B. C. P.

**A LITERATURE REVIEW OF ZINC-65 IN BIRDS
WITH
SPECIAL REFERENCE TO THE MALLARD **MASTER**
by Richard D. Curnow**

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**A Literature Review
of
⁶⁵Zinc in Birds
with Special Reference to the Mallard**

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A Literature Review of ⁶⁵Zinc in Birds With Special Reference to the Mallard

Part I. Introduction

Trace element investigation has evolved from studies of pigments in living organisms to quests into the mineral composition of both plant and animal materials (Kehoe, Cholak, and Story, 1940). Development of high energy accelerators and nuclear reactors in the 1950's brought trace element studies into a new phase. All elements could be made radioactive and thus potentially hazardous to living matter (Underwood, 1962).

Radionuclides entering the natural environments from power reactors, weapons testing, and waste disposal are of particular importance to scientists since

radiation from high concentrations of these materials may induce mutations, cancer and death. In lesser concentrations, the effects of environmental radioactive substances, as well as the manner in which these materials are spread within natural environments, are essentially unknown (Odum, 1959). Knowledge of the distribution of elements in humans and other animals, as well as in the food materials which are the source of these elements, has become necessary for determination of the maximum safe levels of radionuclides and their movements in the food chain (Underwood, 1962).

Part II. Zinc in Animal Nutrition

Zinc is a physiologically essential element represented in part by unstable isotopes (Table 1). Such unstable isotopes (radionuclides) of zinc offer a precise method for following the element within biological systems because the chemical behavior of zinc radionuclides is "essentially the same as the non-radioactive element" (Odum, 1959, 468).

Raulin (1869) found zinc to be essential in the growth of black mold, *Aspergillus niger*. The presence of zinc was reported in all organs of the cat, rat and man by Lutz (1926). After much work in the 1920's during which Lutz proved the essentiality of zinc in animal nutrition, Stirn (1935) and Todd et al. (1934) demonstrated that zinc was necessary for the growth and development of rats. The ability of zinc to prevent porcine parakeratosis in swine was later elucidated by Tucker and Salmon (1955). Zinc requirements for birds were not shown until much later when O'Dell et al. (1957) demonstrated reduced growth rate in chicks fed low zinc diets.

inorganic and organic radicals allows the number of possible interactions of zinc compounds to be nearly infinite (Vallee, 1959).

TABLE 1. Zinc isotopes with corresponding physical data (Prasad, 1966).

Isotope	Atomic Mass	Half Life	Mode of Decay Radiation	Mode of Decay Energies (Mev)
62		9.3 hrs.	B ⁺ G K	0.66 0.42
63		38.3 min.	B ⁺ G K	2.36, 1.4, 0.47 0.969, 1.89, 2.60
64	63.95356		Stable	
65		250 days	B ⁺ G	0.32 1.14, 0.201
66	66.94667		e ⁻ Stable	0.439
67	66.9482		Stable	
68	67.9488		Stable	
69		13.8 hrs.	e ⁻	0.439
Isomeric transition 70	69.9470	52 min.	B ⁺ Stable	0.86
71		2.2 min.	B ⁻	2.1
72		49 hrs.	B ⁻ G	0.3, 1.6
73		2 min.	B	

B⁻ beta particle; B⁺ positron; G gamma ray; e⁻ internal electron conversion; K orbital electron capture

ZINC BIOCHEMISTRY

In biological systems, zinc exists as organic ligand complexes rather than the free metallic ion. The ubiquitous distribution of zinc in the biosphere as

Other writers have reported in depth on zinc chelation mechanisms (Martell and Calvin, 1952; Bailar, 1956); biochemical (Lehninger, 1950), and physical chemical properties (Gurd and Wilcox, 1956; and Williams, 1959); and their significance to zinc biological activity.

ABSORPTION AND EXCRETION

Savage et al. (1964) stated there was limited information regarding the distribution of zinc in the tissues and essentially nothing known about zinc absorption and excretion in birds. The presence and spread of various radionuclides in the food web are of immediate concern to man. ^{65}Zn absorption and excretion not only has been studied from the radioecological point of view but also from the nutritional aspect. Absorption of ^{65}Zn from the gastro-intestinal (G.I.) tract generally is low, but due to its long biological half life small assimilated quantities are of physiological significance (Rubini et al., 1961). In attempting to learn more of the role of zinc in the metabolism of vertebrates, much laboratory work has been carried out on chicks (Roberson and Schaible, 1958; Rahman et al., 1959; Kienholz et al., 1964; Turk, 1963, 1964; and Ziegler et al., 1964), rats (Stirn, 1935; Feaster et al., 1955; Ballou, 1958, 1960; Taylor, 1961; and Furchner, 1962), and mice (Sheline et al., 1943; Cozias et al., 1962; and Richmond et al., 1962).

Environmental ^{65}Zn

Concentrations of ^{65}Zn which accumulate in natural water systems, aquatic and terrestrial plants and animals, and hence in man are found in the effluent coolant waters from nuclear reactors. A prominent source of such waste radionuclides is the Hanford reactors on the Columbia River in central Washington. As stated by Soldat and Essig (1966, 12), "Migratory waterfowl, such as ducks and geese, that have utilized the Hanford section of the Columbia River and the swamps and ponds within the project boundaries may contain ^{32}P , ^{65}Zn and small amounts of other radionuclides." In studies conducted on the Hanford Reservation during 1960-1961, approximately 200,000 waterfowl were found using this section of the Columbia River and neighboring vicinity as a wintering ground; of which 41% were tagged with radionuclides which associated the birds with the effluent of the Hanford reactors (Hanson and Davis, 1959). The mallard duck (*Anas platyrhynchos*) is a highly mobile, migratory bird known to act as a vector in transporting ^{65}Zn (Glover et al., 1967). Mallards utilizing the Columbia River Basin of Washington, Oregon and Idaho during January, as determined from surveys conducted by the Bureau of Sport Fisheries and Wildlife, have averaged over

600,000 birds for the past twelve years (Pacific Waterfowl Flyway Report 55, May, 1966). The mallards in Washington at this time represented over 38% of the total mallard population of the Pacific flyway, with most of the birds being in the Columbia Basin area (Glover et al., 1967).

Affecting Factors

Because of the limited research on ^{65}Zn metabolism in mallard ducks, much of the literature discussed in this review relates to domestic fowl. In addition, references to small mammals and livestock are included.

Dietary Protein Protein in the diets of domestic fowl is known to influence zinc absorption and excretion. Proteins containing phytic acid (hexaphosphoric acid ester of myo-inositol), a compound widely distributed in plants, have less capacity for supplying zinc than do those not containing the acid (Forbes, 1960; Oberlease et al., 1960; and Plumbee et al., 1960). Phytic acid appears to be capable of binding zinc, thus reducing its availability in the intestinal tract. Protein sources from soybeans and sesame seeds contain phytic acid (Underwood, 1962). Cotton seed also contains zinc in an unavailable form according to Graham and Telle (1967). Rabbits fed a soybean protein diet (25ppm zinc) retained 58% of orally administered ^{65}Zn after 16 days, while rabbits fed a dietary supplement of ZnO (54ppm zinc), retained only 14 percent of the oral ^{65}Zn (Graham and Telle, 1967). This experiment revealed that the soybean protein diet without supplemental zinc led to greater retention of body zinc than the diet which was supplemented with zinc. In chicks surgically prepared so that urinary and fecal products could be collected separately, phytic acid was found to have little effect on urinary zinc while fecal zinc excretion was increased greatly (Savage et al., 1964). Casein is a protein source not containing phytic acid (Underwood, 1962). Turk (1965) reported that in fowl fed casein rations (71ppm zinc), zinc concentrations in blood plasma were significantly higher than in birds fed soybean (65ppm zinc) or sesame (62ppm zinc) diets. However, zinc concentrations of different tissues in the fowl were not changed by the various dietary protein sources.

Table 2 lists common foodstuffs and their respective phytate content. Both stable and radioactive zinc absorption in wild mallard ducks may be affected depending upon the phytate abundance in mallard food.

In summary, the literature indicates that protein containing phytic acid is a much less effective source of zinc than is protein containing no phytic acid.

Dietary Calcium Forbes (1960) considered the level of dietary calcium and the protein source to be the two major factors which affect zinc requirements. He proposed increased calcium levels may affect intestinal absorption of zinc. McDonald et al. (1966, 144) states, "Calcium is believed to inhibit the

absorption of zinc." According to Newland et al. (1958), increased calcium intake increases zinc elimination, both in urine and feces. Calcium-zinc antagonism in animal nutrition has been pointed out by Tucker and Salmon (1955) and Newland et al. (1958) with pigs. These researchers stressed the detrimental effects of calcium and the controlling effect of zinc on parakeratosis. O'Dell et al. (1958) reported a similar relationship in studies on growing chickens. They found zinc requirements were decreased with decreased calcium (from 1.6% to 1.1%) but zinc requirements were not increased by excess dietary calcium (2.1%).

TABLE 2. Percent phytate in footstuffs (Prasad, 1966, 234).

Foodstuff	%Phytate
Barley	1.07-1.19
Oats	0.77
Rye	1.04-1.87
Wheat Bran	4.53
Wheat Flour	0.68-1.23
Soybeans	1.87-2.58
Peanuts	1.34-2.17
Corn	0.78-0.84
Sesame Oil Meal	3.30-5.46
Infant Cereals	1.30-1.45
Soybean-Based Formula	1.00-1.14

From the literature, it may be postulated that calcium has an influence on zinc requirements; however, the exact interrelationship appears difficult to identify. One "fact" that has been isolated, in contrast to O'Dell's findings, is that, "with a borderline zinc level" (bordering deficiency levels) high calcium levels increase zinc requirements (Prasad, 1966, 216). Forbes (1960) wrote a critical assessment of the conflicting data on zinc and calcium.

Vitamin D. Gross effects of vitamin D in zinc metabolism of the chick have been revealed (Martin and Patrick, 1961; Worker and Migicovsky, 1961; Kienholz et al., 1964). These workers have reported most extensively on influences of vitamin D on zinc concentrations in bone tissue. ^{65}Zn movement into bone tissue of rachitic chicks was found markedly increased by vitamin D (Worker and Migicovsky, 1961). Kienholz et al. (1964) found zinc levels in bone to be greater in hens fed higher concentrations (1000 IU/kg) of vitamin D than in hens fed a lesser amount (150 IU/kg).

Vitamin D₃, when added to a diet deficient in this vitamin, decreased ^{65}Zn in the liver regardless of dietary zinc (Martin and Patrick, 1961). They also noted ^{65}Zn in the tibia increased in the presence of 1.6% calcium.

Worker and Migicovsky (1961) believed competition might exist between divalent ions in the presence of vitamin D. Zinc absorption as influenced by the vitamin may reflect the presence of other divalent

ions. However, since a small percentage of zinc is absorbed from the G.I. tract, zinc availability probably is not affected except under vitamin D deficient conditions (Worker and Migicovsky, 1961).

Dietary Zinc. Much investigation has been devoted to determining the effect of various zinc levels and chemical complexes on the absorption, retention, and excretion of ingested zinc. An animal's ability to absorb zinc varies with the ingested chemical form or combination. Edwards (1959) reported that zinc oxides, carbonates, and sulfates, as well as metallic zinc, seem to be equally available to the chick; while zinc ores such as sphalerite (largely zinc sulfide), and franklinite (zinc, iron, and manganese oxides) contain zinc in a less available form. Animals ingesting diets low in zinc absorb and retain a larger percentage of dietary zinc than animals fed diets adequate in zinc (Ziegler et al., 1964; and Miller et al., 1967). Prasad (1966) asserted that increased dietary zinc levels definitely raised the absolute amount of zinc absorbed by the animal.

Utilization of ^{65}Zn in metabolism studies has added much to the knowledge of zinc metabolism. Cotzias et al., (1962) believed that zinc, when used as a metabolic load, is the only element which will accelerate ^{65}Zn excretion. Chick and adult fowl studies have shown that increased dietary zinc causes increased loss of ^{65}Zn from the liver (Martin and Patrick, 1961). Turk (1963) and Ziegler et al. (1964) found the tissues of chicks maintained on diets deficient in zinc to have a ^{65}Zn content higher than like tissues from birds fed a zinc supplemented diet. Graham and Telle (1967) reported dietary zinc levels also influenced ^{65}Zn retention in rabbits. Table 3 presents absorption and retention data for several animals, including man. Zinc retention in rats also has been shown to decrease when dietary zinc levels were increased (Furchner and Richmond, 1962). Kienholz et al. (1965) found zinc-deficient hens contained only 30% as much radioactivity per unit of bone and 150% as much radioactivity per unit of soft tissue as did the zinc supplemented hens (Table 4). The effect of both zinc deficient and zinc supplemented diets on zinc fecal loss in hens is shown in Figure 1.

Greater retention of ^{65}Zn in the soft tissues than in the bones of hens deficient in zinc, as found by Kienholz et al. (1965), is an indication of the body's response to maintain zinc levels in soft tissue. Elevated dietary zinc levels were reported to increase concentrations of zinc in tissues even though the capacity of the body to store excess zinc is limited to bones (Lutz, 1926). Turk (1965), in contrast, found no significant differences in zinc tissue concentrations due to dietary zinc levels. These findings may explain the results of Ziegler et al. (1964) and Kienholz et al. (1965) in which stable and radioactive zinc concentrations of bone were lowered in hens fed diets deficient in zinc.

TABLE 3. ^{65}Zn absorption and retention in various animals.

Animal	^{65}Zn Dosage		Absorbed %	Retained %	Time after dose	Reference
	Chronic	Acute				
Calf (5-12 mos.)	X		20	—	—	Miller and Cragle, 1965
Calf (week old)	X		55	—	—	Miller and Cragle, 1965
Cow	X		12	—	—	Miller and Cragle, 1965
Mallard		X	13.5	6.8	72 days	Curnow et al., 1967
Man			—	50 avg. (20-80)		Hoekstra, 1964
Rabbit (Soybean protein diet; 25ppm Zn)		X	—	58	16 days	Graham and Telle, 1967
Rabbit (Soybean protein diet; 56ppm Zn)		X	—	14	16 days	Graham and Telle, 1967
Rat		X	—	5	24 hrs.	Feaster et al., 1955
Rat		X	—	16	24 hrs.	Ballou, 1960
Rat		X	—	7	200 days	Ballou, 1960
Rat	X		—	20	200 days	Ballou, 1960
Rat			—	40	200 days	Ballou, 1960

Zinc stored in bone may be removed for utilization in zinc deficient body systems thus lowering the bone concentration of zinc in favor of zinc maintenance in soft tissues. Studies of ^{65}Zn metabolism in mice, rats, and dogs (Rubini et al., 1961) indicated that absorption of the radionuclide was hindered by feeding amounts of carrier zinc in excess of nutritional re-

quirements. In the same light, Ballou (1958, 3) found, from his work on zinc metabolism in rats, "... ^{65}Zn absorption to be significantly decreased only after addition of 100 to 1000 times the normal zinc intake." However, Furchner and Richmond (1962) reported ^{65}Zn retention in rats fed only 6-10 times the normal zinc intake to be reduced by a factor of three.

TABLE 4. Effect of dietary zinc level on ^{65}Zn distribution and total zinc concentrations in some tissues from hens 70 days after ^{65}Zn injection (Kienholz et al., 1965, 350).

Hen Tissues	Level of Dietary Zinc				⁶⁵ Zn in Zn-def. / ⁶⁵ Zn in Zn-suppl. x 100, %
	10ppm		70ppm		
	⁶⁵ Zn count/min per mg fresh wt.	Zinc ppm fresh wt.	⁶⁵ Zn Count/min per mg fresh wt.	Zinc ppm fresh wt.	
Keel bone	8.9	84	23.0	92	39
Humerus	5.7	130	25.1	118	22
Tibia	5.0	82	19.4	104	26
Femur	11.2	90	22.2	91	50
Mean	7.7	97	22.4	101	34
Telencephalon	3.0		2.1		150
Gizzard muscle	7.9	31	4.6	27	170
Heart muscle	5.2	29	3.8	33	140
Breast muscle	1.4	6	1.2	6	120
Liver	3.4	21	2.6	42	130
Ovary	3.0	13	2.0	22	150
Kidney	3.8	21	2.2	23	170
Uterus	2.0	14	1.1	13	190
Spleen	4.3	30	2.7	31	160
Preventriculus	3.7	28	2.2	17	170
Lung	1.6	23	1.1	24	150
Mean	3.7	22	2.3	24	155

FIG. 1. Fecal losses of ^{65}Zn from hens during short intervals following intramuscular injection of ^{65}Zn (Kienholz, et al., 1965, 348)

To summarize, birds with low dietary zinc intakes retain radioactive and stable zinc more tenaciously than those ingesting adequate zinc diets. Excess dietary zinc appears to reduce retention. Bone is reported as the only storage tissue for zinc. During low zinc intake, zinc concentrations in bone drop while other tissue concentrations appear to remain constant. Thus,

stored zinc is sacrificed in favor of zinc maintenance in other tissues

Other minerals and physiological factors believed to possibly influence zinc and thus ^{65}Zn absorption and excretion in birds are enzymes, ion competition, chelates, solar radiation, and numerous physiological factors

Enzymes Zinc has recently been shown to be a constituent of various metalloenzymes (Table 5). Keilin and Mann (1940) were the first to show the mode of action of zinc in biological materials. They isolated and purified the metalloenzyme, carbonic anhydrase, containing 0.33 percent zinc. In all, "fifteen to twenty" enzymes containing zinc have been identified (Prasad, 1966, 68). Zinc's essentiality in the activity of various enzymes seems a reason for its necessity in animal and plant metabolism. In addition to these metalloenzymes, arginase, carnosinase, enolase, histidine deaminase, lecithinase, yeast aldolase, oxaloacetic decarboxylase and several peptidases are enzymes increased in activity by zinc ions (Valle, 1959).

TABLE 5 Zinc metalloenzymes in various animals and plants (adapted from Prasad, 1966, and Underwood, 1962)

Enzymes	Source
Alcohol Dehydrogenase	Yeast
Alcohol Dehydrogenase	Equine liver, Human liver
Lactate Dehydrogenase	Rabbit muscle
Milate Dehydrogenase	Porcine heart
D(-) Lactate cytochrome C Reductase	Yeast
D-Glyceraldehyde- β -phosphate Dehydrogenase	Rabbit and bovine muscle, yeast
Glutamate Dehydrogenase	Bovine liver
Alkaline Phosphatase	<i>E. coli</i> , Mammalian kidney, Human leukocyte
Carboxypeptidase A	Bovine pancreas
Carboxypeptidase B	Bovine pancreas, Porcine pancreas
Neutral Protease	<i>B. subtilis</i>
Aldolase	Yeast, <i>Aspergillus</i>
Carbonic Anhydrase	Bovine erythrocytes, Human erythrocytes
Metallothionein	Horse kidney

Ion Competition Ions which compete with zinc for binding sites in protein molecules may influence zinc availability to the animal. Smith and Larson (1946) reported that competition between zinc, iron and copper might exist. Rats fed high levels of zinc developed hypochromic microcytic anemia, suggesting impaired iron utilization. The anemia was later shown to have resulted from a three way competition in which zinc interference in copper metabolism depressed iron utilization (Van Reen, 1953, and Duncan et al., 1953). Also, zinc and copper antagonism was postulated to result from competition for protein binding sites, with high dietary copper acting to reduce zinc availability (Davis, 1966).

The competing relationship between zinc and other ions is further exemplified by antagonism between zinc and cadmium (Parizek, 1960, and Gunn et al., 1963a). Strain and Pories (1966) stated that cadmium added to the diet of poultry produced zinc deficiency symptoms. High levels (640 ppm) of dietary cadmium have resulted in suppression of growth in the bovine (Powell et al., 1964). Gunn et al. (1961) have shown testicular damage due to cadmium poisoning in the rat to be prevented by increased zinc intake. Physiological abnormalities correlated with zinc deficiency in the chick are decreased rate of growth, shortening and thickening of long bones, enlargement of hock joints, frizzy feathers, labored respiration, and in severe deficiency conditions, keratinization of epidermal tissues (O'Dell et al., 1958).

Chelates Chelating agents also influence zinc metabolism. Ethylenediaminetetraacetic acid (EDTA) increased zinc availability when used as a dietary supplement (Kratzer et al., 1959). Chelates have been used to remove ^{65}Zn from "various organs" (Prasad, 1966, 955) of the body. Treatment of heavy metal poisoning (lead) with large amounts of chelates has met with good results in humans (Bessman et al., 1952, and Kneller et al., 1955). Two chelates which had a high affinity for zinc were EDTA and diethylenetriaminepentaacetic acid (DTPA). ^{65}Zn was more effectively removed from man by DTPA. The findings of Spencer et al. (1966) indicated chelate administration in humans resulted in increased ^{65}Zn excretion in urine.

Egg Production Egg production is known to be a route of zinc loss in birds (Kienholz et al., 1964, and Turk, 1964). Kienholz et al. (1964) found the zinc concentration of dried yolks from hen eggs was approximately 60 ppm. With the mallard duck laying 6.15 eggs per complete clutch and an average egg measuring 2.3 inches by 1.6 inches (Collins, 1959), a significant amount of ^{65}Zn might thus be excreted. A positive correlation appeared to exist between zinc in the lemur and egg production of hens (Turk, 1964). Kienholz et al. (1965) reported egg yolks from hens fed zinc supplemented diets contained three times the zinc levels of egg yolks from hens deficient in zinc. The influence of dietary zinc on ^{65}Zn loss via hen eggs is shown in Table 6.

TABLE 6 Zinc 65 loss in feces and eggs during 70-day experiment (Kienholz, 1965, 348)

Zinc Conc. of Diet ppm	No. eggs laid per hen	Percent of Total Zinc 65 Dose			
		In avg egg	In all eggs	In all feces	Lost from birds
10	33	1.06	35.0	16.2	51.2
	36	1.07	37.6	19.7	51.3
70	15	1.63	24.5	24.8	49.3
	24	1.44	34.2	25.5	59.7

The above data indicate that the level of zinc in the diets had little effect on total ^{65}Zn loss in the eggs. Approximately 35% of the injected ^{65}Zn was excreted via the eggs over a 70-day period while fecal loss was 15% and 25% of the dose in hens on low and high zinc diets, respectively.

Solar Radiation Solar radiation (ultraviolet) exposure in wild birds could be a factor in zinc metabolism due to its effects on vitamin D production in the bird. "The effect of the ultraviolet radiation in promoting proper bone calcification is approximately eight times as great from April to August as from November to January (Jull, 1938, 309)."

Feather Molt Adult mallard ducks molt once a year during early summer. Feathers are known to be among the tissues highest in zinc concentration in the chick (Savage et al., 1964). Thus a large percentage of ^{65}Zn assimilated and incorporated in feathers might be lost during the annual molt.

Hormones Strain and Pores (1966) suggested that hormones might play a role in zinc metabolism due to their effects on specific reactions and systems in the animal body. Glucagon and insulin are two hormones, produced in the pancreas, believed related to zinc metabolism (Orten, 1966). Zinc concentrations of the alpha and beta cells of the islets of Langerhans parallel respectively the glucagon and insulin content of these cells (Lowry et al., 1954; and Weitzel et al., 1956). Orten (1966) reported a relationship between zinc and the adrenocorticotrophic hormone (ACTH). He stated that treatment of ACTH with zinc as $\text{Zn}_3(\text{PO}_4)_2$ or $\text{Zn}(\text{OH})_2$ increased its physiological action. Also, gonadotropic hormones in the males have been implicated with zinc concentrations in the prostate, seminal fluid, and spermatozoa due to the degenerative changes of these tissues during zinc deficiency (Orten, 1966).

Age Age has been reported as an influencing factor in zinc turnover and distribution in animals. Berfenstam (1952a) stated that the level of zinc in whole blood varied with the age of the animal. In newborn infants, zinc content of the erythrocyte is only one-quarter the adult value; progressively rising over the first 12 years of life (Berfenstam, 1952b). This appears to correlate with the low level of carbonic anhydrase found in erythrocytes of infants (Stevenson, 1943).

In mice, Gunn et al. (1963b) found the ^{65}Zn excretion rate to be dependent on age (Table 7).

Ballou's (1960) findings, in which the ^{65}Zn retention by immature rats was as much as 5.5 times that of adults, agreed with those of Gunn (1963) for mice. Net absorption from the abomasum and small intestine was reported as 12% in mature cows, 20% in calves five to twelve months old, and 55% in week-old calves (Miller and Cragle, 1965).

Sex Underwood (1962) and Gunn et al.

TABLE 7. Retention of injected ^{65}Zn in mice in relation to time (Gunn et al., 1963b).

Age injected Days	Percent Retention 6 Days Post Injection
5	90
11	85
25	70
40	45
60	45
180	45

(1963b) stated that no sex differences existed in distribution and retention of zinc in humans and mice. However, work on adult fowl revealed that ovulating females had significantly more zinc in plasma, brain, femur, and skin and significantly less zinc in thigh muscle than males (Turk, 1964).

Curnow et al. (1967) found ^{65}Zn retention in mallard ducks to be significantly different between sexes (Table 8). Longer half life values found in male mallards were believed associated with lower metabolic rate due to larger body mass.

The ducks referred to as feed-spiked were force-fed gelatin capsules containing feed granules into which $^{65}\text{ZnCl}_2$ had been absorbed. The mallards designated as water-spiked were placed in a tank containing a ^{65}Zn solution (10 $\mu\text{Ci/l}$) of sufficient depth for the birds to float.

Body Weight Data on body weights of mallards whose ration or water was dosed with ^{65}Zn suggested the mean body weight differences of the two sexes might have a bearing on the ^{65}Zn half-time in mallards. Mean drake and hen weights were 1.25 kg and 1.11 kg, respectively, for the birds in Table 8 (Curnow et al., 1967). Greater ^{65}Zn half-times are displayed in the heavier birds (males). A similar relationship of radionuclide retention and body size (mass) has been reported between the mouse, rat, dog and man by Richmond et al. (1962). He found greatest ^{65}Zn retention time in the largest species studied (man), and the least in the smallest (mouse). Retention of ^{65}Zn in the rat and dog was also proportional to relative body mass. Golley et al. (1965) substantiated the findings of Richmond et al. (1962) in mice and rats.

Radioisotopes of phosphorous (^{32}P) and cesium (^{137}Cs) also exhibit greater retention in organisms of larger mass (Johannes, 1964; and Eberhardt, 1967).

Metabolic Rate Species size (mass) has been suggested to have some effect on zinc metabolism as reported by Richmond et al. (1962). He found ^{65}Zn was retained longer as species size increased. The basic ecological concept of metabolic rate being inversely related to the mass of the organism (Odum, 1959) presents a reasonable explanation for differences in zinc retention in animals of various sizes. Odum (1959) asserted that secondary to size other

TABLE 8. ^{65}Zn half life in the mallard (Curnow et al., 1967).

Treatment	No. ducks/ treatment	Biological half life (days)		Effective half life (days) ¹	
		Mean short component	Mean long component	Mean short component	Mean long component
FEED SPIKED					
Male Female	19	10.8 ± 10.0	219.8 ± 81.7	10.3	115.9
Male	9	7.6 ± 1.3	250.6 ± 98.9	7.4	123.9
Female	10	14.1 ± 10.1	188.9 ± 82.9	13.3	106.7
WATER SPIKED (Limited H ₂ O)					
Male Female	7	2.8 ± 0.2	74.5 ± 16.9	2.8	57.1
Male	3	2.9 ± 0.0	87.5 ± 12.52	2.9	64.5
Female	4	2.7 ± 0.3	61.5 ± 10.0	2.7	49.1
WATER SPIKED (Free H ₂ O Access)					
Replication 1					
Male Female	10	2.4 ± 0.4	126.9 ± 59.2	2.4	83.6
Male	5	2.6 ± 0.5	149.0 ± 61.7	2.6	92.7
Female	5	2.2 ± 0.3	104.8 ± 53.5	2.2	73.4
Replication 2					
Male Female	10	3.1 ± 0.8	59.9 ± 15.3	3.1	48.1
Male	5	3.1 ± 0.8	67.1 ± 17.0	3.1	52.7
Female	5	3.1 ± 0.7	52.6 ± 8.2	3.1	45.3

$$^1 \text{Effective half life} = \frac{T_b \times T_p}{T_b + T_p}$$

T_b = Biological half life

T_p = Physical half life (245 days)

²Significantly different ($P < .005$) from T_b of Female

factors affect the rate of metabolism. The metabolic rates of mallard ducks was found by Hartung (1967) to be inversely related to ambient temperature (Figure 2). One implication of these findings is that ^{65}Zn turnover in mallard ducks and other migratory waterfowl could be affected by ambient temperature.

FIG. 2. Metabolic rates of normal mallards at various ambient temperatures (Hartung, 1967, 799).

^{65}Zn TISSUE DISTRIBUTION AND TRANSPORT

It is accepted generally that only a small percentage of ingested zinc is absorbed via the intestinal tract (Underwood, 1962). McDonald et al. (1966) stated that zinc resembles iron; being poorly absorbed from the G.I. tract. Absorbed zinc in the chick has been reported as probably below 10% of that ingested (Worker and Migicosky, 1961).

Once ^{65}Zn has been absorbed from the G.I. tract and entered the blood stream, zinc complexing occurs. Due to binding within the animal body, the rate of zinc excretion is reduced greatly. The absorbed radionuclide is transported via the circulatory system to different compartments of the body at various rates (Figure 3). Rahman et al. (1959, 1239).

FIG. 3. Zinc kinetics in the animal body (Underwood, 1962, 169).

referring to hens, stated, "The rate of disappearance of activity from the blood was very rapid for the first ten minutes, and decreased for the next ten. Thereafter, the rate of loss was approximately linear, 0.2% of the original activity being detectable after two hours." Two hours past ^{65}Zn injection 28% of the dose had accumulated in the liver. During this same period, no fecal ^{65}Zn was found.

McDonald et al. (1966) presented the zinc concentration in the animal body along with other essential elements (Table 9).

TABLE 9. Approximate concentration of elements in the animal body (McDonald, 1966, 76).

Essential Elements		Probably Essential Elements	
	%	ppm	Trace
Calcium	1.5	Iron	20-80
Phosphorous	1.0	Zinc	10-50
Potassium	0.2	Copper	1-5
Sodium	0.16	Molybdenum	1-4
Chlorine	0.11	Iodine	0.3-0.6
Sulfur	0.15	Cobalt	0.2-0.1
Magnesium	0.04	Selenium	—
		Manganese	0.2-0.1
		Fluorine	Trace
		Bromine	Trace
		Barium	Trace
		Strontium	Trace

Sheline et al. (1943) and Rubini et al. (1961) found ^{65}Zn injected in the mouse and dog was accumulated first in the liver, pancreas and spleen, with later transfer to bone, skin, and skeletal muscles. As liver, pancreas, spleen and kidney tissue declined in ^{65}Zn activity, other tissues slowly began to accumulate radioactive zinc. Among these slow-buildup tissues were bone, skeletal muscles, hair, red blood cells, and nervous tissue (Sheline et al., 1943; and Luecke, 1965).

^{65}Zn excretion from the liver did not appear to be via bile fluid (Sheline et al., 1943). The major ^{65}Zn loss from the body was reported to be via pancreatic juice into the intestinal tract where reabsorption could occur (Underwood, 1962). Reabsorbed ^{65}Zn might be deposited in other organs or recycled and eventually lost from the body.

Prenatal transfer might play an important role in ^{65}Zn loss from the animal body. ^{65}Zn excreted in hen eggs over a 70-day period was 35% of an injected dose (Kienholz et al., 1965). ^{65}Zn loss in mallard eggs, from birds receiving an oral dose of the isotope,

was reported (Curnow et al., 1967). Postnatal transfer in mammals also contributes to ^{65}Zn net excretion. Fetuses from ewes fed the zinc radionuclide during a two week period exhibited tissue concentrations of ^{65}Zn $\frac{1}{10}$ to $\frac{1}{2}$ those in comparable tissue in the ewes. Colostrum of the same ewes was found to contain 20 to 30 times more ^{65}Zn than the plasma, in comparison to only 10 times as much three days after the parturition (McKenney et al., 1960).

The progeny of female rats bred to male rats injected with ^{65}Zn were radioactive at birth (Cunn et al., 1936b). This is an enlightening example of paternal transfer of ^{65}Zn through semen.

Fecal ^{65}Zn is the major excretory pathway for the isotope (Sheline et al., 1943; Ballou, 1958; Underwood, 1962; and Luecke, 1965). Milk and egg production may represent an important excretory pathway in mammals and birds (Prasad, 1966). Urinary loss is slight in normal animals (Ballou, 1958; and Underwood, 1962). Radiozinc excretion in steers (from an intravenous dose) was demonstrated to be 20% in feces and 0.25% in urine, while excretion from an oral dose of radiozinc was 70% and 0.3% for feces and urine respectively (Feaster et al., 1954).

CANCER

Very interesting reports of abnormal zinc concentrations in cancerous tissue have been published (Olson et al., 1954; Mawson and Fisher, 1952; Caruthers and Sontzeff, 1945; and Vallee, 1959). Blood from cancerous patients generally showed subnormal zinc concentrations (mean, 4.5 $\mu\text{g Zn/ml}$) compared with normal values (6.5 $\mu\text{g Zn/ml}$) and favorable progress against the disease was accompanied by an increased zinc concentration in the blood (Underwood, 1962).

Leukemic leucocytes which contained only $\frac{1}{10}$ the normal amount of zinc, increased in zinc content as the number of leucocytes fell due to therapy (Gibson et al., 1950). Zinc concentrations below normal also have been discovered in malignant liver (Olson et al., 1954) and prostate tissue (Vallee, 1959) while zinc concentrations of intestinal tumors have exceeded the concentrations of normal intestines by several fold (Vallee, 1959).

Part III. ^{65}Zn in the Food Web

Radionuclides in natural environments are known to be assimilated by migratory waterfowl populations, of which the mallard duck is a major component. Nelson (1962, 11) stated that ^{65}Zn exposure in humans might result from

"... ingestion of Columbia River water; ingestion of sanitary water derived from the river; ingestion of fish and waterfowl which inhabit the river; consumption of agricultural and dairy products derived from land irrigated with water drawn from the river..."

Whitefish and waterfowl associated with the Columbia river were thought to be "the most probable route whereby significant amounts of radioisotopes from Hanford Operations could potentially be available to man via the food web . . ." (Hanson and Davis, 1959, 139). Willard (1960, 148) believed radionuclides found in birds were of significance to man not only from the radiological hazard viewpoint but also because the omnivorous diet of many bird species "is ecologically comparable to the mixed diet of man," and thus, uptake of radioisotopes by birds might furnish an assay of amounts of radionuclides to be expected in the trophic level of interest to man.

Birds also might act as a transport vector of radionuclides which are not ingested but rather are carried on feather surfaces. Penned mallard ducks contaminated externally with a ^{65}Zn solution and later given

free access to uncontaminated water carried significantly ($P < 0.05$) less (20%) ^{65}Zn than did similarly contaminated ducks without free access to uncontaminated water (Glover et al., 1967). These findings indicate feathers might act as a vector for the rapid transport of radionuclides in the ecosystem. Much of the feather radioactivity of birds netted in White Oak Lake bed (a low-level atomic waste pond) was found to be from external contamination with soil and water. Most of the feather radioactivity could be removed by washing (Willard, 1960). Data have been published on ^{65}Zn concentrations in oysters (Soldat and Essig, 1966; Nelson, 1962), fish (Hanson and Davis, 1959; Foster, 1963, and Skidmore, 1964), man (Foster, 1963), and in waterfowl (Hanson and Case, 1963; and Glover et al., 1967).

Part IV. Application of ^{65}Zn in Research

The recognition of low-level radioactivity in migratory waterfowl has stimulated investigation into uses of radioactive tracers in waterfowl migrations. ^{65}Zn because of its strong gamma ray (1.11 Mev) and 245 day half-life (Radiological Health Handbook, 1960) has been studied in connection with waterfowl movements by Hanson and Case (1963) and Glover et al. (1967).

^{65}Zn as a trace radionuclide has a multitude of possible applications in various phases of biological science. Ecological studies of food web interaction (i.e. mineral kinetics through trophic levels) offer knowledge of the utilization and concentration of zinc in different trophic levels.

An excellent bibliography of radionuclides in ornithology has been written by Schultz (1963).

Prasad (1966, 222) expressed the complexity of zinc in the animal body when he wrote: "Any study of the function of zinc within an animal must take into careful consideration the multiplicity of interrelationships and interactions which involve zinc."

SUMMARY

Radioactive zinc, ^{65}Zn , entering natural environments is important since it may eventually reach man via the food chain. The mallard duck (*Anas platyrhynchos*) is a highly mobile, migratory bird known to act as a vector in transporting ^{65}Zn , either by ingestion or surface (feather) retention.

Since radioactive elements behave chemically the same as those which are not radioactive, it is possible to apply what is presently known about the metabolism of stable elements to those which are radioactive. To understand truly the role the mallard duck plays in ^{65}Zn movement through the food web, familiarity with factors affecting zinc metabolism is necessary.

Zinc is a physiologically essential element having a

concentration of 10-50 ppm in the animal body. Absorption of ^{65}Zn from the intestinal tract into the body is generally low, but due to its long biological half-life small assimilated quantities are significant.

The organic nature of ingested zinc affects its absorption. Proteins which contain phytic acid are a much less effective source of zinc than are proteins containing no phytate.

Calcium is believed to inhibit the absorption of dietary zinc. Zinc requirements were decreased in chickens when dietary calcium was decreased.

Vitamin D is known to affect ^{65}Zn kinetics within the chick. Bone concentrations of ^{65}Zn were increased with added vitamin D₃.

Dietary zinc levels also affect zinc kinetics within birds. Increased dietary zinc has been shown to increase excretion of ^{65}Zn . Zinc absorption is affected by the mineral complex in which zinc is ingested, for example; zinc sulfide has been reported as less available to the animal than zinc carbonate, sulfate or metallic zinc.

Other factors which affect zinc and therefore ^{65}Zn metabolism in birds are ion competition among dietary elements, dietary chelating agents, and various physiological conditions (i.e. egg production, feather molt, age, and metabolic rate).

Tissues have varying affinities for ^{65}Zn . Liver, pancreas, spleen, and kidney are the tissues first to contain ingested ^{65}Zn . However, these tissues also rapidly lose the radioisotope as bone, muscle, nerves, sex organs, and hair or feathers begin to accumulate ^{65}Zn . The latter tissues retain radio-zinc much longer than the rapid turnover tissues mentioned.

^{65}Zn associated with free-roaming waterfowl has been used to study movements and behavior of waterfowl in ecological research.

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