Copulatory Plugs in Snakes: Enforced Chastity

Abstract. *The male of some snake species forms a copulatory plug which occludes the oviductal parts of the female's cloaca for a few days. The plug, apparently formed from secretions of the kidney immediately after insemination, probably prevents rival males from copulating with the same female.*

Although the formation of a mating plug in certain mammals (*1*) and insects (*2*) is reasonably well known, the existence of a similar phenomenon in any reptile is suggested only by a single report of pluglike gelatinous material in garter snakes (*3*). During the spring of 1974, I discovered copulatory plugs in the cloacae of nine females of the natricine snakes *Thamnophis sirtalis, Thamnophis butleri,* and *Natrix taxispilota.* The plug noticeably distends the female's cloacal region, and part of it may be visible directly through the anus, but it is most easily detected by palpation as a hard mass. A plug dissected from one female *T. sirtalis* was located in the urodaeum anterior to the openings of both the ureter and the intestine (Fig. 1). The somewhat translucent plug material appeared gelatinous, but was quite tough and resisted cutting with a scalpel. Figure 1 shows a transition zone between the firm translucent plug matrix and an opaque, creamy white, sperm-dense fluid which covered the oviductal orifices and extended at least 10 cm up the oviducts. None of this sperm-dense fluid escaped until I cut through the anterior cloacal wall because the lateral surfaces of the plug formed a tight seal affixed to the cloacal mucosa.

The plug apparently remains in place for only a few days before the female expels it. Because laboratory snakes have refused to mate and natural matings are rarely observed, the duration of the plug in a female can only be estimated from the following observations. On 4 April, only 2 days after the arrival of suitable weather for snake activity around Ann Arbor, I secured four plugged *T. sirtalis* females and maintained them at 5°C to approximate the cooler weather that prevailed in the days following their capture. One expelled her plug after 2 days, and the others were free of plugs after 4 days. The lateral surfaces of expelled plugs seemed smoother than those of the plug obtained through dissection, which retained a fairly regular texture of minute bumps. This suggests that active or passive chemical breakdown occurs along the female's cloacal walls. Because of the adhesion of fresh plugs to the cloacal mucosa, movement by a female to expel one would tend to force sperm into the oviducts.

The kidneys of breeding male snakes and lizards have a sexual segment consisting of the hypertrophied and glandular preterminal segment of individual nephrons. Histological evidence indicates that this tissue secretes the substrate from which the plug matrix is formed, and supports one of Volsøe's three hypotheses on the function of the sexual segment (*4*). A preliminary series of standard histochemical tests (*5*) were applied to 5-μm paraffin sections of a *T. sirtalis* plug and similar sections of the three male structures which are believed to contribute to the seminal fluid of snakes (*6*): the ductus deferens (containing secretions from the epididymis as well as sperm), the dorsal cloacal gland, and the kidney. Sperm were sparsely scattered and trapped in the plug matrix except at the anterior transition zone where there were masses of sperm which had once been continuous with the sperm-dense fluid. A far more homogeneous distribution of sperm in the plug would be likely if the contents of the ductus deferens contributed significantly to the plug material. Acid mucopolysaccharides occur in the dorsal cloacal gland (Alcian blue positive), but are not apparent in the plug matrix. The plug material appears to be predominantly a tryptophan-rich protein (positive staining with dimethylaminobenzaldehyde and nitrite) with a net basic charge (strongly acidophilic), and it has a lipid component (positive staining with Sudan black B). These qualities are shared, even to the same intensity of staining, by the sexual segment of the kidney. Furthermore, occasional cavities in the plug matrix contain a few spherical bodies (1 to 2 μm in diameter) which are similar in size and staining characteristics to the secretion granules of the cells composing the sexual segment. Since granules of the sexual segment are secreted intact (*7*), these cavities may represent pockets of secretion which, for some reason, failed to complete chemical transformation.

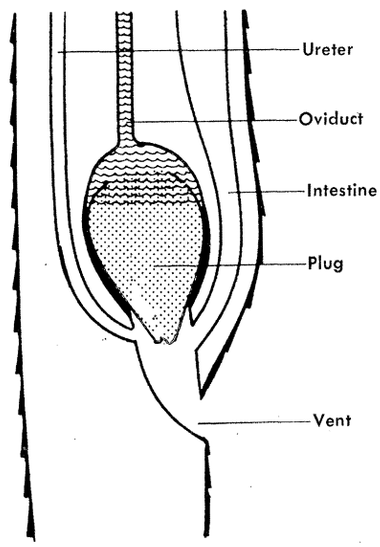

Fig. 1. Sagittal section through the cloacal region of a female *Thamnophis sirtalis* showing semen (wavy lines) anterior to the plug (stippling) in situ. Diagrammatic reconstruction from gross dissection.

The male snake evidently ejaculates a part of the sperm stored in the ductus deferens, followed by a stream of intact sexual segment secretion granules which subsequently lose their integrity and precipitate to form the plug matrix. The general shape and surface texture of the plug suggest that it starts to form anteriorly and progresses posteriorly, filling in the space previously occupied by the male's expanded hemipenis which is withdrawn by gradual involution. Implantation of a plug may account for a significant part of the 10 to 20 minutes (*8*) required for a successful copulation.

Without denying the adaptive value of a plug that reduces sperm leakage, Parker (*2*) emphasized the importance of intrasexual competition among males to fertilize ova as a major selective force in the evolution of insect plugs. Sperm leakage would be disadvantageous to both the male and the female. Selection acting on the female should favor a simple mechanism for sperm retention, such as cloacal muscular control, as opposed to a large cloacal obstruction. Competition among males for females probably played the major role in the evolution of snake plugs. Many snakes, including *Thamnophis* and *Natrix*, often form courtship aggregations consisting of one female and several males. A male that effectively blocked the female's cloaca with a plug would preclude the possibility of an immediate rival diluting his sperm with

a second copulation. This competitive effectiveness is indicated by observations that unsuccessful males of such an aggregate cease courting and begin to disperse soon after their successful rival has achieved intromission (*9*). The plugs do not remain in place long enough to assure males of sole paternity.

In *Thamnophis*, mating generally precedes ovulation by several weeks (*10*), and a female could mate again after expelling a plug. However, the female may leave the area of high male density at the mating site before the plug is expelled. Also, while the plug is in place, the first male's sperm may reach and occupy the specialized sperm storage crypts in the walls of the anterior oviducts, where they would be in the most favorable position to survive until ova enter the oviducts (*10*). If the plugs impede further copulations, they should be prevalent in those species in which there might otherwise be a high probability of two or more matings in succession. While this is true of the three taxa considered here, some reptiles have behavioral attributes, such as territoriality or male combat, which may isolate a female from the advances of other males, thus eliminating the selective advantage for a plug. If the plugs function merely to retain sperm, they should be equally common in either group of reptiles.

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References and Notes

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Cyclic AMP and Cyclic GMP May Mediate Opposite Neuronal Responses in the Rat Cerebral Cortex

Abstract. *Electrophysiologically identified pyramidal tract neurons in the rat cerebral cortex were tested with norepinephrine, acetylcholine, adenosine 3',5'-monophosphate (cyclic AMP), and guanosine 3',5'-monophosphate (cyclic GMP) applied by microiontophoresis. The neurons were usually inhibited by norepinephrine and cyclic AMP, but excited by acetylcholine and cyclic GMP. These opposing responses of pyramidal tract neurons to cyclic AMP and cyclic GMP suggests that these two nucleotides could function as reciprocal intracellular second messengers for norepinephrine and acetylcholine, respectively.*

Extensive evidence now indicates that responses of cells in many tissues to hormones may be mediated intracellularly by the cyclic nucleotide adenosine 3',5'-monophosphate (cyclic AMP) or guanosine 3',5'-monophosphate (cyclic GMP) (*1*). In central nervous tissues, elevations of cyclic GMP content have been correlated with muscarinic cholinergic receptors (*2*), while cyclic AMP concentrations can be increased through beta-norepinephrine receptors (*3*) or responses to dopamine (*4*). Tests of the possible physiological actions of these cyclic nucleotides in neurons have relied heavily on the technique of iontophoretic application (*5*). These studies have led to conflicting interpretations of the actions of cyclic AMP because of different standards for the utilization and assessment of iontophoretic tests (*6–8*). In the rat cerebellar cortex, norepinephrine (NE) and cyclic AMP produce similar changes in discharge rate and membrane properties; furthermore, the proportion of Purkinje cells which give positive immunocytochemical reactions for cyclic AMP is increased only by topical application of NE or activation of the noradrenergic synaptic pathway arising in the locus coeruleus (*5, 9*). We now report that iontophoretic studies on cells electrophysiologically identified as pyramidal tract (PT) neurons in the rat cerebral cortex show distinctly opposite responses to cyclic AMP and to cyclic GMP: while NE and cyclic AMP predominantly depress all cells, acetylcholine (ACh) and cyclic GMP are mainly excitatory.

Adult male rats were anesthetized with urethane (1.25 g per kilogram of body weight, intraperitoneally) and prepared for recording in the parietal cortex (*10*). Standard microiontophoretic procedures (*11*) and five-barreled micropipettes with an overall tip diameter of 5 to 10 μm were employed. They were filled immediately before the experiment by centrifugation (*12*). Our iontophoresis circuitry minimizes polarizations of the electrode tip during drug ejection by use of automatic balancing (*11*); prevention of undesired diffusion of drugs between tests was effected by use of holding currents. Only spontaneously active cells were studied. Artificial activation of cells by excitatory amino acids was not employed in order to prevent complex drug interactions (*7*).

Spontaneously active cortical neurons were identified as PT cells on the basis of the antidromic action potential

Table 1. Responses of rat cerebral cortical neurons to cyclic nucleotides. Values are the numbers of cells (*N*) exhibiting excitation, inhibition, and no response. Unidentified cells are those which could not be identified as PT cells. For the effects of cyclic AMP and NE the correlation coefficient r is .97523 for PT cells and .96429 for unidentified cells; for cyclic GMP and ACh, $r = .99094$.

Response to NE or ACh	Response to cyclic AMP or cyclic GMP					
	PT cells (*N*)			Unidentified cells (*N*)		
	Excitation	Inhibition	No response	Excitation	Inhibition	No response
	NE and cyclic AMP					
Excitation	0	0	0	0	0	0
Inhibition	3	11	0	1	4	0
No response	1	1	2	0	0	2
	ACh and cyclic GMP					
Excitation	22	2	6			
Inhibition	2	6	2			
No response	0	0	1			