

Plasma Calcium, Magnesium and Protein of Viviparous Colubrid Snakes During Estrous Cycle. (22456)

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During the season of rapid follicular growth in cod and puffer fish(1), clawed toad(2), and in many species of birds(3), increases in concentration of plasma calcium have been observed. At this same stage of the estrous cycle, a phosphoprotein has been detected in the plasmas of a trout, a turtle, and a number of species of birds(4). This paper presents evidence concerning plasma changes which occur in viviparous colubrid snakes during estrus.

Materials and methods. Specimens of 10 kinds of water snakes, *Natrix*, and 14 kinds of garter snakes, *Thamnophis*, were collected locally or were obtained from collectors in other areas of the United States. Animals were fasted for one week and then anesthetized with ether and bled from a cardiac puncture. Blood was collected in heparinized tubes. Calcium plus magnesium was determined directly upon a plasma sample; calcium was measured upon an oxalate precipitate of plasma; magnesium was calculated by taking the difference between the two values. A modification of Schwarzenback's ethylenediamine-tetraacetic acid method was used in these analyses(5). Plasma protein was determined with a biuret method(6). Reproductive stages of animals were determined by direct observation of reproductive organs and embryos.

Results. Average plasma levels of calcium, magnesium, and total protein are given in Table I. The concentration of calcium and magnesium of nonestrous females, males and immature specimens differs very little. The plasmas of females of both genera studied have higher protein concentrations than those of males during all seasons. Average calcium and protein levels are higher in *Natrix* than in *Thamnophis*. No distinct seasonal differences

occur in calcium, magnesium, or total protein concentrations of male specimens. In contrast to males, marked increases in all three substances accompany estrus in females of both genera. The increase in calcium may be as much as 10 times or as little as twice as great as the increase in magnesium. The rise in total plasma protein of estrous females averages about 25% in *Thamnophis* and about 15% in *Natrix*. The absolute, average increase is about 1 g/100 ml of plasma in both genera. Preliminary observations of paper electrophoretic patterns of plasma proteins indicate that in some of the species studied a specific fraction is associated with this increase in total protein. Plasma protein concentration of estrous females is less variable than plasma calcium or magnesium levels.

To show the time relationships of these plasma changes, the sum of the concentrations of calcium plus magnesium in plasma of individual specimens of female garter snakes relative to their stage of the estrous cycle is shown in Fig. 1. Calcium plus magnesium is of low concentration until the period of rapid yolk deposition in spring. The initiation of this process is noted when the translucent follicles characteristic of anestrous snakes become opaque and begin to enlarge. When these follicles have about doubled their anestrous size, the plasma of the snake becomes slightly opalescent and contains an increased concentration of alkaline earth elements in comparison to values characteristic of other stages of the estrous cycle. Plasma level of calcium plus magnesium increases steadily as the follicles grow and attains a concentration of between 10 and 15 mM/l during the late stages of yolk deposition. Most extreme values are found in animals bled about the time

egg laying season has been reported by some investigators, but this reported rise is questioned by others(7). Estrogens will induce a rise in plasma calcium in male birds and in females during anestrus(3). The rise in plasma calcium of birds(3) during estrus is greater than increases found in vertebrates previously studied(1,2) but is still less than half the average concentration characteristic of these snakes.

As the eggs of viviparous snakes are not covered by a calcareous shell, it remains to be determined whether the estrous rise in calcium and magnesium represents a transport of these minerals to the developing follicles or whether the phenomenon is merely a physiologic response carried over from forms which used such inorganic material in egg shell formation. Follicles of birds develop in sequence and ovulation is at the rate of one egg/day or less. Viviparous snakes, unlike birds, develop large numbers of follicles simultaneously, reaching a maximum of 85(8). These follicles progress at approximately the same rate and are ovulated within a few minutes or at most a few hours of each other. The great metabolic demands of such large numbers of simultaneously developing follicles may be related to the greatly elevated protein, calcium

and magnesium levels in the plasma of these snakes during estrus. The sharp decrease in these substances following ovulation probably is correlated with the uniform time of ovulation.

Summary. A marked increase in concentrations of calcium, magnesium and total protein occurs in plasma of female specimens of viviparous snakes during estrus. The magnitude of these estrous changes greatly exceeds any such data reported for other vertebrates. Following ovulation these plasma values rapidly fall to concentrations characteristic of nonestrous animals.

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Relationship of Blood Pressure to Mortality in Chickens.* (22457)

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Mortality losses in poultry flocks during the first laying year range from 20 to 40%. Autopsy reports indicate that 50% or more of these deaths result from non-specific disorders or undetermined causes(1-4). Whether or not any portion of the mortality was attributable to circulatory disturbances has not

been ascertained. Sturkie, Weiss, and Ringer (5) previously determined systolic blood pressures on a large population of chickens, ranging in age from 10 to 54 months. At any age the pressures were widely distributed, but no attempt was made to relate the level of pressure to mortality, which is the object of this study.

Procedure. Arterial blood pressures were determined on hens hatched in 1950, 1952, and 1954 and on males in 1954 by an indirect method(6). Three consecutive determinations per bird were made at 7 to 10 months of

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