

Comparative Aspects of Steroid Biosynthesis in Fish

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It is generally accepted that the first Chordates emerging in the Ordovician must have been water-dwelling, fish-like creatures and the ancestry of Vertebrata goes back to this presumably jawless, worm-like "fish". Thus, steroidogenesis in fish relates directly to the evolution of steroidogenesis by the whole group of Craniata. As judged from modern-day representatives of the different fish, steroidogenesis is present in all classes, though corticosteroidogenesis in Agnatha is rudimentary as compared to other vertebrates. Once Gnathostomata had evolved, steroid biosynthesis must have assumed its modern features. Cartilaginous fish, already a true-jawed vertebrate but lacking internal ossification exhibits most features of general vertebrate steroidogenesis. Amongst Selachii, the dominant adrenocorticosteroids are corticosterone and 1α -hydroxy-corticosterone, the latter one of the few unique class-specific vertebrate steroids and testicular testosterone and ovarian estrogen secretion has been established. The Holocephali, the other living order of Chondrichthyes, secrete cortisol as major interrenal steroid and the gonadal steroids are the mammalian-type C_{18} and C_{19} substances. 17-hydroxylated corticosteroids and aldosterone, the tetrapod mineralocorticoids, have yet to be demonstrated in the elasmobranch adrenocortical secretion. Numerically, the bony fish are the most important fish class. Osteichthyes are one of the most speciated vertebrates (teleosts with some 23,000 species). In bony fish, the production of cortisol as major corticosteroid and the mammalian-type gonadal steroids have been established. In addition, it seems that some bony fish gonads contain steroid 11-hydroxylase and 21-hydroxylase, resulting in the synthesis of 11-oxygenated testosterone derivatives and in the ovarian synthesis of 11-deoxycorticosteroids. Aldosterone secretion by bony fish interrenal tissue has been demonstrated occasionally only and not in all the species investigated. The biosynthesis of steroids shows some peculiarities in fish. Bony fish and holocephalean adrenal tissue are the only vertebrate adrenals capable of transforming corticosterone to cortisol. Cytochrome P-450 has been shown to be present in interrenal mitochondria and microsomes of elasmobranchs and teleosts, though adrenodoxin could not be detected in the interrenal mitochondria of the tiger-shark. The intracellular distribution of steroidogenic enzymes in fish adrenal tissue seems to be essentially the same as in tetrapods and recent evidence suggests that some teleost adrenals can utilize exogenous acetate for corticosteroid synthesis *in vitro*. It can be concluded that the vertebrate steroid biosynthetic system must have originated with the ancestral fish and is being utilized, with minor modifications, by all extant vertebrates. However, there is some recent evidence that some aspects of steroid biosynthesis might have been inherited by the ancient craniate from its invertebrate ancestors.

Introduction

Fish, a large and heterogenous group of vertebrates, have a venerable ancestry. Indeed, it is now generally accepted that the first representative of Craniata, emerging in the Ordovician seas some 450 million years ago, must have been an obligatory water-dweller and the origin of Vertebrata and of course ours as well, goes back to this presumably segmented, jawless, worm-like creature. During their evolution, fish have "invented" a number of remarkable physiological and biochemical features of high survival value, all of them transmitted to modern vertebrates. One of them is the maintenance of homeostasis of water and electrolytes through a variety of structures. It seems that in all vertebrates, homeostatic structures are, at least in part, controlled by the steroid hormones of the adrenal cortex or its homologues and it is common knowledge, that this is true for fish as well. Thus, unless evidence emerges to the contrary, we owe to the fish the modern adrenocortical tissue elaborating the corticosteroids which have not changed qualitatively for the last 450 million years or so, except in very

minor details. Another mechanism, which no doubt goes back to piscine origins is the interaction of these adrenocorticosteroids, amongst others, with the structures maintaining homeostasis. This interaction, adrenal cortical tissue → adrenocorticosteroids → appropriate target organs → synthesis of new protein → regulation of homeostasis called for either a novel set of biochemical rules or for the adaptation of old rules to new use. Whichever way it happened, it seems that it did happen in the Ordovician and Silurian seas.

The other steroidogenic organs, namely the gonads, have also attained their modern secretory form in fish, hardly any different from that of mammals. Indeed, for quite a long time it was believed that gonadal steroid secretion was a vertebrate attribute, originating with the piscine classes. Though we know now that this is not necessarily true (*vide infra*), vertebrate-pattern gonadal steroid secretion and utilization has evolved with fish and has been passed on, with minor modifications, to other craniates, including man.

Fish constitute a diverse group and their phylogeny is of some interest (see table 1).

Table 1 *Simplified phylogeny of fish*

Class	Subclass	Infraclass	Order
AGNATHA (CYCLOSTOMATA) Jawless fish			Petromyzontia: lampreys Myxinoidea: hagfish
CONDRICTHYES Cartilaginous fish	Elasmobranchii		Selachii: sharks Batoidea: skates, rays Chimaeriformes: ratfish
	Holocephali		
OSTEICTHYES Bony fish	Actinopterygii: ray-finned fishes	Chondrostei	Acipenseriformes: sturgeons
		Holostei	Amiiformes: bowfin
		Teleostei	Advanced modern ray-finned fishes
	Sarcopterygii: fleshy-finned fishes		Crossopterygii: Coelecanth
			Dipnoi: lungfishes

It can be seen that amongst extant fish, there are still representatives of the ancestral jawless type, originating from the Ostracoderms, extinct since 345 million years. Amongst the present-day fish, the Teleostei are the most numerous, represented by some 22,000 species and occupying various environments, be it fresh water, salt water or brackish water. They may be surface-dwellers or live miles deep in the ocean.

This brief introduction serves only to underline the importance of the biochemistry of these classes of vertebrates in the understanding of the tetrapod vertebrate systems.

Occurrence of Steroids

(A) *Agnatha*

(i) *Corticosteroids*

The ancestral *Agnatha*, the ostracoderms became extinct at the end of the Devonian but the present-day lampreys and hagfishes (*Petromyzones* and *Myxini* respectively) are specialized remnants of these jawless fish. It has to be mentioned that amongst vertebrates, only the myxinoids are iso-osmotic with their environment in contrast with the lampreys which are hypo-osmotic animals (Maetz 1968). Myxinoids spend all their life in salt water and their osmoregulation is similar to that of marine invertebrates. In contrast, most lampreys spend part of their life in fresh water and part in sea water and are able to maintain an electrolyte balance below that of sea water.

The anatomical localization of the adrenocortical tissue in cyclostomes has been controversial. In the sea lamprey (*Petromyzon marinus*) the presumptive adrenal tissue (PAT) has been located tentatively in the pronephric region (Lofts & Bern 1972, Weisbart & Youson 1975).

Though early studies have reported the presence of cortisol and corticosterone in the plasma of the Atlantic hagfish (*Myxine*

glutinosa) and the sea lamprey (*Petromyzon marinus*) (Chester Jones & Phillips 1960, Phillips et al. 1962), confirmation of these results took almost a decade by the then more sophisticated methodology. In 1971, Idler and coworkers succeeded in demonstrating the presence of cortisol (74 ng/100 ml plasma), cortisone (23 ng/100 ml plasma) and that of corticosterone (23 ng/100 ml plasma) in the Atlantic hagfish. These values showed significant increase following treatment of the animals with mammalian ACTH (123, 986 and 23 ng/100 ml plasma respectively). In addition, following ACTH treatment, 11-deoxycorticosterone could also be detected (270 ng/100 ml plasma). Recently, Idler and Burton (1976) localized the adrenocortical tissue in the pronephroi of the hagfish.

In lampreys, plasma corticosteroids could not be detected. However, after some unsuccessful attempts (Weisbart & Idler 1970), incubation studies with the pronephric tissue of the larval and adult parasitic sea lamprey, the transformation of exogenous progesterone to 11-deoxycortisol, 17 α -hydroxyprogesterone and androstenedione was reported (Weisbart & Youson 1975). A subsequent study on the incorporation of exogenous cholesterol into steroids by the presumptive adrenal tissue of sexually mature sea lampreys yielded negative results (Weisbart et al. 1978). It should be realized, however, that anandromous lampreys undergo important degenerative changes.

From the above studies, it can be accepted, that *Agnatha* most probably produce adrenocorticosteroids of the cortisol-cortisone-corticosterone types (table 2).

(ii) *Gonadal steroids*

The occurrence of gonadal steroids has not been investigated exhaustively in this class of vertebrates. Testosterone was extracted from the testes of adult river lampreys (*Lampetra fluviatilis*; Barnes & Hardisty 1972).

Table 2 Corticosteroids of Agnatha

Order and species	Steroid detected	Source	Reference
Petromyzones			
<i>Petromyzon marinus</i> (sea lamprey)	None	Peripheral plasma	(1)
	11-Deoxycortisol, 17-hydroxyprogesterone, androstenedione	Larval form, adult parasitic form: incuba- tion of PAT* with (¹⁴ C) progesterone	(2)
	None	Sexually mature form: incubation of PAT with (³ H) cholesterol	(3)
<i>Lampetra fluviatilis</i> (river lamprey)	None	Peripheral blood ± ACTH; adult females	(4)
Myxini			
<i>Myxine glutinosa</i> (Atlantic hagfish)	Cortisol, cortisone	Peripheral blood	(5)
	Cortisol, cortisone, corticosterone	Peripheral blood	(6)
	Cortisol, cortisone, corticosterone, 11-deoxycortisol	Peripheral blood after ACTH treatment	

*PAT, presumed adrenocortical tissue

[References: (1) Weisbart and Idler 1970; (2) Weisbart and Youson 1975; (3) Weisbart et al. 1978; (4) Buus and Larsen 1975; (5) Phillips et al. 1962; (6) Idler et al. 1971]

In nonspawning sea lampreys, exogenous progesterone yielded 11-deoxycorticosterone but not testosterone, following incubation *in vitro* with testicular tissue (Weisbart & Youson 1975). Testicular tissue from anandromous sea lamprey failed to produce any known gonadal steroid from exogenous cholesterol *in vitro* (Weisbart et al. 1978).

An extract of mature ovaries from the sea lamprey was reported to contain estradiol-17 β and estrone (Boticelli et al. 1963). Incubation of mature ovarian tissue from the same species failed to transform exogenous cholesterol to any known steroid hormone (Weisbart et al. 1978).

The above data give suggestive evidence that Agnatha probably do produce gonadal steroids but, this production might be

episodic and related to their complicated reproductive cycle.

(B) *Chondrichthyes*

(i) *Corticosteroids*

In contrast to Agnatha, cartilaginous fish have discrete adrenal tissue (interrenal gland), separate from chromaffin tissue. Extant cartilaginous fish exist in two subclasses: the Elasmobranchii and the Holocephali. Elasmobranchii may be further subdivided into selachians (sharks) and batoids (rays and skates). Most of our information concerns the elasmobranchs, with scant data on the Holocephali.

Phillips and Chester Jones (1957) showed that the plasma of rays and dogfish contain substances isopolar with mammalian corticosteroids when subjected to paper partition

chromatography. In 1959, Phillips examined the plasma of a large number of sharks and reported the presence of substances behaving like C₂₁ corticosteroids. A few years later, Bern et al. (1962) examined the corticosteroid production of dogfish (*Squalus acanthias*), the skate (*Raja rhina*) and the ratfish (*Hydrolagus colliiei*) interrenals *in vitro* and concluded that these adrenal tissues did indeed produce mammalian type corticosteroids. The elaboration of corticosteroids was further confirmed by Lupo di Prisco et al. (1967).

A systematic study of corticosteroids in cartilaginous fish has been performed by Idler and colleagues. This study resulted in 1966 in the isolation and identification, both from peripheral plasma and from interrenal incubation studies *in vitro* of 1 α -hydroxycorticosterone (Idler & Truscott 1966a). This substance, specific for elasmobranchs

and not found in any other animal, has been shown to be present in all species investigated, whether selachian or batoid (Truscott & Idler 1968a, Idler & Truscott 1972). The corticosterone 1 α -hydroxylating system is heat labile *in vitro* being completely inactivated above 30°C, which might account for the fact that it escaped earlier detection (Idler & Truscott 1967). It is now well accepted that 1 α -hydroxycorticosterone is the major circulating corticosteroid in the elasmobranchs. This has been confirmed quite recently. Kime (1977) has developed a radioimmunoassay procedure for the measurement of this steroid and the preponderance of 1 α -hydroxycorticosterone in elasmobranch plasma was also validated by RIA.

Table 3 gives a partial overview of corticosteroids found *in vivo* and *in vitro* in selachians and batoids. It should be pointed out that, in some instances, in addition to

Table 3 Corticosteroids of Chondrichthyes

Order and species	Steroid detected	Source	Reference
ELASMOBRANCHII			
Selachii			
<i>Squalus acanthias</i> (Spiny dogfish)	Corticosterone	Incubation of adrenal tissue <i>in vitro</i>	(1)
	1 α -Hydroxycorticosterone	Incubation of adrenal tissue <i>in vitro</i>	(2)
<i>Scyliorhinus caniculus</i> (dogfish)	1 α -Hydroxycorticosterone, corticosterone	Peripheral plasma	(3)
Several species of sharks	1 α -Hydroxycorticosterone, corticosterone, DOC	Peripheral plasma and incubation of adrenal tissue <i>in vitro</i>	(2)
Batoidea			
<i>Raja clavata</i> (thornback ray)	1 α -Hydroxycorticosterone, corticosterone	Peripheral blood	(3)
Several species of genus <i>Raja</i>	1 α -Hydroxycorticosterone, corticosterone, 11-dehydrocorticosterone, DOC	Peripheral blood, incubational of adrenal tissue <i>in vitro</i>	(4)
<i>Raja brachyura</i> (sandskate)	1 α -Hydroxycorticosterone, corticosterone, DOC	Incubation of adrenal tissue <i>in vitro</i>	(5)
HOLOCEPHALI			
<i>Hydrolagus colliiei</i> (ratfish)	Cortisol, 11-deoxycortisol, corticosterone	Incubation of adrenal tissue <i>in vitro</i>	(6)

References: (1) Bern et al. 1962; (2) Truscott and Idler 1968; (3) Kime 1977; (4) Idler and Truscott 1972; (5) Sandar et al. 1976 (6) Idler et al. 1969

1 α -hydroxycorticosterone, 11-deoxycorticosterone and corticosterone were also isolated and identified by both methodologies. These latter two steroids are intermediaries in the biosynthesis of 1 α -hydroxycorticosterone from earlier precursors and might also be secreted (*vide infra*).

It seems that 17-hydroxylated corticosteroids are not elaborated by elasmobranch adrenal tissue. Truscott and Idler (1972) failed to detect cortisol in the plasma of seven elasmobranch species by double isotope derivative assay, and Kime (1977) could not detect measurable amounts of cortisol (≤ 40 ng/100 ml plasma) in the plasma of the skate (*Raja clavata*) and the dogfish (*Scyliorhinus caniculus*). Similarly, the presence of aldosterone in elasmobranch adrenocortical secretion has yet to be established.

Holocephali, in contrast to elasmobranchs have been investigated to a much lesser extent. Early work (Bern et al. 1962) has suggested the synthesis of cortisol by ratfish adrenal tissue *in vitro*. This same species was subsequently reinvestigated by Idler and his group (Idler et al. 1969, 1971). Though member of the class of Chondrichthyes, the ratfish does not secrete 1 α -hydroxycorticosterone. According to available evidence, the ratfish is a cortisol secretor and as it will be shown later, its adrenocortical secretory pattern is very similar to that of bony fish (Sandor et al. 1976).

(ii) Gonadal steroids

The first estrogen isolated from nonmammalian vertebrate sources was from the ovary of the dogfish (*Squalus suckleyi*). Subsequent work on other elasmobranch ovaries yielded also, as in the case of *S. suckleyi*, estradiol-17 β and in some instances, estrone. These same steroids and occasionally estriol were found in the peripheral plasma of elasmobranchs (Ozon 1972).

Mammalian-type androgens have also been shown to be elaborated by cartilagin-

ous fish. In 1961, Chieffi and Lupo di Prisco isolated testosterone and androstenedione from the testes of the dogfish, *Scyliorhinus stellaris*. Testosterone in elasmobranch circulating plasma was first identified by Idler and Truscott (1966b). It should be noted that the above authors found testosterone in the plasma of both male and female *Raja radiata*, and *R. ocellata*. The production *in vitro* of testosterone from exogenous precursors was reported by Simpson et al. (1964) using testes of the dogfish, *Squalus acanthias*. Further work has confirmed the production of testosterone by elasmobranch testes (Ozon 1972a).

A special feature of the elasmobranch testis is the elaboration of testosterone in conjugated form. This was first noticed by Idler and Truscott (1966b) and recently confirmed by Kime (1978). He has shown that dogfish testes transformed exogenous progesterone and pregnenolone to free testosterone, small amounts of testosterone glucosidurinate and relatively large amounts of a testosterone conjugate, hydrolyzed by solvolysis and presumed to be sulfate esters. The physiological significance of the esterified testosterone is not clear.

(C) Osteichthyes

(i) Corticosteroids

In bony fish, cortisol is quantitatively the most important adrenocortical steroid hormone secreted and it has been demonstrated in the peripheral plasma of all bony fish investigated (Sandor et al. 1976). In addition to cortisol, variable amounts of cortisone, 11-dehydrocortisol and corticosterone have also been demonstrated (table 4).

Studies *in vitro* of adrenocortical tissue (head, kidney, posterior cardinal vein preparations) utilizing endogenous or exogenous precursors also show cortisol to be quantitatively the most important end product. Cortisone was isolated as a companion of

Table 4 Principal corticosteroids in Osteichthyes

Species	B*	F	E	ALD	DOC	S	Reference
<i>Acipenser oxyrinchus</i> (Atlantic sturgeon)	+	+	+				(1)
<i>Amia calva</i> (Atlantic bowfin)		+	+				(1)
<i>Anguilla anguilla</i> (European eel)	+	+	+		+	+	(1)
<i>Fundulus heteroclitus</i> (killifish)		+		+			(1)
<i>Salmo gairdneri</i> (rainbow trout)	+	+					(2)
<i>Salmo trutta trutta</i> (sea trout)	+	+		+	+	+	(3)
<i>Clupea harengus</i> (herring)	+	+	+	+			(1)
<i>Pseudopleuronectes americanus</i> (winter flounder)	+	+	+		+	+	(4)
<i>Hypoglossus hypoglossus</i> (Atlantic halibut)		+	+				(1)
<i>Lepidosiren paradoxa</i> (South American lungfish)	+	+		+		+	(1)

*B, corticosterone; F, cortisol; E, cortisone; ALD, aldosterone; DOC, 11-deoxycorticosterone, S, 11-deoxycortisol.

(1) Sandor et al. 1976; (2) Kime 1977; (3) Fuller et al. 1976; (4) Campbell et al. 1976

+ indicates presence

cortisol together with significant amounts of 11-deoxycortisol and smaller amounts of corticosterone (Idler & Truscott 1972, Sandor et al. 1976).

Some years ago, the nature of corticosteroids secreted by the lungfish has been examined. These fish are often regarded as transitional forms between bony fish and amphibians. Idler et al. (1972) analysed the peripheral blood of the South American lungfish (*Lepidosiren paradoxa*) and found cortisol, corticosterone, 11-deoxycorticosterone and aldosterone. These findings in part confirmed previous data reporting the transformation of progesterone to corticosterone by African lungfish (*Protopterus* sp.) adrenocortical tissue *in vitro* (Janssens et al. 1965). In this fish, rather large amounts of aldosterone were found (0.58 µg/100 ml as compared with 0.60 µg cortisol/100 ml and 0.16 µg corticosterone/100 ml), and this fact raises the still

controversial question of aldosterone production by bony fish adrenocortical tissue. For some time it was believed that bony fish adrenals do not produce 17-deoxycorticosteroids due to the intense 17-hydroxylating activity. Aldosterone was not readily detectable either *in vivo* or *in vitro* and the question of the production of this mineralocorticoid was rather hotly debated. Though Phillips et al. (1959) reported the presence of aldosterone in the plasma of the salmon, *Oncorhynchus nerca* and in the same year, Phillips and Mulrow reported the *in vitro* aldosterone biosynthesis from exogenous progesterone by killifish (*Fundulus heteroclitus*) adrenocortical tissue, these findings were questioned by Idler et al. (1959). In the following years *in vitro* experiments, mostly using the adrenocortical tissue of the European eel (*Anguilla anguilla*) consistently failed to demonstrate in a chemically convincing way aldosterone

biosynthesis from a variety of exogenous substrates (Sandor et al. 1966, 1967 a, b, Sandor 1969). In addition, 18-hydroxycorticosterone, the constant and obligatory companion of aldosterone, in all vertebrates proven to produce aldosterone (Sandor 1972), was absent in these experiments.

In the last 10 years, these questions were re-examined. Truscott and Idler (1968b) identified aldosterone and 18-hydroxycorticosterone as conversion products of exogenous corticosterone by herring (*Clupea harengus*) adrenocortical tissue. Subsequently, Truscott and Idler (1969) identified aldosterone in the peripheral plasma of the herring. Chavin and Singley (1972) found aldosterone in the goldfish and Pillai et al. (1974) isolated aldosterone and 18-hydroxycorticosterone from trout embryo incubates. In 1975, Whitehouse and Vinson reported the biosynthesis *in vitro* of aldosterone by whitefish (*Coregonus clupeoides*) interrenals and Fuller et al. found in 1976 aldosterone and 18-hydroxycorticosterone in the plasma of a number of salmonid fish. At present, the general consensus of opinion is that, though bony fish seem to have the biochemical equipment for 18-hydroxylation (Sandor et al. 1970), aldosterone is not a regular and obligatory biosynthetic product of the bony fish adrenocortical tissue (Chester Jones 1976). In addition, it might be possible that the ability for aldosterone biosynthesis is not present in all bony fish.

Table 4 summarizes some of the salient data on bony fish corticosteroid secretion.

(ii) Gonadal steroids

Estrogens have been identified in bony fish ovary by direct extraction (Ozon 1972b). The nature of these estrogens was reported as estradiol-17 β and estrone. Estradiol-17 β was also found as one of the biosynthetic products following the incubation of ovarian tissue with exogenous precursors. More recently, Yaron et al. (1977) have measured

estradiol-17 β in the peripheral plasma of the cichlid, *Tilapia aurea* by radioimmunoassay and concluded that the ovary was the main source of this steroid (See also Wingfield & Grimm 1976).

Interestingly, the ovary of teleost fishes is a rich source of a variety of steroids other than estrogens. Indeed, it seems that C₂₁ and C₁₉ steroids are more important secretory products, quantitatively and physiologically, than are C₁₈ phenolic steroids. Thus, the ovary of a number of teleosts has been shown to produce 11-deoxycorticosterone, 11-deoxycortisol, androstenedione, testosterone, 11-ketotestosterone and 11 β -hydroxytestosterone (Colombo et al. 1973, Colombo & Belvedere 1976, Katz & Eckstein 1974, Yaron et al. 1977, Truscott et al. 1978). It is now well established, that in teleost fish, gonadotropins induce oocyte maturation through the synthesis of C₂₁ steroids. In many species, the source of these C₂₁ steroids is the ovary though in some, like the catfish (*Heteropneustes fossilis*) it is believed that gonadotropins stimulate the adrenals to secrete the necessary C₂₁ compound (Sundararaj & Goswami 1977).

In bony fish and especially in teleosts (these have been most extensively investigated), testosterone, 11-ketotestosterone and 11 β -hydroxytestosterone are the major androgens. Originally, 11-ketotestosterone was isolated from salmonid plasma and shown to be biosynthesized by salmonid testicular tissue (for early references, see Ozon 1972). Subsequent works seem to indicate that 11-oxo testosterone derivatives are being secreted by all teleost testes and apparently, by teleost ovaries as well (Campbell et al. 1976).

It is apparent from the above discussion, that fish gonadal steroid secretion is somewhat different from that of other vertebrates. One has to underline especially the occurrence of 11-ketotestosterone which seems to play an important role as the major androgen in teleosts and which has not been

readily identified from gonads of other classes of vertebrates.

Biosynthesis of Steroids

In the preceding sections, the nature of steroids secreted by the different classes of fish has been established. It is apparent, that qualitatively, fish steroidogenic tissue elaborates, on the whole, the same type of compounds as does corresponding tissue from other vertebrate classes, including mammals. However, identity of a substance, originating from two different biological sources, does not necessarily mean that the biochemical mechanisms involved in the production of this substance are identical as well. Thus, it has been felt necessary to give an overview on our present knowledge on the biosynthesis of steroids by fish adrenal tissue and gonads.

(i) Corticosteroids

Very little is known about the biosynthesis of corticosteroids by jawless fish adrenocortical tissue and further studies will have to be conducted to establish, beyond reasonable doubt, the nature and site of corticosteroids elaborated by Agnatha.

The biosynthetic pathways operating in the chondrichthyan interrenal are better

known. In their original experiments, Idler and Truscott (1966a) have reported that 1α -hydroxycorticosterone was formed freely from exogenous corticosterone. Subsequently, Simpson and Wright (1970) have shown the transformation of pregnenolone to progesterone and that of progesterone to 11-deoxycorticosterone, corticosterone and 1α -hydroxycorticosterone by dogfish adrenocortical tissue preparations. We have, some time ago, repeated these experiments with an adrenal homogenate from the sand skate, *Raja brachyura* (Sandor et al. 1976). A homogenate, fortified with NADPH was incubated with ^{14}C -progesterone, ^{14}C -11-deoxycorticosterone or ^{14}C -corticosterone. These experiments (table 5) show beyond doubt that the biosynthesis of 1α -hydroxycorticosterone proceeds through the pathways: progesterone \rightarrow 11-deoxycorticosterone \rightarrow corticosterone \rightarrow 1α -hydroxyprogesterone. As the transformation of pregnenolone to progesterone by elasmobranch interrenal tissue has been previously reported (*vide supra*) the above pathway can be extended to include pregnenolone as the precursor of progesterone. Thus, the various *in vivo* experiments, together with the studies *in vitro* leave little doubt that the elasmobranch adrenal tissue operates with the same

Table 5 Cytochrome P-450 in fish adrenocortical tissue

Species	Cytochrome P-450 Content in nmol/mg Protein		References
	Mitochondria	Microsomes	
<i>Squalus acanthias</i> (dogfish)	0.43	0.51	(1)
<i>Galeocerdo cuvieri</i> (tiger shark)	0.10	Not measured	(2)
<i>Raja brachyura</i> (sand skate)	0.31	0.52	(1)
<i>Anguilla anguilla</i> (European eel)	0.72	0.74	(1)

(1) Lehoux et al. 1972; (2) Kimura et al. 1976

pathway as do other vertebrate adrenals (Sandor 1972). We have no ready knowledge on the precursors earlier than pregnenolone.

In the mid-sixties, it was established, that adrenal steroid hydroxylation requires a mixed function oxidase, denoted as cytochrome P-450 and an associated electron transport chain. In adrenal mitochondria, which contain in most vertebrates the cholesterol side-chain cleavage system, the 11 β and 18-hydroxylases, the reducing equivalents travel through the sequence: NADPH \rightarrow flavoprotein (FAD) \rightarrow adrenodoxin (nonhem iron protein) \rightarrow P-450 \rightarrow O₂. This system differs distinctly from that of adrenal cortex microsomal steroid hydroxylases, such as steroid-21-hydroxylase and steroid-17-hydroxylase: NADPH \rightarrow flavoprotein (FAD-FMN) \rightarrow P-450 \rightarrow O₂. The obvious difference is the absence of adrenodoxin in the microsomal system. (For leading references, see Sandor et al. 1976).

The above systems have been demonstrated at first in mammalian adrenal cortices. Subsequently, several groups, including ours, have shown that cytochrome P-450, indistinguishable in its spectral characteristics from the mammalian one, is present in non-mammalian vertebrate adrenal tissue and is necessary for steroid hydroxylation (Sandor 1972, Sandor et al. 1972a, 1975, 1976). In 1972, evidence was presented that dogfish (*Squalus acanthias*) and skate (*Raja brachyura*) adrenocortical mitochondria and microsomes contain P-450, in quantities similar to that of other vertebrate organelles (Lehoux et al. 1972, Sandor et al. 1972b). The presence of P-450 and the mitochondrial electron transport chain was further investigated in nonmammalian vertebrates by Kimura et al. (1976). Working with the adrenal tissue of the tiger-shark, they confirmed the presence of P-450 in mitochondria. However, they were unable to detect mitochondrial adrenodoxin by EPR spectrometry.

They drew the preliminary conclusion that in the tiger-shark, mitochondrial steroid hydroxylation might utilize an electron transport chain similar to the one detected in mammalian and avian microsomes.

Studies on corticosteroid biosynthesis by bony fish adrenocortical tissue are very numerous (for reviews, see Sandor 1969, Sandor et al. 1976, Ilder & Truscott 1972, Chester Jones et al. 1974). All studies, and these include the ones performed on the more primitive bony fish, such as the Atlantic sturgeon and the bowfin, indicate quite clearly that in Osteichthyes, cortisol-cortisone is the most important end product and that the general biosynthetic pathway on the whole is cholesterol \rightarrow pregnenolone \rightarrow progesterone \rightarrow 11-deoxycortisol \rightarrow cortisol, familiar from cortisol-producing mammals. In addition, it seems that 17-deoxycorticosteroids are synthesized as well through the pathway described for mammalian and other vertebrate adrenals (Sandor 1972, Sandor et al. 1976). Circumstantial evidence indicates that the so called Δ^5 -pathway also operates in bony fish adrenal tissue (Sandor et al. 1966, 1967a, b). Figure 1 gives a general overview of the authenticated biosynthetic sequences from cholesterol to corticosteroids in teleost adrenal tissue.

For a number of years, it was believed that bony fish adrenal tissue is not capable of biosynthesizing cholesterol from acetate and that the cholesterol, necessary for corticosteroid biosynthesis originated exclusively from plasmatic precursors. However, more recent results seem to indicate that at least in some species of teleosts, exogenous acetate is transformed, though in low yield, to cholesterol and corticosteroids (Colombo et al. 1972, 1977, Whitehouse & Vinson 1975).

Bony fish adrenocortical tissue shows a peculiarity not encountered in other species, except the holocephalean, the ratfish. Teleost and ratfish adrenals are capable, with quite

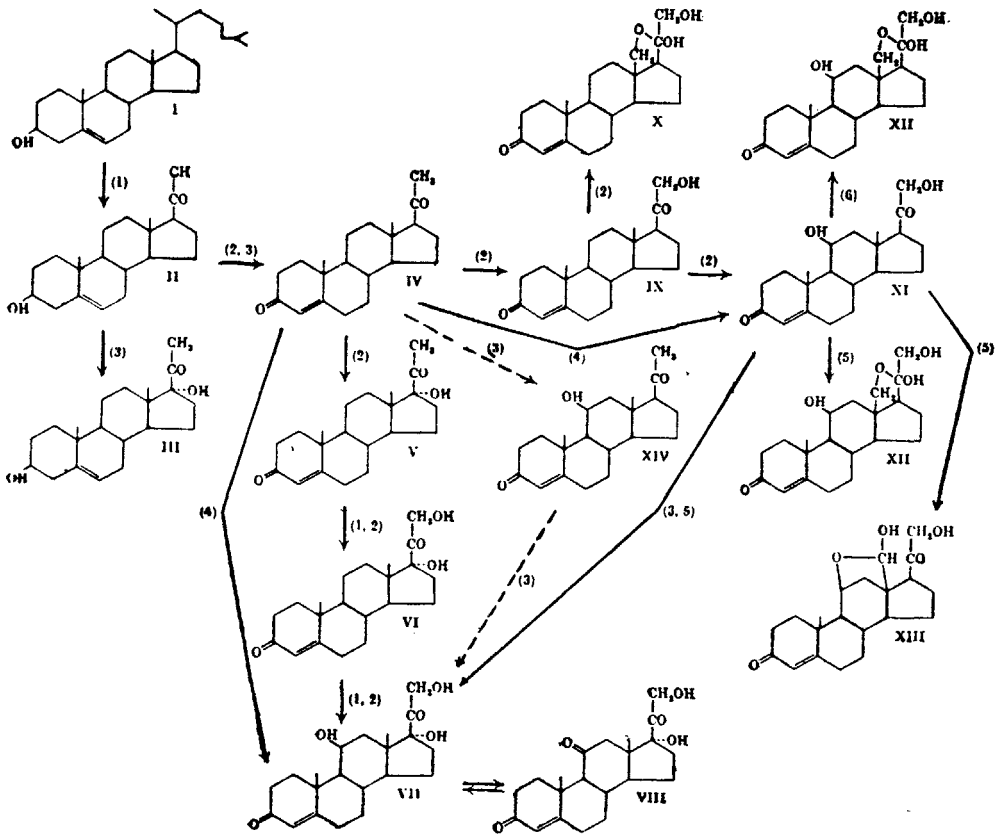


Figure 1 Schematic representation of corticosteroid biosynthetic pathways in teleost fish adrenocortical tissue, from cholesterol to corticosteroids. I, cholesterol; II, pregnenolone; III, 17 α -hydroxy-pregnenolone; IV, progesterone; V, 17 α -hydroxy-progesterone; VI, 11-deoxycortisol; VII, cortisol; VIII, cortisone; IX, 11-deoxycorticosterone; X, 18-hydroxy-11-deoxycorticosterone; XI, corticosterone; XII, 18-hydroxycorticosterone; XIII, aldosterone; XIV, 11 β -hydroxycorticosterone.

[References : (1) Sandor et al. 1967a,b; (2) Arai et al. 1969; (3) Sandor et al. 1966; (4) Leloup-Hatey 1966; (5) Truscott and Idler 1968; (6) Sandor et al. 1970].

good yield, to transform exogenous corticosterone to cortisol and exogenous 11-deoxycorticosterone to 11-deoxycortisol (Sandor et al. 1966, 1970, Truscott & Idler 1968b, Sangalang et al. 1972). It has been theorized that the only episodic presence of aldosterone in bony fish might be related to the corticosterone \rightarrow cortisol transformation, which effectively removes much of the biosynthesized corticosterone, the obligatory precursor of aldosterone.

Similar to cartilaginous fish, evidence has been presented relating to the occurrence of cytochrome P-450 in the adrenocortical mitochondria and microsomes of a teleost, the European eel, *Anguilla anguilla* (Lehoux et al. 1972, Sandor et al. 1972b). Table 5 shows a comparative picture of the quantitative aspects of the P-450 in fish adrenocortical mitochondria and microsomes. Unfortunately, we have no information about the presence of adrenodoxin in bony fish mitochondria.

Table 6 Steroidogenesis in vitro by adrenal tissue of the sand skate
(After Sandor et al. 1976)

Substrate	Products	Percentage Conversion
¹⁴ C Progesterone	11-Deoxycorticosterone	16.2
	Corticosterone	25.0
	1 α -Hydroxycorticosterone	10.1
¹⁴ C 11-DOC	Corticosterone	70.0
	1 α -Hydroxycorticosterone	43.0
¹⁴ C Corticosterone	1 α -Hydroxycorticosterone	67.0

Incubation was performed with a whole homogenate fortified with excess NADPH. Per cent transformation expressed as transformation per 41.7 mg of wet adrenal tissue. The incubation was performed at 25°C for 5.5 hours

A short note should be added relating to the presumptive endocrine role of the corpuscles of Stannius. For a number of years it was claimed that these organs act as additional adrenals and secrete corticosteroids. Their steroidogenic function was not always apparent (Chester Jones et al. 1965), though in some species the production of progesterone from pregnenolone and 11-deoxycorticosterone from progesterone was reported (Idler & Freeman 1966). Subsequently, Colombo et al. (1971) re-examined the steroidogenic capabilities of the corpuscles. Though they found 21-hydroxylase activity in the corpuscles of the salmon (*Salmo gairdneri*), this hydroxylase activity was less than that found in the kidney of the animal. In addition to 11-deoxycorticosterone, both the corpuscles and the kidney transformed exogenous progesterone to 5 α - and 5 β -pregnandione. They have concluded that to assign specific corticosteroidogenic activity to the corpuscles was probably unjustified.

(ii) Gonadal steroids

From available evidence it seems probable that fish ovaries and testes biosynthesize estrogens and androgens by the same biosynthetic path as do gonads of other vertebrates. The biosynthesis of 21-hydroxylated steroids by teleost ovaries follows the same route as

the biosynthesis of these compounds in vertebrate adrenals.

It was mentioned earlier that teleost gonads, both ovaries and testes, biosynthesize 11-ketotestosterone, apparently the major androgen of teleosts. This synthesis is not occurring in any other vertebrate gonads which are not capable of the 11 β -hydroxylation of C₁₉ precursors. The synthesis of 11-ketotestosterone is probably proceeding through acetate—cholesterol—pregnenolone—progesterone—17-hydroxyprogesterone—androstenedione—testosterone—11 β -hydroxytestosterone—11-ketotestosterone. Another pathway, presumed of less importance was shown to proceed through pregnenolone—17 β -hydroxypregnenolone—dehydro-epiandrosterone—5-androstenediol—5-androstene—3 β , 11 β , 17 β -triol—11 β -hydroxytestosterone—11-ketotestosterone (Ozon 1972a, b).

There is no report on the involvement of cytochrome P-450 in fish gonads.

Conclusions

This brief survey, which does not claim to be complete, underlines the fact that the steroidogenic mechanisms in all classes of fish are basically the same as those of other vertebrates. The two specific reactions, occurring only in fish adrenal tissue and not yet

documented in any other class of vertebrates are shown in figure 2. These are the 1α -hydroxylation of corticosterone to 1α -hydrocorticosterone by elasmobranch adrenal

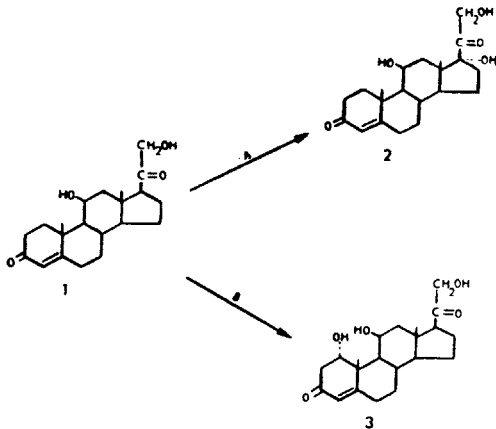


Figure 2 Biosynthetic reactions specific to piscine adrenocortical tissue. 1, Corticosterone; 2, cortisol; 3, 1α -hydroxycorticosterone

Reaction A: Validated in adrenocortical tissue of teleost and the holocephalean, the ratfish

Reaction B: Validated in all elasmobranch adrenocortical tissue. 1α -Hydroxycorticosterone does not seem to be synthesized by any other vertebrate adrenal.

tissue (Idler & Truscott 1966a) and the 17β -hydroxylation of 17-deoxycorticosteroids by teleost adrenal tissue (Sandor et al. 1966). Another reaction, peculiar to fish is the 11β -hydroxylation of C_{19} steroids by bony fish gonads. And finally, we should mention the observation of Kimura et al. (1976) regarding the possible lack of adrenodoxin in the adrenocortical mitochondria of a cartilaginous fish, the tiger shark. From these data, it is difficult to decide whether steroidogenesis in vertebrates did undergo evolutionary changes. The exceptions in biosynthesis and the compounds found only in fish are, as mentioned, few and the possibility always exists that these are by no means primitive features but acquired through the adaptive

radiation of the obligatory water dwellers.

The enzyme "deficiencies", such as the apparent lack of 17-hydroxylation and 18-oxygenation in elasmobranch interrenals should be treated with caution. It was only recently shown that rat adrenal might produce cortisol (Vinson et al. 1978) and the original report on cortisol production by birds adrenals (Sandor & Lantheir 1963) was substantiated only 15 years later with the explanation that cortisol is a secretory product of embryonic and young birds only (Nakamura et al. 1978).

It was mentioned in the introduction that it is believed that a large part of the vertebrate steroid biosynthetic system, as we know it now, has originated with Ordovician fish. But did it really originate with the craniate ancestor? Recent evidence shows that invertebrates, and especially marine invertebrates are capable of steroid and especially androgen and estrogen biosynthesis and one should open one's mind to the possibility that our distant ancestor carried already in his genetic make-up coding for steroid biosynthesis and utilization, inherited from its invertebrate antecedents (Sandor et al. 1975, Sandor & Mehdi; in press).

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