The length of hibernation affects temperature-induced (25 °C) spermatogenic multiplication in *Helix aspersa* Müll.

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Summary. In hibernating snails an increase in temperature from 5 °C to 25 °C induces DNA synthesis in the gonad but not in the albumen gland and intestine. Spermatogenic multiplication augments in response to increased temperature and this is dependent upon the duration of hibernation.
Key words. DNA synthesis; spermatogenesis; hibernation; snail; *Helix aspersa.*

Hibernation is an intriguing form of adaptation that enables animals "to pass the winter in a torpid or lethargic state"[1]. "Many aspects of hibernation remain unexplained, and researchers continue to investigate its effects, as well as potential biochemical applications"[2].
Among invertebrates, snails from temperate regions start hibernation at the end of summer and autumn and return to activity the following spring. Studies carried out to discover the causes of start and duration of hibernation in snails have shown that environmental factors (photoperiod, temperature, humidity) and endogenous factors[3–7] intervene at the same time.
Currently, the dearth of edible snails in nature requires the development of commercial raising methods which take into account the snails' physiological characteristics[8]. In this connection, it has been found that snails of the species *Helix aspersa,* which begin natural hibernation in the autumn, are capable of returning to feeding and movement if they are awakened after 1.5 months of hibernation. However, these snails start mating only 7 weeks later and lay eggs after 12 weeks[9]. In the same environmental conditions, but after twelve months of hibernation the snails mate immediately, followed by egg-laying in the fourth week; in this latter case the number of eggs deposited in eighteen weeks was thirteen times higher than in the former. These observations show that internal changes occurred in snails during hibernation although these took place at a constant temperature of 5 °C. In this study we analyzed response to spermatogenic multiplication (DNA synthesis) of snails in dormancy exposed to 25 °C after three periods of hibernation. (1, 6 and 12 months in length).

Materials and methods

Pulmonate snails *Helix aspersa* of the same age were used in this study. They were born in the spring of 1987 and raised to adulthood that summer under natural conditions. In the fall the snails went into natural hibernation and until their use they were maintained in a cold chamber (5–6 °C), in total darkness and at a relative humidity of 50 %. Three experimental groups were formed with snails after 1, 6 and 12 months hibernation. For each experimental group, six snails were maintained for four weeks at 25 °C in a dark, non-humidified container. Under these conditions, the snails remained inactive and withdrawn in their shells. The control snails were maintained under similar conditions but at 5 °C. Synthesis of DNA of the ovotestis, intestine and albumen gland was measured in both experimental and control groups.
For ^{3}H-thymidine incorporation and autoradiography, we employed the techniques previously described[10]. Cellular multiplication was evaluated by measuring ^{3}H-thymidine incorporation in the three above-mentioned organs. 5 µCi of ^{3}H-thymidine (sp. act. 1 Ci/mmole; CEA: Commissariat à l'Energie Atomique, 91191 Gif-sur-Yvette) per gram of wet weight was injected into the hemocoel 5 h before the removal of organs. Following the sacrifice, three pieces of tissue from each organ were prepared as follows: piece one for cytology and autoradiography; piece two for the measurement of incorpora-

Incorporation of ^{3}H-thymidine in tissues of control snails at 5 °C and in three experimental groups at 25 °C

Organ	DNA synthesis: dpm/pg DNA ± SEM			
	Control 5 °C	Experimental groups (25 °C 4 weeks) After 1 month hibernation	After 6 months hibernation	After 12 months hibernation
Gonad	20 ± 2	103 ± 27* a b	351 ± 59* a	519 ± 153* b
Intestine	53 ± 13	75 ± 11	111 ± 34	85 ± 19
Albumen gland	24 ± 6	13 ± 5	22 ± 4	13 ± 6

* Significantly different from control values. Values showing a common letter vary significantly.

tion of ^{3}H-thymidine; and piece three for detection of total DNA in the tissue in order to express specific activity of ^{3}H-thymidine per unit of DNA (dpm/pg DNA). The comparison of average values of ^{3}H-thymidine incorporation was done by the non-parametric U-test of Mann and Whitney[11].

Results

1) Incorporation of ^{3}H-thymidine in snails kept at 5 °C. Irrespective of the length of hibernation, the values of ^{3}H-thymidine incorporation in all three organs studied were low and of the same order. At this temperature, autoradiographic examination did not show any ^{3}H-thymidine incorporation in the cells of the gonad. The male germ cells were represented only by spermatogonia and spermatocytes scattered in the lumen of acini (fig. 1), whereas the female germ line consisted of oocytes at different stages of development and they were located at the edge of the acini.

2) Incorporation of ^{3}H-thymidine after four weeks at 25 °C. The incorporation of ^{3}H-thymidine in the ovotestis of inactive snails subjected to 25 °C was significantly higher than that in either the intestine or the albumen gland (table). Autoradiography examination showed that this increase in incorporation occurred only in the male germ line, e.g. spermatogonia and spermatocytes. This allowed us to consider the values of incorporation measured by scintillation counter as a spermatogenic multiplication coefficient (SM).

We observed that SM is sensitive to a higher temperature (25 °C) from the beginning of hibernation. After one month of hibernation, the SM at this temperature was five times higher than at 5 °C while after six and twelve months it was 17 and 25 times higher respectively.

The observations suggest an increased temperature sensitivity of the control mechanism regulating SM during hibernation. This increase is significant during the first six months of hibernation (SM6/SM1 = 351/103 = 3.4) with a further increase between six and twelve months (SM12/SM6 = 519/351 = 1.5). Over twelve months the total increase of SM is 5.

Autoradiographic examination confirmed the increase of SM (figs. 2 and 3). Cytological observations showed that at 25 °C meiosis proceeded normally and spermatogenesis was completed in four weeks in all three hibernation periods considered.

Discussion

These experiments show that cellular multiplication in different organs differs in response to increase in temperature during hibernation as has also been shown in other hibernating animals[12,13]. Incorporation of ^{3}H-thymidine in the gonad and albumen gland, organs of the reproductive system, differ significantly. In fact, in hibernating snails cell multiplication in the albumen gland is completely insensitive to ambient temperatures while the

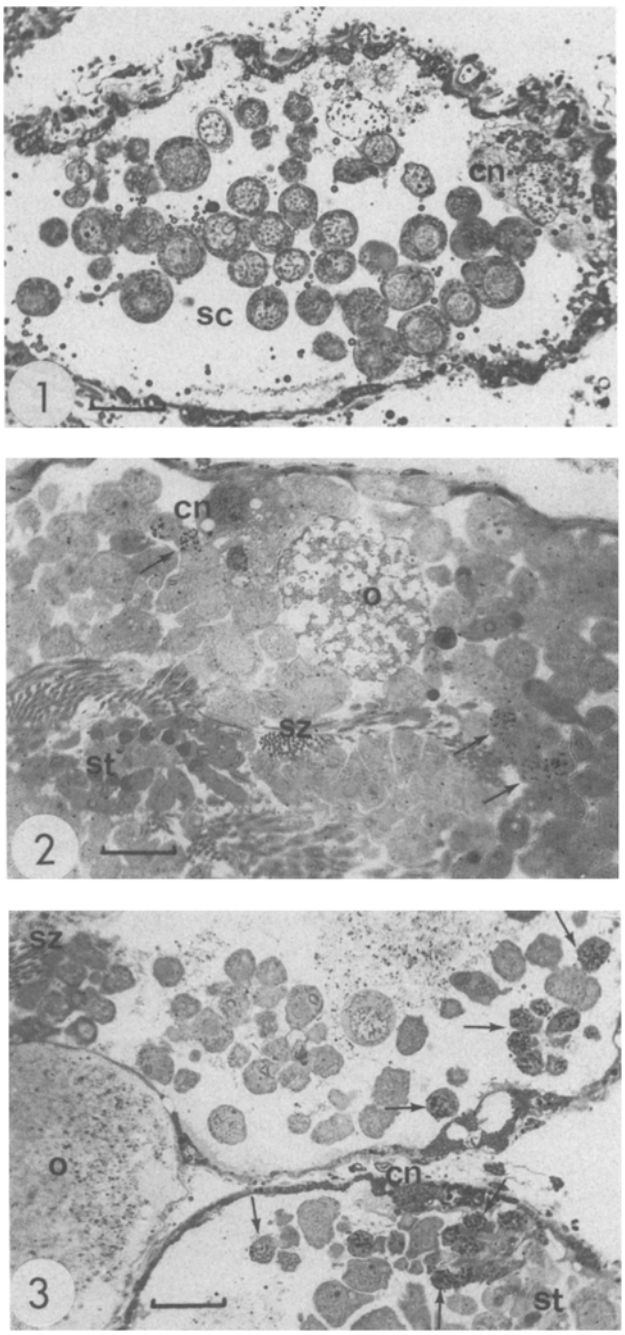

Figure 1. Section of an acinus of the gonad (ovotestis) of a snail after one month in hibernation at 5 °C. The spermatogenesis is arrested at spermatocyte stage.

Figure 2. An acinus of a gonad of a snail kept for four weeks at 25 °C following one month in hibernation. Note the presence of a few spermatids (st) and spermatozoa (sz). The number of labelled spermatocytes (→) is about 8 per mm^2.

Figure 3. Acini of the gonad of a snail kept at 25 °C for four weeks following twelve months in hibernation. The number of labelled spermatocytes (↗) increases nearly fourfold, i.e. 30 spermatocytes per mm^2. cn, nurse cells; o, oocyte; st, spermatids; sz, spermatozoa. Bar equals 25 μm.

male germ cells of the gonad multiply and differentiate when the temperature is increased from 5 °C to 25 °C. It has been shown that cell multiplication in the albumen gland of *Helix* is linked to photoperiod [14]. It is negligible during short days (L8 : D16) but significantly higher during long days (L18 : D6).

The use of ^{3}H-thymidine allowed us to show that multiplication of spermatogonia and spermatocytes is a selective response to increased temperature (25 °C) during hibernation and that the multiplication enhances with the increased length of hibernation. The weak response of SM to temperature at the start of hibernation (1 month) is without doubt one of the causes of the long delay in starting reproduction of snails at this point in their annual cycle. This state represents an adaptive mechanism which prevents onset of spermatogenesis in the event of a temporary increase in temperature during winter months. From the physiological point of view of reproduction this means that at the beginning of hibernation in snails there is a kind of refractory period comparable to that which exists in the reproductive cycle of vertebrates with photoperiod sensitivity [15]. This relative temporary insensitivity is without doubt triggered by information furnished by a decrease in the photoperiod and by unfavorable autumn environmental conditions. The factors giving rise to this decreased sensitivity of SM to temperature at the start of hibernation must come from the brain, because brain extirpation leads to a great increase in SM [16].

The purpose of this work is to show that hibernation in snails is a complex biological phenomenon during which synthesis of DNA is hardly modified by temperature in most organs (intestines, albumen gland), while in the ovotestis SM is controlled by ambient temperature which acts through the brain as an intermediary. These analyses support an increase in temperature sensitivity of SM during lengthened hibernation. This is translated by changes in the endogenous regulation of spermatogenesis during time (biological clock) in an environment where conditions are maintained constant (5 °C and permanent darkness).

The consequence of these results is important for the development of snail rearing methods because they show the need to use, as stock, animals having hibernated for 6–12 months to obtain abundant SM and rapid reproduction.

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Is glycogen a major energy source in avian gizzard smooth muscle contraction?

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Summary. In comparison to other avian smooth and striated muscles, chicken gizzard contains very low levels of glycogen. This myoglobin-rich muscle seems to derive the energy for its powerful contraction from other sources.
Key words. Birds; gizzard; smooth muscle glycogen.

Glycogen is a readily mobilized reservoir of glucose in muscle, providing energy for contraction. The breakdown of glycogen is initiated by the enzyme phosphorylase kinase. This enzyme has been most thoroughly studied in skeletal and cardiac muscle; much less information is available about its function in smooth muscle contraction [2, 3]. Sotiroudis et al. [4, 5] have recently purified phosphorylase kinase from chicken gizzard smooth muscle; they found a very low concentration of the enzyme, which also differed from the striated muscle isoform in subunit composition and regulatory properties. In addition, the role of glycogen as an energy source for smooth