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Dietary calcium and zinc effects in unilaterally neutron irradiated swine

Hermel Rosas

To the Graduate Council:

I am submitting herewith a dissertation written by Hermel Rosas entitled "Dietary calcium and zinc effects in unilaterally neutron irradiated swine." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

C.S. Hobbs, Major Professor

We have read this dissertation and recommend its acceptance:

M.C. Bell, H.J. Smith, J.K. Bletner, K.M. Barth, C.C. Chamberlain, J.A. Martin

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(Original signatures are on file with official student records.)

June 15, 1967

To the Graduate Council:

I am submitting herewith a dissertation written by Hermel Rosas entitled "Dietary Calcium and Zinc Effects in Unilaterally Neutron Irradiated Swine." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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Major Professor

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DIETARY CALCIUM AND ZINC EFFECTS IN UNILATERALLY NEUTRON IRRADIATED SWINE

A Dissertation

Presented to

The Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Hermel Rosas

August 1967

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CHAPTER I

INTRODUCTION

The importance of zinc in animal nutrition has been increasingly recognized in recent years. Numerous experiments have demonstrated that this trace element is an important constituent of rations for swine, poultry, ruminants, and some data are available on humans.

No information was found regarding the metabolism of stable zinc and radiozinc in tissues of neutron irradiated swine.

It has been widely demonstrated that a calcium and zinc relationship exists and that an inadequate amount of zinc in the diet has induced growth retardation and parakeratosis in swine (Lewis et al., 1956), as well as a health problem in cattle, goats, and chickens. Zinc is known to be a constituent of a number of metalloenzymes (Orten, 1966 and Vallee, 1959 referred to by Prasad, 1967). These include: carbonic anhydrase, pancreatic carboxypeptidase, liver, and yeast alcohol dehydrogenase, alkaline phosphatase, malic dehydrogenase, and glutamic and lactic dehydrogenase. In addition zinc increases the activity of a number of other enzymes apparently as a "cofactor" in a nonspecific manner.

The gross and histological features of swine skin resemble that of the human, and the radiosensitivity of the pig is within the range estimated for man. Hence, investigations using X-ray and mixed neutron-gamma irradiated swine have been conducted.

Since neutron irradiation can produce changes in the animal body, this work was initiated to study: (1) the excretion and retention of radiozinc and stable zinc in the organs of neutron irradiated swine fed different levels of calcium and zinc; and (2) to observe any gross and/or histological changes caused by neutron exposure.

CHAPTER II

LITERATURE REVIEW

Effects of Neutron Radiation on Animals

Gamma and X-ray irradiation have been used extensively in radiobiological studies by many researchers. However, meager information has been found pertaining to neutron radiation in animal and human research.

Mraz (1965) using neutron doses of 175 and 275 rads on male chicks 3, 10, 17, or 24 days of age found that at the 275 rads exposure, chicks started to die within 5 hr. and approximately 50 per cent of them were dead within 24 hr. Based on preliminary studies, Rosas and Bell (1965) stated that swine receiving three different unilateral neutron doses (632, 736, and 742 rads) retained more zinc in the hair than the non-irradiated swine and hair had a greater selective retention of zinc when the hair and skin were compared.

Thomas and Brown (1961) and Kuhn, Kyner, and Brown (1964) observed nervous system symptoms and epilation on burros with whole-body exposure to 145 rads neutron and 35 rads gamma irradiation and from a nuclear detonation, respectively.

Swine subjected *in utero* to neutron irradiation averaging 367 rads on the 21st day of gestation produced deformities of the thoracic leg bones (McFee, Murphree, and Reynolds, 1965).

Fowler (1964) noted that the first skin erythema in pigs appeared about 15 days after irradiation to the flank with X-ray dose of 2,910,

3,430, and 3,950 rads and neutron doses of 915, 1,100, and 1,240 rads. After the first peak of erythema had subsided at 30 days after irradiarion, a second broader peak occurred between 40 and 80 days. Some of the pigs were observed for 3 years with the result that no progressive increase in damage has been seen in the neutron irradiated pigs.

Sheep and goats following whole-body neutron irradiation of approximately 400 rads showed epilation, diarrhea, and vascularization of the cornea (Batchelor and Edmondson, 1964; and Quaife et al., 1964).

Myelocytic leukemia occurring in a rhesus monkey (Macaca mulatta) after exposure to a cumulative dose of 565 rads of whole-body neutron irradiation has been reported (Zalusky et al., 1965). Chromosome aberrations in humans induced by neutron and X-ray irradiation have been studied (Gooch, Bender, and Randolph, 1964).

Spalding, Sayed, and Johnson (1964) working with mice stated that the resultant $LD_{50/30}$ for fission neutrons was 204 $\pm$ 2.7 rads. When the fractionation method was used in fission neutron exposures the MLD was 616 $\pm$ 21.7 rads. The author concluded that the acute $LD_{50/30}$ for ^{60}Co gamma rays was 739 $\pm$ 12 rads, but when delivered by the fractionation method, the MLD was 2,670 $\pm$ 31.6 rads. Ainsworth (1964) mentioned that in acute mortality studies conducted with unilaterally neutron irradiated dogs, no significant differences in $LD_{50/30}$ were found between groups irradiated at 23 rads/min. or exposed to pulsed dose rates in excess of 1.5×10^5 rads/min. Cragle et al. (1965) studied the effects of lethal doses of gamma or neutron irradiation in cows exposed unilaterally during the lactation period. The total rads of neutron plus

gamma radiation used in cattle were 730, 755, and 502, respectively; and for gamma 650 rads. They observed a reduced feed intake and milk production following neutron irradiation.

Zinc in Animal Metabolism

Research concerning zinc metabolism in mammals, including the human has been gradually increasing. Miller et al. (1965a) observed that blood plasma zinc concentration increased with increasing level of supplemental zinc in cows; and zinc concentration increased at a more rapid rate in plasma than in milk. This suggests that the udder discriminates against zinc at the higher dietary and blood levels.

In spite of the fact that chicks fed a low zinc diet showed typical deficiency symptoms, zinc concentration of most of the soft tissues was not affected. However, pancreas and feathers showed a significant decrease in zinc concentration as a result of dietary zinc deprivation (Savage et al., 1964). Prasad (1967) and Lewis, Hoekstra, and Grummer (1957) reported a reduction of stable zinc in hair and blood plasma in swine and man on zinc deficient diets. Miller et al. (1967) reported that tissue zinc level in hair, liver, and pancreas was increased, and liver copper level was decreased with increasing dietary zinc level. Berry et al. (1961) mentioned that ^{65}Zn retention in the lung, heart, spleen, kidney, pancreas, small intestinal wall, muscle and skin was decreased by additional dietary zinc.

Fecal excretion following intravenous doses of ^{65}Zn was higher in normal ruminants fed a normal diet containing 46 p.p.m. zinc than

in comparable animals fed a zinc deficient diet containing 6 p.p.m. zinc (Miller et al., 1966). However, the deficient animals excreted more ^{65}Zn in the urine, suggesting a possible pathological effect of the deficiency on the kidneys. Cotzias, Bord, and Selleck (1962), working with ^{65}Zn in the mouse, found that the addition of various amounts of zinc to the diets in the form of zinc sulfate accelerated the turnover (rapid excretion) of ^{65}Zn. Similar results in rats have been observed (Furchner and Richmond, 1962).

Oral administration of ^{65}Zn in humans and dogs showed the greatest retention of the radioisotope to occur in the liver. Other tissues containing high radioactivity included the pancreas and kidneys (Spencer et al., 1965a; and Robertson and Burns, 1962). Van Campen and Mitchell (1965) using ^{65}Zn in rats observed that ^{65}Zn was taken up most rapidly from the duodenum, somewhat more slowly from the ileum, and with the least absorption occurring from the stomach.

Hair is rich in zinc, and the guinea pig and kitten, which are covered with hair or fur when they are born, contain more zinc per kilogram than the rat, rabbit, pig, or human baby (Spray and Widdowson, 1950, referred to by Comar and Brooner, 1964).

Syndrome of Zinc Deficiency

Zinc is known to be an essential trace element for plants and animals. Hence, an absence or inadequate amount in rations of laboratory and farm animals has caused severe symptoms in these subjects.

The rat was the first specie in which unequivocal zinc deficiency

was produced. Failure of growth was observed in rats placed on a purified diet containing only 0.16 mg. zinc/100 gm. In addition, alopecia and loss of pigment of the hair, mild dermatitis, scaling, and cracking of paws was also seen (Todd, Elvehjem, and Hart, 1934. referred to by Prasad, 1967; and Forbes and Yohe, 1960). Millard et al. (1958) observed a depressed growth and development of testes, epididymis, accessory sex organs, and in many cases severe atrophy of testicular germinal epithelium in weanling rats fed a poor zinc diet.

Pierson (1966) and Mills et al. (1965) reported that the clinical signs attributed to zinc deficiency in sheep and young lambs were complete loss of wool over their body, development of thick, wrinkled, and pink skin, scaly lesions around the eyes, nose, and mouth, excessive salivation, and parakeratotic lesions around the hocks and hooves.

In cattle the zinc deficiency symptoms observed included: anorexia; lower feed efficiency; breaks in the skin with deep fissure formation around the hoof; alopecia, especially on the rear legs; edematous soft swelling of the feet in front of the fetlocks with accumulation of fluid; extensive dermatitis between the legs and behind the elbows; red, scabby, and shrunken skin on the scrotum; undersized testicles; reduced serum alkaline phosphatase, and lower blood hemoglobin. Parakeratosis was also observed in the following areas: muzzle; vulva; anus; top of tail; ears; flanks; and neck (Miller et al., 1965b; Miller and Miller, 1962; and Legg and Sears, 1960).

Robertson and Burns (1962) reported that signs of zinc deficiency in dogs were similar in many respects to those seen in other

animals studied. However, the skin alteration was different than that mentioned in other animals, with no evidence of parakeratosis followed by thickening of the epidermis.

Kernkamp and Ferrin (1953) reported a dermatosis in swine that was characterized by hard, dry, crusted proliferations of the superficial layer of the epidermis. They termed the syndrome as parakeratosis and attempted to produce the disease by feeding several different rations, without success. Finally, Tucker and Salmon (1955) concluded that parakeratosis is primarily a zinc deficiency disease. Histological studies from zinc deficient pigs revealed parakeratosis and hyperkeratosis in sections from the tongue and skin of the tail, eyelid, and coagulation necrosis in the gastric mucosa (Beardsley, 1958).

Many investigators have mentioned the importance of zinc in chicks for the prevention of enlarged hocks, for maximum length to width ratio of the leg bones, and for good feathering and egg hatchability (Young et al., 1958; and Berg, Bearse, and Merrill, 1962). Similar symptoms were described by Klussendorf and Pensack (1958) on turkeys.

Prasad (1967) described zinc deficiency in man on the following basis: a decrease of zinc concentrations in plasma, red cell, and hair; retardation of skeletal maturation; and testicular atrophy.

Zinc Interrelationships with Factors Affecting Availability of Zinc

Several investigators have demonstrated interference in zinc availability can occur in different ways. Tucker and Salmon (1955) established that an excess of calcium and/or phosphorus in the ration

affected the incidence and severity of parakeratosis in swine by decreasing the availability of zinc.

Savage et al. (1964) mentioned that phytic acid (hexaphosphoric acid ester of myo-inositol) may simply form an insoluble zinc phytate salt which is not absorbed from the intestinal tract of the growing chick. This could occur whether the zinc arises from an exogenous source, or as endogenous zinc which is secreted into the intestine. He concluded that, in addition to its effect in decreasing zinc absorption, it is conceivable that phytic acid is absorbed and increases zinc loss by chelation and subsequent excretion by way of the urine. O'Dell et al. (1963) mentioned that there was a clear calcium-zinc-phytate interaction which decreased biological availability of zinc in the chick. However, ethylene-diaminetetra-acetate added along with phytic acid largely counteracted the detrimental effect of the phytic acid.

Neilsen et al. (1966) reported that soy protein contains a complicating factor other than phytic acid which affects zinc metabolism, especially in bone.

Nutritional interactions with zinc have also been demonstrated for copper, cadmium, iron, and molybdenum (Hoekstra, 1964). The presence of only one case of parakeratosis in the iron group and none in the copper-fed pigs suggests that these elements may be related to this disease (Hoefer et al., 1960). They further stated that the combination of 50 p.p.m. of zinc and 100 p.p.m. of iron was less effective than zinc alone suggests an antagonism between these two elements. However, work

at Wisconsin (Smith, 1959, referred to by Hoekstra, 1964) has repeatedly shown no beneficial effect of copper in curing or preventing zinc deficiency in swine. Dynna and Havre (1963) mentioned that copper administered alone caused slight improvement in cattle exhibiting poor growth, depigmentation of the hair, thickening of skin, and alopecia.

Copper sulfate, when added to a high calcium (1.3 per cent) basal diet at the rate of 125 to 250 p.p.m. of copper, stimulated pig growth and prevented parakeratosis during all but the last week of a 15-week trial. The 250 p.p.m. level of copper was much more effective in preventing parakeratosis than the lower level. However, several pigs exhibited symptoms of copper toxicity (Ritcher et al., 1963).

Many studies have been conducted to demonstrate a cadmium and zinc antagonism. Parizek (1960) and Gunn, Gould, and Anderson (1961) demonstrated in rats that cadmium alone produced a destruction of the testes and atrophy of sex organs, but feeding of zinc prevented these changes, with no gross or microscopic alterations. There was a reduction in growth, feed intake, and water consumption in calves fed as much as 160 p.p.m. cadmium without the addition of zinc. However, the addition of zinc with the added cadmium in the diet tended to increase feed consumption, weight gains, testicle size, hemoglobin, and blood zinc values (Powell et al., 1964).

Britton and Hill (1967) stated that the effects of cadmium on growth and phosphate activity and the effect of copper on growth of zinc deficient chicks indicate that copper as well as cadmium may act as a zinc antagonist.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

A total of 24 pigs were selected from 41 weanling barrows averaging approximately 15 kg. To facilitate this work, two separate trials using 12 pigs in each were conducted during the winter of 1965 and the summer of 1966. For the first trial 12 animals, averaging 30 kg. were randomly selected from a group of 20 pigs of mixed breeding, predominantly Yorkshire and Duroc, purchased at a feeder pig sale. The second trial involved 12 Duroc pigs selected from a group of 21 from the swine herd at the UT-AEC Agricultural Research Laboratory. These pigs averaged approximately 26 kg.

Pigs in both tests were weighed, ear tagged, and allotted into three dietary treatment groups, and placed in 1.22 x 2.44 meter pens with two pigs per pen. Uniformity of weight was considered in the allotment of the swine. To avoid zinc consumption from other sources, the feeders and waterers were painted with three coats of enamel (R-K-190, DuPont de Nemours and Co., Wilmington, Delaware). After the pigs were placed in the pens they were treated for internal and external parasites. For helminthic treatment, piperazine (Hexanthelin) in a single dose of 0.65 ml. per kilogram of body weight was administered in the feed. For ectoparasitic treatment, the pigs were sprayed with a single dose of 25 per cent lindane.

Experimental Design and Treatment

The experimental design employed in this study is presented in Table I. The three rations determined by chemical analysis were: Basal (0.6 per cent Ca and 29 p.p.m. Zn); Basal + 0.6 per cent Ca; and Basal + 0.6 per cent Ca + 71 p.p.m. Zn. All ration contained 0.5 per cent phosphorus. The composition of the dietary treatments is shown in Table II (Berry et al., 1961).

All three dietary rations and distilled H_2O were supplied ad libitum. Immediately following the pre-experimental period, 12 animals were transferred to a swine metabolism unit (as described by Mayo, 1961) for a 4-day adjustment period, and for quantitative separation and collection of feces and urine.

Body weight was recorded once a week, and feed intake values were determined weekly until the experiment was completed.

Fast Neutron Irradiation Procedure

During the fifth day in the metabolism units, six of the pigs, two from each dietary ration were placed into an adjusted aluminum crate for fast neutron irradiation at the Health Physics Research Reactor (HPRR) located at Oak Ridge National Laboratory. The remaining six nonirradiated swine served as controls. The reactor core used for neutron radiation is a circular cylinder of enriched uranium (93.14 per cent ^{235}U) alloyed with 10 per cent by weight of molybdenum (Auxier, 1965). All animals were unilaterally irradiated with 268 rads of fission spectrum neutrons at midline in air. The fast neutron dose was administered during a single 20-min. exposure at a rate of approximately

TABLE I

EXPERIMENTAL DESIGN

	Treatment					
	Basal		Basal + Ca		Basal + Ca + Zn	
	Irrad.	Nonirrad.	Irrad.	Nonirrad.	Irrad.	Nonirrad.
No. of pigs (Trial 1)	2	2	2	2	2	2
No. of pigs (Trial 2)	2	2	2	2	2	2
Oral dose ^{65}Zn (mCi.)	0.5	0.5	0.5	0.5	0.5	0.5
Neutron dose (rads)*	268	0	268	0	268	0
Gamma dose (rads)*	37	0	37	0	37	0

*Unilateral exposure.

TABLE II

PERCENTAGE COMPOSITION OF LOW ZINC RATIONS

	Basal	Basal + Ca	Basal + Ca + Zn
Corn	74.50	72.99	72.96
Soybean oil meal	18.00	18.00	18.00
17% dehy. alf. meal	2.81	2.81	2.81
Fish meal	2.50	2.50	2.50
$CaHPO_4$	1.04	1.04	1.04
$CaCO_3$	0.23	1.74	1.74
$ZnSO_4$	0.00	0.00	0.03
Salt	0.50	0.50	0.50
Antibiotic supplement[a]	0.28	0.28	0.28
Vitamin premix[b]	0.08	0.08	0.08
Trace mineral mix[c]	0.06	0.06	0.06
Total	100.00	100.00	100.00

[a]Supplied 60 mg. oxytetracycline hydrochloride per kilogram of the ration.

[b]Vitamin premix supplied 284 I.U. vitamin A, 94 I.U. vitamin D, 2.27 mcg. vitamin B_{12}, 0.54 mg. riboflavin, 2.27 mg. calcium pantothenate, 2.81 mg. niacin, 0.27 mg. pyridoxine, 4.26 mg. choline chloride, 28.35 mg. butylated hydroxytoluene, and 102.97 mg. methionine per kilogram of ration.

[c]Trace mineral mix supplied 0.17 mg. cobalt, 0.05 mg. iodine, 10.10 mg. manganese, 17.42 mg. iron, and 4.40 mg. copper per kilogram of ration.

13 rads per minute. During neutron irradiation, the subjects were located 3.00 meters from the core with a reactor height of 1.28 meters.

Besides the neutron dose of irradiation, the pigs received an additional 37 rads of gamma radiation giving a total of 305 rads. The neutron-to-gamma ratio in the irradiated animals was approximately seven-to-one.

Sampling and Analysis Procedures

Twenty-four hours postirradiation, all 24 test pigs were dosed orally by stomach tube with 0.5 mCi. of $^{65}ZnCl_2$ in HCl solution with specific activity of 7,636 mCi./gm. of stable zinc. Whole blood samples were taken from the jugular vein at 1, 4, 8, 24, 72, 96, and 120 hr. postdosing with a 30 ml. hypodermic glass syringe and a 5 cm. x 18 gauge bleeding needle. Sodium heparin, as an anticoagulant, was used to moisten the internal wall of the syringe prior to bleeding. A sufficient amount of the same anticoagulant was added to a graduated test tube to prevent blood coagulation. After dosing, urine and excreta were collected, weighed, recorded, and sampled every 24 hr. for 6 consecutive days to measure ^{65}Zn activity.

Blood samples were obtained 9 and 44 days after neutron irradiation for stable zinc analysis, and hair 88 days after exposure.

After 18 weeks on the experimental rations, the irradiated and nonirradiated pigs were sacrificed and a complete necropsy was performed. The following tissue samples were taken to determine the chemical zinc content: liver, pancreas, heart, spleen, kidney, hoof, muscle, and rib. All of these tissues except muscle and rib were weighed and

recorded. They were sectioned and aliquot samples were placed in glass jars and refrigerated for later analysis.

To study in vitro uptake of ^{65}Zn by red blood cells, 5 ml. of heparinized fresh whole blood were obtained from each of the 24 pigs and dosed with 0.4 μCi. of high specific activity ^{65}Zn. These blood samples were incubated under an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide at 38° C. for a 2-hr. period, then the red blood cells were washed twice in 0.85 per cent saline solution and separated from blood plasma by refrigerated centrifugation at 4° C. (methods of Wright and Bell, 1963). The radioactivity of the packed erythrocytes was counted in a well-type 51 x 51 mm. NaI crystal with a gamma spectrometer. A pulse height selector setting of 0.5 Mev. was used. The counts were expressed as per cent radioactivity uptake after adjusting the packed cell volume to 40.

For radioactivity studies in whole blood and blood plasma, 3 ml. of whole blood were placed into a scintillation tube for direct counting in the automatic gamma spectrometer. Following centrifugation of the blood at 2,000 revolutions per minute, 3 ml. of plasma were obtained for radiochemical counting.

Since ^{65}Zn is a gamma emitter, 3 ml. of urine and 3 gm. of excreta were used for direct counting in the gamma spectrometer. A single sample of whole blood and plasma was used for radiochemical studies because blood samples were drawn at intervals and a small amount of blood was obtained each time. However, urine and feces radiozinc determinations were conducted in duplicates.

Dose standards were counted at the time of the radioassays of the samples of blood, urine, plasma, and feces. The ^{65}Zn concentrations of the whole blood and plasma was expressed as percentage of dose per milliliter. The accumulated urinary and fecal ^{65}Zn excretion was reported as per cent of dose.

Approximately 88 days after neutron exposure, hair samples were collected from the pigs for stable zinc analysis. The hair was obtained by clipping the lateral area between the scapula and crest of the ilium of both the irradiated and nonirradiated sides, as well as from the controls. Prior to clipping, the hair was brushed and washed with 1 per cent Sterox (Monsanto Chemical Co., St. Louis, Mo.) a nonionic detergent. The sample was then placed into a glass jar for further analysis. Subsequently, the hair was covered with a 1 per cent solution of the detergent and the jar was covered with a cheesecloth (grade-10) and rinsed with distilled H_2O for 10 min. This washing procedure was repeated twice. Thereafter, the hair was squeezed with a glass rod and transferred to a Büchner funnel containing a filter paper for three additional washings with the nonionic detergent previously prepared with double distilled H_2O. Later the hair was rinsed three times with double distilled H_2O and squeezed again. The hair was dried in an oven at 70° C. for 72 hr. and allowed to come to equilibrium with the air for three days. The sample was then weighed (approximately 1 to 3 gm.) and ashed at 450° C. overnight. After ashing 5 ml. of N. HCl was added for 2-hr. period, followed by dilution to a volume of 10 ml.

Thereafter, a final dilution was prepared and the zinc content

was determined by atomic absorption spectrophotometry. The atomic absorption instrument was set according to the specifications recommended by Perkin-Elmer handbook (Perkin-Elmer Manual, 1964). A noise suppression of 4 and scale expansion x 1 was used. Standards were prepared from analytical reagent granular zinc, (assay zinc 100.0 per cent, J. T. Baker Chemical Co., Phillipsburg, N. J.). These standards were made to contain 0.1, 0.2, 0.4, 0.7, 1.0, 2.0, 3.0, and 5.0 p.p.m. of zinc. Double distilled water used for sample dilution and for preparation of standards was tested to assure a low zinc content.

All glassware used was cleaned with 1 per cent nonionic detergent and immersed overnight in 6 N. HCl. Afterwards, the glassware was rinsed with double distilled water and dried in an oven at 110° C. for a 3-hr. period.

Approximately 9 and 44 days after neutron irradiation blood samples were taken from each of the animals. After separating the blood plasma from the erythrocytes by centrifugation, 3 ml. of each sample were placed in a crucible and ashed at 450° C. overnight. Following ashing, stable zinc was determined by atomic absorption spectrophotometry.

The soft organs such as liver, pancreas, heart, spleen, muscle, and kidneys were weighed as fresh tissue, dried overnight at 100° C. and transferred to a furnace set at 450° C. for a 24-hr. period. The temperature in the furnace was increased 50° C. every 30 min. until it reached 450° C. to prevent loss of the oligo-elements. After ashing, stable zinc analysis was determined by atomic absorption spectroscopy.

The same procedure was used for the hard tissue such as bone and

hoof, except that the ashing temperature was 600° C. overnight to insure complete ashing.

Aliquots were sectioned from the kidneys, liver, skin and hair of irradiated and nonirradiated pigs; placed in a jar containing 10 per cent formalin buffered with sodium acetate for histological studies. Subsequently, the tissues were subdivided and processed by the method described by Russell and Shell (1966), and stained with Weigert's iron hematoxylin and eosin solution (A.F.I.P., 1957).

All data were recorded and subjected to statistical analysis (Variance analysis, Duncan's New Multiple Range Test, and "t" test) according to procedures outlined by Steel and Torrie (1960).

CHAPTER IV

RESULTS AND DISCUSSION

The Effect of Fast Neutron Exposure on Swine Pathology

The swine exposed to fast neutron irradiation (Figure 1) suffered a macro- and microscopic change in the hair shaft. The neutron irradiation apparently produced an absence of the pigment in the hair cortex without affecting the pigment in its medulla. This demonstrates that the change of the normal red pigment of hair to a white-gray color is due to the absence of pigment in the cortex. Hence, it is the pigment in the cortex (Figure 2) that gives color to red hair. The length of time required to change the pigment in hair was approximately 40 days. Ham (1953) reported that melanin was responsible for the color of the hair, and is formed by the epithelial cells of the matrix of the follicle. However, in this study no histological difference in the cells of the matrix or in any other tissue of skin was noticed at the time of sacrifice between the neutron irradiated and the control animals. Archambeau et al. (1964) found no histological changes in the skin of pigs at time of sacrifice after neutron irradiation with a neutron fluence of 5×10^{12} N./cm.^{2}. This suggests that some blocking of enzymatic factors or the absence of an enzyme are involved in the change of hair color. Chase (1949) indicated a variable effect of X rays on greying in mice due to the stage of hair growth at the time of X-raying and to the dose employed. Thomas and Brown (1961) observed in one burro

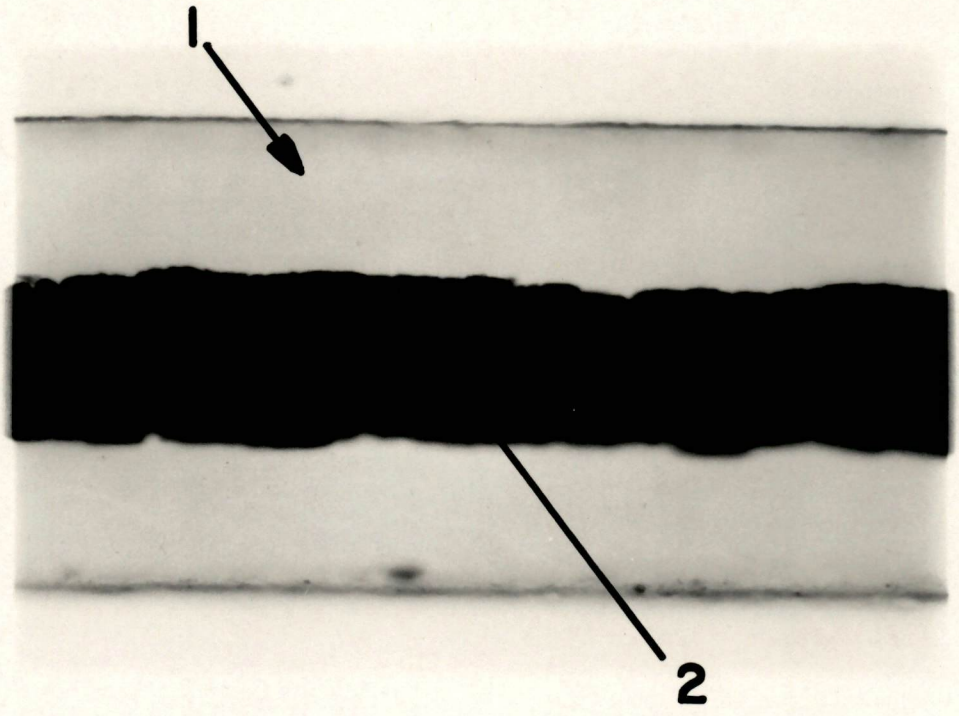

Figure 1. Photomicrograph of the hair shaft of fast neutron irradiated swine, in vivo. Observe the absence of pigment in the cortex (1) and the presence of hair pigment in the medulla (2).

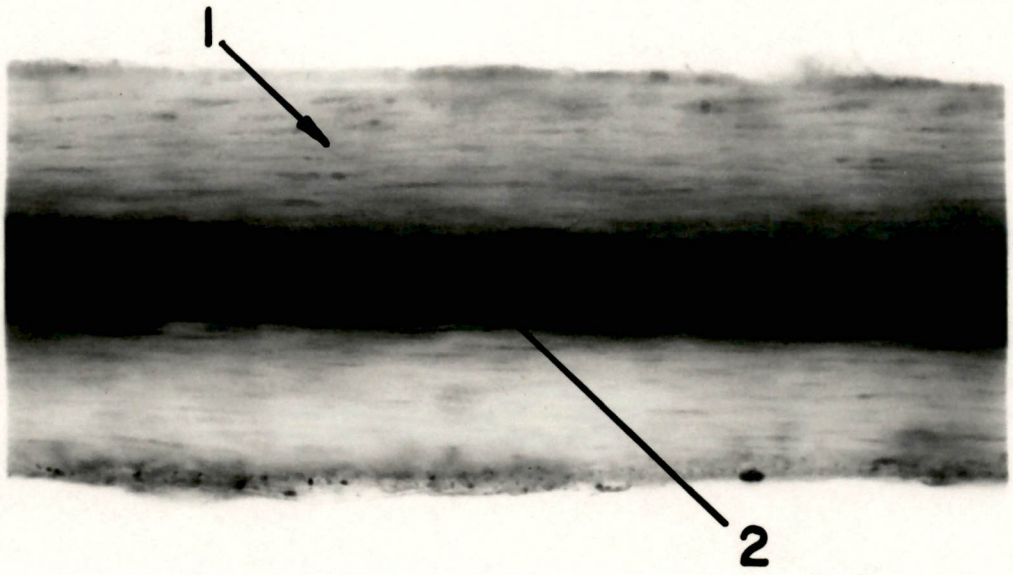

Figure 2. Photomicrograph of a normal hair shaft. Note the presence of pigment in the cortex (1) and in the medulla (2).

exposed to neutron gamma radiation a normal hair regrowth with no apparent change in color in 3 months. However, clipping the hair in animals before they were exposed to neutron irradiation, the immediately following hair generation being gray and never appearing with a normal pigmentation.

Chase, Straile, and Arsenault (1963) mentioned that a delay in hair growth was an indication of follicular damage in mice exposed to X rays. In this study no histopathological changes were observed in the hair follicles in the 30 slides that were examined.

Galbraith (1966) reported that X-ray doses exceeding 1,000 R. delivered to resting hair follicles of mice caused a reasonable graying response. Chase and Rauch (1950) mentioned a hypothesis that radiation affects the melanoblast source of pigment granules for each follicle in mice, and the smallest type, the zigzag hair, is the most sensitive.

A rather striking difference ($P<.01$) in hair growth was observed (Figure 3) in irradiated pigs. The hair growth on the left side of the pig near the core was retarded in comparison with the right side, which was farther away from the reactor.

No epilation in the neutron irradiated side was observed. This was probably due to the sublethal dose that each pig received during neutron irradiation. However, epilation has been observed in burros beginning about the second week postexposure to neutron irradiation (Thomas and Brown, 1961). Sheep and goats exposed to neutron irradiation with a dose of 400 R. suffered an epilation with the former but not with the latter (Quaife et al., 1964). Complete individual data

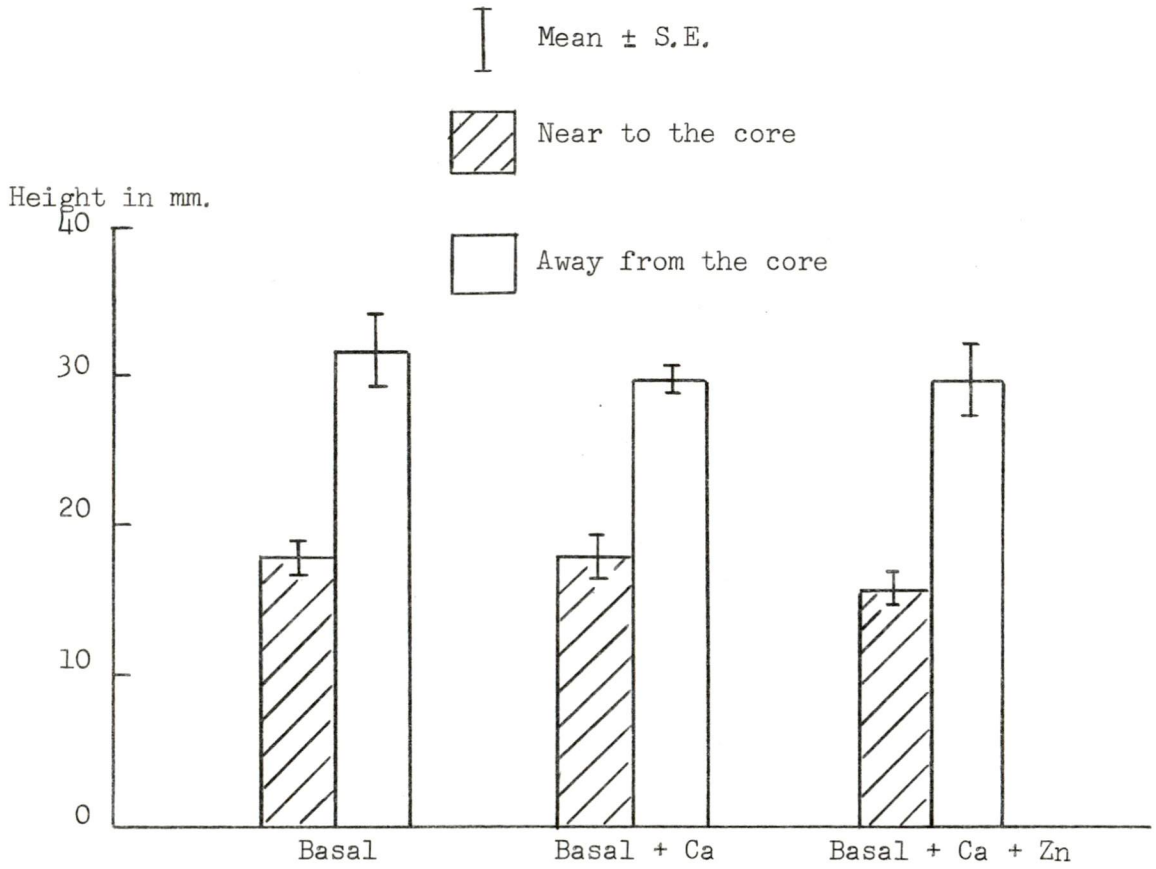

Figure 3. Effect of radiation and ration on hair growth.

in hair length with standard error of means appear in Table V of the Appendix.

Prasad (1967) noticed an increase in pubic hair growth in dwarfs receiving zinc supplement. In this experiment no significant difference in hair growth was observed in pigs receiving a zinc supplement in the ration.

The nature of hair growth is not well understood. Many questions are still unanswered, such as the nature of the stimulus for the hair growth cycle and the other factors controlling the length of time of growth and resting (Chase, 1954).

Following necropsy and unexpected atrophy of the kidney of the left side nearest the core (Figure 4) was found in pigs under the three different regimens. Left kidneys weighed less than controls' kidneys ($P<.01$) in both trials (Figure 5). Ration effects on kidney weights were tested for significance by using Duncan's New Multiple Range Test (Steel and Torrie, 1960); and the results were as follows: Basal + Ca was greater than Basal ($P<.01$), but not significantly greater than Basal + Ca + Zn ($P>.05$). Basal + Ca + Zn also was not significantly greater than Basal ($P>.05$).

This reduction in size and weight of the kidneys nearest to the reactor shows that kidney cells are sensitive to neutron irradiation. No compensatory hypertrophy in the kidney away from the reactor was observed. Ranninger (1967) estimated the relationship between the atrophy of the irradiated kidney and the compensatory hypertrophy of the normal kidney in dogs administered 3,500 rads, and concluded that the degree of

Figure 4. Comparison of kidneys in the same animal exposed to unilateral neutron irradiation. The left kidney was nearest to the reactor and shows atrophy.

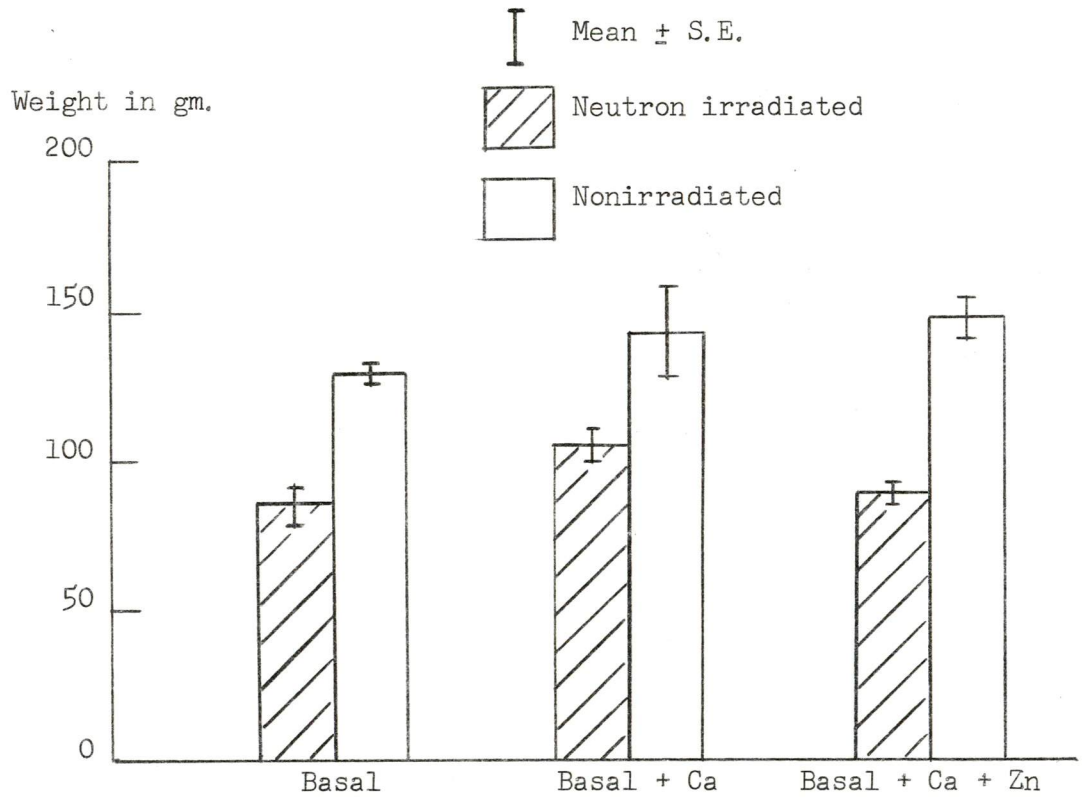

Figure 5. Effect of radiation and rations on the left swine kidneys.

the hypertrophy is not proportional to the extent of the atrophy of the irradiated kidney.

The average weight of the left atrophic kidneys in both trials was 94 gm. compared with 141 gm. of the left nonirradiated kidneys of the controls. The average length for neutron irradiated kidneys was 11 cm. compared with 13 cm. in the nonirradiated animals. Individual kidney weight and measurement data are reported in Table VI of the Appendix.

After the pigs were sacrificed the following organs were taken: liver, spleen, pancreas, heart, and hoof. With the exception of hooves and pancreas, a marked reduction in organ size was noticed in swine exposed to neutron irradiation. The data for fresh organ weights are shown in Table III.

Radiation significantly decreased liver and spleen weights ($P<.01$) and heart weight ($P<.05$). The hoof and pancreas (Table III) were not significantly influenced ($P>.05$) by neutron exposure. Ration was not a significant source of variation ($P>.05$) in any of the previously mentioned organs.

These results seem to indicate that cells of the liver, spleen, and heart were radiosensitive to sublethal levels of neutron irradiation. However, the pancreas and hoof did not differ from controls, suggesting a greater resistance to irradiation. Presumably, the reduction in weight of the liver, spleen, and heart was caused by the neutron exposure and not by the dietary effects. Shikita and Tamaoki (1963) observed that spleen and kidney decreased in weight in mice receiving a single 400 R. whole-body dose of X irradiation.

Histological studies of muscle, liver, and kidney revealed no differences between tissues exposed to neutron irradiation and

TABLE III

FRESH ORGAN WEIGHTS AS AFFECTED BY FAST NEUTRON IRRADIATION AND RATION

	Treatment					
	Basal		Basal + Ca		Basal + Ca + Zn	
	Nonirrad.	Irradiated	Nonirrad.	Irradiated	Nonirrad.	Irradiated
Liver[a]	1560 ±88.64[c]	1223 ±81.32	1532 ±111.90	1382 ±44.18	1646 ±89.42	1174 ±163.38
Spleen[a]	122 ±10.04	76 ±19.88	113 ±2.64	72 ±11.32	123 ±14.61	64 ±7.68
Heart[b]	290 ±16.66	254 ±32.21	319 ±13.68	267 ±13.62	312 ±12.83	276 ±20.92
Kidney[a]	130 ±2.87	86 ±6.56	144 ±15.34	106 ±5.56	148 ±6.65	90 ±3.94
Pancreas	143 ±41.74	124 ±17.59	120 ±26.08	130 ±16.99	154 ±11.92	86 ±25.16
Hoof	14 ±1.99	12 ±0.86	14 ±3.06	12 ±1.82	27 ±2.22	26 ±1.29

[a]Means between nonirradiated and irradiated significantly different ($P<.01$).

[b]Means between nonirradiated and irradiated significantly different ($P<.05$).

[c]Mean weight in gram ± standard error.

nonirradiated tissues. Apparently, the sublethal dose used in pigs did not produce any change in the cells of the above organs measurable at time of necropsy.

Zalusky et al. (1965) reported an extensive infiltration of leucocytes in the interstitial tissue of the kidney in monkeys exposed to whole-body neutron irradiation with a cumulative dose over a 3-year period.

Claudication (lameness) of the left rear leg, which was facing the core, was observed in all pigs approximately 76 days postneutron irradiation. Both rear legs of the same animal were measured immediately following necropsy to determine any differences in length. The average length and weight of the left rear leg from the third phalange to tibial tarsal bone was 15.6 cm. and 301.5 gm., respectively, compared with 18.0 cm. and 364.8 gm. for the right rear leg away from the reactor. In this case, soft and hard tissues were included. These differences indicate that the cause of claudication in neutron irradiated pigs is the shortening of the leg bones and atrophy of the muscles in the leg nearest the reactor. These data were analyzed statistically and demonstrated significantly lower values for weight ($P<.05$) and length ($P<.01$) in legs near the reactor. Individual data for length and weight of the rear leg are shown in Table VII of the Appendix.

A congestion of the conjunctiva in both eyes of the neutron irradiated pigs was observed. This condition began within the first postirradiation week. Also, a similar congestion of the palpebra tertia (third eyelid) located at the medial angle of the eye was noticed in two of the subjects. About two weeks postexposure the congested conjunctiva

and third eyelid of the eye disappeared. No other syndrome of the eye was observed in pigs receiving neutron irradiation. Quaife et al. (1964) reported a vascularization of the cornea in sheep receiving 600 neutron rads. The nutritional status of the swine on the three treatments did not affect the severity of the congested area in the eye.

In vitro Uptake of ^{65}Zn by Erythrocytes in Neutron Irradiated Swine Fed Different Rations

The effect of fast neutron irradiation in swine fed three different rations upon in vitro uptake of ^{65}Zn by red blood cells is presented in Figure 6. The uptake of ^{65}Zn averaged higher in the irradiated than in nonirradiated animals; however, the neutron radiation had no significant effect ($P>.05$) upon in vitro uptake of ^{65}Zn by packed red blood cells.

The effect of ration on uptake of ^{65}Zn was highly significant ($P<.01$) with the highest uptake on the Basal + Ca and the lowest on Basal + Ca + Zn. All treatment differences were highly significant ($P<.01$). Individual data with the standard error of a mean appears in Table VIII of the Appendix.

The high uptake of ^{65}Zn by packed erythrocytes from the Basal + Ca treatment indicates that these cells were deficient in zinc due to an interference between calcium and zinc. Similar results in the effect of calcium and zinc upon in vitro uptake of ^{65}Zn by porcine blood cells was found by Berry, Bell, and Wright (1966). They demonstrated that calcium increased whereas zinc decreased in vitro uptake of ^{65}Zn throughout the experiment.

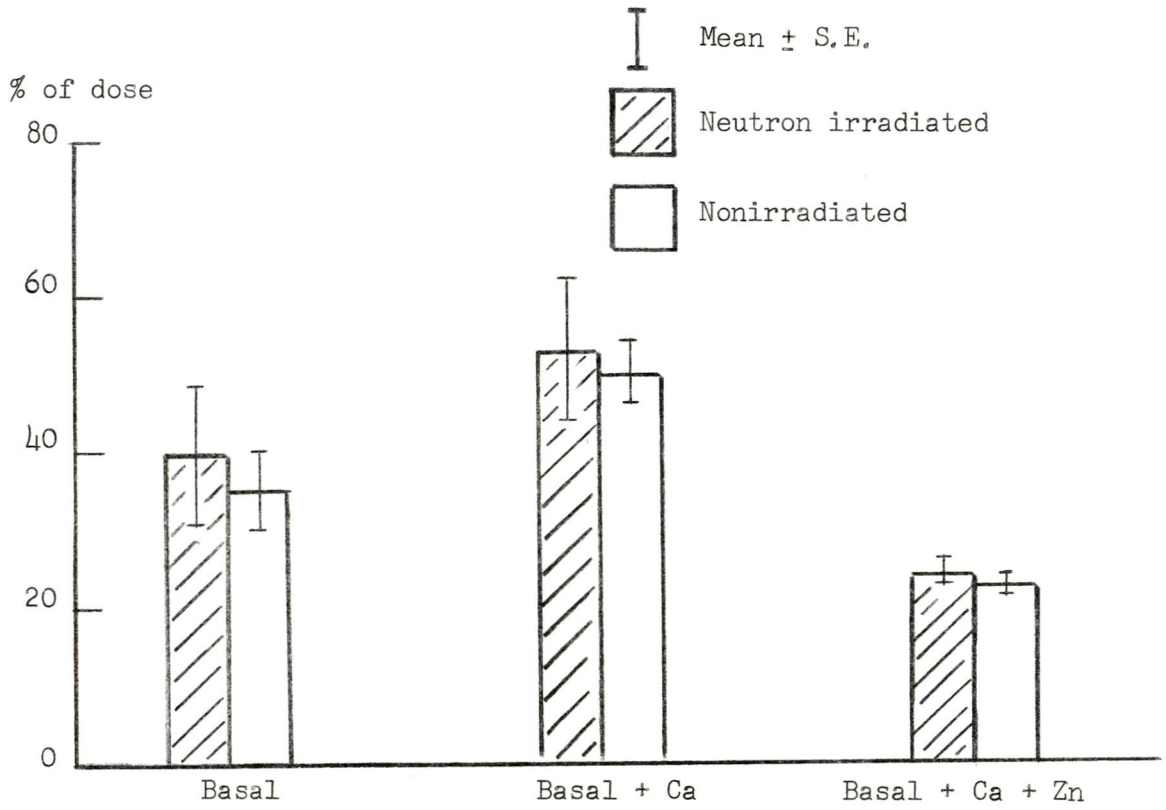

Figure 6. <u>In vitro</u> uptake of ^{65}Zn by swine red blood cells.

Wright and Bell (1963) reported that an increase of cellular uptake of ^{75}Se in ovine red blood cells was influenced by selenium. Weswig et al. (1966), working with low selenium forage supplemented with various levels of selenium, reported similar results.

In this study zinc added to the ration decreased in vitro uptake of ^{65}Zn and is in agreement with the findings of Berry et al. (1961) and Furchner and Richmond (1962). However, Pearson, Schwink, and Reich, 1965 (referred to by Prasad, 1966) failed to reduce ^{65}Zn uptake by feeding a high zinc diet in rats. Presumably, this discrepancy could be attributed to species difference and the use of intestinal segments instead of red blood cells to determine the uptake of ^{65}Zn.

Possibly, the in vitro uptake of the radiochemical element by red blood cells can be used as a diagnostic tool to determine trace element deficiencies in animals and humans. The advantages of this technique are that a small amount of blood is required and it can be obtained by vena puncture. Hence, no radioactivity is given the animal and necropsy of the subject is not required.

The slight increase in the uptake of ^{65}Zn by red blood cells in neutron irradiated animals (Figure 6, p. 32) is not well understood. Presumably, neutron irradiation causes a transitory damage of the erythrocytic membrane and increases the permeability of the radioelement in red blood cells. Hirose (1964) stated that the effects of fast neutrons for tissue damage in mice are over 20 per cent more powerful than those of X rays. Myers and Tribe (1967) found that the external membrane of rat erythrocytes is altered after X irradiation.

^{65}Zn in Blood, Plasma, Urine, and Feces of Irradiated and Nonirradiated Pigs Receiving Different Rations

The results of ^{65}Zn retention in whole blood are presented in Figure 7. After the subjects were dosed orally with ^{65}Zn, a rapid absorption occurred through the intestinal tract and reached a peak in the whole blood between 24 and 48 hr. after ingestion of ^{65}Zn in the irradiated and nonirradiated animals. Thereafter, the retention of ^{65}Zn declined gradually in controls and neutron exposed animals. Radiozinc retention in whole blood from irradiated animals was lower ($P<.05$) than in that from nonirradiated animals. The effect of ration on ^{65}Zn activity in whole blood was greater in both Basal and Basal + Ca than in Basal + Ca + Zn ($P<.01$) while Basal and Basal + Ca did not differ ($P>.05$).

As shown in Figure 7, the retention of ^{65}Zn in whole blood by Basal + Ca + Zn was the lowest compared with Basal and Basal + Ca rations. This trend could possibly be explained by the addition of stable zinc producing a dilution effect of the trace radiochemical element. Similar results of ^{65}Zn retention in the whole blood of pigs fed Basal + Zn + Ca were reported by Berry et al. (1961).

The nonirradiated pigs (Figure 7) fed high calcium showed a lower ^{65}Zn retention on the whole blood at 72, 96, and 120 hr. after dosing than the nonirradiated swine receiving a low calcium regimen during the same period. Similar results in man orally administered tracer doses of ^{65}Zn (Spencer et al., 1965b) showed that during high calcium intake the ^{65}Zn levels in whole blood were slightly lower at 8 hr. and at 24 hr. than at the corresponding time intervals with low calcium intake.

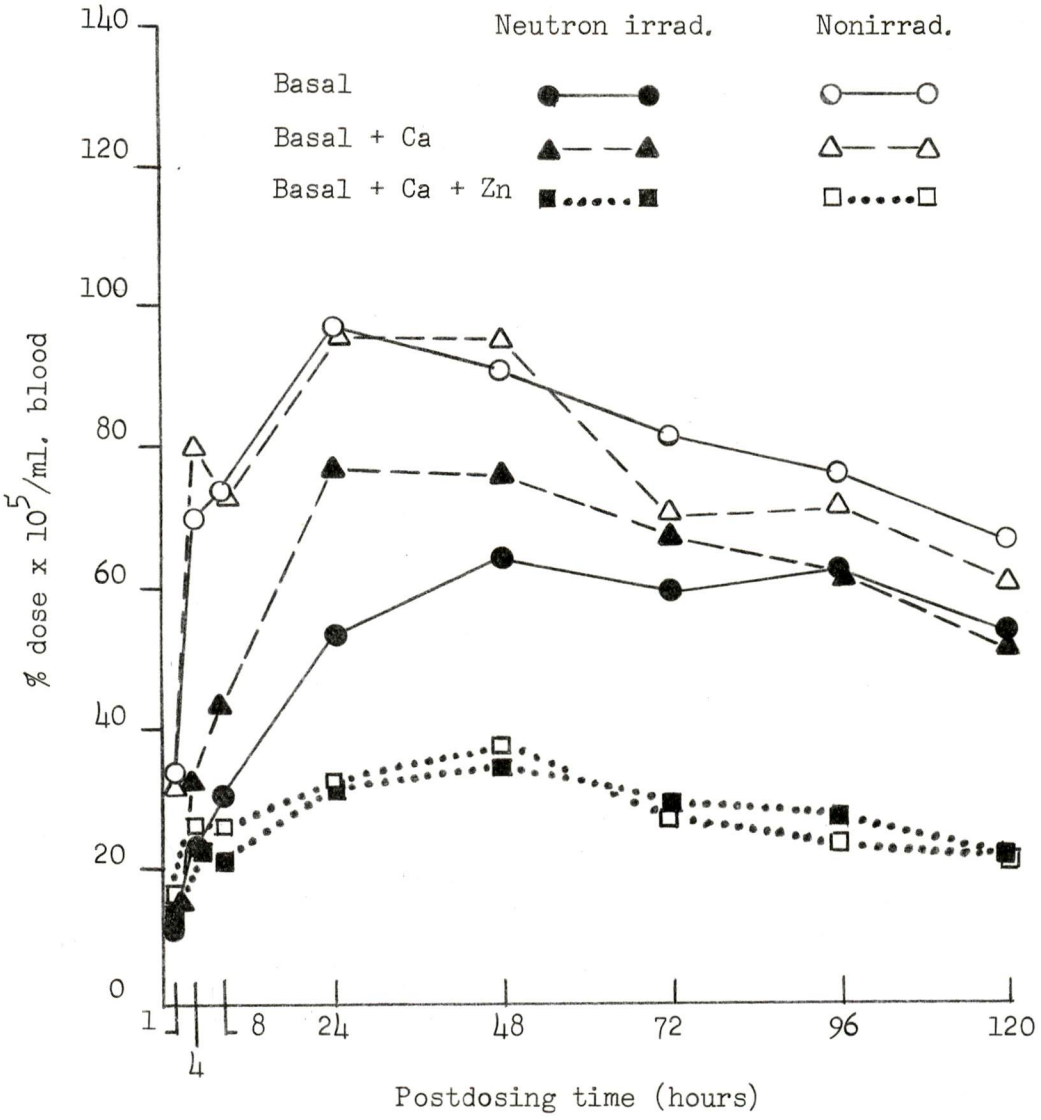

Figure 7. Radioactivity of ^{65}Zn in whole blood of swine.

The fact that ^{65}Zn whole blood level was highest between 24 and 48 hr. after dosing in irradiated and nonirradiated animals, followed by a gradual decrease, indicates that most of the absorption of the radioelement from the intestinal tract was obtained during the first two days. A decrease of ^{65}Zn at 120 hr. postdosing may be attributed to the uptake of this radiochemical element by organs, tissues, and enzymes important in metabolic processes and/or by partial excretion via the urine and feces of the radioelement not in use by the organism.

The blood plasma (Figure 8) illustrated that ^{65}Zn entering the blood stream reached a peak 4 hr. postdosing in neutron irradiated animals followed by a gradual decrease. The ^{65}Zn levels in blood plasma were lower than in whole blood in both controls and neutron irradiated swine. Radiation resulted in significantly lower ($P<.01$) blood plasma levels of ^{65}Zn. Radiozinc activity was greater in both Basal + Ca and Basal than in Basal + Ca + Zn ration ($P<.05$). However, the Basal + Ca was not significantly higher than Basal ($P>.05$). The probabilities of significance and the treatment mean values of plasma and whole blood on ^{65}Zn activity are indicated in Table IX of the Appendix.

The retention of ^{65}Zn by blood plasma in animals fed Basal + Ca + Zn showed the lowest activity of the three treatments compared. A similar result appeared in the whole blood ^{65}Zn activity. Therefore, the same explanation previously mentioned for the whole blood can be applied in the retention of ^{65}Zn by blood plasma. It seems to indicate (Figures 7, p.35, and 8) that the disappearance of ^{65}Zn is more rapid in plasma than in whole blood. The reason for the low levels of

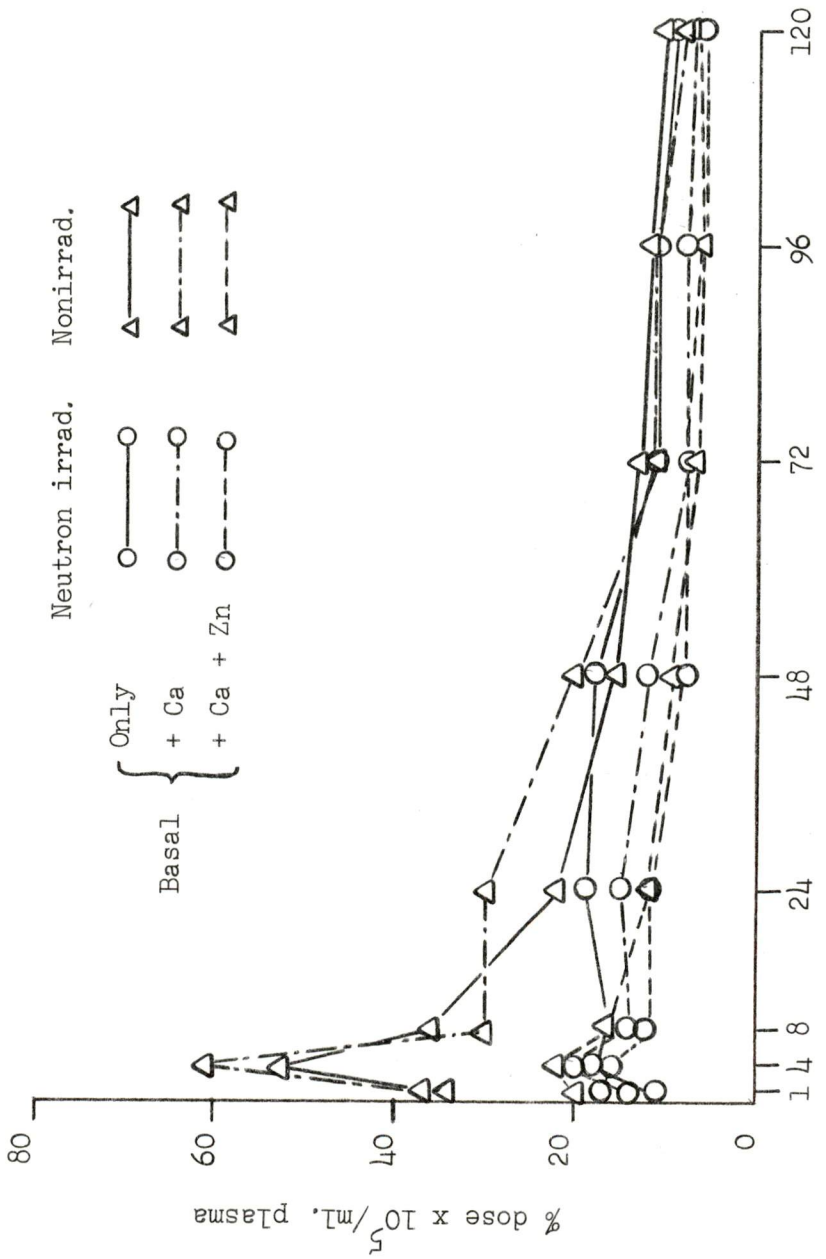

Figure 8. Radioactivity of ^{65}Zn by blood plasma in swine.

^{65}Zn in the plasma and whole blood of neutron irradiated swine is not readily apparent. Presumably, this phenomenon is due to zinc metabolic disturbances in subjects submitted to fast neutron irradiation. The reduction in growth rate of swine exposed to neutron irradiation was accompanied by a decrease in organ weight (Table III, p. 29).

In the human following an intravenous injection of ^{65}Zn, a lower concentration of the isotope was found in the plasma 4 hr. postdosing, than in whole blood (Spencer et al., 1965a).

Quastler and Hampton (1962), using 200 rads X ray in the mouse, observed a softening of the epithelium in the intestinal membrane within 10 min. after irradiation indicating a radiosensitivity at a low dose. They concluded that after two days the cells appearing on the villus showed a decrease in absorption and minimal transport across the epithelium. Baker and Perrotta (1967) found in rats that X irradiation produced an early reduction in both crypts and villus cell counts reaching minimum values at about 48 hr. In dogs, Alpen, Shill, and Tochilin (1960) described more extensive damage to the intestinal epithelium after neutron irradiation than after X irradiation.

Lowery and Bell (1964) reported that the per cent dose of ^{89}Sr in plasma of pigs dosed 1 day postirradiation reached a peak 4 hr. after dosing in both the controls and irradiated groups. Although the per cent dose appeared higher in irradiated compared to nonirradiated, the difference was not significant ($P>.05$). In a later experiment Bell, Lowery, and Withrow (1966) showed that whole-body gamma irradiation affected both ^{89}Sr and ^{45}Ca in blood plasma of pigs.

A slight increase in urinary excretion of the isotope in nonirradiated pigs fed Basal and Basal + Ca was observed. However, this increase was not significant ($P>.05$) when compared with neutron irradiated swine (Figure 9) receiving the same diets. The accumulative average per cent dose of ^{65}Zn recovered during the six days collection period was 1.04 in nonexposed swine fed a Basal ration. Meanwhile, the neutron irradiated pigs demonstrated an excretion of 0.80 per cent of dose during the same period.

The amounts of ^{65}Zn excreted in the urine by neutron irradiated swine varied greatly among subjects, and the excretion of ^{65}Zn ranged from 0.06 per cent to 2.23 per cent of the administered dose in six days. In the controls, the range observed was from 0.10 per cent to 1.90 per cent of the administered dose in six days. Individual amounts of ^{65}Zn excreted in the urine are given in Table X of the Appendix.

Presumably, the decrease in urine excretion of the radioelement observed in neutron irradiated animals could be due to kidney atrophy caused by fast neutron irradiation. However, further studies will be required before a definite conclusion can be made.

Ration affected ^{65}Zn urinary excretion in pigs ($P<.05$). Both Basal + Ca and Basal were significantly greater ($P<.05$) than Basal + Ca + Zn. However, Basal + Ca was not significantly greater ($P>.05$) than Basal. As shown in Figure 9, the addition of calcium to the ration increased the excretion of ^{65}Zn in the urine. However, the incorporation of chemical zinc to the diet decreased the excretion of the radioisotope in the urine, in neutron, and nonirradiated pigs. Beardsley

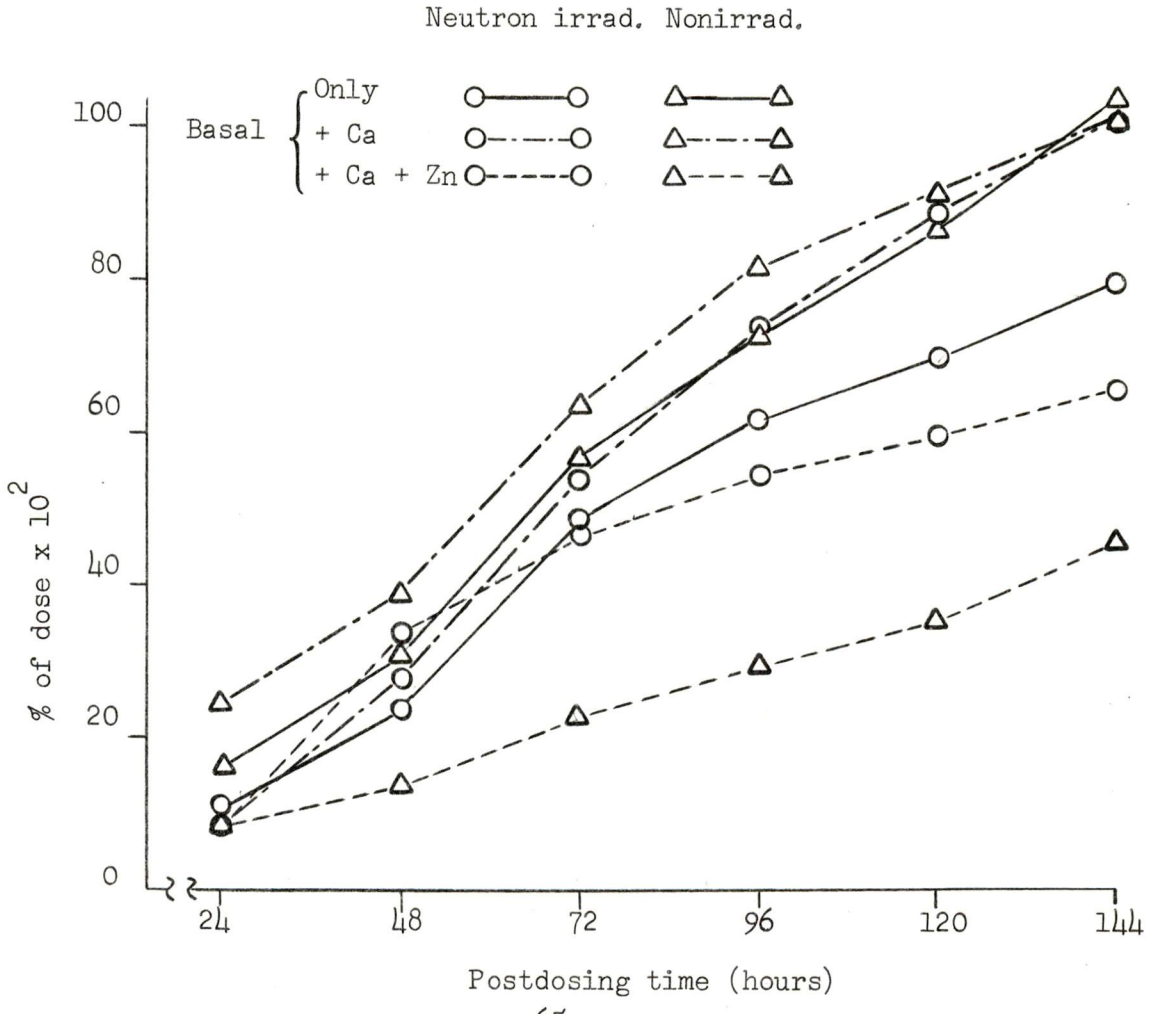

Figure 9. Accumulative ^{65}Zn urine excretion from oral dose in swine.

(1958) stated that extra calcium increased the excretion of zinc in the urine in baby pigs.

Individual data of ^{65}Zn excretion in the feces of irradiated and nonexposed swine are presented in Table XI of the Appendix. These results demonstrated that the principal pathway of ^{65}Zn excretion after oral administration of the radioelement was via the digestive tract and and not via the urinary tract. Similar observations have been found in man (Spencer et al., 1965a), dogs (Robertson and Burns, 1962), and rats (Kinnamon and Bruce, 1965).

The highest excretion of ^{65}Zn in the feces was observed in animals fed Basal + Ca with an accumulative average of 38 per cent of dose, and the lowest fecal excretion of the radioisotope was obtained in the pigs fed Basal + Ca + Zn and Basal with 37 per cent of dose, respectively. These trends in ^{65}Zn fecal excretion within treatments were not significantly different ($P>.05$). The increase of ^{65}Zn fecal excretion in pigs receiving Basal + Ca may be attributed to the calcium-zinc interaction. However, Cotzias, Bord, and Selleck (1962) observed in the mouse that zinc used as a dietary load accelerated the elimination of ^{65}Zn.

The accumulative average of ^{65}Zn fecal excretion in pigs receiving neutron irradiation was not significant ($P>.05$) when compared with nonirradiated swine. Lowery and Bell (1964) observed in pigs that after the second oral dosing at 21 days postirradiation there was no difference in the fecal excretion of ^{89}Sr between irradiated and control animals.

Stable Zinc Concentrations in Porcine Tissues Affected by Neutron Irradiation and Dietary Treatments

The stable zinc analysis performed on blood plasma and erythrocytes demonstrated slight but nonsignificant ($P>.05$) differences in zinc concentration between neutron irradiated and nonirradiated animals. However, the ration effect on blood plasma (Figure 10) was highly significant ($P<.01$) with the highest stable zinc content in the Basal + Ca + Zn and the lowest in Basal + Ca. Stable zinc in blood plasma of pigs fed Basal + Ca + Zn was significantly greater than Basal + Ca ($P<.01$) and Basal was greater than Basal + Ca ($P<.05$). Basal + Ca + Zn was not significantly higher than Basal ($P>.05$).

These results show that calcium exerts a depressing effect on the availability of zinc. Also, the slightly different stable zinc content in packed erythrocytes and blood plasma in irradiated swine is presumably based on the utilization of zinc in tissue repair. Zinc is an essential element for tissue repair (Pories and Strain, 1965) and is also involved in many enzyme systems (Prasad, 1967).

There was a tendency for zinc concentration in the blood and injured skin to increase in rabbits following radiation (Prokopchuk and Sosnoskii, 1966). Miller et al. (1965b) reported an increase of zinc blood plasma in cows fed an excess of zinc. Zinc concentrations in erythrocytes and blood plasma from the ration-irradiation subgroups are shown in Table XII of the Appendix.

A difference in zinc content of hair from neutron irradiated swine fed Basal and Basal + Ca was observed (Figure 11). The neutron irradiated

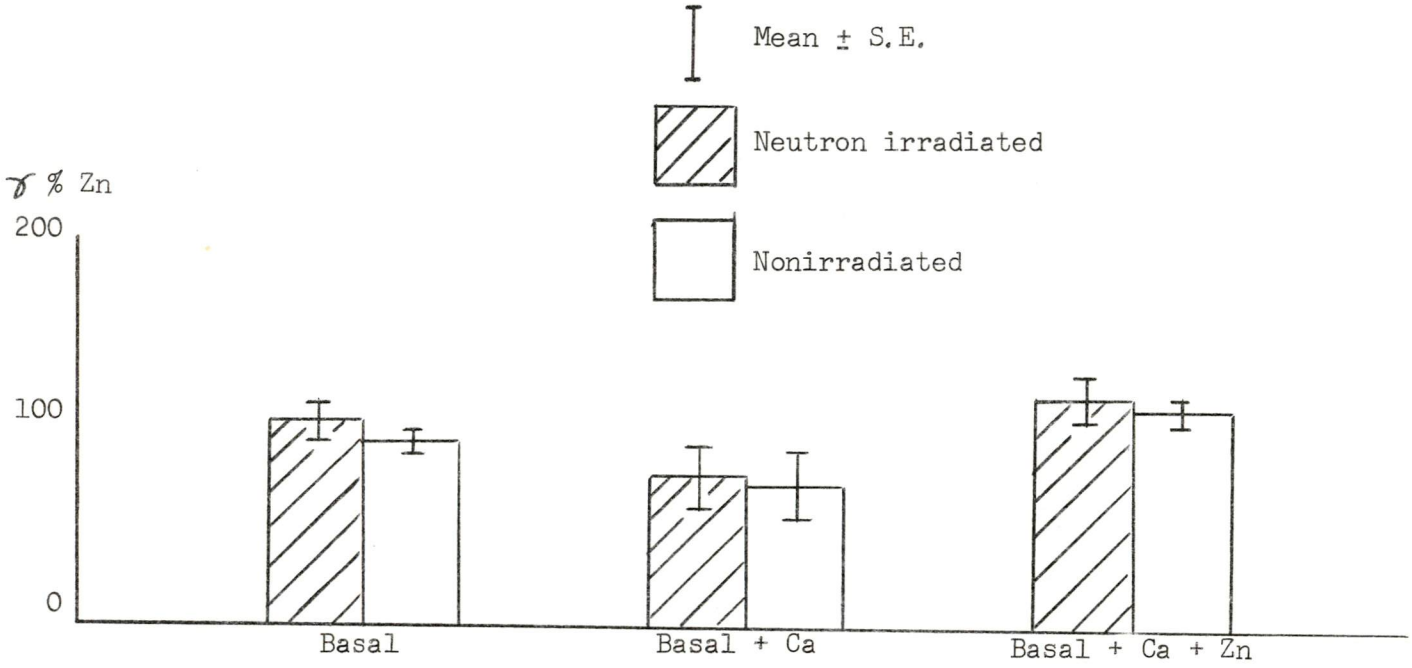

Figure 10. Stable zinc in swine blood plasma.

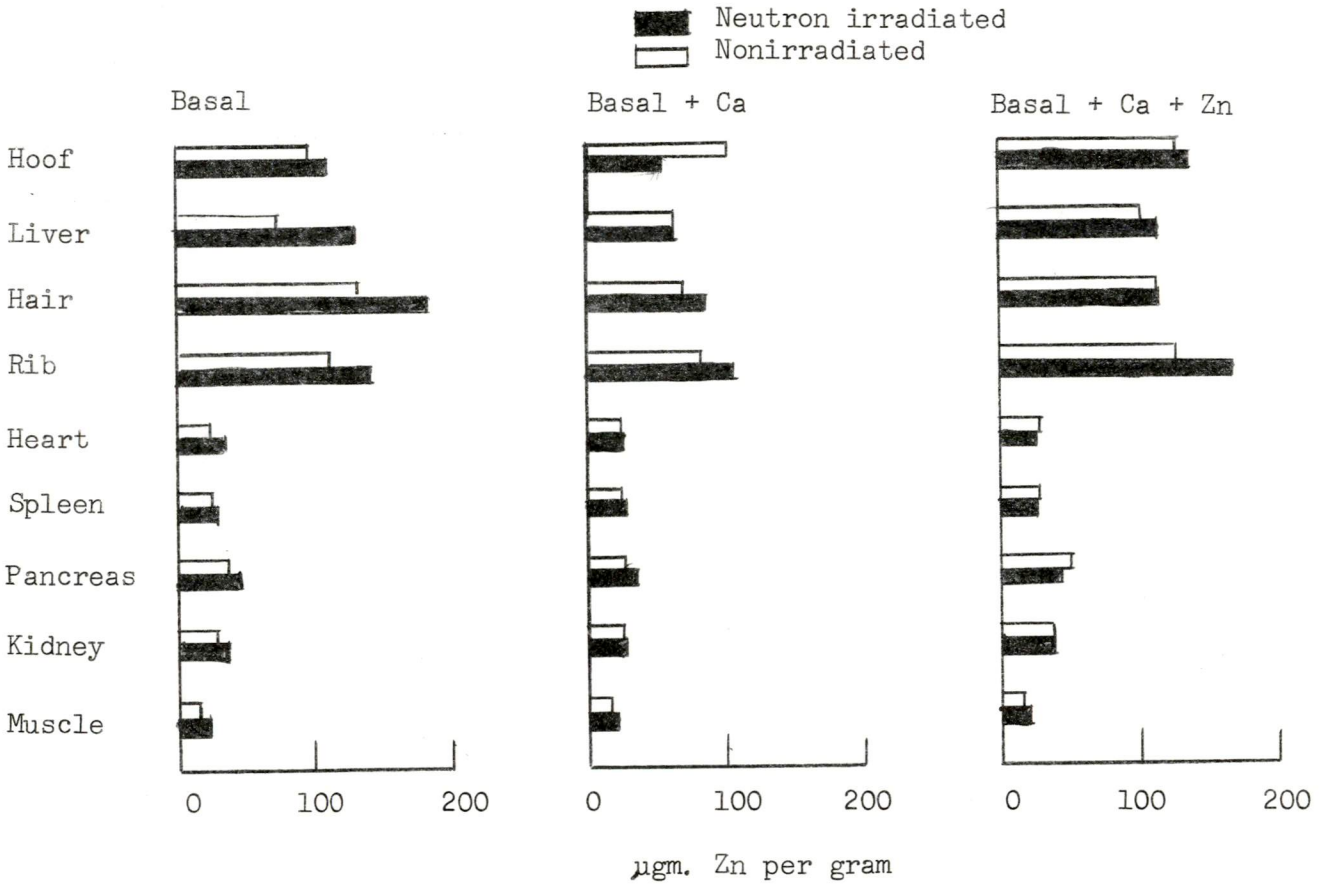

Figure 11. Effect of radiation and ration on zinc tissue concentration.

animals receiving the Basal diet (Table XIII of the Appendix) showed a zinc hair concentration of 186 ± 38.10 µgm. per gram. However, the non-irradiated pigs fed the same regimen demonstrated a lower ($P<.05$) zinc hair content of 134 ± 23.29 µgm. per gram. These results suggest the utilization of zinc in tissue repair. A positive correlation between zinc level and the speed of human healing has been demonstrated (Pories and Strain, 1965). The lowest zinc hair retention was found in animals fed the Basal + Ca ration. This could be explained by the zinc-calcium antagonism.

A significant ($P<.05$) ration X irradiation interaction was observed between levels of zinc in the hair in this experiment. This interaction resulted from a differential response to irradiation between rations. Addition of calcium to the basal ration resulted in a 96 µgm. per gram drop in hair zinc level in the irradiated animals contrasted to a drop of 61 µgm. per gram in the nonirradiated animals. The addition of both zinc and calcium to the basal ration resulted in a 72 µgm. per gram reduction in stable zinc in irradiated groups versus an 18 µgm. per gram reduction in nonirradiated groups. Also when the Basal + Ca is compared to the Basal + Ca + Zn, then greater retention in response to the addition of zinc was noted in the nonirradiated animals. A significant ($P<.05$) ration X irradiation interaction between levels of zinc in the hoof was observed.

Since the significant interaction was not observed in any of the other organs considered except hair and hoof, it could be concluded that zinc hair and hoof levels are more sensitive to zinc ration levels and

irradiation than the other tissues.

Lewis, Hoekstra, and Grummer (1957) found in swine an increase in hair zinc content when an excess of the supplemental zinc was included in the diet. In this experiment, however, the addition of zinc to the ration did not increase the hair zinc concentration. Miller et al. (1965c) observed that zinc content of bovine hair can be appreciably altered by a severe zinc deficiency.

An increase in zinc concentration in the pancreas, spleen, heart, kidney, and hair of irradiated swine fed Basal and Basal + Ca was noticed (Figure 11, p. 44). Also, an increase in zinc retention in the rib of neutron irradiated animals fed the three different regimens was observed. Stable zinc accumulated in greater amounts in the rib, hair, liver, and hoof of neutron and nonirradiated pigs (Figure 11, p. 44) fed Basal and Basal + Ca + Zn, than in those fed Basal + Ca.

Muscle (Longissimus dorsii), kidney, pancreas, spleen, and heart presented lower stable zinc concentration than other tissue tested. Miller and Miller (1962), in calves receiving a low zinc diet, found a high retention of zinc in liver, hoof, and rumen mucosa when compared with pancreas, heart, spleen, kidney, bone, and feces. When an excess of zinc was added to the pigs ration a high zinc content in the liver, pancreas, and hair was noticed (Lewis, Hoekstra, and Grummer, 1957).

It is important to mention that when no zinc was added to the Basal ration, the hair and liver remained high in zinc concentration in neutron irradiated pigs. These results suggest a slow turnover rate of zinc in tissue.

Macapinlac, Pearson, and Darby, 1966 (referred to by Prasad, 1966) stated that the hair zinc concentrations in rats remained unchanged during deficiency, but progressively increased in the animals which received zinc.

Differences among tissue zinc content (Table XIII of the Appendix) in swine fed three different rations have been noticed. The lowest zinc concentration in the previously mentioned porcine organs was observed in animals receiving Basal + Ca diet, while the highest zinc content was found in organs of swine fed Basal + Ca + Zn ration. Muscle tissue (Longissimus dorsii) is noted as an exception. Similar results in the breast (chicks) and thigh (rats) muscles were reported by Savage et al. (1964) and Macapinlac, Pearson, and Darby, 1966 (referred to by Prasad, 1966).

The average zinc concentration in the ribs of pigs receiving a Basal diet was 143 ± 29.43 µgm. per gram and 112 ± 19.06 µgm. per gram, respectively, for the irradiated and nonirradiated groups.

Liver, hoof, hair, heart, spleen, pancreas, kidney, and muscle present a higher zinc concentration in neutron irradiated swine fed the same regimen than in the nonirradiated animals. However, the zinc concentration of these tissues from the pigs fed the other two diets were not consistent.

Yendell, Tupper, and Wills (1966) reported a significant change in zinc concentration in the liver and spleen of the mouse after X irradiation.

Neutron Irradiation and Dietary Ration Effects on Swine Growth and Feed Efficiency

The average daily gain by neutron irradiated animals (Table IV) was lower ($P<.01$) than by nonirradiated swine.

The average daily gain by neutron irradiated pigs fed Basal was 0.51 ± 0.02 kg. compared with 0.67 ± 0.02 kg. for nonirradiated animals. The neutron exposed pigs fed the Basal + Ca ration had an average daily gain of 0.52 ± 0.02 kg. while the controls gained 0.72 ± 0.03 kg. The average daily gain for neutron irradiated pigs fed the Basal + Ca + Zn ration was 0.52 ± 0.06 kg. versus 0.67 ± 0.02 kg. for controls.

No significant ($P>.05$) difference in average daily gain of pigs receiving the three different nutritional regimens was noticed. Mraz (1965) stated that both neutron and gamma irradiation reduced the rate of growth in the chick. However, Lowery and Bell (1964) reported that the growth rate in young pigs was not affected by 450 R. of whole-body irradiation.

Feed conversion in non-irradiated animals (Table IV) was more efficient than in neutron exposed pigs ($P<.01$). The highest average feed conversion for neutron irradiated animals fed Basal + Ca was 4.35 ± 0.18 kg. compared with 3.22 ± 0.23 kg. for nonirradiated pigs fed the same regimen. No significant difference ($P>.05$) in feed conversion for the three dietary treatments was observed.

Prasad (1967) reported an increase in height in dwarfs who received zinc supplement. Coble et al. (1966) stated that zinc deficiency in animals results in a retardation of growth and sexual

TABLE IV

AVERAGE DAILY GAIN, FEED CONVERSION, AND DAILY FEED CONSUMPTION AFFECTED BY NEUTRON IRRADIATION AND RATION IN SWINE

	Basal		Basal + Ca		Basal + Ca + Zn	
	Neut. Irrad.	Nonirrad.	Neut. Irrad.	Nonirrad.	Neut. Irrad.	Nonirrad.
	values in kg.					
Average daily gain	0.51 ± .02[a]	0.67 ± .02	0.52 ± .02	0.72 ± .03	0.52 ± .06	0.67 ± .02
Feed conversion	3.85 ± .06	3.33 ± .16	4.35 ± .18	3.22 ± .23	4.21 ± .06	3.38 ± .36
Daily feed consumption	1.96 ± .10	2.24 ± .09	2.23 ± .06	2.29 ± .06	2.22 ± .24	2.28 ± .18

[a]Mean ± standard error.

development. Klussendorf and Pensack (1958) stated that zinc added to the ration increased feed utilization and caused more rapid growth in poultry.

Berry et al. (1961) reported that dietary levels of calcium or zinc did not influence rate of gain or feed efficiency in pigs which is similar to results found in the present experiment. However, Berry, Bell, and Wright (1966) stated that dietary calcium significantly decreased ($P<.05$) daily feed consumption and rate of gain in swine; but feed efficiency was not significantly affected by diet.

No significant difference ($P>.05$) in daily feed consumption between neutron and nonirradiated animals was observed. Similar results were noticed for the three different dietary treatments. This study shows that the low average daily gain and feed conversion in irradiated pigs was due to neutron irradiation and not to ration effects.

CHAPTER V

SUMMARY AND CONCLUSIONS

Twenty-four weanling male pigs were selected according to body weight and assigned to three dietary treatments: Basal (0.6 per cent Ca and 29 p.p.m. Zn); Basal + 0.6 per cent Ca; and Basal + 0.6 per cent Ca + 71 p.p.m. Zn. Unilateral neutron irradiation (268 rads) was administered to a sample half of each treatment group. Twenty-four hr. postirradiation, all pigs (n=24) were orally dosed with 0.5 mCi. of ^{65}Zn.

In vitro uptake of ^{65}Zn by erythrocytes averaged higher in the irradiated pigs. The effect of ration on uptake of ^{65}Zn was significant ($P<.01$) with the highest uptake on Basal + Ca and the lowest on Basal + Ca + Zn. However, the effect of ration in stable zinc analysis was not significant ($P>.05$). These findings show that in vitro uptake of ^{65}Zn by erythrocytes as a measure of zinc deficiency was advantageous over the stable zinc analysis in that results were obtained more rapidly with greater accuracy and less equipment. Consequently, the in vitro uptake method has a greater potential for use as a clinical diagnostic tool than does the stable zinc method.

Retention of ^{65}Zn in whole blood and plasma was lower ($P<.05$ and .01, respectively) in the neutron exposed swine. Also, the radiozinc retention in whole blood for both Basal + Ca and Basal rations was greater than Basal + Ca + Zn ($P<.01$).

Radiation significantly decreased left kidney, liver, and spleen weights ($P<.01$); heart weight ($P<.05$) and hair growth ($P<.01$). An absence of pigment in the hair cortex was observed on the side of the animal exposed to the reactor.

Stable zinc accumulated in greater amounts in the hoof, hair, rib, and liver of both irradiated and nonirradiated pigs fed Basal and Basal + Ca. In the first two organs a significant ($P<.05$) ration X-irradiation interaction was noticed.

Average daily gain in neutron irradiated animals was lower ($P<.01$) than in nonirradiated animals. Feed conversion was significantly greater ($P<.01$) in controls.

This experiment determined some of the effects of neutron radiation on metabolic changes, postirradiation syndrome, in vitro uptake of radiozinc, average daily gain, and feed conversion in swine fed variable calcium and zinc levels. This investigation should help promote an understanding of neutron irradiation on animals, thereby facilitating and stimulating further study in this area.

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APPENDIX

TABLE V

INDIVIDUAL HAIR LENGTH MEASUREMENT, IN SITU, NEAR AND AWAY FROM THE REACTOR CORE

Treatment	Swine number	Near core	Away core
		millimeter	
Basal	635	15.2	38.0
	642	17.2	33.2
	677	19.4	28.0
	675	19.8	29.2
	Mean	17.9 ± 1.06[a]	32.1 ± 2.26
Basal + Ca	644	15.8	30.0
	652	15.8	28.2
	684	21.6	31.6
	687	20.6	31.8
	Mean	18.4 ± 1.54	30.4 ± 0.84
Basal + Ca + Zn	639	12.8	28.6
	649	14.4	32.2
	676	16.8	23.2
	693	18.6	35.4
	Mean	15.6 ± 1.28	29.8 ± 2.62

[a]Plus or minus standard error of a mean.

TABLE VI

INDIVIDUAL KIDNEY WEIGHT AND LENGTH IN SWINE AFFECTED BY RATIONS AND NEUTRON IRRADIATION

Treatment	Swine number	Neutron irradiated		Swine number	Nonirradiated	
		Weight	Length		Weight	Length
		gm.	cm.		gm.	cm.
Basal	635	84	11.00	645	130	11.50
	642	102	8.00	640	136	11.00
	677	70	11.00	678	122	13.00
	675	88	11.50	685	130	12.50
	Mean	86 ± 6.56[a]	10.38 ± 0.80	Mean	130 ± 2.87	12.00 ± 0.46
Basal + Ca	644	118	13.00	636	106	12.00
	652	92	11.00	647	134	11.50
	684	102	12.00	686	176	15.50
	687	110	11.00	683	160	14.00
	Mean	106 ± 5.56	11.75 ± 0.48	Mean	144 ± 15.34	13.25 ± 0.92
Basal + Ca + Zn	639	94	11.00	646	148	12.00
	649	100	10.50	650	140	12.00
	676	84	11.00	680	166	14.50
	693	84	11.50	688	136	13.50
	Mean	90 ± 3.94	11.00 ± 0.20	Mean	148 ± 6.65	13.00 ± 0.61

[a]Plus or minus standard error of a mean.

TABLE VII

INDIVIDUAL WEIGHT AND LENGTH OF THE REAR LEG, NEAR AND FAR AWAY FROM THE REACTOR[a]

Swine number	Facing toward reactor		Facing away from reactor	
	Weight	Length	Weight	Length
	gm.	cm.	gm.	cm.
677	286	16.0	392	19.0
675	285	15.0	385	18.5
684	288	16.0	394	19.0
687	390	17.0	396	18.0
676	270	14.5	318	17.5
693	290	15.0	304	16.5
Mean	301.50 ± 18.00[b]	15.58 ± 0.37	364.83 ± 17.25	18.08 ± 0.97

[a]Samples from first trial were not considered.

[b]Plus or minus standard error of a mean.

TABLE VIII

INDIVIDUAL IN VITRO UPTAKE OF ^{65}ZN BY SWINE RED BLOOD CELLS

Treatment	Swine number	Neutron irradiated	Swine number	Non-irradiated
		% dose		% dose
Basal	635	47.34	645	48.40
	642	60.20	640	35.28
	677	23.12	678	25.22
	675	28.59	685	31.22
	Mean	39.81 ± 8.54[a]	Mean	35.03 ± 4.91
Basal + Ca	644	79.86	636	50.31
	652	49.11	647	60.30
	684	39.38	686	43.10
	687	43.07	683	44.86
	Mean	52.86 ± 9.22	Mean	49.64 ± 3.86
Basal + Ca + Zn	639	22.96	646	25.00
	649	26.20	650	21.46
	676	20.81	680	20.67
	693	27.43	688	25.30
	Mean	24.35 ± 1.51	Mean	23.11 ± 1.19

[a]Plus or minus standard error of a mean.

TABLE IX

EFFECT OF RATIONS IN PLASMA AND WHOLE BLOOD ON ^{65}ZN ACTIVITY

	Blood plasma			Whole blood		
	% dose/liter[a]			% dose/liter[a]		
Treatment	Basal + Ca + Zn	Basal	Basal + Ca	Basal + Ca + Zn	Basal	Basal + Ca
Mean	0.12	0.19	0.19	0.25	0.59	0.63

NOTE: Values underlined by the same line are not significantly different ($P \geq .05$).

[a]Mean of 12 irradiated and 12 control swine.

TABLE X

INDIVIDUAL ^{65}ZN URINARY EXCRETION IN SWINE AFFECTED BY DIET AND UNILATERAL NEUTRON EXPOSURE

Treatment	Swine number	Neutron irradiated (Cumulative % dose)	Swine number	Non-irradiated (Cumulative % dose)
Basal	635	1.25	645	1.45
	642	0.97	640	1.07
	677	0.42	678	1.21
	675	0.57	685	0.42
	Mean	0.80 ± 0.32[a]	Mean	1.04 ± 0.22
Basal + Ca	644	1.41	636	1.90
	652	2.23	647	1.68
	684	0.25	686	0.37
	687	0.16	683	0.10
	Mean	1.01 ± 0.50	Mean	1.01 ± 0.46
Basal + Ca + Zn	639	0.89	646	0.45
	649	1.56	650	0.88
	676	0.14	680	0.26
	693	0.06	688	0.24
	Mean	0.66 ± 0.35	Mean	0.46 ± 0.14

[a]Plus or minus standard error of a mean.

TABLE XI

INDIVIDUAL ^{65}ZN FECAL EXCRETION IN SWINE AFFECTED BY DIET AND UNILATERAL NEUTRON EXPOSURE

Treatment	Swine number	Neutron irradiated (Cumulative % dose)	Swine number	Non-irradiated (Cumulative % dose)
Basal	635	55.11	645	65.41
	642	71.02	640	39.70
	677	21.60	678	7.87
	675	19.21	685	14.75
	Mean	41.74 ± 12.74[a]	Mean	31.94 ± 13.08
Basal + Ca	644	43.27	636	68.30
	652	39.38	647	65.39
	684	23.13	686	19.21
	687	20.65	683	21.04
	Mean	31.61 ± 5.68	Mean	43.48 ± 13.50
Basal + Ca + Zn	639	53.84	646	66.35
	649	23.52	650	64.94
	676	21.27	680	22.61
	693	25.99	688	18.07
	Mean	31.16 ± 7.62	Mean	42.99 ± 13.11

[a]Plus or minus standard error of a mean.

TABLE XII

INDIVIDUAL ZINC CONCENTRATION IN ERYTHROCYTES AND BLOOD PLASMA AFFECTED BY RATIONS AND NEUTRON IRRADIATION

Treatment	Neutron irradiated			Nonirradiated		
	Swine number	Erythrocytes	Plasma	Swine number	Erythrocytes	Plasma
		µgm./ml.			µgm./ml.	
Basal	635	4.35	1.00	645	5.33	1.00
	642	5.58	1.00	640	5.33	1.00
	677	5.50	1.32	678	5.00	0.82
	675	7.58	0.94	685	5.00	1.00
	Mean	5.75 ± 0.67[a]	1.06 ± 0.08	Mean	5.16 ± 0.10	0.96 ± 0.04
Basal + Ca	644	5.08	0.50	636	4.83	0.75
	652	4.58	0.50	647	4.83	0.50
	684	6.66	1.06	686	5.50	0.50
	687	6.16	1.06	683	5.83	1.19
	Mean	5.62 ± 0.48	0.78 ± 0.16	Mean	5.25 ± 0.25	0.74 ± 0.16
Basal + Ca + Zn	639	4.83	1.00	646	4.58	1.00
	649	5.58	1.00	650	4.33	1.00
	676	6.00	1.32	680	5.50	1.25
	693	6.75	1.44	688	6.58	1.25
	Mean	5.79 ± 0.40	1.19 ± 0.11	Mean	5.25 ± 0.51	1.12 ± 0.07

[a]Plus or minus standard error of a mean.

All analyses are expressed on fresh weight basis.

TABLE XIII

EFFECT OF NEUTRON RADIATION AND RATION ON ZINC TISSUE CONCENTRATION

Organs	Basal		Basal + Ca		Basal + Ca + Zn	
	Neutron irradiated	Non-irradiated	Neutron irradiated	Non-irradiated	Neutron irradiated	Non-irradiated
	µgm./gm.					
Liver	133 ± 43.22[a]	76 ± 20.15	64 ± 22.98	65 ± 11.69	113 ± 15.10	103 ± 11.02
Hoof[c]	111 ± 8.32	102 ± 8.36	56 ± 8.70	107 ± 11.02	136 ± 5.24	134 ± 8.08
Hair[c]	186 ± 38.10	134 ± 23.29	90 ± 10.06	73 ± 8.26	114 ± 8.24	116 ± 6.44
Rib	143 ± 29.43	112 ± 19.06	106 ± 22.26	85 ± 11.96	169 ± 11.56	126 ± 24.38
Heart	31 ± 6.92	21 ± 3.60	26 ± 6.18	21 ± 0.98	24 ± 0.88	25 ± 4.02
Spleen	29 ± 3.02	26 ± 2.34	28 ± 1.94	24 ± 1.16	29 ± 1.40	29 ± 4.48
Pancreas	47 ± 17.34	36 ± 9.82	36 ± 16.45	26 ± 2.56	44 ± 4.21	52 ± 7.66
Kidney	36 ± 4.91	27 ± 5.34	27 ± 1.92	27 ± 3.18	34 ± 3.81	39 ± 7.34
Muscle	22 ± 4.44	16 ± 2.32	16 ± 1.52	17 ± 1.72	19 ± 1.18	18 ± 3.50

[a]Mean ± standard error.

[b]All analyses are expressed on fresh weight basis.

[c]Expressed on air-dry basis.