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**Ultrastructural Changes of Testicular Interstitial Cells of Silver
Japanese Eels, *Anguilla japonica*, Treated with Human
Chorionic Gonadotropin**

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Abstract

Testicular interstitial cells of silver males of the Japanese eel, *Anguilla japonica*, are present as prominent clusters of several cells in the interlobular spaces. They are characterized ultrastructurally by having relatively evident cytoplasmic organelles such as a poorly developed smooth endoplasmic reticulum, mitochondria with lamellar cristae, a large amount of free ribosomes and an ill-developed Golgi complex.

Administrations of human chorionic gonadotropin (HCG) to the immature silver males could induce a rapid occurrence of spermatogenesis resulting in a complete maturation of testes. The treatment caused simultaneous changes in the ultrastructure of testicular interstitial cells. Mitochondria, which showed a considerable increase both in size and in number, underwent conspicuous elongation and became provided with a large number of tubular cristae. Smooth endoplasmic reticulum with tubular and vesicular cisternae was increased in amount gradually as HCG injections were repeated. Numerous free ribosomes came to be scattered in rosettes throughout the cytoplasm, and the Golgi complex appeared to be increased in size and in complexity in the HCG-stimulated interstitial cells. Thus it is concluded that testicular interstitial cells of silver males of the Japanese eel are apparently responsive to HCG to develop their steroidogenic function.

To date, considerable attention has been paid on testicular steroidogenesis in teleost fishes, and ultrastructural characteristics of testicular steroidogenic cells have been elucidated in various species of teleosts.¹⁾²⁾³⁾ There is, however, little information from an ultrastructural point of view regarding the differentiation and functional development of testicular steroidogenic cells in teleost fishes. So far as we know, the stickleback, *Gasterosteus aculeatus*⁴⁾, and the cichlid fish, *Cichlasoma nigrofasciatum*⁵⁾, are only those in which the differentiation and development of testicular interstitial cells have been examined by electron microscopy during their reproductive cycle.

Silver males of the Japanese eel, *Anguilla japonica*, captured in the river, usually have immature testes in which spermatogonia are the preponderant constituent. During the course of experiments carried out in our laboratory on artificial maturation of the eels, the administration of human chorionic gonadotropin (HCG) to the male was shown to be effective in causing full testicular maturation. The treatment with HCG also caused prominent melanogenesis on the pectoral

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fins and other parts of the body in treated males, which led us to surmise a probable contribution of androgenic hormones secreted from the stimulated testes. However, no report has hitherto been concerned with ultrastructural observations on testicular steroidogenic cells of the Japanese eel as well as other species of eels, though there are some which deal only with ultrastructural studies on spermiogenesis and spermatozoa in eels⁶⁾⁷⁾⁸⁾.

Thus the present study was conducted to determine ultrastructural characteristics of testicular interstitial cells of silver Japanese eels at the commencement of their catadromous migration and, in addition, to examine the possibility of functional stimulation of these cells under the influence of the HCG treatment.

Material and Methods

Silver males of the Japanese eel, *Anguilla japonica*, ranging from 55.5 to 61.2 cm in body length and from 225 to 370 g in body weight, were collected in rivers around Hiranuma, Aomori Prefecture, Japan, during the late autumns of 1976 and 1977. The fish were transported to the laboratory and were then acclimated stepwise to sea water. They were kept, without feeding, in concrete tanks containing sea water circulated with aeration and regulated at about 18°C under natural daylength. They were subjected to intramuscular injections of 250 IU of human chorionic gonadotropin (HCG; Gonatropin, Teikoku Hormone Mfg. Co., Tokyo) per fish once a week until up to full testicular maturation.

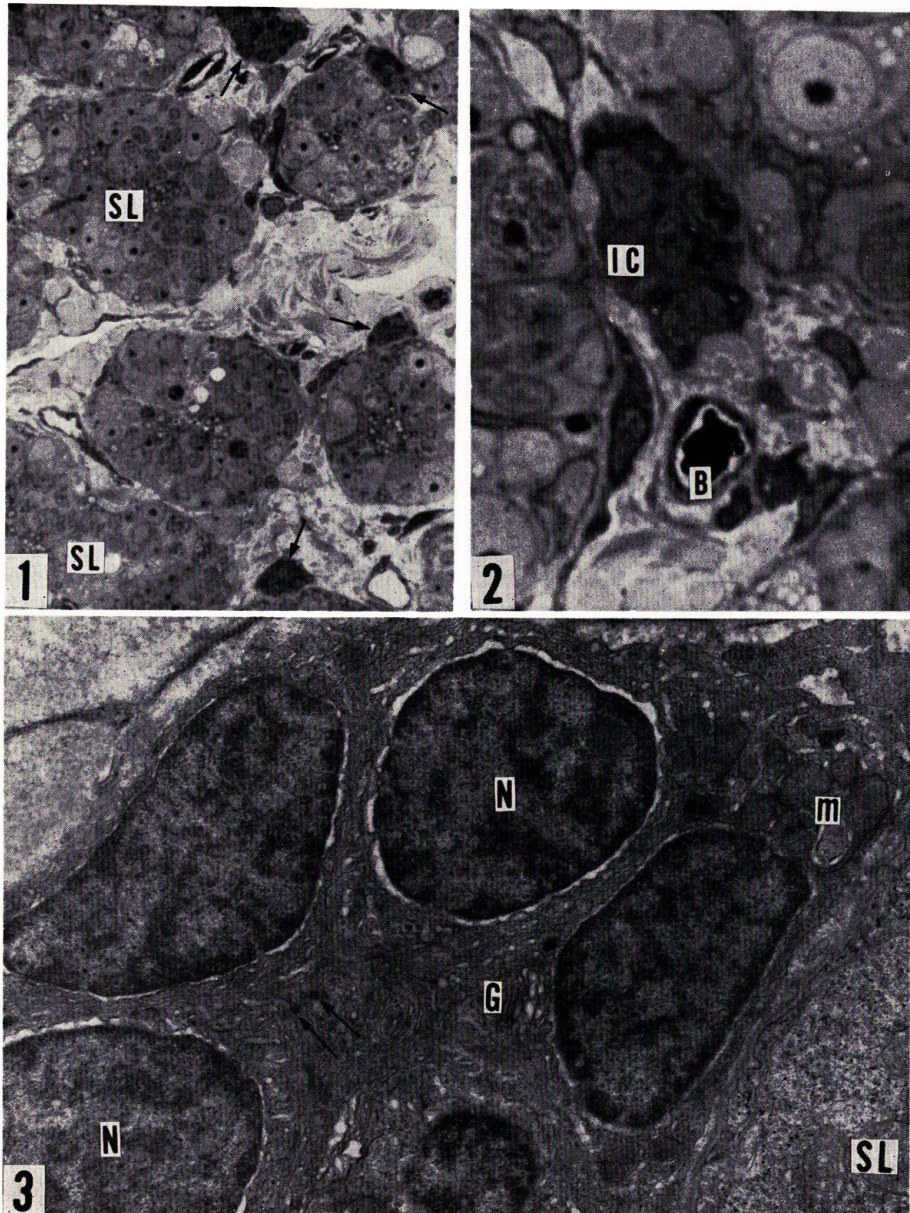
At the beginning of the HCG treatment and on the 3rd and the 7th day after each of the weekly injections of HCG, small pieces of the testis of the treated fish were sampled by laparotomy so as to explore ultrastructurally the changes of testicular interstitial cells in each individual fish which was marked by different colored tags.

The excised pieces of the testis were fixed immediately in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C or in Karnovsky's fixative⁹⁾ at room temperature for 3 hours, and then postfixed in Millonig's osmium tetroxide solution or in 1% osmium tetroxide in 0.2M cacodylate buffer (pH 7.4) for 2 hours at 4°C. Ultrathin sections of Epon-embedded specimens were cut with glass knives on a Porter-Blum ultramicrotome, stained with uranyl acetate followed by lead citrate, and observed with a Hitachi HU-12 electron microscope. Parallel sections cut at about 1 μ m were stained with methylene blue for light microscopic observations.

Results

Testicular interstitial cells of eels at the beginning of treatment

The testes of silver males of the Japanese eel at the commencement of their downstream migration were still thin and rather transparent in external appearance, but showed a distinct lobation along their whole length. Gonadosomatic indices (GSI, gonad weight \times 100/body weight) of 2 fish autopsied at the beginning of treatment were 0.27 and 0.39%, respectively.



Figs. 1 and 2. Light microscopic figures of sections through Epon-embedded testes of silver eels at the beginning of treatment. Seminal lobules (*SL*) contain cysts of spermatogonia together with some cysts of primary spermatocytes. Interstitial cells (*IC* and arrows) are present as clusters of a few cells located in an interlobular space. *B*, blood capillary. Methylene blue stain. Fig. 1, $\times 570$; Fig. 2, $\times 2,100$.

Fig. 3. Electron micrograph of testicular interstitial cells of a silver eel at the beginning of treatment. Arrows indicate desmosomal junctions between the neighbouring cells. *G*, Golgi complex; *m*, mitochondrion; *N*, nucleus; *SL*, seminal lobule. Fixed with 2.5% glutaraldehyde and Millonig's OsO_4 . $\times 12,350$.

The testes consisted of numerous seminal lobules containing many cysts of spermatogonia together with some cysts of primary spermatocytes (Fig. 1). The progress of the initial spermatogenetic process appeared to vary in different specimens and even in different seminal lobules of the same testis. Seminal lobules, which were confined each by a thin wall, were separated from each other by interlobular spaces of varying widths in which blood capillaries, elongated fibroblast-like cells and clustered interstitial cells existed among collagen fibrils (Fig. 2). The interstitial cells were present as aggregates of a few, darkly stained cells near blood capillaries. The cell had a roundish, oval or polygonal nucleus with indistinct nucleoli and a relatively narrow cytoplasm. The boundaries of the cells were usually indistinct light microscopically. Numerous large granular bodies (probably mitochondria), which were stained deeply with methylene blue, were invariably encountered in the cytoplasm (Fig. 2).

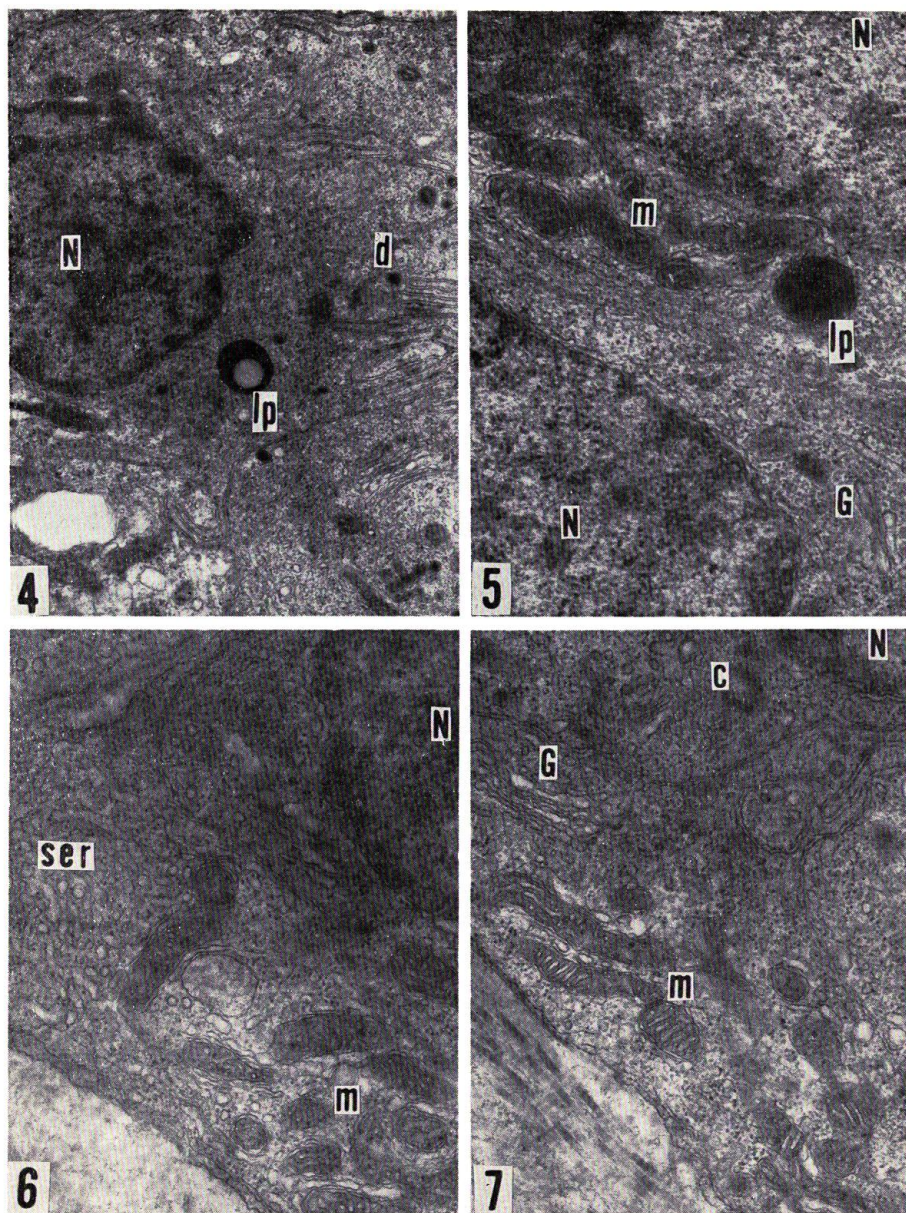
By electron microscopy, aggregated interstitial cells were found generally in close contact with the basal lamina of seminal lobules (Fig. 3). The cells were generally polyhedral in shape but often irregular in contour. They made compact clusters leaving obscured intercellular spaces. The neighbouring cells usually showed a complicated interdigitation of their plasma membranes which were connected with desmosomal junctions (Figs. 3 and 4). In the nucleus of these cells, the chromatin was observed to be partly dispersed finely and partly aggregated especially adjoining the nuclear envelope.

In the cytoplasm of these cells, mitochondria were the most conspicuous organelles. They were generally round or oval in form, but were irregularly elongated in some cases (Figs. 5-7). They possessed a few, usually indistinct cristae of apparently lamellar configuration, though some of them appeared to have a few tubular cristae. The mitochondrial matrix was higher in electron density than the surrounding cytoplasm and contained a small number of much dense, minute granules (Figs. 5-7). Golgi complexes were only a few in number and meager in development displaying a parallel array of a few flattened sacs and small vesicles gathering around the sacs (Figs. 5 and 7). Centrioles were occasionally observed to be present adjacent to the Golgi elements.

The endoplasmic reticulum was poorly developed in most of the cells. It was composed exclusively of smooth-surfaced cisternae which usually assumed either a vesicular or a short-tubular aspect. In the interstitial cells of the testis which contained a larger amount of spermatocytes, there appeared to be a somewhat increased amount of smooth endoplasmic reticulum as compared with that of the testes showing less advanced spermatogenesis (Fig. 6). Free ribosomes were abundant and were distributed uniformly throughout the cytoplasm. Besides these organelles, lipofuscin pigment granules and dense bodies were also found in some interstitial cells (Figs. 4 and 5).

Testicular interstitial cells of eels treated with HCG

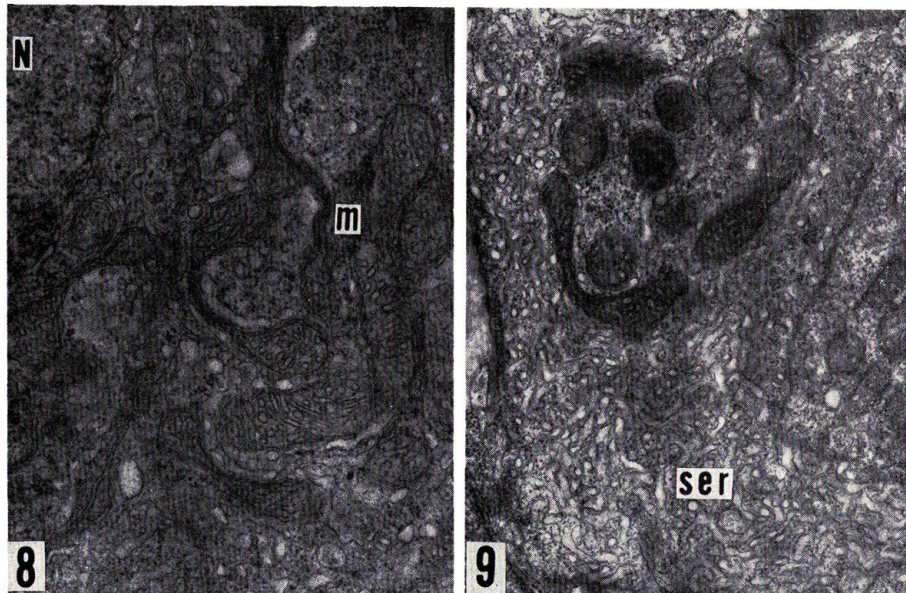
Injections of HCG could effectively stimulate the spermatogenesis in all treated fish. After 3 weekly injections with HCG, mature spermatozoa began to appear in the lobule lumina of the affected testes, and after 5 injections, a considerable amount of milt could be obtained by hand-stripping from these fish.



Figs. 4-7. Electron micrographs of testicular interstitial cells of silver eels at the beginning of HCG treatment, showing a complicated interdigitation between the two neighbouring cells (Fig. 4), mitochondria (*m*) with lamellar cristae and intramitochondrial granules (Figs. 5-7), a relatively large amount of smooth endoplasmic reticulum (*ser*) (Fig. 6), and relatively well-developed Golgi complex (*G*) and centriole (*c*) (Fig. 7). *d*, dense body; *lp*, lipofuscin pigment granule; *N*, nucleus. Fixed with Karnovsky's fixative and 1% OsO₄. Fig. 4, $\times 11,300$; Fig. 5, $\times 20,900$; Figs. 6 and 7, $\times 21,500$.

As testicular maturation proceeded, interlobular spaces in the testes became increasingly narrow, without showing any notable change in the number of testicular interstitial cells in those spaces.

As early as 3 days after the 1st injection of HCG, some changes were observed to occur in the testicular interstitial cells of the treated eels. Mitochondria under-



Figs. 8 and 9. Electron micrographs of testicular interstitial cells of silver eels subjected to the HCG treatment. Fig. 8 shows considerably elongated and distorted mitochondria (*m*) with numerous tubular cristae. In Fig. 9, the cytoplasm of the cell is filled with numerous tubular cisternae of smooth endoplasmic reticulum (*ser*). *N*, nucleus. Fixed with Karnovsky's fixative and 1% OsO₄. Fig. 8, $\times 18,500$; Fig. 9, $\times 14,100$.

went a considerable increase both in size and in number. Many of them were seen to be elongated to become very thin at their middle portion, assuming in many cases a bilobed or a horseshoe-shaped feature (Fig. 8). They were provided with a large number of cristae which were evidently of the tubular type. Intramitochondrial granules, which were ordinarily present in the mitochondria of the interstitial cells of immature testes, were no more encountered in the cells at that period of HCG treatment. These states of mitochondria were subsequently kept unaltered in the interstitial cells throughout the HCG treatment.

Another change observed in the affected cells was an increase in amount of smooth-surfaced cisternae of the endoplasmic reticulum, which tended to be augmented gradually in relation to the repeated HCG injections. The cisternae were mostly tubular in form in the majority of the affected interstitial cells (Fig. 9), though they were largely vesicular in form in some of them.

Free ribosomes were still abundant and were scattered in rosettes throughout the cytoplasm (Fig. 8). Centrioles were prominent in some of the HCG affected cells. A mitotic figure of a testicular interstitial cell was found 7 days after the 1st injection of HCG (Fig. 10).

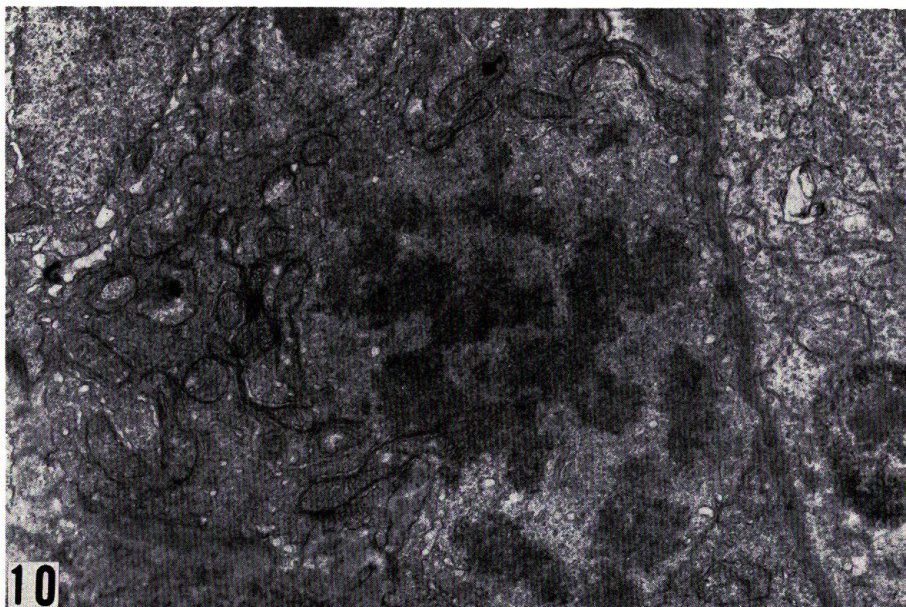
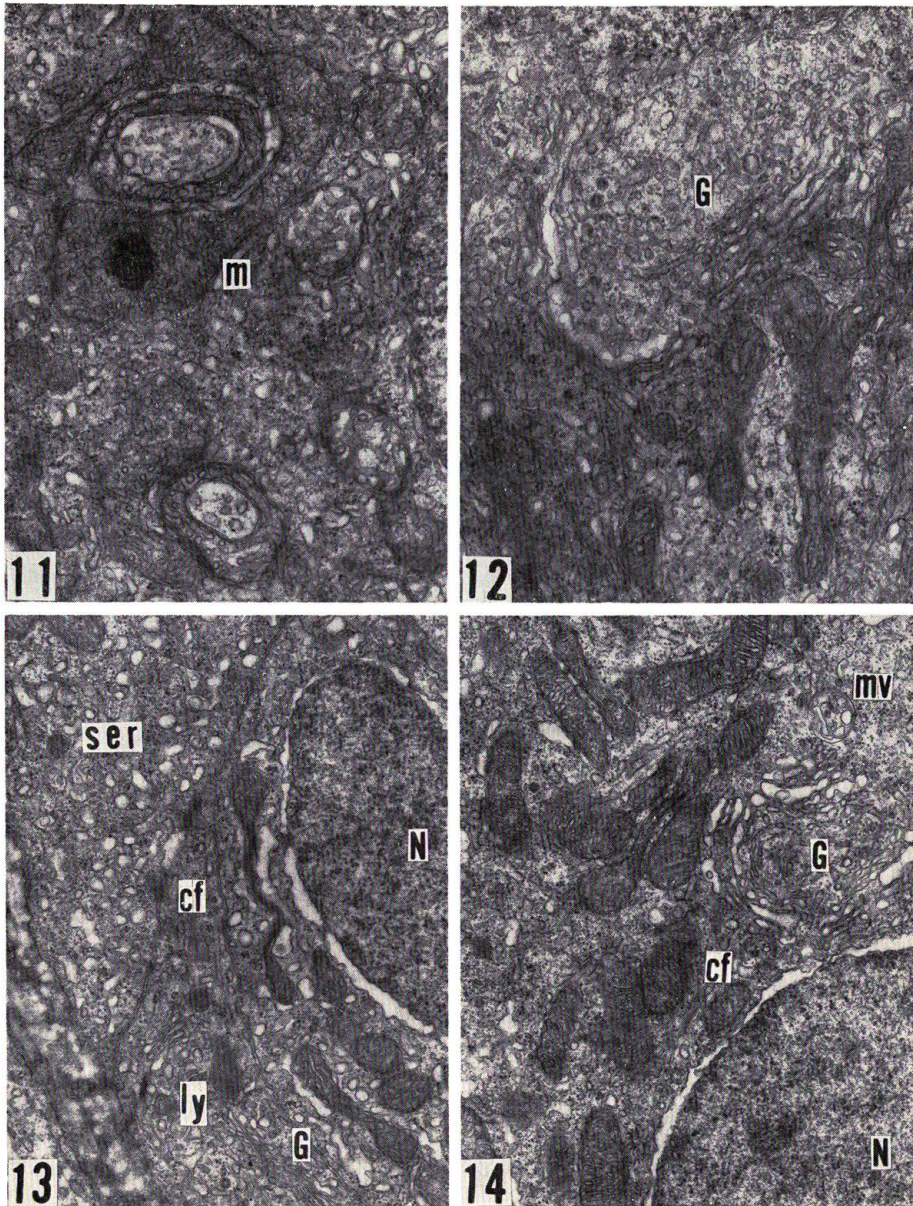


Fig. 10. A mitotic figure of a testicular interstitial cell of a silver eel examined 7 days after the 1st injection of HCG. Fixed with Karnovsky's fixative and 1% OsO₄.
× 11,800.

In the testes of the eels treated with HCG, aggregated interstitial cells which possessed nearly spherical nuclei with prominent nucleoli could be observed frequently. They were joined by desmosomes with one another, but displayed no remarkable interdigitation of plasma membranes of neighbouring cells. Accumulations of osmiophilic material, which were presumed to be intramitochondrial lipid, were frequently seen in the mitochondria of these cells (Fig. 11). The Golgi complex in some of the cells appeared to be increased in size and in complexity; although their basic organization composed of parallel lamellae and vesicles was retained, both components were more plentiful and more extensively distributed in these cells than those in the ordinary interstitial cells (Fig. 12). Other organelles which could be occasionally found in the cytoplasm were membrane-bound lysosomal bodies and small dense bodies. In addition, cytofilaments and multivesicular bodies were also present in a few HCG-affected interstitial cells (Figs. 13 and 14).



Figs. 11-14. Electron micrographs of testicular interstitial cells of silver eels subjected to the HCG treatment, demonstrating a stimulated development of mitochondria (*m*) with accumulation of osmiophilic material (Fig. 11), Golgi complex (*G*) (Figs. 12 and 14), and vesicular cisternae of smooth endoplasmic reticulum (*ser*) (Fig. 13). Cytofilaments (*cf*), multivesicular bodies (*mv*) and lysosomes (*ly*) are encountered occasionally in the cells. *N*, nucleus. Fixed with Karnovsky's fixative and 1% OsO₄. Fig. 11, $\times 17,500$; Fig. 12, $\times 19,700$; Figs. 13 and 14, $\times 14,000$.

Discussion

Ultrastructural features of steroidogenic cells of the testis have been described to date in a variety of teleost fishes such as *Poecilia reticulata*¹⁰⁾, *Salmo gairdneri*¹¹⁾, *Gasterosteus aculeatus*⁴⁾, *Cichlasoma nigrofasciatum*⁵⁾, *Gobius joso*¹²⁾ and *Mollienisia latipinna*¹³⁾. These investigations have clarified several common features of functional steroidogenic cells in fish testes, i.e. the presence of a large amount of smooth endoplasmic reticulum and the occurrence of mitochondria with tubular or vesicular cristae.

The testis of silver eels at the beginning of treatment had distinct interstitial cells which were distinctly different in appearance and in fine structure from other cells appearing in interlobular spaces; they were aggregated in small clusters and contained poorly developed smooth endoplasmic reticulum, mitochondria with lamellar cristae, a large amount of free ribosomes and ill-developed Golgi complex. The interstitial cells appear to be comparable in ultrastructural organization to immature Leydig interstitial cells of other vertebrate animals¹¹⁾¹⁴⁾¹⁵⁾.

Since it has been shown that testicular interstitial cells of immature rainbow trout, whose testes have seminal lobules filled with many spermatogonia only, possessed mitochondria with tubular cristae along with a poorly developed smooth endoplasmic reticulum¹¹⁾, it seems likely that the testicular interstitial cells of silver eels at the beginning of treatment are in a much indifferent state as to their functional activity. In the testes of the eel, however, the transformation of spermatogonia into spermatocytes had already begun in most seminal lobules. It is uncertain at present whether the initiation of spermatogenesis in these testes may reflect some secretory activity of the interstitial cells in view of the reported facts which suggest the involvement of male sex steroids in the transformation of spermatogonia into spermatocytes¹⁾¹⁶⁾.

In the present study, the treatment of silver eels with HCG could bring about a rapid stimulation of spermatogenesis with an ensuing complete maturation of the testis. In parallel with such an accelerated development of the testis, testicular interstitial cells were also activated in function. In the cells of the eels administered with a single dose of 250 IU of HCG, notable changes took place first in the shape, size and structure of mitochondria which seemed to be the first to react to the exogenous hormone. A similar case was reported by Russo and Sacerdote¹⁷⁾ for the Leydig cells of adult mice subjected to the HCG treatment. These results may suggest that the mitochondria of testicular steroidogenic cells are a target for gonadotropins. This suggestion seems to receive support from the observation of Christensen¹⁸⁾ who showed that the mitochondria of Leydig cells in hypophysectomized rats decreased in size and lost their internal structure.

It has been suggested from morphological studies that the smooth endoplasmic reticulum is implicated closely in steroid production.¹⁹⁾²⁰⁾²¹⁾ The smooth endoplasmic reticulum of testicular interstitial cells of HCG-treated eels became gradually increased in amount with the repetition of HCG injections. HCG has been shown to be capable of causing a proliferation of Leydig cells accompanied with an increase in amount of their smooth endoplasmic reticulum in some mammals¹⁵⁾²²⁾²³⁾. The presence of prominent centrioles and of mitotic figures in testicular interstitial cells of the treated eels is indicative of an accelerated proliferation of the cells

affected by the gonadotropin. It has been demonstrated that, in the testis of the stickleback, *Gasterosteus aculeatus*, mitoses occur in interstitial cells undergoing structural differentiation by the influence of pituitary hormones⁴⁾.

The development of the Golgi complex and the disappearance of lipid droplets in HCG-affected Leydig cells have been described in some mammals¹⁷⁾²²⁾. In some testicular interstitial cells of HCG-treated eels, the development of the Golgi complex was also evident. On the other hand, the interstitial cells of the eels were completely devoid of lipid droplets at the beginning of, and during, the treatment. The absence of lipid droplets in testicular interstitial cells has been noted also in other teleost species⁴⁾¹⁰⁾¹¹⁾²⁴⁾, but lipids are apparently present as intramitochondrial inclusions in *Salmo gairdneri*¹¹⁾ and *Oryzias latipes*²⁴⁾. Intramitochondrial granules of a similar aspect were also detectable in the interstitial cells of the eels at the beginning of the treatment, and they disappeared quickly following the HCG treatment. Thereafter, accumulations of osmiophilic material appeared in the mitochondria of the affected cells. These changes are similar to those observed by Aoki and Massa²⁵⁾, who showed that lipid droplets of testicular interstitial cells in LH-treated adult mice decreased in number and dense bodies resembling lipids appeared in the mitochondrial matrix.

The treatment with HCG brought about a change in the free ribosomes in affected testicular interstitial cells. While they were seen scattered singly throughout the cytoplasm before the HCG treatment, they became assembled into rosettes after the treatment. Similar changes of free ribosomes were observed also in the Leydig cells of LH-treated chick²⁶⁾.

Thus it may be concluded from the ultrastructural evidence obtained in the present study that the Japanese eels possessed interstitial cells of possible steroidogenic nature in the testis at the initial phase of their catadromous migration, and that the interstitial cells are apparently responsive to HCG to develop their steroidogenic function. It seems most likely that the secretion of sex steroids may also be stimulated by the HCG treatment. However, exact correlation between the stimulated testicular interstitial cells and the accelerated spermatogenesis in the eel is open to question at present.

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