be offered at the present time.

Exp VI Table VII

BODY, PITUITARY AND OVARIAN WEIGHT
Female rats

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
BODY (g)	N	9	2	7	11	24	7
	MEAN± SEM	44.5±1	46±2	86.3±2.7	53±2.4	66.5±2.7	117.3±3.6
	P	↘	NS	<0.0000	↘	0.004	<0.0000
PITUITARY (mg)	N	7	2	7	6	3	6
	MEAN± SEM	2.7±0.2	2.9±0.4	3.4±0.4	2.2±0.2	3±0.5	5.3±0.4
	P	↘	NS	NS	↘	NS	<0.0000
OVARY (mg)	N	4	2	6	11	12	5
	MEAN± SEM	11.6±1.0	11.9±0.3	16.1±1.3	11.2±1.1	18±1.2	15.1±2.5
	P	↘	NS	< 0.03	↘	0.0004	NS

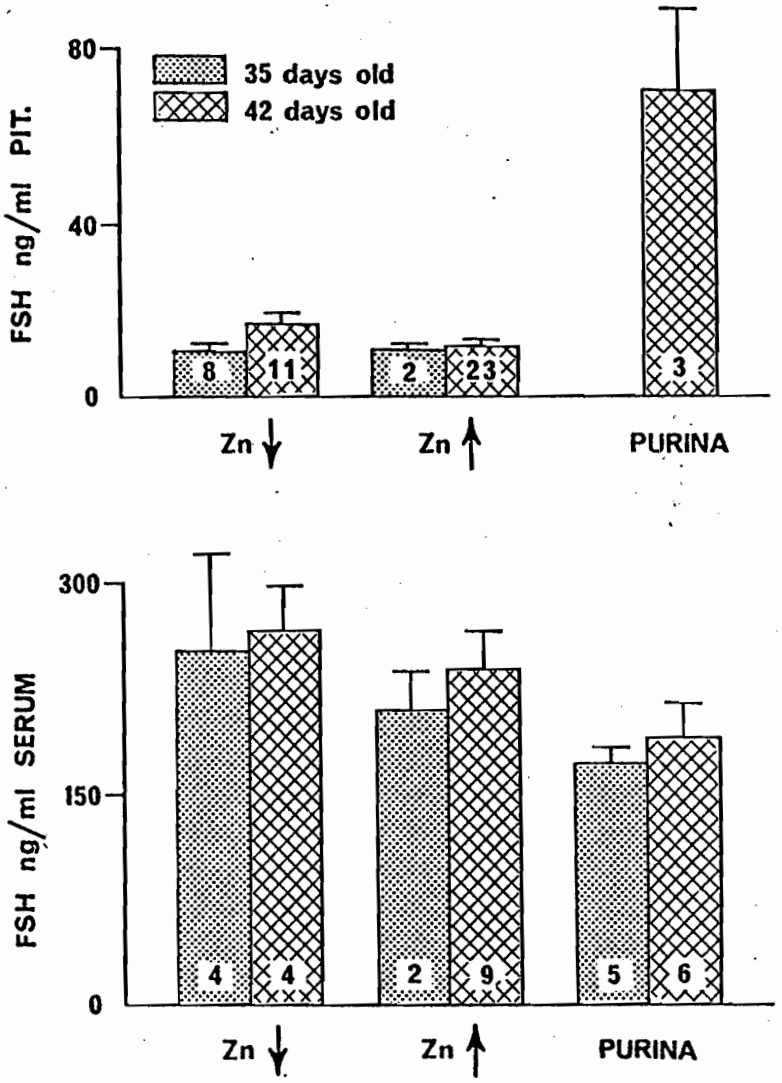
Exp VI Table VIII

PITUITARY AND SERUM FSH CONCENTRATIONS (ng/ml)

Female rats

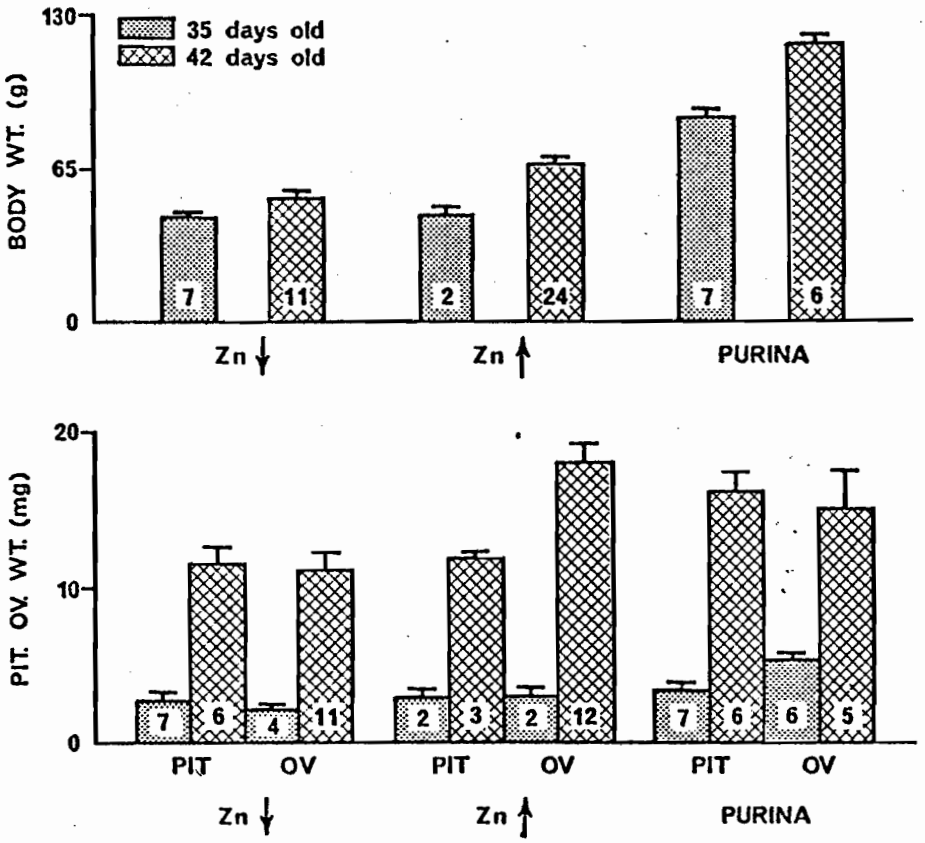
		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
PITUITARY	N	8	2	7	11	23	3
	MEAN± SEM	2706 ± 265	2811 ± 164	943 ± 232	4328 ± 412	2945 ± 375	18174± 4644
	P	→	NS	< 0.04	→	< 0.03	< 0.0001
SERUM	N	4	2	5	11	9	6
	MEAN± SEM	253 ± 68	209 ± 28	173 ± 10	267 ± 31	240 ± 27	189 ± 26
	P	→	NS	NS	→	NS	NS

Exp. VI. Histogram 5



Pituitary and Serum FSH Concentrations in 35 and 42 day old rats.

Exp. VI. Histogram 6



Body, Pituitary and Ovarian Weights in 35 and 42 day old female rats.

Exp VI Table IX

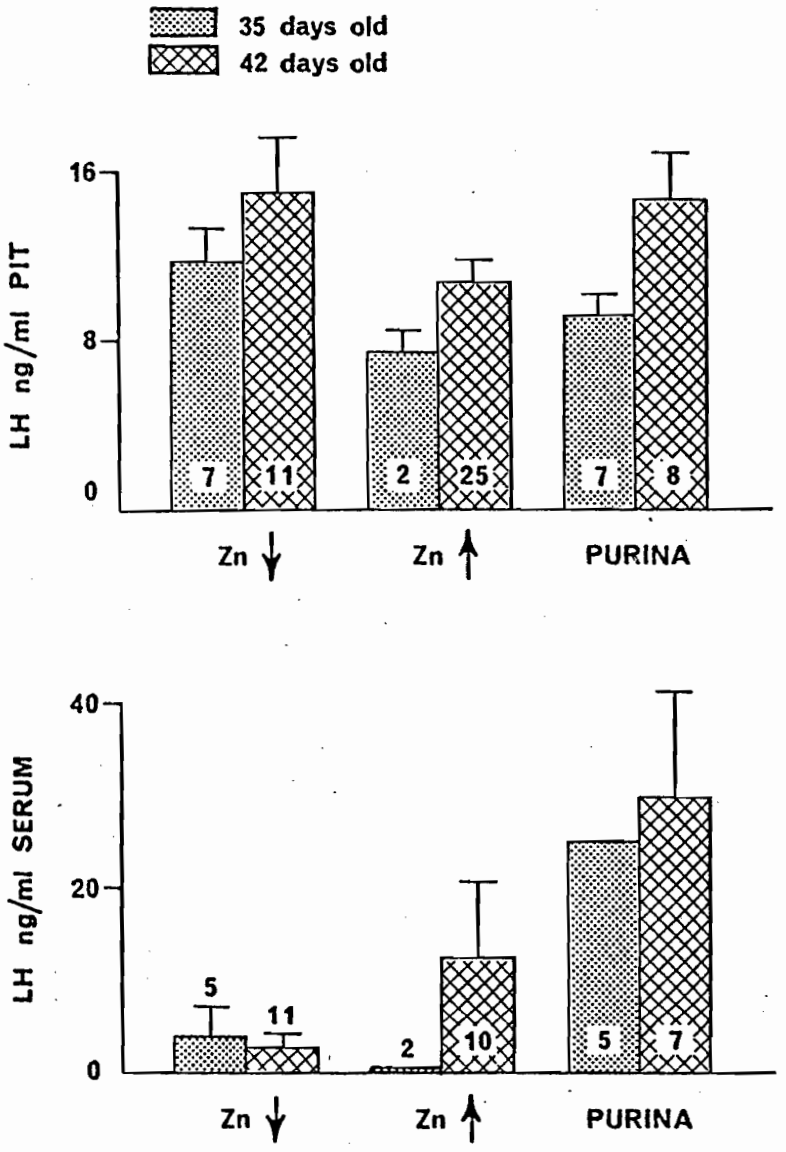
PITUITARY AND SERUM LH CONCENTRATIONS

LH (ng/ml)

Female rats

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
PITUITARY	N	7	2	7	11	25	8
	MEAN± SEM	2994± 385	1865± 250	2314± 284	3800± 640	2700± 256	3721 544
	P	↘	NS	NS	—	NS	NS
SERUM	N	5	2	5	11	10	7
	MEAN± SEM	4.0± 3.5	0.5±0	24.9± 24.4	2.5±1.6	12.9± 8.1	29.6± 11.7
	P	↘	NS	NS	↘	NS	< 0.01

Exp. VI. Histogram 7



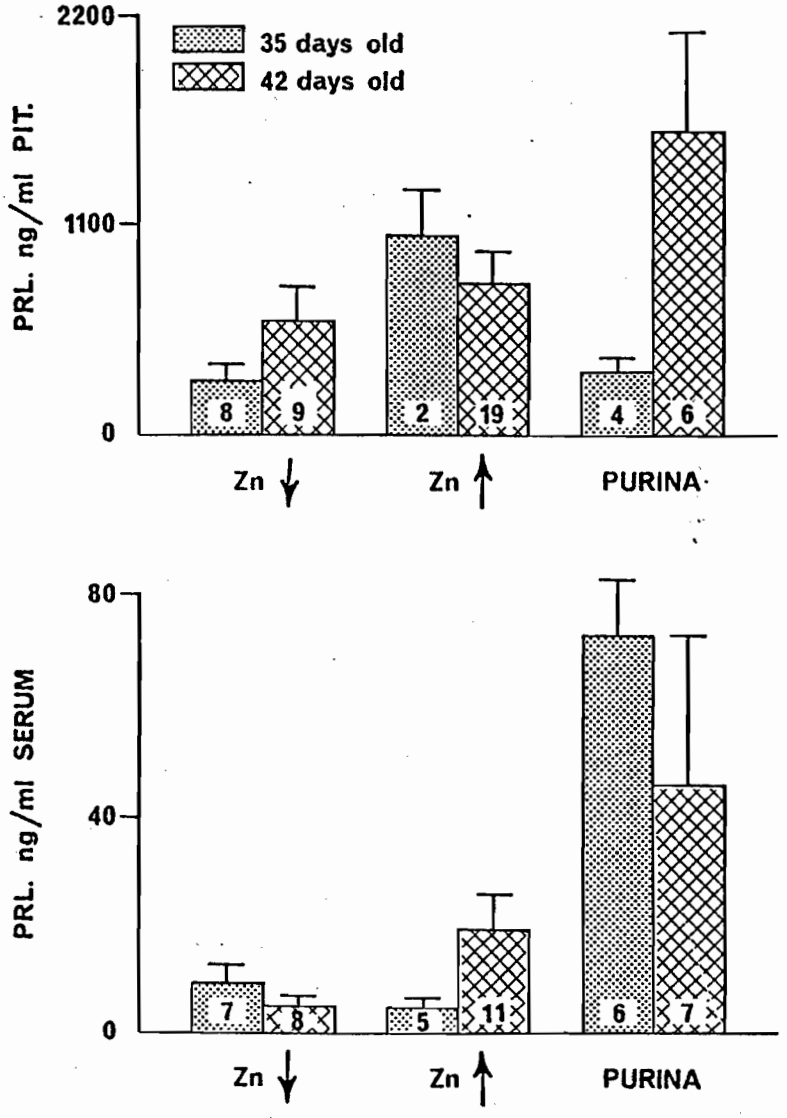
Pituitary and Serum LH Concentrations in 35 and 42 day old rats.

Exp VI Table X

PROLACTIN, PITUITARY AND SERUM CONCENTRATIONS (ng/ml)

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
PITUITARY	N	8	2	4	9	19	6
	MEAN± SEM	75.3 ± 18	267.0± 59	82.2±18	153.6± 41	199.9± 41	404.2 ± 127
	P	→	< 0.002	NS	→	NS	< 0.05
SERUM	N	7	5	6	8	11	7
	MEAN± SEM	9.3 ±3.1	4.7±2.1	71.9 ± 15.4	1.0 ± 0	18.9 ± 6.3	45.3 ± 27.5
	P	→	NS	< 0.001	→	< 0.02	NS

Exp. VI. Histogram 8



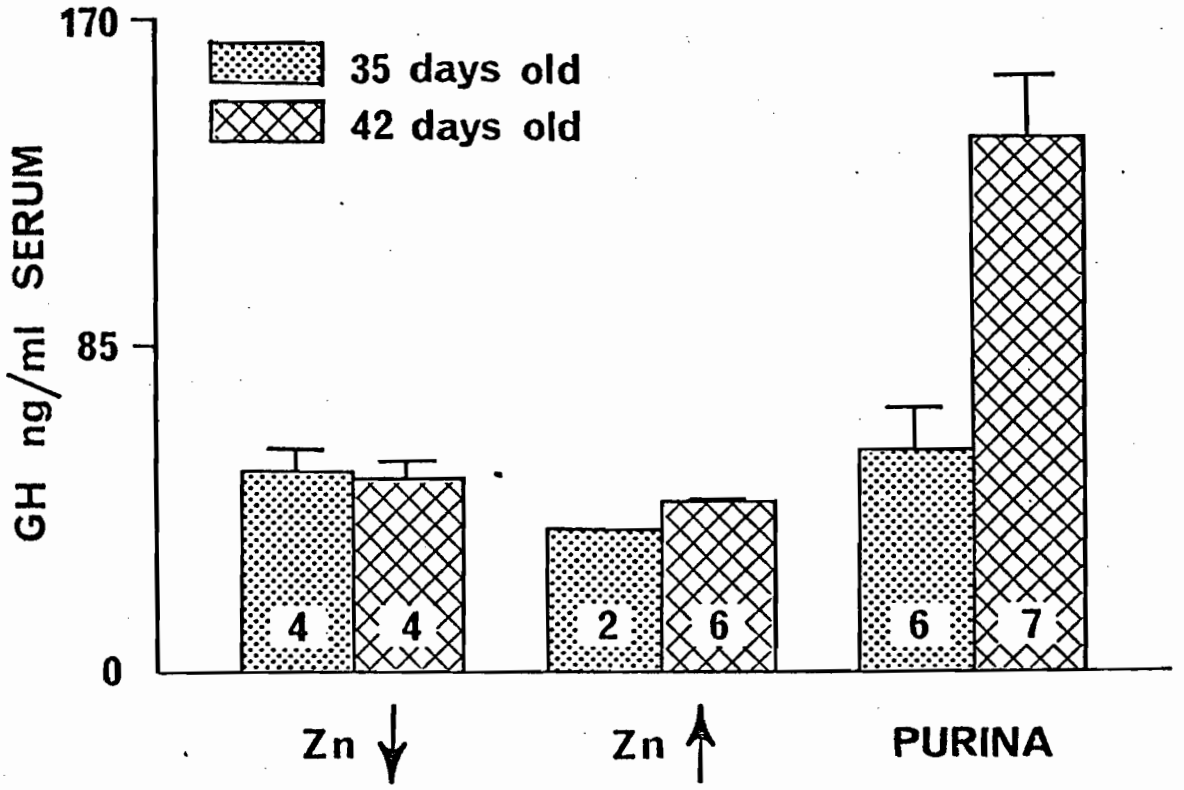
Pituitary and Serum Prolactin Concentrations in 35 and 42 day old rats.

Exp. VI. Table XI

GROWTH HORMONE, SERUM CONCENTRATIONS, ng/ml

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn ↑	Purina
SERUM	N	4	2	6	4	6	7
	MEAN± SEM	53.2± 6.3	38± 0	59 ± 8.9	52 ± 2.2	45 ± 1.3	139.7 ± 15.2
	P	→	NS	NS	→	< 0.01	<0.002

Exp. VI. Histogram 9



Serum Growth Hormone Concentrations at the ages 35 and 42 days

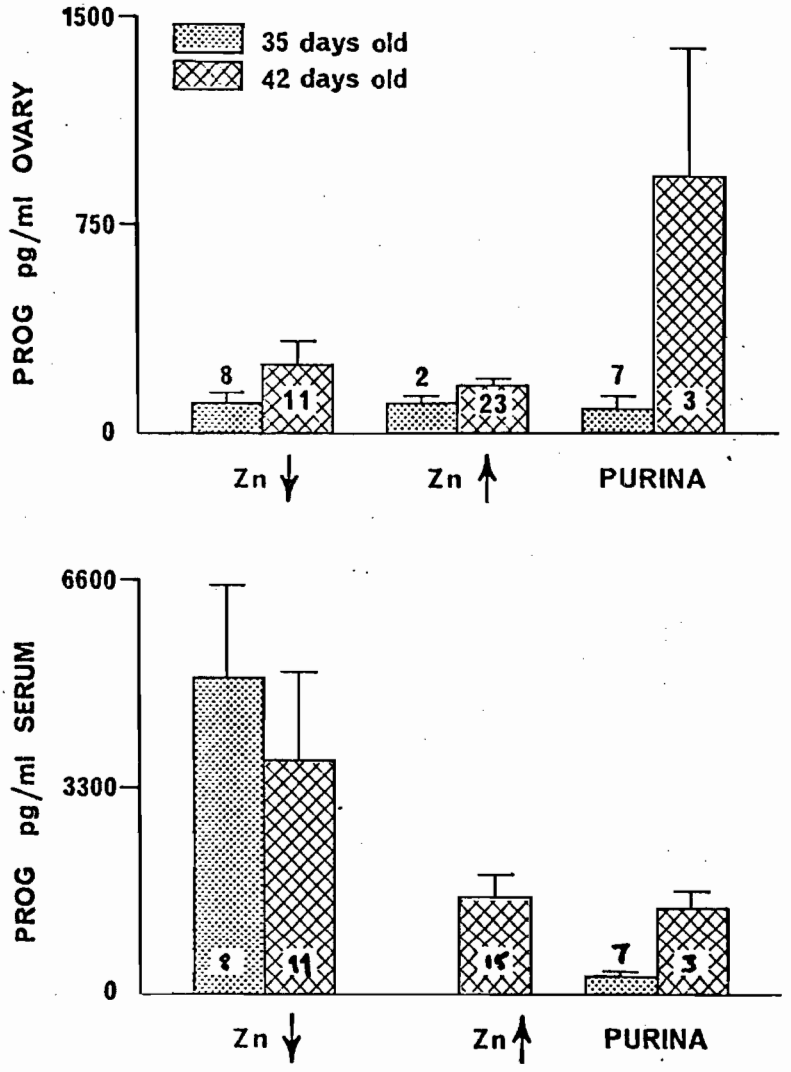
Exp VI Table XII

PROGESTERONE, OVARIAN AND SERUM CONCENTRATIONS (ng/ml)

Female rats

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
OVARY	N	8	2	7	11	23	3
	MEAN± SEM	110±24	109±12	84±38	242 ± 79	169±21	933±447
	P	→	NS	NS	→	NS	p<0.02
SERUM	N	4	—	6	8	8	7
	MEAN± SEM	5038± 1471	—	274 ± 20	3742 ± 1390	1591 ± 321	1367 ± 278
	P	→	—	>0.005	→	NS	NS

Exp. VI. Histogram 10



Ovarian and Serum Progesterone Concentrations
in 35 and 42 day old rats

Exp VI Table XIII

ESTRADIOL (E_2), OVARIAN CONCENTRATIONS (ng/ml) AND CORTICOSTERONE (B), SERUM CONCENTRATIONS (ng/ml)

Female rats

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
(E_2) OVARY	N	9	2	7	11	23	4
	MEAN ± SEM	43.4±4.9	23 ± 4	35 ± 3	55.9±12	39±5.5	224±122
	P	↘	NS	NS	↘	NS	< 0.03
(B) SERUM	N	9	2	6	11	10	7
	MEAN ± SEM	668 ± 86	1304± 107	90 ± 42	396±70	371±82	217± 39
	P	↘	> 0.01	> 0.0002	↘	NS	NS

Exp VI Table XIV

BIOCHEMICAL DATA - STATISTICAL SUMMARY

	35 d.o.			42 d.o.		
	N=6 Zn↓	Zn↑	N=4 PURINA	N=5 Zn↓	N=8 Zn↑	N=5 PURINA
Alkaline Phosphatase	—	—	<0.0000	—	—	<0.0001
SGPT	—	—	N.S.	—	—	NS
Cholesterol	—	—	< 0.02	—	< 0.02	NS
Ca^{++}	—	—	< 0.04	—	NS	NS
CO_2 TOTAL	—	—	NS	—	< 0.02	NS
Albumin	—	—	NS	—	NS	NS
Uric Acid	—	—	NS	—	NS	NS
Glucose	—	—	NS	—	NS	NS

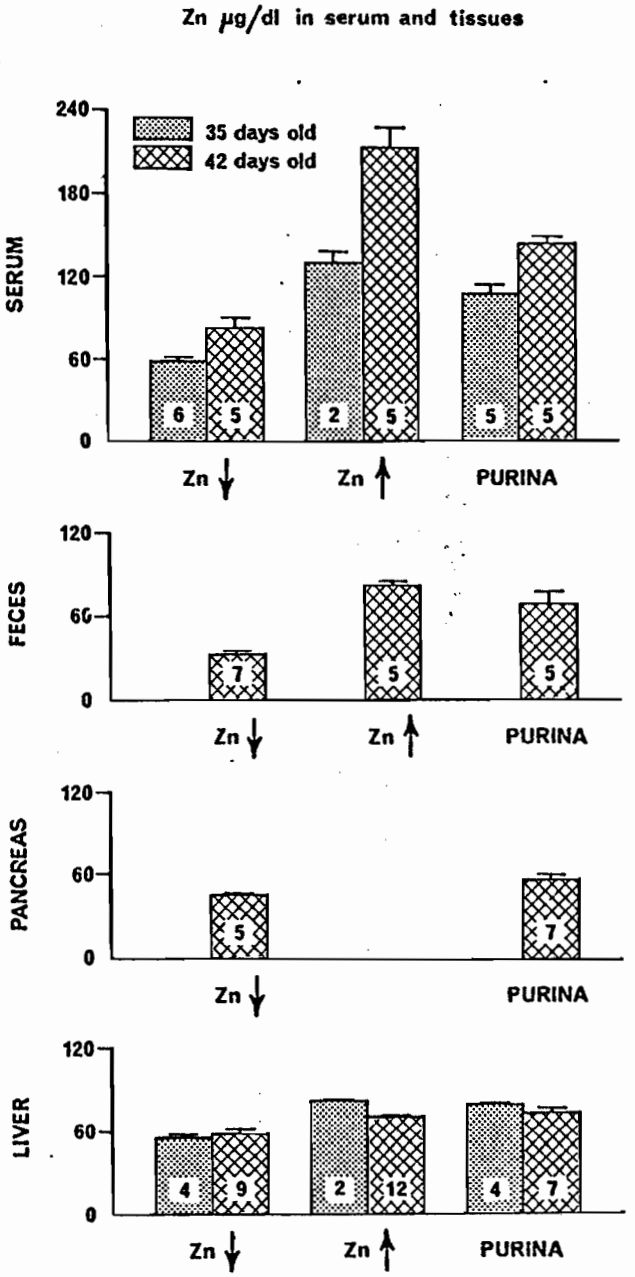
Exp VI Table XV

Zn CONCENTRATIONS IN SERUM AND TISSUES (µg/dl)

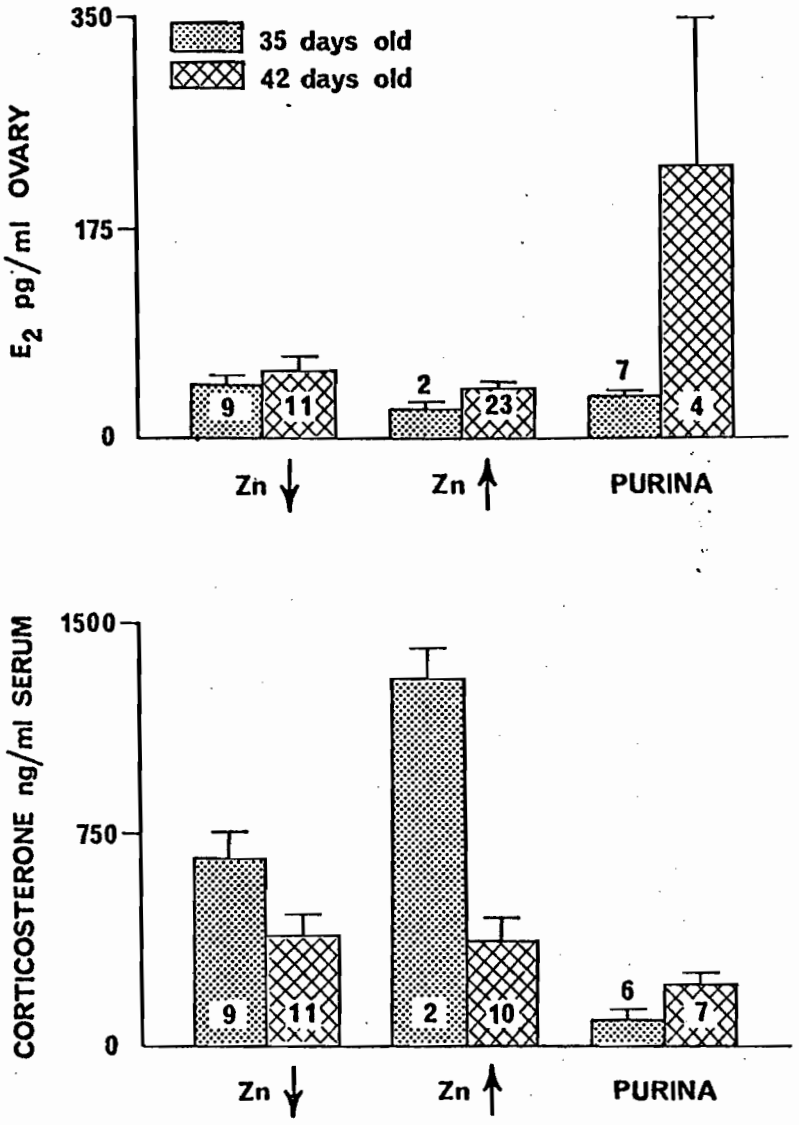
Female rats

		35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
SERUM	N				5	5	5
	MEAN± SEM				82 ± 7.1	211 ± 3.9	143 ± 4.1
	P				→	<.001	<.005
FECES	N				7	5	5
	MEAN± SEM				32.5 ± 2.5	82 ± 3.4	69.2 ± 7.4
	P				→	<0.01	<0.02
PANCREAS	N				5	—	7
	MEAN± SEM				45.2 ± 1.2	—	56.1 ± 2.8
	P				→	—	0.01
LIVER	N	4	2	4	9	12	7
	MEAN± SEM	54.6 ± 2.4	81.7 ± 0.7	78.9 ± 1.1	58.3 ± 2.8	70.4 ± 1.8	72.2 ± 2.3
	P	—	0.001	0.0001	→	<0.001	<0.002

Exp. VI. Histogram 11



Exp. VI. Histogram 12



Ovarian, Estradiol and Serum Corticosterone Concentrations in 35 and 42 day old rats

Exp VI Table XVI

STATISTICAL SUMMARY OF DATA ACCRUED

		Female rats 35 d.o.			42 d.o.		
		Zn↓	Zn↑	Purina	Zn↓	Zn↑	Purina
Body wt (g)		↓	NS	<0.0000	↓	<0.004	<0.0000
Pituat.wt (mg)		↓	NS	NS	↓	NS	<0.0000
Ovary wt (mg)		↓	NS	<0.03	↓	<0.0004	NS
PITUITARY	FSH	↓	NS	> 0.04	↓	< 0.03	< 0.0001
	LH	↓	NS	NS	↓	NS	NS
	PRL	↓	<0.002	NS	↓	NS	< 0.05
OVARY	E_2	↓	NS	NS	↓	NS	<0.03
	P_4	↓	NS	NS	↓	NS	<0.02
	B	—	0.06 (NS)	NS	—	NS	NS
SERUM	GH	↓	NS	NS	↓	<0.01	< 0.002
	LH	↓	NS	NS	↓	NS	<0.01
	FSH	↓	NS	NS	↓	NS	NS
	PRL	↓	NS	<0.001	↓	<0.02	NS*
	P_4	↓	NS	<0.005	↓	NS	N.S.
	B	↓	>0.009	>0.0002	↓	NS	NS

Exp VI Table XVII

PANCREAS, INSULIN AND SOMATOSTATIN CONCENTRATIONS (ng/ml)

	Zn↓	Zn ↑	Purina
Insulin N	6	6	7
MEAN ± SEM	342 ± 27.1	96 ± 16.6	396 ± 18.9
P	→	>0.0001	NS
Somatostatin N	6	6	7
MEAN ± SEM	5.8 ± 0.4	0.37 ± 0.05	4.5 ± 0.32
P	→	>0.0001	NS

Experiment VII:

The results from this experiment are tabulated as follows:

1. Table XVIII presents a statistical summary of the body weights of 56 day-old females.
2. Table XIX presents a statistical summary of the body weights at the ages 21 and 56 days old.
3. Table XX depicts the ovarian, adrenal and serum progesterone concentrations of the groups concerned.
4. Table XXI depicts the ovarian estrogen concentrations.
5. Table XXII depicts the pituitary and serum FSH concentrations.
6. Table XXIII presents the serum zinc of the groups investigated.
7. Histogram 13 depicts the body, ovarian and adrenal weights of the groups investigated.

One finding in this experiment is considered of specific interest: 5 out of the 10 zinc deficient females and 4 of the zinc deficient plus Placebo females - i.e. > 60% of the animals in these groups put together, had the vagina closed (sexually maturity), on the 56th day of life. In all the other cases, the vaginal opening occurred between the 35th and 38th day of life in all the animals involved. Sexual immaturity on day 56 of life in conditions of only relative zinc deficient regimen that allowed the body growth to low-normal size for sexual maturity to occur, can be considered as a direct, specific effect of zinc deficiency upon the process of sexual development. None of the 7 zinc deficient plus EFA females exhibited a closed vagina. This is taken to be a direct result of the effect of essential fatty acids upon zinc metabolism and certain

processes dependent on it. The growth pattern of the zinc deficient plus EFA females however was rather similar to that of the other zinc deficient groups. This result indicates on one hand that zinc supplementation is paramount to growth and on the other hand that the addition of essential fatty acids cannot counter all the effects of zinc deficiency.

Exp VII Table XVIII

BODY, PITUITARY, ADRENAL AND OVARY WEIGHTS OF 56 d.o. FEMALES

		Zn↓	Zn↓ + Placebo	Zn↓ + EFA	Zn↑	Zn↑ + EFA	Purina	Purina + EFA
BODY	N	10	4	8	8	8	8	8
	MEAN± SEM (g.)	113.5 ±3.9	105.5 ±7.2	168.3 ± 3.9	198.5 ± 5.4	195.2 ±3.7	199 ± 6.3	207 ± 3.5
	P	↘	NS	<0.0000	<0.000	<0.000	<0.000	<0.0000
PITUIT	N	9	4	7	7	7	7	7
	MEAN± SEM mg.	5.8 ± 0.8	5.2 ± 0.4	7.5 ± 0.4	9.6 ± 0.6	10.1± 0.4	8.6 ± 0.6	9.0 ± 0.5
	P	↘	NS	NS	<0.002	<0.0005	<0.01	<0.005
OVARY	N	5	4	7	7	7	7	7
	MEAN± SEM mg.	15.5 ± 1.7	18.9 ± 2.1	27.6 ± 1.6	30.4± 3.0	43.4 ± 3.5	33.9 ± 3.1	30.8 ± 5.1
	P	↘	NS	<0.0004	<0.003	<0.0001	<0.0009	<0.03
ADRENAL	N	5	4	7	7	7	7	7
	MEAN± SEM mg.	14.6 ± 1.6	18.8 ± 1.9	25.4 ± 1.5	24.7 ± 1.8	26.8 ± 1.5	29.7 ± 2.2	31 ± 1.3
	P	↘	NS	<0.0007	<0.002	<0.0003	<0.004	<0.0000

Exp VII Table XIX

BODY WEIGHTS AT AGE 21 AND 56 DAYS (GMS)

Age	21days	56days						
	Purina	Zn↓	Zn↓ + Placebo	Zn↓ + EFA	Zn↑	Zn↑ + EFA	Purina	Purina + EFA
N	54	10	4	8	8	8	8	8
Mean SEM	39.3± 1.3	113.5± 3.9	105.5± 7.2	168.3± 3.9	198.5 ± 5.4	195.2 ± 3.7	199± 6.3	207 ± 3.5

Exp VII Table XX

SERUM, OVARIAN AND ADRENAL PROGESTERONE CONCENTRATIONS (ng/ml)

	Zn ↑	Zn↓+Plac	Zn↓+EFA	Zn ↑	Zn ↑+EFA	Purina	Purina+EFA
OVARY							
N	0	3	6	7	7	7	7
MEAN ± SEM	3.3±1.1	4.7±2.74	20.6±1.97	11.2±2.7	6.11±0.5	4.6±.5	38.5± 6.92
P	→	NS	<0.0001	<0.007	<0.06(NS)	NS	<0.0000
ADRENAL							
N	10	4	7	7	7	7	7
MEAN ± SEM	20.6±4.6	10.9±1.6	29.4±6.1	34.7±2.9	20.0±2.7	30.9±3.03	26.3±2.9
P	→	NS	NS	<0.03	NS	NS	NS
SERUM N	12	4	6	6	7	6	6
MEAN ± SEM	1.9±0.7	1.8±0.7	5.3±1.9	3.1±0.5	3.1±0.8	3.1±0.2	3.1±0.5
P	→	NS	<0.05	NS	NS	NS	NS

Exp VII Table XXI

OVARIAN ESTRADIOL (pg/ml)

	Zn↓	Zn↓ + Placebo	Zn↓ + EFA	Zn↑	Zn↑ + EFA	Purina	Purina + EFA
N	9	2	5	6	7	5	7
Mean + SEM	33.2 ±5.2	26.7 ±8.6	160.6 ± 86.5	311.1 ± 181.2	504.8 ± 182.7	256.6 ± 153.3	179.6 ± 100.1
P	—	NS	NS	NS	0.01	NS	NS

Exp VII Table XXII

FSH, SERUM AND PITUITARY CONCENTRATIONS (ng/ml)

		Zn↓	Zn↓ + Placebo	Zn↓ + EFA	Zn↑	Zn↑ + EFA	Purina	Purina + EFA
SERUM	N	7	3	5	6	6	5	5
	Mean ± SEM	394 ± 38	416 ± 51	387 ± 38	381 ± 34	453 ± 53	433 ± 48	441 ± 64
	P	→	NS	NS	NS	NS	NS	NS
PITUITARY	N	5	2	4	3	4	4	4
	Mean ± SEM	2689 ± 264	2583 ± 100	1888 ± 154	1901 ± 428	1993 ± 154	1660 ± 351	1917 ± 157
	P	→	NS	>0.05	NS	NS	>0.05	>0.05

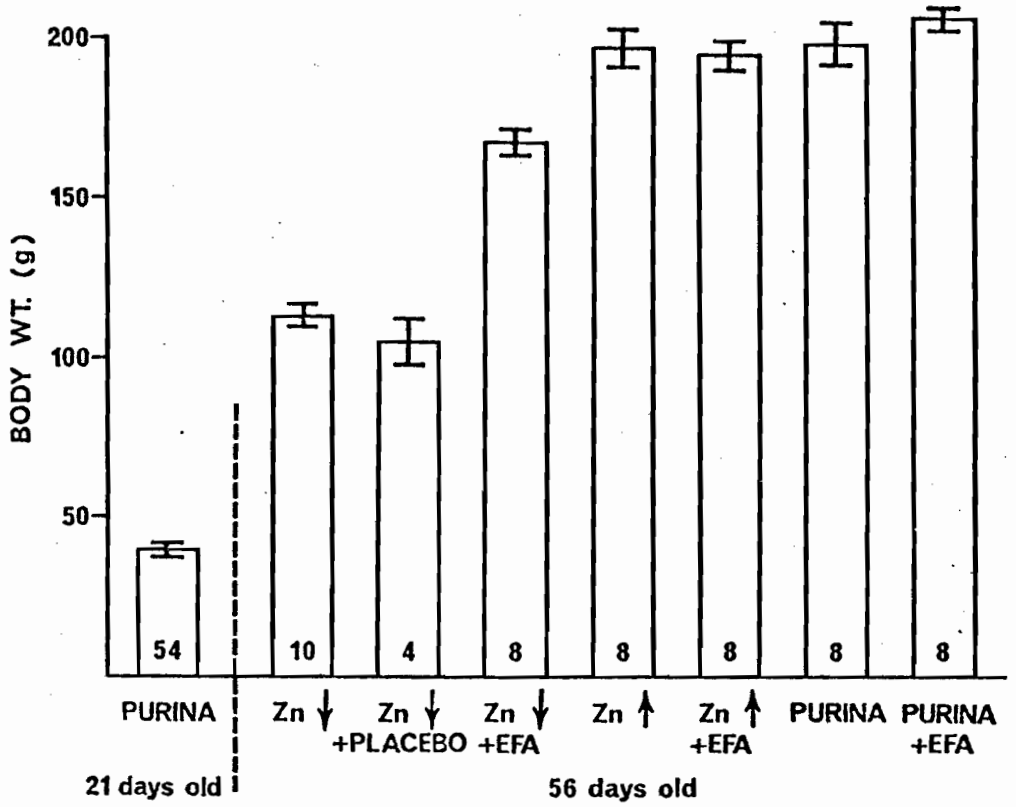
Exp VII Table XXIII

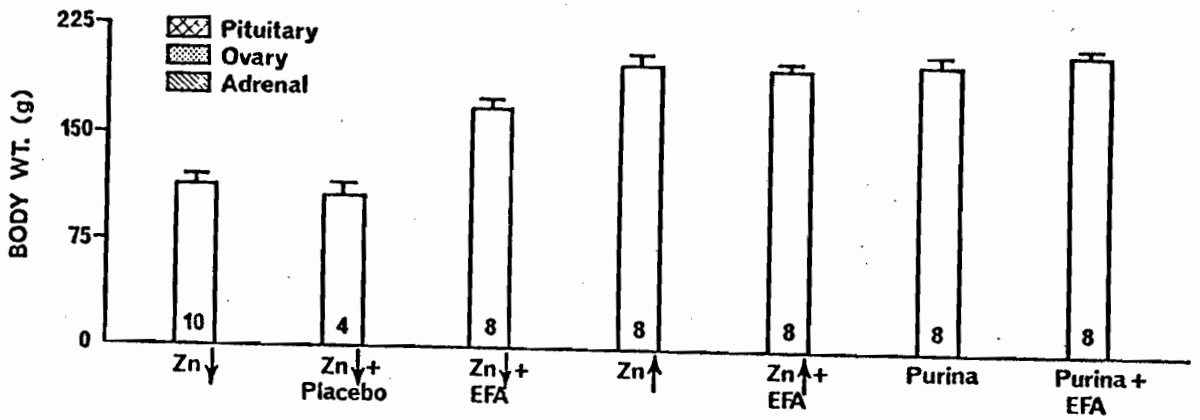
SERUM [Zn] (μg/dl)

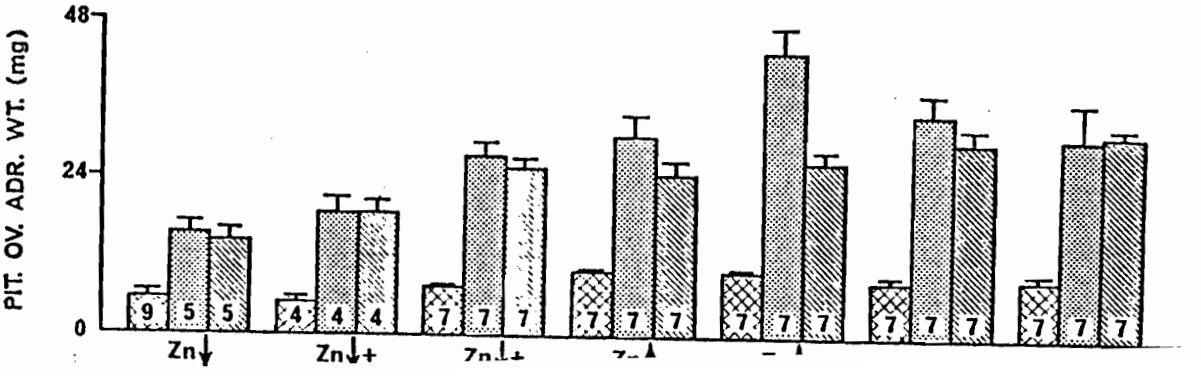
	Zn↓	Zn↓ + Placebo	Zn↓ + EFA	Zn↑	Zn↑ + EFA	Purina	Purina + EFA
N	5	4	5	5	5	5	5
MEAN± SEM	30 ±	27.5 ±	49.6±	153 ±	167 ±	142 ±	123 ±
P	→	NS	< 0.1(NS)	<0.000	<0.000	<0.000	<0.000

Exp. VII. Histogram 13

Body, Pituitary, Ovarian and Adrenal Weights







DISCUSSION

The experimental model of conditioned nutritional zinc deficiency provides a useful tool for the study of the processes of growth and sexual maturation and activity in rats. The model itself consists of ad libitum feeding of the experimental animals with a diet containing adequate amounts of all essential components of nutrition with the exception of zinc. The food contained less than 2 ppm zinc and the drinking water less than 0.05 ppm (demineralized water). If adequate care is taken to eliminate zinc from the "edible" environment, within 10 - 12 days the animals' appetite is determined to decrease and concomitantly, body size and weight reach and maintain a plateau. Sexual activity is arrested in adult female rats in such a condition (i.e. pregnancy does not occur or is not maintained beyond 2 - 3 days). Sexual maturity (i.e., vaginal opening) cannot be reached after 3 weeks of zinc deficient regimen (less than 2 ppm zinc) and can be significantly impeded (60% of the cases studied) if the animals are maintained on a zinc reduced diet (less than 10 ppm zinc) from weaning to maturity (i.e. age 56 days).

It has been established by several investigators that a minimal zinc concentration of 15 ppm is essential for normal growth (74, 76, 81). The rate of somatic growth is significantly reduced in a direct proportion to the zinc intake. The process of sexual maturation in female rats is significantly slowed down or completely arrested, again in proportion to the subminimal level of zinc available and ingested.

Precocious sexual maturation can be induced pharmacologically

by PMSG and/or estrogen-progesterone treatment in 26 day old Sprague-Dawley female rats born of dams kept on a zinc supplement diet (restricted in quantity as to equal that ingested by zinc deficient-regimen peers), and themselves kept on a regimen similar to their mothers'. Precocious sexual maturation could not be induced in the zinc deficient peers of the above group. The pharmacologic stimulants did have a utero-trophic effect, but ovulation per se could questionably be noted in only one fallopian tube among several investigated in repeated experiments. The detrimental effect of zinc deficiency upon both somatic growth and sexual maturation has brought under investigation the empiric hypothesis that a critical minimal body mass is paramount to sexual maturation(82). It is apparent from our investigations that there is a relationship between the "zinc factor" and the processes of growth and sexual maturation and activity. The relationship is rather "triangular", meaning that zinc deficiency inhibits on one hand growth and on the other, sexual maturation and activity. The degree of inhibition of each process is qualitatively and quantitatively inversely proportional to the zinc bioavailability. The "side" of the "triangle" relating directly growth to sexual maturation and activity is not quite clarified by the use of the experimental model of zinc deficiency. Adopting this model, at zinc levels less than 2 ppm, growth is skewed and arrested within 10 - 12 days, concomitantly with the appearance of alopecia, keratosis, and hemorrhages. Sexual maturation is inhibited only if the duration of the experiment extends beyond 14 days, as in the case of our protocol (i.e., to 21 days). If only a relative level of zinc deficiency is employed (zinc less than 10 ppm) a plateau in growth is reached within 28 - 35 days, no alopecia or keratosis is noted and sexual maturation occurs in about 40% of cases. It ought to

be further clarified that in the latter protocol, all the females in the zinc reduced group had reached the size at which typically the vagina opens by the age 35 - 37 days. Yet, at age 56 days, circa 60% of them had not reached that state, although no concurrent symptoms and signs of zinc deficiency (i.e., hemmorrhages, keratoses, alopecia) were observable. Thus, the postulate that sexual maturation does not occur unless a critical minimal body weight is reached is partly satisfied if one is not biased in the favor of considering that if sexual maturation is delayed by 3 weeks and only about 40% of the animals undergo it, the postulate is not quite valid.

It is probably more appropriate to describe the effects of zinc deficiency upon growth and sexual maturation and activity in terms of the effects zinc deficiency has upon the hormones involved, as discussed later in this section).

Restricted food intake, in the case of the pair-fed, zinc supplemented animals, clearly resulted in a reduced somatic development. However, in no case did it result in lack of sexual maturation or delay of it. Feeding the zinc supplemented group ad libitum, as in Exp. VII, resulted in a growth rate similar to that of the purina group. This is taken as undisputable evidence as to the quality of the nutrition employed. The effects of zinc deficiency and EFA treatment are elegantly demonstrated in this last experiment. Zinc deficiency in combination with EFA treatment are conducive to a significant increment in growth as compared to the zinc deficient and/or zinc deficient plus placebo (olive oil). However, the lack of zinc itself results in a significant difference in growth when compared to the zinc supplemented group. If zinc is provided, growth is not significantly increased by the addition of EFA. Although at this stage of the investigation, the actual biochemical

mechanisms of action are not clear, it is possible to state that the EFA treatment,exerts its effects on Zn retention in the tissues and in the serum (i.e., the liver and serum zinc concentration are significantly higher in the zinc deficient plus EFA group than in the zinc deficient or zinc deficient plus placebo groups). By the same mechanism(s) of zinc retention, sexual maturation is facilitated in animals having a sub-minimal zinc intake. In effect, all the females in the zinc deficient plus EFA group had the vagina opened by the age 56 days of life. Thus, it is possible to postulate that the effects of zinc on growth and on sexual maturation are separately achieved. That does not exclude the probability that the two processes are otherwise related and dysfunctions in one may influence the metabolism of the other.

Once adult size is achieved, zinc deficiency will inhibit further growth within 10 - 12 days. That amount of time seems to be necessary also for the inhibition of mating and/or capability of gestation (resorption of fetuses observed by several investigators, including us). During our investigation on pregnancy and lactation in conditions of zinc deficiency, induced after mating, not described in the previous section we noted that most dams reached the end of the gestation without exhibiting a significant increment in weight. Parturition is very stressful in all zinc deficient gestant rats. Based on a total of 36 deliveries, six rats died during the process of parturition, with the pups unborn (i.e., vaginal dilatation but no pups born). A peculiar phenomenon that could be defined as PARTUM BOTRUDON has been noted with regards to three deliveries. These animals were found to have died, evidently during the attempt of

delivery. The peculiar phenomenon was that the pups were half-born, all in a cluster, the umbilical cords serving as "branches to a common stalk". Two other rats managed to actually deliver "pup-clusters" and stay alive. In one of the latter cases, the pups thus born were dead (N=9), suffocated, as the mother took no care to cleanse them and allow respiration to occur. In the other case, the mother consumed the placentae of 3, (N=10) while the others died suffocated. All the other parturient rats (zinc deficient) observed had stressful and prolonged deliveries (4-5 hours) however, most pups were born alive and survived. The zinc deficient mothers took less care of the sucklings with respect to cleansing, covering or defending them against "intruders". Lactation was not observed to be different in the zinc deficient group but more accurate measurements are to be done in the future before precise statements can be made on the matter. On the whole, one can state that the processes of gestation and parturition are more stressful in the zinc deficient dams than in their pair-fed zinc supplemented peers. The pups of the former group grew to only about 60% the size of those of the latter group and exhibit during lactation the typical signs of zinc deficiency (i.e., relative lack of fur growth and keratosis including corneal keratosis, etc). After they are weaned, if placed on a zinc deficient diet, within 6-8 days these weanlings exhibit the typical relative anorexia and concomitantly arrest in growth. If placed on an ad libitum zinc supplemented or purina diet after weaning, the pups born and lactated by zinc deficient mothers, grow a normal fur within about 10 days and achieve normal size for the age within three weeks. (Experimental data of 12 males, not presented in the previous section). Thus, the effects of

zinc deficiency on growth and fur formation are not irreversible.

As described in the "Results" section, the levels of several hormones were determined in the serum and in organs such as the pituitary ovary and adrenals of the zinc deficient, zinc supplemented and purina fed animals.

Growth hormone was found to be significantly lower in the serum of the zinc deficient groups than in the zinc supplemented (pair-fed -20%) or the purina (ad libitum fed) group, after 3 weeks on the zinc deficient regimin. This finding provides a direct explanation for the lack of somatic growth in these animals. Insulin too was found to be significantly decreased in the serum of the zinc deficient animals ($P < 0.001$) when compared to that of the other two groups. It was also lower in the serum of the zinc supplemented pair-fed group ($P < 0.05$) when compared to the ad libitum purina fed group. Interestingly, the glycemia of the three groups did not differ. It is considered that in the former case the low insulin level was due to the shorter half-life of the hormone in the condition of nutritional zinc deficiency in these animals and perhaps also to the inability of the pancreas to synthesize appropriate amounts of insulin in the condition of zinc deficiency (i.e., the insulin hormone contains 3 zinc molecules in its structure).

The thyroid hormones T_4 and T_3 were determined. T_4 was significantly lower in the serum of the zinc deficient animals than in the serum of zinc supplemented peers. T_3 was not found to differ in the two experimental conditions. Considering the three hormones, GH, insulin, and T_4, abnormally low values of those could explain a variety of symptoms and signs in the zinc deficient animals: e.g., growth arrest keratosis (corneal, periocular, etc) hemorrhages, stressful parturition, and others. Relative deficiency of these hormones by itself could have a

significant effect on the processes of sexual maturation and activity as well as on the general metabolism. The gonadotropins were determined both in the pituitary and in the serum of the zinc deficient animals and the control groups. The pituitary FSH concentration was found to be significantly lower in the zinc deficient group than in the others after 3 weeks of zinc deficient regimen. The attempt of pharmacologic induction of precocious sexual maturation via estradiol-17β-progesterone treatment results in no FSH serum surge in the zinc deficient animals. However, the serum FSH levels after two or three weeks of zinc deficient regimen were not different among the zinc deficient group and the zinc supplemented or purina peers.

The LH concentrations in the pituitary were not different among the three groups, either 2 weeks or 3 weeks after the differential zinc regimen was instituted. No serum LH surges could be induced with either PMSG or estrogen-progesterone treatments, in the zinc deficient animals. The serum LH levels were not different among the three groups before the occurrence of sexual maturation, at age 35 days. At the age of 42 days, the serum LH levels were significantly higher in the purina group than in the zinc deficient group and the zinc supplemented (pair-fed -20%)group.

Prolactin was found to be significantly lower in the pituitary in the zinc deficient group at age 35 days in comparison with the zinc supplemented group and at 42 days of age in comparison with the purina group. In the serum, PRL was found to be significantly decreased with regards to the purina group both at the age 35 and 42 days. It was significantly lower at 42 days of age also with regards to the zinc supplemented

group (pair-fed or pair-fed -20%) groups. The pituitary and serum PRL concentration results seem to indicate that the PRL secretion is significantly depressed in the conditions of zinc deficiency. The PRL levels may seriously affect the lactation level and ... quality of the zinc deficient mothers. Accurate measurements of PRL and milk levels in this condition are projected. The ovarian levels of progesterone and estrogen were found to be significantly lower after 3 weeks of zinc deficient regimen when compared to those from the ad libitum, purina fed animals. The results obtained with regards to progesterone from the PMSG experiments, indicated that the serum levels were significantly lower in the zinc deficient animals when compared to the zinc supplemented peers. The results obtained from the estrogen-progesterone experiment indicated that the rate of catabolism of the progesterone was significantly decreased, probably as a result of hepatic dysfunction. The serum corticosterone and progesterone levels were not found to be generally different among the zinc deficient, zinc supplemented and purina groups after three weeks of the regimen. One week earlier than that, perhaps as a result of adrenal stress in the zinc deficient animals, the serum progesterone concentrations and corticosterone concentrations were significantly higher than those of the purina controls. Serum corticosterone at that age was also significantly higher in the zinc deficient group as compared to the zinc supplemented (pair fed -20%), group however the progesterone levels were not different.

To summarize the hormonal profile studied, zinc deficiency is generally associated with significantly decreased levels of several hormones. Not

all hormones determined were equally affected. This is taken to signify that zinc has a rather specific effect on each hormone studied so far (eg. insulin). As compared to other essential trace metals, the effect of zinc seems to be significantly at the neuro-endocrine level. This effect may be direct, at the hypothalamic-hypophysial level (e.g. hypopituitarism), it may be indirect, i.e., via more than 80 zinc enzymes, or probably is a combination of both. In my opinion, one ought not consider that there is only one mechanism of action of zinc upon the affected processes, but rather a combination of 1)direct zinc effects 2) zinc affected hormones and 3) zinc dependent enzymes.

It is hoped that the present investigation has contributed to a better understanding of the role of this essential trace metal in metabolism in general and in the process of growth and sexual maturation and activity in particular.

SUMMARY

Certain parameters of growth and sexual maturation and activity in female rats were investigated, based on an experimental model of chronic nutritional zinc deficiency. The results obtained indicate that the trace element zinc has an essential function in the metabolic processes studied. Zinc deficiency is associated with and causal to growth arrest. The hormones GH, insulin and T_4 are significantly reduced in the serum of the zinc deficient animals. The administration of essential fatty acids to such an experimental group results in a significant increase in growth, probably via the zinc mobilization effect of this compound.

Sexual maturation in female rats is inhibited in the condition of zinc deficiency. A slightly minimally depressed zinc concentration can be conducive to sexual maturation after a significant delay. The concentration of the hormones LH, FSH and PRL are reduced by zinc deficiency. Ovarian production of estrogen and progesterone is also significantly reduced. Attempts of pharmacologic induction of precocious sexual maturation via PMSG and estradiol-17β-progesterone treatment in zinc deficient female rats lead to a uterotrophic effect, but not to significant gonadotrophin surges nor to the expected rise in sex steroids. Ovulation probably can not occur in this condition. Pregnancy cannot occur or be maintained if mature females are fed on a zinc deficient diet for more than 7 days. If mature females are mated and then placed on such a regimen, gestation can continue but is stressful and delivery is very difficult. A peculiar phenomenon of stressful delivery has been noted and described. The growth of the pups of zinc deficient mothers is severely inhibited. The

condition can be reversed after weaning, following a long period of normal nutrition. The biochemical (SMA-24) profile is not specifically effected by zinc deficiency with the exception of alkaline phosphatase, a zinc enzyme. The stress of a partial or total zinc deficient diet is not reflected in the serum levels of corticosterone and glucose.

It has become evident from the investigation presented that zinc deficiency detrimentally affects several hormones at the secretory and circulatory levels, growth, sexual maturity and activity in female rats.

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PART VIII

STATISTICAL ANALYSIS

The significance of the data accrued was assessed by the use of student's t-test (one-tailed).

The computations were executed via a program set on a 9162-0050 Digital Cassette, and on a 9866A Model Printer, Hewlett-Packard.

The print-out expressed the means, standard deviations, standard errors, T-values and p-values of the groups submitted to statistical evaluation.

At all times the statistical significance was construed as a comparison between the zinc deficient groups and the others evaluated. The point of significance was taken at all times to be a value of $p \leq 0.05$.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

The following hormonal determinations, concomitant or not, are original and novel to the field of zinc deficiency investigated:

- Growth Hormone, serum concentration
- FSH, LH, and Prolactin, pituitary and serum levels.
- Tetraiodothyronine (T_4) and Triiodothyronine (T_3) serum levels.
- Insulin and somatostatin, pancreatic concentrations; insulin, serum levels.
- Progesterone, estrogen and corticosterone, ovarian levels.

The concomitant biochemical analysis - SMA-24, is novel to the field.

The attempt of pharmacological induction of precocious sexual maturation via Pregnant Mare Serum Gonadotropin (PMSG) and Estradiol-Progesterone (E_2-P_4) treatments is original and novel.

The treatment with essential fatty acids and the effects on growth and sexual maturation is novel (in collaboration with Dr. D. Horrobin and Mr. S. Cunnane, Montreal Research Institute).

- the observation of an atypical, stressful type of parturition defined as PARTUM BOTRUDON, is originally described in this investigation.
- this was the first investigation in the field of zinc studies in which the processes of growth, pregnancy, lactation, precocious induction of sexual maturation, and sexual maturation per se with the underlying hormonal and biochemical (SMA-24) profile were assessed together (i.e. eliminating the inherent variability of different strains of rats, nutrition, environmental conditions, investigative methods, etc.).

The results obtained from Experiment II have been submitted to the 13th Annual Conference of Trace Substances in Environmental Health (June 4-7, 1979, University of Missouri, Columbia).

The results obtained from Experiment V have been submitted to the Second International Symposium on Clinical Psycho-Neuro Endocrinology in Reproduction (Venice, June 1979).

The results obtained from Experiment VI have been submitted to the Congress of the Society For The Study of Reproduction (August 21-24, Université Laval, Quebec).