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CALCIUM METABOLISM IN EMBRYOS OF THE OVIPAROUS SNAKE *COLUBER CONSTRICTOR*

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SUMMARY

Total calcium in embryos of an oviparous, colubrid snake (*Coluber constrictor* L.) rises rapidly during the last half of incubation as the embryos increase in size. Although most of this calcium is drawn from stores in the yolk, hatchlings contain more calcium than was present in yolk of eggs at oviposition. Because shells from eggs incubated to hatching contain less calcium than do shells from freshly-laid eggs, the extra calcium appears to be drawn from the eggshell. Indeed, approximately 20% of the calcium required for development in this snake is obtained from the eggshell, with the remainder coming from the yolk. Thus, embryos of oviparous snakes, like embryonic chelonians, crocodilians and birds, withdraw calcium from their eggshells and do not rely exclusively on calcium supplied in their yolk for support of growth and development.

INTRODUCTION

Two distinct groups of oviparous, amniotic vertebrates are currently recognized on the basis of sources of calcium for embryonic development (Simkiss, 1967; G. C. Packard, Tracy & Roth, 1977). In one group, containing chelonians, crocodilians and birds, the amount of calcium in the yolk and albumen of eggs at oviposition is insufficient to meet the needs of embryos, and developing young resorb calcium from the eggshell to satisfy part of their requirement for this element, thus leading to increases in the calcium content of eggs during incubation (Johnston & Comar, 1955; Simkiss, 1962, 1967; Bustard, Jenkins & Simkiss, 1969; Crooks & Simkiss, 1974; Jenkins, 1975; Dunn & Boone, 1977). In the second group, comprised of squamate reptiles (lizards, snakes and amphisbaenians), the calcium content of eggs is thought not to change during incubation, and all of the calcium required for embryogenesis apparently comes from stores present in the yolk at oviposition (Simkiss, 1967; Jenkins & Simkiss, 1968).

Unfortunately, most studies of calcium content of squamate eggs and embryos have been performed using viviparous forms that have no calcareous material in the eggshell, thereby precluding resorption of calcium from this site (Simkiss, 1967; Jenkins & Simkiss, 1968). Because such studies are unlikely to provide an adequate model for

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calcium metabolism in embryos of oviparous species, we undertook the present study of calcium content of eggs and embryos of the oviparous, colubrid snake *Coluber constrictor* to determine the source(s) of calcium for embryonic development and to characterize the pattern of mobilization of calcium by embryos. Data on water balance of eggs and on growth of embryos were also gathered.

METHODS AND MATERIALS

Three gravid female *Coluber constrictor* were collected in June 1983 in the Valentine National Wildlife Refuge, Cherry County, Nebraska, brought to the laboratory, and maintained under appropriate thermal and photic conditions until oviposition. One fertile egg from each clutch was used to estimate calcium contained within eggs (exclusive of the shell) as well as calcium content of eggshells at oviposition. None of the eggs contained an albumen layer, but each contained a small embryo. Because it was not possible to separate the embryo from the yolk at this stage of development, the contents of each egg were simply emptied into pre-weighed tares, and the inside of the shell was rinsed with a known mass of distilled, deionized water to remove remnants of yolk adhering to the inner surface of the shell membrane. The egg contents were weighed, wet mass was calculated by subtracting the mass of wash-water, and the sample was dried to constant mass at 50 °C.

The remaining eggs were incubated at 29 ± 0·4 °C on vermiculite substrates having a water potential of −150 kPa (M. J. Packard, G. C. Packard & Boardman, 1980). The substrates were prepared by mixing 333·8 g of distilled water with 300 g of dry vermiculite (grade 3, Terra Lite, W. R. Grace & Co., Cambridge, Mass.). Small quantities of water were added to boxes at regular intervals to replace water taken up by eggs or lost from boxes by evaporation (G. C. Packard, M. J. Packard & Boardman, 1981). All eggs were weighed on day 0, again on day 7, and at weekly intervals thereafter.

On days 20, 30 and 35 of incubation, a sample of eggs was removed from the incubator and opened. Each embryo was separated from its yolk, and the yolk and carcase were weighed individually and dried to constant mass. Snakes emerging from a sample of eggs incubated to hatching were killed by freezing. Retracted yolk was dissected from the abdominal cavity of each hatchling, and the yolk, carcase and eggshell were weighed and dried.

For calcium analyses, samples less than 250 mg dry mass were digested intact; samples greater than 250 mg dry mass were ground to a powder, and a 250–270 mg subsample of the material was added to a 16×150 mm polystyrene tube. Two ml of reagent grade nitric acid (concentrated) were added to each tube. The tubes were capped finger tight and left at room temperature for a few hours to effect an initial digestion. Tubes were then transferred to a water bath at 60 °C for 16–20 h. After that interval, the tubes were removed from the water bath and allowed to come to room temperature. One ml of reagent grade hydrogen peroxide (30 %) was added to each tube, and all tubes were loosely capped. After 1–1·5 h at room temperature, the caps were tightened slightly and all tubes were returned to the water bath for another 16–20 h. When digestion was complete, the caps were screwed on tightly and the samples were stored until used for calcium analyses. Several reagent blanks containi

ly nitric acid and hydrogen peroxide were prepared in parallel with each digestion.

Calcium analyses were performed with a Perkin-Elmer model 306 atomic absorption spectrometer using an acetylene/nitrous oxide flame. Each tube containing digestate was brought to volume, and subsamples were diluted to bring the calcium concentration into the working range of the instrument. Each dilution was made in duplicate, and the concentration of calcium in the dilution was determined. Total calcium was calculated from data for concentration, and values based on paired dilutions were averaged to yield a single representative value for total calcium in each of the samples.

Standards were prepared using appropriate dilutions of a stock solution containing 500 μg Ca ml^{-1}. The stock solution was prepared with reagent grade calcium carbonate. Appropriate standards, standard blanks and reagent blanks were run with each analysis.

Eggs of squamate reptiles typically absorb large quantities of water from moist substrates (M. J. Packard *et al.* 1980), and solutes such as calcium presumably could be taken up with this water (G. C. Packard *et al.* 1977). To address this question, we soaked 25 g of vermiculite in 300 ml of distilled water for 12 days in a tightly stoppered flask. Three samples of water from this mixture were analysed for calcium concentration as described previously, and the data were used to estimate the quantity of calcium in 1 g of water absorbed by eggs from substrates.

Data on change in mass of eggs during incubation were analysed using a two-way analysis of variance without replication (Sokal & Rohlf, 1969). Data for mass, water content, relative hydration, total calcium content and calcium concentration of yolks and carcases were analysed with one-way analyses of variance, using sampling date as the classification variable (Snedecor & Cochran, 1967). Comparisons of calcium content of samples at the beginning and end of incubation were made using one-way analyses of variance with clutch as a blocking factor (Snedecor & Cochran, 1967).

RESULTS

Changes in mass of eggs during incubation

Eight eggs, representing two clutches, were incubated to hatching. Analysis of variance of values for mass of these eggs revealed significant variation during incubation [$F(5,35) = 465{\cdot}18$, $P < 0{\cdot}001$]. Eggs experienced a net increase in mass of 3·6 g between day 0 and day 28 of incubation, but declined in mass by an average of 0·4 g between days 28 and 35 (Fig. 1). Nonetheless, eggs weighed about 3 g more on day 35, the last weighing before hatching began on day 40, than they did at the beginning of incubation (Fig. 1).

Absorption of liquid water and loss of water vapour occur simultaneously (see Discussion), so absolute quantities of water absorbed cannot be determined using net change in mass between oviposition and hatching as the index to uptake of liquid from the substrate. We estimated the absolute quantity of liquid likely to have been absorbed by eggs in this study by calculating the daily increment in mass over the linear portion of the curve in Fig. 1, i.e. between oviposition and day 21, because exchanges of vapour probably were of minor importance during this interval (see G. C. Packard *et al.* 1981). The daily increment during these 21 days was 0·15 mg. Assuming that

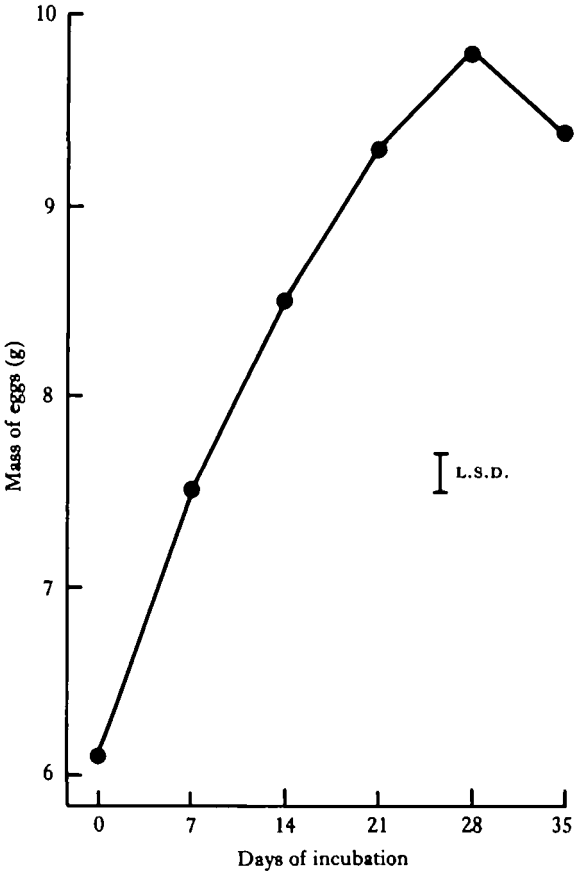

Fig. 1. Mean values for mass of eight eggs of *Coluber constrictor* at different times during incubation. Eggs were incubated to hatching in contact with substrates of −150 kPa water potential. The L.S.D. is the least significant difference for multiple comparisons of sample means (Snedecor & Cochran, 1967); means differing by the L.S.D. are significantly different at alpha = 0·05.

this value accurately reflects the rate of uptake of liquid water throughout all of incubation, eggs absorbed approximately 6 g of water between oviposition and hatching.

Growth of embryos and consumption of yolk

Dry mass of both embryos [$F(3,10) = 41{\cdot}07$, $P < 0{\cdot}001$] and yolks [$F(4,12) = 44{\cdot}48$, $P < 0{\cdot}001$] varied with time during incubation. Dry mass of embryos was about 0·1 g on day 20, but had increased to approximately 1·0 g by the time young emerged from eggs on days 40–42 (Fig. 2). Assuming that dry mass of embryos was essentially nil at oviposition, the average daily increment in dry mass between oviposition and day 20 was 0·005 g. In contrast, the increment in dry mass of embryos between day 20 and hatching was 0·04 g day^{-1}.

At oviposition, yolks contained approximately 1·4 g of solids (Fig. 2). There was no significant change in total solids of yolks between oviposition and day 20, but dry mass of yolks declined more-or-less linearly thereafter (Fig. 2). The average dai

decrement in yolk solids over the linear portion of the curve was 0·05 g, and yolk dissected from hatchlings contained approximately 0·2 g of solids (Fig. 2).

Movements of water inside eggs

Analysis of variance revealed significant variation in water content of yolks during incubation [$F(4,12) = 46{\cdot}52$, $P < 0{\cdot}001$]. The amount of water in yolks increased from 3·6 g at oviposition to 4·6 g on day 20 of incubation, but declined appreciably thereafter (Fig. 3). On day 30 of incubation, water content of yolks had been reduced

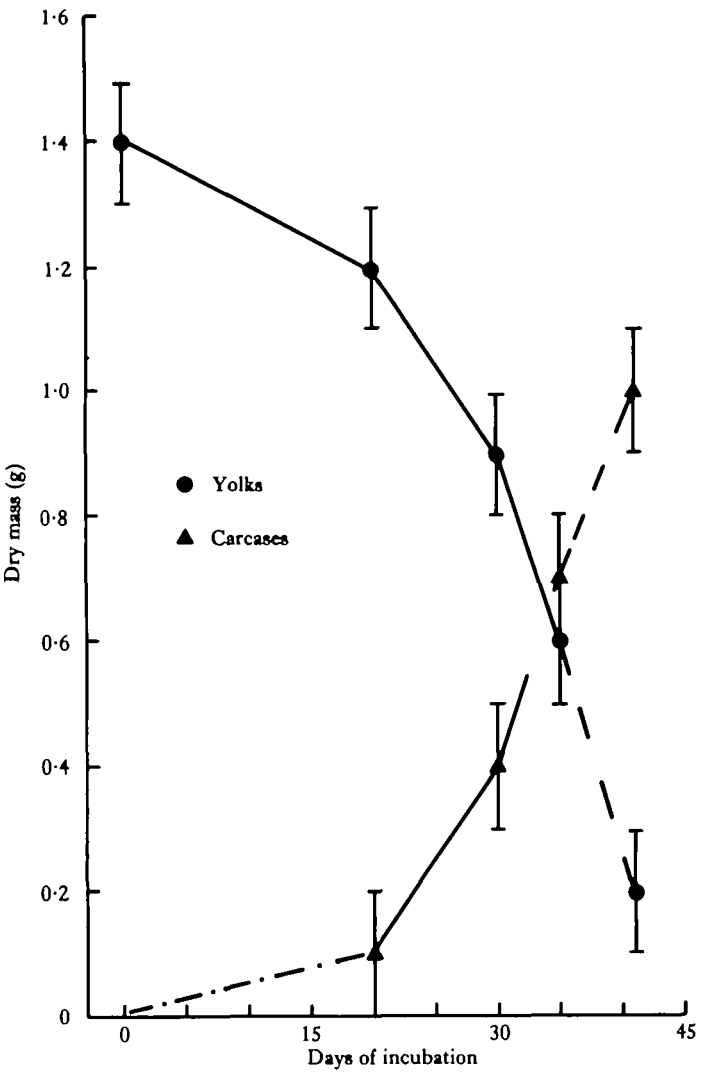

Fig. 2. Mean values for dry mass of yolks and of carcases of embryos/hatchlings from eggs of *Coluber constrictor*. Eggs were sampled at oviposition (day 0), on days 20, 30 and 35 of incubation, and at hatching. Sample sizes are 3 on day 0, 2 on day 20, 3 on day 30, 4 on day 35, and 5 at hatching. Vertical lines represent ± one-half the least significant difference (L.S.D.) for multiple comparisons of sample means (Snedecor & Cochran, 1967); means differing by the L.S.D. are significantly different at alpha = 0·05.

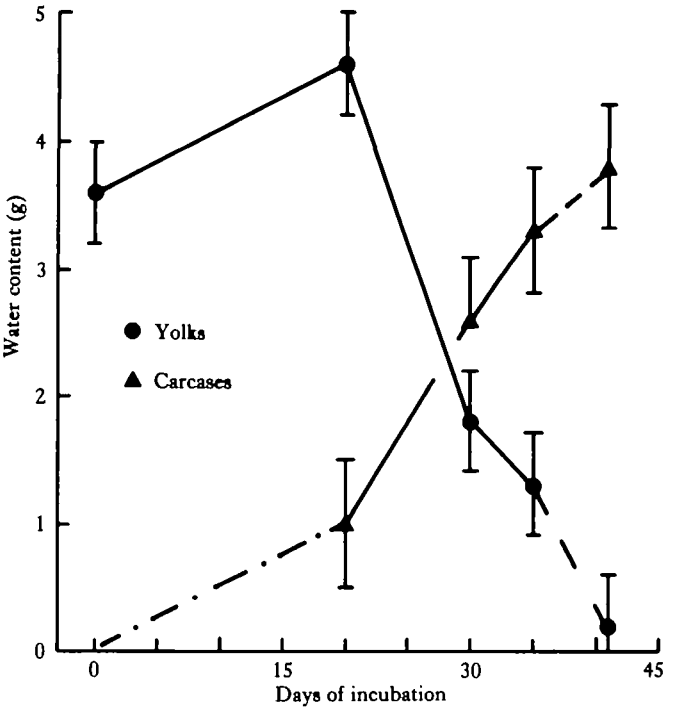

Fig. 3. Mean values for water content of yolks and of carcases of embryos/hatchlings from eggs of *Coluber constrictor*. Eggs were sampled at oviposition (day 0), on days 20, 30 and 35 of incubation, and at hatching. Sample sizes are 3 on day 0, 2 on day 20, 3 on day 30, 4 on day 35, and 5 at hatching. Vertical lines represent ± one-half the least significant difference (L.S.D.) for multiple comparisons of sample means (Snedecor & Cochran, 1967); means differing by the L.S.D. are significantly different at alpha = 0·05.

to 1·8 g, and the quantity of water present in yolk dissected from hatchlings was only 0·2 g (Fig. 3).

The percentage water content, or relative hydration, of yolks also varied during incubation [$F(4,12) = 8{\cdot}83$, $P = 0{\cdot}002$]. Approximately 70 % of the mass of yolks at oviposition was attributable to water whereas this proportion had increased to 80 % by day 20 of incubation (Fig. 4). The proportion of water in yolks declined between day 20 and hatching, and the relative hydration of yolks removed from newly hatched young was approximately 60 % (Fig. 4).

Analysis of variance revealed significant temporal variation in water content [$F(3,10) = 13{\cdot}25$, $P = 0{\cdot}001$] and relative hydration [$F(3,10) = 138{\cdot}86$, $P < 0{\cdot}001$] of embryos. On day 20, embryos contained approximately 1·0 g of water and relative hydration was 90 % (Figs 3, 4). Water content of embryos increased and relative hydration declined thereafter. Hatchlings contained an average of 3·8 g of water and had a relative hydration of approximately 80 % (Figs 3, 4).

Calcium in yolks, embryos and eggshells

The total quantity of calcium in yolks of eggs of *Coluber constrictor* varied significantly with time [$F(4,12) = 45{\cdot}17$, $P < 0{\cdot}001$]. Yolks contained an average of 30 mg of calcium at oviposition, and the amount of calcium available from the yolk d[illegible]

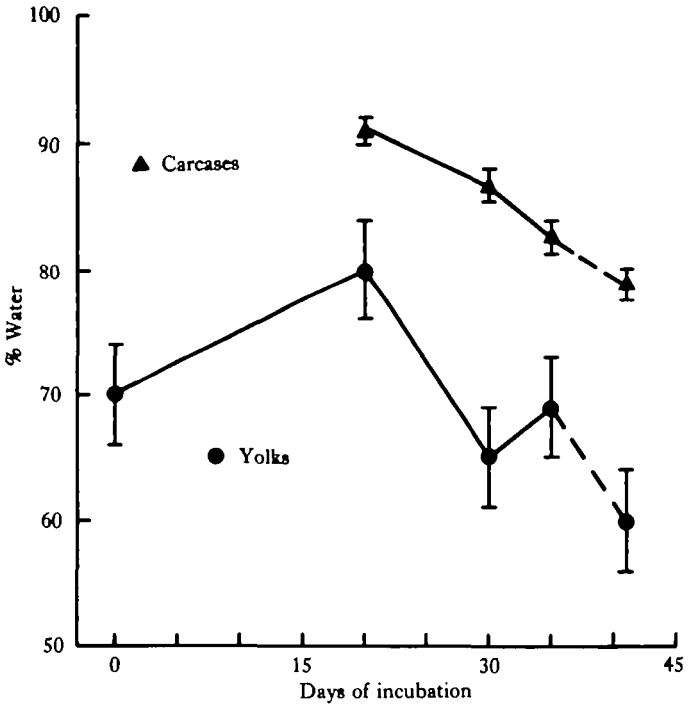

Fig. 4. Mean values for relative hydration of yolks and of carcases of embryos/hatchlings from eggs of *Coluber constrictor*. Eggs were sampled at oviposition (day 0), on days 20, 30 and 35 of incubation, and at hatching. Sample sizes are 3 on day 0, 2 on day 20, 3 on day 30, 4 on day 35, and 5 at hatching. Vertical lines represent ± one-half the least significant difference (L.S.D.) for multiple comparisons of sample means (Snedecor & Cochran, 1967); means differing by the L.S.D. are significantly different at alpha = 0·05.

not change appreciably during the first half of incubation (Fig. 5). However, the quantity of calcium in this compartment declined more-or-less linearly between day 20 and hatching at an average rate of 1·3 mg day^{-1} (Fig. 5). Yolk removed from hatchlings contained only about 3 mg of this element (Fig. 5).

The concentration of calcium in yolks also varied significantly with time [$F(4,12) = 11{\cdot}91$, $P < 0{\cdot}001$]. Yolks of freshly laid eggs had a calcium concentration of around 20 mg g^{-1} dry mass. There was little variation in calcium concentration of yolks for most of incubation (Fig. 5). However, late in incubation, the concentration of calcium in yolks declined significantly (Fig. 5), and the concentration of calcium in yolk removed from hatchlings was 16 mg g^{-1} dry mass (Fig. 5).

Analysis of variance also revealed significant temporal variation in total calcium content [$F(3,10) = 60{\cdot}29$, $P < 0{\cdot}001$] and in calcium concentration [$F(3,10) = 150{\cdot}19$, $P < 0{\cdot}001$] of embryos. Both total calcium and calcium concentration increased more-or-less linearly with time (Fig. 6). Embryos contained approximately 1 mg of calcium and had an average calcium concentration of 12 mg g^{-1} on day 20 of incubation (Fig. 6). Hatchlings contained significantly more calcium and had a higher concentration of this element than characterized embryos at the mid-point of incubation (Fig. 6). Average calcium content of yolk-free hatchlings was 36 mg and average calcium concentration was 36 mg g^{-1} dry mass (Fig. 6). The average daily increment in total

calcium content of embryos between oviposition and day 20 was 0·06 mg, assuming that calcium content of embryos at oviposition was essentially nil. During the second half of incubation, the average rate of increase in calcium content was 1·6 mg day^{-1}.

The quantity of calcium available in 1 ml of water in the water/vermiculite mixture used for analysis of calcium content was 0·024 mg. Thus, eggs absorbing an average of 6 g of water from the substrate could obtain a maximum of 0·14 mg of calcium in this manner.

From an examination of Figs 5 and 6 it appears that hatchlings contain more calcium (36 mg) than can be accounted for by that present in egg contents at oviposition (30 mg), and analysis of variance with blocking by clutch supports this contention [$F(1,4) = 30{\cdot}89$, $P = 0{\cdot}005$]. Moreover, a similar analysis revealed a significant increase in total calcium contained within eggs (Table 1). Total calcium content of eggs (exclusive of the eggshell) at oviposition was approximately 30 mg, but at the end of incubation total calcium content (i.e. calcium in yolks and in carcases) had increased to 38 mg (Table 1). Conversely, shells from eggs incubated to hatching contained significantly less calcium than shells from eggs at oviposition (Table 1). There was, however, no change in calcium contained within entire eggs (Table 1).

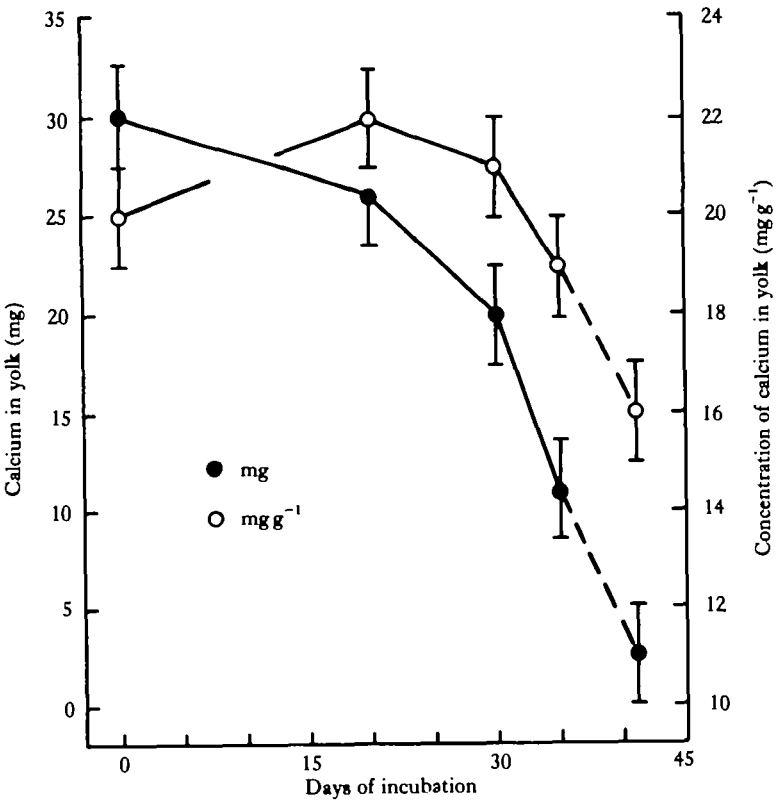

Fig. 5. Mean values for total calcium and calcium concentration in yolks from eggs of *Coluber constrictor*. Eggs were sampled at oviposition (day 0), on days 20, 30 and 35 of incubation, and at hatching. Sample sizes are 3 on day 0, 2 on day 20, 3 on day 30, 4 on day 35, and 5 at hatching. Vertical lines represent ± one-half the least significant difference (L.S.D.) for multiple comparisons of sample means (Snedecor & Cochran, 1967); means differing by the L.S.D. are significantly different at alpha = 0·05.

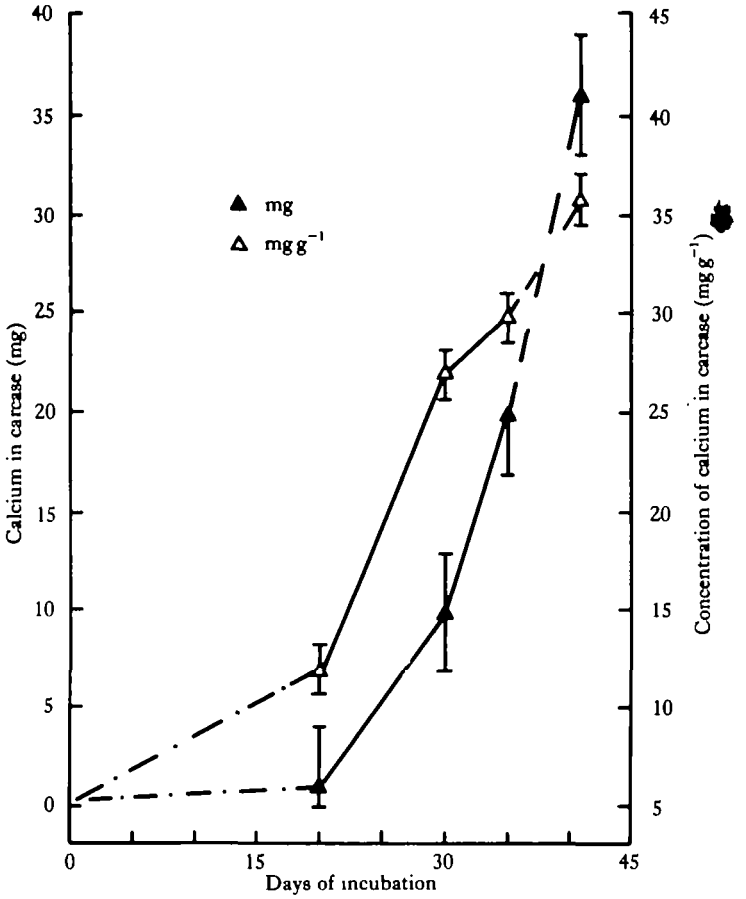

Fig. 6. Mean values for total calcium and calcium concentration in carcases of embryos/hatchlings from eggs of *Coluber constrictor*. Eggs were sampled at oviposition (day 0), on days 20, 30 and 35 of incubation, and at hatching. Sample sizes are 3 on day 0, 2 on day 20, 3 on day 30, 4 on day 35, and 5 at hatching. Vertical lines represent ± one-half the least significant difference (L.S.D.) for multiple comparisons of sample means (Snedecor & Cochran, 1967); means differing by the L.S.D. are significantly different at alpha = 0·05.

Table 1. *Mean values (S.E. in parentheses) for calcium in contents of eggs (yolk plus embryo), in eggshells, and in entire eggs (yolk plus embryo plus shell) of the snake* Coluber constrictor *at oviposition (N = 3) and at hatching (N = 5)*

	Calcium (mg) at			
Variable	Oviposition	Hatching	F(1,4)	*P*
Egg contents	29·6 (3·0)	38·3 (2·1)	43·61	0·003
Eggshell	10·5 (2·4)	6·4 (1·1)	37·70	0·004
Entire egg	40·2 (5·2)	44·7 (3·1)	0·96	0·384

Data are from fertile eggs only. However, when data for five infertile eggs were included in the sample of eggs at oviposition, results of the analyses were similar, and the conclusions were not changed. Data were examined by analyses of variance with clutch as a blocking factor to control for variation among eggs produced by different males.

DISCUSSION

Changes in mass of eggs during incubation

During incubation, eggs contacting substrates exchange water with their environment in both the liquid and vapour phases (G. C. Packard *et al.* 1981; Tracy, 1982). If the quantity of liquid water absorbed by eggs exceeds the quantity of water vapour that is lost, eggs increase in mass. Conversely, if eggs lose vapour in excess of liquid absorbed from the substrate, they decline in mass.

Eggs used in this study increased in mass for 28 days of incubation, indicating net absorption of water (Fig. 1). Although eggs declined in mass between day 28 and day 35, they weighed 3 g more, on average, at the end of incubation than at oviposition. Thus, net storage of about 3 g of water occurred during development. However, the absolute quantity of water absorbed by eggs was probably closer to 6 g because some of the liquid absorbed undoubtedly was subsequently lost from eggs as water vapour. The pattern of change in mass of eggs in this study is similar to that reported for flexible-shelled eggs of other squamates incubated under favourable hydric conditions (M. J. Packard *et al.* 1980; Tracy, 1980; Andrews & Sexton, 1981; Muth, 1981).

Growth of embryos and consumption of yolk

Small embryos were present in freshly laid eggs, but were too small and fragile to be isolated and weighed. However, development occurring prior to oviposition probably emphasized differentiation rather than growth (Shine, 1983), so dry mass of embryos in recently laid eggs can be assumed effectively to be zero. Consequently, growth of embryos subsequent to oviposition is a reasonable approximation to growth overall.

Our estimates of rate of increase in dry mass indicate that embryos grow slowly during the first half of incubation. During the second half of incubation, however, the pattern of growth is roughly linear and occurs at a much higher rate (Fig. 2). Moreover, the change in total solids of yolk is small for the first 20 days of incubation, but dry mass of yolk declines dramatically during the second half of development as embryos consume yolk to support growth and metabolism (Fig. 2). Comparable data for change in dry mass of squamate embryos and yolks with time are not available, but other measures of metabolic activity, such as oxygen consumption, confirm that growth and metabolism of squamate embryos increase dramatically during the latter half of incubation (Dmi'el, 1970).

Movements of water inside eggs

Some of the water absorbed by eggs during incubation was apparently stored temporarily in the yolk sac, because water content and relative hydration of this compartment increased between oviposition and day 20 of incubation (Figs 3, 4). Data for water content of yolks for other squamate eggs are not available, but similar changes in water content have been reported for yolks of turtle eggs incubated under favourable hydric conditions (Morris *et al.* 1983; G. C. Packard *et al.* 1983). In the turtle eggs, water content of yolks increases early in incubation owing largely to transfer of water from the albumen to the vitelline sac (Morris *et al.* 1983; G. C

Packard *et al.* 1983). However, albumen was not apparent in freshly laid eggs (nor in eggs at other sampling periods) in this study, and therefore the increase in water content of yolks reflects only storage of water absorbed from substrates.

Between day 20 of incubation and hatching there was a progressive decline in both water content and relative hydration of yolks (Figs 3, 4). A portion of the water extracted from yolks was presumably incorporated into growing embryos, because the water content of carcases increased over the same interval (Fig. 4). Thus, in snake eggs as in turtle eggs, the yolk sac acts as an intermediate store for water absorbed from the environment and is the proximate source of water during development (Morris *et al.* 1983; G. C. Packard *et al.* 1983).

Water content of embryos increased during incubation in conjunction with increases in mass of embryos and withdrawal of water from yolks to support embryonic development (Figs 2–4). As embryonic solids increased, however, the relative hydration of embryos declined from a high of 90% on day 20 to about 80% at hatching (Fig. 5). These changes in relative hydration are similar to those reported for embryos of the green iguana *Iguana iguana* (Ricklefs & Cullen, 1973). Indeed, young iguanas have about the same proportion of body water at hatching as characterized the snakes examined in this study (Ricklefs & Cullen, 1973).

Calcium in yolks, embryos and eggshells

Eggs of *Coluber constrictor* are similar to eggs of other squamates in that the yolk is a relatively rich source of calcium (Simkiss, 1967; Jenkins & Simkiss, 1968). Indeed, the quantity of calcium in the yolk of *Coluber* eggs is within the range of values reported for the much larger eggs of domestic fowl (Simkiss, 1967; M. J. Packard & G. C. Packard, 1984). In contrast, shells of *Coluber* eggs are a relatively poor source of this element (Table 1).

Mobilization of calcium by embryos of *Coluber constrictor* occurred relatively slowly during the first half of incubation, but calcium metabolism increased dramatically during the second half of development as calcium was withdrawn from yolks and incorporated into embryos (Figs 5, 6). The major requirement for calcium during embryogenesis is for ossification of the skeleton, and calcium metabolism generally increases dramatically once skeletal formation commences (Simkiss, 1967). We have no information on the timing of bone formation in embryos of *Coluber constrictor*, but the increase in calcium metabolism characterizing the second half of incubation indicates that skeletal formation is probably confined largely to the latter half of incubation in this species as it is in embryos of other oviparous vertebrates (Figs 5, 6; Simkiss, 1967).

The concentration of calcium in embryos examined in this study increased during incubation in parallel with the increase in total calcium content of carcases (Fig. 5). In contrast, the concentration of calcium in yolks did not change in concert with the changes in total calcium content of this compartment (Fig. 6). The concentration of calcium in yolks was essentially unchanged during the first 35 days of incubation, but declined significantly between day 35 and day 41, the average day of hatching (Fig. 6). Changes in concentration of calcium in the yolk indicate that embryos withdraw calcium selectively from this compartment, particularly during the last few days of incubation.

The total quantity of calcium within eggs increased significantly during incubati (Table 1). Eggs may have absorbed calcium along with water from the substrates on which they were incubated, but the quantity of calcium available in water absorbed by eggs (0·14 mg) is too small to account for these changes (Table 1). Thus, we conclude that embryos of *Coluber constrictor* obtain a portion of their calcium requirement from the eggshell. This interpretation is supported by the observation that shells from eggs at oviposition contain more calcium than do shells from eggs incubated to hatching (Table 1).

The decline in calcium content of shells does not match exactly the increase in calcium within eggs in part because of the variability inherent in analyses of this sort and in part because it is not possible to follow changes in calcium content of individual eggshells throughout incubation. Thus, we emphasize the significant decline in calcium in eggshells, the significant increase in calcium within eggs, and the lack of significant change in calcium content of entire eggs (Table 1), and suggest that undue emphasis should not be placed on absolute values.

These observations indicate that embryos of *Coluber constrictor* are similar to embryos of chelonians, crocodilians and birds in that calcium used during embryogenesis is obtained from both yolks and eggshells. Thus, the dichotomy placing embryonic squamates in a group separate from embryos of other oviparous, amniotic vertebrates seemingly requires revision (Simkiss, 1962, 1967; Jenkins & Simkiss, 1968; Bustard *et al.* 1969; Crooks & Simkiss, 1974; Jenkins, 1975; Dunn & Boone, 1977; M. J. Packard & G. C. Packard, 1984). Admittedly, the investment by squamates of relatively large quantities of calcium in egg contents at oviposition has made it unnecessary to postulate additional sources of calcium for embryos (Simkiss, 1967; Jenkins & Simkiss, 1968). Moreover, the poorly calcified eggs laid by most squamates have made it seem unlikely that embryos would mobilize calcium from this source (M. J. Packard, G. C. Packard & Boardman, 1982). Nonetheless, squamate embryos clearly have the capacity to withdraw calcium from eggshells even though this compartment furnishes a relatively small proportion of the calcium used during development.

Snake embryos examined in this study relied on the eggshell for about 20 % of the calcium used during embryogenesis with the remaining 80 % coming from the yolk. In contrast, embryonic crocodilians, chelonians and birds rely on the shell for 50–80 % of their need for this element (Simkiss, 1967; Jenkins & Simkiss, 1968; Bustard *et al.* 1969; Jenkins, 1975; M. J. Packard & G. C. Packard, 1984). These differences in the degree to which embryos rely on the eggshell for a portion of their calcium requirements may reflect, in part, differences in the availability of calcium from the shell. Eggs laid by most squamate reptiles, including *Coluber constrictor*, have poorly calcified shells compared to crocodilian, chelonian and avian eggs (Board, 1982; Ferguson, 1982; M. J. Packard *et al.* 1982), and the quantity of calcium available from this compartment may have placed constraints on the evolution of calcium metabolism in embryos of oviparous amniotes.

Control of calcium metabolism during embryogenesis

Calcium metabolism in *Coluber constrictor* embryos is similar in general to calcium metabolism in embryos of other oviparous reptiles but differs considerably from calcium metabolism in embryos of domestic fowl (M. J. Packard & G. C. Packar

84). Calcium content of the yolk of hens' eggs increases as some of the calcium absorbed from the shell is stored in the yolk, and yolk retracted into the abdominal cavity of chicks contains more calcium than was present in yolks of eggs at oviposition (Johnston & Comar, 1955; Crooks & Simkiss, 1974; Dunn & Boone, 1977). In contrast, embryonic snakes apparently store none of the calcium removed from the eggshell in the yolk, for there is no increase in yolk calcium during incubation, despite the fact that the amount of calcium in egg contents (yolk plus embryo) does increase.

Both sources of calcium used by snake and bird embryos (the yolk and eggshell) are separated from embryos by the cellular epithelia of the yolk sac and chorioallantois, respectively, and these extraembryonic membranes are potential target organs for control of calcium metabolism during embryogenesis (Clark & Simkiss, 1980). The chorioallantois is presumably the more important of the two sites in avian embryos because it transports more calcium during embryogenesis than does the yolk sac (M. J. Packard & G. C. Packard, 1984). On the other hand, the yolk sac may be the more important target organ in snake embryos in that these embryos obtain most of their calcium from the yolk. These differences between chicken and snake embryos may indicate fundamentally different mechanisms for the regulation of calcium mobilization from yolk and eggshell and for the distribution of calcium to egg compartments during development, but these mechanisms and their role have not yet been identified.

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