ZINC CONCENTRATIONS IN SELECT TISSUES FROM ACUTE ALLOXAN-DIABETIC MALE RATS

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

by

Kent D. Slemmons

December 1983

ZINC CONCENTRATIONS IN SELECT TISSUES FROM ACUTE ALLOXAN-DIABETIC MALE RATS

by

Kent D. Slemmons

APPROVED BY:

CO-DIRECTOR OF THESIS M. Evelyn McNeill
M. Evelyn McNeill, Ph.D.

CO-DIRECTOR OF THESIS Everett C Simpson
Everett C. Simpson, Ph.D

COMMITTEE MEMBER Gerhard W. Kalmus
Gerhard W. Kalmus, Ph.D

COMMITTEE MEMBER Takeru Ito
Takeru Ito, Ph.D.

CHAIRMAN OF THE DEPARTMENT OF BIOLOGY

Charles E. Bland, Ph.D

DEAN OF THE GRADUATE SCHOOL

Joseph G. Boyette, Ph.D

ABSTRACT

Kent D. Slemmons. ZINC CONCENTRATIONS IN SELECT TISSUES FROM ACUTE ALLOXAN-DIABETIC MALE RATS. (Under the direction of M. Evelyn McNeill, Ph.D. and Everett C. Simpson, Ph.D.). Department of Biology, December 1983.

Current evidence suggests that the diabetic condition may be accompanied by alterations in zinc metabolism. This study compared the zinc levels in select tissues from acute alloxan-diabetic and normal adult male rats. Of particular interest were tissues associated with reproduction and the complications of diabetes mellitus.

Experimental diabetes was induced in male Sprague-Dawley rats by intravenous alloxan injection (40 mg/kg body weight). Four weeks after alloxan administration, tissue zinc levels were determined by atomic absorption spectrophotometry. Zinc concentrations were significantly higher in the adenohypophysis ($P < 0.03$), brain ($P < 0.03$), and prostate ($P < 0.01$) of the diabetic group. Zinc concentrations in the lens, retina, pineal, neurohypophysis, hypothalamus, aorta, liver, kidney, and pancreas were similiar to controls.

These results, along with the results of other studies (Failla and Kiser, 1981; McNeill et al., 1981), suggest that experimental diabetes results in a buildup of zinc in select tissues.

ACKNOWLEDGMENT

I want to give my sincere thanks to my thesis co-directors, Dr. M. Evelyn McNeill and Dr. Everett C. Simpson for the help and encouragement they gave me while I completed this thesis. I also want to thank Dr. Gerhard W. Kalmus and Dr. Takeru Ito for their help with the manuscript. In addition, I want to thank the trio from the Trace Elements Lab, Dr. John Bray, Mr. Alex Webb, and Ms. Jessica Howells for their help with the zinc assay. And finally, I wish to thank Dr. Jacqueline F. McGinty for her time and assistance.

Supported in part by United Way Grant 0-58026 and Mary C. Helms Research Grant.

TABLE OF CONTENTS

PAGE

LIST OF TABLES v

LIST OF FIGURES vi

INTRODUCTION 1

A. Zinc Metabolism 1

B. Zinc and Insulin 6

C. Insulin and Diabetes Mellitus 9

D. Experimental Diabetes 10

E. Zinc and Diabetes Mellitus 10

MATERIALS AND METHODS 18

A. Animals 18

B. Tissue Collection 18

C. Trace Element Analysis 20

D. Miscellaneous 21

RESULTS 22

DISCUSSION 32

BIBLIOGRAPHY 39

APPENDIX A 47

Body weight and blood glucose levels

APPENDIX B 48

Wet weights and dry weights of tissue samples from alloxan-diabetic rats

APPENDIX C 49

Wet weights and dry weights of tissue samples from control rats

APPENDIX D..... 50

Zinc content and zinc concentration in tissue samples from alloxan-diabetic rats

APPENDIX E..... 51

Zinc content and zinc concentration in tissue samples from control rats

LIST OF TABLES

PAGE

Table I. Body weight, weight gain, and blood glucose levels of alloxan-diabetic and control rats..... 25

Table II. Wet weights and dry weights of tissues from alloxan-diabetic and control rats..... 26

Table III. Relative weights of tissues from alloxan-diabetic and control rats..... 27

Table IV. Percent weight loss due to freeze-drying tissues from alloxan-diabetic control rats..... 28

Table V. Zinc content of tissues delineated from alloxan-diabetic and control rats..... 29

Table VI. Zinc concentrations of tissues from alloxan-diabetic and control rats..... 30

LIST OF FIGURES

PAGE

Figure 1. Rat weight vs. time..... 24

Figure 2. Zinc concentrations of tissues from alloxan-diabetic and control rats (4 weeks duration).....31

INTRODUCTION

A. Zinc Metabolism

The interest in studying trace element metabolism has increased enormously in the past few decades. Primary reasons for this include the discovery of specific roles for many of the trace elements and the recognition of deficiency symptoms in humans and animals. Currently, fourteen trace elements are considered essential in animals, and this list includes the much studied element zinc (Mertz, 1981).

Zinc was first shown to be necessary for the growth of the fungus Aspergillus niger (Raulin, 1869). In 1934, Todd et al. demonstrated that zinc was required for the normal growth of the laboratory rat. Since then, the growth of a wide variety of plants and animals has been shown to be zinc dependent. The first specific biochemical role for zinc was described in 1940 by Keilin and Mann, who showed it to be a constituent of the enzyme carbonic anhydrase. Today, approximately 90 zinc-metalloenzymes have been discovered, and it is in this enzyme-related role that zinc is believed to have its greatest function (Prasad, 1982). Zinc-metalloenzymes are represented in every phyla, in each of the major enzyme systems, and affect such processes as nucleic acid, protein, carbohydrate, and lipid metabolism (Prasad, 1979). Zinc, like most naturally occurring metal ions, is primarily protein bound (Hughes, 1981).

Studies with rats have shown that zinc absorption takes place via the small intestine, primarily in the duodenum (Methfessel and Spencer, 1973; Davies, 1980). Factors which influence the amount of zinc ab-

sorbed include: 1) the amount of zinc ingested; 2) the tissue levels of zinc; 3) the presence of ligands or chelators in the gut which facilitate absorption; and, 4) the presence of substances in the diet itself that deter absorption (Sandstead, 1981). Picolinic acid (Evans, 1980) and prostaglandin E2 (Song and Adham, 1980) have been suggested to be endogenous facilitators of zinc absorption. Phytate (inositol hexaphosphate) and dietary fiber are exogenous substances (readily present in unrefined cereals, bran, nuts, and seed) that are known to inhibit zinc absorption (Reinhold et al., 1976).

The mechanism(s) by which zinc is absorbed remain(s) controversial. However, there appear to be at least three steps involved in the absorption process: 1) the attachment of zinc to specific receptors on the brush border surface; 2) the intracellular transfer of this zinc to the basolateral membrane of the absorptive cell; and, 3) the removal of zinc from the basolateral membrane to carrier molecules in the blood (Evans et al., 1975). The intracellular transport of zinc is believed to take place by an active transport system, not by simple diffusion (Davies, 1980). Current evidence suggests that a low molecular weight metalloprotein, metallothionein (MTN), may play a regulatory role in zinc absorption. Richards and Cousins (1976) proposed that when serum and tissue zinc levels are high, the intestinal mucosal cells increase their synthesis of MTN. This protein actively binds zinc, making it unavailable for absorption. This MTN-bound zinc is eventually excreted in the feces, as intestinal mucosal cells are continuously being sloughed and replaced by new cells. Metallothionein has already been

implicated in the storage and detoxification of trace elements in the liver and kidney (Prasad, 1982).

Vallee and Gibson (1948) have shown that in normal human blood, 75-85% of the zinc is found in erythrocytes; 12-22% in the plasma; and 3% in leukocytes. About two-thirds of the plasma zinc is loosely bound to albumin; about one-third is tightly bound to alpha-2 macroglobulin (Giroux, 1975; Chesters and Will, 1981). Other plasma proteins which have been shown to bind zinc are transferrin, ceruloplasmin, haptoglobin, and gamma globulins (Prasad and Oberleas, 1970). In addition, a small percentage of the plasma zinc is bound to amino acids, such as histidine, glutamine, threonine, cysteine, and lysine (Prasad and Oberleas, 1970).

Tissues known to have relatively high amounts of zinc include the liver, kidney, bone, retina, prostate, and muscle (Prasad, 1979). The biologically available stores of zinc are probably small, have a high turnover rate, and are most likely found in the soft tissues (Harper et al., 1979). In conditions of acute stress, however, tissue zinc stores are mobilized and redistributed within the body (Beisel et al., 1976; Spencer et al., 1976). Hyperzincuria is often observed in conditons characterized by a hypercatabolic state, such as surgery, burns, major fractures, diabetes mellitus, protein deprivation, and starvation (Casey and Hambridge, 1980).

The excretion of zinc occurs mainly through the gastrointestinal tract (Sandstead, 1981). Zinc is found in secretions of the salivary glands, intestinal mucosa, pancreas, and liver (Sandstead, 1981). It

is estimated that the endogenous loss of zinc via the gastrointestinal tract is 1-2 mg/day for adult humans (Prasad, 1982). Compared with feces, relatively little zinc is present in urine (Underwood, 1977; Spencer et al., 1979). Zinc bound to amino acids and other small ligands is able to be filtered out by the glomeruli of the kidney; zinc bound to larger substances such a plasma proteins is not normally present in urine. Adult human urinary zinc loss is about 0.5 mg/day (Prasad, 1982). Approximately 0.5 mg of zinc is excreted each day in sweat (Prasad, 1982), and in hot climates, such zinc losses may be considerably greater (Sandstead, 1981).

It is estimated that a normal 70 kg man contains about 1.5-2.0 g of zinc, and that with a well-balanced diet, the intake of zinc is about 10-12 mg/day (Prasad, 1982). In general, foods high in protein contain relatively high amounts of zinc. Meat, liver, eggs, seafood, and milk are good sources (Harper et al., 1979). Zinc deficiency is often associated with inadequate protein intake and/or the presence of substances in the diet which may inhibit zinc absorption (Prasad, 1982).

As of yet, there is no single reliable method in which to assess the zinc nutriture of an individual (Solomons, 1979; Phillips, 1982; Shapcott, 1982). Plasma (or serum) and hair are readily accessible tissues, but their zinc concentrations are unreliable guides to zinc status (Phillips, 1982; Shapcott, 1982). This is particularly true in the conditions of infection, myocardial infarction, and acute stress, as zinc may be mobilized from the plasma and redistributed to other

tissues (Prasad, 1982). Zinc concentrations have also been measured in erythrocytes, saliva, and fingernails, but the significance of such measurements is unknown (Solomons, 1979). In addition, the lack of set procedural standards for the collection and preparation of tissues for zinc analysis make comparisons between laboratories extremely difficult (Phillips, 1982; Shapcott, 1982). Presently, the only way to accurately determine the existence of a zinc deficiency in a subject is by a therapeutic response to zinc and zinc alone (Mertz, 1981; Sandstead, 1981).

Symptoms characteristic of zinc deficiency have been reported for many animals and man (Miller and Neathery, 1980). In animals, effects of zinc deficiency include reduced consumption of food, growth retardation, perakeratosis, brittle hair, impaired sexual function, and reduced wound healing (Miller and Neathery, 1980). In man, clinical manifestations of chronic zinc deficiency have been described as poor appetite, growth retardation, mental lethargy, and skin changes (Prasad, 1982). Two genetic disorders, acrodermatitis enteropathica and sickle-cell disease, have been shown to be associated with zinc deficiency (Prasad, 1982).

The reduced growth and impaired wound healing seen in zinc deficiency are believed to be related to the availability of zinc, in as much as zinc is required for DNA, RNA, and protein synthesis (Prasad, 1982). Zinc supplementation has been shown to accelerate wound healing in zinc-deficient rats, but not in normal rats (Prasad, 1982). Pories and Strain (1966) reported beneficial effects of oral zinc supplemen-

tation on wound healing in surgical patients. It now appears that these patients may have been zinc deficient, as zinc supplementation will not accelerate wound healing in zinc-sufficient patients (Hallbook and Lanner, 1972).

Studies with zinc deficient rats have shown reduced zinc levels in the liver, kidney, testes, bone, and pancreas (Prasad et al., 1967; Roth and Kirchgessner, 1981), and reduced activities in the zinc metalloenzymes alkaline phosphatase, carboxypeptidase A, carbonic anhydrase, and alcohol dehydrogenase (Prasad, 1982). However, zinc deficiency has no consistent effect on tissue zinc or zinc metalloenzymes, and the manifestations of zinc deficiency have not been conclusively related to a zinc loss from particular tissues or enzymes (Cunnane and Horrobin, 1980).

Despite all of the known functions of zinc, its overall metabolism in the body is not understood. Likewise, the specific biochemical abnormalities responsible for the symptoms of zinc deficiency are little known (Kirchgessner and Roth, 1980). Because the neuroendocrine system is the ultimate integrator of biochemical processes leading to homeostasis, relationships between zinc and the neuroendocrine system have been sought. It has become increasingly evident that zinc-hormone interactions are bi-directional, i.e., zinc has effects on hormone metabolism and, conversely, hormones have an effect on zinc metabolism (Henkin, 1980; Kirchgessner and Roth, 1980).

B. Zinc and Insulin

Insulin is the most studied hormone in terms of its relationship

to zinc. Insulin is produced by the pancreas, a gland having both endocrine and exocrine functions. The endocrine portion of the pancreas consists of scattered clusters of cells called islets, which make up 1 to 2% of the pancreatic mass (Turner and Bagnara, 1976). Certain islet cells called B-cells function in the synthesis, storage, and secretion of insulin. Insulin is first formed on the B-cell ribosome as preproinsulin, a single chain peptide of about 109 amino acids (Steiner, 1976). Preproinsulin is quickly coverted into proinsulin via proteolytic cleavage. Structurally, proinsulin consists of an A-chain (21 amino acids in length), a B-chain (30 residues), and an interconnecting C-peptide (about 33 residues). Additionally, there are three disulfide bonds in the proinsulin molecule: two interchain (A7-B7; A20-B19) and one intrachain (A6-A11). Once formed, proinsulin is transported by the endoplasmic reticulum to the Golgi apparatus, where it is packaged into secretory granules (Steiner, 1976). In the granules, proteolytic enzymes cleave the C-peptide from proinsulin (along with a pair of basic residues on either side of the C-peptide), forming insulin (Steiner, 1976; Roth and Kirchgessner, 1981). The A- and B-chains of the insulin molecule are held together by the aforementioned disulfide bonds. By complexing with zinc, insulin is able to take on an insoluble, osmotically inactive, and biochemically stable crystalline form (Roth and Kirchgessner, 1981). This zinc-insulin complex is a way of storing "ready-made" insulin until it is needed by the body. Upon stimulation by glucose (or certain other chemical factors), the zinc-insulin complex dissociates and the storage granules

are released, via exocytosis, to the blood. Along with insulin and C-peptide, small amounts of proinsulin, peptide intermediates, and zinc are released from the granules (Steiner, 1976). The extent, if any, to which serum zinc influences the integrity of serum insulin is not known (Roth and Kirchgessner, 1981).

Insulin is, without a doubt, one of the most important anabolic hormones of the body, as shown by its profound effects on carbohydrate, lipid, and protein metabolism. An outstanding feature of insulin is its hypoglycemic effect, i.e., its ability to mediate glucose transport from the blood into target cells. Insulin also acts to stimulate the formation of glycogen (glycogenesis), fats (lipogenesis), and proteins (proteogenesis), while at the same time, inhibiting the breakdown of these substances (Turner and Bagnara, 1976). In view of the fact that insulin is such an important anabolic hormone, it seems likely that any alterations in the normal metabolism of insulin would affect, at least indirectly, zinc metabolism. This idea is supported by the fact that hormones are not "islands" in and of themselves. Rather, the effects of one hormone are counterbalanced by the effects of other hormones, with homeostasis being achieved through harmonious hormonal interplay and regulation. Thus, an upset in insulin metabolism would affect not only those processes directly associated with insulin, but also those of other hormones as well. Glucagon, somatotropin, glucocorticoids, and epinephrine all have metabolic effects opposed to those of insulin (Lehninger, 1982).

C. Insulin and Diabetes Mellitus

An upset in normal insulin metabolism is seen in diabetes mellitus. Although the etiology of diabetes mellitus is not known, it is characterized by the insufficient production of insulin relative to the body's needs (Harper et al., 1979). Many tissues of the body depend on insulin to facilitate glucose uptake. When insulin levels are too low, these tissues are unable to absorb glucose effectively, resulting in elevated blood glucose levels (hyperglycemia). In effect, these tissues are "starving" for glucose, despite its being present in excessive amounts in the blood. The body compensates for this glucose starvation by converting available energy resources into glucose, as evidenced by increases in the breakdown of glycogen (glycogenolysis), adipose tissue (lipolysis), and the conversion of amino acids into glucose (gluconeogenesis). The increased glycogenolysis, lipolysis, and gluconeogenesis are in part due to the effects of glucagon, a hormone of pancreatic A-cell and gastrointestinal origin. The secretion of glucagon is increased in diabetes mellitus (Ganong, 1981), resulting in a high glucagon:insulin ratio. As glucagon and insulin have opposite metabolic effects, this high ratio results in overall catabolic (glucagon) activities (Unger et al., 1978). Other characteristics of diabetes mellitus include glycosuria (glucose in urine), polyuria (increased urine production), polydipsia (increased water intake), and polyphagia (increased food intake).

The most important factor predisposing to the chronic complications of diabetes mellitus is generalized vascular disease

(Kefalides, 1981). Complications involve large and medium arteries (macroangiopathy), and capillaries (microangiopathy). Microangiopathy is characterized by endothelial cell proliferation, loss of pericytes, and thickening of the basement membrane; however, the pathogenesis of these processes is not known (Kefalides, 1981). The capillaries of the glomerulus, retina, skin, and striated muscle are particulary affected (Kefalides, 1981). Even in insulin-treated patients, chronic complications affect the eyes, kidneys, nerves, and arteries (Brownlee and Cerami, 1981).

D. Experimental Diabetes

Alloxan and streptozotocin (STZ) are of particular use in the study of diabetes mellitus. When injected into laboratory animals, each of these compounds is reported to selectively destroy the B-cells of the pancreas, with minimal damage to other body tissues (Rerup, 1970). These two drugs are, in effect, highly diabetogenic, greatly reducing insulin secretion. The severity of the diabetic condition depends upon the species of the animal, and the dosage given. The duration of the diabetes is variable; most rats and mice eventually recover, i.e., become normoglycemic (Rerup, 1970).

E. Zinc and Diabetes Mellitus

Current evidence strongly suggests that the diabetic condition is accompanied by alterations in zinc metabolism. In recent studies, male STZ-diabetic rats exhibited significant increases in dietary zinc absorption (Craft and Failla, 1983) and urinary zinc excretion (Lau and Failla, personal communication), as compared to normoglycemic rats. In

studies with humans, insulin treated diabetics showed higher urinary zinc levels than normal controls (Meltzer et al., 1962; Kumar and Rao, 1974; McNair et al., 1981). Streptozotocin-diabetic rats have been reported to have significant increases in hepatic and renal zinc concentrations as compared to controls (Failla and Kiser, 1981, 1983; Lau and Failla, personal communication). It has been suggested that increased tissue zinc concentrations are due, in part, to the increased absorption of dietary zinc in the STZ-diabetic rat (Craft and Failla, 1983). On the other hand, zinc levels in the spleen and muscle were reported similiar in both STZ-diabetic and normal rats (Failla and Kiser, 1981). Zinc concentrations in both the duodenum and plasma have been reported to be higher (Failla and Kiser, 1981), or similiar (Failla and Kiser, 1983; Lau and Failla, personal communication) in STZ-diabetic vs. control rats.

It is interesting to note that some of the symptoms of zinc deficiency (i.e., growth retardation, delayed wound healing, and sexual dysfunction) are similiar to those found in diabetes mellitus. Recent evidence suggests that the reduced growth seen in both of these conditions may be due, in part, to reduced somatomedin activity. Somatomedins are peptide hormones, believed to be of hepatic and renal origin (Ganong, 1981), which are secreted in response to growth hormone (somatotrophin) from the anterior pituitary (adenohypophysis). Somatomedins have insulin-like activity, but do not bind to anti-insulin antibodies (Ganong, 1981). They have a positive effect on cartilage synthesis and cell proliferation (Ganong, 1981). In a study using

zinc-deficient and control rats (Oner and Bor, 1978), the zinc-deficient group had significantly lower serum somatomedin-A and serum insulin levels. In another study, male acute STZ-diabetic rats were shown to have significant decreases in both serum somatomedin and cartilage growth activity (Phillips and Young, 1976). In the latter study, the administration of insulin, but not growth hormone, increased the activities of both somatomedin and cartilage growth in the STZ-diabetic rats. In view of these results, it was concluded that: 1) insulin deficiency resulted in reduced somatomedin and cartilage growth activities, and 2) growth failure in diabetes may be due to decreased somatomedin activity (Phillips and Young, 1976).

Impaired wound healing has been observed in both zinc deficiency and diabetes mellitus. The speed with which a wound is repaired depends greatly on the ability of fibroblasts to produce collagen. Collagen is the major extracellular structural protein in connective tissue and bone, and constitutes about one-third of the total body protein (Lehninger, 1982). Collagen synthesis requires the presence of zinc, as does the manufacture of other proteins (Prasad, 1982). Zinc deficient rats, which have a reduced ability to heal incised wounds (Prasad, 1982), have exhibited a significant reduction in the collagen and non-collagenous protein content of skin and loose connective tissue (Fernandez-Madrid et al., 1973; Prasad, 1982). It has been suggested that the impaired healing seen in some diabetic ulcers may be related to zinc deficiency (Sandstead, 1981; Prasad, 1982). Zinc supplementation has improved wound healing in some diabetic patients

(Henzel et al., 1970). An increase in skin collagen catabolism has been observed in STZ-diabetic rats (Schneir et al., 1982).

Impaired sexual function is another malady common to both zinc deficiency and diabetes mellitus. Reduced testicular androgen production has been observed in male rats in both conditions (Cusan et al., 1980; Howland and Zebrowski, 1976; Lei et al., 1976), although the reason(s) for the reduction are still not clear. As normal testicular function is dependant upon an intact hypothalamo-pituitary-gonadal axis, researchers have looked at all three organs as possible sites for the endocrine lesion.

The pituitary has long been called the "master gland" of the body, secreting a number of hormones, most of which have been shown to act at specific target glands. However, it is now known that the release of the anterior pituitary (adenohypophysis) hormones is under the direct influence of the hypothalamus. Two of the regulatory hormones that the hypothalamus secretes to the adenohypophysis are follicle stimulating hormone-releasing hormone (FSH-RH) and luteinizing hormone-releasing hormone (LH-RH). These releasing hormones prompt the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH), respectively, from the adenohypophysis. Once in the bloodstream, FSH and LH travel to the testes, where they attach to specific cellular receptor sites. Follicle stimulating hormone (FSH) acts on the seminiferous tubules to initiate spermatogenesis. Luteinizing hormone (LH) stimulates the interstitial cells of Leydig to secrete male sex hormones (androgens), which are steroidal in nature. Testosterone is

the major androgen produced and, like insulin, is primarily an anabolic hormone.

The reasons why zinc deficiency causes reduced testicular androgen production is not known. Some findings indicate that the hyposecretion of pituitary gonadotrophins (FSH and LH) may be the cause (Henkin, 1976), while others point to an impaired ability of the testes to carry out steroidogenesis (Lei et al., 1976). The depressed androgen production in diabetes mellitus also has been attributed to reduced gonadotrophin release (Cusan et al., 1980; Howland and Zebrowski, 1976, 1980; Hutson et al., 1983) or of the reduced ability to produce gonadal steroids (Adashi et al., 1982). In the latter study, the authors concluded that adequate levels of insulin are needed for androgenesis, as insulin was shown to indirectly augment testosterone production at the testicular level.

Divalent cations present in fractions of bovine hypothalamic tissue have been reported to have effects upon the release of adeno-hypophysial trophic hormones (La Bella et al., 1973). Copper had the greatest effect, stimulating the release of all hormones; zinc and nickel stimulated the release of all hormones except prolactin.

Another factor which may play a part in the regulation of the hypothalamo-pituitary-gonadal axis is the pineal gland. The pineal produces melatonin, an indole hormone suggested to have antigonadal effects. Melatonin is synthesized from serotonin, a process utilizing the enzymes N-acetyl-tranferase (NAT) and hydroxyindole-O-methyl-transferase (HIOMT).

Suggestions have been made that the pineal may act as a neuroendocrine transducer, mediating (via melatonin in some species) the effects of light conditions on the gonads (Turner and Bagnara, 1976). The site(s) at which melatonin may exert its effects upon the hypothalamo-pituitary-gonadal axis are not known. The pineal gland functions in a diurnal rhythm, with its highest activity found during the dark phase of the photoperiod (Johnson and Reiter, 1978). Of all the laboratory animals studied, the golden hamster is the most sensitive in terms of the effects of environmental lighting on reproductive function; rats are much less sensitive (Johnson and Reiter, 1978). A recent study has demonstrated that male rats between 20 and 40 days of age are more sensitive to the effects of exogenous melatonin administration than male rats 5-20 or 70-90 days old (Lang et al., 1983). The 20-40 day old group exhibited reduced plasma FSH, LH, and testosterone levels, as compared with controls. Also, the number of gonadotrophin hormone-releasing hormone (GnRH) receptors were fewer in number in the pituitaries of the melatonin-injected 20-40 day old rats. The adult rats (70-90 days old) in the study were relatively unresponsive to melatonin administration.

The pineal receives its information on light conditions through an indirect connection with the retina. Information travels from the retina to the suprachiasmatic nucleus of the hypothalamus, to the preganglionic sympathetic neurons in the upper thoracic intermediolateral cell column, to the superior cervical ganglia, and finally (via postganglionic sympathetic fibers) to the pineal (Moore, 1978).

The pineal has been reported to have a high concentration of zinc (Wong and Fritze, 1969; Demmel et al., 1982), the significance of which is not known. It has been suggested that zinc may play an enzymatic role in pineal biochemistry, or perhaps be involved as a constituent of a so far undetected neurotransmitter (Demmel et al., 1982).

Pinealectomy has been reported to affect tissue zinc levels in male rats. Eight weeks following pinealectomy, zinc in the thoracic aorta was found to be significantly increased, while pituitary, adrenal, heart, lung, and hair zinc levels were significantly reduced (Cunnane et al., 1979). Interestingly, impaired wound healing has been observed following pinealectomy (Weichselbaum et al., 1975), in zinc deficiency (Prasad, 1982), and in some cases of diabetes mellitus (Henzel et al., 1970).

In summary, sexual dysfunction is a symptom of both zinc deficiency and diabetes mellitus. The pineal gland, which has a high concentration of zinc, has been implicated in photoperiodic regulation of reproduction in rodents. Impaired wound healing has been observed in zinc deficiency, diabetes mellitus, and in pinealectomy. Although diabetes mellitus has been studied in great detail, the etiology of the disease is still unknown. Current evidence suggests that diabetes mellitus may be accompanied by alterations in zinc metabolism. With this in mind, the present study was undertaken to determine the effects of acute alloxan-diabetes on tissue zinc concentrations in adult male rats (adult female rats were not used in order to avoid the additional biochemical variables estrous cycling would incur). Of particular

interest were tissues known to be affected by diabetes, and tissues associated with reproduction. Zinc levels in the lens, retina, pineal, adenohypophysis, neurohypophysis, hypothalamus, brain, aorta, liver, kidney, pancreas, and prostate were measured.

MATERIALS AND METHODS

A. Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 205-256 g were used. The rats were housed individually in stainless steel cages, in a temperature-controlled room, with a light/dark cycle of 12 hours/12 hours (lights on at 0700 hours). Tap water and rat chow were available ad libitum. In order to induce a diabetic state, half of the rats received a single tail vein injection of a freshly prepared solution of alloxan (Eastman Kodak Co., Rochester, NY), at a dose of 40 mg alloxan/kg body weight. Control rats were not treated in any manner. Body weights and blood glucose levels were measured four weeks later, one day before sacrifice. The criterion for considering a rat as diabetic was a blood glucose level in excess of 300 mg/dl.

B. Tissue Collection

Eight alloxan-diabetic and 8 control rats were sacrificed, November 10, 1982, 30 days after alloxan injection. The specific date of sacrifice is noted because many animals exhibit seasonal variation in their reproductive physiology. The rats were sacrificed by cervical dislocation at 1000-1230 hours. Following dislocation, the thoracic cavity was opened ventrally, and blood was drawn from the exposed heart into a heparinized syringe. Collected blood samples were placed in heparinized test tubes, covered with Parafilm, and temporarily stored on ice. Centrifugation of the samples was performed the same day, at 700 X g for 15 min. The samples were then frozen (-18 degrees C) until

analysis. After the blood was drawn, the sacrificed rats were decapitated and each carcass was placed into a plastic bag and stored in a refrigerator (1.5 degrees C) until dissection. From each rat head, the retina from the right eye and the lens from the left eye were removed. After weighing, these tissues were placed into acid washed collection vials and frozen (- 18 degrees C). Upon removal of the eyes, each rat head was immediately placed into a plastic bag and frozen.

The carcasses were dissected the day of sacrifice, with each carcass being removed from the refrigerator and dissected individually. The dissected tissues were immediately wet weighed, placed into acid washed collection vials, and frozen. The following tissues were collected from each rat: 1) aorta: the arch of the aorta was removed, with branches arising from the arch being cut close to their origin. After flushing with distilled-deionized water, the aorta was gently blotted dry and wet weighed; 2) liver: a sample was removed from the median lobe; 3) kidney: a cross-sectional slice, caudal to the hilus, was taken from the right kidney; 4) pancreas: a sample was taken from the gastro-splenic portion of the pancreas; and, 5) prostate: a portion of the ventral lobe was collected.

The day after sacrifice, each rat head was removed from the freezer and allowed to slightly thaw. The soft tissue was removed, and the cranium was opened by separating the occipital and parietal bones. The pineal, adenohypophysis, and neurohypophysis were collected. In the process of dissection, the pars intermedia was fragmented between

the adenohypophysis and neurohypophysis, with the majority being associated with the latter. The remaining brain was refrozen and dissected the following day, with the following tissues being collected: 1) hypothalamus: this tissue was excised using the optic chiasma, mammillary bodies, and anterior commisure as anterior, posterior, and dorsal landmarks, respectively; and, 2) brain: this sample consisted of all tissue anterior to the pyramidal decussation minus the hypothalamus and olfactory bulbs.

C. Trace Element Analysis

Tissues were freeze-dried for 4 days except for the brain, pancreas, and prostate, which were freeze-dried for 6 days. All freeze-dried tissues were weighed on a Cahn 26 Electrobalance (Cahn Instruments, Cerritos, CA) and recorded as dry tissue weight.

To each sample vial, 0.5 ml redistilled nitric acid (G. Frederick Smith Chemical Co., Columbus, OH) was added. The samples were then sequentially heated at 70 degrees C for 1 hour, 120 degrees C for 1 hour, and then at 140 degrees C until dryness. Next, 1.0 ml of 2% nitric acid was added to each sample vial and the zinc content of the samples (along with appropriately diluted standards) were determined by atomic absorption spectrophotometry (Model 951, Atomic Absorption Spectrophotometer, Instrumentation Laboratory, Inc., Decator, GA).

The serum samples were of very small volume and upon visual inspection, were seen to be contaminated with red blood cells. For these reasons, the serum zinc levels were not measured.

D. Miscellaneous

1) Glassware: All glassware was initially rinsed 7X in distilled-deionized water (18 megaohm) (Sybron-Barnstead NANO-pure A water purification system, Wilimington, MA), then acid washed 24 hours in 2% nitric acid, rinsed again 7X in distilled-deionized water, and oven dried. Glass vials (Kimble No. 60975-L) were used in collecting and freeze drying samples; after weighing, freeze-dried samples were transferred to glass test tubes (Pyrex No. 9800-12) for zinc analysis.

2) Surgical instruments: All were stainless steel, and were initially washed in detergent (Alconex, Inc., NY, NY), then rinsed 7X with distilled-deionized water, soaked in a 1% EDTA solution for 1 hour, rinsed 7X with distilled-deionized water, and oven dried.

3) Blood glucose: Blood glucose was measured with a Technicon Auto Analyszer, using the alkaline ferricyanide method (Hoffman, 1937).

4) Statistics: Data were analysed using a Hewlett-Packard System 45-B Desktop Computer and General Statistics program. Data are presented as mean +/- standard error and were evaluated by the Mann Whitney test.

RESULTS

Rats injected with alloxan (40 mg/kg body weight) gained weight at a slower rate than control rats (Figure 1) and had lower terminal body weights than controls (Table I). The alloxan-diabetic rats characteristically exhibited increased consumption of water and food, along with polyuria and diarrhea. The alloxan-diabetic group also had much higher blood glucose levels (Table I).

The wet weights and dry weights of the lens, retina, and adenohypophysis were significantly lower in the alloxan-diabetic group ($P = 0.05$ or less) as compared to controls (Table II). The wet weights and dry weights of the pineal and neurohypophysis were not significantly different between the two groups. At the time of collection, the prostates in the alloxan-diabetic rats were notably edematous.

As alloxan-diabetic animals characteristically have reduced body weights, it is useful to represent the weight of a particular tissue as a percentage of the total body weight. Relative weight of a tissue = [wet weight (g)/body weight (g)] X 100. Of the delineated tissues studied, the relative weight of the neurohypophysis was greater in the alloxan-diabetic rats ($P<0.02$) than in controls (Table III). This was in spite of the fact that the wet weight of the neurohypophysis was not significantly different between the two groups (Table II). The relative weights of the lens, retina, and adenohypophysis, were not significantly different.

As diabetic animals appear to be in a state of dehydration, a check was made to see whether there were differences in the percentage

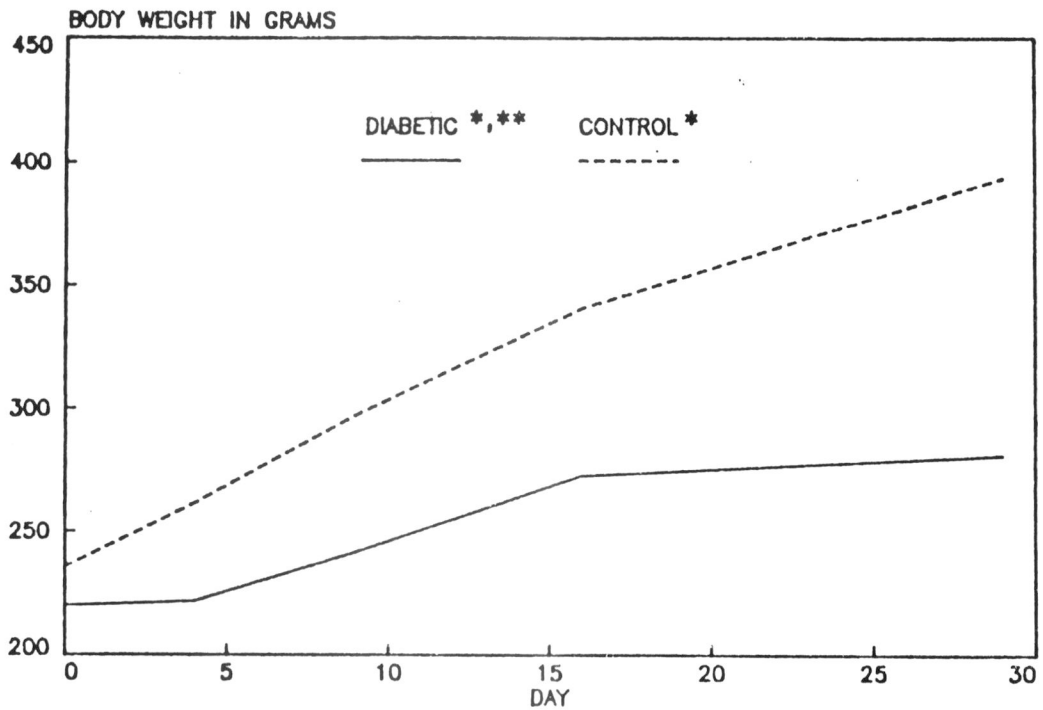
of weight that each tissue lost in the freeze-drying process. The percent weight loss was higher in the hypothalamus ($P<0.01$) for control rats while higher in the prostate ($P<0.01$) for the alloxan-diabetic group (Table IV). The percent weight loss was not statistically different for the other tissues studied.

The total zinc content of the tissues delineated as neural or endocrine were calculated (Table V). Value differences between the two groups were not significantly different.

The zinc concentrations (ug Zn/g tissue) were significantly higher in the adenohypophysis ($P<0.03$), brain ($P<0.03$), and prostate ($P<0.01$) of the alloxan-diabetic group (Table VI). Zinc concentrations in the other tissues assayed were not statistically different. The zinc concentration in the prostate for both the diabetic and control groups (55.5 and 36.8, respectively) were considerably lower than was originally expected. A possible reason for this is that the prostate sample consisted of a portion of the ventral lobe, and not the whole tissue. Autoradiographic study (Dencker and Tjalve, 1979) has shown the dorsolateral prostate to exhibit much greater Zn-65 labelling than the ventral lobe. Zinc concentrations are graphically represented in Figure 2.

Raw data are presented in tabular form in Appendices A-E.

FIGURE 1
RAT WEIGHT VS. TIME



* n = 8

** injected day 0 with alloxan
(40 mg/kg body weight)

TABLE I

BODY WEIGHT, WEIGHT GAIN, AND BLOOD GLUCOSE LEVELS OF ALLOXAN-DIABETIC AND CONTROL RATS[*,**]

GROUP	FINAL BODY WEIGHT[a]	BODY WEIGHT GAIN[b]	FINAL BLOOD GLUCOSE[c]
ALLOXAN-DIABETIC	280.9 ± 13.3[***]	2.1 ± 0.5[***]	451.2 ± 9.8[***]
CONTROL	394.0 ± 4.5	5.5 ± 0.1	95.1 ± 0.8

[*] data are presented as MEAN±SEM

[**] n = 8

[***] P < 0.001

[a] grams

[b] grams/day

[c] milligrams/100 ml

TABLE II

WET WEIGHTS AND DRY WEIGHTS OF TISSUES FROM ALLOXAN–DIABETIC AND CONTROL RATS *,**

TISSUE	ALLOXAN–DIABETIC [t]	CONTROL [t]	ALLOXAN–DIABETIC [tt]	CONTROL [tt]
Lens	18.2 ± 3.0 [a]	27.1 ± 4.0	8.76 ± 1.11 [b]	12.71 ± 1.46
Retina	15.1 ± 1.2 [c]	24.1 ± 2.6	1.48 ± 0.19 [a]	3.04 ± 0.67
Pineal	0.45 ± 0.09	0.69 ± 0.16	0.19 ± 0.02	0.23 ± 0.03
Adeno–hypophysis	5.75 ± 0.60 [c]	8.41 ± 0.39	1.47 ± 0.17 [a]	1.97 ± 0.11
Neuro–hypophysis	0.98 ± 0.09	1.04 ± 0.07	0.29 ± 0.03	0.31 ± 0.02
Hypo–thalamus	109 ± 5 [b]	140 ± 9	27.5 ± 2.0	30.3 ± 2.6
Brain	1476 ± 31	1540 ± 37	347 ± 9	361 ± 10
Aorta	40 ± 5	42 ± 7	7.9 ± 1.0	7.7 ± 0.9
Liver	254 ± 30	276 ± 40	97 ± 10	105 ± 11
Kidney	185 ± 16	190 ± 28	46.2 ± 4.5	43.5 ± 4.6
Pancreas	312 ± 177 [a]	367 ± 32	100 ± 5	140 ± 16
Prostate	113 ± 12 [a]	142 ± 11	26.1 ± 2.8 [c]	51.7 ± 3.3

* data are presented as MEAN ± SEM

** n = 8 except for alloxan–diabetic pineal where n = 7

[t] wet weight in milligrams

[tt] dry weight in milligrams

[a] $P < 0.05$ [b] $P < 0.01$ [c] $P < 0.005$

TABLE III

RELATIVE WEIGHTS OF TISSUES FROM ALLOXAN-DIABETIC AND CONTROL RATS *,**

TISSUE	ALLOXAN-DIABETIC [a]	CONTROL [a]
Lens	0.0063 ± 0.0007	0.0070 ± 0.0010
Retina	0.0054 ± 0.0005	0.0061 ± 0.0006
Pineal	0.0002 ± 0.0000	0.0002 ± 0.0000
Adenohypophysis	0.0020 ± 0.0001	0.0021 ± 0.0001
Neurohypophysis	0.0004 ± 0.0000 ***	0.0003 ± 0.0000

* data are presented as MEAN ± SEM

** n = 8 except for alloxan-diabetic pineal where n = 7

*** $P < 0.02$

[a] relative weight = $\frac{\text{wet weight (g)}}{\text{body weight (g)}} \times 100$

TABLE IV
PERCENT WEIGHT LOSS DUE TO FREEZE-DRYING TISSUES FROM ALLOXAN-DIABETIC AND CONTROL RATS *,**

TISSUE	ALLOXAN-DIABETIC [a]	CONTROL [a]
Lens	49.7 ± 2.9	50.6 ± 3.1
Retina	89.9 ± 1.1	88.2 ± 1.4
Pineal	50.2 ± 8.0	57.7 ± 7.5
Adenohypophysis	74.6 ± 1.0	76.5 ± 1.0
Neurohypophysis	70.1 ± 1.7	68.2 ± 4.1
Hypothalamus	74.9 ± 0.8 ***	78.4 ± 0.8
Brain	76.5 ± 0.4	76.6 ± 0.3
Aorta	79.0 ± 2.5	77.3 ± 4.2
Liver	60.9 ± 1.4	57.3 ± 7.5
Kidney	74.8 ± 1.7	75.4 ± 2.2
Pancreas	67.8 ± 0.9	60.9 ± 4.0
Prostate	73.2 ± 6.6 ***	62.8 ± 2.3

* data are presented as MEAN ± SEM

** n = 8 except for alloxan-diabetic pineal where n = 7

*** $P < 0.01$

[a] $\text{percent weight loss} = \dfrac{\text{wet weight (mg)} - \text{dry weight (mg)}}{\text{wet weight (mg)}} \times 100$

TABLE V

ZINC CONTENT OF TISSUES DELINEATED FROM ALLOXAN-DIABETIC AND CONTROL RATS *,**

TISSUE	ALLOXAN-DIABETIC ^{t}	CONTROL ^{t}
Neural or endocrine tissues		
RETINA	0.10 ± 0.02	0.11 ± 0.05
PINEAL	0.02 ± 0.00	0.02 ± 0.01
ADENOHYPOPHYSIS	0.14 ± 0.03	0.14 ± 0.01
NEUROHYPOPHYSIS	0.04 ± 0.02	0.03 ± 0.01
HYPOTHALAMUS	1.19 ± 0.05	1.21 ± 0.03
BRAIN	17.28 ± 0.47	17.52 ± 0.43
Subtotal	*18.77*	*19.03*
LENS	0.16 ± 0.02	0.14 ± 0.03
TOTAL	*18.93*	*19.17*

* data are presented as MEAN ± SEM

** n = 8 except for alloxan-diabetic pineal where n = 7

^{t} micrograms of zinc

TABLE VI

ZINC CONCENTRATIONS OF TISSUES FROM ALLOXAN-DIABETIC AND CONTROL RATS*,**

TISSUE	ALLOXAN-DIABETIC***	CONTROL***
Lens	20.0 ± 2.9	14.0 ± 1.2
Retina	72.8 ± 15.7	41.2 ± 5.8
Pineal	137.5 ± 33.6	83.3 ± 20.1
Adenohypophysis	90.6 ± 10.4 [a]	70.6 ± 3.0
Neurohypophysis	123.8 ± 35.7	85.4 ± 36.4
Hypothalamus	44.2 ± 2.1	41.4 ± 2.5
Brain	49.9 ± 1.4 [a]	48.7 ± 0.7
Aorta	50.6 ± 2.5	48.8 ± 2.0
Liver	126.4 ± 5.3	118.9 ± 7.4
Kidney	179.2 ± 13.8	180.4 ± 34.2
Pancreas	83.6 ± 9.0	75.2 ± 10.5
Prostate	55.5 ± 5.0 [b]	36.8 ± 2.8

* data are presented as MEAN±SEM

** n = 8 except for alloxan-diabetic pineal where n = 7

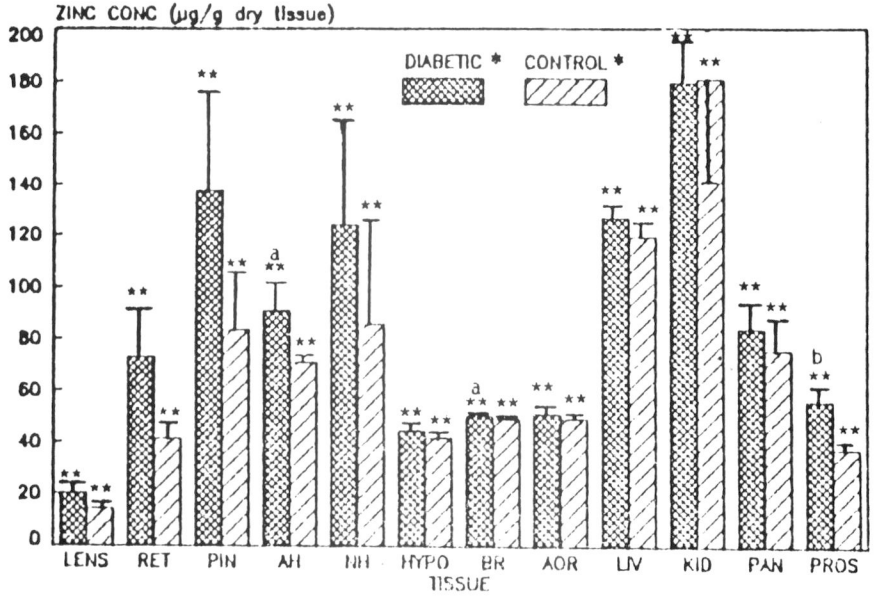
*** micrograms zinc/gram dry weight

[a] $P < 0.03$

[b] $P < 0.01$

FIGURE 2

ZINC CONCENTRATIONS OF TISSUES FROM ALLOXAN-DIABETIC AND CONTROL RATS (4 WEEKS DURATION)



* n = 8 except for alloxan-diabetic pineal where n = 7

** standard error of the mean

[a] $P < 0.03$; [b] $P < 0.01$

LEGEND: LENS = lens; RET = retina; PIN = pineal;
AH = adenohypophysis; NH = neurohypophysis;
HYPO = hypothalamus; BR = brain; AOR = aorta;
LIV = liver; KID = kidney; PAN = pancreas;
PROS = prostate

DISCUSSION

The alloxan-diabetic rats in this study had lower terminal body weights ($P < 0.001$) and lower growth rates ($P < 0.001$) than controls. These findings are similiar to those reported elsewhere (Howland and Zebrowski, 1976; Cusan et al., 1980). The wet weights and dry weights of the lens, retina, and adenohypophysis were found to be significantly lower in the alloxan-diabetic group (Table II). Significantly reduced adenohypophysial wet weights have been similiarly reported in alloxan-diabetic male rats (Howland and Zebrowski, 1976).

In this study, significantly elevated concentrations of zinc were found in the adenohypophysis ($P < 0.03$), brain ($P < 0.03$), and prostate ($P < 0.01$) of acute alloxan-diabetic male rats (Table VI). Zinc concentrations in the lens, retina, pineal, neurohypophysis, hypothalamus, aorta, liver, kidney, and pancreas were not significantly different. Although most of the alloxan-diabetic and control tissues had similiar zinc concentrations, it is interesting to note that, except for the kidney, all of the alloxan-diabetic tissues had higher zinc concentrations than did the controls (Table VI, Figure 2). Similiarly, in a study with acute streptozotocin(STZ)-diabetic male rats (Failla and Kiser, 1981), zinc concentrations were always either higher or the same as compared to controls - never significantly lower (tissues studied were the liver, kidney, duodenum, spleen, muscle, and plasma). The same was true even when the STZ-diabetic and control rats were fed equivalent amounts of zinc (Failla and Kiser, 1981).

Collectively, these findings indicate that a buildup of zinc may

occur in particular tissues in acute experimental diabetes. Furthermore, as none of the diabetic tissues in these studies had significantly lower zinc concentrations, the possibility exists that diabetes mellitus results in an overall positive zinc balance. A positive zinc balance would result from an increase in zinc absorption and/or a decrease in zinc excretion. Increases in zinc absorption (Craft and Failla, 1983) and urinary zinc excretion (Lau and Failla, personal communication) have been observed in acute STZ-diabetic male rats. Whether or not this increased urinary zinc excretion is indicative of, or has a significant effect upon the total body zinc excretion remains to be determined. Normally, relatively little zinc is excreted via the urine as compared to the gastrointestinal tract (Spencer et al., 1979, Sandstead, 1981).

Tissue zinc concentrations have been determined in chronic alloxan-diabetic male rats (10 months duration) (McNeill et al., 1981). Compared to normal controls, zinc concentrations were significantly increased in the adenohypophysis ($P < 0.02$), hypothalamus ($P < 0.01$), and aorta ($P < 0.038$), while decreased in the brain ($P < 0.0002$). The zinc concentrations in the pancreas, testes, pineal, retina, and serum were similiar for the two groups. In comparing the results of this study (chronic alloxan-diabetes) with the results of the present study (acute alloxan-diabetes), it is noted that: 1) the zinc concentration in the adenohypophysis was significantly increased in both the acute and chronic diabetic animals; 2) the zinc concentration of the brain was increased in acute-alloxan diabetes, while decreased in chronic alloxan-

diabetes (as compared to controls); 3) the zinc concentration of the aorta was similiar for acute alloxan-diabetic vs. control rats, but was significantly elevated in chronic alloxan-diabetic animals (as compared to controls); and, 4) the zinc concentrations in both the retina and pineal were not significantly different in either acute or chronic diabetic animals, as compared to their controls.

It has been suggested that changes in the tissue distribution of zinc during periods of chronic diabetes mellitus may contribute to the onset of the secondary complications of the disease (McNeill, 1981; Failla, personal communication). As previously mentioned, acute STZ-diabetic male rats have exhibited increased hepatic and renal zinc concentrations (Failla and Kiser, 1981), and these increases were correlated with an increase in the protein metallothionein. Currently, much interest has been expressed into the possibility that an increase in the formation and accumulation of non-enzymatically glycosylated proteins may mediate, in part, the secondary complications of diabetes mellitus (Stevens et al., 1978; Cerami et al., 1979; Brownlee and Cerami, 1981; Karasik et al., 1982; Monnier et al., 1982). Intriguingly, there is some evidence suggesting that the physiology of zinc may be altered by non-enzymatically glycoslylated molecules (Freeman et al., 1975).

Non-enzymatic glycosylation is a post-translational reaction in which a reducing sugar (such as glucose) covalently binds to a protein (or amino acid), without the aid of an enzyme (Adrian, 1974). The formation of glycosylated proteins has been studied in the food pro-

cessing industry (where they are known as Maillard-type reactions), as such reactions can lead to the browning of stored foods, and a reduction in the biological value of dietary protein (Adrian, 1974; Miller, 1979). Interestingly, hemoglobin (Bunn et al., 1978), erythrocyte membranes (Miller et al., 1980), plasma proteins (Yue et al., 1980), lens crystallins (Stevens et al., 1978), renal glomerular proteins (Chang and Noble, 1979), and collagen (Monnier et al., 1982) are known to form non-enzymatically glycosylated compounds, and their formation is enhanced in the diabetic state. Furthermore, non-enzymatic glycosylation is known to result in the altered physiology of hemoglobin (McDonald et al., 1978) and lens crystallins (Stevens et al., 1978; Brownlee and Cerami, 1981). It has been suggested that the accumulation of physiologically altered proteins may help potentiate the accelerated basement membrane thickening (Brownlee and Cerami, 1981; Kefalides, 1981) and cataract formation (Stevens et al., 1978; Brownlee and Cerami, 1981) so often seen in diabetes.

There is some evidence to indicate that zinc may form complexes with non-enzymatically glycosylated molecules. Patients receiving total parental nutrition (TPN) often excrete relatively large amounts of zinc in their urine (Freeman et al., 1975; Phillips, 1982). According to Freeman et al., (1975), this phenomenon is due to the chelation of zinc by sugar-amine compounds, the latter being formed by the non-enzymatic glycosylation of amino acids during the autoclaving of solutions containing protein hydrosylates (or crystalline amino acids) and glucose. Such complexes are filtered into the urine, but

are not reabsorbed, resulting in a significantly increased urinary zinc loss (Freeman et al., 1975). Significant increases in urinary zinc were not observed when the TPN solutions were administered nasogastrically, due to the fact that sugar-amine compounds are not absorbed by the gut (Freeman et al., 1975).

Additional information concerning the interactions between zinc and non-enzymatically glycosylated proteins is meager. However, the possible importance of such interactions are too provocative to ignore when one considers: 1) the formation and accumulation of non-enzymatically glycosylated proteins may help mediate the secondary complications of diabetes mellitus; 2) zinc may become chelated to non-enzymatically glycosylated molecules; and, 3) minerals may increase the rate of non-enzymatic glycosylation of some proteins (Miller, 1979).

In light of the previous discussion, I would like to suggest the use of the lens of a diabetic animal as a model for studying the interactions between zinc and non-enzymatically glycosylated proteins *in vivo*. The lens, like other tissues that develop complications due to diabetes, does not use insulin for intracellular glucose transport (Brownlee and Cerami, 1981). High glucose concentrations *in vitro* and *in vivo* have been shown to increase the non-enzymatic glycosylation of lens crystallins (Stevens et al., 1978). Furthermore, as the major lens crystallins undergo little or no replacement, the amount of glycosylated proteins within the lens continually increases during the life of an individual (Brownlee and Cerami, 1981). *In vitro*, high glucose levels lead to enhanced disulfide cross-linking and aggregation

of lens crystallins (Stevens et al., 1978), and these crystalline aggregates may be responsible for the opalescence of cataractous lenses (Stevens et al., 1978; Brownlee and Cerami, 1981).

The extent to which zinc takes part in the formation of cataracts is not known; however, indications are that zinc may play an important role in the process (Karcioglu, 1982). The possibility exists that zinc may be involved in the formation and/or aggregation of non-enzymatically glycosylated lens crystallins. If this is so, then one can speculate that the concentration of zinc may be increased in the diabetic cataractous lens. In this study, the concentration of zinc in the lens was higher for the acute alloxan-diabetic group as compared to controls. However, this difference was not statistically significant ($P< 0.13$).

Additional studies need to be carried out concerning the *in vitro* and *in vivo* interactions between zinc and non-enzymatically glycosylated proteins. The most useful *in vivo* animal models would be those with chronic diabetes, as non-enzymatic glycosylation is a time-dependent process. It would also be useful to look at other diabetic models besides the rat. For example, rats do not develop classic diabetic retinopathy, and the reason for this may be because their relatively short life-span does not allow sufficient time for restrictive increases in basement membrane thickness (Sosula, 1974).

In addition to the lens crystallins, the possible interactions between zinc and collagen is of great interest. Collagens, like lens crystallins, are relatively long lived proteins (Brownlee and Cerami,

1981), and the normal cross-linking of collagen may involve the use of zinc (McClain et al., 1973). Diabetes mellitus is known to result in the altered physiology (Schneir et al., 1982) and increased non-enzymatic glycosylation of collagen (Monnier et al., 1982).

In summary, acute alloxan-diabetes resulted in significant increases in the zinc concentrations in the adenohypophysis ($P < 0.03$), brain ($P < 0.03$), and prostate ($P < 0.01$) of adult male rats. Furthermore, the overall trend was for the alloxan-diabetic tissues to exhibit higher zinc concentrations than those of the control animals. Current evidence suggests that experimental diabetes alters zinc metabolism and may result in increased zinc concentrations in select tissues. However, the exact consequences of such increases remain unclear because the intracellular physiology of zinc is not understood. Within the cell, zinc is believed to be bound primarily by metalloenzymes (Di-Silvestro and Cousins, 1983). How such Zn-metalloenzymes are affected by an intracellular excess of zinc is uncertain. In such an excess state, the possibility exists that zinc may be "stockpiled" by certain proteins in such a manner as to make zinc unavailable in sufficient quantities to other intracellular ligands. Thus, a functional zinc deficiency could exist within a cell, even though the overall cellular zinc concentration is increased. In addition, one must consider the possibility that high amounts of zinc may alter the physiology of particular intracellular and/or intercellular proteins, leading to impaired biological function (i.e., zinc toxicity).

BIBLIOGRAPHY

Adashi, E. Y., Fabics, C., and Hsueh, A. J. W. 1982. Insulin augmentation of testosterone production in a primary culture of rat testicular cells. Biol. Reprod. 26: 270-280.

Adrian, J. 1974. Nutritional and physiological consequences of the Maillard reaction. World Rev. Nutr. Diet. 19: 71-122.

Beisel, W. R., Pekarek, R. S., and Wannamacher, R. W. 1976. Homeostatic mechanisms affecting plasma zinc levels in acute stress. In: "Trace Elements in Human Health and Disease, Volume 1: Zinc and Copper" (Prasad, A. S., ed.), pp. 87-106. Academic Press: New York.

Brownlee, M., and Cerami, A. 1981. The biochemistry of the complications of diabetes mellitus. Ann. Rev. Biochem. 50: 385-432.

Bunn, H. F., Gabbay, K. H., and Gallop, P. M. 1978. The glycosylation of hemoglobin: relevance to diabetes mellitus. Science 200: 21-27.

Casey, C., and Hambidge, K. 1980. Epidemiological aspects of human zinc deficiency. In: "Zinc in the Environment, Part II: Health Effects" (Nriagu, J. O., ed.), pp. 1-27. John Wiley and Sons: New York.

Chesters, J. K., and Will, M. 1981. Zinc transport proteins in plasma. Br. J. Nutr. 46: 111-117.

Cerami, A., Stevens, V. J., and Monnier, V. M. 1979. Role of nonenzymatic glycosylation in the development and sequelae of diabetes mellitus. Metabolism 28: 431-437.

Chang, A. Y., and Noble, R. E. 1979. Estimation of HbAlc-like glycosylated proteins in the kidneys of streptozotocin-diabetic and control rats. Diabetes 28: 408.

Chesters, J. K., and Will, M. 1981. Zinc transport proteins in plasma. Br. J. Nutr. 46: 111-117.

Craft, N. E., and Failla, M. L. 1983. Zinc, iron, and copper absorption in the streptozotocin-diabetic rat. Am. J. Physiol. 244: E112-E128.

Cunnane, S. C., Horrobin, D. F., Manku, M. S., and Oka, M. 1979. Alteration of tissue zinc distribution and biochemical analysis of serum following pinealectomy in the rat. Endocr. Res. Comm. 6: 311-319.

Cunnane, S. C., and Horrobin, D. F. 1980. Parenteral linoleic and gamma-linolenic acids ameliorate the gross effects of zinc deficiency. Proc. Soc. Exp. Biol. Med. 164: 583-588.

Cusan, L., Belanger, A., Segun, C., and Labrie, F. 1980. Impairment of pituitary and gonadal functions in alloxan-induced diabetic male rats. Mol. Cell Endocrinol. 18: 165-176.

Davies, N. T. 1980. Studies on the absorption of zinc by rat intestine. Br. J. Nutr. 43: 189-203.

Demmel, U., Hock, A., Kasperek, K., and Feinendegen, L. 1982. Trace element concentration in the human pineal body. Sci. Total Environ. 24: 135-146

Denker, L., and Tjalve, H. 1979. An autoradiographic study on the fate of Zn-65 in zinc-rich tissues in some rodents. Med. Biol. 57: 391-397.

DiSilvestro, R., and Cousins, R. 1983. Physiological ligands for copper and zinc. Ann. Rev. Nutr. 3: 261-288.

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

Evans, G. W. 1980. Normal and abnormal zinc absorption in man and animals: the tryptophan connection. Nutr. Rev. 38: 137-141.

Failla, M. L. 1983. Personal communication.

Failla, M. L., and Kiser, R. A. 1981. Altered tissue content and cytosol distribution of trace metals in experimental diabetes. J. Nutr. 111: 1900-1909.

Failla, M. L., and Kiser, R. A. 1983. Hepatic and renal metabolism of copper and zinc in the diabetic rat. Am. J. Physiol. 244: E115-E121.

Fernandez-Madrid, F., Prasad, A. S., and Oberleas, D. 1973. Effect of zinc deficiency on nucleic acids, collagen, and noncollagenous protein of the connective tissue. J. Lab. Clin. Med. 82: 951-961.

Freeman, J. B., Stegink, L. D., Meyer, P. D., Fry, L. K., and Denbesten, L. 1975. Excessive urinary zinc losses during parenteral alimentation. J. Surg. Res. 18: 463-469.

Ganong, W. F. 1981. "Review of Medical Physiology", 10th edition, pp. 283, 324. Lange Medical Publications: Los Altos, CA.

Giroux, E. L. 1975. Determination of zinc distribution between albumin and alpha-2 macroglobulin in human serum. Biochem. Med. 12: 258-266.

Hallbook, T., and Lanner, E. 1972. Serum zinc and healing of venous leg ulcers. Lancet 2: 780-782.

Harper, H. A., Rodwell, V. W., and Mayes, P. A. 1979. "Review of Physiological Chemistry", 17th edition, pp. 477, 591. Lange Medical Publications: Los Altos, CA.

Henkin, R. I. 1976. Trace metals in endocrinology. Med. Clin. North Am. 60: 779-797.

Henkin, R. I. 1980. Copper-zinc-hormone interrelationships. In: "Zinc and Copper in Medicine" (Karcioglu, Z. A., and Sarper, R. M., eds.), pp. 126-159. Charles C. Thomas: Springfield, Ill.

Henzel, J. H., De Weese, M. S., and Lichti, E. L. 1970. Zinc concentrations within healing wounds. Arch Surg. 100: 349.

Hoffman, W. S. 1937. A rapid photoelectric method for the determination of glucose in blood and urine. J. Biol. Chem. 120: 51-55.

Howland, B. E., and Zebrowski, E. J. 1976. Some effects of experimentally-induced diabetes on pituitary-testicular relationships in rats. Horm. Metab. Res. 8: 465-469.

Howland, B. E., and Zebrowski, E. J. 1980. Pituitary response to gonadotropin-releasing hormone in diabetic male rats. Experentia 36: 610-611.

Hughes, M. N. 1981. "The Inorganic Chemistry of Biological Processes", p. 2. John Wiley and Sons: New York.

Hutson, J. C., Stocco, D. M., Campbell, G. T., and Wagoner, J. 1983. Sertoli cell function in diabetic, insulin-treated diabetic, and semi-starved rats. Diabetes 32: 112-116.

Johnson, L. Y., and Reiter, R. J. 1978. The pineal gland and its effects on mammalian reproduction. Prog. Reprod. Biol. 4: 116-156.

Karasik, A., Modan, M., Halkin, H., and Lusky, A. 1982. Prevalance of lens cataract-- independent positive association with glucose intolerance and with glycosylated hemoglobin level. Diabetes 31: 92A.

Karcioglu, Z. A. 1982. Zinc in the eye. Surv. Opthalmol. 27: 114-122.

Keilin, D., and Mann, J. 1940. Carbonic anhydrase. Purification and nature of the enzyme. Bioch. J. 34: 1163-1176.

Kefalides, N. A. 1981. Basement membrane research in diabetes mellitus. Collagen Rel. Res. 1: 295-299.

Kirchgessner, M., and Roth, H.-P. 1980. Biochemical changes of hormones and metalloenzymes in zinc deficiency. In: "Zinc in the Environment, Part II: Health Effects" (Nriagu, J. O., ed.), pp. 77-103. John Wiley and Sons: New York.

Kumar, S., and Rao, K. S. J. 1974. Blood and urinary zinc levels in diabetes mellitus. Nutr. Metabol. 17: 231-235.

La Bella, F., Dular, R., Vivian, S., and Queen, G. 1973. Pituitary hormone releasing or inhibiting activity of metal ions present in hypothalamic extracts. Biochem. Biophys. Res. Comm. 52: 786-791.

Lang, U., Aubert, M. L., Conne, B. S., Bradtke, J. C., and Sizonenko, P. C. 1983. Influence of exogenous melatonin on melatonin secretion and the neuroendocrine reproductive axis of intact male rats during sexual maturation. Endocrinology 112: 1578-1584.

Lau, A. L., and Failla, M. L. 1983. Personal communication.

Lehninger, A. L. 1982. "Principles of Biochemistry", pp. 135, 816, 848. Worth Publishers: New York.

Lei, K. Y., Abbasi, A., and Prasad, A. S. 1976. Function of pituitary-gonadal axis in zinc-deficient rats. Am. J. Physiol. 230: 1730-1732.

McDonald, M. J., Shapiro, R., Bleichman, M., Solway, J., and Bunn, H. F. 1978. Glycosylated minor components of human adult hemoglobin. J. Biol Chem. 253: 2327-2332.

McClain, P. E., Wiley, E. R., Beecher, G. R., Anthony, W. L., and Hsu, J. W. 1973. Influence of zinc deficiency on synthesis and cross-linking of rat skin collagen. Biochim. Biophys. Acta. 304: 457-465.

McNair, P., Killerich, S., Christiansen, C., Christiansen, M. S., Madsbad, S., and Transbol, I. 1981. Hyperzincuria in insulin treated diabetes mellitus-- its relations to glucose homeostasis and insulin administration. Clin. Chem. Acta. 112: 343-348.

McNeill, M. E. 1981. The pineal, zinc, and complications in diabetes mellitus. NIH Grant Application 1R23AM 30946-01.

McNeill, M. E., Simpson, E. C., Bray, J. T., Smith, C., Webb, L. A., and Morgan, C. R. 1981. Zinc levels in tissues from alloxan-diabetic rats. Soc. Neurosci. 7: 950.

Meltzer, L. E., Rutman, J., George, P., Rutman, R., and Kitchell, J. R. 1962. The urinary excretion pattern of trace elements in diabetes mellitus. Am. J. Med. Sci. 244: 282-289.

Mertz, W. 1981. The essential trace elements. Science 213: 1332-1338.

Methfessel, A. H., and Spencer, H. 1973. Zinc metabolism in the rat. I. Intestinal absorption of zinc. J. Appl. Physiol. 34: 58-62.

Miller, J. 1979. Effect on growth and hemoglobin regeneration in anemic rats of interactions between glucose and minerals during heating. Nutr. Rep. Int. 19: 679-688.

Miller, J. A., Gravallese, E., and Bunn, H. F. 1980. Nonenzymatic glycosylation of erythrocyte membrane proteins. J. Clin. Invest. 65: 869-901.

Miller, W. J., and Neathery, M. W. 1980. Manifestations of zinc abnormalities in animals. In: "Zinc in the Environment, Part II: Health Effects", (Nriagu, J. O., ed.), pp. 61-70. John Wiley and Sons: New York.

Monnier, V. M., Kohn, R. R., and Cerami, A. 1982. Accelerated browning of collagen in diabetic humans. Diabetes 31: 28A.

Moore, R. Y. 1978. The innervation of the pineal gland. Prog. Reprod. Biol. 4: 1-29.

Oner, G., and Bor, N. M. 1978. Serum somatomedin-A activity and insulin levels in zinc deficiency. Nutr. Rep. Int. 18: 749-753.

Phillips, L. S., and Young, H. S. 1976. Nutrition and somatomedin. II. Serum somatomedin activity and cartilage growth activity in streptozotocin-diabetic rats. Diabetes 25: 516-527.

Phillips, G. D. 1982. Zinc in total parenteral nutrition. In: "Clinical Applications of Recent Advances in Zinc Metabolism" (Prasad, A. S., Dreosti, I. E., and Hetzel, B. S., eds.), pp. 169-180. Alan R. Liss, Inc.: New York.

Pories, W. J., and Strain, W. H. 1966. Zinc and wound healing. In: "Zinc Metabolism" (Prasad, A. S., ed.), pp. 378-394. Charles Thomas: Springfield, IL.

Prasad, A. S., Oberleas, D., Wolf, P. L., and Horwitz, J. 1967. Studies on zinc deficiency. J. Clin. Invest. 46: 549-557.

Prasad, A. S., and Oberleas, D. 1970. Binding of zinc to amino acids and serum proteins in vitro. J. Lab. Clin. Med. 76: 416-425.

Prasad, A. S. 1979. "Zinc in Human Nutrition". CRC Press, Inc.: Boca Raton, FL.

Prasad, A. S. 1982. Clinical and biochemical spectrum of zinc deficiency. In: "Clinical, Biochemical, and Nutritional Aspects of Trace Elements" (Prasad, A. S., ed.), pp. 3-62. Alan R. Liss, Inc.: New York.

Raulin, J. 1869. Etudes Cliniques sur la Vegetation. Ann. Sci. Nat. Biol. Veg. 11: 93.

Reinhold, J. G., Faradji, B., Abadi, P., and Ismail-Beigi, F. 1976. Binding of zinc to fiber and other solids of wholemeal bread. In: "Trace Elements in Human Health and Disease, Volume 1: Zinc and Copper" (Prasad, A. S., ed.), pp. 163-180. Academic Press: New York.

Rerup, C. C. 1970. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol. Rev. 22: 485-518.

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

Roth, H.-P., and Kirchgessner, M. 1981. Zinc and insulin metabolism. Biol. Trace Element Res. 3: 13-32.

Sandstead, H. H. 1981. Zinc in human nutrition. In: "Disorders of Mineral Metabolism" (Bronner, F., and Coburn, J. W., eds.), pp. 93-157. Academic Press: New York.

Schneir, M., Ramamurthy, K. H., and Golub, L. 1982. Skin collagen metabolism in the streptozotocin-induced diabetic rat. Diabetes 31: 426-431.

Shapcott, D. S. 1982. Hair and plasma in the diagnosis of zinc deficiency. In "Clinical Applications of Recent Advances in Zinc Metabolism" (Prasad, A. S., Dreosti, I. E., and Hetzel, B. S., eds.), pp. 121-139. Alan R. Liss, Inc.: New York.

Solomons, N. W. 1979. On the assessment of zinc and copper nutriture in man. Am. J. Clin. Nutr. 32: 856-871.

Song, M. K., and Adham, N. F. 1980. Evidence for an important role of prostaglandins E2 and F2 in the regulation of zinc transport in the rat. J. Nutr. 109: 2152-2159.

Sosula, L. 1974. Capillary radius and wall thickness in normal and diabetic rat retinae. Microvascular Res. 7: 274-276.

Spencer, H., Osis, D., Kramer, L., and Norris, C. 1976. Intake, excretion, and retention of zinc in man. In: "Trace Elements in Human Health and Disease, Volume 1: Zinc and Copper" (Prasad, A. S., ed.), pp. 345-361. Academic Press: New York.

Spencer, H., Asmussen, C. R., Hotzman, R. B., and Kramer, L. 1979. Metabolic balances of cadmium, copper, manganese, and zinc in man. Am. J. Clin. Nutr. 32: 1867-1875.

Steiner, D. F. 1976. Insulin today. Diabetes 26: 332-340.

Stevens, V. J., Rouzer, C. A., Monnier, V. M., and Cerami, A. 1978. Diabetic cataract formation: potential role of glycosylation of lens crystallins. Proc. Natl. Acad. Sci. 75: 2918-2922.

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

Turner, C. D., and Bagnara, J. T. 1976. "General Endocrinology" 6th edition, p. 258. W. B. Saunders Co.: Philadelphia.

Underwood, E. J. 1977. "Trace Elements in Human and Animal Nutrition" 4th edition, pp. 208-209. Academic Press: New York.

Unger, R. H., Dobbs, R. F., and Orci, L. 1978. Insulin, glucagon, and somatostatin secretion in the regulation of metabolism. Ann. Rev. Physiol. 40: 307-343.

Vallee, B. L., and Gibson, J. G. 1948. The zinc content of normal whole blood, plasma, leucocytes, and erythrocytes. J. Biol Chem. 176: 445-457.

Weichselbaum, R., Patel, M., and Das Gupta, T. K. 1975. Influence of the pineal gland on wound healing. Nature 254: 349.

Wong, P. Y., and Fritze, L. 1969. Determination by neuron activation of Cu, Mn, and Zn in the pineal body and other areas of brain tissue. J. Neurochem 16: 1231-1234.

Yue, D. K., Morris, K., McLennan, S., and Turtle, J. R. 1980. Glycosylation of plasma protein and the relation to glycosylated hemoglobin in diabetes. Diabetes 29: 296-300.

Appendix A. Body weight and blood glucose levels.

ANIMAL NUMBER	BODY WEIGHT IN GRAMS Day 0	Day 4	Day 9	Day 16	Day 29	TERMINAL BLOOD GLUCOSE *
6	227	238	268	280	278	464.1
9	222	231	255	299	336	480.9
10	219	224	220	255	230	408.4
12	213	207	220	253	253	440.6
13	224	208	237	258	278	495.0
18	208	221	254	304	334	434.4
20	222	207	227	258	255	445.6
23	224	238	252	274	283	440.6
24	225	256	290	337	405	99.6
25	230	259	287	328	386	95.6
26	226	251	287	333	378	95.6
32	241	267	303	350	403	93.5
34	244	265	299	339	385	95.6
35	228	255	295	329	384	93.5
37	250	279	323	362	414	93.5

Animals 6-23 were injected Day 0 with alloxan (40 mg/kg body weight); Animals 24-37 were not treated in any manner and served as controls.

*milligrams/deciliter

Appendix B. Wet weights and dry weights of tissue samples from alloxan-diabetic rats.

TISSUE		ANIMAL NUMBER 6	9	10	12	13	18	20	23
Lens (mg)	wet	13.78	36.89	14.20	11.49	13.26	23.48	16.23	16.07
	dry	8.36	16.37	7.81	6.79	7.24	8.70	7.39	7.39
Retina (mg)	wet	9.64	10.46	14.37	17.20	16.31	19.01	16.51	16.88
	dry	1.26	1.31	1.69	0.93	1.26	2.68	1.42	1.25
Pineal (mg)	wet	0.40	0.42	0.77	0.77	0.12	0.45	---	0.24
	dry	0.18	0.25	0.22	0.26	0.11	0.18	---	0.12
Adenohypo-	wet	5.84	8.62	4.94	3.28	5.25	7.66	5.71	4.73
physis (mg)	dry	1.58	2.00	1.18	0.82	1.37	2.14	1.71	0.97
Neurohypo-	wet	0.95	1.48	0.92	0.63	0.82	0.96	1.18	0.93
physis (mg)	dry	0.33	0.43	0.29	0.20	0.26	0.33	0.29	0.20
Hypothala-	wet	128	111	111	110	111	77	108	117
mus (mg)	dry	36.4	30.6	25.5	26.1	24.7	19.1	28.9	28.8
Brain (g)	wet	1.384	1.583	1.512	1.497	1.521	1.561	1.412	1.340
	dry	0.305	0.355	0.373	0.322	0.366	0.384	0.338	0.326
Aorta (mg)	wet	34	53	28	31	65	31	35	40
	dry	4.5	9.7	8.7	6.8	8.7	7.0	10.8	6.8
Liver (mg)	wet	342	402	279	176	252	236	198	145
	dry	120.7	150.1	93.5	74.1	102.9	95.7	74.1	66.7
Kidney (mg)	wet	259	150	173	250	183	164	165	134
	dry	72.0	49.6	31.1	51.0	50.3	41.4	38.3	35.7
Pancreas (mg)	wet	375	367	262	236	308	346	280	320
	dry	121	116	94	82	84	113	92	97
Prostate (mg)	wet	132	141	137	140	95	127	47	84
	dry	25.1	36.7	19.5	25.7	15.4	34.3	33.6	18.8

Appendix C. Wet weights and dry weights of tissue samples from control rats.

TISSUE		ANIMAL NUMBER 24	25	26	32	34	35	37	39
Lens (mg)	wet	24.98	39.09	40.58	12.44	25.76	21.01	38.89	14.05
	dry	12.94	17.43	18.33	8.01	10.33	10.11	16.14	8.40
Retina (mg)	wet	24.03	27.00	15.17	20.10	17.18	20.37	31.47	36.72
	dry	3.23	4.08	1.02	2.28	1.98	1.62	3.02	7.05
Pineal (mg)	wet	0.38	0.43	0.47	0.79	0.85	1.65	0.16	0.75
	dry	0.22	0.27	0.22	0.24	0.19	0.41	0.12	0.14
Adenohypophysis (mg)	wet	8.47	8.59	7.99	9.27	7.84	6.29	9.93	8.90
	dry	1.85	1.69	1.69	2.30	2.05	1.64	2.13	2.42
Neurohypophysis (mg)	wet	0.69	1.16	0.90	1.06	1.01	1.23	0.94	1.30
	dry	0.38	0.23	0.28	0.28	0.29	0.38	0.39	0.27
Hypothalamus (mg)	wet	120	200	138	130	123	150	127	128
	dry	30.5	47.7	30.0	28.9	24.4	28.4	26.9	25.6
Brain (g)	wet	1.701	1.572	1.589	1.589	1.459	1.590	1.395	1.422
	dry	0.383	0.389	0.379	0.368	0.326	0.377	0.341	0.321
Aorta (mg)	wet	29	17	53	19	53	81	43	42
	dry	10.5	5.6	7.0	6.8	6.0	7.9	5.6	12.5
Liver (mg)	wet	155	370	439	121	196	317	254	355
	dry	54.9	121.1	148.2	115.1	79.6	118.8	8.31	120.8
Kidney (mg)	wet	192	301	130	322	166	120	127	162
	dry	46.8	72.7	36.1	36.8	36.3	32.2	38.2	49.1
Pancreas (mg)	wet	404	319	427	330	390	499	193	375
	dry	106	128	123	98	197	225	111	132
Prostate (mg)	wet	143	185	137	108	170	89	144	161
	dry	44.0	56.6	46.3	43.7	67.6	42.6	61.4	51.4

Appendix D. Zinc content and zinc concentration in tissue samples from alloxan-diabetic rats.

TISSUE		6	9	10	12	13	18	20	23
Lens	µg Zn	0.200	0.169	0.190	0.200	0.186	0.079	0.086	0.190
	µg Zn/g	23.92	10.32	24.28	29.45	25.71	9.11	11.57	25.66
Retina	"	0.169	0.169	0.110	0.048	0.031	0.090	0.052	0.134
		134.07	128.95	65.28	51.90	24.62	33.45	36.42	107.56
Pineal	"	0.017	0.028	0.007	0.014	0.026	0.045	---	0.021
		95.76	110.32	31.34	53.04	250.72	248.98	---	172.37
Adenohypo-	"	0.148	0.317	0.097	0.055	0.097	0.193	0.121	0.090
physis		93.82	158.58	81.80	67.27	70.46	90.21	70.56	92.40
Neurohypo-	"	0.048	0.141	0.028	0.041	0.010	0.028	0.010	0.010
physis		146.25	328.71	95.10	206.84	39.78	83.57	35.66	54.43
Hypothala-	"	1.325	1.220	1.231	1.214	1.179	0.986	1.038	1.358
mus		36.40	39.85	48.26	46.49	47.73	51.62	35.91	47.16
Brain	"	15.982	18.230	15.198	16.557	18.543	18.962	17.812	16.975
		52.40	51.35	40.75	49.87	50.66	49.38	52.70	52.07
Aorta	"	0.293	0.421	0.362	0.328	0.455	0.345	0.569	0.353
		65.12	43.36	41.61	48.16	52.31	49.25	52.67	51.96
Liver	"	13.077	16.903	13.525	8.974	15.145	11.767	10.284	7.699
		108.34	112.61	144.65	121.11	147.18	122.95	138.79	115.42
Kidney	"	12.629	8.182	7.940	8.314	6.081	7.458	7.975	5.906
		175.40	164.95	255.31	163.12	120.90	180.13	208.21	165.44
Pancreas	"	11.868	10.384	8.528	7.073	7.274	12.594	7.442	2.421
		98.08	89.51	90.73	86.25	86.59	111.45	80.90	24.96
Prostate	"	1.764	2.106	1.448	1.396	0.967	1.816	1.071	0.758
		70.27	57.37	74.27	54.31	62.80	52.95	31.88	40.29

Appendix E. Zinc content and zinc concentration in tissue samples from control rats.

TISSUE		ANIMAL NUMBER 24	25	26	32	34	35	37	39
Lens	µg Zn	0.137	0.297	0.169	0.151	0.123	0.131	0.016	0.123
	µg Zn/g	10.57	17.01	9.22	18.85	11.86	12.96	17.30	14.59
Retina	"	0.124	0.103	0.059	0.148	0.114	0.041	0.103	0.186
		38.42	25.35	57.46	64.98	57.46	25.54	34.25	26.41
Pineal	"	0.010	0.021	0.014	0.010	0.010	0.017	0.017	0.028
		47.01	76.61	62.68	43.09	54.43	42.04	143.64	196.99
Adenohypo-physis	"	0.159	0.128	0.110	0.152	0.152	0.107	0.159	0.141
		85.72	75.48	65.28	65.95	73.99	65.16	74.45	58.41
Neurohypo-physis	"	0.021	0.003	0.010	0.010	0.014	0.021	0.041	0.090
		54.43	14.99	36.94	36.94	47.55	54.43	106.07	331.97
Hypothala-mus	"	1.341	1.210	1.265	1.143	1.210	1.255	1.131	1.131
		43.97	25.37	42.17	39.53	49.59	44.19	42.04	44.18
Brain	"	18.491	17.768	18.543	19.066	16.139	17.341	16.923	15.669
		48.28	46.19	48.93	51.81	49.51	46.00	49.63	48.81
Aorta	"	0.414	0.255	0.359	0.324	0.348	0.414	0.283	0.565
		39.40	45.56	51.29	47.66	58.03	52.37	50.48	45.23
Liver	"	8.147	14.214	12.215	11.077	10.250	14.973	11.077	14.490
		148.40	116.42	82.42	96.24	128.77	126.03	133.30	119.95
Kidney	"	5.596	8.630	5.079	6.113	6.596	13.318	5.734	7.492
		119.57	118.70	140.69	166.11	181.70	413.61	150.10	152.59
Pancreas	"	8.502	8.671	10.479	9.151	6.941	8.437	14.111	9.913
		80.21	67.74	85.20	93.38	35.23	37.50	127.13	75.10
Prostate	"	1.386	2.763	2.120	1.527	1.632	1.448	2.368	1.895
		31.51	48.81	45.79	34.95	24.15	34.00	38.57	36.87