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## STEROIDS IN MALE REPRODUCTION

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### Summary

Key words: Teleostean fish testis - Steroidogenesis - Steroid activity - Spermatogenesis - Spermiation

In common with other vertebrate classes, the Leydig cells of teleostean fish are the main site of steroidogenesis, although some steroidogenic enzymes possibly occur in Sertoli cells. Other organs, especially the interrenal and the liver, are able to metabolize steroids. The importance of their contribution to the establishment of circulating levels of sex steroids is still unknown.

Testis steroid biosynthesis occurs mainly via the  $\Delta 4$ -pathway. However, the  $\Delta 5$ -pathway present in the male gonad has been little explored. Attention needs to be drawn to the occurrence of  $5\alpha$ -reductase and aromatase activity. Besides, conjugation might be an important step in the modulation of androgen activity, under the direct control of temperature.

Maturational gonadotropin is probably a major factor in testis steroidogenesis regulation; however some facts show that other ways of regulation must be explored, such as other pituitary hormones or paracrine and autocrine factors.

The biological significance of sexual steroids in testis function has been studied mainly concerning testosterone,  $11\beta$ -keto-testosterone and  $17\alpha$ -hydroxy- $20\beta$ -dihydroprogesterone. Their possible actions on spermatogenesis and spermiation are discussed.

### Introduction

Several recent reviews deal with the endocrine function of fish testis and the control of male reproduction (Billard et al., 1982; Fostier et al., 1983; Kime, 1980; Lofth, 1987; Nagahama, 1986; Norris, 1987). In this paper mainly controversial topics will be discussed together with some matters open to further research. No exhaustive citation can be given in such a short paper. Gonochoric teleost species will be focused upon and a few references will be cited as examples of the numerous studies done in the field.

### Tissues and cells identified as able to synthesize or to metabolize steroids in male fish

#### Testis and genital tract

The site of steroid production in the testis of teleosts has been a topic of controversy until recently. This was mainly a consequence of confusion concerning the identity and homology of somatic cells due to low resolution of histochemical techniques used in early studies. The use of ultrastructural techniques has now enabled the identification of the cell types (Billard et al., 1972; Grier, 1981). "The interstitium of all teleosts contains Leydig cells. Previous reports indicating that Leydig cells were lacking in testes of some teleosts were incorrect...Within the tubule basement membrane, only germ cells and their associated Sertoli cells exist" (Grier, 1981).

There is now more evidence that the Leydig cells are the main source of gonadal steroids in fish, as in mammals. The Leydig cells are usually distributed singly or in small to large groups, according to the species. They lie in the interstices between the lobules from which they are separated by layers of collagen and myoid cells. Enzymes involved in steroid biosynthesis, mainly  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ -HSD), have been histochemically demonstrated in the testicular interstitial cells of numerous teleosts. Since 1960 (Follenius & Porte), it has been shown in many species that these cells have ultrastructural typical features of active producing cells, although in some species Leydig cells do not accumulate lipids and lack lipid droplets (Van Den Hurk et al., 1974).

Direct evidence of the steroidogenic activity of the testicular interstitial cells has been obtained in two cases. Firstly, the glandular part of the testis of some gobidae produce androgens (Bonnin, 1977). Secondly, populations of pure Leydig cells, isolated from trout testis and maintained in primary culture, produce androgens and progestins and these secretions can be stimulated by pure salmon gonadotropin (s-GTH) (Loir, in preparation).

The steroidogenic capacities of Sertoli cells are still uncertain. Ultrastructural and histochemical data generally do not suggest such a capacity, although numerous lipid droplets are sometimes present in their cytoplasm (Billard et al., 1972;

Hoar and Nagahama, 1978). No significant amounts of 11-ketotestosterone (11K-T) or 17 $\alpha$ -hydroxy-20 $\beta$ -dihydroprogesterone (17,20-P) have been detected in primary cultures of pure populations of Sertoli cells prepared from spermiating trout testes (Loir, in preparation). However hydroxysteroid dehydrogenases have been localized in Sertoli cells of some species (Bara, 1969), sometimes only during a limited period of the sexual cycle (Van Den Hurk et al., 1978). Rarely ultrastructural investigations have shown steroidogenic capacities (Nicholls and Graham, 1972).

It has been proposed that spermatozoa participate in the biosynthesis of active steroids: washed spermatozoa were able to convert oestradiol-17 $\beta$  into oestrone (Hathaway, 1965), adrenosterone into 11K-T (Idler and Macnab, 1967), and 17 $\alpha$ -hydroxyprogesterone (17-P) into 17,20-P (Ueda et al., 1984). Because of the possibility of sperm contamination by somatic cell components, such ability needs to be carefully further explored.

With respect to the genital tract, 3 $\beta$ -HSD activity has been detected in epithelial cells around the vas deferens of various species (Van Den Hurk et al., 1978). Furthermore isolated trout spermiduct (Schulz, 1986a) or catfish seminal vesicles (Schoonen and Lambert, 1986a) can produce or metabolize sexual steroids in vitro.

#### Interrenal

Several hydroxysteroid dehydrogenase activities have been detected in the interrenal tissue and they can vary during the male sexual cycle (Sufi et al., 1980). The teleost interrenal could participate in the production of male sexual steroids at least by producing precursors usable by the testis and possibly by synthesizing active androgens (Idler and Macnab, 1967).

#### Peripheral metabolism of steroids

In at least three species the liver is able to metabolize cortisol into androstenedione ( $\Delta$ 4) and 11 $\beta$ -hydroxyandrostenedione (11 $\beta$  OH- $\Delta$ 4) which could be further transformed into 11K-T by the testis (Kime, 1978). However, according to Schulz (1986b) this concept of an interrenal-liver-gonadal axis might be of minor relevance considering the low rate of conversion permitted by the liver. Besides 17 $\beta$ -HSD, transforming  $\Delta$ 4 into T, is present in the liver and its activity varies with the male sexual cycle and is maximal at spawning season (Hansson and Gustafsson, 1981). Finally, 5 $\alpha$ -reduction and glucuronidation of androgens occur in the liver (Kime and Saksena, 1980).

Recently, Schulz (1986b) has proposed that the plasma androgen levels found in mature

male rainbow trout result from synthesis and metabolism not only in the testis, but possibly in the interrenal and liver, and also in blood cells, which possess 17 $\beta$ -HSD and glucuronyltransferase activities. However plasma steroid protein binding could protect steroids from this metabolism.

Various reductase, hydroxysteroid dehydrogenase and glucuronyltransferase activities have been detected in the male skin (Ali et al., 1987). Their roles are probably local, and their participation in the control of steroid plasma levels non-significant.

The same conclusion could be drawn from the finding of steroidogenic enzymes in the teleost brain and pituitary.

#### Steroid biosynthesis in the testis

Since the author's review edited in 1983 (Fostier et al.) a few studies have been published on steroid metabolism by the testis of gonochoric teleostean species. The tilapia Saratherodon mossambicus (Kime and Hyder, 1983), the eel-pout Zoarces viviparus (Teraevaeinen, 1983), the sailfin molly Poecilia latipinna (Kime and Groves, 1986), the catfish Clarias gariepinus (Schoonen and Lambert, 1986b) and the rainbow trout Salmo gairdneri (Saad and Depeche, 1987). The following discussion will refer to the papers already cited in the previous review and to these more recent studies.

#### $\Delta$ 4 and $\Delta$ 5 Pathways

The biosynthesis of steroids in fish testis has been shown to follow mainly the  $\Delta$ 4-pathway, but it must be pointed out that most of the metabolism studies were performed with labelled precursors belonging to this pathway (progesterone, 17 $\alpha$ -hydroxyprogesterone, androstenedione, testosterone), and that unlabelled references (carriers) used to characterize metabolites were also mainly chosen within the various  $\Delta$ 4 - steroids. However some studies have demonstrated that, at least partly, testicular steroids may be metabolized via the  $\Delta$ 5 - pathway in fish. This pathway has been established and is followed more or less depending on the species: pregnenolone  $\rightarrow$  17 $\alpha$ -hydroxypregnenolone  $\rightarrow$  dehydroepiandrosterone  $\rightarrow$  5-androsten-3 $\beta$ ,17 $\beta$ -diol.

When investigated, the  $\Delta$ 5-pathway does appear quantitatively less active than the  $\Delta$ 4 pathway. However, these studies were often performed at only one physiological stage. Thus, in rainbow trout ovaries, Lambert and Van Bohemen (1979) found that the relative importance of  $\Delta$ 4 and  $\Delta$ 5 - pathways changes during ovogenesis. Besides, incubation duration was relatively long in most cases (a few hours), while in the pike, dehydroepiandrosterone conversion into androstenediol could only be detected in

incubations lasting one hour or less (Lupo di Prisco et al., 1970). Furthermore, the existence of a  $\Delta 5$ -pathway in rat testes has recently been shown by using very short incubation times (Weusten et al., 1987).

#### 5 $\alpha$ / $\beta$ -Reduced androgens

Recent works have confirmed the production of 5-reduced compounds, mainly 5 $\beta$ -reduced androgens, in various species. In the sailfin molly significant quantities of such steroids can be synthesized, and it has been postulated that they might play a role in the reproductive endocrinology for this species (Kime and Groves, 1986).

#### Biosynthesis of 11-oxoandrogens

The pathway for biosynthesis of 11-oxoandrogens has been studied in various species and recently reinvestigated (Teräsväeinen, 1983; Leitz and Reinboth, 1985; Schoonen and Lambert, 1986b). The sequence:  $\Delta 4 \rightarrow T \rightarrow 11\beta OH-T \rightarrow 11K-T$  seems to predominate; but other routes have been proposed:  $\Delta 4 \rightarrow 11-OH-\Delta 4 \rightarrow$  androstosterone  $\rightarrow 11K-T$   $\Delta 4 \rightarrow 11-OH-\Delta 4 \rightarrow 11-OH-T \rightarrow 11K-T$

Furthermore, 11 $\beta$ -hydroxylation can occur before the cleavage between carbons 17 and 20: 17 $\alpha$ -hydroxyprogesterone  $\rightarrow$  21 deoxycortisol  $\rightarrow$  11-OH- $\Delta 4$ .

#### Biosynthesis of 17 $\alpha$ -hydroxy-20 $\beta$ -dihydroprogesterone

17,20-P was first detected in male fish in the plasma of salmon (Schmidt and Idler, 1962), then later identified by double isotopic dilution assay in rainbow trout (Campbell et al., 1980). The potential of fish testis to produce this steroid was discovered in a selachian species (Simpson et al., 1964) and has now been confirmed in various teleostean species. Ueda et al. (1984) suggested that, at the end of the cycle, spermatozoa, bearing 20 $\beta$ -HSD, were involved in 17,20-P synthesis. However this synthesis can occur during the whole sexual cycle (Saad and Depeche, 1987; Le Gac and Fostier, 1987) and isolated Leydig cells can secrete 17,20-P (Loir, in preparation).

#### Biosynthesis of oestrogens

Low levels of oestradiol-17 $\beta$  have been detected by radioimmunoassay in various species, but, when investigated, no aromatase activity could be shown in teleost testis, although it has been recently found in the dogfish (Callard et al., 1985).

We have reinvestigated the capacity for rainbow trout testis to produce oestradiol. In the first experiment testicular fragments (spermiation) were incubated (24 h, 14°C) with increasing concentrations of testo-

sterone or of s-GtH. In both cases oestradiol secretion, measured by RIA, increased with the level of testosterone used as a precursor, or with the level of GtH (Fig. 1). In the second experiment (beginning of spermatogenesis) microsomes were prepared by differential centrifugation, then incubated with tritiated testosterone. At the end of incubation (10 minutes to 4 hours) tritiated oestradiol and oestrone were purified, then identified by successive crystallisation to constant isotope and specific activity (Fig. 2).

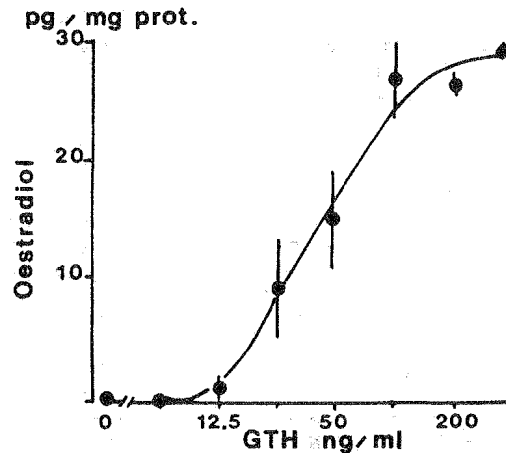


Fig. 1. Oestradiol secretion by testicular explants, incubated (24 hrs, 14°C) with increasing concentrations of s-GtH.

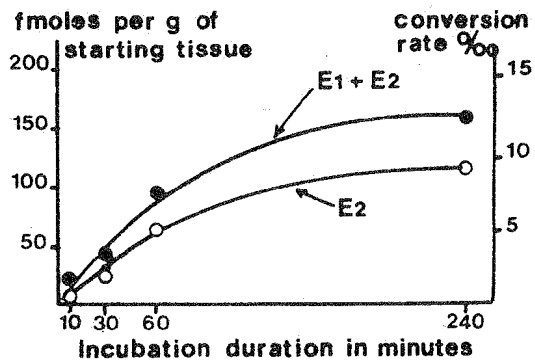


Fig. 2. Aromatase activity in microsomes of rainbow trout testis (beginning of spermatogenesis):  $^3H$ -oestradiol (E2) and  $^3H$ -oestrone (E1) produced from 1,2,6,7- $^3H$ -testosterone (12.8 picomoles/g starting tissue).

#### Conjugation

Testicular conjugation of steroids is an original phenomena in vertebrates (Kime and Hyder, 1983). Since the detection of testosterone glucuronide in the peripheral plasma and testes of sockeye salmon (Grajcer and

Idler, 1963), glucuronyl transferase activity has been found in the testis of other species. Very high levels of testosterone glucuronide were found in salmon plasma during spawning (Truscott et al., 1986). Sulphotransferase activity has been reported more rarely (Kime and Groves, 1986).

#### Regulation of testicular sex steroid production

Since the early study of Pickford and Atz (1957) the prominent role of the pituitary in the regulation of the male steroidogenic function, notably of the maturational hormone (Yamazaki and Donaldson, 1969; Ng and Idler, 1969) has been demonstrated in various species of fish.

In vitro studies have confirmed and prolonged in vivo results, allowing investigators to isolate the testis from other potential sources of steroids. Thus, association of pituitaries with Gobius niger glandular tissue greatly increases the secretion of free and conjugated testosterone (Bonnin, 1977). Various fish gonadotropins stimulate androgen production by isolated testicular tissue (Schulz, 1986b; Le Gac and Fostier, 1987). Ueda et al. (1984) first reported GtH stimulation of 17,20-P production by salmonid testicular explants. Recently Saad and Depeche (1987) suggested that several enzymatic steps of steroidogenesis are under positive GtH control: cholesterol side chain cleavage, 3 $\beta$ -HSD, 17 $\alpha$ -hydroxylase, 20 $\beta$ -HSD. They did not find an inhibition of C17-C20 lyase as hypothesized by Scott and Baynes (1982). Finally, using isolated and cultured testicular cells, Loir (in preparation) found that GtH acts mainly on Leydig cells by increasing the secretion of 11K-T and 17,20-P.

Binding of GtH on target cell membranes may be the first step of the hormone action since the concentrations of GtH necessary to induce half-maximum 11K-T response in vitro is in the same order of magnitude as the concentration necessary to saturate 50% of the testis GtH binding sites (Le Gac et al. 1985). Cyclic AMP is probably an intracellular mediator of GtH action (Chang and Huang, 1982; Schulz, 1986b).

However, not only positive effects of GtH on 11K-T production have been reported: in silver eel, 11K-T levels increase significantly following hypophysectomy (Khan et al., 1986). Besides, there is not always a clear relationship between GtH and plasma steroid levels during the cycle (Fostier et al., 1982). Such apparent discrepancies led to the consideration of factors other than GtH levels being involved in the regulation of the steroidogenic cells. For example changes in the receptivity to GtH, action of other hormones, paracrine and autocrine

intratesticular regulations and the effects of environmental factors.

Up until now testicular receptivity to trophic hormones has not been widely studied. We have shown recently that, in rainbow trout, in vitro sensitivity to purified s-GtH is maximum at the beginning of spermiation for 11K-T production, or during spermiation for 17,20-P production. Increase in sensitivity is concomitant to a rise in the number of testicular high affinity binding sites (Le Gac and Fostier, 1987). Such events might favour increased plasma levels of these two steroids at the end of the sexual cycle.

Few studies have been concerned with the action of other hypophysial hormones. According to Ng and Idler (1980) the "vitellogenic" gonadotropin (Con A I) has no effect on testicular steroidogenesis. On the other hand, the results obtained by Pickford et al. (1972) may be interpreted as a synergic effect of bovine growth hormone (GH) and b-LH on 3 $\beta$ -HSD activity in the interstitial tissue of the hypophysectomized killifish.

The high concentration of binding sites found in tilapia testicular membranes for prolactin (Edery et al., 1984) show the need for investigation of its potential role in steroidogenesis regulation.

Using isolated populations of somatic cells Loir (1987) found that the morphology and function of Leydig cells are better conserved when cocultured with Sertoli cells, suggesting the existence of paracrine regulation in the testis. Besides, steroid short-loop feed back can be also an important regulatory step. Recently, the direct effect of 11K-T on 20 $\beta$ -HSD (17-P  $\rightarrow$  17,20-P) has been evidenced in the rainbow trout testis (Lepretre, 1985 cited in Saad and Depeche, 1987). These findings show the need to explore intratesticular regulations.

Finally, considering the poikilothermy of fish, temperature may modulate directly the testis function. In fact, the recent studies of Kime and coworkers have demonstrated the temperature dependancy of testicular steroidogenic enzymes, especially the glucuronidation (Manning and Kime, 1985).

#### Biological activities of sex steroids in male fish reproduction

Numerous studies have shown the role of androgens on the development of sexual secondary characters, sex accessory structures and gonoducts in male fish (Norris, 1987), but as far as the control of testis function is concerned, data are still relatively scarce.

#### Action of steroids on spermatogenesis

Although the highest plasma levels are found during the spawning season, androgens

are present all along the sexual cycle, and therefore, are potential regulators of spermatogenesis. 11K-T might be implicated in the last stages of spermatogenesis that are partly concurrent with the beginning of spermiation (see below). This is supported by the results of Cochran (1987) who finds the 11K-T increase in killifish to strongly coincide with the rise in "sperm-index", which partly reflects the progress of spermiogenesis.

Studies using intact fish are difficult to interpret in terms of a direct action of steroids in the testis, even when treatments are performed in immature fish (Magri et al., 1985); thus studies using hypophysectomized animal or in vitro culture of testis explants have been focused on. Testosterone, testosterone propionate or methyltestosterone administered in hypophysectomized adult fish are able to maintain (catfish; Nayyar et al., 1976; goldfish: Billard, 1974) or to restore (catfish: Sundararaj et al., 1967) all the stages of spermatogenesis, except spermatogonia mitoses. However the effect may be quantitatively small, as in the killifish (Lofts et al., 1966). Further evidence of the role of testosterone in spermatogenesis was furnished by Remacle (1976). He showed in vitro completion of spermatogenesis in undeveloped testis maintained in culture with testosterone isobutyrate crystals.

Biological significance of oestrogens in male fish is still unclear. When plasma oestradiol levels were measured at various sexual stages the highest values were found during spermatogonia mitoses or spermiation. However no effect of oestradiol was detected in vivo in hypophysectomized killifish (Sundararaj and Nayyar, 1967) or in vitro on goldfish testicular explants (Remacle, 1976).

Activity of 17,20-P is only known in relation with spermiation (see below), although the testicular potentiality for its synthesis is present during the whole sexual cycle.

#### Action of steroids on spermiation

Several descriptive or experimental studies have been reported that suggest a possible relationship between 11K-T and milt production. Higher plasmatic concentrations of 11K-T are generally found just before or during milt production. Furthermore, Fostier and coworkers (1982) found a significant correlation between 11K-T levels and volumes of milt collected during the beginning of spermiation. However the 11K-T peak levels are not maintained throughout the whole period of "spermiation". According to Schulz (1984) some discrepancies between studies could be the result of sperm stripping regime.

Exogenous administration of androgens can

induce spermiation in hypophysectomized goldfish (Yamazaki and Donaldson, 1969; Billard, 1976). But Ueda and coworkers (1985) found that injections of low doses of 11K-T or T failed to induce spermiation in mature amago salmon and was relatively ineffective in goldfish. However high doses might be necessary to significantly increase intratesticular androgen concentrations. For Yamazaki and Donaldson (1969), but not for Billard and coworkers (1982), 11K-T was more effective than other androgens. When tested, progesterone (Billard, 1976) and 17,20-P (Ueda et al., 1985) were more effective than androgens.

Elevated 17,20-P plasma are generally found in males with running milt or in spawning males (Scott and Baynes, 1982; Truscott et al., 1986). Furthermore one injection of gonadotrophin preparation (SGA) in mature goldfish or salmon induced a stimulation of both milt production and 17,20-P plasma levels (Ueda et al., 1985). Studies performed by Scott and Baynes in various rainbow trout strains have shown positive correlation between 17,20-P plasma levels and volume of milt expressed manually, or sperm count. There is also evidence that the progesterin may control the ionic composition of the seminal plasma, notably the  $K^+/Na^+$  ratio (Scott and Baynes, 1982; Baynes and Scott, 1985).

However, two different studies have described males with running milt, but in non-spawning conditions, displaying undetectable or low 17,20-P plasma levels. On the other hand similar fish in spawning condition (courtship behavior and ejaculation) show elevated 17,20-P levels (Liley et al., 1986; Kobayashi et al. 1986).

Finally, from the results presented here, a major role of 11K-T in the induction of spermiation cannot be excluded. One of its further actions could be to push the steroidogenic flux towards 17,20-P production by acting directly on 29 $\beta$ -HSD activity (Lepretre, 1985, cited in Saad and Depeche, 1987). The rise of 17,20-P in male plasma could then increase milt production during spawning with a view to synchronizing sperm release with oviposition. This phenomenon could be amplified by the presence of ovulatory females as proposed by research groups working on social regulation of reproduction (Liley et al., 1986; Dulka et al., 1987).

#### Conclusion

Numerous data are now available on circulating levels of some hormones, mainly GTH and sex steroids, during the sexual cycle. Such results are very useful and need to be complemented with measurements of other hormones suspected to participate in the regulation of reproductive physiology. However it is increasingly obvious that a

greater understanding of this regulation requires investigation within the testis itself. Thus, further work has to be done on intratesticular levels of steroids, hormone sensitivity of the different cell categories and cooperation between these cells in the maintenance of the testis function.

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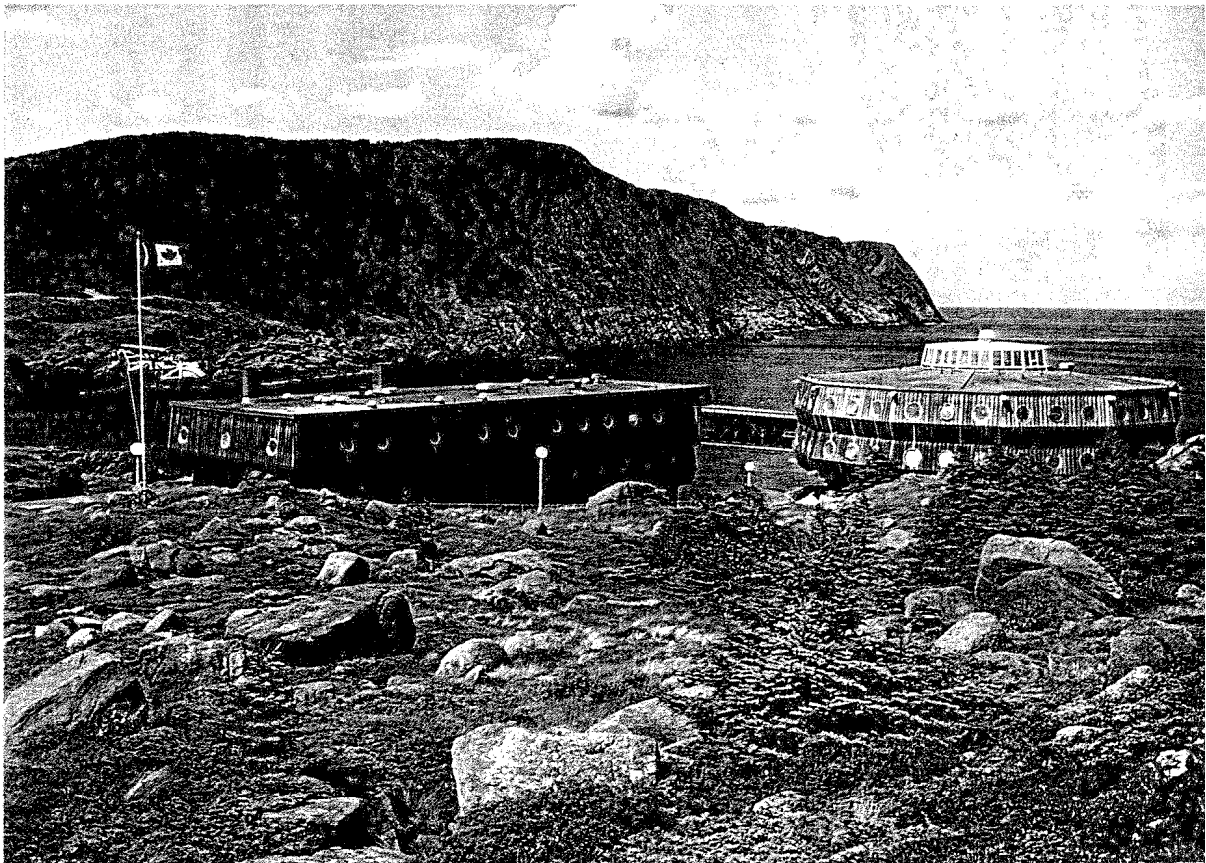
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