Intestinal Absorption of Zinc or Calcium-Ethylenediaminetetraacetic Acid Complexes in Chickens. (29614)

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Ethylenediaminetetraacetic acid (EDTA) improves the availability of zinc for poults (1,2), chickens(3,4,5), and rats(6) fed purified diets containing isolated soybean protein. This suggests the possibility of the absorption of zinc-EDTA complexes from the intestines of these animals. This report describes the recovery of Zn^{65}, Ca^{45}, and C^{14} from venous blood draining an isolated segment of the small intestine of chickens following the placement of Zn^{65} or Ca^{45}-EDTA-C^{14} complexes into the lumen of the isolated segment.

Methods. Three White Leghorn cockerels weighing approximately 500 g, allowed water but starved for 20-27 hours, were anesthetized with sodium pentobarbital and prepared by a modified procedure of Neame and Wiseman(7). A (9-18 cm) segment of the small intestine, approximately 6 cm caudal to the duodenal loop was severed, drained of any contents, and tied at each end. A polyethylene cannula (O.D. 0.0585 cm) was quickly inserted and tied into the mesenteric vein between the gastroduodenal and cranial mesenteric veins after tieing and cutting all vessels not draining the isolated loop. Blood from the cannula drained continuously into centrifuge tubes graduated to 0.1 ml. Heparin (1.5 ml/kg) was injected into a jugular cannula previously prepared [before the mesenteric cannula was in place]. The birds were artificially respired after puncturing the right abdominal air sac by passing a constant stream of air at 40°C, saturated with water at 20-25°C, into the trachea at a rate of about 100 ml/kg/minute. Blood volume was maintained by infusion of warmed (40°C) heparinized blood, obtained from anesthetized donor chickens, into the jugular vein at a rate of approximately 0.3 ml/minute by means of a peristaltic pump. The isolated intestinal segment, kept outside the body to avoid kinking, was wrapped in surgical gauze kept moistened with warm 0.15 M NaCl solution.

Test solutions (1.0 ml calcium complex or 2.0 ml zinc complex) were injected into the loop and blood was collected every 20 minutes for 140-180 minutes.

Preparation of complexes. Zn^{65}-EDTA-C^{14} complex: Equimolar (0.2 millimols) quantities of $Zn^{65}Cl$ and EDTA-$C^{14}OOH$ were dissolved in 10 ml H_2O. The solution was filtered and evaporated to dryness to yield 64.2 mg of the complex. An aqueous solution containing 8.94 mg complex per ml (24.5 mM) was used in this study.

Ca^{45}-EDTA-C^{14} complex: $Ca^{45}(OH)_2$ (0.2 millimols) and disodium salt of EDTA-2-C^{14} were dissolved in 10 ml H_2O. The solution was filtered and evaporated to dryness to give about 60 mg of the Ca^{45}-EDTA-2-C^{14} complex. The aqueous solution used in this study contained 7.4 mg complex per ml (21.5 mM).

Radioactivity determinations: Zinc-65 solutions containing carbon-14 were counted by the use of a flat crystal, scintillation detector (P-20 D, Tracerlab Inc.), after evaporating a known volume of the sample to dryness in polyethylene planchets.

The samples for Ca^{45} measurement were ashed at about 900°C and the ash was dissolved in 10% HCl. The solution was evaporated to dryness in polyethylene planchets and counted under a thin window detector (TGC3, 1.9 mg/cm^2, Tracerlab Inc.).

Carbon-14 was counted in a gas flow counter as $BaC^{14}O_3$ after a wet combustion of the sample according to a modified procedure of Van Slyke and Folch(8). Necessary corrections for self-absorption were applied.

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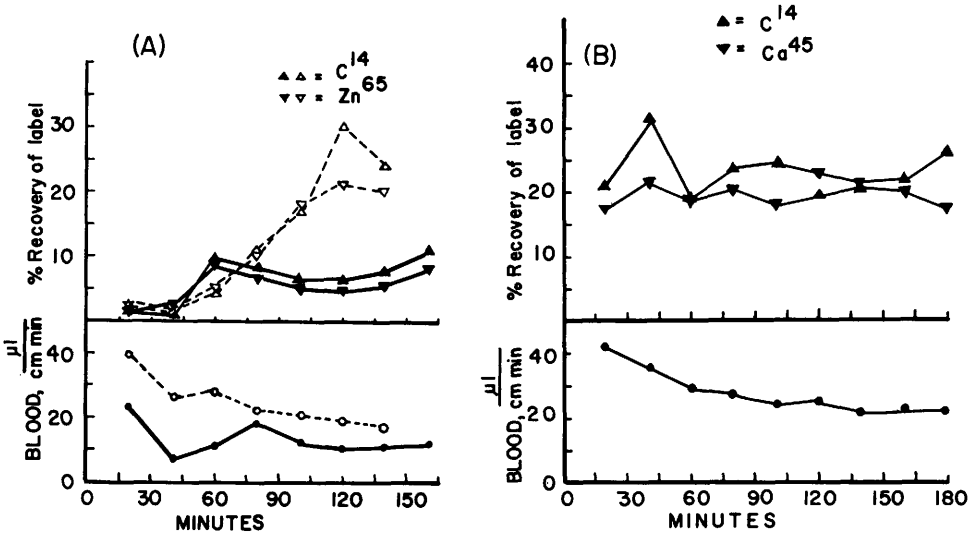

FIG. 1 (A & B) Blood flow and rate of appearance of C^{14} and Zn^{65} or Ca^{45} in the blood perfusing isolated intestinal segments. Rate of appearance expressed as % of injected label appearing in blood per cm min^{-1} ($x10^3$).

The radioactivity was measured in counts per minute for Zn^{65} or Ca^{45} and C^{14} in the solutions placed in the intestinal segments, the residual solutions, and the various blood samples.

After centrifugation, the packed red cell volumes of the blood samples were determined and a measured volume of the blood plasma was used for radioactivity determinations. Little or no activity was detected in the red cell fraction. The radioactivity was calculated as counts per minute per ml whole blood.

Results and discussion. The blood flow through the intestinal segments and the rates of appearance of C^{14}, Zn^{65}, and Ca^{45} in the venous blood draining this segment are summarized in Fig. 1 (A and B). Blood flow rates were high initially, but approached the rates at which blood was infused into the birds. Due to differences in rates of input of blood as well as the length of the intestinal segments used, blood flow varied between 10-25 µl per minute per centimeter length of the segment. Judged by the color of the preparations, the perfusion of the tissues did not appear to be insufficient even at the lowest rates.

Generally, the levels of C^{14} in the venous blood paralleled that of the labeled cations and were apparently independent of the blood perfusion rates with the possible exception of one preparation (Fig. 1A, solid symbols). In contrast to the Ca^{45} experiment in which the rate of appearance of the labels remained constant from the initial collection period (Fig. 1B), relatively small amounts of Zn^{65} were detected in the first 40 minutes, and reached a reasonably steady state after 40 (closed symbols) or 80 minutes (open symbols, Fig. 1A).

The C^{14}/Zn^{65} ratios in the luminal contents divided by similar ratios of the injected complexes gave values > 1.0 indicating a relative increase in C^{14} activity over that of Zn^{65} (Table I). There is also somewhat less Zn^{65} than C^{14} in the blood which suggests that some of the Zn^{65} was absorbed on the lumen wall. In contrast to this C^{14} from EDTA-Ca^{45} complex appeared to be transferred more rapidly through the mucosa than the Ca^{45} as shown by the lower C^{14}/M^{+2} ratio in the lumen and a higher ratio in the blood. Also, the recovery of Ca^{45} was on the order of 2-3 times greater than that for Zn^{65} over comparable periods.

The close association as evidenced by the parallel appearance of C^{14} with Zn^{65} or Ca^{45} in the blood would suggest that EDTA and the cations pass through the intestinal mu-

cosa together. However, on the basis of our data, no definite statement can be made as to the absorption of the EDTA-metal complexes as such through the mucosa or the prior metabolism of EDTA by the intestinal mucosa before the appearance of the labels in the blood. Darwish(9) found that in the laying hen about 70% of the C^{14} in the urine after administering EDTA-2-C^{14} was in the EDTA molecule. Earlier reports, on the other hand, indicate that very little of the C^{14} activity of an orally administered EDTA-C^{14} appeared in blood of rats and man(10,11,12).

TABLE I. Recoveries of C^{14}, Zn^{65} or Ca^{45} in Blood Perfusing an Intestinal Segment Containing Zn^{65} or Ca^{45} Complexed with EDTA-C^{14}.

Bird	EDTA complexed cation	Initial conc (mM)	Collection time (min)	Segment length (cm)	Total recovery (%) of injected		Lumen C^{14}/M^{+2}	Blood C^{14}/M^{+2}	Blood (ml/min)	
					C^{14}	Cation	Inj C^{14}/M^{+2}	Inj C^{14}/M^{+2}	Infusion	Collection
A	Zn^{65}	25	160	18	17	15	1.18	1.26	.26	.23
B	Zn^{65}	25	140	9	16	14	1.33	1.15	.26	.22
C	Ca^{45}	22	180	13.5	56	50	.75	1.14	.34	.37

The labeled complexes were dissolved in distilled water and were hypotonic to the body fluids. The osmotic gradients existing across the intestinal mucosa, initially, would cause net movement of water from the lumen to blood. However, it would appear unlikely that net flow of water (solvent drag) contributes significantly to the movement of Zn^{65} or Ca^{45} complexes with EDTA in our preparations since osmotic equilibrium would probably be attained in a relatively short time(13). In dogs, Ca absorption is not altered by changes in osmolarity of luminal fluid(14). Although the rate of appearance of C^{14} and Zn^{65} appeared to be correlated with blood flow in one experiment (Fig. 1A, solid symbols), injection of 0.5 ml KF (2×10^{-3}M) into the lumen in this particular preparation (not shown in the Figure) lowered the rates of appearance of both C^{14} and Zn^{65} without a comparable reduction in blood flow. The values for the rate of appearance of C^{14} and Zn^{65} in the fourth collection period following KF administration were 0.7 (units as in Fig. 1) while the blood flow was 7 μl/cm min.

The efflux (movement from lumen to blood) of Ca^{45} calculated from our data was 2.7 μM/hr cm, which would be the minimum value for labeled and unlabeled calcium efflux. This value is within the range of the efflux values reported in literature for chicks(15).

It is apparent that Zn^{65} efflux rates were considerably more variable. The average values for the 2 experiments were 0.6 and 0.2 μM/hr cm (open and solid symbols) using the data from the final 3 and 6 collection periods, respectively.

Summary. Carbon-14 and zinc-65 or calcium-45 appeared in the blood perfusing an isolated segment of the small intestines of chickens following placement of EDTA-2-C^{14} complexed with Zn^{65} and Ca^{45} in the lumen. The lumen tended to adsorb the zinc from its complex but not calcium. An

efflux of 2.7 μM/hr cm was observed for calcium-45. The values for zinc-65 varied from 0.2-0.6 μM/hr cm.

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Chromosomes of the Nevada Ground Squirrel *Spermophilus richardsonii nevadensis* (Howell).* (29615)

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Taxonomic characters currently used for investigation of the 3 geographically isolated subspecies of *Spermophilus richardsonii* include cranial and body measurements, pelage and geographic distribution(1,2). Although these characters differentiate each subspecies as a whole, they are not always reliable for individual specimens and they do not clarify evolutionary relationships between the subspecies(3).

Chromosomes from 2 of the 3 isolated subspecies of *S. richardsonii* have been analyzed previously(3). The Montana (*S. richardsonii richardsonii*) and Wyoming-Colorado (*S. r. elegans*) populations differed consistently in chromosome number and morphology. Chromosomal characters accurately identified individual specimens and, in addition, suggested that the *elegans* karyotype evolved from a chromosomal polymorphism which probably once existed in the more ancestral *richardsonii* population(3). Specimens of *S. r. nevadensis* from Nevada representing the third isolated subspecies, were not available previously for chromosomal analysis.

The purpose of the present study is to determine the chromosome number and morphology of *S. richardsonii nevadensis* and utilize this data to provide a unified concept regarding subspecies relationships within *S. richardsonii.*

Methods. Two male and two female *Spermophilus richardsonii nevadensis* from Jiggs (Elko Co.) Nevada were trapped alive and studied in the laboratory. Specimens were identified by cranial and pelage characters and geographic distribution(1,2).

Chromosomes were analyzed from femoral bone marrow using a Colcemide, hypotonic citrate, acetic orcein squash technique(4,5). Sixteen karotypes were constructed to determine the chromosomal pattern for *S. r. nevadensis.*

Results. Chromosome number. The data are listed in Table I. The modal diploid (2n) number was 34 in all 4 specimens of *S. r. nevadensis.*

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