REPRODUCTIVE BIOLOGY OF BENSONIES JACQUEMONTI (MARTENS 1869)

BY

REHANA PARVEEN AURANGZEB

M. Sc, M. Phil (Pesh)

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بسم اللہ الرحمن الرحیم

All praises to Allah, who Guides Us in Darkness and Helps us in Difficulties

AND

All Respects to His Holy Prophet (Peace Be Upon Him). Who Enables Us to Recognise our Creator.

Saif Printing Press Nothia Peshawar.

DEDICATED TO

MY SELF
whose will power, only, has enabled this work a success

MY HUSBAND
for his encouragement and devotion to the family

MY CHILDREN
for their generous cooperation to complete this study

THESIS OF MRS. REHANA PARVEEN AURANGZEB
IS APPROVED

1. RESEARCH SUPERVISOR 

2. EXTERNAL EXAMINER 

DATED_____199

ABSTRACT

Bensonies jacquemonti is a medium sized terrestrial pulmonate snail which is commonly available at the campus of University of Peshawar, N.W.F.P., Pakistan.

A detailed study of the reproductive activity, anatomy, histology and function of the different parts of the reproductive tract is worked out. There are two breeding seasons and theseare related to seasonal rainfall, temperature and humidity. The genital organs retain the basic pattern of stylommatophores. Different portions of reproductive tract assist in different processes of reproduction e.g. male tract helps in sperm movement in two ways, one, upward movement of home sperms to reach penial complex and second; downward movement of foreign sperms from bursa to reach fertilization pocket. Penial complex forms the spermatophore. The female tract secretes different nutritive and protective coverings for the egg. The dart stimulates the excitement for the copulation.

Formation of spermatophore, role of bursa copulatrix, and mating behavior are observed. Premating behavior is very intricate and follows a complex courtship. Eggs are laid in egg chamber dug inside the soil.

Developmental embryology from oviposition to hatching takes place 15-21 days, depending upon the temperature. In

cold weather the incubation period is 18-21 while in summer it is 14-15 days. Post embryonic growth till maturity have been worked out.

The effect of neurosecretory hormones on reproduction is demonstrated; it shows relation with cell differentiation. Optic tentacular hormone assists male sex cells while ablation of optic tentacles promotes oogenesis.

During unfavourable condition they undergo dormancy, the process of under going dormancy and re-activation from dormancy has been observed to be the direct effect of the external factors.

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CLAIM OF ORIGINALLY AND CONTRIBUTION TO KNOWLEDGE

The study under-taken is the first of its kind on the reproductive biology of Bensonies jacquemonti (Martens 1869) and effect of neurosecretory hormones on reproduction, dormancy and seasonal rhythm.

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CHAPTER - I

INTRODUCTION

Molluscs includes numerous species only a step behind in this respect to the Arthropoda. Of the estimated 100,000 species, gastropods alone includes over 80,000 different species. Recently discovered species from some obscure habitats is a challenge for scientist for finding others. Pulmonates form half of the species of gastropoda because of its rich adaptability. They have invaded diverse habitats like, marine, fresh water and terrestrial.

Man has always been attracted by gastropods as they are easily collected and form an easy supply of food and decorative material. Because of their abundance, number, their easy collection, availability and successful captivity in laboratory, they are used extensively for experimental purposes. Pulmonates are easily reared in the laboratory, some aquatic snails have short life cycle, therefore it is easy to keep these forms for many successive generations under observation as laboratory research animal.

In Pakistan little attention has been given to faunistic studies and particularly to the snails, Zoology Department, University of Peshawar initiated a study on Morphology and Ecology of Bensonies jacquemonti a terrestrial snail. This work is a continuation of the previous research in the form of reproductive biology, reproduction, effect of endocrine system on reproduction and the phenomenon of dormancy. Observations have been recorded from laboratory reared snails

as well as from field in their natural environment. Neuro-secretory cells originally demonstrated in insects have been demonstrated in many groups of invertebrates including molluscs in many parts of the world. But, in Pakistan, for the first time the presence of neurosecretory cells in terrestrial snail Bensonies jacquemonti is observed. The probable role of neurosecretory cells on the reproduction of snail is worked out.

Dormancy being an out standing phenomenon to overcome the extreme conditions of weather, the factors responsible for establishing dormancy are also studied.

CHAPTER - 2

REVIEW OF LITERATURE

A critical review of literature shows that literature on molluscs and particularly on gastropods is extensive. Only the relevant literature on reproduction, reproductive biology, neuro-secretion and dormancy related with present work is summarised here.

The high degree of organization of pulmonate molluscs is reflected in their complex reproductive system.

The reproductive system of the pulmonate has attracted many workers who studied the system from different aspects and different factors effecting the working of the system.

Much work is done on the reproduction of the fresh water snail Lymnaea stagnalis (a basommatophoran pulmonate) because of its short life cycle, and terrestrial slug Arion (a stylommaphoran pulmonate) because of its easy availability.

Crabb (1927) showed that the reproductive systems of Lymnaea stagnalis and Lymnaea stagnalis appressa say had similar anatomy. In both normal mode of reproduction was self fertilization and not cross fertilization. He suggested that in the process of fertilization foreign sperms had less or no chance to compete successfully with the sperms that had developed and matured alongwith ovum in the same acinus of ovotestis.

Hoff (1939) studied the anatomy of reproductive system of Ferrissia tarda (ancylid snail). He concluded

that like other aquatic pulmonate snail, the reproductive system was hermaphrodite, with a single gland, ovotestis, leading into hermaphrodite duct. He made numerous corrections in the description of various sex organs i.e. a single seminal vesicle and the number of follicles in ovotestis were much less than formerly described, the most important correction was, that the flagellum duct enter the free end of the pr-eputium and not the penis sheath.

Holm (1946) gave the histological picture of the reproductive system of the pond snail Lymnaea stagnalis say. He also worked out the functioning of the reproductive tract and reported that his finding agrees with that outlined by Baudelot (1863) and Crabb (1927).

Vander Mohr (1949) published his notes on Achatina fulica that had two distinct breeding periods and self fertilization was observed repeatedly. Incubation period was 1-10 days. Very short incubation period, perhaps indicated a tendency towards ovoviviparity. Fertility of eggs were more and so was the hatching rate.

Abdel-Malek (1954 a,b) in two separate studies described the gross morphology of the genital organs of Helisoma trivolvis (sub-family Helisomatinae) and Biomphalaria boissyi (sub-family Planorbinae). He stated that morphological studies showed that the reproductive organs were similar but there were some significant histological differences in both snails. In both self fertilization and cross fertilization

occured. They observed the sperms in the acini of the ovotestis at all times of the year even during hibernation and aestivation.

Duncan (1958) in a study gave a description of the structure and function of the reproductive system of the same snail Physa fontinalis. The reproductive system was based on typical basommatophoran plan. The system was fully developed in the breeding season only. In winter male organs remain mature while female organs were small in size. Sperms passed by the penis into the copulatory partner. Duncan suggested that P. fontinalis was largely self fertilizing, with cross fertilization occurring less frequently and only when there was some physiological barrier to the snail's own sperm. After fertilization the ova received its different covering as it passed down the oviduct to form the egg capsule.

Duncan (1959), described the annual cycle, copulation and oviposition of fresh water snail Physa fontinalis. He concluded that temperature was the most important factor that governed the annual cycle. Copulation was induced readily by a sudden congregation of the snails. It appeared that any environmental variation such as a rise in temperature or change of water, was sufficient to initiate oviposition.

Duncan (1960) traced out the evolution of the pulmonate genital system and stated that in both Ellobiidae and Chilinidae lie close to the base of the pulmonate stock and

the Stylommatophora and the Hygrophilla appeared to have originated from their allied forms of these families.

In a later study Duncan (1975) worked out the full details of the reproduction in pulmonates. His studies were based on general organization, functional anatomy and physiology of the genital system, egg capsule, reproductive cycle, copulation and oviposition.

Ghose (1960-63) studied the reproductive tract of Achatina fulica. He gave a detail histological description of the tract and function of the different portions. He observed gamate formation, fertilization, oviposition, embryogenesis and larval organs of Achatina fulica.

Berry (1963-65) studied reproduction and breeding in different snails found on Malayan lime stone-hill. These snails were Gyliotrachela depressis pira (helicid); Opisthostoma reptrovertens (cyclophrod) and Hydrocena monterosatiana (archaeogastropod). There were slight differences from the common pattern but not significant enough. In all types the rainfall had a direct effect on the reproductive cycle.

Kugler (1967) described the morphological and histochemical study of the reproductive tract of the slug Philomycus carodinianus. He stated that glycogen and alkaline phosphate were in close association with each other in the epithelial lining of the whole reproductive tract.

Meenakshi and Scheer (1968) gave their classical observations on the carbohydrates of the slug Ariolimax

columbianis and its distributions in the reproductive system. They summerized that Glucose formed 84% and was the major component of carbohydrate. Beside glucose no traces of fructose or galactose were found in the blood. The blood sugar level normally reflected carbohydrate intake i.e. when the animal were starved the level decreased and when animals were fed the blood sugar level increased. Glucose and galactose were stored in albumen gland. Mucopolysaccharide were present in the oothecal and muciparous glands.

Fantin et al (1968) studied the histochemistry of the glands associated with reproductive tract of Lymnaea stagnalis. They observed galactogen, and acid polysaccharide in albumen gland; mucopolysaccharide in muciparous gland; and acid mucopolysaccharide in upper zone and mucopoly-saccharide, sulphated mucopolysaccharide, protein and glycogen in the lower zone of the oothecal gland. They further observed that the composition of polysaccharide changed from gland to gland as the secretion of the gland received acid in accordance with the structure of different enveloping layers of the egg.

Clampitt (1970) gave a comparison of two closely related species of fresh water pulmonate snails Physa gyrinasay and P. physintegra. He concluded that apart from differences in size, there appeared to be no morphological differences between the two species. Food of both species was varied; so it did not affect either species limiting for other.

Lambert and Dehnel (1974) worked out the seasonal variations in biochemical composition during the reproductive cycle in Thais lamellusa gmelin (Gastropoda, Prosobranchia). They analysed the digestive gland, foot muscle and gonad for protein, glycogen, and lipid, and found that digestive gland was mainly formed of lipid but the maximum glycogen level coincided with major feeding period. The lipid reserve were for use during winter when glycogen level was low due to its consumption by developing gonad. During winter the digestive gland index decreased and the ovary index remained constant and few oogonia reached maturity. They pointed out that starved animals used more protein from ovary than the fed one.

Manganelli, et al (1989) described two land snails Helix parlatoris and Helix reinael (Pulmonata; Hygromiidae) these were small helicoid species living in Sicily (Italy) and resemble other helicoid species in morphology but anatomical research confirmed the validity of the two as distinct species and also new genus. They differed from other well known Hygromiidae e.g. Xeromicra and Microxeromagna their genital duct were with well-developed dart complex. In these snails vagina was short without any trace of dart sac complex and digitiform gland. The penis and epiphallus was equal in length, while flagellum was variable. They were without Hygromiid like penial papilla, right ommatophore retractor, passed between penis and vagina.

Giusti and Manganelli (1990) described Ciliellopsis oglasae a new Hygromiid having very much reduced vagina, without dart complex or digit form gland, the penis much longer than epiphallus. Penis covered by a thin muscular sheath, penial flagellum was very short. A very reduced papilla was present. Right ommatophore retractor passed between penis and vagina.

Formation of sperm, egg, their fertilization and then safe deposition of eggs is a necessity for the development of terrestrial and fresh water pulmonate snails like other oviparous forms. In some forms the eggs are even retained inside uterus for safe incubation. To study these details many workers have maintained snails in their laboratory for many generation.

Gatenby (1919) had collected notes on various modern technique to find out the cytoplasmic inclusions of the germ cells in invertebrate. In his later study in 1920, Gatenby, summerized the detail of the oogenesis and spermatogenesis in Lymnaea stagnalis that agreed with that of other pulmonata. In the mature ovum the golgi rods had increased enormously as compared to young oocyte. In fully grown sperm, the head was very small as compared to very long mitochondrial tail.

Prashad (1925) worked out the anatomy of the common Indian apple snail, Pila globosa. He described histology of reproductive system and stated that rudimentary copulatory organ was similar in structure to the penis.

Bahl (1928) studied the reproductive process and development of Pila globosa with special reference to copulation and oviposition. Copulation occured in water or on ground near the edge of the pond, it took about 3-4 hours. Oviposition occured 2 or 3 days after copulation eggs were laid on ground. Apparently there seemed to be no incubation period as eggs hatched soon after the oviposition.

Hickman (1931) in an investigation of the spermiogenesis of Succinea ovalis revealed that the germ cells were differentiated from different germinal epithelium in the form of progerminative cells with large and rounded nuclei. Forty chromosomes were found in the spermatogonial division and twenty in the maturation division. At maturation divisions mitochondria were grouped into peculiar thread-like structure. Golgi apparatus was banana shaped rods closely surrounding idiosome. The mature sperm had a spiral structure both of head and tail region.

Noland and Carriker (1946) reared Lymnaea stagnalis for twenty generations in the laboratory and found the snails in good health and size as compared to their wild forms. In laboratory sexual maturity reached within three months in summer while in the winter it took upto four months. Egg production was doubled when snails were fed on boiled wheat cereal rather than on its normal diet of lettuce. Snails kept in group started egg laying earlier than in solitary culture.

Raven (1948) described the insemination in Lymnaea stagnalis and stated that sperms received at copulation were stored in the seminal vesicles of hermaphrodite duct, and that insemination occured in hermaphrodite duct. Oocyte had an irregular and elongated shape, poly spermy occured but the sperm which was most centrally located fertilized the egg and others dis-integrated.

Lilly (1953) gave a description of four distinct habitat from which Bithynia tentaculata a fresh water prosobranch gastropoda had been collected. He studied the biology, the structure and functioning of the reproductive duct.

Graham (1954) studied the reproductive tract of Janthina janthina and stated that in male phase many oligopyren and eupyren spermatozoa were formed. The last part of male genital duct was prostatic and with out copulatory organ. In female phase the ov um was fertilized within ovary which also contained developing eggs and young embryos. The last part of oviduct formed an albumen gland and mucus gland. The secretion of these glands was eaten by older embryos which wandered about within duct.

Cain (1956) studied cross and self fertilization in Lymnaea stagnalis and observed that cross fertilization greatly exceeded self fertilization if snail were allowed to cross copulate. The transferred sperm may remain viable in the body of the recipient snail for as long as 116 days.

Barrand (1957) described copulatory behaviour in Lymnaea stagnalis and observed different types of copulation. Copulation by two partners; mutiple copulation or chain two copulation in which four or five snails participated, the upper ones acting as male only, the intermediate ones as both male and female (female to snail above and male to snail below), the last one acted as female only; and lastly self copulation performed by a single snail inserting its penis into vagina.

Fried and Good-child (1963) worked on a small planorbid snail Menetus buchanensis (an experimental host for a spirochiid trematode). The adult had a life span of 5 months in laboratory and reached sexual maturity in 50 days and laid more eggs than wild snails. The laboratory record indicated rapid growth in first four weeks followed by less rapid for six weeks and reached maximum at twentieth week. Snails fed on boiled lettuce had transparent shell, while the wild snails had dark and opaque shell as of laboratory reared on maple leaves.

Duncan (1964) studied the penial complex of Lymnaea stagnalis and stated that isolated penis preparation showed rhythmic activity and this activity was not affected by presence of central nervous system. Duncan further revealed the fact that there was marked difference between the response shown by isolated penial complex preparation of Helix aspersa by Goddard (1962) and his findings.

Smith (1965) described the structure of the reproductive tract and histochemistry of the various glands associated with this system in Arion ater (a garden slug). He concluded that spermatophore was secreted by male flask cells and prostate gland.

Elwell and Ulmer (1971) studied Anguispira alternata, an intermediate host of the trematode Postharmostomum helicis, in lowa in nature and also reared in the laboratory for four years. They observed that sperms were not produced until greatest diameter was about 9 mm and did not oviposit uptil 13 mm. They copulated only once. Egg were deposited in soil at depth of 1.5 to 2.5 cm. Hatching occured after 28-32 days.

Lind (1973) while working on the genital tract of Helix pomatia suggested that spermatophore formation was necessary to ensure the function of the female system at copulation. He further stated that spermatophore formation started 40-50 seconds after start of copulation and sperm transfer from hermaphrodite duct initiated 5-10 second later.

Vander LAAN (1978) observed seasonality in breeding, copulation, oviposition, egg hatching and variation in reproductive rates of coastal population of the terrestrial snail Heliminthoglypta arrosa in North Central California. He suggested that breeding coincided with reactivation from long term aestivation and occured within 24 hours. In low temperature embryogenesis was prolonged.

Takaichi (1979) irradiated a pulmonate snail,

Buharda Hichonis with x-ray in early spermiogenesis, when the gonad contained mostly spermatids. Irradiation changed some spermatids while others showed affect on the various components. In the affected cell the nuclear envelop was missing, mitochondria and reticulum had abnormal appearance, and sperm flagellum had upto 4 axonemes within a single flagellar membrane.

Terakado (1981) described spermiogenesis as a complex phenomenon of cellular differentiation. It involved the structural changes in some specialized cells. His main study was on chromatin arrangement and axis formation in the spermiogenesis of a pulmonate snail physa acuta.

Bulman, (1990) observed the life cycle of Carychium tridentatum in laboratory and showed that morphology of the reproductive system was subjected to the seasonal changes. The animal collected in summer had copulatory organ and were called euphallic while those captured during remaining time of the year had lost penis and vasa deferentia and were called as aphalic. He was unable to observe copulation and suggested that self fertilization might have occured during the period when C. tridentalum was supposed to be aphallic. He found that in laboratory there was rapid growth, short life span and distinct reproductive period. The mechanism of oviposition, the effect of different factors on oviposition and to retain the eggs in uterus during aestivation by oviparous pulmonate snails has been described by many workers.

Bretschneider (1948) studied mechanism of oviposition in Lymnaea stagnalis. He observed that egg cell was surrounded by pervivetalline fluid which was enclosed within capsular membrane. Many egg capsules were joined together by a gelatinous substance to form the egg-mass. He further noted that the shape size of the egg mass was similar to the shape and size of the lower part of oviduct (the pars nidamentaria) of which it was cast off. The egg mass was highly species specific and could be used as a character in identification of the species. He confirmed that renewal of the aquarium water and addition of aquatic plant Hydrocharis acted as stimulus to oviposition.

Dewitt (1954) studied reproduction in Physa gyrina and stated that in nature the reproduction was directly related to temperature while in laboratory the seasonal periodicity of oviposition disappeared. The egg-laying started when the snail reached a certain morphological and physiological state. The percentage of hatching of eggs in field collected snails were greater as compared to laboratory isolated individual. There was no difference in total life span in laboratory reared individual and field population.

According to Vander Steen (1968) excessive amounts of food supplied to adult Lymaea stagnalis decreased oviposition without affecting egg production. This showed that the frequency of the endogenous rhythm in oviposition was liable to changes by non-rhythmical environmental agents.

Owiny (1974) stated that in equatorial land snail Limicolaria martensiana (Pulmonata) the breeding continued throughout the year but the peak breeding occured during the dry season and it was low during the rainy season. The evolution towards ovoviviparity enabled the snail to retain its egg and youngs in uterus during aestivation. This seemed to be the reason for peak breeding during dry season. Incubation period was 10-14 days in rainy season but in dry season eggs youngs only deposited when rain returned. Oogenesis was observed at all times.

Harris and Charleston (1977) demonstrated that under similar laboratory condition Lymnaea columella produced more eggs than L. tomentosa. Both oviposited at $5^{o}C$, and a single egg capsule of L. columella was produced at $2^{o}C$. The minimum temperature at which egg could develop and hatch was 5-10 ^{o}C. In both species at $5^{o}C$ eggs started development but failed to hatch. $30^{o}C$ was lethal for eggs of L. tomentosa while $34.5^{o}C$ was normal temperature for eggs of L. Columella.

Hodasi (1979) studied the life cycle of Achatina achatina under laboratory condition and observed that snail matured at age of 21 months and started oviposition in their second and third year. In fourth and fifth years the number of eggs deposited were few. Eggs were laid in chamber made by the snail. He observed that A. achatina with large shell laid large eggs, but the number of eggs laid had no direct relationship with the size of the shell (animals).

Incubation period ranged between 10-31 days. In the field the eggs even hatched after 1 or 2 days. Thus eggs were retained inside uterus for longer time, a sign showing a step toward evolution to oviviparity.

Ohtsuki and Takahashi (1982) reported that Euphaedusa tau (Bottger) was a hermaphrodite and ovoviviparous snail. They used growth of shell whorls as a parameter for growth of the snail. According to the growth of whorls number, the growth of snail was divided into eight stages, i.e. stage 3,4-8. As the snail was born with the shell of three whorls i.e. at stage 3. They observed spermiogenesis at stage 7 and oviduct with embryoes and young snails were found at stage 10.

In another study in the same year Ohtsuki and Takahashi (1982) studied the development of germ cells in Euphaedusa tau and stated that at stage 4 ovotestis was visible as a mass of germ cell with distinguishable small perivetellogenic oocytes. Full grown mature oocytes were seen only in stage 10. Spermiogenesis occured at stage 7,8 and at stage 9 ovotestis was full of mature sperms. After the discharge of sperms and oocytes the acini of ovotestis remained empty for some time and the spermiogenesis started again.

Siddiqi and Aurangzeb (1984) described the general anatomy and reproduction of land snail Bensonies jacquemonti. They observed accessory sex organ in the form of dart while muciparous gland was missing in this species. Seasonal

changes affect reproduction. The volume and colour of albumen gland and ovotestis changed during active and resting periods.

Aurangzeb and Siddiqi (1984) reported that in Bensonies jacquemonti there was no superfecial seperation of male and female system, but histological study revealed that they were of seperate entity. Seasonal histological changes were observed in gonad, albumen gland and nidamental gland.

Siddiqi and Ali (1988) worked out the morphology of the reproductive system of the land snail Monacha obstructa. The genital system was on the common stylommatophoran pattern, muciparous gland and dart gland in the form of accessory sex organs were present. During active breeding season the oviduct and albumen gland were well-developed and bursa was filled with red pigment.

Chaudhry (1988) isolated snail pheromones or growth modulating compounds from snail conditioned media and found them in the form of small molecules that inhibited oviposition in Bullinus tropicus (a fresh water snail). He suggested that these inhibitor factors accumulated in the holding water of closed system where these fresh water snails were cultured. He further found the inhibitor compound in water conditioned by Fossaria cubensis and Physa sp. and reported that the inhibitory factor might be destroyed by incubation with trypsin. He concluded that most probably inhibitor was a protein.

Bulman (1990) observed the life history of Carychium tridentatum (Pulmonata) in the laboratory. His data illustrated that eggs were laid one per day and 1-8 eggs were deposited; an individual in its life time laid 1-13 eggs, incubation period varied from 7-24 days, egg mortality was 65%. Depending upon season the snail reached sexual maturity in 37-129 days.

Aparicio et al (1991) described four pairs of dart sacs in the genital system of Helicopsis turcica. He further stated that Helicopsis Xeroleuca as a sub-genus of Helicopsis. According to the author it was difficult to decide whether the structure of dart sac complex is an advance stage of evolution or a primitive stage. Currently some authors have used this genital character to deduce phylogeny while still others are trying to interpret the evolution of the genitalia, using phylogenetic relationship.

Young and Evans (1992) described modern land molluscan communities on island of Flat Holm, South Glamorgan. These were disturbed by breeding gulls and human activity. Three main molluscan species prefered unstable habitats. There appeared to be little correlation between the communities and habitat factor. Thus different communities reflected the generally unstable nature of the island's environment.

Ali and Suleman (1992) described seasonal variation in abundance, reproduction, dormancy and activity of Monacha obstructa at Peshawar University Campus, N.W.F.P., Pakistan. Spring was the peak breeding season, while hot summer was

resting season. They undergo dormancy in groups. The breeding season was closely related to temperature, humidity and food.

The development of egg cells in different classes of pulmonates from oviposition till hatching, and the embryonic food and post embryonic growth has been worked out by many workers. The function of different cytophasmic inclusions, vitalline fluid, egg layers and the safe deposition and hatching of the eggs were also worked out. In all sub-classes of pulmonates at the time of hatching most of the larval organs have disappeared and the hatchings snail has resemblance with its adult externally.

Crabb (1929) described a method for rearing snails in the laboratory. His finding were that, boiled wheat grain and fresh lettuce as food gave best growth, over crowding retarded growth, introduction of Daphnia into the media retarded fouling in the stock culture, aeration promoted growth, direct sunlight did not seem to increase growth rate.

Ranjah (1942) studied the embryology of Pila glubosa as a typical gastropod. The cleavage of the Pila resembled other gastropods in which eggs had less yolk. The characteristic gastropods spiral cleavage was seen at 4-cell stage.

Raven (1945-75) contributed a lot in the field of embryology of the pulmonate snails. His investigation included the normal development of gametes (ovum and sperm), insemination, mechanism of oviposition, development of the fertilized egg uptil trochophore stage. He concluded that

the differences in the mode of development between a gastropod egg and a "regulation egg" like those of Amphibia, was much less pronounced as was previously assumed.

Fraser (1946) worked out the embryonic development of the reproductive tract of Lymnaea stagnalis appressa say in detail. The system arose from three primary enlagen. The mesodermal rudiment appeared on the 6th day and reproductive tract was completed on the 13th day. The basic pattern of the development of reproductive system resembled that of the dioecious prosobranch.

Greek (1950) described the anatomy and histology of reproductive tract, structure of the egg capsule and hatching of the egg in a terrestrial prosobranch pomatia cleguns. He stated that the drastic departure from the course of development from other prosobranch was found in the process of torsion. Torsion was the result of differential growth alone, as at that time muscles capable of contraction were not differentiated.

Dewitt (1954) observed reproduction, embryology and growth in the pond snail Physa gyrina say oviposition usually was abundant in the early hours of morning. A rise in temperature induced oviposition, while at low temperature the oviposition stoped. Freshly laid egg masses were transparent in appearance. Development was direct without a larval stage.

Ghose (1960-1962) gave a detailed account of cleavage, germ layer formation of Achatina fulica. He

observed that at gastrula stage, some of the ectoderm cells were enlarged, elongated and laiden with yolk to utilize the egg albumen as ovum had small amount of albumen. Cleavage was total and spiral, gastrulation occured by invagination.

Later observation of Ghose (1963) revealed that eye and stotocyst were ectodermal in origin, eyes appeared as invagination on dorso-lateral side of the snout while statocyst on either side of the junction of foot and visceral mass. Morphogenesis of mantle cavity and lung revealed that lung was an independent structure and mantle cavity was formed by an invagination near the lung rudiment. Pedal gland was also ectodermal in origin but developed as a pair of invagination which united afterwards to form a single median gland. Thus, pedal gland development in Achatina fulica was different from other gastropods.

Burch (1965) formulated a method for suitable medium to culture snail cells and tissue. He stressed on the importance of sterility, proper antibiotic mixture, addition of snail extract, temperature and pH, in culturing snail tissues. The gonadal tissue were maintained for 60 days. Active motile mature sperms were evident continually through the 60 days of observations.

Bayne (1966 and 1967) gave detailed composition of egg the layers and extracts of the reproductive glands of pulmonate slug Agriolimax reticulalus. Egg had three layers, the perivetelline sac forms the main bulk. Different

portions of female reproductive tract were involved in the formation of different egg layers.

Kapur and Gibson (1967) gave a histological account of the development of mantle edge and shell in Helisomaduri eudiscus. The mantle edge originated as peripheral thickening of the evaginated shell gland. The protoconch was secreted initially by the entire mantle edge but at later development its secretion was limited to the mantle edge-gland.

In a further study in (1968) Bayne plotted a desiccation curve of egg capsule of eight gastropod species. He concluded that differences in the desiccation rate coincided with size of the experimental eggs, the larger egg-masses had slow water loss as compared to the smaller ones.

Brisson (1968) stated that the embryonic development of Achatina marginata and Achatina fulica could be distinguished into two phases; Intra-genital phase upto the laying of the egg and the second phase starting from the laying of the egg to its hatching. The first phase was similar in two species but the second phase showed some diverged differences.

Sathananthan (1970) studied the structure, distribution, cytochemical nature and functional significance of the mitochondria during the embryogenesis in slug Arion ater rufus. Mitochondrial activity was indicated by its enlargement, increase in number and increase in its complexity. The main function of mitochonolria beside energy-link was a directive role in development.

Demian and Yousaf (1973) presented embryonic development and organogenesis in the aquatic gastropod Marisa cornuarietis (Mesegastropoda; Ampullariidae). The importance of this snail was in the biological control of schistosom-transmitting snails. Egg showed total spiral cleavage, torsion occured on 3½ days, through differential growth of two sides of the embryo. The development of different organs was completed except reproductive system which developed mainly after hatching. Their findings regarding origin and development of different organs such as mantle, mantle cavity heart, pericardium, kidney etc. diversed from the related Ampullariid snails.

Fironi (1977) reported that during development in pulmonate the albumen (Perivetelline fluid) resorption was through ectodermal cells and was stored in ectodermal albumen vacuoles through pinocytosis. The resorption occured in three phases i.e. during early development, at gastrulation and finally in later development stages. In this later development the peripheric albumen resorption, was stopped and it alone lead to the formation of the extensive albumen sac, thus endodermal resorption started.

Tompa (1979) reported that no pulmonate snail was demonstrated to be truely viviparous. Majority of them were oviparous and some showed tendancy towards ovoviviparity by retaining eggs and even young snails, after they had hatched from eggs, inside the uterus. In later study Tompa (1980)

described that Veronicella ameghini laid their egg in calcium rich soil or covered them with faecal material (calcium rich pellets). to provide calcium for the developing embryo.

Stephenson (1980) described the life history of Arion subfuscus. Slug were never seen in copulation, so no data on pre-oviposition was available. Eggs laid in soil incubation period was 25 - 38 days and 62% eggs were fertile. The mucus of young slug was clear and sticky while that of older was yellow. Longevity was 340 - 403 days. They died between 3-20 days after deposition of last batch of eggs.

Bigas (1981) demonstrated the appearance of polyvitellinity (in extreme cases upto 47 egg cells pro-entire eggs) and germ fusions (upto 6 germs) in Physa acuta during four years observations in laboratory. Both were independent of each other and occured in the last one third of fertility period and no germfusion reached the adult stage.

Fironi (1982) further discussed the peripheric resorption in five Basommatophora, ten Stylommaphora and a Prosobranchia. The conclusion drawn from this was that process of peripheric resorption was similar in these forms and permitted embryonic nutrition and growth from segmentation. This seemed contrary to the standard theory that the sole aim of segmentation was the multiplication of nuclei and the formation of blastomeres. The peripheric resorption required an intermediate transformation of endodermal cells, so that these might accumulate albumen. Fironi further added that the classic concept of "The protective ectoderm and nutritive endoderm" is to be changed.

Pekkarinen (1986) studied the egg cleavage and early development of the bivalve Nacoma balthica and found that early larvae of this species resembeled other bivalve larvae thus making it difficult to distinguish them. The larvae developed by chance in another experiment in the laboratory. Early cleavage resembled other bivalve types. Some larvae lived for one week and by that time had a small membranous larval shell. Intra ovarian egg cleavage was noted in some diseased (inflammatory response) female.

Siddiqi and Ali (1988) studied embryonic development of Monacha obstructa. The cleavage was total and spiral. At 56 hours stage velum in the form of ciliated of epithelial cells was observed. Gastrulation was completed on third day. Differential growth of the two sides caused by muscle contraction was very slow and resulted in a symmetry of embryo. Incubation period was 7-9 days. The development was direct without any valiger larva.

Naggs (1989) reported Guelella bicolor (Hutton) a carnivorous land pulmonate widely distributed in tropics and sub-tropical areas. This snail had attracted many naturalist firstly because it had the capability of actively expanding its population in a given area and secondly it fed on soft bodied invertebrates including juveniles of Achatina fulica which itself was a serious pest.

Phorson (1990) observed the development of juvenile shell of Trivia. He found dead shells of adult T. monacha and T. arctica were very common but the juvenile shells were rare in the field.

He disclosed that the adult and juvenile specimens of Trivia were very different with successive developmental stages, the small delicate shell, having small Slot aperture was transformed into adult shell. The great strength was added by vertically surface ridges and horizontally thick columella with a longer slot aperture.

Phorson (1991) observed juveniles and growth series of Leucophytia bidentata and Ovatella myosotis. As these two species were found together, it was essential to recognise their protoconch. In L. bidentata the smallest specimen had small chink for an umbilicus while in O. myosotis the bulb was visible on under side. Further distinctions between juveniles of the two species were from the two growth series. e.g. in L. bidentata, the apex was very small, looked comparatively blunt and whorls were flatter as compared to O. myosites.

The knowledge about the neuro-secretion in molluscus is limited as compared to other groups of invertebrates.

The nerve cells are regarded as "neuro-secretory" only when they are a part of endocrine system. A nerve cell can be part of the endocrine in a different way and evidence for its being a neuro secretory cell may be obtained by morphological and histo-chemical tests.

Lane (1961) revealed that Flamming as early as (1870-1872) pointed the existance of two cell types of secretory nature in optic tentacle of Helix aspersa.

In Stylommatophora the differentiation of sex cells or gametogenesis is under endocrine control. Many investigators had focussed their studies on the site of production of the gonadotrophic hormones and found that optic tentacles and cerebral ganglia (brain) were involved in secreting these hormones as quoted below.

Pulluet and Lane (1961) had suggested relation between neurosecretion and cell differentiation in the ovotestis while working on two slugs Arion ater and Arion subfuscus. They stated dual hormonal control, first the tentacular hormone produce male sex cells, this was then followed by egg production assisted by brain hormone. When the ocular tentacles of slugs were removed they produced more eggs as compared with the control ones. The injection of brain and tentacular homogenate were given to control snails, they showed that brain homogenate accelerated egg production and that of optic tentacle suppressed oogenesis and promoted sperm production.

Lane (1962) observed neurosecretory cells in the optic tentacles of certain stylommatophoran pulmonates. She distinguished three type of cells; 'collar cells' surrounding digitate ganglion and eye,'lateral oval' and 'lateral processed' cells lie laterally in the tentacle i.e. on the inner side of the dermomuscular sheath. All these cells appeared to

form an active neurosecretory area in the tentacles. These three types of cell were found only in order Stylommatophora of Pulmonata. She examined the head region and tentacles of Helix aspersa in detail. Beside this other animals used were Agriolimax, Limax, Arion, Cepaea, Oxychilus and Milax. She also studied other gastropods including Archidoris and Creilada (Opisthobranchs); Patella. Crepidula and Buccinum (Prosobranch); and Planorbis and Limnaea (Basommatophora).

All these three types of cells were absent in the Basommatophora as well as in other sub-classes of the gastropoda.

Lane (1963) in histochemical and electron microscopical investigation reported the presence of thiamine pyrophosphatase, acid phosphatase, and alkaline phosphatase in the neurones of Helix aspersa. All three phosphatases were found to be localized mainly in the cortices of bodies that have resemblence with 'blue' and 'yellow' lipid globules in their size, shape and distribution. Examination of neurons coloured with Sundan IV and then incubated for enzyme activity, showed a sudanophilic positive reaction for lipids.

Lane (1964 a) worked out the fine structure of the secretory cell in the optic tentacle of Helix aspersa. The cytoplasm of the collar cells that surrounded the tentacular ganglion contained spheroidal granules of low electron density and contained electron dense vesicles that were within the range of elementary neurosecretory granules. The lateral cells, that lie on the inner side of dermo-muscular sheath encasing

the optic tencles, had endoplasmie reticulum, with ribosome, mitochondria etc. in their cytoplasm. There were also electron dense inclusions which may be lysosome. The structural details of these cells showed their resemblance with some mucus-secreting cells.

Lane (1964 b) during a re-examination of the fine structure of the neurones of Helix aspersa, made an attempt to investigate the ultra structural localization of the elementary neurosecretory granules. She reported the presence of these granules in the cytoplasm of certain neurones. The granules appeared within lamellae of the golgi complex by budding and vesiculation.

Lane (1964 c) after using vital stain observed the secretory cells in the optic tentacle of Helix aspersa, no perinuclear bodies were present in collar and lateral oval cells as described previously. In addition the lateral-oval and lateral-processed cells had unicellular calcium glands, lying immediately under the epithelial covering.

In a later study the same author Lane (1964 d) using histo-chemical and electron microscopical investigation on semper's organ (a cephalic gland) compared it in two genera i.e. Limax and Helix. In both cases the organ lies on each side and under the buccal mass. The major difference was that in the Limax the organ was much larger.Semper's organ was composed of three cell-types. In the first type, the cytoplasm possessed lipochondria; in the cytoplasm of the second type of cell there were polysaccharide and lipid

while the cytoplasm of the third cell type contained mucopolysaccharide. In structure these cells showed resemblance with the secretory cells of the covering sheath of optic tentacle.

Inspite of their resemblance with the collar cells of optic tentacle, the cells of the semper's gland did not correspond structurally or cytochemically to any tentacular cell. Though these cells were stained with 'neurosecretory' dye at the light microscopical level, there was no significant evidence that elementary neurosecretory granules were present.

Menon (1966) demonstrated that in cerebral, parietal and visceral ganglia of Oncidium verraculatum, neurosecretory cells were present. During breeding period conspicuous neurosecretory activity was shown by neuronal cells of the cerebral ganglia.

Newman et al. (1968) described the location of large neurons in the ganglia of the brain of Helix aspersa. They further stated that at synaptic junction in the neuropil esterase was present while at these location on presynaptic position were present clear vesicles. The chromaffin reaction indicated that certain vesicle contain amines.

Sedden et al (1968) demonstrated the presence of dopamine and 5-hydroxytryptamine in the neurons of Helix aspersa. They used fluoresence microscopy. They stated that their observation agreed with the finding of Dahl et al. (1962).

Walker et al. (1968) stated that spontaneous activity of certain neuron may be blocked by the acetylcholin in the brain of Helix aspersa.

According to Boer et al. (1968) in the cerebral ganglion of Lymnaea stagnalis "Gomeri-positive" neurons i.e. caudo-dorsal cells occur. All these cells were true neuro secretory cells as they produce neuro hormones, specially at synaptic termination of axon.

Joosse et al. (1968) worked out the influence of Y-irradiation and hunger on gametogenesis and oviposition in Lymnaea stagnalis. By Y-irradiation oviposition stoped, and within one month the developing sex cells were degenerated. L. stagnalis withstand starvation for 6 weeks but oviposition stoped after two weeks although gametogenesis appeared to be continued but at a lower rate. They also revealed that in small hermaphrodite duct the sperms were stored as well as resorbed by gland cells of the duct. The mature oocyte were found only for a short period for ovulation after that they were absorbed by their nurse cell. Gametogenesis and oviposition was affected by radiation and starvation.

The effect of cutting off the optic tentacles on reproductive capacity has been observed by many authors. The results are summerized here.

Berry and Chan (1969) demonstrated that when ocular tentacles were removed in Achatina fulica it produced more shelled eggs than normal snail. The num of oocytes in

the gonad was also increased. They suggested that production of oocytes in gonad was under the hormonal control involving the tentacles.

In an experimental study Bierbauer (1978) showed that removal of optic tentacle of Helix pomatia at mid time of the winter hibernation and during natural revival in the spring resulted in stimulation of oogenesis within two weeks, there was a significant increase of first oocyte, second oocytes and ova as compared to the control. On the other hand the first and the second spermiocytes were decreased considerably over the control.

Kulkarni (1980) demonstrated that removal of the optic tentacle in land slug Laevicaulis alte had marked increase in albumen gland ratio in immature slug over mature as well as control one.

In a later study Kulkarni and Hazari (1983) worked out the effect of optic tentacle ablation on the growth of the reproductive tract in the land snail Cerastus moussonianus. In mature snail the reproductive tract index remained constant while in immature ones there was an increase specially after the optic tentacle ablation. They suggested that optic tentacle hormone controled the growth of reproductive tract.

Mortoja (1972) concluded that several type of neuro-secretory cells existed in Mollusca and their distinction could only be based on morphological or cyto-metrical criteria. He stated that Gastropoda possessed neuro-secretory cells as well as the neuro hemal system and

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endocrine glands. In addition to principal ganglia of nervous system, there are present a number of necessary ganglia i.e. tentacular ganglion and ospharadial ganglion. These had some-how connection with endocrine system.

Mortoja (los.cit) further pointed that neuro-secretory cells were found in cerebral, parietal and visceral ganglia. Among accessory ganglia, the tentacular ganglion of stylommatophora was given main attention as it contained certain cells which had the neuro- secretory nature.

Endean (1972) stated that inspite of the fact that Molluscan brain is simple, it resembles a vertebrate brain in basic structural and functional features. He studied two aspects of molluscan pharmacology i.e. one concerning chemical transmission within the nervous and neuro-muscular system and the other concerned with toxic material elaborated by molluscs. He found giant neurons in gastropods, and stated that 5-Hydroxy trypamine caused excitory action on neurons. The 5-HT established a synaptic transmitter in the central nervous system. In contrast to 5-HT, the Dopamine had a marked inhibitory effect on neurons as a synaptic transmitter. In molluscan biotoxins, the choline esters were prominent and active and found in hypobranchial gland, while tetramine was present in salivery gland of some species. Both of them inhibited the normal function.

Gainer (1972) demonstrated the effect of an experimentally induced diapause, which stimulated the natural conditions of hibernation and aestivation in land snail

Otala lactea. He stated that in 4 out of the 5 identified neurons examined in the isolated brain, of Otala lactea, one neurons, cell 11 had specific pattern of protein synthesis and electrophy-siological changes during diapause.

Parmentir (1973) identified giant neurons in the brain of Helix aspersa. Certain morphologically symmetrical neurons were found to be part of a functional network of cells, which received simultaneous input from at least one unidentified source, either directly or through a series of interneurons. The symmetrical neurons had firing pattern of endogenous rhythmic burst activity.

Bianchi (1973) examined the histochemistry of the neuro-secretions in the cerebral and sub-esophageal ganglia of Enchytraeus albidus (Enchytraeidae). The cerebral ganglia contain two types of neuro-secretory neuron; P cells and Q cells; while in subesophageal ganglion two neuro-secretory cells, the U cells were present, Q and U cells contained disulfide group and there was no indication of the presence of carbohydrate, lipid and tryptophan in these cells. The P cell secretion had proteinaceous character.

Barker and Gainer (1974) calculated that seasonal rhythm in the land snail Otala lactea was the result of a vasopressin and related peptides that induced bursting pace maker potential activity and altered the relations of the membrane in a specific neurosecretory cell. They suggested membrane regulatory role for these peptides, which might be indicative of a new form of information transfer in the

nervous system.

Ifshin et al (1975) suggested that the long term changes of a specific neuro-secretory cell in the land snail Otala lactea was caused by long term changes in the membrane properties of this cell. The regulatory hormone was found to be a peptide. The regulatory effects observed involved the induction or potentiation of bursting pacemaker potential (BPP) activity, resulting the excitation of the cell. They had isolated this peptide factor from molluscan ganglia, which might induced or enhanced BPP activity in a specific neuro-secretory cell in Otala lactea. The activation effect of this peptide factor is similar to certain neuro-hypophysial peptides of vertebrate nervous tissue.

Pengloh and Gainer (1975) had identified the low molecular weight specific proteins in the molluscan neurons, where they were synthesized and stored.

Barker et al (1975) studied the bursting pace maker potential activity in molluscan neurons. They applied vertebrate peptides and hormones to a number of identified neuro-secretory and non-neurosecretory cells in two molluscan preparation. These peptides induced regulatory changes in membrane properties unlike produced by conventional neuro-transmitters. The peptide stimulated cell terminals to release more neuro-secretory material while steroids caused a decrease of this materials. By the effect of vertebrate hormones on invertebrates it appeared possible that similar forms of inter cellular communication were present in the vertebrate nervous system and neuro-endocrine system.

Chase and Goodman (1977) found homologous neuro-secretory cell groups in the land snail Achatina fulica and the sea slug Aplysia californica. In Achatina these cells were located on right parietal ganglion and claimed homology with "rostral white cells" in the parieto-visceral ganglion of Aplysia.

Goudsmit (1978) explained that the brain of Helix pomatia when immersed in high potassium medium release a hormone from neuro-secretory cell terminals. This hormone stimulated galactogen synthesis in albumen gland. He regarded this stimulatory substance as a neuro-chemical messanger that acted over a period of several weeks and in specific season. Based upon its calcium dependent mode of release. He was unable to point out its ganglionic site of production, the route from the brain to albumen gland and how it affects the enzyme of the galactogen synthetic pathway.

Kononenko (1979) supported the conclusion of earlier workers about the existance of a factor modulating the endogenous electrical activity of the bursting neuron in the snail brain. This factor, as is accepted, was a peptide and had effect only on specific cell but no effect on electrical activity of other neurons.

Jong Brink et al (1979) investigated the histology of the reproductive tract of Bulinus truncatus in order to study the possible effect of castration upon the accessory sex glands. The female part contained 13

while male part contain 12 histochemically different secretory cells. The effect of castration was in the form of rapid growth of snail. The value (growth) of albumen gland and prostate gland were not arrested by castration. The results suggested that stimulating effect of the dorsal body hormone on the growth were not exerted in. B. truncatus, as were supposed for Lymnaea stagnalis.

Kit (1980) studied the neuro-endocrine cells in the caudo-dorsal (CDC) in the cerebral ganglia of Lymnaea stagnalis. These cells release an ovulation hormone at the periphery of the cerebral commissure. He further stated that CDC exhibited three states of excitability with distinct electrophysiological characteristics. CDC produced ovulation hormone and stated of excitability were associated with egg-laying cycle. According to it finding the active state started two hours before egg laying and there was release of hormone, it lasted for one hour. After this there was an inhibited state, without any activity egg laying occured and this state lasted about four hours after oviposition. The last resting state in which an after-discharge was evoked, thus the cells entered the active state again. The transition between these states occured spontaneously. These states were 'Resting State', 'Active State' 'Inhibited State'.

Geraerts and Mohamed (1980) demonstrated the role of the lateral lobes connected with cerebral ganglia and the ovotestis of Bulinus truncatus in the control of

body growth and reproduction. Extirpation and implantation showed that neuro secretion of lateral lobe (LL) decreased body growth and accelerated egg production. By removal of LL, body growth was increased and egg production was decreased.

Van-Minnen and Reichelt (1980) reported two droplet cells (DC) and one large canopy cell (CC) in the lateral lobe of cerebral ganglia in Lymnaea stagnalis. Photoperiodic stimuli control the neurosecretory activity of the CC but that of DC was not affected by the differences in photoperiod. Thus photoperiod dependent CC affect the endocrine control of reproduction.

Van-Minnen and De-Vries (1980a) demonstrated that ablation of one lateral lobe of the Lymnaea stagnalis resulted in significantly higher neurosecretory activities of the CC and DC in the remaining lobe.

Van-Minnen and Reichelt (1980b) in an other experiment showed the effect of photoperiod on neurosecretion of the lateral lobe specially of the canopy cells (CC). They reported that under long day (LD) condition CC were more active than under short day (SD) condition. No difference was in the DC activities. They further noted that on LD condition reproductive activities was high and growth rate was low while on SD reverse was observed.

Nagabhushanam et al (1981) studied the hormonal control of left pleuro-visceral ganglia (PV) on

egg laying in the marine pulmonate, Onchidium verruculatum. Their observations revealed that the removal of the left but not the right pleural ganglion blocked the egg-laying. Reimplantation of PV or injection of a PV homogenate restored egg-laying capacity in PV-less animal.

McCrone et al (1981) stated that reproductive maturation hormone in slug was effected by photoperiod. In short day light cycle the slugs remain immature and the transition from short day to long day photoperiod would have induced reproductive maturation in Limax maximus.

Bohlken and Joosse (1982) observed that at long day (LD) photoperiod, egg laying of fresh water snail Lymnaea stagnalis started 2 weeks earlier than animals kept at short day. The high ovipository activity of the LD animals was also associated with the proportional wet weight of female tract and its accessory sex organs.

Jong-Brink et al (1982) studied secretory activity in albumen gland of Lymnaea stagnalis and reported that the albumen gland produced the galactogen rich perivetalline fluid which envelops the oocyte. The neuroendocrine cells of the caudo-dorsal cells (CDC) secreted a hormone that in turn stimulated galactogen synthesis in the albumen gland.

Cobbs and Pinsker (1982) demonstrated the role of Bag cells of Aplasia brasiliana. According to his finding the bag cells spontaneously discharge a neurosecretory hormone and this in turn caused the release

of eggs. They further stated that overt behavioral component (rhythmic head movement) of normal egg deposition in A. brasiliana was preceded by some unknown event that initiated but it did not effect the movement of the egg through the reproductive duct.

Saleuddin et al (1983) reported effect of dorsal bodies on oocyte maturation in Helisoma and Helix. They stated that in virgin and non egg-laying Helisoma the DB are in-active. When virgin animals were allowed a single mating, egg mass production started. In single and multiple mating the, result of egg production was nearly equal. Cauterization of medio-dorsal bodies MBD in Helisoma resulted in an increase of oocytes but the ratio of immature to mature was higher. They stressed that in Helix also there was a cellular source (endocrine control) of the ovulation factor.

Notle (1983) investigated the dorsal bodies of stylommatophoran snails including Cernuella variabilis, Eobania vermiculata, Helix pomatia, Oxyloma elegans, Strophocheilus spec., Theba pisana, and Sphincterochila candidissima. He demonstrated that Dorsal body (DB) cells were most abundant in connective tissue sheath surrounding the inter-cerebral region on its dorso-frontal side. The place was called neurohaemal area and was supplied with neuropeptides by small nerve branches from cerebral commissure. Extirpation of DB in stylommatophoran snails resulted in decreased ovulation and oviposition.

Geraerts et al. (1983)proved that in Lymnaea stagnalis an ovulation hormone was secreted by the Caudo-dorsal cells (CDC). This hormone was stored and released at the periphery of the inter-cerebral. Electrically active CDC, pre-incubated with radio active amino acids, released at least four labelled compounds presumably peptides one of these is probably identical to Caudo-dorsal Cell Hormone (CDCH) and the role of the others in connection with egg laying was still unknown.

Dogterom et al (1983) demonstrated that a rapid in vivo bioassay of the ovulation hormone Caudo-dorsal Cell Hormone (CDCH) in Lymnaea stagnalis when established was found to be based on the dose-dependent relationship between the quality of injection of (CDCH) and the number of snail effected at the first stage process of egg-mass production. At low dose the oviposition took more than 30 minutes while at high dose it was less than 30 minutes (apprx. 20 min). The size of egg mass was not affected by the quantity of the dose. Other factors which influenced bioassay were refractory period, photoperiod, and daily variability of the assayed snail. Refractory period was refered to a period of 6-20 hours after oviposition during which the snail could not be induced to produce an egg mass.

Sokolove and Minen (1983) presented evidence that annual reproductive cycle was under photoperiodic mechanism in the Giant garden slug Limax maximum. They

stated that slugs when raised on short days remained immature indefinitely while transition of them to long days showed full reproductive maturation within 4 weeks. They demonstrated that photoperiodic stimulating secretion (hormone) was from cerebral ganglion near the cerebral commissure. This area called an "area Z" indicated highest activity.

Dogterom et al (1984) observed that Caudo 'dorsal' cells (CDC) in Lymnaea stagnalis produced an ovulation hormone (CDCH). After transfer of snail from 20 degree to low temperature, spontaneous ovi-position stopped. This was due to reduction of the CDCH. The reduction in the hormone was probably caused by a reduction in the activities of the endocrine dorsal (DB), which controlled vitellogenesis and the activities of the female accessory sex organs. After a change from 4-20 degree, egg mass production and spontancous oviposition restarted quickly thus suggesting a rapid increase in DB and CDC activities in the form of CDCH (ovulation hormone).

Bierbauer and Fazekas (1984) analyzed and tested the extract of albumen gland in Helix pomatia. The albumen gland extract contained a weak tris buffer and a high salt concentration, the weak tris buffer inhibited oocyte production but accelerated sperm production by 17.25%. The high concentration of the extract effected formation of oocyte (inhibited) and increased sperm by 3.73%.

They also suggested the possibility that the substance responsible for this biological effect on gametogenesis was not the product of albumen gland but the neuro-secretion of the cerebral and tentacular ganglion might have acted indirectly on the albumen gland.

It is a well known fact that snails react to extreme conditions of cold and hot weather by retreating inside the shell and undergo dormancy. The dormant state in winter is called as hibernation while of summer is refered as aestivation. The opening of the shell is close by a temporary lid formed by dried mucus. The vital processes are reduced and it is said that the content of carbon and uric acid are increased in lung and digestive gland.

This dormant state may be compared with that of some mammals which undergo dormancy in severe cold. This dormancy is the result of in sudden transformation from warm-blooded (homoiothermic) type to cold blooded (Poikil-othermic) type.

Formerly, it was believed that hibernation and daily sleep were strikingly similar but differ only in degree, while many still believed that they were entirely different things (Rasmussen, 1914).

Rasmussen (1914) stated that dormancy in certain mammals was caused by sudden fall of temperature, which made them sleepy and sluggish. He reported that since last one hundred years the survey of literature

showed awareness about hibernation and that there were much differences of opinion with regard to the factors and mechanism involved. Rasmussen quoted many authors stating different theories of hibernation. According to him Buffon (1849) and Lacepede (1829) were of the opinion that blood became cold by the surrounding cold weather and the cold blood in turn produced changes characteristic of dormancy.

Rasmussen (1914) quoted Saissy (1808) that certain anatomical pecularities caused torpidity. The external cold caused the surface blood diversion towards the centre of the body and thus heart and thoracic blood vessels were enlarged, which interfered with the respiration and thus heat production was decreased. The animal became cold and dormant.

Lawson (1925) noticed aestivation in H.hortensis. The previous survival period for this snail was six months. He recorded survival of H. hortensis without food for full eight month.

Hora (1925) discovered four species of snail aestivating in peculiar manner in western Ghats. The first type was Succinea arboricola and the snails were found aestivating in group on the bark of mango trees at Lonavia. Cremnoconchus syhadrensis aestivates in small pits that were protected from direct mid-day sun. Neritina perottetiana lived on rocks that were kept moist by spray from a fall. This species when removed from its natural

habitat died within few hours. Hora also found Lithotis rupicola and paludomus in hibernation on rocks, trees etc in the Ghats.

The dormancy and its effect, in relation to internal and external factors are studied by many investigators, some of which are summerized here.

Hora (1928) concluded that hibernation and aestivation had been independently acquired by various groups of snails and under different conditions. He quoted Prashad (year) that the origin of lung or lung sac for direct aerial respiration, was evolved in different classes at different time as a result of terrestrial habit.

According to Hora the state of dormancy seemed to be the direct outcome of the terrestrial habit.

Baldwin and Needham (1934) confirmed the presence of arginase and urease in Helix, discovered by previous worker. The uricotelic character of the metabolism of Helix pomatia was also confirmed. He was of the opinion that the snail hepatopancreas contained no precursor (found in vertebrates) which could convert the ammonia of protein into uric acid. He suggested that the formation of uric acid might have some different course. They concluded that molluscan hepatopancreas and vertebrate liver could be homologised. During dormancy concentration of urea and uric acid were found in hepatopancreas of snail (later finding).

Baldwin (1936) reported arginase a widely distributed enzyme in animal tissue. He called it a "urea-producing ferment". It was present in the liver of mammals, reptiles, frogs and several fishes but not in birds. The birds and gastropod molluscs resembled each other in the formation of urea and the production of uric acid followed quite distinct paths in the two groups. Baldwin disclosed that during starvation and aestivation the urea excretion output fell down 20 mg/day as to a normal diet average output of 70 mg. in adult hen.

Eisenburg (1966) discussed that there was relationship between role of food and snail destiny. He stressed that food limitation would force the snail to the dormant state.

Little (1968) studied aestivation and ionic regulation in two species of Pomacea i.e. Pomacea lineata and Pomacea depressa. The osmotic pressure (O.P) of haemolymph though high in one snail, the proportion of ion was found same in both cases. The concentration of protein and uric acid in the haemolymph was compared between active and dormant snails. Long aestivated snail had lost an average 79.11% of original body weight. There was much accumulation of uric acid around blood vessels in lungs as compared with that in the two chambers of the kidney. Recovery of snail occured after 24 hour when placed in water.

Volovirta (1968) described the relationship of land molluscs to the pH of the soil and stated that the

increase in pH resulted in increase in number and abundance of land molluscs.

Pomeroy (1968) stated that land snail Helicella virgata spent much time in dormancy, and the snail selected conspicuous position above the ground, at heights upto 9 m from the ground, fully exposed to sun. He found that in laboratory they lived much longer at 20-30°C but as the temperature was increase there was a decrease in the life.

Peake (1968) said that during unfavourable condition or when the hydrostatic pressure of skeleton was lowered, the snail retreated completely inside the shell thus reduced or eliminated evaporation from the body surface. He further quoted Machin (1964, 1966). The inactive Helix aspersa was able to regulate the evaporative water loss from the mantle. He described this as a unique property of mantle tissue.

Grime and Blythe (1969) studied plant-snail relationship in field and laboratory. He selected two large snails i.e. Cepaea nemoralis and Arianta arbustorum for his study. The two snails had diurnal pattern of emergence. A. arbustorum even on exposed situation was found fully or partially emergent from the shell on majority of days while C. nemoralis was found to retreat within shell during the day. During low humidity C. nemoralis tended to retreat readily into the shell and secreted the epiphragm more rapidly than Arianta

arbustorum. In field A. arbustorum was found attached to the stem and leaves of tall herbaceous plant. The epiphragm seemed to be thin as they were easily dislodged from the foliage by wind and possessed remnant of an epiphragm.

C. nemoralis was found beneath the grass tussocks or on the under-side out crops while A. arbustorum was found on the floor of tall vegetation. Cepacea fed on grass leaves and A. arbustorum on leaves of herbaceous plants.

Dejorge and Petersen (1970) demonstrated that urea and uric acid contents were present in the hepato-pancrease, kidney and lung of active and dormant snails, Strophochellus and Thaumastus (Pulmonata; Mollusca) He further added that during dormancy (aestivation and hibernation) there was an increase in urea in hepato-pancrease of both species. In hibernation, in both species the urea content increased. While in aestivation the kidney of Strophocheilus lose urea. The uric acid had increased in the lung and hepato-pancreas of both species during hibernation but was diminished in the kidney.

Hunter and Popovich (1977) found Cepaea nemoralis in hibernation under fallen leaves, vegetation and stones. In winter months snail fully with drawn with aperture oriented upward and covered by 1-3 epiphragms. Adult snails in state of hibernation when provided with

favourable condition became active for short period but soon entered hibernation again. During summer the growth of the digestive gland was faster than other tissues collectively (tissue-less gland). Both these organs showed marked increase in carbon contents before hibernation and then gradually showed decline in winter and spring. In kidney no carbon or nitrogen content change was noticed.

Hakala (1979) described seasonal variation in the carbon and energy content in Mysis relicta. The store energy was of less importance for maintenance of life. The organic carbon content varied from 50-63%. The values were high in young. In mature animal carbon content was high before breeding season while in male it was high before copulation and was totally lost in copulation.

Hodasi (1979) observed that seasonal and physiological change and abiotic factor resulted in aestivation in Achatina achatina. The snail emerged from aestivation after few rain falls even though the snails were indoor, they might have some sensing device to detect change in atmospheric content.

Stephenson (1980) observed that in dry weather Arion subfuscus constructed cells in the soil in which later on it aestivated.

Storey (1983) quoted his previous work of 1972 that Lymnaea peregra had a peculiar pattern of behaviour. The snail rotated its foot and applied it to the inside of the shell in such a way that the aperture of the shell

was closed by the side of the body rather than the foot. The evaporative water loss was reduced by this behavior. In 1983 he observed that under controlled laboratory conditions, when subjected to drying out, the juvenile of Lymnaea truncatula, L. stagnalis, and L. palustris adopted same behavior as previously observed in L. peregra. Such behavior was presumed to be "of adaptive value" and showed the state of dormancy in these snails inhabiting areas that dry up.

Dimitriadou and Saunder (1986) described that dormancy of Helix lucorum was controlled primarily by low humidity, photoperiod and temperature. The duration of hibernation was slightly short at high temperature.

Davies (1988) has confirmed the fact that land snail could bore into limestone rocks. He found Cepaea nemoralis Cepaea hortensis and Helicigonia lapicida were resting in circular cavities. He called the cavity a hibernacula, the enterance lead to a tubular hole which was bore upward into the rocks. These snails lived there for several generations. Helix aspersa had not occupied these "snail hole" but appeared to like more exposed site or larger hole with larger diameter.

CHAPTER - 3

MATERIALS AND METHODS

The terrestrial snail Bensonies jacquemonti is herbivorous and feedsalso on soil detritus. It is widely distributed at the campus area of University of Peshawar. For the present study the snails were collected from the garden and kitchen vegetable field of F.18 house near "Masjid-e-Wasta" while selecting the adult snails preference was given to egg laying and copulating snails. As they provided material for further detailed study of the oviposition and mating behavior.

Present study required a continuous supply of snails of known individual history and the knowledge of reproductive status (ovipositing, copulating,post copulating, young, and adult etc.)

In the laboratory the adult snails were kept either isolated or in groups in flower pot (having a layer of pebbles covered with 2 inches of soil) and covered by wire guaze, they were fed generally on fresh mulberry leaves, occasionally supplied with potatoes, carrots etc.

Newly hatched Bensonies jacquemonti were also collected recognized by their tiny and fragile shell and the young one, recognized by number of their shell whorls. These were kept in separate groups.

A record was maintained to note the growth rate of the shell whorls and size of the snail. Measurements were taken after every week and from time to time they ...

dissected to note the development of genital organs.

In the laboratory the incubation was observed, the egg container also acted as the first home for the neonate.

Some snails were kept as experimental population in cages kept under shady places in open, in inner court-yard of the house. Thus providing the snail with its natural environment. The experimental snails were examined daily in breeding season to observe oviposition and copulation.

Snail were killed by prolong drowning in water. They were kept in 70% alcohol for a day for slight hardening and then dissected.

Histological section of ovotestis were made to find out various developmental stages of spermatogenesis and oogenesis. The tissues were fixed in formo-saline, sections were stained with Haematoxyline and Eosine. For quick count of mature sperms and ovum the squash technique was applied. For early stages of development the eggs were obtained before oviposition from the uterus of egg laying snails by dissection (Fig. 19).

To study embryogenesis and organogenesis five eggs were fixed after every twenty four hours. Starting from oviposition upto the hatching of the youngs and after hatching the dissection of the youngs upto the age of 5-6 months. The eggs were de-shelled with the

help of fine needle and forceps. Care was taken not to damage, the perivitelline membrane. The eggs were then fixed in the fixative; Formo-saline (5 percent formaline, fixation time 6 hours) and Kauli's fluid. (fixation time 4-6 hours).

Kauli's fluid was prepared as follows:

Distilled water	:	30 parts
95% alcohol	:	15 parts
Conc HCL	:	6 parts
Glacial acetic acid	:	1 part

Five percent formaline showed good results for the histological studies of the embryos. Parafin wax was used for embedding. Section were cut 6-8 microns thick and were stained with Delafield's Haematoxyline and Eosin, Cedar Wood Oil was used as a clearing agent.

To determine the time taken by the egg to hatch, freshly laid clutches of eggs from the field as well as from the laboratory were collected. Each clutch was marked with the date when it was laid, the number of eggs in the clutch, the shell size of the parent snail, the size and external appearance of the eggs and the hatching date (Table I).

For incubation purpose the egg clutches were kept in the laboratory in separate small mud pots, (Fig. 46) small flower pots, petridishes or in a large flower pot, several egg clutches were kept along the periphery.

Each individual clutch was marked and wrapped in soft cloth and kept in moist earth (soil) inside the container. In case of small containers, they were kept collectively inside a large container having little water (just to damp the bases of the small containers). In all cases the soil was kept moist by placing the container of the egg inside a water container. The eggs were inspected daily and a record was maintained to note the date of hatching. The newly hatched snails were kept in egg container (if it contained only one clutch of eggs) which also served as their initial home. The neonates were maintained on diet of small foliage leaves of mulberry and potatoes. The food was changed on alternate days.

If several clutches hatched on the same day they were kept together in a big container, other wise not. The hatching of egg was observed under a binocular microscope to study the process of hatching.

The egg clutches, which become dry accidentally i.e. desiccated or the medium in which they were kept were flooded with water, such eggs were not counted in the record and were regarded as the discarded lot.

Apart from laboratory hatching record, some egg clutches of unknown age were collected from the field, which were about to hatch. The purpose was to maintain

the growth record, the eggs which were about to hatch (Fig.47) were recognized by their gross appearance and colour. The colour of such eggs were yellow and when touched with needle, these eggs show hard structure due to the presence of under lying shell of the baby snails.

Some baby snails of unknown age were also collected from the field and record was maintained to measure the size of the shell and number of whorls to know the rate of the increase in size, number of whorls and time (days) (Table IV-IX). This is a safe process because in the laboratory sometime due to negligence snails undergone aestivation or hibernation and thus upset the daily record.

The material and methods for specific experiments have been described in relevant sections.

RESULTS

CHAPTER - 4

REPRODUCTIVE SYSTEM

In the previous study Siddiqi and Aurangzeb (1984) and Aurangzeb and Siddiqi (1984). we have traced out the morphology and histology of the reproductive tract of Bensonies jacquemonti. In this chapter the structure of reproductive tract is being described briefly in order to have a base for understanding the position of different organs, their secretions and functions, and conditions of development of organs. For the description of reproductive tract only mature snails were used.

4.1 GENERAL ANATOMY

In mature Bensonies jacquemonti the reproductive tract is a large and prominent structure and about 25-35 percent of the total weight of the snail. Like other hermophroditic snails, the reproductive system consists of several quite seperate and easily distinguishable parts. Generally, the whole reproductive system is composed of Three Parts.

a. The hermaphrodite system composed of hermaphrodite gland, its duct and the fertilization pocket, a small blind chamber present on inner side of albumen gland.

b. The male system consisting of four components; vasdeferens, prostate gland, penial complex (including epiphallus,

flagellum, epiphallus, penial caecum and penis with its muscular sheath) and the dart sac (amatorial organ).

c. The Female system: The female system is composed of albumen gland, oviduct, shell gland, vagina and bursa copulatrix. (Fig.1).

4.2 HERMAPHRODITE SYSTEM

Hermaphrodite system is composed of ovotestis and its duct. Ovotestis is embedded in the posterior lobe of the digestive gland towards the inner side of the spiral. It is multi-lobular in structure and enclosed in the same connective tissue which covers the digestive gland. The size and colour of the ovotestis vary greatly during different periods of the year. Normally the ovotestis of the mature Bensonies jacquemonti were observed to be of medium size and white in colour but in breeding season and specially when they are about to copulate, the size and the volume of the ovotestis is greatly increased and have nearly covered the underlying digestive gland. The colour of ovotestis changes from white to dark cream. In still mature forms i.e. egg laying snail the colour of the ovotestis was observed to be light brown. Each lobule is composed of numerous small follicles opening in a central cavity leading to a small duct, the vasa efferentia or efferent duct. The small

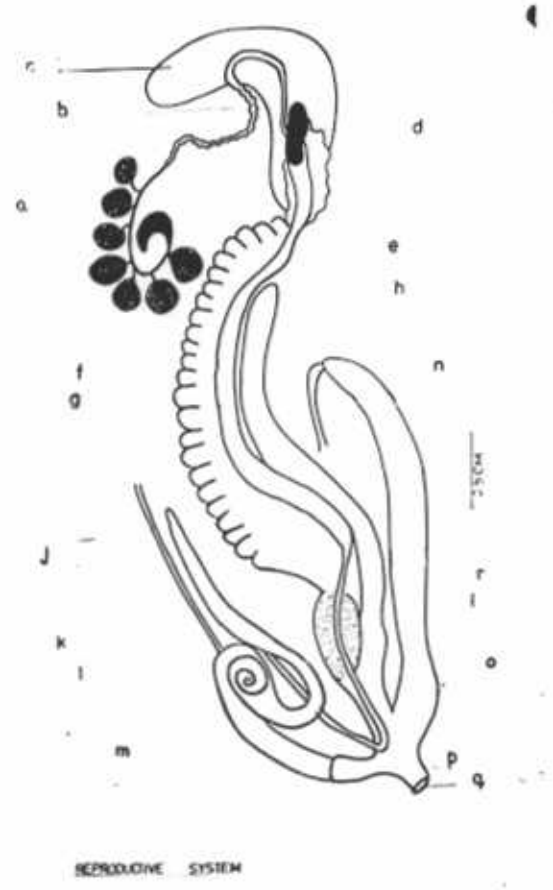

Fig.1: Reproductive system of Bensonies jacquemonti

a. Ovotestis
b. Small hermaphrodite duct
c. Albumen gland
d. Fertilization pocket
e. Large hermaphrodite duct
f. Oviduct
g. Prostate
h. Bursa copulatrix
i. Shell gland
j. Epiphallus flagellum
k. Epiphallus
l. Penial complex
m. Penis
n. Amatorial organ
o. Vagina
p. Genital atrium
q. Geital pore
r. Vasa deferentia

ducts join together to form the larger duct called small hermaphrodite duct.

It was observed that the number of lobule and the number of follicles in each lobule is fixed from the very beginning. However, the over all size of the ovotestis is increased or decreased in different periods which is related to the increase or decrease of the volume of the tissue.

Each lobule is covered by a layer of flattened germinal epithelium which in turn is bounded externally by a an Ancel's layer with inconspicuous nuclei as observed by Hickman (1931).

The sex cells are usually detached in isolated groups from the germinal epithelium or some time they may be seen aggregated. Spermatogenia are always found in different developing stages, while ova is liberated only in the breeding and active period.

4.2.1 SPERMATOGENESIS

The male sex cells are budded off singly or in groups from undifferentiated cells of the germinal epithelium of the ovotestis. In contrast to oogenesis, spermatogenesis proceeds without pause. Thus, there is no seasonal cycle of production of sperms as mature sperms are observed throughout the year.

Two stages of developing spermatids are noted:

a. An early stage with a small central nucleus and large, rounded cell.

b. Older stage with a large, rounded nucleus in terminal upper portion and elongated cell.

The last stage i.e. the mature sperm has an elongated pointed sickle - shaped head with a central nucleus and a long spiral tail.

According to Runge (1977) the development of sperm may be divided into three stages:

a. Primordial germ cells: These cells divide mitotically an d later develop into gonads.

b. Pre-spermatogenesis: This stage of development occurs after the germ cells have established in the gonad.

c. Spermatogenesis: The process begins with meiosis and leads to differentiation of the spermatozoan.

Runge (1977) was of the opinion that in the development of male sex cell if there is a pause, it is usually before proper spermatogenesis i.e. in pre-spermatogenic stage. Once the sex cell has certain activation, the development proceeds through spermatogenesis. The spermatogenesis occurs in a regular sequence of steps i.e. Germinal epithelium - spermatogonia - primary spermatocyte - secondary spermatocyte - spermatid-sperm. B.jacquemonti undergoes the regular sequence of events in spermatogenesis as mentioned by Runge (1977) (Fig 2).

The sperm of Bensonies jacquemonti has a sickle-shaped pointed head with a central nucleus (resembling an almond) and a long spiral tail. Hundreds of sperms are seen in the cavity of a follicle of ovotestis, while their tails are still attached to the residual cytoplasm (Fig.2).

4.2.2 OOGENESIS

The undifferentiated cells giving rise to ova seems to remain inactive in resting period and becomes active when breeding season approaches. The mature ova are comparatively very large in size but their number is found to be less as compared to the mature sperms. Observations of different stages of germ cells have revealed that in the follicles of ootestis the central portion acts as male gonad while the peripheral as female gonad. The mature sperms occupied the central part and the ovum were found in the periphery of the ovotestis. However in rare cases all stages of spermatogenesis may be found in a single follicle.

According to Hyman, (1967) the spermatogenesis and oogenesis occur simultaneously without any arrangement in follicles of the ovotestis. Abdul Malek, (1954a) observed in Helisoma trivolvis that oocytes are usually found towards the periphery, while sperms, attached in clusters to the basal sertoli cells near the lumen of the acinus. More detail of oogenesis is given in sc
development.

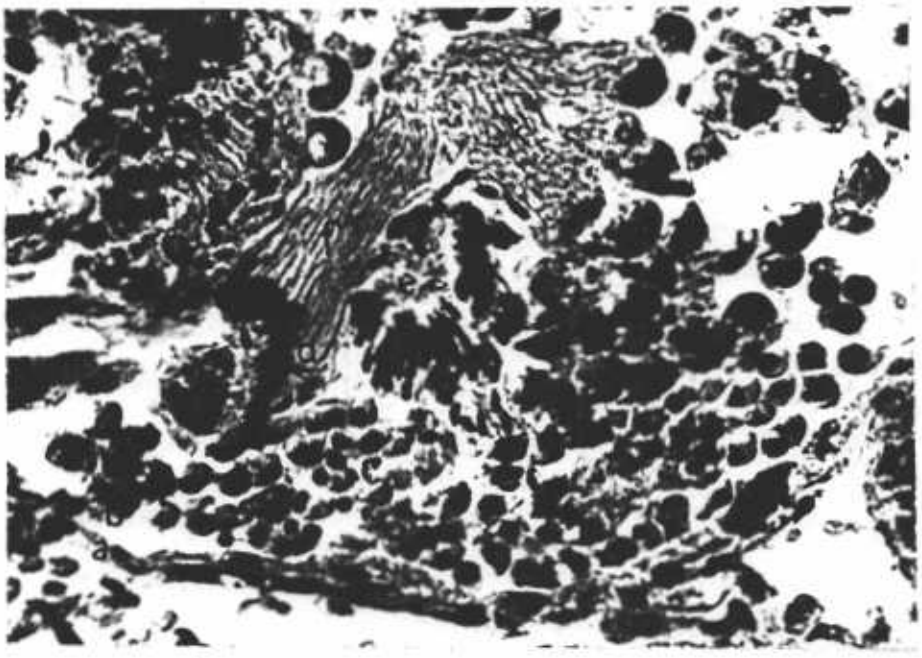

Fig.2: Microphotograph of a section of ovotestis of Bensonies jacquemonti showing stages of spermatogenesis. Note long sperm tail (X 1000).

a. Germinal epithelium
b. Spermatogonia
c. Spermatocyte
d. Spermatid
e. Sperm
f. Sperm tails.

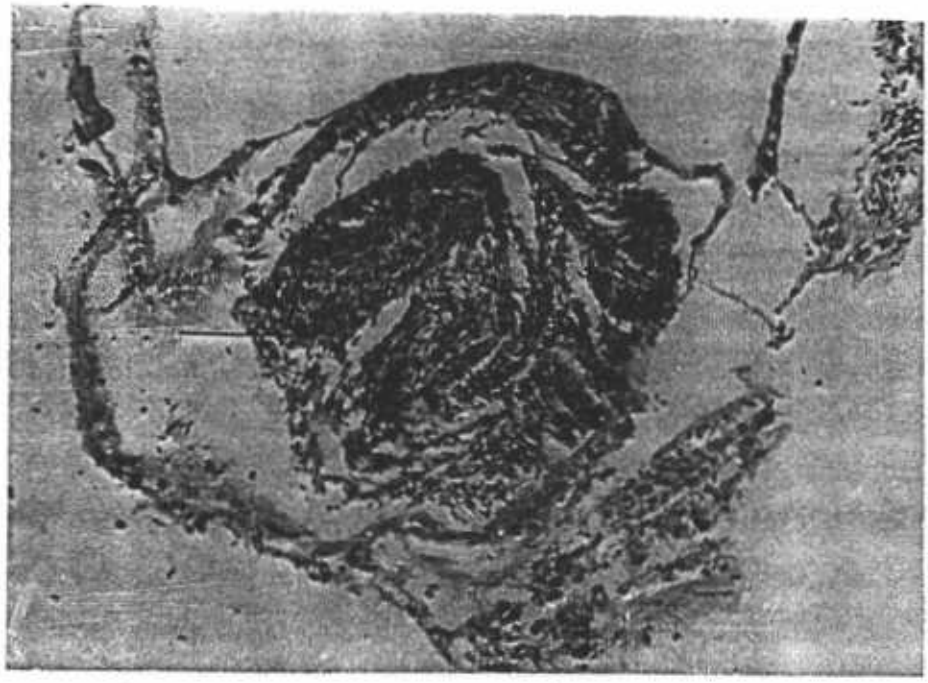

Fig.3: Microphotograph of a section of Bensonies jacquemonti of the small hermaphrodite duct opening in fertilization pocket showing masses of sperms in different acini (X 40).

From the ovotestis sperms pass to small hermaphrodite duct which leads to carrefour (see Fig.1,3). In the carrefour the two systems i.e. male and female separate. The sperms pass to the male system and lastly enter penial complex through vas deferens. In the posterior portion of the penial complex i.e. epiphallus and its flagellum, the formation of spermatophore occurs.

In Bensonies jacquemonti the spermatophore is transferred to the copulatory partner in an elaborate courtship. The spermatophore is stored inside the bursa copulatrix and later on the sperms are released to enter the carrefour to fertilize the egg (Fig. 4) or the foreign sperms are stored in seminal vesicles (small evaginations) of the small hermaphrodite duct.

4.2.3 SMALL HERMAPHRODITE DUCT

As described earlier, each lobule has a central cavity into which lead the follicles. The cavity leads into smaller duct the vasa efferentia. Vasa efferentia join together to form the small hermaphrodite duct. This duct runs forward along the inner border of lobule to open in the carrefour. Small hermaphrodite duct has three obviously distinguishable parts:

a. The first portion which is straight and it runs along the inner side of ovotestis. It measures 7 mm with a diameter of 0.01 m, it is narrow and rounded.

b. The second part is highly folded or convoluted. The folds are tightly held together by a thin sheet of connective tissue. When these folds are stretched out then this area measures about double the length of the first part. This means that this area is three or even four times longer than the first part, and so is its diameter. These fold are in a peculiar pattern i.e. out. pushing and in-pushing of the duct. In some snails the out-pushing or (upper fold) were smaller rounded and measured 1 mm in length while the in-pushing or lower folds were somewhat straight and measured 2 mm. The total number of these folds were 8/8 i.e. upper and lower folds. In still other forms the folds on either side were of equal size. These folds are similar to seminal vesicles of other snails as they are always loaded with mature sperms in adult snails in breeding season. As this area is folded upon itself, it gives the appearance of a short, broad compact structure. Usually it is dark cream to light brown in colour.

c. The third part is again straight and rounded and covered by a thin sheath. It runs forward in a zig-zag manner along the inner concave side of albumen gland. It then takes a sh[illegible] turn and bear a small swelling giving rise

narrow blind pouch, which Duncan (1975) called 'talon'. It lies burried in the substance of the albumen gland. Earlier this pouch was called 'fertilization pocket or chamber' by Duncan (1960) and Carrefour by Baker (1945) as quoted by Hyman (1967). Actually, the carrefour represents a conspicuous chamber receiving three ducts i.e the duct of albumen gland, the small hermaphrodite duct and aperture of the fertilization pocket. Actual fertilization takes place in fertilization chamber (see Fig.4).

From the carrefour leads the large hermaphrodite duct which soon divides into two, the oviduct and the sperm duct. Before division it takes a turn, so that the oviduct comes to lie on outer and sperm duct on the innerside. The two ducts are united superfacially but could be distinguished by their gross appearance. (see fig.5,6). The oviduct is much coiled and broad while sperm duct is narrow and contains thick secretory product.

4.3 MALE SYSTEM

The male system has four parts i.e. vas deferens and prostate gland, penial complex, dart sac and spermatophore. (Fig 1).

4.3.1 VAS DEFERENS AND PROSTATE GLAND

Superfecially it seems that they form a combine structure which lies on the inner concave side of the large hermaphrodite duct. When seen grossly it appears

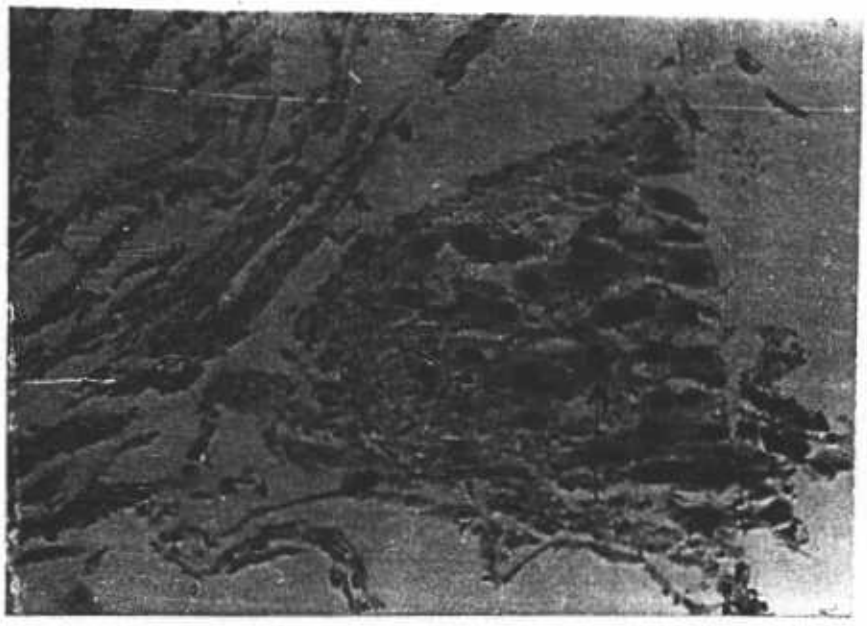

Fig.4: Microphotograph of a section of fertilization pocket of Bensonies jacquemonti showing process of fertilization (X 100).

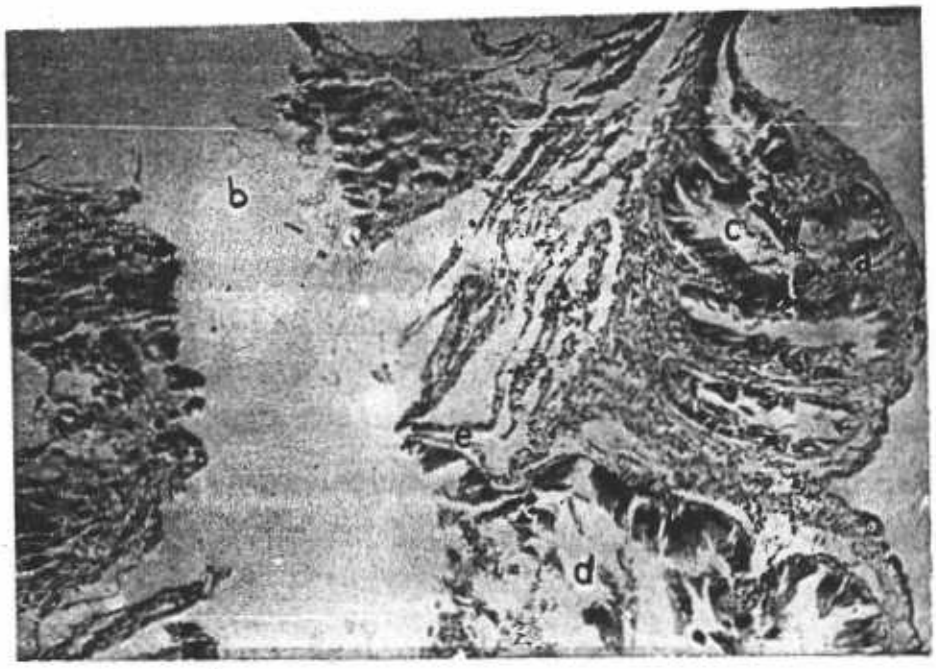

Fig.5: Microphotograph of a L.S. of genital tract of Bensonies jacquemonti showing junction of small and large hermaphrodite duct, fertilization pocket and albumen gland (X 40).

a. Small hermaphrodite duct
b. Large hermaphrodite duct
c. Acini of small hermaphrodite duct
d. Albumen gland
e. Fertilization pocket

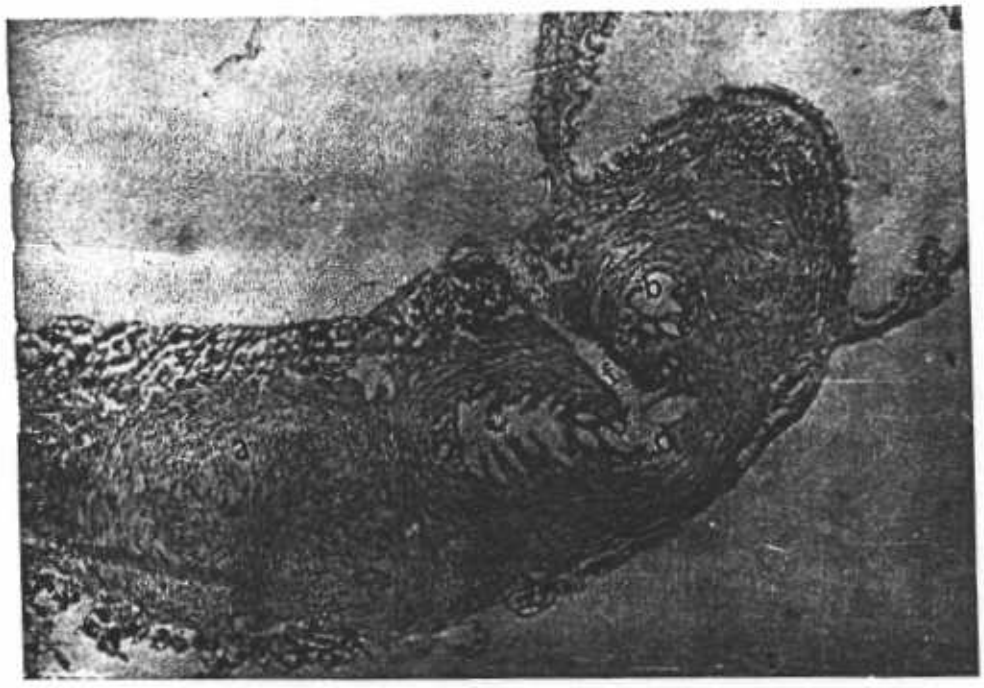

Fig.6: Microphotograph of a L.S. of genital organ of Bensonies jacquemonti showing union of different ducts at carrefour (X 100).

a. Albumen gland
b. Small hermaphrodite duct
c. Large hermaphrodite duct
d. Fertilization pocket
e. Seminal vesicle
f. Carrefour.

to be a granular structure of white colour. In the adult snail the colour changes from dark cream to light brown in the breeding season. Histological study revealed their seperate entity. Vas deferens is lined by flattened epithelial cells with prominent nuclei, followed by circular muscle fibres and patchy inner ciliated lining.

Prostate gland is also lined with flattened epithelium but here the nuclei are large. Following the circular muscle fibre are the large number of acini which are connected with the vas deferens by their narrow ends. In the acini the cells are arranged radially around a central cavity. These cells have granular cytoplasm which stains deeply. The lumen of acini join one another and ultimately open in large spaces.

4.3.2 PENIAL COMPLEX

It is composed of epiphallus, epiphallus flagellum, penial caecum, penis and penis sheath.

i. Epiphallus is a short muscular organ about 5 mm in length, with a narrow lumen. Epiphallus flagellum seems to be a continuation of the epiphallus. Both are supported externally by the supporting tissue with large prominent nuclei, followed by muscular layer. The lumen of epiphallus is with villi while these are absent in epiphallus flagellum. Epiphallus

and its flagellum takes part in the formation of spermatophore.

ii. Penial caecum, penis and penis sheath. The epiphallus continues into a coiled structure the penial caecum. This in turn opens into a thick short, muscular organ the penis. The penis is covered by a penis sheath which is connected with the genital atrium. The penis has a deep and narrow lumen. It is bounded by a muscular layer and also surrounded by the connective tissue.

4.3.3 DART SAC OR AMATORIAL ORGAN

It is tough, cylindrical and muscular organ and slightly bend on its self at its posterior part. This is also connected with the retractor muscle. Inside the sac is present the pointed dart. During copulation the dart is pierced into the body of the copulating partner and thus it stimulates the excitement for the copulation. The whole organ is composed of several layers of muscle fibres around a central core. The central core is having finger-like projection around a central canal, followed by two or three rows of circular muscle fibres and longitudinal fibres. All this is covered by epithelial lining. As there are no glandular element in the structure of the dart sac it seems that it cau[illegible]physical stimulation to partner du[illegible]ing copulation as t[illegible]gan is highly muscular.

Paricio et al (1992) studied systematics of family Helicidae (Gastropoda: Pulmonata) and described the dart sac complex in Helicopsis. They found four clearly distinguishable dart sacs on either side of vagina. The external dart sacs contain a dart while the internal sacs were empty. The function of the inner dart sac is unknown as it does not contain a dart. The authors were of the opinion that as the inner dart sac opens downwards towards the vagina, it may have a glandular tissue. According to them it is difficult to decide whether the dart sacs complex represents a primitive or an advanced stage in its evolution in Helicidae.

4.3.4 SPERMATOPHORE

Spermatophore is an elongated structure and has serrated edges. Its upper part is divisible cylindrical thin walled sac which is spiked at its tip, and is usually of a dark brown colour. It bends on itself and then enters the elongated gutter-like posterior portion (Fig.7). Which is spined on its sides. From this portion spines are given out at regular intervals. Each spine divides into two branches and each branch bifurcates in turn. Thus each spine has four smaller branches. The posterior tip of the spinous portion is sharply curved and without spines. It is the last portion that enters the genital atrium of the copulating partner and is observed hanging for a

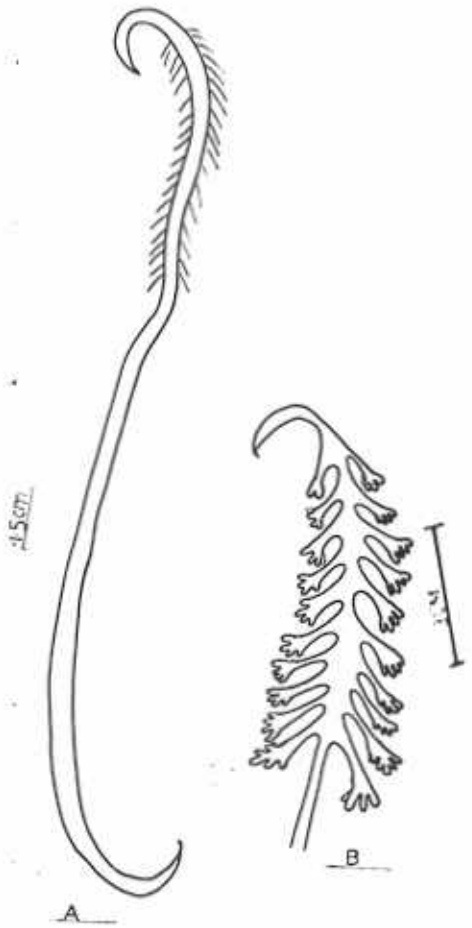

Fig.7[a]: A. An entire spermatophore of Bensonies jacquemonti

B. The spinous portion enlarged

while through the genital aperture of a copulating snail (when it is disturbed in last movements of copulation , otherwise not). The sperms were seen escaping through the tail of the spermatophore.

The spermatophore is formed inside the epiphallus and epiphallus flagellum. It looks like the mirror image of both of them. Epiphallus forms the anterior sac of the spermatophore and the epiphallus flagellum forms the posterior spinous portion of the spermatophore. It was observed by dissection that during copulation the spermatophore formation begins in Bensonies jacquemonti.

For this experiment, copulating snails were sited in the field. They were watched in love play and when the penis were inserted in the partner, and if one or both of the snails were touched with a needle, the penis were at once retracted. These snails were picked up from the ground. The major portion of their shell were broken with the help of a forcep so that they could not retreat inside the shell. They were killed by drowning into water or by adding one to two drops of chloroform to water to anaesthetise them. They were dissected and their penial complex and bursa copulatrix were cut open for search of the spermatophore. Usually the bursa copulatrix was found empty. In penial complex, the epiphallus flagellum was found with spinous portion of the spermatophore while the anterior sac-like portion of spermatophore was present inside the epiphallus. The penis and penial caecum were

loaded with thick dense milky secretion resembling the secretion of prostate gland. This secretion may have lubricating function during copulation. In some snails one complete spermatophore was observed inside the penis or in the penial caecum, while in the epiphallus flagellum only the spinous portion of the spermatophore was present, which was a developing spermatophore. It was further recorded that in a single copulation two and some times even three spermatophores are transferred to the partner.

As stated earlier, spermatophore is formed in epiphallus and its flagellum. In Bensonies jacquemonti it is observed that if these organs are pressed with a needle or forceps the secretory product inside them is seen clearly moving. This fluid is milky white in colour and resembles, in appearance, the content of sperm duct and prestate gland. Internally, the epiphallus flagellum is provided with rib-like structure which presses the secretion and molds it in the form of the spinous portion of spermatophore. Later on, the lumen of spermatophore may be filled with sperms.

Lind (1973) stated that in Helix pomatia the the spermatophore formation started 40-50 seconds after copulation and the sperms transfer from spermioviduct started after 5-10 seconds later. In present study it was observed in some sections of epiphallus of copulating snail the epiphallus contained spermatophore but it was

empty of sperms. This observation confirms the statement of Lind (1973).

The spermatophore passes directly into the bursa copulatrix during copulation for the simple reason that its opening is in direct line with the vagina and genital atrium while egg shell gland followed by large hermaphrodite duct is a side branch of the vagina.

Regarding the function of the spermatophore, one can say that it is for the safe transfer of sperm from male to female organ. In animals without copulatory organ this function seem to be true but in most stylommatophora with a muscular penis the function of the spermatophore may be more than that.

Lind (1973) suggested that in Helix pomatia the function of the spermatophore is not to protect the enclosed sperm during transfer from one snail to another but to ensure that majority of sperms can escape safely into the oviduct from the digesting bursa copulatrix. He found that the tail of spermatophore is long and sometime has a connection with the oviduct, as sperm are released already before the body of the spermatophore reaches the bursa copulatrix. The sperms thus coming out of tail, will escape through bursa stalk without coming in contact with bursa.

It is generally agreed that the spermatophore are passed inside bursa copulatrix by the peristaltic movements of the bursa stalk and that the sperms come out from the tail canal.

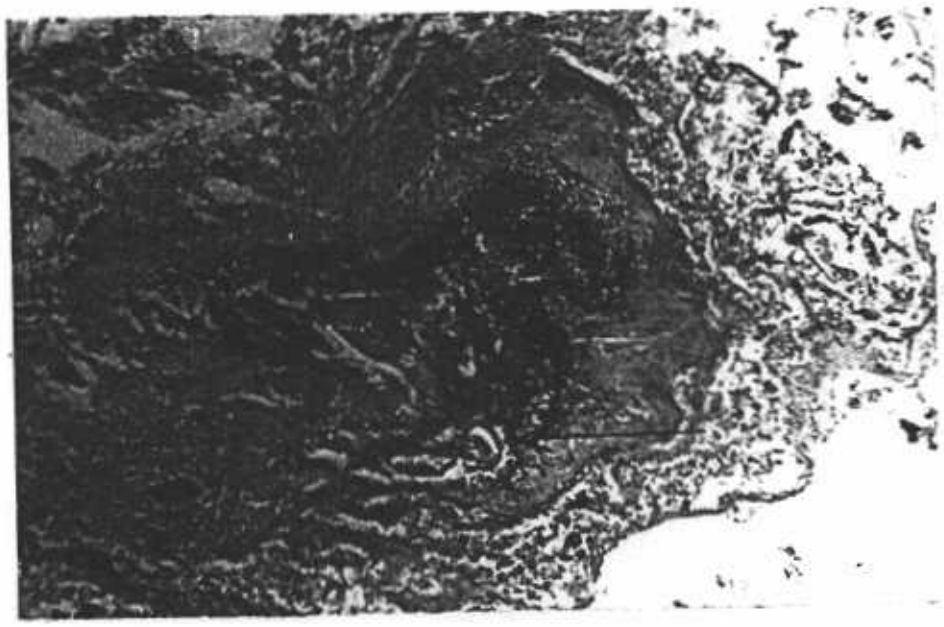

Fig.7[b]: Spermatophore in the process of absorption in bursa of B. jacquemonti

In the light of the finding of Meisenheimer (1907), Rigby (1965), Lemehe (1956), Fretter (1941), Linke (1933), Fretter and Graham (1964) as quoted by Lind (1973), we can assume that the working and function of the spermatophore inside a digestive bursa copulatrix, is to ascertain safe migration of sperms.

The spermatophore passes inside the bursa copulatrix by the peristalsis of the stalk, which carries the spermatophore upward. The sperms that have come out the spermatophore are moving down-ward i.e. against the peristalsis of the stalk. There is a chance that they may be traped and killed before they escape the bursa copulatrix. Keeping in view, the peculiar structure of the spermatophore and the way it is transferred, the major portion of sperms may succeed to migrate in spermatheca.

During spermatophore transfer the anterior sac-like portion is the first part to enter the bursa copulatrix, this end is closed at its tip. The spinous posterior portion enter the bursa afterward and the sperms are already in the process of release, so many of them escape to the oviduct. The remaining sperms during their migration downwards have to face the upward movement of the spermatophore and bursa. Lind (1973), suggested that longitudinal ridges and furrows in the wall of spermatophore (formed by flagellum and epiphallus) may have the specific function to offer elastic resistance against the muscular pressure of the bursa stalk. Otherwise the pressure could

have pressed the body of the spermatophore and squeezed out all sperms too early and then pressed into the bursa before their escape.

In Bensonies jacquemonti the spermatophore has anterior sac-like portion and a posterior spinous portion, the branches of this portion divide and sub-divide thus forming greater area to resist the muscular pressure of the bursa. As the tail enters the bursa lastly so majority of sperms have a chance to pass unchecked from the bursa. The remaining sperms are in a way safe from the upward movement of bursa in the shade of the ridges. It seems that only active sperms are allowed to pass to spermatheca and majority of them are lost. Further more long tail or long spermatophore seems to facilitate sperms migration from the bursa stalk.

The measurement of bursa copulatrix and spermatophore showed that bursa copulatrix is 18-21 mm long while spermatophore varies from 16-20 mm in length. The spermatophores are usually found near the stalk region of the bursa and not in its anterior tip. This means that the tail of spermatophore is always near the bursa stalk. It is concluded that the main function of spermatophore in members of Helicidae (to which B. jacquemonti belongs) is that perhaps the most active and limited number of sperms are allowed to pass the bursa and enter the female duct. Majority of sperms entering bursa are killed rapidly. Dissolution of the spinous portion

of spermatophore in the bursa copulatrix, as observed, may take several days after the migration of sperms.

4.4 FEMALE SYSTEM

Female system is composed of albumen gland, oviduct, shell gland, vagina, and bursa copulatrix(Fig.1).

4.4.1 ALBUMEN GLAND

It is a large gland measuring between 11x15 to 17x6 mm and is of cream white colour. During breeding season the size of the albumen gland is enlarged considerably and its colour also becomes dark and changes to mustard or light brown. The surface of the gland is smooth but slightly curved, giving the appearance of convex dorsal and flat ventral surface. Both ends are rounded but the anterior is narrower as compared with the pasterior broad end. Histological studies shows that gland is composed of a large number of vesicles, covered externally by a single-layered thin compressed cells. The vesicles are elongated or rounded. The vesicles have gland cells arranged radially in a single layer around the central lumen. The lumen open into small ductule which join together to form larger ducts. In the mature state the albumen gland cells are extremely enlarged and packed with secretory product. The albumen is stored in the ducts temporarily and then after fertilization of egg, it is deposited around the egg at the carrefour. The

albumen gland duct is lined with cubical epithelium having large cilia. The size of albumen gland and the size of its component cells were larger in the specimens which were about to copulate or have just copulated.

4.4.2 OVIDUCT

The large hermaphrodite duct which emerges from the 'talon' may be seperated as spermo-prostatic duct and oviduct by their gross appearance. The sperm duct is granular and narrow in appearance while oviduct is broad glandular, wider and much coiled structure of milky colour. Its upper and lower region are compressed and narrow while the middle region is much wider and provided with transverse folds (fig.8,9).

The large hermophrodite duct as it comes out of the talon takes a turn in the anterior direction. So that the oviduct is now on the outer side and the sperm duct is on its inner side. The oviduct becomes much wider and may be referred to as uterus. It is in the form of a loop with inner concave margin towards sperm duct. Uterus has numerous transverse folds. Each fold is bounded by epithelial tissue with some cells having prominent nuclei. Numerous gland cells, broader towards their outer ends are present. They pour their secretions in the inter cellular spaces. It seems that the secretion is stored there and later on are passed in the lumen of the uterus through the small

Fig.8: Microphotograph of a T. S. of genital organ of Bensonies jacquemonti showing albumen gland, oviduct and sperm duct (X 40).

a. Albumen gland
b. Oviduct
c. Sperm duct

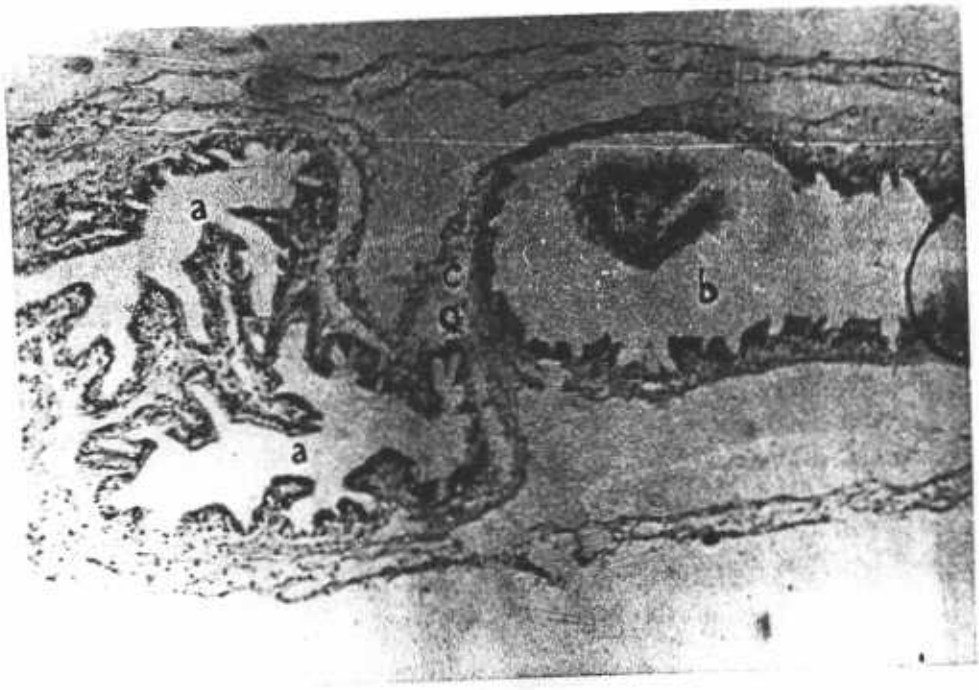

Fig.9: Microphotograph of a L.S. of genital organ of Bensonies jacquemonti showing seperation at fertilization pocket of sperm duct and oviduct (X 400).

a. Oviduct
b. Sperm duct
c. Fertilization pocket

channels present in between the epithelial cells. The secretion of the uterus is more abundant in the breeding season specially in egg-laying snail. In this state, it is difficult to dissect out the entire reproductive system intact (specially the uterus). As the uterus is packed with the secretion and has gained enormous size, its wall gets ruptured by the load and a milky white secretion is noticed coming out from the lumen. According to Bayne (1967) the fertilized egg, after getting its albumen, when enters the upper oviduct is surrounded by the egg jelly in this region. As the egg moves towards middle and then towards lower oviductal region egg shell is secreted around it (see fig.19). The present study agrees with the observation of Bayne (Loc.cit.).

The normally laid egg is resistant to water absorption. When eggs removed from oviduct are placed in water, their shells swell enormously, as does the normally laid egg when its shell is removed i.e. the jelly swells. To show this an experiment was performed and from a normal healthy egg, shell was removed and the de-shelled egg and shell was placed in a dilute aqueous solution of methylene blue just visibly coloured. The diameter of the egg shell, jelly and perivitelline sac were measured by a caliberated binocular. It was noticed that the volume of the jelly increased three folds, perivitelline layer had slight increase in size while shell had negligible change. The jelly reached the maximum size within 10 minutes after it was placed in water and no further increase was

noticed in its volume even after thirty minutes.

The last portion of large hermaphrodite duct completely bifurcates externally into sperm duct and free oviduct.

4.4.3 SHELL GLAND OR CAPSULE GLAND

The free oviduct is modified into egg shell gland. During active breeding season it resembles bursa copulatrix in its pink colour but normal period the colour of shell gland is light brown. This is the region where the final covering of the egg, in the form of shell is secreted. The egg from here is then conveyed to genital atrium through vagina to be laid in suitable place.

The shell gland is lined by epithelial tissue followed the supporting structure, the connective tissue and muscle fibre.

Inside the shell gland the lumen is quite wide which becomes narrow at some places. The inner wall of which is folded resembling the structure of a vertebrate stomach (Fig.10).

4.4.4 VAGINA

Posteriorly, the egg shell gland continues into vagina. It is a muscular tube with folded wall. Which is supported by muscle fibres. Its average length is 3 mm. Vagina also receives the duct of bursa copulatrix and

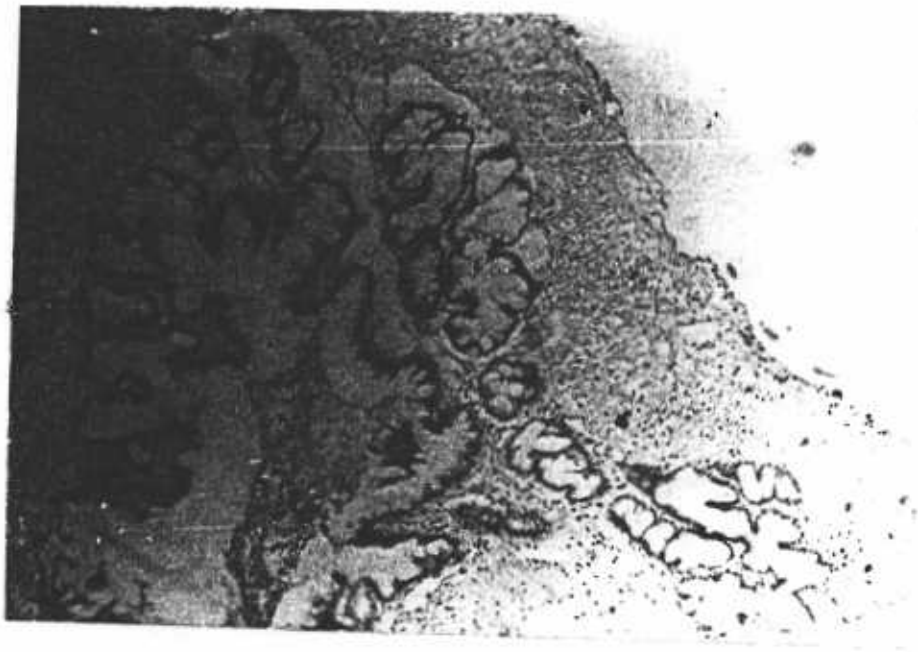

Fig.10: Microphotograph of L.S. of shell gland of Bensonies jacquemonti (X 40)

leads posteriorly into genital atrium.

4.4.5 BURSA COPULATRIX

It is an elongated cylindrical structure present in between large hermaphrodite duct and penial complex (Fig.11). It is attached to the inner side of the large hermaphrodite duct. Anteriorly it directly leads into vagina. The posterior portion of bursa is a bit dilated as compared with the anterior region. In active season, in a freshly killed and dissected Bensonies jacquemonti it was observed as a prominent dark pink organ. On close examination it was found that the posterior region is filled with a thick secretory product of dark pink colour, in the middle there are also some milky white secretory product. The bursa is covered by a thin covering sheath through which the internal components are seen easily, e.g. spermatophore and internal secretory product. There are no cilia in bursa or its duct.

When dissected out the bursa is seen to contain spermatophore (Fig.11). Usually one or two spermatophores are found inside the bursa but some time even three spermatophore are present inside bursa copulatrix. In egg laying snails the bursa copulatrix is observed to contain complete and incomplete spermatophore i.e. one complete spermatophore (with both spinous and sac-like portion) and two incomplete spermatophores (only spinous

Fig.11: Microphotograph of L.S. of bursa copulatrix of Bensonies jacquemonti showing spermatophore inside (X 40).

portion is present while sac like portions are missing). This indicates that when sperms are released, (to be passed to fertilization pocket to fertilize the egg), the empty sac-like portion of the spermatophore is absorbed first by the surrounding secretory material and in the second step the spinous portion may be absorbed. This fact is proved by the presence of only two spinous portion of spermatophores as their sac-like portions which have released the sperms were missing i.e. they might have been absorbed by the surrounding material. The spermatophores are passed inside the bursa copulatrix in such a way that the anterior sac-like portion is lodged first in side the bursa and posterior spinous end of spermatophore is passed in the second step in the bursa copulatrix.

Another interpretation of this peculiar type of transfer of the spermatophore may be in the light of the observation by Lind (1973). According to him in pulmonate snails there is a digestive bursa copulatrix. After or even during copulation as soon as the sperms escape the spermatophore many of them are dissolved by the bursa secretion and so is the fate of the body of spermatophore afterwards. In present study it was observed that sperms escaped from the posterior part of the spermatophore and this part was the last portion to enter the bursa copulatrix. Thus the tail of the spermatophore in a way facilitates the sperm migration from the

bursa stalk to the oviduct. In other words the sperms have to leave the bursa quickly as they are killed there (fig.12)

In Bensonies jacquemonti like other pulmonate snails with well developed penis it seems that the function of spermatophore is not protection of the sperms during the transfer to the other copulant, but it ensures that the majority of sperms can escape quickly into the oviduct before they come in contact with the digestive bursa. During and after copulation the bursa becomes more pink in colour. This suggests that it is ready to dissolve or absorb anything that enters it. During copulation penis may contain some debris of the soil or something that may get entery into the genital atrium and then to bursa. So beside excess of sperms it dissolves these extra invaders. Lind (1973) reported that bursa content beside absorption also activate the sperms. Anterior bursa is more active in absorption and by the time sperms reach there they are also more active, by coming in contact with bursa fluid.

The generally agreed function of the bursa is to receive and store the sperms during copulation. In mature Bensonies jacquemonti the bursa is always filled with a mixture of sperm cells, prostatic secretion and the secretion of bursa itself. The secretion of bursa are of central red mass surrounded by a more fluid whitish mass. The main functions of bursa copulatrix seem to be the reception of the spermatophore, release of the sperms from the spermatophore and to assist sperm migration from

Fig.12: Microphotograph of L.S. of a portion of genital system of Bensonies jacquemonti showing bursa copulatrix containing spermatophore with sperms inside (X 100).

a. Bursa copulatrix
b. Spermatophore
c. Sperms.

bursa to spermatheca. The results of the studies of Smith (1965) on Arion, Lind (1973) on Helix and Duncan (1975) on Lymnaea, strongly suggested that the role of bursa is to digest the excess of sperms. As the foreign sperms received during copulation are disintegrated in the bursa, so they must be stored somewhere else in the interval between copulation and fertilization. They suggested that this storage of sperm may be for weeks or even years and the storage site is carrefour or fertilization pocket.

Holm (1946), observed that once the sperms reach the bursa copulatrix, its epithelial cells begin activity. Small bulbs of clear cytoplasm appear at the distal ends of the cells of the bursa. These bulbs are pinched off, pass into the lumen, disintegrate here, and contribute to the fluid of the lumen. He further reported that this apocrine method of secretion of bursa is wide spread in the pulmonates (described in Helix, Physa and Lymnaea). Holm suggested that sperm possibly migrate, from bursa copulatrix to oviduct, by their own swiming movement as the bursa and its duct is without cilia.

Lind (1973) autopsied Helix pomatia at different intervals of time after their copulation and examined the preparation of spermathecae. He observed, the time required by the sperm to reach the spermatheca. The sperms have not reached the spermatheca in the snail killed after 1 hour and 45 minutes but peak of migration of sperms are found about two or three hours after copulation. He

also added that the sperms are in a state of temporary inactivation in the spermatophore and after coming in contact with the secretion of the bursa, they are activated.

Duncan (1958) suggested that in Physa fontinalus the bursa secretion has a stimulating effect on the motility and power of fertilization of the sperms. Duncan (1958) and Holm (1946) observed a short time storage of sperms before migration in the bursa copulatrix.

4.5 DISCUSSION

Bensonies jacquemonti retains the basic pattern of stylommatophoran reproductive system with slight changes in accessory sex organs i.e. muciparous gland is absent. The genital pore is present near the base of the right optic tentacle. It is monoecious or hermaphrodite. Ovotestis (hermaphrodite gland) lies in visceral mass buried in the posterior lobe of the digestive gland (liver).

Ovotestis is follicular composed of numerous lobules, ova and sperms are liberated from the inner lining of the ovotestis. Ova is rounded and large while sperm is with small head and long spiral tail. From the ovotestis leads a convoluted duct the small hermaphrodite duct carrying ova and sperm. The small hermaphrodite duct enter albumen gland but at the junction is the enlargement or swelling called "talon or fertilization pocket" from here the two ducts male and female are seperated. In Bensonies jacquemonti, the ducts run side by side but

superfecially they look single and is called as large hermaphrodite duct. After a considerable distance the two ducts seperate completely into oviduct and sperm duct (Vasa deferens). Oviduct forms a spongy structure, the shell gland and then leads through vagina into genital atrium. The genital atrium also receives the duct of an elongated sac the bursa copulatrix. The Bursa receives the spermatophore during copulation from the other snail. The sperm duct or vas deferens carries the spermatic content to the male organ. The male organs are called as penial complex and is composed of muscular penis, penial caecum, epiphallus and epiphallus flagellum. The vas deferens after forming a loop over the shell gland and genital atrium joins epiphallus. Epiphallus continues posteriorly into epiphallus flagellum which leads anteriorly into penial caecum. From the penial caecum emerges the penis and its sheath which are protruded externally during mating.

The histology of the ovotestis of pulmonates is studied by many workers and the detail varies in different species. Hickman (1931) found that the volume of the ovotestis , varied at different time of the season in Succina ovalis but the gland was not inactive at any time. Duncan (1975) quoted many authors including Archie (1942) Mahoney (1940), Abdul Malek (1954a), Duncan (1958) and Ancel (1902) that there are three developmental stages in the ovotestis of Helix pomatia. In first instance the undifferentiated cell form the male gametes, in

second step nurse cells are formed in third step these nurse cells stimulated the formation of female gametes. Morton (1968) observed the consecutive sexuality with protandry in Ianthina and Clathrus and rhythmical sexuality in Valvata tricarinata. Morton (loc. cit) also stated the famous discovery of Orton (1909) that Crepidula fornicata starts life as a male, then passes through a hermaphrodite stage and dies as a pure female. Graham (1954) described distinct male and female phase in Ianthina janthina, Grabb (1927) stated that sperms and ovum mature in the same acinus in L. stagnalis and L. stagnalis appressa say.

Abdul Malek (1954) found sperms in the acini of ovotestis for all the time even during dormancy in B. Biossy while in H. trivolvis ova and sperm are found during all seasons of the year. The present observations of the ovotestis of Bensonies jacquemonti, show that spermatogenesis is a continuous process but oogenesis is seasonal.

The acini of ovotestis open into small hermaphrodite duct and thus convey the reproductive product in it. The small hermaphrodite duct has three portions, the middle portion is convoluted and as it is always filled with mature sperms, so it acts like seminal vesicles of other gastropods. Abdul Malek (1954) in another study contradicted the nature of these convoluted portions as

seminal vesicle. He studied the histological sections of the duct in Helisoma trivolvis and found them different from seminal vesicles. He suggested that these convolution may serve to increase or dilate the cavity of the duct, as the eggs too pass through this passage and are retained here occasionally.

Duncan (1958) observed that in Physa anterior and posterior portions of the duct are ciliated while the middle one (Seminal vesicle) is glandular. He quoted Holms (1946), Aubry (1956) that in Lymnaea the seminal vesicles have a clear cavity while remaining duct is ciliated. Duncan (1958), and Lind (1973) suggested that not only the seminal vesicles but whole duct may serve as a store for the sperms. They also reported that the gland cells may resorb some of the sperms, as in pulmonates the resorption of gamete is common. Joosse et al (1968) frequently observed degenerating oocyte in the acini of the ovotestis. They mentioned the theory of Morton (1930) that ripe oocyte must lie there and wait for ovulation stimulus. This period may be short after which the ripe oocytes are resorbed by their nurse cells. They observed degenerating as well as ripe oocytes in the ovotestis of Lymnaea stagnalis. Joosse et al (1968) also found that sperm cells are resorbed by the gland cells of the spermoviduct. They mentioned similar finding by Breucker (1964) in Helix Pomatia.

The small hermaphrodite duct leads into a blind dilated pouch, the carrefour. In fact this area receives the duct of albumen gland, aperture of a small fertilization pocket and the small hermaphrodite duct. From this area the snail's own sperms are directed into the male duct and the foreign sperms, as a result of copulation, also arrive here. In this area egg receives its albumen coat.

Fertilization of the ova either by home sperms or by foreign sperms takes place in this region. In histological sections of the fertilization pocket it is observed that the fertilization of ovum occurs inside the fertilization pocket in Bensonies jacquemonti (see fig.4). There are several views regarding fertilization of ovum either inside the fertilization pocket or at the terminal portion of the hermaphrodite duct. In fact fertilization pocket is in the form of a blind caecum at the end of hermaphrodite duct in Bensonies jacquemonti, while Duncan (1975) quoted several authors giving description of several diverticulae at posterior end of hermaphrodite duct in different snails. e.g. in Succinea, Rigby (1965) in Laevapex, Basch (1959), in Acroloxus, Hubendick (1963), Berry et al (1967) on Ellobidae; and Lind (1973) on Helix Pomatia).

After fertilization the egg is surrounded by the secretion of the albumen as it passes the carrefour. The albumen gland secretes the nutritive material

in the form of albumen. The main constituent of which is galactogen, a galactose polymer (Holm, 1946; Duncan, 1958). As the egg passes down the large hermaphrodite duct, in its female part (oviduct) it receives the additional protective layers. Bayne (1966) suggested that perivitelline fluid is secreted by the albumen gland and that the perivitelline membrane is formed of the same biochemical constituent as the perivitelline. There is an outer thin and inner thick fibrous layer in Agriolimax, it is formed by the contact of perivitelline fluid with the surrounding jelly.

As egg passes through different portions of the convoluted oviduct it receives these layers and then the layer of jelly-like substance that maintains the water balance and mechanical support (Bayne, 1966). Smith (1965) reported that the secretions of the reproductive tract are complex showing different staining properties. From the oviduct egg leads into a spongy gland, the egg shell gland, which forms the outer most layer of the egg in the form of shell. Finally the completed egg is expelled to exterior through vagina, genital atrium and out of genital pore.

CHAPTER - 5

MATING BEHAVIOR AND FERTILIZATION

5.1 MATING BEHAVIOR

Bensonies jacquemonti is hermaphrodite with both systems opening by a single genital aperture to exterior. The bursa copulatrix is in straight line with the aperture while the oviduct is on the side.

The copulation occurs in breeding season when the snails have emerged from aestivation and hibernation. After a rain fall many of them are seen in the act of love play. Copulation can be observed at any time but the maximum number are found from dusk to dawn. The process can be divided into following stages:

5.1.1 PRE COPULATION

In the pre-copulatory behavior two, three or even four snails are found approaching each other with everted dart sac, but ultimately two will copulate and the remaining will move away to find a new mate (Fig.13). The two snails will come close to each other with the aperture of the shell pointing in opposite direction, so that the heads of the snails are also in opposite direction. The two snails will caress each other with their optic tentacles. The dart (love dart) is everted and is pierced in the body of the partner at any point (fig.14,16) this piercing causes the excitement and the caudal gland secretes the secretion. It seems as if this secretion is the identity of sexually excited snails. The two snails lick the caudal secretion of each other

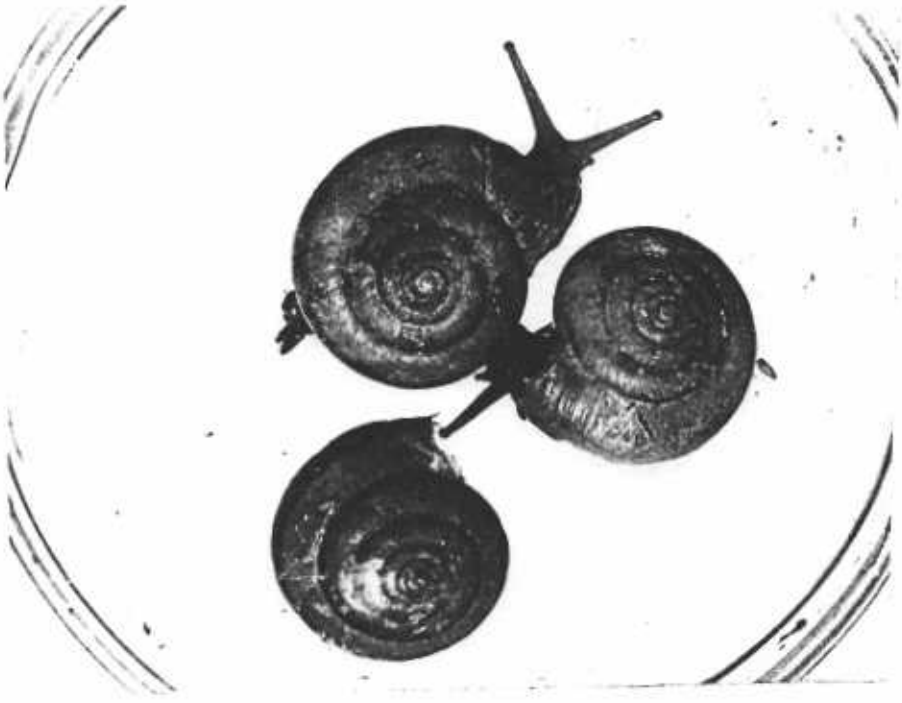

Fig.13: Bensonies jacquemonti precopulation, three snail in love play I dancing in clock wise II dancing in anti-clock wise direction.

Fig.14: Bensonies jacquemonti in pre-copulation note the love dart of I is visible.

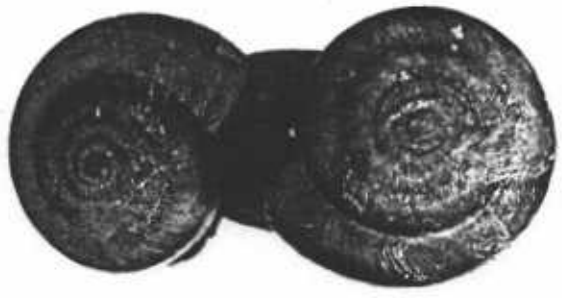

Fig.15: Bensonies jacquemonti (Dorsal view) in copulation, both snails are extremely close to each other feet are under the shell, no disinction of heads or tentacles note penis inside genital pore, this marks the exchange of spermatophore.

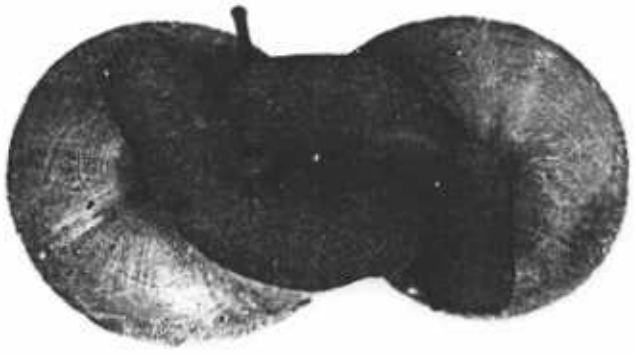

Fig.16: Bensonies jacquemonti (Ventral view) in copulation, love dart seen from the ventral side. Posterior tip of the foot is curved to lodge the head of the partner. Note love dart of both snails piercing in the body of the partner.

and continue caressing with tentacles. Both snails move in circle as if they are dancing. Usually one dances in clock-wise and the other in anti-clockwise direction. The area covered in this dancing state is not more than 40-50 mm. Sometimes one snail remains stationery while the other with the foot fixed on the same place moves in circle (As if some-body takes a circle or moves around on the heel of one leg). After that it comes close to its partner and again pierce the dart in its body and will lick the body of the partner specially the posterior foot region. By now on the posterior dorsal side of the dart a small knob like protru-berance is seen, it is the appearance of the penis. This love play continues for 3-4 hours.

5.1.2 COPULATION

The two snails are now very close to each other (fig.15) the foot of each is bend towards the right side to form a curve making an angle of 20^{o} (fig.16). The heads are inside the curve and licking of caudal secretion is continued. The right optic tentacles are fully retracted and the left one, if protruded, is directed back ward. The surface of the body shines as if vapours are given out, it looks as if it is sweating. The genital pores are near each other but not touching each other. The dart of one snail is out while that of the other is retracted. This snail remains calm and motionless. The

dart is pierced in the body of the partner and if it touches or is inserted (a little) in the genital aperture of the partner, it suddenly moves away with a jerk and again comes close to the partner. If the process is repeated, occasionally the snail simply takes a round turn but do not leave the place. In the last moments the darts are pierced in the head region of each other like hooks thus holding the partners close. The rhythmic movements of the penial complex and dark pink bursa copulatrix are clearly seen through the skin.

The dart of one snail is placed over the head region of its partner as if embracing and holding it fast and kissing the partner as clear caressing is observed.

After sometimes the darts are protruded under the foot. The genital pores are near and opposite each other (fig.17), the heads are inside the curve of the tail region under the shell, so that both snails have a firm hold of each other (see fig.16). The penis of each is inserted in the genital pore of the partner (fig. 18). The two snails remain calm and somewhat motionless for a while. During this perioa, sometimes one snail takes a jerk and gives stroke to its shell only, in clock wise and anticlock wise direction, i.e. leaving the foot and head region motionless, the shell is rotated in forward and backward direction. It seems that if this snail is

Fig.17: Bensonies jacquemonti copulation preceding. The tip of the foot is slightly visible under the shell. Note the genital pore (white) prominent in one snail.

Fig.18: Bensonies jacquemonti in copulation. The tip of the penis of upper snail is about to touch the genital pore of the lower snail. Note the genital atrium of the lower snail is fully everted with love dart seen on left side.

exerting some pressure on itself, may be relieving the spermatophore. This process is repeated two or three times. Sometimes the second snail also repeats this process. Then the two remain calm for 10-15 minutes and it is assumed by the rhythmic movement of the penial complex that transfer of spermatophore is taking place. Ultimately they seperate from each other. Sometimes the tail of the spermatophore is seen hanging from the genital aperture of one of the snail for a while but soon it is sucked in. The snails move away from each other. In the field they move uptil 10-15 feet and then are seen retiring in some safe place while in the laboratory they move toward the edge of the petridish and remain calm.

5.2 FERTILIZATION

In several stylommatophore pulmonates the transfer of sperms take place by means of spermatophore. In these forms either there is mutual transfer of spermatophore between the copulating pair or only one receives the spermatophore (that acting as female) and after a pause the sex role is reversed.

The role of spermatophore and penis is of great significance in the transfer of sperms from male to female. In many forms without penis or copulatory organ the spermatophore formation is essential. As the spermatophore protect sperms and ensure its safe transfer. In Bensonies

jacquemonti there is mutual transfer of spermatophore between the two copulant and the spermatophore is received by the bursa copulatrix.

The fate of the sperms and the detail of the insemination of ovum in this hermaphrodite snail is not clearly established. In Bensonies jacquemonti the mature ovum reaches the fertilization pocket by some unknown mechanism and is fertilized here by the sperm. Poly-spermy is observed as many sperms surround ovum but only one succeeds in fertilizing the ovum while other disintegrate. No actual fusion of ovum and sperm is observed. When the two meet, they are simply applied close to each other (see fig.24, 25). On the bases of these observations it is assumed that the egg is fertilized by the foreign sperm received during copulation. It is reported in the literature that after leaving the spermatophore, the sperms are thought to have descended from the bursa then into vagina and have continued their journey upward in the sperm oviduct to reach the sperma-theca (small evaginations near the fertilization pocket). The foreign sperms are stored here and are used to fertilize the ovum.

5.3 DISCUSSION

The study of the copulatory behavior in pulmonate is best observed in Basommatophora. The aquatic forms are easily reared and copulation may be

induced, by placing isolated individuals in groups, changing the water of the container or providing some special food etc. But in terrestrial forms it is not easy to induce copulation by artificial methods although in some cases success have been achieved.

Seshaiya (1927) observed mating behavior in Lymnaea luteola and found the individual that are slightly bigger in size act as male. Mating is not reciprocal. The two copulant come to the surface of water, the female attaches itself to some object or rim of jar by its foot and male gets hold of the female's shell. Copulation takes about forty minutes. He also observed occasionally three snails in copulation.

Grab (1927) found three snails in copulation one on the upper side acts as male the middle one as both (i.e. female to upper one and male to lower one) and the lower one as female only. He also observed self fertilization in Lymnaea stagnalis. Though in the present study 3-4 Bensonies jacquemonti are observed in love play before copulation but only two succeed in pairing and the remaining move away. Copulation of more than two snails are not seen.

Noland et al (1946) observed that Lymnaea stagnalis appressa say copulated shortly before oviposition. Snails is monoecious and there is no indication that a snail is exclusively (male or female)

or has tendency toward "maleness or femaleness". They observed copulation in detail and found that the first snail which is able to assume the upper or top position will act as male and the lower one as female. During copulation male's foot is firmly attached to the right side of body whorl of female. The penis and preputium after forming an arc over the female shell's edge reach the female genital pore. From the ventral surface of the male foot, an extra quantity of mucus is secreted which helps to bind the two snails, so strongly, that they cannot be seperated without causing injury to the male. The penis remain for 3-4 hours inserted in the female genital pore. These snails may copulate again after completion of first copulation but with reverse sex role.

Lilly (1953) observed copulation in Bithynia tentaculata after their emergence from inactive period. The copulating pair do not extend fully from the shell and head is almost covered by the mantle fold. Penis of male is protruded and inserted into female genital pore. The sperms received are stored inside the bursa copulatrix.

Abdel Malek (1954) studied mating in Helisoma trivolvis. In these snails copulation is proceeded by eversion of penial complex. During mating the verge is protruded and is seen moving from side to side this movement requires large amount of fluid which is provided by secretion of flask-shaped cells in the wall of the verge sac.

Graham (1954) reported the absence of copulatory organ in Ianthina janthina and suggested that there is no real copulation.

Cain (1956) also observed self- and cross-fertilization in Lymnaea stagnalis. Barraud (1957) gave a very detailed description of the copulatory behavior in Lymnaea stagnalis. He observed in preliminary behavior that the snail moves in anti-clock wise direction in search of mate. When it meets the partner, it crawls or climbs on its shell and the foot is drawn up like a cup around the shell of the female. If the female snail moves she carries him on her back. Sometimes in attempt to overcome the female, the female may loose hold of the foot and both may fall to the bottom of the pond or float to the surface, but male still have the grip on her back. He further observed chain or multiple copulation, self fertilization and even false fertilization in this species.

In Stylommatophora the copulation is usually preceeded by an intricate "love play"courtship. Hyman (1967) stated observation of many authors on mating behavior. Some of them are described here:

Meisenheimer (1907, 1912) observed Helix pomatia before copulation crawls about in seeking manner and lifts the anterior part of the body at short intervals. When two such snails meet each other, the anterior foot

soles are firmly pressed against each other while posterior foot is fixed to the ground. They exchange caresses with their tentacles after a pause of 15-30 minutes The love dart is not ejected simultaneously in the two partners but when shot in the partner's body seems to cause considerable pain. Ultimately the penis is inserted into the gonopore of the partner and long spermatophore is transferred in about 3-4 hours. The dart is lost and a new one is formed in next few days.

In Bensonies jacquemonti it is observed that the crawling and searching behavior are similar. The love dart is shot at random and at the end of the 3-4 hours love play, the love dart is not lost but is retracted inside its sac. The penis deliver the spermatophore mutually.

Hyman (1967) observed copulation in Arion empiricorum which move in circle and lick caudal gland of each other. Finally the right sides are pressed against each other. The genital atrium are everted and touch each other and then the spermatophores are exchanged. She also quoted (Kun Kel, 1900) that in Limacidae sexual behavior follows the usual courtship, moving, seeking, trailing each other in circle and licking the caudal gland. The anterior part of the body of the snail winds spirally around each other, the penes are protruded and mutual exchange of spermatophore takes place.

Bensonies jacquemonti also dance in circle in anticlock wise or clock wise direction and there is continuous caressing with optic tentacles and licking of the caudal secretion but no winding up of the body.

Hyman gave description of (Adam, 1898; Gerbardt, 1933, 1934; and Chace, 1952) . Limax maximum climb a tree trunk or wall and after courtship for 1-3 hours suddenly wind spirally around each other and hang down-wards by a long thick mucus strand (10-25 cm long). The heads are pointing down-wards.The penis unrolled in long cord and form a tight knot. Sperm mass is exchanged. The penis is retracted and they ascend after eating the mucus cord.

Duncan (1975) quoted Runham and Hunter (1970) that Agriolimax retriculatus crawls and caresses its partner and periodically thrust its head to strike the partner. It moves in circle, then the right sides of the body are pressed together. The genital pore touch each other and there is exchange of spermatophore.

Abdul Malek (1954) observed two types of ova in the fertilization pocket of Biomphalaria Biossyi. Ova of first type are smaller and similar in size as found in acini of ovotestis. They apparently have left the ovo-testis and are not inseminated. The second type of ova are large irregular in out-line and seem that they have been inseminated earlier before reaching fertilization pocket. These ova are found in fertilization pocket and also in

terminal oviduct. Abdul Malek was of the opinion that insemination usually occurs in the terminal portion of hermaphrodite duct as fertilized ova are observed in this portion. He further added that fertilization by foreign sperms (received during copulation) occurs in fertilization pocket or in terminal portion of hermaphrodite duct while insemination of ova with home sperm (animal's own sperm) only occurs in the terminal portion of hermaphrodite duct.

Graham (1954) stated that in Ianthina janthina fertilization takes place in the ovary and in the same acinis in which the egg matures and soon after its release from the germinal epithelium. He suggested, as there is no bursa copulatrix, seminal vesicle and male copulatory organ (Penis), there is no real copulation. A large number of oligopyren spermatozoa of complex structure along with eupyren spermatozoa are formed in the male phase. Eupyren becomes fastened to the tail of oligopyren thus forming complex structure called as spermatozeugmata. The oligopyren sperms act as a kind of carrier of the eupyren and can swim through water carrying them. Fertilization is achieved when carrier sperm enters oviduct. Graham also quoted Ankel (1930) who found similar male phase in I. pallida and formation of spermatozeugmata. Morton (1968) described eupyren sperm as fertile while oligopyren un-fertile. Eupyren are small and normal sperms while oligopyren are much larger, may lack flagella or bear a tuft of flagella at one end.

Duncan (1958) observed that Physa fontinalis under laboratory condition is capable of self fertilization. The eggs are fertilized in the hermaphrodite duct. As in the fertilization pocket the eggs were found with albumen covering while some fertilized eggs in hermaphrodite duct were undergoing division.

Lind (1973) traced the migration of the sperms from the bursa to the spermatheca. He also pointed out as, there is frequent occurrence of recipient's own sperms in the sperm oviduct, so these may be confused with the foreign sperms. He found migrating sperms in the duct of fertilization pocket and later inside the spermathecal sac. The orientation of foreign sperms are such that their heads rest against epithelial cells. He further added that the function of the secretion of bursa, beside resorption of excess sperms, is to activate the sperms.

Whether self fertilization, cross fertilization or both types of fertilization that takes place in pulmonates snails, the insemination probably occurs at two loci i.e. either in the fertilization pocket or in the terminal portion of the hermaphrodite duct.

Raven (1958) and Duncan 1975 observed that at the time of fertilization sperm passes through egg cytoplasm and rests near the egg cortex. The sperm head becomes rounded and tail is resorbed in egg cytoplasm in Lymnaea, Limax and Bulinus.

In pulmonates the variability of fertilization depends both upon species and on environmental conditions. The foreign sperms are generally found more effective, one cause of this may be, that they have exposed to prostatic secretion and bursa secretions.

CHAPTER - 6

OVIPOSITION

Large number of snails are seen depositing eggs clutches in two breeding seasons i.e. April-June and then in September-November. During breeding seasons the oviducts are full of eggs (see fig.19).

6.1 PRE-OVIPOSITION

The egg laying is usually proceeded by making a hole or egg chamber in the soil into which the eggs are deposited. The egg hole is only dug when the soil is soft and loose otherwise the eggs are deposited just under fallen leaves and under stones. Very rarely the eggs are found exposed on the surface of soil.

EGG CHAMBER

The snails are not observed in actual process or act of making the egg chamber but it is presumed that they dig egg chamber with the help of head and anterior foot region. A completely finished egg chamber is 20-35 mm in depth and 4-6 mm diameter. The chamber is usually rounded corresponding with the shape of anterior foot and head of the snail. It is further observed that the chamber is not dug straight into the soil but slightly bent (diagonal). Thus forming an angle of 10^{o}. Apparently there is no correlation between the size of the clutch (i.e. number of eggs) and the depth of the egg chamber. It looks like that if the soil is loose the hole will be more deep.

Fig.19: Genital system of Bensonies jacquemonti showing oviduct filled with eggs. Note the ruptured oviduct and the already secreted protective covering of the egg.

The actual period for egg laying is not observed in the field (natural environment). But a record was maintained to note the time spent by a snail at the site of egg chamber i.e..when the snail is first observed at the site and then when it leaves the site. This time interval only signifies the time in which the snail had laid the eggs. But there was no way to ascertain that egg chamber was already made or this time included the construction of chamber and egg laying. In present observation time varied from 60-150 minutes.

The egg laying snails are easily detected in the field as they are firmly attached to the soil, with the aperture of the shell pointing towards the soil. They are usually mistaken as empty shells but when tried to pick them up they were found to be attached to the soil and some force has to be applied to dislodge them.

Such egg laying snails were collected for the observation of oviposition and watched under a binocular microscope.

6.2 OVIPOSITION:

The snail looks calm and motion less in the petridish. The head region is slightly bend towards the left side. The following steps are observed during egg laying:

1. Great dilation occurs in the head region which can be judged by the prominent muscular action of this area (Fig.20).

2. Both pairs of tentacles (anterior as well as optic tentacles) are fully retracted inside head region.
3. The snout and the tip of foot are sudden-ly pulled upward (toward shell).
4. The genital pore becomes prominent as white spot.
5. The egg appears at the genital pore (Fig.20,21)
6. The egg is pushed out rapidly until it is fully expelled.
7. The genital aperture subsides to complete the process.

The egg remains at the genital pore until next egg comes out and pushes it or the snail moves its snout away. Usually the egg is held at the genital pore for 7-8 seconds.

The time taken in appearance of the egg at the genital aperture and its final extrusion is noted to be 3 to 5 seconds.

Though deposition of an egg clutch apparently seems to be a continuous process and eggs are laid one by one, still the time interval between succeeding eggs varies from 1-25 seconds.

6.3 POST OVIPOSITION

In the field the mother snail after oviposition covers the enterance of the egg chamber with the loose soil. Because there is no sign of open hole on the site of

Fig.20: Bensonies jacquemonti during oviposition the head is dilated, both pairs of tentacles retracted. Note the fourth egg making its exit at the genital pore.

Fig.21: Bensonies jacquemonti during oviposition with almost complete egg clutch (10 eggs) the last egg is still attached to genital pore after its extrusion.

Fig.22: Bensonies jacquemonti as seen after oviposition. The snail remains at the site in the same position.

oviposition previously marked. The site of oviposition was marked by flag marks stating the date and time of egg-laying. The snails, are left undisturbed in the process. Later on when the holes are searched there was no superfecial sign of the hole except the flag mark. When the site is dug the eggs are found in the form of small heap of pearls inside the egg chamber. The eggs are laid in the bottom of the chamber and a small air space is observed above the eggs.

In the laboratory it is observed that the snail after it has deposited eggs remains at the site in the same position for 10-20 minutes. (Fig.22). Afterwards the tentacles are protruded and it moves away from the site. Some snails feed and move about while others simply secrete a thin slim covering around the aperture of the shell after retreating inside.

When the snails are disturbed in the process of egg-laying in the field, some of them will withdraw their head from the egg chamber and will simply move away. These snails seem to be in initial stage of egg laying, therefore they have retreated from the egg chamber. On other occasions it is observed that the snail when disturbed still remains at the site and will continue the process. If the head of such snails is pulled out from the ground, an egg is found attached near the genital pore. This shows that this snail was under the process of actual oviposition after making the

egg chamber. When such snails are picked during process of oviposition the entire head and anterior foot region is surrounded by loose soil which is the result of making egg chamber. Egg laying snail were collected from the field and in the laboratory they were isolated individually into petridishes for observations. Some of them started egg laying immediately while others paused for 20-40 minutes and in some cases the time interval was even more than one hour. Thus again making up their mind to lay eggs.

In the laboratory while observing oviposition many interesting events were noted:

In one instance instead of one egg, two eggs were pushed out one behind other from the genital pore. Then there was an interval of 20 minutes and again two eggs are pushed together. After 10 minutes there appeares a single egg at the genital pore and this is followed by two more eggs laid one by one at an interval of 10 minutes thus making the number of clutch 14, whole oviposition is completed in 3-4 hours. In an other observation the snail laid three eggs at time and then there was a pause of ten minutes and after this six eggs are pushed rapidly in the form of chain. The total time covered is less than three seconds. The eggs in this clutch were nine. After completing the clutch the snail with the help of its anterior tentacles tried to move the eggs under its foot perhaps to arrange them, but later on left them as

such because she has come across the caudal secretion and starts licking it and then move, away from that place leaving the eggs as such. One snail showed a queer behavior. This individual laid one egg and did not lay further eggs till 12 hours period of observation and then laid another egg. The snail was kept under observation for three days and then released in the breeding area after marking it. Next morning it was found copulating alongwith other snail in the field. From this interesting observation it may be hypothesised that copulation may be repeated as sperms in this prolonged detaining may have been absorbed and fresh sperm are needed to fertilize the egg. Thus eggs can be detained till recopulation occurs.

Recopulation does occur in the field when those snails which have already oviposited in the laboratory were marked and released in the breeding area.

Some snails use to eat their own eggs as is observed in present study. Nine eggs were deposited by a snail in a petridish and after 30 minutes only two empty shells of eggs were found near the mouth of the snail. This shows that during eating eggs the snail first cracks the egg shell sucks the inner content (albumen etc) and lastly the egg shell is eaten. This was observed on three different occasions. When the snail were collected during oviposition, kept in a close petridish and even after the oviposition when the snail is left inside the petridish alongwith the eggs.

In the laboratory it is observed that snail likes to lay eggs near solid margin or edge of petri dishes and flower pots. There is no correlation between the size of egg, size of clutch (egg number) and size of shell (see Table 1).

During breeding season at least two clutches of egg are laid. This is confirmed as egg laying snails were marked on first oviposition and if such marked snails were observed depositing eggs again this shows that at least two clutches of eggs are laid during a breeding season. The laboratory study showed that almost all eggs are fertile when they are deposited as only 1.09% eggs failed to hatch due to some unknown reason (see Table II).

6.4 DISCUSSION

Hyman, (1967) quoted a number of authors, Meisenheimer (1912) for Helix pomatia; Herzberg and Herzberg (1962) for Helix aspersa and Dasen (1933) for Ariophanta ligulata that the snail dig 1 to 2 inches deep hole for eggs and uses its foot for pushing out the soil from the hole. They stated that several hours are spent in making the hole. B. jacquemonti is observed in the field spending several hours during oviposition but there was no extra soil on the sides of the egg chamber to mark that soil is being pushed out. It seems that the egg chamber is formed simply by pushing in the head and anterior foot region in the soft soil.

Owiny (1974) reported that Limicolaria martensiana deposits eggs only in rainy season while retains them in uterus in dry season. The eggs are laid in a "nest" dug by the parent with the help of the foot. The foot is drawn out gradually as it lays eggs. After completion of egg laying the "nest" is covered with soil. Observations in the present study agree with the findings of Owiny (1974). Bensonies uses its foot and anterior head region to dig the egg chamber and after completion of oviposition covers the chamber with soil. The difference is that L. martensiana withdraws its foot gradually as it lays the eggs while B. jacquemonti withdraws its foot once for all after completion of the clutch as eggs are found in one group at the end of the chamber and not scattered.

Plummer (1975) observed that in Archachatina marginata the two individual after mating produce their own eggs clutch in one spherical mass under the soil surface. Oviposition takes several hours.

Duncan (1975) described his personal observation and that of (Palseneer 1901; Berry, 1931, 1965) that many Stylommatophora are ovo-viviparous. The eggs are retained inside the uterus until they develope into young ones.

Vander Laan (1978) found egg mases of Helminthoglypha arrosa in shallow holes in soil or below fallen leaves. The oviposition is positively correlated with total rainfall. In rainy season egg production is more.

Hodasi (1979) quoted (Mohr 1949; Rees, 1951; Ghoose, 1959; Kekauha, 1966) that in Achatina fulica the clutch size varies from 37-305 eggs while he has recorded 35-87 eggs in 24 clutches in first breeding season and 6475 eggs in second breeding season in 36 clutches. He calculated the average of 60 clutches as 167.7 eggs per clutch. He was not sure of the actual clutch size (number) of some of the snails as occasionally two snails have used the same chamber jointly for egg-laying.

Hodasi (1979) also observed egg-laying in Achatina and stated that during breeding season the genital aperture of mature snail becomes prominent and protruded. In present observation in B. jacquemonti the genital aperture was not prominent or protruded during breeding season. He further noticed that right anterior tentacle is often busy in directing the eggs as they are deposited. No such use of anterior tentacle in B. jacquemonti is observed during egg laying. Hodasi further stated that there is no direct relationship between size of egg and size of shell. But larger snail tended to lay larger eggs.

Tompa (1980) stated that Pomatia elegans releases only one egg at a time, so that each one is coated with soil (calcium) because if many eggs are deposited inside an egg chamber most of the eggs would not be in direct contact with the soil calcium.

In B. jacquemonti there is only one egg clutch and the number of eggs in a clutch is very small i.e it

varies from 4-14 eggs per clutch as compared to other. Helix arrosa, 60-75 eggs, Limax martensiana 100-200, Achatina fulica 200-300. Achatina achanina-300-500 (Hyman, 1967).

Pawson and Chase (1984) stated that in Achatina fulica clutches of eggs are found in pairs in seperate location and they exhibit similar hatching dates. They also observed that Achatina fulica retain eggs in uterus and lay them either shortly before hatching or 2 or 3 days before hatching.

CHAPTER - 7

DEVELOPMENT (EMBRYOLOGY)

In Bensonies jacquemonti the developmental period (oviposition to hatching) varies from 14 to 18 days. Incubation period varies with temperature and humidity. During high temperature (90° - 100°F) in summer the incubation period is observed to be 14 days while in winter or in rainy season when temperature decreases (60 - 80°F), the incubation period is noted to be 18-21 days (Table I,III).

Apparently, all eggs are fertilized and are capable of development. Very rarely one or two failed to develop because of unknown reasons (see Table II). Different eggs of the same clutch showed embryoes in different stages of development, which shows that development started as soon as the egg is laid. It is observed that soon after ovulation and fertilization, while the egg is on its way through genital tract, certain changes occur in its cytoplasm. In other words cleavage begins two or three hours before egg is laid.

7.1 EGG STRUCTURE

The freshly laid egg of B. jacquemonti is rounded, galatinous, pure white in colour and covered by an elastic envelop which becomes stiff, to give a firm round shape to the egg. The embryo is white spherical structure which lies centrally and occupies 1/100 of the egg volume.

Table-I Size and number of eggs in each clutch and their incubation period in B. jacquemonti under laboratory conditions

Shell size	Clutch size	Egg size	Incubation period
	(No.of eggs)		
21.9 mm	12	2.8 mm	14 days
18.8 "	15	3.5 "	14 "
21.8 "	12	2.8 "	13 "
21.0 "	10	2.8 "	14 "
18.8 "	10	2.8 "	15 "
20.6 "	14	3.0 "	16 "
18.8 "	10	3.4 "	13 "
19.2 "	10	4.0 "	15 "
21.2 "	12	4.0 "	18 "
20.9 "	13	3.9 "	18 "
19.5 "	10	4 "	21 "
20.0 "	8	3.6 "	21 "
20.8 "	12	3.5 "	7 "

Average incubation days = 15.30

Table-II Showing mortality rate in each clutch observed under laboratory conditions.

Clutch No	Eggs clutch	No. of unhatched egg
1	14	Nil
2	15	"
3	14	"
4	18	"
5	16	1
6	10	Nil
7	11	"
8	13	"
9	10	1
10	8	Nil
11	9	"
12	13	"
13	9	"
14	14	"
15	10	"

Mortality rate = 1.09%

Table-III Incubation period (time from oviposition to hatching) in different seasons in B. jacquemonti

Clutch No.	Egg Number	Laid on	Hatched	Incubation period
1	14	15 May	29 May	14 days
2	15	16 "	30 "	14 "
3	14	Ist June	13 June	13 "
4	18	2 May	17 May	15 "
5	16	14 June	27 June	13 "
6	10	7 "	20 "	13 "
7	13	12 Nov.	Ist Dec.	18 "
8	11	20 "	8 "	18 "
9	10	5 Dec.	26 "	21 "
10	8	6 "	27 "	21 "
11	12	24 Sept.	30 Sept.	7 "

Average Incubation days = 15.1

For isolation of the main egg layers freshly laid eggs are taken singly i.e. one at a time and washed (if any soil is attached to it) and placed under a binocular microscope. The egg is held in position by a forceps pierced by a fine entomological needle from one side and care being taken not to puncture the perivitelline membrane. The aperature is then widened with great care. If the albumen membrane is ruptured, the damaged eggs are discarded. The egg shell is held on one side with the help of inserted needle and the perivitelline sac is squeezed out of the shell very carefully with forceps.

The albumen membrane is difficult to be seperated from the albumen sac, but if the egg is boiled or treated by a fixative, it is easily detached from the albumen.

The egg is found to be composed of outer shell, a thin shell membrane, jelly and perivitelline or albumen sac. Shell forms the outer protective covering of the eggs. It is white in colour, when freshly laid it is soft, porous, but becomes semi rigid when exposed to air. The shell is about 0.05 mm thick. It is lined internally by a very delicate transparent shell membrane.

The jelly occupy the space between shell membrane and perivitelline sac. It is in the form of a layer. It absorbs water very rapidly and become swollen when the egg is placed in water. If jelly is teased with a needle

and squeezed, while holding the perivitelline sac with a forceps, it adhers to the watch glass.

The perivitelline or albumen sac forms the thick globular fluid mass which fills the entire space inside the shell. A thin transparent albumen membrane firmly covers the albumen sac. It also gives mechanical strength to the egg along with egg shell, as if the egg is deshelled the membrane has enough strength to maintain the round shape of the albumen. This membrane is also permeable to solutes.

Albumen forms the transparent fluid sphere, inside which floats the embryo. If the albumen membrane of a freshy laid egg is pierced with needle the fluid albumen flows out carrying the embryo with it. There is no free larval stage in the development. Therefore the amount of albumen is more than those having free larval forms.

Bayne (1966) stated that extract from the albumen gland of Helix Spp. can be used to diagnose blood group A of man (.T.E. Thompson, personal Communication). The statement was confirmed by testing the human blood group A with the extract of albumen gland of Bensonies jacquemonti. The test was positive as the blood clotted by the addition of albumen extract.

7.2 OOGENESIS

Like other pulmonates in Bensonies jacquemonti egg formation is of follicular type. The egg cells are budded off from the germinal epithelium of the gonad. These cells then pass to the upper portion of the acini of the gonad by their amoeboid movement and settle there. The oocyte is still connected with connective tissue of the wall of acini. Oocyte begins to grow and is surrounded by a follicle. (Follicle appear to be composed of two distinct layers see Fig.23). The older oocyte (after attaining a certain size) is surrounded by the inner layer of follicle consisting of 6 - 7 cells.

In Bensonies jacquemonti, in the beginning the follicle cells are closely applied to the surface of the oocyte but later on a groove appears in its upper side and extends in lateral direction thus forming a follicular cavity between oocyte and follicle cells. At the approach of ovulation this cavity extends and oocyte is detached from the wall of the gonad. Now by autolysis of the follicle cells, the full grown oocyte is set free (see fig. 25 also).

7.3 EGG MATURATION

As soon as the oocyte has left the gonad and it enters the first part of small hermaphrodite duct; egg maturation begins. It is assumed that first and second maturation division has taken place here.

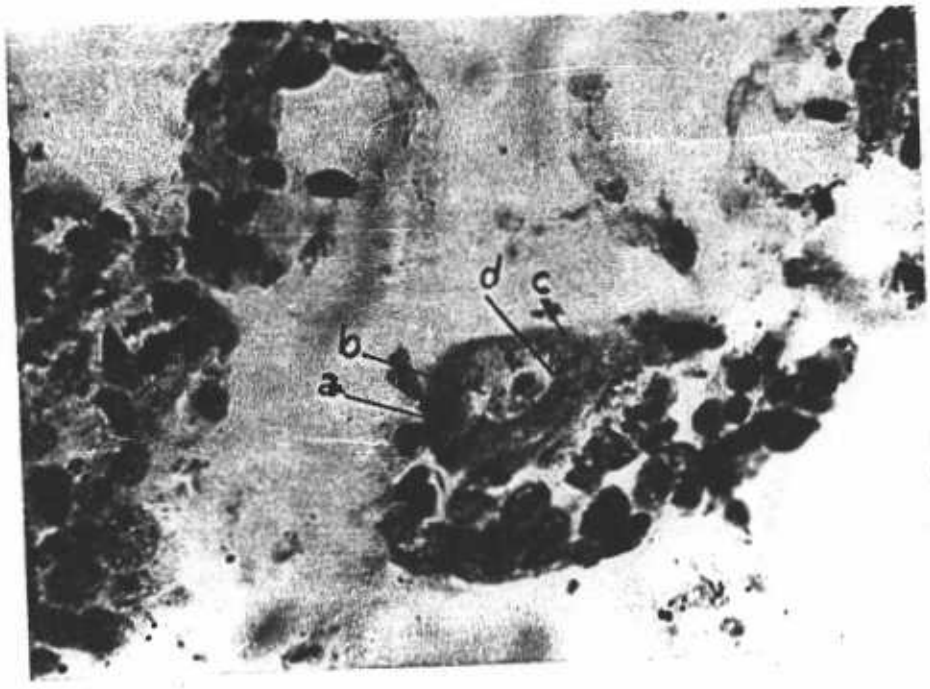

Fig.23: Microphotograph of the ovotestis of Bensonies jacquemonti showing all stages of oogenesis. Note 2 layers of follicle, follicular cavity and cells arrangement (X 1000).

a. Follicle cell
b. Follicular cavity
c. Outer layer
d. Inner layer

7.4 FERTILIZATION

Due to the secretion of some stimulus the mature egg reaches fertilization pocket or carrefour. The sperms released by the spermatophore received during copulation are stored in spermatheca, from there the foreign sperms arrive at the fertilization pocket and fertilizes the mature egg. Polyspermy is observed (fig. 24) but only one sperm is successful in getting entry into the ovum while other disintegrate. No actual fusion of the two pronuclei is observed. When the two met, they are simply applied close to each other (fig. 25).

7.5 OOPLASMIC SEGREGATION

When full grown ovum is ready for ovulation its cytoplasm is filled with numerous granules of different size. These granules are generally more or less evenly distributed through out the egg. Immediately after ovulation and fertilization when the egg is passing through the genital tract and ready for oviposition, a clear "ooplasmic segregation" takes place (fig. 26). By ooplasmic segregation the cytoplasmic composition becomes different in different parts of the egg i.e. unequally distributed.

In Bensonies jacquemonti an egg which is fixed before oviposition the accumulation is in the form of a ring of 8-9 patches. It may be assumed that these sub-cortical, patches correspond to the positions of the nuclei of a whorl of six or seven cells in the inner

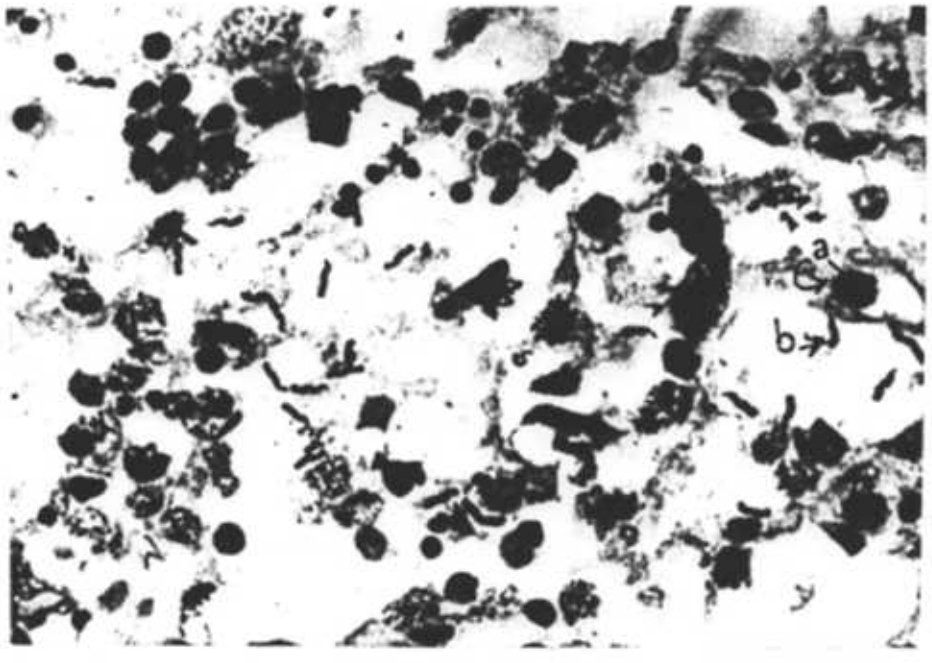

Fig.24: Microphotograph of a portion of carrefour of Bensonies jacquemonti showing sperms travelling toward ovum (X 400).

a. Ovum
b. Sperm

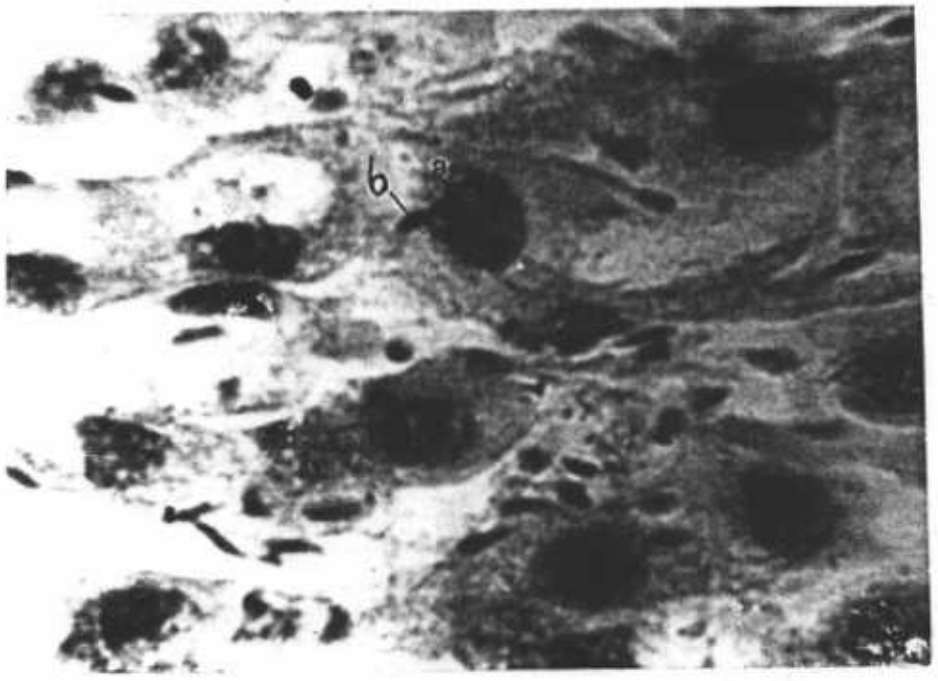

Fig.25: Microphotograph of a portion of fertilization pocket of Bensonies jacquemonti showing union of egg and sperm. Note sperm has entered the egg membrane (X 1000).

a. Ovum
b. Sperm

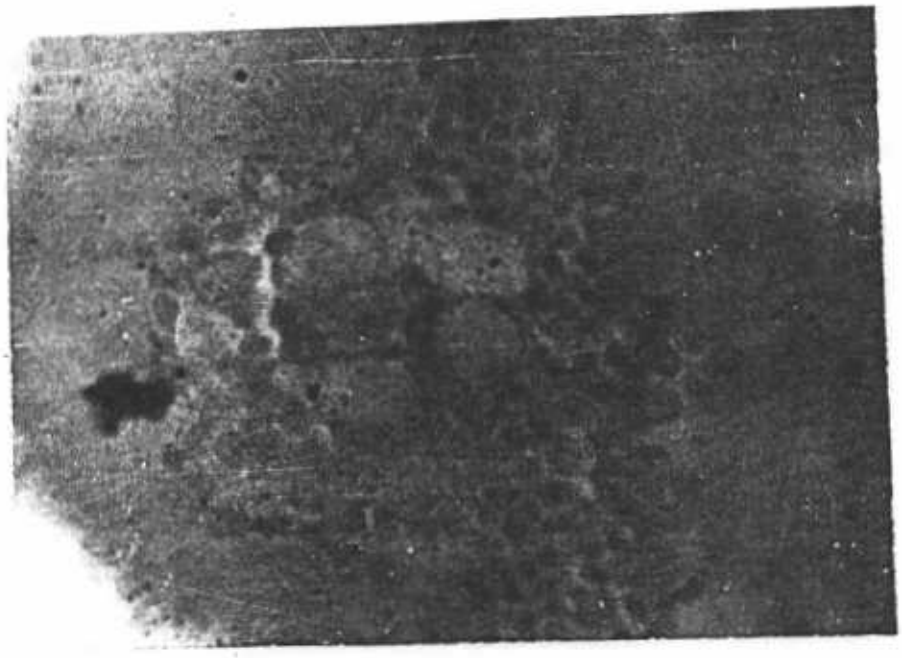

Fig.26: Microphotograph of an egg of Bensonies jacquemonti taken from the oviduct before oviposition showing ooplasmic segregation (X 1000).

layer of follicle, surrounding the growing oocyte (fig. 27, 29). It is obvious that cell determination is dependent upon the material composition of the cytoplasm, and ooplasmic segregation is important for cell determination.

7.6 EMBRYOLOGY

EGG BEFORE OVIPOSITION

The very small fertilized egg or oocyte while still inside oviduct shows a vacuolated cytoplasm and a nucleus on one side i.e. animal pole. Thus the fertilized ovum forms a germinal disc which lies on one side of the egg. The remaining egg is filled with albumen which forms the dominant extra embryonic source of nutrition for the developing embryo. Average diameter of an egg is 0.2-0.3 mm. Oo-plasmic segregation takes place by concentration of certain substances at vegetative pole which forms vegetative pole plasm, later on this extends in the form of a uniform layer around the egg forming the sub-cortical plasm. Oo-plasmic segregation continues, result is in the form of 7-9 lenticular patches of cytoplasm (see fig. 27, 28).

DAY I EGG

The egg observed just after oviposition has started segmentation. Due to scanty yolk, segmentation is more or less equal. The first cleavage furrow that

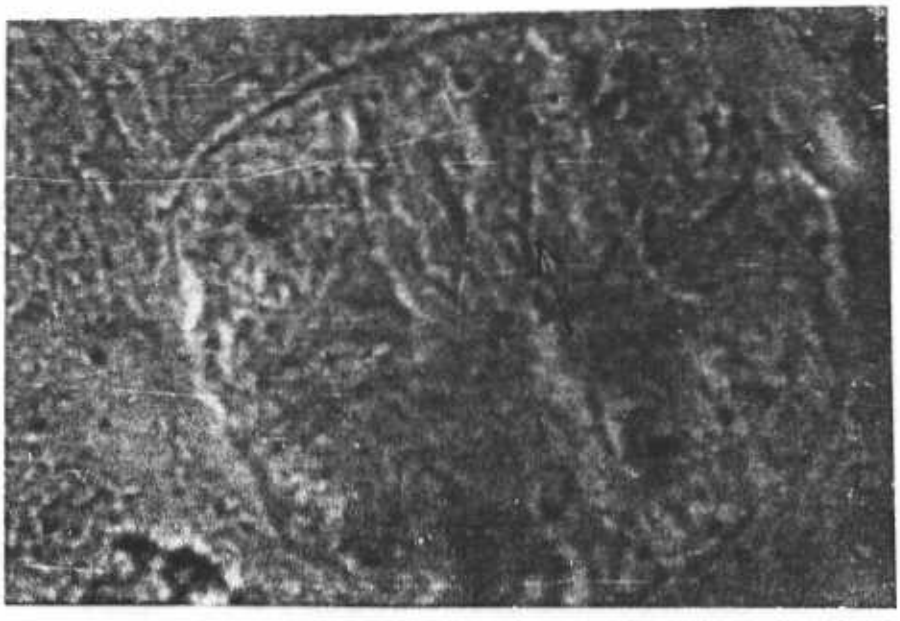

Fig.27: Microphotograph of an egg of Bensonies jacquemonti taken from the oviduct before oviposition. Note 7-9 lenticular patches (X 1000).

Fig.28: Microphotograph of a section of an egg of Bensonies jacquemonti before oviposition showing formation of vegetative pole and sub-cortical plasm (X 1000).

a. Sub-cortical plasm
b. Vegetative pole

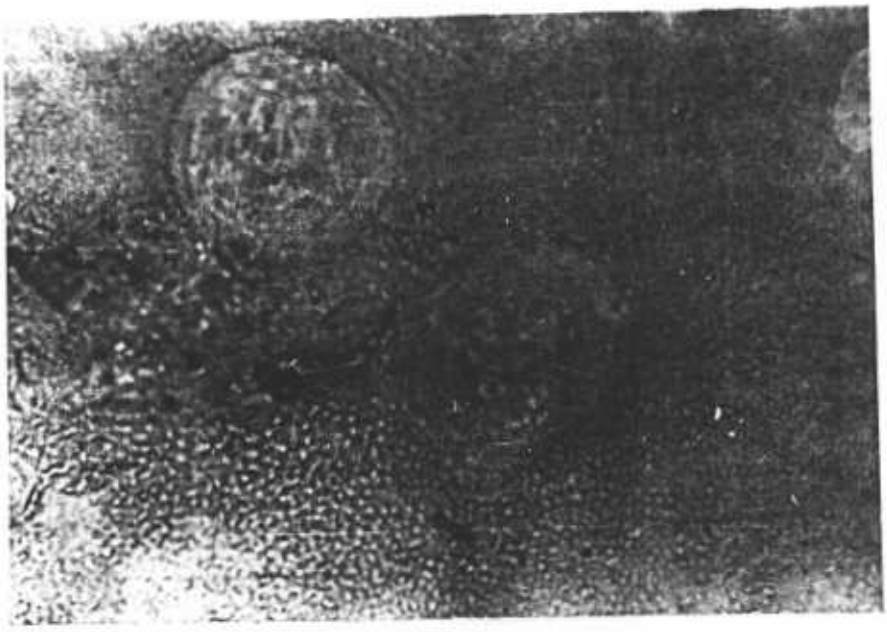

Fig.29: Microphotograph of an egg of Bensonies jacquemonti just after oviposition showing two cell stage (X 1000).

divides the egg into two equal halves is observed (fig.29).

7.6.1 MORULA

Two or three hours after oviposition the germinal disc with numerous nuclei without definite cell boundry was observed. This probably represented the morula stage. The cells appeared equal in size. Without distinction of micro-or mega-meres. The cleavage seems to be total and spiral (fig. 30).

7.6.2 BLASTULA

In the cells, without differentiation of micro-and megameres a cavity is formed the segmentation cavity, which seems to increases in size gradually. In pulmonates the cleavage gives rise to a coeloblastula with a wide cleavage cavity (Raven, 1975). In embryo of about 1½ day, there is distinction of upper micro-meres and lower macro-or megameres. The cells increase in number and the cleavage cavity expands till a blastula with large blastocoel is formed (Fig. 31).

At this stage absorption of the albumen has started but the process was not very clear. The special cells called pinocytes (fig. 31) begin albumen ingestion. Before gastrulation pinocytosis has started. Pinocytosis is marked by circular cells inside which are vacuoles (pinosomes) usually several pinosomes fuse and form bigger vacuole with albumen inside (Fironi, 1977; Raven, 1946). In Bensonies these fused albumen vacuoles are of very large size.

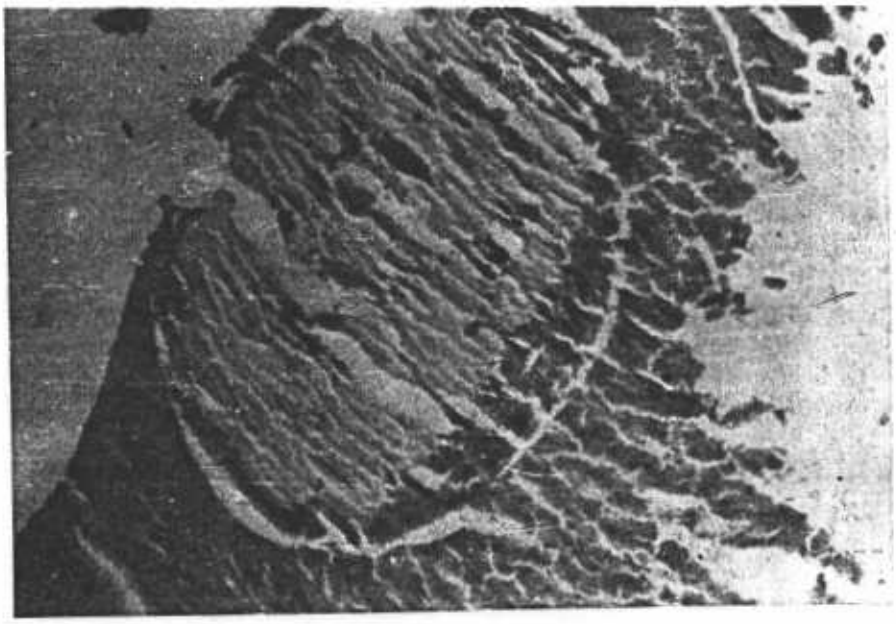

Fig.30: Microphotograph of an egg of Bensonies jacquemonti 2-3 hours after oviposition showing morula like structure (X 1000).

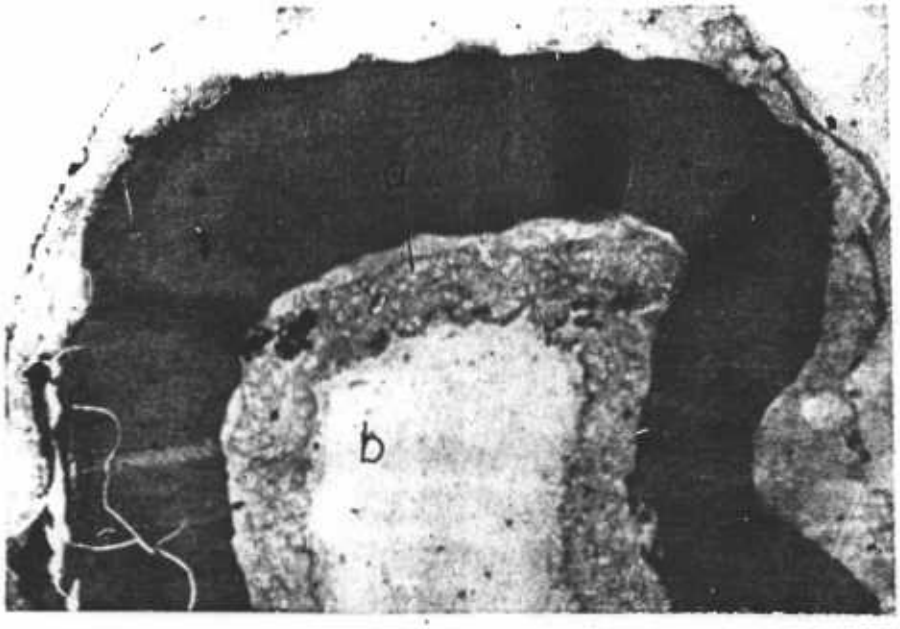

Fig.31: Microphotograph of day I embryo of Bensonies jacquemonti showing blustula. Note pinocyte with ingested albumen and wide blastocoel (X 40).

a. Pinocyte
b. Blastocoel

7.6.3 GASTRULATION

In day-II embryo the cells have arranged themselves in layers. Until now pinocytosis was rapid at ectoplasmic part in special albumen vacuoles. Gastrulation takes place by embolic invagination of the cells at (fig. 32) vegetative pole. The albumen ingestion is now more or less restricted t o endoderm. The albumen is taken in and digested in albumen sac or larval digestive gland that has developed from endoderm. The embryo is now more or less pear-shaped in out-line. Between ectoderm and endoderm the mesodermal cells penetrate to form a loose network (Fig.33).

7.6.4 LARVAL DEVELOPMENT (PRETORSION)

DAY-III

The day-III embryo is bilaterally symmetrical and anterio-posteriorly elongated. The typical molluscan trochophore or veliger larvae is not present as the whole development is passed inside the egg capsule from which they hatch as young crawling snails.

In typical trophophore larva, there is a prototroch and post-trochal regions. In further development the prototroch grows into velum. However in B. jacquemonti the prototroch is greatly reduced and lack the apical tuft and is represented by the velum. The foot and shell gland primordia are formed. Larval

Fig.32: Microphotograph of day II embryo of Bensonies jacquemonti showing gastrula formation by emboly at vegetative pole. Note blastopore and large pinosomes (X 40).

a. Blastopore
b. Pinosome

Fig.33: Microphotograph of day II embryo of Bensonies jacquemonti showing formation of mesodermal cells (X 100).

body has made its appearance near the side of digestive gland, its wall is many cell thick. The foot rudiment starts as ectodermal thickening on the mid ventral side of the embryo behind the mouth. Later on foot rudiment elongates, flattens and then bends dorsally to form the podocyst. (Fig. 34). Primordia of cerebral ganglion appears.

DAY-3½

The embryo is a-symmetrical, foot and podocyst have elongated considerably. Podocyst is hooked at its anterior end while enlarged (fig. 35) larval body is spoon - shaped at the posterior end.

There is the appearance of a short neck-like structure connecting the larval body to visceral hump. Ghose (1963) stated resemblance between larval body and cephalic vesicle of the other Stylommatophora. Shell gland is dome-like. Cerebral, pedal, pleural and visceral ganglion are distinct by their gross appearance and position. Kidney makes its appearance along with pericardium. Mesenchyme cells are scattered in between ectoderm and endoderm layers.

DAY-IV (POSTTORSION)

This stage follows immediately after torsion. Torsion occurs through differential growth of two sides of the embryo. The shape of the embryo has become more

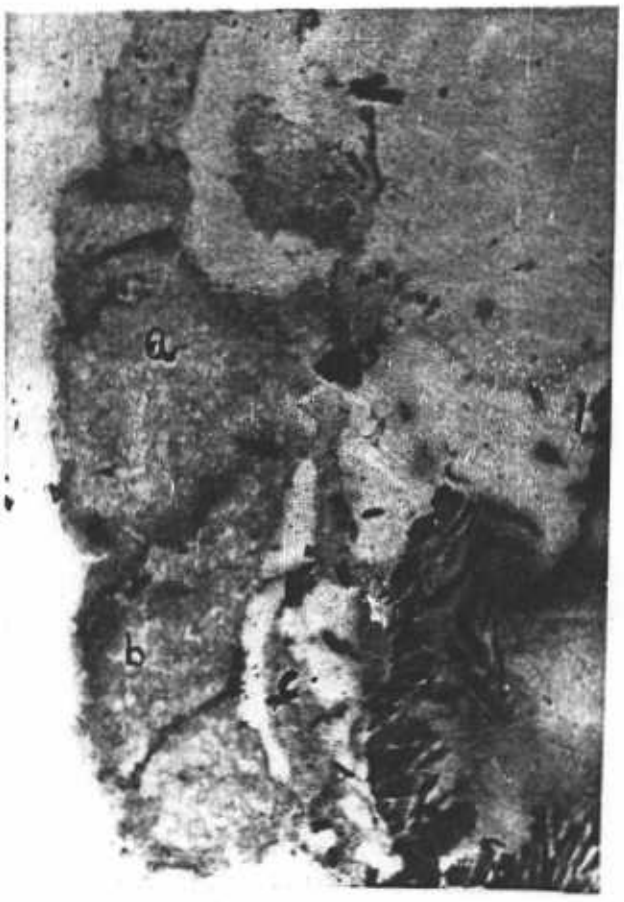

Fig.34: Microphotograph of a day III embryo of Bensonies jacquemonti showing formation of podocyst larval body and visceral hump, note neck like structure connecting larval body to visceral hump. (X 40).

a. Podocyst
b. Larval body
c. Visceral hump

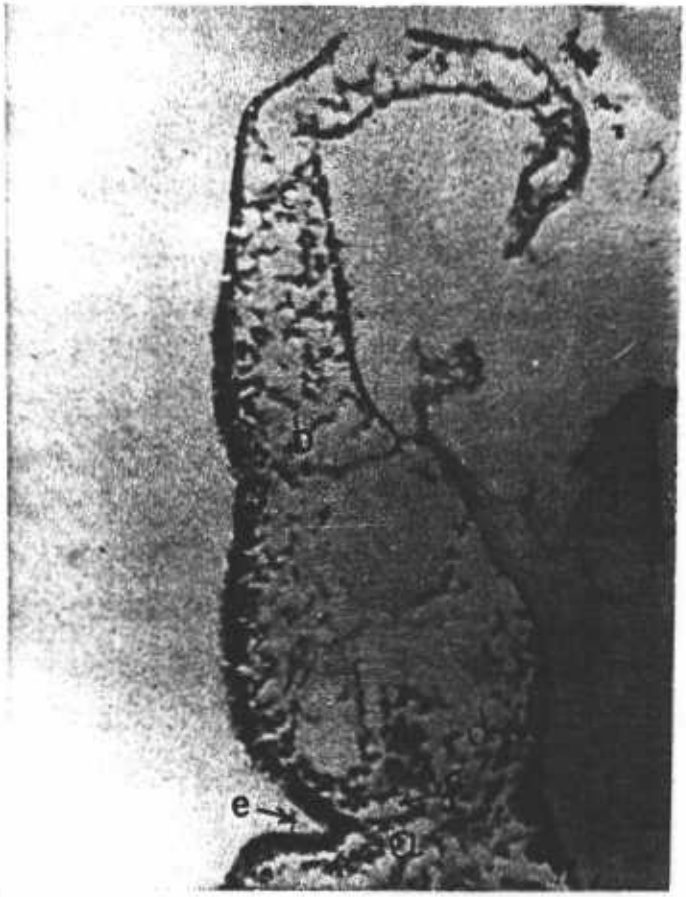

Fig.35: Microphotograph of a day III embryo of Bensonies jacquemonti showing hook-like podocyst and spoon-shaped larval body (X 100).

a. Larval body
b. Foot
c. Podocyst
d. Shell gland
e. Mouth
f. Prototroch

asymmetrical due to torsion. Foot has assumed triangular shape with pedal gland (fig. 36). Immediately after torsion, it seems that a number of structures whose analgens could not be traced previously make their sudden appearance rather in advanced stage of development. They are abdominal gland, buccal cavity, sub-lingual cleft, subo-esophagèal cleft, mantle collar and mantle fold. Radular sac along with radula and odontophore are more prominent, mesenchye cells fill major portion between organs and within the foot. Podocyst and foot makes half the total length of the embryo.

DAY-V

Anterior and posterior lobes of the adult digestive gland have started differentiation (fig. 37). Radular sac enlarges further. Radula with very small radular teeth develops from the floor of the radular sac. Odontophore is observed below radular sac oesophagus makes its appearance. Archenteron can also be discerned as a sac-like structure. Mesenchyme cells greatly increase in size and occupy space between ectoderm and endoderm. They are more crowded towards the foot and podocyst region. The foot with podocyst is now more prominent. Mantle is raised in the form of ridges to form the mantle collar. Salivary gland appears for the first time.

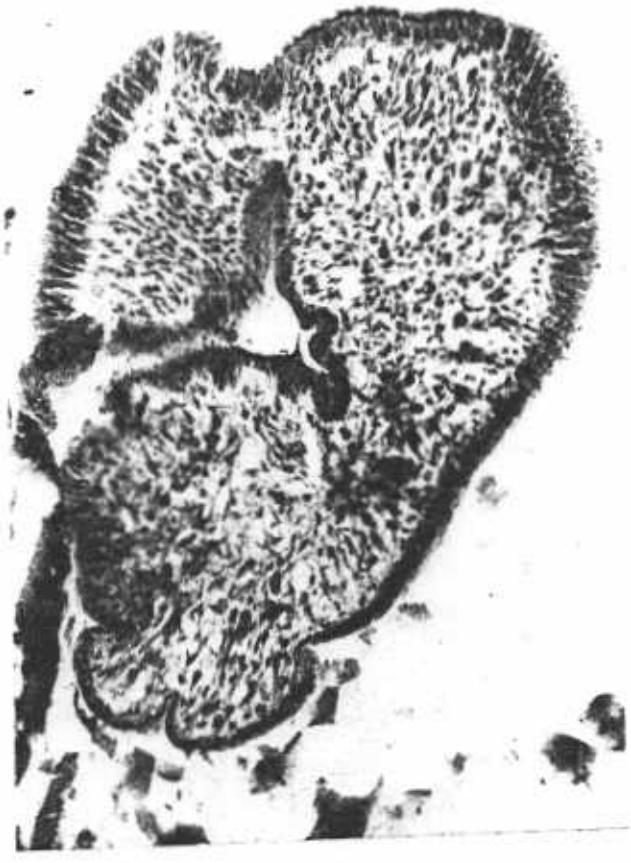

Fig.36: Microphotograph of a day IV embryo of Bensonies jacquemonti showing triangular foot with pedal gland (3 lobes) and pedal gland duct (X 100).

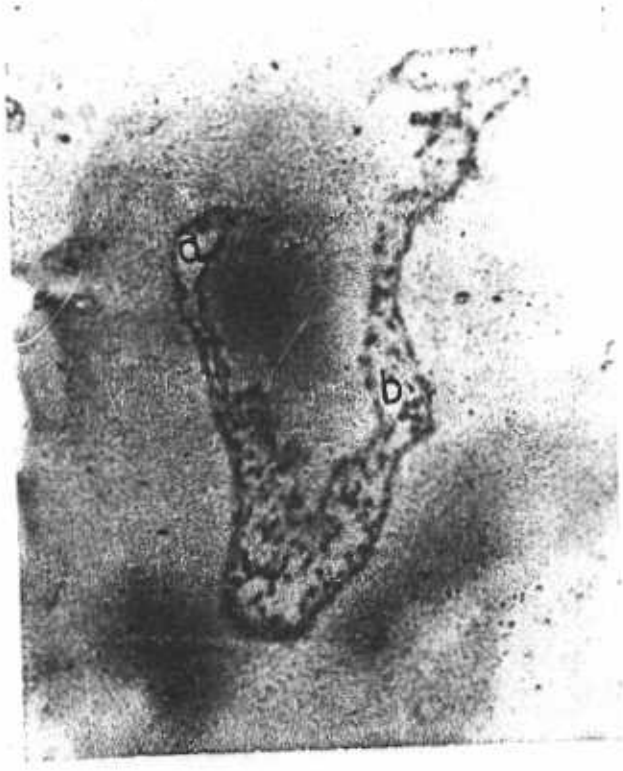

Fig.37: Microphotograph of a day V embryo of Bensonies jacquemonti showing anterior and posterior lobes of digestive gland. Posterior lobe more elongated and has started spiral twist (X 40)

a. Anterior lobe
b. Posterior lobe

DAY-VI

The shell gland has secreted a rounded shell. Pericardium alongwith heart and ureter are visible. On the tip of the dorso-lateral side of velar area there appears small evagination, this marks the formation of the optic tentacle as in later embryo the development of eye was observed on it. The eye starts its appearance as small ectodermal invagination, made up of large cells (Fig.38) arranged radially. With increase in size it becomes constricted off from the ectoderm and lies in the centre.

Intestine appears as a loop-like structure inside the digestive gland. Reno-pericardial aper-ture is just visible.. Nuchal cells are seen for the first time. The nuchal cells are rounded, oval, polyhedral or irregular in shape with a central nucleus and can be detected easily as they take deep stain. These cells are found scattered within the body cavity. They are also found near posterior part of cerebral ganglion and near the lower end of radular sac.

DAY-VII

The gonad rudiment proliferates and extends along the wall of digestive gland in the form of a cord. (fig. 39).

Nuchal cells not only increase in number but also in size. Rudiment of lung has appeared as an ectodermal invagination at the posterior side.

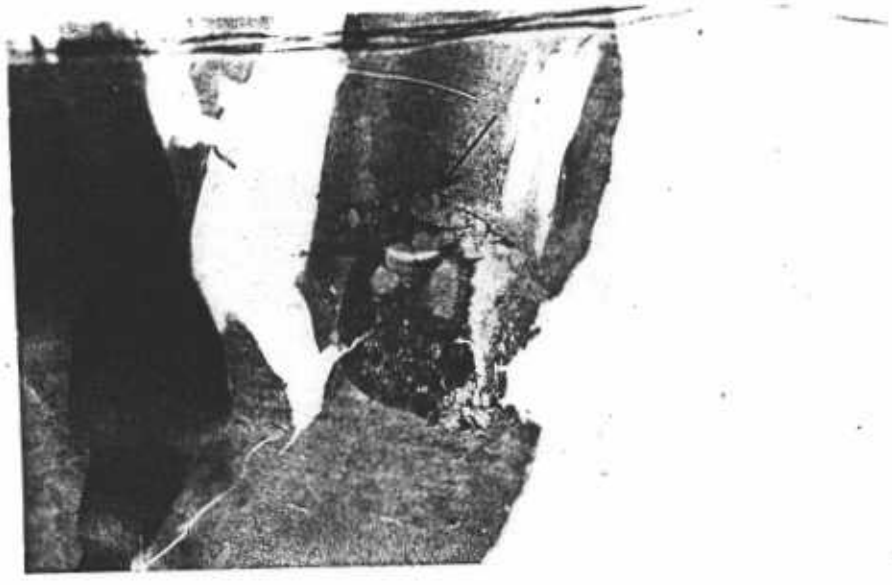

Fig.38: Microphotograph of a day VI embryo of Bensonies jacquemonti showing formation of optic tentacle and eye (X 100).

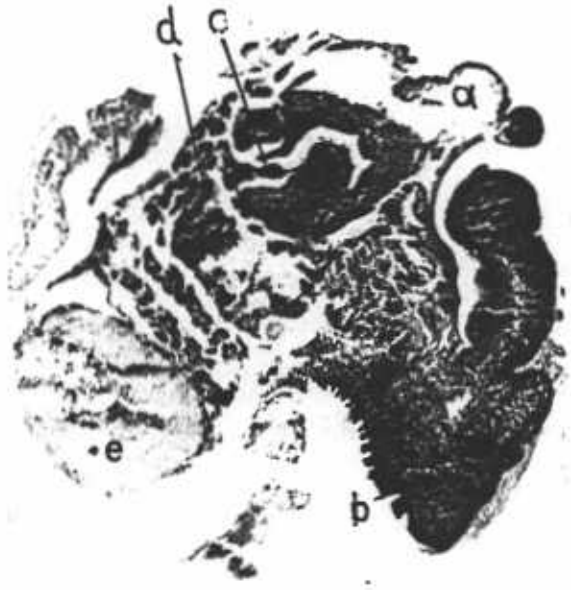

Fig.39: Microphotograph of a day VII embryo of Bensonies jacquemonti showing tentacle, foot, shell, gonad and nuchal cells (X 40).

a. Tentacle
b. Foot
c. Gonad
d. Shell
e. Nuchal cells

DAY-VIII

Odontophore is well marked. The radular sac is cup-shaped and surrounds the radula for most of its length except the anterior end where it is exposed towards mouth. The small radular teeth hardly visible on the posterior end of the radula initially are now quite prominent (fig. 40). Odontophore is in the form of aggregated tissue surrounding the base of radular cells Gonad completely emerges from the kidney rudiment and is visible within acini. Small vacoules appear within nuchal cells and due to those vacoules the central position of nucleus is disturbed and the shape of the cells get distorted and irregular. Salivary gland is in the form of lobules on the sides of well marked œsophagus mesenchyme fills the interior of the body.

DAY-IX

All nerve ganglia increase in size. Radular teeth though not counted, show obvious increase in size and number. The foot has assumed its normal shape, its sole is thick and is formed of several layers of ectoderm. In 4-day embryo, the pedal glands appear as a paired invagination slightly near the anterior end of the foot. The two sides then had united in the middle to form a single structure in the older embryo. As these invaginations meet, they become narrow on sides and broad in the middle thus dividing this region into three incomplete

Fig.40: Microphotograph of day IX of Bensonies jacquemonti showing advanced development of radula, radular sac adontophore and buccal ganglia (X 100).

a. Padular sac
b. Radula
c. Odontophore
d. Buccal cavity
e. Buccal ganglia
f. Salivery gland
g. Nuchal cells.

lobes. With further development the gland is moved towards the middle of the foot with a single duct (see fig. 36). The podocyst is enlarged more reaching its almost maximum size. Mantle cavity and lung have expanded in the form of cavity.

DAY-X

The posterior lobe of the digestive gland has elongated as compared to anterior lobe and show beginning of twisting (see fig. 38). The junction of the foot and the podocyst has become distinct. Shell is only distinguishable along mantle edge. Shell forms a dome over the visceral mass (fig. 42).

DAY-XI

Heart is very well-developed, auricle, ventricle and aorta are quite distinct structures with surrounding pericardium (fig. 41). Kidney forming two limbs occupies lateral position to the heart. The Radula is more ribbon-like, jaw cartilage can be differentiated. Lung forms a spacious cavity. Larval digestive gland begins to degenerate. Larval kidney is filled with vacuoles containing excretory granules, mouth opens directly into the presumptive buccal cavity that is in the form of a wide cavity. In the floor of buccal cavity lies radular sac surrounded by the muscle fibres and odontophore. The cells lining the buccal cavity and oesophagus seem to be columnar but

Fig.41: Microphotograph of day XI embryo of Bensonies jacquemonti showing heart, aorta, kidney and renopericardial aperture (X 100).

a. Auricle
b. Ventricle
c. Aorta
d. Kidney
e. Renopericardial aperture.

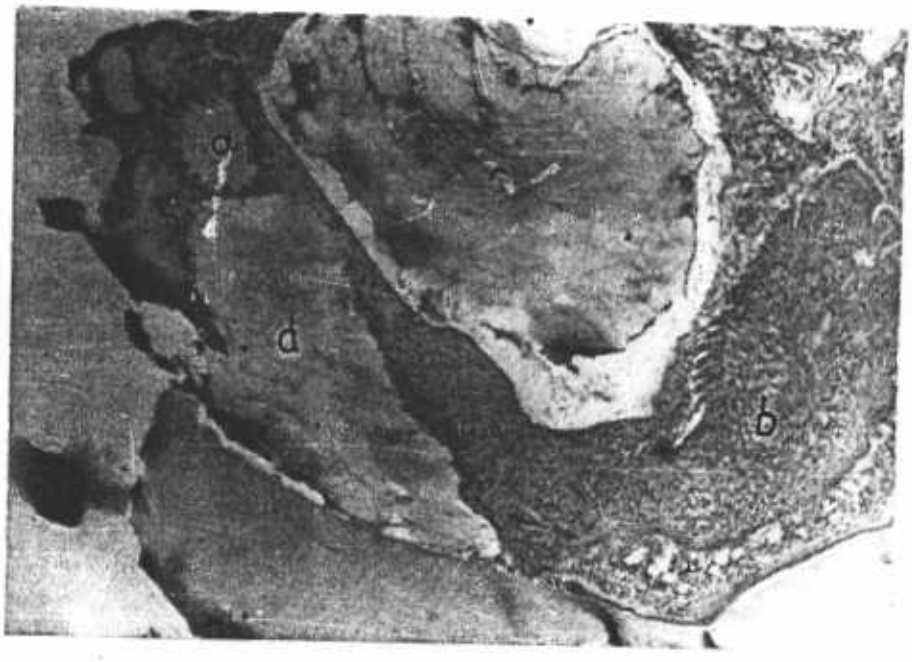

Fig.42: Microphotograph of a day XII embryo of Bensonies jacquemonti showing microvilli in intestine (X 40).

a. Intestine
b. Foot
c. Shell
d. Lung.

cilia are not traceable on them. Mantle surround the head portion. About this time haemocoelic chambers make their appearance as small spaces.

DAY-XII

Foot has become more adult-like in appearance with thick sole having developed musculature. The muscle fibre running in all direction forming a loose spongy structure. The margin of foot is warty in appearance (Fig. 42). The shell is in the form of thin hyaline transparent structure and has almost completed its one turn. Heart with distinct renopericardial aperature is prominent. Posterior lobe of the digestive gland develops several lobes, which may be sub-divided into smaller lobes and take up the function of digestion of albumen, which is present inside the lobules. Statocyst-like structure is observed in well developed form near the base of pedal ganglion (see fig. 44).

DAY-XIII

Pedal gland increases in size and sub-divides into lobes lined by glandular cells. Mantle edge is composed of large compactly arranged columnar, cubiodal cells with basophilic granules. Mantle edge is clearly developed in the form of dorsal, ventral and median lobes. Internal core of mantle is filled with scattered mesenchymal cells (fig. 43).

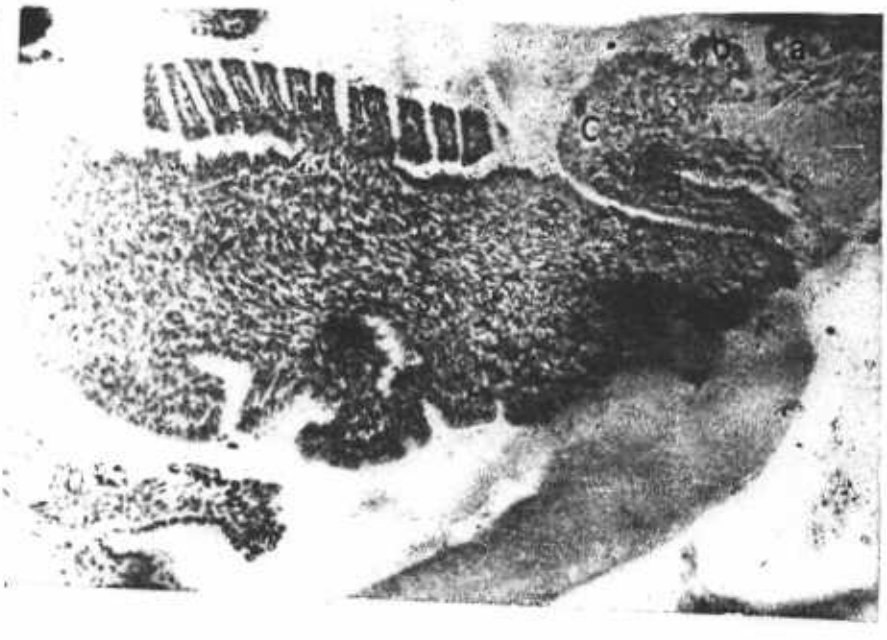

Fig.43: Microphotograph of a day XIII embryo of Bensonies jacquemonti showing mantle collar and mantle lobes (X 100).

a. Mantle collar
b. Dorsal lobe
c. Anterior lobe
d. Ventral lobe

Some of mantle edge cells i.e. columnar cells, form the primordia of mantle edge gland. These cells are also basophilic and show secretory vesicles. Others with large vacuoles, suggesting that secretory product has been liberated. Posterior to mantle edge gland, the dorsal lobe is composed of cubo-columnar epithelial cells. Median lobe is anterior and ventral to mantle edge gland and is lined with simple cuboidal epithelial cells. Ventral edge is also covered by simple cuboidal epithelium. Within mesenchyme connective tissue are few uni-cellular mucous cells along ventral edge. Intestine begins coiling as in adult.

DAY-XIV

Both anterior and posterior tentacles are elongated tubular structure lined with columnar cells having basal nuclei. Eyes are in the form of well-formed double-walled closed vesicles. The inner cells have developed into retina while outer form the cornea of the eye. In the centre of each eye is a clear crystalline lens lower to it are the retinal cells. The body resembles adult form i.e. head and foot in a line and visceral hump quite distinct. The inner epithelial lining of intestine show infolding to form microvilli, in some portion muscular layer is apparent (fig. 44,45).

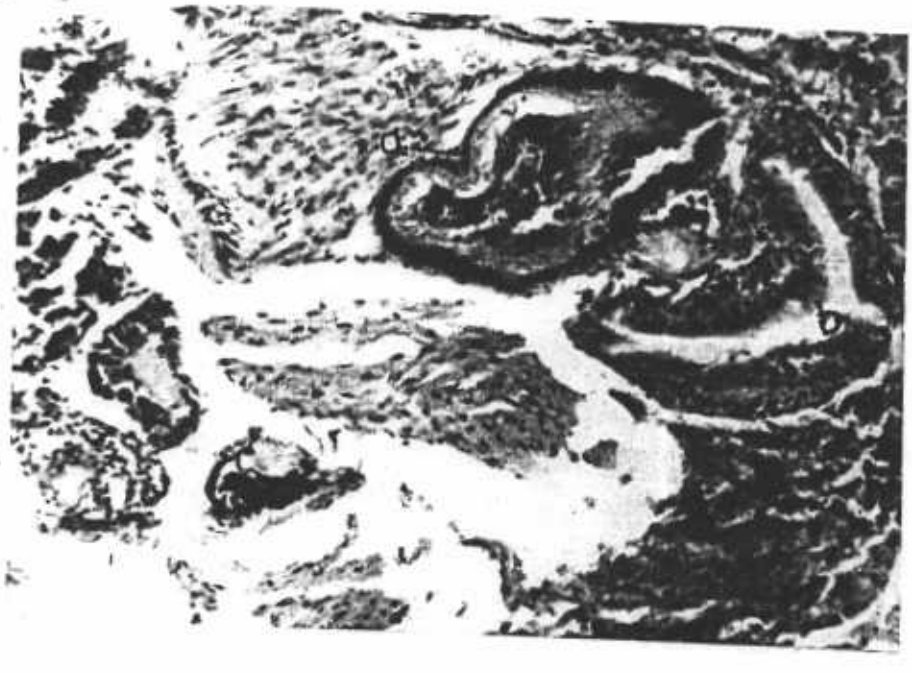

Fig.44: Microphotograph of a day XIV embryo of Bensonies jacquemonti showing radula, jaw and eye (X 400)

a. Radula
b. Jaw.
c. Eye.

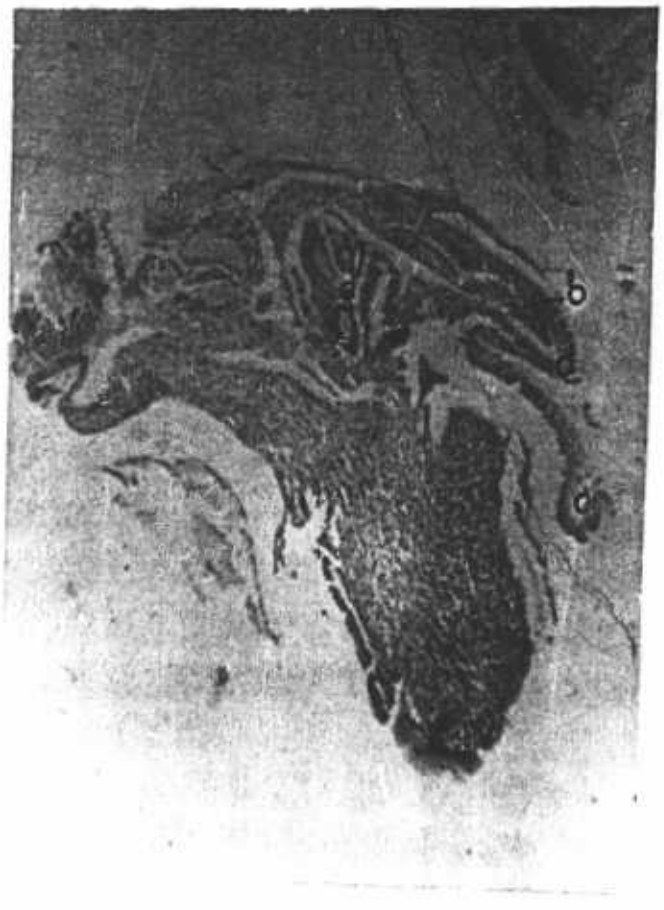

Fig.45: Microphotograph of I-day young of Bensonies jacquemonti showing visceral chain of ganglia, eye, anterior and posterior tentacles (X 40).

a. Visceral chain
b. Eye
c. Anterior tentacle
d. Posterior tentacle

DAY-XV

No new structure are formed after this stage but most of the embryonic organs are degenerated and absorbed. Embryo fills the entire egg and its movement are visible within the egg, with further development the shell has developed another half turn and appears as transparent glossy shell of one and half whorls. Albumen is almost utilized.

7.7 PREHACHING

About two days before hatching the baby snail is visible through transparent envelop, and its shell and its movement can be observed. In this prehatching condition the embryo fills the entire volume of the egg. At this time the heart beat and movement of the foot can be observed.

7.8 HATCHING

The egg shell divides into two pieces from its middle region the two halves nearly equal in size. The newly hatched snail has a light yellow delicate transparent protoconch of one and half whorls. During hatching (fig.46,47) first of all the head along with tentacles comes out and then the foot, which is soon drawn in the shell, and then after a pause the process is repeated and this time it remains out as the egg shell is now broken into two pieces and the baby snail hatches out. Usually in each clutch all eggs do not hatch out simultaneously but some eggs hatch out one or two days later. Neonates fully

Fig.46: Photograph of a small earth (mud) pot used for incubation of eggs of Bensonies jacquemonti Note eggs are wrapped in moist cloth.

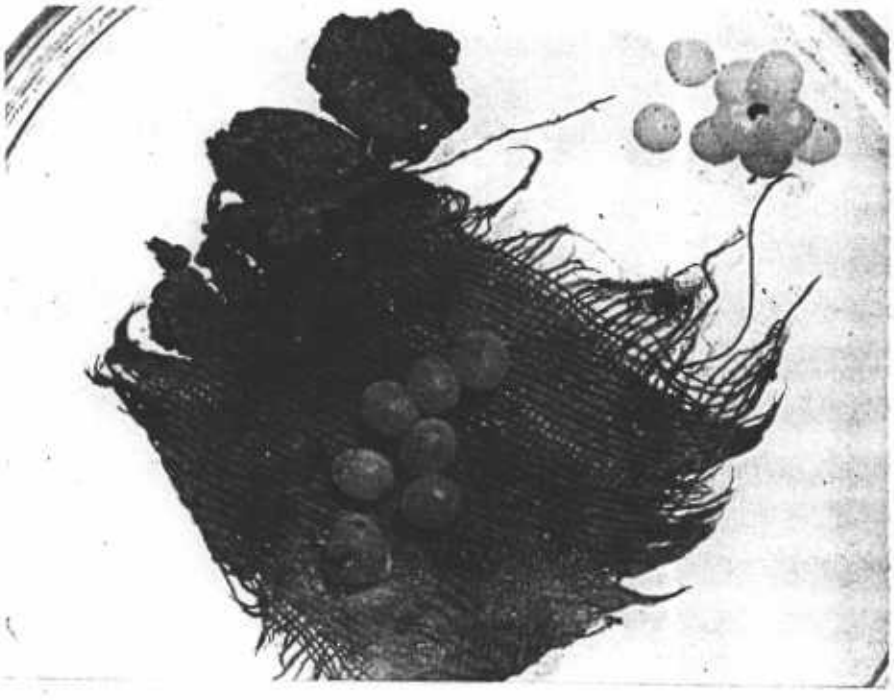

Fig.47: Photograph of eggs of Bensonies jacquemonti during incubation. Note the eggs in cloth are just about to hatch as black spots on eggs indicate that they have cracked on these spots.

resemble the adult but are small and delicate.(Fig.45).

7.9 POST-HATCHING

The newly hatched (neonate) snail remains at its birth place in aggregated condition alongwith other (Fig.48) members of the clutch for 7-15 days, without eating food, although food is provided. Soon after hatching it is observed that the young snail remains calm for few minutes and then starts eating the egg shell and albumen most probably as its first food. It looks like that the newly hatched snail eats the egg shell to utilize its calcium (of egg shell). Tompa (1980) stated that high concentration of calcium in the egg albumen is toxic for the developing embryo therefore the calcium salts are deposited on the outside of the egg i.e. in egg shell. The young snail is capable of retracting within the transparent shell. The rhythmic movement of body could be seen through the shell.

All the systems of the adult are developed except the reproductive system which is rudimentry and an albumen gland is entirely lacking. Digestive system is complete, the post hatching event is the addition of radular teeth in the radula. Musculature of foot is further strengthened.

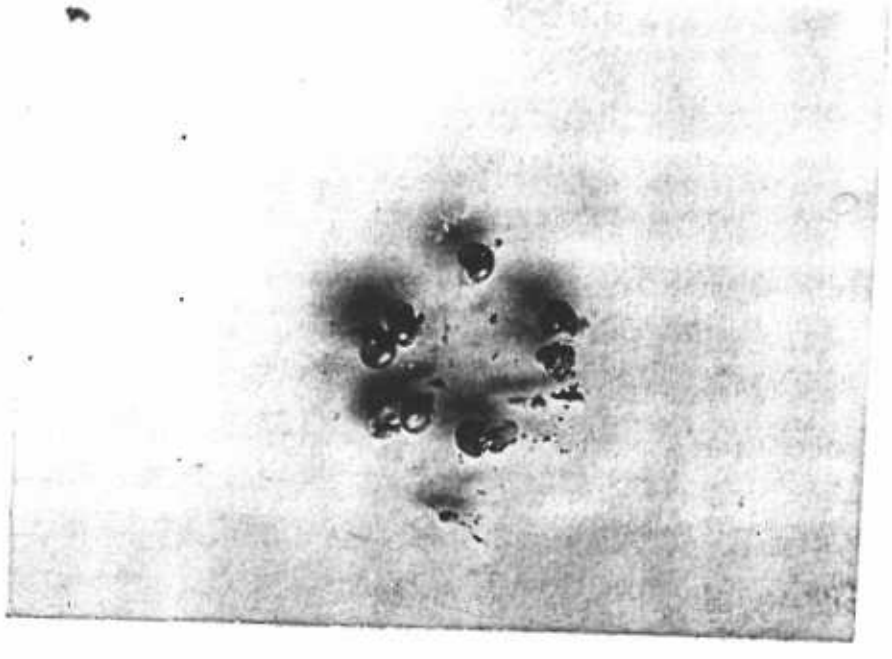

Fig.48: Photograph of neonates of Bensonie jacquemonti

7.10 POST HATCHING GROWTH AND EVENTS

The young snail maintains a rapid growth in its first months (Table IV-VI). The size of shell, foot length and the whorl increases. The baby snail has a shell of 1½ whorls and it took only 3-4 weeks to become 2 whorls. The growth in the following two months is rather slow and gradual but not very slow. Between 4-7 months the growth rate slows down considerably as the snail moves towards sexual maturity (fig. 49, Table VII-IX).

The reproductive system is not clearly visible until 3½ whorl stage with-in a dissected snail. At this stage only penial complex is detected as small sac-like structure with a narrow protrubarance. The ovotestis and albumen gland are not distinguishable and so is the female system.

At 4 whorls stage which is achieved after 4-4½ months the penial complex is well developed as the penial caecum, epiphallus and epiphallus flagellum are quite prominent. Spermatheca appears like a small tubular structure and large hermaphrodite duct is in the form of a narrow and delicate duct. No albumen gland is detected. Ovotestis is now visible in the form of small dots on the upper most portion of the digestive gland (Table-X).

In some Stylommatophora, the presence of a reflected shell lip in post embryonic development, indicates the termination of growth and the start of adult phase.

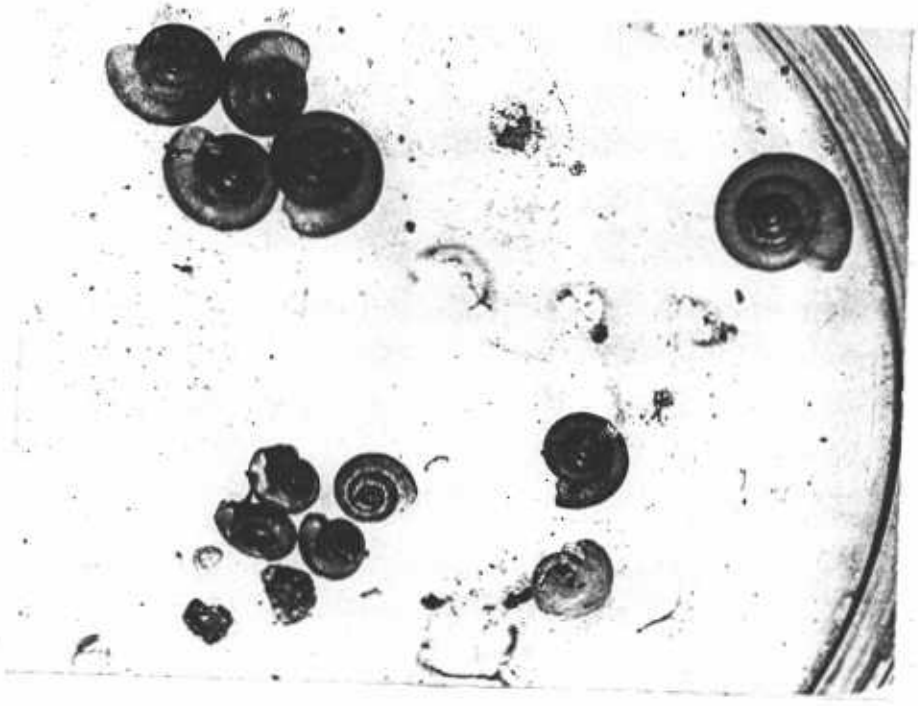

Fig.49: Photograph of young Bensonies jacquemonti with different shell whorls.

Table-IV Showing size of shell and foot at the time of hatching in B. jacquemonti

Individual number	Whorls	Shell size	Foot size
1	1½	3 mm	4.5 mm
2	"	"	5 "
3	"	"	5 "
4	"	"	5 "
5	"	"	6 "
6	"	4	5 "
7	"	3	5 "
8	"	"	"
9	"	"	6 "
10	"	4 mm	5 "

Average size foot = 5.2 mm
shell = 3.2 "

Table-V Showing size of foot and shell at 2 whorls stage and time interval between hatching and 2 whorls in B. jacquemonti

Individual number	Whorls	Shell size	Foot size	Time (days)
1	2	4 mm	5 mm	19
2	"	"	6 "	21
3	"	"	7 "	22
4	"	"	6 "	20
5	"	5 "	5 "	19
6	"	6 "	6 "	22
7	"	5 "	5 "	19
8	"	6 "	6 "	20
9	"	4 "	5 "	22
10	"	5 "	5 "	23
11	"	4 "	6 "	24
12	"	4 "	6 "	25
13	"	5 "	5 "	25

Average number of days = 21.8
Average foot size = 5.6
Average shell size = 5.4

Table-VI Showing size of foot and shell at 3 whorls and time interval between 2 whorls and 3 whorls stage of B. jacquemonti

Individual number	Whorls	Shell size	Foot size	Time (days)
1	3	8 mm	12 mm	31
2	"	7 "	11 "	30
3	"	6 "	10 "	31
4	"	6 "	12 "	32
5	"	8 "	11 "	33
6	"	11 "	10 "	34
7	"	8 "	10 "	30
8	"	9 "	11 "	30
9	"	8 "	10 "	31
10	"	12 "	11 "	31

Average number of days = 31.3
Average shell size = 8.3
Average foot size = 10.8

Table VII Showing size of foot and shell at 4 whorl stage and time interval between 3 whorls and 4 whorls in *B. jacquemonti*

Individual number	Whorls	Shell size	Foot size	Time (days)
1	4	9 mm	19 mm	56
2	"	11 "	18 "	53
3	"	11 "	16 "	52
4	"	10 "	16 "	55
5	"	11 "	16 "	52
6	"	9 "	17 "	46
7	"	10 "	20 "	45
8	"	10 "	18 "	46
9	"	13 "	13 "	49
10	"	13 "	18 "	48

Average days = 49.2
Average shell size = 10
Average foot size = 17

Table-VIII Showing time interval between 4 whorls and 5 whorls i.e. shell completion in B. jacquemonti

Individual number	Whorls	Time (days)	Whorls number in adult individual
1	5	58	5
2	"	60	5.3
3	"	64	5.2
4	"	64	5.1
5	"	70	5.3
6	"	62	5.5
7	"	67	5.4
8	"	54	5.1
9	"	68	5.3
10	"	63	5.4

Average number of days = 59 days

Table-IX Showing size of foot and shell at shell completion stage in B. jacquemonti

Individual number	Whorls	Shell size	Foot size
1	5.1	19	30
2	5.3	20	32
3	5.2	19	30
4	5.5	21	31
5	5.2	20	29
6	5.1	18	28
7	5.0	19	26
8	5.2	20	26
9	5.4	21	30
10	5.3	19	32

Average shell size = 19.6

Average foot size = 29.4

Table-X Developmental status of different portions of reproductive organs in different whorl stage of Bensonies jacquemonti after dissection.

Whorl No.	Albumen gland	Ovotestis	Penial caecum	Epiphallus	Large hermaphrodite duct	Bursa copulatrix
3	Not distinguishable	Not distinguishable	Just visible	Just visible	Thread like	Just visible
4	-do-	Dot-like	Prominent	Distinguishable	Delicate duct	Prominent but small
4½	Just visible	Prominent	-do-	-do-	-do-	-do-
5	Prominent	-do-	-do-	-do-	Developed	Developed

In B. jacquemonti there is no reflected lip in the adult, so the adult phase is assumed by the number of whorls and size of the animal. Usually at 4½ whorls stage when the minimum size of the shell approximately 15 mm, the reproductive organs are fully developed except the albumen gland which is still rudimentary (small in size) (see Table X) The snails with complete 5 whorls and shell size 16 mm or above are observed in copulation and oviposition.

7.11 SEXUAL MATURITY

By the time the snail has reached 4-4½ whorls and 5-6 months of age, the ovotestis and albumen gland are recognisable (see Table X). The sexual maturity is attained when the snails are aged between 7-9 months and this corresponds with a shell measuring at least 16 mm in length. The individuals with shell length less than 16 mm have never been found in copulation. As there are two distinct reproductive periods it is observed that full sexual maturity is not reached until the second-reproductive period. For example those hatch and mature in spring will reproduce in autumn breeding season.

7.12 LIFE SPAN

The life span of Bensonies jacquemonti in field observation is found between 3-4 years, while in captivity it lives for 5-6 years. The snails in continuous successful dormancy are observed to survive for six years (Table XVIII).

7.13 DISCUSSION

The pulmonate eggs of aquatic or terrestrial forms is enclosed in an egg capsule. These eggs in aquatic forms are placed in a gelatinous substance and enclosed in further protective layers. In terrestrial forms the eggs are either laid singly or in batches. In B. jacquemonti the fertilized ovum is surrounded by albumen, albumen membrane, jelly and shell layers (membranes) and egg shell.

Raven (1958) in an egg of Lymnaea found that the primary membrane around the fertilized egg i.e. the vitalline membrane, is secreted by the egg itself. The secondary membrane (Chorion) in fresh water pulmonates has not been described but a number of tertiary membranes are found. He stated that in all cases the embryo and albumen are surrounded by an inner perivitelline membrane. In fresh water forms there is another second individual layer followed by external layer.

Duncan (1975) stated that in Stylommatophora, the zygote is surrounded by albumen and perivitelline membranes, these in turn are surrounded by the jelly and shell layers. The last important covering is in the form of shell which is composed of crystals of calcium carbonates. He quoted (Bayne 1966, 1968; Chernin and Adler, 1967; Morton, 1955) that albumen is composed of neutral polysaccharide (galactogen) and proteins. Bayne (1966) found that albumen of Agriolimax reticulatis contains glycoprotein, water, free amino acid and calcium but no lipid.

The structure and physio-chemical composition of the pulmonate egg and the changes that occur before and after oviposition are studied by different methods by different authors. In the light of these findings it was possible to differentiate various parts of the egg and to some extent the early changes in the components of egg of Bensonies jacquemonti.

In Bensonies jacquemonti by the gross appearance of the egg and by difference in the staining reaction of the different parts, vegetative pole plasm and sub-cortical plasm are differentiated. No segmentation is started in the egg, taken from oviduct before oviposition (see fig. 26, 28).

Raven (1945) described that in Lymnaea stagnalis the cytoplasm of egg has two distinct parts; the ectoplasm and endoplasm. They differ in their staining reaction. At oviposition the ectoplasm covers a portion at vegetative pole while the endoplasm occupies the rest of the egg. The ectoplasm after maturation division spreads beneath the egg cortex and surrounds the whole egg. At this stage animal pole plasm and vegetative pole plasm are formed by concentration of local substances.

In Bensonies jacquemonti after ooplasmic segregation in early blastula stage, cells with vacuoles are considered as pinosome, as some of them are observed with albumen granules inside (see fig. 31). In later blastula stages smaller pinosomes have fused to form the larger

macropinosomes. In the beginning of the gastrulation macro- and micro-pinosomes could be differentiated. Even after gastrulation when archenteron or albumen sac is found the peripheric resorption is carried out by pinocytosis of ectodermal cell.

The developmental process proceed uniformly in pulmonates. All pulmonates have an egg with little or no yolk and large extra-embryonic food reserve in the form of albumen. Raven (1945), Brisson (1960) and Fironi (1977, 1982) gave first information about the peripheric resorption of albumen in Lymnaea stagnalis. They found that peripheric resorption by pinocytosis begins during segmentation and continues during and after gastrulation. Ghose (1963) and Brisson (1968) confirmed this statement with different species of Achatina.

The resorption of albumen takes place by micro- and macropinocytosis. Later on several macropinosome fuse and form the bigger vacuole. Inside these vacuoles albumen is accumulated. After gastrulation resorption is decreased, pinocytosis stops and albumen vacuoles are digested (Raven, 1975). All this depends upon species. In Lymnaea vacuoles disappear on seventh embryonic day while in Achatina the peripheric resoroption results in large cephalacyst and resorption is carried out for long period. In Helix the reduction of these vacuoles is slow.

Fironi (1982) stressed that the classical concept of protective ectoderm and nutritive endoderm is to be changed.

The resorption of albumen is not strictly limited to the endoderm but ectoderm also shares with it the nutritive function. So the role of germinative layers is not fixed and but can be modified according to the need of the embryo with its surrounding.

The gastrulation is formed by emboly and very clear invagination of cells was observed in Bensonies jacquemonti in the present study (fig. 32).

Mean while the invagination of the endodermal surface occurs and archenteron is formed. Albumen is accumulated inside the archenteron, so its resorption is not restricted to ectodermal vacuoles but also happens in endodermal cell. Later on the peripheric resorption ceases in ectodermal cells but continues in endodermal cells and results in the formation of albumen sac or larval digestive gland.

Raven (1946 & 1975), and many others have observed that after gastrulation, when albumen sac or larval digestive gland has developed from endodermal cell, the uptake and digestion of albumen is more or less limited to the endoderm.

Ranjah (1942) described mesoderm formation in Pila globosa and traced out that posterior macromere named as 3D form the mother mesoderm cells. Thus mesoderm is teloblastic in origin in Pila globosa. In addition to teloblastic type, there are two other types of the origin of mesoderm i.e. ectodermal and enterocoelic in

gastropoda. The ectodermal origin of mesoderm was described by Tonniges (1896) and Otto and Tonniges 1905 (as quoted by Ranjah, 1942). They found that in Paludina the mesoderm originates by a wandering in of the ectodermal cells into the segmentation cavity, while Dautert (1929) as quoted by Ranjah (1942) confirmed the ectodermal origin of mesoderm and found that mesoderm originates from the ectodermal wall just below velum.

The second type i.e. enterocoelic type of mesoderm formation is also studied in Paludina only. Ranjah (1942), quoted Erlanger (1891), that near the blastoporal end, from the archenteric wall a ventral pouch-like outpushing is given out. This by further development expands laterally into a bilobed out growth and is detached completely from the archenteron. He described this bilobed sac as the primary coelomic sacs. The cells detach from the wall of coelomic sac which wander in the segmentation cavity as mesenchyme cells in later stage.

The mesoderm in Bensonies jacquemonti is formed by enterocoelic type. As near the blastoric end, from the outer archenteric wall small pouch-like evagination is observed. This is marked as the beginning of the coelomic pouch (see fig. 32,33). In previous studies mentioned above particular attention has been paid to the study of cell-lineage to trace the mother mesoderm cell, for confirming the origin of mesoderm. To trace the cell lineage was beyond the scope of the present study and it cannot be

said with certainity that in Bensonies jacquemonti the mesoderm is ectodermal or enterocoelic in origin.

After gastrulation in some pulmonates there is a free living larval stage in the form of a veliger or trochophore larva. While in others, that pass their development within egg capsule, hatch out as young crawling snail. In some Basommatophora the stages comparable to typical trochophore and veliger are still recognized. In Stylommaphora there is greater deviation from the typical course of development. The typical trochophore has a prototrochal band above the mouth, a post-trochal region and there is an apical tuft. The prototroch in further development grows into velum.

In B. jacquemonti, the prototroch is without apical tuft, greatly reduced and represented by the velum. Ghose (1963) stated that in Achatina due to the presence of a large larval body and the Podocyst, the embryo remains folded inside the egg and cannot move. Therefore the velum is greatly reduced and its cilia instead of help in locomotion takes up the secondary function to drive albumen into gut and larval digestive gland, as during development the albumen has become thick. In Stylommatophora the prototroch is greatly reduced in some (Helix), while in others it remains for a short time (Arion) and in still others no prototroch develops at all (i.e. Limax,Raven,1975).

In pulmonata in pretrochal region a large head vesicle is present on the dorsal side. In Stylommatophora

the head vesicle is greatly developed and temporarily becomes bigger than the rest of the body and show no active contractivity (Raven, 1975).

In B. jacquemonti cephalic vesicle is missing but there is present a large larval body, the foot and digestive gland are the first to appear. The foot rudiment elongates, flattenes ,bends on its self to form a hook like structure the podocyst (see fig. 34,35).

Ghose (1963) observed that head or cephalic vesicle is absent in Achatina and instead a large larval body, (a special portion of archenteron for digestion of albumen) is present. Larval body gives the embryo, an elongated shape. He further suggested that larval body and cephalic vesicles are homologous organs as both of them develop in similar manner from the anterior end of embryo.

Raven (1975) quoted Carrick (1935) for Agriolimax; Balsubramaniam (1952) for Ariophanta and Ghose, (1963) for Macrochlamys that podocyst contains muscular element and by its powerful contraction and relaxation helps embryo to rotate within albumen mass. Due to large size of podocyst the embryo is unable to rotate within shell, so podocyst help in circulating the body fluid in early larval life. Raven quoted Brisson (1968) that in Archachatina the head vesicle possesses muscle cells that show active contraction in later development.

Torsion in the form of rotation of visceral hump occurs at an early stage of development in gastropods. In

some forms it is very quick and lasts for seconds while in other it is a slow process.

In the present study, inspite of great efforts, the exact time of torsion is not observed. However on 3½ embryonic day the embryo becomes asymmetrical by the elongation of foot and podocyst, larval body is enlarged and spoon-shaped and visceral hump is seen towards right side. These changes occur during short time and last till on the 4th day. The asymmetry of body seems to be result of the differential growth as stated by Raven (1975) for pulmonates and presumably marks the torsion in Bensonies jacquemonti (fig. 35).

Ranjah (1942) stated that in Pila globosa the rotation of organs is observed in early stages of development. The rotation causes asymmetry of the embryo, the organ rotates from left to right side (Pila, being a dextral form while in sinistral it is from right to left) and torsion starts on 3rd day and ends on 4½ to 5½ day. Ghose (1963) observed that in Achatina fulica at stage 2 i.e. 3½ day torsion occurs as the embryo is asymmetrical both externally and internally. Demian and Yousaf (1973) observed that in Monacha cornuarietis torsion occurs through the differential growth of the two sides it starts in 3½ days old embryo and lasts for two days. Raven (1975) described rotation as a slow process in pulmonates. The rotation is due to differential growth of two sides rather than the muscle contraction.

In pulmonates stomodium is formed by an invagination on the ventral side near the anterior end. The

anterior portion of the gut gives rise to a tubular structure that in later development forms buccal cavity, oesophagus and stomach. In B. jacquemonti the gut portion or the rudiment of alimentry canal is the endodermal archenteron and resembles that of Pila globosa as described by Ranjah (1942). In Bensonies jacquemonti the stomodeal invagination gives rise to buccal cavity and aesophagus while endodermal cells become vacuolated and encircle the albumen to form albumen sac or larval digestive gland. Later on, the hind gut rudiment is differentiated in the form of a band of cells. The archenteron communicates with the stomodeum as the development proceeds.

Smith (1935) described that in Patella vulgata a constriction seperates the adult stomach and aesophagus from the larval stomach. Ranjah (1942) described the formation of adult alimentary canal from endodermal archenteron in Pila globosa, the anterior blind end of archenteron gives rise to stomach and digestive gland. Greek (1951) stated that the rudiment of digestive tract in the form of a blind tube in the dorsal half of the visceral mass. Anteriorly the ectodermal half forms buccal region by a constriction and the posterior endodermal sac gives rise to the rest of the digestive gland. Hyman (1967) stated that a part of archenteron is reserved to form the mid gut and intestine, while remaining portion becomes vacuolated to form the wall of the albumen sac.

Raven (1975) described thatoesophagus and stomach are formed by the ventral and lateral wall extension of the archenteron respectively.

In the present study the mantle fold was visible on the fourth embronic day. During its later stages of development, the mantle edge grows in the form of dorsal, ventral and median lobes. These lobes are mainly lined with cubo-columnar epithelium cells with few mucous cells. The mesenchyme tissue fills the internal core (see fig.43).

Ranjah (1942) described that mantle rudiment is differentiated from the part of visceral sac rudiment that is bounded by the mantle fold and the mantle groove at its free end. The mantle is lined by tall columnar cells. He stated that in Pila globosa mantle skirt develops as two ectodermal folds, that in later development meet to form the roof of mantle cavity. Gho-se (1963) found that mantle appears in the form of small invagination on the right side of the embryo at stage II i.e. 3½ day. Later on, it grows obliquely, anteriodorsally and form the large mantle cavity. Hyman (1967) observed that the shell gland becomes the mantle. The mantle cavity is formed by invagination under the mantle, this cavity deepens and after expanding fuse under the neck region. Kapur and Gibson (1967) stated that mantle edge originates as a peripheral thickening of the evaginated shell gland. During prehatching it develops into different folds and internally the connective tissue cells contain amoebocyte, yellow body cells and some muco-protein cells.

Raven (1975) described that mantle fold grows forwards and then is elevated from the body forming an arch-like that of mantle cavity.

Just after gastrulation in B. jacquemonti the shell gland rudiment arises as an ectodermal thickening on the dorsal side of visceral hump. The centre of shell gland invaginates to assume a shallow cup like shape. The first shell is secreted in the form of a thin layer of cuticle just inside the shell fold. Ranjah (1942) observed shell gland as an ectodermal thickening at the aboral pole. The thickening first invaginates and later on evaginates. During eversion the central cells of the everted shell gland becomes thin and flat and form the outer covering of the visceral sac while its marginal cells that form the shell gland remain tall and columnar. Hyman (1967) observed that shell gland rudiment appears as a circular thickened area of ectoderm on the dorsal side of velum. It secretes a thin cap-like shell. Demian and Yousaf (1973) observed that ectoderm thickens in small circular area on the posterior side to form the rudiment of shell gland in Marisa cornuarietis.

In the present investigation, in B. jacquemonti the lung rudiment is observed on the seventh embryonic day, as ectodermal invagination at the right side. (see fig. 42). In pulmonate the development of lung is observed by Ghose (1963) for Achatina; Brisson (1968) for Arion; Raven (1975) for Limnaea, all agreed that the lung primordium

arises as an ectodermal invagination. Slightly later the mantle cavity is formed, which surrounds the now deepened lung invagination. Lung communicates with mantle cavity by an opening.

In B. jacquemonti the heart, pericardium and kidney arise from the common mesodermal primordia. The common mesodermal cell divides into anterior and posterior parts. The anterior portion gives rise to heart and pericardium while posterior part forms the kidney. Pericardium is formed by splitting of mesodermal cells. The kidney communicates with heart by reno-pericardial aperture (see fig. 41).

Ranjah (1942) stated that in Pila globosa, the right and left kidneys arise as two thickening from the right and left pericardial cavities on its postero-ventral walls. The right one develops into adult functional kidney (left) while the left one in later development forms the genital duct. Ghose (1963) stated that in Achatina the kidney primordium becomes tubular and this kidney tubule communicates with the pericardium by the reno-pericardial aperture. Demian and Yousaf (1973) observed that the common rudiment of heart and kidney divides incompletely into two portions. The larger and thin walled anterior portion develops into heart and pericardium while a small, thick-walled posterior portion forms the rudiment of kidney. The rudiment of renal vestibule and ureter deepens and

connects with the cavity of kidney. Raven (1975) stated that in other pulmonates the kidney breaks through secondarily into pericardium.

In the present study it was observed that just after gastrulation, the cerebral ganglia are formed. They appear on the dorso lateral side near the mouth by the proliferation of ectodermal cells.

Ranjah (1942) observed that all ganglia arise seperately as ectodermal thickening by delamination and later connects with connectives and commissures. Raven (1975) described that ganglion are formed first by proliferation and later delamination from the ectoderm. The main body of the ganglia arises by proliferation while connectives and commissure are formed by secondary out growth from the ganglia.

In Bensonies jacquemonti, the eyes and tentacles appear on the same day and are ectodermal in origin. The eyes develop as small invagination that is pinched off and passed into the vesicle. The inner wall thickens and by elongation forms retina while outer wall develops into the inner part of the cornea. Tentacles originate as outpushing of ectoderm. Ranjah (1942); Hyman (1967) and Raven (1975) stated that eyes in pulmonates are formed from ectodermal invagination.

A statocyst-like structure is observed in advanced stage near the pedal ganglia. Raven (1975) stated the statocysts are ectodermal inv tions near

the boundary of foot and body [illegible]. By delamination of a solid cluster of cells, it is marked off. The cavity inside soon becomes wide and develop cilia.

In the present investigation in B. jacquemonti the gonad rudiment is observed along the wall of digestive gland in the form of a cord. The reproductive system is rudimentary at time of hatching and develops fully at 4 whorls stage (fig. 39, Table X).

Hyman (1967) described that the entire reproductive system arises by a single ectodermal invagination in the inner part of mantle cavity. Gonad develops from proximal part while distal half forms the spermoviduct in which folds partially seperate the male and female passages. The copulatory organ develops from an ectodermal invagination and secondarily gets connected with the anterior end of the male duct.

Raven (1975) stated that gonad as a rule is formed by the mesodermal cell mass, that have isolated and passed in the body cavity. The gonoduct and copulatory organs arise from common primordium, a swelling near the opening of invagination (now shifted to its definative place i.e. side of head) form rudiment of copulatory organ.

In B. jacquemonti the neonate (newly hatched snail) resembles the adult in general appearance (see fig.48). Internally all systems of adult are developed except reproductive system that is rudimentary and albumen gland is entirely lacking.

Mohr (1949) stated that in Achatina fulica full sexual maturity corresponds with a shell measuring 80 mm at least. In smaller species like Physa fontinalis, Dewitt (1955); Physa gyrina and P. integra, Cãampitt (1970) the young snails produced in late summer under favourable conditions mature rapidly and start egg laying in early August.

Duncan (1975) stated that snails hatching in the spring in England have mature systems by September.

The mortality of eggs of B. jacquemonti in laboratory was 1.09% (Table II). The average egg size range from 2.8-4.0 mm (Table I). The newly hatched snail has a protoconch of 1½ whorl. The mortality of the neonate is moderate. The young snail maintains a rapid growth rate in its first month. In the next two months the growth is a bit slow. The snail reaches 1½ to 3 whorl shell in 7 weeks while 3-4 whorl stage alone takes 7 weeks. With the increase in shell whorl there is visible increase in the length and size of the shell and body (see Table IV—IX). The time between 4-5 whorl is 8 weeks. The reproductive organs during hatching are rudimentary and a snail of 3½ whorl when dissected, the organs are found as sac-like penial complex, small tubular spermoviduct and a cord like bursa copulatrix. At 4 whorl stage the penial complex shows distinct portions but albumen gland and ovotestis are still indistinguishable. By the time, the snail reaches 4½ whorl and have attained the shell size 15 mm all

structures of adult reproductive system has developed including albumen gland. At the age of 7-9 month when shell size is 16 mm B. jacquemonti has reached sexual maturity and can be observed in copulation (Table X).

Grabb (1927) found that L. stagnalis started oviposition two and half months after hatching under laboratory conditions. Fraser (1946) observed that L. stagnalis reaches maturity at age of 18 weeks, while Noland et al (1946) stated that in laboratory L. stagnalis reaches sexual maturity a little less than three months in summer and upto four months in winter. Grabb, Fried and Good Child (1963) reported that Menetus dilatalus becomes sexually mature in 50 days.

In Stylommatophora the longer life span is observed. Duncan (1975) reported in wild form longevities of three years and in captivity uptil 15 years have been recorded. He quoted several authors stating different life span and time of maturation in different species of snail. According to him Agriolimax reticulatus, the spring generation, matures in 5 months and autumn generation in 7 months (Hunter, 1968); the smaller helicides mature quickly with life span from 12-18 months (Chat-field, 1968).

Hodasi (1979) observed that in Achatina achatina the growth rate of young snail is rapid but variable for the first six months (0.21 mm/day). The growth is then suppressed for the next two months (aestivated) then high growth rate is resumed (0.22 mm/day)

until snail is one year old. A sharp decline in growth rate (0.06 mm/day) follows until snail is 18 months old. The growth is again suppressed for three months (aestivated again) just after aestivation the growth rate is increased slightly and this is followed by normalization of growth rate. The snail reaches maturity at age of two years. In young snail the columella and outer lip of the shell are similar in colour as that of the shell, but in adult snail the columella and the parietal wall become vinaceous red and outer lip assumed a bluish white colour.

Chase et al (1984) studied growth rate of Achatina fulica. The snail's growth rate is rapid in first month followed by slow rate for next two months. Between 4-6 months the snail is near sexual maturity. He found mature sexual organs in snail of 150 days of age and also observed precopulatory behavior in some.

Siddiqi and Ali (1988) stated that mature Monacha obstructa are recognized by the presence of a maturation lip around the mouth. The young snails were at their peak number in the month of May. Duncan, (1959) disclosed that Physa fontinalis that hatch in June have developmental history of about 6-7 weeks. The growth is very rapid and male and female reproductive systems are matured by the end of this period.

The life span of Basommatophora is usually short i.e. between 8-14 months. Bulman (1990) stated that in

Carychium tridentatum the life span ranges from slightly less than 4-19 months. The sexual maturity is indicated by completion of shell whorls and shell growth. He calculated an average of 79 days for completion of shell.

All previous literature show scattered account of the life cycle and reproduction of the pulmonate. Observations were made on most Basommatophora (Physa, Lymnaea etc) and some Stylmmotaphora mainly (Achatina,). None of these reports provide the account of development i.e. from egg to sexual maturity of Bensonies jacquemonti. No precise data exists in the literature on the growth rate, while on the incubation, egg production per individual, there is no information at all. The present work is an attempt to provide this aspect of the life history of Bensonies jacquemonti.

CHAPTER - 8

NEUROSECRETION

In pulmonata neuro-secretory cells are extensively studied as compared to other gastropods. In this group these cells are present in well defined areas of the central nervous system and some organs associated with central nervous system e.g. the optic tentacles and cerebral gland. The most classical method of identification of neuro-secretory product and its control on reproduction were best studied by processes, such as ablation, implantation and injection of homogenate of the related organs.

But in Bensonies jacquemonti the single gonad is deeply embedded in the digestive gland and its complete removal was impossible. Therefore associated organ such as optic tentacles are removed to test its endocrine role.

To detect the occurance of neuro-secretory cells and its location, cytological and histological structure of the brain and presumed endocrine organs are studied.

MATERIAL AND METHODS

To demonstrate the presence of neurosecretory material the periodic acid Schiff (PAS) reaction is employed.

The tissues (brain and optic tentacle) from alive or freshly killed animal are dissected out as quickly as possible and fixed in 10% Formo-alcohol (10% formuline 90% alcohol) for 12-18 hours. Before embedding the tissue is processed in the following steps:

Formo-alcohol (10, 90%) → Formo-alcohol (50, 50%) →
Alcohol 50% → Alcohol 70% →
Alcohol 90% → Alcohol 100% →
I change
Alcohol 100% → Alcohol+Xylene 50, 50% → Xylene I change →
II change
Xylene → Wax → Wax → Embedding (in paraffin)
II change I change II change

The sections are cut 6-8 u thick and are stained with periodic acid Schiff. (PAS).

Method:

1. Paraffine sections are dewaxed and brought through Xylene and Alcohol to water.
2. Treated with 1% periodic acid solution to oxidise (8 minutes).

3. Washed in several changes of distilled water
4. Covered with Schiff's solution (15 minutes)
5. Washed with running tap water several changes (5-10 minutes)
6. Stained nuclei with Harris's haemotoxylin (nuclei-blue).
7. Treated with acid alcohol to differentiate
8. Treated with ammonia water then tap water
9. Dehydrated and brought to xylene
10. Mounted in canada balsom

To test the working of the stain, slides of chicken's liver section are treated with PAS stain it showed positive results. The glycogen and other periodate reactive carbohydrate took Magenta (Pinkish red) colour.

For observing the endocrine role of the optic tentacles mature Bensonies jacquemonti of nearly equal size and weight are selected. The size of shell ranged from 19.5-20 mm and weight 1.8 to 2.0 gms/snail. Seperate sets of snails are kept and divided into experimental and control lots. They are kept in identical flower pots containing moist soil and covered by wire guaze. They are fed on mulberry leaves.

The snails are allowed to move on a flat surface or table. When tentacles are fully extended and the snail moves unaware, one of the optic tentacle is cut with sharp fine scissors. When the snail has

recovered the second optic tentacle is cut in the similar manner. After a pause when head is protruded any anti-septic powder (cicatrin) is poured on the wounds and the snail are returned to their group.

It is not possible that whole of the optic tentacle is cut off from the base while snail is alive. For experimental work it is enough that tip of the optic tentacle is cut, thus removing, along with it, the eye, tentacular ganglion, tentacular and optic nerves.

For preparation of ovotestis smear (control and experimental) alive snail is taken the shell is broken gently from the body whorl and then the columella is cut. The body of the snail is pulled out with great care and finally the last portion of the digestive gland is pulled out slowly the snail body is weighed. The ovotestis along with small hermaphrodite duct is cut from the rest of the body.

The larger/oldest lobule of the ovotestis, nearer the small hermaphrodite duct, is removed carefully and placed in a solution of 1% aq. methylene blue 5-10 minutes. The nuclei of the eggs and sperms are thus stained and easily recognisible. The ovotestis is placed on a slide and covered by a cover slip. The cover slip is gently pressed to flatten the ovotestis.

The eggs and sperms are counted with the help of an oculometer per square millimeter (area) of the

gonad (Table XI, XII). Count are made carefully in good preparation. If stained preparation are not successful then simple plane slide of ovotestis is prepared and an average of two or three count is taken. The result is then multiplied by ten to convert it into centimeter.

For preparation of homogenate of optic tentacle 30 (thirty) snails are collected and their optic tentacles are removed and kept in a seperate bowl. The contents of bowl is cut into small pieces crushed in a cavity block with a glass rod and mixed with 1ml of distilled water. The homogenate is stored in a refrigerator for further use. Normal saline water is prepared by dissolving 0.7 Nacl in 100 ml of distilled water. For injection fine 1.0 cc disposible surgical syringes are used. The injection is given in the body cavity. 0.1 cc i.e. equivalent to two optic tentacles is used as a single dose/animal. Two injections are given at weekly interval after ablation of optic tentacles.

During period of captivity the eggs laid by any snail in the flower pot are counted and discarded. The snails are dissected at weekly interval and following observation are made.

1. Colour and size of ovotestis white or light brown, conspicuous or reduced.
2. Number of shelled eggs in oviduct (uterus) i.e. female part of large hermaphrodite duct.

3. Large oocytes visible or not in the ovotestis smear (i.e. more than 50 in diameter).

4. Spermatozoa filling the ovotestis lobule to what extent (1/2, 1/3 or 1/4).

5. Colour and volume of albumen gland (white or light brown, reduced or very enlarged).

6. Weight of albumen gland, reproductive tract and of the snail's body was recorded.

The index for albumen gland and reproductive tract (Tables XIII-XVIII) are calculated by the formula used by Kulkarni (1980) and Pelluet and Lane (1961) for ovotestis following Wells and Wells (1959).

For reproductive tract index.

$$\text{Index} = \frac{\text{wt of rep. tract}}{\text{wt of the animal}} \times 100$$

For albumen gland index

$$\text{Index} = \frac{\text{wt of albumen gland}}{\text{wt of the animal}} \times 100$$

8.1 OCCURANCE OF NEURO-SECRETORY CELLS

It is observed that Bensonies jacquemonti have a number of neuro-secretory cells found in the cerebral ganglia, visceral chain, optic tentacles and dorsal bodies. (figs. 50 - 54). The neuro-secretory cells of the cerebral ganglion are found in the peripheral layers of medial lobe (meso - cerebrum). These cells are

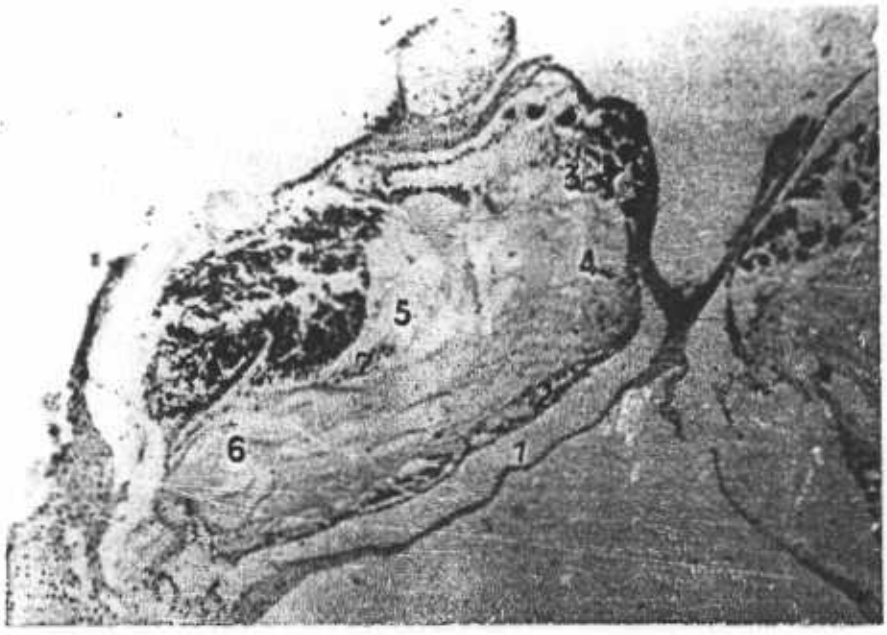

Fig.50: Microphotograph of L.S. of cerebral ganglion of Bensonies jacquemonti showing neurosecretory cells of meso cerebrum (X 400).

1. Outer thick layer
2. Inner thin layer
3. Dorsal bodies
4. Pro-cerebrum
5. Meso-cerebrum
6. Meta-cerebrum
7. Neuro-secretory cells of mesocerebrum

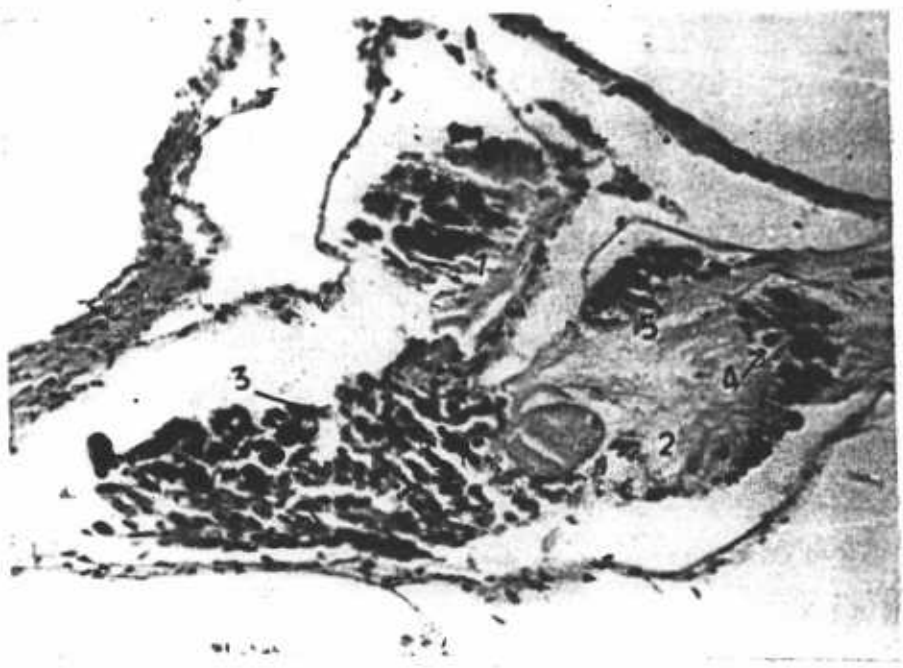

Fig.51 Micro photograph of T.S. of a portion of visceral chain of Bensonies jacquemonti showing dark neurosecretory cells in Pleural, Parietal and visceral ganglion (X 400)

1. Right pleural ganglion
2. Right parietal ganglion
3. Visceral ganglion
4. Cell body
5. Neurosecretory cells

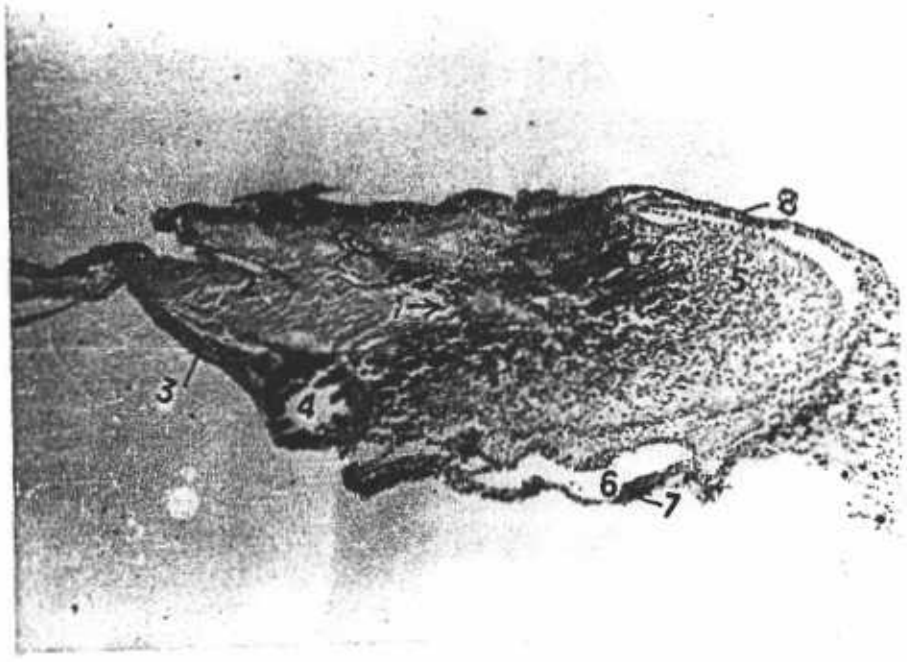

Fig.52 Microphotograph of L.S. of optic tentacle showing wavy margin and smooth tip on Bensonies jacquemonti note dense neurosecretion (X 400).

1. Tentacular ganglion
2. Tentacular nerve
3. Optic nerve
4. Eye
5. Neurosecretion
6. Haemocoel
7. Wavy margin
8. Smooth tip

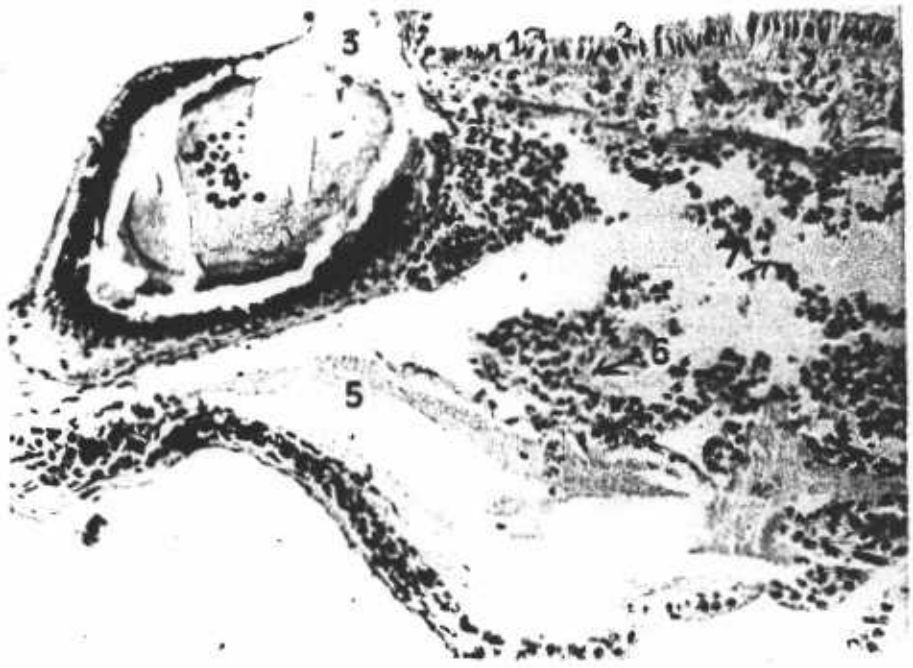

Fig.53 Micro Photograph of L.S. of optic tentacle of Bensonies jacquemonti showing structure and position of different cells note the collar cells (X 400).

1. Cuticle
2. Dermomuscular layer
3. Optic nerve
4. Eye
5. Tentacular nerve
6. Tentacular ganglion
7. Collar cells

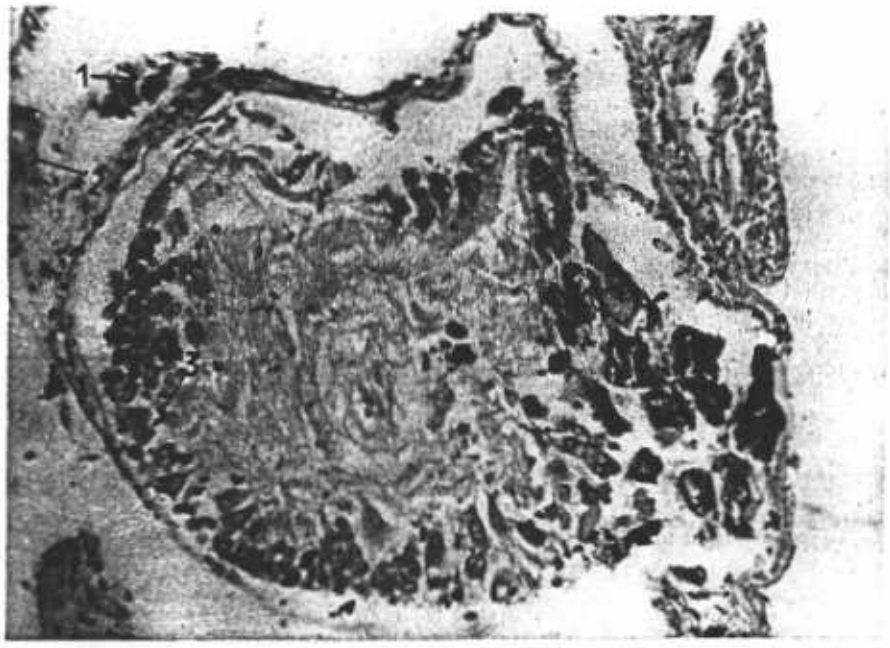

Fig.54 Microphotograph of T.S. of cerebral ganglion of Bensonies jacquemonti showing dorsally located dorsal bodies in the surrounding connective tissue (X 400).

1. Dorsal bodies
2. Connective tissue
3. Neurosecretory cells

present in groups on the dorsal part of meso-cerebrum (fig. 50). In visceral chain (parietal, pleural and visceral ganglion) the neurosecretory products are located in the form of cluster of white cells (fig. 51). These cells resemble the staining characteristic of the neurosecretory cells. Neurosecretory cells are also observed in optic tentacles of B. jacquemonti. They are more abundant near the optic ganglion and eye and form an incomplete ring around these structures (fig. 53). These cells secrete a hormone which plays important role in gametogenesis.

Dorsal bodies occur as clusters of pear-shaped cells. They are found dispersed in between double layers of connective tissue that enclose the brain. (fig. 54). Histochemical tests have proved them to be neurosecretory. It is observed that the volume of dorsal bodies increase during active period of growth and return to its normal size in resting phase. Thus they show seasonal or cyclic activity.

8.2 THE ENDOCRINE ROLE OF OPTIC TENTACLES

The effect of tentacular hormone and neuro-secretory cells of the cerebral ganglia on gametogenesis are studied in various forms of stylommatophora e.g. in Arion, Milax, Helix and Ariolimax.

Pelluet and Lane (1961) published their first report about endocrine control on gametogenesis in

pulmonate. They suggested that normal differentiation of the cells of the ovotestis may be regulated by a chemical substance, perhaps a hormone. They further pointed out that the site of their origin is in the brain and associated structures. Brain hormone controls egg production while tentacle hormone stimulates the male cells production. In their experimental work, the optic tentacles of the slug are removed and the effect is found significant as the number of oocytes are increased. The object of the present investigations is to study the effect of optic tentacle hormone on gametogenesis (Table XI, XII) and reproductive system in B. jacquemonti (fig. 55, Table XIII-XVIII).

8.2.1 EFFECT OF OPTIC TENTACLE HORMONE ON GAMETOGENESIS

Effect of optic tentacle removal on cyto-differentiation in the ovotestis of Bensonies jacquemonti shows a noticeable change. There is an increase in the number of large oocytes on one hand and a decrease in the number of motile sperms on the other hand, when compared with the normal snail (Table XI, XII).

The optic tentacle as described earlier has neuro-secretory cells surrounding the optic ganglion and eye as an incomplete ring called "collar" cells (fig.53). These cells produce a hormone which has an important effect on gameto-genesis.

Fig.55 Photograph of Reproductive system of Bensonies jacquemonti showing effect of tentacle ablation on reproductive tract and albumen gland note the small sizé on right side.

To varify the finding of pelluet and Lane (1961) on gametogenesis in slugs, experimental work on Bensonies jacquemonti is carried out. The snails are divided into experimental and normal groups. In the experimental snails either one or both of the optic tentacles are cut off.

Pelluet and Lane (1961), Kulkarni (1980) and Kulkarni and Hazara (1983) while observing the effect of optic tentacle hormone have removed both of the optic tentacles of the experimental slugs. In present experiment Bensonies jacquemonti is divided into two groups. I with single optic tentacle removed and II with both optic tentacles removed.

Effect of optic tentacle ablation has shown very interesting results in gametogenesis while observing the smear of gonad. The results are illustrated in Table XI and XII, the experiment is carried out during peak breeding season, so ovotestis is in full swing of gametogenesis.

At the beginning of the experiment it is observed that all lobules of the ovotestis are filled with masses of sperms and there are very few eggs (1-2) in each lobule.

EFFECT ON SPERMIOGENESIS

a. AFTER ONE HOUR

After ablation of optic tentacle the result of spermiogenesis is similar in control as well as in experimental groups. All stages of spermiogenesis along with mature sperms are found in abundance.

b. AFTER ONE WEEK

The count of motile sperms in control is fairly constant. There is slight rise (1.4%). In snails with ablation of one optic tentacle, the number is decreased by 37% while in snails with ablation of both optic tentacles the values are fallen down by 73% (Table XI).

c. AFTER TWO WEEKS

The effect is shown in Table XI there is 2.9% increase in the control snails while sperm level remained almost constant in the snails with only one optic tentacles removed i.e. the decrease is 38% after second week. In contrast to this in the snails with both optic tentacles removed the decrease is very high (86%) as compared to the control snails. From this experiment it is concluded that optic tentacle hormone is responsible for spermiogenesis. By ablation of one optic tentacle the sperm production is decreased by 37% in the first week but then the balance is maintained some how. As in the second week the fall is only 1% i.e. 38% relative to the control.

When both optic tentacles are cut off there is a sudden decline, 73% in the first week followed by a further descend in the second week making the total decrease of 86%. The snails with one optic tentacle still have secretion of the hormone and the spermiogenesis, though decreased, is not suppressed all together.

EFFECT ON OOGENESIS

a. AFTER ONE HOUR

The average number of large oocyte per square centimeter (i.e. per unit area assessed) is identical in both, control and experimental snails. There is no evident change in the oogenesis. (Table XII)

b. AFTER ONE WEEK

The effect of tentacle ablation is pronounced. The number of large oocytes rises to 35% in snails with one optic tentacle and in snails with both optic tentacle removed the increase is 60.5%. It is evident from the result that the secretion of hormone by optic tentacle suppresses oogenesis and as the optic tentacle is removed the oogenesis is increased.

c. AFTER TWO WEEKS

The oocytes are much more abundant in the gonad of snails with both optic tentacles removed. The average number of oocytes is 72.3% as compared with 38.8% in snails with only one optic tentacle ablation. The later

still have one optic tentacle to secrete the oogenesis suppressing hormone. The control snails have the usual smaller number of oocytes i.e. 11% (Table XII).

It is thus summerized that optic tentacle hormone has an important role of promoting spermiogenesis and suppressing oogenesis. Removal of the optic tentacles increase oocyte production in ovotestis and tends to promote the production of shelled eggs. It is observed that inspite of abundant number of oocytes in the ovotestis the number of shelled eggs deposited is fairly small.

The size of the ovotestis also seems to be effected by the ablation of the optic tentacle. In peak breeding season the size and volume of the ovotestis is increased and its colour changes from cream to light brown. It is observed that after removal of the optic tentacle the colour of the ovotestis remains the same but its size and volume is reduced inspite of increase in the number of oocytes. It is apparent that ablation of optic tentacle has resulted in inhibiting the growth of ovotestis. In other words the growth of ovotestis is under hormonal control of optic tentacle.

Table-XI Average number of motile sperms per square centimeter of gonad of Bensonies jacquemonti with one or both optic tentacles removed.

	Motile sperms per square centimeter		
	1 hour	1 week	2 weeks
Normal	272	276	280
One optic tentacle removed	276	172	170
Both optic tentacles removed	272	72	38

Table-XII Average number of visible large oocytes per square centimeter of gonad of Bensonies jacquemonti with one or both optic tentacles removed

	Large oocytes per square centimeter		
	1 hour	1 week	2 week
Normal	18	18	20
One optic tentacle removed	18	82	88
Both optic tentacles removed	17	120	140

8.2.2 EFFECT OF OPTIC TENTACLE HORMONE ON GROWTH OF REPRODUCTIVE TRACT (REP.TRACT) AND ALBUMEN GLAND (AL.GLAND)

The present study is undertaken to find out whether the reproductive tract and albumen gland of Bensonies jacquemonti is under neurosecretory control of optic tentacle. For 'cutting off' the optic tentacle Bensonies jacquemonti are divided into two groups.

1. Adult with shell size 19.5-5.20 mm and body weight 1.8-2.0 gms/snail
2. Sub-adult with shell size 16-16.7 mm and 1.0-1.3 gms/snail.

The sub-adult thus selected have complete 5 shell whorls with shell size 16 mm and above. These snails have reached the range of maturity (in the light of previous observation on post embryonic development. (Table VIII and X).

For experiment the snails are divided into three group:

1. Normal, with intact optic tentacle injected with 0.1 cc saline (0.7% Nacl) (Control)
2. Deprived of both optic tentacles injected with 0.1 cc saline (Control)
3. Deprived of both optic tentacle injected with tentacular solution. (homogenate)

EFFECT ON REPRODUCTIVE TRACT:

a. AFTER ONE HOUR

The effect of various injections after one hour shows the reproductive tract index is not altered in both types sub-adult and adult snails. In sub-adult the ratio is 7.5-7.7 while in adult it is between 24.59-24.69 as shown in table-XIII.

b. AFTER ONE WEEK

Table-XIV shows that after one week there is no effect of various injection on the reproductive tract index in adult and it remains fairly constant. In sub-adult group, the snails with optic tentacle removed shows an increase over the normal one. In them the value is 8.0 as compared with 7.54 of normal snail. Thus the increase is 6.10%. The snail with injection of optic tentacle solution has also increase of 1.45% over the normal one but it is very slight. Normal 7.54 and injected with tentacle solution 7.65. (Table-XIV).

c. AFTER TWO WEEKS

In adult snails the reproductive tract index remained constant, In sub-adult with optic tentacle ablation forms there is an increase. Normal snail have 7.75 and with optic tentacle removed form 8.18. So over all increase is 5.5%. Snails with injection of tentacle solution has increase of 3.2% (normal 7.75 tentacle solution injected form 8.0) (Table-XV).

From above experiments it is concluded that the removal of the optic tentacle shows no effect on the reproductive tract index in adult form but has an effect on the sub-adult snails. It is assumed that hormone of optic tentacle.may have an inhibitory action on the reproductive tract. As in the adult snail the reproductive tract has reached its full size and the sub-adult forms still have to grow therefore the reproductive tract index in sub-adult is more as compared with the adult. Even the result of sub-adult with optic tentacle ablation and injected with optic tentacle homogenate shows an increase over the normal although it is less than those with optic tentacle removed.

EFFECT ON ALBUMEN GLAND

a. AFTER ONE HOUR

As indicated in Table-XVI there is no effect of various injections after one hour on the albumen gland index in adult as well as in sub-adult snails. In adult Bensonies jacquemonti range of albumen gland index is 9.48-9.69 and in sub-adult from 0.910 to 0.947.

b. AFTER ONE WEEK

The effect of various injections is illustrated in Table-XVII. The albumen gland index in adult Bensonies jacquemonti is not altered. In sub-adult group an increase is observed in snail with optic tentacle removed

as compared with the normal one. In normal snail albumen gland index is 0.96 and with tentacles ablation snail it is 1.05, making about 9.3% increase. The snail with ablation of optic tentacle and injected with optic tentacle homogenate there is also an increase of about 5.2% (normal - 0.96 tentacle homogenate injected - 1.01).

c. AFTER TWO WEEKS

The effect of various injections after two weeks is more pronounced in the sub-adult form while in adult snails the index remains fairly constant. In sub adult with optic tentacle removed the ratio has increased normal = 1.00 and of optic tentacle ablation = 1.09 making = 9% increase. In snails with optic tentacle homogenate injections albumen gland index is 1.07, normal = 1.0 so an increase of 7% is recorded which is less from those with tentacle ablation but more than normal (Table XVIII).

It appears that the neurosecretory cells of the optic tentacle have control over the growth of albumen gland specially in sub-adult forms. As the sub-adult Bensonies jacquemonti in which optic tentacles are removed show increase in the index of albumen gland.

From these facts we can conclude that hormone of optic tentacle has an inhibitory effect on reproductive tract and albumen gland. When the optic tentacles are removed, the inhibitory effect is no longer there, so these organs grow in size specially in sub-adult, having more provision for growth.

Table-XIII The reproductive tract (rep.tract) index one hour after removal of optic tentacles in Bensonies jacquemonti (After various injections)

–	Adult			/gms	Sub-adult		
	No.of animals	Av.Body wt.	Av.wt. of the rep. tract.	Rep. tract index	Av.wt. of body	Av.wt. of the rep. tract.	Rep. tract. index
Normal injected with saline	5	9.8	2.41	24.59	5.6	0.43	7.68
Optic tentacles removed (saline)	5	9.8	2.42	24.69	5.6	0.42	7.50
Optic tentacles removed injected with tentacle solution	5	9.7	2.39	24.63	5.7	0.44	7.72

$$\text{Reproductive tract index} = \frac{\text{wt. of rep. tract}}{\text{wt. of body}} \times 100$$

Table-XIV The reproductive tract (rep.tract) index 1 week after removal of optic tentacles in Bensonies jacquemonti (After various injections)

	Adult /Gms				Sub-adult		
	No.of animals	Av.body wt.	Av.wt. of the rep. tract.	Rep. tract index	Av.wt. of body	Av.wt. of the rep. tract.	Rep. tract index.
Normal injection with saline	3	9.7	2.38	24.05	5.7	0.43	7.54
Optic tentacles removed (saline)	3	9.9	2.42	24.4	5.5	0.44	8.0
Optic tentacles removed injected with tentacle solution	3	9.8	2.42	24.6	5.6	0.42	7.65

$$\text{Reproductive tract index} = \frac{\text{wt. of rep. tract}}{\text{wt. of body}} \times 100$$

Table-XV The reproductive tract (rep.tract) index 2 weeks after removal of optic tentacles in Bensonies jacquemonti (After various injections)

	Adult /Gms				Sub-adult		
	No.of animals	Av. body wt.	Av.wt. of rep. tract.	Rep. tract. index	Av.wt of body	Av.wt. of rep.tract	Rep.Tract
Normal injected with saline	3	9.7	2.41	24.84	5.8	0.45	7.75
Optic tentacles removed (saline)	3	9.7	2.40	24.74	5.5	0.45	8.18
Optic tentacles removed injected with tentacle solution	3	9.9	2.43	24.54	5.5	0.44	8.0

$$\text{Reproductive tract index} = \frac{\text{wt. of rep. tract}}{\text{wt. of body}} \times 100$$

Table-XVI Result of treatment with various injections on the albumen gland (Al.gl) index one hour after removal of optic tentacles in Bensonies jacquemonti.

	Adult /Gms				Sub-adult		
	No.of animals	Av. body wt.	Av.wt. of Al.gland	Al-gland index	Av.body wt.	Av.wt of Al.gland	Al.gland index
Normal injected with saline	5	9.8	0.95	9.69	5.6	0.051	0.910
Optic tentacles removed (saline)	5	9.8	0.93	9.48	5.6	0.053	0.946
Optic tentacles removed injected with optic tentacle solution	5	9.7	0.92	9.48	5.7	0.054	0.947

$$\text{Albumen gland index} = \frac{\text{wt. of Al. gland}}{\text{wt. of body}} \times 100$$

Table-XVII Result of treatment with various injection on the albumen gland (Al.gland) index one week after removal of optic tentacles in Bensonies jacquemonti.

	Adult				Sub-adult		
	No.of animals	Body wt.	Wt. of Alb. gland	Al.gland index	Body wt.	Wt. of Al.gland	Al.gland index
Normal injected with saline	3	9.7	0.94	9.69	5.7	0.055	0.96
Optic tentacles removed(saline)	3	9.9	0.97	9.79	5.5	0.058	1.05
Optic tentacles removed, injected with optic tentacle solution	3	9.8	0.96	9.79	5.6	0.057	1.01

$$\text{Albumen gland index} = \frac{\text{wt. of Al. gland}}{\text{wt. of body}} \times 100$$

Table-XVIII The result of treatment with various injection on the albumen gland (Al.gland) index two weeks after removal of optic tentacles in Bensonies jacquemonti

	Adult				Sub-adult		
	No.of animals	Body wt.	Wt.of Al.gland	Al.gland index	Body wt.	Wt. of Al.gland	Al.gland index
Normal injected with saline	3	9.7	0.95	9.79	5.8	0.058	1.00
Optic tentacles removed (saline)	3	9.7	0.96	9.89	5.5	0.060	1.09
Optic tentacles removed injected with optic tentacle solution	3	9.9	0.97	9.79	5.5	0.059	1.07

$$\text{Albumen gland index} = \frac{\text{wt. of Al. gland}}{\text{wt. of body}} \times 100$$

8.3 DISCUSSION

B. jacquemonti like most pulmonates maintains a permanently active gonad. The sperms are produced throughout the year and different stages of spermiogenesis are observed for most of the time. The production of large oocyte is relatively few as compared with the masses of sperms produced. The apparent annual cycle of reproduction is a direct response to rainfall. Pelluet and Lane (1961) put forward the concept of dual hormonal control system. According to this theory tentacle hormone stimulates the production of sperms in first place, followed by the egg production under the control of brain hormone. They suggested that these hormones act on germinal epithelium of the ovotestis for the formation of gametes. They assumed that in young slug both hormones are present, but tentacle hormone (TH) suppress egg hormone (EH) and only sperms are formed. When the animal is mature the EH is no longer suppressed by the TH and it becomes active and egg production ensues.

In Bensonies jacquemonti also there is a balance between these two neurosecretory product. There is a long period of sperm production followed by a short period of egg production. Thus a balance between 'maleness' and 'femaleness' is maintained. It seems that the tentacular hormone either inhibits the production of oocytes until maturity or it regulates neurosecretory output of the brain. The ablation of optic tentacle results in an increase in the number of oocytes and a decrease in the number of sperms. Beside this there is a reduction in size and

volume of ovotestis inspite of increase in the number of oocytes. Thus by removal of the optic tentacle actual growth of ovotestis is effected and degree of femalesness as indicated by marked increase in oogenesis.

The decrease in size of the ovotestis may be associated with the malnutrition because of the removal of the optic tentacle which help in finding of food. Hyman (1967) observed that in terrestrial pulmonates the olfactory sense are present in tentacles and specially in posterior tentacles.

Martoja (1972) quoted Vanmol (1960, 1962, 1967) Jossee (1964) and Menon (1966) that "The neurosecretory cells of the cerebral ganglia of pulmonata also show a maximum of activity at the time of gametogenesis" He also stated that according to Smith (1967) the variation of the neurosecretory activity according to functional state of reproduction are more prominent in visceral ganglion than in the cerebral ganglion.

The relation between the neurosecretion of optic tentacles and growth of reproductive tract and albumen gland is observed. The optic tentacles of B. jacquemonti are cut off and the snails are maintained for different length of time. Injection of optic tentacle homogenate is given at different interval.

Observation revealed that reproductive tract and albumen gland is under control of endocrine system of optic tentacle. Kulkarni and Hazari (1983) stated that

Abeloos (1943) was of the opinion that albumen gland is under the control of the gonad. They concluded from the results that growth of albumen gland is controlled by the optic tentacles and not by gonad.

The present investigations have established that after cutting off the optic tentacles the neurosecretory product of optic tentacle has effect on:

1. Cytodifferentiation or gametogenesis in gonad. The hormone promotes spermiogenesis and suppress oogenesis.
2. The size and volume of the ovotestis increases with the presence of optic tentacle hormone, while in the absence of this hormone there is decrease in volume and size of ovotestis inspite of the fact that the number of oocytes has increased considerably.
3. The neurosecretion of the optic tentacle effect the growth of reproductive tract. The hormone has inhibitory action on the growth specially in the immature snails. The mature snails are least effected by the hormone.
4. The optic tentacle hormone has effect on the growth of the albumen gland. Just like reproductive tract, the albumen gland of the sub-adult B. jacquemonti is increased in the absence of this hormone.

The optic tentacle hormones therefore controls the growth of albumen gland and reproductive tract. The effect is more prominent in sub-adult as compared to

adult forms.

The fact is that in young B. jacquemonti the secretory activity is very intensive because of the rapid growth rate. So, removal of the optic tentacle, results in the release from the inhibitory action of this hormone. This in turn causes the rapid growth of the reproductive tract, albumen gland and also the ovotestis to some extent.

When B. jacquemonti reaches maturity it is expected that the neurosecretion of the optic tentacle is less intensive than in the growing animal.

Therefore the reproductive tract and albumen gland of the mature snail is least effected by the removal of neurosecretory product of the optic tentacle (in form of its ablation).

The weight and volume of the albumen gland varies greatly during different seasonal cycle. It appears that it gives a direct response to these climatic conditions when food is abundant and the general nutritional condition is good, the albumen gland is full.

Although optic tentacle hormone controls gametogenesis and its absence results in increased number of oocytes in the gonad. It does not effect the growth of albumen gland in adult. However when the albumen gland is full or heavy and there are increased oocytes in the gonad, it tends to promote the production of shelled eggs, on the other hand when the albumen gland

is light due to nutritional conditions the snail produces few shelled eggs inspite of the fact that there are more oocytes in the gonad.

These observations also coincide with the fact that the albumen gland forms a heavy layer of albumen (about 80%) around the ovum as it passes by the duct of the gland. Thus the production of oocyte is not effected by the general nutritional state of the snail but it do effect the albumen gland and production of shelled eggs.

CHAPTER-9

DORMANCY

Dormancy (Hibernation and Aestivation) is referred to the period of any inactivity in which the snail retreats inside the shell completely and then the aperture of the shell is closed by a temporary lid or seal of dried mucus. Usually the aestivation indicates the lengthy period of dormancy occuring in summer while hibernation is referred to winter sleep. (Fig. 56).

According to Rasmussen (1914) the German Called hibernation as "Winter Schiaf" French called it "Sommcil hivernal" and Italian used "letargo" for this winter sleep.

Hibernation, aestivation and tolerance of dehydration are well achieved in higher Stylommatophora (Morton, 1967).

It is difficult to note the process of dormancy in natural condition because during severe cold and hot weather they are usually found in dormant state. What happens before, during and after dormancy was not observed.

To observe the actual process step by step Bensonies jacquemonti are forced to pass in dormant state. The procedure may be divided into three steps.

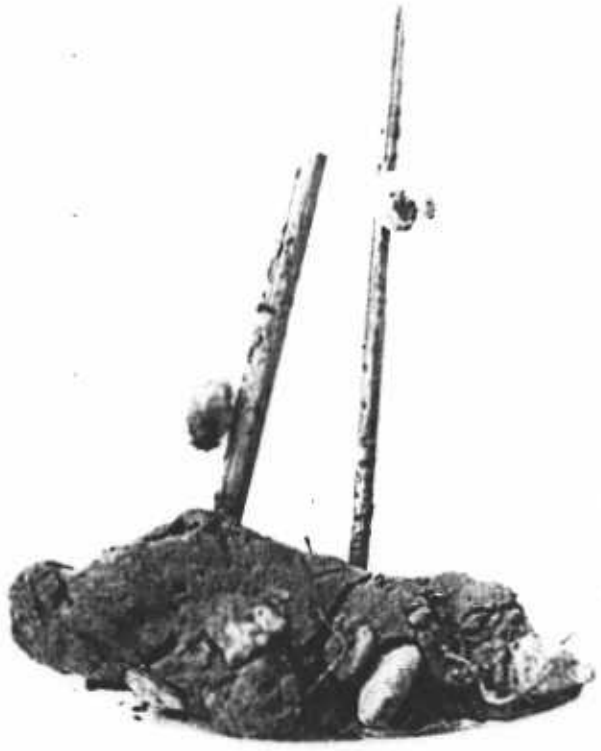

Fig. 56 Photograph showing Bensonies jacquemonti attached to a stick inside a flowerpot in the laboratory during aestivation note the aperture of the shell is directed upward and one snail is attached to the shell of its fellow snail.

1. Pre dormancy
2. Dormancy
3. Post dormancy

9.1. PRE-DORMANCY

Bensonies jacquemonti of two age groups, young (shell size 12-14 mm) and adult (shell size 19-21mm) are collected and placed in separate petridishes without providing them food. The petridishes are covered by glass covers. In this pre-dormant period they are quite active and crawled and moved about in the dish. Faeces is produced constantly upto 36 hrs (1½ days) and more infrequently upto about 2 days. After 24 hrs. their activities are slowed down, the tentacles are retracted and they remained fixed to the petridish. It is worth mentioning that they undergo hibernation or aestivation separately not in groups attached to each other. Although many of them are found in dormant state near each other in the same place in nature. In the field they select a place for aestivation which is not in direct line with the sun rays and usually the site selected is under side of stone, bark of tree, corner of wall which ever are the shady sides.

In laboratory on the first 2 days, the snails are found active during the night only. For the next

1-3 days the tentacles are protruded and some time they may move about in petridish, but after this they tend to pass in dormancy as they become fixed at one place, tentacles are retracted and they remain motionless.

9.2. ONSET OF DORMANCY

During extreme conditions of season Bensonies jacquemonti reacts more or less positively and passes into a dormant state. Following steps are observed during the process of hibernation and aestivation in the laboratory while watching through the glass surface of petridish.

1. The snail becomes in-active, the tentacles are retracted completely.
2. The foot is very much contracted(reducing its size to 1/4 almost) and sticks to the surface.
3. The mantle surrounds the foot on all sides leaving a small portion near the aperture of the pulmonary sac.
4. Mantle starts secreting epiphragm, the mucus is deposited along the borders only, which becomes hard and milky white on exposure to air (this is the beginning of epiphragm).
5. First of all the edge, opposite the columella, is attached to the substratum, so that it is hard enough to fix the shell properly

to the object. (The aperture of the shell is always directed upward (Fig. 56).

The formation of epiphragm may be divided into following steps.

a. As the mucus secreted by the mantle comes out, it flows around the foot and thus forms the outer boundry of the epiphragm.

b. It looks like that the foot size determines the size of the aperture of the epiphragm. As in the beginning the size of the foot is 1/4 of the original length and so is the boundry of the epiphragm. In later stages the foot is further contracted making its size 1/8.

c. At this stage the size of the epiphragm opening is also reduced and it nearly encircles the foot.

d. Clear movement is observed in the mantle in the form of contraction and expansion as if mucus is secreted (it shines).

e. the foot also shows some kind of movement from time to time as if spreading the mucus secreted by the mantle.

f. The foot is attached to the object by its sole and the mantle surrounds its upper border which has become elongated and is for away from sole.

g. The mucus as it comes out from mantle, runs along the sides of the foot to make the boundry of the epiphragm more thick.

h. By now the shell is properly fixed to the secretion of the mantle.

i. The foot slowly starts detaching from the object, as is assumed by the very thin covering of mucus above the foot's sole. (as said earlier it seems that foot is spreading the mucus and mantle is secreting it).

j. Above the foot is a very thin covering of the mucus through which the movement of the mantle and foot can be observed.

6. The snail withdraws its whole foot inside the shell and it is covered by the mantle.

7. The mantle secretes the remaining epiphragm and the aperture near the pulmonary opening is also closed. So a complete lid-like epiphragm is formed.

8. The body of the snail in first instance was retracted 0.5 mm inside the shell and now it has shifted further deep for about 8-10 mm. (Fig. 57-60).

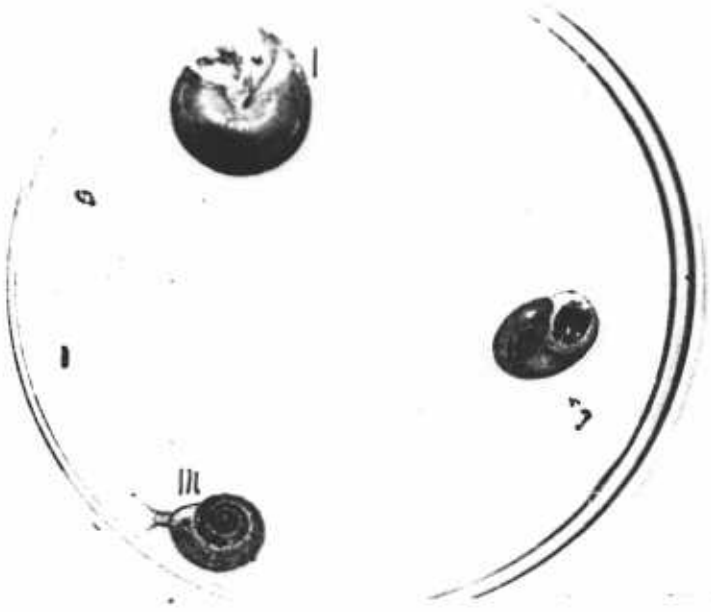

Fig.57 Photograph of Bensonies jacquemonti during aestivation as seen through Petri dish. Snail I with complete epiphragm,in snail II the foot is just retreated in and covered by thin layer of mucus while snail III is activated by ambient temperature during photography.

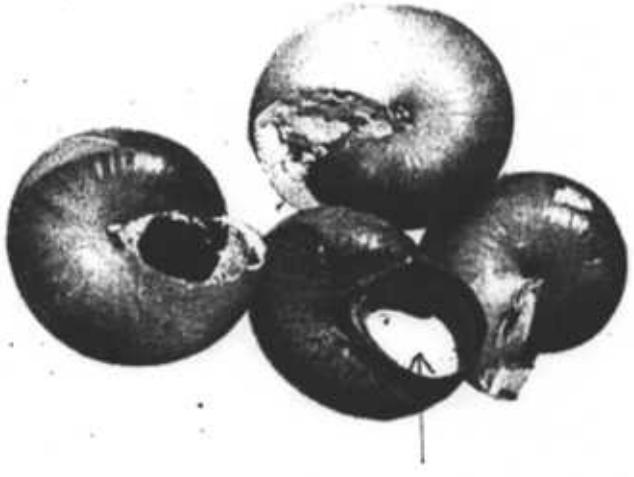

Fig.58 Photograph of Bensonies jacquemonti during aestivation as seen through petridish. In snail I foot is much reduced but still attached to the dish, snail two has complete epiphragm note the free margin of shell and shape of epiphragm.

Fig.59 Photograph of aestivated Bensonies jacquemonti note the epiphragms are cracked by disturbance.

Fig. 60 Photograph of aestivated Bensonies jacquemonti in three snails the epiphragm formation is almost finished while in the upper snail the foot is attached to the dish.

9. The newly formed epiphragm is thin and membranous and it becomes thick and hard after addition of more secretion and more exposure to air. Thus forming a hard and complete lid.

When the snails have completely retracted inside the shell, and the mouth of the shell is covered by epiphragm this is the state known as dormancy.

The epiphragms formed in winter are thick as compared to those of summer which are more thin. In severe condition the snail retreats further inside the shell but a second epiphragm is not formed.

The epiphragm is not rounded but some what oblong in appearance, in initial stages, it gradually becomes rounded in shape, as more mucus is added on larger sides. After 36 hours the shape is almost round it is fixed to the object (in this case to the petridish).

After 48 hours the inner circle measures 5 mm in diameter. The outer margin of the circle is in the form of white membranous material. In due course this aperture continues to become narrow and ultimately it closes leaving no connection with the exterior. Observations of several dormant *B. jacquemonti* show that none is found with a perforated epiphragm. All of them whether

they are hibernating singly, attached to their fellows, shell or to some support have complete epiphragms. In adult snail with a shell size 20 mm the average large diameter is 9 mm and smaller diameter is 5mm of the shell aperture.

In the laboratory observations on the third day the epiphragm is almost complete leaving no pore. If this epiphragm is damaged by needle or the snail is detached from the dormancy place and kept in petridish (uncovered). It was noticed that usually the snail remains motionless for the whole day and in the evening or next morning the second epiphragm is found, with some space, on the inner side of the first one (the first epiphragm is left as such i.e. damaged). On one occasion the snail remained motion-less for the whole day but next morning it was found missing from the petridish. When searched for it, it was found to have travelled a distance of 45 cm-180 cm (1 1/2 ft-6 ft) and has fixed to some object on wall in dormant state. The escaped snail usually like to travell towards concealed places i.e. behind the curtains under side of table, corners of walls and behind the books.

As snail is reactivated from the dormancy, this time it under-goes dormancy quickly. It fixes itself to the object and the epiphragm is completed rapidly

(within 30-45 minutes). The old epiphragm is never repaired and if the snail is unable to secrete a new and complete epiphragm after the damage of the first one, it desiccates completely and dies.

The probable function of the epiphragm is to protect the body against the unfavourable condition of the season. In winter it prevents the body from extreme cold and thus helps the snail to exclude the cold. In summer as the waather is extremely dry and hot it forms a shield against draught and does not permit evaporation thus helps the snail to retain its own moisture.

Temporary epiphragm which is thin and membranous is formed in those snails which under go short period of dormancy. Such epiphragm are formed, broken and reformed. These short periods of dormancy are in early summer when days are hot but nights are cold and on most nights fall of dew occurs. During this season the snail undergoes temporary or short dormancy during the day time by secreting a thin epiphragm but is active every night.

9.3. POST DORMANCY(AWAKENING)

The Recovery or Awakening from dormancy in Bensonies jacquemonti occurs after the return of favourable conditions. This is followed by rainfall. The presence of epiphragm is no longer needed so it is rejected and the snail once more awakens from its sleep and is re-activated.

Morton (1967) quoted Well (1944)"that rain drops knock at the door and the snail comes out. Hydration follows after, when it has eaten and drunk" Morton was of the opinion that this Sudden reactivation after rain is not due to hydration but may be caused by some sensory stimulation effect.

The process of reactivation may be summarized as following:

1. The rain drops fall on the epiphragm and this may cause gentle striking, shaking or rotation of Bensonies jacquemonti.
2. The animal thus produces responses which are necessary for initial stages of reactivation.
3. The mantle shows some signs of life and slight movement is observed, this is followed by muscular movement of the mantle.
4. The pneumostome opens.
5. The heart beat becomes visible.
6. The posterior tip of the foot is protruded.
7. The foot and head is pressed against the membranous epiphragm which ruptures and thus the snail is activated.

During winter the epiphragm is thick calcarcous and solid lid like. In this case the method of reawakening differs slightly.(After number six). When the foot is

protruded, it seems to secrete some secretion which softens the margins of the epiphragm, or the atmospheric water loosens the sides of the epiphragm. The whole epiphragm is then lifted like a lid and may be seen for some time on the posterior tip of foot like false operculum. During active period after rain-fall many epiphragms in the form of Solid lids may be seen near the bases of hedges or at the resting place of the snail. These are the cast off epiphragms of snails after awakening from dormancy(Fig. 61).

In the laboratory three dormant snails of equal size are selected for experiment, their epiphragms are removed, one is kept in a dry petridish while the second in a petridish containing small quantity of cold water and a third one in a dish with little warm water. The snail of the first dish remained un-changed till next morning, that of second dish is activated after 2-3 hours while the snail in the third dish containing warm water revives within a short time. Food in form of mulberry leaves is provided to both of them. The activated snails have come out of their shells and have crawled about. In the evening the snail of cold water is found reaestivated without eating food, while that of warm water was still active. In the morning it is observed that this snail has eaten food, passed some faeces and then has undergone dormancy.

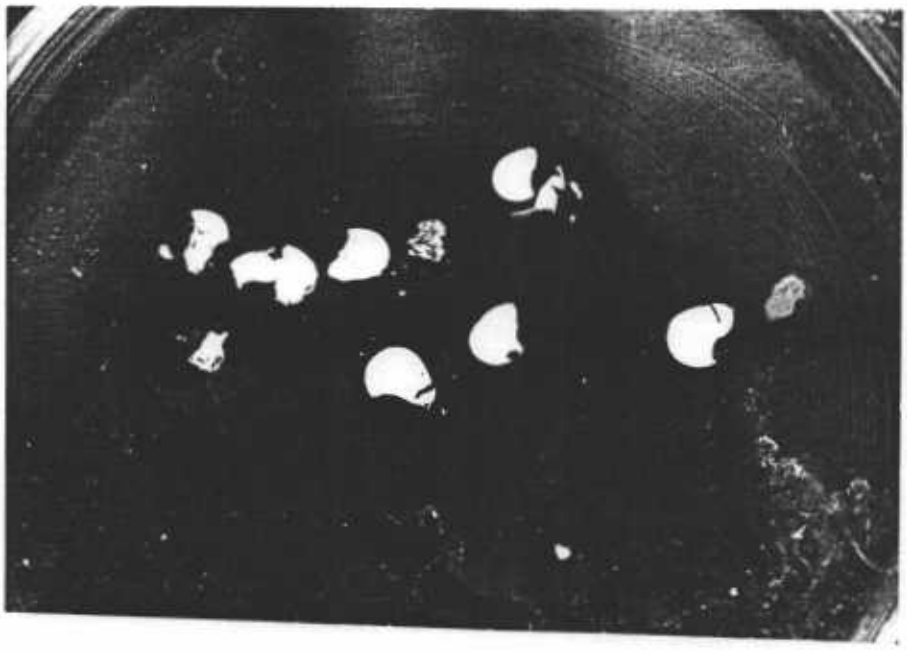

Fig.61 Photograph of the epiphragms of Bensonies jacquemonti as they are removed from aestivated snails. Note the peculiar shape of epiphragm corresponding with the size and shape of shell aperture.

From above experiment it is calculated that for reactivation, the first and important factor is direct contact with water and next a certain amount of warmth and humidity is also needed.

Weight loss of dormant Bensonies jacquemonti is supposed to be due to evaporative loss. The data shows that the loss is environmentally corelated and depends on temperature, humidity and even the size of the snail. (Table XIX - XXII). The continous weight record of inactive Bensonies jacquemonti, with epiphragm shows gradual loss of weight upto 1 mg (Table XIX -XX), in initial days. There may be occasonal increase in weight. The loss may be related to release of humid air from the lung and the gain is corelated with the absorption of water by the dehydrated mantle surface. (Table XXII - XXIV).

The normal heart beat rate is 72/min of the active Bensonies jacquemonti (Table XXV). A record was maintained to observe how much the heart beat/decreases during dormancy. During onset of dormancy the rate slowed down from 66/min to 62/min(when only the out-line of the epiphragm is secreted).

As the epiphragm formation is in progress and the snail is firmly attached to the object the heart beat is recorded to be 42-40/min. Further observation was not possible, because the heart was indistinguishable

Table- XIX. SHOWING WEIGHT LOSS (g) DURING ONSET OF DORMANCY IN Bensonies jacquemonti(Juvenile)

Individual	Shell size	Initial wet weight(g)	Wet weight loss after different duration				
			0-6 hrs	6-12 hr	12-18 hr	18-24 hr	24-30 hr
1	15 mm	0.9	0.88	0.79	0.75	0.70	0.70
2	13	0.81	0.80	0.62	0.60	0.60	0.59
3	14	0.81	0.80	0.78	0.70	0.65	0.64
4	12	0.60	0.58	0.58	0.50	0.50	0.48
5	13	0.80	0.79	0.75	0.69	0.67	0.65
	Mean	.78	.77	.70	.64	.62	.61

Average weight loss as % initial wet weight = 21.79

Table-XX. SHOWING WEIGHT LOSS(g) DURING ONSET OF DORMANCY IN Bensonies jacquemonti (adult)

Individual	Shell size (mm)	Initial wet weight(g)	Wet weight loss after different duration				
			0-6 hr	6-12 hr	12-18 hr	18-24 hr	24-30 hr
1	21.0	1.9	1.8	1.7	1.69	1.65	1.61
2	20.0	1.89	1.8	1.7	1.69	1.69	1.61
3	21.0	2.0	1.9	1.8	1.7	1.7	1.65
4	18.0	1.6	1.5	1.39	1.3	1.25	1.21
5	19.0	1.8	1.7	1.59	1.5	1.42	1.4
6	Mean	1.83	1.74	1.63	1.57	1.54	1.49

Average weight loss as % initial wet weight = 18.57.

Table-XXI. PERCENTAGE OF LOSS OF WEIGHT (g) DURING AESTIVATION IN Bensonies jacquemonti FOR A DURATION OF 120 DAYS.

Age	Shell size (mm)	Initial weight(g)	Days of aestivation							
			10 dys	20 dys	50 dys	60 dys	80 dys	90 dys	120 dys	% loss
Adult	22 (8 snails)	13.3	13.1	12.9	12.64	12.54	12.4	12.3	10.9	18.0
Sub-adult	15 (8 snails)	8.55	7.8	7.5	7.2	7.1	7.02	6.8	6.2	27.48

Average weight loss as % of initial wet weight.

In adult = 18.0

In sub-adult = 27.48.

Table-XXII. PERCENTAGE OF LOSS OF WEIGHT(g) DURING HIBERNATION IN Bensonies jacquemonti FOR A DURATION OF 120 DAYS.

Age	Shell size (mm)	Initial weight(g)	Days of hibernation							
			10 dys	20 dys	50 dys	60 dys	80 dys	90 dys	120 dys	% loss
1.Adult	22 (8 snails)	14.7	13.4	13.2	13	12.9	12.7	12.6	10.9	25.8
2.Adult	16 (10 snails)	9.0	8.1	7.9	7.8	7.7	7.59	7.5	6.4	28.8
3.Sub-adult	10 (10 snails)	4.0	3.4	3.28	3.2	3.2	3.2	3.1	2.6	35.0
Sub-adult	6 (6 snails)	0.9	0.7	0.7	0.64	0.64	0.62	0.6	0.6	33.3

Table- XXIII. SHOWING GAIN IN WEIGHT(g) AFTER UPTAKE OF WATER DURING RECOVERY FROM DORMANCY IN YOUNG Bensonies jacquemonti.

Individual	Initial dry weight(g)	Wet weight gain by water after different duration				
		0-4 hr	4-6 hr	6-18 hr	18-30 hr	30-44 hr
1	0.6	0.65	0.62	0.62	0.6	0.6
2	0.4	0.5	0.5	0.5	0.5	0.6
3	0.5	0.5	0.6	0.5	0.5	0.55
4	0.3	0.4	0.4	0.4	0.4	0.42
5	0.5	0.61	0.61	0.61	0.6	0.65
Mean:	0.46	0.53	0.54	0.52	0.52	0.56

Average gain wt. after 44 hours in 21.7% of initial dry weight.

Table- XXIV. SHOWING GAIN IN WEIGHT(g) AFTER UPTAKE OF WATER DURING RECOVERY FROM DORMANCY IN Bensonies jacquemonti (adult)

Individual	Initial dry weight(g)	Wet weight gain by water after different duration				
		0-3 hr.	4-6 hr	6-18 hr	18-30 hr	30-44 hr
1	1.5	1.6	1.7	1.7	1.6	1.55
2	1.0	1.4	1.5	1.5	1.4	1.3
3	1.5	1.8	1.9	1.8	1.7	1.62
4	1.2	1.6	1.62	1.85	1.62	1.5
5	1.4	1.8	1.7	1.8	1.65	1.6
Mean:	1.32	1.64	1.68	1.73	1.59	1.51

Average gain in wt. after 44 hrs is 14.39% of initial dry wt.

Table-XXV. SHOWING RATE OF HERAT BEAT DURING DIFFERENT TIME AND AGE OF Bensonies jacquemonti AND OTHER PULMONATE SNAILS

Species	Age	Condition	Heart beat per minute	Source
1. Bensonies jacquemonti	Adult	Active	72/min	Present observation
2. "	Young	"	"	"
3. "	Adult	Summer day sleep (active)	46/min	"
4. "	Young	"	60/min	"
5. "	Adult	Onset of dormancy	56/min	"
6. "	Adult	"	40/min	"
7. "	Adult	Egg laying	50/min	"
8. "	Adult	Reactivated after removal of epiphragm	88-90/min	"
9. Lymnaea palustris	"	Active	80-81/min	Hyman(1967)
10. Physa	"	"	70/min	"
11. "	Young	"	73/min	"
12. Helix pomatia	Adult	"	50/min	"
13. "	"	Dormant	35/min	"
14. Cepaea hortensis	Adult	Active	43/min	"
15. "	Young	"	74/min	"

Table-XXVI. SHOWING SURVIVAL TIME WITHOUT FOOD AND WATER IN Bensonies jacquemonti of different age group under laboratory conditions

Age	Condition	Temperature °C	Duration of desiccation period	Survival(%)
15 days 1½ whorls	Laboratory	16-22	24 hours	100
20 days	"	"	54 hours	33
			3-4 days	
Sub-adult(10 mm)	"	15-32	15 days	100
Sub-adult(12-13 mm)	"		60 days	90
Sub-adult(12-13 mm)	"		90 days	55
Adult(shell size 15 mm)	"		90 days	75
"	"		150 days	55
" 20 mm)	"		150 days	62
" 22 mm)	"		150 days	75
Adult shell size(17-21 mm)	"		3½ years	60
Sub-adult(10 mm)	"		150 days	70
Young (6 mm)	"		90 days	40

at this stage. Whether the area previously occupied by heart is concealed under the epiphragm or the snail has contracted in such a position that heart is no longer visible now.

In the present study the heart-beat of B. jacquemonti of different age group, and during different time is recorded. The snail during dormancy is collected, its epiphragm is removed and the body is touched with the forceps (2-3 times). Heart starts beating at once with very high i.e. 80/min as compared to normal 72/min. During dormancy the heart beat seems to be ceased. To observe whether the heart beat ceases or is slowed down during dormancy, the epiphragm of a dormant snail is removed. Leaving the dorsal side intact, the main body whorl is broken from the ventral side. The purpose was that the position of the heart remains un-concealed for observations.

The snail is left in this condition in the petridish In the evening it is observed that a new epiphragm is secreted,the body of the snail has shifted deep in the shell. The position previously occupied by the heart is not covered by the epiphragm.

The heart beat on the same spot is not located. This means that during dormancy the heart stops or its beat is negligible. The heart beat during day time (when the snail retires, takes rest or day sleep) also slows

down. In young snail the heart beat is fast than adult (Table heart rate XXV).

During dormancy it is observed that an average of about 20% of the normal wet weight of the tissue is lost. In adult snail the loss is low while in the young it is a bit high from the average mean (Table XIX -XXII).

Inspite of this weight loss many snails continue dormancy for over one year. A record of dormancy in B. jacquemonti is 3½ years (Table XXVI). During recovery from the dormancy when the snail is kept in water for activation, it is observed that snail after awakening may crawl away from the water. The gain of weight during reactivation by the uptake of water is recorded.

The snail has completely absorbed the lost water within four hours of its activation. During early hours there is a gradual increase in weight by the water as no food is provided at this stage. There may be occasional loss in weight which may be related with the water loss by evaporation from exposed body surface or through lungs (Table XXIII- XXIV).

It is interesting to mention that at the end of the experiment it is found that weight loss during dormancy and weight gain at the recovery from dormancy nearly equals.

Hyman (1967) stated old analysis of Wicke (1863) about the content of epiphragm in Helix pomtia these are:

Calcium carbonate	= 86.75%
Magnesium carbonate	= 0.96%
Alkaline earth phosphate.	= 5.36%
Iron	= 0.16%
Silica	= 0.35%
Organic material	= 6.42%

As it is observed that when hibernating B. jacquemonti is brought indoor, its epiphragm is removed. It is placed in warm water to be activated, and food is provided. The snail becomes active it may or may not feed but soon secretes a new epiphragm and passes into hibernation again. In contrast to this the aestivated snail (in summer) after reactivation (by force) when provided with food and favourable condition remains active.

The field as well as the laboratory observations show that the adult snails undergo dormancy earlier than their young forms. The young snails are found active for longer period during unfavourable condition.

In contrast to this it is worth mentioning that adult individual survives longer than the young one.

Adult snail usually stays longer in dormancy but the young snail may be tempted out earlier on warm days. It seems that juvenile is subjected more to physiological stress, and adverse condition.

9.4. DISCUSSIONS

In Bensonies jacquemonti the dormancy and activity is directly related to season. The method of dormancy is also different from other pulmonates. The slugs spend their dormant period burried in the ground while some pulmonate snail may climb high up the trees and rocks and many other may face the sun. These snails may have conspicuous and exposed position/or hidden places during dormancy.

B. jacquemonti is cryptic during the period of inactivity. This snail aestivates above the gorund but in concealed places. The usual height of dormancy ranges 8-20 cm above the ground. During unusual circumstances, when the ground is very wet, flooded with water or when kept in the laboratory away from their natural and favourable environment, the snail may climb a considerable height upto 10-15 feet. Their favourit sites

are back sides of flower pot, tree trunk, the portion near the ground, back side of stones etc. B. jacquemonti usually avoid tree climbing and aggregation in dormancy. Many of them are found near each other in the same place but aestivated singly or 2-3 may be found attached to the shells of their fellow snails. (Fig. 56).

Hora (1925) found Succinea arboricola aestivating on the bark of mango tree, in groups. Pomeroy (1968) stated that Helicella virgata tend to be gregarious and form conspicuous white "caps" on tree trunks, ranging between 300-400 H. virgata aestivating on it, at heights of 0.9-2.5m.

In present studies young and mature B. jacquemonti are found traped in pools of water, (after heavy rainfall or when the area occupied by them is watered by small canals) due to over hydration. As by prolonged contact with water the body has swollen excessively and has caused hinderance in efficient crawling.

In Succinea arboricola and Hygromia fusca as observed by Hora (1928), the young individuals of these snails have the tree climbing habit during dormancy. The probable reason for this pecular habit of young snail may be that the young ones are more liable to be drowned than adults after heavy rain when the ground is flooded with water.

When the weather is unfavorable B. jacquemonti hides itself in a variety of ways. The snail closes the mouth of the shell with mucus (epiphragm) and fastens it to some object. If the snail is isolated then the aperture of the shell is closed by the mucus in the form of a complete lid. During present work it is observed that even in severe seasonal conditions, a single epiphragm is secreted (Fig. 57-61). Although the body of the snail is found continuously retreating further and further as a reaction to the weather, a second epiphragm is secreted only when the first one is damaged or cracked accidentally. During lengthy dormancy the epiphragm is thick and calcarous.

Hora (1928) quoted many authors stating that many snails secret a second, third or even fourth epiphragm in severe conditions of weather and that the outer epiphragm is thick as compared to the inner ones. He observed lime salts deposition in the epiphragm of Roman snail (Helix pomatia) thus forming a sort of solid lid.

Hunter and Popvich (1977) stated that hibernating Helix pomatia are most commonly found with either 2 or 3 epiphragms (heavily calcified).

A Bensonies jacquemonti (dormant) with perforated epiphragm is never found. The newly formed epiphragms is thin and delicate and is liable to be damaged if the object to which it is fixed is shaken.

In most individual snails which are roughly handled or removed from their places of dormancy, the epiphragm is found cracked(Fig. 59).

Hora (1928) quoted Allman (1986) who found that the epiphragm of Helix aspersia is perforated by a small pore over the site of pulmonary aperture to enable its access with atmospheric air. Hora (1928) himself was unable to find Helix aspersa with perforated epiphragm;

During dormancy in B. jacquemonti the heart beat is missing or negligible and it is not certain whether it beats or stops beating in this state (Table XXV).

Little (1968) made a hole in the shell of an aestivated terrestrial snail Pomacea and found the heart beating. He was not sure whether the heart beats through out dormancy or this happened due to stimulation by the operation. Dejorge and Petersen (1970) observed that in Strophocheilus the Pneumostome is kept open for respiration during dormancy.

To avoid dehydration the snails usually select such sites which are shady, cooler and away from sun rays. These areas are of high humidity, moderate temperature and low light intensity Further loss of water is reduced by sealing the shell aperture by epiphragm. Although the epiphragm is thought to be permeable to water to some extent, it still forms the major barrier for water loss from the shell aperture.

In Bensonies jacquemonti during dormancy the weight loss ranges from 18.57-21.79% within first two days while initial gain in weight 14.39%-21.7% occurs within 24-36 hours (Table XIX - XXIV). A record of 120 days showed that in young B. jacquemonti during aestivation as well as in hibernation, the weight loss is more i.e. 27.48% and 35.5% respectively. While adults have weight loss of 18.0% and 28.8% in two conditions (Table XXI-XXII).

Dejorge and Petersen (1970) noticed 24 to 39% loss of original weight after 60 days of hibernation in Thaumastus.

Machin (1975) stated the view of Warburg (1965) that air space behind the epiphragm is saturated with water vapours and acts as insulation between the snail and external environment. He stated that amongst different workers the growing realization that the principal barrier to the water loss from the shell aperature is in the form of mantle tissues of the snail's body.

For reactivation from dormancy B. jacquemonti is observed to need direct contact with water, a certain amount of warmth and humidity.

Machin (1975) suggested that activation of snail involves two distinct phases of response: The first is a reaction to external stimulus and the second is readily activation when the high ambient humidity is sensed.

Brady(1983), stated that the termination of dormancy is often accellerated by some optimum temperature but delayed by either higher or lower temperatures.

REFERENCES

Abdul Malek, E.T. 1954a.

Genital organs of Helisoma trivolvia.

Trans. Amer. Micr. Soc. 73: 103-124.

Abdul Malek, E.T. 1954b.

The genital organ of Biomphalaria biosayi.

Trans. Amer. Micr. Soc. 73: 285-296.

Ali, N. and Suleman, M. 1992.

Seasonal variation in population density and activity of the land snail, Monacha, obstructa

Pak. Jour. Zool. 24: 86-88.

Altonaga, K. 1990.

A new species from the Iberian peninsula. (Pulmonata: Zonitidae)

J. Conch. 33: 281-289.

Aparicio, M.T., Seddon. M.B. and Holyoak. D.T. 1991.

Systemic and distribution of Helicopsis in Morocco.

J. Conch. 34: 47-58.

Aurangzeb, R.P. 1984.

Morphology and Ecology of Bensonies jacquemonti

M.Phill Thesis Zoo.Deptt.Univ.Pesh. PP.160.

Aurangzeb, R.P. and Siddiqi, M.N. 1984.

Histological studies of thegenital tract of Bensonies jacquemonti

Pak.J. Agric. Res.5(4): 264-268.

Bahl, K.N.B. 1928.

On reproductive process and development of Pila globosa part-I copulation and oviposition Memoirs of the Indian Museum, 9: 1-11.

Baker, F.C. 1945.

Molluscan family planorbidae.

Univ. 111 Press 530 pp.

Baldwin, E. 1936.

Arginase.

Biol. Rev. Woods Hole. 11: 247-268.

Baldwin E. and Bell, D.I. 1938.

Preliminary investigation of galactogen from the albumen gland of Helix pomatia.

Jour. Chem. Soc.:1461-1465.

Baldwin, E. and Needham J.1934.

Problem of nitrogen catabolism in invertebrates (The snail).

Biochem. Jour. 28: 1372-1392.

Bancrofti, J.D. 1982.

Theory and practice of Histological techniques.

Churchil livingston Edinburgh, London, Newyork pp. 184-192.

Barker, J. I. and Gainer, H. 1974.

Peptide Regulation of Bursting Pacemaker activity in a Molluscan Neurosecretory Cell.

Beha. Biol. Sci. 184: 1371-73.

Barker, J.L. Ifshin, M.S. and Gainer, H. 1975.
Studies on bursting Pacemaker potential activity in molluscan neurons III.
Brain Research, 84: 501-513.

Barraud, E. 1957.
Copulatory behaviour of Lymnaea stagnalis
Brit. Jour. Ani. Beh. 5: 55-59.

Bayne, C.J. 1966.
Observations on the composition of the layers of egg of Agriolimax reticulatus.
Comp. Biochem. Physiol. 19: 317-338.

Bayne, C.J. 1967.
Studies on the composition of extracts of the reproductive glands of Agriolimax reticulatus, the grey field slug (Pulmonata, Stylommatophora).
Comp. Biochem. Physiol., 23: 761-773.

---------1968.
A study of the desiccation of egg capsule of eight gastropod species.
Jour. Zool. Lond. 155: 401-411.

Berry, A.J. 1963.
The genital system of the Malayan limestone hill snail with notes on breeding.
Proc. Zool. Soc. Lond. 141: 361-369.

Berry, A.J. 1964.

The reproduction of opisthoma from a lime stone hill.

Proc. Zool.Soc.Lond. 142: 655-663.

---------- 1965.

Reproduction and breeding fluctuation in Hyrocena monteros atiana, a Malayan limestone archaeogastropod.

Proc.Zool.Soc.Lond. 144: 219-227.

---------- and Chan, L.C. 1969.

Reproductive condition and tentacle extirpation in Malayan Achatina fulica (pulmonata).

Aust. Jour. Zool. 16(5): 849-855.

Bianchi, S. 1973.

A histochemical investigation of neurosecretion in Enchytraeus albidus.

Gen. Comp. endocrin. 22: 454-458.

Bianchi, S. 1969.

The Histochemistry of the neurosecretory system in Carebratulus.

Gen. Comp. Endocrin., 13: 206-210.

Bierhauer, J. 1978.

An experimental study of the regulation of gametogenesis in Helix pomatia by the end of hibernation.

Biologia, 26 (1): 99-104.

Bierbauer, J. and Fazekas, s. 1984.
The effect of the albumen gland extract containing a weak trias buffer on gametogenesis in Helix Pomatia
Allanttani-Kozl 71(1-4): 31-38.

Bigus, V.L. 1981.
Polyvitellinity and fusion of germs in Physa acuta.
Zool. Jr. Anat., 105: 526-550.

Boer, H.H; Douma, E. and Koksma, J.A. 1968.
Electron microscope study of neurosecretory cells and neurohaemal organs in Lymnaea stagnalis.
Symp. Zool. Soc. Lond. 22: 237-256.

Boer, H.H. and Joosse, J. 1975.
Endocrinology in Pulmonates I.
Academic Press Inc. London: 245-307.

Bohlken, S. and Joosse, J. 1982.
The effect of photoperiod on female reproductive activity and growth of the fresh water pulmonate snail Lymnaea stagnalis kept under laboratory breeding condition.
Int. Jour. Invert. Reprod., 4: 213-222.

Brady, J. 1983.
Photoperiodism, or seasonal timing in Biological Clocks.
Edward Arnold Pulish., Ltd. Lond:
pp. 1-60 (41-46).

Bretschneider, L.H. 1948a.

Insemination in Limnaea stagnalis.

Proc. K. Ned. Akad. Wet., 51: 357-363.

----------1948b.

The mechanism of oviposition in Limnaea stagnalis

Proc. Acad. Sci. Amst., 51: 616-626.

Brisson, P. 1968.

Development of embryos and Annexes of Achatina and their study from in vitro culture.

Arch. d.Anat. Micros., 57(4): 345-368.

Bulman, K. 1990.

Life History of Carychium Tridentatum in the laboratory.

J.Conch. 33: 321-333.

Burch, J.B. and Cuadros, C. 1965.

A culture medium for snail cells and tissues.

Nature, Lond. 206: 637-638.

Cain, A.J. 1980.

Whorl number, shape and size of shell in some pulmonate fauna.

Jour. Conch., 30: 209-221.

Cain, G.L. 1956.

Studies on cross-fertilization and self fertilization in Lymnaea stagnalis.

Biol. Bul. III.:45-52.

Cambion, M. 1961.

The structure and function of the cutaneous glands in Helix aspersa.

Quart. Jour. Microsc. Sci.:102-105.

Carriker, M.R. 1943.

Variability, developmental changes and denticle replacement in the radula of Lymnaea stagnalis app.

Nautilus. 57: 52-59.

Chase, R. and Goodman, H.E. 1977.

Homologous neuro-secretory cell groups in the land snail Achatina fulica and sea slug Aplysia.

Cell Tiss. Res. 176: 109-120.

Chatfield, J.E. 1968.

The life history of the helicid snail Monacha cantians with reference also to M. cartusiana.

Proc.Malac.Soc.Lond., 38: 233-245.

----------1972.

Observation on the ecology of Monacha cantiana and associated molluscan fauna.

Proc.Malac.Soc.Lond., 40: 39-69.

Chaudhry, M.A. 1988.

The seperation of an oviposition inhibitory factor from Bullinus tropicus

Pak.J.Zool.20(1): 87-98.

Clampitt, T.P. 1970.

Comparative ecology of the snails Physa gyrina and Physa integra (Basommatophora). Malacologia, 10(1): 113-151.

Cobbs, J.S. and Pinsker, H.M. 1982.

Role of bag cells in egg deposition of Aplysia brasiliana

J.Comp.Physiol. 147: 537-546.

Crabb, E.D. 1927.

Anatomy and Function of the reproductive system in the snail L. stagnalis appressa say

Biol. Bull, 53(2): 55-67.

Crabb, E.D. 1929.

Growth of a pond snail, Lymnaea stagnalis as indicated by increased in shell size.

Biol. Bull.,56: 41-63.

Davies, S. 1988.

Rock boring land snails.

Conch. News letters, 105: 104.

De Jorge, F.B. and Petersen, J.A. 1970.

Urea and Uric acid contents in the Hepatopancrease, kidney and Lung of active and dormant snails.

Comp. Bioch. Physiol.35: 211-219.

Dewitt, R.M. 1954.

Reproductive capacity in a pulmonate snail Physia gyrina Amer. Nat., 88: 159-164.

Dimitriadou, M.L and Saunders, D.S. 1988.

The influence of Humidity, Photoperiods and temperature on the Dormancy and activity of Helix lucorum

J.Moll.Stud. 52: 180-189.

Dogterom, G.E; Bohlken, S. and Geraerts, W.P.M.1983.

A Rapid Vivo Biossay of the Ovulation Hormone of Lymnaea stagnalis

Gen.Comp.Endocrin, 50(3): 476-82.

Dogterom, G.E; Hofs, H.P.;Wapenaar, P.; RouBos, E.W. and Geraerts, W.P.M. 1984.

The effect of temperature on spontaneous and ovulation hormone induced female reproduction in Lymnaea stagnalis.

Gen. Comp. Endocrinol. 56(2): 204-209.

Domian, E.S. and Yousäf, F. 1973.

Embryonic development and organogenesis in the snail Marisa cornuarietis.

Malacologia, 12(1): 123-150.

Duncan, C.J. 1958.

The anatomy and physiology of the reproductive system of the fresh water snail Physa fontinalis.

Proc.Zool.Soc.Lond., 131: 55-84.

Duncan, C.J. 1959.
The life cycle and ecology of the fresh water snail Physa fontanalis.
J.Anim.Ecol.28: 97-117.

---------- 1960.
The evolution of the pulmonate genital system.
Proc.Zool.Soc.Lond. 134: 601-609.

---------- 1964.
Rhythmic activity in an isolated penis preparation from the fresh water snail Lymnaea stagnalis
Jour.Vergl.Physiol., 48: 295-301.

---------- 1975.
Reproduction. In Pulmonates. 1: 309-365.
Academic Press Inc. Lond. Ltd.

Eisenberg, R.M. 1966.
The role of food in the regulation of the pond snail, Lymnaea elodes.
Ecol. 51: 680-684.

Elwell, A.S and Ulmer. M.J. 1971.
Biology of Anguispira alternata
Malacologia, 11(1): 199-215.

Endean, R. 1972.
Aspect of Molluscan pharmacology. In chemical Zoology.
Acad. Press. Lond. 421-460.

Fantin, A.M. and Vigo, E. 1968.
Histochemistry of the glands associated with the reproductive tract of Lymnaea stagnalis.
Histochem. Ist. Anat. Comp. Univ. Povici Italy. 15: 300-311.

Fioroni, V.P. 1977.

On the peripheric resorption of albumen in gastropods and its significance for general embryology.

Zool.Jr.Anat. Bd., 98: 181-202.

---------- 1982.

The peripheric resorption of albumen during ontogenesis of Gastropoda Pulmonata.

Bull.dela Soc. Zool. de France., 107(1) 87-100.

Florkin, M. and Scheer, B.T. 1972.

Chemical Zoology Vol.VII. Mollusca.

Academic Press New York: 467 pp.

Fraser, L.A. 1946.

The embryology of the reproductive tract of Lymnaea stagnalis appressa.

Trans.Amer.Micr.Soc., 65: 279-298.

Fried, B., Good Child, G. 1963.

Studies on reproduction, growth and survival of Planorbid Snail.

Trans,Amer. Micros. Soc 89: 143-149.

Gainer, H. 1972.

Effect of induced diapause on protein synthesis patterns of identified molluscan neurons.

Brain Research, 39: 387-402.

Gatenby, J.B. 1919.

The cytoplasmic inclusion of the germ cell. Part V. Gametogenesis and early development of Lymnaea stagnalis.

Quart.Journ.Mior.Sci. 63: 445-491.

---------- 1920.

The cyloplamic inclusion of germ cell. Part VII. Modern technique of Cytology.

Quart. Journ. Micr. Sci. 64: 267-303.

Geraerts, W.P.; Maat, A. and Hogenes, T.M. 1983.

Studies on the release activities of the neurosecretory caudo-dorsal cells of Lymnaea stagnalis

Biosyn. Meta. Inv. Hormone. 44-50.

Gerartes, W.P.M. and Mohamed, A.M. 1980.

Studies on the role of lateral lobes and the ovotestis of pulmonate snail Bulinus truncatus in the control of body growth and reproduction.

Int. Jour. Invert. Reprod., 3: 297-308.

Ghose, K.C. 1960.

Observation on the gametes, fertilization and gonadal activities of two land pulmonate Achatina fulica and Macrochlamys indica.

Proc.Zool.Soc.Calculta, 13: 91-96.

---------- 1962a.

The cleavage gastrulation and germ layer

formation in the giant land snail Achatina fulica.
Proc.Zool.Soc.Calcutta, 15(1): 47-55.

---------- 1962b.
Morphogenesis of the eye in the giant land snail Achatina fulica.
Jour.Univ.Bombay, 30: 73-76.

---------- 1962c.
Morphogenesis of the statocyst in the giant land snail Achatina fulica.
Jour.Univ.Bombay, 30: 77-79.

- - - - - - 1962d.
Morphogenesis of the shell gland, lung,mantle and mantle cavity of the giant land snail Achatina fulica.
Proc. /Malac. Soc. Lond., 35: 119-126.

---------- 1963a.
Morphogenesis of the pedal gland in the giant land snail Achatina fulica.
Curr. Sci., 4: 167-168.

---------- 1963b.
Embryogenesis and larval organs of giant land snail Achatina fulica.
Proc. Roy. Soc. Edin., 68: 237-260.

---------- 1963c.
Reproductive system of the snail Achatina fulica.
Proc.Zool.Soc.Lond., 140: 681-697.

Giusti, F. and Manganelli, G. 1990.

Ciliellopsis oglasae, A new Hygromiid.

J.Conch. 33: 269-277.

Ghraham, A. 1954.

Some observations on the reproductive tract of 'Ianthina janthina (L).

Proc.Malac.Soc.Lond. 31: 1-6.

Grabb, E. 1927.

The fertilization process in snail Lymaea stagnalis appressa say

Biol. Bull. 53: 67-108.

Graham, A. 1954.

Some observation on the reproductive tract of Ianthiana janthina.

Proc. Malac. Soc. Lond., 31: 1-6.

Greek, G.A. 1951.

The reproductive system and embryology of the snail Pomatia elegans.

Proc.Zool.Soc.Lond., 121(3): 599-640.

Grime, J.P. and Blythe, G.M. 1969.

An investigation of the relationship between snails and vegetation.

Jour.Ecol. 57: 45-66.

Goudsmith, E.M. 1978.

Calcium-dependent release of a neurochemical messenger from the brain of Helix pomatia

Brain research, 151: 418-423.

Hakala, I. 1979.

Seasonal variation in the carbon and energy content of Mysis relicta

Ann.Zool.Fennici. 16: 129-137.

Harris, R.E. and Charleston, W.A.G. 1977.

Some temperature responses of Lymnaea tomentosa and L. columnela (Mollusca gastropoda) and their eggs.

New Zeal. Jour. Zool. 4(1): 45-49.

Heller, H.C. 1979.

Hibernation: Neural Aspects.

Ann. Rev. Physiol. 41: 305-21.

Hickman, C.P. 1931.

Spermiogenesis of Succinea ovalis Say with special reference to the components of the sperm.

Jour.Morph. 51: 243-289.

Highnam, K.C. and Hill, L. 1978.

The comparative endocrinology of the Invertebrates.

Publisher Edward Arnold Lond. pp.357 (15-20), 84-103).

Hodasi, J.K.M. 1979.

Life History Studies of Achatina (achatina) achatina

J.Moll.Stud. 45: 328-339.

Ifshin, M.S.; Gainer, H. and Barker, J.L. 1975.
Peptide factor extracted from molluscan ganglia that modulates bursting pacemaker activity.
Nautre, 254: 72-73.

Jong-Brink, M.; Borg,J.P.; Marion, J.M.; Bergamine-Sassen and Boer, H.H. 1979.
Histology and histochemistry of the reproductive tract of pulmonate snail, Bulinus truncatus, with observations on the effects of castration on its growth and histology.
Int.Jour.Invert.Reprod., 1: 41-56.

Jong-Brink, M; Koop, H.M.; Roos, W.F.de; and Bergamin-Sassen, J. M. 1982.
Regulation of secretory activity in the albumen gland of the pulmonate snail Lymnaea stagnalis.
Int.Jour.Invert.Reprod., 5: 207-219.

Joosse, J., Boer, M.H. and Cornellisse, C.J. 1968.
Gametogenesis and oviposition in Lymnaea stagnalis
Symp.Zool.Soc.Lond., 22: 213-235.

Kapur, S.P.P. and Gibson, M.A. 1968.
A histological study of the development of mantle edge and shell in the fresh water gastropod,Helisoma duryi edudiscus (Pilbry).
Canad.Jour.Zool. 45: 481-491.

Kerkut, G.A. and Walker, R.J. 1975.
Nervous system, eye and statocyst.
In Pulmonates 1: 165-244.
Acad. Press Inc. Lond. Ltd.

Kit, K.S. 1980.
States of exciteability in ovulation Hormone producing neuro endocrine cells of Lymnaea stagnalis and their relation to the egg lying cycle.
J. Neurbio. 2(4): 397-410.

Kononenko, N.I. 1979.
Modulation of the endogenous electrical activity of the bursting neurons in Helix Pomatia
Neuroscience, 4: 2055-2059.

Kugler, O.E. 1967.
Reproductive system of slug philomycus carolinianus
J.Morph. 116: 117-132.

Kulkarni, A.B. 1980.
Effect of optic tentacle on the growth of Albumen gland in the Land slug, Laeviculisalte
Ann. Zool (Agra) 14: 171-179.

---------- and Hazari, V.S. 1983.
Effect of optic tentacle on the growth of reproductive tract in the land snail, Cerastus moussonianus

Lambert, P. and Dehnet, P.A. 1974.
Seasonal variation in biochemical composition during the reproductive cycle of the intertidal Gastrodthias lamellosa Gmelin (Gastropoda prosobranchia).
Canad. Jour. Zool. 52(3): 306-317.

Lane, N.J. 1962.
Neurosecretory cells in optic tentacles of certain pulmonates.
Quart.J.Micr.Sci. 103(2): 211-226.

---------- 1963.
Thiamine pyrophosphatase, acid phosphatase and alkaline phosphatase in neurones of Helix aspersa.
Quart.J.Micr.Sci. 104(3): 401-412.

---------- 1964.
Elementary neurosecretory granules in the neurones of the snail, Helix aspersa
Quart.J.Micr.Sci.,105(1): 31-34.

---------- Further observation on the secretory cells in the optic tentacle of Helix aspersa
Quart.J.Micr.Sci.105(1): 61-71.

Lane, N.J. 1964.
Semper's organ, a cephalic gland in certain gastropods.
Quart.J.Micr.Sci. 105(3): 331-342.

Lattaud, C. 1982.

Stimulation of testicular Androgen in the annelid.

Int.J.Invert, rep. 4: 223-237.

Lawson, A.K. 1926.

Aestivation of H. hortensis.

Jour.Conch.Leed. 18:10

Lilly, M.M. 1953.

The mode of life and the structure and functioning of Bithynia tentaculata.

Proc.Malac.Soc.Lond. 30: 87-110.

Lind, H. 1973.

The functional significance of the spermatophore and the fate of spermatozoa in the genital tract of Helix pomatia (Gastropoda: Stylommatophora).

Jour.Zool.Lond., 169: 39-64.

Little, C. 1968.

Aestivation and ionic regulation in two species of Pomacea (Gastropoda,Prosobranchia).

Jour.Exp.Biol.48: 569-585.

Machin, J. 1975.

Water relationship.

In pulmonates Vol.I Functional Anatomy and Physiology.

Acad.Press.Lond. 105-163.

Manganelli, G.; Sparacio, I. and Giusti, F. 1989.
New Data on the systemics of two sicillian Land Snails.
J.Conch, 33: 141-156.

Martoja, M. 1972.
Endocrinology of Mollusca.
In chemical Zoology VII Mollusca.
Acad. Press, New York: pp. 349-392.

McCrone, E.J; Minnen, J.V.; Sokolove, P.G. 1981.
Slug reproductive maturation hormone from brain and cerebral ganglia.
J.Comp.Physiol, 43: 311-315.

Meenakshi, V.R. and Scheer B.T. 1968.
Studies on carbohydrates of the slug
Ariolimax columbianis
Comp.Biochem.Physiol. 26: 1091-97.

Menon, K.R. 1966.
Observations on Neurosecretory activity during reproductive period in Oncidium.
Gen.Comp.Endoc. 7: 186-190.

Morton, J.E. 1967.
Molluscs.
Hutchinson & Co.Ltd., Great Portland Street, London
pp.244.

Nagabhushanam, U.D.; Deshpande and Hanumante,M.M. 1981
Hormonal control of egg laying in the marine pulmonate Onchidium verruculatum.
Hydrobiologia, 80(3): 277-281.

Naggs, F. 1983.
Perforatella. The helicid snail newly recorded in Britain
J.Conch, 31: 201-206.

Naggs, F. 1989.
Gulella Bicolor (Hutton) and its implication for the taxonomy of streptaxids.
J.Conch, 33: 165-168.

Newman, C.; Korkute, G.A. and Walker, R.J. 1968.
The structure of the brain of Helix aspersa electron microscope localization of Cholinesterase and amines.
Symp.Zool.Soc.Lond. 22: 1-17.

Noland, L.E. and Carriker, M.R. 1946.
Observations on the biology of the snail Lymnaea stagnalis appressa during twenty generation in laboratory culture.
Amer.Midl.Nat. 36: 467-493.

Nolte, A. 1983.
Investigation on the dorsal bodies of stylommatophoran snail
Zool.Inst.Wilhelms-Univ: 142-147.

Ohtsuki, M. and Takahashi, S. 1982.
Studies on the hermaphroditism of the ovoviviparous pulmonate, Euphaedusa tau I. Shell whorls as a Growth Parameter.
Venus,Jap.Jour.Malac., 41(2): 141-145.

Ohtsuki, M. 1982.
Studies on the hermaphroditism of ovoviviparous pulmonate, Euphaedusa tau.III. Development of germ cells.
Venus,Jap.Jour.Malac., 41(3): 221-226.

Owiny, A.M. 1974.
Some aspect of breeding biology of the equatorial land snail L. martensiana
J. Zool.Lond.172: 191-206.

Parmentier, J. 1973.
Mapping studies of a gastropode brain,
Brain Research, 59: 201-210.

Pawson, P.A. and Chase, R. 1984.
The life cycle and reproductive activity of Achatina fulica
J.Moll.Stud. 50: 85-91.

Peake, J.F. 1968.
Habitat distribution of solomon Island land molluscs.
Symp.Zool.Soc.Lond, 22: 319-346.

Pekkarinen, M. 1986.

Notes on the spawning, egg cleavage and early development of the bivalve Macoma balthica.

Ann.Zool. Fennici, 23: 71-75.

Pellute, D. and Lane, N.J. 1961.

The relation between neuro-secretion and cell differentiation in the ovotestis of slugs Gastropoda, Pulmonata.

Canad.Jour.Zool. 39: 789-805.

Pengloh, Y and Gainer, H. 1975.

Low molecular weight specific protein in identified molluscan neurons I.

Brain Research, 92: 181-192.

Phorson, J.E. 1990.

Some observations on the development of juvenile shells of Trivia.

Conch. News letter, 113: 279-283.

Phorson, J.E. 1991.

Some observations on juveniles and growth series of Leucophytia Bidentata and Ovatellaa Myosotis.

Conch. News letter, 117: 366-370.

Plummer, J.M. 1975.

Observation on the reproduction, growth and longevity of laboratory colony of Archachatina marginata.

Proceeding of Mol.Soc. Lond.41: 395-413.

Pomeroy, D.E. 1968.

Dormancy in the land snail Helicella virgata.

Aust.Jour.Zool., 16: 857-869.

Prashad, B. 1925.

Anatomy of the common Indian apple snail

Pila globosa.

Mem. Ind. Museum, 8: 91-152.

Prosser, C.L. 1962.

Comparative animal Physiology.

W.B. Saunders Company London,

pp. 1-688.

Ranjah, A.R. 1942.

The embryology of Indian apple snail Pila

globosa (Mollusca: gastropoda).

Rec. Ind. Museum 44(3): 217-322.

Rasmussen, A.T. 1914.

Theories of Hibernation

The American Naturalist, 50: 608-625.

Raven, C.P. 1945.

The development of the egg of Lymnaea

stagnalis from oviposition till first

cleavage.

Arch. Neerl. Zool., 7: 91-121.

---------- 1948.

The chemical and experimental embryology of

Lymnaea stagnalis

Biol.Rev., 23: 333-369.

--------- 1959.
The formation of 2nd maturation spindle in the eggs of Succinea, Physa and Planorbis.
Jour.Embryol.Exp.Morph., 7: 344-360.

- - - - - - 1964a.
Mechanisms of determination in the development of gastropods.
Adv. Morph., 3: 1-32.

---------- 1964b.
The formation of 2nd maturation spindle in the eggs of various Limnaidae.
Jaur.Embr.Exp.Morph., 12(4): 805-823.

- - - - - - 1972.
Chemical Embryology of Mollusca. in chemical Zoology
Aca.Press New York and Lond, 155-180.

---------- 1975.
Development In pulmonates 1: Functional anatomy and physiology.
Academic Press. Lond., 467-400.

Rehm, M. 1959.
Observation on neurosecretory material in nerve terminals in Careinus maenas.
Acta. Histochemica Bd. 7: 88-106.

Roosen-Runge, E.C. 1977.
The process of spermatogenesis in animals.
Camb.Univ.Press Lond. pp. 2

Saleuddine, A.S.; Kunigelis, S.C., Schollen, L.M. and Miksys, S.L. 1983.
Studies on endocrine control of reproduction in Helisoma and Helix
Lever.J. Boer. H.H. Molluscan: 138-142.

Sathananthan, A.H. 1970.
Studies on the mitochondria in the early development of the slug.Arion ater rufus.
Jour.Embryol.Exp.Morph., 24(3): 555-582.

Scharrer, B. 1962.
The fine structure of the neurosecretory system of the insect leucophae maderae
Neurosecretion. Acad. Press. London. pp.170.

Sedden, C.B; Walker, C.B. and Kerkute, G.A. 1968.
The localization of Dophamine and 5-droxy tryptamine in the neuron of Helix aspersa.
Symp.Zool.Soc.Lond. 22: 19-32.

Seshaiya, R.V. 1927.
On the breeding habits and fecundity of the snail Lymnaea luteola.
Jour.Bomb.Nat.His.Soc., 32: 154-162.

Siddiqi, M.N and Ali, N. 1988.
Embryonic development of Monacha obstructa
J.Sc. & Tech. Univ. Pesh. 12: 89-94.

Siddiqi, M.N. and Aurangzeb, R.P. 1984.
General anatomy and reproductive system of the land snail (Bensonies jacquemonti).
Pak.Jour.Agr.Res., 5(2): 118-123.

Smith, F.G.W. 1935.
The development of Patella vulgata. Philos. Trans. (B) 205: 95-125.

Smith, H.J. 1965.
The secretion of the reproductive tract of the garden slug Arion ster.
Ann.N.Y. Acad.Sci.,118(Art.24): 997-1014.

Smith, S.T. 1967.
The ecology and life history of Retusa obtusa (Gastropoda Opisthobranchia)
Canad.Jour.Zool., 45(4): 397-405.

Sokolove, P.G. and Ninnen, J.V. 1983.
Control of reproductive development in the giant garden slug Limax.
J.Comp.Physiol. 101-105.

Stephenson, J.W. 1980.
Notes on the life history of Arion subfuscus.
Conch. News letter, 74: 247-248.

Storey, R. 1983.
Dormancy in Lymnaea.
J. Conch. 31: 257-258.

Takalchi, S. 1979.

Spermatogenesis in the pulmonate snail Euphadra hickonis. IV. Effect of X-rays on the spermatogenesis.

Develop.Growth and Differ., 21(1): 87-98.

Terakado, K. 1981.

Chromatin arrangement and axis formation in the spermatogenesis of a pulmonate snail.

Devel. Growth and Differ., 23(4): 381-399.

Tompa, A. 1979.

Studies on reproductive biology of gastropods part-I Egg retention.

J.Malac.Soc.Aust. 4(3): 113-120.

Tompa, A. 1980.

Studies on reproductive biology of gastropods. Part-III. Calcium provision and the evolution of terrestrial eggs among gastropods.

J.Conch. 30(3): 145-154.

Tyrell, S.T. 1967.

The ecology and life history of Retusa obtusa Montagu (Gastropoda, Opisthobranchia).

Canad. Jour. Zool. 45(4): 397-405.

Valovirta, I. 1968.

Land molluscs in relation to acidity on hyperite hills.

Ann. Zool. Fenn. 5: 245-253.

Van Der Borght, O. and Van Puymbroech, S. 1966.
Calcium metabolism in a fresh water mollusc.
Nature. Lond. 210: 791-793.

Van Der Laan, K.L. 1976.
Terrestrial pulmonate reproduction, seasonal and annual variation and environmental factors in Helminthoglypta arrosa.
Veliger's 23(1): 48-54.

Vander Meer Mohr, J.C. 1949.
On reproductive capacities of the African giant snail, Achatina fulica
Treubia, 20: 1-10.

Van Der Steen, W.J. 1968.
Notes on the effect of excessive amount of food on the reproduction of Lymnaea stagnalis (L) Gastropoda, Pulmonata .
Archs. Neerl. Zool. 18: 411-414.

Van-Minnen, J. and De-vries, G.J. 1980.
Effect of extirpation of Lateral lobe of the Lymnaea stagnalis.
Proc.Biol.Med.Sci.83(2): 187-194.

Van Minnen, J. and Reichelt, D. 1980.
Photo-period dependent neural control in the lateral lobe of Lymnaea stagnalis.
Cell.Tissue.Res. 208 (3): 457-466.

Van-Minnen,J. and Reichelt, D. 1980.
Effect of Photo period on the activity of neurosecretory cells in the lateral lobes of Lymnaea stagnalis
Cell.Biol.Med.Sci. 83(1): 15-24.

Vard court, B. 1988.
The behavior of Achatina Lactea Reeve in Captivity
Conch. News letter, 107: 140-145.

Vard court, B. 1992.
The identity of two Euconuloid snails recently found in the Seychelles.
J.Conch.Lond. 34: 169-174.

Willis, J.S. 1979.
Hibernation: Cellular aspects.
Ann.Rev.Physiol. 41: 275-286.

Walker, Hedges and Wood ruff 1968.
Pharmacology of the neuron of Helix aspersa.
Symp.Zool.Soc.Lond.22: 33-75.

Young.M.S. and Evans J.G. 1992.
Modern Land Molluscs Communities from Flat Holm, South Glamorgan,
J.Conch, 34: 63-70.