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The role of external and internal factors during the phase of reproduction in lampreys, with special regard to the interplay between gonadal and extragonadal (interrenal) steroids.

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Introduction

In 1958 I made the following conclusion about cyclostomes in a review of the interplay between the pituitary gland and the gonads in vertebrates: "Hypophysectomy has not been performed in this group with regard to gonadal function. Neither has anybody investigated whether the pars distalis of cyclostomes has any gonadotropic effect. In the river lamprey (*Lampetra fluviatilis*) mammalian gonadotropin has some effect on gonads and secondary sex characters." (Nielsen 1958).

Hypophysectomy

Soon after, I obtained a number of river lampreys and have continued work on this fascinating group ever since. My first plan was to study effects of hypophysectomy, and then study the effects of transplantation of the gland, in order to define the role of the central nervous system in stimulating or inhibiting the pituitary gland. When performed before sexual maturation, hypophysectomy prevented development of secondary sex characters and prevented spermiation and ovulation (Larsen 1965). Evennett (1963, see Dodd *et al.* 1960; Dodd and Sumpter 1984) found similar effects with regard to secondary sex characters in river lampreys.

Transplantation of pituitary tissue

Heterotopically transplanted pieces of the pro- and mesoadenohypophysis ("pro + meso", corresponding to the pars distalis) allowed sexual maturation (*i.e.* development of secondary sex characters and spermiation/ovulation) to occur normally in 9 river lamprey males (56%) and in 6 females (35%), although often with considerable delay (Jørgensen and Larsen 1967; Larsen 1969a,b; Table 1 and Fig. 1). For comparison, orthotopic transplants allowed normal development in 5 males (50%) and in 5 females (71%), whereas hypophysectomy (pro + meso) prevented normal development in all (17 males and 19 females). Therefore, I no longer paid attention to the central nervous system. These results agreed well with the lack of innervation and portal vessels, and the presence of a layer of connective tissue separating the brain from the pro + meso (Gorbman 1965, 1980, *Lampetra planeri* and *Petromyzon marinus*; Belenky *et al.* 1979, *L. fluviatilis*).

However, the situation changed when a peptide related to other vertebrate gonadotropin releasing hormones (GnRH) was found in the hypothalamus of the sea lamprey (see Sower 1990). Sower and her coworkers have accumulated evidence that GnRH plays a biological role, but precisely how awaits clarification and has to be seen in the light of the data from the transplantation studies. GnRH probably reaches the pro + meso by way of diffusion, so its main effect may be to stimulate the rate of matu-

Table 1. Sexual maturation in *Lampetra fluviatilis* with pro- and mesoadenohypophysial tissue transplanted heterotopically or orthotopically (Larsen 1973).

Time of operation	Site of transplant[c]	Males			Females			Hypophysectomized controls					
								Males			Females		
		I	II	III	I	II	III	I	II	III	I	II	III
Nov.–Dec., 1962	eye	2	0	1	1	2	0	3	0	0	2	2	0
–	muscle	1	0	4	2	2	1						
Nov.–Jan., 1964/65	muscle				0	0	1	2	0	0	0	1	0
–	original site	3	0	1	1	0	0						
Jan. 1967	muscle	4	0	4				12	0	0	1	0	0
–	original site	2	0	4									
Jan. 1969	muscle				2	2	4				9	1	0[a]
–	original site				0	1	5				3	0	1[b]

I no sexual maturation; II only secondary sex characters; III secondary sex characters and spermiation or ovulation; [a] hypophysectomized Oct. 1968; [b] hypophysectomized Febr. 1969 (late enough for ovulation to occur without gonadotropin?); [c] pro- and mesoadenohypophysial tissue, generally from one third to one half of the gland, was transplanted into the anterior chamber of one of the eyes or to a muscle in the pharynx, to deprive the gland of any possible innervation or direct humoral control. Remnants of the pro- and mesoadenohypophysis which had been left *in situ* were removed. As a control a similar piece of hypophysial tissue was reimplanted into the original place in the capsule of connective tissue which separates the pro- and mesoadenohypophysial tissue from the brain; only lampreys surviving until the time when normal lampreys showed sexual maturation were included in the table; the cytological picture of the transplants are described in Larsen (1973) and in Larsen and Rothwell (1972; Table IV).

ration and differentiation of the gonadotropin-producing cells (Larsen 1980). For vertebrates it is generally assumed that GnRH has a key role in allowing or inducing the gonadotropin surge necessary for ovulation (and in some species spermiation too). In this connection, it is worth noting that when hypophysectomy (pro + meso) was performed in March, at a time when gonadal maturation was sufficiently advanced, ovulation and spermiation in river lampreys might occur days or weeks after the operation (Larsen 1969a, 1970), *i.e.* not only without a gonadotropin surge, but probably even when gonadotropin has been absent for several days.

Localization of gonadotropin production

The localization of the site of gonadotropin production was tested in river lampreys by using partial hypophysectomy, and Larsen (1965) found that the pro + mesoadenohypophysis was essential, whereas the proadenohypophysis could be removed without any effect on sexual maturation. Evennett (1963) had similar results in his study. A supplementary production of gonadotropin in the proadenohypophysis was indicated (Larsen 1965), but the evidence is admittedly weak. The cell type responsible has been much discussed (see Larsen and Rothwell 1972). By use of an antibody directed towards the rat LH-beta-subunit Wright (1983) observed a positive reaction in the so-called chromophobes of the mesoadenohypophysis in *P. marinus.*

Treatment with gonadotropin

Treatment of hypophysectomized river lampreys in winter and spring with mammalian gonadotropins or suspensions of lamprey pituitaries induced development of secondary sex characters and ovulation (Evennet and Dodd 1963), although treatments before sexual maturation have not been very effective in eliciting precocious sexual maturation in intact lampreys (see Larsen and Rothwell 1972).

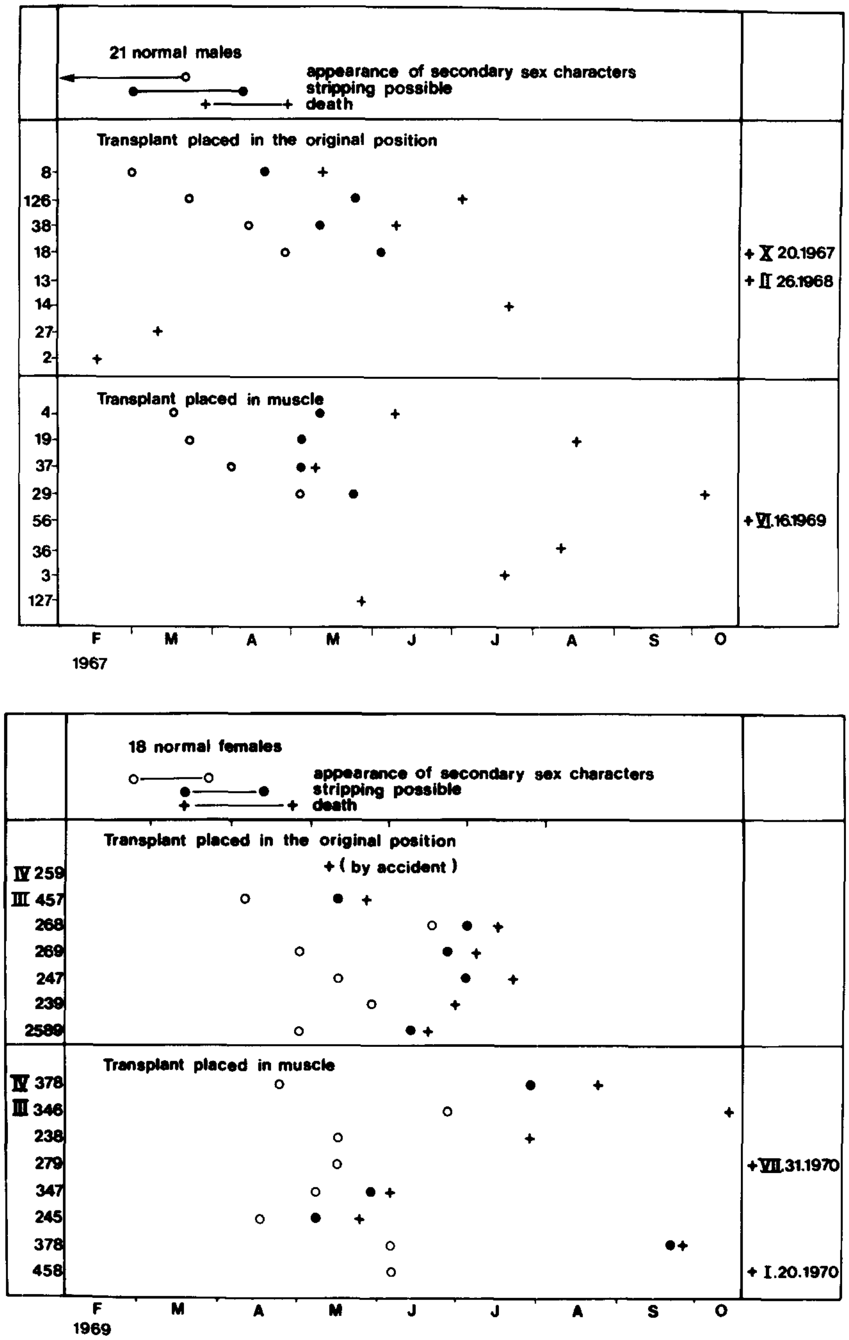

Fig. 1. Sexual maturation in *Lampetra fluviatilis* with transplanted pro- and mesoadenohypophysial tissue (from Larsen 1973). Upper graph males, lower graph females.

Gonadectomy and treatment with sex hormones

Evennett and Dodd (1963) demonstrated that gonadectomy prevented the development of secondary sex characters in river lampreys, and that male characters could be elicited both in males and females by implants of testosterone. Larsen (1974) made a more detailed and quantitative study of secondary sex characters and also treated with implants of estradiol. This hormone elicited female secondary sex characters in females as well as in males. Sex hormone treatment before sexual maturation induces precocious secondary sex characters in intact lampreys to only a limited degree (Larsen 1974, 1987).

Development of sensitivity to hormones

It thus seems typical for (river) lampreys that development of sensitivity (= capacity to respond) to both gonadotropin and sex hormones is essential for initiation of sexual maturation. Larsen (1987) tested whether development of sensitivity for testosterone was dependent on the pro + meso. Testosterone elicited secondary sex characters in hypophysectomized (pro + meso) river lamprey males. However, in some cases there was a delay, and growth of the urogenital papilla was slow, although normal size was reached eventually. Apparently, a genetically based "programmed" development of sensitivity to hormones is important in (river) lamprey reproduction (Larsen 1980, 1982).

Initiation and progression of sexual maturation

Lampreys become sexually mature in early spring; secondary sex characters appear, gametogenesis is finished, nest building and mating take place, release of ovulated eggs and spermiated sperm occurs, and soon after spawning both males and females die, after their first and only spawning season. Such a pattern of reproduction made it natural to investigate whether increased temperature or increased daylength were necessary signals to initiate sexual maturation. As demonstrated in Fig. 2 (row 1,7,8), neither was decisive, and later studies showed that even when the river lampreys were kept in darkness and at 11° constantly from autumn, sexual maturation occurred normally. This does not mean that light intensity or daylength has no effect on for instance behavior, but increasing daylength is not a necessary condition for initiation of sexual maturation. In this connection it is noteworthy that lampreys are nocturnal and photophobic until spawning, which takes place in daytime, often in sunshine (Sjöberg 1977). Neither does it mean that temperature has no effect on sexual maturation; temperature certainly affects sexual maturation as illustrated here by comparison of row 8 with row 1 and 11 in Fig. 2: When temperature is high, maturation is early and well synchronized; when temperature is low, maturation is late and scattered

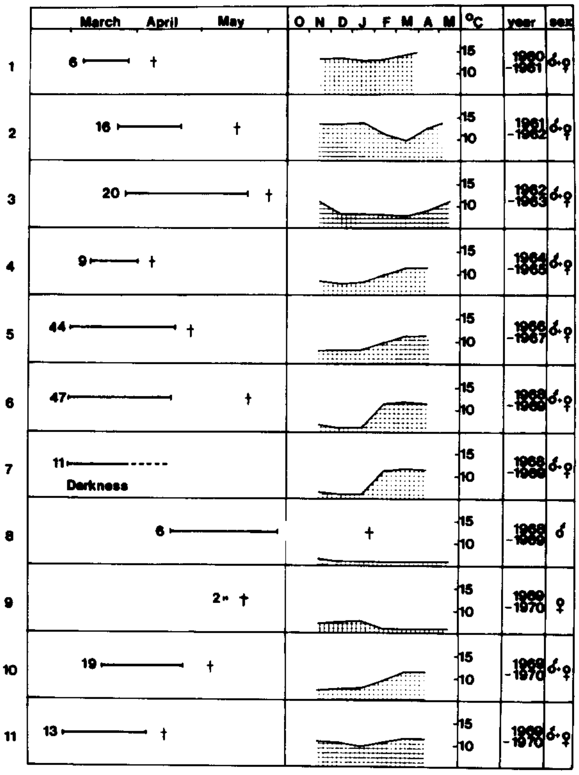

Fig. 2. Sexual maturation under various conditions of light and temperature in intact *Lampetra fluviatilis* (from Larsen 1973). ⊢⊣ Period in which ovulation or spermiation was observed (by stripping) for the first time (number of lampreys indicated); † natural death of the last lamprey in the group; row 7: darkness except at times of inspection. Experiment interrupted on March 28, when it was clear that sexual maturation proceeded normally. On that date 2 males and 4 females had concluded sexual maturation and died; row 8 + 9: in a dark refrigerator from around February 1st. Four of eight females for unknown reasons did not survive the transfer from the tanks to the refrigerator. Two of the four surviving females developed normally; two died before ovulation, but after the anal fin had started to develop. Otherwise dim day light in the room. Row 4–6 and 10 came closest to natural conditions in Denmark.

in time. However, an increase in temperature is not a necessary condition for initiation of sexual maturation. Since the transplantation studies indicated that sexual maturation in river lampreys could be initiated even when the pro + meso was removed from its normal position close to the brain, attention was paid to the possible role of internal signals. Intestinal atrophy and the metabolic pattern connected with the prolonged starvation period, which precedes sexual maturation, were considered (Larsen 1978). The hypothetical explanations were abandoned one by one, and in 1980 I concluded: "With such a complicated phenomenon as sexual maturation it may be naive to look for *an* external signal or *an* internal factor that initiates it." Today I would conclude: Initiation of sexual maturation in (river) lampreys seems to be based on development of sensitivity to gonadotropin and sex hormones. The rate of development of sensitivity is probably influenced by temperature, and sensitivity develops in spite of hypophysectomy (pro + meso), but otherwise nothing is known about mechanisms involved. With regard to progression of sexual maturation, the following conclusion seems warranted: Normal development of secondary sex characters and rapid and well-synchronized (in a given area) gametogenesis and ovulation/spermiation depend on temperature profiles above a certain lower limit and below a certain upper limit and with a pattern whose optimal shape has not yet been defined. Field data on the relationship between temperature profiles and sexual maturation in river lampreys can be found in Sjöberg (1977, 1980). The fact that all lampreys in a given area will be influenced by the same pattern of temperature-increase in spring, alone may explain seasonal reproduction (Larsen and Rothwell 1972), and temperature need not act *via* the GnRH-gonadotropin system but will tend to synchronize all temperature-dependent processes in the body. Sexual maturation in river lampreys does not necessitate day light of a certain intensity or quality or duration. Gonadotropin and sex hormones are necessary, but gonadotropin less essential for gametogenesis, including vitellogenesis (see below) and ovulation, than expected when compared with most other vertebrates. Nothing is known about the role of hormones in spawning behavior.

Interplay between gonadal and interrenal steroids

In a review of the role of hormones in reproduction and death of lampreys, Pacific salmon, and eels (Larsen 1985), I listed a number of processes related to sexual maturation in river lampreys that apparently did not depend strictly on the gonadotropin-sex hormone system: 1. intestinal atrophy, 2. rapid reduction in body length, 3. biliverdin accumulation in the liver, and 4. natural death in males. In a later paper (Larsen 1987), I added 5. the first growth of the urogenital papilla (from less than 3 mm to 5 mm). I can also add 6. swelling of the anal region and 7. increased blood glucose. Some of these processes may occur in gonadectomized lampreys (2, 3, 5, 6), some are typical of stressed lampreys (2, 6, 7) in which sexual maturation may be delayed and mortality high (Larsen 1973, and unpublished), and some are typical of both males and females, although with quantitative differences (1, 2, 3, 6, 7). Typical of all parameters is a lack of knowledge about their endocrine control. In the following I will define what is known in the river lamprey.

Intestinal atrophy takes place in the months before secondary sex characters start to develop, at a rate which can be increased by implants of testosterone or estradiol (Pickering 1976b) and decreased by extirpation of the pro + meso (see Fig. 3, and Pickering 1976b), but in January, when atrophy is nearly complete, gonadectomy leads to a marked hypertrophy, which is prevented neither by hypophysectomy (pro + meso) or by implants of testosterone or estradiol (Larsen 1974; Pickering 1976b). In 1980 I wrote: "This is surprising, since as mentioned treatment in autumn caused atrophy in gonadectomized lampreys. This may indicate that some unknown factor in the gonadectomized January-lampreys counteracted the atrophy-inducing effect of the sex hormones or that the intestine had lost its sensitivity towards sex hormones at this time of the year." The last possibility implies not only that sensitivity towards sex hormones has a reverse pattern of change in the intestine and in the tissues responsible for secondary sex characters, but also that sex hormones are secreted in effective amounts before sexual maturation. It should be remembered

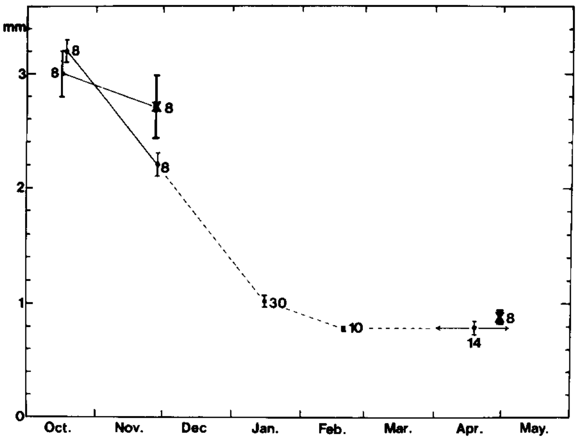

Fig. 3. Diameter of the posterior end of the intestine in *Lampetra fluviatilis* (from Larsen 1973). · Intact controls (males and females); x hypophysectomized (pro + meso) lampreys; SE and number of lampreys indicated; The data from autumn came from two groups of 8 where the intestine was measured on anesthetized lampreys after an incision was made in the body wall. The rate of reduction was slower in the hypophysectomized group ($P \leq 0.005$).

that the true sex hormones may be derivatives of testosterone and estradiol, and that the implants of testosterone and estradiol released steroids at high rates (3–86 μg/day; Larsen 1974). The first possibility implies a growth stimulating factor. This could perhaps be intrinsic to the intestine and might be gastrin or other peptides known to be present (see van Noorden, this issue), since such peptides in mammals have been demonstrated to have growth-promoting activity in gastro-intestinal epithelia (Larsson 1988).

Reduction in body length takes place during the whole period in fresh water, from autumn until death after spawning in spring (Larsen 1962). In the laboratory it was shown to be slow until the time when secondary sex characters start to develop, and then rapid until death after spermiation/ovulation, being most remarkable in females (Larsen 1969a). The rapid reduction was prevented by hypophysectomy (pro + meso) (Larsen 1965, 1969a). Gonadectomy caused rapid reduction in length for some weeks. Sham-operated lampreys showed a similar rapid reduction in length, indicating that this was an effect of the extensive surgery. However, those gonadectomized lampreys which showed prolonged survival eventually had a slow rate of reduction in length (Fig. 4). A slow reduction in body length is correlated with a slow mobilization of fat and protein from the body wall and results in prolonged survival (Larsen 1969a). The rate of reduction in body length is supposed to reflect the rate with which body reserves are mobilized to cover energy requirements and to furnish material for gonadal growth, which is most remarkable in females. Treatment with sex hormones has not been shown to have any effect, so it is an open question through which hormones the pro + meso influence the mobilization processes (Larsen 1980).

Biliverdin accumulation is supposed to be responsible for the green color of the liver often observed in the later phases of sexual maturation, especially in males (Table 2) (NB in *P. marinus* all livers become green, dark green in females and light green in males (Applegate 1950)). Among river lampreys hypophysectomized (pro + meso) in autumn and winter (18 males, 27 females), none had green livers at death in spring or up to about one year later (Larsen 1973). However, Larsen (1973) observed green livers in 9 out of 13 gonadectomized lampreys which survived beyond May 1. Testosterone implants induced green livers in 11 out of 14 hypophysectomized (pro + meso) males (Larsen 1987), and in intact immature males in autumn (Pickering 1976a). However, estradiol in high doses may even protect against this change (Larsen 1974). The possibility that the green color of the liver is caused by corticotropin is supported by the finding that after injection of corticotropin (20 i.u. every second/day for 8 days), the livers became green (they were brown in untreated controls) (Chester Jones, personal communication). In another experiment with 1 i.u. every second/day for 5 days, the livers did not become green, but the serum, the intestine, the kidneys and the ureters did (Larsen, unpublished results). A green color has been observed in the liver of two immature lampreys with fungus infections and in other organs during stress (Larsen, unpublished), a stress probably caused by mono- and dichloramine in the tap water (Larsen 1973). Perhaps this reflects that biliverdin is circulating in the blood in increased amounts under the influence of corticotropin-corticosteroid hypersecretion and is

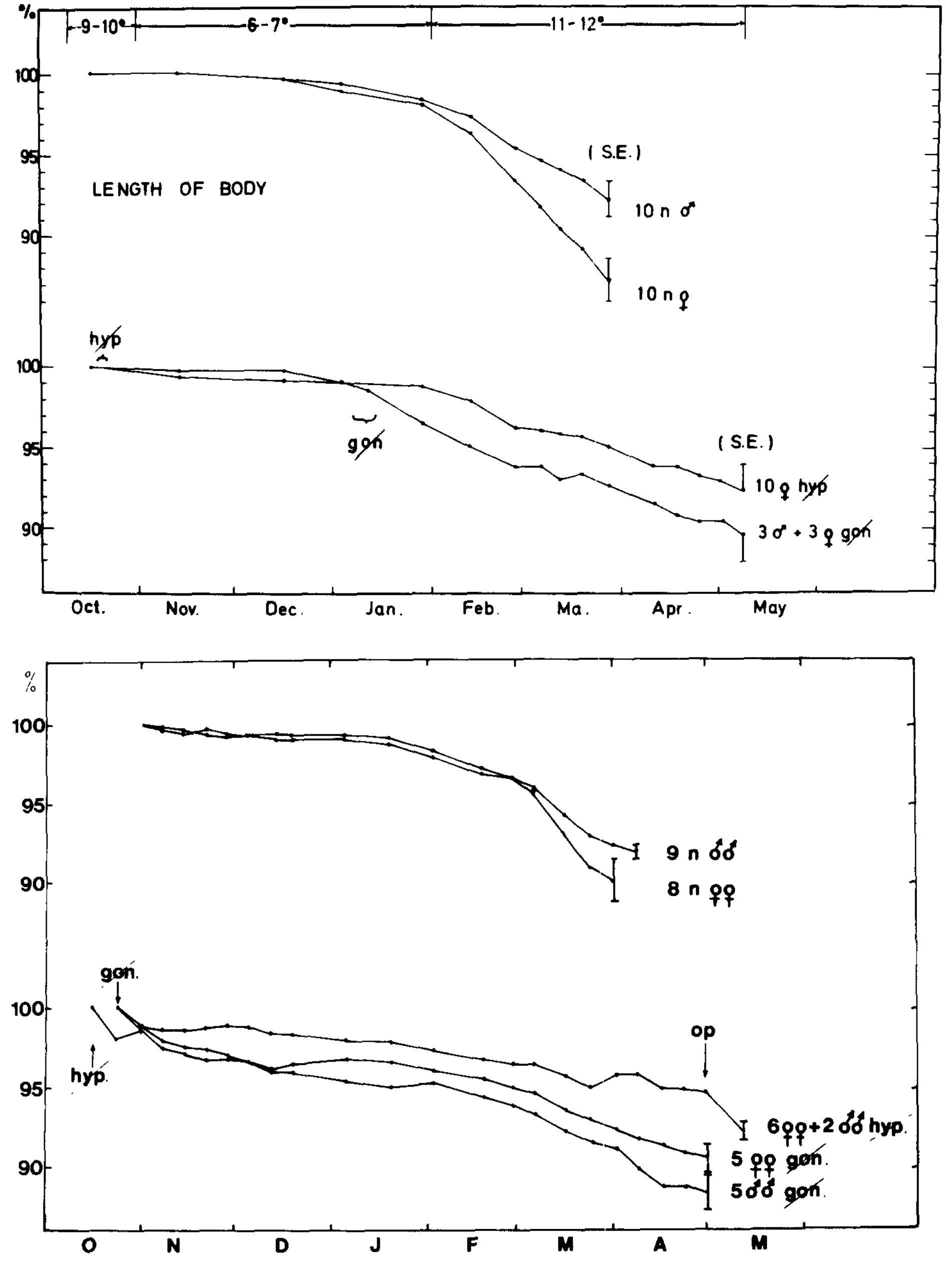

Fig. 4. Length of the body in percentage of initial body length in intact, hypophysectomized (pro + meso), and gonadectomized *Lampetra fluviatilis* (from Larsen 1973). Upper graph 1968–1969, lower graph 1969–1970; the 8 hypophysectomized lampreys were either gonadectomized or shamgonadectomized (4 ♀♀, 1 ♂) on April 30th.

under some circumstances accumulated in other organs than the liver. To explain these data it may be suggested that testosterone in high doses may have similar effects as corticosteroids on the liver color.

Natural death, as mentioned, occurs shortly after spermiation/ovulation, and survival is prolonged for up to about one year in hypophysectomized (pro + meso) lampreys (Larsen 1965, 1969a). By this operation sexual maturation is prevented, and it might be assumed that gonadotropin-sex hormones were involved. However, in two males with extirpation of the mesoadenohypophysis (Larsen

Table 2. Occurrence of green livers in *Lampetra fluviatilis* at natural death (Larsen 1973).

Year	Percent of animals with green livers (total number)	
	females	males
1967	73 (22)	91 (21)
1969	33 (18)	84 (19)
1970	50 (8)	70 (10)

1965) and in two males with transplanted pro + meso tissue (Fig. 1), survival was prolonged in spite of normal sexual maturation. Also gonadectomy in some lampreys caused prolonged survival, up to some months (Larsen 1973, 1974). However, treatment of intact lampreys with implants of testosterone or estradiol did not shorten survival, on the contrary: Estradiol in several cases (in all 19 males and in 19 of 23 females) caused prolonged survival (Larsen 1974) and testosterone in some (5 out of 8 males) (Larsen 1987).

Growth of the urogenital papilla. This is a true secondary sex character for males; never seen in females, abolished by gonadectomy and induced by testosterone implants in gonadectomized males and in intact females (Larsen 1974). In intact males treated with testosterone a supernormal length was sometimes obtained (Larsen 1987). However, in two gonadectomized males and in one gonadectomized female, growth from less than 3 mm and up to 5 mm was observed (Larsen 1973). It was suggested to be correlated with the stress of the extensive surgery (Larsen 1987).

Swelling of the anal region is the first sign of approaching sexual maturation in both sexes (Larsen 1965). It was abandoned as a reliable sex character, since it was often seen in hypophysectomized (pro + meso) lampreys in which there was no reason to suspect any remnants of gonadotropin-producing tissue. It may also be observed in gonadectomized lampreys and occasionally in intact immature lampreys (Larsen 1973). Later in the season the swelling increases because of hyperemia and because of growth of nodus muscularis cloacalis (Larsen 1974) and accumulation of fat (Larsen, unpublished).

Blood glucose levels do not decrease during autumn and winter in spite of the prolonged starvation, and at sexual maturation there is a tendency to a well regulated increase. A marked increase is also seen in stressed immature lampreys; it is not prevented by hypophysectomy (pro + meso) (Larsen 1969a, 1976 a,b). Hitherto, catecholamines have been supposed to be responsible (Larsen 1980).

Pituitary, internal cells, and reproduction. Hypotheses to explain the hormonal control of some of the seven enigmatic events were advanced by Larsen (1985) and by Kime and Larsen (1987). Larsen (1985) suggested that lampreys may secrete 15-hydroxylated corticosteroids, and that these or androstenedione (Katz *et al.* 1982, *P. marinus*) may be responsible for those processes typical of sexual maturation that apparently do not depend on gonadotropin-sex hormones. Kime and Larsen (1987) found that compared with intact lampreys testosterone levels were increased in hypophysectomized (pro + meso) and gonadectomized males and females, and estrogen levels increased in gonadectomized males; none of the experimental groups showed a decrease. They suggested that these substances (or unidentified steroids cross-reacting in the RIA) were produced by the interrenal cells and might be precursors for gonadal production of 15-hydroxylated estradiol and testosterone, which may constitute the true sex hormones of lampreys. This idea is based on previous findings by Kime and Rafter (1981) and by Kime and Callard (1982) that gonadal tissue from *P. marinus* and *L. fluviatilis in vitro* will convert testosterone and progesterone to 15-hydroxylated derivatives. In 1985 Larsen noticed that survival is typically prolonged more by hypophysectomy than by gonadectomy, although the latter operation removes an energy requiring organ. Now it may be added: and perhaps a steroid-transforming organ.

Kime and Larsen (1987) suggested that the proposed interrenal steroid secretion after extirpation of pro + meso might be stimulated by hormones from the metaadenohypophysis. Recent knowledge about the stimulation of the adrenal cortex in mammals by proopiomelanocortin-derivatives from the pars intermedia, especially in fetuses, may draw attention to the metaadenohypophysis also in lampreys in this respect (see Eberle 1988). Another possibility is that the stress of surgery in some way may stimulate the steroid secreting cells without the intervention of the pituitary gland. Catecholamines may be likely candidates (see Hardisty and Baker 1982). Unfortunately, the investigation by Kime and Larsen (1987) lacked sham-operated controls; it cannot be excluded that the large surgical stress of gonadectomy would cause similar increases in plasma testosterone and estradiol. In principle, the source of testosterone and estradiol in the above-mentioned experiment may be other cells than the interrenal cells, for instance gonadal remnants. They are not a likely source, because the gonads are easy to remove. Many data speak in favor of interrenal cells. Interrenal cell volume and cell number were shown by Sterba (1955) to increase dramatically from the time of metamorphosis to the beginning of the spawning period (Fig. 5 and Table 2 in that paper) in *Lampetra (Petromyzon) planeri*, and evidence for their steroid production is rather convincing. Hardisty (1979) reviewed early work. Since then, more evidence has appeared (Seiler *et al.* 1983; Ackermann 1984; Seiler *et al.* 1985). These authors found that sexually mature *L. planeri* of both sexes when compared with immature had very high activity of 3β-hydroxysteroid dehydrogenase in presumed interrenal cells (from pro- and opistonephros) when 3β,17β-dihydroxy-5α-androstane was used as substrate and NADP as coenzyme. In *P. marinus*, Callard *et al.* (1980) showed that kidney tissue incubated with labeled androstenedione formed estrone; the authors suggest that "presumptive adrenocortical" cells embedded in the kidney may be the source. Such *in vitro* test systems may be used for testing of the presented hypotheses. The list of possible steroid hormones was increased by the finding of Adams *et al.* (1987a) of several conjugated or unconjugated steroids in urine from male sea lampreys. Conjugated, water soluble steroids may act as pheromones (Adams *et al.* 1987b).

Below, I will give examples of possible relationships between pituitary, interrenal cells and reproduction and draw on knowledge from several vertebrate groups.

Experience has taught me that in order to understand physiological control mechanisms, it may be as fruitful to compare groups which are considered distantly related phylogenetically as those which are considered closely related. An increase in interrenal cell activity before and during sexual maturation may be a prerequisite for sexual maturation in a way reminiscent of the "adrenarche" preceding puberty in mammals (see Parker 1989) as suggested by Larsen (1987). Adrenarche may be defined as an endogenous activation of the interrenal cells with similarities to a stress-response, but in contrast to this with a pattern of hormones in the blood that stimulates reproduction instead of preventing it. A continued activation of the corticotropin-interrenal system is likely to be important. A few examples of this shall be given as illustration: In vertebrates both corticotropin-producing cells and gonadotropin-producing cells often appear to be activated at sexual maturation. This has caused confusion with regard to identification of the two cell types (see Larsen *et al.* 1971). In teleosts, the involvement of corticosteroids in reproduction has been suggested by Kime (1978) and by Pickering and Christie (1981). A recent paper by Schreck *et al.* (1989) gives information on possible control of interrenal cell secretion in fish. Parkes and Deanesly (1966) review older literature on relationships between gonads and adrenal glands in mammals. In the Australian marsupials (*Antechinus stuartii* and *Phascogale calura*) corticosteroid levels in blood are increased during sexual maturation (Bradley *et al.* 1980; Bradley 1987); the authors focus on exogenous stress factors, but it seems likely that an endogenous activation is involved too. In these marsupials reproduction seems to be followed by death, as in lampreys, eels, and Pacific salmon (Larsen 1985), and in all cases corticosteroids may be involved. In this connection it is interesting that even in the rat, with its cyclic reproductive pattern, corticosteroids influence duration of life. Everitt *et al.* (1980) have shown that hypophysectomized rats treated with cortisone acetate once a week showed delayed aging, of the same type as seen in rats fed on limited rations. Corticosteroids have many functions: Corticotropin – corticosteroids are necessary for survival in some species such as the toad *Bufo bufo* (Jørgensen and Larsen 1963; Jørgensen 1988); in some (all?) species they are involved in reproduction, but in certain species they may end by killing the animal ("programmed death"). Greenberg and Wingfield (1987) discuss "Stress and reproduction: Reciprocal relationships" and add that adrenal activation is not necessarily deleterious. Schreibman (1987) has suggested that the pars intermedia in platyfish (*Xiphophorus maculatus*) may regulate early gonadal development. This fits the hypothesis that the pars intermedia is involved in initation of sexual maturation.

Stress, pituitary, and interrenal cells. In Larsen and Rothwell (1972) some evidence for the involvement of the interrenal cells in stress response has been gathered. In that review evidence of corticotropin-secretion was also presented. Further evidence has been added later, both with regard to corticotropin and other proopiomelanocortin-derivatives (Baker and Buckingham 1983; Eastman and Portanova 1982; Dores *et al.* 1984; Nozaki and Gorbman 1984). Hardisty (1972) found that the estimated number of interrenal cells increased in two hypophysectomized (total ?), two sham-operated, one (of three) osmotically stressed, and in four light stressed river lampreys (sex not mentioned). Frequent mitoses were especially found in hypophysectomized, sham-operated and light stressed lampreys. So stress factors may perhaps act without the intervention of the pituitary gland as mentioned above.

Unidentified corticosteroids. Considering the accumulation of findings pointing to a role of steroids from interrenal cells in the period of sexual maturation, and the increasing evidence that they are not identical to the known corticosteroids (see review by Hardisty and Baker 1982), it is of interest that J. Leloup-Hâtey in 1964 (personal communication) found high amounts of corticosteroids in *P. marinus* with a modified Porter Silber method (Nelson and Samuels 1952) as shown in Table 3. These old fluorescense methods are not as specific as the later methods based on isotope-labeling or RIA, but they may be more valid as long as the precise chemistry of the steroids are unknown. The presence of unknown steroids being produced by the interrenal cells of sexually mature *P. marinus* has in fact been indicated by Weisbart *et al.* (1978), who found that interrenal tissue produced unidentified compounds isopolar and isomorphic with testosterone and androstenedione.

Possible origin and role of estradiol. There is also uncertainty with regard to the chemical identity of sex hormones (see Kime and Larsen 1987; Larsen 1987); they may, as mentioned, be 15-hydroxylated derivatives of estradiol and testosterone. Sower and Larsen (unpublished observations) found that total hypophysectomy in adult female *P. marinus* caused a decrease in plasma estradiol and progesterone; they furthermore showed that substitution therapy with lamprey pituitaries normalized the estradiol and progesterone levels. In intact females injection of lamprey pituitaries had no effect on estradiol, but progesterone was decreased. The data by Kime and Larsen (1987) indicate that at least estradiol (or cross-reacting steroids) may be secreted by the interrenal cells; estradiol is also produced in lamprey males and is present in similar amounts in plasma of both sexes (see Sower this issue). The observation that plasma estradiol is not decreased, but rather slightly increased, after hypophysectomy (pro + meso) in river lamprey females (Kime and Larsen 1987) is at variance with the data just referred to (Sower and Larsen, unpublished observations). The reason may be species differences, different time interval between surgery and blood sampling, different stages of reproduction, and perhaps especially different types of surgery: Sower and Larsen removed the whole pituitary, that is also the metaadenohypophysis, which may support interrenal cell secretion of estradiol as suggested above. It may be worthwhile to investigate whether interrenal estradiol production in hypophysectomized (pro + meso) river lampreys may explain the fact that the slow rate of vitellogenesis, typically seen in autumn and winter, continues after hypophysectomy (pro + meso), which ony abolishes the rapid rate seen in the last weeks before ovulation (Larsen 1965, 1969a, 1973). Interrenal estradiol can perhaps also explain the continued development of secondary sex characters in river lamprey females hypophysectomized (pro + meso) in March (Larsen 1969a,b). In discussing possible biological roles of estradiol not only its effects on vitellogenesis should be considered (Pickering

Table 3. Corticosteroids in plasma of *Petromyzon marinus.*

	Males		Females	
	Plasma content (μg/100 ml)	Gonadosomatic index	Plasma content (μg/100 ml)	Gonadosomatic index
April	2.65 (2)	immature	1.8 (5)	immature
May	15.6 (2)	1.26:1.53	7.4 (4)	10–15
June/July	38.6 (2)	1.10:1.38	11.9 (1)	28
End of July	0.6	1.17		

Data from J. Leloup-Hâtey (personal communication 1967). Method: Nelson and Samuels (1952).

1979a; Fukayama *et al.* 1987), but also its possible effects on the coelomic epithelium in both sexes (Fukayama and Takahashi 1985).

Conclusion

Reproduction in river lampreys does not occur if the pro + mesoadenohypophysis is not present. There is evidence for production of unidentified gonadotropin(s) in the mesoadenohypophysis and perhaps also in the proadenohypophysis in sea and river lamprey. There is also evidence for secretion of unidentified gonadal sex hormones, which are probably closely related to testosterone in males and to estradiol in females. As a working hypothesis, supported by indirect evidence, it is suggested that unidentified steroids from the interrenal cells of lampreys are secreted in increasing amounts in the period before and during sexual maturation. Their secretion may be stimulated by corticotropin and/or by other proopiomelanocortin-derivatives, either from the meso- or the metaadenohypophysis. These corticosteroids may, alone or in concert with gonadal sex hormones, be involved in various processes taking place before, during or after sexual maturation, like intestinal atrophy, swelling of the anal region, early growth of the urogenital papilla, early (and slow) vitellogenesis, increased blood glucose, the green color of the liver, mobilization of tissue from the body resulting in rapidly decreasing body length and finally in death after spawning, especially in males. In some cases, large doses of exogenous estradiol or testosterone may elicit effects which in natural development supposedly are caused by interrenal steroids (intestinal atrophy and the green color of the liver). In other cases, these large doses of estradiol or testosterone may counteract the effect of endogenous interrenal steroids (natural death).

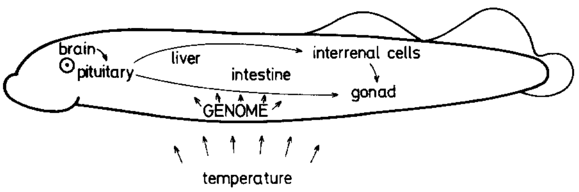

Fig. 5. Survey of important external and internal factors involved in reproduction in lampreys. "Genome" signifies the genetically based development of sensitivity to gonadotropin and sex hormones.

When sexual maturation proceeds with maximal efficiency and a high degree of synchronization in a given area, the pituitary is probably under the influence of substances diffusing from the hypothalamus – in sea lampreys presumably gonadotropin releasing hormones similar to those of other vertebrates. However, the increasing temperature of the water may be the main factor responsible for seasonality and synchronization in lampreys since it acts on all processes related to sexual maturation, including the unexplained increase in sensitivity to gonadotropin and sex hormones in spring. The survey (Fig. 5) of essential external and internal factors involved in reproduction in lampreys helps to stress:

1. The importance of the endogenous control in initiation of sexual maturation.

2. The importance of temperature of the water for seasonality and synchronization of sexual maturation in a given area.
3. The modest role of brain control of pituitary function.
4. The suggested role of interrenal steroids.

Attention should now be directed towards isolation and characterization of the gonadotropin(s) in order to be able to develop antibodies and measure the levels in the blood, and towards establishing the chemical identity of the gonadal sex hormones and analyze further the interactions between the gonad and the interrenal cells. In this interplay also possible control from the metaadenohypophysis should be considered.

It may be wise to continue to concentrate on two species, *L. fluviatilis* and *P. marinus* and to supplement the present extensive biological knowledge of *L. fluviatilis* with studies of GnRH, and to supplement the detailed measurement of hormones in *P. marinus* with parallel studies of biological parameters.

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