

## Steroidogenesis in Corpuscles of Stannius

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The developmental and ultrastructural characteristics were considered to establish any relationship of Stannius corpuscles to the adrenal system. Physiological evidences suggest that the function of corpuscles of Stannius could be similar to that of adrenocortical tissue and favour a calcium-regulating function of these. Biochemical evidence on the possible functions of Stannius corpuscles is lacking. Evidence for a steroidogenic role of Stannius corpuscles in some species of teleosts is available. Evolutionary changes probably account for differences in the results obtained from diverse groups of teleosts so far studied.

### Introduction

Corpuscles of Stannius (CS) are putative endocrine organs located on/in the kidneys of holostean and teleostean fishes. They have no known homologue among other vertebrates. No other endocrine-looking structures were known in the kidneys of fishes when CS were discovered by Stannius (1839), hence they were considered as the adrenals of fishes. The discovery of interrenals (IR) by Giacomini (1902) led to comparative developmental, physiological, biochemical and ultrastructural studies of CS and IR and the controversy regarding the steroidogenic activity of CS is hitherto unsolved. Evidences for other possible functions exist. The evidences for and against considering CS as a steroidogenic tissue are discussed.

### Developmental Studies

Studies of Giacomini (see Garrett 1942) on the development of CS and IR and those of Srdinko (1903), Garrett (1942), De Smet

(1962) and Krishnamurthy (1967) showed that the IR developed from the coelomic epithelium whereas CS developed from the pronephric or mesonephric components or from both in some species of teleosts. Garrett (1942) after an extensive discussion on the homologues of CS among other vertebrate groups concluded that CS should not be considered in any way related to the adrenal system. This was often quoted in support of denouncing a possible steroidogenic activity of CS by other investigators. Intensive search through literature shows that some of the critical investigations on the development of adrenal cortex of mammals have revealed that the developing adrenal cortex receives contributions from the pronephros, mesonephros (especially from the tubular components) and from the Wolffian body (Mitsukuri 1882, Aichel 1900, Crowder 1957). However, homology cannot be a prerequisite for analogy (see Barrington 1968). It will therefore be prudent to

conclude on direct evidences rather than on developmental studies, which although of academic interest, may not help in assessing the function of endocrine structures.

### Ultrastructural Studies

The membrane-bound secretory granules, lamellar cristae of mitochondria and rough endoplasmic reticulum found in the cells of CS of some species of teleosts were considered to ascertain the protein-secretory nature of these cells. In contrast, the cells of IR were shown to possess lipid droplets, tubulovesicular or vesicular cristae of mitochondria and smooth endoplasmic reticulum. Recent studies on the ultrastructure of CS cells (for details and references see Krishnamurthy 1976), however, have shown that the mitochondrial cristae vary in their form. In a number of species the cristae were tubulovesicular. Ogawa (1967) could show rough as well as smooth endoplasmic reticulum in different parts of the cells of CS of goldfish, and on hypophysectomy the rough endoplasmic reticulum completely transformed into smooth endoplasmic reticulum. This was also accompanied by loss of secretory granules making the cells look like steroidogenic ones. Interestingly enough adrenocortical cells of some mammals and of human foetus (which is known to be functional) present ultrastructural features which are generally considered not characteristic of steroid-producing cells (for references see Luse 1967).

The ultrastructural evidences, therefore, do not reveal whether CS are steroidogenic or not.

### Physiological Studies

Changes in the cytology of CS cells have been studied after hypophysectomy, interrenalectomy, administration of ACTH, corticosteroids, metopirone, aldactone, angiotensin II and saralasin acetate (for details see Krishnamurthy 1976, 1977). Some

results were interpreted to suggest an adrenocortical-like response of CS to such experiments. Although interactions between CS and other endocrine structure were shown, an axis between CS and pituitary (ACTH cells) or between CS and renin-angiotensin system was not established. Cytological changes such as increase/or decrease of secretory material in the cells or changes in the nuclear diameter could be interpreted differently and hence are not reliable indices of a specific response. Studies on the effects of Stanniectomy on other endocrines were also of no much help because conclusions were again based on interpretation of cytological changes.

Changes in the plasma electrolytes following Stanniectomy seemed more or less uniform in a number of teleost species (table 1). In general, there was a fall in plasma sodium (Na) and increase in plasma potassium (K) and calcium (Ca). Although there had been some inconsistencies in the results obtained in some forms (e.g. yellow eel in fresh water and sea water) the effect on Na in most of the cases was similar to that caused by interrenalectomy. However, the effect of Stanniectomy on plasma Ca was more uniform in several species studied. In the absence of a convincing biochemical evidence it becomes difficult to rely and ascribe any significance to the results of physiological studies (particularly the effect on plasma Na and K) and it would be better to treat them with reserve.

### Biochemical Studies

To verify the steroidogenic ability of CS, total extraction and incubation (without added precursors) techniques were attempted (table 2). Corticosteroids such as cortisol, cortisone, DOC and 11-deoxycortisol were detected in the CS of some teleost species, while no steroid could be found in the CS of other species. However, presence of these corticosteroids in a tissue may just

Table 1 Effect of stamiectiony on plasma electrolytes

Species	Na	K	Cl	Ca	Author(s)
<i>Sicyases sanguineus</i>	—	*	*	*	Vargas & Concha (1957)
<i>Anguilla anguilla</i>	—	+	*	+	Fontaine (1964)
	—	*	—	+	(1967)
	—	+	*	*	Leloup & Leloup-Hatey (1964)
	—	=	*	*	Leloup-Hatey (1964 a)
	—	+	*	*	(1964 b)
	—	=	*	*	(1964 c)
FW yellow	—	+	—	+	Chan et al. (1967)
FW silver	—	+	—	+	
SW yellow	+	=	*	+	
SW silver	+	=	+	+	
DW yellow	—	+	*	—	
FW yellow	—	+	*	+	(1969)
SW silver	—	+	*	+	
	=	*	*	+	Fenwick & Forster (1972)
<i>Anguilla japonica</i>	—	*	*	+	Chan (1967)
	—	+	=	+	(1972)
<i>Anguilla rostrata</i>	—	+	—	+	Butler (1969)
<i>Carassius auratus</i> (FW)	=	=	=	+	Ogawa (1968)
(DW)	+	—	+	=	
<i>Fundulus heteroclitus</i> (1/3 SW)	—	+	+	+	Pang (1971)

—, decrease; +, increase; =, no change; \*, not studied

mean storage or cooperative steroidogenesis similar to the one occurring in foetoplacental unit (Nandi 1967). Therefore, it would be inevitable to demonstrate the enzyme activity if the steroidogenic role of a tissue is to be

established, i.e. the catalysis of a reaction shortly after the formation of steroid nucleus during the synthesis of steroid hormone will have to be demonstrated. Thus, demonstration of  $3\beta$ -Hydroxysteroid dehydrogenase activity in CS, either by biochemical or histochemical method, would be the only solution to the present problem.

Table 2 Steroidogenic activity of teleostean CS: Tissue extraction or incubation without added precursors

Species	Steroids	Reference
<i>Cyprinus carpio</i>	None	Bondy (1957)*
<i>Oncorhynchus gorbuscha</i>	None	Ford (1959)
<i>Salmo salar</i>	Cortisol Cortisone	Fontaine & Leloup-Hatey (1959)
<i>Carassius auratus</i>	DOC	Ogawa (1963)
<i>Colisa lalia</i>	Cortisol 11-deoxy-cortisol	Krishnamurthy (1968)
<i>Gadus morhua</i>	Cortisol	Freeman & Idler†

\*cited by Pickford & Atz (1957)

†cited by Idler & Truscott (1972)

Biochemical conversion of Pregnenolone- $^{14}\text{C}$  was demonstrated in *Gadus morhua* (Idler & Freeman 1966) and *Salmo gairdneri* (Colombo et al. 1971). In addition to  $3\beta$ -HSDH activity,  $21\beta$ -hydroxylase activity was also demonstrated in the CS of these fishes (table 3). However, Colombo et al. (1971) showed that CS of *Salmo gairdneri* could convert much less of the substrate than the kidney tissue could. Thus, in the opinion of Colombo et al. (1971) the significance of steroidogenesis in CS was questionable.

**Table 3** Steroidogenic activity of teleostean CS: Biochemical conversion and demonstration of enzyme activity

Species	Substrate	Product	Enzyme	Reference
<i>Pseudopleuronectes americanus</i>	Prog.- <sup>3</sup> H	None	None	Phillips & Mulrow (1959)
<i>Anguilla anguilla</i>	Prog.- <sup>3</sup> H	„	„	Chester Jones et al. (1965)
	Prog.- <sup>3</sup> H	„	„	„
<i>Gadus morhua</i>	Estradiol-17 $\beta$	Estrone	17 $\beta$ -HSDH	Breuer & Ozon (1965)
	Prog.- <sup>14</sup> C	Prog.- <sup>14</sup> C	3 $\beta$ -HSDH	Idler & Freeman (1966)
	Prog.- <sup>14</sup> C	DOC- <sup>14</sup> C	21 $\beta$ -hydroxylase	„
<i>Salmo gairdneri</i>	Prog.	5 $\alpha$ -Prog.	5 $\alpha$ -hydrogenase	Arai et al. (1969)
	„	5 $\beta$ -Prog.	5 $\beta$ -hydrogenase	„
	11-DOC	None	None	„
	Prog.- <sup>14</sup> C	5 $\alpha$ - & 5 $\beta$ -Prog.	5 $\alpha$ - & 5 $\beta$ -hydrogenase	Colombo et al. (1971)
„	„	DOC- <sup>14</sup> C	21 $\beta$ -hydroxylase	„

The literature shows that demonstration of enzyme activity was not attempted in the CS of the same or closely related species in which corticosteroids were shown to be pre-

sent. Histochemical tests uniformly showed a negative reaction for 3 $\beta$ -HSDH activity in the CS of a number of teleost species (table 4).

**Table 4** Steroidogenic activity of teleostean CS: Histochemical demonstration of enzyme activity

Species	Substrate	Reaction	Reference
<i>Anguilla anguilla</i>	DHA	None	Chieffi & Botte (1963)
	DHA	None	Botte et al. (1964)
	DHA	None	Hanke & Chester Jones (1966)
<i>Conger conger</i>	DHA	None	Chieffi & Botte (1963)
<i>Gambusia holbrooki</i>	DHA	None	Botte et al. (1964)
<i>Syngnathus acus</i>	DHA	None	Botte et al. (1964)
<i>Gadus morhua</i>	Pregnenolone sulphate	None	Grimm (in Idler & Freeman 1966)
	DHA	None	„
	DHA sulphate	None	„
<i>Fundulus heteroclitus</i>	Several	None	Bara (1968)
<i>Colisa fasciata</i> (FW) (1/3 SW)	DHA	None	Krishnamurthy (1978)
	DHA	Positive	„

An attempt to demonstrate 3 $\beta$ -HSDH activity in the CS of *Colisa fasciata* (as cortisol and 11-deoxycortisol could be found in the CS of *C. lalia*) was met with some success. Although CS of *C. fasciata* under normal conditions did not show 3 $\beta$ -HSDH activity, CS of fish kept in a hypertonic medium (1/3 sea water) for 25 min showed an intense enzyme activity as demonstrated by histochemical method. The intensity of

reaction diminished 30 min after transfer of the fish to the hypertonic medium. Presence of enzyme activity in the CS of fish subjected to experimental alteration was further confirmed by separating the enzyme by acrylamide gel electrophoresis (0°C) and by incubating the gel tubes in the medium containing DHA, NAD and Nitro BT in phosphate buffer (pH 7.4 at 37°C).

## Conclusions

Developmental and ultrastructural studies throw no significant light on the steroidogenic role of CS. It is known that CS have different origin and constitute different types of cells. Thus, there is a possibility of CS secreting different active principles controlling different functions. Results of physiological experiments, although seem to show some similarities of CS and IR in the control of Na, they are, however, not convincing enough in the absence of a biochemical evidence. Considering the enigma presented by CS in spite of considerable research on it, it is clear that demonstration of enzyme activity alone, especially that of  $3\beta$ -HSDH, would confirm the steroidogenic role of CS, if any. Fragmentary evidences are available to suggest that CS in some species of teleosts

may undertake a steroidogenic role under some conditions of necessity. Histochemical and biochemical tests for  $3\beta$ -HSDH activity of fish subjected to various experimental alterations may be rewarding. The teleosts have evolved into such a diverse group in their form, function, habit and habitat that structures responsible for some functions (e.g., osmoregulation) might have aggregated with structures responsible for other functions or might have undergone drastic changes along with the evolution of the function itself. Inconsistencies in the experimental results may be due to the evolutionary process that different groups of fishes have undergone. A speculative approach in designing experiments is necessary which alone would show whether CS are truly steroidogenic or not.

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