CHAPTER 4

CONTROL OF REPRODUCTION

(i) ENDOCRINE CONTROL OF REPRODUCTION

(11) ENVIRONMENTAL CONTROL OF REPRODUCTION
INTRODUCTION

Like other stylommatophoran pulmonates, *Laevicaulis alta* has an ambuscual hermaphroditic gonad in which oocytes and spermatozoa are produced simultaneously in close proximity within each acinus. Although young slugs tend to develop oogonia slightly earlier than spermatogonia, the further development of spermatogonia to spermatozoa is very rapid. In stylommatophora, the existence of a tentacular hormone seems to be well demonstrated. Pelluet and Lane (1961) and Pelluet (1964) had shown that the gonads of the slugs showed ambuscual hermaphroditic condition. Moreover they showed shifts of sexuality and at any stage of development or at any given movement either spermatogenesis or oogenesis was predominant. According to these authors the tentacular hormone stimulated spermatogenesis in the slug *M. ilax*, on the other hand Gottfried et al. (1968) had shown that in *Agriolimax*, the tentacular hormone had an inhibitory influence on the functioning of gonad. Choquet (1965) had also reported similar findings in the limpet, *Patella vulgata*. Differentiation of the germ cells is under the influence of both internal (hormonal) and external (environmental) factors. Apart from the neurosecretory cells of the
central nervous system, the neurosecretory principle elaborated by optic tentacle has been implicated in the control of gametogenesis. In general, the opinion is divided among workers on the role of optic tentacular principle in promoting/inhibiting spermatogenesis in gastropods.

It was observed that in *Ariolimax californicus* (Gottfried *et al.* 1963), the tentacular hormone inhibited spermatogenesis, whereas in *Helix aspersa* (Kuhlman and Nolte, 1967) it was shown to be unaffected and in *Achatina fulica* (Berry and Chan, 1968), it had promoted spermatogenesis. According to Gottfried and Dorfman, (1968) this discrepancy in the physiological role of optic tentacular principle was due to species specific differences.

Removal of optic tentacle in *Arion subfuscus* resulted in an increase in number of oocytes in the regenerating gonad (Wattez and Durban, 1972 and Wattez, 1973). This situation seems to be somewhat different in the prosobranch, (Choquet, 1971), *Patella vulgata*. Here the spermatogenesis was inhibited by an inhibitory tentacular factor during the male phase. According to Wattez (1973, 1975 and 1978), the optic tentacular factor inhibited auto-differentiation of oocytes.
This finds some support from the work of Nagabhushanam and Kulkarni (1974), who showed that optic tentacle removal resulted in an increment in the number of eggs in the land slug, *Laevicaulis alte*. Takeda (1977) got similar results while working on the slugs, *Limax flavus* and *Deroceras reticulatus*.

Contrary to the above observations, Gottfried and Dorfman (1970 b) had proposed an inhibitory control of optic tentacular factor on spermatogenesis in the protandrous slug, *Agriolimax reticulatus*. The recent work by McCrone and Sokolove (1979) claimed that the optic tentacular principle was now no longer a hormonal principle, affecting either spermatogenesis or oogenesis in *Limax maximus*. At the same time, they proposed optic tentacles to be the organs of transducers or photoreceptors which regulated the reproductive activity. The role of optic tentacular principle in the maintenance of male germ cell line has been proposed (Sriramulu and Sukumaran, 1977) by histological and culture methods. These finds mere support in the sense that whether the optic tentacles have, if any, the neurosecretory or hormonal cells, is in dispute. Many aspects of reproduction such as gamete maturation, maturation of reproductive tract, oviposition and ovulation had been demonstrated
to be under hormonal control in pulmonates (Boer and Joosse, 1974). The maturation of male and female gametes were under the control of separate hormonal principle in the land slug, *Agriolimax reticulatus* (Runham et al. 1975). Organ culture studies on slugs, such as, *Ariolimax californicus* (Cottfried and Dorfman, 1970) and *Arion subfuscus* (Wattes, 1976, 1979), have proposed two antagonistic hormones originating from the optic tentacles and the brain. It was suggested that the optic tentacular hormone inhibited the autodifferentiation of female gametes or female genital cells, in these slugs. In *Agriolimax reticulatus* (Wijdenes and Runham, 1976) it has been shown to promote vitellogenesis. Recently Saleuddin et al. (1980) had shown that the dorsal body hormone induced vitellogenesis in *Helisoma dujardini* and *H. trivolvis*. Evidence for the endocrine control over gametogenesis has been given by Nagabhushanam and Hanumante, (1978) in various prosobranchs. Arch (1976) presented neurohormonal induction of egg laying behaviour in Opisthobranchia. A good deal of work for hormonal control over oocyte maturation and reproduction in pulmonates has been furnished by Boer and Joosse (1975).
There are indications that the gonads of pulmonates produce one or more hormones. Up till now, no secretory phenomenon have been described for any type of secretory cells present in the gonad. Bailey (1973) reported for the first time, the in vitro culture of the gonadal material of *Agricolimax reticulatus* and did not find any differentiation of the sex cells in an hormonal medium. But the close association of a cerebral ganglia and optic tentacle with gonadal material caused onset of spermatogenesis. The effects on the differentiation of female cells was less clear.

Since then a great number of investigations have been carried out on tentacle extirpations in various species of *Stylommatophora*. Sanchez and Sablier (1962) extirpated the optic tentacle of *Helix aspersa*, but could not confirm the earlier results of Pelluet and Lane (1961). Guyard (1971) while working on the same species observed no changes in the gametogenesis after optic tentacle extirpation. Kuhlman and Nolte (1967) and Renzoni (1969) obtained negative results with *Helix pomatia* and *Vaginulus borellianus* respectively.

From the above mentioned literature it is clear that the optic tentacle extirpation has presented contradictory results with respect to control of
gametogenesis. These contradictions can be explained on the grounds by considering not only different species were used but the experimental conditions also may not be uniform. Then if the optic tentacle are important for day light perception and for orientation of food, a significant indirect effect of the extirpation of optic tentacle on the reproductive activity cannot be excluded for any reason.

Various physiological processes are linked with metabolic processes for maintenance, growth, food, consumption etc. Reproductive cycles are said to be under the control of exogenous and endogenous factors. Most probably endogenous factors in synchrony with exogenous factors control the process of reproduction. Number of environmental factors like temperature, photoperiod, food availability are known to affect reproductive cycle.

Henderson and Pelluet (1960) studied the effect of visible light on the ovotestis of the slug, *Dermoceras reticulatus*. It was stated that photoperiod can affect the reproduction. McCrone and Sokolove (1979) while working on the slug *Limax maximus* had demonstrated that the male maturation was under the photoperiodic control and might be manifested through photoreceptors. In *Haliotis postulata* (Pearse, 1968).
suggested the relationship between the seasonal increase in temperature and gametogenesis, wherein the gametogenesis was restricted to summer. However, Webber and Giese (1969) did not support this hypothesis. According to them it was quite possible that temperature influence depends only upon geographical locations.

Generally sexual maturity is reached before maximum size and age. Since gametogonial activity starts well before final size is reached, there seems to be no correlation between reproduction and growth. The literature concerning the endocrine and environmental control of reproduction in gastropods is rapidly increasing during recent years. However, the role of environmental and endocrine factors in the control of reproduction, especially on the gametogenesis of L. alte is not known. Here an attempt has been made to study the role of optic tentacle in gametogenic activity of this slug. The chapter also gives an information regarding the probable environmental cues regulating the gametogonial activity of the slug.
MATERIAL AND METHODS

Fresh specimens of *L. alte* were collected from the cultivated fields in and around Aurangabad and maintained under laboratory conditions in moist soil. Healthy animals were selected for the experimental purpose.

(i) **Endocrine control of reproduction** :-

To ascertain the effect of optic tentacular principle on the gametogonic activity of the gonad of the slugs, the animals were divided into following groups :-

Group I  -  Control

Group II  Experimental - Tentacle ablated

II $A_1$  Immature, tentacle ablated for 7 days and 15 days.

$A_2$  Mature, tentacle ablated for 7 days and 15 days.

Group III  Experimental - Tentacle homogenate injected.

III $B_1$  Immature, tentacle homogenate injected for 7 days.

III $B_2$  Mature tentacle homogenate injected for 7 days.
(A) **Tentacle ablation** :-

The animals were allowed to crawl on a glass plate illuminated with light. When they have fully stretched their body with fully extended tentacles, the tentacles were excised with the help of a fine sterilized scissor. Antiseptic powder was applied to the wound and the animals were returned to their respective groups. The food was given once in a day and the soil was changed with a period of three days interval. At the end of experiment, the slugs were sacrificed and the gonads were fixed in Bouin's fluid. After routine histological procedure, they were observed under light microscope.

(B) **Tentacle homogenate preparation** :-

Optic tentacles from twenty to forty mature slugs were excised and homogenized in a chilled glass mortar in molluscan saline. The homogenate was centrifuged and supernatant was used as optic tentacle homogenate. The immature and mature slugs were received 0.1 ml of the homogenate containing '2' optic tentacles per slug. The injections were made in foot region. The slugs were kept for 7 days, after which they were dissected to fix gonadal tissue for microscopic examination.
(2) **Environmental control of reproduction**:–

The data collected over 2 years and which was utilized in "Chapter 1" was utilized here to determine the physical factors influencing the reproductive activity in the land slug, *L. alte*.

Laboratory experiments were performed to ascertain the effect of long day photoperiod, short day photoperiod and complete dark on the ovotestis of the slug. The data for humidity, rainfall and temperature was collected for a period of one year i.e. from March 1980 to February, 1981.

**Effect of photoperiod**:–

For the present experiment the field collected animals were provided light with the help of a overhang, 100 watts tungsten filament bulb for 18 to 20 hrs. They were kept under such a condition for a week (7 days). At the end of the experiments, the slugs were sacrificed and the gonads were fixed in Bouin's fluid and were processed histologically. Controls were provided 12:12 light and dark regimes.

**Tentacle ablated; exposed to long day photoperiodic treatment**:–

The optic tentacles of the slugs were removed and then they were subjected to long day photoperiodic
treatment. The experimental period ranged to 15 days. Gonads were fixed, histologically processed and paraffin sections were cut at 6 to 7 μ. Histological observations were made under light microscope.

**Tentacle ablated; exposed to short day photoperiod:**

The optic tentacletomized slugs were subjected to short duration of the photoperiod i.e. short day photoperiodic treatment is about 18 to 20 hours dark. The fixation and histological procession was made as usual.

**Effect of complete dark:**

The animals were kept in a complete dark. The food was provided once in a day. The gonads of the animals were excised at the end of 7 and 15 days.

**RESULTS**

(A) **Effects of tentacle ablation:**

In case of immature control slugs (Fig.1) the gonads showed gametogenial cells, supporting cells etc. There was no trace of differentiation of spermatogonia. However, in some follicles the oogonial proliferation or differentiation was slightly noticed.
Hormonal control of reproduction in L. alte

Fig. 1: Immature - control for tentacle ablated slugs, gonad follicle X 150.

Fig. 2: Mature - control for tentacle ablated slugs. Gonad follicle X 150.

GSS = Gonad stem cells,
SS = Supporting cells,
O = Oocyte (Proliferating),
SP = Spermatozoa.
In case of immature tentaclemised slugs, (Fig. 3) after 7 days, large number of darkly stained cells were observed, whose sexual nature was unknown. They might be the oogonial proliferating cells. Only one or two follicles showed primary oocyte and in some cases spermatogenic stages were noticed. All the features of the gonads were normal, there being no effect on spermatogenic process. 

In the immature tentaclemized slugs, after 15 days (Fig.4) the follicular membrane of the gonad was thick. Vitellogenic and maturing oocytes were noticed with their nucleus and nucleolus. Follicles were packed with gonad stem cells, supporting cells and sperm bundles. There was an enhancement in the oogonial activity but the spermatogenic activity and spermiogenesis also were not affected at all.

The control mature slugs showed well developed oocytes as well as sperms. The follicles were packed with all types of cells (Fig.2).

At the end of 7 days, the mature tentacle extirpated slugs showed 3 to 4 previtellogenic oocyte, mature oocytes some of which were in a releasing condition. The spermatogonia, spermatids and sperms were in normal course of development (Fig.5).
Hormonal control of reproduction in *L. alte*

**Fig. 3:** Immature - tentacle ablated (7 days)  
Gonad follicle X 150.

**Fig. 4:** Immature - tentacle ablated (15 days)  
Gonad follicle X 150.  
(Note: increase in the number of oocytes).

GSS = Gonad stem cells,  
SS = Supporting cells,  
O = Oocyte (Proliferating),  
VO = Vitellogenic oocyte,  
N = Nucleus,  
nu = Nucleolus  
UC = Cells of unknown nature,  
MO = Mature oocyte.
After 15 days, the tentacletomized mature slugs, the gonad showed thick follicular membrane and vitellogenic oocytes were noticed. In some oocytes vacuolisation was already started. The process of spermiation was not affected and sperm bundles were observed attached to sertoli cells at the peripheral region (Fig. 6).

Thus the results of the tentacle ablation in immature as well as mature slugs caused slight enhancement in the oogonial activity. However, the process of spermatogenesis and spermiation were normal and were not affected at any stage of development. The results were more pronounced in the immature slugs compared to mature ones.

(B) **Effect of tentacle homogenate injection (Fig. 7):**

In case of immature tentacle homogenate injected slugs, after 7 days, the gonad showed normal male and female gamete development. The follicles showed pre-vitellogenic oocytes and stages of spermatogenesis. Supporting cells were much more in number than the gonad stem cells. The follicular membrane was thick.

In mature tentacle homogenate injected slugs (Fig. 8) after 7 days, the gonad showed all stages of oogonia and spermatogonia. The development of both
Hormonal control of reproduction in *L. albo*.

Fig. 5: Mature - tentacletomized (7 days)  
Gonad follicle X 400.

Fig. 6: Mature - tentacletomized (15 days)  
Gonad follicle X 400.  
(Note: thick follicular membrane).

SS = Supporting cells.  
SP = Spermatozoa,  
VO = Vitellogenic oocyte,  
N = Nucleus,  
m = Nucleolus,  
ST = Spermatids,  
SC = Spermatocytes.
Hormonal control of reproduction in *L. albite*

(For immature control slide, Refer Fig.1).

**Fig. 7:** Immature, tentacle homogenate injected
Gonad follicle X 150.

(For mature control, refer Fig.2)

**Fig. 8:** Mature - tentacle homogenate injected.
Gonad follicle X 400.

CSS - Gonad stem cells,
SS - Supporting cells
O - Oocyte.
MO - Maturing oocyte
N - Nucleus
nu - Nucleolus
ST - Spermatids
SC - Sertoli cells.
the gametogenesis was normal. In one case release of ova can be noticed. Sperm bundles were recognized at peripheral border. At any stage of development neither oogonia nor the spermatogonia were affected. A core was formed between the follicular layer and centre of the follicle.

Thus tentacle homogenate injection could not brought about any significant change in the development of gonadal follicle. This may be because of the low dose (0.1 ml = \( \frac{20}{100} \)), the animal has received. Higher doses were not tested.

(ii) Effect of photoperiod on gametogenic activity in *L. alte* :-

In case of immature control slugs receiving 12:12 dark and light regimes the gonad has small follicle with various gametogenic cells of unknown nature (Ref. Fig. 1).

In the immature slugs receiving 20 hrs. of photoperiod or long day photoperiodic treatment, the gametogenic development was arrested. The epithelial border was disturbed and the follicular layer was thin and zigzag. The number of gonadal stem cells was much more and the follicles showed over stimulation of spermatogenic activity. The developmental processes were disturbed. (Fig. 9).
Environmental control of reproduction in *L. alba*

Fig. 9: Immature slugs exposed to long day photoperiod.
Gonad follicle X 400.
(Note: Overstimulation of spermatogenesis).

Fig. 10: Mature slugs exposed to long day photoperiod.
Gonad follicle X 150.

SP = Spermatozoa,
ST = Spermatids.
In case of mature slugs exposed to long day photoperiodic treatment, overstimulation of the spermiogenesis with large number of sperm bundles were noticed. The oocyte showed a vacuolization at early stage (Fig. 10).

(iii) Tentacle ablated; exposed to long day photoperiodic treatment:

Long day photoperiodic treatment in immature and mature tentacleotomized slugs induced male maturity. The histological observations of gonadal tissue revealed over stimulation of spermatogenesis following long day photoperiodic treatment against short day or control photoperiodic cycle (Fig. 11).

(iv) Tentacle ablated; exposed to short day photoperiod:

Tentacle ablated immature and mature slugs exposed to short day photoperiodic regimes significantly affected the spermiation process (Fig. 12).

However, there was no effect of long day or short day photoperiodic treatment on oogenesis. The spermatogenic process was enhanced by exposing slugs to long day photoperiod treatment where as short day photoperiod blocked the spermatogenic process.
Environmental control of reproduction in *L. alte*

**Fig. 11:** Immature tentaclemised slugs exposed to long day photoperiod. 
Gonad follicle X 150. 
(Note: the core formation and overstimulation of spermatogenesis).

**Fig. 12:** Immature tentaclemised slugs exposed to short day photoperiod. 
Gonad follicle X 150. 
(Inhibition of gametogenial activity).

SP - Spermatozoa,  
O - Oocyte,  
ST - Spermatids,  
SC - Sertoli cells,  
CSS - Gonad stem cells,  
C - Core formation.
(v) **Effect of continuous dark on gametogenesis in**

*L. alte*:

As compared to the control slugs, immature slugs, after 7 days, showed large number of gametogonial cells. The germinal tissue was affected and the follicular membrane was thin. Proliferation of the oogonial cells and supporting cells were noticed. The sperm stages were not predominant (Fig. 13).

At the end of 15 days experimental period, there was an arrest in spermatogenic activity. However, the follicular structure was disturbed and follicular membrane was zigzag. Predominance of oogonia was noticed but the gonad appearance was unhealthy (Fig. 14). In case of mature slugs after 7 days the gland possessed thin follicular membrane, zigzag in appearance. The oogonial proliferation attained a high degree than in the previous stage. The retardation of spermatogenesis was noticed. Many darkly stained basophilic oocytes were also observed (Fig. 15).

At the end of 15 days experimental period the gonad had no stages of development but there was a resorption of all the gonial cells. The oogonia and spermatogonia were in resorbing condition. The
Environmental control of reproduction in *L. alte*

**Fig. 13:** Immature slugs exposed to 24 hrs. dark (7 days)
Gonad follicle X 150.
(Note: stimulation of female gonial activity. Thin follicular membrane).

**Fig. 14:** Immature slugs exposed to 24 hrs.
dark (15 days).
Gonad follicle X 150.
(Note: Disturbed gametogonial prosses and thin follicular membrane).

O - Oocyte
SS - Supporting cells,
FM - Follicular membrane,
SP - Spermatozoa.
Environmental control of reproduction in L. alte

Fig. 15: Mature slugs exposed to 24 hrs.
dark (7 days).
Gonad follicle X 60
(Note: thin follicular membrane,
Zigzag in appearance).

Fig. 16: Mature slugs exposed to 24 hrs.
dark (15 days).
Gonad follicle X 150.
(Note: the resorbed or degenerated
follicle. Thick follicular
membrane).

O - Oocyte,
VO - Oocyte (Vitelligenic),
RS - Resorptive cellular structure
FM - Follicular membrane.
oocyte and spermatozoa were in retarding or disintegration phase. The gonad showed an appearance of a senile animal (Fig. 16).

Thus long-day photoperiodic treatment induced male maturity in immature slugs. The histological observations of the gonadal tissue revealed an over stimulation of spermatogenesis. The results were pronounced in immature slugs than mature ones. The tentacle ablated slugs exposed to long day photoperiodic treatment showed enhancement in spermatogenic activity but short day photoperiod however blocked the spermatogenesis.

Continuous dark firstly brought about a stimulation in female gonial cells but afterwards there was a resorption of gonadal tissue and early stages of maturation of the male germ cells were affected considerably.

**DISCUSSION**

It is only in recent years that some elegant studies have been appeared on various aspects of endocrinology of pulmonates, such as endocrine control, over vitellogenesis (Geraerts and Joosse, 1976) and endocrine control of maturation of reproductive tract (Boer and Joosse, 1975).
The first report about the endocrine control of gametogenesis in pulmonates was published by Pelluet and Lane (1964), in terrestrial slugs. They have demonstrated a balance of secretions from the tentacles and brain which controlled the state of gonad. Berry and Chan (1968) found that, in Achatina fulica, number of oocytes and production of egg capsules were increased after tentacle extirpation. In Laevicaulis, as reported by Nagabhushanam and Kulkarni (1971) and in the present investigation, there was an enhancement in the oogonial proliferation after optic tentacle extirpation. However, optic tentacle ablation and its subsequent injections could not have any effect on the spermatogenic process.

Gottfried and Dorfman (1970 a) found an increase in spermatogenic activity in Agriolimax californicus, after removal of optic tentacles. It has been demonstrated for number of slugs that optic tentacles exert an inhibitory influence on the differentiation and maturation of the female cell line. In the stylommatophoran slug, like Arion ater, A. subfuscus and Limax sp. optic tentacular factor exerted an influence on the maturation and differentiation of female gametes (Pelluet and Lane, 1961; Pelluet, 1964; Wattez, 1973; 1975; 1978; 1979). Gottfried and Dorfman
(1970 a,b) found precocious development even within 1-3 weeks in immature snails from which optic tentacles have been removed. Therefore, it was suggested by them that the principle elaborated by neural tissue of optic tentacle exercised a controlling influence on the male phase of the ovotestis. Péllet and Lane (1961) suggested spermatogenic progress by optic tentacle extirpation and decrease in gonad weight but had not mentioned the fate of spermatogenic activity. Kuhlman and Nolte (1967) in Helix pomatia could not find any effect on spermatogenic process activity following 'OT' removal. Renzoni (1964) while working on Vaginulus borellianus, also got negative results for spermatogenic process. Indicative presence of neurohormonal control over egg laying behaviour has been noticed in the land slugs, Deroceras reticulatus and Limax flavus (Takeda, 1977). The culture techniques employed for the differentiation of the sex cells have demonstrated that the addition of the optic tentacles to the culture medium with gonadal material suppressed autodifferentiation of female cells (Guyard and Gomot, 1964). The first in vitro culture results by Bailey (1973) reported that in an un hormon al medium, differentiation of the sex cells could not occur. However, addition or close association of the cerebral ganglia and optic tentacle
with gonadal material caused onset of spermatogenic activity. The effects on the differentiation of female cells were not so clear.

In the land slug, *Laevicaulis atra*, also tentacle extirpation lead to the enhancement of oocyte production. However, the spermatogenic process was not affected. Injections of the optic tentacle also could not exert any summative or retroactive effect on gonadal cells. As reported by Sukumaran and Sriramulu (1977) that the injection of 'OT' caused overperispermation and disturbance in spermatogenic process, our results did not corroborate with them.

One thing should be 'always' kept in mind that the results cited in different slugs are all protandrous species where male maturity occurs before the female one. Also these authors did not indicate any experimental period (i.e. whether the experiment was done in male phase or female phase). The land slug, *Laevicaulis* is a simultaneous hermaphrodite, having no xp protandric period or so. Then the results obtained here and the discrepancies in the role of optic tentacle should be considered due to species specific differences (Gottfried and Dorfman, 1970 a) and may be the results of rather unphysiological conditions.
From the available literature up to date, at this moment no cell type from the optic tentacle should be considered as 'hormone source', since on the other hand the experimental results are in favour of a production of a hormone source by optic tentacles, a reinvestigation of the cellular structure of optic tentacle with new staining techniques and electron microscopy is feasible and in such cases attention should be paid to the tentacular ganglion itself as the ultrastructure of the neurons has hardly been investigated (Guyard, 1971).

The vaginulidae slugs are shown to have no neurosecretory cells in their optic tentacles. Then from functional point of view also the cellular structure of the optic tentacle should be tested (Guyard, 1971).

Thus, there appears to be two hormones, one active at male phase and another during the female phase or it is quite possible that the gonadotropins of cerebral ganglia act via the gonad on the sex organs in L. alte. Such a possibility of existence of two hormones, one stimulating the growth of the male reproductive tract and another one controlling female growth and functioning of gonad and reproductive tract is gleaned out in Agriolimax reticulatus.
(Runham and Hunter, 1970) and Runham et al. (1973). McCrone and Sokolove (1979) with in vitro studies in mature gonads of A. reticulatus, failed to induce maturation of the juvenile tract. However, the presence of a brain stimulating factor for maturation of gonads have been worked out, Wijdenes and Runham (1976) observed that the stylommatophoran slug, A. reticulatus secreted a hormone which controlled the development of female accessory organs.

There are indications from experimental work that gonad of the pulmonates produces one or more hormones, up till now no definite secretory phenomenon have been described in any of the cell types of the ovotestis. Though it is not known whether the trophic hormones of the brain act directly or via gonadal hormones, the latter possibility at least for the present investigation supports the view that the pulmonate gonad produces two hormones which control the male and female cell line of the reproductive tract. (Boer and Joosse, 1975, Joosse, 1976).

In Laevicaulis, it has been reported that the optic tentacle extirpation and injections of central nervous system lead to an increase in the number of egg capsules (Nagabhushanam and Kulkarni, 1971). Taking into account the latter one, it can be postulated,
though too premature to conclude here, that ablating the optic tentacle resulted in secreting a trophic hormone from the CNS and consequently resulted in an increase in the number of oocytes in the gonad. The present probe shows that optic tentacle extirpation and its subsequent injection could not able to exert any effect on the spermatogenic activity of the slug. These results corroborates with those of Kuhlman and Notle (1967) in Helix pomatia and Renzoni (1964) in Vaginulus borellianus. Moreover, if the optic tentacles are important for day light perception, the significant indirect effect of the extirpation of optic tentacles on the reproductive activity obviously cannot be excluded.

Evidences also suggest that the hormones involved with growth and differentiation of the reproductive tract in pulmonate gastropods originate from the gonad. Apparently the source being the dorsal bodies and neurosecretory cells of the cerebral ganglia (Geesaerts and Algera, 1972). The hormones may influence the reproductive tract via gonad. The recent work of McCrone and Sokolove (1979) have entirely ruled out the endocrine status of the optic tentacle at least in the species, they have studied, Limax maximus.
Studies on the reproductive physiology of the land slug, *L. alte* revealed that it breeds during summer (the maturation and growth). Spawning occurs at the onset of monsoon. The temperature increases steadily from March onwards up to June in Marathwada Region. Thus there occurs an increase in photoperiod or day length. This suggests that control of gametogenesis in *L. alte* occurs through different or multiple environmental factors.

Histological observations of gonadal follicles of different months revealed that oogonial proliferation occurs through late winter phase. The gonad in the month of January showed some proliferating oogonia, stem cells and supporting cells but there was no trace of early or late spermatogenic stages. Increased day length can be correlated with increased photoperiod. Nearly March gonad revealed precocious development of male gametes/gonial cells. The spermatogenic process was also enhanced. Thus rapid gonadal growth occurred through summer season. Then the experimental results obtained also coincides very well to the reproductive cycle of the slug. Long day photoperiodic treatment and OT ablated and exposed to long day photoperiod, induced male maturity and over spermiation in the slug. However, short day
photoperiod treatment inhibited spermatogenic stages. Thus the role of photoperiod in the regulation of spermatogenic activity as a probable environmental factor can not be denied for any reason. The various reproductive organ indices also showed gradual increase in their indices from March onwards. (Chapter I)

Active period of animal does not mean necessarily vitellogenesis, spawning, breeding or egg laying. The animal remains apparently mature upto 2-3 months. In abalone, *Halocynthia postulata* (Pearse, 1968) it has been suggested that there is a relationship between seasonal increase in temperature and gametogenesis. The later being restricted to summer. *Laevicaulis* also stands good for the same. Though the animal inhabits moist places, it has to face various drastic environmental conditions. The spermatogenic process is very rapid and completed within pre-reproductive period. As compared to this oogenesis is a slow process. The prevailing conditions of monsoon initiates spawning. Characteristically reproduction and egg laying occurs at a specific time of the year but abnormal environmental conditions can alter the time of reproduction. *Laevicaulis* is thus a restricted breeder, it breeds from March to October in a restricted part of the year.
Segal (1960) has reported that the annual reproductive cycle appears to persist in absence of any external cues and therefore a photoperiodic control mechanism is unnecessary. In *Laevicaulis*, long day photoperiodic treatment induced male maturity i.e. over-spermiation. Generally one assumes that a nocturnal animal like slug, would produce normal cell organization with no light at all, but our results suggest that complete darkness has resorptive effect on gonad. This again explains the role of photoperiod in controlling the reproductive activity of *L. albo*. These results are in agreement with those of the land slug, *Limax flavus* (McCrone and Sokolove, 1979) where the male maturation was found to be under photoperiodic control. The role of photoperiod on female part is not clear. Probably it may have some role in controlling the female reproductive activity also. The egg laying period of the slug is marked by rainfall, humidity, low temperature, sample food etc. This also supports the view that reproductive activity in the slug is governed by multiple environmental factors.
It is still not known how environmental i.e. external and internal or intrinsic factors synchronize the secretions of hormones concerned and so swing the gonad towards maleness or femaleness.
SUMMARY

The endocrine control of reproduction and environmental cues regulating the reproductive activities have been warranted.

The endocrine function of optic tentacle has been discussed. It was found that they were important organs for daylight perception. Cutting of the 'OT' resulted in the increment of the oocytes. The process of spermatogenesis was not affected at any stage of development, by extirpation of 'OT' and its subsequent injections. The release of a gonadotrophic hormone has been suggested.

The photoperiod is an important factor in the regulation of the reproductive activity. Long day photoperiodic treatment induced male activity while short day photoperiod blocked spermatogenesis. Complete dark had resorptive effect.

These results coincides very well with the reproductive plan of Laevicaulis.
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