IV. DISCUSSION

1. Prostate gland

The histological, histochemical and biochemical investigation of polysaccharides, proteins and cholesterol elaborated by the prostate gland of the terrestrial pulmonate pest slug under the effects of extracts of optic tentacles, cerebral ganglia and ovotestis was undertaken with a view to augment the understanding of the biochemical nature of the prostate gland in general and the metabolism of these nutrients under the influence of neuro-hormones and gonadal hormones in the prostate gland and their probable function in physiology of reproduction of the pulmonate gastropods in particular.

It has been well established that the control of accessory sex organs in the stylommatophoran pulmonates is under gonadal hormones (Runham et al.,1973; Gottfried and Dorfman, 1970; Takeda, 1979; Wijdenes, 1981). But Bailey (1973), Garaerts and Joosse (1975), Garaerts and Algera (1976), Veldhuijzen and Cuperus (1976) and Wijdenes and Runham (1976) have shown that the control of accessory sex organs is under the neurohormones of cerebral ganglia and optic tentacles along with the gonadal hormones. Thus, it can be stated that our knowledge of the endocrine control of reproduction of the pulmonate gastropods is rapidly increasing. With the help of this information we are in a position to formulate the most complete picture of this subject in these animals. In brief, the state of the reproductive tract is governed by gonadal hormones and that the gonad might itself be governed by hormones originating in or near optic tentacles and cerebral ganglia or other ganglia or central nervous system. But to date no study is available to show the exact effects of these various hormones on the accessory sex organs particularly the male ASOs. Therefore, three important accessory sex organs prostate gland, dart gland and penis mainly attached to the male reproductive part of a locally available pest slug, S.maculata were studied histologically, histochemically and biochemically to see the effects of optic tentacular and cerebral ganglionic neurohormones and ovotesticular hormones on the glycogen, proteins and cholesterol elaboration of the prostate gland.

Histology and seasonal alterations in the prostate gland of this slug has studied by Nanaware (1974) and Nanaware and Varute (1976). The present study confirms the observations of both the investigators on the two types of glandular cells - secretory cells and non-secretory cells in the lobules of the prostate gland. These investigators have claimed that during seasonal changes, there were third type of cells showing a combination of both the types of cells. According to them one type of cell could be transformed into another. But such season specific occurance of third type of intermediary cells were not noticed in our study. We could observe only two types of cells.

Interestingly there was an increase in the number of both the types of cells after the injections of extracts of optic tentacles, cerebral ganglia and ovotestis. This indicated the stimulus for the increase of cells must have received through the neurohormones of the optic tentacles and cerebral ganglia and also through the gonadal hormones. This particular speculation gets support from the observations on the optic tentacles ablation experiment. When optic tentacles were ablated, their neurohormones were not received by the slugs. Therefore, the number and size of the prostate gland cells were reduced. This also indicated the acceleratory effects of neurohormones of optic tentacles on the prostate gland cell differentiation and growth.

The present detailed histochemical investigation confirms the presence of polysaccharides and also the nature of polysaccharide. The polysaccharides elaborated by the secretory cells of this gland are of neutral polysaccharide type and glycogen of the other non-secretory cells. Thus, there are cells which elaborate only neutral mucosubstances and others only glycogen. Thus, the prostate gland of S. maculata affords an example of biochemical division of labour at cell level for its secretory function.

The neurohormones of optic tentacles and cerebral ganglia brought about the alterations in the concentration of the cellular mucopolysaccharide contents of both the cell types of prostate gland. The concentration of mucopolysaccharides of the two types of cells is very low, after the ablation of optic tentacles. On the other hand, the concentration increases in both the cell types after the injections of neurohormones of the optic tentacles and the cerebral ganglia, indicating their function for the synthesis and elaboration of mucopolysaccharides in the prostate gland cells.

The observations on the biochemical investigation also confirm the histological and histochemical observations. It is observed that per cent glycogen, proteins and cholesterol contents of the prostate gland decreases than the control values after optic tentacles ablation. The decreasing values reached the lowest levels of 2.97, 2.58 and 2.69 mg, respectively on 25th day after ablation. On the contrary, the values of these nutrients increased after the injections of extracts of optic tentacles. It is well-known that the optic tentacles of pulmonate gastropods contain the neurohormones, responsible for reproductive activity. The results indicated that these neurohormones stimulated the synthesis of elaboration of glycogen, proteins and cholesterol in the prostate gland of this slug. In this context, the observations of Muthe and Naik-satam (1972) on the effects of cerebral ganglionic extract on the glycogen content of foot, mantle and digestive gland of Pila globossa, Srinivasa Reddy et al. (1978) on the effects of cerebral, pleuropedal and visceral ganglionic extracts on the free amino acids of the foot and digestive gland of active and aestivated snail, Pila globossa and Lomte and Godhamgaonkar (1982) on the effects of cerebral ganglionic extract on the protein and amino-acid levels in the foot, mantle and digestive gland of V. bengalensis, are important. According to these investigators, two possibilities exist. The ganglia might be releasing two types of neurohormonal principles, one is acceleratory and the other is inhibitory. In certain cases, the activation reached with cerebral ganglionic extract surpassed the pleuropedal ganglionic extract indicating the influence of secretions of this ganglion on biochemical contents of these tissues. Similar accelatory active neurohormonal principles might be affecting the concentrations of glycogen, proteins and cholesterol in the prostate gland of S. maculata. Therefore, their concentrations are increased after the injections of extract of cerebral ganglia and optic tentacles in this gland.

Just opposite effects of the bilateral ablation of optic tentacles were noted in the male crab, Scylla serrata by Rangnekar et al. (1971). They found an increase in the diameter of the seminiferous tabules and the number of cells per tubule and also an increase in the diameter of vas deferens after the bilateral ablation of the eyestalks. As pointed out by Lomte and Godhamagaonkar (1982), this might be due to the dominance of inhibitory neurohormonal principle in the eye-stalk tentacles of this crab. The accelerating roles of active principles in the cerebral ganglia and ovotestis also function in a similar fashion for the synthesis and elaboration of glycogen, proteins and cholesterol in this gland.

Lusis (1961) was doubtful about the functional role of the prostate gland in the pulmonates. The position of its opening at the distal end of the spermoviduct might indicate that this gland may take part in the copulatory process or in the formation of spermatophores. On the other hand, the nature of its secretion suggests that it may be a source of nutritive material for the spermatozoa. In support of the above observation, work of Smith (1966) for the spermatophores formation and of Plesch et al (1971) for the sperm nutrition may be taken into consideration. According to Runhan and Hunter (1970) sperms are coated with secretions from the prostate gland into the male groove during the copulation. Kenny and Inamdar (1969) have attributed lubricating role to these secretions at the time of gliding of sperms along the vas deferens. According to Nanaware (1974) and Nanaware and Varute (1976) the mucosubstance secretion of this gland might help in formation of the spermatophores and provide nutrition for the sperms which are stored for long time in the hermaphrodite duct till the copulation. The fact that there are secreting cells containing large amount of neutral mucopolysaccharides, proteins and cholesterol which may be contributing to the formation of the spermatophores. The other cells seem to synthesize and secrete glycogen and glucose, indicates that these energy materials supply the necessary nutrients to the sperms. These functions of the prostate gland are under the direct control of gonadal hormones and under the direct or indirect control of neurohormones elaborated by the optic tentacles and cerebral ganglia.

2. Dart gland :

As in case of prostate gland, the histological, histochemical and biochemical investigation of polysaccharides, proteins and cholesterol elaborated by the dart gland of the slug, S. maculata under the effects of extracts of optic tentacles cerebral ganglia containing neurohormones and of extract of ovotestis containing gonadal hormones was undertaken with a view to augment the understanding of the biochemical nature of the dart gland in general and the metabolism of these nutrients in this gland and their probable function in the physiology of reproduction of pulmonate gastropods in particular.

In pulmonate gastropods, the dart gland, also known as the multifed gland, has been reported to play an important role during the courtship behaviour in evoking heightened sexual excitement and serving as a releaser of stimulus for courtship (Hyman, 1967). During the copulation, the dart gland ejects watery secretion into the partner's body which acts as a lubricant and the dart - a fine pointed calcareous shaft - is also shot into the partner's body where it remains and is resecreted newly by the dart sac in the next few days after the copulation (Morton, 1967).

Many investigators have studied this gland from the morphological (Hyman, 1967; Morton, 1967), biochemical (Hollande, 1968; Cercos, 1968), ultrastructural (Ovtracht, 1967; Hollande, 1967) and seasonal (Nanavare, 1974; Nanaware and Varute, 1973) points of view. These investigators have pointed out that this glandular structure secreted secretory granules composed of sulfated acid mucopolysaccharides and glycoproteins (Hollande, 1968) or of muco-proteins (Ovtracht, 1967) which were collected by the intercellular lacunar system (Hollande, 1967) and then glycoproteins were reabsorbed by the lysosomes (Hollande, 1969). According to Nanaware (1974) and Nanaware and Varute (1973a) the secretion of the glycoproteins of this gland was season dependent which begins by the end of the pre-breeding season and attains a maximum in the breeding season. During aestivation period they are not synthesised.

However, the control of the secretory activities of this gland has not been paid much attention. Runham et al. (1973), Gottfried and Dorfman (1970), Takeda (1979) have pointed out the role of gonadal hormones in the control of accessory sex organs of some pulmonate slugs. According to Bailey (1973), Garaerts and Joosse (1975), Garaerts and Algera (1976), Veldhuijzen and Cuperus (1976) and Wijdenes and Runham (1976), along with gonadal hormones, neurohormones elaborated by the optic tentacles and cerebral ganglia were also involved in the control of the activity of accessory sex organs in pulmonates. But no investigator has studied in detail the effects of these hormones on these ASOs in this group. Therefore, the present investigation first time provides the information on the effects of hormones on the male accessory sex organs.

The histological observations of the present investigation showed that the neurohormones of the optic tentacles and cerebral ganglia accelerate the growth, cellular differentiation and secretory activities in the peripheral glandular cells of this gland. On the basis of the elaboration of the secretory material, these cells could be differentiated into five different types. The number and staining reactivities towards the histological staining method were increased, indicating their role in the synthetic function of this gland.

The detailed histochemical investigations unrevealed some significant facts about the elaboration of mucosubstances by the peripheral glandular cells of the dart gland under the influence of hormones. On the basis of their capacity to elaborate mucosubstances, they could be classified into five types. The first type of cells are endowed with a capacity to elaborate only neutral mucosubstances (glycosaminoglycans). At a comparative level numerically they were more in number. The type 2 cells are endowed with a capacity to elaborate the neutral mucosubstances and sulfomucins. Numerically, these cells were much less than the type 1 cells. The type 3 cells contained only sulfomucins and their number was maximum amongst the various types of cells. The type 4 cells are endowed with a capacity to elaborate sialomucins. They were less than type 1, 2 and 3 but more than type 5 cells. The type 5 cells exhibited histochemical reactivities of both the type 3 and type 4 cells indicating simultaneous presence of sulfomucins and sialomucins. Their number was minimum in the peripheral glandular cells.

Interestingly, the neutral mucosubstances in the type 1 and type 2 cells were localised in the entire body of the cells but in case of type 3, 4 and 5 cells the acidic mucosubstances were localised in the basal portion of the cells.

These types of cells secreted their mucosubstances in the form of secretory droplets which could also be classified into five types corresponding to the five types of cells of the peripheral glandular cells. The distinct histochemical reactivities of these granules, indicate that the various cell types pour their secretion separately into the lumen where they seem to retain their chemical individuality.

The very fact that the peripheral glandular cells can be classified into five distinct types each having its own peculiar mucosubstance content, shows that all the glandular cells in a dart gland are not endowed with a capacity to synthesise all the mucosubstances found in the lumen. Instead, for its secretory function, the dart gland seems to have evolved a specialised division of labour at cell level.

The present detailed histochemical investigation confirms the results of Nanaware (1974) and of Hollande (1967, 1969). But in addition, the present investigation shows the hormone-dependent variations in the elaboration of mucosubstances in the various glandular cells of dart gland. In the absence of neurohormones (afterablation of optic tentacles), the mulosubstances elaboration was stopped and it was increased after injections of the neuro-hormones through the extracts of optic tentacles andcerebral ganglia. Such increase in the elaboration of mucosubstances was also observed after injections of gonadal hormones through the extracts of ovotestis. The effects of these hormones was not only on the elaboration of mucosubstances but also on the cellular multiplication and interconversion from the neutral to acidic, sulfated mucosubstances. Because their concentration was increased after injections of extracts of optic tentacles and cerebral ganglia containing the neurohormones and due to injections of extracts of ovotestis containing the gonadal hormones. The increased concentrations of muscosubstances seem to be completely employed in the absence of neurohormones of optic tentacles, since these mucosubstances decrease to a minimum level after ablation of optic tentacles. They must have ejected into partner's body, where it acts as a lubricant. Since recently, Goudsmit (1972) has attributed the role as lubricant to the molluscan mucosubstances. Thus, the hormones elaborated in the optic tentacles, cerebral ganglia and ovotestis bring out changes in the mucosubstances in the dart gland which play important role in the reproductive physiology of this group of animals.

The speculation based on the histological and histochemical observations of the present investigation gets strengthened if we look at the biochemical results. The biochemical results showed that the normal per cent concentrations of glycogen, proteins and cholesterol of the dart gland were 7.65 mg, 5.01 mg and 6.75 mg, respectively. These values were droped after ablation of optic tentacles and showed increase after injections of extract of optic tentacles. Similar alteration patterns were observed after the injections of extracts of cerebral ganglia and ovotestis. Thus, the effects of these hormones seemed on the elaboration and storage of various nutrients in the dart gland of S. maculata.

The stimulatory effects of hormones on the galactogen synthesis in albumen gland of H. pomatia (Goudsmit, 1975, 1978), and polysaccharide synthesis in albumen gland of L. stagnalis particularly by cerebral ganglia hormones (Wijdenes et al.,1983) has been well established. Similarly such stimulatory effects on elaboration of mucosubstances and storage of glycogen, proteins and cholesterol must be bringing observed effects in the dart gland of this slug.

3. Penis

In pulmonates partial or complete atrophy of male copulatory apparatus occurs in a surprisingly large number of species mostly in the stylommatophoran. It is also exhibited by a small number of basommatophorans. So long as self-fertilization can occur, atrophy, partial or complete, of the male organs does not embarrasses the species and may permit the establishment of local races showing higher ecological adaptation. Even if self-fertilization is not possible aphallic animals can always act as females, euphallic animals behaving as males. This supposes a rather different copulatory behaviour in those species from the other stylommatophorans in which copulation may be abandoned if both animals do not act together as male and female (Fretter and Graham, 1962). Riedel (1955) started that the absence of copulatory organs and male duct may not be an absolute barrier to copulation because sperm may be passed down the vagina which can evert to act as an intromittent organ allowing some transfer of sperm.

The earliest suggestion of hormonal control was that of Sereni (1929) and Giese (1915). They pointed out that the secondary sexual characters were under the endocrine control. Rohlack (1959) and Jenner and Chamberlain (1955) noticed production of estrogen (not identical with vertebrate estrogen) in the ovary of L. littorea and it's effect on the resorption and redevelopment of the penis in the neogastropod Nassa obsoleta. But according to Laviolette (1950) gonadal hormones did not affect penis in A. subfuscus during reproduction and gets regenerated towards breeding season. According to Linke (1934) this phenomenon is controlled by male hormone. Harrey (1965) showed that the growth and maturation of accessory sex glands of Taphius were controlled by hormones in the ovotestis.

From survey of the literature it seems that in stylommatophora, a dual endocrine control of the ASO by the gonad is well established. According to Bailey (1973) gonads, cerebral ganglia and optic tentacles control growth of the prostate gland in D. reticulatum. But these organs cannot control the development and growth of female ASO. Geraerts and Joosse (1975) showed control of cellular differentiation and growth of the male part only by the dorsal body hormones. Because of the contraversal reports in various stylommatophoran animals, Joosse and Geraerts (1983) in the concluding remarks of the topic on hormonal control of reproduction in the chapter endocrinology have mentioned that there are no indications of a neuroendocrine control of the gonadal hormonesecretion in stylommatophora. An endocrine organ analogous to the DB. for the control of male activity probably does not exist.

As pointed out above the interesting phenomenone, the atrophy of male organs is especially found in stylommatophora. Another significant aspect of male accessory organs of this group is seen from the results of present study which pointed out that the administration of neurohormones in the optic tentacles and cerebral ganglia and the gonadal hormones of the ovotestis brings about hypertrophy of the penis. The histological observations showed the increase in the size, in the number of unicellular and multicellular glands and staining reactivities of various tissues of the penis. The histochemical findings also pointed out that the elaboration of various mucosubstances in various cells were enhanced, the number of mucosubstances elaborating cells and their staining reactivities were increased.

In addition to making the above significant addition to our present knowledge of the effects of neuro-endocrine hormones on the male accessory sex organs, this investigation also confirms the presence of acid mucopolysaccharides, sulfomucins and sialomucins in the elaboration of mucins by the various tissues and glands in the penis. These results coincide with the observations of Plesch et al.(1971), Nanaware (1974) and Nanaware and Varute (1973b). The penial glands of this slug have evolved a specialised biochemical division of labour for their secretory function, certain glands being specialized to elaborate certain mucosubstance. Some glands secrete only sulfomucins and other only sialomucins. Therefore, this forms an excellent example of biochemical division of labour for secretory function in the penis.

The biochemically observed changes were parallel with the histochemical changes after the administration of the neuro-hormones of the optic tentacles and cerebral ganglia also after the administration of gonadal hormones of the ovotestis. After injections of extracts of optic tentacles the concentration of glycogen was increased from 6.65 mg% to 11 mg%, the concentration of proteins was increased from 4.01 mg% to 10.12 mg% and the concentration of cholesterol was increased from 5 mg% to 10.5 mg% on the 25th day after administration of extracts.

Similarly, concentrations of glycogen, proteins, and cholesterol after injections of extracts of cerebral ganglia and these were increased upto 13.75 mg%, 12 mg% and 13.03 mg% after the injections of extract of ovotestis on the 25th day after injections. These numerical changes and the fluctuations in the concentrations of these nutrients in the penis after injections of optic tentacles, cerebral ganglia and ovotestis, brings out two interesting functional features of the metabolism of these nutrients in the penis under the influence of neuroendocrine hormones : (1) The functioning of penis is influenced by the neurohormones elaborated by the optic tentacles and cerebral ganglia and gonadal hormones of the ovotestis, (2) During hypertrophy of penis nutrients like glycogen, proteins and cholesterol are very much essential to provide energy required.

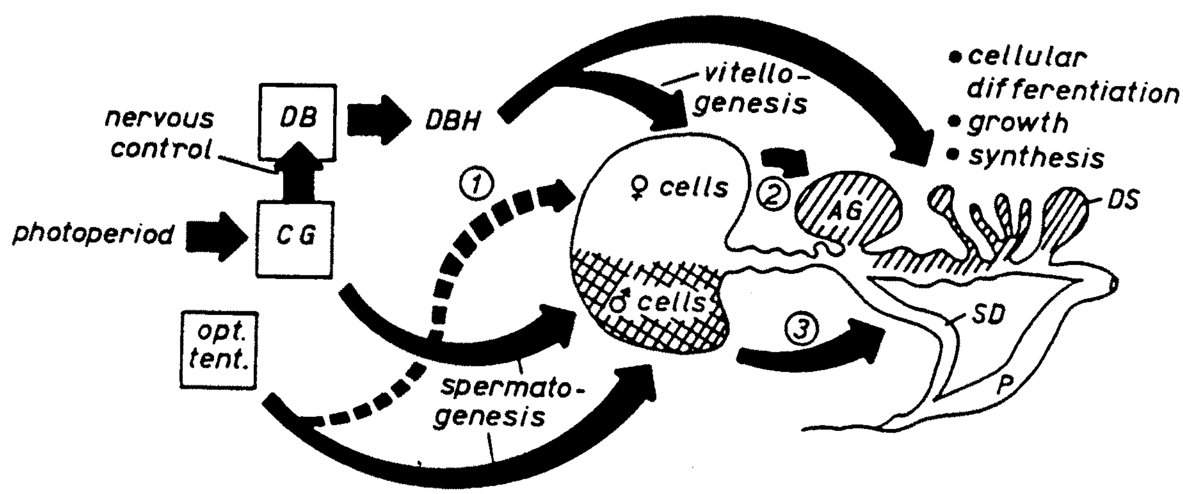
The above described changes seem to be related with the functions of the penis. During the copulation the penis is protruded and it helps in the release of sperms (Beeman, 1970) and the acid mucopolysaccharides probably play a role during copulation (Plesch et al.,1971). The sulfomucins and sialomucins found in the present investigation might be functioning as a biological lubricant to avoid the friction at sites of copulatory organs and smooth transfer of the sperm strand during the copulation (Goudsmith, 1972) or in the formation of seminal strand (Beeman, 1970). During these functions of penis changes occured due to the effects of hormones must be essential.

V. GENERAL SUMMARY AND CONCLUDING REMARKS :

It is well established that the reproduction in pulmonate gastropodes is influenced by environmental, genetic and hormonal factors. There are several studies to show effects of hormones on the gonads and female accessory sex organs. But Joosse and Geraerts (1983), in their recent review on this subject have expressed doubt about the neuroendocrine control of the gonadal hormone secretion in stylommatophoran and about the control of male activity in male accessory sex organs by the dorsal body hormones in this group of animals.

Therefore, in order to confirm the present data on the endocrine control of reproduction in stylommotophoran pulmonates, in general (as shown in the scheme given below), and to confirm the effects of neuroendocrine hormones, if any, on the male accessory sex organs (ASO) of a terrestrial, stylommatophoran slug S. maculata, in particular, the present investigation was undertaken.

Scheme on the endocrine control of reproduction in stylommatophoran pulmonate molluscs



In the present investigation the effects of neurohormones elaborated in the optic tentacles and cerebral ganglia and gonadal hormones elaborated in the ovotestis were studied on the three important male accessory sex organs : prostate gland, dart gland and penis. The study is concerned with the histological changes, histochemical alterations in the mucosubstances and biochemical variations in the concentrations of glycogen, protgeins and cholesterol in the above mentioned organs in the absence (i.e. after ablation of bilateral optic tentacles) of neurohormones of the optic tentacles and after administration of neurohormones of the optic tentacles and cerebral ganglia and gonadal hormones of the ovotestis through the injections of extracts of these organs.

Well known and recent histological, histochemical and biochemical techniques were employed for the present investigation.

In the histological observations showed an increase in size, number and staining intensity of various cells in the prostate gland, the dart gland and penis after injections of neurohormones in the extracts of optic tentacles, and cerebral ganglia and of gonadal hormones in the extracts of ovotestis. But these changes were reversed in the slugs with the extirpation of the bilateral optic tentacles.

The results of the histochemical study showed an increase in the neutral mucosubstances and glycogen in the secretory and non-secretory cells of the prostate gland respectively, an increase in neutral acidic, sulfated and carboxymucins and mixed muco-substances in the peripheral glandular cells and in the secretory droplets in the amorphous waters secretion in the lumen of tubules and glycogen in the muscular coat of the dart gland and increase in sulfomucins in the type 1 - unicellular and multicellular gland and sialomucins in the type 2 - unicellular and multicellular glands, glycogen and sulfomucins in the muscles and loose connective tissue of the penis, respectively, under the effects of neurohormones and gonadal hormones after their administration through the extract of optic tentacles and cerebral ganglia and ovotestis respectively. All these results were reversed in the slugs with the ablation of their optic tentacles.

The observations on the biochemical studies showed 76.10%, 102.21% and 81.36%; 62.78%, 68.76% and 64.38% and 22.54%, 34.88% and 36.45% increase in glycogen; 98.63%, 106.94%, and 68.10%, 100%, 121.74% and 100%, 31.30%, 40.17% and 46.48% increase in the proteins and 128.61%, 104.32% and 94.79%; 54.54%, 56.81% and 60.17% and 28.77%, 44.77% and 44.44% increase in the cholesterol of prostate gland, the dart gland and penis, after injections of extracts of optic tentacles, cerebral ganglia and ovotestis, respectively, on the 25th day after administration. Whereas the concentrations of glycogen, proteins and cholesterol were decreased by 182.92%, 211.24% and 172.86%, respecively, in the prostate gland, 257.37%, 260.07% and 243.75% respectively, in the dart gland and 24.23%, 13.22% and 11.66%, respectively, in the penis of the slugs with ablated optic tentacles on the 25th day after extirpation.

Thus, it can be concluded that the endocrine control of reproduction is identical to the earlier proposed scheme for other stylommatophoran pulmonates, where cerebral ganglionic and optic tentacular neurohormones bring about activation for male activity in the ovotestis. The increased ovotesticular activity increases gonadal hormones consequently bring about cellular differentiation, growth and synthetic activity in the male accessory sex organs. Again the cerebral ganglionic neurohormones activate dorsal body hormones which directly and indirectly via gonadal activation bring about changes in the prostate and dart glands in this group of animals.

Secondly, the neurohormones of the optic tentacles and of the cerebral ganglia and ovotesticular gonadal hormones bring about similar changes in all the male ASOs, which are concerned with the histological changes in the size and number of various cells, histochemical alterations in the quality and quantity of muco-substances elaboration in the various tissues and biochemically observed numerical variations in the glycogen, proteins and cholesterol of the prostate gland, dart gland and penis of S. maculata. All these hormones induced changes are seem to be related to the functions of the prostate gland, the dart gland and the penis in the physiology of reproduction of S. maculata.